30 June - 5 July ○ Palacio Euskalduna

info@euromar2024.org

www.euromar2024.org

POSTERS ABSTRACT BOOK

Organizing Committee

Oscar Millet, Chair
CIC bioGUNE
Ignacio Alfonso
CSIC
Jesús Angulo
CSIC
Ganeko Bernardo-Seisdedos
CIC bioGUNE
Eurico Cabrita
Universidade Nova de Lisboa
Sonia Cabral de Menezes
AUREMN- NMR
Tammo Diercks
CIC bioGUNE
Nieves Embade
CIC bioGUNE
Marga Gairí
University of Barcelona
Douglas Laurents
CSIC
Ana Poveda
CIC bioGUNE

Scientific Commitee

Óscar Millet
CIC bioGUNE
Joseba Alonso
MRILab
Christian Bonhomme
Sorbonne-Univ./CNRS
Melanie Britton
University of Birmingham
Matthias Ernst
ETH Zurich
Jesús Jiménez-Barbero
CIC bioGUNE
Anne Lesage
CNRS
Joanna R. Long
UFHealth
John Morton
University College London
Miquel Pons
bioNMR
Michael Tayler
ICFO
Christina M Thiele
Centre for Environmental Science
Anna Zawadzka-Kazimierczuk
University of Warsaw

BCH-002: Benchtop NMR Spectroscopy Distinguishes Metabolic Profiles in Stratifying COVID-19 Patient Severity

Pilar Alonso-Moreno[1], Antonio Ferruelo[2,3], MD Carolina Gotera[4], Raquel Murillo[2,3], MD Maria de los Angeles Zambrano[4], Zuriñe Blasco[2,5], MD Raquel Herrero[2,3], MD Germán Peces-Barba[2,4], PhD Jesus Ruiz-Cabello[1,2,5], MD Jose Angel Lorente[2,3], Phd Jose Luis Izquierdo García[1,2]
[1]Universidad Complutense, Madrid, Spain, [2]CIBER Enfermedades Respiratorias (CIBERES), Madrid, Spain, [3]Hospital de Getafe, Getafe, Spain, [4]Hospital Fundación Jimenez Diaz, Madrid, Spain, [5]CIC biomaGUNE, Donostia, Spain

Introduction: The COVID-19 pandemic has posed an unprecedented global health challenge, straining healthcare systems worldwide in their efforts to manage and treat patients suffering from this viral respiratory illness. A critical aspect of this challenge is identifying and stratifying COVID-19 patients early in their disease course to optimize clinical decision-making.
Aims: To investigate the potential of a benchtop NMR (bNMR)-based metabolomic approach for stratifying individuals based on their clinical outcomes.
Methods: Serum samples were collected upon hospital admission from a cohort of COVID-19 positive patients. To assess disease severity, patients were classified into three groups:
- Discharged (n=83): No longer required hospitalization.
- General Ward (n=21): Admitted to general medical wards and did not receive mechanical ventilation, except for four patients receiving High Flow Oxygen Therapy (HFOT).
- ICU (n=32): Required intensive care and commenced mechanical ventilation within 48 hours of admission, except for three patients who did not receive mechanical ventilation.

Prior to analysis, samples underwent ultracentrifugation to remove lipids and proteins that could interfere. Metabolite profiles were then analyzed using a Magritek Spinsolve 80 MHz NMR spectrometer.
Results: Principal component analysis (PCA) of the NMR spectra effectively distinguished all three patient groups (discharged, general ward, ICU) based solely on the first two principal components. Notably, samples from patients receiving HFOT outside the ICU and non-ventilated ICU patients clustered at the boundary between ICU and non-ICU groups, suggesting a potential novel metabolic cluster. This finding reinforces the correlation between the observed metabolic profile and the severity of the disease.
Conclusions: This pilot study demonstrates the potential of bNMR for stratifying COVID-19 patients based on their metabolic fingerprints. The observed correlation between metabolic profiles and disease severity warrants further investigation. Additionally, the affordability and portability of bNMR technology hold promise for wider implementation of metabolomics in clinical settings.

BCH-003: Benchtop nuclear magnetic resonance-based metabolomic for the diagnosis of caprine tuberculosis

PhD Candidate Pilar Alonso-Moreno[1,2], Paula Ortiz[1], Javier Ortega[3,4], Carlos Velasco[3], Alba López[1], Javier Bezos[3,4], Jose L. Izquierdo-García[1,2,5]
[1]NMR and Imaging in Biomedicine Group, Complutense University of Madrid, Madrid, Spain, [2]Department of Chemistry in Pharmaceutical Sciences, Pharmacy School, Complutense University of Madrid, Madrid, Spain, [3]VISAVET Health Surveillance Centre, Complutense University of Madrid, Madrid, Spain, [4]Department of Animal Health, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid, Spain, [5]CIBER de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain

Introduction: Goats are one the main reservoir of tuberculosis (TB) in animals, a mycobacterial disease and with a significant sanitary and economic impact in public and animal health. The ante mortem diagnosis of this disease is complicated due to the limited sensitivity of the diagnostic techniques. Nuclear Magnetic Resonance (NMR)-based metabolomics is a potential diagnostic tool for identifying biomarkers and understanding metabolic changes associated with different diseases. However, high-field NMR has several limitations due to the high cost and large size. Benchtop NMR spectrometer is proposed as a compact, low-cost alternative to facilitate the use of this technique in livestock farms.

Aims: This study aims to identify biomarkers for the diagnosis of TB in goats and to demonstrate the effectiveness of benchtop NMR instruments in diagnosis.

Methods: Three experimental groups were used in this study: TB-infected (n=26), healthy controls (HC, n=25) and paratuberculous-infected (PTB, n=16) goats. PTB is a chronic disease caused by non-tuberculous mycobacteria, but it may cause similar clinical signs and diagnostic interferences. Groups were selected based on previous analysis using the gold standard. A metabolomic study of 67 serum samples was conducted using NMR (700 MHz Bruker AVIII NMR spectrometer and 80 MHz Magritek NMR spectrometer), and multivariate statistical analyses were performed on the results.

Results: Multivariate statistical analyses demonstrated metabolic differences in the different experimental groups with both spectrometers. Results compared three groups: 1) TB VS HC; 2) TB VS PTB; 3) PTB VS HC. The unsupervised analysis (Principal Component Analysis; PCA) managed to separate the different groups based solely on their metabolic profile. The qualifying models demonstrated 100% accuracy and cross-validation values for confirmation of diagnostic biomarkers.

Conclusions: We demonstrated benchtop NMR instruments had similar effectiveness that high-field NMR spectrometers and we promote it to be used in diagnostic routines.

BCH-004: Low-micromolar quantification of fluorinated analytes using SABRE-hyperpolarised 19F benchtop NMR

Mrs Ana I. Silva Terra[1], Dr Meghan E. Halse[1]
[1]University of York, York, United Kingdom

Benchtop NMR spectrometers (1 – 2.4T) present a more accessible and sustainable alternative to their high-field analogues (≥ 7T). However, this comes at a cost in terms of sensitivity and chemical shift resolution limiting this technology's full analytical potential. Here were present a combination of strategies aimed at improving the sensitivity of benchtop NMR spectrometers to aid the development of analytical applications.

This increase in sensitivity is achieved by using the hyperpolarisation technique SABRE (Signal Amplification By Reversible Exchange). In SABRE, the high spin order of parahydrogen is used as a source of polarisation which is transferred to a target molecule through reversible binding to an iridium catalyst. [1] This hyperpolarisation technique is relatively cheap and easy to implement, which is well suited in combination with benchtop NMR as it does not compromise its accessibility. In order to reduce signal overlap issues commonly seen at lower magnetic fields, we exploit the wider chemical shift range of ^{19}F NMR measurements. The analytical performance of SABRE-hyperpolarised benchtop NMR was further improved by using the sensitivity-enhancing SHARPER pulse sequence and by using co-substrates to decrease limits of detection and enable quantification [2].

In this work, the analytical potential of SABRE-SHARPER ^{19}F benchtop NMR (1T) is showcased through the low-micromolar accurate quantification of 3,5-difluoropyridine and detection at 800 nM in a single scan. This was extended to a molecule with exchangeable protons, 2,4,6-trifluorobenzylamine, to understand the impact on sensitivity when hyperpolarisation is diverted from the analyte to the solvent through proton exchange. Results show that, by minimising hyperpolarisation loss through solvent deuteration, detection and accurate quantification in the low-micromolar regime can be achieved. This work paves the way towards accurate, sensitive and accessible analytical applications using SABRE-enhanced benchtop NMR spectroscopy.

[1] Adams et al., Science., 2009, 323.
[2] Eshuis et al., JACS, 2014, 136.

BCH-005: Solvent-suppressed pure-shift NMR for highly resolved spectra of complex mixtures on compact spectrometers

Mr Joris Mandral[1], Dr Jonathan Farjon[1], Dr Patrick Giraudeau[1], Dr Jean-Nicolas Dumez[1]
[1]Nantes Université, CNRS, CEISAM, UMR 6230, F-44000, Nantes, France

Nuclear magnetic resonance (NMR) spectroscopy is a powerful technique for the analysis of complex mixtures. Most applications rely on high-field spectrometers that require the use of costly cryogenic fluids, dedicated lab space and expert operators. In contrast, compact spectrometers are less expensive and work with permanent magnets requiring minimal maintenance. The main drawbacks of these spectrometers are their low sensitivity and resolution. In order to improve the performance of benchtop spectrometers, a major challenge is to improve the resolution while keeping maximum sensitivity for high throughput conditions.

The advent of gradient coils on benchtop spectrometers has triggered a number of pulse sequence developments for benchtop NMR, including solvent suppression pulse schemes or pure-shift NMR spectroscopy. Our aim is to combine, optimize, and further improve these methods for the analysis of increasingly complex mixtures and to evaluate their ability to decipher individual analyte signals.

First, we tested and compared different solvent suppressed-based homodecoupling strategies in terms of sensitivity, resolution, repeatability and spectral purity on a model mixture of 17 metabolites, using an 80 MHz spectrometer. Second, we used these methods to characterize extracts from different fish food formulations, containing alternative feeds such as insect larvae, spirulina microalgae or yeast. Our results show that more sensitive and cleaner pure-shift acquisitions can be achieved on such samples on a benchtop system in several tens of minutes, providing rich spectral fingerprints.

Future work will aim at establishing whether a non-targeted 1H NMR metabolomics analysis of these sample makes it possible to observe differences in water soluble compounds between these feed formulations, and relate these to differences in fish growth performance. This was shown previously using high-field NMR. A benchtop NMR approach would open up considerable prospects for metabolomic investigations on compact spectrometers.

BCH-008: Portable, versatile, low-noise instrumentation for hyperpolarization-enhanced nuclear magnetic resonance experiments at zero- to ultralow fields

M.Sc. Román Picazo-Frutos[1,2], M.Sc. Ekaterina V. Pokochueva[3], M.Sc. Anne M. Fabricant[1,2], MS. c. Gary P. Centers[1,2], M.Sc. Erik Van Dyke[1,2], Ph. D. Raphael Kircher[1,2], M. Sc. Jingyan Xu[1,2], Ph. D. James Eills[4], M. Sc. Liubov Chuchkova[1,2], Ph. D. Danila Barskiy[1,2], Prof. Alexej Jerschow[1,2,6], Prof. Sami Jannin[3], Prof. Dmitry Budker[1,2,5]
[1]GSI Helmholtzzentrum für Schwerionenforschung, Helmholtz-Institut Mainz, Mainz 55128, Germany, [2]Institute for Physics, Johannes Gutenberg-Universität Mainz, 55128 Mainz, Germany, [3]Université Claude Bernard Lyon 1, CRMN UMR-5082, CNRS, ENS, Lyon, Villeurbanne 69100 , France, [4]IBEC - Institute for Bioengineering of Catalonia, Barcelona Institute of Science and Technology, 08028 Barcelona, Spain, [5]Department of Physics, University of California, Berkeley, USA, [6]Department of Chemistry, New York University, New York, United States

Introduction:
Zero- to ultralow-field (ZULF) nuclear magnetic resonance (NMR) is an alternative to conventional high-field NMR that operates without strong magnets. In the absence of magnetic fields chemical shift vanishes, so this technique depends on the indirect J-coupling interaction for molecular structure elucidation.
Well below Earth's magnetic field, two relevant characteristics emerge: (i) extremely narrow linewidths (~mHz) due to the low absolute magnetic field inhomogeneities caused by the magnetic susceptibility of the sample, and (ii) deep penetration of electromagnetic waves through conductive material at low frequencies due to the skin depth phenomenon. This regime is also especially well suited to studying exotic spin-spin interactions and nonstandard relaxation effects.
Since thermal polarization is prohibitively low at such low fields, hyperpolarization techniques are often synergic to ZULF NMR.

Aims:
To construct a portable experimental setup capable of carrying out a plethora of experiments in a remote location.

Methods:
We use commercial optically pumped magnetometers (OPMs) to detect the signal emanating from the sample. We employ a total of 4 sensors for exquisite common-mode noise removal using a gradiometric configuration, as well as increasing resolution via quadrature detection. Furthermore, the transition between thermal and hyperpolarization modalities is seamlessly achieved.

Results:
Such experimental setups allowed us to novelly study quadrupolar nuclei at zero field. To date, we have performed experiments using hyperpolarization based on parahydrogen, and dynamic nuclear polarization. We additionally discuss theoretical considerations of optimization relevant only to ZULF NMR, serving as an analog to the Ernst Angle in conventional high-field NMR.

Outlook:
This modular hardware is capable of probing exotic spin-dependent interactions beyond the standard model, such as the coupling of gravity to nuclear spin, as well as for the direct observation of electrolytes through the housing of batteries, due to the ability to penetrate through conductive material at such frequencies.

BCH-009: A Low-Cost Magnetic Measurement System for Low-Field MRI Magnets based on a Motion Tracked Flexible-Joint Robot with 5 Degrees of Freedom

Master Of Science Pavel Povolni[1], Master of Science Robin Bendfeld[2], Bachelor of Science Sergej Maltsev[1], Dr Kai Buckenmaier[1], Dr Klaus Scheffler[1]
[1]Max-Planck-Institute For Biological Cybernetics, Tuebingen, Germany, [2]Institute for Nonlinear Mechanics, University of Stuttgart, Stuttgart, Germany

Introduction

Magnetic resonance imaging (MRI), nowadays a fundamental instrument in diagnostic imaging in Western industrialized countries, causes enormous costs for clinical devices. This significantly limits access in low- and middle-income counties (LMICs). As an alternative, open source MRI devices using permanent NdFeB magnets in a Halbach-Array have been developed and already used successfully in LMICs.

Aims

Halbach-Magnets show high inhomogeneities in the FOV due to manufacturing errors. Shimming becomes necessary. Typical mapping robots are expensive due to mechanics (bearings, etc.) and commercial field probes. We present a new approach by using an inexpensive flexible joint robot in combination with a self-developed Hall-Sensor.

Methods

A robot arm with 5 Degrees of Freedom is used, whereby the joint angles can be set via Servo Motors. Motion tracking based on ArUco markers, a standard camera and the OpenCV-Python-library was implemented to increase the accuracy of the system. A self-developed magnetic field sensor based on commercial Hall-Sensors is mounted on the robot and is moved inside the magnetic field of a 45mT-Halbach-Magnet (13 disks with 2 circular Halbach-Arrays each, 536 NdFeB magnets) with a length of 300mm and an aperture of 160mm.

Results

The mapping of the magnetic field in a cubic 75x75x75mm³ FOV with a step size of 5mm (4096 points) was performed in 2.8 hours. Due to the motion tracking a position accuracy <0.1mm was achieved in the xy-plane, which is comparable to standard mapping systems.

Conclusions

The use of the system was demonstrated for a Halbach-Magnet. Although the accuracy in the xy plane (camera image plane - CIP) is accurate, the position accuracy in the yz plane (perpendicular to CIP) still needs to be improved in order to achieve efficient shimming.
The revision of the software and hardware remains pending in order to make it available as open source.

BCH-010: Earth-field and high-field 1H relaxation for real-time detection of free-radical formation

Mr Alexandru Topor[1,2,3], Dr Aude Sadet[1,2,4], Dr Mihai Adrian Voda[1,2], Dr Arnaud Comment[2,5], Prof Paul Vasos[1,3,4]
[1]Biophysics and Biomedical Applications Lab, ELI-NP, Extreme Light Infrastructure - Nuclear Physics, IFIN-HH, Bucharest-Magurele, Romania, [2]Institute for Organic and Supramolecular Chemistry, ICOS, Bucharest, Romania, [3]University of Bucharest, ISDS Doctoral School and Chemistry Doctoral Schoo, Bucharest, Romania, [4]Institute of Biological Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria, [5]Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge, United-Kingdom

Aims
The formation of free radicals is at the center of both curative and toxic effects in biomedicine. NMR relaxation-based methods for following the course of redox reactions can be used to understand the mechanism of therapeutic approaches. Recently-introduced FLASH radiotherapy relies on high dose-rate radiation delivery to reduce toxicity. We propose a twofold 1H-relaxation-based Earth-field and high-field approach to understand oxidative processes dependent on free-radical generation rates.

Methods
Earth-field 1H-relaxation experiments were performed in an open-coil Terranova-MRI to detect free-radical formation in water, as generated via: (i) H2O2 in presence of CsI as catalyst, or (ii) the addition of tritiated water, HTO, as an in-situ radiation source.
High-field NMR experiments using dissolution-DNP for sensitivity enhancement, magnetisation storage in long-lived spin-order, and multiple detection were performed to follow the oxidation of glutathione (GSH) by H2O2 in real time.

Results
Earth's magnetic field H2O 1H relaxation was used to follow the formation of H2O2 oxidation products in real time. The 1H relaxation rate constants detect spin-trapped intermediate *OH radicals. Earth-field MRI was then used to visualize radiation effects in tritiated water samples, H2O/HTO.
In high magnetic fields, 1H-based long-lived states (LLS) and coherences (LLC's) of an endogenous antioxidant present in high-concentration in cells -glutathione (GSH)- were optimised to detect oxidation processes. Dissolution-DNP can be used to obtain sensitivity-enhanced LLS in GSH-Cys,Gly aliphatic protons and LLC's in GSH-Gly-Ha protons. LLS follow GSH oxidation by H2O2 in real time during 30 s.

Conclusions
Oxidation processes were detected on-the-go by 1H relaxation in Earth's magnetic field and in high field using new methods. Earth-field detection in open-coil spectrometers of 1H relaxation effects as *OH are spin-trapped opens new perspectives for imaging radiation effects. In high magnetic fields, hyperpolarised LLS in endogenous antioxidants can be used to probe oxidation kinetics in real time.

BCH-011: A compact and mobile stray-field NMR sensor

Belal MK Alnajjar[1], Professor Bernhard Blümich[1], Dr Jürgen Frick[1], Professor Jens Anders[1,3,4]
[1]Universität Stuttgart, Stuttgart, Germany, [2]RWTH Aachen University, Aachen, Germany, [3]Center for Integrated Quantum Science and Technology , Stuttgart, Deutschland, [4]Institute for Microelectronics Stuttgart , Stuttgart, Deutschland

Aim
This work aims to develop a compact stray-field NMR sensor with microscopic spatial resolution, suitable non-destructive testing of materials by depth profiling including cultural heritage studies. The sensor, consisting of a magnet, rf coil, spectrometer, and translation stage, is characterized by 20 µs dead time and light weight for ease of operation and transportation. By use of genetic algorithms, the magnetic stray field has been optimized to minimize lateral field variations perpendicular to the magnet surface, resulting in a homogenous slice at 9 mm depth of access at 18 MHz.
Method
To achieve microscopic spatial resolution in depth profiles, the sensitive slice is aligned parallel a planar object by CPMG measurements. The assembled sensor exhibits a measured magnetic field gradient of 20 T/m and inhomogeneity of 10000 ppm within a 10 mm × 10 mm region. The desired resolution defined by the thickness of the excited slice is adjusted via the acquisition of the CPMG echoes. Two acquisition times have been employed: tacq = 110 µs and tacq = 64 µs, while maintaining the step size of a translation stage at 50 µm. Each profile was fitted with the integral of a Gaussian point-spread function. The spatial resolution changes with the acquisition time, transitioning from Δrlong = 61 µm to Δrshort = 101 µm for tacq = 120 µs to tacq = 64 µs, respectively.
Results and Conclusions
The optimization and fabrication of a compact stray-field NMR sensor with a gradient of 20 T/m, is reported which enables microscopic spatial resolution for depth profiling. The findings validate both the employed alignment approach and the utilized measuring scheme. Ongoing efforts focus on reducing the sensor's dead time and power consumption to enable battery operation, on miniaturizing the electronics, and on the integration of distance sensors for precise object alignment.

BCH-012: Relaxometric charaterization of proteins tagged with Gd(III) complexes as potential MRI theranostic agents

Mr Adam Kubrak[1], Ms Jlenia Bindi[1], Professor Marco Fragai[1], Professor Pedro Sebastiao[2], Professor Giacomo Parigi[1]
[1]Magnetic Resonance Center (CERM), University of Florence, and CIRMMP, Sesto Fiorentino, Italy,
[2]Center of Physics and Engineering of Advanced Materials, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

Introduction
Field-Cycling NMR Relaxometry is a widely used technique that enables one to study the molecular dynamics of macromolecules as proteins by measuring relaxation rates R1 in different magnetic fields. The characterization of paramagnetic compounds potentially useful as contrast agents in MRI is an important application of 1H FFC-NMR Relaxometry, providing structural and dynamic properties important for the characterization of their mechanism of action. Contrast agents based on gadolinium(III) complexes are widely used in clinical diagnostics, but due to the high toxicity of that metal ion, there are concerns about their safety.

Aims
Striving towards a decrease of the injected Gd(III) complex, several studies aim to enhance the relaxivity and thus the efficacy of the MRI agents. To this aim, low molecular weight Gd(III) complexes have been developed to be covalently bound to macromolecules with molecular reorientation times in the nanosecond time scale. Here, we investigate human transthyretin (TTR), which is a carrier protein for the delivery of cytotoxic drugs, as a potential theranostic agent. The paramagnetic Gd-C4-IA complex is used as a tag.

Methods
The analysis of the acquired relaxometry (or Nuclear Magnetic Relaxation Dispersion, NMRD) profiles is performed through the application of relaxation models able to describe interactions and motional processes occurring in the system by taking into account defined parameters and conditions. A fitting software with a graphical interface is under development, that will easily allow for the analysis of the relaxation profiles of paramagnetic molecules, proteins and nanoparticles.

Results and Conclusions
The relaxometry profile of TTR tagged with the paramagnetic Gd-C4-IA complex shows that this adduct represents a promising theranostic agent. The analysis of the profile with the available relaxation models provides information on the parameters describing the dynamic processes responsible for relaxation.

Acknowledgements
This project was supported by HORIZON-MSCA-DN-2021 grant agreement No. 101072758.

BCH-013: Insight into dynamics of binary systems by means of Nuclear Magnetic Resonance Relaxometry

Dr Elzbieta Masiewicz[1], Dr Adriane Consuelo Leal Auccaise[1], Prof Danuta Kruk[1]
[1]Department of Physics and Biophysics, University of Warmia and Mazury in Olsztyn, Oczapowskiego 4, 10-719, Olsztyn, Poland

Introduction: Relaxation experiments performed versus the resonance frequency provide an exceptional opportunity to study dynamical processes occurring on different time scales and to reveal the underlying mechanisms of motion. These unique advantages are hampered by a relatively "low resolution"-relaxation behaviour is observed that results from the presence of multiple pools of NMR-active nuclei. This implies that to fully exploit the potential of NMR relaxometry, considerable effort is required in terms of appropriate theoretical models and carefully performed experiments.

Aims: The work has two intertwined aims. The first is to reveal the dynamical properties of binary mixtures containing amino acids and glycerol. The underlying question concerns the translational and rotational dynamics of the individual fractions compared to the dynamical properties of the solvent depending on the structure of the amino acid. The second aim is to provide a theoretical framework for the use of NMR relaxometry for binary mixtures [A. C. Leal Auccaise, et. al, J. Chem. Phys. 160, 144116 (2024)].

Methods: ^{1}H spin-lattice relaxation experiments were performed in the frequency range from 10kHz to 10MHz using an FFC-NMR relaxometer (STELAR s.r.l., Mede, Italy) for amino acid-glycerol mixtures containing glycine, l-aspartic acid and alanine with different deuteration levels, versus a broad temperature range.

Results: The data have been interpreted in terms of a theoretical model including intramolecular and intermolecular relaxation contributions and taking into account isotopic substitution effects. The consistent interpretation has led to a comprehensive picture of the motion performed by the individual molecular fractions.

Conclusions: The proposed theoretical models have been successfully tested against several combinations of molecules with different deuteration levels, providing a robust framework for exploiting NMR relaxometry for systems including several molecular fractions.

Acknowledgments/Funding: This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 899683 (project “HIRES-MULTIDYN”).

BCH-014: Spectral assignment transfer using a solvent gradient

Msc Paulina Putko[1]
[1]University of Warsaw, Warsaw, Poland

Nuclear Magnetic Resonance (NMR) is a powerful tool for identifying small molecules and analyzing their structures. The first step of an NMR analysis is usually the resonance assignment i.e. connecting chemical shifts with specific atoms in the molecule. Interestingly, NMR spectra of the same compound dissolved in different solvents reveal different chemical shifts. On one hand, this might be perceived as a problem—the assignment of resonances achieved in one solvent is not easily transferable to another solvent. On the other hand, changing solvents may help to resolve peaks and avoid ambiguities [1]. We demonstrate how to solve the former problem and use the latter possibility by creating a binary solvent composition gradient along an NMR tube.
We show the potential of a new technique using 43 MHz benchtop NMR equipped with SWAPE (Sweeping Apparatus for Polarization Enhancement) [2] to mechanically shift the NMR tube. Recently, we have demonstrated a similar approach using a pH gradient [3]. We measure alternately a series of 1H and HSQC NMR spectra at different solvent proportions. As a result, we obtain a stack of spectra with smoothly varying peak positions. This allows us to find the direction of movement of peaks in different ratios of DMSO and D2O, and transfer the assignment between these solvents. We demonstrate this approach in the study of small molecules - i.e., α-Asarone, and ε-caprolactone.

[1] P. Laszlo, Prog. NMR Spectrosc., 1967, 3, 231-402.
[2] J.R. Romero, K. Kazimierczuk, D. Gołowicz, Analyst, 2020, 145, 7406-7411.
[3] P. Putko, J.R. Romero, K. Kazimierczuk, Analyst, 2024, 149, 1998-2003.

BIO-001: Protein unfolding and aggregation at all stages as seen by NMR

MSc Luis Gardon[1,2], MSc Leonardo Levorin[1,2], MSc Melinda Jaspert[1,2], BSc Neomi Rüter[1], Dr. Lothar Gremer[1,2], Dr. Nils Lakomek[1,2], Prof. Dr. Henrike Heise[1,2]
[1]University Of Düsseldorf, Düsseldorf, Germany, [2]Forschungszentrum Jülich, Jülich, Germany

Protein misfolding and aggregation is closely linked to many neurodegenerative diseases like Alzheimer's or Parkinson's disease, and understanding the underlying processes like protein (un)folding, aggregation and fibril formation is a rewarding challenge. Here we demonstrate the enormous potential of NMR-Spectroscopy at all time-scales ranging from solution NMR, HR-MAS via room-temperature solid-state NMR to DNP enhanced low-temperature NMR in frozen solutions for the study of protein folding, unfolding and aggregation [1].
We apply a multidisciplinary approach combining solution-NMR, in-situ MAS NMR-spectroscopy and DNP-enhanced NMR solid-state NMR-spectroscopy of frozen solutions to follow and characterize the process of unfolding, oligomerization and protein aggregation of the model protein PI3K SH3 in real time and with high resolution. Structural ensembles of backbone and side-chain conformations seen in frozen solution [2-3] are linked to aggregation kinetics and products at different conditions. Stepwise lowering the pH leads to reversible unfolding of the protein, particularly at high temperatures. Lowering the pH also enhances the conformational space sampled by the protein, thus leading to line broadening of DNP-enhanced spectra in frozen solution. At the same time, the dispersion of chemical shifts, which in solution NMR represent the ensemble average, reduces upon protein unfolding. Under conditions which favor protein unfolding, aggregation into aggregates and protein fibrils is observed.
With concerted high-resolution and solid-state NMR spectroscopy at different temperatures we have obtained valuable insight into the concerted unfolding/aggregation process, which also plays a role in many degenerative diseases associated with protein misfolding.

[1] Gardon L et al. Frontiers in Molecular Biosciences. 2023;10.
[2] König A et al. Solid State Nucl Magn Reson. 2019;98:1.
[3] Uluca B et al. Biophys J. 2018;114:1614.

BIO-002: Unlocking the molecular structure of complex lipid membranes using solid-state NMR: towards myelin in nervous tissue

Tiago Mendes Ferreira[1]
[1]Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

Lipid membranes are fundamental cellular components and the basis of various biological materials such as myelin and lung surfactant. The combination of solid-state nuclear magnetic resonance (ssNMR) experiments with molecular dynamics simulations has been providing structural and dynamical details from various lipid membrane models as evidenced in the open collaboration NMRlipids (1,2). I will present our main ssNMR contributions in the NMRlipids project, and introduce our most recent work towards applications to complex lipid membranes (3). Namely, I will describe how accounting for the effect of RF inhomogeneity in dipolar recoupling ssNMR experiments will facilitate the measurement of structural details from membranes close to their native environment such as myelin in nerve tissue.

(1) A. Kiirikki et al., Nat. Commun., 15, 1136 (2024)
(2) A. Bacle et al. J. Am. Chem. Soc., 143, 13701–13709 (2021)
(3) A. Wurl, K. Saalwächter and T. Mendes Ferreira, Magn. Reson., 4, 115–127 (2023)

BIO-003: Conformation and dynamics of lutein in Light-Harvesting Complex II

Dr. Emanuela Crisafi[1], Dr Karthick Sai Sankar Gupta[1], Dr Francesco Buda[1], Dr Anjali Pandit[1]
[1]Leiden University, Leiden, Netherlands

The most abundant light-harvesting 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. This process is key to plant photoprotection and its fundamental understanding would allow for fine tuning to optimize conditions for production of solar biomass. The LHCII light-harvesting switch is proposed to rely on changes in lutein conformation and interactions, which hitherto have not been resolved. LHCII contains 8 Chla, 6 Chlb, 2 lutein, 1 neoxanthin and 1 violaxanthin per protein monomer, complicating characterization of individual pigments. In this work, we faced those challenges by combining pigment-protein reconstitution, selective lutein isotope labelling and Magic Angle Spinning (MAS) NMR spectroscopy. Ring-current shifts induced by lutein-Chl interactions reveal the orientation of the two lutein in LHCII with their ionone e-ring head faced towards the stromal side and b-ring towards the lumen. Doubling of signals was observed for some head group chemical shift correlations, suggesting that there are only small differences between the ground-state electronic structures of the two lutein molecules.
LHCII forms light-harvesting states in isolated form, while it adapts excitation-quenched states under aggregated conditions. To gain insight in a potential lutein switch, 1H-13C Hetcor and 13C-13C PARIS experiments were performed on LHCII in frozen detergent micelles and on LHCII aggregates. A comparison of the two protein states suggests conformational flexibility and a re-orientation of the carotenoid head groups. This outcome demonstrates that lutein, a key chromophore in tuning light harvesting, can adapt different conformations inside the LHCII protein scaffold, associated with specific photophysical states.

BIO-004: Biophysical characterization of a viral-host protein complex using DNP and MAS-NMR

Mr. Abel Cherian Varkey[1], Dr. Evgeny Nimerovsky[1], Dr. Karin Giller[1], Dr. Stefan Becker[1], Dr. Loren Andreas[1]
[1]Max-Planck Institute For Multidisciplinary Sciences, Goettingen, Germany

Proteins encoded in the viral genome tend to be spatially constrained due to the small size of the viral genome. Therefore, such proteins are often multifunctional and carry protein-protein interaction surfaces that can help in hijacking host cellular machinery. Determining the structure and understanding the biophysical characteristics of these viral proteins can help to elucidate functional mechanisms and to develop strategies to counteract infection.
We aim to biophysically characterize a viral membrane protein in complex with a soluble host protein.
We used magic angle spinning NMR and dynamic nuclear polarization NMR as the primary tools for characterization of the complex.
We reconstituted a viral membrane protein in a native-mimetic lipid bilayer along with a bound host protein. We assigned the two proteins using differential labelling strategies and sensitivity-enhanced assignment spectra in solids. MAS-NMR was used to determine the dynamics in the complex in the nanosecond-microsecond timescale which showed that the entire complex was relatively rigid. The soluble protein was also shown to be immobilised near the surface of the membrane bilayer. We further assessed the oligomeric state of the viral protein when bound to the dimeric host protein using CODEX measurements at a temperature of 95K with DNP-NMR.
In conclusion, we characterised the structure and structural dynamics of a full-length viral membrane protein in complex with a soluble host protein and found the complex to be surprisingly rigid in the context of a lipid bilayer.

BIO-006: Investigating CALM Binding with PtdIns(4,5)P2 Model Membranes Using NMR and Neutron Reflectometry: Uncovering Novel Structural Details in Endocytic Adaptor Protein

Ildefonso Marín-Montesinos[1]
[1]CICECO-Aveiro Institute of Materials/University Of Aveiro, Aveiro, Portugal

This work explores the structural basis of the interaction between Clathrin assembly lymphoid myeloid leukemia protein (CALM) and the cell membrane in clathrin-mediated endocytosis. Although CALM is known to bind to phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2) in the plasma membrane via electrostatic interactions, the mechanism of protein insertion into the membrane has not been experimentally proven. A biophysical approach combining solid-state NMR and neutron reflectometry (NR) was employed to investigate the CALM-PtdIns(4,5)P2 interaction. Results of the study shed light on the three-dimensional orientation of CALM upon lipid binding and its insertion into the membrane, contributing to a better understanding of the vital process of endocytosis.

BIO-008: New applications of solid-state NMR to extracellular matrices with relevance to human health and disease

Dr Wing Ying Chow[1], Ananya Singh[1], Dr Darius Koester[2], Sabeeha Malek[2], Teresa Massum-Wu[2], Professor Mohan Balasubramanian[2]
[1]Department of Physics, University Of Warwick, Coventry, United Kingdom, [2]Centre for Mechanochemical Cell Biology, University of Warwick, Coventry, United Kingdom

The extracellular matrix (ECM) is a necessary component of life, especially multicellular life, including humans. For cells to function beyond simply proliferating, the ECM has to provide the correct biochemical and mechanical environment. Complex and heterogeneous, the ECM can consist of proteins, glycans, lipids, and even nucleic acids. The crosslinked and insoluble nature of the ECM means that there are unique advantages to investigating such samples using solid-state NMR, despite the underlying chemical and biological complexities. To illustrate the strength of this approach, we will demonstrate recent results in two examples.

Firstly, building on previous work on probing collagen proteins in the ECM in tissue such as bone and cartilage, we are now using a similar approach to investigate Ehlers-Danlos syndrome (EDS). EDS patients commonly suffer from overly mobile joints and stretchy skin. For the most common subtype of EDS, the genetic cause is not known. Using ^{13}C-labelling of EDS fibroblast culture, we aim to identify molecular differences that can explain the key symptoms of this disease, with the eventual aim of improving diagnosis and treatment.

Secondly, inspired by others working in this area, we have begun an investigation of the fungal cell wall of the fission yeast Schizosaccharomyces pombe. While S. pombe is not a pathogenic fungus, as a model organism, it is amenable to genetic manipulation. Therefore, we can hope to achieve a mechanistic understanding of fungal cell wall formation, especially involving different forms of α- and β-glucans and compensatory pathways, which may drive the development of new antifungal drugs.

BIO-009: Structure of Membrane-mediated Amyloid Fibrils of human islet amyloid polypeptide (hIAPP)

Dr. Venus Singh Mithu[1], Karin Giller[1], Dr Loren Andreas[1], Dr Stefan Becker[1], Prof Christian Griesinger[1]
[1]Max Planck Institute For Multidisciplinary Sciences, Goettigen, Germany

Type II diabetes mellitus (T2DM) is characterized by insulin resistance, progressive islet β cell failure, and the presence of extracellular fibrils (islet amyloid) of a 37 amino acid long peptide hormone 'human islet amyloid polypeptide' (hIAPP) in the pancreatic islets of Langerhans. Evidence suggests that the interaction between hIAPP and phospholipid membrane plays a pivotal role in causing β cell failure. A mechanistic understanding of this interaction is thus essential for understanding the therapeutic activity of small molecules. The molecular structure of membrane-mediated hIAPP fibrils, crucial for understanding their role in T2DM, is still unknown. This study aims to determine their structural fold in the presence of model phospholipid membranes.

Small unilamellar vesicles (SUVs) composed of POPC and POPS phospholipids were used as models for β cell membranes in growing hIAPP fibrils. Unlike the evenly dispersed hIAPP fibrils seen without lipids, we observed bundles of fibrils mixed with misshapen lipid vesicles. This complexity challenges structure determination using cryo-EM, thus making solid-state NMR (ssNMR) the preferred method for studying these lipidic hIAPP fibrils. Solution-state NMR and Thioflavin-T (ThT)-based fluorescence assays were used to characterize the aggregation kinetics of hIAPP. The assignment of backbone and side-chain resonances of hIAPP was accomplished through a series of 1H-detected 3D and 13C-detected 2D correlation ssNMR spectra, recorded at 55 kHz magic-angle-spinning (MAS). Chemical shift data was utilized to predict secondary structural and torsional angles using TALOS-N, revealing significant differences from previously reported structures of non-lipidic fibrils.

13C-13C correlation proton-driven spin diffusion spectra at slow MAS were used to gather distance constraints. NMR-derived torsional angles and distance constraints helped construct a model for hIAPP fibrils in membrane environments. These backbone and side-chain assignments will aid in identifying potential binding sites of therapeutic and diagnostic molecules.

BIO-010: Investigating structure and folding of the lipoprotein BamC within a native bacterial settings

Mr Ajit Kumar Bishoyi[1], Dr. Marc Baldus[1]
[1]Utrecht University, Utrecht, The Netherlands

The β-barrel assembly machinery (BAM) complex controls the insertion and folding of outer membrane proteins (OMPs) of gram-negative bacteria and plays a fundamental role in cellular viability[1][2]. Elucidating the structure and dynamics of the complex components is important to understand the mechanism underlying membrane insertion and folding of OMPs and aids the design of antimicrobial peptides against pathogenic gram-negative bacteria.
Previous in-vitro solution and solid-state NMR studies suggest that BamC is the most dynamic lipoprotein of the BAM complex. While the N-terminus of BamC is unstructured, the N- and C-folded domains adopt structured conformations both in the free form [3] and as part of the entire reconstituted complex [4]. We are currently interested to use solid-state NMR to obtain a more detailed understanding of the structure and dynamics of BamC in its native membrane environment. For this purpose, we have prepared BamC within bacterial cell envelopes [5] and employed dipolar and scalar-based 1H-detected two – and three-dimensional ssNMR experiments to investigate the rigid and flexible domains in native settings. In our residue-specific analysis, we make use of previous in-vitro data [3],4]. In addition, our ssNMR studies are supported by molecular simulations to obtain a comprehensive view of the fold and possibly the function of BamC in native bacterial settings.

1. O'Neil, P.K., et al., Biochemistry, 2015. 54(41): p. 6303-11.
2. Brown, D.G., Bioorg Med Chem, 2016. 24(24): p. 6320-6331.
3. Knowles, T.J., et al., Biomol NMR Assign, 2009. 3(2): p. 203-6.
4 Xiang, S. et al., Angew. Chem. Int Ed. (2022), 61, e202203319.
5. Narasimhan, S., et al., Nat Protoc, 2021. 16(2): p. 893-918.

BIO-011: Light responsive peptide chromophore complexes for artificial photosynthesis.

Miss Padmaja Kar[1], Dr Karthick Babu Sai Sankar Gupta[1], Dr Anne Wentink[1], Dr Agur Sevink[1], Prof. Dr. Huub de Groot[1]
[1]Leiden University, Leiden, Netherlands

In nature photosynthetic antenna system 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 a baseplate, which is a scaffolding super structure, formed by protein CsmA and bacteriochlorophyll a, Bchla.1 In this project, we use truncated CsmA construct, tCsmA containing the central alpha-helix and Zinc protoporphyrin IX, ZnPP as the chromophore binding partner, to put forth a simple 2D charge separator complex mimicking the natural CsmA-BChla baseplate. 2
Here we present the protocol for overexpression, purification of authentic N terminal and non-amidated C terminal recombinant tCsmA peptide and its subsequent complexation with the chromophore ZnPP. We are currently interested to use solid-state NMR to obtain an understanding of the structure and dynamics tCsmA-ZnPP complex in native condition. We will use 13C, 15N residue specific assignments from BMRB entries of PDB 5lcb and PDB 2k37 to corroborate with our assignments. We will employ dipolar and scalar-based 1H detected two dimensional ssNMR experiments to study the rigid and flexible regions of tCsmA in bound and unbound state with ZnPP.
References:

1. Nielsen, J. T. et al. In situ high-resolution structure of the baseplate antenna complex in Chlorobaculum tepidum. Nat Commun 7, (2016).
2. Sun, Z., Diebolder, C. A., Renault, L. & de Groot, H. A Semisynthetic Peptide−Metalloporphyrin Responsive Matrix for Artificial Photosynthesis. ChemPhotoChem 3, 630–635 (2019).

BIO-012: Topology of phospholipid membranes under DNP conditions

Professor Emeritus Frances Separovic[1], Dr David Keizer[1], Dr Marc-Antoine Sani[1]
[1]University Of Melbourne, Melbourne, Australia

Introduction
Lipids are a key component of cellular systems. Their vast chemical diversity provides biological membranes with a mechanism for structural adaptability and functionality that play important roles in cell physiology. As dynamic nuclear polarization (DNP) NMR is used to investigate membrane-active biomolecules such as peptides or proteins reconstituted in lipid membranes, the topology of these model systems at 100 K and under cryoprotection conditions needs to be determined.
Aims
Determine the topology of phospholipid lipid assemblies in glycerol/D_2O/H_2O solution at ca. 100 K. Initially, simple systems made from either palmitoyloleoylphosphatidyl -choline (POPC), -ethanolamine (POPE), -serine (POPS) or brain sphingomyelin (bSM) were investigated. The impact of the lipid structure and hydration level on the signal enhancements, relaxation properties and packing are reported.
Methods
^{31}P, ^{1}H, ^{15}N NMR experiments were performed under DNP conditions and in the fluid phase at natural abundance. ^{15}N detected ^{31}P dephased REDOR experiments were employed to determine the packing of the lipid headgroups.
Results
Multilamellar vesicles (MLVs) made of POPC or bSM had similar signal enhancements, relaxation times (T_1, $T_1\rho$, Tcp) and ^{15}N-^{31}P distances. Both MLVs systems behaved similarly at 33%wt or 50%wt hydration. Static ^{31}P NMR experiments confirmed a lamellar phase at 33%wt hydration.
However, MLVs of POPE or POPS had longer relaxation times and poorer enhancements. NMR parameters were more sensitive to the hydration level for POPE but not POPS. Both lipids showed closer proximity between nitrogen and phosphorous atoms and formation of a lamellar phase at 33%wt hydration.
Conclusions
The characterization of the propensity for particular headgroup arrangement under DNP conditions in phospholipid bilayers are an important insight and a first step towards determining the topology of biological membranes. Complex bilayers and addition of metabolites or membrane-active biomolecules will be examined in future studies.

BIO-013: Allosteric Modulation of Microsecond Dynamics at a Protein-Protein Interface

Mr Tye Gonzalez[1], Mrs Sara Medina Gomez[1]
[1]TU Dortmund, Dortmund, Germany

The dynamic behaviour of proteins across various timescales is crucial in dictating their functional properties and interactions. This study explores the microsecond dynamics of the SH3 domain using Near Rotary Resonance Relaxation Dispersion (NERRD) and Molecular Dynamics (MD) simulations. We employ fast-MAS solid-state 15N R1ρ NERRD to reveal microsecond dynamics in specific residues, which are significantly altered by the R21A mutation. Specifically, we elucidate dynamic interactions at the interface of a crystal lattice corresponding to the SH3 binding pocket and compare it to the monomeric protein in solution where mutations lead to isolated changes. By comparing the wild type and R21A mutant, MD simulations allow atomistic interpretation of the molecular mechanisms governing the motion detected by NMR. In addition, Markov state modelling allows identification of mechanisms underlying molecular motion and back-calculation of NERRD profiles. This work provides new understanding of how dynamic interactions, formed from previously separate elements, can influence intramolecular allostery through intermolecular contacts.

BIO-014: Effects of model peptides on lipid membranes by ssNMR

Hr Nicolai Alsaker1, PhD Mahmoud Moqadam2, Phd Tiago Ferreira3, Prof. Øyvind Halskau4, Prof. Bengt Erik Haug1, Prof. Nathalie Reuter1,2, Prof. WIlly Nerdal1
1University Of Bergen, Department of Chemistry, Bergen, Norway, 2University of Bergen, Computational Biology Unit, Bergen, Norway, 3University of Halle, Department of Physics, Halle, Germany, 4University of Bergen, Department of BIological sciences, Bergen, Norway

We investigated the interactions of the model peptide Ac-WL-X-LL-OH, where X = Leu (L), Tyr (Y), Phe (F), or Trp (W), with a phospholipid membrane using solid-state NMR (ssNMR). Our ssNMR 13C and 31P data indicated that the phospholipids retained a lamellar phase in the presence of each of the peptides with an aromatic X residue, whereas the Leu peptide perturbed the bilayer to an extent where an additional isotropic phase was observed. The strength of the effect of the peptide on lipid dynamics depend on the X residue. The magnitude of effect is consistent with the relative amino acid interfacial hydrophobicity reported by Wimley and White,1 increasing throughout the order L > Y ~ F > W.

CEL-001: Molecular-level model of a microalgal cell wall by in situsolid-state NMR

Dr. Alexandre Poulhazan[2], Dr. Alexandre Arnold[2], Dr. Frederic Mentink-Vigier[3], Dr. Tuo Wang[4], Dr. Isabelle Marcotte[2], Dr. Dror Warschawski[1]
[1]CNRS, Paris, France, [2]Université du Québec à Montréal, Montréal, Canada, [3]National High Magnetic Field Laboratory, Tallahassee, USA, [4]Michigan State University, East Lansing, UQA

Recent progress in in-cell solid-state NMR (ssNMR) has allowed the in situ structure determination of starch (1), but also order determination of membranes in living bacteria (2) or red blood cell ghosts (3). Studying the cell wall of microorganisms brings new challenges to structural biology, especially since polysaccharides are organized as polymers with no unique sequence or structure.

Precise glycan composition of Chlamydomonas reinhardtii has been determined using mostly ssNMR, following a protocol recently described (4,5). A similar protocol was applied to the amino-acid composition determination of the microalga. The first Dynamic Nuclear Polarization study of a microalga also allowed us to detect preferential contacts within and between amino acids and glycans.

We further identified a strong heterogeneity in flexibility and hydration, depending on the nature of the glycan or amino-acid, providing hints towards molecular organization and biophysical properties of C. reinhardtii polysaccharides and glycoproteins in the cell wall, that can be compared to extensins in higher plants.

(1) A. Poulhazan, A. A. Arnold, D. E. Warschawski and I. Marcotte (2018) Int. J. Mol. Sci. 19:3817
(2) Z. Bouhlel, A. A. Arnold, J.-S. Deschênes, J.-L. Mouget, D. E. Warschawski, R. Tremblay and I. Marcotte (2021) Biochim. Biophys. Acta Biomembr. 1863:183642
(3) K. Kumar, M. Sebastiao, A. A. Arnold, S. Bourgault, D. E. Warschawski and I. Marcotte (2022) Biophys. J. 121:1512-1524
(4) A. Poulhazan, M. C. Dickwella Widanage, A. Muszyński, A. A. Arnold, D. E. Warschawski, P. Azadi, I. Marcotte and T. Wang (2021) J. Am. Chem. Soc. 143:19374-19388
(5) A. Poulhazan, A. A. Arnold, F. Mentink-Vigier, A. Muszyński, P. Azadi, A. Halim, S. Y. Vakhrushev, H. J. Joshi, T. Wang, D. E. Warschawski and I Marcotte (2024) Nat. Commun. 15:986

CEL-002: Topological heterogeneity of protein kinase C modulators in human T-cells resolved with in-cell dynamic nuclear polarization NMR spectroscopy

Dr Sarah Overall[1], Ms Sina Hartmann[1], Dr Quang Luu-Nguyen[2], Dr Patrick Judge[3], Dr Dorothea Pintosi[1], Ms Lea Marti[1], Prof Snorri Sigurdsson[4], Prof Paul Wender[2], Prof Alexander Barnes[1]
[1]ETH Zurich, Zurich, Switzerland, [2]Stanford University, , USA, [3]Washington University in St. Louis, St. Louis, USA, [4]Universit of Iceland, Reykjavik, Iceland

Phorbol ester analogs are a promising class of anti-cancer therapeutics and HIV latency reversing agents that interact with cellular membranes to recruit and activate protein kinase C (PKC). However, it is unclear how these esters interact with membranes and how this might correlate with the biological activity of different phorbol ester analogs. Here, we have employed dynamic nuclear polarization (DNP) NMR to characterize phorbol esters in a native cellular context. The enhanced NMR sensitivity afforded by DNP and cryogenic operation reveals topological heterogeneity of 13C-21,22-phorbol-myristate-acetate (PMA) within T cells utilizing 13C-13C correlation and double quantum filtered NMR spectroscopy. We demonstrate the detection of therapeutically relevant amounts of PMA in T cells down to ~60.0 pmol per million cells and identify PMA to be primarily localized in cellular membranes. Furthermore, we observe distinct 13C-21,22-PMA chemical shifts under DNP conditions in cells compared to model membrane samples and homogenized cell membranes, that cannot be accounted for by differences in conformation. We provide evidence for distinct membrane topologies of 13C-21,22-PMA in cell membranes from 13C-13C DARR spectroscopy, corroborated by 31P dephased REDOR distance estimates, that are consistent with shallow binding modes. This is the first of its kind in-cell DNP characterization of small molecules dissolved in the membrane of living cells, establishing in-cell DNP-NMR as an important method for the characterization of drug-membrane interactions within the context of the complex heterogeneous environment of intact cellular membranes. This work sets the stage for the identification of the molecular determinants that govern the biological activity of phorbol esters.

CEL-004: Exploiting the sensitivity of 19F to probe membrane interactions with whole cells by solid-state NMR

Dr. Kiran Kumar[1], Dr. Alexandre Arnold[1], Raphaël Gauthier[2], Marcus Mamone[2], Prof. Jean-François Paquin[2], Dr. Dror Warschawski[1,3], Professor Isabelle Marcotte[1]
[1]Université du Québec à Montréal, Montreal, Canada, [2]Université Laval, Quebec, Canada, [3]Laboratoire des Biomolécules, CNRS UMR 7203, Sorbonne Université, Paris, France

Whole-cell solid-state (ss) NMR serves as a valuable tool for investigating the action mechanism of membrane-active molecules like host-defense peptides (HDPs). To maintain sample integrity and viability in whole-cell and in vivo ssNMR studies, it is crucial to employ approaches that minimize acquisition time. In this study, we leveraged the high sensitivity of fluorine-19 to probe HDP interactions with erythrocyte ghosts. Fluorine-19 possesses several advantages, including low occurrence in biological samples minimizing background signals, and a broad chemical shift range sensitive to minor structural changes.
We introduced fatty acids monofluorinated at various positions in ghost membranes to examine HDP interactions at different membrane sites. Specifically, we used palmitic acid with a 19F atom at positions 4, 8, or 14 on the acyl chain. Our results showed that changes in the 19F chemical shift anisotropy (CSA), assessed via a C-F bond parameter, reflect changes in lipid bilayer dynamics. This information was also provided using magic-angle spinning 19F ssNMR with or without decoupling, by monitoring variations in the second spectral moment (M2), isotropic chemical shift, linewidth, and relaxation times.
Upon addition of caerin 1.1 to monofluorinated erythrocyte ghosts, the appearance of an additional isotropic peak with reduced CSA, narrower linewidth, and shorter T1 revealed the presence of high-curvature regions in erythrocytes indicative of pore formation, similar to its antimicrobial mechanism. These results were obtained at least twice as fast as 2H ssNMR. Altogether, our work shows that by incorporating fatty acid monofluorinated probes at different depths within the membrane, changes in dynamics can be mapped across various locations in the membrane hydrophobic core. Our approach offers a promising avenue for studying whole cells using 19F ssNMR.

COM-001: Exploring Alternative Approaches for Meaningful Results after Automatic Peak Assignment and Structure Verification of NMR Data

Dr Dimitris Argyropoulos[1], Dr. Sergey Golotvin[1], Dr. Rostislav Pol[1]
[1]Advanced Chemistry Development Inc., Toronto, Canada

The development of modern NMR spectrometers and methodologies has made it possible for scientists to acquire numerous 1D and 2D spectra in a very short time. Consequently, the subsequent processing and interpretation of this data has now become the bottleneck of many structure elucidation and verification workflows. This has left chemists and NMR spectroscopists searching for a reliable way to accelerate this analysis. Furthermore, with the escalating requirements of regulatory agencies and publishers, this quickly becomes a daunting task. As a result, the adoption of Automated Structure Verification (ASV) systems has witnessed a recent surge in popularity.

In an ASV system, the conventional outcome is presented as the Match Factor (MF), a numeric value ranging from 0 to 1 that indicates the level of agreement between the proposed structure and the recorded spectra. The determination of the MF involves the evaluation of various criteria encompassing multiple factors. These factors include the agreement of the observed chemical shifts, integral values, multiplicities, and 2D correlation peaks in relation to those predicted or expected for the structure.

This presentation delves into a comprehensive examination of these criteria, assessing their reliability, practical applicability in real-life spectra, and potential limitations, alongside the possibility of extracting more insightful information beyond a numerical value. We also take into account the presence or absence of multiple spectra and evaluate the impact of additional spectra on the final outcome. Ultimately, we propose supplementary metrics aimed at generating more meaningful outcomes from the ASV procedure with illustrative examples to substantiate our findings.

COM-002: The Collective Value of NMR Data in the 21st Century

Professor Jeffrey Hoch[1]
[1]UConn Health, Farmington, United States

NMR spectroscopy stands on a precipice with a clear view toward unprecedented impact in all areas of molecular discovery. This vantage point has been reached through remarkable advances in superconducting magnets that attain higher magnetic fields and are more sustainable, RF probes with improved sensitivity and astonishing sample spinning speeds, and pulse sequences that manipulate spin systems with exquisite precision. No less significant are developments in allied fields that heighten the impact of NMR, especially the ability of NMR to probe dynamically disordered systems with atomic resolution. In the realm of biomolecular NMR, this window of opportunity is framed by advances in cryogenic electron microscopy – the so-called resolution revolution – and machine learning systems that are now capable of predicting protein structure with accuracy comparable to experimental methods. The 40% of the human proteome that is disordered and "invisible" to machine learning, diffraction methods, or electron microscopy constitutes a very large window.

The collective value of data, as opposed to data from an individual study, is well known to the NMR community. Applications of machine learning to curated NMR data enabled developments such as Talos, Sparta, ShiftML, CS-Rosetta and Deep Picker. Curated data collections were also critical to the development of ChatGPT (the collective works of publishing houses) and AlphaFold (the Protein Data Bank). There are, however, areas where the absence of collections of curated NMR data currently limit the ability to exploit the window of opportunity posed by the unique abilities of NMR to characterize dynamics and disorder. These include the lack of empirical (1) time-domain data and (2) relaxation data. Three concrete actions are described that the European scientific community can undertake to close the curated data gap and help fully realize the potential of NMR to expand our knowledge of disordered and dynamic biomolecules:

- Establish a European branch of the Biological Magnetic Resonance Data Bank (BMRBe).
- Join the US Network for Advanced NMR in creating a World-wide Network for Advanced NMR (wwNAN).
- Participate in the BMRB data challenge by uploading time-domain is support of existing BMRB entries

These actions would leverage the leadership of Europe in the development and application of advanced NMR technology and the historically collaborative European approach to research infrastructure. NAN (usnan.org) is a collaboration among the Universities of Connecticut, Georgia, and Wisconsin-Madison, supported by the US National Science Foundation grant 1946970. BMRB (bmrb.io) is supported by the US National Institutes of Health grant R24GM150793.

COM-003: Quantum-mechanical treatment of thermal effects on NMR of buckminsterfullerene: negative thermal expansion, chemical shift and isotope shifts

M.Sc. Tiia Jacklin[1], Dr. Petr Štěpánek[1], Dr. Perttu Lantto[1]
[1]NMR Research Unit, University of Oulu, Oulu, Finland

C_{60} is the most commonly occurring fullerene. Because of its simple structure it is often studied as an example case of fullerenes or carbon nanostructures in general.

One interesting property of fullerene is its possible negative thermal expansion (NTE) predicted in a computational study [1] and indirectly observed with a few experimental methods [2]. Another interesting experimental observation are the secondary isotope peaks in the ^{13}C NMR spectrum of C_{60} fullerene [3], due to a second ^{13}C atom sharing a bond.

In this study we, for the first time, describe temperature dependence of the volume of a C_{60} molecule and ^{13}C chemical shift in a few isotopomers at fully quantum-mechanical level of theory. This enables detailed analysis of both the NTE and temperature dependence of ^{13}C secondary isotope shifts.

We use density functional theory calculations to produce energy and shielding surfaces for quantum-mechanical rovibrational averaging. Three different isotopomers of C_{60} are considered in the temperature range T=0-500 K, one with only one ^{13}C and two cases with ^{13}C atoms sharing one of the two different kinds of bonds.

While our treatment confirms the NTE, it is much smaller than the prediction by classical molecular dynamics [1]. Additionally, the region of NTE appears around room temperature, higher than reported previously. The ^{13}C chemical shift and isotope shifts are practically unaffected by the NTE and change monotonically with temperature.

The results show that quantum-mechanical treatment of thermal effects is necessary for both the magnitude and thermal range of the NTE of C_{60} fullerene. Reasonably good agreement with very small and sensitive experimental ^{13}C isotope shifts and their temperature dependence [3] substantiates the methodology.

[1] Kwon et al., PRL 2004, 92, 015901. Ibid. 2005 94, 209702.
[2] Brown et al., PRB 2006, 73, 125446.
[3] Bacanu et al., PCCP 2020, 22, 11850.

COM-004: Protein NMR assignment by isotope pattern recognition

Mr Uluk Rasulov[1], Mr Harrison Wang[2,3], Dr Thibault Viennet[2,3,4], Mr Maxim Droemer[2,3], Mr Srdan Matosin[2,3], Dr Luca Mureddu[5], Professor Geerten Vuister[5], Dr Scott Robson[2,3], Professor Haribabu Arthanari[2,3], Professor Ilya Kuprov[1]
[1]Department of Chemistry, University Of Southampton, Southampton, United Kingdom, [2]Department of Biochemistry and Molecular Pharmacology, Harvard Medical School, Boston, USA, [3]Dana-Farber Cancer Institute, Boston, USA, [4]Department of Chemistry and iNANO,Aarhus University, Aarhus, Denmark, [5]Department of Molecular and Cell Biology, Institute for Structural and Chemical Biology, University of Leicester, Leicester, United Kingdom

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 only be generated by high-fidelity simulations.

In this communication, we offer a solution to this problem. We propose polyadic decompositions to store millions of synthetic three-dimensional NMR spectra, on-the-fly simulation of instrumental artefacts during training, a probabilistic way to incorporate prior and posterior information, and integration with the industry standard CcpNmr software framework.

The resulting neural nets take [1H,13C] 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) are rapidly assigned from just the HNCA spectrum.

COM-005: Towards Integrative Spin and Molecular Dynamics Modelling of Relaxation Mechanisms: A case study of electrolyte solutions

Florin Teleanu[1,2,3], Prof. Alexej Jerschow[1]
[1]New York University, New York City, United States, [2]Extreme Light Infrastructure - Nuclear Physics, "Horia Hulubei" National Institute for Physics and Nuclear Engineering, Bucharest, Romania, [3]Interdisciplinary School of Doctoral Studies, University of Bucharest, Bucharest, Romania

Introduction
Understanding dynamic processes in liquids such as molecular tumbling, conformational changes or solvent interaction is essential for converting nuclear polarization-based information to structural constraints. Usually, this is done by statistical models describing molecular phenomena by a priori defined correlation functions. However, precious information might be lost when running such approximations, especially in cases where correlations between multiple phenomena or motions on different timescales are at play. Here, we present the first steps in integrating molecular dynamics trajectories for predicting nuclear spin relaxation rates of NaBF4 electrolytes. This multi-spin system requires that cross-correlated interactions must be considered and further comparison with multi nuclear experimental data points towards additional intermolecular interactions.

Aims
To provide a methodology for implementing molecular dynamics trajectories in predicting relaxation rates of multi-spin systems with correlated interactions.

Methods
We employ several computational resources to predict both tumbling rates and molecular properties such as internuclear distances, chemical shielding and electric field gradient tensors in electrolyte solutions at different concentrations and temperatures. A comparison between predicted and measured multi nuclear relaxation rates is provided.

Results
Apart from the known dominant quadrupolar contribution to 23Na relaxation, we show that solvent collisions induce an averaged non-zero electric field gradient around the two boron isotopes despite the anticipated tetrahedral geometry. Furthermore, we describe secondary isotope effects related to the spin-spin interactions in the BF4 unit which displays both concentrations and temperature dependency pointing towards additional intermolecular interactions.

Conclusions
Our work paves the way towards corroborating spin and molecular dynamics on a more general scale compared to previous work by incorporating several relaxation mechanisms and their correlations within a model-free description of correlation functions in multiple spin systems. The proposed methodology can be extended to improve the analysis of relaxation rates in molecules with highly complex spin and conformational dynamics such as proteins.

COM-006: The application of optimal control pulses for magnetisation transfer in a microfluidic chip

Miss Anupama Acharya[1], Dr Tom R. Robertson[1], Dr Yael Ben-Tal[1], Dr Sylwia J. Barker[2], Prof. Malcolm H. Levitt[1], Prof. Marcel Utz[2], Prof. Ilya Kuprov[1]
[1]University Of Southampton, Southampton , United Kingdom, [2]Karlsruhe Institute of Technology, Karlsruhe, Germany

Pulse design using optimal control is well implemented in various aspects of NMR spectroscopy and imaging [1,2,3]. However, its application to microfluidic lab-on-a-chip (LoC) devices is still novel because both B_0 and B_1 fields are non-uniform, and the sample undergoes simultaneous flow and chemical reactions. Such microfluidic chips are becoming an important means of monitoring mammalian reaction pathways [4], within which a constant stream of hyperpolarised substrate such as pyruvate can be delivered to cultured tissue, and their downstream metabolites can be detected via NMR – all on the same device. In this communication, we present the initial steps towards simulating observable hyperpolarised magnetisation on [1-^{13}C]pyruvate by transferring spin order from para-enriched hydrogen using the gradient ascent pulse engineering (GRAPE) algorithm in Spinach. We simulate their performance on two different pyruvate molecules, and achieve fidelities of around 50%. Additionally, we report the challenges faced in calibrating the simulated pulses onto a Bruker instrument, since achieving the same fidelities experimentally remains a work in progress.

[1] T.E. Skinner, T.O. Reiss, B. Luy, N. Khaneja and S.J. Glaser. Application of optimal control theory to the design of broadband excitation pulses for high-resolution NMR. Journal of Magnetic Resonance, 163(1), pp.8-15, 2003.
[2] N. Khaneja, T. Reiss, C. Kehlet, T. Schulte-Herbrüggen, and S.J. Glaser. Optimal control of coupled spin dynamics: design of NMR pulse sequences by gradient ascent algorithms. Journal of magnetic resonance, 172(2), pp.296-305, 2005.
[3] E. Van Reeth, H. Ratiney, M. Lapert, S.J. Glaser, and D. Sugny. Optimal control theory for applications in Magnetic Resonance Imaging. Pacific Journal of Mathematics for Industry, 9, pp.1-10, 2017.
[4] S.J. Barker, L. Dagys, W. Hale, B. Ripka, J. Eills, M. Sharma, M.H. Levitt, and M. Utz. Direct Production of a Hyperpolarized Metabolite on a Microfluidic Chip. Analytical Chemistry, 94(7), pp.3260-3267, 2022.

COM-008: Spin-rotation relaxation: separation of contributions from internal and overall motion

Professor Alexej Jerschow[1], Professor Malcolm Levitt[2], Dr. Mohamed Sabba[2], Harry Harbor Collins[2], Professor Alexander Auer[3]
[1]New York University, New York, United States, [2]University of Southampton, Southampton, UK, [3]Max-Planck Institute for Coal Research, Mülheim, Germany

Spin-rotation (SR) relaxation, arising from the coupling of the angular momentum with spin is typically discussed in the context of gas molecules due to their fast motion, but it can be observed as a significant contribution in liquids as well. SR may become a dominant relaxation mechanism in low-field NMR, but also in nuclear singlet order relaxation.

We discuss here several situations where effective SR relaxation is observed experimentally, and aim to model these relaxation interactions using MD simulations and ab initio calculations. We show examples of where SR becomes a dominant relaxation contribution in the study of nuclear spin singlet order, and examine in particular the situation of free and hindered methyl rotations.

The separation of internal motion from global motion contributions to SR relaxation is a particularly tricky problem. We discuss several implications of such effects, including the study of how spin-rotation tensors should be calculated in such a case.
In the particular case of methyl SR relaxation, we also model the SR interaction with MD simulations and ab initio calculations, and find that the unhindered methyl rotation is not modeled well by the simulations as is evidenced by the discrepancy between experiment and simulations.

At present, calculations do not fully account for the observed effects, potentially due to quantum rotor effects which alter spin-rotation rates. This study aims to produce a solid foundation for the use of spin-rotation relaxation mechanisms for providing structural and dynamic information.

COM-009: SpinDETR: Direct Spin System Elucidation through Detection Transformers in NMR Spectroscopy

Nicolas Schmid[1,2], Marc Wanner[3], Giulia Fischetti[4], Ph.D. Andreas Henrici[1], Meshkian Mohsen[1], Ph.D. Flavio De Lorenzi[1], Ph.D. Simon Bruderer[5], Ph.D. Björn Heitmann[5], Ph.D. J.D. Wegner[2], Ph.D. R.K.O. Sigel[2], Ph.D. Dirk Wilhelm[1]
[1]Zurich University of Applied Sciences (ZHAW), Winterthur, Switzerland, [2]University of Zurich (UZH), Zurich, Switzerland, [3]Swiss Federal Institute of Technology (ETH), Zurich, Switzerland, [4]Ca' Foscari University of Venice, Venice, Switzerland, [5]Bruker Switzerland AG, Fällanden, Switzerland

Introduction:
1D (^1H) Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful tool for revealing molecular structures and dynamics, but interpreting NMR spectra, especially those with intricate signal patterns, requires significant expertise and time.

Aims:
Our study aims to enhance the efficiency and accessibility of NMR spectroscopy analysis by harnessing spin system simulations and advanced deep-learning techniques.

Methods:
We adopt detection transformer models (state-of-the-art deep learning algorithms) to interpret complex NMR spectra. Therefore, we generate a dataset via spin system simulations that emulate common structural motifs. The dataset is used to train our model to learn the correlations between spectral patterns and their corresponding spin system features.

Results:
Our approach advances the interpretation of complex experimental NMR spectra, particularly those characterized by strong coupling or overlapping resonances. Our model can directly predict key spin system parameters such as chemical shifts, proton numbers, and coupling constants from experimental spectra. This achievement underscores the potential of our approach as a complementary asset to traditional analysis methods, promising to streamline the interpretation workflow and broaden the application scope of NMR analysis.

Conclusions:
Integrating relational deep learning via transformer models with spin system simulations signifies an important step in the automated analysis of NMR spectroscopy. This research contributes to the evolving landscape of computational spectroscopy analysis, aiming for a future wherein NMR analysis becomes more universally accessible and efficient.

COM-010: NMR studies of mixed surfactant solutions

M.Sc. Pau Mayorga Delgado[1], Dr. Ritu Ghanghas[1], Dr. Anne Selent[1], Dr. Sanna Komulainen[1], B.Sc. Ilari Ainasoja[1], B.Sc. Kalle Koitila[1], Dr. Pär Håkansson[2], Prof. Nønne Prisle[3], Dr. Perttu Lantto[1], Dr. Jiří Mareš[1], Prof. Ville-Veikko Telkki[1]
[1]NMR Research Department, University Of Oulu, Oulu, Finland, [2]Dispelix Oy, Oulu, Finland, [3]Center for Atmospheric Research (ATMOS), University of Oulu, Oulu, Finland

Surfactants are long carbon chain molecules with a hydrophilic and hydrophobic group. At low concentration in water, they tend to distribute in the bulk and in the droplet interface, lowering the surface tension of the solution. As their concentration increases, they start clustering and at a certain concentration threshold, the so called critical micelle concentration (CMC), bigger aggregates are formed, called micelles. The distribution of surfactants in the bulk affects the water activity. This is an important property to understand how the atmospheric water droplets are formed, which would allow us to gain a deeper understanding on cloud formation and the impact of the surfactants on the climate.

This study focuses on computational simulations to understand the structure and dynamics of two mixtures of surfactants in water: sodium decanoate with hexanol and sodium hexanoate with SDS. These are examples of surfactants present in the atmosphere. Our results are compared with NMR experimental data.

Molecular dynamics simulations are used to characterize these mixed systems at several concentrations above and below their CMC. The Stokes-Einstein model has been used to compute the diffusion of the surfactants, along with state-of-the-art NMR relaxation modelling, to gain insight on their dynamics.

Our computational results are the following. For the first mixture, we obtained similar diffusion coefficients for both surfactants. The relaxation rates are also similar, agreeing with the experimental trend. In contrast, the diffusion of the hexanoate and SDS from the second mixture differed by an order of magnitude and relaxation pointed towards slower dynamics.

The surfactants from the first mixture tended to self-assemble forming common aggregates. In the second mixture, below the CMC of the hexanoate, only the SDS formed micelles, while above the CMC both surfactants form a variety of aggregate sizes. This work provides a detailed understanding of their size distribution.

COM-011: Software field locking and post-acquisition correction of high resolution NMR spectra

Dr Eugeny Kryukov[1], Dr Alexander Karabanov[1], Dr Denis Langlais[1], Dr Jeremy Good[1]
[1]Cryogenic Ltd, London, United Kingdom

The resolution of NMR data often suffers from spectral distortions due to instabilities of the external magnetic field. There can be various reasons for such instabilities, from a field drift and mechanical vibrations to a magnetic field ramp or even the quench of a nearby magnet. The standard way to correct these distortions is the use of a field lock. We introduce an alternative way to overcome this problem based on applying a software correction. This method is universal and particularly useful for cryogen-free magnets where the mechanical nature of the cryocooler can cause temporal field distortions. Such magnets can be used at different magnetic fields, so by the method we suggest, the field drift after the field change ceases to be a problem.

Our method is based on recording a large set of single shot spectra. All individual spectra are software processed in three steps. First, the field distortions during single acquisitions are removed from each recorded spectrum. Second, all individual spectra are brought to the same frequency. Third, the average of the corrected individual spectra is obtained to get the final spectrum.

With the application of the software correction, high resolution spectra of liquid ethanol were obtained by accumulating single shot measurements during more than twelve hours. We also showed that this correction remains accurate if the magnetic field changes due to nearby magnet ramps and quenches. Several 2D spectra were also recorded to demonstrate the performance of our 400 MHz cryogen-free magnet.

Our software correction can be used as an alternative to a deuterium field lock. It does not require a special acquisition channel and works for high and low frequency distortions related to the time of the signal acquisition.

COM-012: MuSe Net: a deep learning framework for trustworthy multipletsegmentation in one-dimensional proton NMR spectra

Ms. Giulia Fischetti[1], Mr. Nicolas Schmid[2,3], Dr. Andreas Henrici[2], Prof., Dr. Dirk Wilhelm[2]
[1]Ca' Foscari University Of Venice, Mestre - Venezia, Italy, [2]Zurich University of Applied Sciences (ZHAW), Winterthur, Switzerland, [3]University of Zurich (UZH), Zurich, Switzerland

Introduction: Numerous research fields, including organic chemistry, metabolomics, drug discovery, battery technology, and environmental sciences, rely widely on one-dimensional proton NMR spectroscopy to investigate the structural properties of compounds, thanks to its simplicity and reduced acquisition times. However, spectra annotation can be time-consuming and prone to interpretation biases, due to artefacts and overlapping signals.
Aim: To address this issue, we exploited the potentiality of deep learning in pattern recognition and predictive uncertainty estimation methodologies to build a reliable tool to assist NMR practitioners in the annotation process.
Methods: We present MuSe Net (Multiplet Segmentation Network), a new supervised probabilistic deep learning framework designed to identify and categorize multiplets within phenotypes with up to four coupling constants based on the splitting pattern. Without any information on the compound, MuSe Net produces a segmentation of the spectral range, paired with a confidence score. The confidence score both assesses classification reliability and detects signals that do not fit in any other phenotype class: this is the case of overlapping multiplets. Our algorithm was trained on a wide collection of synthetic spectral segments, automatically produced and labelled to ensure consistency of the multiplet phenotypes represented.
Results: MuSe Net performance was evaluated against 48 experimental proton NMR spectra of small molecules annotated by experts. MuSe Net produced an accurate prediction in a large variety of experimental conditions, dealing effectively with solvent peaks, anomalies, and unclear signals while correctly classifying multiplets and detecting overlapping peaks. Interestingly, MuSe Net prediction and confidence score remained robust even at reduced signal-to-noise ratios.
Conclusions: In conclusion, MuSe Net holds the potential to significantly shorten the annotation process of one-dimensional proton NMR spectra, providing automatic reliable predictions of multiplet splitting patterns.

COM-013: Gaussian processes for regression and guided slice selection in non-uniformly sampled NMR data – A concept demonstration

Dr James Ellison[1,2], Prof Dame Clare Grey[2]
[1]ETH Zurich, Hönggerberg, Switzerland, [2]University of Cambridge, Cambridge, United Kingdom

As long NMR measurements are costly, extracting as much information as possible from a given NMR dataset is crucial, particularly for high-dimensional data. Gaussian processes (GPs) are a non-parametric fitting technique that are an attractive yet relatively unexplored method for NMR processing.
This presentation demonstrates GPs' application in regression, accurately estimating the true noiseless NMR spectrum in one or multiple dimensions from noisy, finitely (under-)sampled data. GPs are highly amenable to this goal, as their primary assumption is that all variables are drawn from a multivariate normal distribution, which NMR noise excellently approximates. By considering an infinite set of potential fitting functions, defined by a kernel, and weighted by their likelihood, GPs provide an estimate of the true underlying NMR signal and, novelty, a reasonable estimate of the error bars, mitigating the risk of noise overanalysis and assisting in identifying statistically significant changes.
In non-uniform sampling, GPs enable interpolation or extrapolating data points, facilitating the estimation of data points not originally sampled and enabling a conventional Fourier transform to give the desired spectrum, again with error estimates. Further, it is shown that one can calculate the expected information gain (EIG) for each potential future slice in the NUS schedule. Using the EIG, one can create the sampling schedule on the fly to maximise the information gained from each slice. Utilising prior knowledge of the high dimensional spectrum, such as 1D spectra of each axis, further enhances GP performance. The application of GPs in analysing peak intensities from operando 17O NMR spectra of batteries demonstrates their utility in avoiding the trade-off between time resolution and signal-to-noise when selecting the number of transients to average over when acquiring operando data.
GPs are shown to compare favourably to existing regression and reconstruction methods and are an exciting avenue for further investigation.

COM-014: An analytical formulation of dynamic magic-angle spinning NMR

Dr Jérôme Hirschinger[1]
[1]CNRS, Strasbourg, France

The treatment of chemical exchange is well known in liquid-state NMR. A similar approach is applicable for static solid-state spectra where the exchange occurs between different anisotropic frequencies. Under magic-angle spinning (MAS), however, numerical time-ordered integration of the stochastic Liouville equation (SLE) [1] or a relatively complex mathematical treatment using Floquet theory [2] is necessary to deal successfully with the additional time dependence of the resonance frequencies due to MAS. Indeed, simplified procedures based on approximations to the time-ordered integration method [3] or the Anderson-Weiss theory [4] exhibit large gaps of validity.

In this work, we present a simple analytical model to compute the full side-band spectra of exchanging nuclei under MAS for arbitrary rates of exchange and spinning frequencies.

Our method is deduced from an insightful paper by A. J. Vega [5] presenting a generalized approach to relaxation under coherent averaging. The basic recipe followed in our line shape calculations is then to take the longer of the anisotropic T_2 values approximated by relaxation [6] and Floquet [2] theories in the fast and slow exchange limits.

As an example, this method is employed for analyzing the case of two-site exchange in ^{2}H MAS NMR. Comparison with exact calculations through numerical integration of the SLE justifies the application of our approach even in the intermediate exchange regime.

References

1. M. J. Duer, M. H. Levitt Solid State Nucl. Magn. Reson. 1, 211 (1992)
2. A. Schmidt, S. Vega J. Chem. Phys. 87, 6895 (1987)
3. L. Frydman, B. Frydman Magn. Reson. Chem. 28, 355 (1990)
4. J. Hirschinger Concepts in Magn. Reson. 28A, 307 (2006)
5. A. J. Vega J. Magn. Reson. 65, 252 (1985)
6. P. Schanda, M. Ernst Prog. Nucl. Magn. Reson. Spectrosc. 96, 1 (2016)

COM-015: NMR quantum simulation on a NISQ computer

Mr Artemiy Burov[1]
[1]FHNW, Zurich, Switzerland

Here we investigate the application of noisy intermediate-scale quantum devices for simulating nuclear magnetic resonance experiments in the high-field regime. In this work the NMR interactions are mapped to a quantum device via a product formula with minimal resource overhead, an approach that we discuss in detail. Using this approach, we show results of simulations of proton NMR spectra on relevant molecules with up to 11 hydrogen spins, for a total of 47 atoms, and compare them with real NMR experiments. Despite current limitations we show that a similar approach will eventually lead to a case of quantum utility, a scenario where a practically useful computational problem can be solved by a quantum computer but not by conventional means. We provide an experimental estimation of the amount of quantum resources needed for solving larger instances of the problem with the presented approach. The polynomial scaling we demonstrate on real processors is a foundational step in bringing practical quantum computation closer to reality. Apart from this conceited interest from the quantum computing community, we believe that our results are also of profound interest to the NMR community, in particular for applications such as chemical structure verification and elucidation via NMR spectroscopy.

COM-016: Progress in NMR data sharing and online data analysis

Mr. Thomas Vosegaard[1], Dr. Armin Afrough[1]
[1]Interdisciplinary Nanoscience Center, Aarhus University, Aarhus C, Denmark

It remains surprisingly challenging to share NMR data in a simple and self-contained fashion despite many years of this need, and this limits the availability of FAIR (findable, accessible, interoperable, and reusable) data in the NMR community. Given the enormous success of databases in other disciplines (e.g. the protein database), it seems like there is a great unexploited potential for this in the NMR community.

This presentation adresses our attempts to provide easier (and online) access to all aspects of the pipeline from recording NMR spectra to the NMR processing and data analysis. Remote access to NMR spectrometers (typically running old operating systems) and their data in a highly secured IT setup and different approaches to data sharing are discussed.

Finally, new approaches online data processing and analysis of NMR spectra using our in-house developed web platform EasyNMR is presented.

COM-017: Characterising Aromatic Side Chains in Proteins through the Synergistic Development of NMR Experiments and Deep Neural Networks

Dr Vaibhav Kumar Shukla[1], Dr Gogulan Karunanithy[1], Prof Pramodh Vallurupalli[2], Prof D Flemming Hansen[1]
[1]The Francis Crick Institute and University College London, London, United Kingdom, [2]Tata Institute of Fundamental Research Hyderabad, Hyderabad, India

Introduction: Nuclear magnetic resonance (NMR) spectroscopy has become an important technique in structural biology for characterising the structure, dynamics and interactions of macromolecules. While a plethora of NMR methods are available to inform on backbone and methyl-bearing side-chains of proteins, a characterisation of aromatic side-chains is more challenging and often requires specific labelling or 13C-detection.

Aims: The aim of the research is to develop a deep neural network (DNN), named FID-Net-2, which transforms NMR spectra recorded on simple uniformly 13C labelled samples to yield high-quality 1H-13C correlation spectra of the aromatic side chains.

Methods: FID-Net-2 was developed based on our previous FID-Net DNN architecture, but with the ability to transform entire 2D planes in one shot and also to produce uncertainties of the transformation. Key to the success of FID-Net-2 is the design of complementary NMR experiments that produce spectra with unique features to aid the DNN produce high-resolution aromatic 1H-13C correlation spectra with accurate intensities.

Results: We first evaluate the trained FID-Net-2 model on synthetic data and show that the reconstructed aromatic 13C-1H spectra can be used for quantitative purposes as FID-Net-2 predicts uncertainties in the resulting spectra. We also validated the new methodology experimentally on protein samples ranging from 7 to 40 kDa in size. We demonstrate that the method can accurately reconstruct high resolution two-dimensional aromatic 1H-13C correlation maps, high resolution three-dimensional aromatic-methyl NOESY spectra to facilitate aromatic 1H-13C assignments, and that the intensities of peaks from the reconstructed aromatic 1H-13C correlation maps can be used to quantitatively characterise the kinetics of protein folding.

Conclusions: We believe that the strategy of devising new NMR experiments specifically for analysis using customised DNNs represents a substantial advance that will have a major impact on the study of molecules using NMR in the years to come.

COM-019: Improved interoperability of chemistry data with CHEMeDATA

Dr. Damien Jeannerat[1,2], Dr. Felipe Seoane[2], Dr. Carlos Cobas[2]
[1]NMR Process, Geneva, Switzerland, [2]Mestrelab Research, Santiago de Compostela, Spain

The CHEMeDATA project aims to introducing a better interoperability of chemistry data at the level of key chemistry concepts. In NMR, these include spectra, 3D molecular structures, but also calculated or experimental sets of NMR parameters. A simple mechanism will enable any scientist dealing those concepts (spectra, NMR parameter, peak lists, etc.) to derive, if necessary, the CHEMeDATA core concepts according to their own specific needs and benefit from the existing tools for visualization, conversion, metadata extraction, etc.

In the CHEMeDATA environment, pasting the output of a DFT/GIAO calculation into a browser opened on the ingesting tool should display a list of icons representing the objects found in the data: the 3D structure of the compound, the predicted NMR parameters, but also derived objects such as the spectrum corresponding to the predicted data and any other user-defined objects, such as a measure of the similarity of NMR data. Each object could be readily opened in the user's preferred desktop-software (such as Mnova) for the visualization of the synthetic spectrum, use the default browser-based preview, or any user-defined action to validate, reformat, share, archive, etc., the data.

CHEMeDATA is being designed for researchers and software developers having no interest in data ontology or overwhelming data schemas. It consists of small units of data specifications with illustrative examples, guidance on how to modify, extend, test and eventually share visualizers, data converters, etc. with the community.

The CHEMeDATA can also be used to make data hidden in zip files within repositories readily searchable and reusable thanks to the metadata and relevant data extraction performed during ingestion. Those AI-compatible metadata could be easily made accessible to search engines.

We will exemplify the CHEMeDATA approach to the new open data format of the Mestrelab Mnova software.

COM-020: Using Machine Learning to Improve the Hard Modeling of NMR Time Series

Jan Hellwig[1,2], Prof. Klaus Neymeyr[1,2]
[1]University Of Rostock, Rostock, Germany, [2]Leibniz Institute for Catalysis, Rostock, Germany

It has recently become common practice to use neural networks for peak detection in single NMR spectra. However, applying neural networks to time series of NMR spectra goes beyond a mere application to several single spectra. The problem of peak shifts and possible crossings requires a correct peak tracing through time. Furthermore, situations where multiple peaks overlap may lead to multiple viable solutions for current neural networks, even though the mathematical solution is unique [1]. Using additional information given by the second dimension helps to resolve this type of ambiguity. Our proposed solution for modeling time series of NMR spectra assumes that the peak parameters are smooth functions in time, which allows their interpolation by cubic spline functions, thus limiting the number of parameters at given points in time that need to be optimized [2]. The smoothness of the splines guarantees robust peak tracing and deconvolution of overlapping peaks, provided the peak parameters are correctly optimized. However, this is not always the case. A large number of peaks, and thus peak parameters, may not only lead to inaccurate tracing, but also to long runtimes. To address the shortcomings of nonlinear optimization algorithms we use neural networks to provide more accurate initial estimates from which the optimizer only needs to fine-tune the predicted parameters to achieve numerical convergence. We present results for constructed as well as experimental data sets and achieve an improvement in both runtime and accuracy.

[1] J. Hellwig, K. Neymeyr, submitted (2024)

[2] D. Meinhardt, H. Schröder, J. Hellwig et al., J. Magn. Reson. 339 (2022) 107212.

COM-021: Combining Molecular Dynamics with Deep Neural Network Architectures for Realistic Simulations of Porous Liquids

Dr. Ouail Zakary[1], Dr. Perttu Lantto[1]
[1]NMR Research Unit, University Of Oulu, Oulu, Finland

Aims:

Porous materials serve diverse applications, including molecular separations and catalysis, facilitating the efficient capture of greenhouse gases (CO_2 and CH_4) and valuable noble gases (Xe, Ar, and Kr). Xenon, crucial in optics, medicine, and nuclear processes, presents extraction challenges due to its low atmospheric abundance and inert nature, leading to high commercial costs. Effective xenon isolation demands materials with precise selectivity and high adsorption capacity. Porous liquids (PLs) with porous organic cages (POCs) offer a promising solution to these challenges. Understanding the interaction between host (PL/POC) and guest (Xe), including binding, occupancies, dynamics, and equilibrium, is pivotal for designing POCs tailored to specific functionalities. Combined with ab initio methods, molecular dynamics simulations have proven essential for understanding the physicochemical processes governing these systems. However, this combination is limited to tens of picoseconds and a few hundred atoms, falling short of capturing realistic timescales and size of these porous systems. In recent years, machine learning, particularly neural networks (NNs), has emerged as a promising solution to these limitations by learning accurate interatomic potentials from a set of high-fidelity reference calculations while maintaining computational efficiency.

Methods and Results:

We introduce precise and data-efficient machine learning interatomic potential (MLIP) models developed using Allegro, a local equivariant deep neural network architecture. These models, trained, validated, and tested on DFT-level data, encompass energies, forces, and virials in structures comprising 600 to 1170 atoms (H, C, N, O, F, Cl, Xe). Encompassing various configurations of xenon atoms in different PLs and POCs, the dataset comprises 1.7 million atoms and 12 million data points.

Conclusion:

The MLIP models enable simulations of large-scale porous liquids under realistic physicochemical conditions. They facilitate microscopic interpretation of experimental ^{129}Xe NMR data, a local probe critical for understanding the condition-dependent dynamic processes within these systems at both static and dynamic levels.

COM-022: Optimal control flow encoding for time-efficient magnetic resonance velocimetry

Dr. Mehrdad Alinaghian Jouzdani[1], Dr. Mazin Jouda[1], Prof. Jan Korvink[1]
[1]KIT, Eggenstein-leopoldshafen, Germany

Phase contrast velocimetry relies on bipolar gradients to establish a direct and linear relationship between the phase of the magnetic resonance signal, and the corresponding fluid motion. Despite its utility, several limitations and drawbacks have been reported, the most important being the extended echo time due to the encoding after the excitation. In this study, we elucidate a new approach based on optimal control theory that circumvents some of these disadvantages. An excitation pulse is designed to encode velocity into phase already during the radiofrequency excitation. As a result of concurrent excitation and flow encoding, and hence elimination of post-excitation flow encoding, it achieves a shorter echo time than the conventional method. This achievement is a matter of significance not only because it decreases the loss of signal due to spin–spin relaxation and B0 inhomogeneity, but also because a shorter echo time is always preferred in order to reduce the dimensionless dephasing parameter and the required residence time of the flowing sample in the detection coil. The method is able to establish a non-linear bijective relationship between phase and velocity, which can be employed to enhance the resolution over a specific range of velocities, for example along flow boundaries. A computational comparison between the phase contrast and optimal control methods reveals that the latter's encoding is more robust against remnant higher-order-moment terms of the Taylor expansion for faster voxels, such as acceleration, jerk, and snap.

COM-023: Scheme for defending gradient field spillover by coherence locking

Mr. Mengjia He[1], Dr. Neil MacKinnon[1], Prof. Jan Korvink
[1]Karlsruhe Institute Of Technology, Eggenstein Leopoldshafen, Germany

Introduction
The implementation of parallel NMR detection was intended to enhance measurement throughput, thereby expediting drug screening processes[1]. However, the local gradient coils in parallel NMR probes introduce field spillover in adjacent channels, leading to spin dephasing and signal suppression. The inter-channel coupling through gradient field spillover remains to be addressed.
Aims
This study proposed a pulse compensation scheme employing an optimized RF pulse to achieve coherence locking during gradient pulse periods.
Methods
The design of spin-locking pulses utilizes optimal control in which gradient-induced field inhomogeneity was addressed. In the optimal control, the specified spin coherences were protected against the field gradient. Multiple spin locking pulses obtained from the single spin model can be applied simultaneously considering that J coupling was effectively averaged out, which was verified by using the average Hamiltonian theory. The optimal control was implemented in Spinach v2.8[2].
Results & Conclusions
These pulses are applied to the parallel HMQC and HSQC sequences, demonstrating their effectiveness in spin locking. These pulses exhibit robustness to an extensive coupled gradient (15 Gauss of B0 inhomogeneity) and resonance offsets, with reasonable RF amplitude. This compensation scheme presents a valuable solution for using NMR probes with parallel gradient coils.
Reference
[1] Kim, M. Liu, C. Hilty, Parallelized ligand screening using dissolution dynamic nuclear polarization. Analytical Chemistry 88, 11178–11183 (2016).
[2] H. J. Hogben, M. Krzystyniak, G. T. Charnock, P. J. Hore, I. Kuprov. Journal of Magnetic Resonance 208, 179–194 (2011).
Acknowledgments
The authors are supported by the Joint Lab Virtual Materials Design (JLVMD) of the Helmholtz Association, Germany. M.H. thanks the state of Baden-Württemberg through the bwHPC BwUniCluster2.0.

COM-024: Magnetstein: a novel tool in qNMR and monitoring chemical reactions

Barbara Domżał[1], Magdalena Grochowska-Tatarczak[1], Anna Gambin[1], Krzysztof Kazimierczuk[1]
[1]University Of Warsaw, Warszawa, Poland

Monitoring chemical reactions using quantitative NMR spectroscopy can be a challenging task. Common difficulties include overlapping peaks of substrates and products, shifting of peak positions over time and distorted lineshapes due to magnetic field inhomogeneities. Here, we present Magnetstein: a novel tool for quantification of spectral components, offering solutions to these problems. The algorithm is based on the Wasserstein metric — a purely mathematical concept that has proven to be remarkably successful in the analysis of spectroscopic data. Magnetstein has already been widely tested on a task of estimating proportions of components in a mixture given the spectrum of this mixture and a library of components' spectra [1, 2]. Presently, we demonstrate its effectiveness in monitoring chemical reaction dynamics as well.

[1] Domżał, B., Nawrocka, E.K., Gołowicz, D., Ciach, M.A., Miasojedow, B., Kazimierczuk K. & Gambin, A. (2023). Magnetstein: An Open-Source Tool for Quantitative NMR Mixture Analysis Robust to Low Resolution, Distorted Lineshapes, and Peak Shifts. Analytical Chemistry. 96. 10.1021/acs.analchem.3c03594.

[2] https://github.com/BDomzal/magnetstein

COM-025: Combination of a Novel Reduced Matrix STD NMR Approach with Molecular Modelling and Machine Learning to generate 3D models of weak protein-ligand complexes

Gabriel Rocha Domínguez[1], Adelyn Betances[1], M. Carmen Padilla Perez[2], Elena Sánchez-Fernández[2], Juan Carlos Muñoz-García[1], Carmen Ortiz-Mellet[2], Jesus Angulo[1]
[1]IIQ-csic, Sevilla, España, [2]Departamento de Química Orgánica. Facultad de Química. Universidad de Sevilla, Sevilla, España

Introduction

STD NMR is one of the most powerful approaches to detect and characterise low to medium affinity receptor-ligand interactions in solution. The relative distribution of STD intensities on the ligand protons allows mapping of the ligand binding epitope, revealing structural details of the interaction. This allows an understanding of the molecular basis of biomolecular recognition processes, which is fundamental for drug discovery. A proper, quantitative, combination of this technique with Molecular Docking calculations and Molecular Dynamics simulations can lead to 3D molecular models of weak ligand-protein complexes. In this work, the binding of a fluorinated derivative of the sp2-iminosugars type (α-D-gluco configuration) to the enzyme α-glucosidase from Saccharomyces Cerevisiae (a model used for in vitro inhibition studies) has been studied. The results demonstrate the validity of the combined approach for generating experimentally validated 3D models of weak protein-ligand complexes.

Aims

To develop a protocol combining NMR and computational techniques to obtain structural information on weak ligand-protein interactions.

Methods

STD NMR, Molecular Docking, Molecular Dynamics Simulations, Machine Learning.

Results

The results show recognition of the iminosugar ring, although preference for interaction with the attached aromatic moiety of the ligand is observed. In addition, competition experiments demonstrated that there exist different binding sites available for ligand binding, in agreement with the literature. The combination of molecular modelling, fast STD NMR validation of 3D models of the protein-ligand complexes via our RedMat software, and Machine Learning techniques for cryptic pocket identification (PocketMiner), allowed us to propose a 3D molecular model of the complex validated by experimental data from STD NMR spectroscopy.

Conclusions

The proposed protocol, combining NMR spectroscopy, molecular modelling and machine learning, is a robust method to reveal the molecular recognition in systems of weak affinity for which X-ray crystallographic data cannot be obtained.

COM-026: Improving Preparation Pulse Design through Hyperparameter Tuning for GRAPE

Amanda Nicotina[1], Dr. Steffen J. Glaser[1]
[1]Techinal University Of Munich, Garching, Germany

Magnetic Resonance Imaging (MRI) pulse design can bring optimal imaging results, particularly by enhancing contrast from intrinsic tissue properties, such as relaxation times. However, tuning the hyperparameters involved in this process is highly sensitive and challenging to perform manually. We applied the BOHB (Bayesian Optimization with Hyperband) algorithm to automate hyperparameter optimization to address this. BOHB combines the probabilistic modeling of Bayesian Optimization to guide the search process with the dynamic resource allocation of Hyperband, ensuring efficient and robust optimization. In this work, we applied BOHB as a hyperparameter tuning technique for GRAPE (Gradient Ascent Pulse Engineering) in designing pre-acquisition pulses to improve saturation contrast in MRI. This approach is computationally intensive but particularly beneficial for complex optimizations, such as those involving B0 and B1 inhomogeneities, because it automates the task. Our hyperparameter search focused on selecting the optimal protocol for adjusting the scalar ε, which scales the gradient element for the field update within the GRAPE algorithm and the final control time. The BOHB method has significantly improved manual tuning and grid search, offering a more efficient and automated solution for hyperparameter tuning. We aimed to design a pulse sequence that simultaneously introduces contrast and considers B0 inhomogeneity. This optimization was performed using the new implementation of the GRAPE algorithm in Julia. By defining a cost function that rewards good contrast and robustness against B0 inhomogeneity, the algorithm iteratively designs the optimal pulse for each scenario. The following steps involve integrating this work with KomaMRI, a software designed to simulate MRI, to create a suite that implements the optimally designed pulses and evaluates their performance in improved contrast and inhomogeneities by simulating MRI experiments in a end-to-end manner. This method holds potential for various applications, such as in the food industry.

COM-027: BEYOND TRADITIONAL NMR DATA PROCESSING WITH AI

Mr. Amir Jahangiri[1], Professor Vladislav Orekhov[1]
[1]University Of Gothenburg, Gothenburg, Sweden

Artificial Intelligence (AI), specifically Deep Neural Network (DNN), has found new applications in NMR data processing [1] in recent years. Rapid and high-quality Non-Uniform Sampling (NUS) reconstruction [2-4], efficient homo-decoupling, and automated peak picking are examples of novel AI-based tools that can be utilized as alternatives to traditional methods in NMR data processing.

We recently presented a novel architecture, WNN, specifically designed to capture distinct patterns within 2D NMR spectra [4]. In comparison with other methods, our architecture, WNN, benefits from pattern recognition of the corresponding Point Spread Function (PSF) pattern produced by each peak on a fixed non-uniform sampling (NUS) schedule, resulting in higher-quality and more robust NUS reconstruction. WNN is also able to successfully perform virtual homo-decoupling in both indirect and direct spectral dimensions.

In this work, we first introduce a generalized version of the WNN architecture for high-dimensional spectra, enabling AI tools in high-dimensional NMR, and then present novel "smart" NMR signal processing tasks that go beyond the traditional methods. Overall, our results underscore the potential of deep learning techniques for enhancing NMR data analysis and outperforming traditional NMR processing methodologies.

References

[1] D. Chen, Z. Wang, D. Guo, V. Orekhov, X. Qu, Chemistry – A European Journal 2020, 26, 10391.
[2] X. Qu, Y. Huang, H. Lu, T. Qiu, D. Guo, T. Agback, V. Orekhov, Z. Chen, Angewandte Chemie 2020, 132, 10383.
[3] G. Karunanithy, D. Hansen, Journal of Biomolecular NMR 2021, 75.
[4] A. Jahangiri, X. Han, D. Lesovoy, T. Agback, P. Agback, A. Achour, V. Orekhov, Journal of Magnetic Resonance 2023, 346, 107342.

COM-028: A Bottom Up Approach: Frequency Dependent19F-NMR Relaxation Rates from Molecular Simulations

Ms. Angel Mary Chiramel Tony[1], Mr. Lennart Kruse[1], Dr. Anne Strate[1], Prof. Ralf Ludwig[1,2,3], Dr. Dietmar Paschek[1]
[1]Physical and Theoretical Chemistry, University Of Rostock, Rostock, Germany, [2]Department Life, Light & Matter, University of Rostock, Rostock, Germany, [3]Leibniz-Institut für Katalyse, Rostock, Germany

Introduction:
By using Fast Field Cycling (FFC) NMR spectroscopy, dynamical processes can be studied over many orders of magnitude. However, interpreting FFC-NMR data requires motional models that often use simplified analytical forms of the relevant time correlation functions.

Aim:
A novel approach for computing the inter- and intramolecular contributions to the magnetic dipolar relaxation from molecular dynamics (MD) simulations, enabling us to predict NMR relaxation rates addressing the full FFC frequency range, covering many orders of magnitude, while also avoiding influences due to limitations in system size and the accessible time interval.

Method:
Our approach is based on combining the analytical theory of Hwang and Freed (HF) for the long-range intermolecular contribution of the magnetic dipole-dipole correlation function employing MD simulations.

Results:
We successfully computed the inter- and intramolecular NMR relaxation of 19F nuclei in the ionic liquid n-pentyl- pyridinium bis-(trifluoromethyl-sulfonyl)imide (C5Py-NTf2) to study the dynamics of the NTf2 anion. We show that the correlation functions due to the HF-theory do asymptotically converge with our MD simulation results at long times.

Conclusion:
We are able to study both the influence of the translational dynamics and the rotational dynamics of the anions. Moreover, by using our MD simulation based approach, we can disentangle the different contributions to the intramolecular 19F NMR relaxation rate due to the complex intramolecular dynamics of the anion.

COM-029: A Deep Learning Approach for Removing Vibration Artifacts in Shuttled NMR Experiments

Rajka Pejanovic[1], Dr. Lucas Siemons[1], Dr. Philippe Pelupessy[1], Mr. Jorge Garibay[2], Prof. Fabien Ferrage[1], Prof. Flemming Hansen[3]
[1]Ecole Normale Supérieure, Paris, France, [2]Bruker BioSpin GmbH, Wissembourg, France, [3]University College London, London, United Kingdom

Introduction

The principle of high-resolution relaxometry is to measure 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. This approach combines high-field NMR's high sensitivity and resolution with low-field measurements of relaxation. This technique is useful for studying ligand/protein interactions and internal dynamics in biomolecules. In motor-driven sample shuttle systems, vibration artifacts can propagate through NMR magnet and detection coils, leading to peak distortions in the spectrum.

Aim

Our study aims to develop a machine-learning signal processing technique to eliminate these vibration artifacts, while ensuring the processed data remains quantitative, preserving the original signal intensities and fitted relaxation rates (R2). We also aim to estimate errors to validate the accuracy of our method.

Methods

A series of models based on the FidNet architecture, utilizing Convolutional Neural Networks (CNNs), were implemented to process and analyze signal data. FidNet employs dilated convolutional layers to connect long- and short-term patterns, ensuring a large receptive field for analyzing free induction decays while maintaining small filter sizes. This design captures detailed temporal and special features overcoming the typical limitation of small receptive fields in CNNs that only detect localized features.

Results

A functional form was developed to model vibration artifacts accurately, and synthetic datasets were generated for model training. These trained models effectively removed artifacts from both synthetic and experimental data. Experiments on samples with few signals demonstrated the robustness of the models, even in the absence of stabilization delays, which are commonly used to mitigate vibration artifacts. This feature streamlines experiments and improves sensitivity to low-field relaxation rates.

Conclusions

Our machine-learning framework shows significant potential in accurately processing and interpreting complex signal data, with the potential to enhance the quality and reliability of NMR relaxometry measurements.

DRG-001: Ex-situ and In-situ Solid State NMR Studies of Mechanochemical Transformations of Active Pharmaceutical Ingredients (APIs) and Related Products.

Prof. Marek Potrzebowski[1]
[1]Polish Academy of Sciences, Centre of Molecular and Macromolecular Studies, Lodz, Poland

Mechanochemistry is an innovative, versatile platform for the production of a variety of compounds that in many cases are difficult to obtain using classic "wet" methods. Mechanosynthesis eliminates the need for solvents, which reduces waste and minimizes environmental impact and typically operates at room temperature leading to energy savings. Recently, it has been shown that modern analytical tools facilitate the study of solid-state processes under real operating conditions (operando). Various analytical probes such as X-rays or laser beams, vibrational spectroscopy (Fourier transform infrared spectroscopy, Raman spectroscopy) were used to control the reaction mechanism and the formation of intermediates. Unfortunately, high-resolution solid-state NMR spectroscopy has not been used very often until now, and its potential is not fully apparent. Recently, we have proven that NMR spectroscopy, in particular the 1D and 2D Magic Angle Spinning (SS MAS NMR) techniques, can be treated as a complementary tool to other analytical methods. [1,2] SS MAS NMR is particularly suitable for in situ monitoring processes when substances during transformation form low-melting eutectic mixtures (e.g. cocrystal formation). If the final products obtained by the ball mill method and the thermal process are exactly the same, Variable Temperature (VT) SS NMR is the bridge between the two methods and provides unique information about the mechanism of transformations. In this sense, MAS NMR can be thought of as a "prepanostic" device (preparation and diagnostics). The application of 19F MAS NMR as probe for investigation of mechanochemical loading of APIs into the mesoporous carriers[3] and 133Cs MAS NMR as support in mechanosynthesis of peptides will be discussed.

References

1) M. K. Dudek et al. Chem. Eur. J., 2023, e202302138.
2) M. K. Dudek, et al. Chem. Eur. J., 2020, 26, 13264.
3) K. Trzeciak et al. J. Phys Chem. C, 2021, 125.10096

DRG-002: Towards multiplexing photo-CIDNP NMR for ultrafast fragment based drug screening

Alicia Hongler[1], Matthias Bütikofer[1], Dr. Félix Torres[1], Prof. Dr. Roland Riek[1]
[1]ETH Zürich, Zürich, Switzerland

Nuclear magnetic resonance (NMR) is established as the gold standard for fragment based drug discovery (FBDD). Due to inherently low sensitivity, however, it requires extensive experimental time and sample consumption. We recently showed that photochemically induced dynamic nuclear polarization (photo-CIDNP) NMR overcomes these limitations enabling high throughput and reducing sample consumption by an order of magnitude[1]. Additionally we have been able to automatize the screening process[2] and enable drug discovery by photo-CIDNP on benchtop spectrometers[3]. Mixing fragments to multiplex FDBB is a common approach, however some compounds show incompatibility when being combined in mixtures for photo-CIDNP screens. The issue presents itself by decrease or cancellation of photo-CIDNP signal enhancement of these fragments when combined with others in solution. To enable optimal multiplexing and thus maximize throughput, compound compatibility must be predicted a priori requiring an adequate physicochemical model and parameters with predictive power.

The current study investigates the underlying kinetics aiming to determine parameters and a physicochemical model which enables the prediction of compound compatibility for fragment mixtures in photo-CIDNP NMR. Experiments were conducted to elucidate the influence of parameters including compound and photosensitizer concentrations, chemical structure as well as temperature and solvent effects. The data supports a major influence of temperature on photo-CIDNP efficiency, the stronger signal enhancement at lower temperatures is likely due to diffusion effects. Furthermore the results shed light onto the poorly understood kinetics requiring a revised physicochemical model and suggest redox potentials are a determining factor in compound compatibility prediction. Using these insights we want to derive a full kinetic model of the photo-CIDNP process. This is currently under investigation and subsequent results will be presented.

[1] F.Torres et al. JACS 2023,145(22),12066-12080

[2] W.Wüster et al. An automated NMR platform with light-coupled cryogenic probes to detect low micromolar samples, Magn.Reson.Discuss.[preprint]

[3] G.R.Stadler et al. Angew.Chem.Int.Ed.2023,62,e2023086

DRG-003: Mechanism of Tau R3 Aggregation and InhibitionRevealed by NMR-based Chemical Kinetics

Dr. Virginia Casablancas-Antras[1,2], Sylvain Demanze[3], Caroline Kerridge[3], Sarah Pearce[3], Annalisa Cavallini[3], Gary Sharman[3], Suchira Bose[3], Professor Andrew J. Baldwin[1,2]
[1]Physical and Theoretical Chemistry Laboratory, Department of Chemistry, University of Oxford, South Parks Road, Oxford OX1 3TA, , United Kingdom, [2]The Kavli Institute for Nanoscience Discovery, Dorothy Crowfoot Hodgkin Building, South Parks Road, Oxford OX1 3QU, , United Kingdom, [3]Eli Lilly & Co., Erl Wood Manor, Windlesham, Surrey GU20 6PH, , United Kingdom
Outline: We developed an algorithm to fit detailed chemical kinetic aggregation models to NMR data. We applied the algorithm to determine the aggregation mechanism (including nucleus size) of an amyloid forming protein, and how this is inhibited by a literature peptide.

Introduction: Protein misfolding diseases are becoming increasingly prevalent and constitute a global health challenge. Macroscopic deposits of aggregated protein are associated with diseases such as Alzheimer's (tau and β-amyloid) and Parkinson's (α-synuclein). The mechanisms of protein aggregation are poorly understood, hindering drug discovery. Chemical kinetics analyses are limited by approximations in the models, and limited information content and reproducibility of the data.

Methods: We used a label-free NMR assay to quantify the kinetics of aggregation. Fitting the NMR data to successively more complex detailed models can account explicitly for the effects of drugs and molecular chaperones. Here, we extended this method by systematising the model building, selection and testing routine, yielding a 'winning' best-fitting model of minimal complexity.

Results: Tau R3, corresponding to tau's third microtubule region repeat, aggregated in the absence of cofactors or agitation. R3 aggregates were shown to form via a tetrameric nucleus. We found that the peptide D-TLKIVW has a multifaceted mode of inhibition that includes direct targeting of the nucleation step, which extends its action beyond the originally proposed mechanism as an elongation blocker.

Conclusions: The detailed mechanism of aggregation and inhibition of tau R3 presented in this work highlights the potential of NMR-based chemical kinetics for drug discovery in the field of protein aggregation, a hallmark of severe neurodegenerative diseases lacking effective treatments.

DRG-004: Intricate recognition of dopamine and ochratoxin A by DNA aptamers

Dr. Cameron Mackereth[1]
[1]Inserm / Univ. Bordeaux, Bordeaux, France

Atomic information provides a key aspect in revealing the mode of recognition by aptamers, and aids in the strategic development of these DNA- and RNA-based tools for diagnostic and therapeutic use. We have primarily used NMR spectroscopy, and in particular a biomolecular NMR spectroscopy approach, to characterize the high-resolution binding of dopamine and ochratoxin A to their respective DNA aptamers. In the dopamine-binding aptamer, NMR spectroscopy has first enabled a more accurate picture of the DNA secondary structure, with a surprising discovery of a highly complex arrangement of the nucleotides that is distant from the initial predicted stemloop-type model, or even the possible G-quadruplex hinted by an early 1D NMR analysis. Unusual for DNA, our structural study reveals unexpected arrangements of nucleotides to define specificity for dopamine that incorporates base-triples and extended planes of nucleotide bases. The NMR spectroscopy study also helped generate new truncations and indeed one such modification allowed for a minimal design with high affinity. In both cases, the structural data has complemented additional binding and biophysics studies in order to probe ligand preferences. In the case of the ochratoxin A-aptamer complex, the NMR-based investigation has highlighted a mechanistic role for the divalent calcium ion in the formation of the bound complex. This was demonstrated by the atomic data as well as NMR spectroscopy with cobalt hexammine. To further investigate the role of calcium binding, we have additionally performed native mass spectrometry to capture the various ligand plus ion complexes that show an absolute requirement for calcium in the formation of the complex under the initial conditions used to generate the aptamer. The combined data highlight our approach to derive crucial insight from NMR structural studies of small-ligand/aptamer complexes to aid in modifying the properties of these and other aptamers for a range of end uses.

DRG-005: Profiling the dsDNA:Thiazotropsin and dsDNA:MGB-BP3 complex binding using NMR spectroscopy approaches

Mr Bekir Caglar Celikkaya[1], Prof Hasan Y Alniss[2], Dr Fraser J Scott[1], Ms Joanna Hoffman[1], Ms Bryony Kemp[1], Ms Elizabeth Holmes[1], Mr Rafal Ploch[1], Dr John A Parkinson[1]
[1]Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, United Kingdom, [2]Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, Sharjah, United Arab Emirates

The structure-activity relationship of biomolecular ligand-target complexes is being determined using NMR methods (1, 2). There are two types of minor groove binder ligands, sequence-specific such as thiazotropsin-class and multi-sequence targeting such as the anti-infective S-MGBs (3, 4). The thiazotropsin-class are based on the lexitropsin minor groove binders, members of this class of compound have thiazole groups (5, 6). Thiazotropsins have exhibited different thermodynamic, chemical, and binding properties in biomolecular interactions with targeted DNA sequences (7). By contrast, anti-infective S-MGBs such as MGB-BP-3 are intended to target multiple DNA sequences to circumvent the problem of anti-microbial resistance present in single-targeted approaches.

With the thiazotropsin-class ligands DNA binding is screened via 1D – 1H NMR studies by further data acquisition using standard 2D NMR procedures. Different types of nucleic acid sequences have been screened for binding by the thiazotropsin-class ligands. A sequence preference emerges, specifically d(CGCAGTACTGCG)2.

MGB-BP-3, a promising S-MGB compound which is active against C. difficile, and undergoing clinical phase trails (8). It is thought to have a binding preference for AT-rich sequences, but limited specificity. It has previously been shown to bind to d(CGCATATATGCG)2 DNA oligonucleotide duplex as an example target (3). In this study, the AT-rich binding site length has been investigated using DNA oligonucleotide targets with different numbers of ATs in the proposed, central binding region, in a range between 5 W (A/T) and 0 W (A/T) using a 1D – 1H NMR titration assay. The data have also been supported by native mass spectrometry data for all ligand-oligonucleotide complexes. Moreover, some restriction enzyme assays have been applied to further investigate the mode of action for MGB-BP-3.

Keywords: Thiazotropsin, S-MGB, DNA Minor Groove, MGB-BP-3

DRG-006: Mapping hydrogen atom positions in powdered Folic acid systems using solid-state NMR

Dr Piotr Paluch[1,2], Dr Inês C. B. Martins[3], Dr Mariana Sardo[1], Dr Ildefonso Marin-Montesinos[1], Dr Marta K. Dudek[2], Dr Luis Mafra[1]
[1]Ciceco - Aveiro Institute Of Materials, Department Of Chemistry, University Of Aveiro, Aveiro, Portugal, [2]CMMS, Polish Academy of Sciences,, Lodz, Poland, [3]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark
Piotr Paluch,[1],[2] Inês C. B. Martins,[3] Mariana Sardo,[1] Ildefonso Marin-Montesinos,[1] Marta K. Dudek[2], Luis Mafra[1]
[1] CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal
[2] CMMS, Polish Academy of Sciences, Lodz, Poland
[3] Department of Pharmacy, University of Copenhagen, Denmark

The location of hydrogen atoms in molecular crystals is a significant challenge for crystal structure determination techniques. This is mainly because single-crystal X-Ray diffraction, the method of choice in establishing crystal structures is less sensitive to hydrogen atoms, due to their low electron density. This is the case of the hydrogen atoms located between two electronegative atoms, such as nitrogen and/or oxygen, leading to even lower electron density around hydrogens. On the other hand, finding a correct protonation state can be crucial for the understanding of the formation, nature and energetics of a given crystal form, as well as from the regulatory perspective, in particular in pharmaceutical industry. In this contribution we showcase the many ways in which solid-state NMR (ssNMR) can be used to determine hydrogen atom position(s) in binary crystals/salts and in crystals built by different tautomeric forms [1-2], featuring in particular the case of folic acid (FA) dihydrate and a new mesylate salt of folic acid. FA (vitamin B9) exists in several tautomeric forms (pteridine ring) in addition to zwitterionic forms (glutamyl moiety). This diversity results in the difficulty to establish the protonation state in FA solid forms.. Herein, we combine multiple ssNMR experiments, including 1H-14N T-HMQC, 1H detected 1H-15N HETCOR to probe the structure of FA dihydrate, in addition to establishing the protonation state of a new FA mesylate salt.

Molecular structure of folic acid (FA)

[1] Cryst. Growth Des. 2023,
[2] IUCrJ 2023, 10, 448.

DRG-007: Drug- like small molecules binding to RNA: NMR structure and binding assessment of the complex

Dr. Bhawna Chaubey[1]
[1]University Of Washington, Seattle, United States

Here we present two projects under investigation in our group. The first project reports the discovery of a small series of drug-like small molecules that satisfy all Lipinski rules of pharmacologically viable small molecules and bind to the precursor of the pro-oncogenic and pro-inflammatory non-coding RNA miRNA-21 with mid-nM affinity and specificity. These molecules target a local structure at the Dicer cleavage site and induce distinctive structural changes in the RNA which correlate with specific inhibition of miRNA processing. Among all the molecules investigated, compound 52 showed numerous intermolecular NOE connections with mir-21 along with closing its loop by forming G: U base pairs, suggesting that the entire 52 molecule is in direct contact with the RNA. Structurally conservative single nucleotide substitutions eliminate the conformational change induced by the small molecules, which is not observed in other miRNA precursors. The most potent one reduces cellular proliferation and miR-21 levels in cancer cell lines without inhibiting kinases or classical receptors, thereby providing an avenue towards therapeutic development in multiple diseases where miR-21 is abnormally expressed. The second project reports NMR structure of a specific complex between Palbociclib and HIV-1 TAR RNA. Palbociclib binds to the TAR with nM affinity with specificity. Palbociclib recognizes a site spanning the UCU bulge and induces formation of a new G36-C29.C24+ base triple and a structure never observed before. The C24 base of UCU bulge is deeply inserted into the major groove of stem I and is in close contact with G36. The aromatic protons of Palbociclib depict gigantic chemical shift changes and exhibit intermolecular NOEs with G21, A22, G26, U40 and C41 residues. Furthermore, 2-OH and NH2 groups for all residues of Stem I are observed in NOESY and 1H-15N HSQC indicating the stabilization of the RNA structure.

DRG-008: Chemical Composition of leaves extract from Ceratopetalum hylandii Rozefelds & R.W.Barnes. an Australian Tropical Plant

Mr. Andres Felipe Ruiz Alvarez[1]
[1]James Cook University, CLIFTON BEACH, Australia

Ceratopetalum hylandii, an endemic plant of northeastern Australia, is part of the Cunoniceae family. Initial investigations suggest its potential for antioxidant and anti-inflammatory properties. This study aims to isolate and identify compounds derived from the leaves of C. hylandii. Techniques such as column chromatography, size exclusion, and multiple rounds of preparative high-performance liquid chromatography (HPLC) were employed to isolate compounds. The isolated compounds were determined using mass spectroscopy and Nuclear Magnetic Resonance. 1D NMR (1H and 13C NMR) and 2D NMR (COSY, HMBC, HSQC, NOESY) experiments were used in this identification. The analysis revealed the presence of Trans-Melilotoside, cis-Melilotoside, 3-Dehydroshikimic acid, Shikimic acid, and Gallic acid. The physiochemical properties of these compounds agreed with Lipinski rule of 5 for small molecule therapeutics, making them promising candidates for the development of new drugs.

DRG-009: CLIC1: A Perplexing Drug Target for Glioblastoma Multiforme

Miss Victoria Cheung[1]
[1]University of Kent, Canterbury, United Kingdom

Glioblastoma multiforme (GBM) is the most aggressive and prevalent form of primary brain cancer and its poor prognosis makes GBM a public health concern. Increasingly, evidence points towards Chloride Intracellular Channel 1 (CLIC1) promoting oncogenic development with its high level of activity and expression during tumorigenesis. CLIC1's unique 'moonlighting' abilities means that it may serve separate functions at both the cytoplasm and membrane. Intriguingly, the metamorphic nature of CLIC1 serves as a biological switch for malignant transformation in which only the membrane-bound form is carcinogenic. This distinct feature could pave way for a new selective, conformation-specific cancer therapy which would potentially spare normal cells making CLIC1 a highly promising pharmacological target.

Our research focuses on understanding the membrane-bound structure of CLIC1 as this is currently unknown and developing selective CLIC1 inhibitors with antiproliferative activity for the treatment of glioblastoma multiforme. To explore the structure of the membrane-bound form, we have used CryoEM with CLIC1 inserted into nanodiscs. To detect drug binding, we have performed solution-state NMR and X-ray crystallography of the soluble form of CLIC1 to a panel of FDA-approved compounds derived from in-silico and AI-based drug screens. To test drug inhibition, we performed viability assays in human glioblastoma cells. This integrative approach using a plethora of biophysical and cellular techniques sheds some light on CLIC1 behaviour in lipidic environments and begins to reveal CLIC1 interactions with drugs for the treatment of glioblastoma multiforme.

DRG-010: Discovery of a novel HTT ligand with preferential binding to polyQ expanded HTT Exon1

PhD Martina Bischetti[1], PhD Alessandro Piai[1], PhD Elizabeth Doherty[2], PhD Julien Boudet[2], PhD Edith Monteagudo[2]
[1]IRBM S.p.A., Roma, Italy, [2]CHDI Management, Los Angeles, United States

Huntington's disease (HD) is a neurodegenerative disorder in which genetic mutations lead to the expansion of the polyQ region of the N-term of Huntingtin (HTT) protein resulting in its pathological aggregation due to protein misfolding. Because of the need to identify small molecule binders for diagnostic applications (e.g., PET radioligands) as well as for developing therapeutic drugs (e.g., aggregate blockers, target protein degraders) CHDI has conducted a massive screening campaign using a combination of different Affinity Selection-Mass Spectroscopy (AS-MS) platforms to identify small molecule binders to the full-length Q48 HTT/HAP40 protein complex or related constructs such as the N-term fragment Q48 HTT(2-171) as an MBP fusion protein.
Here, we describe how we used Nuclear Magnetic Resonance (NMR) spectroscopy to characterize CPD-936, a novel HTT ligand exhibiting a binding affinity in the low µM range. Comparison of binding results with progressively shorter N-terminal HTT constructs revealed that this compound interacts preferentially with the HTT Exon1 domain. Binding experiments with monomeric, native sequence, human HTT Exon1(2-90) fragment (with no artificial tags) unequivocally confirmed the occurrence of protein-ligand binding which, based on currently available data, appears dependent on the length of the polyQ tract. Preliminary SAR conducted on several analogs provided some initial clues to guide further optimization of the compound. These results offered new opportunities for targeting pathological mHTT in diagnostic and/or therapeutical applications.

DRG-011: NMR in the discovery of novel PROTAC molecules for the DCAF1 and TRIM58 E3 ligases

Dr. César Fernández[1]
[1]Novartis Biomedical Research, Basel, Switzerland

PROTAC molecules that promote targeted protein degradation (TPD) through the ubiquitin proteasome system constitute an emerging therapeutic approach that has recently gained significant attention. In contrast to conventional ligands, PROTACs offer benefits, especially by their ability to regulate targets that are typically deemed "undruggable". Currently, the number of available E3 ligase recruiters remains limited in comparison to the >600 E3 ligases present in human cells, with CRBN and VHL standing out as the most frequently ones. Increasing the variety of E3 ligases accessible for PROTACs, for instance, by including DCAF1 and TRIM58, is extremely valuable for modulating tissue specificity and overcoming emerging drug resistance.
The immense power of NMR is illustrated with two recent successful applications to our TPD drug discovery portfolio. In the first example [1,2], we describe the rapid identification of potent binders for the WD40 repeat domain (WDR) of DCAF1. In the second example [3], we describe the discovery of novel ligands to the PRY-SPRY domain of the RING ligase TRIM58. In both examples, hit to lead optimisation was carried out by an integrated approach, where in-silico methods, NMR, X-ray crystallography, computer-aided drug design and biochemical assays played a crucial role. In both cases, NMR was the only technique capable of detecting early, very weak ligands, and it played a central role in the hit-to-lead optimisation flowchart.

[1] A. Vulpetti et al. Discovery of new binders for DCAF1, an emerging ligase target in the Targeted Protein Degradation field. ACS Med. Chem. Lett. 2023, 14, 949–954. DOI: 10.1021/acsmedchemlett.3c00104
[2] M. Schroeder et al. DCAF1-based PROTACs with activity against clinically validated targets overcoming intrinsic and acquired-degrader resistance. Nat. Commun. 2024, 15, 275–292. DOI: 10.1038/s41467-023-44237-4
[3] K. Hoegenauer et al. Discovery of ligands for TRIM58, a novel tissue selective E3 ligase. ACS Med. Chem. Lett. 2023, 14, 1631–1639. DOI: 10.1021/acsmedchemlett.3c00259

DRG-012: Optimized NMR-based fragment screening platform in academia

Dr Christian Richter[1], Dr. Sridhar Sreeramulu[1], Verena Linhard[1], Kerstin Witt[1], Prof. Dr. Harald Schwalbe[1]
[1]Institute for Organic Chemistry and Chemical Biology; Center of Biomolecular Magnetic Resonance (BMRZ); Goethe-University Frankfurt, Frankfurt am Main, Germany

Fragment-based screening (FBS) is a well-established method towards discovering potent drug-like molecules. Using FBS, low-molecular weight (~150 Da) molecules are tested for their ability to bind to a high-molecular weight biological target molecule. NMR-based fragment screening (NMR-FBS) is particularly promising to detect binding of compounds over a broad range of affinities and can simultaneously ensure the chemical purity and constitution of the fragments[1]. We have assembled the accessibility of fragment libraries at BMRZ as well as characterized the recently established European Fragment Screening Library (EFSL)[2] for open-science approaches to accelerate structure-based drug discovery across diverse screening technologies both in academia and industry. FBS has not only become a widely used technology in pharmaceutical and biotechnology industry but has been also successfully adopted in academia. As an example, during the SARS-CoV-2 (SCoV-2) pandemic, we undertook a massive fragment screening campaign involving 20 RNA elements[3] and 25 proteins[4] from SCoV-2. These screens resulted in the identification of 69 and 311 high-quality fragment hits against RNA and proteins, respectively. These data not only assist and accelerate medicinal chemistry efforts even beyond COVID-19 but also allowed us to efficiently streamline the protocols for running such large-scale screens in an academic setting[5]. Previously, screening efforts have been undertaken manually typically involving only a small number of fragments. Screening infrastructure often had remained limited not only in the drug development process but also in the context of chemical probe development. To meet the requirements economically, advanced workflows will be presented. These include the latest state-of-the-art hardware; high-throughput NMR acquisition of traditional ^{1}H and optimized ^{19}F ligand-observed screening experiments[6]; Advanced NMR screening experiments such as the dual detection techniques that allow the detection of ^{1}H and ^{19}F at the same time[7] (there by reduces overall measurement time) and 3D-filtered experiments assisting structure calculation of target ligand complex[8].

DRG-013: Interrogating covalent ligand protein complexes by NMR

Dr Eiso AB[1], Dr Stephan Theisgen, Johan Hollander, Dr Harish Balasubramanian, Dr Marta Carneiro
[1]ZoBio BV, Leiden, Netherlands

The use of covalent ligands has been gaining popularity for developing drugs against undruggable targets and many campaigns rely on X-ray crystallography to obtain structural information on these systems. Yet, in cases where the target is hard to crystallize or the binding mode observed in the crystal lattice may be unconvincing, NMR is an attractive alternative, offering a rich source of information on target ligand complexes in a wide variety of circumstances and levels of structural detail. This ranges from validating binding to full structure determination. We will show how NMR has been used at ZoBio to characterize covalent complexes in various stages of the drug development process. In particular, we will show how NMR allows the rapid assessment of the most promising early stage binders by delivering information about important characteristics of the covalent binding mode and about the quality of the non-covalent interactions, looking at both the protein and the ligand side. We will also discuss our experiences solving a large series of 3D solution structures of covalent KRAS complexes, to validate and complement structural information from crystallography, and discuss improvements in the speed and information content of this process that we were able to achieve through data collection strategies and automated interpretation of spectra.

DRG-014: Conformational analysis of heparan sulfate mimetics and molecular recognition by NMR

Ms. Paula González Gómez[1]
[1]Cicbiogune, Derio, Spain

As components of the glycocalyx glycosaminoglycans (GAGs), a family of linear, highly negatively charged polysaccharides, are considered relevant biomolecules. In particular, heparan sulfates, composed of repeating units of GlcNAca1-4GlcA disaccharides, are involved in a plethora of biological processes such as cell growth, proliferation, adhesion, anticoagulation and wound repair. Importantly, the extraordinary structural and conformational plasticity of GAGs, which are key for their biological activity, offers fascinating opportunities for drug design.

Here, we present the structural, conformational, and molecular recognition studies of an array of fluorinated GlcNAcα1-4GlcA disaccharides. The effect of fluorine incorporation as well as sulfation pattern on the conformation and binding features of these newly designed molecules has been evaluated by employing advanced NMR techniques together with computational approaches.

DRG-015: Probing Protein-Ligand Methyl-π Interaction Geometries Through Chemical Shift Measurements of Selectively Labeled Methyl Groups

Andreas Beier[1], Dr. Gerald Platzer[2], Dr. Theresa Höfurthner[1], Aleksandra L. Ptaszek[1], Dr. Roman J. Lichtenecker[1], Dr. Leonhard Geist[3], Dr. Julian E. Fuchs[3], Dr. Darryl B. McConnell[4], Dr. Moriz Mayer[3], Dr. Robert Konrat[1]
[1]University of Vienna, Vienna, Austria, [2]MAG-LAB, Vienna, Austria, [3]Boehringer Ingelheim RCV GmbH & Co. KG, Vienna, Austria, [4]Curie.Bio, Boston, USA

Fragment based drug design is heavily dependent on the optimization of initial low affinity binders and feedback on design iterations via NMR is key in this process. One of the most prominent forms of interaction for small fragment-like molecules is the formation of weak hydrogen bonds between aromatic π-systems and aromatic or aliphatic CH-groups. This interaction can be readily detected using simple NMR chemical shift perturbation measurements, as the additional magnetic field induced by the aromatic ring currents leads to a large anisotropic shift change in the surrounding nuclei. We introduce an approach that uses selective labeling of methyl groups in Leucine and Isoleucine sidechains to directly probe methyl-π contacts. We find good agreement with a commonly used model of the ring-current effect as well as overall interaction geometries extracted from the Protein Data Bank. By combining both interaction geometries and chemical shift calculations as fit quality criteria, we can position dummy aromatic rings into an alpha fold model of the protein of interest. The proposed method can therefore provide medicinal chemists with important information about binding geometries of small molecules in a fast and iterative manner even in the absence of high-resolution experimental structures.

DRG-016: Identifying interactions between proteins and excipients using NMR, and their consequences on viscosity and injectability

Liza Boeffard[1,2], Camille Dagalier[2], Géraldine Eudier[2], Sophie Zinn-Justin[1], François-Xavier Theillet[1]
[1]IntGen Team, I2BC, Gif-Sur-Yvette, France, [2]Formulation and Process Development lab, Sanofi, Vitry-Sur-Seine, France

Higher concentrated forms of biopharmaceutical treatments allow for better injection deliveries and patients' quality of life. However, increasing the concentration have consequences on the stability and properties of the treatment. It comes with increased viscosities, which impacts the injection. The knowledge is very limited on how the most common excipients stabilize and fluidify highly concentrated therapeutic proteins.
In this context, we sought to examine the correlation between the strength of excipients' interactions with concentrated proteins and the resulting viscosity. NMR spectroscopy appears as an excellent technique in this context, because of its ability to report low affinity interactions. Different NMR methods exist for this, based on non-redundant physical mechanisms related to weak interactions. These approaches are commonly used for structural biology purposes, but pharmaceutical formulations are conspicuously different from the standard solutions used in academic labs. Here, we present the first results of our work. We tested the adaptation of various standard pulse sequences to fit our need and biopharmaceutical samples. These NMR methods were developed to report on protein/ligand interactions. We will show how they can complement each other and their adaptability to our protein/excipient samples. We will finally discuss the use of other NMR experiments and the complementarity with other techniques, using size-exclusion chromatography, light-scattering methods and capillary electrophoresis.

EPR-001: CW-EPR spectroscopy as a powerful tool for complex binding studies on fatty acid binding proteins

Sebastian Michler[1], Florian Schöffmann[1], Prof. Dr. Dariush Hinderberger[1]
[1]Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

Continuous-wave (CW) electron paramagnetic resonance (EPR) spectroscopy is a time-efficient, economical and material-saving technique that offers superior options for studying complex binding mechanisms of biomolecules. We applied this technique to analyze the binding properties and thermodynamics of the fatty acid binding proteins FABP3, FABP4 and FABP5 under different physical conditions. FABPs are a family of cytosolic transport proteins showing a high diversity in terms of their amino acid sequences and binding preferences. Many aspects regarding their binding mechanism and biological functions remain unclear. In our study, the FABPs were loaded with amphiphilic spin probes as model ligands. The CW EPR spectra of non-covalently bound 5- and 16-DOXYL stearic acid (5/16-DSA) provide in-depth information about the dynamics and chemical environments of ligands inside the binding pockets of the FABPs. The proportion of bound 5/16-DSA strongly depends on the protein concentration and the temperature, but with remarkable differences between the three FABPs. Subsequent EPR simulations enabled the construction of binding curves, revealing two different binding states with distinct rotational dynamics and environments ('intermediately' and 'strongly' bound). The more dynamic state ('intermediately bound') seems to dominate at body temperature with thermodynamic preference. Our utilization of CW EPR spectroscopy provides new insights into molecular-level mechanisms of protein-ligand interactions in general. We could successfully combine classic CW EPR spectroscopy with advanced binding studies and thermodynamics, demonstrating the power and efficiency of even basic magnetic resonance experiments.

EPR-002: Simulation-Independent Approach to ESR Spectral Analysis

Dr. Aritro Sinharoy[1], Dr. Madhur Srivastava[1]
[1]Cornell University, Ithaca, United States

Introduction: Accurate analysis of continuous-wave electron spin resonance (cw ESR) spectra of biological or organic free-radicals and paramagnetic metal complexes is key to understand their structure-function relationships and electrochemical properties. However, in the presence of g- and/or A-anisotropy and/or large number of hyperfine lines, spectral analysis becomes highly challenging. Either high-resolution experimental techniques are employed to resolve the spectra in those cases or a range of suitable ESR frequencies are used in combination with simulations to identify the corresponding g and A values.

Aims: To develop a direct spectral analysis method to obtain g and A values to overcome the limitations of simulation approach. Simulation-based analysis often fails to extract spectral information fully and accurately from non-ideal spectra. In addition, such analyses are highly sensitive to spectral resolution and artifacts, users' defined input parameters and spectral complexity.

Methods: We introduce a simulation-independent wavelet packet transform-based analysis which extract g values and hyperfine (A) constants directly from cw ESR spectra, enabling broader application of ESR. The method decomposes the ESR spectra into its constituent components to reveal the hidden features.

Results: Our method overcomes the challenges associated with simulation-based methods for analyzing poorly / partially resolved and unresolved spectra, which is common in most cases. The accuracy and consistency of the method are demonstrated on a series of experimental spectra of organic radicals and copper-nitrogen complexes.

Conclusion: We show that for a two-component system, the method identifies their individual spectral features even at a relative concentration of 5% for the minor component.

EPR-004: Membrane Composition Drives Sidechain Ionization and Assembly of Transmembrane Protein Domains: Potential Implications to TCR assembly.

Prof Tatyana Smirnova[1], Dr. Maxim Voinov[1], Dr. Sergey Milikisiyants[1], Prof. Gabriel Cook[2], Prof. Alex Smirnov[1]
[1]North Carolina State University, Raleigh, United States, [2]Oklahoma State University, Stillwater, USA

Introduction. Ionization states of amino acid residues play significant roles in membrane protein assembly and function; however, they are difficult to decipher experimentally. The analysis becomes especially complicated for membrane proteins because of the dearth of data on transmembrane gradients in polarity, electric potentials, and hydration at the protein-membrane interface.
Aims. Here we examine how electrostatic interactions, suggested to be essential for T-cell receptor (TCR) membrane assembly, could be manipulated by modifying the membrane lipid composition.
Methods. Novel pH-sensitive ionizable EPR labels were employed to profile heterogeneous dielectric environment along the transmembrane protein-lipid interfaces. A series of model transmembrane α-helical WALP peptides was employed to derive the profile of effective pK(a) as a function of membrane depth. A peptide corresponding to the transmembrane domain of TCRα was labeled with pH-sensitive nitroxide and incorporated into liposomes and effects of charges on the domain agglomeration were quantified by Double Electron-Electron Resonance (DEER).
Results. EPR of pH-sensitive labels reported on the protonation state, membrane insertion, and association of the helices within the membrane. It was found that an increase in negative change density at the membrane surface significantly alters the protonation state of membrane-buried ionizable sidechains in the transmembrane domain of TCRα. Turning the charge of the sidechain “off” increases tendency of the transmembrane domain to agglomerate - a phenomenon that could be critical for the TCR assembly and its degradation.
Conclusions. Overall, EPR of ionizable probes uncover the mechanisms of how the changes in local environment along the protein-lipid interface may “tune” pK(a) of the ionizable sidechains and drive the structural changes in membrane proteins including TCRα.

EPR-006: Indirect Measurement of Proton Spectrum via Electron Detection

Gian-Marco Camenisch[1], Nino Wili[2], Matthias Ernst[1], Gunnar Jeschke[1]
[1]D-CHAB ETH Zurich, Zurich, Switzerland, [2]Interdisciplinary Nanoscience Center (iNANO) and Department of Chemistry, Aarhus, Denmark

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. Simultaneous electron decoupling during the inversion pulse reduces the proton line width significantly. Under optimized π-pulse decoupling on the electrons the FWHM is reduced to 300 kHz. We carried out our experiments using OX063 Trityl radical in glycerol-d_8:D_2O:H_2O, (v/v 6:3:1) at X-band (0.35 T) and temperature of 80 K.

[1] A. Henstra et al. “Nuclear spin orientation via electron spin locking (NOVEL)”. In: Journal of Magnetic Resonance (1969) 77.2 (1988), pp. 389–393. issn: 00222364. doi: 10.1016/0022-2364(88)90190-4.
[2] Nino Wili, Jan Henrik Ardenkjær-Larsen, and Gunnar Jeschke. “Reverse dynamic nuclear polarisation for indirect detection of nuclear spins close to unpaired electrons”. In: Magnetic Resonance 3.2 (2022), pp. 161–168. issn: 26990016. doi: 10. 5194/mr-3-161-2022.

EPR-007: Q-band probehead for pulsed ENDOR spectroscopy with enlarged sample volume

Dr. Vasyl Denysenkov[1], Dr. Andrei Kuzhelev[1], Prof. Thomas Prisner[1]
[1]Institute of Physical and Theoretical Chemistry, Goethe University Frankfurt, , Frankfurt am Main,, Germany

Here we describe the design and performance of a probehead for pulsed ENDOR experiments on 1H 19F 31P 13C and 2H nuclei in frozen samples with a volume of up to 15 microliter. The probehead is designed as a combination of a sapphire dielectric resonator [1] for microwave excitation and a Helmholtz coil for RF excitation. Optimal RF pulse lengths in the range of 50-100 microsec been estimated for the nuclei by nutation experiments. The performance of the probehead has been tested with trityl radicals in frozen solutions containing various nuclei of interest by using Mims and Davies ENDOR pulse sequence with stochastic RF excitation. All experiments were accomplished on a Bruker ELEXYS Q band EPR spectrometer.

[1] J.S. Hyde, J.W. Sidabras, R.R. Mett (2019) Cell Biochemistry and Biophysics 77:3–14.

EPR-008: Quantum rotor EPR spectroscopy: Investigating the sensitivity of methyl rotors towards their local environment

Andrea Eggeling[1], Gunnar Jeschke[1]
[1]ETH Zürich, Zürich, Switzerland

Site-directed spin labelling is a powerful technique to investigate the conformations and dynamics of biomolecules using electron paramagnetic resonance (EPR) spectroscopy. Typical spin-labels are based on nitroxides that contain geminal methyl groups to shield and stabilize the unpaired electron. The low-temperature two-pulse electron spin echo envelope modulation (ESEEM) signal of such nitroxides contains two contributions on different time scales. The slower contribution arises from nuclear pair ESEEM,[1] whereas the faster ESEEM contribution is caused by methyl tunneling.[2] The latter contribution contains valuable structural information since the rotation barrier V_3 of the methyl rotors is sensitive to the local environment. In a glassy matrix, this information manifests in a distribution of rotation barriers $P(V_3)$, which can be extracted using the methyl quantum rotor (MQR) model.[3]
In this work, we aim to study the influence of the nitroxide backbone structure and the glassy matrix on the rotation barrier distribution. Therefore, we analyze low-temperature two-pulse ESEEM signals of three commonly used nitroxides (MTSL – pyrroline, IAP – proxyl, TEMPOL – piperidine) in different glassy matrices using the MQR model to extract their rotation barrier distributions. By systematically studying the resulting local environment information, we find that mainly the nitroxide backbone structure dictates the mean value of the rotation barrier distribution. We suspect that the distribution width depends on both the nitroxide conformation as well as solvation of the nitroxide in the glassy matrix, however further investigation in this direction is required in the future. In general, we believe that quantum rotor EPR spectroscopy can become a powerful tool to gain additional local structural information from spin-labelled proteins, which can be used a constraint in ensemble modelling.

[1] G. Jeschke, JMRO 2023, 14-15, 100094
[2] S. Soetbeer et al., PCCP 2021, 23, 21664-21676
[3] A. Eggeling et al., PCCP 2023, 25, 11145-11157

EPR-009: EPR Based Distance Measurements Reveal the Low Structural Heterogeneity of the Binding Site in a Fluoride Riboswitch

Dr Laura Remmel[1], Dr Andreas Meyer[1,2], Prof. Dr Marina Bennati[1,2], Dr Bela E. Bode[3,4]
[1]Research Group EPR Spectroscopy, Max Planck Institute For Multidisciplinary Sciences, Goettingen, Germany, [2]Institute of Physical Chemistry, Georg-August University, Goettingen, Germany, [3]EaStCHEM School of Chemistry and Centre of Magnetic Resonance, University of St Andrews, St Andrews, United Kingdom, [4]Biomedical Science Research Complex, University of St Andrews, St Andrews, United Kingdom

Riboswitches control gene regulation upon external stimuli such as environmental factors or ligand binding. The fluoride sensing riboswitch from Thermotoga petrophila is thought to be involved in resistance to cytotoxicity of high fluoride concentration by controlling production of fluoride exporters.

To counteract the electrostatic repulsion between the negatively charged fluoride ion and the negatively charged phosphate backbone the riboswitch coordinates a triangle of magnesium ions which in turn encapsulate the fluoride ion.[1]

To date, only the crystal structure of the fluoride-bound riboswitch is known. Using a combined approach of site-directed spin labelling with nitroxide spin labels, PELDOR measurements, and ^{19}F ENDOR[2] measurements at 34 and 94 GHz we could gather insight into the global structure elements and the local structure in the region of the fluoride binding site in the solution structure.

Pulse dipolar EPR measurements demonstrate the preorganisation of the global structure elements of the sensing domain in the free state and the retention of these structural elements in the magesium-bound apo and the fluoride-bound holo forms.

For the ^{19}F ENDOR spectra we have developed an analysis combining simulations based on a model- and bias-free approach with Gaussian distributions of distances and a rotamer library-based simulation approach that retains all orientation information. ^{19}F ENDOR experiments reveal low structural heterogeneity around the ligand binding site as well as deviations between solution and crystal structures at the 5' end. This approach even allows us to identify the dominant spin-labelling product in a diastereomeric pair and further to identify a distance in excess of 20 Å, well above the previous limit of 15 Å for nitroxide spin-label based ^{19}F ENDOR.

[1] A. Ren, K. R. Rajashankar, D. J. Patel, Nature 2012, 486, 85–89.
[2] A. Meyer, S. Dechert, S. Dey, C. Höbartner, M. Bennati, Angew. Chemie 2020, 132, 381–387.

EPR-010: Biophysical and Pharmaceutical Applications of CW EPR Spectroscopy under Ultraviolet and Visible Light Irradiation

Florian Lehmann[1], Dariush Hinderberger[1]
[1]Martin Luther University, Halle (Saale), Germany

Continuous wave electron paramagnetic resonance (CW EPR) spectroscopy enables the investigation of paramagnetic species, offering insights into electronic and structural dynamics. Under ultraviolet (UV) and visible light irradiation, CW EPR spectroscopy facilitates the study of light-induced processes, including the formation and behavior of transient radical species, photochemical reactions, and photo-induced conformational changes. This can be experimentally achieved by the combination of an EPR spectrometer and a fiber-coupled irradiation setup connected via the opening for the Mn-standard. With this setup, the photodegradation of monoclonal antibodies and polysorbates are investigated, light-induced Bergman-cyclisation in enediyne-polymers can be observed, and photochemically triggered release of a spin probe from arylazopyrazole polymers is examined.

EPR-011: EPR characterization of intrinsically disordered regions in the RNA-binding protein SRSF1

Dr Laura Galazzo[1], Noémie Kociolek[2], Dr Maxim Yulikov[1], Prof. Dr Frédéric Allain[2], Prof. Dr Gunnar Jeschke[1]
[1]Institute of Molecular Physical Science, ETH Zürich, Zürich, Switzerland, [2]Institute for Biochemistry, ETH Zürich, Zürich, Switzerland

Over the past decade, the importance of disordered regions in the correct assembly and function of RNA-binding proteins has been demonstrated1. These biomolecules undergo liquid-liquid phase separation in response to stress, and the reversibility of such process is connected to pathological disfunction2. In order to understand the mode of action of such biomolecules, knowledge on their structural and dynamic properties is required.
For systems that do not adopt a well-structured three-dimensional fold, EPR is a powerful technique to investigate conformational landscapes and derive structural information from distance distribution restraints3.
Here we address the RNA-binding protein SRSF1 (serine/arginine-rich splicing factor 1), a member of the SR protein family of gene regulators; it contains two RRMs (RNA-recognition motifs) connected by a flexible linker and a disordered serine/arginine-rich tail (RS domain) located at the C-terminus. While the RRMs are responsible for protein-RNA interactions, the RS domain is involved in protein-protein interactions and aspecific protein-RNA interactions and is responsible for phase separation. The phosphorylation of this domain is fundamental for the protein correct localization and function. Here we use EPR and NMR to investigate the conformational ensemble of full-length SRSF1 in its free and phosphorylated form in dispersed state. Our results suggest an interaction between the RRMs and the RS domain in the absence of RNA, but this interaction does not prevent RNA binding. Moreover, SRSF1 phosphorylation leads to a destabilization of this interaction. Experiments in the condensed phase are complex due to high local protein concentration and fast relaxation times; in this regard, optimization of experimental conditions for sample preparation and data analysis in droplets are underway.

[1] Calabretta, S., Richard, S., Trends Biochem. Sci., 2015, 40 (11), 662-672.
[2] Babinchak, W.M., Surewicz, W.K., J. Mol. Biol., 2020, 432 (7), 1910-1925.
[3] Jeschke, G., Esteban Hofer, L., Meth. Enzymol., 2022, 666, 145-169.

EPR-012: Increased sensitivity in Electron Nuclear Double Resonance spectroscopy with chirped radiofrequency pulses

Julian Stropp[1], Dr. Nino Wili[2], Prof. Niels Chr. Nielsen[2], Dr. Daniel Klose[1]
[1]Department of Chemistry and Applied Biosciences, ETH Zürich, 8093 Zurich, Switzerland,
[2]Interdisciplinary Nanoscience Center, Aarhus University, 8000 Aarhus C, Denmark

Electron Nuclear Double Resonance (ENDOR) spectroscopy is an EPR technique to study the hyperfine coupled nuclei close to paramagnetic centres, which have interactions that are not resolved in continuous wave EPR spectra and may be fast relaxing on the time scale of NMR. Since typical samples of interest are non-crystalline solids, i.e. powders or frozen solutions, the anisotropy of the hyperfine and nuclear quadrupole interactions renders NMR lines often several MHz broad, thus diminishing intensity. In commonly used pulsed ENDOR setups with radiofrequency (RF) amplifiers of 500 W nominal, the minimal π pulse length is 5 µs with an excitation bandwidth of 150 kHz (FWHM). Hence, only a small fraction of the NMR line is excited, and this limits the sensitivity in conventional ENDOR experiments.

In this work, we show the benefit of chirped RF excitation, previously introduced for sharp lines of crystals in time domain ENDOR [1], as a simple yet effective way to improve sensitivity in frequency domain ENDOR. The RF pulses are generated by a dedicated arbitrary waveform generator (AWG) on a home-built X-band EPR spectrometer. We demonstrate on a frozen solution of Cu(II) tetraphenylporphyrin that the broad copper and nitrogen ENDOR lines increase up to 5-fold compared to single frequency RF excitation. Furthermore, the chirp pulses help to reduce RF amplifier overtones, since lower RF powers suffice to achieve intensities matching conventional ENDOR. The sensitivity increase is also observed for nuclei with small hyperfine couplings in Mims ENDOR, although a smaller RF chirp bandwidth must be used to maintain the resolution compared to single frequency Mims ENDOR.

In conclusion, we show that chirped RF pulses significantly enhance the signal intensity, and thus sensitivity, especially of broad lines in Davies ENDOR experiments.

[1] Jeschke, G., Schweiger, A., J. Chem. Phys. 103, 8329 (1995)

EPR-013: Rotational dynamics of a globular protein undergoing liquid-liquid phase separation

M. Sc. Dominik Gendreizig[1], M. Sc. Abhishek Kalarikkal[2], Dr. Saumyak Mukherjee[2], M. Sc. Simon L Holtbrügge[2], Dr. Laura Galazzo[1,2], Dr. Svetlana Kucher[1], Dr. Arnulf Rosspeintner[1], Dr. Sashary Ramos[2], Prof. Dr. Martina Havenith[2], Prof. Dr. Lars V. Schäfer[2], Prof. Dr. Enrica Bordignon[1]
[1]University Of Geneva, Geneva, Switzerland, [2]Ruhr University Bochum, Bochum, Germany

Liquid-liquid phase separation (LLPS) is a commonly observed phenomenon which may occur in vitro on water-soluble proteins. Changing the concentration of the proteins in the aqueous solution and the environmental properties with cosolutes, molecular crowders, protein partners, temperature, etc., can fine-tune the water-water, water-protein and protein-protein interactions, thereby favouring or disfavouring the formation of protein droplets. To understand how this tuning is performed, a detailed analysis of the physicochemical properties of the proteins in the droplets is required. Here, we use MD simulations and continuous wave EPR to investigate the change in the rotational diffusion of a globular protein, γD-crystallin undergoing LLPS in vitro in aqueous solutions in absence and presence of cosolutes. The rotational diffusion of pure crystallin in water is found by MD simulations to be slowed down by a factor 15-60 when droplets are formed at 273 K. To validate the theoretical findings, spin-labeled crystallin proteins were used as viscosity nanoprobes. A direct correlation was obtained between effects induced by bulk viscosity on isolated proteins with different concentrations of sucrose and by the molecular crowding induced by neighbouring proteins in the condensed phase. The EPR-derived changes in rotational correlation times were found to be in agreement with the MD data. This study further validates the predictive power of MD simulations and highlights the relevance of using sensitive nanoprobes to extract the viscosity of condensates

EPR-014: Electron spin resonance spectroscopy of the trans-envelope lipopolysaccharide transport system

Ms. Malavika Pramod[1]
[1]Freie Universität Berlin, Berlin, Germany
Malavika Pramod, Marina Dajka, and Benesh Joseph
Department of Physics, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany
malavip07@zedat.fu-berlin.de; m.dajka@fu-berlin.de

In Gram-negative bacteria the outer membrane is composed of lipopolysaccharides (LPS) which are crucial for antibiotic resistance. The LPS transport complex constitutes seven proteins, LptB2FG-C-A-DE, that act as a bridge to translocate LPS from inner to outer membrane. The inner membrane ATP binding cassette (ABC) transporter sub-complex is made of LptB2FG that drives the transport by the energy of ATP binding and hydrolysis.1-3 In this work, pulsed electron-electron double resonance (PELDOR/DEER) spectroscopy studies show the conformational heterogeneity of LptB2FG in presence and absence of LptC in micelles and liposomes. Along with that LPS binding and release is studied using laser-induced bead ion desorption mass spectrometry. The distance distribution studies in apo and vanadate trapped states show that the β-jellyroll of LptF interacts stably with LptG and LptC β-jellyrolls. The ATP binding induces closed conformation of the nucleotide domains in LptB2FG, which leads to the collapse of the first lateral gate. While in the second lateral gate where LPS entry occurs, a heterogenous conformation is observed. LptC binding limits the flexibility of this gate to two conformations, likely representing the helix of LptC as either released from or inserted into the transmembrane domains. Thus, the transmembrane helix of LptC serves a regulatory role in the transport process. These results reveal the dynamic basis of LPS transport by LptB2FGC and establish a foundation to further elucidate the intricate details of LPS transport.4

References:
1. Li, Y. et al. Nature 2019, 567, 486–490
2. Owens, T. W. et al. Nature 2019, 567, 550–553
3. Thélot, F. et al. Curr Opin Struct Biol 2020, 63, 26–33
4. Dajka, M. et al. bioRxiv 2024.05.20.594984

EPR-015: Conformational space of the major periplasmic chaperone SurA

Pratyusha Chakraborty[1]
[1]Freie University Berlin, Berlin, Germany

Pratyusha Chakraborty, and Benesh Joseph
Department of Physics, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany
pchakraborty@zedat.fu-berlin.de

The survival protein A (SurA) plays significant role in the biogenesis of outer membrane proteins (OMPs) in Gram-negative bacteria. SurA is a periplasmic chaperone that works with the beta-barrel assembly machinery (BAM) complex to facilitate the folding and insertion of OMPs into the outer membrane(1). Its three-domain architecture consists of two peptidyl-proline isomerase domains (named P1 and P2) and an N- and C-terminal domain that together form the core domain. P2 domain and core domain are connected via a flexible loop in the structures that are currently available. P1 domains adopt P1-closed or P1-open conformations(2). SurA may switch between co-populated conformations. SurA's mechanism may be based on its flexibility and conformational dynamics because ATP is not present in the periplasm (3,4). It is proposed that SurA binds substrate in a cradle between the core and P1 domains4 or the substrate might spread throughout SurA following the initial interaction (5,6). It is yet unclear how these domains are oriented in relation to one another or how their conformations change upon binding with the unfolded OMP (uOMP) or the BAM complex. We investigated the structural and dynamic basis for SurA function using pulsed ESR spectroscopy techniques. Our observations show that P1 and P2 domains exhibits large flexibility in relation to the core domain.

1. Konovalova et al., Annu. Rev. Microbiol. (2017)
2. E. Bitto et al., Structure (2002)
3. Schiffrin et al., Commun. Biol. (2022)
4. Calabrese et al., Nat. Commun. (2020)
5. Marx et al., PNAS (2020)
6. Gao et al., Elife (2022)

EPR-016: AWG Pulses in EPR Spectroscopy

Dr. Burkhard Endeward[1,2], Mr. Paul Trenkler[1,2], Prof. Dr. Thomas F. Prisner[1,2]
[1]Goethe University Frankfurt, Frankfurt, Germany, [2]BMRZ, Frankfurt, Germany

About 10 years ago, arbitrary waveform generators (AWG) got available, which are fast enough to modulate pulses on the timescale of typical pulses used in EPR. These AWG pulses could be used to increase the overall excitation bandwidth of pulses compared to rectangular pulses. The excitation bandwidth depends on the switching speed an AWG can accomplish, however typical switching speeds are blow 1ns and therefore could reach bandwidth up to 1GHz[1].
Pulsed EPR spectrometer have a certain bandwidth, which do differ depending on the possible bandwidth of used pulses. Especially for AWG pulses with higher bandwidth also need similar bandwidth of its receiver system. However, the frequency dependency of EPR spectrometer cannot neglected anymore for AWG pulses and it is necessary to correct pulses for such frequency dependences[2].
An obvious application of broadband AWG pulses is inverting a whole spectrum like a nitroxide spectrum, which is not possible with rectangular pulses in common EPR spectrometer. Such inversion of a whole nitroxide spectrum amplifies effects like electron-electron dipole coupling, which are visible for dimes (multimers) in experiments like in PELDOR/DEER/SIFTER. However, with broad bandwidth pulses the dipolar modulation is well detectable in a 2-pulse echo experiment.
In our contribution, we show several experiments demonstrating the above-mentioned effects. Including the effect of electron-electron dipolar modulation on nitroxide biradicals in a 2-pulse echo experiment.
References:
[1] Spindler, P.E., Schöps, P., Bowen, A.M., Endeward, B., Prisner, T.F., 2016. Shaped Pulses in EPR, in: eMagRes. American Cancer Society, pp. 1477–1492.
https://doi.org/10.1002/9780470034590.emrstm1520
[2] Endeward, B., Bretschneider, M., Trenkler, P., Prisner, T.F., 2023. Implementation and applications of shaped pulses in EPR. Prog. NMR Spectrosc. 136–137, 61–82.
https://doi.org/10.1016/j.pnmrs.2023.04.003

EPR-017: Electron spin resonance spectroscopy of the β-barrel assembly machinery (BAM) complex

Elisabetta Di Gregorio[1], Aathira Gopinath[1], Prof. Dr. Benesh Joseph[1]
[1]Freie Univeristaet Berlin, Berlin, Germany

Despite the tremendous advancements in structure determination and prediction for membrane proteins, observing them in their native surroundings remains a major challenge. The β-barrel assembly machinery (BAM) complex, which consists of the central β-barrel BamA and four other lipoproteins (BamB–E), performs protein folding and insertion into the outer membrane of gram-negative bacteria. It's crucial role makes it a potential target for new antibiotics [1]. Essential to its functionality is the gating of BamA between an inward-open (IO) and a lateral-open (LO) conformation [2]; however, owing to the conformational plasticity of BAM, this mechanism is not fully understood. Here we addressed these questions by characterizing the conformational heterogeneity of BamA, BamAB, BamACDE, and BamABCDE complexes using pulsed dipolar electron spin resonance spectroscopy (PDS), and also established an in situ approach to directly observe BAM in the native outer membranes and intact Escherichia coli [3, 4]. We show that while BamA has an inward-open IO conformation in micelles, the native membrane environment induces a large conformational heterogeneity. In micelles, BamAB also adopts an IO conformation, whereas the binding of BamCDE subcomplex induces the opening of the lateral gate. Experiments using intact E. coli cells validated the latter observation, but also revealed an equilibrium between open and closed conformations of the lateral gate. Our results demonstrate that the BamCDE complex plays a key role in the functionality by regulating lateral gating in BamA and that a more native environment can significantly modulate the conformational heterogeneity.

1. Wu et al., Cell 121, 235-45 (2005).
2. Noinaj et al., Structure 22, 1055-62 (2014).
3. Gopinath et al., PNAS Nexus 3, pgae019 (2024).
4. Gopinath A. & Joseph B, Angew. Chem. Int. Ed. (2021).

HRD-001: Homebuilt Low-temperature DNP Setup for Ultra-Wideline NMR in Solid Materials

Mr Jake Lumsden[1]
[1]École Normale Supérieure, Paris, France

We present here a custom-built low-temperature DNP setup (< 10 K for helium and < 80 K for nitrogen) to probe ultra-wideline (UW) nuclei.[1] This custom-built setup allows us to probe, for example, ^{63}/^{65}Cu, ^{91}Zr, ^{195}Pt, ^{47}/^{49}Ti, ^{207}Pb, ^{35}Cl, ^{119}Sn, and ^{33}S, which have ultrawide NMR linewidths (~MHz) that are well beyond the magic-angle spinning frequencies (~ kHz) due to large quadrupolar couplings, chemical-shift, or Knight shift anisotropies. Thus, these UW nuclei are usually investigated in static conditions to avoid complicated NMR spectra. Besides the broad lines, the sensitivity is further hampered by low natural abundance and/or low gyromagnetic ratios. Hence, our hardware can solve this poor NMR sensitivity issue by performing DNP at low temperatures and at high fields. [2, 3] Additionally, it is a modular setup that can be easily adapted for any wide-bore (89 mm) NMR spectrometer (9.4 T or 18.8 T in our case). We have built a static DNP probe coupled with a commercial Helium (He) cryostat (Lake Shore Janis), which will boost the sensitivity due to (i) larger sample volume, (ii) gain in Boltzmann factor, (iii) lower noise, and (iv) better coil geometry (perpendicular to B_0, not 54.7°).

As a proof of principle, we have performed variable temperature experiments (80-300 K) to investigate the ^{35}Cl and ^{91}Zr nuclei on ammonium chloride and zirconocene dichloride ($ZrCp_2Cl_2$), respectively. We found the ^{35}Cl nuclei on ammonium chloride exhibit unusually long T_2 relative to its T_1. Furthermore, it shows a 3x signal improvement (without DNP) at 80 K, or 10x saving in experimental time for the same-quality spectrum. Hence, we expect further signal improvement when the setup is extended for 10 K operation with DNP soon.

HRD-004: Ultrafast High-Resolution Relaxometry

Fabien Ferrage[1], Ana Paula Aguilar Alva[1], Daria Astapovych[3], Éric Beyer[2], Sébastien Breham[2], Guillaume Bouvignies[1], Frank Engelke[3], Letizia Fiorucci[5], Jorge Garibay[3], Agnès Glémot[4], Lukas Haenichen[3], Ulric le Paige[1], Giulia Licciardi[5], Claudio Luchinat[5], Volker Niemann[3], Giacomo Parigi[5], Philippe Pelupessy[1], Rainer Pietig[3], Daniel Podadera[2], Enrico Ravera[5], Alessandro Ruda[1], Rainer Sattler[4], Lucas Siemons[1], Didier Varney[2], Duc-Duy Vu[1], Tobias Werner[3], Philip Wurm[3], Jean-Max Tyburn[2]
[1]Ecole Normale Supérieure - PSL, Paris, France, [2]Bruker Biospin FR, Wissembourg, France, [3]Bruker Biospin DE, Ettlingen, Germany, [4]Bruker Biospin CH, Fällanden, Switzerland, [5]CERM - CIRMMP, Florence, Italy

Introduction: NMR relaxation is a fascinating phenomenon to probe molecular dynamics experimentally. Methods such as fast field-cycling relaxometry measure relaxation rates over several orders of magnitude of magnetic field, providing information on dynamics over many timescales. High-resolution relaxometry combines the analytical power of high-resolution NMR and the information of relaxometry, by using a sample shuttle on a high-field magnet, exploiting the stray field as a variable field. Yet, physically moving the sample leads to strong limitations: very low fields are too distant and fast relaxation cannot be probed as polarisation would not survive shuttle transfers.

Aims: Our objective is to design a new approach to overcome the limitations of high-resolution relaxometry and obtain information from relaxation on dynamics from picoseconds to microseconds, while preserving high-resolution and sensitivity.

Methods: We introduce ultrafast high-resolution relaxometry (UHRR). A commercial high-field magnet is equipped with a sample shuttle to move at high speed the sample to a field-cycling coil. To preserve magnetization during the sample-shuttle transfer, the magnetic field is maintained above 1 T by a magnetic tunnel positioned inside the bore of the high-field magnet. Field cycling down to 100 µT is achieved in about 1 ms, allowing the measurement of fast relaxation. Detection is performed at high field with high sensitivity and resolution.

Results: We show examples of UHRR measurements down to 100 µT. Nuclear magnetic relaxation dispersion profiles of water protons in solutions of Mn^{2+} cations are in excellent agreement with fast-field cycling relaxometry. Transient binding of tryptophan to serum albumin is probed and analyzed quantitatively from proton longitudinal relaxation. Site-specific nitrogen-15 relaxation in a disordered protein enables the determination of pico-to-nanosecond backbone dynamics.

Conclusions: We show that UHRR is a powerful approach to determine molecular motions over orders of magnitude of timescales in a broad range of complex systems.

HRD-005: High-Q Photonic Band Gap Resonators for mm-Wave EPR and DNP are Getting Bigger

Dr Alex Smirnov[1], Dr. Sergey Milikisiyants[1], Dr. Antonin Marek[1], Dr. Alexander Nevzorov[1]
[1]North Carolina State University, Raleigh, United States

Introduction. Time domain EPR and DNP NMR experiments are more informative at high magnetic fields. Both methods require high electronic B1 fields on the sample, but this becomes challenging above 90 GHz as nonresonant losses increase and output of power components falls. The current technical solution involves low-Q probeheads of milliliter volumes together with high power gyrotrons and extended interaction klystrons (IEKs).
Aims. We demonstrate a radically new solution to the long-standing problem of generating high electronic B1 field over large volumes by developing high-Q/high-finesse resonators based on one-dimensional photonic band gap (PBG) crystals that effectively separate electric and magnetic field components.
Methods. 1D PBG crystals were assembled from λ/4 low-loss layers with alternating dielectric constants. The resonators were optimized by choosing dielectric materials, geometry of the reflecting mirror and increasing sample diameter.
Results. PBG resonators with Q-factors from 3,000 to 8,000 at 94 GHz and up to Q=1,500 at 197 GHz were developed. The use of low loss /high-ε materials improved finesse to ca. 50% of Q-factor. Conversion factors of these resonators were evaluated in nutation experiments using 100 µm thick polystyrene film doped with 1 mM of BDPA. While the shortest 90o pulses for PBG at 95 GHz were still longer (34 ns) vs. those (23 ns) achieved with cylindrical TE012-type cavity of comparable Q when using only 0.6 W of incident power, an increase in sample volume from 0.8 to 120 µl resulted in >60-fold signal gain for the same spin concentration. PBGs were employed for DNP at 197 GHz/300 MHz frequencies using 130 W pulsed EIK amplifier. The record 92 MHz electronic B1 fields were observed in three pulse echo nutation experiment.
Conclusions. PBG resonators enable high B1 fields over several microliter volumes resulting in dramatically improved EPR sensitivity and pave the way to pulse DNP.

HRD-006: Frequency multiplexing enables parallel multi-sample EPR

Mr Chun Him Lee[1], Prof Jan Korvink[1], Dr Mazin Jouda[1]
[1]Karlsruhe Institute of Technology, Eggenstein-leopoldshafen, Germany

Electron paramagnetic resonance (EPR) spectroscopy is an outstanding analytical technique appearing comprehensively in the fields of biology, chemistry, physics, and material sciences. It provides crucial information in understanding the molecular structure and reaction mechanisms of substances containing unpaired electrons, such as metal complexes, organic and inorganic radicals, and intermediate states in chemical reactions. Currently, EPR spectroscopy is limited by the sample throughput, as current commercial systems only target sequential analysis. Here, we introduce a novel scheme for conducting ultra-high frequency (UHF) continuous-wave EPR (CW EPR) targeting the parallel EPR spectroscopy of multiple microliter volume samples. Our proof-of-principle prototype involves two decoupled detection cells equipped with high-quality factor Q=104 solenoidal coils tuned to 488 MHz and 589 MHz, ensuring a significant frequency gap for effective radio frequency (RF) while limiting to a typical EPR scan range and an orthogonal arrangement to optimize the decoupling between the channels. This work further presents an innovative RF circuit concept that utilizes a single physical RF channel with the use of digital lock-in amplification and a custom field modulation coil to house up to eight cells for simultaneous measurements. Parallel EPR experiments on two 18.3 µL BDPA samples have registered signal-to-noise ratios of 255 and 252 for the two respective EPR measurement cells. Furthermore, parallel EPR spectroscopy of BDPA and TEMPO samples was achieved with no measurable coupling despite the significant difference in signal strength (a factor of 1000) between the two samples. The showcased prototype, built using cost-effective commercially available fabrication technology, is readily scalable and represents an initial step with promising potential for advancing sample screening with high-throughput parallel EPR.

HRD-007: 250 GHz Solid-State Source for Electron Paramagnetic Resonance and Dynamic Nuclear Polarization Applications

Phd Alisa Leavesley[1], PhD Eric Bryerton[1], PhD Theodore Reck[1], PhD Jeffrey Hesler[1]
[1]Virginia Diodes Inc, Charlottesville, United States

Microwave sources are an integral part of any electron paramagnetic resonance (EPR) or dynamic nuclear polarization (DNP) compatible spectrometer. Solid-state (ss) sources are ideally suited for EPR and DNP applications due to their compatibility with complex signal generation via arbitrary waveform generators (AWG) and pulsing. Additionally, ss-sources have wider tuning bandwidths and are relatively affordable compared to the vacuum tube-based alternatives. The primary disadvantages of ss-sources are their low output powers and their incompatibility with amplitude modulation due to the amplification-multiplication methods used to reach 100s of GHz. The aim of this work is to demonstrate a ss-source at 250 GHz that closes the power gap between ss-sources and the vacuum tube-based alternatives and take the first steps to alter the standard architecture of ss-sources to be more compatible with amplitude modulation via a mixer-amplifier based design approach. To obtain higher microwave power, our 125 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 125 GHz – 1.3 W. To reach our target frequency of 250 GHz, the source architecture was modified to be mixer-based, where signal at 62 GHz was mixed with an AWG and doubled to 125 GHz, where it was amplified via the eight-way amplifier block and finally doubled again to reach 250 GHz. Each doubler 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 should enable amplitude modulation at >100 GHz. Here we present a 250 GHz source that can generate complex signals with 500 mW of power via a mixer-doubler-amplifier-doubler design.

HRD-008: Implementation of Machine Learning in Assisting NMR Microcoil Design Optimization

Dr. Bing Wu[1], Prof. Arno Kentgens[1]
[1]Radboud University Nijmegen, Geleen, Netherlands

As a promising way to achieve superior mass sensitivity in RF coils, NMR Microcoil has been frequently implemented in various probehead designs. It has been shown that microcoil-based NMR probes can obtain a mass sensitivity of more than 10 times better than a conventional 5mm Helmholtz coil NMR probe.
On the other hand, with the rapid development of modern wireless communications and radar, antennas are becoming more complex, therein having, e.g., more degrees of design freedom, integration and fabrication constraints, and design objectives. Though utilizing different properties of RF waves, some design concepts can be interchanged between NMR RF microcoil and telecommunication antennas. In both designs, fullwave electromagnetic simulation can be very accurate and therefore essential to the design process. However, it is also very time-consuming, which leads to many challenges for coil design, optimization, and sensitivity analysis (SA).
Recently, machine-learning-assisted optimization (MLAO) has been widely introduced to accelerate the design process of telecommunication antennas. Machine learning (ML) methods, including Gaussian process regression, support vector machine (SVM), and artificial neural networks (ANNs), have been applied to build surrogate models of antennas to achieve fast response prediction. With the help of these ML methods, various MLAO algorithms have been proposed for different applications.
In this study, we first introduce this concept into the design of RF microcoil for NMR application. Through utilizing MLAO, several RF coil designs were optimized theoretically for samples with different geometries. Some simple RF microcoils are also manufactured to validate the simulation.

HRD-009: Simultaneous Multinuclear MRI via a Single RF Channel

M.sc. Mohammadrasool Vaezikakhki[1], Prof. Jan G. Korvink[1], Dr. Mazin Jouda[1]
[1]Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

Magnetic resonance imaging (MRI) is one of the most advanced noninvasive and non-destructive imaging techniques, widely used in not even medical but industrial fields. Its capability to measure multiple nuclei enhances its strength, allowing for the acquisition of multi-dimensional datasets of the object being examined. Traditionally, this requires specialized hardware for detecting each nucleus. In this paper, we present a method using a digital lock-in amplifier to perform simultaneous multi-nuclear MRI with a single radio frequency (RF) channel. We demonstrate this concept by producing fully parallel (TX and RX) 1H and 19F MRI images, highlighting that it can be extended to include additional nuclei without increasing hardware costs or scan time. The scalability of this method is virtually unlimited, constrained only by the processing speed of the digital unit. Additionally, our results show that the quality of parallel imaging, with a signal-to-noise ratio (SNR) of 54, is comparable to which of a commercial single channel system with an SNR of 43. Therefore, this approach offers significant reductions in scan time, system complexity, and hardware expenses without compromising imaging quality and signal-to-noise ratio (SNR).

HRD-010: Localised shimset for mutli-nuclei NMR spectroscopy

Mr Hossein Esmaeilizadshali[1], Professor Jan Korvink[1], Dr Mazin Jouda[1]
[1]KIT, Eggenstein-Leopoldshafen, Germany

In NMR spectroscopy, one of the main requirements to obtain an acceptable high-resolution spectrum is to have the B0 field as homogeneous as possible. Localized shimming coils, integrated in close proximity to the NMR detector, hold great potential to facilitate reaching a more homogeneous B0 field while consuming less power. In this report, an intelligent localized shimset is used to achieve high-resolution multinuclear NMR spectroscopy. The shimset, only with the first-order spherical harmonics (x, y and z), could show an improvement in the NMR spectral linewidth from 84 Hz to 3.5 Hz in a 1.05 T permanent magnet. Additionally, a new method is introduced here to perform multinuclear spectroscopy using a single RF transceiver. In this method, a high-sensitivity NMR signal was used as a reference to correct the problem of field drifting of the permanent magnet, thereby enabling the measurement of low-sensitivity nuclei.

HRD-011: AI-driven shimming for parallel multi-sample NMR spectroscopy

Dr. Moritz Becker[1], Yen-Tse Cheng[1], Achim Voigt[1], Ajmal Chenakkara[1], Mengjia He[1], Dr. Sören Lehmkuhl[1], Dr. Mazin Jouda[1], Prof. Jan Korvink[1]
[1]Karlsruhe Institute of Technology - KIT, Eggenstein-Leopoldshafen, Germany

Achieving high-throughput parallel multi-sample NMR spectroscopy requires each detector to be equipped with a localized set of shim coils to homogenize the B0 field in the corresponding sample. This setup increases the complexity of the NMR array and presents significant technical challenges, such as maintaining sufficient decoupling between the RF detectors and ensuring the orthogonality of the shim sets relative to each other's sample. Beyond these technical difficulties, parallel NMR spectroscopy poses another major shimming challenge due to the large number of shim lines and the lack of complete orthogonality between the shim sets. In this work, we present the hardware of a two-sample parallel NMR spectroscopy array designed for operation in a 15.2T preclinical MRI scanner. The array includes two stripline RF detectors, each equipped with six localized shims (x, y, z, z2, z3, z4). Additionally, we introduce a deep learning-based approach to simultaneously shim the two samples.

HRD-012: Towards Tabletop Recyclable Hyperpolarization with a Compact Freeze, Melt, and Flow DNP Polarizer

Nathan Rougier[1], Ewoud Vaneeckhaute[1], Charlotte Bocquelet[1], Yang Wang[1], Julien Dieterich[2], Laurent Veyre[2], Chloe Thieuleux[2], Roberto Melzi[3], Daniel Banks[4], James Kempf[4], Sami Jannin[1]
[1]Univ. Lyon, CNRS, ENS Lyon, UCBL, Université de Lyon, CRMN UMR 5082, 69100 Villeurbanne, France, [2]Univ. Lyon, Institut de Chimie de Lyon, CP2M UMR 5128 CNRS-UCBL-CPE Lyon, 69100 Villeurbanne, France, [3]Bruker Italia S.r.l., Viale V. Lancetti 43, , 20158 Milano, Italy, [4]Bruker Biospin, Billerica, United States

Hyperpolarization methods provide a way to tackle the inherently low NMR sensitivity and acquire a higher signal intensity in a shorter time. Twenty years ago, dissolution Dynamic Nuclear Polarization (d-DNP) was introduced and is now one of the hyperpolarization methods providing a 10’000-fold boost of sensitivity in solution on a routine basis.

However, this method suffers from one major drawback narrowing its applicability. The overall dDNP experiment is destructive and therefore single shot in operation. The frozen hyperpolarized sample, once polarized, is dissolved and diluted before being analyzed in liquid state NMR. Afterward, the hyperpolarized signal relaxes within seconds, and the sample must be thrown away. On the other hand, most NMR experiments rely on acquisitions of multiple scans coherence selection through phase cycling and multidimensional analysis, thus requiring numerous consecutive acquisitions which is impossible today.

We are presently working on turning d-DNP into a new version that will be widely compatible with NMR spectroscopy [1]. It consists of replenishing the DNP hyperpolarization of a sample flowing through a closed loop, without dilution nor contamination using hyperpolarizing silica-based material (HYPSO) as polarizing matrices in a compact and helium-free DNP polarizer coupled to a benchtop NMR spectrometer for liquid-state detection.

Here, we will present: i) our current DNP polarizer performing at 77K and with a 1T magnetic field now equipped with a cryostat for static measurements, a double-tuned 1H/13C probe, a 30 GHz and 5W microwave generator, and a nitrogen auto-refill station, ii) our latest progress on implementing fast freeze and melt of the sample with the concept of a dedicated probe composed of a dual cryostat and heating unit that will be placed in our polarizer and iii) our preliminary results obtained on water samples.

[1] Bocquelet, C. et al. https://doi.org/10.26434/chemrxiv-2024-3nlnd (2024)

HRD-013: Ultra High-Field Miniature Magnets

Dr. Jasmin Schönzart[1,2], Dr. Alexander Barnes[1]
[1]ETH Zürich/Resonance Explorations AG, Zurich, Switzerland, [2]Resonance Explorations AG, Zurich, Switzerland

We propose a strategy to build magnets above 50 Tesla for wide dissemination to the NMR community. High temperature superconductor (HTS) with high current densities will readily provide a viable path to a new generation of magnets for NMR which are smaller, better, and cheaper. A key innovation we foresee is the reduction of NMR magnet mandrels (a.k.a. winding bobbins) from typically employed diameter of ~150 mm to smaller than 15 mm. The magnets will be comprised of pancake coils each charged to different, and controllable, currents which will facilitate magnetic field homogenization. Conventional NMR probes are far too large in diameter to fit into such magnet bores, and are unfavorably long due to the distance from the homogenous region of the magnet to edge of the bore. Instead, we envision narrow (< 10 mm diameter), short (<20 mm) extensions of the probe inside the magnet bore. The magnets will improve dissemination as the production costs and required space will be significantly lower. The small magnets will have 5 Gauss radii of less than a meter without active shielding—making them easier to cite and easier to manufacture. Such technology, if successful, will catapult magnetic resonance into a brave new world. With a new generation of enhanced technology, NMR spectroscopists will be well-positioned to push the frontiers of science.

HYP-001: R3-Noria-methanesulfonate: Extremally Sensitive Novel Molecular Imaging Agent for Hyperpolarized Xenon-129 MRI

Phd Yurii Shepelytskyi[1,2], PhD Vira Grynko[1,2], PhD Viktoriia Batarchuk[1,2], PhD Carson Hasselbrink[3], Antal Kovacs[1], PhD Iulian Ruset[4], Karla Rodriguez[1], PhD Nedal Al Taradeh[1], Tanu Talwar[1], PhD Brenton DeBoef[5], Mitchell Albert[1,2,6]
[1]Lakehead University, Thunder Bay, Canada, [2]Thunder Bay Regional Health Research Institute, Thunder Bay, Canada, [3]California Polytechnic State University, San Luis Obispo, USA, [4]Xemed LCC, Durhan, USA, [5]University of Rhode Island, Kingston, USA, [6]Northern Ontario School of Medicine, Thunder Bay, Canada

Molecular imaging (MI) is a rapidly evolving medical imaging field that has the potential to become a stepping stone for personalized medicine. Due to the overall lack of sensitivity, conventional magnetic resonance imaging (MRI) does not perform well in MI settings. Utilization of hyperpolarized (HP) xenon-129 (^{129}Xe) MRI reveals the potential for MI via the implementation of the HP Chemical Exchange Saturation Transfer (HyperCEST) phenomenon. HyperCEST relies on the reversible binding of HP 129Xe to a supramolecular host molecule that confers a specific chemical shift to the encapsulated ^{129}Xe with subsequent depolarization of the encapsulated ^{129}Xe by selective radiofrequency pulse train application. Despite the existence of multiple HyperCEST active molecules, complex synthesis and challenges in functionalization significantly limit their utilization for clinical applications.

Our research aims to introduce a novel water-soluble resorcinarene trimer methanesulfonate (R3-Noria-$MeSO_3H$) as a highly sensitive agent for ^{129}Xe MI with a dual contrast mechanism.

Synthetized R3-Noria-$MeSO_3H$ was dissolved in deionized water (DI H_2O), phosphate-buffered saline (PBS), and saline. 3mL of R3-Noria-$MeSO_3H$ solutions (concentrations up to 5 mM) was drawn into the frit phantom vessel and placed inside a custom-built quadrature dual-tuned ^{1}H/^{129}Xe MRI coil within a clinical Philips Achieva 3.0T MRI scanner. Natural abundant (for MR spectroscopy) and enriched (~93%) (for MRI imaging) ^{129}Xe was polarized up to 56% using Xemed XeBox-10E polarizer.

HP ^{129}Xe MRS and MRI demonstrated that R3-Noria-MeSO3H produces not only a strong HyperCEST effect (up to 85%, depletion peak at +87 ppm with respect to dissolved-phase ^{129}Xe) but also extremely strong negative T_2^* contrast (reduction in T_2^* by a factor of 5.7,6.8, and 5.9 for DI H_2O, PBS, and saline respectively). The position of the HyperCEST depletion peak and the strong negative contrast can be explained by a complex aggregation dynamic of R3-Noria-$MeSO_3H$ in aqueous solutions corroborated by dynamic light scattering experiments.

HYP-003: Magnetic resonance pH measurements enabled by decarboxylation of [1-13C]pyruvate-d3 enhanced via parahydrogen

PhD Maria Daniela Santi[1,2], Theresa Hune[1,2], Lisa Fries[1,2], PhD Gonzalo Rodriguez[1,2], PhD Mei Ruhuai[1,2], PhD Sonja Sternkopf[1,2], PhD Stefan Glöggler[1,2]
[1]Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany, [2]Center for Biostructural Imaging of Neurodegeneration, University Medical Center Göttingen, Göttingen, Germany

Introduction: Alterations in pH are a hallmark of several pathologies including, cancer, ischemia, and inflammation. Non-invasive magnetic resonance methods to measure pH offer a new approach to early diagnosis of diseases characterized by acid-base imbalances. Hyperpolarization with parahydrogen-induced polarization (PHIP) enhances inherently low signals in magnetic resonance experiments by several orders of magnitude and offers a suitable platform to obtain biocompatible markers in less than one minute.
Aim: Develop and validate a PHIP-derived pH sensor suitable for in vivo studies.
Methods: A 13C carbonate pH sensor was achieved via non-enzymatic decarboxylation with H2O2 of [1-13C]pyruvate-d3 obtained by PHIP-SAH at 7 T. Validation was performed by measuring pH values in phantoms after the addition of hyperpolarized (HP) H13CO3-/13CO2. The integrals of HP H13CO3-/13CO2 at the steady state and the Henderson−Hasselbalch equation were used to calculate pH.
Results: An optimized 13C polarization of purified [1-13C]pyruvate-d3 in D2O with 36.65 ± 0.06 % polarization was obtained starting from 50 mM precursor. Subsequent decarboxylation, H13CO3-/13CO2 exhibited 12.46 ± 0.01 % of polarization at physiological pH 45 seconds after the reaction started. Considering the dilution factor that [1-13C]pyruvate-d3 exhibits in vivo, we optimized our methodology to test the accuracy of the pH sensor at singlet digit millimolar concentration. In vitro pH estimations on phantoms demonstrated accurate pH calculations with uncertainties of less than 0.07 units. Moreover, the calculated biosensor-pH values did not show significant differences compared with the electrode-measured.
Conclusions: Purified [1-13C]pyruvate-d3 with a notable 13C polarization in aqueous solution and at physiological pH was decarboxylated non-enzymatically to obtain a pH biosensor. We attribute the remarkable polarization to optimizations performed in our purification and pH adjustment procedures. The promising results highlight the utility of a pH sensor generated via PHIP that we envision can be rapidly adopted for preclinical investigations and future clinical patient imaging.

HYP-004: Hyperpolarized 13C NMR metabolomics of urine samples at natural abundance applied to chronic kidney disease

Mr Victor Ribay[1], Mr Benoît Charrier[1], Dr Mikaël Croyal[2], Dr Bertrand Cariou[3], Dr Samy Hadjadj[2,3], Dr Julien Boccard[4], Dr Jean-Nicolas Dumez[1], Dr Marine Letertre[1], Pr Patrick Giraudeau[1]
[1]Nantes Université, CNRS, CEISAM UMR 6230, Nantes, France, [2]M-SHARK Mass Spectrometry Core Facility, Nantes, France, [3]L'institut du thorax, INSERM, CNRS, Nantes Université, Nantes, France, [4]School of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland

NMR-based metabolomics provides important information on complex biological mixtures. but mostly relies on 1D 1H experiments for sensitivity reasons. However, strong peak overlap is a limitation for the analysis of inherently complex biological mixtures. To overcome this limitation, 13C NMR benefits from a 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) offers an opportunity to improve significantly the sensitivity of 13C NMR. Recently, 13C d-DNP has been successfully incorporated into an untargeted metabolomics workflow on plant extracts pools, paving the way towards hyperpolarized 13C NMR metabolomics. Moreover, we recently reported the first hyperpolarized 13C NMR experiments on urine samples at natural abundance in conditions which are compatible with metabolomics studies.
This communication presents the first application of this promising hyperpolarized approach to a clinical metabolomics study. The analysis of urine samples from patients with different stages of chronic kidney disease (CKD) was performed using 13C d-DNP NMR and conventional 1H NMR metabolomics at 600 MHz to explore the complementarity between the two methods. Data were meticulously acquired and processed according to metabolomics standard guidelines to ensure trustful results.
Supervised analysis of the d-DNP NMR dataset provided a valid statistical model separating patients with CKD stages 3 and 4 from patients with CKD stage 1. Moreover, 13C d-DNP NMR highlighted several biomarkers known to be biologically relevant. Some of them were in agreement with those obtained with conventional 1H NMR, while the results also highlighted some degree of complementarity between hyperpolarized and conventional NMR metabolomics.
These results highlight the promising potential of 13C d-DNP NMR at natural abundance for metabolomics, and its complementarity with established methods. We will discuss the challenges that remain to be tackled for a general application to metabolomics.

HYP-005: Towards personalized medicine via parallel-microfluidics metabolic MRI

PhD David Gomez-Cabeza[1], Marc Azagra[1], Alba Herrero-Gomez[1], Lluis Mangas-Florencio[1,2], PhD James Eills[1], PhD Alejandro Portela[2], Gergo Matajsz[1], Phd Irene Marco-Rius[1]
[1]Institute For Bioengineering Of Catalonia, Barcelona, España, [2]Vitala Technologies, S.L., Barcelona, España

INTRODUCTION: To address the complexity of metabolic diseases, the integration of organs-on-a-chip (OoC) technology with hyperpolarised magnetic resonance spectroscopic imaging (HP-MRSI) offers a novel approach to study cell metabolism in real-time. This method enables the development of complex, patient-derived biological models for disease and treatment studies, potentially reducing the reliance on animal testing and facilitating personalized medicine through lower costs and patient-specific models.

METHODS: Our study combined microfluidics with HP-MRSI, utilizing 13C labeled pyruvate to observe its conversion into lactate in HepG2 cell suspensions, thus allowing for the real-time monitoring of metabolism. By employing a dissolution DNP HyperSense polarizer (Oxford Instruments) for substrate polarization, we enhanced sensitivity and significantly cut acquisition times for these rapid metabolic processes.

To emphasize the parallelisable potential of combining microfluidics with HP-MRSI, we evaluated 8 different conditions by using Chemical Shift Imaging (CSI) with our 3T MRI scanner (BioSpec, Bruker). The base sample was cells in EMEM media (1% P/S, 10% FBS). We supplemented two samples with Glucose (Glu, 25mM) and Glutamine (Gln, 4mM) and two samples with NADH (33mM) (reaction cofactor). We also lysed three samples with RIPA buffer. Finally, we left two samples as positive (commercial enzyme) and negative (only media) controls.

RESULTS: With this type of voxel spectroscopic metabolic imaging provided by CSI, we observed three different phenomena in a single experiment. Our findings reveal that media richness and extracellular NADH significantly influence lactate production, highlighting the importance of glycolytic activity and redox balance in cellular metabolism.

CONCLUSION: Our results are the first to show such level of parallelisation with high spatial resolution for cellular studies using microfluidics and HP-MRSI. Better detection coils with large areas (we employed a volume coil) and better k- space sampling with low pulsing are required to improve the resolution and aim for temporal imaging.

HYP-006: An ESR signature for DNP samples benefiting from microwave frequency modulation.

Dr Thanh Phong Lê[1], Dr Quentin Stern[3], Dr Jean-Noël Hyacinthe[1], Dr Sami Jannin[3], Dr Andrea Capozzi[1,2]
[1]EPFL, Lausanne, Switzerland, [2]DTU, Kgs. Lyngby, Denmark, [3]Université Lyon 1, Lyon, France

Introduction: In recent years, dissolution DNP has moved towards higher magnetic field because of the greater maximum achievable polarization that can be obtained. At this new experimental conditions, microwave frequency modulation (FM) has proven to be very useful for increasing the DNP enhancement on many systems. Often, a beneficial effect was reported for samples doped with radicals characterized by short T1 (T1e) and broad linewidth (e.g. nitroxide radicals). Nevertheless, the reason why FM works only on certain systems and experimental conditions is still unclear.

Aim: in this work, we provide experimental proof of why and when FM yields higher polarization.

Methods: as a function of different FM values, we performed DNP measurements (frequency sweeps and enhancements) and LOngitudinal Detected (LOD)-ESR measurements (spectra and saturation/relaxation times) at 5T and 6.7T on two systems characterized by different T1e and radical linewidth (i.e. 30mM AH111501 dissolved in [1-13C]pyruvic acid and 30mM TEMPOL dissolved in a solution of glycerol:water 1:1 (v/v) and 3M sodium [1-13C]acetate). All experiment were performed at 1.15K.

Results: in good agreement with the fact that FM is more remarkable at high field, we observed that moving from 5 to 6.7T made the T1e shorter and the spectrum linewidth broader in both samples. Most importantly, only the TEMPOL sample at 6.7T showed a real benefit from FM. This observation correlates nicely with the fact that, in this sample at high field, the saturation transient of the electron spins became slower than the relaxation one, when using no FM. Employing FM, saturation was again faster than relaxation, as observed in the other cases already with no FM.

Conclusion: short T1e and broad ESR lines hamper spectral diffusion and thus saturation. FM, engaging more spins in the first place, re-establishes a correct saturation/relaxation balance in the electron spin system yielding efficient DNP.

HYP-007: Solid-Effect Dynamic Nuclear Polarization at High Magnetic Fields: From Viscous Liquids to Aqueous Solutions

Dr. Andrei Kuzhelev[1], Dr. Vasyl Denysenkov[1], Iram Ahmad[2], Prof. Dr. Elena Bagryanskaya[3], Dr. Victor Tormyshev[3], Prof. Dr. Snorri Sigurdsson[2], Prof. Dr. Thomas Prisner[1]
[1]Institute of Physical and Theoretical Chemistry, Goethe University Frankfurt am Main, Frankfurt am Main, Germany, [2]University of Iceland, Reykjavik, Iceland, [3]N. N. Vorozhtsov Institute of Organic Chemistry, Siberian Branch of Russian Academy of Sciences, , Russia

In recent years, the solid-state effect of dynamic nuclear polarization (DNP) has attracted new attention in liquid-state NMR. This mechanism often competes against the Overhauser effect, especially in viscous liquids. In this work, we demonstrate that narrow-line polarizing agents (1,3-bisdiphenylene-2-phenylallyl or tris(2,3,5,6-tetrathiaaryl)methyl radicals) can be used to hyperpolarize lipid bilayers in the fluid phase [1] or tripeptides dissolved in glycerol [2] through the solid-effect mechanism at a magnetic field of 9.4 T and ambient temperature. In addition, the enhanced proton polarization can be transferred to carbon nuclei via insensitive nuclei enhanced by polarization transfer (INEPT) [3]. The solid effect can be also expanded not only to viscous liquids but also to aqueous solutions. We show a beyond 30-fold DNP enhancement for adenosine triphosphate and single-/double-stranded oligonucleotides dissolved in water for proton and phosphorus nuclei at a magnetic field of 9.4 T and near-physiological temperatures.

This work was supported by the Deutsche Forschungsgemeinschaft (DFG, grant 518679424).

References:
[1] Kuzhelev, A., Dai, D., Denysenkov V., Prisner, T. J. Am. Chem. Soc, 2022, 144 (3), 1164-1168.
[2] Kuzhelev, A., Denysenkov V., Ahmad, I., Rogozhnikova, O., Trukhin, D., Bagryanskaya, E., Tormyshev, V., Sigurdsson, S., Prisner, T. J. Am. Chem. Soc, 2023, 145, 18, 10268–10274.
[3] Dai, D., Denysenkov, V., Bagryanskaya, E., Tormyshev, V. Prisner, T. Kuzhelev, A. J. Phys. Chem. Lett., 2023, 14 (31), 7059-7064.

HYP-008: On the measurement of chemical exchange and J-coupling constants driving SABRE

Master Science Charbel Assaf[1], Dr. Oleg Salnikov[2], Master Science Xin Gui[3], Master Science Anna Yi[2], Prof. Dr. Simon Duckett[4], Prof. Dr. Eduard Chekmenev[5], Dr. Alexander Auer[3], Prof. Dr. Igor Koptyug[2], Prof. Dr. Jan-Bernd Hövener[1], Dr. Andrey Pravdivtsev[1]
[1]Section Biomedical Imaging, Molecular Imaging North Competence Center (MOIN CC), Department of Radiology and Neuroradiology, University Medical Center Kiel, Kiel University, Am Botanischen Garten 14, 24118, Kiel, Germany, E-mail: jan.hoevener@rad.uni-kiel.de, , , [2]International Tomography Center SB RAS, 3A Institutskaya St., 630090 Novosibirsk, Russia, , , [3]Max-Planck-Institut für Kohlenforschung, Kaiser-Wilhelm-Platz 1, 45470 Mülheim an der Ruhr, alexander.auer@kofo.mpg.de, , , [4]Centre for Hyperpolarization in Magnetic Resonance (CHyM), University of York, Heslington YO10 5NY, UK, E-mail: simon.duckett@york.ac.uk, , , [5]Department of Chemistry, Integrative Biosciences (Ibio), Karmanos Cancer Institute (KCI), Wayne State University, Detroit, MI 48202, United States, ,

Introduction:
Observing pyruvate metabolism in vivo has become a focal point of metabolic magnetic resonance imaging. Signal amplification by reversible exchange (SABRE) has recently emerged as a cost-efficient technique for hyperpolarizing pyruvate. Pyruvate is hyperpolarized via transient interaction with parahydrogen (pH_2) in an Ir-complex. The chemical exchange rate of the substrate to be polarized, pH_2, and the size of the propagating spin-spin interactions influence SABRE efficiency.
Aims:
In this study, we aim to measure the small ^{1}H-^{13}C J-couplings that connect hydride protons in SABRE catalysts to 1- and 2-^{13}C nuclei in pyruvate. Also, we seek to produce a toolkit for quantifying the underlying exchange rates to ensure catalyst optimization and the accuracy of spin dynamics simulations.
Methods:
An experimental method using frequency-selective excitation of parahydrogen-derived PASADENA polarization followed by frequency-selective pulsed insensitive nuclei enhanced by polarization transfer (SEPP-SPINEPT) is shown to be able to measure key couplings. Additionally, three kinetic models for substrate exchange are developed to determine ligand exchange rates by rationalization of experimental observations obtained by ^{1}H 1D, and 2D NMR.
Results:
By incorporating variable temperature analysis, we probe eight ^{1}H-^{13}C interactions that drive pyruvate-SABRE, estimate exchange rates and determine energies of activation for pyridine, nicotinamide, and 4-aminopyridine loss. The processes' activation energies are then compared with density functional theory computations.
Conclusion:
A novel approach to analyzing the spin topology of short-lived organometallic complexes with the ability to measure spin-spin interactions < 1 Hz is described. Furthermore, a process wherein ligand exchange rates can be simply determined is rationalized against more complex models to facilitate the optimization of SABRE.

HYP-009: Detecting intermediates during peptide guided biomineralization by Dissolution Dynamic Nuclear Polarization and >100-fold enhanced multidimensional NMR.

MSc Ertan Turhan[1,2], Assoz. Prof. Dennis Kurzbach[1]
[1]University Of Vienna, Department of Biological Chemistry, Vienna, Austria, [2]University of Vienna, Vienna Doctoral School in Chemistry (DoSChem), Vienna, Austria

Introduction:
Osteopontin (OPN) plays an essential role in the biomineralization of human calcium phosphate in bone. However, its effect on modulating bone stability and flexibility remains unclear. To obtain a more detailed understanding, methods need to be developed that can capture the templating effect of OPN during the mineralization event. However, due to the low sensitivity of 2D NMR spectra, it is not possible to adequately track this very fast reaction with conventional methods.
Aim:
The goal was to expand the use of dissolution-DNP hyperpolarized water in combination with signal-enhanced 2D NMR [1] to track the conformational adaptions of OPN during calcium phosphate precipitation. By enhancing the protein spectrum >100-fold, high-resolution 2D spectra could be recorded within ca. 10 s, which allowed the recording of a fingerprint of an intermediate OPN state.
Methods:
OPN was preincubated with an excess of phosphate ions, leading to self-assembly of calcium phosphate templating structures, and subsequently mixed with hyperpolarized calcium containing water using a dedicated mixing device [2]. Immediately after mixing a 1H-15N BEST-HMQC was recorded within 10 s and compared with spectra before and after completion of the mineralization process.
Results:
We achieved signal enhancements >100-fold compared to a thermal equilibrium reference. This enabled us to identify signals stemming from intermediates forming during calcium-phosphate nucleation and to identify key residues involved in OPN’s mineral templating function.
Conclusion:
Hyperpolarized solution-state multidimensional NMR allows for the identification and characterization of intermediates in (bio)mineralization processes at residue resolution, thus allowing for rationalization of the effect of peptides such as OPN on the morphology of a material such as calcium phosphate. Such a methodology might thus help rationally design peptides that can tune material properties.

[1] Hilty, Kurzbach, Frydman, 2022, Nature Protoc. 17, 1621-1657
[2] Negroni, Turhan, Kress, Ceillier, Jannin, Kurzbach, 2022, JACS 144 (45), 20680-20686

HYP-011: The RASER Approach: Exploiting Stimulated Emission in NMR and MRI

Dr. Sören Lehmkuhl[1], Jing Yang[1], Simon Fleischer[1], Moritz Becker[1], Prof. Dr. Eduard Y. Chekmenev[2], Prof. Dr. Thomas Theis[3], Prof. Dr. Stephan Appelt[4], Dr. Mazin Jouda[1], Prof. Dr. Jan G. Korvink[1]
[1]IMT, Karlsruhe, Germany, [2]KCI, Detroit, USA, [3]Department of Chemistry, Raleigh, USA, [4]ZEA2, Jülich, Germany

Conventional NMR and MRI rely on radio frequency (rf) pulses for excitation. An alternative to external rf-excitation is self-excitation using a RASER (Radiofrequency Amplification by Stimulated Emission of Radiation). A RASER requires a population inversion on the nuclear spin states, which can easily be provided by hyperpolarization techniques. We employ Para-Hydrogen Induced Polarization (PHIP) for different types of RASER experiments. Here, I would like to discuss the potential of the RASER approach in NMR and MRI looking at the most recent results and highlight its advantages and disadvantages.
First, I will focus on the RASER “regime”, understanding the different nonlinear effects that can be observed during RASER activity. They can be controlled experimentally and predicted based on theoretical considerations. We measured and simulated the bifurcation diagram of a RASER system.
Second, I would like to focus on the precision in frequency resolution RASER-based sensing can achieve. Both J-coupling as well as chemical shifts could be detected with a RASER in the past, while the RASER allows for unprecedented precision due to the long measurement times. However, for very long measurement times, the limiting parameter can be the drift of the magnetic field during the experiment. We developed ways to process RASER spectra to correct for the effects from magnetic field drifts.
Finally, I would like to discuss the potential of RASER MRI. Using stimulated emission for MRI can have three advantages. The signal is inherently background-free, can be higher resolved and does not require rf-excitation. The potential for higher resolution, however, comes at the cost of nonlinear artifacts that blur the spectrum. We trained a convolutional neural network to remove these artifacts. As a faster alternative, we recorded an image within one second and without the need for rf-pulses using the RASER approach.

HYP-012: Characterisation of [1-^{13}C]Pyruvate and [2-^{13}C]Pyruvate at Zero to Ultralow Field Enabled by SABRE-SHEATH and SQUID Sensors

John Z. Myers[1], Dr. Andrey N. Pravdivtsev[2], Dr. Kai Buckenmaier[3], Prof. Markus Plaumann[4], Dr. Rainer Körber[1]
[1]Physikalisch-Technische Bundesanstalt, Berlin, Berlin, Germany, [2]Molecular Imaging North Competence Center, Kiel, Germany, [3]Max Planck Institute for Biological Cybernetics, Tübingen, Germany, [4]Otto-von-Guericke University, Magdeburg, Germany

Introduction

One of the greatest limitations of NMR is its lack of sensitivity, achieving a thermal polarisation of only ppm at clinical field strengths. This limits the use of NMR for high sensitivity applications, including spectroscopy at zero to ultra-low field (ULF) and characterisations of mechanisms, such as spin-spin relaxation. Hyperpolarisation techniques, such as SABRE-SHEATH make these applications feasible by increasing nuclear polarisation to the order of percent.

Aims

Using SABRE-SHEATH, we aim to empirically characterise the relaxation processes of [1-^{13}C]pyruvate and [2-^{13}C]pyruvate in comparison to what would be predicted from the evolution of their product operators, derived for arbitrary magnetic field.

Methods

We hyperpolarised a 50 mM solution of 13C labelled pyruvate salt with the IrIMes catalyst and co-ligand DMSO in methanol, using SABRE-SHEATH. We controlled the guiding BHyp and detection BDet fields in a home-built ULF MRI setup, inside a moderately shielded room. Hyperpolarisation was performed with the BHyp field along the detection axis. For detection, the BHyp field was switched off and an orthogonal BDet field in the range of nTs-µTs was switched on non-adiabatically. Detection was performed, using an ultra-sensitive SQUID system. The NMR spectra were then modelled, using a density matrix approach.

Results

The results from the strong-coupling regime (zero-field) and from the weak-coupling regime (µTs) were then compared with the model. Both the empirical data and model agreed well with the FID evolution of [1-^{13}C]pyruvate, although there were some unexpected signals detected from [2-^{13}C]pyruvate. However, the empirical data also revealed unexpected behaviour of the spin-spin relaxation, manifested by the variable linewidths of the ^{13}C multiplets.

Conclusions

The implication of this is that hyperpolarisation enables single shot long acquisitions, through increased SNR that reveal features otherwise impossible to detect, enabling a better understanding of ULF spin evolution and future optimisation of hyperpolarisation.

HYP-013: Elucidating 129Xe sublimation DNP: from gas to gas again

Ms Emma Wiström[1], Dr Andrea Capozzi[1,2], Dr Jean-Noel Hyacinthe[1], Dr Thanh Lê[1]
[1]EPFL (Swiss Federal Institute of Technology in Lausanne), Lausanne, Switzerland, [2]Technical University of Denmark, Kgs. Lyngby, Denmark

Introduction

Hyperpolarized 129Xe via Dynamic Nuclear Polarization (DNP) is very little explored. Nevertheless, because of peculiar experimental conditions, this technique offers very interesting spin physics insights into the DNP process. Moreover, a dDNP polarizer can in theory enhance the NMR sensitivity of any non-zero nuclear spin, representing a multi-purpose hyperpolarization platform.

Aims

We demystified the cause of poor DNP performance of 129Xe samples with respect to 13C, despite very close gyromagnetic ratios. We developed a new method of gas extraction after solid-state polarization.

Methods – Results

Solid state investigations include LOD-ESR and DNP measurements at 1.15K and 5T. Samples: 2.6M xenon and 30mM/50mM TEMPO in isobutanol (sample A/B), 3M sodium [1-13C]acetate and 30mM TEMPOL in water:glycerol (50:50) (sample C). The admixture of the gas in the solvent is promoted by 20kHz ultrasonication while submerged in a bath of melting ethanol (-110°C).
The electron relaxation time (T1e) of nitroxyl radicals in isobutanol is at least 5-times shorter than in water:glycerol. Moreover, it is not affected by the presence of [1-13C]acetate in the sample, differently to what happens when adding xenon. This effect is reflected in the buildup times and polarization levels: sample B/C reach 14-15% after 2h, sample A reaches only 8% after over 4h. However, applying frequency modulation greatly improves sample A, resulting in similar polarization levels as sample B, the modulation has minimal effect on sample C.
Finally, we are implementing a cryocollection-free method to collect the gas after solid-state polarization.

Conclusions

Our exploration of solid-state xenon DNP shows the importance of the T1e parameter in the polarization process. We foresee that a cryogen-free collection of the hyperpolarized gas will reduce polarization losses because of reduced number of phase transition of the sample, notably a process characterized by short nuclear T1.

HYP-014: High-field optically induced NMR hyperpolarization in solids

Dr. Federico De Biasi[1], Máté Visegrádi[1], Dr. Marcel Levien[1], Dr. Ganesan Karthikeyan[2], Dr. Michael A. Hope[1], Dr. Yunfan Qiu[3], Paige J. Brown[3], Prof. Michael R. Wasielewski[3], Prof. Olivier Ouari[2], Prof. Lyndon Emsley[1]
[1]Institut des Sciences et Ingenierie Chimiques, École Polytechnique Fedérale de Lausanne (EPFL), Lausanne, Switzerland, [2]Aix-Marseille University, CNRS, Institut de Chimie Radicalaire, Marseille, France, [3]Department of Chemistry, Center for Molecular Quantum Transduction, Paula M. Trienens Institute for Sustainability and Energy, Northwestern University, Evanston, USA

Poor sensitivity is still one of the main barriers in cutting-edge applications of NMR spectroscopy for structural and chemical analysis. Hyperpolarization alleviates the sensitivity issue by enhancing the population imbalance between the nuclear spin states, and thus the NMR signal. For solid-state NMR spectroscopy, optical hyperpolarization methods would be particularly enticing. They exploit transient electronic excited states as the spin polarization source, allowing very large nuclear spin polarizations to be generated. One such optical technique is solid-state photochemically induced dynamic nuclear polarization (photo-CIDNP), where light is used to excite a donor–acceptor system, creating a spin-correlated radical pair whose evolution and decay build nuclear hyperpolarization.

We recently showed that bulk optical ^{1}H hyperpolarization can be observed at low magnetic field (0.3 T) by polarization relay from synthetic donor–acceptor molecules.[1] Here, we extend the concept of photo-CIDNP from synthetic donor–acceptor systems to the high magnetic fields required for high-resolution NMR spectroscopy, demonstrating optically enhanced ^{1}H and ^{13}C NMR at 9.4 T and 21.1 T.

[1] De Biasi et al., J. Am. Chem. Soc. 2023, 145, 27, 14874 – 14883

HYP-015: Enhancing Sensitivity of Dynamic Nuclear Polarization Utilizing a Helium Spinning System at 40 K: Case of Halogenated Perovskite Nanocrystals

Shadi Alarab[1]
[1]Cea_uga, Grenoble, France

The discovery of CsPbBr3 nanocrystals (NC) in 2015 and their unique light-emitting properties sparked interest in colloidal NC synthesis. CsPbBr3 NCs; with an ionic core, differ from other semiconductors and are prone to degradation. To address this, researchers have used ligands which play a key role in controlling the growth, shape, and colloidal stability. Investigations into surface chemistry, utilizing both liquid-state and solid-state NMR techniques, have provided valuable insights into ligand binding thermodynamics and potential surface composition. Nevertheless, progress in understanding the surface chemistry using ssNMR is clearly impeded by the current lack of sensitivity. Only a few studies made use of DNP to improve this sensitivity limitation but so far, with a limited efficiency compared to model systems. In this presentation, we discuss the main problems encountered when using DNP to polarize perovskite NCs at around 100 K and shows that ×10-20 improvement in sensitivity can be demonstrated using state-of-the-art polarizing agents and a home-built closed-cycle cryogenic helium spinning system enabling DNP down to 30 K. The work was conducted on CsPbBr3 NCs that were synthesized using a slightly modified version of the hot injection synthesis, in order to improve the quality and stability of the monodisperse cuboidal structures. More precisely, we first discuss the sample preparation protocol that was used to conduct DNP experiments on NCs, considering ligand lability and potential surface modifications. We also discuss the effect of various experimental parameters such as dissolved oxygen, sample storage; microwave absorption, sample heating, as well as glass quality and 1H T1 to explain why such systems are so difficult to polarize at 100 K. In order to improve this limitation, we discuss three strategies relying on the use of KBr to dilute the sample, state-of-the-art polarizing agents (cAsymPol-TEK and PyrroTriPol-OMe) and access to ultra-low temperature ~ 30 K.

HYP-016: Rubidium Hyperfine Coupling in Spin-Exchange Optical Pumping of Xenon: A Multiscale Simulation

Dr. Juha Vaara[1]
[1]University Of Oulu, Oulu, Finland

Xenon nuclei may be hyperpolarised through collisions with optically pumped rubidium [1]. While the spin exchange with the unpaired electrons is due to the Xe hyperfine coupling (HFC), also the Rb HFC turns out to play a decisive role. We model the polarisation transfer to xenon-129/131 in short binary collisions and long van der Waals (VDW) complexes between gaseous Rb and Xe. The simulations feature molecular dynamics of the Rb-Xe mixture, spin-Hamiltonian parameters from relativistic quantum chemistry, and explicit spin dynamics [2].

Oscillation in the VDW bond-length R strongly modulates the Xe HFC, imposing steps in Xe polarisation [3]. Rb nucleus and the unpaired electron constitute a strongly coupled two-spin system, with practically constant Rb HFC regardless of R. These two spins undergo a Rabi-like oscillation in a timescale determined by the Rb HFC, similar to the lifetime of the longest VDW complexes. The role of the Xe HFC is a perturbation oscillating at the much faster timescale of the VDW oscillation. The result is an overall reduction of the resulting Xe polarisation as compared to the simple model without Rb HFC. The Rabi oscillation constitutes an envelope on which the steps in the Xe polarisation are superimposed, restricting the achievable Xe polarisation in long VDW events.

Diagonalisation of the spin Hamiltonian with either rubidium-85 (spin 5/2) or -87 (3/2) isotopes at different values of R and the field strength B, suggests larger efficiency of transfer with rubidium-85, as well as its persistence up to higher B. Level anticrossings render the field interval between 1 and 10 mT efficient.

[1] T. G. Walker and W. Happer, Rev. Mod. Phys. 69, 629 (1997).
[2] P. Hilla, M. Tuomela, J. Rantaharju, and J. Vaara, in preparation (2024).
[3] J. Rantaharju, M. Hanni, and J. Vaara, Phys. Rev. A 102, 032813 (2020).

HYP-017: dDNP at PHIP speed with a fully portable 0.9T/4.2K dissolution DNP polarizer

Thanh Phong Lê[1], Jean-Noël Hyacinthe[1], Andrea Capozzi[1,2]
[1]Laboratory of Functional and Metabolic Imaging, École polytechnique fédérale de Lausanne (EPFL), Lausanne, Switzerland, [2]Department of Health Technology, Center for Hyperpolarization in Magnetic Resonance, Technical University of Denmark (DTU), Kgs Lyngby, Denmark
Introduction: MR metabolic imaging provides unique information for some diseases (e.g. cancer). Dissolution DNP is the most widespread technique for producing hyperpolarized metabolic contrast agents, but challenged by a limited sample throughput at high expenses. Recently, PHIP hyperpolarizers suggested similar performance as dDNP, but at a faster rate and with a smaller footprint. For most preclinical applications, 10% carbon polarization may be sufficient to obtain relevant biomarkers. In theory, this polarization value can be achieved using dDNP by employing much simpler, smaller, and less expensive hardware.

Aims: We design and characterize a fully portable dDNP polarizer.

Methods: The device is based on a small liquid helium bath cryostat, modified to couple a WR28 waveguide to the 12mm ID sample loading tube's side and using a 0.9T/16mm SmCo halbach array. DNP was initiated with a custom-built 15-40GHz/200mW microwave source, and NMR detected using a portable spectrometer (LapNMR, Tecmag). All devices are battery-operated and loaded on a transportation cart (50×65×95cm^3, W×L×H, 30kg total weight). ^{1}H DNP of H_2O:D_2O:glycerol: d_8-glycerol 1:9:1:9 v:v doped with 50mM TEMPOL was performed at 77K and 4.2K.

Results: The hold time is 24h at 77K and 10h at 4.2K with 1L of cryogen. At 77K, microwave frequency sweeps identified the positive/negative DNP peaks and zero-crossing at 25.18GHz, 25.29GHz and 25.23GHz respectively. A 100-fold enhancement within seconds was measured on the negative peak. DNP was observed at 4.2K as well. However, no quantitative measurements were performed due to lack of time.

Conclusions: We present a portable dDNP polarizer, which can perform ^{1}H DNP within seconds. By improving microwave power and irradiation, as well as working at 4.2K, where the electron polarization is 15% and ^{1}H DNP buildup time is less than 1min, the DNP enhancement will be significantly increased.

HYP-018: Bullet-DNP enables in-situ observation of multiple short-lived calcium carbonate precursors

Ertan Turhan[1], Dr Masoud Minaei[1], Dr Pouja Narwal[1], Dr Benno Meier[1], Dr Karel Kouril[1], Assoc. Prof. Dr. Dennis Kurzbach[1]
[1]University of Vienna, Vienna, Austria

Introduction

The discovery of (meta)stable pre-nucleation species (PNS) that challenge the conventional nucleation-and-growth paradigm has triggered a revival in crystallization research. This phenomenon, observed in various solidification mechanisms, including calcium phosphates and carbonates, inspired the exploration of novel experimental approaches. While stable PNS with longer lifetimes are readily accessible experimentally, identifying and characterizing early-stage precursors with short lifetimes remains challenging.

Aims

Herein, we aim to demonstrate that PNS with lifetimes << 5 s can be characterized by nuclear magnetic resonance (NMR) spectroscopy when boosted by ‘Bullet’ dynamic nuclear polarization (Bullet-DNP), which, as we show, renders the application of DDNP to pressure-sensitive samples possible.

Methods

With Bullet-DNP, we investigated the previously elusive early-stage prenucleation of calcium carbonates in the highly supersaturated concentration regime, characterizing species that form within milliseconds after the encounter of calcium and carbonate ions at high millimolar concentrations.

Results

Thus, we show that ionic pre-nucleation species not only govern the solidification of calcium carbonates at weak oversaturation but also initiate very rapid precipitation events at high concentrations. In particular, we report a transient co-existence of two PNS with distinct molecular sizes.

Conclusions

Using Bullet-DNP, it is possible to record spectra enhanced by over four orders of magnitude even for very temperature or pressure-sensitive samples. This feature could significantly widen the scope of ex-situ hyperpolarization techniques. For the case of hyperpolarized carbonate, the first seconds of Ca2+-bound precipitation could thus be accessed under high oversaturation by 13C-NMR, which remained out of the scope of traditional dDNP or any other high-resolution method.
Further, pre-nucleation species could be identified and characterized by NMR immediately after exposing calcium and carbonate ions to each other. The presence of these PNS signals indicates a non-classical two-step precursor pathway – as reported for mild oversaturation – responsible for CaC formation also under high oversaturation conditions.

HYP-019: Rapid and Simple 13C-Hyperpolarization by 1H Dissolution Dynamic Nuclear Polarization Followed by an Inline Magnetic Field Inversion

Dr. Quentin Stern, Quentin Renard-Feytis, Dr. Morgan Ceillier, Dr. Stuart Elliott, Dr. Olivier Cala, Prof. Konstantin Ivanov, Prof. Sami Jannin
[1]Universite Claude Bernard De Lyon 1, Villeurbanne, France, [2]Imperial College London, London, United Kingdom, [3]International Tomography Center, Novosibirsk, Russia

Introduction. Dissolution dynamic nuclear polarization (dDNP) is a method of choice for preparing hyperpolarized ^{13}C metabolites such as 1-^{13}C-pyruvate used for in vivo applications, including the real-time monitoring of cancer cell metabolism in human patients.

Problem. Common dDNP protocols use direct hyperpolarization of ^{13}C spins reaching polarizations of >50% in ∼1–2 h. Alternatively, ^{1}H spins are polarized before transferring their polarization to ^{13}C spins using cross-polarization3, reaching polarization levels similar to those of direct DNP in only ∼20 min. However, it relies on more complex instrumentation, requiring highly skilled personnel.

Solution. Here, we explore an alternative route using ^{1}H dDNP followed by inline adiabatic magnetic field inversion in the liquid state during the transfer. ^{1}H polarizations of >70% in the solid state are obtained in ∼5–10 min. As the hyperpolarized sample travels from the dDNP polarizer to the NMR spectrometer, it goes through a field inversion chamber, which causes the ^{1}H → ^{13}C polarization transfer. This transfer is made possible by the J-coupling between the heteronuclei, which mixes the Zeeman states at zero-field and causes an anti-level crossing.

Results. We report liquid-state ^{13}C polarization up to ∼17% for 3-^{13}C-pyruvate and ^{13}C-formate. The instrumentation needed to perform this experiment in addition to a conventional dDNP polarizer is simple and readily assembled.

Reference. Journal of the American Chemical Society 2023 145 (50), 27576-27586
https://doi.org/10.1021/jacs.3c09209

HYP-020: Hyperpolarization Chemistry and Spin Physics for Precision Measurement and Molecular Imaging

Professor Thomas Theis[1,6,7], Professor Eduard Chekmenev[2], Professor Stephan Appelt[3], Professor Matthew Rosen[4], Professor Yi-Fen Yen[4], Professor Boyd Goodson[5], CSO Patrick TomHon[6], CEO Carlos Dedesma[6], Group Leader Kai Buckenmaier[7], Mr Stephen McBride[1], Ms Megan Pike[1], Mr Nicolas Kempf[1,7], Mr Mustapha Abdulmojeed[1], Ms Erica Curran[1], Mr Chris Nelson[1], Mr Adam Ortmeier[1], Mr Brojo Dhali[1], Mr Seth Dilday[1], Mr Nick Volya[1], Ms Rielly Harrison[1], Dr Austin Browning[1], Dr. Keilian MacCulloch[1], Dr Iuliia Mandzhieva[1]
[1]North Carolina State University, Raleigh, United States, [2]Wayne State University, Detroit, United States, [3]RWTHAachen University, Aachen, Germany, [4]A. A. Martinos Center for Biomedical Imaging, Charleston, United States, [5]Southern Illinois University, Carbondale, United States, [6]Vizma Life Sciences, Chapel Hill, United States, [7]Max-Planck Institute for Biological Cybernetics, Tübingen, Germany

Introduction

Over the last century, advances in Magnetic Resonance (MR) technologies have revolutionized modern science and technology. Despite the tremendous progress, typical MR experiments only measure a tiny fraction of the available nuclear spins in samples. In this contribution we present particularly simple-to-implement parahydrogen-based hyperpolarization chemistry to boost NMR and MRI signals by several orders of magnitude. Such technology is intriguing because unexplored opportunities and new physical effects arise.

Aims

The presented work aims for
(A) scalable molecular imaging via low-field hyperpolarized MRI, which can be made broadly available and for
(B) precision NMR via the parahydrogen pumped RASER (Radiofrequency Amplification by Stimulated Emission of Radiation).

Methods

(A) We advance parahydrogen-based Signal Amplification By Reversible Exchange (SABRE) to hyperpolarize a wide range of molecules and process them into a biocompatible solution, which is injected into rodents at low magnetic fields to observe metabolism non-invasively.
(B) We use both hydrogenative parahydrogen induced polarization (PHIP) and SABRE to attain polarization levels that exceed the RASER threshold to yield everlasting NMR signal associated with unprecedented precision.

Results

(A) In vivo hyperpolarized NMR and MRI are enabled to observe metabolic conversion of pyruvate to alanine, lactate and bicarbonate in liver and kidney at 1.5 T magnetic fields. In phantom hyperpolarized MRI of 13C pyruvate is shown all the way down to 120 uT.

(B) parahydrogen pumped RASER NMR is demonstrated for both 1H and 13C systems showcasing more than 100-fold improvements in resolution over conventional NMR while retaining the critical J-coupling and chemical shift information.

Conclusion

This contribution illustrates how detailed, fundamental spin physics and chemistry can be translated to major advances, with a keen eye on making modern technology broadly available and providing affordable tools that give the most precise information possible.

HYP-021: High-throughput Hyperpolarization with Bullet-DNP

Dr Masoud Minaei[1], Dr Kajum Safiullin[1], Marie Harder[2], Dmitrii Zasukhin[1], Prof. Dr. Arno Kentgens[2], Dr Karel Kouril[1], Dr Benno Meier[1]
[1]Karlsruhe Institute Of Technology, Eggenstein-Leopoldshafen, Germany, [2]Radboud University, Nijmegen, The Netherlands

Nuclear spins may be polarized to near unity polarization in solids by transferring polarization from free electron spins to nuclear spins using dynamic nuclear polarization (DNP). In dissolution-DNP (D-DNP) and bullet-DNP (B-DNP), the sample is subsequently liquefied and spectra with highly enhanced signals may be recorded. The rate at which these signals may be recorded however has thus far been limited by long buildup times and the need for manual sample insertion into the polarizer. Here we show that the throughput of the method can be increased considerably through full automation of the experiment, including sample preparation, polarization, detection and cleaning stages. Moreover, by using a microfluidic stripline probe, multiple spectra maybe recorded in series using a single bolus of hyperpolarized solution, facilitating the acquisition of data of higher dimensionality.

HYP-022: Solution to the limiting effect of distant dipolar field in ParaHydrogen-Induced Polarization

Phd Laurynas Dagys[1,2], PhD Martin C. Korzeczek[3], PhD Anna J. Parker[1], PhD James Eills[1,4], PhD John W. Blanchard[1,5], PhD Christian Bengs[6,7], Prof. Maclolm H. Levitt[6], PhD Stephan Knecht[1], PhD Ilai Schwartz[1], Prof. Martin B. Plenio[3]
[1]NVision Imaging Technologies, Ulm, Germany, [2]Vilnius University, Vilnius, Lithuania, [3]Universität Ulm, Ulm, Germany, [4]Institute of Bioengineering of Catalonia, Barcelona, Spain, [5]University of Maryland, College Park, USA, [6]University of Southampton, Shouthampton, United Kingdom, [7]University of California, Berkeley, USA

Introduction

ParaHydrogen-Induced Polarization (PHIP) is an efficient route to achieve hyperpolarization and to overcome the challenges imposed by the limited sensitivity of NMR spectroscopy. The process involves a chemical reaction between a target molecule and parahydrogen gas with high nuclear singlet spin order which can be transformed into magnetization of a designated nucleus. Although the singlet-to-magnetization polarization transfer process works effectively at moderate concentrations, it is observed to become much less efficient at high product concentrations. We associate this limit with interference from a distant dipolar field produced by strongly polarized nuclear spins and demonstrate a method to mitigate this effect.

Methods

Polarization transfer from singlet order to magnetization is performed at low magnetic field by adiabatic Spin-Lock Induced Crossing (adSLIC) protocol. We also combine this sequence with Lee-Goldburg (LG) decoupling to average direct dipolar interactions between the polarized spins. These techniques are applied on (1-^{13}C,d_6)-dimethyl maleate (DMM) which is produced by reacting (1-^{13}C,d_6)-dimethyl acetylenedicarboxylate with parahydrogen in acetone-d_6.

Results

Experiments involving LG decoupling combined with adSLIC protocol illustrate that suppression of the dipolar field alleviates its effect and successful implementation results in 0.45 mol/L molar ^{1}H polarization in organic solution of DMM. This displays a striking boost to molar polarization over 0.06 mol/L achieved using the unmodified adSLIC sequence and opens promising prospects for the further PHIP utilization with new hyperpolarization methodologies.

Conclusions

Our work highlights that further improvements in hyperpolarization can lead to circumstances where NMR pulse sequences can be disrupted by high internal sample magnetization. Sequences which incorporate averaging of the dipolar interaction can help to reduce this phenomenon and may become increasingly relevant.

HYP-023: Pairwise Addition of Parahydrogen to Triple Bonds Leads to Single-Compound Multimodal RASER

Ivan Trofimov[1,2], Dr. Oleg Salnikov[2], Dr. Andrey Pravdivtsev[3], Henri de Maissin[1,4], Anna Yi[2,5], Prof. Eduard Chekmenev[6,7], Prof. Jan-Bernd Hövener[3], Dr. Andreas Schmidt[1,4,6], Prof. Igor Koptyug[2]
[1]Division of Medical Physics, Department of Diagnostic and Interventional Radiology University Medical Center Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany, [2]International Tomography Center SB RAS, Novosibirsk, Russia, [3]Section Biomedical Imaging, Molecular Imaging North Competence Center (MOIN CC), Department of Radiology and Neuroradiology, University Medical Center Schleswig-Holstein and Kiel University, Kiel, Germany, [4]German Cancer Consortium (DKTK), partner site Freiburg and German Cancer Research Center (DKFZ), Heidelberg, Germany, [5]Novosibirsk State University, Novosibirsk, Russia, [6]Department of Chemistry, Integrative Biosciences (Ibio), Karmanos Cancer Institute (KCI), Wayne State University, Detroit, United States, [7]Russian Academy of Sciences, Moscow, Russia

Radio Amplification by Stimulated Emission of Radiation (RASER) is a phenomenon observed during NMR acquisition of strongly negatively polarized samples. Pairwise addition of parahydrogen to unsaturated precursors in PHIP (ParaHydrogen-Induced Polarization) experiments has been shown to yield RASER of the introduced protons in the products. RASER has garnered interest in the NMR community because sensing very narrow NMR signals and chemical transformations has become possible without application of excitation pulses. However, there is a knowledge gap in PHIP RASER of complex spin systems. Here we aim to investigate the RASER of molecules containing allylic and methyl-substituted allylic spin systems, which have not been studied in this regard before. Allyl alcohol, 3-buten-2-ol, 2-methyl-3-buten-2-ol, and allyl pyruvate were hyperpolarized at high concentrations (200-600 mM) at high (PASADENA) or Earth's (ALTADENA) magnetic field and subsequently ^{1}H RASER signals were recorded in the bore of a 300 MHz NMR spectrometer without application of excitation pulses. In both PASADENA and ALTADENA experiments, strong ^{1}H RASER signals were observed in all investigated molecules – up to 3 different types of protons contributed to the RASER signals simultaneously. Spectral-temporal analysis of the RASER signals unveiled RASER activity of non-parahydrogen-nascent protons, indicating polarization transfer. In both PASADENA and ALTADENA conditions, PRINOE (Parahydrogen and RASER-Induced NOE) effect was the key route of polarization transfer. In case of ALTADENA experiments, initial distribution of polarization via J-coupling networks led to RASER-activity of protons not observed in PASADENA RASER signals. Acquired RASER signals lasted for 1.5-2.5 minutes without active parahydrogen pumping. PRINOE effects were also observed when other analytes were added to the sample containing hyperpolarized allylic compounds enhancing their signal by up to 45 times. This study unveils the complex interplay of ½-spins producing multimodal RASER. PHIP RASER was shown possible for non-parahydrogen-nascent protons.

HYP-024: Overcoming the challenges of hyperpolarizing substrates with parahydrogen-induced polarization in an MRI system

Henri de Maissin[1,2], Vladislav Ivantaev[1], Obaid Mohuiddin[1], Prod. Dr. Valerij Kiselev[1], Prof. Dr. Dominik von Elverfeldt[1], Prof. Dr. Maxim Zaitsev[1], Dr. Andreas Schmidt[1]
[1]Division of Medical Physics, Department of Radiology, University Medical Center Freiburg, Freiburg, Germany, Freiburg im Breisgau, Germany, [2]German Cancer Consortium (DKTK), partner site Freiburg, German Cancer Research Center (DKFZ), Heidelberg, Germany

Introduction
Parahydrogen-induced polarization (PHIP) is a promising route to fast and cost-effective hyperpolarization of biomolecules. RF-induced spin-order transfer (SOT) sequences inside a spectrometer have demonstrated extremely high ^{13}C polarization (55 %). If the polarization process could be performed in the same MRI system where the subject will receive the hyperpolarized agent, it could reduce costs and minimize polarization loss during transfer. Nevertheless, MRI bores often lack field homogeneity and radiofrequency field strength. Korchak et al. introduced a modification to the ESOTHERIC pulse sequence, exploiting pulsed field gradients and multiple composite refocusing pulses to mitigate radiation damping.
Aims
Our study aims to explore why the modified ESOTHERIC scheme is more robust and assess whether its implementation on a preclinical MRI could achieve similar polarization levels as seen in a spectrometer.
Methods
We evaluated the benefit of using the modified SOT when performed simultaneously with molecular motion in an inhomogeneous magnetic field, using the density matrix formalism. The impact of using multiple composite refocusing pulses with miscalibrated RF pulses was simulated. Deuterated vinyl acetate (50 mM) polarization experiments were conducted within the bore of a 7 T preclinical MRI (Bruker) using various modifications of ESOTHERIC sequence.
Results
Simulations indicated that, while the multiple refocusing pulses are needed to alleviate the effects of molecular motion in an inhomogeneous B_0 field, the composite pulses are necessary to mitigate the flip angle error accumulation. Polarization experiments not only validated the simulated results but also led to the remarkable ^{13}C polarization of 44.5 % for ethyl acetate in under 13 seconds.
Conclusion
The modified ESOTHERIC sequence was simulated in silico and the results were validated by experimental evidence leading to high ^{13}C polarization. This outcome may enable a novel approach to the generation of hyperpolarized agents without the need for a dedicated polarizer.

HYP-025: 1H and 15N hyperpolarization of a nitroimidazole derivative in under 15 seconds using hydrogenative PHIP

Henri de Maissin[1,2], PhD Adriana Sacristán-Martín[3], Ivan Trofimov[1], Juanita Ferreira[3], Prof. Dr. Maxim Zaitsev[1], Prof. Dr. Dominik von Elverfeldt[1], Michael Keim[4], Prof. Dr. Max von Delius[3], Dr. Andreas Schmidt[1]
[1]Division of Medical Physics, Department of Radiology, University Medical Center Freiburg, Freiburg, Germany, Freiburg im Breisgau, Germany, [2]German Cancer Consortium (DKTK), partner site Freiburg, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, Heidelberg 69120, Germany, Heidelberg, Germany, [3]Insitute of Organic Chemistry, Ulm University, Albert-Einstein-Allee 11, 89081, Germany, Ulm, Germany, [4]NVision Imaging Technologies GmbH, 89081 Ulm, Germany, Ulm, Germany

Introduction
The ongoing quest to efficiently polarize ^{15}N-labeled biomolecules is motivated by several factors: isotopic ^{15}N labeling of endogenous nitrogen sites enables nuclear spin hyperpolarization of biomolecules, of which certain sites are known to feature long relaxation times. Hyperpolarization offers means to overcome the inherently low gyromagnetic ratio of ^{15}N. Although the polarization of metronidazole, an FDA-approved drug, was demonstrated with dDNP and SABRE, these methods suffer from long build-up and activation time, respectively. PHIP hyperpolarization of derivatives of nitroimidazole has not been attempted to date and may enable non-invasive probing of hypoxic metabolism.
Aims
In this work, we investigate hydrogenative parahydrogen-induced polarization (PHIP) of custom-synthesized precursor 2-methyl-4-nitro-1-(prop-2-en-1-yl)-imidazole, ^{2}H and ^{15}N labeled, and explore rapid polarization transfer to ^{15}N.
Methods
PHIP precursor (50 mM) was hydrogenated in the presence of a homogeneous Rh-based catalyst (5 mM) in acetone-d_6 under PASADENA conditions (400 MHz). The experiment was repeated for different hydrogenation durations with 8 bar parahydrogen and 80 °C. Polarization transfer to the ^{15}N nucleus was executed in three steps: hydrogenation in tens of mT, magnetic field cycling (MFC) in a µ-metal shield, and transfer to the spectrometer for ^{15}N signal acquisition. Para-enrichment of hydrogen was approximately 50%.
Results
From the hydrogenation curve of the precursor was determined the rate of hydrogenation of the probe (5.5±2.8 s), the relaxation time of parahydrogen-derived protons (57.5±9.4 s), and the maximum proton polarization obtained (1.3 %). MFC yielded 0.9 % hyperpolarization on one ^{15}N site.
Conclusion
The probe features a very fast hydrogenation even at 50 mM concentration and a relatively long proton polarization lifetime. Higher parahydrogen fraction and pulsed polarization transfer methods may improve ^{15}N polarizations. In this contribution, we demonstrate the fast (<15s) polarization of a ^{15}N-labeled nitroimidazole derivative, a potential biomedical probe for hypoxia.

HYP-026: Direct detection of 10 MHz 1H NMR spectra of hidden spins using DNP @ 1.4 K

Dr Zhenfeng Pang[1], Dr Kirill Sheberstov[1], Dr Bogdan Rodin[1], Jake Lumsden[1], Dr Utsab Banerjee[1], Dr Daniel Abergel[1], Prof Geoffrey Bodenhausen[1], Prof Kong-Ooi Tan[1]
[1]Laboratoire des Biomolécules, LBM, Département de Chimie, École Normale Supérieure, PSL University, Sorbonne Université, CNRS, Paris, France

Introduction
Paramagnetic species widely exist and play crucial roles in catalysts, semiconductors, batteries, and polarizing agents in dynamic nuclear polarization (DNP). Due to the hyperfine-broadened linewidths or enhanced relaxation rates, in NMR spectroscopy the nuclei close to unpaired electrons are almost unobservable using conventional NMR, and, hence, they are also referred to as hidden spins. Here, we present the direct NMR observation of these hidden spins in TEMPOL-doped samples using DNP at 1.4 K.

Methods
We first characterized the hidden spins by introducing a new pulse sequence known as spin diffusion enhanced saturation transfer (DEST), inspired by the CEST experiment. Then, we directly acquired the NMR signals across the 10 MHz tuning range of the RF probe. Finally, a 2D NOESY-like experiment was performed to directly quantify the spin diffusion rate between the hidden spins and the bulk protons.

Results
Both DEST and directly observed NMR spectrum of the hidden spins yield very broad profiles (> 5 MHz), and the profiles were confirmed by simulated NMR spectrum of hyperfine-coupled protons in TEMPOL. The 2D NOESY-like experiment confirmed polarization transfer between the hidden spins and the bulk protons separated by ~ MHz frequency difference.

Conclusions
We have directly observed the protons in TEMPOL using a homebuilt 6.7 T DNP-NMR spectrometer at 1.4 K. Direct observation of spin diffusion between the hidden spins and bulk protons proves that the hidden spins shifted by ~MHz away are outside the spin diffusion barrier. We anticipate that this methodology could evolve into a novel form of spectroscopy—DNP-enhanced paramagnetic NMR—potentially leading to the development of more efficient DNP polarizing agents or a deeper understanding of chemical moieties in paramagnetic systems.

References
[1] Stern et al., Sci. Adv. 2021; 7: eabf5735
[2] King et al., Phys. Rev. Lett. 1972; 28: 1099–1102

HYP-027: Hyperpolarization mechanisms in a 1 T benchtop DNP polarizer

Mr Ewoud Vaneeckhaute[1], Mrs Charlotte Bocquelet[1], Mr Nathan Rougier[1], Mrs Shebha Anandhi Jegadeesan[2], Mr Sanjay Vinod-Kumar[2], Mrs Guinevere Mathies[2], Mr Roberto Melzi[3], Mr Daniel Banks[4], Mr James Kempf[4], Mr Quentin Stern[1], Mr Sami Jannin[1]
[1]CRMN, Universite Claude Bernard Lyon 1, Villeurbanne, France, [2]Department of Chemistry, University of Konstanz, Konstanz, Germany, [3]Bruker Biospin Italia, Milano, Italy, [4]Bruker Biospin, Billerica, United States

Boosting nuclear magnetic resonance (NMR) sensitivity in harmony with its general applicability, high resolution and non-destructiveness remains a major scientific challenge. Dissolution dynamic nuclear polarization (dDNP) emerged as a front runner in the development towards tackling this challenge. Despite this advancement, dDNP implies the use of complex cryogenic and high-field instrumentation which is not only costly but remains an intrinsically destructive operational tool and thus irreversible from a practical point of view. [1]

A way to expand the application horizon of dDNP can therefore be to find a recyclable DNP alternative. To pursue this ambitious goal, we introduced the concept of recyclable hyperpolarized flow (HypFlow) DNP which comprises a closed loop cycle where first hyperpolarization happens in a compact benchtop DNP polarizer at a magnetic field of 1 T and a temperature of 77 K. With enhancement factors of more than 100 in proton, hyperpolarization in frozen solution has been shown to be effective in such a benchtop polarizer. However, to optimize DNP even further, a closer look into which polarization transfer mechanisms are dominant at these conditions is essential. [2]

Here, we present a solid-state DNP study comparing the hyperpolarization performances of TEMPOL and TRITYL radicals on a partially protonated frozen solution measured in the 1 T benchtop DNP polarizer. Specifically, experimental DNP spectra, buildup rates, and nuclear spin relaxation rates were measured and the properties of the unpaired electrons of TEMPOL were more closely investigated using Q-band EPR. From this, we were able to derive that DNP is dominated by solid effect for TRITYL radicals and cross effect for TEMPOL radicals under HypFlow DNP conditions.

[1] Ardenkjaer-Larsen, J. H. et al. Proc. Natl. Acad. Sci. 100, 10158–10163 (2003)
[2] Bocquelet, C. et al. https://doi.org/10.26434/chemrxiv-2024-3nlnd (2024)

HYP-028: NMR Detection of Picomoles of Pyruvate at Nanomolar Concentrations Using Bullet-DNP

Pooja Pooja[1], Nils Lorz[2], Dr. Masoud Minaei[1], Dr. Sami Jannin[3], Dr. Karel Kouřil[1], Dr. Alvar Gossert[2], Dr. Benno Meier[1,4]
[1]Institute For Biological Interfaces 4, Karlsruhe Institute Of Technology, 76344 Eggenstein-Leopoldshafen, Germany, [2]Institute of Molecular Biology and Biophysics, ETH Zurich, 8093 Zürich, Switzerland , [3]Centre de Résonance Magnétique Nucléaire à Très Hauts Champs – FRE 2034 Université de Lyon / CNRS / Université Claude , Lyon, France, [4]Institute of Physical Chemistry, Karlsruher Institut Für Technologie, Karlsruhe, Germany

In bullet DNP, a sample is hyperpolarized at cryogenic temperatures and rapidly transferred to a second magnet where it is dissolved and liquid-state nuclear magnetic resonance (NMR) spectra are recorded [1]. We have recently reported 13C spectra with polarization levels of approximately 30 % with a resolution of 2 Hz, and less than 10-fold dilution [2]. Hyperpolarized pyruvate is a widely used marker to track metabolism in vivo, and a benchmark molecule for hyperpolarization methods. Here, we show how a combination of improved bullet-DNP instrumentation, optimized sample preparation, and a further sensitivity increase via a 13C-1H polarization transfer after dissolution enables the observation of pyruvate at a concentration of 250nM immediately after dissolution. The experiment exhibits excellent mass sensitivity employing a total mass of pyruvate of only 8 ng, or 70 pmol. This high sensitivity is possible because the solvent volume in bullet-DNP can be chosen to match the detector volume such that most of the hyperpolarized material is observed in the detection. If the concentration of pyruvate is increased to 45 µM, pyruvate may be detected one minute after dissolution with a signal-to-noise ratio exceeding 50. The procedure can be used for studying protein-ligand interactions at nanomolar protein concentrations.

References :

[1] Kouřil, K., Kouřilová, H., Bartram, S., Levitt, M. H., and Meier, B.: Scalable Dissolution-Dynamic Nuclear Polarization with Rapid Transfer of a Polarized Solid, Nat. Commun., 10, 1733, https://doi.org/10.1038/s41467-019-09726-5, 2019.
[2] Kouřil, K., Gramberg, M., Jurkutat, M., Kouřilová, H., Meier, B.: A cryogen-free, semi-automated apparatus for bullet-dynamic nuclear polarization with improved resolution, Magnetic Resonance, 2, 815, https://doi.org/10.5194/mr-2-815-2021, 2021.

HYP-029: Imaging strategies for hyperpolarized contrast agents within the ultralow-field regime

Kai Buckenmaier[1], Friedemann Bullinger[1], Nicolas Kempf[1], Richard Neumann[1], Pavel Povolni[1], Jörn Engelmann[1], Klaus Scheffler[1,2], Felix Mysegaes[3], Charbel Assaf[4], John Z. Myers[5], Adam Ortmeier[6], Rainer Körber[5], Philipp Pohlmann[7], Andre F. Martins[7], Thomas Theis[6], Andrey N. Pravdivtsev[4], Markus Plaumann[3]
[1]High-Field Magnetic Resonance Center, Max Planck Institute for Biological Cybernetics, Tuebingen, Germany, [2]Departement of Biomedical Magnetic Resonance, Eberhard-Karls University, Tuebingen, Germany, [3]Institute for Molecular Biology and Medicinal Chemistry, Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany, [4]Section Biomedical Imaging, Molecular Imaging North Competence Center (MOIN CC), Department of Radiology and Neuroradiology, University Medical Center, Kiel University, Kiel, Germany, [5]Physikalisch-Technische Bundesanstalt, Berlin, Germany, [6]Departement of Chemistry and Physics, NC State University, Raleigh, USA, [7]Werner Siemens Imaging Center and Cluster of Excellence iFIT (EXC 2180) “Image-Guided and Functionally Instructed Tumor Therapies”, Eberhard Karls University Tuebingen, Tübingen, Germany

Introduction:
Nuclear spin hyperpolarization dramatically increases the sensitivity of magnetic resonance, enabling many new applications, including real-time metabolic imaging. Importantly, this enhancement renders signal intensity independent of field strength, enabling in vivo MRI applications in the ultra-low field regime. Parahydrogen-based signal amplification by reversible exchange (SABRE) and Overhauser dynamic nuclear polarization (ODNP) are two methods that allow continuous hyperpolarization generation and can be combined with a superconducting quantum interference device (SQUID)-based MRI system.[1]
Aims:
Our goal is to develop efficient imaging techniques tailored for hyperpolarized contrast agents in the ultralow-field regime.
Methods:
Two different techniques were used for hyperpolarization: SABRE and ODNP.
For SABRE, the sample was hyperpolarized before each k-space line was acquired. Readout was performed with a simple spin-echo sequence.
For ODNP, we modified a bSSFP sequence by adding two 180-degree pulses to eliminate the usual banding artefacts. The sample was rehyperpolarized during phase encoding.
Results:
In this study, 13C MRI at 120 µT of hyperpolarized [1-13C]pyruvate under ULF conditions was demonstrated for the first time.[2]
In addition, we used a modified bSSFP sequence to efficiently hyperpolarize the sample during phase encoding and to speed up the imaging process itself.
Conclusions
Although this study demonstrated the feasibility of ultralow field 13C imaging with SABRE, in vivo application remains impractical due to slow imaging speed. However, replacing the readout with a more efficient turbo-spin-echo or bSSFP readout could significantly increase the imaging speed.
A modified bSSFP sequence combined with ODNP accelerated imaging compared to spin echo readout with no observed banding artefacts.

Literature:
[1] K. Buckenmaier et al., Rev. Sci. Instrum. 2018, 89, 125103.
[2] N. Kempf et al., Sci. Rep. 2024, 14, 4468.

HYP-030: Understanding 129Xe DNP at 1.2K/5T using ELDOR and multinuclear solid-state NMR

Dr Jean-noël Hyacinthe[1], PhD Thanh Phong Lê[1], Prof Andrea Capozzi[1,2]
[1]EPFL, Lausanne, Switzerland, [2]DTU, Lyngby, Denmark

Introduction:

Hyperpolarized ^{129}Xe is a powerful MRI/NMR probe. The gold standard spin exchange optical pumping performance is, as of today, unmatched. Nevertheless, the intrinsic dDNP versatility may allow a multi-nuclei hyperpolarization platform, and xenon is an interesting system to study DNP mechanisms. Interestingly, despite very close gyromagnetic ratios, polarization enhancement obtained for ^{129}Xe have been limited when compared to ^{13}C.

Aims:

To better characterize the DNP process at play in typical xenon samples and dDNP experimental conditions and to understand whether ^{129}Xe only is affected or also the other nuclei in the system, we implement ELDOR spectroscopy and multinuclear (^{1}H/^{13}C/^{129}Xe) solid-state NMR.

Methods:

We used a purpose-built reusable fluid path for the whole procedure. The sample was prepared by incorporating natural xenon in isobutanol doped with 33mM TEMPO. [1,^{13}C]- d5-Ethanol was previously added to the solvent to obtain the same final stoichiometric ratio than ^{129}Xe. Admixture of natural xenon to the radical-doped glassing solvent takes place directly in the sample cup. The latter is sonicated in a bath of melting ethanol until complete dissolution of the gas. ELDOR spectroscopy and multinuclear solid-state NMR were performed during DNP at 5T and 1.2K.

Results:

ELDOR spectra suggested very different spectral diffusion behavior when compared to typical TEMPO-doped- (e.g. water/glycerol) or trityl-doped-matrices. We confirmed earlier demonstrations of very short radical $T_{1}e$. DNP sweeps were similar for ^{13}C and ^{129}Xe nuclei, but not for ^{1}H. Microwave frequency modulation was beneficial to improve the enhancement of all nuclei, but with different gains.

Conclusions:

Those preliminary results using ELDOR spectroscopy and multinuclear solid-state NMR of xenon matrices at dDNP operating conditions shed new light on the involved DNP mechanism, opening potential performance improvement strategies.

HYP-031: Hyperpolarizing Small Molecules using Parahydrogen and Solid-State Spin Diffusion

Mr Martin Gierse[1], Dr Laurynas Dagys[1], Dr Michael Keim[1], Dr Sebastian Lucas[1], Dr Felix Josten, Prof Martin B. Plenio[2], Dr Ilai Schwartz[1], Dr Stephan Knecht[1], Dr James Eills[1]
[1]NVision Imaging Technologies, Ulm, Germany, [2]Institute for Theoretical Physics, Universität Ulm, Ulm, Germany

Introduction
Parahydrogen-induced polarization (PHIP) offers a fast and low-cost approach to boost the NMR signals of molecules and materials by many orders of magnitude. PHIP requires chemical reactions or interactions with molecular H2, which strongly limits what targets can be polarized. To make hyperpolarization technology more widely accessible, we aim to keep the experimental simplicity of PHIP, but make it possible to polarize arbitrary molecules. We introduce a new approach named PHIP-SSD (PHIP with solid-state spin diffusion), in which we highly polarize a source molecule via PHIP, then precipitate it out of solution with target molecules. Spin diffusion in the solid state carries 13C polarization from the source molecules to the target molecules.

Methods
We polarize [1-13C]fumarate via PHIP, obtaining approximately 200 mM concentration at 40% 13C polarization. We add a molecular target to the solution, such as [13C4]-fumarate or [1-13C]-benzoic acid, then acidify the solution to precipitate out both the source and target molecules. This is done in a 100 mT magnetic field, and we allow a short waiting period (5-90 s) for spin diffusion. The solids are then redissolved in aqueous solution and we detect the hyperpolarized 13C signals in a benchtop NMR spectrometer.

Results
We hyperpolarized the molecular targets [1-13C]-acetylene dicarboxylate, [13C4]-fumarate, and [1-13C]-benzoic acid using PHIP-SSD. We carried out a series of experiments, and achieved 13C signal enhancements on [1-13C]-benzoic acid of between 50 and 17000 at 1.9 T, depending on the molar ratio of fumarate:benzoate in the solid state. This corresponds to up to 3% 13C polarization on the target.

Conclusions
PHIP-SSD is a new approach to hyperpolarization, combining the simplicity of PHIP with the generality of spin diffusion as a means to spread polarization from source to target molecules. These experiments are reproducible and can be repeated on a timescale of a few minutes.

HYP-033: Solvents effects in SABRE-based nuclear spin hyperpolarization

Prof. Dr. Markus Plaumann[1], Dr. Felix Mysegaes[1], Till Houben[1], John Z. Myers[2], Friedemann Bullinger[3], Nicolas Kempf[3], Charbel D. Assaf[4], Prof. Dr. Dr. Johannes Bernarding[5], Dr. Rainer Körber[2], Dr. Andrey N. Pravdivtsev[4], Dr. Kai Buckenmaier[3]
[1]Otto-von-Guericke-University, Medical Faculty, Institute of Molecular Biology and Medicinal Chemistry, Magdeburg, Germany, [2]Physikalisch-Technische Bundesanstalt, Department 'Biosignals', Berlin, Germany, [3]Max Planck Institute for Biological Cybernetics, High-Field Magnetic Resonance Center, Tübingen, Germany, [4]University Medical Center Kiel, Section Biomedical Imaging, Molecular Imaging North Competence Center (MOIN CC), Kiel, Germany, [5]Otto-von-Guericke-University, Medical Faculty, Institute of Biometry and Medical Informatics, Magdeburg, Germany

Introduction
One of the most promising signal enhancement techniques with respect to future biomedical applications is parahydrogen-based nuclear spin hyperpolarization.[1] In particular, the method Signal Amplification by Reversible Exchange (SABRE) allows for reproducible hyperpolarization.[2] Here, we investigate the effect of solvent on SABRE.

Aims
The solvent and co-solvent dependency of hyperpolarization is an important aspect for understanding the mechanism and estimation of achievable signal enhancement. We have an aim to find a most suitable solvent for hyperpolarization and subsequent two-phase separation of hyperpolarized media.

Methods
^{1}H and ^{19}F hyperpolarization were performed on 3-fluoropyridine. Acquisition was on a Bruker wide bore 7T NMR spectrometer. 2mL solvent (methanol, chloroform, toluene, or xylene) were added into a 10mm NMR tube. Around 1.7mg of non-activated Ir-IMes complex was dissolved with 4µL of 3-fluoropyridine. Both catalyst activation and ^{19}F signal amplification were investigated, using 50% enriched parahydrogen (para-H_2) at 6 bar pressure shook with the sample for 10s at 6mT.

Results
Hyperpolarization in methanol, chloroform and toluene can already be detected after the first introduction of para-H_2. Solvent molecules interacting with the Ir cation influence the coupling system between 3-fluoropyridine and hydrides which leads to a positive phase of the enhanced ^{19}F signal.
In pure xylene no signal enhancement was detected. Directly after addition of 500µL methanol to xylene solution, no hyperpolarization was observable. However, after waiting several hours a clear ^{19}F signal enhancement with negative phase was measured.

Conclusions
The solvent has a significant impact on SABRE reactions. Phase and enhancement of the detected signal depend on the specific coupling system. Steric reasons prevent direct hyperpolarization in pure xylene.

Literature
[1] Hövener J.-B. et al. 'Parahydrogen-Based Hyperpolarization for Biomedicine', Angew. Chem. Int. Ed., 2018, 57:11140-11162.
[2] Adams R.W. et al. 'Reversible Interactions with para-Hydrogen Enhance NMR Sensitivity by Polarization Transfer', Science, 2009, 323(5922):1708-1711.

HYP-034: Hyperpolarization of Long-Lived States of Protons in Short Aliphatic Chains by Bullet Dissolution Dynamic Nuclear Polarization

Aiky Razanahoera[1,2,3], Anna Sonnefeld[1,2,3], Dr. Kirill Sheberstov[1,2,3], Prof. Geoffrey Bodenhausen[1,2,3], Pooja Narwal[4], Dr. Masoud Minaei[4], Dr. Karel Kouřil[4], Dr. Benno Meier[4]
[1]École Normale Supérieure, Paris, France, [2]PSL University, Paris, France, [3]Sorbonne Université, Paris, France, [4]Karlsruhe Institute of Technology, Karlsruhe, Germany

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, ^{1}H 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 LLS lifetimes 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.

HYP-035: Structural investigation of lipid nanoparticles by relayed Dynamic Nuclear Polarization

Adrienn Rancz[1], Judith Schlagnitweit[1], Landry Gayet[1], Frédéric Ronzon[2], Loïc Salmon[1], David Gajan[1], Guido Pintacuda[1], Anne Lesage[1]
[1]Centre de RMN à Très Hauts Champs, Université de Lyon (CNRS/ENS Lyon/UCBL), Villeurbanne, France, [2]Sanofi Pasteur, Marcy l'Étoile, France

Lipid nanoparticles (LNPs) have recently emerged as the most advanced and effective vehicles to deliver mRNA in cells, as demonstrated with the development of vaccines against SARS-CoV-2. However, due to the wide panel of lipid compositions in use and the complexity of the LNP self-assembly process itself, the architecture of mRNA-loaded LNPs is still poorly understood. In a recent contribution, Viger-Gravel et al demonstrated that the structure of LNPs could be probed by relayed Dynamic Nuclear Polarization (R-DNP) experiments in solid state.[1] In this approach, the hyperpolarization produced by exogenous dinitroxides in the bulk solution surrounding the particles is transported within the radical-free LNPs through proton spin diffusion.[2] This enables the identification of the spatial distribution of the different components based on the evolution of the proton DNP enhancement factors (εH) as a function of polarization times. Leveraging this pioneer study, we investigate the molecular and nanostructure of mRNA-loaded LNPs of pharmaceutically-relevant composition and concentration.

DNP enhanced ^{13}C cross-polarization (CP) spectra of LNPs in frozen solutions were acquired at 9.4 T, 10-12.5 kHz Magic Angle Spinning (MAS) and 100 K. The CPMAS spectra were assigned from the comparison with spectra recorded on individual lipid or mRNA components. Enhancement values and polarisation build-up times were measured in the LNP mixture for each of these components, allowing one to elucidate their distribution with respect to the particle surface. The low DNP enhancement of the mRNA resonances suggests that the mRNA strand is located in the inside of the nanoparticle. Structural changes were also observed as a function of LNP concentration.

1. J. Viger-Gravel et al., J. Phys. Chem. B, 2018, 122, 2073-2081.
2. A. C. Pinon et al., J. Phys. Chem., 2017, 121, 29, 15993–16005.

HYP-037: Monitoring and analysis of complex mixed chemical and enzymatic reaction pathways: challenges and solutions

Second Year Phd Student At Ens Paris Mehdi Soussi-Therond[1], First year PhD student Léa Gutierrez[2], Professor Nicolas Giraud[2], Maître de conférence Emeric Miclet[3], Research engineer Mathieu Baudin[1,2], Professor Daniel Abergel[1]
[1]Ecole Normale Superieure, PARIS, France, [2]Université Paris-Cité, Paris, France, [3]Sorbonne-Université, Paris, France

Glucose metabolism is known to be affected in various pathological contexts, including tumors, where cell proliferation is accompanied by an increased glycolysis activity (Warburg effect). This deregulation is often associated with the stimulation of the oxidative stage of the pentose phosphate pathway (PPP), thus increasing the production of NADPH, the main source of reducing power in the cell. The PPP plays a key role in the response to oxidative stress, a well-known factor involved in oncogenesis. Using dissolution Dynamic Nuclear Polarization (D-DNP), [1] we investigated in-vitro kinetics of key enzymes enabling the production of NADPH: Glucose-6-Phosphate dehydrogenase (G6PDH) and 6-Phosphogluconic dehydrogenase (6PGDH). The sensitivity gains provided by D-DNP allow to monitor enzymatic reactions with sufficient signal-to-noise ratio to achieve a time resolution limited by the acquisition time (~500 ms), compatible with near-physiological conditions.

The study of these reactions and their pathways raised complex challenges. First, achieving the reproducibility necessary to extract reliable quantitative information. Then, obtaining a sound kinetic model to interpret these time-resolved kinetic experiments to get meaningful kinetic parameter values. Thus, first order kinetic models systematically used in D-DNP experiments become questionable.

Using these enzymatic systems, we illustrate the prospects and limitations of D-DNP enzyme kinetics. We have used G6PDH as a model system for a complex reaction pathway, as its substrate undergoes fast mutarotation interfering with the oxidation reaction. We were able to disentangle both reactions in our experiments using enzymatically produced doubly labeled [13C, 2H]-G6P. Our strategy also allowed us to provide the first direct and quantitative description of the substrate selectivity of G6PDH towards G6P. This study was complemented by kinetic studies of 6PGDH.

[1] Ardenkjær-Larsen et al., PNAS (2003), 100, 10158-63
[2] Bornet et al. J. Phys. Chem. Lett. (2013) 4, 1, 111–114
[3] Sadet et al. Chem. Eur. J. (2018) 24, 5456-5461

HYP-038: Probing Branched-Chain Amino Transferase in Cancer Cells Using [1-^{13}C]-α-Ketoisocaproate Hyperpolarized via Reversible Exchange with Parahydrogen

Robert Willing[1,2], Phillip R. Groß[1,2], Henri de Maissin[1,2], Stefan Petersen[1], Prof. Dr. Leif Schröder[3,4], Prof. Dr. Maxim Zaitsev[1], Prof. Dr. Eduard Y. Chekmenev[5], Prof. Dr. Thomas Reinheckel[2,6], Dr. Stephan Knecht[7], Dr. Andreas B. Schmidt[1,2,5]
[1]Division of Medical Physics, Department of Radiology, University Medical Center Freiburg, Freiburg Im Breisgau, Germany, [2]German Cancer Consortium (DKTK), partner site Freiburg, German Cancer Research Center (DKFZ), Heidelberg, Germany, [3]Translational Molecular Imaging, DeutschesKrebsforschungszentrum (DKFZ), Heidelberg, Germany, [4]Molecular Imaging, Leibniz-Forschungsinstitutfür Molekulare Pharmakologie (FMP), Berlin, Germany, [5]Department of Chemistry, Integrative Biosciences (Ibio), Karmanos Cancer Institute (KCI), Wayne State University Detroit, Detroit, USA, [6]Institute of Molecular Medicine and Cell Research, Faculty of Medicine, University of Freiburg , Freiburg im Breisgau, Germany, [7]NVision Imaging Technologies GmbH , Ulm, Germany

Signal amplification by reversible exchange in shield enables alignment transfer to heteronuclei (SABRE-SHEATH) is an efficient para-Hydrogen (pH_2) based method to hyperpolarize α-keto acids such as pyruvate and α-ketoisocaproate (Kic). Especially Kic is metabolized by branched-chain amino acid aminotransferase (BCAT) to leucine (Leu) or β-hydroxy-β-methylbutyrate (HMB). Interestingly, an upregulation of BCAT has been linked to c-MYC signaling and metastatic cancer. Therefore, non-invasive imaging of BCAT activity could be of high clinical value.

This work aims for the purification of the SABRE-hyperpolarized Kic to extract hyperpolarized Kic in aqueous solution and its application to cancer cells.

30mM [1-^{13}C]-Kic was hyperpolarized via SABRE-SHEATH similar to the protocol reported by Adelabu et al. (90s polarization build up in presence of 5mM iridium pre-catalyst, 20mM dmso-d6 in 600µL methanol-d4 at 5°C). For Purification PBS in D_2O was added and the solution was evaporated by 12-s exposure to vacuum and a 96°C water bath at 100-mT. Precipitated catalyst was removed using a glass-microfiber filter with 0.45-µm pore size. Purified solution was injected into a 5-mm NMR tube containing 10^7 HeLa wild type cells suspended in ~500 µL medium kept at 37°C. NMR measurements were conducted using a 1T Magritek NMR spectrometer.

[1-^{13}C]-Kic was hyperpolarized to P(^{13}C)= ~3.7% using ~50% pH_2. After purification, 15mM Kic with P(^{13}C)=0.4% in ~240µL D_2O were extracted. Upon administration to the cells, ^{13}C-signals of Kic, and products Leu and HMB were observed in the NMR spectra.

SABRE-hyperpolarized Kic was successfully purified and applied to living cells for the first time. Polarization levels before and after purification can likely be improved using 100%-enriched pH_2, perdeuterated [1-^{13}C]-Kic and dedicated polarization transfer schemes. Successful observation of hyperpolarized downstream metabolites Leu and HMB already demonstrates utility of SABRE-hyperpolarized Kic for probing BCAT-activity in cancer in vivo, which we are currently preparing for in our lab.

HYP-040: Live magnetic observation of parahydrogen hyperpolarization dynamics

Dr James Eills[2], Ms Ariadna Gomez del Pulgar[1], Prof Morgan Mitchell[1], Dr Sven Bodenstedt[1], Dr Irene Marco Rius[2], Dr Michael Tayler[1]
[1]ICFO - The Institute Of Photonic Sciences, Castelldefels, Barcelona, España, [2]IBEC - Bioengineering Institute of Catalonia, Barcelona, España

INTRODUCTION
Hyperpolarized nuclear spins in molecules exhibit high magnetization that is unachievable by classical polarization techniques, making them widely used as sensors in physics, chemistry, and medicine, together with NMR or MRI for detection. Looking closer, we have identified "missed opportunities" to study hyperpolarized systems, arising from limits imposed by conventional NMR, namely: pulse-acquire approaches detecting transverse magnetization (1) necessarily involve (the partial) destruction of longitudinal magnetization, and (2) involve compromising spectral resolution between indirect (longitudinal) and direct (transverse) sampling dimensions.

AIM
Here we show that both of these limitations are bypassed by using direct, passive sensors of longitudinal magnetization, e.g. atomic magnetometers. These enable the complex dynamics of hyperpolarized materials to be studied without disturbing or interrupting the process.

RESULTS
As an example of dynamics that are impossible to detect `live' by conventional means, we examined parahydrogen-induced 1H and 13C magnetization during adiabatic eigenbasis transformations at µT-field avoided crossings in [1-13C]-fumarate. Magnetometry reveals previously unseen spin dynamics with ms time resolution, and informs about leakage mechanisms and fidelity limits. We also observe direct signatures of the "strong magnetization" regime, where magnetization back-action effects enter play, and must be accounted for or decoupled during magnetogenesis procedures. General application to a broader range of observation scenarios involving production, transport and systems-interaction of hyperpolarized compounds is discussed. The approach is particularly useful for monitoring "single-shot" samples like those destined for in vivo imaging with strict quality control requirements, or those exhibiting complex/chaotic spin dynamics.

As a second example, we apply atomic magnetometry during a variety of SABRE-SHEATH scenarios. We demonstrate feedback hyperpolarization of 13C spins in [1-13C]-pyruvate -- the most important spin tracer for clinical metabolic imaging -- where parahydrogen spin-order transfer pulse sequences (including optimal control sequences) can be adjusted in situ, on-the-fly, to maximize hyperpolarization yield.

HYP-041: Hyperpolarization Transfer Experiments for Liquid-state Overhauser Dynamic Nuclear Polarization

Dr Alex Van Der Ham[1], Dr Luming Yang[1], Prof Marina Bennati[1]
[1]Max Planck Institute, Göttingen, Germany

Liquid-state Overhauser-effect DNP (OEDNP) at high fields is emerging as a tool to enhance 13C NMR spectra by up to two orders of magnitude. In OEDNP, enhancements on nuclear sites tend to, however, be non-uniform; different sites within the same molecule show different degrees of signal increase or decrease. Moreover, different nuclei (1H, 19F, 13C etc.) show varying degrees of hyperpolarization, ultimately dependent on the exact balance of dipolar and scalar interactions between the target nucleus and polarizing agent. We use existing and are designing new NMR experiments, which transfer (hyper)polarization from nuclei that experience large DNP enhancements to those experiencing little direct enhancement or even signal attenuation. Here we present a variety of 1D and 2D NMR experiments that exploit sites of large OEDNP enhancement, to obtain structural information on small molecules. For example, isotropic mixing in combination with OEDNP, now allows us to resolve one-bond carbon–carbon scalar coupling constants (1JCC), at natural 13C abundance, in one tenth of the time, and requiring only one tenth of the amount of material, compared to a conventional 1D INADEQUATE experiment at thermal Boltzmann population. Overall, our work shows that Overhauser DNP has the potential to open up new avenues in liquid-state NMR, by making accessible experiments whose otherwise poor sensitivity, made them unattractive until now.

HYP-042: Application of machine learning to steady-state photo-CIDNP

Ms. Marta Stefańska[1], Dr. Thomas Müntener[1], Prof. Dr. Sebastian Hiller[1]
[1]Biozentrum, University Of Basel, Basel, Switzerland

Hyperpolarization such as photochemically induced dynamic nuclear polarization (photo-CIDNP) can help to overcome the low sensitivity inherent to Nuclear Magnetic Resonance (NMR) spectroscopy. The photo-CIDNP effect is based on radical pairs formed between a photosensitizer and the molecule of interest upon light irradiation, which results in signal enhancement due to the spin-sorting mechanism. The obtained degree of hyperpolarization is quantitatively well understood for time-resolved photo-CIDNP experiments. On the other hand, a quantitative interpretation of the gained polarization enhancement in steady-state photo-CIDNP experiments has not been established so far. Moreover, individual molecules have to be experimentally checked for their suitability in photo-CIDNP experiments as their performance is highly dependent on the interaction between the molecule and the dye.
In this work, we attempt to correlate the experimentally measured steady-state photo-CIDNP signal-to-noise enhancement (SNE) with calculated molecular properties. We calculated nucleophilicity, adiabatic ionization potential, Fukui indices, hyperfine interactions and Landé factors using density functional theory. The hydrophobicity of the molecule was taken into account through a predicted partition coefficient. The measurements and calculations were done for a series of indole derivatives with the following substituents: fluorine, methyl, hydroxy, methoxy, and amino group. We observed a huge variety in SNE depending on the substituent and its position. Moreover, the negative Fukui index turned out to be a great local indicator of the position with the highest SNE within a given molecule. No straightforward correlation was found between one single molecular property and the SNE, indicating that the enhancement depends on the interplay between multiple properties. Therefore, we employed various machine learning models for qualitative and quantitative predictions of the SNE based on the molecular properties. We identify the quantitatively best predicting models and based on their performance, we discuss the potential of such an approach for prediction of the steady-state photo-CIDNP effect.

HYP-043: Cocrystalline matrices for hyperpolarization at room temperature using photoexcited electrons

Dr. Munehiro Inukai[1], Mr. Haruki Sato[1], Dr. Koichiro Miyanishi[2,3], Dr. Makoto Negoro[3,4,7], Dr. Akinori Kagawa[3,7], Dr. Yuta Hori[5], Prof. Yasuteru Shigeta[5], Prof. Koichi Nakamura[1], Dr. Takuya Kurihara[6]
[1]Tokushima University, Tokushima, Japan, [2]Graduate School of Engineering Science, Osaka University, Osaka, Japan, [3]Center for Quantum Information and Quantum Biology, Osaka, Japan, [4]Institute for Quantum Life Science, National Institutes for Quantum and Radiological Science and Technology, Chiba, Japan, [5]Center for Computational Sciences, University of Tsukuba, Tsukuba, Japan, [6]Graduate School of Natural Science and Technology, Kanazawa 315 University, Kanazawa, Japan, [7]Premium Research Institute for Human Metaverse Medicine, Osaka, Japan

We present that cocrystals are utilized as polarization matrices for triplet-DNP at room temperature. We focused on solid-solutions of cocrystals and small amount of polarization source to dope polarization sources into cocrystals without the loss of crystallinity. Polarization source was able to be uniformly doped in cocrystals formed by acid-acid, amide-amide, and acid-amide synthons. Dense-packing crystal structures through multiple hydrogen boding and π- π interactions provide enough long T_1 relaxation time to diffuse polarization in the crystals. We also demonstrated the polarization of DNP-MRI molecular probe such as urea in cocrystal matrix at room temperature using Triplet-DNP.

HYP-044: Hyperpolarization dynamics of SABRE in fluorinated pyridines

Mr. Joni Eronen[1], Dr. S. Karl-Mikael Svensson[1], Mr. Nazmul Hossain[1], Dr. Vladimir V. Zhivonitko[1], Dr. Juha Vaara[1], Dr. Anu M. Kantola[1]
[1]University Of Oulu, Oulu, Finland

Introduction

Signal amplification by reversible exchange (SABRE) has quickly established its position alongside other popular hyperpolarization methods in NMR. SABRE allows the hyperpolarization of different molecules in a cost- and time-efficient manner. At its core, SABRE makes use of the magnetic properties of parahydrogen by transferring its spin order to the substrate-to-be-polarized in a temporary organometallic complex.

Aims

Spin-order transfer in SABRE is a complicated process and having a proper understanding of it is crucial for developing practical applications. The primary objective of this study is to delve deeper into the spin-order transfer process and to explain the key mechanisms leading to the hyperpolarization.

Methods

SABRE experiments were conducted using benchtop NMR spectrometer together with a custom-built SABRE system. Sample preparation involved dissolving either 3-fluoropyridine or 3,5-difluoropyridine (15 mM) and IrCl(COD)IMes (0.5 mM) into deuterated methanol. Information about the spin-order transfer was obtained by studying the efficiency of SABRE at different fields and by performing build-up and relaxation experiments.

Spin dynamics simulations were conducted using Spinach which is an open-source spin dynamics package developed for MATLAB. The simulations incorporated a 14-spin model including the hydride protons and the two equatorial ligands.

Results

Optimum spin-order transfer for fluorine-19 was obtained at 5 µT for both molecules. Smaller polarization levels were observed at mT fields. In qualitative agreement with the experiments, spin dynamics simulations show that the spin-order transfer in SABRE can be mostly explained by the coherent mechanisms. Incoherent mechanisms are required to understand the polarization transfer at mT fields.

Conclusions

Results of this study have led to a better understanding of the mechanisms behind the spin-order transfer in SABRE for fluorinated compounds. This work has shown that most of the spin-order transfer can be attributed to the coherent dynamics whereas a smaller but significant contribution may arise from dissipative mechanisms.

HYP-045: On the way to a hyperpolarizable Ca-Sensor

Philip Saul[1,2], Charbel Assaf[1,2], Dr. Kolja Them[1,2], Prof. Dr. Jan-Bernd Hövener[1,2]
[1]University Medical Center Schleswig-Holstein (UKSH), Kiel, Germany, [2]Kiel University, Kiel, Germany

Introduction

Although nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) are powerful tools in analytics and medical diagnostics, there still is room for improvement. In fact, no more than a few parts per million of all spins in a given sample effectively contribute to the signal. Hyperpolarization techniques allow for an increase of this fraction to the order of unity, providing 10.000 – 100.000-fold signal enhancement. Here, we present an approach to a hyperpolarizable Ca-sensor which will be a valuable tool for the determination of the presence and concentration of Ca, with plays a role in, amongst others, neuronal diseases.

Results and discussion

Herein we introduce a method for the synthesis and subsequent polarization of Tetraallyl-2,2',2'',2'''-{[(ethane-1,2-diylbis(oxy))bis(2,1-phenylene)]bis(azanetriyl)} tetraacetate. The compound features a polarizable and cleavable allyl-sidearm, allowing for polarization transfer to a close-by ^{13}C.
Since not all materials for the synthesis are available with ^{13}C label, an emphasis was put onto the development of a synthetic route which allows for the synthesis of the 2-^{13}C labelled compound. The compound was successfully synthesized and hyperpolarized by hydrogenating the allyl sidearm with para-hydrogen (>90%) at 7 bar at 9.4 T. Strong ^{1}H signal enhancement of several orders of magnitudes was observed on the added hydrogens.

Conclusion

The herein shown results pave the way for the synthesis and hyperpolarization of ^{13}C labeled Tetraallyl-2,2',2'',2'''-{[(ethane-1,2-diylbis(oxy))bis(2,1-phenylene)]bis(azanetriyl)} tetraacetate. The isotopic labelling would allow for the transfer of the obtained proton polarization to ^{13}C, leading to longer T_1 and thus longer observability of the hyperpolarized compound. Potential perdeuteration should enhance T_1 even further, making the thus hyperpolarized Ca-sensor an excellent candidate for the detection of e.g. early stage neuronal diseases using MRI. Based on the herein proposed compound, polarization via Parahydrogen-Induced Polarization Relayed via Proton Exchange (PHIP-X).

HYP-046: Enhancing ^{19}F-NMR in liquid state with Overhauser dynamic nuclear polarization at high magnetic fields

Mr Maik Reinhard[1,2], Dr Marcel Levien[2,3], Dr Luming Yang[2], Dr Alex van der Ham[2], Dr Igor Tkach[2], Dr Tomas Orlando[2,4], Prof Marina Bennati[1,2]
[1]Institute of Physical Chemistry, Georg August University of Göttingen, Göttingen, Germany, [2]Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany, [3]Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, [4]National High Magnetic Field Laboratory, Tallahassee, USA

Nuclear magnetic resonance (NMR) signals can be enhanced using dynamic nuclear polarization (DNP). Magnetization is transferred onto nuclei of interest by microwave irradiation of unpaired electron spins located on organic radicals. In the liquid state, cross-relaxation enables this polarization transfer, which is known as the Overhauser effect. The DNP efficiency crucially depends on the time modulation of the electron-nuclear hyperfine coupling.
We investigated Overhauser DNP on ^{19}F-NMR, which has important applications in drug analysis and protein-ligand interactions. To date, there have been no reports on Overhauser ^{19}F-DNP beyond a magnetic field of 5 T [1]. Here, we employed our new liquid state DNP setup operating at 9.4 T equipped with a frequency agile gyrotron as microwave source [2] to perform ^{19}F liquid state Overhauser DNP at high magnetic field.
We studied the ^{19}F-NMR enhancement of hexafluorobenzene using galvinoxyl and TEMPO as polarizing agents (PA). We complemented our data with previously reported data [3] at other fields to obtain the field dependence for hexafluorobenzene. With aid of DFT calculations, we identified key features of the interaction between the PA and target molecule necessary for efficient high field ^{19}F-DNP.
Using galvinoxyl as PA, positive ^{19}F-NMR enhancements up to 25 were observed on various target molecules in DNP at 9.4 T. Finally, we were able to show that it is possible to transfer the increased polarization on fluorine to scalar coupled carbon atoms on a model system, resulting in large indirect ^{13}C enhancements.

[1] N. M. Loening, M. Rosay, V. Weis, R. G. Griffin, J. Am. Chem. Soc., 2002, 124, 30, 8808-8809
[2] M. Levien, L. Yang, A. van der Ham, M. Reinhard, M. John, A. Purea, J. Ganz, T. Marquardsen, I. Tkach, T. Orlando, M. Bennati, submitted
[3] C. George, N. Chandrakumar, Angew. Chem. Int. Ed., 2014, 126, 8581-8584

HYP-047: Glucose as a versatile glassing agent for Dissolution-DNP sample formulation and a probe of metabolic pathways for biological applications.

Léa Gutierrez[1]
[1]Université Paris Cité, Paris, France, [2]Ecole Normale Supérieure, Paris, France

Glutamine is an abundant amino acid that participates in essential cellular metabolic pathways. It has garnered growing interest for its role in various diseases like cancer and cardiovascular conditions. Studying its metabolism is valuable for identifying its metabolic products as biomarkers, stratifying patients in new therapies, and accelerating the translation of experimental drugs into clinical practice.

Dynamic Nuclear Polarization (D-DNP) allows to increase magnetic resonance sensitivity by several orders of magnitude using the transfer of polarization from highly polarized electrons to the nuclei. This increased sensitivity creates opportunities to analyze 13C metabolites at low concentrations in glutamine metabolism [1].

For this purpose, it is essential to replicate physiological conditions as closely as possible during experimentation. However, some compounds used in D-DNP as glassing agents like glycerol or DMSO [2],[3] can be toxic and potential obstacles during data extraction and analysis. We explored an alternative sample formulation where glycerol is replaced by sugars, which also permits the co-polarization of both acetate or glutamine along with glucose.

We will show that glucose is a promising glassing agent which has enabled a 1H polarization up to 71% in solid state. We have also been able to monitor the enzymatic conversion of glutamine into glutamate by achieving a liquid-state 13C polarization up to 24.5 % for 5-13C-glutamine.

We think that this new formulation paves the way to future simultaneous analyses of multiple enzymatic reactions or metabolic pathways in vitro or in live cellular cultures.

[1] Dos Santos, K and al. ChemPhysChem 24, no 12 (2023): e202300151.
[2] Lumata, L and al. Phys. Med. Biol. 2011, 56 (5), N85–N92.
[3] Leavesley, A and al. Phys. Chem. Chem. Phys. 2018, 20 (15), 9897–9903.

HYP-048: Straightforward Method for the Generation of Hyperpolarized Orthohydrogen with a Partially Negative Line

Phd Adam Mames[1], PhD Mariusz Pietrzak[1], PhD Tomasz Ratajczyk[1]
[1]Institute Of Physical Chemistry PAS, Warszawa, Poland

Hydrogen gas at thermal equilibrium at 298K consists of 75% orthohydrogen (o-H2) and 25% parahydrogen (p-H2). This mixture can be p-H2-enriched by cooling with a catalyst, enabling para-ortho conversion. A p-H2-enriched mixture can be heated to 298K, but without the catalyst, the composition remains unchanged. Equilibration will begin when a p-H2-enriched mixture is introduced to a system with a conversion catalyst. In some cases, it has been observed that the NMR signal of o-H2 converted from p-H2 can reveal a partial negative line (PNL).
We aim to investigate PNL. We focus on PNL generated via Signal Amplification by Reversible Exchange (SABRE). We investigate the interplay between the type of the solvent and PNL.
We employed SABRE for the conversion of p-H2 into o-H2. In SABRE, a catalyst interacts with p-H2 and a ligand. During activation, the catalyst is rearranged into its active form. The active form, p-H2, and a ligand create a labile complex. We conducted our experiments with C6D6, C6F6, or C6D6/C6F6 mixtures (solvent), pyridine (ligand), and the Ir-IMes (catalyst). Our experimental protocol is as follows. The sample was exposed to p-H2 in the Earth's magnetic field. The sample was shaken, placed in the spectrometer, and the 1H(45°) NMR spectrum was recorded (the entire procedure is called a cycle). Cycles were conducted one after another.
PNL was observed in both solvents only during catalyst activation. PNL decreased with each cycle because the activation progressed. Finally, PNL was not observed anymore. PNL showed a positive-negative mode in C6D6, while in C6F6, PNL showed a negative-positive pattern. Our research explored the effect of the mixing of these solvents. At the specific ratio of C6D6/C6F6, PNL was not observed.
Switching of the PNL pattern was observed in C6D6 and C6F6. In a mixture at a specific C6D6/C6F6 ratio, PNL was not observed.

HYP-049: Adiabatic DNP with shaped chirp pulses

Dr. Shebha Anandhi Jegadeesan[1], Dr. Yujie Zhao[2], Dr. Hassane El Mkami[2], Dr. Robert Hunter[2], Dr. Graham Smith[2], Dr. Ilya Kuprov[3], Dr. Guinevere Mathies[1]
[1]University of Konstanz, Konstanz, Germany, [2]University of St Andrews, St Andrews, United Kingdom, [3]University of Southampton, Southampton, United Kingdom

Pulsed dynamic nuclear polarization (DNP) with amplitude or frequency sweeps has been reported to improve the sensitivity of nuclear magnetic resonance (NMR) at high magnetic fields [1,2]. A microwave chirp pulse can give rise to the integrated, stretched, or adiabatic solid effect (SE), depending on bandwidth and position of the chirp with respect to the electron paramagnetic resonance (EPR) spectrum. So far, DNP by adiabatic passage has only been reported with linear chirps of constant amplitude.

We extensively investigated adiabatic DNP with shaped chirp pulses through numerical simulations based on the kernel of Spinach [3]. Previously, numerical simulations with a single polarization transfer from 1 electron to 2 protons proved useful in predicting the conditions for pulsed DNP, but deviations between experiment and simulation were also observed [4]. To address these, we have extended the simulations to include more proton spins and the build-up of bulk polarization. We show that the steady-state of a dynamically polarized spin system, which is typically only reached after thousands of polarization transfers, can be simulated directly, without explicit propagation. Simulations of adiabatic DNP at W band (94 GHz/3.4 T/144 MHz) suggest that it is advantageous to shape the chirps, for example, by amplitude modulation, as in WURST, or by non-linear sweeping, as in a hyperbolic secant.

Experimental field profiles at W band are in very good agreement with the simulations. Experiments confirm that, given an average microwave power, the DNP efficiency can be improved by truncating frequency chirps immediately after the polarization transfer by adiabatic passage is complete.

1. Can et al. Angew. Chem. Int. Ed. 2017, 56, 674.
2. Quan et al. J. Phys. Chem. Lett. 2022, 13, 5751.
3. Hogben et al. J. Magn. Reson. 2011, 208, 179.
4. Redrouthu et al. J. Chem. Phys. 2023, 159, 014201.

HYP-050: SABRE Hyperpolarisation of [2-13C]pyruvate in non-alcoholic solution.

Dr Oksana A. Bondar[1], Dr Gamal A.I. Moustafa[1], Dr Thomas B.R. Robertson[1]
[1]University of Southampton, Southampton, United Kingdom

Signal amplification By Reversible Exchange (SABRE)[1] can provide strong signal enhancement of different molecules through the use of parahydrogen, a nuclear singlet state. An iridium catalyst may then reversibly bind pH_2 derived hydrides and a ligand of interest, allowing polarisation transfer. Among the substrates that can be used as a probe for hyperpolarised NMR and MRI, pyruvate has gained much attention since it is a natural metabolite that is converted to lactate by lactate dehydrogenase (LDH), it is completely harmless to the body. SABRE 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-d_4 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 with further easy purification methods. This work has shown hyperpolarization of [2-^{13}C]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 ^{13}C resonance corresponding to a free pyruvate at 205 ppm 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. For developing future purification methods a study of the relaxation times for [2-^{13}C]pyruvate has been done for various type of solutions used for hyperpolarized experiments.
Our work opens new possibilities for obtaining an aqueous solution of pyruvate for potential in vivo applications.

[1] Adams R.W. et al. Science, 2009, 323(5922),1708-1711.
[2] Patrick TomHon et al. J. Am. Chem. Soc.2022,144,1,282–287.
[3] Manoharan A. et al. ChemMedChem, 2018, 13(4), 352-359.
[4] Liberski S. et al. Archives of Toxicology, 2022,1-21.

HYP-051: Systematic study of hyperpolarization with hybrid polarizing solids – moving towards pure hyperpolarized solutions after dissolution-dynamic nuclear polarization

M.sc. Ekaterina Pokochueva[1], PhD Nghia Le[1,2], Sylvie Guibert[1], M.Sc. Chloé Gioiosa[3,1], PhD Quentin Stern[1], PhD James Tolchard[1], M.Sc. Charlotte Bocquelet[1], Olivier Cala[1], Mathieu Cavailles[2], Laurent Veyre[2], Prof. Chloé Thieuleux[2], Prof. Sami Jannin[1]
[1]Universite Claude Bernard Lyon 1, CRMN UMR-5082, CNRS, ENS Lyon, Villeurbanne, France,
[2]Université de Lyon, Institut de Chimie de Lyon, Laboratory of Catalysis, Polymerization, Processes and Materials, CP2M UMR 5128 CNRS-UCB Lyon 1-CPE Lyon, CPE Lyon, Villeurbanne, France,
[3]TotalEnergies, Centre de recherche de Solaize, BP 22, Solaize, France

Dissolution dynamic nuclear polarization (dDNP) [Ardenkjær-Larsen 2003] is one of the most powerful hyperpolarization techniques, able to provide proton polarization close to unity and 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 ^{1}H is very effective, the presence of the radicals in the final solution can cause several problems. It contaminates the sample, leads to paramagnetic relaxation and causes significant paramagnetic lines broadening at low magnetic fields.[Picazo-Frutos 2023] As a solution, mesoporous silica-based matrices with radicals covalently immobilized on the surface (HYPSO, hybrid polarizing solids) were proposed.[Gajan 2014, Cavaillès 2018]
Here we report a first comparative ^{13}C hyperpolarization study with HYPSOs of different pore sizes (4, 12 and 28 nm) and concentrations of radicals (from 9 to 67 µmol.cm^{-3}). We show that the concentration of polarizing agents can be tuned to achieve an optimum dDNP conditions. With such optimization, liquid-state ^{13}C polarization of 15% can be achieved on a small molecule (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, which was also confirmed by EPR measurements.
We believe that with further optimization, HYPSO materials may compete with standard sample formulation. For now, HYPSOs already offer the possibility to effortlessly remove hyperpolarizing agents while retaining significant hyperpolarization, which may be used for many applications in the near future.

HYP-052: 400 MHz/263 GHz Ultra-Low Temperature MAS-DNP Using a Closed-Cycle Helium Gas Cooling System and a Solid-State Microwave Source

Dr Hiroki Takahashi[1], Dr Fumio Hobo[1], Mr Yusuke Tanimoto[1], Mr Yuki Endo[1]
[1]JEOL Ltd., , Japan

Dynamic nuclear polarization (DNP) dramatically enhances sensitivity of solid-state NMR. A conventional magic angle spinning DNP (MAS-DNP) system utilizes a gyrotron as a microwave source since it generates high output power (>10 W) at high frequency (>200 GHz), and operates at 100 K with nitrogen gas. However, a gyrotron has disadvantage in introduction and running costs and in footprints. Furthermore, an NMR magnetic field needs sweeping to match the DNP condition when a sample (polarizing agent) is changed because the range of frequency tuning of a gyrotron is very narrow. A solid-state microwave source, on the other hand, is a very small device and capable of tuning wide range of frequency (>5 GHz for the 263 GHz system) but its output power is very small (a few hundred milliwatts at 263 GHz) thus it gives only a small DNP enhancement.

Here, we show a 400 MHz/263 GHz MAS-DNP system utilizing a compact solid-state microwave source (160 mW) combined with an ultra-low temperature (ULT) MAS probe where a sample is cooled and spun by a closed-cycle helium gas cooling system. The efficiency of DNP drastically increases at 30 K compared to 100 K since the relaxation times of polarizing agents become much longer at ULT, and therefore it compensates the reduction of microwave power. Moreover, a preamplifier is placed inside the DNP probe and cooled to gain a further SNR enhancement. By combining a ULT-MAS probe and a cold preamplifier, an MAS-DNP system using a solid-state microwave source provides great sensitivity even compared to a conventional 100 K gyrotron DNP system.

This work is supported by Adaptable and Seamless Technology transfer Program through Target-driven R&D (A-STEP) from Japan Science and Technology Agency (JST) Grant Number JPMJTR204D.

HYP-053: Diffusion anisotropy of Helium-3 gas in ordered Al2O3 aerogels

Mr Hadi Loutfi[1], Ms Geneviève Tastevin[1], Mr Pierre-Jean Nacher[1], Mr Vyacheslav Kuzmin[2], Mr Kajum Safiullin[3]
[1]Laboratoire Kastler Brossel, ENS-PSL University, CNRS, Sorbonne Université, Collège de France, Paris, France, [2]Laboratory of Magnetic Radiospectroscopy, Institute of Physics, Kazan Federal University, Kazan, Russia, [3]Until 11-2022: see affiliation 2. Currently: Institute for Biological Interfaces 4-Magnetic Resonance, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany

Introduction: Helium-3 was used for comparison of NMR measurements of gas diffusion along different directions within nanoporous ordered Al_2O_3 aerogels at room temperature in Paris and low temperature in Kazan [1,2].

Aim: This work aimed at a test of the validity and limit of the Knudsen model of gas diffusion within native and compressed aerogel samples [2] for a wide range of gas densities and associated mean free paths.

Methods: At 300 K, 10-30 mbar of laser-polarised ^{3}He gas mixed with 0-1 bar of N_2 (effective pressure, Peff : 10-2500 mbar) was used for multiple CPMG (or XY-16) spin-echo studies at 2.6 mT with pulsed gradients G of fixed or variable directions and strengths. At 4.2 K, diffusion was probed at 0.5 T with Boltzmann-polarised ^{3}He gas (10-1000 mbar), Hahn echoes, and static gradients.

Results: Within aerogel, diffusion was strongly restricted at all gas densities and a “free diffusion regime” was achieved (negligible effects of sample boundaries). At 300 K, ^{3}He polarisation ($\simeq$50%) typically yielded SNRs around 200. Diffusion anisotropy (change in apparent diffusion coefficient, Dapp, with the angle between G and the aerogel fibers) was highest at low Peff (up to a factor of 2). At 4.2 K, we have observed that the degree of diffusion anisotropy depends significantly on the aerogel porosity, notably in the compressed samples. We have also observed a strong decrease of gas diffusion and a deviation from the expected (Knudsen) behaviour, attributed to the low-T enhanced influence of the wall attractive potential on gas dynamics.

Discussion: Our measurements of anisotropy and pressure dependence of Dapp at 300 K support and complement the 4.2 K findings. They significantly extend the range of gas densities used to probe restricted gas diffusion in such complex materials.

[1] anr.fr/Projet-ANR-19-CE30-0023 ; RSF project: 20-42-09023
[2] Kuzmin et al.(2023) doi.org/10.1021/acs.jpcb.2c08251

HYP-054: Where does hyperpolarization go? Tracking the evolution of a hyperpolarized spin system

Dr Bonifac Legrady[1], Dr Mohamed Sabba[1], Ms Chloe Gioiosa[2,3], Dr Quentin Chappuis[2], Dr Stuart Elliott[4], Professor Sami Jannin[2], Professor Malcolm Levitt[1]
[1]University Of Southampton, Southamton, United Kingdom, [2]University of Lyon, Lyon, France, [3]TotalEnergies, Solaize, France, [4]Imperial College London, London, United Kingdom

Hyperpolarisation techniques can provide very large spin polarisation and huge NMR signal enhancements. Most of us have seen this statement in some form countless times, but what does it actually mean? Why is that? Is hyperpolarisation signal enhancement then? What kind of polarisation do we generate? Where does it go? These questions are relatively straightforward to answer for isolated spins, but far less easy for coupled multi-spin systems.
In this work, we investigate this issue using dissolution DNP (d-DNP) experiments on a strongly coupled 13C two-spin system. To address this, one needs to determine the precise state of the spin-system, which is possible even from the simplest experiments via careful analysis of the data. A straightforward method will be presented that allows quantification of all reasonably possible spin states immediately after dissolution, and after two different preparation sequences. In the present system, a single spectrum contains sufficient information, and thus, the evolution of the entire density operator can be followed until signal can be detected, for approximately 5 minutes. It is shown experimentally that after dissolution, the three triplet states rapidly reach an internal quasi-equilibrium, while the singlet population persists in a non-equilibrium state for approximately 25 times longer.
The precise experimental population-operator trajectories not only enable one to finely tune or fit parameters of any relaxation model, but a “model-free”, purely experimental determination of the zero-quantum block of the complete Liouvillian is also possible. This is achieved by a numerical optimisation procedure that relies only on some of the most fundamental results of the theory of open quantum systems, known as the Kossakowski conditions.
The presented techniques may be extended to any multi-spin system, when resonances are well-resolved and precise peak intensities can be extracted.

HYP-055: Probing DNP Driven Transitions in Methyl Rotors using Acetate 13C NMR Spectra

Ms. Pooja Singh[1], Dr. Michael Jurkutat[1], Dr. Benno Meier[1]
[1]Karlsruhe Institute Of Technology, Karlsruhe, Germany

Dynamic Nuclear Polarization (DNP) has emerged as a powerful technique to enhance NMR signals, significantly improving the sensitivity and resolution of NMR spectroscopy. In the present study, we focus on the simulation of the acetate carbon spectrum, specifically examining the influence of the methyl rotor on the lineshape under different DNP conditions. Using quantum mechanical simulations, we model the dipolar couplings and Zeeman interactions of the methyl group rotor. Experimental observations reveal a noticeable distinction between spectra of 2-13C labeled acetate under positive and negative DNP enhancement [1,4]. Our simulations elucidate the significant differences in the NMR spectra, demonstrating how the population distribution of the methyl rotor states varies with the DNP condition. In the positive DNP-enhanced spectra, the A state of the methyl rotor is predominantly populated, while in the negative DNP-enhanced spectra, the E state shows higher population. This disparity in state populations can be attributed to the underlying tunneling splitting of the methyl rotor, which is comparatively high in molecules like acetate. Our findings corroborate previous studies that have reported similar phenomena in systems with significant tunneling splitting [1,2,3]. These results underscore the critical role of methyl rotor dynamics in interpreting DNP-enhanced NMR spectra and provide a deeper understanding of the molecular behavior under different polarization conditions.

References :

[1] Elliott, S. J. et al.,.: Solid-state 1H spin polarimetry by 13CH3 nuclear magnetic resonance, Magn. Reson., 2, 643–652, DOI: 10.5194/mr-2-643-2021, 2021.
[2] Dumez, J.-N. et al ., Jannin, S., Levitt, M. H. Dynamic Nuclear Polarization of Long-Lived Nuclear Spin States in Methyl Groups. J. Phys. Chem. Lett. 2017, 8 (15), 3549– 3555, DOI: 10.1021/acs.jpclett.7b01512
[3] M. Šimėnas et al.,Magnetic excitation and readout of methyl group tunnel coherence.Sci.Adv.6,eaba1517(2020). DOI:10.1126/sciadv.aba1517
[4] Meier, B. Quantum-rotor-induced Polarization. Magn. Reson. Chem. 2018, 56 (7), 610– 618, DOI: 10.1002/mrc.4725

HYP-056: Bullet-DNP with UV-induced radicals for the study of UV-sensitive samples

Mr. Dmitrii Zasukhin[1], Mr. Benno Meier[1]
[1]Institute of Biological Interfaces 4, Karlsruhe Institute Of Technology, Karlsruhe, Germany

Bullet-DNP is a modified form of dissolution-dynamic nuclear polarization (D-DNP) in which the samples are placed into a plastic bullet, hyperpolarized under standard D-DNP conditions, and then, still being in the solid phase, transferred from the polarizer into an injection device for liquid state NMR detection [1].

The presence of homogeneously distributed radicals is essential for the DNP process. However, the radicals also reduce the technique's sensitivity by accelerating relaxation prior and during liquid-state NMR detection. One way to eliminate that problem is to use UV-induced radicals that are annihilated by heating above 190 K [2]. But UV-light harms some substances, making this approach problematic for studying UV-sensitive samples.

This study is focused on the design and development of Bullet-DNP experiments using UV-induced pyruvic-based radicals for UV-sensitive target molecules. This technique should allow to perform DNP on rapidly relaxing substances.

The samples with UV-radicals were prepared using original home-built setup. The setup is based on a rotating N_2-cooled copper disk, similar to [3]. First, a layer of radical precursor solution is placed on the surface of the disk in which radicals are formed with the help of UV-light. Then a second solution containing the target substance is sprayed onto the surface. This allows us to separate the steps of radical generation and spreading of the test substance.

We tested and established a procedure for preparing samples with UV-induced radicals. Using EPR spectroscopy we found that the formed radicals survive during sample preparation. Concentration of the radicals is estimated to be in the range of 30–40 mM.

The first obtained results confirm the validity of the proposed technique to prepare and use UV-radicals for DNP experiments. Current progress of this work will be reported.

[1] K.Kouřil et al. DOI:10.1038/s41467-019-09726-5
[2] A.Capozzi et al. DOI:10.1038/ncomms15757
[3] J.Jeon et al. DOI:10.1073/pnas.1908006116

HYP-057: 129Xe Hyperpolarization using the Bullet-DNP Method

Dr. Kajum Safiullin[1], Dr. Benno Meier[1]
[1]Institute for Biological Interfaces 4-Magnetic Resonance, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany

Introduction
The use of dynamic nuclear polarization (DNP) to achieve ^{129}Xe hyperpolarization (HP) has emerged as a possible alternative to spin-exchange optical pumping [1-3]. In bullet-DNP a sample hyperpolarized by DNP is shuttled rapidly as a solid into a liquid-state NMR magnet for melting and liquid state experiments at ambient temperature [4]. Thus it represents a promising and convenient approach for ^{129}Xe DNP and HP ^{129}Xe gas NMR.

Aims
This work is aimed on the design, development and evaluation of ^{129}Xe Bullet-DNP experiments. This original technique should allow to perform studies at room-T using HP ^{129}Xe as a probe.

Methods
A home-built filling station was used to: (1) fill a bullet with a selected mixture of solvent and radical; (2) condense xenon into the bullet at up to 9 bar; (3) cool the bullet to 160-165 K at which both solvent and xenon are in liquid state for homogenous redistribution of spins; (4) cool the bullet down to 77 K for further transfer to the bullet-DNP setup. Our standard bullet-DNP setup was used for DNP experiments at 1.4 K and 6.7 T [5].

Results
The first DNP results were obtained for several solvents which impact differently on the xenon solubility, i.e. its final concentration in the bullet, and the achieved ^{129}Xe polarization. The results stimulate the search of a suitable solvent that exhibits a high solubility and affords a high polarization.
Current progress of this work will be reported.

[1] A.Comment et al. doi:10.1103/PhysRevLett.105.018104
[2] A.Capozzi et al. doi:10.1021/jp5124053
[3] E.Wiström et al. doi:10.1021/acs.jpclett.4c00177
[4] K.Kouřil et al. doi:10.5194/mr-2-815-2021
[5] K.Kouřil et al. doi:10.1038/s41467-019-09726-5

HYP-058: Longitudinal Relaxation Times of [1-13C] pyruvate: the effects of pH, field strength and chelating agent addition

MSc. Zumrud Ahmadova[1], Dr. Salvatore Mamone[1,2], Martin Gierse[1], Dr. Laurynas Dagys[1,3], Dr. Sebastian Lucas[1], Dr. Michael Keim[1], Dr. Ilai Schwartz[1], Dr. Stephan Knecht[1]
[1]NVision Imaging Technologies GmbH, Ulm, Germany, [2]University of L'Aquila, L'Aquila, Italy, [3]Vilnius University, Vilnius, Lithuania

Introduction

Hyperpolarized [1-13C] pyruvate is widely used as a metabolic imaging probe, particularly in the study of cancer [1]. The sensitivity of hyperpolarized carbon-13 imaging is strongly influenced by the level of hyperpolarization maintained up to the point of injection. This level highly depends on the spin-lattice relaxation time (T1), as longer T1 allows the probe to maintain higher polarization levels during preparation and quality control procedures. This study focuses on the dependence of [1-13C] pyruvate T1 on pH and static magnetic field strength and the effect of ethylenediaminetetraacetic acid (EDTA), a chelating agent.

Methods

We measured carbon-13 T1 relaxation times of [1-13C] pyruvate in deoxygenated, buffered D2O solutions at pH5 to 10. T1 times were sampled at ten different B0 fields, ranging from 0.1mT to 9.4T, using a custom-built sample shuttling system on top of Bruker 400MHz NMR Spectrometer. Each measurement was repeated with samples containing 2.8mM EDTA.

Results

For all pH values, the pyruvate T1 shows a distinct maximum of 100-150s in the range of 0.1 to 1T. We observed a significant drop in T1 down to 50s when the static field is below 0.1T, depending on the pH of the sample. At fields higher than 1T, relaxation times decrease as expected due to an increasing CSA contribution to relaxation. Relaxation times of approximately 150-200s over the field range of 0.0001-1T are achieved with 2.8mM EDTA, for all studied pH values.

Conclusions

Our data demonstrate that an accurate choice of conditions is critical for maintaining the hard-gained nuclear spin polarization in [1-13C] pyruvate during the purification and quality control steps necessary for in vivo MRI applications. This is especially important for emerging PHIP modalities, which require elaborate steps to produce hyperpolarized [1-13C] pyruvate.

References

[1] Brindle, K.M., 2015. Imaging metabolism with hyperpolarized 13C-labeled cell substrates. J.Am.Chem.Soc., 137(20), pp.6418-6427.

HYP-059: Benchtop Hyperpolarization with functional porous polarizing polymers

Julien Dieterich[1,2], Charlotte Bocquelet[1], PhD Théo El Daraï[1], PhD Quentin Stern[1], PhD Roberto Melzi[3], PhD James Kempf[4], PhD Ewoud Vaneeckhaute[1], PhD Damien Montarnal[2], Pr Sami Jannin[1]
[1]Université Claude Bernard Lyon 1, ENS Lyon, CNRS UMR 5082, Villeurbanne, France, [2]Université Claude Bernard Lyon 1, CPE Lyon, CNRS UMR 5128, Villeurbanne , France, [3]Bruker Italia S.r.l., Milano, Italy, [4]Bruker Biospin, Billerica, United States

Hyperpolarization techniques such as dissolution dynamic nuclear polarization (dDNP) have been developed to address the lack of sensitivity in NMR. Few years ago, we showed the performances of hyperpolarizing polymers (HYPOPs) [1], where nitroxide radicals are embedded in a porous polymer matrix loaded with solutions to be hyperpolarized. These HYPOPs were designed to generate and transport hyperpolarized samples over long distances.

However, dDNP remains a difficult task given the complex instrumentation including low temperatures (1-4 K) and high fields (5-7 T) and its applications are narrowed as the hyperpolarized samples are diluted and can only be used once for < 10 s, thus making it incompatible with multidimensional NMR.

With the design of a benchtop DNP polarizer operating at 1 T and 77 K [2] together with our hyperpolarizing polymer matrices, we introduce the concept of recyclable hyperpolarization (HypFlow) with the goal of repeating DNP multiple times on a pure and undiluted sample circulating in a closed loop. Due to their structure, HYPOPs are believed to prevent important paramagnetic relaxation that would occur upon melting, and preserve hyperpolarization. Their geometry and radical loadings are crucial factors that need to be optimized in our conditions to be used as polarizing matrices for HypFlow.

Here we present i) the benchtop DNP setup, ii) the chemistry and synthesis of HYPOP and iii) the first 1H DNP performances of the dry polymer and impregnated with a solution. In particular, we present how to optimize solid-state DNP measurements and we highlight the presence of different mechanisms of polarization build-up as well as a promising signal enhancement.

[1] El Daraï, T. et al. https://doi.org/10.1038/s41467-021-24279-2. (2021)

[2] Bocquelet, C. et al. https://doi.org/10.26434/chemrxiv-2024-3nlnd (2024)

HYP-060: Bullet DNP With a Stripline Flow Probe

Karel Kouril[1], Kajum Safiullin[1], Masoud Minaei[1], Pooja Singh[1], Marie Harder[3], Hans Janssen[3], Arno P. M. Kentgens[3], Benno Meier[1,2]
[1]Institute for Biological Interfaces 4, Karlsruhe Institute of Technology, Eggenstein-leopoldshafen, Germany, [2]Institute of Physical Chemistry, Karlsruhe Institute of Technology, Karlsruhe, Germany, [3]Magnetic Resonance Research Center, Institute for Molecules and Materials, Radboud University, Nijmegen, The Netherlands

Dissolution dynamic nuclear polarization is a method that provides liquid state samples with high spin polarization of target molecules. The liquid is then transferred into a NMR tube for observation. While the polarization inevitably decays due to relaxation it can be depleted much faster by the applied pulse sequence thus making any subsequent experiments impractical.

In flow NMR a fresh sample can be pushed into the sensitive region after each experiment. This means that even with a pulse sequence that fully depletes the polarisation multiple observations are possible on single batch of hyperpolarized liquid.

We combined a bullet DNP system [1,2] with a stripline flow NMR probe [3]. A bullet DNP polarizer is connected to a modified injection dock via a magnetic tunnel. The dock is connected to the inlet of the probe with a short piece of 1/16" tubing. The outlet of the probe is connected to a syringe pump which is used to control the flow of the sample during the experiment.

We report results obtained with this system.

[1] Kouřil, K., Kouřilová, H., Bartram, S., Levitt, M. H., and Meier, B.: Scalable Dissolution-Dynamic Nuclear Polarization With Rapid Transfer of a Polarized Solid, Nat. Commun., 10, 1733, https://doi.org/10.1038/s41467-019-09726-5, 2019.
[2] Kouřil, K., Gramberg, M., Jurkutat, M., Kouřilová, H., Meier, B.: A cryogen-free, semi-automated apparatus for bullet-dynamic nuclear polarization with improved resolution, Magnetic Resonance, 2, 815, https://doi.org/10.5194/mr-2-815-2021, 2021.
[3] Vries, A. J. O., Nieuwland, P. J., Bart, J., Koch, K., Janssen, J. W. G., Bentum, P. J. M. v., Rutjes, F. P. J. T., …, Inline reaction monitoring of amine-catalyzed acetylation of benzyl alcohol using a microfluidic stripline nuclear magnetic resonance setup, Journal of the American Chemical Society, 141(13), 5369–5380 (2019). http://dx.doi.org/10.1021/jacs.9b00039

HYP-061: Photo-CIDNP MAS NMR analysis of a helioabacterial reaction center

Mrs Yunmi Kim[1], Prof. A. Alia[1,2], Prof. Jörg Matysik[1]
[1]Leipzig Univ., Leipzig, Germany, [2]Leiden Univ., Leiden, The Netherlands

Heliobacteria are among the oldest living organisms on earth. Their photosynthetic machinery dates back to the geological age when the earth's atmosphere was still oxygen-free. This is why these bacteria have only been able to survive in extreme habitats. The photosynthetic reaction centres (RCs) are simple, symmetrical and small. When exposed to light, they form radical pairs that enable the solid-state photo-CIDNP effect, i.e. enormous signal amplification. Using this effect, we have analysed the electronic structure and dynamics of these cofactors. The result is a coherent picture of the causes of the effect and the spin dynamics in this RC. Here we will update on recent work:

P. Kurle-Tucholski, et al. “Red-shift in the absorption spectrum of phototropin LOV1 upon formation of semiquinone radical: Reconstructing the orbital architecture”, J. Phys. Chem. B (2024) online. DOI: 10.1021/acs.jpcb.4c00397

Y. Kim, et al. “Electronic structures of radical pair forming cofactors in a heliobacterial reaction center”, Molecules 29, 1021 (2024). DOI: 10.3390/molecules29051021.

P. Kurle-Tucholski. et al. “Stabilization of a flavoprotein for solid-state photo-CIDNP MAS NMR at room temperature by embedment in a glassy sugar matrix”, Journal of Magnetic Resonance 353, 107497 (2023). DOI: 10.1016/j.jmr.2023.107497 (2023).

HYP-062: Parahydrogen hyperpolarized detection of ATP, ADP and AMP in human blood

Kerti Ausmees[1], Helen Pais[1], Indrek Reile[1]
[1]KBFI, Tallinn, Estonia

The study of human small molecule metabolomics focuses on blood, due to its central role as the body's transport system. Its accessibility for routine collection makes it clinically relevant for the detection and monitoring of a range of health conditions. Nuclear magnetic resonance (NMR) is widely used for studying the composition of blood, but it suffers from poor sensitivity and signal resolution in case of complex biofluids. Sensitivity of NMR, however can be improved by implementing iridium N-heterocyclic carbene complex catalyzed parahydrogen hyperpolarizaton (nhPHIP) [1][2].

Despite being the most valuable biofluid for diagnostic applications, blood had not been previously studied by hyperpolarized NMR due to its complex matrix. The high protein content, cellular material and various high molecular weight compounds insoluble in methanol (the best solvent for nhPHIP) present significant challenges. Raftery et al. [3] addressed these blood matrix issues by developing a sample pretreatment technique that uses extraction with a mixture of methanol and chloroform for the detection of small molecules by regular 1D NMR.

Expanding from our work on nhPHIP analysis of urine [2], we present herein the results of our work in increasing sensitivity for blood NMR. Combining an adaptation of the abovementioned sample preparation technique with nhPHIP allows to detect N-heterocyclic small molecules, including the most important cellular energy metabolites (ATP, ADP, AMP), in human blood by parahydrogen hyperpolarized NMR. We demonstrate a considerable gain in sensitivity for these metabolites, presenting a promising step forward in studying human health by NMR analysis of blood.

1. N. K. J. Hermkens, N. Eshuis, B. J. A. van Weerdenburg, M. C. Feiters, F. P. J. T. Rutjes, S. S. Wijmenga, M. Tessari. Anal. Chem., 88, 3406 (2016)
2. K. Ausmees, N. Reimets, I. Reile. Chem. Comm., 58(3), 463–466 (2022).
3. GA Gowda, D. Raftery Anal. Chem., 8, 4620 (2017)

HYP-063: Nitrogen-15 hyperpolarization and pitfalls at low fields

Dr Andrey Pravdivtsev[1], Mr Josh Peters[1], Mr Charbel Assaf[1], Dr Arne Brahms[2], Dr Kolja Them[1], Prof Rainer Herges[2], Prof Jan-Bernd Hövener[1]
[1]SBMI, MOIN CC, UKSH, Kiel University, Kiel, Germany, [2]Otto Diels Institute of Organic Chemistry, Kiel, Germany

The hyperpolarization of nuclear spins boosts the MR signal of selected molecules and has enabled real-time metabolic imaging in vivo. Hyperpolarized nicotinamide (NAM) derivatives are promising imaging targets as they play a critical role in glycolysis and metabolism of fatty acids. NAM can, therefore, be used to assess the activity of several enzymes. We present our studies of 15N hyperpolarization of tracers: NAM, pyridine, 1-methyl NAM, pyrimidine, metronidazole, and urea by dynamic nuclear polarization (dDNP).
We found that some 15N of NAM does not survive the sample transfer at low magnetic fields. Moreover, the effect is pH-dependent. It is our aim to explore the mechanism of polarization loss of 15N at low magnetic fields, generality of the effect, and strategies for circumventing this loss.
15N signals were acquired in the polarizer or after dissolution using a 1T benchtop NMR, a 9.4T WB NMR, and a 7 TMRI. dDNP was performed using a cryogen-free dDNP system (SpinAligner, POLARIZE) operating at ~1.4K and 6.7T and ~50mg samples with different compositions of the tracer with trityl radical (AH111501) in deionized water and trehalose.
The polarization of NAM, pyridine, and N3 nitrogen of metronidazole are greatly affected by pH as their polarization is reduced or completely vanishes after sample transfer at neutral pH. The highest polarization values were reached using dissolution media with basic pH. Urea, NO2, and pyrimidine do not have such effects. One of the solutions to this issue is basic pH transfer and a rapid pH neutralization at a high magnetic field above 1T.
When the pH value is close to the pKa of the protonated pyridine nitrogen, chemical exchange is rapid and can destroy magnetization at low fields. This effect on relaxation can be used to study rapid proton interactions, which are not visible by conventional NMR.

MRI-002: Rapid quantification of epicardial adipose tissue volume based on magnetic resonance imaging for predicting coronary artery disease

Jingji Ma[1], 13309882177 Xinrui Wang[1], 13309886610 Benqiang Yang[1], Jia Shang[1]
[1]General Hospital of the People's Liberation Army Northern Theatre, Liaoning, China

Purpose: To evaluate epicardial adipose tissue volume index (EATVI) for predicting coronary artery disease (CAD) using magnetic resonance images.
Methods: This study prospectively included 41 patients with CAD and 41 without CAD, all of whom underwent both coronary CT angiography (CCTA) and CMR imaging. CAD patients were classified into two groups based on the degree of coronary stenosis: significant (stenosis ≥ 50%, n = 25) and non-significant (n = 16). Semi-automatic segmentation of pericardial fat was used to quantify epicardial adipose tissue volume (EATV). EATVI as well as epicardial adipose tissue thickness and the cardiac function indexes were also measured. Intra- and inter-observer consistency was evaluated, and inter-group differences in the measured parameters were compared. Logistic regression and multiple regression analyses were used to screen for risk predictors for CAD occurrence. The diagnostic efficacy of the predictors was assessed using ROC curve.
Results: Intra- and inter-observer agreement was good (ICC > 0.83). EATVI was significantly higher in CAD patients than in those without CAD (P < 0.001). Additionally, EATVI was higher in patients with significant CAD compared to those with non-significant CAD (P < 0.01). EATVI remained independently associated with CAD using multiple regression analysis. The diagnostic efficacy of a combination of EATVI and EAT thickness for CAD was excellent (AUC of 0.990, 95% CI: 0.977-1.00, P < 0.001).
Conclusions: In this study, EATVI was demonstrated to be an independent predictor of CAD. It may serve as a novel non-invasive index for improving the diagnosis and efficacy evaluation of CAD.

MRI-003: Clinical comparison of 3.0T compressed sensing-sensitivity encoding (CS-SENCE) accelerated mDixon-based non-contrast whole-heart coronary MR angiography against coronary CT angiography for detection of coronary artery stenosis

Jingji Ma[1], Xinrui Wang[1], Benqiang Yang[1], Jia Shang[1]
[1]General Hospital of the People's Liberation Army Northern Theatre, Liaoning, China

Objectives To evaluate the diagnostic accuracy of a compressed sensing-sensitivity encoding (CS-SENCE) accelerated mDixon-based non-contrast whole-heart coronary MR angiography (CMRA) technique against coronary CT angiography (CCTA) for assessing significant coronary disease (CAD).
Methods One hundred and fifty consecutive participants with suspected CAD were enrolled. All participants underwent routine CCTA and CMRA on a 3.0T scanner. Additional X-ray coronary angiography was performed in 18 patients with high risks of CAD. Diagnostic accuracy of CMRA for detecting significant coronary stenosis (≥ 50%) was assessed using CCTA and X-ray coronary angiography as reference standard in patients with low-to-intermediate and high risks of CAD, respectively.
Results: 3.0T mDixon CMRA were completed within 7.0 ± 2.6 minutes with adequate diagnostic image quality (overall image quality score of 3.38 ± 0.71). In patients with low-to-intermediate risks of CAD, the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were: per patient (81%, 77%, 52%, 93% and 78%), per vessel (77%, 89%, 50%, 97% and 88%) and per segment (52%, 94%, 33%, 9 7% and 92%) respectively. CMRA and CCTA also showed good correlation and agreement in the measurements of stenosis. In patients with high risks, CMRA showed comparable diagnostic efficiency with CCTA for detecting clinically relevant CAD.
Conclusions: The noninvasive, radiation and contrast-free mDixon CMRA technology achieved adequate image quality and good diagnostic accuracy for the detection of significant coronary stenosis. It may serve as a promising screening tool and further be integrated into clinical protocols with myocardial perfusion and scar imaging for a comprehensive evaluation of CAD.

MRI-004: Cardiac Magnetic Resonance T2 Mapping Early Detects the Increased Myocardial Inflammatory Change in Experimental Diabetic Pigs

Doctor Lu Zhang[1], Professor Yingkun Guo[1]
[1]West China Second University Hospital, Chengdu, China

Introduction
Myocardial inflammation has been described as a key pathophysiological trigger for the hallmark changes in diabetes myocardial alterations.
Aims
The aim of the present study was to histopathologically validate the T2 mapping parameters for the evaluation of diffuse myocardial changes in pig models of diabetes.
Methods
Eleven diabetes pig models were generated by administering streptozotocin (150 mg/kg, intravenous injection). Three controls were age and weight matched healthy pigs. CMR T2 mapping was performed to evaluate global T2 in the left ventricular. The correlation between histological alterations and global T2 was evaluated following histopathological investigations.
Results
Diabetes pigs had higher T2 values at 2-, 6-, 10-, and 16-month post-modeling (all $p < 0.05$) compared to controls. In the subgroup analysis of diabetes pigs, T2 value was increased at 2 months (44.4±5.1 ms, $p > 0.001$) and 6 months (44.5±6.7 ms, $p = 0.03$) post-modeling, and maintained an increasing trend at 10 months (42.3±3.6 ms, $p = 0.057$) and 16 months (43.9±3.9 ms, $p = 0.066$), compared to the baseline (37.5±2.9 ms). Both the inflammation score ($r = 0.587$, $p = 0.0187$) and the edema score ($r = 0.507$, $p = 0.04$) determined from HE staining had significant associations with the T2 value.
Conclusions
Our findings in the experimental pig model of diabetes suggest that CMR T2 mapping may have potential as a non-invasive tool for the early detection and dynamic evaluation of myocardial edema and inflammation.

MRI-005: Nonlocal Effects and Quantitative Mass Flow Rates in Granular Flows as Studied with Flow-MRI and Rheo-NMR

Dr. Daniel A. Clarke[1,2,3], Dr. Maral Mehdizad[1,2], Dr. Petrik Galvosas[1,3], Dr. Daniel J. Holland[2]
[1]Victoria University Of Wellington, Wellington, New Zealand, [2]University of Canterbury, Christchurch, New Zealand, [3]MacDiarmid Institute for Advanced Materials and Nanotechnology, Wellington, New Zealand

Establishing and validating models for granular flows – ubiquitous in nature and industrial processes – is challenging because of the different conditions these flows maybe found in, from gaseous in one limit to near solid-like motion in the other. Magnetic Resonance Flow Imaging (Flow-MRI) has improved our understanding of particulate flows by providing 3D velocity information even for opaque samples. However, a full description of the flow mechanics requires the knowledge of the solid volume fractions (3D density maps) which vary along with the granular velocities themselves.

While spatially resolved signal intensity is most commonly obtained from MRI, it is not straightforward to translate it into solid volume fractions. This is due to sample dilation occurring during flow and instrumental factors. The aim of this work is to establish methods for 3D mapping of solid volume fractions in the context of flowing particulate matter.

Recorded MRI signal intensities have been calibrated against stationary samples with known densities. Furthermore, resting sample material measured simultaneously with matter under flow provides a reference for signal normalisation and compensation of electronic and environmental instabilities. Single-point imaging protocols make recorded signal intensities less sensitive against relaxation time weighting and flow artefacts.

Spatially resolved mass flow rates have been determined via the knowledge of solid volume fractions [1]. This allowed the experimental 3D cross-validation of proposed hopper flow models and the confirmation of self-similarity for flows in hoppers of different dimensions [2]. Furthermore, nonlocal granular fluidity models [3] were investigated and tested experimentally [4], thus providing cross-validation to simulations and guidance for future research.

[1,2] M. Mehdizad, et al., J. Magn. Reson. 325 106935 (2021) and Powder Technol. 392 69–80 (2021)
[3] K. Kamrin, G. Koval, Phys. Rev. Lett. 108, (2012).
[4] D.A. Clarke et al., Phys. Fluids, under revision

MRI-006: The relationship between abnormal myocardial alterations and glycemic control validated by cardiac magnetic resonance in a long-term pig model of diabetes mellitus

Doctor Lu Zhang[1]
[1]West China Second University Hospital, Chengdu, China

Introduction : The persistent poor glycemic control significantly enhances the increase in glucose toxicity, leading to cumulative, persistent, and largely glycemic damage effects on the cardiovascular system. However, the specific impact of different extent of glycemic control on cardiac alterations is still unclear.
Aims: To investigate the relationship between hemoglobin (HbAlc) and abnormal myocardial alterations as determined by cardiac magnetic resonance (CMR) T1 mapping and feature tracking in a pig model of diabetes mellitus (DM).
Methods: Eight DM models were successfully established and were divided into two groups based on their HbAlc median value (high HbAlc ≥ 6.07 %, $n = 5$; low HbAlc < 6.07 %, $n = 3$). As controls, five healthy age-matched pigs were used. Over the diabetic course of 16 months, CMR cine, T1 mapping, and late enhancement gadolinium (LGE) were performed. Afterward, three pigs were then sacrificed and stained with masson trichrome and advanced glycation end products (AGEs) from control, high, and low HbAlc groups, respectively.
Results: Diabetes and control groups did not differ in terms of left ventricular ejection fraction ($p = 0.784$) and showed no evidence of LGE. However, when compared to controls, pigs with high HbAlc had higher native T1 and ECV as well as lower longitudinal strain and strain rate (all $p < 0.05$). Furthermore, HbAlc correlated well with native T1, ECV, longitudinal strain, and diastolic strain rate ($R2 = 0.470$ to 0.849, all $p < 0.05$). Histological staining showed more significant deposition of myocardial interstitial fibrosis and AGEs in the pig with high HbAlc than others.
Conclusions: High HbAlc was associated with increased myocardial native T1 and ECV and decreased longitudinal myocardial deformation in DM pigs.

MRI-007: Diffusion MRI and localised spectroscopy of organoids on 1.2 GHz

Dr. Chantal Tax[1,2], Dr. Tatiana Nikolaeva[4], Mr. Rico Singer[5], Ms. Channa Jakobs[3], Dr. Alia Alia[5,6], Prof. Aldrik Velders[4], Prof. Jeroen Pasterkamp[3], Dr. Julia Krug[4]
[1]Center for Image Sciences, University Medical Center Utrecht, Utrecht, Netherlands, [2]Cardiff University Brain Research Imaging Centre, Cardiff University, Cardiff, United Kingdom, [3]Department of Translational Neuroscience, University Medical Center, Utrecht Brain Center, Utrecht University, Utrecht, Netherlands, [4]Laboratory of BioNanoTechnology, Wageningen University & Research, Wageningen, The Netherlands, [5]Leiden Institute of Chemistry, Leiden University, Leiden, The Netherlands, [6]Institute for Medical Physics and Biophysics, University of Leipzig, Leipzig, Germany

Introduction: Organoids are gaining importance for detailed study of tissue during development and disease progression and therapy. MRI can provide a novel means for live-imaging of organoids and, in contrast to microscopy, could be translated to human imaging. In particular, diffusion MRI (dMRI) can provide non-invasive estimates of e.g. fraction, size, shape, and orientation of spaces within and around cells, whereas localised spectroscopy can characterise metabolism.
Although promising, the small dimensions require high-resolution imaging for detailed assessment, which are challenging to achieve with low magnetic field strengths and large radiofrequency coils (d ~2.5–30 cm). Ultra-high field MRI setups with small radiofrequency coils (d ~1.5–5 mm) can achieve unprecedented high-resolution dMRI and separation of metabolites.

Aim: To take the first steps in developing MRI at 1.2GHz in organoids, by investigating the longitudinal structural and metabolic stability of living organoids in the scanner, and assessing structural measures derived from dMRI.

Methods: Cortical organoids (DIV55-110, 108 days) were positioned atop an agar substrate and the MR tube filled with medium. dMRI data was acquired with a pulsed-gradient spin-echo (PGSE) sequence at 1.2GHz, equipped with a microimaging probe. One organoid underwent high-resolution dMRI 45x45x100μm^3, $b \leq 3 ms/\mu m^2$, δ=1.5ms, and Δ=4.5-20ms. Another organoid underwent longitudinal measurement with medium replacement during the first 8 hours: PGSE (35x35x100μm^3, b=0-1$ms/\mu m^2$, δ=1.5ms, and Δ=4.5ms) and PRESS (1.3 ppm, 800x800x800μm^3, 256 averages) to characterise the apparent diffusion coefficient (ADC) and metabolites over time.

Results: The estimated ADC and kurtosis were 0.53+-0.16μm^2/ms and 1.12+-0.56 across the organoid at δ=1.5ms/Δ=4.5ms. The largest ADC-decrease with diffusion time occurred between Δ=4.5-10ms (0.47-0.35μm^2/ms). No significant longitudinal ADC-change was observed, yet a difference in metabolite composition was visible.

Discussion & Conclusion: Organoid MRI provides opportunities to study its microstructure and metabolism non-invasively and longitudinally. Future work will endeavour to increase the resolution and perform subsequent microscopy.

MRI-008: Intrathecal mobility assessment of the cauda equina on postural change by dynamic MR imaging

Dr. Mizuho Sumitani[1], Dr. Rikuhei Tsuchida[1], Dr. Kenji Azuma[1], Dr. Masahiko Sumitani[1]
[1]The University Of Tokyo Hospital, 7-3-1, Japan

Background: Magnetic resonance imaging (MRI) provides the most accurate delineation of soft-tissue and osseous structures. Conventional MRI examinations of the spine are usually performed in supine position. However, dynamic (in other words, kinematic) MRI can image patients in flexed and extended positions, revealing abnormalities of spinal instability. We assessed intrathecal mobility of the cauda equina on postural change by using MRI.
Methods: Seventeen patients with a high-risk of lumbar adhesive arachnoiditis, which is susceptible to decreased mobility of the cauda equina, and 18 no-risk patients with chronic low back pain and/or leg pain participated in this study. The patients underwent MRI examinations in both the supine and prone positions. Eleven axial T2-weighted images between the L2 and L5/S levels were obtained, and the proportion of the low-intensity area in the dorsal half to the total low-intensity area in the dural sac was calculated for each axial view.
Results: At some levels of lower lumbar spine, the low-intensity area in the dorsal half of the dural sac was relatively larger in patients with a high risk of lumbar adhesive arachnoiditis than in the no-risk patients. In the no-risk group, the proportion of the low-intensity area in the dorsal half in the supine position was significantly higher than that in the prone position at all lumbar levels. However, in the adhesive arachnoiditis group, at some lumbar levels, the proportions were not significantly different in the dorsal half of the dural sac between the supine and prone positions.
Conclusion: Dynamic MRI examination on postural change could assess the potential of the cauda equina to migrate in the dural sac in the gravitational force direction.
Biography: Graduation from Faculty of Medicine, Tsukuba University (2000), Working at Yokohama City University, Kamio Memorial Hospital, and The University of Tokyo Hospital.

MRI-009: Endogenous 31P CEST: A tool for monitoring Glycolysis without contrast agents

Ms Giulia Vassallo[1], Ms Cecilia Fiorucci[1], Mrs Francesca Garello[1], Mr Silvio Aime[2], Ms Daniela Delli Castelli[1]
[1]Department of Molecular Biotechnology and Health Science, University of Turin, Via Nizza 52, 10126Turin, Italy, Turin, Italy, [2]IRCCS SDN SynLab, Via E. Gianturco 113, 80143 Napoli, Italy, Napoli, Italy

Introduction-Monitoring metabolic alteration in vivo is a powerful tool to diagnose different pathologies. In particular, glycolysis deregulation phenomena have gained a lot of attention in the last years. Numerous Imaging methodologies are being developed in clinical and preclinical settings for imaging glucose metabolism. Despite their promising results, these techniques share the limitation of the use of exogenous probes.

Aim-The aim of this work is the development of a novel and non-invasive MRI method for the in vivo visualization of glycolysis deregulation in tumoral cells through the use of heteronuclear CEST-MRI based on endogenous 31P resonance.

Methods-Three murine Mammary carcinoma cell lines (TS/A - 4T1 - 168 FARN) having different aggressiveness were cultured to reach about 40-60* 10^6 cells. Cells were detached, washed in HEPES buffer and finally centrifuged into a capillary for the NMR measurement. Z-spectra (Irradiation power: 3uT, Irradiation time: 2s), centred on 31P inorganic phosphate signal, were performed on a Bruker Avance 600 operating at 14T at a temperature of 37°C. Control experiments were performed by incubating cells with glycolysis inhibitors.

Results-Z-spectrum of the three cell lines differs significantly among them, revealing the efficacy of the method in discriminating between phenotypes having different aggressiveness. Moreover, measures carried out on control cells displayed a markedly different shape from the untreated one especially where ST from glycolysis substrate is expected.

Conclusions-The method reported based on the acquisition of CEST experiment of 31P belonging to endogenous inorganic phosphate, is a reliable method for monitoring glycolysis activity and to discriminate between tumour phenotypes at different aggressiveness. The advantage over existing methods relies on the possibility of performing the measurement without the use of a contrast agent. Much work has to be done to increase the sensitivity of the method and to optimize the acquisition time.

MRI-010: Contrast Agents Based on Human Serum Albumin and Nitroxides for 1H-MRI and Overhauser-Enhanced MRI

Dr. Alexey Chubarov[1,2], Dmitry Mitin[2], Friedemann Bullinger[3], Dr. Sergey Dobrynin[4], Dr. Jörn Engelmann[3], Prof., Dr. Klaus Scheffler[3], Dr. Olesya Krumkacheva[5], Dr. Kai Buckenmaier Buckenmaier[3], Dr. Igor Kirilyuk[4]
[1]Freie Universitat Berlin, Belin, Germany, [2]Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia, [3]High-Field Magnetic Resonance Center, Max Planck Institute for Biological Cybernetics, Tuebingen, Germany, [4]N.N. Vorozhtsov Institute of Organic Chemistry, Novosibirsk, Russia, [5]International Tomography Center, Novosibirsk, Russia

Introduction
The strategy to reduce the risks associated with the administration of metal complexes for MRI can pass through the use of non-metal contrast agents based on stable organic radicals. Organic radicals are compatible with standard imaging protocols and biocompatible. The incorporation of multiple radicals into supramolecular structures or biopolymers, such as proteins, can enhance their relaxivity, circulation time, and stability.
Aims
We develop macromolecular contrast agents based on nitroxides and human serum albumin (HSA) protein for 1H-MRI, which also can be used for Overhauser dynamic nuclear polarization (ODNP)-enhanced MRI (OMRI). HSA is a major plasma transport protein, which can accumulate in solid tumors due to the enhanced permeability and retention effect, providing delivery of anticancer drugs or imaging probes.
Results
We synthesized albumin conjugates harboring ~ 20 nitroxides. The conjugates retain a secondary structure, absence of the aggregates, and low cytotoxicity of the HSA, which was shown by SDS-PAGE, circular dichroism, and cytotoxicity test. Relaxivities were measured at different magnetic field strengths (1.88, 3, 7, and 14 T). Phantoms were used to demonstrate the potential use of albumin conjugates as a T1- and T2-contrast agent at 3 T and 14 T. Albumin-nitroxide constructions did not show ODNP enhancement in liquids at an ultralow magnetic field (B0 = 92 ± 0.8 μT). However, under the proteolysis conditions mimicking cancer tissue, HSA was cleaved into lower-molecular-weight peptide-nitroxide fragments that activate ODNP capabilities. The maximum achievable enhancement Emax of 50 and a radiofrequency power required to achieve half of Emax, P1/2, of 10 W was obtained. Therefore, albumin-nitroxide conjugates have a high potential to work as protease-responsive, activatable OMRI agents for cancer.
This research was partly funded by the Russian Science Foundation (synthesis of conjugates and MRI, grant No. 23-13-00178) and by the DFG (ODNP studies by K.B., grant No. BU 2694/9-1).

MRI-011: Parallel transmit slice selection in MRI using optimal control with dozens of channels

Mr Uluk Rasulov[1], Prof Ilya Kuprov[1]
[1]University Of Southampton, SOUTHAMPTON, United Kingdom

Magnetic Resonance Imaging (MRI) is a cornerstone in medical imaging, offering unparalleled insights into the body's internal structures without ionizing radiation. However, the hardware used has reached the limits of what is physically, legally or financially possible. Examples include power deposition safeguards in MRI instruments, length of time a patient can be persuaded to stay inside an MRI machine and spatial inhomogeneity of radiofrequency (RF) fields.

In this communication we propose quantum optimal control to overcome some of these limitations. Gradient Ascent Pulse Engineering (GRAPE), a control algorithm originally developed for NMR spectroscopy, emerges as a potent solution. GRAPE operates by iteratively adjusting the control pulses to maximize the fidelity of any defined operation. This approach enables the precise manipulation of the pulse sequence.

We report an application of GRAPE for slice selection of a brain leveraging 16-channel field maps for RF coils. By integrating explicit multi-channel RF field maps into the optimization process, we tailor RF pulses and gradient fields to maximize the uniformity and precision of magnetization transfer, while abiding all limitations imposed. The simulation can be adjusted for any supplied sample, control fields, maximum power per coil and total time, in addition to any other penalties. The optimisation reaches a high fidelity and is calculated in under 10 minutes - fast enough for each new patient.

We implemented GRAPE for the slice selection, but framework of the algorithm can be applied to variety of pulses and experiments in MRI. This advancement holds the promise of extending the capabilities of MRI technology, offering new avenues for clinical diagnosis and research applications.

MRI-012: 1H NMR and MRI markers of phase-separated anisotropic structure formation in plant-protein extrudates

Sam Kuijpers[1], Martijn Gobes[1], Ekaterina D. Garina[2], Dr. Morwarid Mayar[1], John P.M. van Duynhoven[1,3], Dr Camilla Terenzi[1]
[1]Laboratory of Biophysics, Wageningen University and Research, Wageningen, Netherlands, [2]Department of Radiation Science and Technology, Delft University of Technology, Delft, Netherlands, [3]Unilever Global Foods Innovation Centre, Wageningen, Netherlands

1H NMR and MRI methodologies are of great potential for a wide range of applications in food soft matter science, ranging from in vitro/ex situ to in vivo/in situ conditions. In high-moisture extrusion (HME) of plant-based proteins used for meat analogues production, anisotropic fibrous structure is desired to mimic the appearance of animal meat products. Yet, the fundamental understanding of the mechanisms that govern the formation of such phase-separated anisotropic structures inside the extruder remains limited, mainly due to the lack of robust and multiscale measurement approaches. In this work, we demonstrate the use of (i) bulk 1H NMR FID-CPMG and Chemical Exchange Saturation Transfer (CEST) MRI measurements, to unveil the presence of water- and protein-rich phase-separated domains, as well as of (ii) Diffusion Tensor Imaging (DTI) and T2-weighted MRI, to spatially map anisotropic structure formation at µm- up to sub-mm scale in both plant protein extrudates and dead-stop samples. In combination with advanced image analysis, based on Aligned or Rotational Fourier Transform, the fiber alignment degree can be quantitatively mapped by means of weighted order parameters and fractional anisotropy images. These ex situ NMR/MRI results are supported by corresponding nm-scale evidences provided by super-resolution optical microscopy and small-angle X-ray and neutron scattering. The presented ex situ multimodal measurement approach opens the way to future in situ quantification of structure-flow relationships during HME by means of 1H MRI velocimetry.

MRI-013: The Precision of Ultrahigh Field MRI: Detecting intriguing Microstructural Changes in the Brain of Leptin-Deficient Zebrafish

Mr. Rico Singer[1], Ms Alexandra Velthuyzen[1], Dr. Yi Ding[2], Prof Huub de Groot[1], Prof Herman Spaink[2], Prof Alia Alia[1,3]
[1]Leiden Institute of Chemistry, Leiden University, Leiden, Netherlands, [2]Institute of Biology, Leiden University, Leiden, Netherlands, [3]Institute of Medical Physics and Biophysics, Leipzig University, Leipzig, Germany

In this study we used ultrahigh field magnetic resonance microimaging (µMRI) methods at 17.6T to probe changes in the brain of leptin deficient (lep-/-) zebrafish model. Leptin is a hormone that plays a key role in controlling food intake and energy homeostasis. It is currently not clear if leptin deficiency can lead to any alterations in the microstructural features of the brain. In this study we used wild-type (control) and lep-/- mutant zebrafish. Anatomical images as well as chemical shift selective imaging show significant fat accumulation in the brain of mutant lep-/- zebrafish. An elevated T2 relaxation time and significantly reduced apparent diffusion coefficient (ADC) unveil brain-wide microstructural alterations, potentially indicative of inflammation in the brain of lep-/- zebrafish. Diffusion tensor imaging and diffusion kurtosis imaging analysis revealed diminished diffusivity and enhanced kurtosis in various white matter tracks in lep-/- zebrafish compared with control zebrafish, identifying the microstructural underpinnings associated with compromised white matter integrity and axonal degeneration. This study highlights the potential of ultrahigh field MRI at 17.6T in discerning exceptionally fine microstructural details from tiny zebrafish brain and show that leptin deficiency results in severe alterations to the microstructural features of the brain.

Acknowledgements: MRI experiments were supported by uNMR-NL (NWO grant 184.035.207).

MTB-001: Using NMR glycoproteomics as a high-throughput tool for medical diagnostics

Lorena Rudolph[1], Dr. Lina Jegodzinski[2], Dr. Darko Castven[2], Jarne Mölbitz[1], Renia Krellmann[1], Dr. Franziska Schmelter[3], Prof. Dr. Christian Sina[3], Prof. Dr. Jens U. Marquardt[2], Dr. Alvaro Mallagaray[1], Prof. Dr. Ulrich L. Günther[1]
[1]Insitute of Chemistry and Metabolomics, University of Lübeck, Lübeck, Germany, [2]Department of Medicine I, University Medical Center Schleswig-Holstein, Campus Lübeck, Lübeck, Germany, [3]Institute of Nutritional Medicine, University Medical Center Schleswig-Holstein, Campus Lübeck, Lübeck, Germany

Introduction:
We present a fast and accurate method for the quantification of acute-phase inflammation proteins and their associated glycosylation profile from human serum or plasma. The approach combines edited 1D NMR spectroscopy with spectral deconvolution in a fully automated fashion providing as many as 50 new biomarkers sensitive to inflammation and oncogenic transformation. Our methodology delivers top-down results from native proteins in untreated blood samples in < 20 min. The method is illustrated with early detection of metabolic dysfunction-associated steatotic liver disease (MASLD) and its progression to hepatocellular carcinoma (HCC).

Aims:
We aim to identify specific markers of liver disease moving from healthy via simple steatosis, metabolic dysfunction-associated steatohepatitis (MASH), MASH-cirrhosis to HCC, yielding a correlation between disease progression and glycosylation marks in circulating acute-phase proteins.

Methods:
Our integrated NMR strategy combines selective TOCSY (selTOCSY), diffusion difference spectroscopy (DDS), and complementary filtered 1D experiments. Fine-tuning diffusion- and relaxation filters combined with selective spectral irradiation mitigates metabolite and lipoprotein spectral overlapping, revealing quantifiable glycoprotein glycan signals directly from serum. Our method allows to identify altered N-glycosylation patterns, including linkage-specific sialylation levels, galactosylation, fucosylation, and branching complexity of most abundant N-glycans.

Results:
Our analyses reveal distinct glycosylation profiles across MASLD stages, with increased sialylation in steatosis, branch complexity in MASH, and increased concentration of LewisX antigen together with altered α2,3- to α2,6-sialylation in MASH-HCC, as compared to healthy controls. These profiles correlate with disease progression, underscoring the efficacy of our method in identifying subtle variations in glycoprotein concentrations and glycosylation profiles.

Conclusions:
NMR glycoproteomics from serum and plasma samples represent a cost- and time-efficient approach for early detection of MASLD and progression to HCC. By underpinning stage-specific glycoprotein features, our method not only enhances the monitoring of liver disease but also unravels the potential of NMR glycoproteomics as a valuable diagnostic tool.

MTB-002: Exploring the complementarity of fast multi-pulse and multi-dimensional NMR methods for metabolomics: a chemical ecology case study

Aurore Michaud[1,2], Dr. Samuel Bertrand[2], Prof. Serge Akoka[1], Dr. Jonathan Farjon[1], Dr. Estelle Martineau[1], Nicolas Ruiz[2], Thibaut Robiou Du Pont[2], Prof. Olivier Grovel[2], Prof. Patrick Giraudeau[1]
[1]Nantes Université, CNRS, CEISAM UMR 6230, Nantes, France, [2]Institut des Substances et Organismes de la Mer, ISOMer, Nantes Université, UR 2160, Nantes, France

The overwhelming majority of NMR metabolomics studies rely on the acquisition of 1D 1H fingerprints, which are recorded with standard pulse sequences involving solvent signal suppression. 1D 1H spectroscopy has the advantage of being high-throughput (typically a few minutes per sample) and relatively simple to process. However, 1D proton spectra are hampered by severe and ubiquitous peak overlaps arising from the diversity of analytes present in biological samples, associated with a limited frequency range and to the spreading of 1H multiplets. This issue is further compounded by the peak shifting that occurs within sample groups due to pH, concentration or ionic strength variations, making peak integration a complex task.

However, the NMR toolbox includes several promising alternatives for the analysis of complex mixtures, but their application to metabolomics remains scarce. In this study, we evaluate the potential and complementarity of several high-throughput multi-pulse and multi-dimensional NMR methods for metabolomics. Through a chemical ecology case study, three methods are investigated, offering a continuum of methods with complementary features in terms of resolution, sensitivity and experiment time.

Ultrafast 2D COSY, adiabatic INEPT and symmetric adiabatic pure-shift (SYMAPS) HSQC are shown to provide a very good classification ability, comparable to the reference 1D 1H NMR method. Moreover, a detailed analysis of discriminant buckets upon supervised statistical analysis shows that all methods are highly complementary, since they are able to highlight discriminant signals that could not be detected by 1D 1H NMR. In particular, fast 2D methods appear very efficient to discriminate signals located in highly crowded regions of the 1H spectrum.

Overall, the combination of these recent methods within a single NMR metabolomics workflow allows to maximize the accessible metabolic information, and also raises exciting challenges in NMR metabolomics data analysis.

MTB-003: Lipoprotein size and composition variability with sex and age for a better diagnostic of the cardiometabolic risk

Mr Ricardo Conde[1], Mr Ruben Gil-Redondo[1], Ms Maider Bizkarguenaga[1], Ms Angela de Diego[1], Ms Beatriz G. Valle[1], Ms Nieves Embade[1], Mr José M. Mato[1], Mr Óscar Millet[1]
[1]Precision Medicine and Metabolism Laboratory, CIC bioGUNE, BRTA, CIBERehd, Derio, Bizkaia, Spain

Lipoproteins play a pivotal role in cardiovascular health, serving as vehicles for transporting cholesterol and triglycerides through the bloodstream. High-density lipoprotein (HDL), often referred to as 'good' cholesterol, is particularly beneficial in preventing cardiovascular risk. On the other hand, low-density lipoprotein (LDL), known as 'bad' cholesterol, can deposit cholesterol on artery walls, increasing cardiovascular risk. Therefore, maintaining a higher ratio of HDL to LDL is considered protective against heart disease. While this simplistic analysis is useful for assessing cardiovascular risk, it is not sufficiently comprehensive on its own to prevent cardiovascular episodes. NMR spectroscopy allows for a very detailed characterization and quantification of different lipid classes and subclasses including the main apolipoproteins, the particle and sub-particle size distribution and composition; also quantifying phospholipids, cholesterol esters, total and free cholesterol, and triglycerides. This detailed description of the circulating lipoproteins should enable a better prediction for the cardiometabolic risk, but the natural variability with factors such as age and sex are not known. In this work we analyzed more than 20.000 of human serum samples to cluster the lipoprotein parameters according to a criterium of concerted evolution with age and sex in order to define the normal variability ranges. When comparing the non-oriented cohort with a cohort of patients undergoing metabolic syndrome we were able to assign the potential predictive value for the cardiometabolic disease, associated to each lipoprotein parameter.

MTB-004: NMR Metabolomics at CERM

Professor Claudio Luchinat[1]
[1]University Of Florence, Sesto Fiorentino, Italy

Introduction
Metabolomics is the “omic” science focused on measuring the ensemble of metabolites from bio-specimens including urine, serum/plasma, saliva, and tissues. NMR is emerging as an extremely powerful technique to provide metabolomic data through the analysis of biofluids that can be collected non-invasively.

Aims
To improve the technique to provide the basis for an individually tailored screening of pathologies. To do this, we need to scale-up the experimental setting from small pilot studies to large multicentric cohort studies. Therefore, we also need advanced methods for sample collection, spectra acquisition, and metabolite assignment.

Methods
To analyse NMR-metabolomic data, fingerprinting and profiling can be used. In fingerprinting, the entire spectrum is analysed through a bucketing procedure. Fingerprinting needs absolutely optimal standard operating procedures. In profiling, the concentrations of all identifiable and quantifiable metabolites must be determined from the corresponding signal intensities. The accurate identification of the spectral resonances is the bottleneck of this approach.

Results
We have successfully exploited NMR in different pathological contexts, providing significant information on a wide range of diseases, such as cancer, cardiovascular diseases, and COVID-19. On the methodology side, stabilization of urine samples, especially when collected at home, can be achieved by gelification of urines using silica particles. Addition of a small amount of paramagnetic gadolinium chelate permits faster acquisition thanks to the shortening of relaxation delays. A method to provide automated and accurate prediction of chemical shifts in urine was introduced. Now we are further investigating the source of chemical shifts due to metabolite-metabolite interactions.

Conclusions
Methodological advances have shown how speed and information content can be improved and indicate the way to further enhance the value of NMR metabolomic analyses.

MTB-007: Unveiling the Antibacterial Mechanisms of Biogenic Silver Nanoparticles through NMR Analysis

Thyerre Costa[1], Maelson Lacerda[1], Ljubica Tasic[1]
[1]State University of Campinas (Unicamp), São Paulo, Brazil

Systemic infections caused by Staphylococcus aureus (S. aureus), a Gram-positive bacterium, often are triggered by pathogen proliferation or secreted toxins by these microorganisms. Additionally, the escalating resistance to various antibiotics poses a significant threat to public health. Biogenic silver nanoparticles show promise as a safe alternative for topical antimicrobial use compared to conventional agents. This study employs metabolomics via nuclear magnetic resonance (NMR) using a Bruker AVANCE III 600 spectrometer at 25 °C to investigate the effects of two different biogenic silver nanoparticles on pathogenic bacteria, particularly S. aureus. The first type of silver nanoparticles was synthesized using the secretome of the fungus Fusarium oxysporum (AgNP@Fo), while the second one was obtained from the plant extract (Stryphnodendron adstringens) (AgNP@SA). S. aureus metabolite identification involved comparing wild-type cells with those treated with these biological agents, enabling the discovery of altered cellular metabolic pathways and elucidating antimicrobial mechanisms. Both nanoparticles exhibited similar sizes ranging from 20 to 50 nanometers, with zeta potentials of approximately -30 mV. However, AgNP@Fo demonstrated a minimum inhibitory concentration (MIC) of 1.68 μg mL^{-1}, while AgNP@SA had a MIC value of 25.92 μg mL^{-1}. The main metabolic pathways engaged in the antibacterial activity of BioAgNP involve amino acid and lipid metabolism pathways. Through our analysis, we observed a significant reduction in the concentrations of metabolites such as phosphocholine, glycerol, lactate, and glutamate. These metabolites are vital components of key metabolic pathways essential for bacterial survival. Phosphocholine, for example, is crucial in cell membrane synthesis, while lactate contributes to energy production. Additionally, glycerol plays a pivotal role in lipid biosynthesis, and glutamate is involved in amino acid metabolism and the tricarboxylic acid cycle. The observed metabolic changes highlight the significant role of biogenic silver nanoparticles in disrupting essential bacterial pathways.

MTB-008: MAPPING BLOOD SERUM FINGERPRINTS OF VENOUS THROMBOEMBOLISM (VTE) BY NMR

Thyerre Costa[1], Juan Herrera[1], João Pontes[1], Gonzalo Delgado[1], Stephany Huber[1], Joyce Annichino-Bizzacchi[1], Ljubica Tasic[1]
[1]State University of Campinas (Unicamp), São Paulo, Brazil

Venous thromboembolism (VTE) stands as the third leading cause of mortality worldwide, yet remains underexplored through metabolomics. Several studies have suggested a potential link between variations in serum metabolites and the predictive potential of VTE. Nuclear Magnetic Resonance (NMR) emerges as a promising tool for identifying these metabolites. By understanding the metabolic alterations associated with VTE, we can elucidate potential markers allowing for early detection and diagnosis of the condition. This study aims to identify potential metabolite markers for acute venous thrombosis risk using metabolomics. We analyzed approximately 100 serum samples, evenly split between thrombosis-diagnosed individuals and a control group. Each serum sample (300 µL) was mixed with deuterium oxide (300 µL) and transferred into 5-mm NMR tubes. Spectra were acquired using a Bruker AVANCE III 600 spectrometer equipped with a TBI probe at 25 ◦C. We recorded noesy1d one-dimensional 1H-NMR spectra and T2edited spectra using the CPMG pulse sequence with ns = 128. 2D TOCSY and HSQC data were obtained for metabolite identification from randomly selected samples. Multivariate statistical analysis was employed to detect variations in serum metabolome and explore biochemical pathways involved in the disease's pathogenesis. Through receiver operating characteristic (ROC) analyses comparing the healthy control (HC) and VTE groups, we observed significant alterations in glutamine, alanine, glucose, and others, with AUC values exceeding 0.7. From the metabolites, significant for distinguishing the groups, biochemical pathways were analyzed. For example, lactate is a byproduct of anaerobic metabolism, meaning it is produced when cells generate energy without sufficient oxygen, such as during intense exercise or in pathological conditions. In situations of thrombosis, blood circulation may be compromised, leading to oxygen deficiency in affected tissues. This can result in increased production of lactate due to a greater reliance on anaerobic metabolism.

MTB-009. NMR Glycoproteomics Beyond GlycA/B: Quantification of Specific Inflammation Proteins and N-glycosylation Profiles from Serum

Ms. Lorena Rudolph[1], Dr. Lina Jegodzinski[2], Dr. Darko Castven[2], Mr. Jarne Moelbitz[1], Ms. Renia Krellmann[1], Dr. Franziska Schmelter[3], Prof. Christian Sina[3], Prof. Jens U. Marquart[2], Dr. Alvaro Mallagaray[1], Prof. Ulrich Gunther[1]
[1]University of Luebeck, Institute of Chemistry and Metabolomics, Luebeck, Germany, [2]University of Luebeck and Medical Center Schleswig-Holstein, Department of Medicine 1, Luebeck, Germany, [3]University of Luebeck and Medical Center Schleswig-Holstein, Institute of Nutritional Medicine, Luebeck, Germany

We present an integrated NMR strategy for the quantification of acute-phase inflammation proteins and their associated glycosylation profiles from human serum and plasma in less than 20 minutes. Sample preparation involves diluting serum or plasma with a buffer following established protocols, which allows the simultaneous quantification of metabolites, lipoproteins, acute-phase inflammation proteins, and prevalent glycosylation patterns from a single sample.

Our approach combines selective TOCSY (selTOCSY), diffusion difference spectroscopy (DDS), and complementary filtered 1D experiments. Fine-tuning diffusion- and relaxation-based filters, combined with selective spectral irradiation, mitigates spectral overlap from metabolites and lipoproteins, revealing quantifiable signals of proteins and N-glycoprotein glycans directly from serum. Fully automated spectral analysis, based on spectral deconvolution combined with machine learning, identifies as many as 50 new biomarkers sensitive to inflammation and oncogenic transformation.

In a previous study focused on COVID-19 and cardiogenic shock patients, alpha-1-antitrypsin, alpha-1-acid glycoprotein, ceruloplasmin, complement factors C3, C4, and H, haptoglobin, hemopexin, and transferrin could be quantified by NMR. Concentrations showed high correlations with those obtained using standard laboratory methods. Here, we demonstrate how the method can identify altered N-glycosylation patterns, including linkage-specific sialylation levels, galactosylation, fucosylation (Lewis antigens), and the branching complexity of the most abundant N-glycans.

The method is illustrated here with the early detection of metabolic dysfunction-associated steatotic liver disease (MASLD) and its progression to hepatocellular carcinoma (HCC). Our analysis reveals distinct glycosylation profiles across MASLD stages correlating with disease progression, which underscores the efficacy of our method in identifying subtle variations in glycoprotein concentrations and glycosylation profiles.

In conclusion, we demonstrate that by revealing stage-specific glycoprotein characteristics, NMR glycoproteomics offers a cost-effective and time-efficient method for studying pathological processes associated with inflammation and altered N-glycosylation profiles in circulating proteins.

MTB-010: NMR metabonomics of diseases of difficult diagnosis: Perspectives for clinical application

PhD Ljubica Tasic[1]
[1]Biological Chemistry Group, Institute of Chemistry, Department of Organic Chemistry, University of Campinas, UNICAMP, Campinas, Brazil

Our research interests in metabonomics include (i) the use of NMR-based metabonomics in the investigation of clinical and animal model samples; (ii) studying the mechanisms of the alterations provoked because of the disease and/or some drug use through effects on metabolites, and (iii) human biomonitoring studies. Evaluating various human diseases, such as mental health disorders, illicit drug use (crack), cancer in children and adults, and others, using liquid and semi-solid NMR techniques are successfully applied to understand clinical symptoms, which can be nonspecific and variable, yet connected with the biochemical grounds of the investigated diseases.
Any specific problem studied in our research group starts with the sampling, sample preparation for both types of samples - liquid and semi-solid, and well-planned NMR experiments. Further, spectral processing, chemometrics, and an interpretation of the NMR data by linking them with the biochemical molecular basis of the investigated problems are the goals we aim for.
NMR spectra are acquired with different pulse sequences, such as noesy1d, T2-edited, diffusion-edited, TOCSY, and HSQC, allowing for detecting amino acids, organic acids, glucose, lipoproteins, lipids, and others. In most metabonomics applications, biological samples are analyzed by solution NMR (600 MHz, Bruker), on the other side, analysis of intact tissues is performed by HR-MAS NMR (400 MHz, Bruker).

On this occasion, important brain disorders are going to be discussed, which show similar clinical symptoms, not rarely puzzling diagnoses, such as bipolar disorder and schizophrenia. Understanding the prescribed drug effects on metabolome and lipidome can greatly aid in distinguishing the brain disorders' metabolic hallmarks, which are investigated by applying translational studies based on animal models' treatments with drugs commonly used for treating the brain disorders symptoms.
In the end, we show the applicability of metabonomics by NMR in the clinic and share some interesting and exciting findings.

MTB-011: NMR lipidomics in assumed “healthy” and diseased subjects

Dr Calin Deleanu[1,2], Dr Alina Nicolescu[1,2]
[1]"C. D. Nenitescu" Institute of Organic and Supramolecular Chemistry, Bucharest, Romania, [2]“"Petru Poni” Institute of Macromolecular Chemistry, Iasi, Romania

Metabolomics is a well-established topic both in education and in a wide range of research areas. However, NMR lipidomics is less explored in comparison with NMR metabolomics.
We describe a study on the influence of feeding status on advanced NMR lipoprofiling with particular focus on patients hospitalized in a cardiovascular emergency unit. In emergency situations a statin-based treatment is started asap based on first results of LDL levels. In unconscious patients the feeding status is usually unknown. Thus, we evaluated the influence of feeding on lipoprotein fractions and we concluded that treatment may be initiated based on LDL levels regardless of the feeding status.
We describe a second study on healthy subjects for which the blood concentrations of some wide spread environmental pollutants correlate with some lipoprotein fractions. The results rise the question on how we define “controls” in the modern society.
Recent studies have proven impressive interlaboratory reproducibility of NMR spectra when employing trained personnel and the latest industry standard solutions [1,2]. In addition to dedicated metabolomics groups, NMR metabolomics has penetrated many groups with nonexclusive or even marginal metabolomics interests. Thus, we have also evaluated the NMR reproducibility for metabolomics and lipidomics in a “real-life” situation when combining both industry standard NMR solutions and multipurpose NMR equipment and we compared the reliability of NMR metabolomics data when involving both dedicated NMR operators and chemistry users from outside the NMR group. We present expanded reproducibility results based on our previous NMR interlaboratory assessment [3].

[1] S. Monsonis Centelles et al, Anal. Chem. 2017, 89, 8004-8012.
[2] B. Jiménez et al, Anal. Chem. 2018, 90, 11962-11971.
[3] C. Stavarache et al, Diagnostics 2022, 12, 559.

MTB-012: NMR-based metabolomics study of mercury exposure in communities of the Brazilian Amazon

PhD Ljubica Tasic[1], PhD João Guilherme de Moraes Pontes[1], PhD Bruno Alves Rocha[2], PhD Fernando Barbosa Júnior[2]
[1]Biological Chemistry Group, Institute of Chemistry, Department of Organic Chemistry, University of Campinas, UNICAMP, Campinas, Brazil, [2]Faculty of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Brazil

Mercury (Hg) is a toxic element released into the environment and water, mostly accidentally, bringing a great threat to riparian populations through contaminated fish consumption, causing hazards to the cardiovascular and neurological systems. Besides mercury, there are also different fish intake studies evaluating selenium (Se) levels, since the fish consumption is correlated with Se indices status, which could interact with the methylmercury or with inorganic Hg through mechanisms still not understood.

Herein, we aimed to evaluate the impact of mercury on human metabolism through an NMR-based study exploring blood serum samples of riparian populations from Amazonas State, Brazil. Furthermore, we aimed to evaluate their metabolomes in a comparative study with different blood selenium levels.

A total of 351 samples has been analyzed by NMR spectroscopy (Bruker) 600 MHz using an iProbe TBO with 128 scans of acquisition of NOESY (noesygpp1d), CPMG (cpmgpr1d), and 2D spectra. Samples were classified in five groups: 1) high selenium levels ([Se] > 160 µg/L); 2) low selenium and mercury levels ([Se] < 120 µg/L and [Hg] < 20 µg/L); 3) low selenium levels and high mercury levels ([Se] < 160 µg/L and [Hg] > 20 µg/L); 4) high selenium and mercury levels ([Se] > 160 µg/L and [Hg] > 20 µg/L); and 5) control group ([Se] between 120-160 µg/L and [Hg] < 20 µg/L). Partial Least Squares Discriminant Analysis (PLS-DA) was performed using the MetaboAnalyst platform.

Metabolic changes associated with oxidative stress, inflammation, mitochondrial function, endocrine balance, and alteration in metabolic pathways were noticed, and an understanding of exposure risks to Hg and Se shed light on toxicometabolomics in the Amazonian riparian populations. Understanding the changes in metabolites caused by mercury exposure is very important for evaluating health risk factors and our understanding of selenium benefits versus toxicity.

MTB-013: Insights on Venous Thromboembolism and Antiphospholipid antibody syndrome by NMR Metabolomics

PhD Ljubica Tasic[1], PhD Lucas G. Martins[2], PhD Folurunsho Bright Omage[2], Mr Erik S. Braga[1], MSci Bruna M. Manzini[2], PhD Silmara Montalvão[2], Professor Joyce MAnnichino-Bizzacchi Joyce M Annichino-Bizzacchi[2]
[1]Universidade Estadual De Campinas, Campinas, Brazil, [2]Laboratory of Hemostasis, HEMOCENTRO-UNICAMP, University of Campinas, Campinas, Brazil

Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by recurrent arterial or venous thrombosis or obstetrical morbidities accompanied by antiphospholipid antibodies (aPLs). The pathophysiology of APS is complex, containing several pathogenic mechanisms related to coagulation, endothelium, and platelets, which may increase the risk of thromboembolic complications. Metabonomics allows the understanding of the function of metabolites in the organism, giving important information about biological pathways that are compromised by the disease or lead to alterations linked to it.

This study investigated metabolic profiles applying NMR-based metabonomics in serum samples of patients with arterial or venous thromboembolism (VTE) without APS (n = 32), thrombotic primary APS patients (APS, n = 32), and healthy controls (HC) (n = 32). Inclusion criteria were objective diagnosis and age above 16 years. Exclusion criteria were secondary APS, infection, rheumatologic, renal, hepatic, or inflammatory disease, as well as the use of corticosteroids. APS diagnosis was defined by persistently positive aPL. Thrombotic APS patients were matched by age and gender to VTE without APS and HC.

Differences were observed in metabolic profiles between VTE and HC, APS and HC, as well as between VTE and triple-positive APS groups. The changes in metabolites indicated significant alterations in metabolic pathways of glycolysis, TCA cycle, lipid metabolism, and branched-chain amino acid (BCAA) metabolism.

Machine learning (ML) methods were capable of distinguishing the three groups, with comparable performance of Random Forest and XGBoost. Histidine, 3-hydroxybutyrate, and threonine as the top three metabolites with the most substantial impact on model predictions, suggesting these metabolites play a pivotal role in distinguishing between APS, VTE, and HC.

Significant alteration in metabolomic profiles pointed to complex pathogenesis mechanisms of APS and VTE. These metabolites might be potential biomarkers to differentiate triple positive APS and VTE patients, including the parameter of aPLs for APS patients.

MTB-014: Nutrition-induced changes in infants during weaning studied by urinary NMR metabolomics

Simona Rambousek[1], Caroline I. Le Roy[1], Nicholas P. Hays[2], Cathriona Monnard[1], Gonçalo Graça[1], Jose M. Ramos-Nieves[1], Josef K. Vogt[3], Janne M. Moll[3], Elvira M. Estorninos[4], Jean-Philippe Godin[1]
[1]Nestlé Research, Société des Produits Nestlé S.A., Lausanne, Switzerland, [2]Nestlé Product Technology Center – Nutrition, Vevey, Switzerland, [3]Clinical Microbiomics, København, Denmark, [4]Asian Hospital and Medical Center, Muntinlupa City, Philippines

Urine metabolomics using Nuclear Magnetic Resonance (NMR) spectroscopy has emerged as a powerful tool for studying infant metabolic development. We assessed urinary metabolome of infants participating in a controlled trial investigating the impact of a nutritional intervention on infant metabolic development during the weaning period (6-12 months of age).
In the two-arm parallel study, a control group (n=49) received standard follow-up formula (FUF) and infant cereal, while an experimental group (n=51) received a lower-protein FUF and cereal containing whole grains and pulses. Urine samples were collected at 6, 9, and 12 months. Untargeted and targeted ^{1}H NMR metabolomics were performed, and the effect of the diet regimens were compared using univariate and multivariate analyses.
The feeding regimen at 9 and 12 months of age significantly impacted the levels of 26 urinary metabolites (q value < 0.05). The variations observed in the urine of the EXPL group were primarily induced by a decrease in metabolites related to the protein intake. This mainly includes amino acids and their catabolites and metabolites thereof (i.e. branched-chain amino acids, urea, dimethylsulfone and carnitine). Infants were further classified into 4 metabolic phenotypes (metabotypes) based on the urinary metabolite clustering. At baseline (6 months), the majority of infants exhibited the same metabotype. Following the initiation of the intervention, the infants divided into two distinct metabotypes, defined by their respective feeding regimens. This differentiation persisted at follow-up visits (9 and 12 months). This new urinary ^{1}H-NMR metabolomics study performed in infants with different feeding regimens provides further evidence that urinary metabotype is strongly influenced by diet, and thus is a potential tool to monitor effects of nutritional studies. Further evaluation is necessary to assess the short- and long-term effects of such metabolic differences.

MTB-015: Unraveling biological aging: machine learning models for identifying age-related metabolic deterioration from NMR serum data

Alain Ibáñez de Opakua[1,2], Rubén Gil-Redondo[2], Maider Bizkarguenaga[2], Ángela de Diego[2], Ricardo Conde[2], Tammo Diercks[2], Beatriz González-Valle[2], Nieves Embade[2], José María Mato[2], Oscar Millet[1,2]
[1]ATLAS Molecular Pharma, Bizkaia Science and Technology Park, Derio, Spain, [2]Precision Medicine and Metabolism Laboratory, CIC bioGUNE, Basque Research and Technology Alliance (BRTA), Bizkaia Science and Technology Park, Derio, Spain

Aging precipitates a myriad of physiological changes that profoundly impact health outcomes. However, the variability in biological aging among individuals introduces complexity into health predictions. Recognizing and quantifying this variability is imperative for pinpointing those at elevated risk of age-related health deterioration and tailoring personalized interventions to foster healthy aging and prolong lifespan.
To address this challenge, we decided to utilize NMR data from serum samples to develop machine learning models aimed at identifying age-related metabolic deterioration. Leveraging 1H NMR spectra of serum samples from a cohort of over 12,000 healthy patients, we developed machine learning models for predicting chronological age using two approaches: an ensemble stacking model developed using a genetic optimization algorithm and a deep learning model.
Robust associations with chronological age were established, with the ensemble stacking model achieving a commendable correlation coefficient of 0.83 and the deep learning model achieving 0.82. Over 60% of test samples exhibited less than a 5-year error in prediction. Higher age predictions correlated with elevated body mass indices. Additionally, populations afflicted with prostate cancer and inflammatory bowel diseases exhibited higher metabolic age scores on average, with patients with COVID-19 and liver disease showcasing broader distributions.
These findings underscore the potential of our models in unraveling the intricacies of biological aging and highlight the importance of targeted interventions for improved health outcomes across diverse populations.

MTB-016: Hierarchical Maximum Likelihood Estimation for Metabolic Monitoring

Lennart Bosch[1], Jan F. Haase[1], Pernille Jensen[2], Martin B. Plenio[1,3]
[1]Institute of Theoretical Physics, Ulm University, Ulm, Germany, [2]Center for Hyperpolarization in Magnetic Resonance, Technical University of Denmark, Lyngby, Denmark, [3]Center of Integrated Quantum Science and Technology (IQST), Ulm, Germany

In vitro monitoring of metabolic conversions is of immediate relevance to biomedical research and has appplications, e.g., in cell cultivation for anti-body production and immunotherapy.
Experiments have been realized using NMR spectroscopy and hyperpolarized substrate for samples of multiple hundred cells.
To further push the current detection limits, we propose analysis methods based on hierarchical Bayesian models for a joint analysis of hyperpolarized NMR data collected during the metabolic conversion and present a rigorous approach to uncertainty estimation of metabolic rates.

By imposing a kinetic model for the evolution of signal intensitites of individual metabolites over different FID runs, we construct a hierarchical model that exchanges information about observed resonances and intensitites between the runs.
Maxima of corresponding probability distribution are used to derive estimates as well as associated uncertainties of conversion and relaxation rates.
The main result of the present work is the derivation of an analytic expression of the negative log-likelihood as a function of the data and all variable parameters, such as individual resonance frequencies as well as conversion rates, suitable for numeric optimization.

The hierarchical approach is compared to conventional approaches using FT and simple ML estimation on model data as well as experimental data of a pyruvate to lactate conversion.
HML and ML methods are shown to return similar estimates at low SNR, whereas FT estimates exhibit severe statistical fluctuations. The proposed HML method is capable of operation at very low SNR where FT methods are inprecise. Furthermore, it derives estimates of conversion rates directly from the data and does not depend on uncertainty propagation. All these properties makes it particularly interesting for metabolic monitoring with NMR experiments that are typically running close to detection limits, such as single cell studies, where high accuracy and reliable uncertainty estimates are desired.

MTB-017: Metabolomics Studies on the Impact of Glutamine Supplementation on Peripheral Blood Mononuclear Cells Stimulated with Lipopolysaccharide

Patrick Lüer[1,2], Hannah Soergel[1], Lara Stahl[2], Lars Uhlmann[2], Franziska Dopp[1], Dr. Manuel Rodriguez Gomez[2], Dr. Claudia Muhle-Goll[1]
[1]Karlsruhe Institute Of Technology (KIT), Eggenstein-Leopoldshafen, Germany, [2]Max Rubner Institute, Karlsruhe, Germany

Inflammatory processes in humans can be triggered by contact with pathogens, injuries or operations, leading to immunological dysregulations with serious complications. Glutamine, the most abundant amino acid, shows a high potential in boosting the immune system especially in inflammatory states. To monitor changes in different inflammatory states supplemented with various doses of glutamine, we use NMR metabolomics of cell medium. This can give information about the change of metabolism under these different conditions.

To simulate the inflammatory state in vitro, isolated peripheral blood mononuclear cells are stimulated with lipopolysaccharide. Cells were incubated for 48 hours, while supernatant was collected and analyzed at different time points. NMR spectroscopy monitored differences in extracellular metabolites between unstimulated and stimulated as well as glutamine-supplemented and non-supplemented cells, while cytokine concentrations of interleukin 8 were measured to report on the immune status of the stimulated cells.

NMR data of the extracellular medium showed an increased consumption of glucose with increased concentrations of lipopolysaccharide stimulation, independent of the glutamine level. The concomitant increase of lactate production indicates a shift in metabolism towards anaerobic glycolysis. Other metabolites showed LPS induced changes, but independent of glutamine concentration. Formate is a metabolite derived from various cellular processes including one-carbon metabolism, which is known to be enhanced under inflammatory conditions. Omitting glutamine from the medium, formate production stagnated in highly inflammatory states. The same observation was made for interleukin 8, a pro-inflammatory cytokine. These results show that an insufficient supply of glutamine in cell culture experiments can have a limiting effect on immunological reactions. Glutamine deficiency may also negatively immune system interactions in vivo. Thus, supplementation of glutamine under highly inflammatory conditions may be useful.

MTB-018: MetSCORE: a molecular metric to evaluate the risk of metabolic syndrome based on serum NMR metabolomics

Rubén Gil-Redondo[1], Ricardo Conde[1], Chiara Bruzzone[1], Maria Luisa Seco[2], Maider Bizkarguenaga[1], Beatriz González-Valle[1], Angela de Diego[1], Ana Laín[1], Hansjörg Habisch[3], Christoph Haudum[4], Nicolas Verheyen[5], Barbara Obermayer-Pietsch[4], Sara Margarita[6], Serena Pelusi[6], Ignacio Verde[7], Nádia Oliveira[7], Adriana Sousa[7], Amaia Zabala-Letona[8,9,10], Aida Santos-Martin[9,10,11], Ana Loizaga-Iriarte[9,10,11], Miguel Unda-Urzaiz[9,10,11], Jasmin Kazenwadel[12], Georgy Berezhnoy[12], Tobias Geisler[13], Meinrad Gawaz[13], Claire Cannet[14], Hartmut Schäfer[14], Tammo Diercks[15], Christoph Trautwein[13], Arkaitz Carracedo[8,9,10,16,17], Tobias Madl[13,18], Luca Valenti[6,19], Manfred Spraul[13], Shelly C Lu[20], Nieves Embade[1], José M Mato[1], Oscar Millet[1]
[1]Precision Medicine and Metabolism Laboratory, CIC bioGUNE, BRTA, CIBERehd, Derio, Spain, [2]OSARTEN Kooperativa Elkartea, Arrasate-Mondragón, Spain, [3]Molecular Biology and Biochemistry, Gottfried Schatz Research Center, Medical University of Graz, Graz, Austria, [4]Department of Internal Medicine, Medical University, Graz, Austria, [5]Department of Internal Medicine, Medical University and University Heart Center, Graz, Austria, [6]Precision Medicine Lab, Biological Resource Center and Transfusion Medicine, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milano, Milano, Italy, [7]Health Sciences Research Centre (CICS-UBI), Covilhã, Portugal, [8]CIC bioGUNE, BRTA, Derio, Spain, [9]CIBERONC, Madrid, Spain, [10]CIC bioGUNE-Basurto, Biocruces Bizkaia Health Research Institute, , Spain, [11]Basurto University Hospital, Bilbao, Spain, [12]Werner Siemens Imaging Center, Department for Preclinical Imaging and Radiopharmacy, University of Tübingen, Tübingen, Germany, [13]Department of Internal Medicine III, Cardiology and Angiology, University Hospital Tübingen, Tübingen, Germany, [14]Bruker Biospin GmbH, Ettlingen, Germany, [15]NMR Platform, CIC bioGUNE, BRTA, Derio, Spain, [16]Ikerbasque, Basque Foundation for Science, Bilbao, Spain, [17]Biochemistry and Molecular Biology Department, University of the Basque Country (UPV/EHU), Bilbao, Spain, [18]BioTechMed-Graz, Graz, Austria, [19]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milano, Italy, [20]Karsh Division of Gastroenterology and Hepatology, Cedars-Sinai Medical Center, Los Angeles, United States, [21]CIBER Enfermedades Hepáticas y Digestivas, Madrid, Spain

Introduction

Metabolic syndrome (MetS) is a cluster of medical conditions and risk factors correlating with insulin resistance that, when occurring together in an individual, increase the risk of developing life hazardous cardiometabolic health problems. The specific criteria for diagnosing MetS are challenging and vary among different medical organizations but are typically based on the evaluation of abdominal obesity, high blood pressure, hyperglycemia, and dyslipidemia.

Aims

To develop a unique, quantitative, and independent estimation of both the risk and progression of MetS, based solely on quantitative biomarkers.

Methods

We used NMR-based metabolomics on a large cohort of donors (n= 21,323 ; 37.5% female) to investigate the diagnostic value of serum to estimate the MetS risk. Specifically, we have determined a plethora of circulating metabolites and the lipoprotein composition in serum samples, integrating this data to derive MetS biomarkers and train a machine learning model for disease detection.

Results

We have developed MetSCORE, a metabolic model of MetS that combines serum lipoprotein and metabolite information. MetSCORE discriminate patients with MetS (independently identified using the WHO criterium) from general population, with an AUROC of 0.94. This continuous model can quantitatively stratify risk factors according to their contribution to the development of MetS.

Conclusions

We believe that MetSCORE may be an insightful tool for early intervention and lifestyle modifications, potentially preventing the aggravation of metabolic syndrome.

MTB-019: CHARACTERIZATION OF ITALIAN, RED-FLESHED APPLES: NMR INSIGHTS INTO VARIETAL, STORAGE, AND GEOGRAPHIC FACTORS.

Dr. Federico Brigante[1,2], Dr. Pavel Solovyev[1], Dr. Luana Bontempo[1,2]
[1]Edmund Mach Foundation, San Michele A/A., Italy, [2]Fondazione On Foods, Parma, Italy

Introduction: Red-fleshed apples possess outstanding visual appeal, organoleptic properties, and a metabolite profile enriched in compounds that provide benefits for consumers' health, turning them into functional foods. Modified atmospheres during apple storage are used to control oxidative browning and to delay undesirable biological and biochemical changes. Widely targeted metabolomics is an approach that combines untargeted metabolomics and targeted metabolomics to achieve high-resolution, wide-coverage, and quantification. Aim: Study the influence of storage time, variety, and geographic origin in the quality of non-commercial red-fleshed apple varieties. Methods: The juice of different varieties (R204, R205) of Italian, red-fleshed apples from different geographic locations (Pietramurata, Cunevo, Nave San Rocco) stored in a low-oxygen atmosphere at 3 storage timepoints (0, 2, and 4 months) was extracted and analyzed by 1H NMR. 21 metabolites were identified and quantified in the samples. (O)PLS-DA models were performed with the 3 factors and variables selected by VIP score. Results: Classification accuracy of the test set was 100% for time and varietal factors, but decreased for the geographic classification, where less separation was observed between the classes in the scores plot. Among the selected discriminant variables for each factor, 2-hexenal was in higher concentration after 2 and 4 months of storage. Acetaldehyde, lactate, and ethanol showed an increase after 2 months, since they are part of the pyruvate metabolism cycle which is activated in low-oxygen conditions. Regarding the variety, R205 showed a profile enriched in choline, chlorogenic acid, and itaconic acid, while also in sugars. In terms of geographic origin, Pietramurata was clearly separated from the other locations. These apples had lower concentrations of malic acid and higher concentrations of sugars like xylose, arabinose, and sucrose. Conclusions: Variety R205 from Pietramurata showed an enriched profile of flavor, and functional compounds that provide benefits for health after 2 and 4 months.

MTB-020: GENUS SOLANUM: NMR METABOLOMICS INSIGHTS FOR CHEMOTAXONOMICAL CLASSIFICATION

Dr. Federico Brigante[1,2], Dr. Franco Chiarini[3], Dr. Veronica Baroni[3], Dr. Manuela Garcia[3]
[1]Edmund Mach Foundation, Trento, Italy, [2]Fondazione OnFoods, Parma, Italy, [3]Universidad Nacional de Cordoba , Cordoba, Argentina

Introduction: Solanaceae is an important family that comprise over 100 genera and more than 3000 species with considerable economic importance. Steroidal alkaloids and their glycosides from Solanum are gaining a wide interest in various pharmaceutical industries for their validated biological activities. Metabolomics includes studies of a wide range of metabolic intermediaries, and multivariate statistics and machine learning methods are of special importance in this area. NMR techniques bring a lot of structural information from 1D and 2D experiments in a single spectrum.
Aim: Differentiate species of the Solanum genus by means of metabolomics studies by 1H NMR of their alkaloidal extracts.
Methods: Solanum species were collected from some regions of the country. An ethanolic global extract was obtained and a selective extraction of alkaloids in leaves and fruits was performed. Then the NMR spectra were acquired on a 400 MHz instrument and quality control samples were prepared to control the instrumental dispersion. Then, the spectra were processed using the MNova 14.2 software (baseline correction, phase, normalization, referencing, binning) and then the MetaboAnalyst and R Studio platforms for exploratory and classification analyses.
Results: PCA of 10 species of the genus (including leaves, fruits, and quality control samples) showed a 52.5% of the total variance explained between the first two main components with a good clustering of the quality control samples. A classification analysis shows the difference between species without class error according to signals in the 1H-NMR spectra corresponding to different steroidal alkaloids present in this genus. Some of the discriminant variables from the machine learning approach were tentatively identified as part of solanocapsine nucleus.
Conclusion: The genus Solanum could be initially classified based on the presence of steroidal alkaloids. More of these molecules will be identified as chemotaxonomical markers.

MTB-021: Determination of Hesperidin Contents in Citrus Juices by 1D and 2D NMR

Ms Liisi Karlep[1], Ms Adriana Kalmõkova[1], Dr Lauri Toom[2], Dr Ago Samoson[1]
[1]Tallinn University Of Technology, Tallinn, Estonia, [2]University of Tartu, Tartu, Estonia

Hesperidin, a secondary metabolite from citrus fruits, is known for its antiviral, anti-inflammatory and antioxidative properties, preventing cancer and cardiovascular diseases. Most importantly in contemporary context it is also a potential inhibitor against SARS-CoV-2 infection.
In this work we assessed hesperidin content in citrus fruit juices from orange (Citrus sinensis) and tangerine (Citrus reticulata), commercially acquired and homemade juices and peel extract with 1D 1H and 2D NMR.
Tangerine juice pressed from whole fruits including peels was frozen, after defrosting it separated into two fractions, top translucent and bottom opaque. The fractions of the tangerine juice, store-bought orange juice and pieces of tangerine peels were lyophilized. The peels were ground to a powder and all samples were dissolved in DMSO-d6, vortexed and centrifuged. Supernatant of each sample was analysed by 1D 1H NMR and 2D HSQC NMR at 700 MHz.
The opaque fraction of the homemade tangerine juice had the largest hesperidin content of the juices analysed, which was about 13 times higher than for the store-bought juice and that of the translucent fraction of it.
To make the best of the health benefits of hesperidin we recommend choosing freshly squeezed or frozen homemade citrus juice rather than prepackaged commercially available juices, shaken not stirred.

MTB-022: Validation of the software e-COMETA for the identification of congenital metabolic diseases in neonates using NMR analysis of urine samples.

Beatriz González Valle[1], Rubén Gil-Redondo[1], Nieves Embade[1], Maider Bizkarguenaga[1], Chiara Bruzzone[1], Ángela de Diego[1], Ricardo Conde[1], Claire Cannet[2], Manferd Spraul[2], José M. Mato[1,3], Óscar Millet[1,3,4]
[1]Precision Medicine and Metabolism Laboratory, CIC bioGUNE, BRTA, Derio, Spain, [2]Bruker BioSpin, Ettlingen, Germany, [3] Biomedical Research Network on Hepatic and Digestive Diseases (CIBEREHD), Madrid, Spain. , Madrid, Spain, [4]ATLAS Molecular Pharma, Derio, Spain

Inborn errors of metabolism (IEMs) constitute a group of rare disorders, each stemming from deficient activity of a single enzyme within a metabolic pathway. Early diagnosis is crucial to provide appropriate therapy to newborns, a pivotal step in preventing mortality and complications associated with many IEMs. Newborn screening, commonly known as heel prick testing during the first days of life, aims for the early detection of congenital metabolic disorders. However, the number of diseases that can be detected is limited.

Nuclear Magnetic Resonance (NMR) is a highly suitable technique for characterizing biofluids. Through collaboration with Bruker BioSpin and employing the IVDr technique for 1H-NMR spectrum measurement, we can simultaneously identify and quantify 149 metabolites in a urine sample at clinically relevant concentrations for newborns [1].

With the aim of using NMR as a tool for the early diagnosis of IEM, we have developed a database in the form of a software tool called e-COMETA, based on R code, through a bibliographic review of inborn errors of metabolism. This tool can read quantification files extracted from NMR analysis and traduce them to a score that indicates the tendency to have one or more of these 75 diseases.

This work demonstrates the validation of this software through the analysis and quantification of metabolites in more than 400 neonates using NMR, aiming to assess its functionality and capability to identify up to 75 different types of IEMs in the early days of life.

[1] Sci Rep. 2019 Sep 10;9(1):13067.
[2] Adv Exp Med Biol. 2021;1280:19-37.
3] Anal Chem. 2017 Jan 3;89(1):490-510.

MTB-023: Comparative analysis of French artichoke and cardoon leaf extracts by NMR metabolomics

Aurelien Viret[1], Dr Asma Bourafai-Aziez[2], Gwladys Charpentier[2], Emmanuel Cassin[2], Guillaume Rousselot[2], Dr Hassan Oulyadi[1], Dr Corinne Loutelier-Bouhris[1], Dr Laure Guilhaudis[1]
[1]Univ Rouen Normandie, INSA Rouen Normandie, CNRS, Normandie Univ, Mont Saint Aignan, France,
[2]EVEAR Extraction, Coutures, France

Artichoke leaf extracts (ALE) of Cynara cardunculus scolymus, or Globe artichoke, are known to have health benefits such as antioxidant, hepatoprotective and cholesterol lowering effects [1,2]. Cardoon belongs to the same species as the artichoke and its leaf extracts share a number of biological effects with those of artichoke [3]. The main objective of this study is to compare cultivars of each plant, collected in France, to highlight differences in composition with a metabolomic approach.

Dry leaves were first extracted with an infusion method to obtain a cardoon or an artichoke liquor. Two extractions per cultivars were performed. Liquors were then ultrafiltrated to remove remaining macromolecules. Before NMR analysis, an acetate buffer (pH 5.0) solution containing DSS-d6 was added to stabilize sample pH and to obtain standardized chemical shifts. For each sample, a 1D 1H NOESY spectrum with pre-saturation on water signal was acquired.

Spectra were processed and reduced with NMRProcflow [4] and multivariate statistical analyses were performed to investigate sample differences. PCA indicate that, in addition to species differentiation, some samples exhibit different fingerprints within each species. Unsupervised analyses are currently undergoing to explain these differences.
In parallel, a profiling has been started using Chenomx and 2D homonuclear and heteronuclear spectra. About 30 compounds were identified. First analyses show that differences within artichokes are linked to sugars and amino acids while for cardoons, there are due to aromatic compounds.

1. Salem M Ben, Affes H, Ksouda K, et al. Plant Foods for Human Nutrition. 2015;70:441.
2. Wauquier F, Boutin-Wittrant L, Viret A, et al. Nutrients. 2021;13:2653.
3. Zayed A, Serag A and Farag MA. J Funct Foods. 2020; 69:103937.
4. Jacob D, Deborde C, Lefebvre et al. Metabolomics. 2017;13:36.

MTB-024: Metabolomic Profiling in Premenopausal Women by NMR metabolomics

Maider Bizkarguenaga[1], Rubén Gil-Redondo[1], Ángela de Diego[1], Ricardo Conde[1], Tammo Dierks[2], Gary Sánchez-Gordón[3,4], Nerea Fernandez de Larrea-Baz[3,4], Adela Castelló[3,4], Marina Pollán[3,4], Nieves Embade[1], Professor José M. Mato[1,5], Óscar Millet[1,5]
[1]CIC bioGUNE, BRTA, Derio, Spain, [2]NMR Platform, CIC bioGUNE, BRTA, Derio, Spain, [3]CNE-ISCIII, Centro Nacional de Epidemiología–Instituto de Salud Carlos III, Madrid, Spain, [4]CIBER Epidemiología y Salud Pública, Madrid, Spain, [5]CIBER Enfermedades Hepáticas y Digestivas, Madrid, Spain

Research on premenopausal women's health covers a range of topics, including hormonal regulation, metabolic changes, disease susceptibility and lifestyle. Metabolomics allows for the comprehensive analysis of the unique metabolic profiles of premenopausal women and can identify biomarkers that provide insights into various physiological and pathological states, such as cardiovascular risk, metabolic disorders, inflammation and obesity-related health issues in this population[1,2].
To identify specific biomarkers for the premenopausal stage, we have compared a wide range of metabolites and lipoproteins in 41-50 years old premenopausal women with younger (25-40 years old) and elder (>50 years) women of the working population of the Basque Country and Madrid. Serum samples were analyzed using Bruker´s IVDr (In-Vitro Diagnostics research) methodology, a standardized approach used in NMR serum metabolomic analysis. This enables the quantification up to 50 metabolites, such as amino acids, sugars and other small organic molecules. Also, IVDr methodologies allow for the detailed quantification of 120 lipid subclasses.
Through PLS-DA analysis we have identified different metabolite profiles in serum of premenopausal women from both the population of women aged 25-40 and over 50. Additionally, premenopausal women presented higher values of some metabolites involved in the metabolic pathways of ketone bodies than women over 50. On the other hand, metabolic pathways involving several amino acids were increased in women under 40.
The differences found in the metabolic profile of women of different ages supports the move towards personalized medicine. By understanding individual metabolic profiles, treatments and preventive measures can be tailored to the specific needs of each woman, improving efficacy and reducing adverse effects.

[1]Binienda, A., et al. (2019). Metabolomics in Women's Health and Medicine." International Journal of Molecular Sciences; 20(22), 5460.
[2]Del Pozo MDP., et al. (2020). Serum Phospholipid Fatty Acids Levels, Anthropometric Variables and Adiposity in Spanish Premenopausal Women. Nutrients; 12(6):1895.

MTB-025: Investigating the use of low-field NMR for metabolomic studies

Mr. Tomás Martín[1], Dr. Jose Luis Izquierdo-Garcia[2,3,4], Dr. Laura Castañar[1]
[1]Faculty of Chemical Sciences, Complutense University of Madrid, Madrid, Spain, [2]Instituto Pluridisciplinar, Complutense University of Madrid, Madrid, Spain, [3]Ciber de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain, [4]Faculty of Pharmacy, Complutense University of Madrid, Madrid, Spain

NMR-based metabolomics has emerge as a valuable tool for identifying biomarkers and studying the molecular mechanisms involved in specific diseases, aiding to improve diagnosis and treatment.[1] Despite its potential, the expensive cost of high-resolution NMR spectrometers has limited its use in clinical environments. In recent years, there has been growing interest in using low-field (benchtop) NMR spectrometers for biomedical applications due to their small size and low cost.[2] However, benchtop NMR-based metabolomics is mainly limited by two factors: sensitivity and resolution.
In this work, we investigate the impact of both sensitivity and spectral resolution on NMR-based metabolomic analysis of COVID-19. For the former, we conducted a detailed study of the limit of signal-to-noise ratio from which relevant statistical information can be obtained. For the later, we explored the use of pure shift NMR to enhance resolution by removing the effects of homonuclear scalar couplings from the spectra. Preliminary results show that pure shift NMR significantly reduce signal overlap, aiding in metabolite identification and improving statistical analysis. This study utilized both experimental and simulated data, obtained using a Magritek Spinsolve 80 MHz spectrometer and the Spinach software[3],respectively. Our findings suggest that using optimal sensitivity and spectral resolution can enhance the effectiveness of low-field NMR in metabolomic studies, potentially broadening its application in clinical environments.

References:
[1] C. Bruzzone, R. Conde, N. Embade, J.M. Mato and O. Millet, Front. Mol. Biosci. 2023,2, doi.org/10.3389/fmolb.2023.1111482
[2] P. Alonso-Moreno, I. Rodriguez and J.L. Izquierdo-Garcia, Metabolites 2023, 13, doi.org/10.3390/ metabo13050614
[3] H.J. Hogben, M. Krzystyniak, G.T.P Charnock, P.J.Hore,and I. Kuprov, J. Magn. Reson. 2011, 208, doi.org/10.1016/j.jmr.2010.11.008

MTB-026: Generic authenticity screening of Whisky by 1H NMR spectroscopy.

Thorsten Mix[1], Dr Thomas Hackl[1,2]
[1]Institute of Organic Chemistry, University of Hamburg, Hamburg, Germany, [2]HAMBURG SCHOOL OF FOOD SCIENCE - Institute of Food Chemistry, University of Hamburg, Hamburg, Germany

When it comes to high-priced products, counterfeiters repeatedly try to sell inferior goods under a false name as a high-quality product. In order to protect consumers from such attempts, it is now possible to analyze the origin and quality of a large number of foodstuffs based on their metabolite composition using NMR spectroscopy. One advantage is that the NMR spectra of such complex metabolite mixtures represent a multi-parametric system that can contain a wide range of information. In combination with chemometric methods, it is possible to visualize this information and highlight relevant features. The signal intensities of individual features provide direct information about the concentration of the individual metabolites in the sample. This combination of information allows a detailed analysis of products even with highly complex characteristics and enables multiple interpretations based on a single measurement. The higher the number of different parameters, the larger the sample set required. In the case of whisky distilled from grain of different origins, distilled in different stills and stored in a variety of casks in different locations and over different periods of time, there are many parameters that contribute to quality.
In this work ^{1}H NMR spectroscopy with water and ethanol suppression of whisky was used to compare some of these different parameters and identify patterns, in order to make conclusions about the origin of different whiskies. Further aspects include information about the maturation period and the types of casks used for ageing. The results of the study could therefore provide a simple and quick reliable tool to facilitate the detection of counterfeits and mislabelling, even for products with such a complex background.

MTB-027: Advancing Cardiometabolic Risk Assessment: NMR-Based Application for Comprehensive Lipoprotein Profiling in Morbid Obesity

Chemistry Marta González Lázaro[1], Ana Cristina Abreu[1], Elena Martínez-Rosales[3], David Ruiz-González[3], Borja Martínez-Téllez[3], Alberto Soriano-Maldonado[3], Enrique G. Artero[3], Francisco Manuel Arrabal Campos[1,2], Ignacio Fernández[1]
[1]1Department of Chemistry and Physics, Research Centre CIAIMBITAL, University of Almería, Spain, Almería, Spain, [2]2Department of Engineering, Escuela Superior de Ingeniería, University of Almería, Spain, Almería, Spain, [3]Department of Education, Faculty of Education Sciences, and SPORT Research Group (CTS-1024), CiBiS Research Center, University of Almería, Spain, Almería, Spain

Introduction. Obesity is a global epidemic, with dyslipidaemia affecting around 60% of morbidly obese individuals. Accurate measurement of lipoproteins in serum samples is crucial for clinical management. NMR spectroscopy has emerged as a validated method for studying metabolic and lipid profiles in obesity, offering insights for targeted interventions.

Aims. The aim of this study is to develop an application that enables the determination and classification of all lipoprotein subclasses, along with their size (diameter) and density. The application will also predict the fraction of chylomicrons and glycoproteins A and B. Altogether, this will contribute to a paradigm shift in the diagnosis and treatment of cardiometabolic disease and related risks and conditions.

Methods. A total of 77 samples were prepared in a 1:1 ratio of blood serum to buffer and measured using a 600 MHz NMR spectrometer equipped with a quadruple cryoprobe. The deconvolution of the NMR signals corresponding to glycoproteins (1.80 to 2.10 ppm) and lipoproteins (0.65 to 0.95 ppm) was carried out using MATLAB Software. Multivariate statistical analyses based on principal component analysis, hierarchical cluster analysis and partial least squares discriminant analysis were performed using the SIMCA package.

Results. We have developed a new tool capable of automatically determining the fraction of various lipoprotein subclasses (CM, VLDL, IDL, LDL and HDL) and two glycoproteins (A and B). This application also estimates parameters such as diameter and density. Using these variables and after dimensionality reduction, a new classification of patients into three main distinct groups was assessed. This new cauterization differs from the one obtained using traditional risk factors.

Conclusions. A new tool, based on adapted literature regarding glycoproteins A and B and various lipoprotein subclasses along with related variables such as size and density, has been developed. This tool aims to transform the current paradigm of cardiometabolic risk diagnosis.

MTB-028: Exploring Metabolic Biomarkers in Long COVID: Insights from NMR Spectroscopy Analysis

Angela de Diego[1], Rubén Gil-Redondo[1], Maider Bizkarguenaga[1], Beatriz González-Valle[1], Nieves Embade[1], Chiara Bruzzone[1], Ricardo Conde[1], Professor JM Mato[1,2], Oscar Millet[1,2,3]
[1]CIC bioGUNE, Derio, España, [2]Biomedical Research Network on Hepatic and Digestive Diseases (CIBEREHD), Madrid, España, [3]ATLAS Molecular Pharma, Derio, España

Since the beginning of the pandemic caused by SARS-CoV-2, several studies indicate that approximately 10–20% of individuals infected by this virus may progress to develop symptoms aligning with long COVID. Long COVID is characterized by the persistence or emergence of new symptoms three months following the initial SARS-CoV-2 infection, with these symptoms enduring for at least two months without any other discernible cause, irrespective of age or severity of initial symptoms.
Although the etiology of long COVID remains unclear, the involvement of various pathophysiological pathways is postulated, such as chronic inflammation, intestinal dysbiosis, immune and neurological issues, hormonal and metabolic dysregulation, etc
In response to the misinformation surrounding this virus, we conducted a metabolic study on COVID-19 patients. Upon discovering significant differences, between controls, patients in the acute state, and those who had recovered from the disease, our aim was to determine if these differences were also evident in long COVID patients.
To achieve this, we employed the Nuclear Magnetic Resonance (NMR) spectroscopy. We analyzed urine and serum samples from 190 long COVID patients and compared them with samples from individuals who had no prior exposure to the virus, collected from the pre-pandemic period. Both univariate and multivariate statistical analyses were performed.
Several metabolites (predominantly in serum samples), as well as various subfractions of lipoproteins (particularly in LDL), showed significant changes between both cohorts. Additionally, it was observed that some metabolites remained at altered levels since the onset of the infection, while others returned to normal levels and remained so in LC patients.
Therefore, we have successfully observed significant differences primarily in serum samples in long COVID patients using Nuclear Magnetic Resonance (NMR) spectroscopy. The metabolic pathways in which these altered metabolites and lipoproteins are involved are numerous, which also indicates the great complexity of this disease.

MTB-029: NMR Glycoproteomics: Characterising Plasma Glycoproteins directly from serum/plasma samples using selTOCSY NMR experiments

Jarne Mölbitz[1], Lorena Rudolph[1], Renia Krellmann[1], Luna Dechent[1], Dr. Álvaro Mallagaray[1], Prof. Ulrich Günther[1]
[1]University Of Luebeck, Luebeck, Germany

Standard 1D NOESY spectra of serum and plasma samples are dominated by metabolites, proteins and lipoproteins, leaving N-acetyl methyl groups from Neu5Ac and GlcNAc moieties (GlycA and GlycB) as the only observable N-glycan signals. We have recently shown how 1D selective TOCSY experiments (selTOCSY) can be used to extract protein-specific, complex glycosylation profiles from human serum and plasma samples. This approach relies on the identification of unique N-glycan signals associated with specific acute-phase proteins, determined by the N-glycan structure, protein environment, and glycan dynamics. Accurate calibration of this method requires detailed knowledge of serum glycoprotein N-glycan NMR profiles obtained under native conditions.

Methods:
As a first step, we have created a reference spectral database of 43 O-glycans, N-glycans, histo-blood group antigens (HBGAs), and Lewis antigens under defined experimental conditions. Assignment of corresponding NMR fingerprints enabled the identification of structural reporting groups suitable for selTOCSY experiments. Next, a panel of acute-phase glycoproteins was purified from human serum using "soft isolation techniques" that preserve native protein folding, including gradient ultracentrifugation and various chromatographic methods. N-glycan composition assessed by NMR of unfolded samples showed good agreement with previously reported mass spectrometry profiles. Characteristic N-glycan fingerprints facilitated the identification of protein-specific glycan profiles.

Results:
Our study reveals that NMR glycosylation signatures in plasma or serum are influenced not only by the glycosylation type but also by the protein to which the glycans are bound. Fucosylated oligosaccharides, observed in pathological processes such as autoimmune diseases, cancer, and atherosclerosis, show specific fucose signals dependent on the glycan structure (core fucose, HBGA, or Lewis antigens) and the associated protein.

Conclusion:
Our findings support the viability of glycoprotein quantification using simple 1D selTOCSY NMR experiments. Plasma and serum glycosylation profiles can differ between various glycoproteins, enhancing the diagnostic value of glycosylation patterns.

MTB-030: GNAT for Stats: A Comprehensive Module for Chemometrics and Metabolomics for NMR data

Mr Hugo Rocha[1], Prof. Anders Malmendal[2], Dr. Guilherme Dal Poggetto[3], Dr. Paul Bowyer[4], Prof. Gareth Morris[1], Dr. Mathias Nilsson[1]
[1]University Of Manchester, Oxford Rd, Manchester., United Kingdom, [2]Roskilde University, Universitetsvej 1, 4000., Denmark, [3]Merck & Co., Inc., Scott Ave, Rahway, NJ 07065., USA, [4]JEOL (UK) Ltd, Silver Court Watchmead, JEOL House., United Kingdom

The General NMR Analysis Toolbox (GNAT) has established itself as a versatile software suite for processing and analyzing NMR data. Now the toolbox has expanded to encompass comprehensive metabolomics analysis. This expansion is driven by the inclusion of a new suite of powerful statistical tools designed for tasks like classification, discrimination, and correlation analysis, such as Principal Component Analysis (PCA), Partial Least Squares Discriminate Analysis (PLS-DA), Orthogonal Projections for Latent Structure Discriminant Analysis (OPLS-DA) and Statistical Total Correlation Spectroscopy (STOCSY). A set of preprocessing tools before metabolomics analysis for binning and variable selection (iPLS and biPLS) are also available, as well as graphical tools for outlier detection. Additionally, graphical tools for outlier detection are available, allowing researchers to identify and address potential data inconsistencies. Model validation and application to unknown samples are a straightforward process, accompanied by relevant analytical figures of merit. All analyzes done in the toolbox can be exported as reports in various formats (i.e. .txt, .xlsx or .mat). The standard version is intended to be run with MATLAB. Users can also use available standalone compiled versions for Windows, Mac, and Linux operating systems. These new functionalities are demonstrated using a test data set for the classification of edible oils.

MTR-001: Exploring active sites in functional materials throughfluoropyridines as NMR probe molecules

Mr Joseph Hurd[1], Dr Yujie Ma[2], Dr Run Zou[1], Dr Paolo Cerreia Vioglio[3], Dr Dinu Iuga[4], Prof Sihai Yang[2], Prof Xiaolei Fan[1], Dr Daniel Lee[1]
[1]University Of Manchester, Department of Chemical Engineering, Manchester, United Kingdom, [2]University Of Manchester, Department of Chemistry, Manchester, United Kingdom, [3]University of Nottingham, Sir Peter Mansfield Imaging Centre, Nottingham, United Kingdom, [4]University of Warwick, Department of Physics, Coventry, United Kingdom

Porous solid acid catalysts such as zeolites are widely used for the industrial processing of olefins as well as for a variety of other reactions. For the continual improvement of such catalysts, it is vital to characterise the acid sites present and their function to aid smart design. Both Brønstedacid and Lewis-acid sites (BAS/LAS) play a key role in their catalytic properties and selectivity; their locations and proximities to one another are therefore of great interest.

Adsorption of pyridine and pyridine derivatives has been widely used to characterise zeolite acid sites through FTIR spectroscopy and is a standard technique [1]. Pyridine adsorption has also been extensively exploited for solid-state NMR spectroscopy, where 15N chemical shifts give information on the strength and type of acid sites present [2,3]. Generally, 15N-enriched pyridine is used for these studies owing to the low natural isotopic abundance of 15N (~0.4%). Moreover, this 15N-enriched pyridine is often deuterated so that interesting 1H NMR signals from the zeolite are not obscured by overlapping signals from the probe molecule. Therefore, extensive studies using pyridine-15N-D5 are costly.

Fluoropyridine species also bind to zeolite surfaces [4] and the 19F 100% natural isotopic abundance, high gyromagnetic ratio, and large chemical shift range make it a sensitive probe nucleus. The foundations for using fluoropyridines as cheap informative probe molecules has been established using moderate- and high-field MAS NMR, as well as with low temperature (100 K) MAS NMR. Moreover, 19F–X heteronuclear couplings have been investigated to determine hostguest
interactions in metal-organic frameworks (e.g. defected UiO-66(Zr)) and new insights into industrially-relevant acid catalysts (zeolites HY and HZSM-5) and BAS-LAS synergy have been unveiled using this methodology. It is anticipated that fluorinated probe molecules will become ubiquitous for intricate surface studies and this work demonstrates their utility.

[1]J.Phys.Chem.,1995,99,10285
[2]J.Phys.Chem.C,2016,120,28533
[3]Solid.State.Nucl.Magn.Reson.,2013,55,12
[4]J.Am.Chem.Soc.,1996,118,3262

MTR-002: Investigation of lithium in graphite anodes via transverse relaxation rates

Andreas Markert[1], Michail Gerasimov[2], Ulrike Krewer[2], Hermann Nirschl[1], Gisela Guthausen[1,3]
[1]KIT, Institute of Mechanical Process Engineering and Mechanics (MVM), Karlsruhe, Germany, [2]KIT, Institute for Applied Materials –Electrochemical Technologies (IAM-ET), Karlsruhe, Germany, [3]KIT, Engler-Bunte-Institut (EBI), Chair of Water Chemistry and Water Technology, Karlsruhe, Germany

Introduction

Ion mobility in anodes, here Li+ in graphite, is an essential aspect for the performance of batteries. Spectrally resolved transverse relaxation reveals the ion mobility in a technically relevant temperature range ($T \in [273, 333]$ K).

Aims

$^7Li^+$ ($I = 3/2$) experiences quadrupolar interactions with the host. Spectra reveal the quadrupolar splitting of the satellites ($\delta\nu_s$) and width of the central transition due to magnetic interactions ($\delta\nu_c$). Transverse relaxation R_2 studied with three pulse sequences allows for a differentiated analysis and the quantification of the correlation time τ_c (T), leading to the activation energy (E_a).

Methods

Battery cells have been formatted and charged to defined States of Charge (SoC), graphite anodes were harvested for ^7Li NMR. $\delta\nu_c$ (T), $\delta\nu_s$ (T), and R_2 (T) have been measured as a function of T and modelled according to a BPP approach.

Results

As the ^7Li shifts reflect the chemical environments of the ions[1], the spectra as a function of T reflect the occupation of interstitial sites in graphite[2]. The amounts of the phases LiC_6, LiC_{12} and LiC_{18}+ have been quantified depending on the thermal state of the used material. R_2 (T) depends on the pulse sequences refocusing properties, with Hahn-Echo leading to the fastest relaxation and $\pi/2$-β-experiment to the slowest. According to BPP, E_a of Li+ mobility in graphite are $\in [0.5, 0.6]$ eV, a solid-state diffusion coefficient amounts to ~$[10^{-16}, 10^{-15}]$ m²/s at room temperature.

Conclusion

^7Li spectra and R_2 (T) were measured on graphite anodes. $\delta\nu_c$, $\delta\nu_s$, and R_2 (T) were quantified and interpreted in terms of ion mobility in the accessible temperature range. Modelling of these parameters reveals the correlation times τ_c (T), activation energies and in consequence the solid-state diffusion coefficient were derived assuming thermal activation and applying the Einstein-Smulochowski equation.

[1]Letellier M. Carbon 2007
[2]Sole C. FaradayDiscuss. (2014)

MTR-003: Speciation of Lanthanide Metal Ion Dopants in Microcrystalline All-Inorganic Halide Perovskite CsPbCl3

Dr Dominik Kubicki[1], Dr Daniel Prochowicz[2], Dr Albert Hofstetter[3], Dr Amita Ummadisingu[4], Prof. Lyndon Emsley[3]
[1]School of Chemistry, University of Birmingham, Birmingham, United Kingdom, [2]Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland, [3]Laboratory of Magnetic Resonance, Institute of Chemical Sciences and Engineering, Lausanne, Switzerland, [4] Manufacturing Futures Laboratory, Department of Chemical Engineering, University College London, London, United Kingdom

Introduction
Lanthanides are versatile modulators of optoelectronic properties owing to their narrow optical emission spectra across the visible and near-infrared range. Their use in metal halide perovskites has recently gained prominence, although their fate in these materials has not yet been established at the atomic level.

Aims
Our objective was to use paramagnetic solid-state NMR strategies to determine if lanthanides have the capacity to dope and modify the structure of a prominent emerging light emission material, cesium lead chloride, $CsPbCl_3$.

Methods
We use ^{133}Cs MAS NMR to establish the speciation of all nonradioactive lanthanide ions (La3+, Ce3+, Pr3+, Nd3+, Sm3+, Sm2+, Eu3+, Eu2+, Gd3+, Tb3+, Dy3+, Ho3+, Er3+, Tm3+, Yb3+, Lu3+) in microcrystalline $CsPbCl_3$. The materials were prepared using mechanochemistry, a highly efficient way of synthesising solids without the use of solvents.

Results
We found that lanthanide doping changes the atomic-level structure of the host material through aliovalent incorporation and new local environments appear that are characterized by substantially shortened T_1 values.
The nearly linear changes in the ^{133}Cs shifts observed for doping with the ions from La3+ through Gd3+ are dominated by structural rather than paramagnetic effects. They are determined by the radius of the lanthanide ion rather than its magnetic properties, and therefore are a proxy of the local octahedral distortion.
On the other hand, for the lanthanides between Tb3+ and Tm3+, paramagnetic effects dominate, and we attribute the changes in the ^{133}Cs NMR spectrum to pseudocontact shifts. We find no evidence of contact shifts on ^{133}Cs, indicating that there is no significant overlap of the unpaired 4f electron spin density with the Cs+ site.

Conclusions
Our work shows that solid-state NMR of metal halide perovskites doped with lanthanides is a highly sensitive indicator of the dopant incorporation, loading, and interaction with the perovskite structure.

MTR-005: NMR on gas transport in heterogeneous polymer membranes

Dr Leoncio Garrido[1], Dr Mar López-González[1], Ms Nastasia Yuriychuk[1], Ms Esperanza Benito[1]
[1]Instituto de Ciencia y Tecnología de Polímeros, ICTP-CSIC, Madrid, Spain

Glassy polymers mixed with fillers having different intrinsic properties (i.e. structure, chemical functionality and porosity) have been proposed to improve the performance of membranes for applications in gas separation. However, the practical application of these membranes remains elusive and the lack of knowledge on the behavior of gas-membrane systems at the molecular scale often leads to the application of empirical rules, extended serialized experimentation or trial-and-error approaches in pursue of the desired objectives. In this regard, the evaluation of changes in the intrinsic NMR properties of the observed nuclei (e.g., relaxation times, chemical shifts, etc.) with multinuclear NMR spectroscopy could inform on variations in the interactions of the molecular species of interest with their environment. Furthermore, NMR spectroscopy combined with pulsed gradient spin-echo (PGSE) methods allow the determination of the solubility and diffusion coefficients of pure and mixed gases in polymer membranes, providing additional information toward the understanding of the gas transport phenomena in these systems.
In recent work, we have described the gas transport properties of mixed matrix membranes (MMMs) based on glassy polymers with porous organic polymers (POPs). The results of measurements using macroscopic methods showed that the addition of a porous biphenyl-based knitting aryl polymer (K2Ph) as filler lead to changes in the permeability of membranes to pure gases varying from negligible to measurable, depending upon the glassy polymer matrix.
To identifying the causes of the differences observed and attain further knowledge on the behavior of gases in complex membranes at molecular scale, here we report the NMR measurements of transport coefficients of pure and mixed carbon dioxide and methane in MMMs based on poly(2,6-dimethyl-p-phenylene oxide) (PPO) and poly[bisphenol A carbonate-co-4,4'-(3,3,5-trimethylcyclohexylidene) diphenol carbonate] (BPA-TMC-PC) with K2Ph. Moreover, the spin-lattice T_1 NMR relaxation time of gases and polymers in the MMMs are determined.

MTR-006: Solid-state 19F- and liquid-state 1H-NMR methods for screening the influence of catalyst removal techniques on performance of proton exchange membranes (PEMs)

Robert Rameker[1], Mark Pluem[2], Dr. Sven Jovanovic[1], Dr. Gunther Schmid[2], Prof.Dr. Rüdiger-A. Eichel[1,3], Pro.Dr. Josef Granwehr[1,3]
[1]Juelich Research Center, Juelich, Germany, [2]Siemens Energy AG, Munich, Germany, [3]RWTH Aachen University, Aachen, Germany

Proton exchange membrane (PEM) electrolysis is considered as a promising technique to produce green hydrogen at high purity. Although PEMs can be operated in an electrolysis cell at high energy efficiency, this method has not yet been implemented on a large scale in the chemical industry. Additional to the insufficient lifetime and high cost of the components a ban of per- and polyfluoroalkyl substances is being discussed. This forces energy companies to use PEMs efficiently, for which reason the industry is in search of ways to recycle the membrane material. In various studies on this topic, the recycling process is based on the use of alcohols to separate the catalyst material from the PEM, but the influence of the separation mechanism on the structure of the PEM is little discussed.
A screening routine to detect alteration of the performance and molecular structure of PEMs is presented and demonstrated on a short side-chain perfluorosulfonic acid ionomer.
^{1}H PFG NMR measurements revealed that proton diffusion is significantly altered by the separation process while at the same time, two instead of one diffusion coefficients were observed. This shows that even simple measurements provide sufficient contrast to qualitatively estimate the invasiveness of a separation technique, which offers an efficient method for industrial quality control.
Additionally, it was possible to identify the nature of degradation from relative intensity changes of different functional groups in the material, as ^{19}F MAS NMR spectra are highly sensitive to chemical alteration. The ^{19}F T_1 relaxation times were also significantly altered by hydration and the separation mechanism, suggesting a change in the spatial structure of the PEM.
Therefore, this work is of particular interest for industry, as it presents methods that provide clear indicators for assessing the quality of PEMs and quantitative indicators for further assessing of degradation processes.

MTR-008: What are hydrophobic eutectic systems? Investigation from the NMR perspective

Dr Ignacio Delso[1], Dr Fernando Bergua[2], Dr Manuela Artal[2]
[1]University Of East Anglia, Norwich, United Kingdom, [2]University of Zaragoza, Zaragoza, Spain

Deep Eutectic Solvent (DES) is an umbrella term that covers an immense variety of molecular liquids with a growing list of applications, with the common feature that the melting temperature of the mixture is much lower that those of their individual components. Two specific subgroups of DES are attracting much interest in the pharmaceutical industry: Natural Deep Eutectic Systems (NADES) and Therapeutic Deep Eutectic Systems (THEDES) because they offer a totally biocompatible alternative to enhance drug solubility and bioavailability.1 However, very little is known about their molecular structure, due to their highly dynamic behaviour.
Making use of a variety of NMR experiments (such as 1H, 13C, NOE, ROE, relaxation, diffusion) and the measurement of several spectroscopic parameters, we have been able to identify key properties in DES. 2-4 These aid us to firstly identifying eutectic mixtures; and secondly, getting a greater understanding of the structure and molecular interactions that underpin the formation of eutectic phases, in combination with molecular dynamic simulations.
In this communication we will present examples of fast and straightforward NMR experiments useful to determine whether a mixture is eutectic, and our findings in the behaviour, dynamics, and eutectic properties of THEDES and hydrophobic drugs solved in NADES.

1. Alvarez, M. S.; Zhang, Y. F., J. Control. Release 2019, 311, 225-232.
2. Bergua, F.; Delso, I.; Munoz-Embid, J.; Lafuente, C.; Artal, M., Food Chem. 2021, 336, 10.
3. Lopez, N.; Delso, I.; Matute, D.; Lafuente, C.; Artal, M., Food Chem. 2020, 306, 9.
4. Delso, I.; Lafuente, C.; Munoz-Embid, J.; Artal, M., J. Mol. Liq. 2019, 290, 8.

MTR-009: Solid-State NMR as a Toolbox Towards a Full Mechanistic Understanding of Catalytic Butene Dimerization

Mirjam Schröder[1,2], Dr. Thanh Huyen Vuong[1], Dr. Jabor Rabeah[1], Prof. Dr. Angelika Brückner[1,2], Prof. Dr. Björn Corzilius[1,2]
[1]Leibniz-Institute for Catalysis Rostock, Rostock, Germany, [2]Institute of Chemistry and Department Life, Light and Matter, University of Rostock, Rostock, Germany

Butene dimerization on nickel supported mesoporous catalysts has been industrially used for several decades. Despite detailed investigations [1-3], the mechanism of this reaction is not fully understood yet. Here, understanding the involvement of individual atoms with different properties in the attachment of Nickel single sites on the surface of the supporting material is of crucial importance. Of particular interest are the Lewis (LAS) and Brønsted acidic sites (BAS) of the surface.

NMR allows for both, an overall view of the material, as well as the ability to target individual sites on the atomic level using probe molecules such as TMPO for tracking of acidic sites. To tune their acidity, the support (MCM41) was varied in terms of metal type (Al or Ga) and amount. We have developed a spectroscopic toolbox to address different aspects of the catalyst's surface by observing nuclei like ^{1}H, ^{31}P, ^{27}Al and ^{29}Si. This allows to probe directly which acidic sites are occupied by Ni and which are needed for the reaction to take place. By this, we show that Ni is only attached onto the surface of Brønsted acidic supports. Double impregnation with Ni and TMPO reveals that with increasing amount of Ni, the amount of the strongest BAS decreases systematically while LAS are not impacted.

Taken together, we present steps towards fully understanding the affinity of Ni to the surface on an atomic/structural level. A low number of strong BAS is seemingly needed for Ni to bind to the surface while weaker BAS in its proximity stabilize the reaction intermediate. In light of sustainability, this knowledge is crucial for improving the industrial catalysts by increasing the highly important selectivity of the reaction.

[1] Brückner et al., Journal of Catalysis 2009,266,120-128. [2] Rabeah et al., ACS Catalysis 2016,6,8224-8228. [3] Vuong et al., ACS Catalysis 2021,11,3541-3552.

MTR-012: Using NMR to determine how polyelectrolytes and their counterions distribute after polyelectrolyte complexation

Dr. Ricardo Martinho[1], Giulia Allegri[1], Hestie Brink, Prof. Jurriaan Huskens, Dr. Saskia Lindhoud
[1]University of Twente, Enschede, Netherlands

Charged biopolymers are ubiquitous in biological systems, playing a key role in the phenomenon of liquid-liquid phase separation. The electrostatic interactions between negatively charged polynucleic acids and positively charged proteins lead to the formation of membraneless organelles. These organelles are known to be involved with the origin of life but also with numerous diseases. In order to understand these systems in a controlled environment, we have performed systematic studies with different synthetic polyelectrolytes capable of undergoing phase separation.
When aqueous solutions of oppositely charged polyelectrolyte are mixed, an entropically driven phase separation occurs, yielding a dense solid-like polymeric phase and a dilute aqueous phase. In this work, we have developed an NMR methodology to quantify the distribution of the different components in the mixtures, both organic (polymers) and inorganic (counterions). To explore the different affinities among polyelectrolytes, we have conducted experiments with combinations of strong (permanently charged) and weak (with pH-dependent charges) species. The organic components in both phases were assessed by quantitative ^{1}H NMR, aided by the ERETIC method. Subsequently, we have determined the distribution of counterions by quantitative ^{23}Na and ^{35}Cl NMR, also aided by ERETIC, which was validated by elemental analysis. This was tested for multiple compositions, which allowed to determine a relative constant high concentration in polymers and ions in the dense phase. As a result, mole balances and differentiation between ionic pairs was possible. The study of different types of polyelectrolytes also revealed distinct affinities for water encapsulation and towards counterions. The latter was quantitatively assessed by the quadrupolar relaxation properties of sodium and chlorine.

MTR-013: Probing Li-N-H materials using solid-state NMR

Dr. Jennifer S. Gómez[1], MSc. Ruipeng Luo[1], Dr. Evan Wenbo Zhao[1], Professor Arno P. M Kentgens[1]
[1]Radboud University, Nijmegen, Netherlands

The Li-N-H materials have received a lot of attention as potential hydrogen storage materials, especially lithium amide ($LiNH_2$) and lithium imide (Li_2NH). Moreover, with the recent developments of lithium-mediated ammonia synthesis, it has been proposed that these compounds may be a key component of the solid-electrolyte interface which influences the ammonia synthesis performance. However, the Li-N-H materials are extremely complex, and detailed structure information must be resolved by synchrotron-XRD. Therefore, more suitable characterization techniques are needed for an in-depth understanding of Li-N-H. Solid-state NMR spectroscopy is an important tool for the investigation of Li-N-H allowing for high-resolution detection and atomic level information. Although ^{1}H and ^{7}Li NMR have been used for the characterization of Li-N-H materials, very few studies have focused on the N atom. This is probably because its detection is challenging either due to the small natural abundance of ^{15}N or the intricate quadrupolar nature of ^{14}N.

In the present work, the structure and dynamics of $LiNH_2$ and Li_2NH were investigated by a combination of ^{1}H and ^{14}N solid-state NMR at high-field. ^{1}H-^{1}H exchange experiments (EXSY) showed that the protons in $LiNH_2$ are an integral part of the main structure, while for Li_2NH they evidenced the presence of two protons species ($LiNH_2$ and Li_2NH) which are inter-connected in a single phase, as they exchange on the NMR time scale (<1 ms). ^{14}N-^{1}H D-HMQC correlation experiments showed the different nitrogen environments, confirming the presence of one and two local environments for $LiNH_2$ and Li_2NH, respectively. ^{15}N CPMAS experiments were also performed showing similar results. These results indicate that the composition of Li_2NH is more complex than previously thought which may relate to its functional behavior, but this needs further investigation. The results demonstrate the potential of using ^{1}H-detected ^{14}N to study complex Li-N-H materials without resorting to ^{15}N labelling.

MTR-014: Molecular Dynamics assisted NMR Study of morphological heterogeneity in self-assembled star-shaped polypeptides hydrogel

Dr. Bing Wu[1], Prof. Dr. Andreas Heise[2]
[1]Radboud University Nijmegen, Nijmegen, Netherlands, [2]Royal College of Surgeons in Ireland, Dublin, Ireland

Polypeptide as the building blocks of living organisms, is an important class of biopolymers. Like natural proteins, these polymers possess the ability to self-assemble into complex yet highly ordered structures as a result of the precisely controlled sequences and compositions of their constituent amino acid monomers. Hydrogels derived from synthetic polypeptides have attracted huge attention from the scientific community owing to their nontoxicity, biodegradability, and low immunogenicity. By functionalizing their amino acid side chain as well as controlling their block size, these polypeptide-based hydrogels' physical and biochemical properties can be systematically tailored and fine-tuned, which makes these materials very appealing for biomedical applications.
In this study, we report the first systematic study about the impact of the dendrimer core size on the physico-chemical properties of star-like hydrogels. A series of star-shaped polypeptides with different dendritic core sizes and estimated same polypeptide chain lengths were synthesized following the procedure described in our previous publication. They were subsequently characterized by GPC and NMR to determine their corresponding weight average molecular weight (Mw) and degree of polymerization (Xn). The individual polypeptide chains were found by NMR to contain 35 polyglutamic acid (PGA) units and 5 polyvaline (PVL) units on average (Figure 1). Afterward, these star-shaped polypeptides dissolved in DI water, and self-assembled to form physical hydrogels owing to the hydrophobic interaction between valine units in different star-shaped polypeptides.
Systematic NMR relaxometry and diffusometry analyses were used to determine the polymer chain dynamics in these hydrogels, while a series of MD simulations were also performed to rationalize the observed change. Considering star-shaped polypeptides have great potential in biomedical applications, this study can guide chemists to prepare these hydrogels with less inherent structural heterogeneity.

MTR-015: Classifying Quantum Dot Ligands through Diffusion Ordered Spectroscopy

Sebastian Sabisch[1,2], Yuliia Berezovska[1,2], Yesim Sahin[1,2], Leon G. Feld[1,2], Dr. Dimirty Dirin[1,2], Prof. Dr. Maksym V. Kovalenko[1,2]
[1]ETH Zürich, Laboratory of Inorganic Chemistry, Zürich, Switzerland, [2]Empa - Swiss Federal

Laboratories for Materials Science and Technology, Dübendorf, Switzerland

Since their discovery in the 1980s semiconductor quantum dots have been established in a vast array of applications ranging from lighting and display technologies where they are used as downconverters or electrically driven emitters, to quantum communication where they are used due to their ability to emit single photons, all the way to photocatalysis. Implementation of an effective defect passivation of the surface, by either shells or ligands, resulted in the synthesis of quantum dots with narrow banded emission and near unit's quantum yields.1 One material class of interest in the realm of quantum dots are lead halide perovskites (LHP), which can be easily synthesized at room temperature with a low polydispersity using weakly binding ligands which are exchanged for the ligand of interest after the growth.2 Depending on the application, different ligands are better suited than others, which cannot be explained by the widely measured metrics of quantum yield or size dispersity but are better explained by their binding dynamics and surface affinities. Using diffusion ordered spectroscopy (DOSY) we aim to classify ligands based on their binding dynamics by observing the effective diffusion of the ligands throughout multiple washing steps. Using this classification, we can provide a guided selection of a suitable ligand for a given application. Comparing the DOSY spectra we find that the commonly used ligands for LHP QDs cover a range from very fast exchanging to near statically binding ligands and weakly binding to non-removeable ligands. The binding affinity is influenced by the ligand headgroup and its interaction with the quantum dot surface, while the rate of exchange is a function of both the headgroup and the tail of the ligand. Using this approach, we can guide a selection for ligands with a high conversion in photocatalysis or a stable dilution for single-dot spectroscopy.

MTR-016: Topological and Chemical Disorder in Caesium Lead Halides Nanoparticles

Ms Lidiia Dubenska[1], Mr. Sebastian Sabisch[1,2], Dr. Andrii Kanak[1,2], Dr. Victoria Morad[1,2], Dr. Marcel Aebli[1,2], Prof. Dr. Maksym Kovalenko[1,2]
[1]ETH Zürich, Laboratory of Inorganic Chemistry, Department of Chemistry and Applied Biosciences, Zürich, Switzerland, [2]Empa – Swiss Federal Laboratories for Materials Science and Technology, Zürich, Switzerland

Lead halide perovskite semiconductor nanocrystals (NCs) have been attracting attention for the last decade due to their compelling optical properties, such as bright and tunable luminescence, with fast radiative rates and long exciton coherence times. However, understanding their static and dynamic structure governing those unique properties is far from being satisfactory. Since lead halide perovskites are rather soft and dynamic materials, one would ideally opt for the least perturbing experimental methods for structural characterization, for example, nuclear magnetic resonance (NMR) spectroscopy. Besides being nuclear-specific, magnetic resonance is extremely sensitive to the topology and geometry as well as subtle changes in the surroundings of the observed nuclei, both in static and dynamic regards. This work focuses on 133Cs NMR as a main method for the structure elucidation of caesium halide perovskites in different forms, from nanocrystals to large single crystals. We report 133Cs NMR spectra of CsPb(Cl/Br)3 over the entire Cl-Br concentration ratio range. Along with DFT calculations and powder XRD experiments, we show that 133Cs NMR signal has good sensitivity to physical changes in its local environment, shifting by approximately 10 ppm toward lower ppm values when the Pb-Pb distance is increased by just 0.01 Å. The chemical composition also plays a significant role, and it has the opposite effect on the 133Cs NMR signal. Moreover, it is known that upon cooling of caesium lead halides, the volume of the unit cell is reduced, leading to a shorter Pb-Pb distance. This, in turn, should lead to changes in 133Cs chemical shift towards higher ppm values, which is readily observed by low temperature measurements. Experimental results fall in good agreement with computational. These findings showcase the great potential of 133Cs NMR to become a valuable tool for the structure elucidation of caesium lead halide nanocrystals.

MTR-017: 19F MAS NMR studies of highly conductive BaF2–CaF2 composite prepared by thermal plasma processing

Dr. Miwa Murakami[1], Mr. Kazuyuki Sato[2], Professor Kiyonori Takegoshi[2]
[1]Tohoku University, Sendai, Japan, [2]Kyoto University, Uji, Japan

Introduction

Ion conduction in solids has been an important subject due, for example, to its application in all-solid-state batteries. To enhance fluoride ion conduction in metal fluoride, one approach is replacement of the metal ion by other metal ions with different valences. Interestingly, enhanced conduction by replacement by a same-valence ion was reported for alternating BaF_2 and CaF_2 layers [1]. It was further shown that BaF_2-CaF_2 composites prepared by mechanical milling bear even higher conductivity [2]. In this presentation, we report ^{19}F-NMR studies of BaF_2-CaF_2 composites prepared by thermal plasma processing.

Aims

To determine distribution and local configuration of cations in highly conductive BaF_2–CaF_2 composite prepared by thermal plasma processing.

Methods

NMR measurements were done at 14 T. Distance proximities among the ^{19}F spins was examined by 2D-RFDR with XY-16.

Results

It is shown that the relative intensities of the observed five ^{19}F MAS NMR signals, which are assigned to fluoride ions with different combinations of Ba and Ca cations in the first coordination shell, can be described by binominal distribution of cations. Further, the RFDR experiment shows that spatial distribution of both cations is random in a length scale of ca. 2 nm or less. Local rearrangement of both cations occurs well below the decomposition temperature, which relaxes local strain associated with homogeneous mixing of different cations and lowers ionic conductivity.

Conclusions

Homogeneous mixing of vaporized BaF_2 and CaF_2 followed by rapid cooling in the thermal plasma process realizes random distribution of both cations and realizes ion conduction.

1. N. Sata, K. Eberman, K. Eberl, and J. Maier, Nature 2000, 408, 946.
2. B. Ruprecht, et al., J. Mater. Chem., 2008, 18, 5412.

Acknowledgment: This article is based on results obtained from a project, RISING3, JPNP 21006, commissioned by NEDO, Japan.

MTR-018: Cellulose from citrus biorefinery residues: evaluation of crystallinity changes by ssNMR

MSci Symone Costa de Castro[1], Matheus Signorelli Correa[1], Dr. André Marcelo de Souza[2], PhD Ljubica Tasic[1]
[1]Universidade Estadual de Campinas, Institute of Chemistry, Organic Chemistry Department, Laboratory of Biological Chemistry, 13083-970, Campinas, Brazil, [2]Embrapa Solos, Rua Jardim Botânico, nº 1.024, Bairro Jardim Botânico, 22460-000, Rio de Janeiro, Brazil

Cellulose is a linear and main biopolymer on Earth with attractive properties and is widely investigated and applied in many fields. Cellulose units interact through hydrogen bonding and form highly ordered structures. Herein, citrus waste was a source for fabricating cellulose under varied conditions, such as sodium hydroxide concentration, solid/liquid ratio, temperature, and contact time. In total, 14 cellulose samples were fabricated and investigated. The properties of cellulose, like crystallinity indexes (CI), were accessed by attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR), and ^{13}C solid-state NMR spectroscopy. The Cross Polarization Magic Angle Spinning (CP/MAS) ^{13}C-NMR spectra with 2R probe on Bruker 400 MHz were obtained from lyophilized samples. After spectral fitting of the C4 region in the ^{13}C-NMR spectra, the areas of the cellulose crystalline peak (C4, ~89 ppm) and the total area of this carbon (89 ppm plus 84 ppm) were applied to CI determination, 45%. Three parameters were accessed from the ATR-FTIR cellulose spectral data: lateral order index (LOI), total crystallinity index (TCI), and hydrogen-bond intensity (HBI). The LOI values varied between 0.433 to 0.559; TCI from 1.314 to 1.666; and HBI from 0.999 to 1.379. High values of LOI and TCI are related to high crystalline cellulose. Then, the spectral datasets were evaluated by multivariate analysis to understand the behavior of the samples. No correlation was observed between CI values like TCI, LOI, and CI from ^{13}C ssNMR. We observed that variables such as TCI (FTIR) and CI (NMR) have strong positive loadings (PC 1), indicating their importance in capturing the variability along this component. By highlighting the significance of variables like the CI, which played a pivotal role in assessing cellulose quality and understanding its structural changes, this study provides actionable insights for optimizing biorefinery processes, ultimately promoting the efficient and sustainable utilization of biomass resources.

MTR-019: Wettability of (sub)nanopores in C1N1 electrode materials studied by 1H NMR of adsorbed water

Irene Lamata Bermejo[1], Dr Keil Waldemar[2], Dr Janina Kossman[3], Dr Mateusz Odziomek[3], Prof Claudia Schmidt[1], Prof Nieves López Sálas[1]
[1]Paderborn University, Paderborn, Germany, [2]University of Applied Sciences and Arts , Bielefeld, Germany, [3]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

Introduction
Understanding how water interacts with nanopores of carbonaceous electrodes is crucial for green energy storage and conversion applications as carbon–electrolyte (carbon-water) interfaces have a dominant influence on the capacitance and electrocatalytic performance. The standard strategy to increase the affinity of water to pores is doping carbon materials with nitrogen introducing polar sites. Highly nitrogen-doped carbons are expected to increase the hydrophilicity of the pores and the wettability. However, in the case of nanopores, the concept of surface hydrophilicity might be misleading as water confined in pores shows properties different from those of bulk water.
Methods
NMR spectroscopy is a versatile tool to probe local environments of water in pores. The ring currents originating from π-electrons within conjugated carbon ring structures affect molecules within a few tens of ångströms of pore walls. The effect of the ring currents affects the chemical shift of the atoms in their vicinity. The observed average chemical shift of mobile solvents also depends on the pore diameter. As only ions in pores of few nanometers will exhibit a sizeable shift, 1H NMR spectra of water will give us information whether an adsorbate reaches the nanopores or not.
Results and conclusion
We used two C1N1 carbons with different porosity and a pure nanoporous carbon as a reference. The NMR results showed that in C1N1 carbons, liquid water fills only a very small fraction of pores in comparison to the carbon reference. This surprising finding agrees with the cyclic voltammetry experiments in which we do not observe the high capacity in C1N1 materials typical for highly porous N-doped materials. Probing the interaction of water with the surface by water sorption and calorimetric studies revealed the formation of a layer of strongly bound non-freezing water at the surface, preventing the water from diffusing into the pores.

MTR-020: Direct observation of lithium ion migration in sodium ion battery cathodes by nuclear magnetic resonance

Dr. Koji Yazawa[1], Dr. Natalia Voronina[2], Prof. Seung-Taek Myung[2]
[1]Jeol Ltd., Akishima, 日本, [2]Sejong University, Gwangjin-gu, South Korea

Abstract
Oxygen-redox-based-layered cathodes are of great importance in realizing high-energy-density sodium-ion batteries that can satisfy the demands of next-generation energy storage technologies. However, Mn-based-layered materials (P2-type Na-poor Nay[AxMn1-x]O2, where A = alkali ions) still suffer from poor reversibility during oxygen-redox reactions and low conductivity. Here, we present cathode materials in which the Mn sites are replaced by Li or Li and Ni, and conducted direct nuclear magnetic resonance (NMR) observations of Li ion migration associated with the extraction and insertion of Na ions, which are closely related to the electrochemical properties and structural stability. [1]

Experimental
The P2-type layered compounds Na0.67[Li0.22Mn0.78]O2 (LM) and Na0.75[Li0.15Ni0.15Mn0.7]O2 (LNM) were synthesized by combustion method. Solid-state 7Li-NMR spectra were acquired using a JEOL JNM-ECZL600G (14.1 T) with a 1 mm HXMAS probe. pj-MATPASS (projection magic-angle-turning phase-alternating spinning sideband) was used to obtain 7Li isotropic spectra under 50 kHz MAS.

Results and Discussion
Comparing the electrochemical performance of the two electrodes, the discharge capacity of LNM was 160 mAh/g, which was smaller than that of LM (180 mAh/g), but the average operating voltage was 3.3.V, which was higher than that of LM (2.4 V). Voltage hysteresis was also suppressed, and cycle performance was 73% at 300 cycles, a significant improvement in stability compared to LM's 18%.
In the P2-Na0.6[Li0.2Mn0.8]O2 cathode, the migration of Li from the TM to the Na layers is considered to be a cause of capacity reduction. 7Li NMR provided direct evidence of Li migration (TM→Na→TM layers) during charging and discharging. Moreover, less Li migration into the Na layer in LNM than in LM was observed. These results suggest that Ni doping suppresses the migration of Li, leading to improved cycle stability.

References
[1] N. Voronina, K. Yazawa, S.-T. Myung et al., Adv. Energy Mater. 12 (2022) 2103939.

MTR-021: NMR studies of synthetic polymer aerogels

Dr. Attila Forgács[1]
[1]University Of Debrecen, Debrecen, Hungary

The term aerogel is used to describe a family of open porous solids that can be fabricated from a wide variety of base materials, e.g. inorganic oxides, carbon, biopolymers, synthetic polymers. Besides reinforced silicas, the most promising aerogels close to reach the technology readiness level needed for commercialization are synthetic polymer aerogels, mainly polyurea, polyimide and polyamide. Nuclear magnetic resonance (NMR) methods have been proven to be powerful tools for exploring the physical interactions of water molecules with the nanostructured backbones of mesoporous materials. In our research group we have synthesized polyamide and polyimide aerogels. The prepared materials were first characterized with conventional methods, like N2-adsorption-desorption porosimetry, scanning electron microscopy and infra-red spectroscopy. To receive a detailed information about the hydration of these materials we have used non-conventional nuclear magnetic resonance (NMR) methods, namely cryoporometry, diffusiometry and relaxometry, solid-state NMR measurements complemented by SANS measurements. From these measurements, we can get multiple information about aerogels. We can get information about the solvation properties and the structural changes of aerogels both in water and in non-polar solvents. The swelling process of an aerogel and the deformation of the gel structure can also be followed by titrating the solid sample with water or with other organic solvents. The self-diffusion of the molecules can be monitored with NMR diffusiometry. The variation of the effective self-diffusion coefficient as a function the observation time gives information on the pore and/or particle size, and also on the permeability of the pore structure. SANS scattering curves carry information both on the size and on the spatial distribution of the scattering objects.

MTR-022: True 14N NMR signal of perovskite-type oxynitrides

Dr. Yasuto Noda[1], Master Sakurako Ishii[1], Master Sota Shinki[1], Master Tomoya Kamide[1], Dr. Yuji Masubuchi[2], Dr. Kiyonori Takegoshi[1]
[1]Kyoto University, Kyoto, Japan, [2]Hokkaido University, Sapporo, Japan

Metal oxynitrides with perovskite-type structures are a new group of compounds that have attracted attention as novel dielectric materials. Since the relationship between the crystal structure and the mechanism of their dielectric properties is unknown, structural analysis has been performed by solid-state NMR, which is sensitive to the local structure. However, 14N NMR spectra of perovskite oxynitrides with different compositions show little difference, only a single sharp signal around 270 ppm.[1-4]
Here, we report our discovery of the “true” 14N signal of one representative perovskite-type oxynitride, BaTaO2N, revealing that it indeed arises from the BaTaO2N framework by also utilizing DFT calculations, and what was thought to be the 14N signal of the perovskite-type oxynitride is in fact that of residual nitrogen molecules formed during synthesis.

References

[1] T. Bräuniger et al., Chem. Mater. 17 (2005) 4114.
[2] Y-Il Kim and Y. Park, Solid State Sci. 14 (2012) 580.
[3] N. Cordes et al., Eur. J. Inorg. Chem. 2018 (2018) 5019.
[4] Z. Ma et al., Z. Naturforsch. 76 (2021) 275.

MTR-023: Dynamic disorder in monolayer and multilayer 2D Ruddlesden–Popper Lead Iodide Perovskites as seen by solid-state NMR spectroscopy and relaxometry

Dr. Noemi Landi[2], Dr. Silvia Borsacchi[1], Dr. Lucia Calucci[1], Prof. Marco Geppi[2], Dr. Elisa Carignani[1]
[1]ICCOM-CNR, Pisa, Italy, [2]University of Pisa, Pisa, Italy

In the fast-developing research of improved and sustainable materials for optoelectronics, 2D Lead Halide Perovskites (LHP) have attracted considerable attention because they offer the possibility of tunable band gap and enhanced environmental stability with respect to the corresponding 3D perovskites. 2D Ruddlesden–Popper (RP) phases can be prepared by adding a large organic monoammonium cation in order to form a structure with a bilayer of spacer cations between metal halide sheets is formed. For example, butylammonium (BA) is a suitable organic cation to force the archetypical perovskite $MAPbI_3$ into 2D RP perovskites BA_2MAn-1PbnI3n+1, which are the object of the present study. The layer thickness of metal halide sheets is specified by n and can be adjusted by tuning precursor stoichiometry.
Solid-State NMR demonstrated its strength for the characterisation of LHP, in particular for the study of ion dynamics, compositional variations and ion incorporation, chemical interactions, and degradation mechanisms.
In this work, the 2D RP perovskites BA2MAn-1PbnI3n+1 with n=1-4 have been characterized by ^{207}Pb, ^{1}H, and ^{13}C Solid-State NMR. In particular, the reorientational dynamics of butylammonium cations has been selectively and quantitatively characterised by measurement and analysis of ^{13}C nuclear relaxation times. By modelling the dynamic disorder of BA spacers all the samples, interesting differences between the monolayer (n=1) and multilayers (n=2-4) perovskites have been observed.

This work was supported by the European Union “NextGenerationEU” through the PRIN 2022 program promoted by Italian Ministry of University and Research (MUR), project ISOTOPE, 2022EHER2H.

MTR-024: Opportunities for Active Site Identification and Activity Prediction of Zeolite Catalysts using MAS NMR

Dr Sambhu Radhakrishnan[1], Dr. Vinod Chandran Chandrasekharan Nair[1], Dr. Sam Smet[2], Mr Karel Duerinckx[1], Dr. Alysson Morais[1], Prof. Johan Martens[2], Dr. Eric Breynaert[1]
[1]NMRCoRe (NMR/X-ray Platform for Convergence Research), KU Leuven, Leuven, Belgium, [2]COK-KAT, KU Leuven, Leuven, Heverlee

Introduction

With their exceptional structures and versatile properties, zeolites captivate scientific communities and industries. These crystalline (alumino-)silicates with characteristic porosity display high surface area, uniform pore size, and ion-exchange properties. Zeolites are indispensable in numerous applications, pivotal for separation, catalytic processes, environmental remediation, etc. Fundamental understanding of the processes and identification of the active sites will drive innovation towards more optimal materials and processes.

Aims

This research aims to develop ss-NMR strategies to investigate (i) mechanism driving pore-filling adsorption of alcohols in high silica zeolites and (ii) developing a tool for predicting catalytic activity of Cu-zeolites in selective catalytic reduction of NOx.

Methods

Combination of quantitative direct excitation NMR spectra with homonuclear and multinuclear multidimensional NMR spectroscopy, and variable temperature (VT) NMR experiments

Results

Upon liquid phase adsorption of C1–C5 primary alcohols on high silica MFI zeolites (Si/Al = 11.5–140), the concentration of adsorbed molecules largely exceeds the concentration of traditional adsorption sites: Brønsted acid and defect sites. Hydrogen bonding of the alcohol function to oxygen atoms of the zeolite siloxane bridges (Si–O–Si) was shown to drive the additional adsorption. This mechanism co-exists with chemi- and physi-sorption on Brønsted acid and defect sites and does not exclude cooperative effects from dispersive interactions.

Cu atom mobility and dimerization has been implicated by computational chemistry as a key factor in the reduction of NOx to N2 with Cu zeolites. VT 1H NMR revealed Cu induced generation of sharp 1H resonances, and the onset temperature of their appearance was found to strongly correlate with the NH3-SCR activity for a range of catalysts covering multiple frameworks, with different Si/Al ratios and different Cu contents.

Conclusions

Versatility of MAS NMR spectroscopy for active-site elucidation in multiple zeolite-based processes is elucidated.

References: Mater. Horiz., 2023,10, 3702-3711; Molecules 2023, 28(18), 6456

NMR-002: Molecular recognition and human Gai3 protein inhibition

Dr Mariola Ferreras-Gutierrez[1], Ms Marina Minguez-Toral[1], Dr Alain Ibañez de Opakua[2], Dr Sonsoles Martin-Santamaria[1], Prof Mikel Garcia-Marcos[3], Dr Francisco J Medrano[1], Dr Francisco J Blanco[1]
[1]Centro de Investigaciones Biologicas Margarita Salas, CIB-CSIC, Madrid, Spain, [2]CIC bioGUNE, Derio, Spain, [3]Boston University, Boston, USA

INTRODUCTION: The 41 kDa protein Gαi3 is the α subunit of a human heterotrimeric guanine nucleotide-binding protein involved in intracellular signaling cascades. The active GTP-bound Gαi3 protein binds to downstream effectors until GTP is hydrolyzed, returning the protein to its GDP-bound inactive state. Its canonical activators are GPCRs, which accelerate the exchange of GDP for GTP in response to extracellular signals. The protein GIV is a non-canonical activator that is present at high levels in metastatic cancer cells. disrupting GIV binding to Gαi3 might be of therapeutic value. The small molecule IGGi-11 disrupts at high relative concentration.
AIM: Structural characterization of Gαi3 binding to a conserved motif of GIV and to IGGi-11.
METHODS: Solution NMR, crystallography, and computational simulations.
RESULTS: We have found that GIV and IGGi-11 bind to the same cavity of Gαi3 but with a 10-fold difference in affinity. The highly flexible chain ends of Gαi3, identified by 15N-relaxation measurements, do not participate in the molecular recognition. A shortened construct of Gαi3 without these flexible tails binds the two ligands with the same affinity as the full-length protein. Well-diffracting crystals of the shortened Gαi3 grew only in the presence of IGGi-11, but no electron density was observed for IGGi-11 (at a resolution of 3.34 Å). Computational docking indicates that IGGi-11 binding is heterogeneous due to the elongated shape of the cavity and the symmetry of IGGi-11.
CONCLUSIONS: IGGi-11 is a competitive inhibitor but binds Gαi3 with a 10-fold smaller affinity than GIV. Chemical modifications breaking its symmetry could result in a more specific binding and potent inhibitor.

NMR-003: NMR-based measurement Gαi3's guanine nucleotide exchange rate

Dr Mariola Ferreras Gutiérrez[1], Prof Mikel Garcia-Marcos[2], Dr Francisco J Blanco[1]
[1]CIB-CSIC, Madrid, Spain, [2]Boston University, Boston, USA

Introduction:
The rate of guanine nucleotide exchange on the α-subunit of heterotrimeric G-proteins (Gα) is a fundamental aspect of their function, regulating the signaling pathways that mediate in cells. Traditional methods for measuring this rate require the use of radioactivity and meticulous sample manipulation. Alternate approaches employ fluorescent analogs of GTP to measure the Gα binding to GTPγS or indirect measurements via GTP hydrolysis. While these other methods offer benefits in safety and ease of use, they may be limited in accuracy.
Aim:
This study aims to assess the efficacy of NMR spectroscopy as a direct method for measuring the nucleotide exchange rate of Gα subunits, specifically the 41 kDa protein Gαi3.
Methods:
An NMR sample of uniformly 2H-15N labeled GDP-bound Gαi3 protein was prepared and a 10-fold concentration of GTPγS (a non-hydrolyzable analog of GTP) was added before recording a series of 30 minute long 1H-15N TROSY spectra, at 20 ºC and 800 MHz over 15 hours. The intensities of NMR signals corresponding to the indole side chains of Gαi3's tryptophans, distinctive for GDP-bound and GTPγS-bound states, were quantified and fitted to an exponential decay or association to obtain the exchange rate constant.
Results:
Our results indicate an average nucleotide exchange rate of (5.5 ± 0.7) × 10-5 s-1 at 20 ºC for GDP-Gαi3. This value is 0.3 times lower than the rate measured at 30 ºC by traditional radioligand assays. These results demonstrate the feasibility of using NMR spectroscopy to accurately measure nucleotide exchange rates in Gα subunits.
Conclusion:
NMR spectroscopy provides a direct, reliable method for Gα protein nucleotide exchange rate measurement that could be used to monitor the effects of different Gα ligands.

NMR-004: Molecular recognition of the human DNA-clamp PCNA with a phosphomimetic of Y211 phosphorylation

Antonio Ruiz[1], Dr. Belén Chaves-Arquero[1], Dr. Alejandra Guerra-Castellano[2], Prof. Irene Diaz-Moreno[2], Dr. Francisco J. Blanco[1]
[1]Cib Margarita Salas - CSIC, Madrid, España, [2]Instituto de Investigaciones Químicas, cicCartuja-CSIC, Sevilla, España

Introduction:
PCNA is a toroidal 90 kDa homotrimeric protein which encircles the DNA double helix, acting as a docking platform for DNA-editing enzymes in replication and repair. Most PCNA interacting proteins contain a conserved sequence motif (PIP-box) that binds to a defined region on PCNA. However, the catalytic subunit p125 of DNA-polymerase delta binds PCNA through a non-canonical PIP. PCNA also binds regulatory proteins and is further regulated by posttranslational modifications. High levels of PCNA phosphorylation at Y211 increase replication processivity but also nucleotide misincorporation in proliferating cells.

Aim:
Measure the affinity of the p125 PIP sequence for PCNA and the impact of Y211 phosphorylation on the interaction with p125 and the regulatory proteins p21 and p15.

Methods:
PCNA phosphorylation at Y211 was mimicked by incorporating the non-canonical amino acid para-carboxymethylphenylalanine (pCMF) at Y211 through a tRNA complementary to an amber (UAG) stop codon in in E. coli cells. The interactions between uniformly ^{2}H-^{15}N labeled non-phosphorylated PCNA and PIP peptides from p15, p21 and p125 were measured on ^{2}H-^{15}N TROSY spectra, and their affinities to both the unmodified and PCNA_Y211pCMF were measured by calorimetry.

Results:
The PIP motif of p125 binds to PCNA with a dissociation constant of 103 µM at 35 ºC. The interaction of PCNA_Y211pCMF with the PIPs of p21, p15 and p125 are currently being investigated. Preliminary measurements indicate small changes in their affinities.

Conclusions:
The impact of Y211 phosphorylation of PCNA on cellular replication appears to be caused by a mechanism different from disturbing the interaction with the three studied proteins.

NMR-005: Functional disorder in the N-terminus of Phosphoethanolamine Methyltransferase from P. falciparum Studied Using Solution NMR Spectroscopy and MD Simulations

Dr. Alexandra Pozhidaeva[1], Dr. Yulia Pustovalova[1], Dr. Bernd Simon[1], Dr. Irina Bezsonova[1], Oksana Gorbatyuk[1], Prof. Jeffrey Hoch[1]
[1]UConn Health, Farmington , United States

Introduction
Over 500000 people die from malaria every year. The recent emergence of drug-resistant malaria parasites underscores the urgency of developing new treatments to complement existing therapies. Phosphoethanolamine methyltransferase (PMT) is an enzyme that is crucial for synthesizing phosphatidylcholine (PC), a major component of plasmodium membranes. A PC synthesis pathway in which PMT uses S-adenosyl methionine (SAM) to convert phosphoethanolamine to phosphocholine is absent in mammals, making the enzyme an attractive therapeutic target. Crystal structures of the P. falciparum enzyme, PfPMT, with ligands/inhibitors bound have been determined in recent years [1,2]. PfPMT residues involved in ligand interactions are not solvent-exposed in these structures, indicating that conformational changes are necessary for ligand recognition. Furthermore, the structure of the apo protein has never been crystalized, suggesting conformational dynamics in the absence of bound ligands.

Aims and methods
In this work, we aimed to characterize apo PfPMT enzyme using solution NMR methods in combination with molecular dynamics simulations.

Results and conclusions
Our findings revealed distinct conformational differences between the apo and the ligand-bound PfPMT states. Specifically, we found that the N-terminus of the apo enzyme is unstructured and undergoes a disorder-to-order transition during ligand binding, which facilitates tight interaction. We also gained insight into the mechanism of PfPMT inhibition by the known inhibitor amodiaquine (AQ). AQ binds away from the active site of the enzyme and the mechanism of its action is not explained by the crystal structure. Our study revealed that chemical shift perturbations of PfPMT residues upon addition of AQ propagate from the binding site to the active site via the N-terminus, highlighting its role in enzyme function. This work provides a basis for future efforts to develop new potent anti-malarial compounds.

[1] Lee et al., JBC, 287, 1426–1434 (2012)
[2] Lee et al., Bioorg. Med. Chem. Lett, 22, 4990–4993 (2012)

NMR-006: Structure and dynamics of the Sars-CoV-2 NSP3 Ubiquitin-like domain 1 characterized by NMR spectroscopy and further biophysical methods

Katharina Vormann[1,2], M.Sc. Tobias Stief[1,2], Dr. Nils-Alexander Lakomek[1,2], Prof. Dr. Ralf Bartenschlager[3]
[1]Research Center Jülich (FZJ), Jülich, Germany, [2]Heinrich Heine University Düsseldorf, Düsseldorf, Germany, [3]Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany

Novel anti-viral agents are needed in response to the severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2) pandemic. One potential drug target is the multi-domain non-structural protein 3 (nsp3). Nsp3 is involved in forming the Sars-Cov-2 replication organelle (RO), which drives the coronavirus replication in the host cell. Nsp3 is one major constituent of molecular pores embedded in the Double Membrane Vesicles (DMVs) bilayer. These DMV pores allow the import and export of viral RNA. As the largest protein encoded by the coronavirus, nsp3 comprises 16 domains, including the N-terminal Ubiquitin-like domain 1 (Ubl1), forming the cytosolic-faced prongs of the crown-like pore. Considering that ubiquitin-like domains are involved in the regulation of diverse biological processes, the list of potential viral and host cell interaction partners is numerous.
Currently, information on the structure, dynamics, and binding partners of nsp3 is limited. Thus, our in vitro studies aim for structural and dynamic information on nsp3-Ubl1 by nuclear magnetic resonance (NMR) spectroscopy and other biophysical methods. For this purpose, we have expressed and purified the 15N, 13C isotopically labeled Ubl1 domain and characterized its internal and conformational dynamics by NMR spectroscopy. Additionally, circular dichroism (CD) spectroscopy was performed to analyze the secondary structural elements of Ubl1.

NMR-007: Ligand/Receptor Interactions of Receptor Tyrosine Kinases from the Viewpoint of NMR.

Dr Konstantin Mineev[1], Bruno Hargittay[1], Dr Santosh Gande[1], Verena Linhard[1], Sattar Khashkhashi-Moghaddam[1], Kerstin Witt[1], Dr Jing Jin[1], Claudia Catapano[1], Dr Sridhar Sreeramulu[1], Dr Mark Harwardt[1], Dr Christian Richter[1], Dr Hendrik Jonker[1], Dr Krishna Saxena[1], Dr Marta Segarra[1], Dr Marina Dietz[1], Prof. Dr. Mike Heilemann[1], Prof. Dr. Amparo Acker-Palmer[1], Prof. Dr. Harald Schwalbe
[1]Goethe-University Frankfurt, Frankfurt am Main, Germany

Receptor tyrosine kinases (RTKs) represent an important class of membrane proteins, involved in the development of a variety of severe diseases, including cancers. While RTK kinase domains are typical targets for small molecules, such drugs often lack specificity and demonstrate side effects. The extracellular ligand-binding parts are most often targeted by antibodies, which are expensive and their lifespan is limited. In this regard, the search for the small molecules interacting with the extracellular domains of RTKs is a prospective task, and NMR spectroscopy can contribute a lot to its accomplishment.

In this work, we studied the representatives of two RTK families: FGFR and Eph receptors. In the case of FGFR, we investigated the interference between the binding of FGFR native ligand, FGF8b and its small molecule allosteric inhibitor, SSR. FGF8b has a structural element, the N-terminal gN helix, which is unique among FGFs, and it binds to the same pocket as SSR. We expressed a two-domain fragment of FGFR3c and FGF8b and investigated their complex with NMR, to find that gN-helix blocks the SSR binding site on FGFR and converts it from the allosteric inhibitor to orthosteric. However, the functional assays did not reveal similar behavior in the context of full-length FGFR. This inconsistency could be explained only by the effects of the cell membrane, which was supported by the NMR-observed membrane activity of SSR. For Ephrin receptors we managed to express the 20-kDa ligand-binding domain of EphA2 and EphrinA1 ligand. These proteins were later utilized for the fragment-based screening taken separately and in the complex. Together with some known peptide and repurposed small molecule ligands, our data provides the cheminformatics basis for further hit-to-lead optimization. Altogether our results demonstrate clearly the utility of NMR for such classical systems, which are usually explored using other approaches of structural biology.

NMR-008: Probing Dynamics of an Intact Virus Using Pseudo 3D REDOR-Based MAS ssNMR Experiments

Mr. Orr Simon Lusky[1], Mr. Dvir Sherer[1], Professor Amir Goldbourt[1]
[1]Tel Aviv University, Tel Aviv, Israel

The property of dynamics is vital in all biological systems and is ubiquitous to all life forms. Hence, protein dynamics play an important role in the life-cycle of viruses, and can explain many stages in viral morphogenesis including the infection mechanism, protein-DNA/RNA complexation, self-assembly processes and more. Solid-state nuclear magnetic resonance (SSNMR) is a robust method for characterising protein dynamics in large intact systems, thus it is an excellent tool for studying viruses.
We will present an automated protocol for measuring the effective heteronuclear dipolar interaction of multiple spin pairs simultaneously. This method is based on pseudo 3D experiments with a homonuclear mixing scheme and a REDOR dephasing block as the third dimension. We demonstrate this protocol with DARR, RFDR and INADEQUATE mixing schemes to measure the effective C-N dipolar constants, and therefore of dynamic order parameters, for backbone and sidechains in the coat protein of the intact fd-Y21M bacteriophage. Combining the three techniques increases the total number of unambiguously assigned peaks and increases the accuracy of the measurements.
We will show that the helical phage coat protein exhibits uniform motions, and that the non-structured N-terminus undergoes large-amplitude motions. We will further discuss sidechains dynamics, including the lysine residues at the ssDNA binding site, and show two conformations of glycine 3 with a vast difference in their mobility.
This approach pushes the detection window to motions that are slower than the commonly measured C-H and N-H order parameters, and is applicable to a wide range of experimental conditions and biological samples.

NMR-009: Dynamic pre-structuration of lipid nanodomain-segregating remorin proteins

Zeren Xu[1], Adrien Schahl[2], Anthony Legrand[1,3], Mélanie Berbon[1], Marie-Dominique Jolivet[3], Axelle Grélard[1], Estelle Morvan[1], Antoine Loquet[1], Matthieu Chavent[2], Véronique Germain[3], Sébastien Mongrand[3], Birgit Habenstein[1]
[1]Université Bordeaux, CNRS, Bordeaux INP, CBMN, UMR 5248, IECB, Pessac, France, [2]Université de Toulouse, CNRS, Institut de Pharmacologie et Biologie Structurale, UMR 5089, Toulouse, France, [3]Université Bordeaux, CNRS, Laboratoire de Biogenèse Membranaire, UMR 5200, Pessac, France

Abstract: Remorins (REMs) are crucial for plant immunity, development, and symbiosis. They segregate into lipid nanodomains on the membrane surface to regulate f.ex. cell-to-cell connectivity. Classified into six groups based on their domain compositions, REMs feature an intrinsically disordered N-terminal domain and a C-terminal membrane anchor for specific lipid interactions on the plasma membrane. The assembly mechanisms of REMs, the specific functions of their phosphorylation sites, and their role during protein-lipid interactions are not fully understood. Our study employs bioinformatics, molecular imaging, Nuclear Magnetic Resonance (NMR) spectroscopy, structure prediction, and molecular dynamics simulations to explore the structural and dynamic organization of REMs. We reveal that REMs undergo a structuring process in the cytosol, forming anti-parallel coiled-coil dimers with structural fingerprints, which influence their selective nanodomain targeting. The pre-structured dimers have a tunable fuzzy coat and a positive surface charge, facilitating phospholipid binding and the formation of stable, energetically favorable multimers. Electrostatic interactions mediate the interaction between REMs' C-terminal and specific lipids, supporting a 'clip-and-divide' mechanism for lipid-protein nanodomain segregation. Our integrated approach uncovers REMs' complex architecture, providing insights into their essential roles in a diverse set of cellular processes, highly sensitive to local regulation.

NMR-010: Does a Similar 3D Structure Mean a Similar Folding Pathway? An HPP-NMR study of three representative proteins belonging to the structurally conserved MAX effectors family.

Prof. Christian Roumestand[1], Dr Karine De Guillen[1], Dr Mounia Lahfa[1], Dr Thomas Kroj[2], Dr Philippe Barthe[1], Dr André Padilla[1]
[1]University Of Montpellier, Montpellier, France, [2]INRAE, Montpellier, France

Plant diseases caused by fungal pathogens are the main cause of crop loss. When pathogens attack a plant cell, they produce molecular weapons called effectors to promote diseases. These effectors interact with Nucleotides and Leucine-Rich repeat (NLR) proteins which function as key elements in immune receptor networks. Much about the structure and function of pathogen effectors remains unknown. Effectors are indeed usually difficult to identify due to their low sequence homology. However, structural analysis can reveal unsuspected homologies, as in the case of the family of MAX effectors identified in Magnaporthe oryzae, a fungus pathogen of rice. Despite low sequence similarities, MAX effectors share a conserved common fold consisting of a 6-stranded ß-sandwich. Does it mean that they share a common folding pathway? Or, on the contrary, do their highly divergent sequences impose different folding pathways which converge to a similar fold? Trying to answer this question, we used High-Hydrostatic Pressure NMR (HHP-NMR), that proved to be particularly well suited to decipher protein folding pathways. In association with a new modeling protocol, this technique allows for the identification of folding intermediates in the conformational folding landscape of proteins that could be otherwise rubbed out by harsher method like chemical denaturation. We have investigated first the folding pathway of two MAX effectors, AVR-Pia and AVR-Pib, and found that these proteins display a similar folding pathway, with a common folding intermediate made of the ß3ß4 ß-sheet. Then, we analyzed the folding pathway of MAX60, a newly identified MAX effector, and compared it to the folding pathways already described for AVR-Pia and AVR-Pib. MAX60 owns the common core shared by all MAX effectors, but displays an additional decoration: a C-terminal ?-helical extension. This C-terminal extension and seems responsible for drastic changes in the folding pathway of MAX60 when compared to AVR-Pia or AVR-Pib.

NMR-011: Biophysical characterization of the second prion domain of CPEB3

Dr Vinesh Vijayan[1], Ms Faina Pinhero[1], Dr Dhanya Reselammal[1]
[1]IISER THIRUVANANTHAPURAM, THIRUVANANTHAPURAM, India

The maintenance of long-term memory requires sustainable synaptic connections, mediated by the prion-like transformation of the translational regulator protein CPEB3 (Cytoplasmic Polyadenylation Element Binding protein isoform 3) in mammals. The prion form of CPEB3 protein functions as a translational activator, whereas the soluble form actively represses the protein expression critical for maintaining synaptic plasticity. The N-terminal prion domain of CPEB3, composed of the two prion subdomains PRD1 and PRD2 has previously been demonstrated to perform a crucial role in imparting prion-like properties to the protein. We have already reported the amyloid core of the first prion subdomain (PRD1) of the mouse CPEB3. Our current work, reports the structural features of the condensate formed by the second prion domain of the mammalian mouse CPEB3 upon phase separation. Here, we have investigated the aggregation properties and the structural characteristics of the mouse PRD2 (mPRD2) in vitro. Solid-state NMR and other biophysical studies revealed the existence of mixed secondary structures for mPRD2 in condensates. We propose that the distinct phase separation behaviour of the mPRD2 would be due to the conformational changes attributed to the pattern of the mPRD2 amino acid sequence, resulting in the formation of rigid and amyloid-like self-assembly.

NMR-013: Inhibition of fusidic acid resistance through restricting conformational flexibility in domain III of EF-G identified using methyl relaxation dispersion

Dr Jennifer Tomlinson[1]
[1]University of Leeds, Leeds, United Kingdom

Introduction:
Fusidic acid (FA) is one of few remaining antibiotics active against MRSA but resistance is increasing, predominantly through expression of the FusB family of proteins. FA stalls bacterial protein synthesis by binding to elongation factor G (EF-G) once it is bound to the ribosome and preventing its release. FusB binds to EF-G and promotes dissociation of the stalled complexes via allosteric modulation of the dynamics in EF-G domain III, promoting the increase in a more disordered minor state of the domain.

Aims:
With the lack of new antibiotics entering the clinic, an alternative strategy is to try to inhibit existing resistance mechanisms, thereby prolonging the usefulness of current antibiotics. This study therefore aimed to identify key regions controlling the FA resistance mechanism in order to identify a potential druggable site to inhibit resistance.

Methods:
Disulphide bonds were introduced throughout domain III of EF-G, restraining different secondary structure elements. The effects of these restraints on FusB-induced changes in dynamics were measured using methyl CPMG relaxation dispersion and their effect on FA resistance was measured using a fluorescence assay to measure buildup of stalled complexes.

Results:
Using these methods, we were able to identify key regions controlling FA resistance, such that restraints in the central β-sheet of the domain prevented FusB-induced dynamics changes and abolished FusB-mediated FA resistance. Restraint of the second α-helix of the domain promoted increased changes in dynamics in domain III, conferring resistance even in the absence of FusB and highlighting the allosteric mechanism of resistance.

Conclusions:
This study has identified key regions controlling the dynamics of domain III of EF-G that are crucial in causing FA resistance. Using these data, we have identified a potential druggable site to inhibit FA resistance and elucidated the mechanism of resistance to FA mediated by dynamics in the drug target.

NMR-014: PI3K-SH3 Domain: study of an amyloid model protein in its unfolded state with DNP-enhanced ssNMR

Msc Leonardo Levorin[1,2], Msc Luis Gardon[1,2], Msc Melinda Jaspert[1,2], Neomi Rüter[2], Dr. Lothar Gremer[1,2], Dr. Nils-Alexander Lakomek[1,2], Prof. Dr. Henrike Heise[1,2]
[1]Forschungszentrum Jülich GmbH, Jülich, Germany, [2]Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany

A wide variety of degenerative disorders, including Alzheimer's and Parkinson's disease, are associated with protein misfolding and aggregation. The unfolding, oligomerization, and fibrillation of the PI3K-SH3 domain is investigated as a model system to better understand these processes. The protein's native structure [1] unfolds at an acidic pH and assembles into well-folded amyloid fibrils, whose structure was recently revealed by cryo-EM [2]. In this work we want to study the unfolded state of PI3K-SH3 at cryogenic temperatures with DNP-enhanced solid-state (ss) NMR.

At room temperature proteins exhibit substantial conformational freedom resulting in rapid averaging, restricting the ability to provide information about the full proteins' conformational ensemble. Freezing the protein in a cryoprotectant medium stops the exchange between different conformations, and all conformations sampled by each molecule are present with their respective probability. Furthermore, DNP-enhanced ssNMR, in addition to enable the study of PI3K-SH3 in frozen solution at 100K, strongly improves signal-to-noise ratio thanks to the transfer of polarization from highly polarized electron spins to the protein's nuclear spins.

Specifically, we made use of residue selective labelling to analyze the chemical shift value and the linewidth of sidechains 13C nuclei, which strongly reflect the side-chain torsion angles [3] and therefore the protein's conformation.

At acidic pH, in unfolding conditions, we observed broad signals, covering a wide conformational range from α-helical to β-strand. Differently, at neutral pH, when the protein is folded, peaks appear narrower and a conformational distribution in accordance with the proposed structure is observed.

This work, in addition to the investigation of PI3K-SH3 conformational freedom, also elucidates its unfolded state, picturing it as a competition and exchange between a vast range of structures.

References:
[1]-Booker et al. Cell 1993, 73 (4), 813.
[2]-Röder et al. Nature Commun. 2019, 10 (1), 3754.
[3]-Siemons et al. Chem.Commun. 2019, 55 (94), 14107-14110.

NMR-015: Cross-correlated spin relaxation in NMR studies of intrinsically disordered proteins

M.Sc. Paulina Bartosinska-Marzec[1], M.Sc. Bartłomiej Banas[1], PhD Clemens Kauffmann[3], M.Sc. Andreas Beier[2], PhD Daniel Braun[2], Prof Wiktor Kozminski[1], Prof Robert Konrat[2], PhD Anna Zawadzka-Kazimierczuk[1]
[1]Biological and Chemical Research Centre, Faculty of chemistry, University of Warsaw, ul. Żwirki i Wigury 101, 02-089 Warsaw, Poland, [2]University of Vienna, Vienna BioCenter, Campus-Vienna-Biocenter 5, 1030 Vienna, Austria, [3]Wiener Linien GmbH & Co KG, Erdbergstraße 202, 1030 Vienna, Austria

Introduction

Intrinsically disordered proteins (IDPs) are highly dynamic proteins which only transiently adopt certain structural motifs. These properties are significant for signalling and regulation of biological processes.[1] NMR is the only method which provides information on IDPs at atomic scale. Nonetheless, high dynamics leads to a problem of low signal dispersion, which can be solved by increasing spectral dimensionality.[2]
Measurement of cross-correlated relaxation (CCR) rates allows determining dihedral angles distribution of protein's backbone. However it requires measuring of several types of CCR rates.[3]

Aims

Development of a procedure for studying the spatial structural propensities of IDPs with new methods based on measurements of various CCR rates.

Methods

We have developed eight 4D experiments to measure different CCR rates for IDPs. Also, we prepared scripts for easy semi-automatic CCR rates determination, based on spectra supported with a peak list. The obtained list of CCR rates can be subsequently used to determine dihedral angles distributions of protein's backbone using Maximum Entropy method.[3]

Results

We successfully validated experiments on a well-known, folded protein, ubiquitin. We compared the experimental CCR rates with these calculated based on structures deposited in PDB (1d3z, 6v5d, 2k39, 2nr2). We also compared the obtained dihedral angles distributions with the angles extracted from the mentioned structures.

Conclusions

Based on the results for ubiqutin we can confirm effectiveness of our method. Currently, we are conducting the measurements of an IDP, osteopontin.

Acknowledgment

This work was supported by a grant from the Polish National Science Centre (MAESTRO, 2015/18/A/ST4/00270) and Austrian Science Fund FWF (P 35098-B).

References

[1] P. Tompa, FEBS Lett., 2005, 15, 3346–3354
[2] K. Grudziąż et al, Methods, 2018, 148, 81–87
[3] C. Kauffmann et al, ChemPhysChem, 2020, 21, 1

NMR-016: A high-resolution analysis of arrestin2 interactions responsible for CCR5 endocytosis

Ivana Petrovic[1], Samit Desai[1], Polina Isaikina[1], Layara Abiko[1], Prof Anne Spang[1], Prof Stephan Grzesiek[1]
[1]Biozentrum, University Of Basel, Basel, Switzerland

Clathrin-mediated endocytosis (CME) is crucial for regulating G protein-coupled receptors (GPCRs) via phosphorylation-dependent arrestin interactions. However, the interplay between receptor phosphorylation and arrestin coupling to the CME machinery, comprising clathrin and AP2 proteins, remains poorly understood.

Here we have investigated the CME of the chemokine receptor CCR5 induced by native and engineered CCL5 chemokine ligands. Upon CCL5 agonist stimulation, arrestin2 translocates to CCR5 at the plasma membrane forming a long-lived CCR5-arrestin2 complex that is internalized but does not reach the lysosomes. Building on these observations, we then characterized in detail the interplay of molecular forces driving the receptor sequestration from the plasma membrane. Through a combination of solution NMR spectroscopy and size exclusion chromatography, we elucidated the distinct roles of arrestin2 interactions with clathrin and AP2 proteins. The arrestin2 and clathrin backbone, as well as arrestin2 side-chain methyl groups, provide quantitative and high-resolution information about the arrestin-clathrin interaction. We show that arrestin2 interacts weakly with clathrin through a single binding site independent of arrestin2 activation by phosphorylated CCR5 C-terminal tail peptides. This may enable the internalization of receptors with low phosphorylation levels. In contrast, the arrestin2-AP2 interaction is stronger and requires arrestin2 activation depending quantitatively on the CCR5 C-terminal tail phosphorylation. This may indicate a preference towards AP2 complex formation for highly phosphorylated receptors. The in vitro results are corroborated by cellular assays, which establish a crucial role of the arrestin2 interaction with AP2, but not with clathrin for CCR5 internalization.

The NMR spectroscopy provided unique information about the arrestin region that is typically not visible in either crystal or cryo-EM structures due to its high flexibility, shedding light into the intricate mechanisms underlying CME. Moreover, these findings give insights into how variation in receptor phosphorylation propensities leads to the observed differences in arrestin-mediated internalization.

NMR-017: Allostery of the Lac repressor

Prof. Rolf Boelens[1]
[1]Utrecht University, Utrecht, Netherlands

Gene regulation is an essential step in the expression of DNA. A well-known gene regulator is the Lac repressor which regulates the expression of bacterial lactose genes. Thus far, structural understanding of its mode of action is still largely lacking. We analyzed the Lac repressor in distinct states related to gene activation using NMR and show that it exists in a dynamic equilibrium between two conformations [1]. In one conformation the Lac repressor can form a tight complex with DNA. In the other it can bind an inducer, causing destabilization of the helices connecting its core and DNA-binding domains, and thereby weakening DNA binding. Our observations favor a mechanism of activation via the well-known Monod-Wyman-Changeux model [2] as it relies on actively shifting defined structural states upon ligand binding.

[1] Romanuka et al, 2023, Proc Natl Acad Sci USA, 120 (49) e2311240120
[2] Monod et al, 1965, J Mol Biol, 12, 88-118

NMR-018: Deciphering the Structure of SARS-CoV-2 ORF6 Membrane Protein Using Selective Labeling and combination of solution and solid-state NMR

Phd Martí Ninot Pedrosa[1], PhD Gyula Pálfy[2], PhD Marie-Laure Fogeron[1], PhD Hafez Razmazma[1], PhD Bernhard Brutscher[3], PhD Beate Bersch[3], PhD Luca Monticelli[1], Prof. Beat Meier[2], PhD Anja Böckmann[1], PhD Lauriane Lecoq[1]
[1]MMSB - CNRS, Lyon, France, [2]Physical Chemistry, ETH Zurich, Zurich, Switzerland, [3]IBS - CEA/CNRS/UGA, Grenoble, France

SARS-CoV-2, the causative agent of the COVID-19 pandemic, is the coronavirus with the highest number of accessory proteins. These accessory proteins play an important role in the viral pathogenicity, with ORF6 regarded as the most cytotoxic protein of the virus.
While X-ray crystallography and cryo-EM are effective techniques for the structure determination of crystallized proteins and large molecular systems, small membrane proteins present a challenge even for solution NMR due to the size of detergent micelles or lipids. In addition, the limited number of solved structures for this protein's group may result in less accurate predictions by AlphaFold2.

We aim to decipher the structure, oligomeric state and interactions with lipid membrane of the ORF6 protein within detergent micelles and lipid bilayers.

To gain deeper insight into the oligomerisation patterns of ORF6, we employed a range of biophysical techniques, including SEC-MALS, AUC and NMR spectroscopy. To decipher its structure and dynamics, we combined solution and solid-state NMR together with specific amino-acid labelling and PRE as well as CS-Rosseta and molecular dynamics (MD) simulations on membranes.

Our data show that ORF6 aggregates into high order oligomers on lipid membranes, while in detergent micelles the oligomerisation behaviour depends on the protein to detergent ratio. Solution-state NMR spectra of ORF6 in detergent micelles enabled specific assignment of the flexible C-terminal region. Solid-state NMR of ORF6 on proteoliposomes revealed the rigid-part of the protein, corresponding to the region interacting with the membrane. A structural model has been build using CS-Rosetta. MTSL spin-labelling revealed that the dimer forms helices arranged in an antiparallel manner, and MD simulations illustrates the interaction with the membrane and the oligomerisation effect on it.

Our work provides the first structural model of ORF6 peripheral membrane protein, revealing that it forms antiparallel alpha helices involved in multiple contacts at the membrane surface.

NMR-019: How flexible is a DNA duplex? An investigation by relaxometry and molecular dynamics simulations.

Dr Lucas Siemons[1], Dr Emeline Mestdach[2], Dr Sirine Nouri[2], Dr Jorge Garibay[3], Dr Philippe Pelupessy[1], Dr Loic Salmon[2], Dr Fabien Ferrage[1]
[1]Ecole Normale Supérieure, CNRS UMR 7203, Paris, France, [2]Centre de RMN à Très Hauts Champs de Lyon, CNRS UMR 5082, Lyon, France, [3]Bruker BioSpin GmbH, Wissembourg, France

Introduction: Motions in nucleic acids are vital for biological function and determine how and when they interact with their surroundings. While chemical-exchange based methods examine µs-ms motions, few experiments target motions on the nanosecond time scale. High-Resolution Relaxometry (HRR) is a unique technique to probe motions on this timescale by recording low-field longitudinal relaxation rates with high-field NMR.

Aims: This study develops HRR and high-field spin relaxation methods to probe motions in a 12 base-pair DNA helix and interprets them with molecular dynamics simulations. We both quantify the motions present and test the accuracy of modern force-fields.

Methods: We measured carbon-13 relaxation in a 12 base-pair DNA helix at natural abundance at each C1' site: low-field longitudinal relaxation rates from 2T to 10T with a new prototype sample shuttle, and high-field rates (longitudinal R1 and transverse R2 relaxation rates as well as steady-state heteronuclear Overhauser effects 13C-{1H} NOE) at fields from 11T to 23T (500 MHz-1GHz proton resonance frequency). These relaxation rates are compared to ~45 µs molecular dynamics (MD) simulations using three force fields and three different water models.

Results: We have built a framework describing both the high-field and relaxometry experiments using the extended model free approach (EMF), demonstrating that EMF can describe relaxation rates from 2T to 23.5T. The derived time-scales and order parameters show that, despite its simple structure, a DNA helix features a range of local and global dynamics. Comparing experimental results to MD trajectories shows that while MD produces motions compatible with measured relaxation rates, the amplitude of motions derived from simulations are too small.

Conclusions: We have recorded the most extensive set of relaxation rates to date on a DNA duplex and demonstrated that the low- and high-field relaxation rates can be combined with MD simulations to characterize internal motion in nucleic acids.

NMR-020: PI3K SH3 Domain - Amyloid Model Protein (Un)folding investigated by NMR

M.Sc. Melinda Jaspert[1,2], M.Sc. Luis Gardon[1,2], M.Sc. Leonardo Levorin[1,2], Dr. Lothar Gremer[1,2], Dr. Nils-Alexander Lakomek[1,2], Prof. Dr. Henrike Heise[1,2]
[1]Heinrich-Heine-Universität, Düsseldorf, Germany, [2]Forschungszentrum Jülich, Jülich, Germany

The misfolding and aggregation of proteins is associated with neurodegenerative diseases like Alzheimer’s or Parkinson’s disease. In order to get a better understanding of the underlying processes the PI3K SH3 domain is used as a model system. At acidic pH the well folded native structure [1] of the protein unfolds and aggregates into well-folded fibrils. The structure of these PI3K SH3 fibrils was determined recently using cryo-EM [2].

We want to use diverse NMR experiments to get insights into the unfolding process of the PI3K SH3 domain itself and its dependencies. Therefor we apply solution-state NMR experiments, performing secondary chemical shift analyses and relaxation experiments - hence obtaining information about the secondary structure propensities and the dynamics of the investigated protein - to follow the unfolding process with particular regard to its temperature dependency at residue-type resolution.

At acidic pH and lower temperatures an equilibrium between two conformations is present, that upon rising the temperature shifts towards the unfolded conformation.
The same effect can be achieved by increasing the buffer capacity or lowering the pH.
Together with our developed assignment, the secondary chemical shift analyses of the PI3K SH3 domain at acidic pH and high temperature indicate that the molecule indeed is unfolded under these conditions.
This provides a basis for further examinations of the double conformation state at lower temperatures, which will be separated with recently performed experiments, as well as a starting point for ultrafast-MAS-measurements using proton detection, that we want to execute to get further insights into the complexity of the (un)folding process of the PI3K SH3 domain.

References:
[1] Booker, G. W. et al. Cell 1993, 73 (4), 813.
[2] Röder, C. et al. Nature Commun. 2019, 10 (1), 3754.

NMR-021: Molecular basis of specificity in alternative splicing regulation through splicing regulator SRSF6

Julian Von Ehr[1,2], Dr. Andreas Schlundt[1]
[1]Goethe University, Frankfurt Am Main, Germany, [2]IMPRS on Cellular Biophysics, Frankfurt am Main, Germany

The human serine-arginine rich splicing factor 6 (SRSF6) is part of the SR-protein family consisting of 12 members. SRSF6 is involved in (alternative-) splicing regulation and can itself exist in at least three isoforms. It is composed of an N-terminal RRM domain, followed by a pseudo RRM and a C-terminal serine-arginine rich disordered domain. With SRSF6 being an integral part of the splicing machinery, all three domains have been implicated in interacting with RNA and/or proteins, but individual interactions mediating SRSF6 specificity remain poorly understood. Therefore, our goal was to structurally and biochemically analyze single domains as well as their combinations to decipher their RNA interaction sites as well as their sequence requirements. To this end, we used nuclear magnetic resonance (NMR) spectroscopy combined with electrophoretic mobility shift assay, fluorescent polarization, and X-ray crystallography, applied to recombinant SRSF6 variants. In particular, we used RNA Bind-n-Seq to obtain RNA consensus motifs for the single and tandem RRMs. We found the two single RRMs to have significantly different binding affinities and sequence requirements towards RNA: RRM1 binds to cytosine- and adenine-rich RNAs in a canonical way, whereas RRM2 prefers purine-rich sequences in a non-canonical mode of interaction. To understand the latter on an atomistic level, we solved the crystal structure of RRM2 both in the apo- and RNA-bound forms, which confirm our NMR data in non-canonical RNA-binding mediated by RRM2's α-helix 1. Additionally, we found the linker between RRMs to play an important part in increasing affinity towards RNA in concert with RRM2.
Altogether, our data provide a strong structural basis for understanding the functions and target specificity of SRSF6 as opposed to the other 11 members of the SR protein family on a molecular level.

NMR-022: Structural studies of transcription termination complex in E. coli usingSolid-state NMR spectroscopy

Ms Jyothirmayi Unnikrishnan Geethadevi[1], Mrs Johanna Baldus Becker[2], Mr Alexey Sudokov[1], Mrs Anna Wacker[1], Mr Christain Richter[2], Mr Boris Fürtig[1], Mr Albert Weixlbaumer[3,4,5,6], Mr Clemens Glaubitz[2], Mr Harald Schwalbe[1]
[1]Institute for Organic Chemistry and Chemical Biology, Johann Wolfgang Goethe-University Frankfurt, Max-Von-Laue Str. 7, 60438, Frankfurt am Main, Germany, [2]Institute of Biophysical Chemistry, Center of Biomolecular Magnetic Resonance (BMRZ), Johann Wolfgang Goethe-University Frankfurt, Max-Von-Laue Str. 7, 60438., Frankfurt am Main, Germany, [3]Department of Integrated Structural Biology, IGBMC, Institut de Ge´ ne´tique et de Biologie Mole´ culaire et Cellulaire (IGBMC), 67400., Illkirch, France, [4]Universite´ de Strasbourg, IGBMC UMR 7104-UMR-S 1258, 67400., Illkirch, France, [5]CNRS, UMR 7104, 67400., Illkirch, France, [6]Inserm, UMR-S 1258, 67400., Illkirch, France

Transcription termination is a complex process that plays a crucial role in gene expression and regulation. There are two mechanisms underlying this process in bacteria; rho-independent (or intrinsic) and rho-dependent termination. The intrinsic termination is governed by the newly synthesized RNA upon folding of a hairpin loop followed by a uridine-rich tract at the 3’ end. However, the intriguing question is how the nascent RNA terminates its own synthesis in the intrinsic termination process. Here, we use the solid-state NMR to understand the interactions inside the transcription bubble during various stages before termination.

The sample mimicking the intrinsic transcription termination complex is prepared in vitro using E.coli RNA polymerase (RNAP), RNA, and DNA (templates). Various biochemical methods have been applied for specific isotopic labeling of the 3’ end of the RNA. One of them is the 3’ extension of RNA, employing the site-specific ligation using uridine 3’, 5’- bisphosphate. The challenge of observing the single labeled nucleotide in the complex (~ 400kDa) is surpassed by solid-state NMR with dynamic nuclear polarization (DNP). DNP in NMR will increase the signal intensity of the labeled uridine. We successfully implemented this experiment.13C,15N TEDOR and 13C, 13C double quantum single quantum experiments are used to observe the resonances from the uridine.

Since the whole process of transcription is highly sophisticated, the usage of novel methods to unravel aspects of this process is vital. Recent studies using cryo-EM have show that the U-rich tract triggers the clamp module of RNAP. We believe our observation using NMR will shed light on RNA-RNA and RNA-DNA interaction in the transcription termination complex.

NMR-023: Monitoring Cell Culture Growth Using Water NMR

Mr Mark Iain Grimes[1], Dr Cloe Legrand[1], Dr Hassan Rahmoune[1], Dr Matthew Cheeks[2], Dr Felix Roth[2], Ms Jennifer Smith[2], Mr Fabio Zurlo[2], Professor Mick Mantle[1]
[1]University of Cambridge, Cambridge, United Kingdom, [2]AstraZeneca, Cambridge, United Kingdom

Biopharmaceuticals such as monoclonal antibodies (mAbs) accounted for 50% of the ten best selling drugs in 2020, and more than 800 are currently in clinical trials. The majority of antibody-based drugs are produced using Chinese hamster ovary (CHO) cells in bioreactors. Within cell culture, a method to track the state of the culture is to physically count the cells present within the sample under a microscope. Although devices such as haemocytometers have been developed to aid this, it is a time consuming process, as a suitable sample dilution and multiple counts are required to ensure that the results are representative of the original sample.

Water proton nuclear magnetic resonance (wNMR) makes use of the transverse relaxation rate of the water signal [$R_2(^1H_2O)$] to gain understanding about solutes in the sample of interest. To date, it has been utilised in a wide variety of biopharmaceutical-related studies, such as the monitoring of protein concentration/aggregate content.

Here, we describe the first use of wNMR to study CHO cell cultures. $R_2(^1H_2O)$ shows sensitivity to cell line growth over the duration of the culture, at both high- and low-field. Inverted “V” type dependencies were observed for both non-producing and mAb-producing cell lines. By studying this signal, it is possible to carry out live monitoring of biopharmaceutical relevant cell lines in a rapid (< 2 min) and non-invasive manner, with minimal sample preparation.

By using a modern low-field “benchtop” NMR spectrometer in this work, the study of cell cultures by wNMR can be utilised outside of a laboratory setting, such as at the production line, or alternatively as an online measurement. This method provides a faster means by which cell cultures can be monitored, allowing for greater efficiencies and reduction in bioprocess costs, and therefore lowering the cost of antibody-based drugs to patients.

NMR-024: Characterization of food proteins and enzymes - Use of NMR spectroscopy in industrial science & research

PhD Elwin van der Cruijsen[1], Erik van Leeuwen[1], Tim Rietkerk[1], PhD Adriana Carvalho de Souza[1], PhD Klaartje Houben[1]
[1]Analytical Sciences, dsm-firmenich, Delft, Netherlands

Introduction
At dsm-firmenich, a science-based company, we strive to develop innovative and sustainable solutions in food, nutrition, health, and beauty. In that context, our protein products such as enzymes, plant proteins or proteins from precision fermentation are of key interest. At all stages of protein product development different analytical techniques are used and NMR spectroscopy is regularly applied to characterize the protein fold, to follow specific protein modification or to study enzyme kinetics.

Results & Conclusions
This presentation will highlight several examples where NMR has provided key information on our protein ingredients. The first part will show the use of natural abundance 2D NMR methyl fingerprinting to confirm structural similarity between animal-free fermentative food proteins and their mammalian counterparts. Second, the use of natural abundance nitrogen HSQC is presented to follow enzymatic plant protein modifications that correlate with an improved taste profile. Next, we show the use of both solution and MAS solid-state NMR to follow enzymatic conversions of substrates in their actual food matrix such as milk or mashed potatoes. Last, the use of low field time-domain (TD) NMR is presented to characterize water absorption in texturized vegetable proteins (TVPs).

NMR-025: NMR-based secondary structure characterisation of the West Nile Virus Frameshift Element RNA and investigation of small molecule binding

Kathrin Skorodumov[1], Dr. Anna Wacker[1], Dr. Christian Richter[2], Dr. Sridhar Sreeramulu[1], Prof. Dr. Harald Schwalbe[1]
[1]Institute for Organic Chemistry and Chemical Biology, Frankfurt am Main, Germany, [2]Center of Biomolecular Magnetic Resonance (BMRZ), Frankfurt am Main, Germany

Programmed ribosomal frameshift is a regulatory mechanism for the translation of proteins that occurs in many positive-stranded RNA viruses, including many human pathogens.[1][2] It enables the expression of different proteins encoded within a single open reading frame. Programmed ribosomal frameshift is primarily controlled by cis-acting elements in the mRNA sequence, such as pseudoknots or stable stem-loop structures. In West Nile Virus (WNV), the frameshift element (FSE) is predicted to form a pseudoknot structure, which is conserved within the Japanese Encephalitis Virus serogroup.[3] The -1 frameshift induces the translation of NS1' protein, which enhances neuro-invasiveness[4] and plays a role in viral replication.[5]
Structural characterisation and understanding of the function of the frameshift element in WNV and related viruses and identification of frameshift-inhibiting ligands can contribute to the development of new antiviral pharmaceuticals. [6][7]
Solution-state NMR spectroscopy was used to investigate the secondary structure within the frameshift element of the WNV. We confirmed the predicted structure of this FSE as a pseudoknot structure, forming two base pairing stems. The base pairing in the first pseudoknot stem was confirmed by assignment of the imino protons in TROSY and NOESY. The formation of the second pseudoknot stem was shown by titration of a complementary oligonucleotide to a hairpin forming part of the pseudoknot. The assignment of aromatic and sugar resonances, which will follow, will allow high-resolution structural characterization, opening up the possibilities for structure based drug design.
We performed fragment-based NMR screenings to investigate binding of small ligand molecules to the WNV frameshift element RNA. An initial identification of potential binders from a poised library of 607 compounds was carried out based on 1D-1H, waterLOGSY and T_2-CPMG experiments.

NMR-026: pH dependence of the conformation, dynamics and interaction with membranes of diphtheria toxin through the lens of 1H-NMR and HDX-MS

Nicolas Carvalho[2], Mathilde Briday[1], Sadi Mirko[2], Corentin Léger[2], Bruno Vitorge[1], Daniel Ladant[2], PhD Sébastien Brier[1], Alexandre Chenal[2], Phd J. Iñaki Guijarro[1]
[1]Institut Pasteur, Université de Paris Cité, CNRS UMR 3528, BioNMR-HDX Technological Platform, Paris, France, [2]Institut Pasteur, Université de Paris Cité, CNRS UMR 3528, Biomacromolecules Interactions Unit, Paris,

Introduction
The diphtheria toxin (DT) is an amphitropic protein consisting of catalytic (C), translocation (T) and receptor binding (R) domains. The toxin enters the host cell via receptor-mediated endocytosis. The acidic pH of the endosome triggers conformational modifications required for the translocation domain T to insert into the membrane and the subsequent translocation of the catalytic domain C into the cytoplasm where the toxin inhibits protein synthesis.
Aims
To understand the conformational and dynamics transitions triggered by pH required for DT to interact with membranes.
Methods
We use B2LiVe (Binding to Lipid Vesicles), a simple, robust label-free 1H-NMR methodology that we recently described (Sadi et al., Cell Reports Methods 2023) coupled to HDX-MS (Hydrogen-Deuterium eXchange followed by mass spectrometry) to characterise DT at a function of pH. B2LiVe uses the proton SOFAST pulse sequence from Chandra and co-workers to quantify the population of protein in solution at different membrane concentrations and thus determine the partitioning coefficient. With B2Live, we monitor the global conformation and membrane partitioning of DT with SUVs. We use HDX-MS at selected pHs to more finely characterise the conformation and dynamics of the toxin.
Conclusion
As observed by NMR, DT is a globular protein at physiological pH (7.4) that adopts a molten globule-like conformation with exchange broadening (transition pKa=5.9) and completely unfolds at more acidic pH (< 4). As expected, the native state has a very low affinity for membranes and the interaction is favoured by lower pH values. HDX-MS reveals that compared to the native state, at pH 5.9 the three domains of the protein become more dynamic and at pH 5.1, they become highly accessible to the solvent, in agreement with NMR data and in contrast with the rigid picture given by the XR structures of DT at low pH.

NMR-027: Biophysical characterization of SARS-CoV-2 5'-stem loop 3 and its interactions within the viral genome

Matthias Alexander Becker[1,2], Dr. Anna Wacker[1,2], Dr. Christian Richter[1,2], Prof. Dr. Harald Schwalbe[1,2]
[1]Institute for Organic Chemistry and Chemical Biology, Goethe University, Frankfurt am Main, Germany, [2]Center of Biomolecular Magnetic Resonance (BMRZ), Goethe University, Frankfurt am Main, Germany

The pandemic of coronavirus disease 2019 was caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Stem loop 3 is a conserved structural element in the viral 5' UTR and of particularly interest, because it embodies the core of the transcription regulatory leader sequence (TRS-L). This sequence is essential for viral discontinuous transcription. This process generates a subset of subgenomic RNAs that function as a template for translation of all structural and accessory proteins.[1] During discontinuous transcription, SL3 undergoes structural changes as it is involved in RNA-RNA long-range interactions within the SARS CoV 2 genome.[2] Further, SL3 fully opens up for and takes part in cyclization of the viral genome.[2,3] Additionally, A74 of SL3 is m6A methylated by METTL3 in-vitro.
We investigate the structure, dynamics and stability of Stem loop 3 in the local and long-range structural context in presence and absence of the m6A Methylation. We use solution-state NMR-spectroscopy to gain insights about RNA structure and dynamics as well as CD-spectroscopy to obtain information about thermodynamic stability. We found methylation in SL3 to affect thermal stability of SL3 and its long range interactions. As a result, methylation alters relative populations in the equilibrium between SL3 and long-range interactions.[4] Further, our findings indicate that the genome cyclization is stabilized by two base-paired regions that enclose a more dynamic region.
The dynamic equilibrium between local structure of SL3 and long-range structures as well as the influence of viral interaction partners on the equilibrium will be investigated more detailed.

References

[1] I. Sola et al., Annual review of virology 2015, 2, 265.
[2] O. Ziv et al., Molecular cell 2020, 80, 1067-1077.e5.
[3] Y. Zhang et al., Nature communications 2021, 12, 5695.
[4] M. A. Becker et al. pending minor revisions at Angewandte Chemie 2024

NMR-028: Looking at extreme flexibility in RNA by NMR

Dr Sirine Nouri[1], Dr Amin Sagar[2], Dr Lucas Siemons[1,3], Dr Natacha Gillet[4], Dr Judith Schlagnitweit[1], Dr Pau Bernado[2], Dr Loic Salmon[1]
[1]CRMN (CNRS/ENSL/UCBL), Villeurbanne, France, [2]CBS (CNRS/INSERM), Montpellier, France, [3] LBM (ENS/PSL/CNRS), Paris, France, [4]LC (CNRS/ENSL/UCBL), Lyon, France

Due to the pivotal role of RNA in modern life science, there is a strong need to understand their properties at the atomic level. NMR spectroscopy is a unique tool to achieve this goal due to its atomic resolution and sensitivity to all the relevant timescales for biomolecular motions. However, studying large or flexible RNA remains a major challenge for RNA due to the difficulty of measuring sufficient site-specific data to properly constrain structural or dynamic models.
Here, we demonstrate that a combination of ultra-high field and several differently labeled samples allowed us to assign the large let-7 pre-microRNA spin system and access to numerous structural and dynamic site-specific dynamics probe in this system, both in its stem but also in its large 27-nucleotide loop.
The collected experimental data have been used to investigate the conformational dynamics of this system at timescales up to the millisecond in combination with advanced molecular modeling strategies. This allowed to provide a high-resolution conformational ensemble to describe the complex dynamics occurring in this system and illuminate the conformational properties of highly disordered RNA.
This study will provide the methodological basis to describe conformational dynamics in other challenging RNA and at the same time offer a unique picture of large-scale nucleic acid conformational disorder.

NMR-029: NMR investigation of transcriptional riboswitches

M. Sc. Florian Amend[1,2], Dr. Anna Wacker[1,2], Dr. Christian Richter[1,2], Prof. Dr. Harald Schwalbe[1,2]
[1]Institute for Organic Chemistry and Chemical Biology, Goethe University, Frankfurt, Germany,
[2]Center of Biomolecular Magnetic Resonance (BMRZ), Goethe University, Frankfurt, Germany

Riboswitches are cis-regulatory elements found in the 5ʻ-UTR of prokaryotic mRNA. They consist of two domains, a ligand-binding aptamer domain and an expression platform. Ligand-binding to the aptamer domain causes a refolding of the mRNA structure changing also the secondary structure of the expression platform. That mechanism leads to modulation of gene expression. Depending on ligand concentration, either an intrinsic terminator or an antiterminator structure is formed. A more intricate control of gene expression can be achieved by tandem arrangement of either two aptamer domains or two whole riboswitches. The here investigated riboswitch of bacillus megaterium consist of two aptamer domains responding to guanine and PRPP. In that case, the two aptamers domains are overlapping in sequence. Therefore, binding of one ligand influences also the capability to bind the other.
We used solution-state NMR-spectroscopy to investigate the structure of the guanine-binding aptamer domain. In the ligand-free state, we confirmed the formation of the stem loops P2 and P3. We validated hypoxanthine-binding as an analoga to guanine by the observation of canonical and non-canonical interactions within the binding pocket, as well as long-range RNA-RNA tertiary interactions forming a pseudoknot. We showed that ligand binding also stabilizes helix P1 that influences the gene expression.
For further structural characterization, we will look more precisely into the non-canonical hydrogen bonds caused by ligand binding. As known by other riboswitches, these could include NH···N, NH···O=C, NH···2’O, NH···O=P and 2’OH···N. Based on prior results, we will investigate the kinetics of RNA refolding upon ligand addition by Hypersense NMR.

NMR-030: Grb2 structure in solution: unique view of HIStorical artefacts and ErbB2-related signaling by NMR

Dr. Louise Pinet[1], Dr. Nelly Morellet[2], Dr. Aurélien Thureau[3], Dr. Diego Gauto[2], Dr. Nadine Assrir[2], Dr. Carine van Heijenoort[2]
[1]Centre de Resonance Magnétique Nucléaire (CRMN) à Très Hauts Champs de Lyon (UMR 5082 - CNRS, ENS Lyon, UCB Lyon 1), Villeurbanne, France, [2] Institut de Chimie des Substances Naturelles, Université Paris-Saclay, CNRS UPR 2301, Gif-sur-Yvette, France, [3]Synchrotron SOLEIL, Gif-sur-Yvette, France

Grb2 (Growth factor receptor-bound protein 2) is an adaptor protein linking the EGFR family of receptor tyrosine kinase to the Ras signaling pathway. These proteins are important for cell proliferation and are involved in several types of cancer, notably breast cancer. Grb2 is made of three domains, namely two SH3 domains flanking a central SH2 domain. Each domain has been shown to have its own specificity.
Grb2 function lies in its ability to connect different parts of signaling pathways by binding multiple partners via its multiple domains; the connectivity between the domains is therefore critical. Despite the extensive literature on Grb2, very few studies investigated a wild-type, tag-free, and apo form of Grb2 in solution. The different constructs and conditions used lead to apparent contradictions in the results. Confusion remains about the oligomeric state of Grb2, the interactions between its domains, and their dynamic interdependence. Our study aims at examining existing models of Grb2 structure and dynamics and building a biologically relevant one.
Such questions require, in addition to careful experimental conditions, a combination of information at atomic resolution and at the scale of the domains, in terms of both structure and dynamics. Here we used NMR combined with size-exclusion chromatography, small-angle X-ray scattering and molecular dynamics simulations to paint a clearer picture of Grb2 conformational landscape.
We showed that previous studies have been biased by the presence of histidine tags, as well as crystalline contacts not relevant in solution. These have dramatic effects on Grb2 interdomain connections. Indeed, we found tag-free apo Grb2 domains to be widely independent of one another in solution, contrary to previous findings. These conclusions completely changed our view of Grb2's ability to interact with different parts of ErbB2, a unique member of the EGFR family of great therapeutic interest.

NMR-031: Functionality of the Rho guanine nucleotide dissociation inhibitor is based on transient structural properties within an intrinsically disordered region.

Dr Sara Medina Gomez[1], Dr Ilaria Visco[2], Dr Felipe Merino[3], Dr Peter Bieling[2], Prof Dr Rasmus Linser[1]
[1]TU Dortmund University, Dortmund, Germany, [2]Max Planck Institute of Molecular Physiology, Dortmund, Germany, [3]Max Planck Institute of Developmental Biology, Tübingen, Germany

The activity of Rho GTPases, responsible for a wide range of events determining cell morphology, is orchestrated by guanine nucleotide dissociation inhibitors (RhoGDIs), together forming an intensely researched family of regulatory proteins. Their function has been assumed to be based on steric hindrance of nucleotide exchange by their N-terminus, whose structural properties have, however, remained contradictory.
Here we show that this crucial functional element of the inhibitor contains well-defined temporary structural features that increase binding affinity to the client but at the same time maintain a very high degree of plasticity, of benefit for the multi-step recruitment (excavation) of the client from its membrane-bound state. On the basis of the multitude of MD-, NMR- (chemical shifts, relaxation, RDCs, 3J couplings, and NOEs) and FRET-based dynamics data obtained, its sterically exerted inhibitory action previously assumed, by contrast, now seems highly unlikely.
Apart from its importance for molecular regulation of cell morphological events and pharmacological avenues for the Rho family in particular, the study demonstrates the versatility of the enormous spectrum of different degrees of a co-existence of order and disorder that is associated with different kinds of protein interactions.

NMR-032: Biomolecular Condensates Act as a Non-Aqueous Biological Solvent

Mr James Eaton[1,2], Dr Charles Buchanan[3], Miss Emily Haasz[1,2], Dr Virginia Casablancas-Antras[1,2], Mr Ben Naylor[1,2], Professor Andrew Baldwin[1,2]
[1]Physical and Theoretical Chemistry Laboratory, Department of Chemistry, University of Oxford, South Parks Road, OX1 3TA, Oxford, United Kingdom, [2]Kavli Institute of Nanoscience Discovery, University of Oxford, OX1 3QU, Oxford, United Kingdom, [3]Max Planck Institute for Terrestrial Microbiology, Marberg, Germany

Introduction: Biomolecular condensates (membraneless organelles), which form transiently in cells through liquid-liquid phase separation, offer a unique form of compartmentalisation without a membrane. Condensates have been found to partition RNA and modulate chemical reaction rates, but their solvent properties remain relatively unexplored. Understanding the solvent properties of condensates could explain the mechanism behind their compartmentalisation ability. Furthermore, it could open avenues for controlling or manipulating condensate behaviour for molecular transportation and therapeutic purposes. Therefore, this study delves into determining the solvent properties of condensates.

Methods: Nuclear Magnetic Resonance (NMR) is a powerful tool for studying the solution state behaviour of biomolecular condensates. We used a combination of slice-selective imaging and spectroscopic experiments to explore the physico-chemical environment inside condensates.

Results: We determined dramatic variations in water chemical shift, ion concentration, and small molecule concentrations between the interior and exterior of condensates. Furthermore, diffusion and relaxation measurements highlighted a significant increase in viscosity inside the liquid-like condensates. The measurements identified substantial changes to the physico-chemical properties across the interface of condensates, with differences comparable to a change in solvent.

Conclusion: This is remarkable as water is typically assumed to be the uniform solvent of biology. A cell's ability to create small pockets containing distinct solvent environments in the form of membraneless organelles is unusual and likely to substantially impact its biochemistry and metabolism.

NMR-033: A Deeper Understanding of APLF's Role in the NHEJ Pathway Through Intramolecular Interactions Revealed by NMR.

Phd Student Théodore Bellon[1], Duc-Duy Vu[1], Philippe Pelupessy[1], Ludovic Carlier[1], Mauro Modesti[2], Guillaume Bouvignies[1], Fabien Ferrage[1]
[1]Laboratoire des Biomolécules, UMR 7203, CNRS, Département de chimie, École normale supérieure, PSL University, Sorbonne Université, 75005 Paris, France, [2]Cancer Research Center of Marseille, UMR 7258, CNRS, INSERM U1068, Institut Paoli-Calmettes, Aix-Marseille Université UM105, 13273 Marseille, France

Introduction: Non-homologous end-joining (NHEJ) is a predominant pathway for repairing DNA double-strand breaks (DSBs) in eukaryotic cells. Many proteins involved in this pathway often possess intrinsically disordered regions (IDRs), making NMR an ideal tool for their investigation. These IDRs frequently contain specific binding sites for other NHEJ factors, suggesting a role in assembling the NHEJ repair machinery, although the exact mechanism remains unclear. Aprataxin and PNKP-Like Factor (APLF), a so-called accessory NHEJ factor, is a 511-residue protein characterized with high disorder. It contains four functional regions, including the fork-head-associated domain (FHA), the middle domain (MID) and the C-terminal acidic domain (CTAD), each interacting with specific partners.

Aim: The MID domain interacts with the Ku heteromodimer, the initial responder to DSBs, via the Ku80 subunit. It has been proposed that it is through this interaction with Ku that APLF promotes synapsis of the broken DNA ends but the mechanism has not been explained. Furthermore, the FHA domain interacts with the disordered region of XRCC4 via a phospho-threonine residue. Because the XRCC4 scaffolding protein directly interacts with DNA Ligase 4 and with the XLF scaffolding protein, APLF has been implicated in promoting the final ligation step during NHEJ. However, the dynamics of these interactions remain to be elucidated.

Methods and Results: We achieved 95% assignment of the FHA domain's backbone resonances, excluding prolines. Using NMR titrations, CPMG relaxation dispersion and CEST experiments, we identified and characterized the domain's equilibrium between multiple conformations, showing a strong dependence on temperature and concentration. We also revealed an intramolecular interaction between the FHA and CTAD domains, near the XRCC4 binding site and distinct from a putative dimerization region.

Conclusion: These results may explain APLF's ability to form stable synapsis with Ku and help clarify APLF's dynamic role in the assembly of the NHEJ machinery.

NMR-034: NMR unlocks the importance of proline cis/trans isomerization in SLiM-based IDR interactions

Phd Andreas Prestel[1], Frederik F. Theisen[2], Nina L. Jacobsen[2], Oline N. Eriksen[2], Kaare Teilum[1], Karen Skriver[2], Birthe B. Kragelund[2]
[1]cOpenNMR, University of Copenhagen, Copenhagen, Denmark, [2]Repin center, University of Copenhagen, Copenhagen, Denmark

Proline (Pro) is unique among the 20 proteinogenic amino acids in that it forms tertiary amides with a small free energy difference of 1-6 kJ/mol between the cis- and trans-form of the respective Xaa-Pro peptide bond. In folded proteins, the structural context often provides a strong preference for either the extended trans-form or the turn-like cis-form. In intrinsically disordered regions of proteins, however, the two isomers coexist, and the cis-form can be populated up to 40 %, depending on sequence context.
We set out to investigate how the isomerization state of Xaa-Pro peptide bonds affect the binding of disordered short linear motifs (SLiMs) to globular proteins. We selected two model systems: the transcription factor DREB2a from Arabidopsis Thaliana that interacts with transcriptional regulators including the Mediator subunit 25 via a bivalent SLiM[1] and the human prolactin receptor that binds to and is regulated by 14-3-3 proteins through a SLiM in its disordered intracellular domain. In both cases, the SLiM contains a Trp-Pro peptide bond with a high cis-content in the unbound state. NMR-spectroscopy is uniquely powerful to extract isomer-specific information at atomic resolution. Using chemical exchange saturation transfer (CEST) we show that both isomers of DREB2a can bind to Med25 but that the formed complexes are structurally distinct, which also manifests in a two-fold difference in affinity.[1] In the case of the prolactin receptor, we used real-time NMR binding experiments to show that the cis-form of the peptide has a ~500-fold higher affinity for 14-3-3zeta compared to the trans-form.
Our data suggest an additional layer of regulation of cellular processes involving differential binding of proline containing SLiMs. NMR is the key method to unlock the information needed to gain a deeper understanding of these processes on the molecular level.
[1] Theisen, F.F., et al.; NatCommun 15, 592 (2024)

NMR-035: Functional Cycle of Chaperone Hsp70 at Atomic Resolution

Prof. Dr. Sebastian Hiller, Dr. Guillaume Mas
[1]Biozentrum, University Of Basel, Basel, Switzerland

Introduction:
Hsp70 chaperones are molecular machines essential to all kingdoms of live. Their functional cycle is crucial to their role as client processing hubs interacting with multiple co-factors. Segments of the functional cycle have been previously studied by structural and biophysics methods, but the connections between these segments and the interplay with co-chaperones are poorly understood.
Aims:
We want to resolve the complete functional cycle of an Hsp70 protein at atomic level. Such complete description includes the identification of all significantly populated states, elucidation of their structures, determination of the mechanisms and their kinetics connecting them, and the rationalization of all these aspects on the functional biological background.
Methods:
We use an ATP regeneration system to create a non-equilibrium steady-state of Hsp70 BiP under turnover conditions inside the NMR tube. 2D and 3D solution NMR spectroscopy on selectively labeled methyl groups provides a quantitative spatial and temporal description of the underlying energy landscape.
Results:
The data resolve for the first time the clockwork mechanism underlying Hsp70 function. Hsp70 chaperone BiP undergoes a functional cycle comprised of five states. These states include two previously unrecognized high-energy conformations that are essential mechanistic parts of the cycle. One of the novel high-energy conformations serves as a timer of the functional cycle. The other provides a checkpoint for control of ATP hydrolysis by external factors.
Conclusions:
Our study illustrates how the ATP regeneration setup combined with methyl NMR allows studies of the interplay between multiple protein at the atomic level. The technology sets a paradigm for studies of dynamic networks of biomolecules in general and for Hsp70 functional cycles in all kingdoms of life.

NMR-036: From Conventional to High-Power-Laser-Driven Irradiation: Timely Detection of Magnetic Resonance Biomarkers in Cell Cultures

Dr Adrian Voda[1,2], Drd Ioana Fidel[1,2,3], MSc Diana Serafin[1,2], Dr Gabriel Bleofu[1], Dr G. Giubega[1], Dr Liviu Neagu[1], Dr M. Iovea[7], Drd Cezara Zagrean-Tuza[8], Dr. Anamaria Hanganu[2], Drd Silvana Vasilca[1,2,3,4], Dr. Daniel Negut[4], Dr. Miahi Suditu[5], Dr Roxana Popescu[4,6], Dr Bacalum Bacalum[4], Dr Madalin Rosu[1], Dr Ovidiu Tesileanu[1], Dr Aude Sadet[1,2], Dr Ioan C Chiricuta[5], Dr Paul R. Vasos[1,2,3]
[1]Extreme Light Infrastructure - Nuclear Physics (ELI-NP), “Horia Hulubei” National Institute for Physics and Nuclear Engineering (IFIN-HH), Magurele, Romania, [2]"C. D. Nenitzescu" Institute of Organic and Supramolecular Chemistry, Bucharest, Romania, [3]Interdisciplinary School of Doctoral Studies, ISDS, and Departments of Physics and Chemistry, University of Bucharest, Bucharest, Romania, [4]Life Sciences and IRASM Departments – Radiation Processing Centre, IFIN-HH, Magurele, Romania, [5]Amethyst Radiotherapy, Otopeni, Romania, [6]Politehnica University of Bucharest, Faculty of Medical Engineering, Department of Bioengineering and Biotechnology, Bucharest, Romania, [7]AccentPro2000 S.R.L (AP2K), Magurele, Romania

Introduction: Ultra-high dose-rate delivery of radiation emerged as a novel and less invasive radiotherapy approach[1]. A prerequisite for successful clinical applications is the mechanistic understanding of the biomolecular effects of very high doses of radiation delivered on ultra-short timeframes.
Aims: The objective of this work is to identify radiation biomarkers using magnetic resonance profiling from living cells in different irradiation setups: clinical accelerator (X-rays, 6 MV, 5 Gy/min), laboratory irradiator (^{60}Co photons, dose rates up to 35 Gy/min), and high-intensity laser generated radiation (up to Gy/ns)[2].
Methods: We have adapted a protocol to extract and record the NMR metabolic profile of cells grown in-vitro. Furthermore, we have established an irradiation setup using secondary radiation (electrons) stemming from the interaction chamber of a 100 TW, 1 PW and 10 PW lasers operating in pulses.
Results: Changes of certain metabolites concentration could be observed after cells irradiation and are specific to either tumoral or normal cell types behaviour under radiation exposure. First results on cells irradiated in the high-power laser setup will be presented.
Conclusions: We identified Magnetic Resonance biomarkers that can be used for timely detection of radiation dose-rate effects.
An experimental setup to investigate the effects of high dose-rate radiation generated by high-power lasers on cells is presented.

Acknowledgements
Funding from UEFISCDI PN-III-P4-ID-PCE-2020-2642, 545 PED/2020, the Romanian Ministry of Research by ’Objectives of National Interest’ programme (’IOSIN’), support of contract PN 23 21 01 05 funded by the Romanian Ministry of Research, Innovation and Digitalization and NextGeneration EU-PNRR-III-C9-2022-I8 program (contract no.760110).

References
1. Bourhis, J. et al. Treatment of a first patient with FLASH-radiotherapy. Radiother. Oncol. 139, 18–22 (2019).
2. Asavei, T. et al. Laser-driven radiation: Biomarkers for molecular imaging of high dose-rate effects. Med. Phys. 46, e726–e734 (2019).

NMR-037: Towards a molecular understanding of Ddx4N1 phase separation

Mr Eugene Lin[1], Professor Andrew Baldwin[1], Professor Lindsay Baker[1]
[1]Kavli Institute for Nanoscience Discovery, University Of Oxford, Oxford, United Kingdom

Ddx4 is the principal component of 'nuage' membraneless organelles, present in human sperm. Nuage formation is mediated by Ddx4's disordered N-terminus (Ddx4N1) via liquid-liquid phase separation (LLPS). Ddx4N1 phase separates into liquid droplets under certain solution conditions in vitro, and has been used as a model system to study LLPS. While the sequence features driving Ddx4N1 phase separation are relatively well characterised, understanding of the structural basis of Ddx4N1 phase separation remains limited.

Using a combination of structural and biophysical techniques, notably NMR and cryo-electron tomography, we investigate how the sequence features of Ddx4N1 govern its properties across length scales: from its individual chain properties, its ability to form intermolecular interactions, to its propensity to phase separate.

To this end, diffusion NMR experiments were performed on a range of mutants of Ddx4N1 with different phase separation propensities. These experiments, together with measurements of droplet stability, reveal that the hydrodynamic radii of the four Ddx4N1 mutants correlate with their phase separation propensities. Additionally, 15N-1H HSQC spectra were recorded on the four mutants of Ddx4N1 in non-phase separating conditions. The peak intensities in the HSQC spectra of the four mutants hint at regions of Ddx4N1 which are more involved in forming intermolecular interactions for phase separation, and these are used self-consistently to arrive at structural models for the conformation of proteins inside condensates.

Our results allow us to build a mechanistic understanding of how individual Ddx4N1 chains assemble at the atomic scale to form macroscopic droplets.

NMR-039: Structural dynamics of the human copper transporter hCTR1

Zhiyu Sun[1], Claire Ollier[1], Louise Pinet[1], Tobias Schubeis[1], Tanguy Le Marchand[1], Lina Barret[2], Magali Schwob[2], Valerie Kugler[2], Renaud Wagner[2], Guido Pintacuda[1]
[1]Centre de RMN à Très Hauts Champs de Lyon (CNRS/ENS Lyon/UCBL), 69100 Villeurbanne, France, Villeurbanne, France, [2]Plateforme de Chimie Biologique Integrative de Strasbourg (CNRS/Université de Strasbourg), 67412 Illkirch, France, Illkirch, France

In humans, high-affinity copper uptake is modulated by hCTR1, a trimeric membrane transporter which has so far fled from high-resolution x-ray or cryo-EM investigations and is extremely challenging to produce and recover in workable amounts for structural studies. We demonstrate here the power of NMR for the characterization of lipid-bound hCTR1.
We have first developped the methods for preparation of uniformly 15N and 13C-labelled hCTR1 in Pichia pastoris and for reconstitution in lipid bilayers [1]. We have then leveraged MAS NMR at ultra-fast (100 kHz) rate in small-volume rotors to detect and assign 1H, 15N and 13C resonances from the transmembrane domains (TM1, TM2, TM3) of the transporter. In parallel, solution NMR measurements in detergent micelles were used to detect flexible regions comprising the N- and C-termini, as well as the long interconnecting loop between TM1 and TM2. Finally, we have determined and characterized metal binding through experiments at different Cu+ and H+ concentrations. We have notably compared apo and Cu+-bound hCTR1 samples and utilized NMR chemical shifts and spin relaxation rates as sensitive probes for local and global conformational changes.
These data provide the first atomic-level experimental signature of the conformational dynamics associated with the uptake of copper ions by hCTR1, opening the way to understand its transport mechanism.

NMR-041: Mutual Frustration in a Polyproline II Helical Bundle Versus β-Amyloid Tug of War

Rubén López-Sánchez[1], Miguel Mompeán[1], Douglas Laurents[1]
[1]Instituto de Química Física "Blas Cabrera" - Consejo Superior de Investigaciones Científicas (IQF-CSIC), Madrid, España

Introduction:
The PPII helical structure is the basis of collagen, and there are several proteins and protein domains which are composed of bundles of PPII helices (Mompeán et al., 2021). The high conformational stability and the large number of H-bonds that are present in PPII helical bundles is reminiscent of amyloids, which are quasi-infinite β-sheets. In both PPII helical bundles and amyloids, the polypeptide chain extends facilitating self-assembly. Based on our discoveries (Treviño et al., 2018), we predict that PPII helices could also drive biomolecular condensate formation. The tendency of condensates to concentrate proteins like FUS or TDP-43 and spawn harmful amyloids implicated in ALS has attracted much interest (Patel et al., 2015). These proteins contain a PPII helix-forming motif, as well as CPEB3, which adopts an amyloid state key for memory consolidation (De Mingo et al., 2023).

Aims:
We aim to determine the influence of PPII helices on amyloid formation by studying how proline-rich segments adjacent to amyloidogenic sequences affect conformational stability under physiological conditions.

Methods:
Conformational tug-of-wars can provide insight into conformational stability in the absence of high temperatures or chemical denaturants. In this context, we combined NMR and CD spectroscopy, as well as fluorescence-based assays, to characterize the behavior of chimeric peptides. These peptides were designed with amyloid-promoting segments from Aβ and Sup35 fused to sequences favoring PPII helices.

Results:
The results show a mutual structural frustration as no significant evidence for amyloid formation was detected. Moreover, the presence of amyloidogenic segments strongly inhibited the collagen-like triple helix formation characteristic of PPII helices.

Conclusions:
Our study provides novel insights into the role of PPII helices in amyloid formation. The observed structural frustration suggests a complex interplay between PPII helices and amyloidogenic sequences, underlining the importance of PPII flanking segments in modulating both pathological and functional amyloid formation.

NMR-043: Conformational and dynamic properties of proline-rich peptide elucidated by 4,4-difluoroproline incorporation

Ms Anastasiia Trostianetskaia[1,2], Professor Bruno Kieffer[2], Professor Vladimir Torbeev[1]
[1]Ecole Supérieure de Biotechnologie de Strasbourg, CNRS UMR 7242 Biotechnology and Cellular Signalling, University of Strasbourg, Illkirch, France, [2]Institut de Génétique et de Biologie Moléculaire et Cellulaire, University of Strasbourg, Illkirch, France

Protein-protein interactions (PPI) are regulated by the structural properties of binding partners, encompassing post-translational modifications, ligand binding, and conformational changes. PPI are mediated by specialized binding domains such as SH3, SH2, WW, PDZ and others that recognize short linear motifs in the cognate protein sequences. These motifs are often characterized by a large conformational disorder that complicates the understanding and prediction of interactions at a molecular level, hindering precise PPI network descriptions. Proline residues are highly prevalent in such regions, adding an additional layer of complexity due to their unique ability to promote cis-peptide bond. Here, we investigate an interaction between the SH3 domain and a proline rich region of the Bin1 protein, that is involved in centronuclear myopathy.
We conducted a residue scanning analysis of the nine proline positions within the BIN1 SH3 binding peptide by incorporating the non-canonical amino acid 4,4-difluoropoline. Using 19F NMR spectroscopy, our approach facilitated the elucidation of cis/trans isomer ratios, proline pucker isomerization dynamics, and subsequent binding to the SH3 domain. Our findings reveal that the properties of each proline residue are highly sequence-dependent, leading to locally distinct conformational dynamics despite the absence of a defined secondary structure.
This study underscores the difficulties of describing proline-rich regions as disordered and calls for a reassessment of the rules governing molecular recognition between peptide ligands and receptor protein. Our insights shed light on the intricate mechanisms underlying PPIs, paving the way for more accurate predictions and descriptions of interaction networks.

NMR-044: Spontaneous post-translational modification of an ultra-stable computationally designed protein at high temperatures

Dr. Joshua Dudley[1,2], Prof. Colin Smith[1]
[1]Wesleyan University, Middletown, United States, [2]National Institutes of Health, Frederick, United States

The de novo design of miniprotein inhibitors has recently emerged as a new technology to create proteins that bind with high affinity to specific therapeutic targets. Their size, ease of expression, and minimal apparent unfolding at 95°C makes them excellent candidates for a new class of protein drugs. However, little is known about their dynamics, especially at the elevated temperatures they seemingly tolerate quite well. To address that and gain insight for future designs, we have focused on identifying unintended and previously overlooked heat-induced structural and chemical changes in a particularly stable model miniprotein, EHEE_rd2_0005. NMR studies suggest the presence of dynamics on multiple time and temperature scales. Transiently elevating the temperature results in spontaneous chemical deamidation visible in the NMR spectra, which we validate using both capillary electrophoresis and mass spectrometry experiments. High temperatures also result in greatly accelerated intrinsic rates of hydrogen exchange and signal loss in HSQC spectra from local unfolding. These losses are in excellent agreement with both room temperature hydrogen exchange experiments and hydrogen bond disruption in replica exchange molecular dynamics simulations. Finally, continuous real-time NMR observation of the chemical modification at 70-85°C reveals both fast and slow exchange processes happening simultaneously. Our analysis reveals important principles for future miniprotein designs and the potential for high stability to result in long-lived alternate conformational states.

NMR-047: NMR sample optimization and backbone assignment of a stabilized neurotensin receptor

Mariam Mohamadi[1], Dr David Goricanec[1], Prof Dr Gerhard Wagner[3], Prof Dr Franz Hagn[1,2]
[1]Bavarian NMR Center (BNMRZ) and Structural Membrane Biochemistry, Dept. of Bioscience, TUM School of Natural Sciences, Technical University of Munich, Garching, Germany, [2]Institute of Structural Biology, Helmholtz Munich, Neuherberg, Germany, [3]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, USA

G-protein coupled receptors (GPCR) belong to the largest group of membrane proteins encoded by the human genome and are involved in many different cellular and physiological processes. The biological versatility make the GPCRs attractive targets for pharmaceutical drug development. Hence, structural investigation and functional understanding of this protein class became more important in the last decades.
These structural revelations have been crucial in elucidating the mechanisms of GPCR activation and signal transduction. However, high-resolution structures provide valuable information, but NMR spectroscopy offers a unique window into GPCR dynamics.
In this study, we optimized NMR samples of a stabilized neurotensin receptor 1 (NTR1) variant bound to its agonist (neurotensin) for 3D triple resonance experiments. Using size exclusion chromatography, thermal stability assays, and 2D-NMR experiments we identified di-heptanoyl-glycerophosphocholine (DH7PC) as a promising membrane mimic for high-resolution NMR studies. While we achieved a partial backbone resonance assignment, the protein's internal membrane-embedded regions remained invisible due to lacking amide proton back-exchange. Despite this limitation, we could still use NMR and hydrogen deuterium exchange mass spectrometry (HDX-MS) to probe structural changes in the ligand-binding site upon agonist and antagonist binding. To further investigate the transmembrane region, we partially unfolded the receptor, revealing additional NMR signals. However, this approach also increased sample heterogeneity, highlighting the need for alternative strategies for obtaining high-quality NMR spectra of the entire protein.
In summary, the NMR characterization described here represents a critical step toward achieving a more complete resonance assignment of NTR1. Our work paves the way for probing the structural and dynamical features of NTR1 in different functional states, ultimately contributing to a deeper understanding of GPCR function and aiding in the design of more targeted and effective therapeutics.

NMR-048: Protein dynamics during early endocytosis: the example of FCHO proteins

Dr Carlos A. Elena-Real[1], Antonia Körber[1], Andromachi Papagiannoula[1], Kathrin Motzny[1], Dr Sigrid Milles[1]
[1]Leibniz-forschungsinstitut Für Molekulare Pharmakologie (FMP), Berlin, Germany

Thanks to clathrin-mediated endocytosis (CME), cells are able to take up different particles with an astonishing spatio-temporal precision and a very high cargo selectivity. This selectivity is encoded in the early phases of endocytosis, in which a complex sorting mechanism takes place. For this, multiple proteins containing a combination of folded domains and flexible, disordered regions are working together. While the folded domains are known to be essential for membrane binding and bending, and for cargo binding, disordered regions allow for dynamic, multivalent interactions required to recruit and shape the interaction network controlling the early stages of endocytosis. FCHO proteins are the first to arrive at the endocytic pit and are present as two homologs that are expressed in different tissues. While the folded domains are strictly conserved between the two homologs, this is not the case for the intrinsically disordered regions, which must thus be responsible for creating tissue specific function.
We have set out to study the structural dynamics and interaction landscape of both intrinsically disordered domains in FCHO1 and 2 proteins using NMR spectroscopy, which has allowed us to discover the presence of transient secondary structure elements with different levels of structure. Furthermore, spin relaxation point towards different dynamic regimes in both proteins, suggesting evolutionary differences. Interestingly, we have discovered that two of the principal FCHO partners, Eps15 and AP2, compete for binding to the same sequence within the disordered region of FCHO proteins, also affecting the dynamics of their binding sequences. We characterize these competitive interactions, which may be at the core of deciding whether an endocytic pits can mature, and thus shed light at cellular decisions in endocytosis on the molecular level.

NMR-049: Exploiting CLIC1 dynamics to develop Glioblastoma Multiforme inhibitors

Mrs Angela Serrano-Sanchez[1], Dr Joseph Cassar[2], Dr Fisayo Olotu[3], Dr Vahitha Abdul-Salam[3], Dr Jose Ortega-roldan[1]
[1]University Of Kent, Canterbury, United Kingdom, [2]University of Birmingham, Birmingham, United Kingdom, [3]Centre for Cardiovascular Medicine and Device Innovation, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom

Glioblastoma multiforme (GBM) is the most aggressive and prevalent type of brain tumour. Increasingly, evidence points towards CLIC1 promoting oncogenic development with its high level of activity and expression during tumorigenesis. CLIC1 Knockout studies have shown reduced proliferation and a decline in tumorigenic potential, making CLIC1 a highly promising pharmacological target.
CLIC1 displays the unique feature of altering its structure from a cytosolic, soluble form to a membrane-bound chloride channel, with membrane relocalisation linked to endothelial disfunction, tumour proliferation and metastasis. We have recently elucidated the mechanism of CLIC1 membrane insertion (Varela et al, JCS 2022), and subsequently used an integrated structural biology approach combining solution NMR, X-Ray crystallography and SAXS to elucidate structural models of partially open intermediates leading to tetramerization and insertion in the membrane.
15N Relaxation and CPMG relaxation dispersion on Wild Type CLIC1 and different mutants have identified areas of the protein of increased dynamics that enable its membrane insertion as well as potential allosteric sites. Specific targeting of these allosteric areas in an AI in-silico screening approach previously developed by us for the homolog CLIC4 (Olotu et al, CSBJ, 2023) has enabled us to identify a set of inhibitors that reduce CLIC1 membrane insertion. NMR titrations, X-Ray crystallography and 15N dynamics have revealed the allosteric site exploited by these inhibitors and their mechanism of action, reducing CLIC1 dynamics of opening and CLIC1 membrane translocation in cells.
In summary, we have elucidated how protein dynamics are involved in the membrane insertion mechanism of CLIC1 and have exploited this information to develop novel inhibitors of CLICs membrane insertion, paving the way for the development of new treatments of glioblastoma and other types of endothelial disfunction.

NMR-050: Dual client binding sites in the ATP-independent chaperone SurA

Dr Bob Schiffrin[1], Dr Joel Crossley[1], Dr Martin Walko[1], Dr Jonathon Machin[1], Mr G Nasir Khan[1], Dr Iain W Manfield[1], Prof Andrew J Wilson[2], Prof David J Brockwell[1], Dr Tomas Fessl[3], Dr Anton Calabrese[1], Prof Sheena Radford[1], Dr Anastasia Zhuravleva[1]
[1]University Of Leeds, Leeds, United Kingdom, [2]University of Birmingham, Birmingham, United Kingdom, [3]University of South Bohemia, České Budějovice, Czech Republic

The ATP-independent chaperone SurA protects unfolded outer membrane proteins (OMPs) from aggregation in the periplasm of Gram-negative bacteria and delivers them to the ?-barrel assembly machinery (BAM) for folding into the outer membrane (OM). Precisely how SurA recognises and binds its different OMP clients remains unclear. E. coli SurA comprises three domains: a core and two PPIase domains (P1 and P2). Here, by combining methyl-TROSY NMR, single-molecule FRET and bioinformatics analyses we show that SurA client binding is mediated by two binding hotspots in the core and P1 domains. These interactions are driven by aromatic-rich motifs in the client proteins, leading to SurA core/P1 domain rearrangements and expansion of clients from collapsed, non-native states. We demonstrate that the core domain is key to OMP expansion by SurA, but uncover a role for SurA PPIase domains in limiting the extent of expansion and increasing the rates of interchange between expanded and compact states. The results reveal new insights into SurA-OMP recognition and the mechanism of activation for an ATP-independent chaperone, and suggest a route to targeting the functions of a chaperone key to bacterial virulence and OM integrity.

NMR-051: A novel interaction site determines binding between AP180 and AP2 in clathrin mediated endocytosis

Mr. Samuel Naudi-fabra[1,2], Dr. Carlos A. Elena Real[1], Dr. Ida Marie Vedel[1], Mlle. Maud Tengo[2], Mrs. Kathrin Motzny[1], Mr. Pin-Lian Jiang[1], Dr. Peter Schmieder[1], Dr. Fan Liu[1], Dr. Sigrid Milles[1,2]
[1]Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Berlin, Germany, [2]IBS, Université Grenoble Alpes, CNRS, CEA, Grenoble, France

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). AP180 is a CLASP exclusively expressed in neurons and comprises a long IDR of around 600 residues, whose function remains partially elusive.
We set out to characterize how conformational dynamics and interaction patterns within AP180 give rise to function and enable the formation of clathrin coated vesicles.
Using NMR titration studies using different AP180 constructs, comprising also the 600 residue long full IDR, we characterize binding with AP180's major interaction partners, the major adaptor protein complex AP2 as well as the clathrin heavy chain terminal domain. 15N spin relaxation and CPMG relaxation dispersion were performed to assess AP180's backbone dynamics in the absence and presence of the interaction partners as well as exchange dynamics due to binding.
We describe a novel and strong interaction of AP180 with the major adaptor protein AP2 as well as its binding dynamics at atomic resolution. We find that a 70 residue-long site determines the overall interaction between AP180 and AP2 in a dynamic equilibrium between its bound and unbound sates, while weaker binding sites contribute to the overall affinity at much higher concentrations of AP2. Our data suggest that this novel and extended interaction site might play a central role in recruitment of adaptors to the clathrin coated pit, whereas more transient and promiscuous interactions allow reshaping the interaction network until cargo uptake inside a coated vesicle.

This paper meets the conference theme as it makes extensive use of solution NMR for the study of a textbook disordered protein, and highlights novel findings that can only be made through NMR

NMR-053: Structural Insights into High Mannose Recognition by Antibody 2G12: A Paramagnetic NMR approach

M.Paola Oquist-Phillips[1], Adrián Silva-Díaz[2], Javier Ramos-Soriano[2], Javier Rojo[2], Ángeles Canales[1]
[1]Department of Organic Chemistry, Faculty of Chemistry, Universidad Complutense de Madrid, Avd. Complutense s/n., Madrid, Spain, [2]Institute of Chemical Research (IIQ), CSIC - University of Seville , Avd. Américo Vespucio 49., Seville, Spain

Glycoprotein 120 (gp120) plays a crucial role in HIV infection by facilitating viral entry into host cells. gp120 is extensively glycosylated with high mannose glycans, which not only contribute to the structural integrity of gp120 but also serve to shield critical epitopes from host immune recognition. However, the structural characterization of high mannose glycans is still a challenge due to both the inherent flexibility that precludes X-ray studies and the spectral overlapping that hampers NMR elucidation.

In this context, an effective solution to this problem involves the use of paramagnetic NMR spectroscopy by using lanthanide binding tags covalently attached to the glycan. Particularly, the pseudo-contact shift (PCS) induced by the paramagnetic ion, are especially useful since they allow differentiating the three branches of the high mannose glycan (Man9) that are identical in standard NMR experiments. In addition, this method facilitates interaction studies with biologically relevant proteins.

2G12 stands out as one of the first neutralizing antibodies discovered against HIV-1, it has significant implications for the development of immunogens that can induce 2G12-like neutralizing antibodies as part of an HIV vaccine or other viruses. The detailed molecular recognition studies of Man9 by 2G12 are complex due to the inconvenient described above. Elegant attempts have been described by Jesús Ángulo’s group by using Man9 fragments and STD-experiments1. Nevertheless, there is a lack of structural data regarding intact high mannose structures.

Herein, we described novel NMR methods that allow characterizing both intact Man9 conformation and the detailed molecular recognition study of its interaction with 2G12. This development paves the way for a deeper understanding of high mannose interactions with other lectins.

Funding: Spanish Ministry of Science and Innovation (PID2019-105237GB-I00)

[1] Chemistry – A European Journal, 17, 5, 1547–1560. 2011.

NMR-054: Sensitivity-enhanced 15N NMR relaxation experiments with improved water-suppression at high magnetic fields provide novel insights into the structural dynamics of the SNARE protein SNAP25a

Mr Tobias Stief, Ms Katharina Vormann[1], Mr Nils-Alexander Lakomek[2]
[1]Institute of Biological Information Processing (IBI-7), Forschungszentrum Jülich, Jülich, Germany,
[2]Institute of Physical Biology, Heinrich Heine University Düsseldorf, Düsseldorf, Deutschland

SNARE (soluble N-ethylmaleimide-sensitive-factor attachment receptor) proteins are central for synaptic vesicle fusion at the neuronal synapse. The assembly of the SNARE complex provides the energy necessary for membrane fusion. The structural transitions of the SNARE proteins and their membrane interactions are, however, not well understood at the molecular level. We studied SNAP25a (synaptosomal-associated protein of 25kDa), one of the three SNARE proteins, by NMR spectroscopy to obtain new structural insights in the pre-fusion state: SNAP25a is mostly intrinsically disordered and shows high internal flexibility. Two α-helices form the N-terminal part of the first SNARE motif of SNAP25a, but the remainder of the protein is intrinsically disordered, including the second SNARE motif. We hypothesize that the SNAP25a N-terminus may act as a nucleation site for initiating SNARE zippering. [1]
To study the backbone dynamics of SNAP25a and other intrinsically disordered proteins (IDPs), we optimized a set of sensitivity-enhanced backbone relaxation experiments (R1, R1ρ) for improved water-suppression, yielding high signal-to-noise (SNR) for intrinsically disordered proteins (IDPs) and small proteins. We tested these sequences on protonated and deuterated samples and found a strong correlation. [2]

[1] Stief T., Gremer L., Pribicevic S., Espinueva D.F., Vormann K., Biehl R., Jahn R., Pérez-Lara Á., Lakomek N.A., J Mol Biol. 2023 Mar 30;435(10):168069.
[2] Stief T., Vormann K., Lakomek N.A., Methods 2024, 223,1-15

NMR-055: NMR reveals how human serum albumin inhibits Parkinson's disease-relevant Cu(II)-alpha synuclein interactions.

Miss Karla Martinez Pomier[1], Dr. Rashik Ahmed[1], Dr. Jinfeng Huang[1], Dr. Giuseppe Melacini[1,2]
[1]McMaster University, Hamilton, Canada, [2]Department of Biochemistry and Biomedical Sciences, McMaster University , Hamilton, Canada

Introduction

Human serum albumin(HSA), the most abundant protein in plasma and cerebrospinal fluid serves as a crucial carrier of various physiological ligands while also modulating the aggregation of amyloidogenic proteins, including alpha-synuclein(αSyn), associated with Parkinson's disease. HSA decreases αSyn-related toxicity through the direct binding to monomeric and oligomeric αSyn species. Yet, it is possible that HSA also sequesters metal ions that otherwise promote aggregation. Cu(II), for example, enhances αSyn fibrillization in vitro, while also leading to neurotoxicity by generating reactive oxygen species(ROS). However, it is currently unclear if and how HSA affects Cu(II)-binding to αSyn.

Aims

We aim to: (1)elucidate if HSA chelates Cu(II) from αSyn, (2)explore whether glycation of HSA or binding of endogenous fatty acids affects its chelating capabilities, and (3) determine how Cu(II) ions impact previously reported HSA-αSyn interactions.

Methods

Our study primarily relies on low-resolution 1D-13C and high-resolution 2D-1H-15N protein-NMR studies, to monitor the different interactions between HSA, αSyn, and paramagnetic Cu(II) ions. Hence, it fits well with the conference theme.

Results

We show that HSA chelates Cu(II) ions from αSyn more efficiently than standard chelators such as EDTA, revealing an unexpected cooperativity between HSA metal-binding sites. Notably, fatty acid binding to HSA perturbs this cooperativity, thus interfering with the sequestration of Cu(II) from αSyn. We also observed that glycation of HSA diminished Cu(II)-binding affinity while preserving the degree of cooperativity between HSA metal-binding sites. Additionally, we found that Cu(II)-binding to HSA stabilizes the interactions of HSA with αSyn primarily at two different regions, i.e. the N-terminus, Tyr 39 and the majority of the C-terminus.

Conclusions

Our study not only unveils the effect of fatty acid binding and age-related posttranslational modifications, such as glycation, on the neuroprotective mechanisms of HSA, but also highlights the potential of αSyn as a viable NMR-based sensor to investigate HSA-metal interactions.

NMR-056: The two faces of the splicing factor PRPF40A revealed by an NMR-led integrative structural biology approach

Dr Santiago Martínez Lumbreras[1,2], Lena Traeger[2], Niklas Dold[2], Miriam Mulorz[3], Marco Payr[2], Paul Welzl[2], Varvara Dikaya[2], Clara Hipp[2], Dr Julian König[3], Prof. Michael Sattler[1,2]
[1]Helmholtz Munich, Molecular Targets and Therapeutics Center, Institute of Structural Biology, Neuherberg, Germany, [2]Technical University of Munich, TUM School of Natural Sciences, Bavarian NMR Center and Department of Biosciences, Garching, Germany, [3]Institute of Molecular Biology (IMB) , Mainz, Germany

The early assembly of the spliceosome is an essential process for the regulation of pre-mRNA splicing and contributes to alternative splicing. The splicing factor PRPF40A plays a key role in these early steps by promoting bridging between 5´and 3´ splice sites across pre-mRNA introns through protein-protein interactions in spliceosomal E and A complexes. The PRPF40A tandem WW domains are expected to recognize the proline-rich sequences within SF1 and/or SF3A1, while the six FF domains located in the C-terminal region are suggested to interact with U1snRNP-associated proteins. However, the exact binding partners and the molecular details and specificity of these interactions are not known yet.
Here, we present a comprehensive structural characterization of the PRPF40A factor and its various interactions using an integrative structural biology approach led by solution NMR.
We analyzed the structure and dynamics of the N-terminal region of PRPF40A comprising tandem WW domains free and bound to an identified high affinity proline-rich peptide in the C-terminal intrinsically disordered region of splicing factor 1 (SF1) using PRE, RDCs and SAXS. The NMR structure of the complex highlights key contributions by both WW domains including a novel type of proline stacking interaction. Importantly, we show that the N-terminal intrinsically disordered region of PRPF40A mediates intramolecular interactions with the tandem WW domains, that compete with intermolecular ligand binding. Using mutational analysis, we show that these interactions are autoinhibitory and provide a selectivity filter for high-affinity proline-rich motifs. Our data suggest autoinhibitory intramolecular interactions as a general mechanism of WW-containing proteins for enhancing binding selectivity and the regulation of their interacting networks.
Combining X-ray crystallography, SAXS, and NMR, we found that the C-terminal region, composed of six FF domains, presents rigid and structured linkers. We used selective methyl labeling to characterize its interaction with U1snRNP components and guide crystallographic structural analysis.

NMR-057: Unveiling the Mechanism of Action for a Kinase Mutation that Causes Hormonal Resistance Leading to Bone Malformations and Intellectual Disabilities

Leonardo Della Libera[1], Karla Martinez-Pomier[1], Dr. Madoka Akimoto[1], Dr. Bryan VanSchouwen[1], Dr. Giuseppe Melacini[1,2]
[1]Department of Chemistry and Chemical Biology, McMaster University, Hamilton, Canada,
[2]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Canada

Introduction

Protein kinase A (PKA) is a downstream receptor of cyclic-adenosine monophosphate (cAMP) and is essential for propagating and converting the cAMP second messenger signal into tightly regulated cellular responses. cAMP binding to the regulatory subunit of PKA (PKA R) results in kinase activation. Several mutations in the gene encoding the most ubiquitous isoform of PKA R (PKA R1a) are the cause acrodysostosis – a disease characterized by hormonal resistance leading to severe congenital malformations. The loss-of-kinase function R366X mutant causes truncation of C-terminal residues, exhibits severe kinase hypoactivation, and is the most common acrodysostosis genotype observed.

Aim

Though functional studies show drastic losses in activation, the crystal structure of R366X in its kinase-bound form shows only minimal changes compared to the wild type. This structure, however, only captures the most stable inhibitory state of the PKA R1a mutant. We hypothesize that R366X acts by perturbing transient states not visible by crystallography. Our aim is to study R366X intermediates along the activation/deactivation pathway through NMR - enabling us to uncover the allosteric effects of R366X responsible for aberrant behaviour.

Methods

Our approach combines low- and high-resolution studies. We take advantage of the versatility of bio NMR by employing transfer-NOESY, 2D 1H-15N HSQC, and intra-molecular paramagnetic relaxation enhancement (PRE), to comprehensively assess the effects of R366X on ligand binding and the PKA allosteric network.

Results & Conclusions

Our studies show that R366X destabilizes cAMP binding, and disrupts long-range allosteric communication within PKA. Our NMR methods also allow us to prove that R366X not only impedes PKA activation, as seen previously, but also accelerates the deactivation pathway responsible for terminating the cAMP signal. By uncovering these elusive mechanisms we aim to provide an avenue for developing treatments to counter the effects of this inherited acrodysostosis mutation.

NMR-058: Making Relaxation work in the 21st Century: Streamlined Relaxation Analysis in Biological NMR with NEF-Pipelines and the NMR Exchange Format.

Dr Gary Thompson[1], Dr Eliza Płoskon-Arthur[2], Prof Geerten Vuister[2]
[1]University of Kent & CCPN, Canterbury, UK, [2]University of Leicester & CCPN, Leicester, UK

The impressive abilities of AlphaFold2 have clearly radically changed our ability to model the structures of proteins and the speed at which scientific advances are made. However, these advances rely on the large scale deposition of data in the PDB format…

NMR excels at the analysis of dynamics via relaxation across multiple timescales [fs-s]. However, the data and analysis for much of this experimentation is relatively inaccessible to the community because of the lack of an easy way to exchange data between programs and deposit it. Moreover, revolutionary insights from the analysis of large data sets for machine learning into areas such as biological entropy are just not possible [there are less than 400 relaxation data sets in the BMRB overall]. To solve this problem the NMR Exchange Format Consortium is publishing the draft of a format for NMR relaxation series data and analysed rates.

NEF-Pipelines is a set of tools designed to ease the import, manipulation and export of data in the NEF file format. It is a software agnostic tool-set and supports data exchange between a wide and growing number of programs including NMR/Pipe, NMRView/FX, Sparky/Poky, CcpNmr AnalysisAssign, MARS, PALES, iPine, TALOS, CYANA, XPLOR and many more.

As a step in this process NEF-Pipelines is now supporting the assembly and exchange of relaxation data in the draft NEF format. It will be demonstrated that NEF-Pipelines eases the transport of data between spectral display / analysis programs and multiple relaxation analysis programs and repositories in a modular and easy to use manner. It is hoped that this increase in the uniformity of relaxation data pipelines should allow editors of journals to move to _requiring_ the deposition of experimental and analysed data so that the troves of information we need for future research and analysis can become available.

NMR-059: Handle with care - How the cytosol orchestrates the handover of aggregation-prone mitochondrial precursor proteins to achieve competent import into mitochondria.

Mr. Jakob Schneider[1]
[1]ISTA, Klosterneuburg, Austria

Introduction
Mitochondria are essential organelles as they are involved in a plethora of cellular functions, like biosynthesis of amino acids and lipids, energy metabolism, and immune response. Almost the whole mitochondrial proteome is encoded in the nucleus. Therefore, mitochondrial biogenesis and homeostasis require a sophisticated protein import machinery. As these proteins have to be imported in an unfolded state, they are prone to cytotoxic aggregation in the cytosol. To prevent this, the cell employs a tightly controlled network of cytosolic chaperones and membrane-anchored mitochondrial proteins. The important players of this machinery have been identified but the mechanistical details remain elusive.

Aims
We study the interactions of the protein factors involved in the handling of mitochondrial precursor proteins. The interactions of these unfolded substrates with the studied cytosolic proteins are transient, as the substrate has to be bound stable enough to prevent aggregation, but also has to be able to dissociate in order to be sorted to its designated destination. We aim to understand the molecular mechanisms the involved proteins employ to balance this task.

Methods
The studied interactions are highly dynamic. For this reason, we use NMR spectroscopy in combination with computational methodology to identify interaction sites and assess the role of protein dynamics. As these protein complexes can be rather large, we employ a combination of backbone isotope labeling and site-specific methyl labeling.

Results
We show the specific interactions of the members of the mitochondrial Translocase of the Outer Membrane (TOM) complex with each other, cytosolic chaperones, and their substrates. From this, we construct a mechanism of substrate handover, resulting in competent mitochondrial protein import.

Conclusion
The results demonstrate the strength of NMR spectroscopy in the research of transient interactions that are often present in chaperone networks and help to understand the molecular details of mitochondrial protein import.

NMR-060: Partitioning, phase, and pH: exploring structure and dynamics in pulmonary surfactant

Joanna Long[1,2], Diana Tymochko[1], Maria Luiza Nogueira[1,2], Nhi Tran[1], Lauren Schaffer[1], Faith Scott[2], Fred Mentink-Vigier[2]
[1]University Of Florida, Gainesville, United States, [2]National High Magnetic Field Laboratory, Tallahassee, United States

Membrane-active peptides derived from Surfactant Protein B (SP-B) exhibit unique abilities to modulate lipid phase prop-erties. Using both conventional ssNMR and MAS-DNP methodologies, we have been mapping out the properties of model pulmonary surfactant (PS) formulations that mimic the native lung environment and therapeutic formulations used to treat acute respiratory distress in premature infants. By characterizing lipid phases and dynamics in concert with peptide structure, partitioning, and dynamics as a function of pH and temperature, we are mapping out how differential partitioning of amphipathic PS peptides in response to environmental conditions leads to the unique lipid phase behaviors observed in PS. Our observations point to a novel mechanism for trafficking of dipalmitoylphosphatidylcholine (DPPC), the primary lipid in PS, to the air-water interface to lower surface tension and support breathing at ambient pressures. As part of my presentation I will highlight recent advances in MAS-DNP, infrequently utilized ssNMR approaches for mapping out lipid dynamics, and complimentary EPR measurements that enabled us to develop a holistic understanding of the elegant biophysics underlying PS function.

NMR-061: Identification of anticancer peptide CIGB-300 isomer. Influence of racemization in the stabilization of secondary structure.

Celia Gonzalez Moya[1,2], Rafael Rodriguez[2], Prof.Dr Carlos Perez[2], Dr Hilda Garay[3], Dr Silvio Perea[3], Stefan Gröger[1], Prof.Dr Jochen Balbach[1]
[1]Martin Luther University, Halle Saale, Germany, [2]University of Havana, Havana, Cuba, [3]Centre for Genetic Enginiering and Biotechnology, Havana, Cuba

CIGB-300, developed by the Center for Genetic Engineering and Biotechnology, targets Casein Kinase 2 (CK2) and its substrates, implicating significant therapeutic potential in cancer treatment. A key focus of this research was identifying a primary byproduct, CIGB-300iso, which shares the amino acid sequence with CIGB-300 but differs due to the racemization of histidine at position 21. This study explores the synthesis, characterization, and structural elucidation of CIGB-300, a novel anti-tumor peptide, and its isomer, CIGB-300iso. Through comprehensive NMR analysis and comparison of chemical shifts, this work confirms the hypothesis regarding the structural discrepancies between CIGB-300 and CIGB-300iso, attributing them to the racemization process. Notably, the racemization alters the peptide's supersecondary structure, transforming a β-turn type IV3 found in CIGB-300 into a type I β-turn in CIGB-300iso, significantly impacting the peptide's conformations and, potentially, its biological activity. This alteration was further corroborated by synthesizing seven diastereoisomers, pinpointing the racemization site, and detailing the structural implications of this modification. The findings highlight the critical role of peptide structure in its biological function, with implications for the design and synthesis

NMR-062: Screening of Inhibitors of ALR Protein–Protein Interaction Pathway using NMR spectroscopy

Dr Angelo Gallo[1], Dr Riccardo Muzzioli[2]
[1]University of Turin Department of Chemistry, Torino, Italy, [2]Solve Therapeutics, Durham, USA

MIA40 and ALR of the MIA pathway mediate the import of protein precursors that form disulfides into the mitochondrial intermembrane space.[1] This import pathway is suggested to be a linear pathway in which MIA40 first binds to the precursor via a disulfide linkage and oxidizes it.[2] Subsequently, ALR re-oxidizes MIA40 and then ALR transfers electrons to terminal electron acceptors. With a collection of small molecule modulators (MB-5 to MB-9 and MB-13) that inhibit ALR activity, we characterized the import mechanism in mitochondria. NMR studies show that most of the compounds bind to a similar region in ALR.
Here NMR spectroscopy provided a method to investigate protein–ligand interactions in solution, mimicking, even though not fully reproducing, the physiological flexibility of a protein target present in the cell compartment. Appropriate protein preparation protocols coupled with sensitive NMR experiments and systematic data analysing schemes were applied for a drug discovery investigation that clearly showed that the integration of several techniques is needed.[3] Characterization was carried out mainly in vitro and in silico with some information on the inhibitory activity in cellulo by Koehler and coworkers[4].
Mechanistic studies with small molecules demonstrate that treatment with compound MB-6 locks the precursor in a state bound to MIA40, blocking re-oxidation of MIA40 by ALR. Thus, small molecules that target a similar region in ALR alter the dynamics of the MIA import pathway differently, resulting in a set of probes that are useful for studying the catalysis of the redox-regulated import pathway in model systems.

References:
[1] L. Banci, et al. Nat. Struct. Mol. Biol. 2009, 16, 198–206.
[2] L. Banci, et al. J. Am. Chem. Soc. 2012, 134, 1442–1445.
[3] D.V. Dabir, et al. Dev. Cell 2013, 25, 81–92
[4] D.V. Dabir, et al. EMBO J. 2007, 26, 4801–4811

NMR-063: Stable Isotope Labelling of Pseudouridine and N1-Methylpseudouridine Triphosphates: Application for Bio-NMR Spectroscopy

Dr. Filip Sebest[1]
[1]Silantes GmbH, Munich, Germany

Despite commendable advances in biomolecular NMR (Bio-NMR) spectroscopy in recent years, the interpretation of NMR data for larger molecules such as RNAs remains a challenge. While labelling of RNAs with stable isotopes (e.g. ^{2}H, ^{13}C, ^{15}N) can reduce the complexity of NMR spectra, this strategy is often associated with very high costs, particularly for RNA sequences containing modifications. Here, we present a commercially attractive approach for the synthesis of stable isotopically labelled (SI-labelled) pseudouridine (Ψ) and N^{1}-methylpseudouridine ($m^{1}\Psi$) triphosphates containing ^{1}H-^{13}C and ^{1}H-^{15}N isolated spin topologies for application in Bio-NMR spectroscopy.

The purpose of this research was to develop a versatile, convenient, and affordable method for the preparation of SI-labelled Ψ and $m^{1}\Psi$ triphosphates and to test whether these RNA building blocks would be efficiently incorporated into RNA fragments by in vitro transcription.

Uniformly SI-labelled non-modified nucleoside monophosphates (NMPs) were produced from relatively inexpensive reagents by a chemolithoautotrophic fermentation with Cupriavidus necator and used as main starting materials for the chemo-enzymatic synthesis of SI-labelled modified NTPs. Subsequent feasibility studies of incorporating these NTPs into 45mer RNA fragments were performed enzymatically in a T7RNA polymerase system.

Depending on the desired isotopic labelling pattern, the preparation of ΨTPs and $m^{1}\Psi$TPs was accomplished in 3–5 steps and 4–7 steps, respectively. The associated average yields with respect to the most expensive building block (ribose 5’-monophosphate) were 59% and 33%, respectively. The isotopic purity of all NTPs was determined to be > 96% and their incorporation into 45mer RNAs by in vitro transcription proceeded successfully, with kinetics comparable to incorporation of uridine triphosphate.

Overall, we generated differently SI-labelled ΨTPs and $m^{1}\Psi$TPs by developing an efficient, adaptable, and commercially profitable method, and incorporated them into RNA fragments. Future efforts will be directed on producing analogous nucleoside phosphoramidites for the chemical synthesis of site-specifically labelled modified RNAs.

NMR-064: Combining High-Pressure NMR and geometrical sampling to describe protein folding landscapes.

Dr Karine De Guillen[1], Mounia Lahfa[1], Dr Cécile Dubois[1], Dr Thomas Kroj[2], Dr Philippe Barthe[1], Dr André Padilla[1], Dr Christian Roumestand[1]
[1]Centre de Biologie Structurale, UM / CNRS UMR 5048 / INSERM U1054, Montpellier, France, [2]Plant Health Institute, Univ Montpellier, INRAE, CIRAD, Institut Agro, IRD, Montpellier, France

Despite advances in experimental and computational methods, the mechanisms by which an unstructured polypeptide chain regains its unique three-dimensional structure remains one of the main puzzling question in biology. Single-molecule techniques, ultra-fast perturbation and detection approaches and improvement in all-atom and coarse-grained simulation methods have greatly deepened our understanding of protein folding and the effects of environmental factors on folding landscape. But a major challenge remains the detailed characterization of the protein folding landscape. Here, we used high hydrostatic pressure 2D NMR spectroscopy to obtain high-resolution experimental structural information in a site-specific manner across the polypeptide sequence and along the folding reaction coordinate. We used this residue-specific information to constrain Cyana3 calculations, in order to obtain a topological description of the entire folding landscape. This approach was used to describe the conformers populating the folding landscape of two small globular proteins, AVR-Pia and AVR-Pib, that belongs to the structurally conserved but sequence-unrelated MAX effectors superfamily. Comparing the two folding landscape, we found that, in spite of their divergent sequences, the folding pathway of these two proteins involves a similar inescapable folding intermediate, even if, statistically, the routes used are different.

NMR-066: Molecular Interactions of GIP Incretin Hormone with its N-terminal Domain

Prof Chandralal Hewage[1]
[1]University College Dublin, Dublin 4, Ireland

Diabetes is a major threat to the global community. One of the hormones, glucose-dependent insulinotropic polypeptide (GIP) is a gastrointestinal hormone that stimulates insulin secretion by interacting with a G-protein coupled receptor located in pancreatic ?-cell. Due to its glucose lowering and insulinotropic properties, GIP is considered as a potential target for treating type 2 diabetes. In our laboratory, we identified the solution structures of GIP in various solution conditions including membrane mimicking (micellular and bicellular) media using NMR spectroscopy and computational modelling techniques. In order to exploit the potential of GIP for diabetes therapy, our research focus on understanding the GIP hormone-receptor interactions. In this work using NMR based docking approach we have determined the likely docking position of the hormone with its receptor binding region and revealed a likely interaction of GIP amino acid side chains with specific residues on the extra cellular domain from the GIP receptor. These results provide a basic understanding of the interaction mechanism of GIP with its receptor that can be useful for studying the development of peptide drugs for treating type 2 diabetes.

Structural Aspects of Gut Peptides with Therapeutic Potential for Type 2 Diabetes
Hewage CM, Venneti KC. ChemMedChem. 2013, 8(4), 560-567. PMID: 23292985
Conformational and molecular interaction studies of glucagon-like peptide-2 with its N-terminal extracellular receptor domain
Venneti KC, Hewage CM. FEBS Lett. 2011;21;585(2):346-52. PMID: 21167157

NMR-067: Developing solid-state NMR spectroscopy approaches to study extracellular matrices

Ananya Singh[1], Sabeeha Malek[1], Teresa Massam-Wu[1], Mohan Balasubramanian[1], Darius Vasco Koester[1], Wing Ying Chow[1]
[1]University Of Warwick, Coventry, United Kingdom

Introduction

Solid-state NMR (ssNMR) spectroscopy can bring new structural insight to extracellular matrices (ECM). Here, we use ssNMR to illuminate the structure and composition of two kinds of ECMs from two different kingdoms of life:

- cell wall from a fungus, Schizosaccharomyces pombe,
- ECM from human fibroblast culture, relevant to a rare disorder called Ehlers-Danlos Syndrome (EDS), which is characterized by remarkably elastic and fragile skin with unusually flexible joints.

As a first step, we optimized ECM preparation and handling conditions to obtain robust and reproducible samples for ssNMR investigation.

Results

In the fungal project, we observed different α-glucan to β-glucan proportions in the mutants for the unlabelled cell wall samples. In the human fibroblast project, we achieved labelling of glycine and proline in ECM proteins. Variations in the amino acid levels in the three ECM samples- wild-type (WT), hyperplastic EDS (hEDS), and classical EDS (cEDS) were revealed. We also observed an overall decrease in collagen content in the ECM of hEDS and cEDS compared to that of the WT.

Conclusions

The biochemical changes observed by ssNMR in the ECM have not been previously reported in EDS literature. These findings may suggest alterations in post-translational modifications and provide new routes to diagnosis.

In the fungal project, the results demonstrate the strength of combining natural abundance ssNMR with a range of mutations available for S. pombe, a model organism. Further work on 13C-labelled cell walls will improve assignment, quantification, and also enable differences in their dynamics to be probed, eventually leading to the development of new antifungal treatments.

NMR-068: Exploring Variability in Outer Surface Proteins among Borrelia Species: Assessing Structure Versus Function

Mgr. Libor Hejduk[2], PhD Filip Dycka[2], PhD Jan Sterba[2], PhD Ryan O. M. Rego[3], Prof. Norbert Müller[1], PhD Martin Strnad[3], Dr. Adriana Rathner[1]
[1]JKU Linz, Linz, Austria, [2]University of South Bohemia, Ceske Budejovice, Czech Republic, [3]Biology Center CAS, Ceske Budejovice, Czech Republic

Borrelia spirochetes responsible for Lyme disease employ a repertoire of adhesin proteins to infiltrate host cells by interacting with extracellular matrix components such as proteoglycan decorin (decorin-binding proteins, DbpA/DbpB) [1]. The Dbp proteins demonstrate an affinity for glycosaminoglycan (GAG) moieties, and variations in these interactions among Borrelia species have been linked to tissue tropism [2]. Despite limited sequence similarity, Dbps share a conserved 3D structure, except for the "linker" region identified as the primary GAG-binding site in North American species [3].
This study probes impact of subtle conformational differences in homologous adhesins among Dbps from European Borrelia strains on their binding affinity towards GAGs. DbpA and DbpB proteins from B. afzelii, B. bavariensis, and B. garinii were over-expressed as isotopically labeled recombinant proteins in E. coli. Analysis included NMR-based secondary structure predictions and characterization of backbone dynamics of the representative protein variants. Binding affinities to GAGs were explored for Dbp proteins through NMR titration and hydrogen-deuterium exchange mass spectrometry [4].
While Dbp proteins exhibited the ability to bind to all ligands, the specific affinities and residues involved in the interaction demonstrated clear specificity, heavily dependent on the Borrelia species. Interestingly, the affinities of European Dbps significantly deviated from previously determined values for North American adhesins, despite their overall structural similarity [3, 5]. These findings give support to the hypothesis proposing a highly selective interplay between Dbps and GAGs, shedding light on the distinct tissue tropisms observed in various Borrelia species.
References:
1. C.A. Brisette and R.A. Gaultney, Front. Cell. Infect. Microbiol., 2014, 03: 41
2. Y.P. Lin, V. Benoit, X. Yang et al., PLoS Pathog, 2014, 10(7):e1004238
3. A.M. Morgan, X. Wang, Biochem J, 2015, 467(3): 439-451.
4. L. Hejduk, P. Rathner, M. Strnad et al., Biomol NMR Assign, 2021, 15:415-420
5. A.M. Morgan, X. Wang, Biochemistry, 2013, 52(46):8237-45

NMR-069: Glycan recognition mode of Osteopontin, insights from an NMR perspective

Dr. Iris Bermejo[1], MSc Mirane Florencio-Zabaleta[1], Dr. Tammo Diercks[1], Dr. Ana Ardá, Dr. Óscar Millet, Dr. Borja Mateos, Dr. Jesús Jiménez-Barbero[1], Dr. Luca Unione[1]
[1]CICbioGUNE, Bizkaia Technology Park, Building 800 , Derio, Spain

Osteopontin (OPN) is a highly phosphorylated and glycosylated protein found in the extracellular matrix of cells. It contributes to cell spreading and adhesion; being involved in a myriad of processes like bone remodeling, wound healing, inflammation and tumor growth and progression.[1]
The glycosylation pattern of OPN modulates these cellular processes by interacting with glycan binding proteins.[2] However, the precise mechanism through which O-glycosylation of OPN influences these processes remains not fully understood to date.
Here, the OPN protein was expressed in human HEK293F cells with uniformly 13C-labelled glycans.[3] By using state-of-the-art NMR experiments, such as ^{1}H-^{13}C-HSQC, ^{1}H-STD and ^{1}H-STD-^{1}H-^{13}C-HSQC NMR, we elucidate OPN's glycans interactions with diverse glycan-binding proteins, such as Siglecs and Galectins. Altogether, these results shed light on the structural basis of the elusive glycan recognition mode of OPN, aiming to provide a better understanding of the role of OPN post-translational modifications in regulating biochemical processes.

[1] H. E. Miwa, J. Biol. Chem., 2010, 285, 1208-1219
[2] Y. Kariya, Biochem. J., 2014, 463, 93-102
[3] L. Unione, ACS Cent. Sci., 2019, 5, 1554-1561

NMR-070: The Structural Landscape of OxyS Small Non-Coding RNA Involved in Bacterial Stress Response

Dr. Vesna Stih[1], Prof. Janez Plavec[1,2,3], Dr. Peter Podbevsek[1]
[1]National Institute of Chemistry, Ljubljana, Slovenia, [2]University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia, [3]EN-FIST Centre of Excellence, Ljubljana, Slovenia

Small non-coding RNAs are an important class of RNAs in bacteria with regulatory roles in stress response and adaptation to environmental changes. OxyS is a 109 nucleotide long, stable, trans-encoded sRNA found in Escherichia coli. It is regulated by OxyR and is induced in high concentration in response to oxidative stress caused by an elevated concentration of hydrogen peroxide. OxyS is a global regulator affecting the expression of multiple genes, mainly through direct base-pairing with several mRNAs. Despite the clear biological role of OxyS and its significance for the regulation of stress response in E. coli, the structure of OxyS and its complexes with mRNAs have not been studied by high-resolution methods. We adopted the divide and conquer approach to determine the solution structures of the isolated stem-loops as well as full-length OxyS using NMR spectroscopy and SAXS. Our data shows that OxyS adopts a structure with four stem-loops, one of which has not been previously identified. The molecular envelope of OxyS demonstrates that the RNA adopts an extended boomerang-like structure with two arms. The superimposition of 3D models onto the molecular envelope shows that two stem-loops constitute the long arm, whereas remaining two stem-loops coaxially stack and constitute the short arm of the molecular envelope. We performed optimization of the ensemble of OxyS models and demonstrated that OxyS is able to adopt multiple conformations which slightly differ in the relative orientation of the four stem-loops. Furthermore, we employed NMR to investigate the details of the interaction between sRNA OxyS and mRNA fhlA. We identified the specific nucleotides involved in the intermolecular base pairing and demonstrated that the interaction does not involve all s complementary nucleotides from OxyS and fhlA.

NMR-073: MOLECULAR INSIGHTS INTO THE RECOGNITION OF OLIGOSACCHARIDES BY GALECTIN-9

Msc Mirane Florencio Zabaleta[1], Mrs Sandra Delgado[1], Prof Jesús Jiménez Barbero[1,2,3,4], Dr Ana Ardá Freire[1,2], M. Gómez-Redondo[1]
[1]Cic Biogune, Derio, España, [2]Ikerbasque, Basque Foundation for Science, Bilbao, España, [3]Department of Organic and Inorganic Chemistry, Leioa, España, [4]Centro de Investigación Biomédica En Red de Enfermedades Respiratorias, Madrid, España

Galectin-9 is a tandem-repeat galectin that has been reported to have a major role in many biological processes such as cell growth, differentiation, communication, and death.[1] Moreover, it has been proposed as a possible biomarker for many pathologies due to its immunomodulatory capacity.[2] Due to its biological importance, its molecular recognition properties have been analyzed during this work. As a tandem-repeat galectin, Galectin-9 is composed of two carbohydrate recognition domains (CRDs) covalently linked by a peptide linker. Herein, the NMR backbone assignment of both the C-domain and N-domain of Galectin-9 has been achieved, instrumental for defining the protein residues implicated in oligosaccharide binding. Additionally, ligand-observed techniques such as STD and STD-HSQC experiments have performed. In particular, the binding to Lactosamine (LacNAc), the B-antigen tetrasaccharide (B-type 2),[3] 3-Sialyl Lactosamine (3-S'LacNAc), and polylactosamine[4] oligosaccharides has been compared. Affinity values were confirmed by isothermal titration calorimetry (ITC), and molecular dynamics (MD) simulations were performed to obtain 3D atomic resolution complexes.

[1] V. R. Wiersma, M. de Bruyn, W. Helfrich, and E. Bremer, "Therapeutic potential of Galectin-9 in human disease," Med Res Rev, vol. 33, no. SUPPL. 1, Apr. 2013, doi: 10.1002/med.20249.
[2] P. Moar and R. Tandon, "Galectin-9 as a biomarker of disease severity," Cellular Immunology, vol. 361. Academic Press Inc., Mar. 01, 2021. doi: 10.1016/j.cellimm.2021.104287.
[3] A. V. Blenda et al., "Galectin-9 recognizes and exhibits antimicrobial activity toward microbes expressing blood group-like antigens," Journal of Biological Chemistry, vol. 298, no. 4, p. 101704, 2022, doi: 10.1016/j.jbc.2022.101704.
[4] Moure, M. J.; Gimeno, A.; Delgado, S.; Diercks, T.; Geert-Jan Boons; Jesús Jiménez-Barbero; Ardá, A. Selective 13 C-Labels on Repeating Glycan Oligomers to Reveal Protein Binding Epitopes through NMR: Polylactosamine Binding to Galectins. Angewandte Chemie 2021, 60 (34), 18777–18782. https://doi.org/10.1002/anie.202106056.

NMR-074: The effect of the ribosome on the conformational ensemble of an unfolded nascent chain

Dr Julian O Streit[1], Dr Anaïs M Cassaignau[1], Dr Sammy HS Chan[1], Dr Ivana V Bukvin[1,2], Dr Tomasz Wlodarski[1,3], Dr Christopher A Waudby[4], Dr Lisa D Cabrita[1], Dr Angelo M Figueiredo[1], Prof John Christodoulou[1]
[1]Institute Structural & Molecular Biology, University College London, London, United Kingdom, [2]Department of Biology, Stanford University, Stanford, United States, [3]Department of Bioinformatics, Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland, [4]School of Pharmacy, University College London, London , United Kingdom

Most proteins fold co-translationally where the ribosome can play a significant role in regulating nascent chain (NC) folding. Consequently, co-translational folding (coTF) differs from in vitro refolding studies of analogous, isolated counterparts with unique coTF intermediate conformations, folding in the absence of the complete protein sequence and the ability of the ribosome to mitigate misfolding-prone destabilising mutations among the many discriminating observations whose origins remain poorly understood.

In contrast to refolding studies, the unfolded state on the ribosome exists under native conditions and is adopted by all proteins during early biosynthesis. The ribosome-bound unfolded state has been elusive, and its dynamics and structure have not been studied in detail. In this work, we quantitatively examine a series of interactions between the highly charged ribosome surface and the unfolded NC of the immunoglobulin-like FLN5 filamin domain. We combine protein engineering with cross-correlated relaxation rate (CCR) measurements and chemical shift analyses, and identify a C-terminal segment that strongly binds to the ribosome surface and is essential for folding. Mutations in this region reduce the extent of binding and result in a shift in the co-translational folding equilibrium towards the native state, demonstrating a competition between structure acquisition and binding during co-translational folding.

We are also exploring the use of paramagnetic relaxation enhancement (PRE), residual dipolar coupling (RDC) measurements combined with molecular dynamics (MD) simulations to examine structural ensembles of the unfolded state. Applying Bayesian reweighting to the MD structural ensemble we find good agreement with global properties, secondary structure and long-range contacts for isolated, unfolded FLN5. Finally, we validate our reweighed ensemble with SAXS data. Our approach will permit similar measurements of the unfolded state on the ribosome, and, thereby future quantitative comparisons of both dynamics and structures of the unfolded state on and off the ribosome.

NMR-075: Quantifying the Binding of Potential Aggregation Inhibitors to Human Tau Protein

Mr Morgan Wright[1]
[1]University Of Oxford, Oxford, United Kingdom

Introduction: Tau protein assists in maintaining neuronal structure by stabilising microtubules. The aggregation of tau is a hallmark of Alzheimer's disease and other tauopathies, hence the development of potential aggregation inhibitors is seen as a promising strategy to prevent a range of neurological disorders. Aims: Using universal saturation transfer analysis (uSTA), an enhanced form of saturation transfer difference NMR, we can identify at atomic resolution the binding epitope as well as the binding affinity of a variety of small molecules in vitro in order to compare and contrast their potential efficacy as aggregation inhibitors. Methods: Application of a selective saturation pulse to the protein and resultant spin diffusion leads to saturation travelling throughout the protein and to any bound ligand via a dipolar coupling mechanism. Measurement of the amount of saturation observed in the ligand can then be used as a measure for binding efficiency and pose. Results: Through the use of uSTA, the binding of clinically relevant aggregation inhibitors to tau has been measured at atomic resolution, including in varying solvent environments to investigate any effect this may have. Conclusions: uSTA NMR presents itself as a powerful tool for analysing interactions between proteins and clinically relevant small molecules, allowing for potential enhanced development of aggregation inhibitors by unravelling how they interact with relevant proteins at the atomic length scale.

NMR-076: Investigating Interactions Between Lipopolysaccharide and Monoclonal Antibodies Using Novel Differential Fluorination and 19F NMR

Miss Amy Gorman[1], Dr Stephanie Moore[2], Dr Alexander Golovanov[1]
[1]The University of Manchester, Department of Chemistry, Manchester, United Kingdom, [2]Dosage Form Design & Development, BioPharmaceuticals Development, R&D, AstraZeneca, Cambridge, United Kingdom

Lipopolysaccharide (LPS), otherwise known as endotoxin, is a cell-wall component of Gram-negative bacteria that contributes to bacterial toxicity. During processes such as cell division, shedding of outer membrane vesicles or bacterial cell death, LPS is released into the surrounding media. If such contamination got into the bloodstream, it would induce pro-inflammatory immune responses which can result in sepsis and death. Therefore, LPS detection is essential in the pharmaceutical and food industries to prevent exposure of LPS to patients. The Limulus Amebocyte Lysate (LAL) assay is the current major assay used by industry to detect and quantify LPS contamination. However, in recent years the phenomenon of Low Endotoxin Recovery (LER) has gained significant scientific attention. The phenomenon describes the inability of LAL assays, in some cases, to detect LPS due to a masking effect caused by interaction between LPS and formulation excipients. Although the mechanism of LER has not been fully determined, it is widely thought that the origin of the effect is associated with these interactions perturbing the supramolecular formation of LPS aggregates. As such, it is imperative to understand the interplay between the supramolecular structure of LPS and the interactions with the formulation excipients.

Our work demonstrates the successful novel fluorination of LPS molecules to assess their complex aggregation behaviour upon interaction with differentially fluorinated monoclonal antibodies (mAbs), via 19F NMR spectroscopy. Utilising 19F NMR allows simplified data interpretation compared to the intricate deciphering of 1H and 13C NMR spectra that would otherwise be required, due to the complex carbohydrate structure and very large size of LPS. We used 19F 1D and DOSY experiments to demonstrate how the interaction between LPS and mAb results in the uptake of mAb into LPS aggregates.

NMR-077: Unravelling CLIC Protein Dynamics: Insights into Allosteric Regulation and Inhibitor Development

Miss Ángela Serrano Sánchez[1], Dr Ana Isabel Azuaga Fortes[1], Dr Jose Luis Ortega Roldan[2]
[1]Universidad de Granada, Granada, Spain, [2]University of Kent, Canterbury, United Kingdom

CLIC proteins are intracellular proteins that act as chloride ion channels, but unlike others, they are dimorphic, existing in both cytosolic and transmembrane forms. Despite extensive research, understanding the metamorphosis and translocation of CLIC1 into the membrane remains a challenge. Initially, the hypotheses regarding CLIC protein insertion into the cell membrane suggested oxidation as a possible triggering mechanism. However, recent studies have unveiled a more dynamic process, revealing that CLIC1 insertion is facilitated by binding to Zn2+ followed by oligomerization of the protein to form a functional tetrameric channel. This dynamic interplay between protein oligomerization and ion binding highlights the complexity of CLIC1 protein function. A comprehensive study of the dynamics of CLIC1 and mutants with altered membrane insertion properties is essential for understanding the effect of protein dynamics in this process. NMR relaxation data has already determined different relaxation dynamics between WT and transmembrane region mutants. In addition, we have found altered membrane insertion and oligomerization properties in presence of zinc. This alternative perspective is also being exploited for the development of novel inhibitors targeting CLIC1 membrane insertion. An extensive screening of FDA-approved drugs has been carried out using both NMR and other biochemical assays to discover potential inhibitors of CLIC1.
In conclusion, a combination of NMR, SAXS and biochemical assays are being used to reveal conformational changes and elucidate the dynamic mechanisms underlying CLIC membrane insertion. Understanding this process will help identify of key regions of the protein that are involved in membrane translocation in order to target these properties therapeutically.

NMR-078: Regulation of the stress responsive transcription factor DREB2A through formation of intrinsic disorder-based ternary complexes including the MED25 subunit?

Ida Sjøgaard[1], Frederik F. Theisen[1], Bjørn W. Nordsteen[1], Andreas Prestel[1], Steffie Elkjær[1], Birthe B. Kragelund[1], Karen Skriver[1]
[1]University Of Copenhagen, Copenhagen, Denmark

Intrinsically disordered regions (IDR) are integral parts of the majority of transcription factors in addition to their DNA-binding domain (DBD). The regions play important regulatory roles by engaging with a complex network of cofactors needed for transcriptional activity. These interactions may involve activation domains containing short linear motifs (SLiMs).
The stress-associated Arabidopsis thaliana transcription factor DREB2A contains an activation domain in its long IDR that has been shown to interact with the MED25-ACID domain and the αα-hub RST-domain of RADICAL INDUCED CELL DEATH 1 (RCD1) or TBP-ASSOCIATED FACTOR 4B (TAF4B). These factors bind to overlapping motifs in a short sequence stretch where splicing and proline isomerization add another regulatory layer [1].
Here, we have combined structure modelling and biophysical analyses to examine how stress signals are integrated through IDRs. Specifically, we show the formation of distinct ternary complexes of the short DREB2A region with the MED25-ACID domain and the αα-hub RST-domain of RCD1 or TAF4B using nuclear magnetic resonance (NMR) spectroscopy and size-exclusion chromatography. Such ternary complex formation through a short intrinsically disordered interaction hot spot represents a fine-tunable regulatory unit in transcription.

NMR-079: Unconvering a new substrate binding site in the neurotransmitter/sodium symporter homologue LeuT

Alberto Daminato[1,2], Anton Turaev[5], Nathan Adams[5], Letitia Alves[4], Thomas Stockner[4], Claus Loland[3], Eurico Cabrita[1,2]
[1]Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal, [2]UCIBIO, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portogallo, [3]Laboratory for Membrane Protein Dynamics, Department of Neuroscience, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [4]Center for Physiology and Pharmacology, Institute of Pharmacology, Waehringerstr, Vienna, Austria, [5]NanoTemper Technologies GmbH, Munich, Germany

The Neurotransmitter:Sodium Symporters (NSS) found in mammals are complex proteins that play a vital role in facilitating synaptic transmission between neurons by rapid reuptake of neurotransmitters. Given their significance, dysfunctions in these transporters have been linked to various psychiatric and neurological disorders and they serve as targets for both therapeutic and illicit drugs. Almost 20 years ago a breakthrough in the structural investigation of these transporters came with the discovery of the crystal structure of a bacterial protein known as LeuT, which belongs to the neurotransmitter-sodium symporter (NSS) family. This finding not only provided insights into the architecture of NSS transporters but also offered crucial information about their transport mechanism.
Despite the substantial knowledge we have acquired regarding LeuT, ongoing debates persist regarding the number of binding sites and the transport mechanism employed by these proteins. In our research, we employed nuclear magnetic resonance (NMR) techniques and the latest advancements in spectral shift analysis to gain a deeper understanding of the binding mode and transport mechanism of LeuT. Our findings demonstrate that LeuT can bind its substrates even in the absence of sodium ions, contrary to previous demonstrations. This newly discovered binding event occurs within the inner vestibule of the protein when it assumes an inward-facing conformation. Importantly, this binding event triggers conformational changes, leading the transporter to adopt outward conformations as supported by our 19F-NMR experiments and by complementary thermal shift experiments.
In conclusion, by demonstrating that the transporter can bind its subtrates from the inner side our study reconciles the alternating access model proposed for LeuT and the broader class of NSS transporters. By shedding light on the binding mode and transport mechanism of LeuT, our research contributes to the understanding of neurotransmitter transporters and their potential implications in various physiological and pathological processes.

NMR-080: Creating a quantitative understanding of protein entropy changes upon ligand binding: The case of Hsp90-NTD

Julie Maibøll Buhl[1], Simon Holtbrügge[2], Lars Schäfer[2], Frans A. A. Mulder[1]
[1]Institute of Biochemistry, Johannes Kepler University Linz, Linz, Austria, [2]Center for Theoretical Chemistry, Ruhr-University Bochum, Bochum, Germany

It has been known for decades that the driving force of molecular association is a lowering of the total free energy of the system. However, in the field of structure-based drug design, the emphasis has traditionally been placed on optimizing kinetics and favorable interactions, whereas the entropy of the system is often neglegted. Both enthalpic (ΔH) and entropic (-TΔS) contributions significantly influence the total free energy, where even subtle differences can greatly impact binding strength. Ligand binding has long been recognized to induce flexibility in proteins, highlighting the subjective and potentially misleading nature of focusing solely on enthalpic contributions. In this study, we investigate the Hsp90 N-terminal domain (NTD) using NMR spectroscopy coupled with MD simulations to quantitatively assess protein entropy changes upon ligand binding. Our initial aim is to characterize conformational changes in Hsp90-NTD upon binding to a non-hydrolysable analogue of ATP as well as to ADP. We plan to utilize R_1 and R_2 relaxation and hetNOE experiments to estimate protein entropy changes via NMR spectroscopy, aiming to establish a robust, quantitative understanding of how ligand binding influences protein dynamics.

NMR-081: Understanding The Allostery Of P38 Kinase Alpha Using Dynamic Network Analysis And Solution NMR Strategies

Ms Suchandra Roy Acharyya[1]
[1]TU Dortmund, Dortmund, Germany

Allosteric signaling, which involves coordinated responses to specific stimuli, is crucial for the regulation of many proteins. P38 kinase α is particularly ripe for studying these kinds of communications. This protein is important for processes like cell differentiation, inflammation, and autophagy. P38 kinase is targeted by inhibitors like sorafenib, which alter its shape and activity. Recent discoveries have revealed a binding site for lipids at the protein's C-terminal domain. Ligands such as β-octyl-D-glucopyranoside (BOG) are designed to target this site along with the allosteric inhibitor to improve its selectivity. The interaction of BOG with sorafenib induces changes in the structure and behavior of the protein and also induces a ring flip of the sorafenib. Investigating the dynamical changes within the protein for these ensembles can provide insights into how different parts of the protein communicate with each other. Through methods like Molecular Dynamics Simulation and Dynamic network studies, the residual dynamics are explored and certain residues showing more importance in the communications are selected to be mutated in vitro. This understanding is further validated experimentally using relaxation dynamic studies via Nuclear Magnetic Resonance (NMR). Ultimately, this research could uncover new therapeutic strategies for P38 kinase by establishing the communication sites that could be target for drug binding or mutation studies.

NMR-082: NMR spectroscopy of self-hydrolysing DNA

Jessica Schmuck[1], Canyu Zhang[3,4], Dr Manuel Etzkorn[1,2], Dr Hongzhou Gu[3,4]
[1]Institute of Physical Biology, Heinrich-Heine University, Düsseldorf, Germany, [2]Institute of Biological Information Processing (IBI-7) Jülich Center for Structural Biology (JuStruct), Forschungszentrum Jülich, Jülich, Germany, [3]Department of Chemical Biology, School of Chemistry and Chemical Engineering, Frontiers Science Center for Transformative Molecules, and National Center for Translational MedicineShanghai Jiao Tong University, Shanghai, China, [4]Fudan University Shanghai Cancer Center, Institutes of Biomedical SciencesFudan University, Shanghai, China

Since the 1990s, DNA enzymes (DNAzymes) have been gaining popularity as they are applicable in diverse fields of biotechnology and therapeutics. Albeit essential for deciphering their mode of action, acquiring high-resolution structural insights into DNAzymes remains highly challenging. Still, crystallographic studies have been successful for the RNA-cleaving 8-17 DNAzyme and the RNA-ligating 8-17 DNAzyme. Recently, the structure of the RNA-cleaving 10-23 DNAzyme was revealed using NMR spectroscopy. However, so far, no high-resolution structural insights into an autohydrolytic DNAzymes could be obtained. Autohydrolytic DNAzymes are capable to catalytically cleave their own DNA strand with the help of metal-ion cofactors, offering a wide range of biotechnological and therapeutic applications.
Here we report on our recent progress to characterized a set of autohydrolytic DNAzymes. To gather structural insights into metal-ion binding sites, the catalytic cleavage reaction and the influence of temperature on the structure, solution NMR spectroscopy was used. A potential metal ion binding site was identified by real-time NMR and titration experiments with stabilized DNAzyme constructs. Structure prediction tools, such as AlphaFold and RNAfold, predict a defined hairpin structure and NMR experiments at low temperatures are consistent with the predicted Watson-Crick base pairing of this arrangement. Interestingly, our data suggest that the defined hairpin structure is not the active form of the system and that instead conformational plasticity, activated at higher temperatures, is a central element of the catalytic capabilities of the investigated DNAzymes. The observed high level of dynamics could explain why crystallographic studies failed so far and suggest that a certain degree of flexibility is necessary to accomplish effective DNA autohydrolysis.
Our study reveals exciting new insights into autohydrolysing DNAzymes and confirm that NMR spectroscopy is an excellent tool to study DNA catalysts.

NMR-083: Linear discriminant analysis for amino-acid type recognition in intrinsically disordered proteins

Dr. Anna Zawadzka-Kazimierczuk[1], Paulina Putko[2], Dr. Javier A. Romero[2], Dr. Mateusz Urbańczyk[3], Dr. Christian F. Pantoja[4], Prof. Markus Zweckstetter[4], Prof. Krzysztof Kazimierczuk[2]
[1]Faculty of Chemistry, University Of Warsaw, Warsaw, Poland, [2]Centre of New Technologies, University of Warsaw, Warsaw, Poland, [3]Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland, [4]German Center for Neurodegenerative Diseases, Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany

Intrinsically disordered proteins (IDPs) play important roles in many biological processes for which protein chain mobility is crucial, often related to signaling and regulation [1]. Only NMR provides atomic-scale information on these proteins, allowing us to determine detailed structural propensities, dynamics, and interactions of proteins of this class [2]. Nonetheless, the resonance assignment of IDPs can be very challenging. Due to the fast dynamics of these molecules and frequent repetitions in the amino acid sequence, their chemical shifts span a particularly narrow chemical shift range. This leads to ambiguities during the sequentail linking of the spin systems, which in turn causes that the chains of sequentially-linked amino-acid residues are typically very short. Therefore, accurate amino-acid type recognition is essential for correct mapping of the chains on the protein sequence. Statistical values of chemical shifts (CSs) are typically used for this purpose. Also, it has been noticed that temperature coefficients (TCs) are characteristic for different amino acids [3,4]. But manual analysis of multidimensional CSs/TCs space is not very efficient. We present a method for amino-acid type recognition based on linear discriminant analysis of IDPs' chemical shifts [5] and their temperature coefficients. The approach is demonstrated on two highly disordered proteins: alpha-synuclein and a large fragment (1-239) of Tau protein. The employment of LDA allowed more efficient resonance assignment, opening up an avenue for interesting information on IDPs.

[1] Nat Rev Mol Cell Biol 16, 18-29 (2015)
[2] Chem Rev 122, 9331-9356 (2022)
[3] J Biomol NMR 49, 139-149 (2011)
[4] J Biomol NMR 70, 141–165 (2018)
[5] PLOS Comp. Biol. 18, e1010258 (2022)

NMR-084: Progress towards the action of the antibiotic teixobactin in native bacterial membranes.

Msc Rosan de Winter[1], Dr. Rhythm Shukla[1], Msc. Maik Derks[1], Msc. Francesca Lavore[1], Msc. Raj Kumar[1], Msc. Roy van Beekveld[1], Prof. Dr. Eefjan Breukink[2], Dr. Harold Mac Gillavry[3], Dr. Milandip Karak[6], Dr. Stephen Cochrane[6], Dr. Dallas Hughes[5], Dr. Kim Lewis[4], Dr. Markus Weingarth[1]
[1]NMR spectroscopy, Utrecht University, Utrecht,, The Netherlands, [2]Membrane biochemistry and biophysics, Utrecht University, Utrecht,, The Netherlands, [3]Cell Biology, Utrecht University , Utrecht,, The Netherlands, [4]Antimicrobial Discovery Center, Department of Biology, Northeastern University, Boston, USA, [5]NovoBiotic Pharmaceuticals, Cambridge, USA, [6]School of Chemistry and Chemical Engineering Queen's University Belfast , Belfast,, USA

The developing resistance of bacteria to common antibiotics leads to more severe infections and higher mortality rates worldwide. In 2015, the antibiotic teixobactin was discovered in previously unculturable soil bacterium Elefteria terrae. This 11-residue antibiotic has excellent activity against gram-positive bacteria and mycobacteria.

Strikingly, teixobactin does not induce development of resistance, due to its non-proteinogenic and immutable target-site: the peptidoglycan precursor Lipid II. Binding of teixobactin inhibits peptidoglycan synthesis and cell growth. Previously, our group showed that teixobactin uses a dual mode of action. Binding of teixobactin to LII was shown to cause fibrillar suprastructures of teixobactin on the plasma membrane. Additionally, it was found that these suprastructures destabilize the membrane, causing loss of membrane potential. However, the zwitterionic lipid vesicles (DOPC-membranes) used in these studies are not well representative of bacterial membranes.

Here we set out to understand the mechanisms of action of teixobactin in more physiological membranes. Using solid state NMR experiments and ITC, we explore the impact of membrane composition on teixobactin's mode of action. We show that teixobactin binds to anionic phospholipids and propose that interaction with the bacterial membrane facilitates the supramolecular structure formation.

Furthermore, we show a fluorescence microscopy approach that enabled us to study the longevity of teixobactin suprastructures in a strain of Bacillus anthracis, known to cause anthrax disease. The longer the suprastructure persists, the longer the target Lipid II gets trapped, something that is highly relevant for drug activity and dosing. We show that the supramolecular teixobctin-Lipid II structures of teixobactin are highly stable on relevant biological timescales.

NMR-085: CONFORMER SELECTION OF N-LACTOSYLACETAMIDE DERIVATIVES BY GALECTIN-3

Nina Habanová[1], Jakub Zýka[1,2], Jan Choutka[1], Jesús Angulo[3], Radek Pohl[1]
[1]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic, [2]University of Chemistry and Technology, Prague, Czech Republic, [3]Instituto de Investigaciones Químicas (CSIC – Universidad de Sevilla), Sevilla, Spain

Carbohydrates exhibit remarkable structural diversity and play essential roles in living organisms. Besides serving as an energy source and building blocks, they mediate specific recognition processes, such as lectin-carbohydrate interactions, crucial for immune response and pathogen virulence. Since the recognition is driven by the unique molecular geometry of a carbohydrate, understanding of their conformational behaviour both in a free and a bound state is highly desirable.

This study explores the conformational preferences of three N-lactosylacetamide derivatives: N-(3-carboxy-5-hydroxyphenyl)-N-(lactosyl)acetamide, N-(phenyl)-N-(lactosyl)acetamide and N-(6-carboxyindol-4-yl)-N-(lactosyl)acetamide. Variable-temperature NMR experiments performed on the free ligands revealed their structure, population, and the energy barrier between them. This prompted an investigation of conformers selection by the human protein Galectin-3. The recognition process was characterized using experimental techniques such as STD NMR and X-ray crystallography, complemented by computational methods.

Acknowledgement: This work has been supported by the Czech Science Foundation – project 22-17586S.

NMR-086: Understanding protein interactions using solution NMR: Cannabinoid receptor CB1 and intercellular adhesion molecule ICAM1

Mr Daniel Calvo-Marco[1], Ms Angela Franco-Fernandez[1], Dr Miguel A. Treviño-Avellaneda[1], Dr José M. Pérez-Cañadillas[1], Dr Paula Morales[2], Dr David Pantoja-Uceda[1], Dr Maria Angeles Jimenez Lopez[1]
[1]Instituto de Quimica Fisica Blas Cabrera (IQF), CSIC, Madrid, Spain, [2]Instituto de Química Médica (IQM), CSIC, Madrid, Spain

Understanding at the atomic level the complex network of biomolecular interactions, which regulate all the biological processes in healthy living beings, still remains a challenge. Many diseases are caused by mis-regulation of protein interactions. Herein, we focus on two systems involved in physiological processes of relevance in human health: the cannabinoid receptor CB1R, and the intercellular adhesion molecule ICAM1.

CB1R is a G protein coupled receptor (GPCR) whose structure comprises an N-terminal region, seven transmembrane helices connected by extracellular and intracellular loops, and a C-terminal intracellular domain. Different signal cascades are triggered by activation of CB1R via either a canonical pathway by binding to G-proteins or via non-canonical pathways upon binding to non-G-proteins, such as β-arrestins, and CRIP1a. Solution NMR studies on model peptides have provided insights into the interaction between CB1R and β-arrestin.1

ICAM1 is a transmembrane glycoprotein of the immunoglobulin (Ig)-like superfamily, consisting of five extracellular Ig-like domains, a transmembrane helix and a short cytoplasmic tail. Recently, ICAM-1 has been found to interact with NHERF-1, whose structure consists of two PDZ domains followed by a long C-terminal tail.

To gain insights into the CB1R/non-G-proteins and ICAM1/NHERF1 interactions, the structural behaviors of model peptides derived from the cytoplasmic tails of CB1R and ICAM1, as well as their interaction with their respective partners, CRIP1a and a NHERF1 construct containing the PDZ2 domain have been analyzed using solution NMR. Current results will be described.

References

1. Morales, P; Bruix, M.; Jiménez, MA. Int. J. Mol. Sc. 2020, 21, 8111.

Acknowledgements

Financial support from grants PID2020-112821GB-I00 (MCINN/AEI/10.13039/501100011033) and S2022/BMD-7232 (Spanish “Comunidad de Madrid”) is acknowledged. NMR experiments were performed in the “Manuel Rico” NMR laboratory (LMR) of the Spanish National Research Council (CSIC), a node of the Spanish Large-Scale National Facility (ICTS R-LRB).

NMR-087: Kinetic stabilization of translation-repression condensates by a neuron-specific microexon

Dr Jesús García[1], Dr Carla Garcia-Cabau[1], Anna Bartomeu[1], Giulio Tesei[2], Andreea Balaceanu[1], Dr Giuseppe Battaglia[3,4], Dr Kresten Lindorff-Larsen[2], Dr Raúl Méndez[1,4], Dr Xavier Salvatella[1,4]
[1]IRB Barcelona, Barcelona, Spain, [2]University of Copenhagen, Copenhagen, Denmark, [3]IBEC Barcelona, Barcelona, Spain, [4]ICREA, Barcelona, Spain

CPEB4 is an RNA-binding protein that regulates translation through cytoplasmic changes in poly(A) tail length. It carries out its translational regulator function through liquid-liquid phase separation (LLPS). CPEB4 droplets are stabilized by intermolecular interactions driven by its intrinsically disordered N-terminal domain (NTD). The decreased inclusion of the neuron-specific microexon 4 in the NTD of CPEB4 (nCPEB4-NTD) is linked to idiopathic autism spectrum disorder (ASD). Why this microexon is required and how small changes in its degree of inclusion generate a dominant negative effect on the expression of ASD-linked genes is not clear.
Here, we study how the inclusion of microexon 4 in neuronal CPEB4 (nCPEB4) alters the protein properties and its phase transition. Solution NMR experiments were used for backbone assignment and for the identification of specific nCPEB4-NTD regions involved in intermolecular interactions driving protein condensation. CPEB4 droplets were characterized by microscopy imaging, apparent absorbance measurements, and DLS.
We found that nCPEB4-NTD forms reversible multimers on the condensation pathway. Microexon 4, which is rich in Arg residues, increases the kinetic stability of condensed CPEB4 by interacting with a cluster of His residues in the center of the NTD (residues 229–252). In the absence of the microexon, homotypic interactions between aromatic clusters leads to the irreversible aggregation of CPEB4, presumably as a result of the high concentration of this protein in the condensates.
We conclude that microexon 4 in neuronal CPEB4 is required to preserve the reversible regulation of condensation-mediated CPEB4 function.

NMR-088: Unveiling the Role of TNAP in Lipid Metabolism: Insights from NMR Spectroscopy

Eva Drevet Mulard[1], David Magne[1], Gilles Rautureau[1]
[1]ICBMS, Université Claude Bernard Lyon 1, Villeurbanne, France

Hypophosphatasia, a rare genetic disorder caused by Tissue Nonspecific Alkaline Phosphatase (TNAP) deficiency, comes with a range of symptoms from epileptic seizures, bone fractures, and hypotonia in moderate cases (1 in 100,000 individuals) to life-threatening conditions in the most severe cases (1 in 300,000 individuals). Although TNAP is an enzyme distributed throughout the body, the complexities of its action in various organs remain largely unresolved, particularly its potential involvement in energy metabolism.
In this study, we employed Nuclear Magnetic Resonance (NMR) spectroscopy as a versatile tool to identify new potential TNAP substrates, shedding light on their possible role in lipid metabolism. NMR can determine the concentration of molecules both in isolation and in complex mixtures, making it a powerful tool with numerous applications in biochemistry. This poster presents NMR methods (metabolomics, real-time analysis for enzymology, etc.) compatible with biological samples, which are useful for better understanding the role of TNAP: one metabolomic study done on mice organs and serum, a real-time NMR tracking of phosphocholine consumption in serum, and a targeted analysis of cells and supernatants.

NMR-089: Parallel binding-site mapping with multiple receiver NMR

Msc Sonja Knödlstorfer[1,2], PhD Marco Schiavina[4], MSc Maria Anna Rodella[4], ADir. Ing. Karin Ledolter[1], PhD Roberta Pieratelli[4], PhD Isabella C. Felli[4], PhD Robert Konrat[1,3]
[1]Department of Structural and Computational Biology, Max Perutz Laboratories, University of Vienna, Vienna, Austria, [2]Doctoral School in Chemistry (DoSChem), University of Vienna, Vienna, Austria, [3]Christian Doppler Laboratory for High-Content Structural Biology and Biotechnology, Department of Structural and Computational Biology, Max Perutz Laboratories, University of Vienna, Campus Vienna Biocenter, 5, 1030, Vienna, Austria, [4]Magnetic Resonance Center and Department of Chemistry "Ugo Schiff", University of Florence, Sesto Fiorentino, Florence, Italy

Intrinsically disordered proteins, IDPs, play a crucial role in numerous regulatory and cell signaling processes. Malfunctions of these IDPs can predispose many diseases, thus, establishing robust tools targeting these proteins in drug development is of essence. These macromolecules can be studied in near physiological conditions using NMR spectroscopy. When studying IDPs with NMR some challenges arise, like the 1HN exchange with water which results in line broadening. Typically, spectra are acquired on 1HN in the direct dimension to increase sensitivity, but other detection methods such as 13C detection are often beneficial in disordered systems, particularly with higher instrument sensitivity. Such detection methods give another advantage, multiple receivers (MRs), where one records multiple nuclei in the direct dimension within one experiment. MR NMR is beneficial studying complex biological systems, where sample stability is low and sample preparation lengthy and expensive.
Here, MR NMR elucidated the interaction regions between a construct within the disordered region of BRCA1, breast cancer associated protein 1 (residues 219-504), and the MycMAX heterodimer. To measure both binding-sites simultaneously a complementary labeling scheme was employed. BRCA1219-504 was 14N13C1H labeled, whereas MAX was 15N12C2H labeled and Myc was expressed without isotope labeling. Therefore, a 13C detected CACO experiment connecting intraresidue Cα and C' was acquired on BRCA1219-504.Two 1H-15N BEST-TROSY transients were recorded on MAX during the recovery delay .
The binding-site for BRCA1219-504 was mapped between residues 370-430. For MAX the binding seems located within the flexible basic region of the heterodimer. Finally, on BRCA1219-504 a CBCACO was measured as well, permitting the assignment of carboxyl side-chains.
We demonstrated the already established MR NMR, however, with a labeling scheme facilitating simultaneous binding-site mapping on two different proteins. Additionally, the role of charged side-chains in the interaction is highlighted with the side-chain carboxyl assignment of aspartates and glutamates.

NMR-090: Interaction dynamics of ySERF protein and TAR complex from the RNA perspective

Selin Dedeoglu[1], Rishav Mitra[2], James C.A. Bardwell[2], Loïc Salmon[1]
[1]Centre de résonance magnétique à très hauts champs (CRMN), CNRS, ENS de Lyon, UCBL, Université de Lyon, Villeurbanne , France, [2]Howard Hughes Medical Institute and Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, , USA

SERF is a small RNA binding protein, presenting an intrinsically disordered region which plays a crucial role in age-related proteotoxicity. SERF disordered N-terminal region is conserved in homologs of the protein. As an RNA binding disordered protein, SERF forms droplets with RNA in a concentration dependent manner. TAR is a powerful candidate to be a model partner for SERF as a well-characterized, stable and dynamic RNA, in order to investigate the basic biophysical principle underlying such interaction.
A variety of biochemical experiments show that the disordered N-terminal region of SERF interacts with TAR and forms a compact complex. On the other hand, it is very interesting to investigate the interaction from the RNA point of view to understand the behaviour of the complex. Hence, titration experiments of unlabelled SERF into 13C and 15N labelled TAR has been performed with molar ratio of from 0.1 to 5 ([SERF]/[TAR]). Canonical Watson-Crick base-pairs of TAR helices are observed using 15N HSQCs throughout the titration, demonstrating that the secondary structure of TAR is largely conserved. Besides that, high chemical shift perturbations on the bulge region of TAR are pointing towards a principal interaction site in and around the bulge, observed by using 13C HSQCs. Moreover, a decrease in the bending angle between the two TAR helices has been observed by measuring Residual Dipolar Couplings (RDCs) under weak alignment conditions.
In this study it’s been shown that TAR RNA is interacting with SERF protein. While TAR secondary structure is conserved, the bulge region is crucial for this interaction. Also, RDCs of TAR suggest a tendency of the two helices to stack within the complex.

NMR-091: Thermal unfolding and oligomerization of a prion protein studied by solution NMR and Molecular Dynamics

Dr. Manuel Martin-Pastor[1], PhD student Sanaz Sabhezei[2], Dr. Marta Rigoli[3,4], Prof. Emiliano Biasini[3,4], Prof. Víctor Sánchez-Pedregal[5], Prof. Jesús R. Requena[2]
[1]Unidade de Resonancia Magnética, CACTUS, University Of Santiago De Compostela, Santiago De Compostela, Spain, [2]CIMUS Biomedical Research Institute, University of Santiago de Compostela-IDIS, Spain, Santiago De Compostela, Spain, [3]Department of Cellular, Computational and Integrative Biology, University of Trento, Trento, Italy, [4]Dulbecco Telethon Institute, University of Trento, , Trento, Italy, [5]Department of Organic Chemistry, University of Santiago de Compostela, Spain , Santiago De Compostela, Spain

Introduction

The auto-replicative and infectious PrPSc (a misfolded form of the physiological prion protein, PrPc) constitutes a seed for the formation of amyloid fibers and the main effector of prion diseases. Elucidation of the structure of PrPSc has opened the way to understanding the conversion of PrPc into the β strand-rich form PrPSc. For this process to occur, the folded domains of PrPc must partially unfold before it can be templated/refolded into PrPSc. Understanding this mechanism could be a valuable target for therapeutic intervention by preventing or decreasing PrPSc replication. In this work we used solution and solid NMR to study thermal unfolding of Bank Vole (BV)-PrPc 90-231.

Aim

Identification of conformationally labile residues of PrPc and factors contributing to the conversion of PrPc into PrPSc.

Methods

NMR experiments were measured for BV-PrPc and the thermally degraded BV-PrPo oligomeric form. Combined Chemical Shift Perturbations (CCSPs) and Water Accessibility Perturbations (WAPs) were measured for the backbone N-H amides by VT- 1H-15N HSQC and VT 1H-15N SEA-HSQC, respectively. DARR 13C-13C were measured for solid samples of BV-PrPc and BV-PrPo.

Results and conclusions

Thermal CCSPs deviations from linearity identified residues with greater tendency to explore alternative conformations on the way to an unfolded state. The short β1 and β2 strands and many residues in helix α2 opposing these β-strands were labile and with strong thermal WAPs, indicating an unfolding hotspot, compatible with the separation of the α2-β2-β1 and α2-α3 subdomains. Thermal unfolding of BV-PrPc led to an irreversible conformational change at ca. 50 ºC that promoted the formation of a stable and soluble BV-PrPo oligomeric form with a high β -sheet content. The included/excluded core residues of BV-PrPo were identified by their different dynamics. Given the similarities in their structural characteristics, BV-PrPo may constitute an initial step on the pathway to PrPSc.

NMR-092: NMR Studies of a Polyproline II Helix Bundle Protein in the Folded and Denatured States

Dr. Miguel Treviño[1], Dr. David Pantoja Uceda[1], Mr. Rubén López Sánchez[1], Dr. Miguel Mompeán[1], Dr. Douglas Laurents[1]
[1]Institute For Physical Chemistry "Blas Cabrera" CSIC, Madrid, Spain

Introduction: Recently, a protein structure family with domains composed of bundles of interlinked glycine-rich polyproline II (PPII) helices has emerged. These proteins have diverse functions, but the bases of their conformational stability were unclear.

Aims: To understand the bases of PPII helix bundle folding, we used NMR spectroscopy, a theme of this conference, to characterize the folded and denatured forms of the H. harveyi “snow flea” antifreeze protein (sfAFP), a six PPII helix bundle.

Methods: NMR spectroscopy was employed to determine the ^{1}H, ^{13}C and ^{15}N chemical shifts of the folded and reduced-denatured (RD) states. Due to significant signal overlap, the RD state assignment required an unconventional approach based on sequential ^{13}C’, ^{15}N and ^{1}HN connectivities. H/D exchange and {^{1}H}-^{15}N relaxation measurements were performed to determine residue-level stability and dynamics in both the folded and unfolded protein. pKa values and paramagnetic relaxation enhancements (PRE) were determined to assess long-range contacts in the denatured state.

Results: Twenty-eight glycine residues in the folded protein show remarkably different ^{1}Hα2 versus ^{1}Hα3 chemical shift values which arise from weak Cα-H···O=C H-bonds. Two disulfide bonds, interhelical N-H···O=C H-bonds plus hydrophobic surface burial upon dimerization afford additional stability. Despite a high glycine content, the sfAFP folded and denatured states have mobilities similar to those of typical proteins. PRE and pKa values evince a compact denatured state with long-range attractive electrostatic interactions.

Conclusions: Interhelical Cα-H···O=C and N-H···O=C H-bonds are key for PPII-helix bundle protein stability. A typical denatured state dynamics means that PPII helical bundle proteins do not require an exceptional strong set of stabilizing interactions to fold. A “normal” set will do.

Acknowledgements: Funded by PID2019-109306RB-I00 and PID2022-137806OB-I00 from the Spanish Ministry of Science and Innovation: MICIN/AEI/10.13039/501100011033/FEDER, UE. NMR spectra were recorded at the “Manuel Rico” Laboratory (LMR/CSIC), a Spanish Large-Scale National Facility Node (ICTS R-LRB).

NMR-093: Isotopic Dilution-Based Saturation Transfer Difference NMR Approaches to Estimate Affinity Constants Between Carbohydrates and Proteins

Paula Flores-Galán[1], Dr. Hector Martín Merinero[1], Dr. Santiago Rodriguez de Córdoba[1], Dr. June Ereño-Orbea[2], Dr. Ángeles Canales[3], Dr. Jesús Jiménez-Barbero[2,4], Dr Francisco Javier Cañada[1,4]
[1]Centro de Investigaciones Biológicas Margarita Salas, Csic, Madrid, Spain, [2]CICbioGUNE, Bilbao, Spain, [3]Universidad Complutense de Madrid, Madrid, Spain, [4]CIBERES, ISCIII, Madrid, Spain

The Saturation transfer difference (STD) NMR experiment is a powerful technique to explore carbohydrate-protein interactions in solution from the ligand point of view. This experiment can be used to estimate the dissociation constant (KD), either directly or by competition experiments. However, the direct method is time-consuming and the competition approach requires a reference molecule ligand with a known KD and a competitive mechanism must be assumed, i.e., the ligand under study must bind to the same binding site as that of the molecule reference.

We will present an isotopic dilution STD strategy for the estimation of KD, which is based on performing a competition experiment of one ligand with itself but ^{13}C isotopically labelled.

We have applied this strategy to a set of diverse glycan receptors of biomedical interest that recognize sialylated oligosaccharides expressed in cell surfaces with regioisomeric discrimination (α2,3 or α2,6).

For this purpose, the enzymatic synthesis of sialylated α2,3-sialyl N-acetylactosamine and α2,6-sialyl N-acetylactosamine compounds selectively labelled at the C_3 position and methyl group of the acetamide of N-acetyl neuraminic acid moiety has been carried out.[1]

This selective isotopic labelling has enabled us to apply the isotopic dilution strategy and to distinguish between the two acetamido groups in the corresponding sialylated compound. Additionally, the ^{13}C labelling allows to use STD NMR-based competition displacement assays[2] to compare the relative affinities of closely related sialylated glycans.

The cases of complement Factor-H (α2,3 selective),[3] Influenza virus hemagglutinin (α2,6 selective)[4] and Siglec-9 (α2,3 and α2,6)[5] will be presented.

References

1. Unione, L et al, Angew. Chem. Int. Ed. 2022, doi.org/10.1002/anie.202201432
2. Fehér, K et al, J. Am. Chem. Soc. 2008, doi: 10.1021/ja804468k
3. Martín-Merinero, H et al. Blood 2021, doi: 10.1182/blood.2020010069
4. Canales, A et al, JACS Au 2023, doi: 10.1021/jacsau.2c00664
5. Atxabal, U. et al, ACS Chemical Biology 2024, doi: 10.1021/acschembio.3c00664

NMR-094: Structural studies of FOXM1 regulation by β-catenin

MSc Anastasia Rakhimbekova[1], PhD Benjamin Bourgeois[1], Prof Tobias Madl[1,2]
[1]Division of Medicinal Chemistry, Otto Loewi Research Center for Vascular Biology, Immunology, and Inflammation, Medical University of Graz, Graz, Austria, [2]BioTechMed Graz, Graz, Austria

Introduction
Aberrant activation of the canonical Wnt pathway is connected to numerous cancers and is characterized by an increased concentration of the nuclear β-catenin. Thus, transcription factors that synergistically interact with β-catenin are promising cancer therapy targets. Forkhead box protein M1 (FOXM1) is a proliferation-related transcription factor, association of which with β-catenin has been reported in glioma and triple-negative breast cancer. Since FOXM1 and crucial parts of β-catenin are intrinsically disordered, NMR spectroscopy is the key technique to study their interactions with single-residue resolution.

Aims
We aim to resolve the molecular details and regulation of FOXM1/β-catenin interaction in vitro.

Methods
We conducted biophysical and structural studies of FOXM1/β-catenin interaction using liquid-state biomolecular NMR.

Results
So far, we have revealed that the C-terminal part of the overall disordered FOXM1 transactivation domain (TAD) contains a region exhibiting distinct rigidity from the rest of TAD sequence and adopting a transiently folded α-helical conformation. The C-terminus of FOXM1 TAD directly interacts with β-catenin via the N-terminal part of the folded Armadillo repeat region, competing against β-catenin homotypic intermolecular interactions (which are mediated by β-catenin's disordered N/C-termini). Interestingly, binding sites of β-catenin and FOXM1 negative regulatory domain (NRD) overlap with each other at FOXM1 TAD. In addition to intramolecular NRD/TAD interaction, FOXM1 TAD binds FOXM1 DNA-binding domain. Furthermore, we discovered that the structured FOXM1 DNA-binding domain contains a binding site for disordered β-catenin N-terminus.

Conclusions
To summarize, NMR spectroscopy was the key to providing the first insight into the intricate network of intra- and intermolecular interactions within FOXM1 and with the pro-oncogenic co-factor β-catenin. In the next phase, we aim to solve the structure of key complexes and study regulation of the FOXM1/β-catenin interaction by phosphorylation. Our study aims to shed light on the functional relationship of β-catenin and FOXM1 relevant to the cancer-promoting context.

NMR-095: Unraveling the dynamics of p38γ through Ultrafast High-Resolution Relaxometry (UHRR): Insights into Molecular Motion

Miss Ana Paula Aguilar Alva[1], PhD Lucas Siemons[1], Coline Wiame[1], PhD Ulric le Paige[1], PhD Philippe Pelupessy[1], PhD Guillaume Bouvignies[1], PhD Nicolas Wolff[2], PhD Florence Cordier[2], PhD Jorge Garibay[3], Agnès Glémot[3], Philip Wurm[3], Jean-Max Tyburn[3], PhD Fabien Ferrage[1]
[1]École Normale Supérieure , Paris, France, [2]Institut Pasteur, Paris, France, [3]Bruker, , France

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 use high-resolution relaxometry to probe ps-ns motions in great detail in the protein kinase p38γ. The regulation of this protein by phosphorylation has been described from a biological perspective, yet the molecular mechanism is not a simple structural switch and evidence of allosteric pathways has been found [2].

Aims: Our objective is to better understand such allosteric pathways and, in particular, the role of nanosecond dynamics of side-chains in p38γ with NMR relaxation and molecular dynamics simulations.

Methods: We complement 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 reach magnetic fields down to 100 µT in as little as 90 ms.

Results: With these experiments, we obtain and analyze Nuclear Magnetic Relaxation Dispersion (NMRD) profiles to get a further understanding of the dynamics in p38γ. We have assigned methyl resonances in p38γ and have measured a set of relaxation rates at over 20 magnetic fields covering 5 orders of magnitude. A preliminary analysis of relaxometry data will be presented.

[1] C. Charlier, et. al. J Am Chem Soc, vol. 135, no. 49, pp. 18665-18672, 2013. [2] Aoto, P. C. et al. Sci. Rep. 6, 28655, 2016.

NMR-096: NMR studies of biomolecular condensate formation of protein-RNA complexes in Drosophila melanogaster siRNA biogenesis

PD Dr. Gerd Gemmecker[1,2], Clara Hipp[1,2], Dr. Sam Asami[1,2], Dr. Hyun-Seo Kang[1,2], Prof. Dr. Michael Sattler[1,2], Prof. Dr. Job Boekhoven[3], Prof. Dr. Klaus Förstemann[4]
[1]BNMRZ, NAT, TUM, Garching, Germany, [2]STB, Helmholtz Munich, Neuherberg, Germany, [3]Dept. of Chem., NAT, TUM, Garching, Germany, [4]Gene Center, LMU, Munich, Germany

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 protein complex of Dcr-2/Loqs-PD and loaded onto Ago2 protein to form the RNA-induced silencing complex.
Our data demonstrate the formation of biomolecular condensates comprising Loqs-PD, Ago2 and RNA through liquid-liquid phase separation, showing a characteristic behaviour in DOSY experiments. The intrinsically disordered N-terminal region of Ago2, Ago2IDR, forms condensates in vitro which are strongly enhanced in the presence of nucleic acids.
Phase separation also critically depends on the RNA binding capability of the proteins involved. Loqs-PD can co-partition into existing Ago2IDR/dsRNA condensates. The RNA in the condensate remains accessible and if the RNA is enzymatically consumed, phase separation can be reversed.
NMR spectroscopy together with other biophysical experiments and microscopy reveal that the Ago2IDR-RNA condensates are fluid and exhibiting significant peptide backbone flexibility. Phase separation is electrostatically driven by arginine interactions with negative charges in nucleic acids. NMR further reveals an extensive NOE network of protein side chain interactions.
Our data suggest an important role of nucleic acid driven phase separation mediated by the RNA-binding domains of Ago2IDR and Loqs-PD. The observed condensates are probably essential for efficient siRNA processing, and hence antiviral defense in insects.

NMR-097: Engineering Nanobodies for Conformational Signaling

Gabriel Ortega-Quintanilla
[1]CIC bioGUNE, Derio, España

Biosensors play a central role in the future of modern medicine. In contrast to traditional analytical methods, biosensors can measure molecular analytes directly at the point-of-care, and even in vivo, without the need of highly specialized equipment or personnel. Biosensors thus enable monitoring of the health status of patients during their daily routine, achieving fully personalized medicine.

Performing measurements directly in complex biological media, however, poses significant challenges, such as unspecific adsorption, biofouling, and interference from other biomolecules. As a result, many biosensing technologies require complex protocols involving sample dilution, washing, and long incubations, thus complicating their use. In response, we aim to develop a new sensing technology that is reagent-less, selective enough to work in biological fluids, and generalizable to many different targets.

Achieving a truly generalizable sensing technology requires receptors and signal transduction mechanisms easily transferable to many different analytes. To achieve this, we use nanobodies, single domain antibodies capable of binding with high affinity and specificity many different analytes. Inspired by the biophysics of conformational signaling, we engineer nanobodies to couple ligand binding to a folding structural change. To design them, we combine computational prediction and artificial intelligence tools, as well as experimental biophysical and structural characterization, including NMR spectroscopy.

As a proof of concept, we have developed a conformational receptor based on a nanobody for chorionic gonadotropin hormone, a biomarker used to monitor the correct development of pregnancy. We have then implemented optical signal transduction by functionalizing it with a fluorescence probe, and used it to measure hormone concentrations in urine.

The combination of the versatility of nanobodies with the generalizability of conformational signalling will maximize the potential applications of our receptors in real-time health monitoring biosensors, but also in other biotechnologies, such as biomolecular switches for logic circuits, responsive biomaterials, and smart imaging probes.

NMR-098: Towards probing possible long-range interactions and lipid interactions of the SNARE protein SNAP-25a

Sophia Werner[1,2], Tobias Stief[1,2], Katharina Vormann[1,2], Dr. Nils-Alexander Lakomek[1,2], Selcuk Er[2]
[1]Forschungszentrum Jülich, Jülich, Germany, [2]Heinrich-Heine Universität , Düsseldorf, Germany

The SNAP-25a (isoform 2) protein is a part of the SNARE complex, a four-helix bundle composed of SNAP-25a and the two other SNARE proteins synaptobrevin-2 and syntaxin-1a. SNAREs are responsible for the transport of vesicles and membrane fusion to exocytosis. SNAP-25a contributes with two α-helices, the SNARE motifs (SN1: L11-L81) and (SN2: N144-A199), while synaptobrevin-2 and syntaxin-1a contribute with one helix each to the four-helix bundle forming the post-fusion SNARE complex. In its monomeric pre-fusion form, SNAP-25a is disordered except for two α-helices at the N-terminus of the protein (A5-Q20 and S25-V36)1. SNAP-25a lacks a transmembrane region but has four palmitoylation sites that anchor the protein to the cell membrane.

We aim for deeper insights into the structural dynamics and long-range interaction of the pre-fusion SNAP-25a monomer in the presence and absence of a lipid bilayer. Previously, we showed that proteins display high flexibility1. Our current focus is a possible interaction between the N- and C-terminus of SNAP-25a and the palmitoylation site.

NMR spectroscopy is used as the primary technique supported by further biophysical methods. Therefore, the protein was expressed in E. coli BL21 (DE3), isotopically labeled, and purified using affinity, ion exchange, and size-exclusion chromatography. Paramagnetic relaxation enhancement (PRE) data are recorded to obtain long-range distance information relative to a paramagnetic spin-label (MTSL).

High-purity protein samples for the diamagnetic wild-type and the cysteine mutant were obtained, and the paramagnetic spin label was introduced successfully. Both yielded well-resolved NMR spectra. 1H T2 relaxation data are currently recorded for the PREs and will be presented at the conference, allowing first insights into long-range distances. Further experiments in the presence of a lipid bilayer, using lipid nanodiscs and liposomes, are planned.

NMR-099: A comprehensive tetramer database for RNA molecular dynamics validation

Dr Israel Serrano-Chacón[1], Javier González[1], Dr Milosz Wieczor[1], Dra Isabelle Brun-Heath[1], Dr Modesto Orozco[1,2]
[1]Institute for Research of Biomedicine (IRB), Barcelona, Spain, [2]Departament de Bioquímica i Biomedicine. Facultat de Biología. Univeristat de Barcelona, Barcelona, Spain

RNA plays a pivotal role in many biological processes, and it is an attractive target for therapeutic development. The accurate modeling of RNA molecules is of great importance in understanding their structure, function, and interactions within biological systems. The complexity of its conformational space and the intrinsic dynamics of RNA represent a challenge for structural biology. At this point, molecular dynamics (MD) is a powerful tool for studying it, but its accuracy is limited by the quality of the force fields (FF) used to describe it. For these reasons, FF are in continuous development.

Experimental data that represents the flexibility and conformational space of RNA is essential to guiding these new FF. NMR is optimal for the characterization of new structures thanks to its ability to show the dynamics of the biomolecules. In this work, single strand tetramers (four residue RNAs) are chosen to benchmark the FF. Despite their short length, they show the capability to form some right-handed A-form-like helix with sequence dependency. As expected, they are also very flexible biomolecules.

Our objective in this work is to study all the possible threemers combinations of the RNA nucleotides (64 in total). For this, 32 non-self-complementary tetramer sequences were designed and synthesized, where all the threemers are represented. The presence of stacking between the bases of the nucleotides is examined by distance measurements. In addition, sugar puckering equilibrium is quantified by scalar coupling. Both of them help to characterize the presence of right-handed A-form-like helix. In a parallel way, all these RNAs are studied by different MD methods, such as metadynamics or replica exchange, to explore a wide conformation space. The ability of MD to reproduce the experimental data obtained will guide the optimization of the FF parameters.

NMR-100: Structural characterization of Tau protein condensate using Nuclear Magnetic Resonance (NMR)

Dr. Crhistian Felipe Pantoja Rivillas[1], Dr. Alain Ibáñez de Opakua[1], Prof. Markus Zweckstetter[1]
[1]DZNE, Göttingen, Germany

Introduction

Biomolecular condensates have emerged as an essential mechanism for cell organization. Nevertheless, high-resolution characterization by techniques like X-ray and Cryo-EM is unachievable because of their dynamic nature. Instead, NMR spectroscopy can provide atomic-resolution information on biomolecules in the condensate milieu. Here, we present a high-resolution characterization of Tau protein in the condensate medium, uncovering an important role of electrostatics in modulating structural changes.

Aims

The current work aims to provide atomic resolution information of the condensate state of Tau protein, including a detailed description of the structural changes induced by condensation on the low-populated secondary structures and long-distance interactions.

Methods

Structural characterization of Tau condensate was carried out by a collection of triple resonance experiments (HNCO, HNCA, and HN(CO)CA) and prediction of secondary structure using TALOS+. Long-range intra-molecular interactions were studied using Paramagnetic relaxation enhancement (PREs) by the inclusion of paramagnetic probes in Tau protein. Spatially resolved NMR was employed to report composition and pH differences between both phases.

Results

We show that Tau protein adopts a closed conformation under saturation concentration. We observe that the condensate medium drives a pH increase of 0.2 units toward the protein isoelectric point (PI). Consequently, Tau protein undergoes chain extension in the condensate medium, reflecting neutralization of the electrostatic forces. Finally, secondary structure analysis shows that Tau protein remains disordered, with exception of the lowly populated alpha helix in residue 115-119, which seems to be correlated with the extension in the condensate medium.

Conclusion

Altogether, we provide an atomistic characterization of Tau condensate, demonstrating the impact on the remaining secondary structure and long-range intra-molecular interactions. Finally, we provide evidence that the observed changes are closely connected with the electrostatic force balance induced in the condensate medium.

NMR-101: Probing altered receptor specificities of antigenically drifting human H3N2 viruses by NMR

Dr Luca Unione[1], Augustinus Ammerlaan[2], Gerlof Bosman[2], Elif Uslu[2], Ruonan Liang[2], Dr Marcos Gómez-Redondo[1], Dr Iris Bermejo[1], Dr Pablo Valverde[1], Dr Tammo Diercks[1], Dr Ana Ardá[1], Dr Robert de Vries[2], Prof Geert-Jan Boons[2]
[1]CICbioGUNE, Basque Research & Technology Alliance (BRTA), Derio, España, [2]Department of Chemical Biology & Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

Prototypic receptors for human influenza viruses are N-glycans carrying α2,6-linked sialosides. Due to immune pressure, A/H3N2 influenza viruses have emerged with altered receptor specificities that bind α2,6-linked sialosides presented on extended N-acetyl-lactosamine (LacNAc) chains. The binding modes of such drifted hemagglutinin's (HAs) have been examined by chemoenzymatic synthesis of N-glycans having 13C-labeled monosaccharides at strategic positions. The labeled glycans have been employed in 2D 1H-STD-1H,13C-HSQC NMR experiments to pinpoint which monosaccharides of the extended LacNAc chain engage with evolutionarily distinct HAs. The NMR data in combination with computation and mutagenesis demonstrated that mutations distal to the receptor binding domain of recent HAs create an extended binding site that accommodates the extended LacNAc chain. Fluorine containing sialosides have been used as NMR probes to derive relative binding affinities.

NMR-102: NMR Profiling of Chemoselective Protein Modification: Modulating Glycan-Lectin Interactions and Beyond

Doctor Ana Gimeno[1,2], Paula Oroz[3], Claudio, D. Navo[1], Mikel Azkargorta[1], Félix Elortza[1], Jesús Jiménez-Barbero[1,2,4,5], Gonzalo Jiménez-Osés[1,2]
[1]CIC bioGUNE, Basque Research and Technology Alliance (BRTA), Derio, Spain, [2]Ikerbasque, Basque Foundation for Science, Bilbao, Spain, [3]Departamento de Química, Universidad de La Rioja, Logroño, Spain, [4]Departament of Organic & Inorganic Chemistry, Faculty of Science & Technology, University of the Basque Country, Leioa, Spain, [5]Centro de Investigación Biomédica En Red de Enfermedades Respiratorias, Madrid, Spain

Protein modification by means of chemical or chemoenzymatic methods offers a highly valuable tool to modulate protein functionality and efficacy, and results extremely convenient in therapeutic applications.[1] However, achieving controlled protein functionalization poses considerably challenges, especially regarding process efficiency, selectivity, and the need for detailed characterization of the resulting generated biomolecules. Here, we present innovative methodologies for site-selective post-synthetic protein modification that enable modulating glycan-lectin interactions.[2] In combination, comprehensive NMR-based strategies[3] to unambiguously characterize the novel features introduced in the protein and to assess the new molecular recognition processes have been developed. The proof-of-concept has been demonstrated using human galectin-3 as model lectin.

References

[1] E. Hoyt, P. Cal, B. Oliveira, G. Lopes Bernardes, Nat. Rev. Chem. 2019, 3, 147.
[2] Essentials of Glycobiology, 4th edition. Varki A, Cummings RD, Esko JD, et al., editors. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2022.
[3] H. Arthanari, K. Takeuchi, A. Dubey, G. Wagner, Curr. Opin. Struct. Biol. 2019, 58, 294.

NMR-103: Multivalent dynamic colocalization of avian influenza polymerase and nucleoprotein by intrinsically disordered ANP32A reveals the molecular basis of human adaptation

Lefan YU[1], Aldo R. Camacho-Zarco[1], Tim Krischuns[2], Selin Dedeoglu[1], Damien Maurin[1], Guillaume Bouvignies[3], Stephan Cusack[3], Nadia Naffakh[2]
[1]Institut de Biologie Structurale, Université Grenoble Alpes-CEA-CNRS UMR5075, 38000 Grenoble, France;, Grenoble, France, [2]Institut Pasteur, Université Paris Cité, CNRS UMR3569, Unité Biologie des ARN et Virus Influenza, 75015 Paris, France, Paris, France, [3]Laboratoire des Biomolécules, Département de Chimie, E´ cole Normale Supérieur, UPMC Université Paris 06, CNRS, PSL Research University, 75005, Paris, France, [4]European Molecular Biology Laboratory, 38000 Grenoble, France, Grenoble, France

Introduction

Adaptation of avian influenza to human cells requires mutations in the 627-NLS domains of its polymerase (FluPol). An E627K adaptive mutation alone compensates for a 33-amino-acid deletion in the intrinsically disordered domain (IDR) of the human host protein ANP32A, a deletion known to restrict avian FluPol activity in human cells.

Aim

Here we aim to use solution-state NMR to test the putative role of ANP32A in scaffolding the flu nucleoprotein (NP) and FluPol during co-replication assembly of the viral genome and to unveil the molecular basis of E627K mutation compensating the species barrier formed by ANP32A.

Methods

We characterized the exchange between human and avian ANP32A and flu NP using CPMG relaxation dispersion. Further, we characterized ternary complexes formed between human/avian ANP32A, 627NLS domains, and flu NP, using NMR relaxation, PRE, and NMR titration experiments.

Results

Both human and avian ANP32A form ternary complexes with the 627-NLS domains and NP, via two highly distinct binding modes. The IDR of avian ANP32A sterically allows FluPol 627NLS domains and flu NP to simultaneously bind to two distant linear motifs. Such binding mode is restricted in the shorter IDR of human ANP32A with the 33-amino-acid deletion. Instead, NP and human-adapted 627NLS domains share one extended binding motif on human ANP32A IDR and rather than solely competing for binding, they simultaneously bind to this motif via a novel, multivalent electrostatic binding mode. Such multivalent interaction is facilitated by the human-adapted 627K residue on 627NLS domains.

Conclusion

Using solution-state NMR spectroscopy, we revealed the probable molecular basis of adaptive mutations in FluPol: a E627K mutation confers the necessary multivalent properties to allow a shared interaction motif on human ANP32A to colocalize NP and FluPol. Such shared interaction motif is thought to rapidly jump between the two viral proteins, thereby maintaining their colocalization.

NMR-104: A crowded shuttle - An analysis of the effect of crowding agents on the dynamics of intrinsically disordered protein regions

Dr. Ulric Le Paige[1], Dr Duc-Duy Vu[1], Dr. Jorge Garibay[2], Msc. Jean-Max Tyburn[3], Dr. Philippe Pelupessy[1], Dr. Mauro Modesti[4], Dr. Fabien Ferrage[1]
[1]Ecole Normale Supérieure, Paris 5e, France, [2]Bruker Biospin Germany, Karlsruhe, Germany, [3]Bruker Biospin France, Wissembourg, France, [4]Cancer Research Centre of Marseille, Marseille, France

Intrinsically disordered regions (IDR) make up an important part of proteins from the non-homologous end-joining (NHEJ) DNA double-strand break (DSB) repair pathway. They mediate numerous in cis and in trans protein-protein and protein-DNA interactions that are central to the assembly and functioning of the NHEJ machinery.

The use of crowding agents to mimic the cellular environment has a been shown to affect the picosecond-nanosecond motions of proteins, and to even stabilize local conformations. Crowding agents have also been shown to enhance DNA repair activity by NHEJ in vitro.1 However, different crowders act differently, microscopically . In this work, we investigate the difference in effects between PEG and Ficoll on the picosecond-nanosecond dynamics of the C-terminal IDR of the homodimeric NHEJ-scaffolding protein XRCC4 (X4-CTR).

Aims:
To better elucidate the effects of these molecular crowders on the picosecond-nanosecond timescale dynamics of X4-CTR, we combine high-field relaxation experiments with high-resolution relaxometry data recorded with our fast sample shuttle prototype.

Results :
Our fast sample shuttle combined with the MINOTAUR software allowed us to record and analyze relaxation rates measured over two orders of magnitude of magnetic field to compare the effects of PEG and Ficoll on X4-CTR. Differential dynamics due to electrostatic interactions are more pronounced in the presence of Ficoll than PEG.

Conclusions :
Both PEG and Ficoll have microscopic effects on the dynamics of X4-CTR whose nature vary strongly with the protein sequence. Molecular crowders seem to impact interactions within disordered proteins differently which impacts backbone pico- and nanosecond motions.

NMR-105: Investigating the structure and dynamics of the type III connecting segment (IIICS) of fibronectin by NMR spectroscopy.

Miss Paige Banks[1], Dr Matthew Cliff[1], Prof Jonathan Waltho[1,2]
[1]University of Manchester, Manchester, United Kingdom, [2]University of Sheffield, Sheffield, United Kingdom

Introduction
The type III connecting segment (IIICS) is a fibronectin (Fn) domain containing integrin and proteoglycan binding sites, which facilitate the adherence and spreading of melanoma cells and leukocytes. This can lead to disease states such as cancer metastasis and inflammation. Fn is mainly composed of repeating FI, FII and FIII domains, with the IIICS situated between the 14th and 15th FIII domains. The IIICS can be divided into three subdomains (A, B, C), which combine via alternative splicing to give five variants in human Fn.

Aims
Study the structure and dynamics of the IIICS and investigate potential interdomain interactions involving the FIII14, IIICS and FIII15 domains.

Methods
The following constructs were used in this study: H120 (FIII12-14, IIICS, FIII15), H120* (FIII14, IIICS, FIII15), H89 (FIII12-14, IIICS A-B, FIII15), H0 (FIII12-15) and F15 (FIII15). A standard set of triple resonance experiments were recorded on a ^{2}H,^{15}N,^{13}C labelled H120 sample. The dynamics of the IIICS were investigated by recording ^{15}N relaxation experiments on a ^{15}N labelled H120* sample. NH-NH NOE restraints were obtained for a ^{2}H,^{15}N H120 sample by recording a ^{15}N-NOEST-TROSY spectrum. ^{1}H,^{15}N-HSQC spectra were recorded for all constructs.

Results
^{15}N relaxation rates and NOE data show that the IIICS is intrinsically disordered. However, the second half of the IIICS has significantly more restrained dynamics than the first half. Comparison of NHCSPs indicate a transient interaction involving FIII15 which is present in H89, H120* and H120 constructs but absent in H0 and F15.

Conclusions
The IIICS has no areas of well-defined structure in the constructs investigated, making it a difficult therapeutic target. The FIII15 is involved in a transient interaction with either FIII14 or the IIICS, which requires the presence of the IIICS. Spin labelling studies will be carried out to determine the FIII15 interaction partner.

NMR-106: Solid-State NMR for the characterization of biologics and protein-drug conjugates

Luis David Padilla Cortes[1], Dr. Linda Cerofolini[1], Dr. Rizzo Domenico[1], Giulia Licciardi[1], Prof. Giacomo Parigi[1], Prof. Claudio Luchinat[1], Prof. Marco Fragai[1]
[1]Magnetic Resonance Center (CERM), University of Florence, Via Luigi Sacconi 6, 50019, Sesto Fiorentino, Italy

Biologics, unlike small molecules, are produced in living systems, and are characterized by a primary, secondary, tertiary and sometimes quaternary structure. Structural characterization of the higher order structure (HOS) and epitope mapping on therapeutic proteins provides key information to improve affinity and monitor the manufacturing process and drug stability. Given the size of most of such systems, Solid-State NMR comes as a powerful tool for their characterization together with other biophysical techniques, since molecular weight and protein flexibility are not limiting factors.
The aim of the study was the development of new strategies of Solid-State NMR for the characterization of two different systems: a 60 KDa protein-drug conjugate between Transthyretin (TTR) and a cytotoxic drug (Paclitaxel); and a 240 KDa protein-antibody complex between PD-L1 and a biotherapeutic anti–PD-L1 antibody.
The epitope mapping on PD-L1 obtained by this NMR approach matches with the interacting surface previously observed in the X-ray structure of the PD-L1 in complex with Avelumab-scFv, another anti-PD-L1 mAb that shares, with the tested fusion protein, the same Fab sequence. Most of the residues experiencing the largest effects are hydrophobic amino acids: aromatic and aliphatic residues forming a wide hydrophobic patch on PD-L1 that is targeted by the anti-PD-L1 fusion protein.
For the TTR, solid-state NMR was critical for achieving a complete picture about the structural and dynamical features of the system. Indeed, the analysis of the data recorded on the solid-state samples of TTR in the presence and in the absence of two ligands provides information that is out of reach for both X-ray crystallography and solution NMR.
Overall, this approach opens new ways to monitor HOS during pharmaceutical development, allowing us to focus on the structural alterations that may affect target recognition and binding affinity, thus linking HOS assessment to the drug mechanism of action.

NMR-107: Characterisation of the conformational ensemble of microtubule-associated protein 2 c using paramagnetic labelling

Alice Laníková[1,2,3], Séverine Jansen[2], Pavel Kadeřávek[1,2], Lukáš Žídek[1,2]
[1]Faculty of Science, Masaryk University, Brno, Czech Republic, [2]CEITEC, Masaryk University, Brno, Czech Republic, [3]Department of chemistry, ENS-PSL, Paris, France

Introduction
Microtubule-associated protein 2 c (MAP2c) is the shortest isoform of the MAP2 proteins, which regulate the microtubule dynamics in developing brain neurons. Dysregulation of MAP2s has been linked to mental health conditions such as schizophrenia [1]. MAP2s belong to the class of intrinsically disordered proteins (IDPs), which lack a stable three-dimensional structure. Their highly flexible nature makes IDPs challenging systems to study. However, identifying biologically relevant conformational states is crucial for understanding their function.
Aims
Our aim is to extend the existing description of MAP2c's conformational behaviour [2]. We intend to detect long-range contacts between various parts of the MAP2c and reveal potential correlations between them using paramagnetic relaxation enhancement (PRE) and interference (PRI) [3].
Methods
Several MAP2c mutants containing one or two cysteine residues were prepared to enable the attachment of the nitroxide label to their side chains. The prepared samples were employed to measure 1H relaxation rates with the label in paramagnetic and diamagnetic forms. The obtained data were used to calculate the PRE and PRI effects.
Results
Relaxation rates were obtained for five combinations of double cysteine mutants and complementary single cysteine mutants. Moreover, the influence of MAP2c dimerisation on the dataset was detected. To address the contribution of dimerisation to the observed effects, a combination of paramagnetic and isotopic labelling was used.
Conclusions
The current analysis shows that further investigation is required to assess the contribution of dimerisation. Although the interpretation of the data is still partially biased, the preliminary results indicate the existence of multiple uncorrelated long-range contacts throughout the sequence of MAP2c.

[1] Grubisha, M.J. et al., Mol Psychiatry, 26, 5371–5388 (2021).
[2] Melková, K., et al., J. Biol. Chem., 293.34, 13297–13309 (2018).
[3] D. Kurzbach et al., Phys. Chem. Chem. Phys., 19, 10651–10656 (2017).

OTH-002: Symmetry-Based NMR Pulse Sequences in Solids and Liquids

Mohamed Sabba[1], Harry Harbor-Collins[1], James Whipham[1], Bonifac Legrady[1], Moustafa Gamal[1], Nino Wili[3], Markus Leutzsch[2], Christian Bengs[1], Prof. Malcolm H. Levitt[1]
[1]University Of Southampton, Southampton, United Kingdom, [2]Max-Planck-Institut für Kohlenforschung, Mülheim an der Ruhr, Germany, [3]Aarhus University, Aarhus, Denmark

Symmetry-based pulse sequences are sequences of radio-frequency pulses which fulfil well-defined constraints on the symmetry properties of the amplitudes and phases of the radiofrequency fields in time. These symmetries impose strong selection rules on the first-order spin dynamics that they induce. In many cases these selection rules have a simple graphical interpretation, making it relatively easy to design pulse sequences which achieve a desirable effect, at least to a first-order approximation.

Symmetry-based pulse sequences were first designed to achieve selective recoupling of certain classes of nuclear spin interactions in the context of magic-angle-spinning solid-state NMR. For example, the popular C7 pulse sequence achieves selective recoupling of double-quantum dipolar recoupling in solid-state NMR, while suppressing interference from other terms such as the chemical shift anisotropy. The main properties of this pulse sequence are a consequence of the choice of three symmetry numbers (7,2,1), as denoted by the symmetry symbol $C7_2^1$.

Recently, it was found that symmetry-based pulse sequences also have useful applications in solution-state NMR. For example, pulse sequences with the symmetry $R4_3^1$ provide effective singlet-triplet conversion for near-equivalent spin pairs in solution. The resulting pulse sequence is closely related to the PulsePol sequence which is used for electron-nuclear polarization transfer in DNP experiments. It seems that symmetry principles provide an effective design strategy which spans several fields of magnetic resonance.

We have also used symmetry-based pulse sequences for polarization transfer in solution NMR. For example, we have achieved strong enhancements of the ^{103}Rh signals for solutions of various Rh compounds, by applying simultaneous symmetry-based pulse sequences to the ^{1}H and ^{103}Rh channels.

OTH-004: Aqueous Parahydrogen-Derived Hyperpolarization of Nitrogen-13 in Ammonia: Towards Radioactively Detected Zero- To Ultralow-Field NMR in Solution

Dr. Danila Barskiy[1,2], Dr. Valentin Bonvin[3], Prof. Dmitry Budker[1,2,4], Dr. Valentina Garibotto[3], Dr. Andrea Grotzky[3], Dr. Raphael Kircher[1,2], Dr. Magdalena Kowalska[5], Dr. Michael Pesek[5], Dr. Amy Sparks[5]
[1]Johannes Gutenberg University Mainz, Mainz, Germany, [2]Helmholtz Institute Mainz, Mainz, Germany, [3]Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland, [4]University of California - Berkeley , Berkeley, USA, [5]Conseil Européen pour la Recherche Nucléaire (CERN), Meyrin, Switzerland

Introduction
Sensitivity of nuclear signal detection can be significantly improved by harnessing capabilities of radioactively detected NMR (RD-NMR). Indeed, RD-NMR allows monitoring, in principle, single beta-emission events; in practice, measuring down to ~1000 particles is possible with a reasonable signal-to-noise ratio (SNR). In previous studies, RD-NMR was used for detection of nuclear Larmor precession through beta particle counts using radioactive alkali atoms hyperpolarized via spin-exchange optical pumping [1]. Hyperpolarization of radioactive nuclear isotopes in solution so far have not been demonstrated.

Aims
Our goal was to merge the sensitivity of RD-NMR with zero- to ultralow-field-NMR (ZULF-NMR) by hyperpolarizing radioactive nuclear isotopes in solution and measuring asymmetry of their beta-decay. ZULF regime modality eliminates the need for large magnetic fields for signal detection, resulting in a portable and highly sensitive NMR modality [2].

Results
In this work we focused on hyperpolarizing ^{13}N-nuclei in ammonia in aqueous solutions at hypogeomagnetic fields (<5 µT). Parahydrogen-based hyperpolarization SABRE (signal amplification by reversible exchange) was chosen because it can generate significant heteronuclear magnetization in a few seconds [3]. Optimization of the SABRE protocol was performed for a “surrogate” ^{15}N nuclei. Ammonia was polarized in the large excess of water; this was achieved through adjustments of chemical parameters of the system such as solvent and co-ligand composition. Automated setup was constructed for the transfer of radioactive sample, ensuring operator safety. Although the asymmetry as a function of the polarization-transfer field has not been observed yet, initial results show promising responses of the scintillator signals to liquid-gas dynamics in the tube.

Conclusions
Our work has both fundamental and practical implications for RD-NMR as it would eventually allow magnetic control of the beta-decay asymmetry at hypogeomagnetic fields.

References:
[1] Harding, et al. Phys. Rev. X (2020);
[2] Blanchard, Budker. eMagRes (2016);
[3] Kircher, et al. JACS (2024).

OTH-005: SCANS: An open-source modular system for laboratory monitoring, dashboards, and alerts

Dr James Tolchard[1], Dr Anne Lesage[1], Dr Guido Pintacuda[1]
[1]High-Field NMR Center (CNRS/ENS Lyon/UCBL), Lyon, France

NMR laboratories are complex environments that contain hardware, materials, and data representing years of work and many millions of euros in funding. For safe and sustained operations, the monitoring of environmental conditions, spectrometer metrics, IT systems, and general infrastructure is therefore of the utmost importance. However, despite the potential risks, existing monitoring is often overly reliant upon on-site human interactions. Whilst paid laboratory monitoring solutions do exist, they are generally expensive and unable to provide coverage across the heterogeneous hardware environments typical of an NMR setting, comprising off-the-shelf and niche scientific and commercial products, alongside industrial infrastructure, and in-house built equipment.

To this end, we have developed SCANS (Simple Containerised Analysis for NMR Systems), an open source, modular solution for laboratory monitoring (NMR or otherwise) that can collect and store metrics over a local area network, at high time resolution, and from a wide range of hardware and web-connected services. Non-networked equipment is catered for via custom Arduino boards, with data collected from a simple HTTP interface. Data are stored in a central time-series database and showcased in customizable online dashboards, with interactive plots and tables; facilitating both live-reporting and long-term trend analysis. Furthermore, the creation of custom alerts, based on live or projected metrics, is a key feature of the dashboarding platform, allowing for remote notifications (email, SMS, Slack, Teams) as required.

The operation of this interface is illustrated with the monitoring of the CRMN NMR facility in Lyon, France, which houses several high-field NMR spectrometers, gyrotrons, an EPR spectrometer, and a helium recovery system. By leveraging freely available open-source tools (Docker, Prometheus, and Grafana), SCANS' containerised modules, written with Python and human-readable configuration files, provide a versatile and cost-effective monitoring solution in an academic laboratory setting.

OTH-006: Relaxation dynamics of an unlike spin pair system

Dr Adriane Consuelo Leal Auccaise[1], Dr Hugo Danilo Fernández Sare[1], Dr Ruben Auccaise Estrada[2]
[1]Universidade Federal de Juiz de Fora, Juiz de Fora, Brazil, [2]Universidade Estadual de Ponta Grossa, Ponta Grossa, Brazil

Introduction
Dipole-dipole interaction is an essential interaction between two or more nuclei with an internuclear or intramolecular contribution. Studying the dipole-dipole interaction allows us to establish the particle relaxation dynamics. The pioneering works on relaxation were introduced by Bloembergen, Purcell, Pound [1], and Solomon [2]. Two years later, the approach was extended via a master equation by Redfield through concepts from Quantum Mechanics [3].

Aims
Redfield Theory was revisited to analyze a heteronuclear system, where two nuclear species S=1/2 and I=1/2 interacting by dipole-dipole coupling were studied [4].

Methods
Three cases of longitudinal magnetization evolution were revisited. The saturation condition gives the first case, where the populations of the nuclear species S (the most abundant) are equalized. In the second situation, the populations of the nuclear species S are inverted. In the third case, the nuclear species S and I populations are inverted. For the first and second situations, we determine the enhancement time.

Results
The bi-exponential model for the longitudinal magnetization was verified, as was similarly done implementing the projection operator technique [5]. A probable experimental implementation could be figure out at high magnetic field setup.

Conclusions
This work reveals the spectral densities and correlation times values of each system or molecule monitoring the dynamics of magnetizations.

References
[1] Bloembergen, N. E., Purcell, M., Pound, R. V. Physical Review, 1948, 73, 679-712.
[2] I. Solomon, Physical Review, 99, 1955, 559-566.
[3] Redfield, A. G. On the theory of relaxation processes. IBM Journal of Research and Development, 1, 1957, 19-31.
[4] Consuelo-Leal, A., Sare, H.D.F., Auccaise, R. Relaxation dynamics of an unlike spin pair system. Quantum Inf Process, 22, 2023.
[5] Werbelow, G. L., Grant, M. D. Carbon-13 relaxation in multispin systems of the type AXn. J. Chem. Phys. 63, 1975, 544–556.

OTH-007: NMR Relaxation by Redfield equation in a spin system I=7/2

Dr Adriane Consuelo Leal Auccaise[1], Dr Arthur Gustavo Araújo Ferreira[1], Dr Everton Lucas Oliveira[1], Dr Tito José Bonagamba[1], Dr Ruben Auccaise Estrada[2]
[1]Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, Brasil, [2]Universidade de Ponta Grossa, Ponta Grossa, Brasil

Introduction

The Redfield theory is an approach that allows us to describe the relaxation theory by applying quantum mechanics concepts and exploring the density matrix definition [1, 2]. Consequently, all the coherence orders can be determined analytically or numerically. Additionally, the number of relaxation rate constants established by Tsoref-Eliav-Navon et al. [3] was extended, and mathematical expressions were established for the longitudinal and transverse magnetization.

Aims

The Redfield theory must be applied to determine the analytical (order coherence 7,..,2) and numerical (order coherence 1 and 0) solutions for all the density matrix elements of an isolated spin 7/2 system.

Methods

Our research involved two NMR experiments, the spin-echo and inversion-recovery, conducted in a lyotropic liquid crystal Cs-PFO. The characteristic seven spectral lines of a spin 7/2 nuclei were monitored to measure the longitudinal and transverse magnetization in static magnetic field strength of 9.4 Tesla.

Results

All the relaxation rate constants that determine the dynamics of the spin 7/2 system were accurately characterized. This precision in characterizing the dynamics allows us to find the spectral density values and test the theoretical solutions. The amplitudes predicted by the theory were compared with the Laplace Transform tool and the values are remarkably close, further validating the accuracy of our theoretical predictions.

Conclusions

Using the model developed in this work, the theoretical result allows us to describe any spin system 7/2 isolated.

References

[1] Redfield, Alfred G. On the theory of relaxation processes. IBM Journal of Research and Development. 1957, 1, 19-31.
[2] A. Consuelo-Leal, et al. NMR Relaxation by Redfield equation in a spin system I= 7/2. Journal of Magnetic Resonance, v. 349, p. 107403, 2023.
[3] Tsoref, L., U. Eliav, and G. Navon. Multiple quantum filtered nuclear magnetic resonance spectroscopy of spin 7/2 nuclei in solution. The Journal of chemical physics, 1996.

OTH-008: NMR of C60 endofullerenes and endofullerides

Dr George Bacanu[1], Dr Murari Soundararajan[1], Dr Mark Walkey[1], Ms Elizabeth Marsden[1], Dr Vijyesh Vyas[1], Mr Keisuke Matsui[2], Dr Naoya Yoshikane[2], Dr Gabriela Hoffman[1], Dr Sally Bloodworth[1], Dr Francesco Giustiniano[1], Dr Karel Kouril[1], Dr Marina Caravetta[1], Prof Kosmas Prassides[2], Prof Richard Whitby[1], Prof Malcolm Levitt[1]
[1]University of Southampton, SOUTHAMPTON, United Kingdom, [2]Osaka Metropolitan University, Osaka, Japan

Since its discovery[1], the C_{60} fullerene molecule has raised great interest of scientists and mathematicians due to its fascinating truncated icosahedron shape, with a very high degree of symmetry. Endofullerenes are supramolecular complexes where one small endohedral species (atom/molecule) is completely confined within a bigger fullerene molecule.[2],[3] Synthetic chemists have developed a method for making endofullerenes called “molecular surgery”: empty C_{60} through a series of chemical reactions changes its structure and acquires an opening, the endohedral species is inserted through the opening and then the C_{60} is closed back to its original form with the endohedral species completely enclosed.[2],[3]

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

NMR (solution and solid state) measurements of endofullerenes, performed at ambient and cryogenic conditions will be presented. These consist of ^{1}H, ^{13}C and ^{3}He experiments on various endofullerenes: $^{3}He@C_{60}$, $CH_2O@C_{60}$, $NO@C_{60}$, etc.

The fullerene family extends to fullerides, these are ionic salts of negatively charged C_{60} cages counterbalanced by positively charged metal ions.[4] If the C_{60} cages are filled with an endohedral species this leads to endofullerides.[4] Characterisation and solid state NMR measurements will be presented for a selection of endofulleride materials. Endofullerides investigated are of the form Mx($A@C_{60}$), where M is an alkali metal (K or Rb), x is the stoichiometry of the metal with respect to C60 (x = 3, 4, 6) and A is the endohedral species (A = H_2, HD, ^{3}He).

[1] H. Kroto et al., Nature, 1985, 318, 162.
[2] Y. Murata et al., Science, 2005, 307, 238-240.
[3] S. Bloodworth and R. J. Whitby, Commun. Chem., 2022, 5, 1–14.
[4] M. Soundararajan et al., Appl. Magn. Reson., 2023, 54, 1177–1192.

OTH-009: An Artificial Intelligence Approach to Engineer Super-Stable Nanobodies

Alba Pejenaute[1,2], Gabriel Ortega[1,3]
[1]CIC bioGUNE, Bilbao, Spain, [2]Tekniker Basque Research and Technology Alliance (BRTA), Eibar, Spain, [3]Ikerbasque, Basque Foundation for Science, Bilbao, Spain

Nanobodies are the smallest antibodies that retain full antigen-binding function. Derived from the heavy-chain-only antibodies from camelid and sharks, their smaller size, monomeric state and fold simplicity render them more suitable for a diverse range of biotechnological applications, such as drug delivery, immunotherapy, biosensors, and imaging.

Aims

Motivated the biotechnological potential of nanobodies, we aimed to improve their biophysical properties. The improvement of their thermostability and solubility will facilitate their implementation in biotechnological platforms, as well as their durability in the biological and cellular environment. Additionally, it will also facilitate the recombinant production of nanobodies, which is often challenging because of their low expression yield and tendency to aggregate.

Methods

We have developed and validated experimentally a rational engineering strategy to improve the stability, solubility and production yield of nanobodies. For this purpose, we have used phylogenetic and structural analysis, along with new deep learning–based protein engineering tools. To make the approach generalizable to different nanobodies, we have focused our engineering efforts in the conserved region, thus maintaining binding affinity and specificity intact. We have then expressed and purified the resulting variants, and evaluated experimentally their thermostability, folding reversibility, solubility, expression yield and ligand binding

Results

For all nanobodies tested, we have found remarkable improvements in thermostability and, in most cases, also in solubility and expression, while retaining the original binding affinity.

Conclusions

Overall, our approach offers a rational and generalizable strategy to engineer nanobodies with enhanced biophysical properties. Our rational engineering strategy will facilitate the production and use of nanobodies in many different biotechnological applications

SLT-001: Fast dynamics of difluprednate in micelles or swollen micelles revealed by 19F NMR spin relaxation rates

Research Chemist Kang Chen[1]
[1]Us Food And Drug Administration, Silver Spring, United States

Introduction
Amphiphilic surfactant molecules assemble into nanometer (nm) sized micelle globules, dispersed in an aqueous solution to form nano-emulsion solution. Micelles can be utilized during pharmaceutical development to formulate hydrophobic drug molecules. Additionally, oil can be encapsulated in micelle structure to enhance drug solubility, forming swollen micelles (s-micelles). Viscosities of both surfactant and oil are two orders of magnitude higher than that of water, and according to Stokes-Einstein-Debye (SED) equation, solute drug molecules would be in restricted rotational motion in micelles.

Aim
To obtain the knowledge of rotational correlation time of solute molecules inside micelles.

Methods
A 0.5 kDa molecule difluprednate (DFPN) was dissolved in two types of micelle globules, the pure polysorbate-80 (PS-80) micelle and the castor oil swollen-micelle. A DFPN molecule contains two 19F atoms (F28 and F29) that are spin ½ nuclei. The 19F spin relaxation rates R1 and R2 and chemical shift anisotropy (CSA) of both fluorine nuclei were measured at two field strength of 400 and 600 MHz spectrometers. Spectral density functions of the isotropic rigid body rotation model were used to fit the experimental relaxation rates. Rotation correlation (tc) of DFPN in both micelles were obtained through Monte Carlo sampling and data fitting.

Results
Fast nanosecond (ns) rotational correlation time (tc) of 4 and 12 ns were obtained for DFPN in both micelle globules, which unambiguously showed small molecule rotation in viscous PS-80 or oil solvents could deviate from the prediction of SED equation.

Conclusion
Fast nanosecond dynamics of small molecules in viscous media suggests that SED equation was not applicable for soft-core nano emulsion system. The solvent solute interaction could govern the rotational dynamics of small solute molecules, not viscosity. Therefore, NMR could be universally applicable in soft-core nano-emulsion formulations, advancing our knowledge in molecular interactions in complex drug formulations.

SLT-002: Combination of NMR and mass spectroscopy to study the productive encounter complex that results in dysregulation of actin polymerization

Dr. Alexander Shekhtman[1], Mr. Greg Theophall[1], Dr. Sergey Reverdatto[1], Dr. Lisa Ramirez[1], Mr. Aaron Premo[1], Dr. Michaele Manigrasso[2], Dr. Gautham Yepuri[2], Dr. Ravichandran Ramasamy[2], Dr. Ann Marie Schmidt[2], Dr. David Burz[1]
[1]University at Albany, SUNY, Albany, United States, [2]New York University Grossman School of Medicine, New York, United States

Introduction: The diaphanous-related formin, Diaphanous 1 (DIAPH1), is required for the assembly of Filamentous (F)-actin structures. DIAPH1 is an intracellular effector of the receptor for advanced glycation end products (RAGE) and contributes to RAGE signaling and effects such as increased cell migration upon RAGE stimulation. Mutations in DIAPH1, including those in the basic “RRKR” motif of its autoregulatory domain, diaphanous autoinhibitory domain (DAD), are implicated in hearing loss, macrothrombocytopenia, and cardiovascular diseases.

Results: The solution structure of the complex between the N-terminal inhibitory domain, DID, and the C-terminal DAD, resolved by NMR spectroscopy shows only transient interactions between DID and the basic motif of DAD, resembling those found in encounter complexes. Cross-linking studies and mass spectrometric analysis placed the RRKR motif into the negatively charged cavity of DID. Neutralizing the cavity resulted in a 5-fold decrease in the binding affinity and 4-fold decrease in the association rate constant of DAD for DID, indicating that the RRKR interactions with DID form a productive encounter complex. A DIAPH1 mutant containing a neutralized RRKR binding cavity shows excessive colocalization with actin and is unresponsive to RAGE stimulation.

Conclusions: This is the first demonstration of a specific alteration of the surfaces responsible for productive encounter complexation with implications for human pathology. The methodology developed in this work can be used to analyze various productive encounter complexes involved in regulation of protein activity.

SLT-003: Pushing the boundaries of dissolution-DNP applications: methodologies for the study of battery electrolytes

Chloe Gioiosa[1,2], Ekaterina Pokochueva[1], James Tolchard[1], Charlotte Bocquelet[1], Simon Pondaven[2], Ségolène Laage[2], Pr Sami Jannin[1]
[1]TotalEnergies, Centre de recherche de Solaize, Solaize, France, [2]Univ. Lyon, CNRS, ENS Lyon, UCBL, Université de Lyon, CRMN UMR 5082, Villeurbanne, France

Dissolution-DNP (dDNP) has proven itself to be a very powerful hyperpolarizing technique enabling sensitivity gains beyond four orders of magnitude in liquid state NMR. Over the last decades, researchers' efforts led to an extension of dDNP applications in fields such as imaging, early-stage cancer detection, metabolic pathways studies, or drug discovery[1].
One field of research that would heavily benefit from an NMR sensitivity boost is the study of lithium-ion battery electrolytes. Li-ion is the most widespread battery technology, with increasing demand pushing researchers to better understand the different physicochemical mechanisms at stake to make them more efficient, safe, and sustainable. One phenomenon at the heart of several research works is the degradation of the battery electrolyte, which can have a significant impact on the battery's performance. NMR has been used to try and better understand the mechanisms at play, but its lack of sensitivity makes it challenging to detect traces of degradation products[2].
In this work, we propose to use state-of-the-art CP-assisted dDNP methodologies to acquire hyperpolarized battery electrolyte 13C spectra in the liquid state. We show that we successfully detect on a 600 MHz spectrometer hyperpolarized 13C spectra at natural abundance of formulated battery electrolyte solutions with enhancements ranging from 2 to 3 orders of magnitude on different carbon types.
We believe this work will pave the way for studying lithium-ion battery electrolyte degradation after real usage conditions (cycling, thermal aging, air exposure,…) with a 13C detection limit below the micromolar range. With this methodology, we hope to provide new insights into these mechanisms, specifically on the early stages of the degradation processes, and on the role and effectiveness of additives addition to formulas to mitigate these phenomena.

References:

(1) J. Magn. Reson. 2019, 305, 41–50.
(2) Phys. Chem. Chem. Phys, 2016, 18 (38), 26595–26601.

SLT-004: Dissolving the insoluble: aqueous pKa determination of analytes from solvent mixtures using chemical shift imaging 1H NMR

Mr Haider Hussain[1]
[1]University Of East Anglia, Norwich, United Kingdom

Introduction

The acid dissociation constant (Ka or its negative logarithm pKa) of a molecule is an important property that has a significant impact on its chemical and biological activity and is used in drug discovery for predicting the drug-target interactions of pharmaceutical molecules and their respective solubility. However, most pharmaceutical compounds are insoluble in water and are studied instead in mixtures of water and organic solvents such as DMSO. The water pKa is extrapolated from the DMSO-H2O mixtures using the Yasuda-Shedlovsky equation which relates the solvent specific pKa with the dielectric constant of the solution and the respective solvent composition correlated with the dielectric constant. Conventionally this necessitates separate titrations of at least 4 solvent compositions.

Aim

Using a concentration gradient of solvent, we have developed a method that provides the pKa of a compound at different solvent compositions in a single 20 minute 1H chemical shift imaging experiment, thus allowing a titration-free extrapolation to 100% H2O.

Method

We develop a continuous gradient of solvent composition where the pKa is determined at each position along the gradient via the number of protons removed by a basic indicator molecule. The solvent composition is determined accurately from the 1H chemical shift of sodium methanesulfonate. As proof of concept, we measure the pKa values of water soluble and insoluble compounds in DMSO/H2O mixtures and obtain pKa measurements within 0.4 units of the literature pKa in H2O. By investigating the change in line broadening of analyte peaks as a function of solvent composition, we can determine the solvent composition at which aggregation of water-insoluble drugs occurs and from which extrapolation to water becomes impossible.

Conclusion

Our method provides a very fast way to measure the aqueous pKa of water insoluble compounds that avoids many hours of tedious conventional titrations at different compositions of solvent.

SLT-006: Full sensitivity single-scan ultraselective NMR

Dr Margherita Bazzoni[1], Dr Rituraj Mishra[1], Mr Armand Régheasse[1], Ms Aurélie Bernard[1], Prof. Patrick Giraudeau[1], Prof. Ralph Adams[2], Prof. Laura Castañar[2,3], Prof. Gareth Morris[2], Prof. Mathias Nilsson[2], Dr Peter Kiraly[4], Dr Jean-nicolas Dumez[1]
[1]Nantes Université, CNRS, CEISAM, Nantes, France, [2]Department of Chemistry, University of Manchester, Manchester, United Kingdom, [3]Faculty of Chemistry, Complutense University of Madrid, Madrid, Spain, [4]JEOL (UK) Ltd, Long Hanborough, United Kingdom

Selective experiments are an efficient way to address the complexity of NMR spectra and retrieve correlation information in a short time, with applications that include complex stereochemical questions and the analysis of mixtures. Classical selective experiments based on a single shaped radio-frequency pulse are efficient when one well-resolved signal is available. However, in 1H 1D NMR spectra, multiplets due to J couplings frequently overlap. Such cases can be addressed with chemical shift selective filters (CSSF). These sum all the indirect-dimension time increments of a homodecoupled band-selective 2D experiment, but require the acquisition of a large number of such increments.

The GEMSTONE experiment makes it possible to select a 1H multiplet in a single scan, even when it overlaps with other multiplets. These fast “ultraselective” experiments use spatial parallelisation of the indirect dimension of CSSF experiments, through the combined application of frequency-swept pulses and magnetic field gradients. However, long gradient durations result in sensitivity losses due to translational molecular diffusion and convection, which can become prohibitive when high selectivity is needed.

Here we show that single-scan ultraselective experiments can be made less susceptible to the effect of diffusion and thus more sensitive (by a factor which can exceed 10), by splitting the spatial parallelisation process into multiple consecutive blocks. We also describe the parameters governing the choice of pulse bandwidth, and the resulting effects on diffusion losses, through careful analysis of J-coupling effects during the spatial parallelisation process. We compare simple repetitions of the original GEMSTONE block with improved implementations that further reduce diffusion and relaxation losses, and show the resulting benefits for selective correlation experiments on a peptide and on mixtures of small molecules. Overall, these new experiments can extract detailed information from complex spectra in minimal time, with a much more favourable trade-off between sensitivity and selectivity.

SLT-008: High-Resolution Relaxometry (HRR) for Multiscale Dynamics in Complex Systems

Phd Giulia Licciardi[1], L. Fiorucci[1], A. Kubrak[1], J. Garibay[2], E. Ravera[1], G. Parigi[1], C. Luchinat[1]
[1]University of Florence, Sesto Fiorentino, Italy, [2]Bruker BioSpin GmbH & Co, Ettlingeng, Germany

Introduction
High-Resolution Relaxometry (HRR), a cutting-edge NMR technique, offers unparalleled insight into dynamic processes within complex systems. This work builds upon established Fast Field-Cycling (FFC) relaxometry, leveraging the development of Fast Shuttle System (FSS) prototypes for HRR, within the Horizon 2020 "Hires Multidyn" project.

Aims
We aim at exploring novel applications for HRR, also in conjunction with FFC, to unlock deeper understanding of multiscale dynamics in complex systems, but also at the validation of the FSS prototypes for HRR measurements.

Methods
Through the FSS, HRR exploits the high-field spectrometer's stray field for variable-field relaxation measurements, surpassing FFC limitations and enabling high-resolution relaxation measurements over a wide timescale.

Results
Combining FFC, HRR and high resolution NMR measurements, the internal mobility of a complex viscous systems, i.e. olive oil, was investigated in unmatched detail. Moreover, HRR measurements were used for studying interactions occurring in biological systems, as those between proteins and ligands, as well as between metabolites and macromolecules in urine, and for probing the dynamics of intrinsically disordered proteins, highlighting the value of low field relaxation measurements.

Conclusions
This work demonstrates the versatility and effectiveness of HRR for studying multiscale dynamics. FSS prototypes and the synergistic use of HRR with FFC offer significant insights, paving the way for further exploration of complex systems using these powerful NMR relaxometry methods.

Giulia Licciardi obtained her PhD in the “International Doctorate in Structural Biology” program at CERM (Magnetic Resonance Center) in Florence, with a thesis entitled "NMR relaxation of paramagnetic systems and biomolecules". She is a postdoctoral researcher at the University of Florence, working on innovative approaches in NMR.

SLT-009: Combined NMR and molecular dynamics conformational filter identifies unambiguously dynamic ensembles of Dengue protease NS2B/NS3pro

Dr Peter Agback[1], Dr Tatiana Agback[1], Dr Dmitry Lesovoy[4,5], Dr Xiao Han[3], Dr Alexander Lomzov[1,6], Dr Renhua Sun[3], Dr Tatyana Sandalova[3], Prof Vladislav Orekhov[2,4], Prof Adnane Achour[3]
[1]Swedish University Of Agricultural Sciences, Uppsala, Sweden, [2]University of Gothenburg, Gothenburg, Sweden, [3]Karolinska Institute, Solna, Sweden, [4]Swedish NMR Centre, Gothenburg, Sweden, [5]Shemyakin-Ovchinnikov, Institute of Bioorganic Chemistry RAS, Moscow, Russia, [6]Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia

The dengue protease NS2B/NS3pro has been reported to adopt either an ‘open’ or a ‘closed’ conformation. We have developed a conformational filter that combines NMR with MD simulations to identify conformational ensembles that dominate in solution. Experimental values derived from relaxation parameters for the backbone and methyl side chains were compared with the corresponding back-calculated relaxation parameters of different conformational ensembles obtained from free MD simulations. Our results demonstrate a high prevalence for the ‘closed’ conformational ensemble while the ‘open’ conformation is absent, indicating that the latter conformation is most probably due to crystal contacts. Conversely, conformational ensembles in which the positioning of the co-factor NS2B results in a ‘partially’ open conformation, previously described in both MD simulations and X-ray studies, were identified by our conformational filter. Altogether, we believe that our approach allows for unambiguous identification of true conformational ensembles, an essential step for reliable drug discovery.

SLT-010: The latest in the use of paramagnetic NMR data for protein structure refinement

Prof. Giacomo Parigi[1]
[1]University Of Florence, Sesto Fiorentino, Italy

Introduction
Theoretical and computational advancements in quantum chemistry (QC) calculations of paramagnetic NMR observables are opening new routes in structural biology. The presence of a paramagnetic metal ion in a protein can provide structural information at a level often hardly accessible with other techniques. The dependence of the pseudocontact shifts (PCSs) on the nuclear coordinates passes through the paramagnetic susceptibility anisotropy tensor [1], which is very sensitive to the details of the coordination geometry of the paramagnetic metal ion. PCSs can thus be used to drive the refinement of protein structures at the metal coordination site to an unprecedented resolution [2].
Results
Accurate predictions of the susceptibility tensors were performed with ORCA using relativistic CASSCF calculations, with second-order perturbation theory corrections, for the oxalate-bound cobalt(II) human carbonic anhydrase II, with the catalytic zinc ion substituted by the paramagnetic cobalt(II) ion [3]. Machine learning-based protocols were implemented to overcome the limitations of the prohibitive computational power required by these calculations. Experimental PCSs were then compared to PCS calculated from the obtained susceptibility tensors and the protein structure until matching. The correct dependence of PCSs on susceptibility tensor anisotropies was derived using a rigorous QC treatment which also includes previously neglected fourth-order terms accounting for magnetization saturation at very high magnetic fields [4].
Conclusions
PCSs measured for nuclei far from the paramagnetic metal, analyzed with first-principles QC approaches and neuronal network algorithms, allow for picometer-scale structural refinement of the coordination cage of the metal.

1. Lang, Ravera, Parigi, Luchinat, Neese, J. Phys. Chem. Lett. 11, 8735 (2020)
2. Ravera, Gigli, Suturina, Calderone, Fragai, Parigi, Luchinat, Angew. Chem. Int. Ed. 60, 14960 (2021)
3. Gigli, Silva, Cerofolini, Macedo, Geraldes, Suturina, Calderone, Fragai, Parigi, Ravera, Luchinat, under revision
4. Fiorucci, Lang, Parigi, Ravera, Luchinat, in preparation

SLT-011: Exploiting Multiple Receivers at 1.2 GHz to delve into Intrinsically Disordered Proteins

Dr. Marco Schiavina[1], Ms. Sonja Knoedlstorfer[2], Ms. Maria Anna Rodella[1], Dr. Rainer Kuemmerle[3], Prof. Robert Konrat[2], Prof. Isabella Caterina Felli[1], Prof. Roberta Pierattelli[1]
[1]University Of Florence, Cerm, Sesto Fiorentino, Italy, [2]University of Wien, Wien, Austria, [3]Bruker BioSpin AG, Fällanden, Switzerland
Marco Schiavina obtained his PhD in Structural Biology at CERM in 2021, focusing on the development and application of ^{13}C-direct detected NMR experiments for the study of Intrinsically Disordered Proteins (IDPs). In the following period, he shifted his attention to the characterization of modular proteins using NMR spectroscopy. Since December 2022 he has worked as a Researcher at the University of Florence's CERM as part of the ITACA-SB project.

Introduction
Increased magnetic field strengths and console improvements drive progress in biomolecular NMR applications. The availability of Ultra-High-Field NMR instrumentation allows the investigation of complex IDPs. Their study is frequently hampered by the crowding of NMR spectra. Thanks to the excellent resolution that can be achieved at high field exploiting ^{13}C-detection[1] it is possible to partially solve this problem. However, considering the lower ^{13}C sensitivity and the longer longitudinal relaxation time of ^{13}C nuclei with respect to protons, time-consuming experiments are usually required to obtain well-resolved spectra.

Aims
To delve into IDPs at Ultra-High-Fields we are developing Multiple-Receivers NMR sequences (mr_NMR), in which one experiment is acquired during the recycle delay of the second one.

Methods
The recovery delay of ^{13}C nuclei is sufficiently long to enable the acquisition of a second NMR experiment during this period. Combining ^{13}C-detection with mr_NMR permits saving time and obtaining complementary information from the different detected correlations.

Results
Previously developed mr_NMR experiments, mr_CON//HN and mr_CON//HαCAN, were implemented at 1.2 GHz to obtain the fingerprinting and sequence-specific information of the structural heterogeneous CBP-ID4 protein. The newly-designed mr_CACO//btHN pulse sequence provided insights into the interaction between Myc:Max complex and BRCA protein.

Conclusions
This approach allowed us to gather complementary information based on the structural and dynamic properties of the in-studio IDPs as well as to investigate their interactions.

1) M. Schiavina, et al. Nature Protocols, 2024

SLT-012: Which ones are the poisons? Probing complex mixtures of molecules using pH gradients in organic solvents

Dr Matthew Wallace, Dr Serena Monaco
[1]University of East Anglia, Norwich, United Kingdom

Introduction: We show how a combination of pH gradients and spatially resolved NMR analysis can greatly aid the identification of molecules present in complex mixtures. By assessing the pH-dependence of chemical shifts and thus pKa, we can infer the type and proximity of functional groups and whether two resonances on a spectrum belong to the same molecule. When combined with conventional 2D NMR techniques, our approach enables rapid identification of the molecules present without tedious spectral simulations and assignments.

Aims: Complex mixtures arising from microbial fermentation or biomass processing often contain a large number of unknown molecules. Identification of these molecules is vital to optimise extraction processes and reveal metabolic pathways. While NMR is a highly efficient tool for the non-targeted characterisation of mixtures, assignment of resonances can be challenging due to severe overlap of ^{1}H resonances. We demonstrate our pH-gradient approach for the identification of compounds in extracts of poisonous plants.

Methods: We establish working pH scales in methanol and ethanol using our procedure for the determination of pH in aqueous-organic mixtures (1). We can thus determine pH between 1 and 13 via the ^{1}H chemical shifts of a small number of indicator molecules. pH gradients are established via the diffusion of methanesulfonic acid while spatially resolved ^{1}H spectra are recorded along the length of the sample using z-axis gradient phase encoding methods.

Results: pH gradients allow immediate identification of the resonances belonging to pH-active compounds and neutral molecules such as sugars. Combining this information with COSY and diffusion ordered spectroscopy (DOSY) allows identification of the alkaloids present in the extracts.

Conclusions: The combination of pH gradients and localised NMR analysis provides a powerful tool for the identification of compounds in complex mixtures via the pH-dependence of chemical shifts.

1. M. Wallace et al., Anal. Chem., 2023, 95, 15628.

SLT-013: Measuring long-range couplings via singlet NMR

Dr James Whipham[1], Dr Mohamed Sabba[1], Dr Gamal Mustafa[1], Professor Malcolm Levitt[1]
[1]University Of Southampton, Southampton, United Kingdom

Introduction

A pair of nuclear spin-1/2 may host singlet-order; a long-lived population configuration across the singlet and triplet manifolds. This spin-order is immune to the intrapair dipolar interaction as a relaxation mechanism. However, singlet-order is not immune to interactions with out-of-pair nuclei, such as the scalar relaxation of the second kind (SR2K) mechanism. It turns out that this mechanism allows one to probe long-range spin-spin (J-) couplings 4- or 5-bonds from the spin-1/2 pair.

Aims

To measure 4- and 5-bond J-couplings

Methods

Symmetry-based sequences are used to access singlet-order. Resonant irradiation utilising the SLIC condition manipulates the relaxation rate, which is in turn used to estimate J-couplings

Results

4-bond carbon-deuterium couplings are successfully measured to be 18 mHz. Attempts are made to measure 5-bond carbon-proton couplings.

Conclusions

Singlet NMR methods may be used to measure long-range J-couplings using a simple model for the SR2K mechanism. Further, these measurements aren't limited by these couplings being extremely small.

SLT-014: Ultrafast Lapalce NMR for biomaterials

Dr Otto Mankinen[1]
[1]NMR Research Unit, University of Oulu, Finland

Introduction & aim

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 diffusion coefficient and relaxation time 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 provide much higher boost than the loss due to spatial encoding.

Results & conclusions

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.[4-9]

1. Y. Q. Song, J. Magn. Reson. 229, 12 (2013).
2. L. Frydman et al. P.Natl.Acad.Sci.99,15858 (2002).
3. A. Tal and L. Frydman, Prog.Nucl.Mag.Res.Sp. 57, 241 (2010).
4. S. Ahola and V.-V. Telkki, ChemPhysChem. 15, 1687 (2014).
5. S. Ahola et al. Nat.Commun. 6, 8363 (2015).
6. O. Mankinen et al. Nat.Commun. 11, 3251 (2020).
7. M.S. Ullah et al. Phys.Chem.Chem.Phys. 24, 22109 (2022).
8. K. Tolkkinen et al. Phys.Chem.Chem.Phys. 25 13164 (2023)
9. Y. Kharbanda et al. J.DairySci. 106, 1586 (2023).

SLT-015: A Novel Iterative Scaling Method for the Determination of Accurate Molecular Weights of Polymers

PhD. Francisco Manuel Arrabal Campos[1,2], Ms. Marta González Lázaro[1], PhD. Juana M. Pérez[1], PhD. Juan A. Martínez-Lao[2], PhD. Ignacio Fernández[1]
[1]Department of Chemistry and Physics, Research Centre CIAIMBITAL, University of Almería, La Cañada, Spain, [2]Department of Engineering, CIMEDES Research Center (CeiA3), University of Almería, La Cañada, Spain

Introduction: The determination of accurate molecular weights in polymer science is usually a challenge. Traditional methods based on NMR rely on diffusion coefficients estimated through scaling relationships especially sensitive to concentration and solvent viscosity, leading to significant inaccuracies.
Aims: Devise a novel method capable of mitigating dependencies on solvent type and polymer concentration by eliminating the need for Flory conditions, enhancing SNR ratios and thereby improving precision in polymer characterization.
Methods: NMR measurements were performed on a 500 MHz Bruker Avance spectrometer equipped with a TBI 1H/31P/BB probehead and employing stimulated pulse sequences within bipolar pair pulses.
Results: We introduced an iterative method (eq. 1) based on a novel scaling law that incorporates the influence of the molecular weight on the diffusion coefficients, Dη|c=Dη|(1/∞)·e^(-κC^ν)=a·exp^(-(e·Mw+d)·C^ν)·Mw^(-b) (eq. 1). The main inputs include the quantitative diffusion coefficient (D), the viscosity of the chosen solvent, and the selected concentration. Demonstrated across a wide concentration range, the method incorporates two new derived curves (kappa and high dilution curves) to circumvent polymeric interactions that occur as a function of concentration. The method uses an advanced mathematical modelling to iteratively refine molecular weight estimates starting from an assumed value. Validation of the approach employed monodispersed polypropylene glycol and polystyrene samples at various concentrations and solvents confirming its universal applicability.
Conclusions: A novel method based on diffusion NMR has been developed, allowing the estimation of the molecular weight of the polymer in any solvent and at any concentration. Further research is focused in expanding the method to include extracellular polysaccharides and hyaluronic derivatives, thereby broadening its applicability in polymer science.
Acknowledgments: This research has been funded by the State Research Agency of the Spanish Ministry of Science and Innovation (PID2021-126445OB-I00), and by the Gobierno de España MCIN/AEI/10.13039/501100011033/Unión Europea “Next Generation EU”/PRTR (PDC2021-121248-I00, PLEC2021-007774 and CPP2022-009967).

SLT-017: 15N NMR relaxation experiments at high magnetic fields reveal new insights into the ps-ns structural dynamics of intrinsically disordered proteins

Tobias Stief[1,2], Katharina Vormann[1,2], Dr Nils-Alexander Lakomek[1,2]
[1]Heinrich Heine University Düsseldorf, Düsseldorf, Germany, [2]Forschungszentrum Jülich, Jülich, Germany

Intrinsically disordered proteins (IDPs) compose about 30% of the human proteome and are highly dynamic entities, making them challenging to study by X-ray crystallography or cryo-EM. However, IDPs are well suited to be examined by NMR spectroscopy. Recent advances in NMR magnet technology allow for commercially available magnetic field strengths up to 28 T, corresponding to a proton Larmor frequency of 1.2 GHz. Those high field strengths offer substantially improved resolution, with particular benefits for studying IDPs.

We have derived an improved set of 15N NMR relaxation experiments employing a sensitivity-enhanced HSQC read-out scheme and improved handling of the water magnetization. The NMR relaxation experiments have been tested at 1.2 GHz and are suited for operation at high-field magnets and their application on fully protonated IDPs [1].

We use SNARE proteins as a model system. SNARE proteins play a crucial role during neuronal exocytosis by eliciting the fusion of the synaptic vesicle membrane with the presynaptic plasma membrane. In their pre-fusion state, the membrane-anchored SNARE proteins are IDPs [2,3]. To assess the internal dynamics of the SNARE protein SNAP25, we recorded NMR relaxation experiments at different magnetic field strengths, between 600 and 1200 MHz. The field-dependent NMR measurements reveal novel insights into IDP dynamics at the picoseconds to nano-seconds timescale. Our unpublished data will be presented at the conference.

Literature:

[1] Stief T., Vormann K., Lakomek N.A., Methods 2024, 223,1-15
[2] Lakomek N.A., Yavuz H., Jahn R., Perez-Lara A., Proc. Natl. Acad. Sci. U.S.A. 2019, 16(18), 8699-8708.
[3] Stief T, Gremer L., Pribicevic S., Espinueva D.F., Vormann K., Biehl R., Jahn R., Pérez-Lara Á., Lakomek N.A., J Mol Biol. 2023 Mar 30;435(10):168069.

SLT-018: Heteronuclear polarization transfer under steady-state free precession conditions

Dr Rihards Aleksis[1], Dr Elton Montrazi[1], prof. Lucio Frydman[1]
[1]Weizmann Institute Of Science, Rehovot, Israel

Fourier-transform nuclear magnetic resonance (FT NMR) methods are a staple of modern liquid and solid-state 1D spectroscopy. An alternative to FT NMR acquisition was introduced by Carr, who proposed steady-state free precession (SSFP) sequence, which is composed of a train of pulses intermittent with acquisition periods [Phys.Rev. 112, 1693 (1958)]. Although the spectral resolution in this experiment is significantly decreased, the approach gives superior signal to noise ratio per unit time (SNRt) than any other NMR scheme. Further, we have recently demonstrated that resolution can be reinstated into this experiment via phase-incremented strategies [J.Am.Chem.Soc. 146, 3615 (2024)]. Still, regardless of the approach taken, targeted nuclei, particularly low-γ species like ^{13}C or ^{15}N, may exhibit poor sensitivity. The SNR for such species can be enhanced by employing techniques that transfer polarization from highly abundant nuclei with large gyromagnetic ratios. In particular, techniques that rely on the application of a few discrete, well-timed pulses like insensitive nuclei enhanced by polarization transfer (INEPT), can enhance the NMR signal of the low-γ species via J-couplings transfers. In the present study we demonstrate that such coherent polarization transfers can also be combined with SSFP schemes, to impart on the latter further sensitivity gains. A number of variants of what we denote as the INEPT-SSFP experiment, can then provide on ^{13}C ca. 2-3 fold improvements in SNRt over INEPT, and a gain in sensitivity up to 50% compared to conventional SSFP methods. This sensitivity enhancement could be of importance in magnetic resonance imaging of ^{13}C and other insensitive nuclei. Additionally, NMR spectroscopists could benefit from the INEPT-SSFP method if combined with the aforementioned phase-incremented experiment, to achieve these sensitivity gains while reclaiming the high resolution of FT NMR. Furthermore, the new approach described here opens the avenue for developing SSFP-based correlation experiments with substantial sensitivity enhancement.

SLT-020: PRESERVE-TROSY : adding variable flip-angle excitation to TROSY NMR spectroscopy

Dr Bernhard Brutscher[1]
[1]Université Grenoble Alpes, CEA, CNRS, Institut de Biologie Structurale (IBS), Grenoble, France

Transverse-Relaxation Optimized Heteronuclear Correlation Spectroscopy (TROSY) was introduced in 1997 [1]. TROSY comprises three components: an initial INEPT block, a chemical shift editing period (t1), and the final ST2-PT sequence. This experiment possesses some intriguing characteristics. Firstly, it enables sensitivity-enhanced quadrature detection in t1, whereby both orthogonal S-spin components (Sx and Sy), generated during the free evolution delay t1, are transformed into detectable I-spin coherence. Second, the equilibrium polarization of the 2 spin reservoirs (I and S) can be exploited through two parallel polarization-coherence transfer pathways, augmenting the detected NMR signal [2,3]. Lastly, I-spin polarization (Iz) accumulated during t1 is converted by the ST2-PT sequence into S-spin polarization (Sz) that contributes to the detected NMR signal during the subsequent scan [4]. This aspect is particularly pertinent in the context of BEST-TROSY [4–6], where short interscan delays are applied. In order to make TROSY experiments even more versatile, it would be appealing to implement variable flip angle excitation to further increase the steady-state spin polarizations. Here, we introduce the PRESERVE (Polarization Restoring Excitation Sequence foR Versatile Experiments) building block that allows adjustment of the effective excitation flip angle for both the I and S spin polarizations. PRESERVE-TROSY exploits a remarkable array of eight orthogonal polarization-coherence transfer pathways, showcasing the remarkable potential of spin manipulations achievable through the design and optimization of NMR pulse sequences.

[1] K. Pervushin et al., Proc. Natl. Acad. Sci. U. S. A. 1997, 94, 12366–12371; [2] K. Pervushin et al., J. Am. Chem. Soc. 1998, 120, 6394–6400; [3] B. Brutscher et al, J. Am. Chem. Soc. 1998, 120, 11845–11851; [4] A. Favier, B. Brutscher, J. Biomol. NMR 2011, 49, 9–15; [5] J. Farjon et al, J. Am. Chem. Soc. 2009, 131, 8571–7. [6] Z. Solyom et al, J. Biomol. NMR 2013, 55, 311–21.

SLT-021: Time-Resolved Diffusion NMR a tool for reaction monitoring and beyond

Phd Mateusz Urbańczyk[1]
[1]Institute Of Physichal Chemistry, Polish Academy Of Sciences, Warsaw, Poland

Introduction

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. Therefore, it is a perfect tool to study polymerization processes.

Aims

This work aims to present the power of TRD-NMR for studying photochemical processes. The examples would include:

- Photopolymerization of bis-anthracene derivatives studied with interleaved TRD and TR-NUS HSQC NMR methods [1]
- Open-air green-light-driven ATRP with conversion control [2]
- Retinyl Acetate photodegradation was studied on high and low-field NMR spectrometers using TRD, TR-NUS, and UV-VIS spectroscopy.

Additionally, the TRD method will be demonstrated to study the internal structure of the Gelatin Methacrylate used for 3d printing of bionic pancreas by utilizing the restricted diffusion phenomena.

Methods

The method in contrary to other fast diffusion methods, allows us to do this without the sensitivity lost. The TRD-NMR is an adaptation of the Time-Resolved Non-Uniform-Sampling to the world of diffusometry [3]. Thanks to the similar acquisition concept, the two methods can be used in an interleaved way. The technique can be used to study the time dimension in case of the reaction or a parameter space for other approaches, including diffusion time in the PGSTE sequence for the restricted diffusion measurement.

Results and Conclusions

The TRD-NMR method allows the study of time-dependent processes where mass change occurs, providing a powerful tool for chemists and material scientists. We demonstrated the utility of the technique of various systems ranging from photopolymerization and photodegradation to the pore size analysis.

[1] Kristinaityte, K. et al. Journal of the American Chemical Society 144, 13938–13945 (2022).
[2] 1 Szczepaniak, G. et al. Chemical Science 13, 11540–11550 (2022).
[3] Urbańczyk, .M et.al. Analytical Chemistry 92, 9948–9955 (2020).

SLT-022: 13CF3 Chemical Group: Synthesis and Application of Methionine Analogue with a 13CF3 Heteronuclear TROSY Spin System as a Novel Slow Relaxing NMR Probe to Visualize the High Molecular Weight Macromolecules.

Mr Satoshi Kishigami[1], Mr James Eaton[1], Dr Charles Buchannan[2], Ms Virginia Casablancas Antras[1], Professor Andrew Baldwin[1], Professor Ben Davis[3]
[1]University of Oxford, Oxford, United Kingdom, [2]Max Planck Institute for Terrestrial Microbiology, Marburg, Germany, [3]Rosalind Franklin Institute, Didcot, United Kingdom

^{19}F NMR is widely used as an analytical tool to investigate the structures and dynamics of biomolecules. Since fluorine atoms are almost absent in natural biological systems, we can obtain background-free NMR signals from the fluorinated biomolecules in complex biological mixtures. However, one-dimensional ^{19}F NMR has some limitations when applied to high molecular weight biomacromolecules with slow tumbling and fast relaxation rates, which cause a low signal-to-noise ratio and poor resolution. We envisioned that the synthesis of the double-labelled $^{13}C^{19}F_3$ group and the introduction of this novel NMR probe to proteins with large macromolecular structures could address the size limitations of the current solution-state ^{19}F NMR methodologies. Transverse relaxation optimized spectroscopy (TROSY), an advanced signal enhancement technique in biomolecular NMR, could tremendously increase the resolution in heteronuclear two-dimensional (^{13}C, ^{19}F) NMR spectra. Starting from ^{13}C iodomethane ($^{13}CH_3I$), we have developed the synthetic method of Umemoto's reagent with the $^{13}C^{19}F_3$ group, which can be used for site-selective chemical labelling of biomolecules. We have also demonstrated the synthesis and incorporation of a methionine analogue with the $^{13}C^{19}F_3$ group, which can be incorporated into proteins of interest using genetically engineered cell lines. Lastly, we have been conducting a range of different NMR experiments to investigate the potential of the $^{13}C^{19}F_3$ group as a highly sensitive NMR probe using molecular chaperones and virus capsids. $^{13}C^{19}F_3$-methionine was successfully incorporated into a stable chaperone oligomer HSP 16.5 with a molecular weight of 396 kDa and we compared the relaxation properties of $^{13}C^{19}F_3$ and $^{13}CH_3$ within the protein. As a result of the NMR analysis, the relaxation rate of one carbon spin state in the $^{13}C^{19}F_3$ labelled HSP16.5 was significantly reduced for a protein of this size. This shows that the $^{13}C^{19}F_3$ label could provide a gateway for the study of even larger biomolecules using solution-state NMR.

SLT-023: New experimental and computational tools for mixture analysis - spectral "pseudo-dimensions" and Wasserstein metric.

Professor Krzysztof Kazimierczuk[1], Professor Anna Gambin[1], Barbara Domzal[1], Dr. Blazej Miasojedow[1], Paulina Putko[1], Javier Agustin Romero[1], Ewa Klaudia Nawrocka
[1]University Of Warsaw, Warsaw, Poland

Analysis of complex mixtures is an area in which NMR triumphs as an analytical technique.

However, the spectra of such samples require high resolution and sensitivity. Morevover, decomposing them into subcomponent spectra and estimating concentrations is a difficult computational task.

In this presentation, I will discuss new solutions developed in our group to deal with the above problems. I will show how to:

- enhance the spectral resolution by recording experiments with varying temperatures[1,2] and pH[3].
- achieve high efficiency with dedicated data processing methods (Radon transform) and using a pH gradient along the sample.
- use a novel computational approach based on the Wasserstein metric[4] to evaluate concentrations of subcomponents. It is robust to peak distortions caused, for example, by magnetic field inhomogeneities.

References

Nawrocka, E. K., Urbańczyk, M., Koziński, K., & Kazimierczuk, K. (2021). Variable-temperature NMR spectroscopy for metabolite identification in biological materials. RSC Advances, 11(56), 35321–35325. https://doi.org/10.1039/d1ra05626c

Nawrocka, E. K., Jadwiszczak, M., Leszczyński, P. J., & Kazimierczuk, K. (2024). Supporting the assignment of NMR spectra with variable-temperature experiments. Magnetic Resonance in Chemistry. https://doi.org/10.1002/mrc.5433

Domżał, B., Nawrocka, E. K., Gołowicz, D., Ciach, M. A., Miasojedow, B., Kazimierczuk, K., & Gambin, A. (2024). Magnetstein: An Open-Source Tool for Quantitative NMR Mixture Analysis Robust to Low Resolution, Distorted Lineshapes, and Peak Shifts. Analytical Chemistry, 96(1), 188–196.

Putko, P., Romero, J. A., & Kazimierczuk, K. (2024). A pH-enhanced resolution in benchtop NMR spectroscopy. Analyst. https://doi.org/10.1039/d3an02000b

SLT-024: Towards Measuring Molecular Parity Violation with Nuclear Magnetic Resonance

Mr. Erik Van Dyke[1,2,3], Dr. Danila Barskiy[1,2,3], Dr. James Eills[4], Dr. Kirill Sheberstov[5], Dr. Manfred Wagner[6], Dr. Robert Graf[6], Prof. Dr. Rudolf Pietschnig[7], Dr. Denis Kargin[7], Dr. Konstantin Gaul[8], Prof. Dr. Robert Berger[8], Prof. Dr. Dmitry Budker[1,2,3,9]
[1]Helmholtz Institute Mainz, Mainz, Germany, [2]GSI Helmholtz Center for Heavy Ion Research, Darmstadt, Germany, [3]Institute for Physics, Johannes Gutenberg University Mainz, Mainz, Germany, [4]Institute for Bioengineering of Catalonia, Barcelona Institute of Science and Technology, Barcelona, Spain, [5]Ecole normale superieure, Paris Sciences and Letters University, Paris, France, [6]Max Planck Institute for Polymer Research, Mainz, Germany, [7]Institute for Chemistry, University of Kassel, Kassel, Germany, [8]Institute for Chemistry, Phillips University Marburg, Marburg, Germany, [9]Department of Physics, University of California at Berkeley, Berkeley, United States of America

Introduction

The weak interaction has been shown experimentally to exhibit parity nonconservation (PNC) in both nuclear and atomic systems, yet no experiments have been able to observe PNC in a molecular system. This work seeks to build on previously demonstrated use of co-magnetometry and diastereomeric splitting in high-field NMR spectroscopy to resolve energetic differences between chiral molecules from nuclear-spin-dependent contributions of the weak force to the chemical shift tensor [1]. Residual chemical shift differences are expected to be on the order of mHz between enantiomers containing high Z, spin ½ nuclei such as ^{183}W and ^{203}Tl/^{205}Tl [1,2,3].

Aims

Before moving to high Z nuclei, detailed error analysis of NMR experiments involving diastereomeric complexes must be completed. Our goal was to obtain < mHz precision utilizing co-magnetometry with ^{1}H and ^{31}P nuclei of a chiral molecule complexed with a chiral derivatizing agent (CDA) [4].

Methods

A racemic mixture of the chiral probe was combined with enantiomerically pure CDAs to allow titration of enantiomeric excess which is linearly proportional to diastereomeric splitting. Optimization of diastereomeric splitting was performed by testing various solvents, followed by different ratios and concentrations of probe and CDA.

Results

Using linear regression of a co-magnetometry measurement, a resolution of ~100 mHz was obtained. Precision of diastereomeric splitting estimates was improved by using a bilinear rotation decoupling (BIRD) pulse sequence to suppress peaks around the ^{1}H peaks of interest. However, this introduced systematic error likely caused by anti-phase spin order contributions.

Conclusion

The goal of this work was to identify and circumvent possible systematics occurring in co-magnetometry NMR experiments of chiral complexes to maximize chances of resolving mHz energy differences in high Z nuclei resulting from PNC of the weak force. The precision obtained here is ~100 mHz, indicating additional challenges must be met to observe PNC using NMR.

SLT-025: Boosting contrast upon protein-ligand binding by delocalised long lived states

Mr Sebastiaan Van Dyck[1,2,3], Ms Anna Sonnefeld[1,2,3], Ms Lydia Sama[1,2,3], Dr. Kirill Sheberstov[1,2,3], Prof. Dr. Geoffrey Bodenhausen[1,2,3]
[1]Ecole Normale Superieure, Paris, France, [2]Sorbonne Université, Paris, France, [3]Université PSL, Paris, France

Long-lived states (LLS) can have lifetimes that can be longer than longitudinal relaxation times. It also has been shown that, out of all NMR probes, LLS feature the highest contrast upon protein-ligand (PL) binding – that is, the highest difference in the measured relaxation rates between free and bound ligands [1,2]. Recently, it was discovered that delocalized LLS can be excited in short aliphatic chains comprising magnetically inequivalent vicinal protons using polychromatic spin-lock induced crossing (poly-SLIC)[3,4]. Here we aim to investigate the effect of delocalisation of LLS in experiments probing PL binding. To achieve this, we study a model system of sodium trimethylsilylpropanesulfonate that experiences unspecific binding to bovine serum albumin. Different irradiation schemes of poly-SLIC enable us to create LLS that are delocalized to a different extent. We show that the LLS that is uniformly delocalized across the aliphatic chain provides almost two times better contrast compared to an LLS that is highly localized on the end of the chain.

[1] N. Salvi, R. Buratto, A. Bornet, S. Ulzega, I. Rentero Rebollo, A. Angelini, C. Heinis, G. Bodenhausen, J. Am. Chem. Soc. 2012, 134, 11076–11079.
[2] R. Buratto, D. Mammoli, E. Chiarparin, G. Williams, G. Bodenhausen, Angew. Chem. Int. Ed. 2014, 53, 11376–11380.
[3] A. Sonnefeld, G. Bodenhausen, K. Sheberstov, Phys. Rev. Lett. 2022, 129, 183203.
[4] K. Sheberstov, A. Sonnefeld, G. Bodenhausen, J. Chem. Phys. 2024, in press.

SLT-026: Spin-Chain Zero-Quantum NMR Spectroscopy

Dr. Kirill Sheberstov[1,2,3], Anna Sonnefeld[1,2,3], Prof. Dr. Geoffrey Bodenhausen[1,2,3]
[1]Ecole Normale Supérieure, Paris, France, [2]Sorbonne Université, Paris, France, [3]Université PSL, Paris, France

We present a novel form of sparse zero-quantum (ZQ) spectroscopy termed spin-chain ZQ NMR. It is based on the recent discovery of delocalized long-lived states (LLS)[1,2]and collective long-lived coherences (LLCs)[3] in molecules containing a chain of at least three CH_2 groups. Several variants of excitation by polychromatic spin-lock induced crossing (poly-SLIC)[1] are introduced that can excite a non-uniform distribution of the population of the $S_0S_0T_0$, $S_0T_0S_0$, and $T_0S_0S_0$ states in the density matrix. Once the radio frequency fields are switched off, these are not eigenstates, leading to ZQ evolution involving all 6 protons before their reconversion into observable magnetization by a second poly-SLIC pulse that can be applied to any one or several of the CH_2 groups. Here we present an analytical approach to describe the spin dynamics of spin-chain ZQ NMR, showing that the behavior of collective LLCs can be understood as a propagation of spin order along the chain of CH_2 protons during free evolution.

[1] A. Sonnefeld, G. Bodenhausen, K. Sheberstov, Phys. Rev. Lett. 2022, 129, 183203.
[2] A. Sonnefeld, A. Razanahoera, P. Pelupessy, G. Bodenhausen, K. Sheberstov, Sci. Adv. 2022, 8, eade2113.
[3] K. Sheberstov, A. Sonnefeld, G. Bodenhausen, J. Chem. Phys. 2024, 160, in press.

SLT-027: Collective Long-Lived Zero-Quantum Coherences in Aliphatic Chains

Prof. Dr. Geoffrey Bodenhausen[1,2,3], Ms Anna Sonnefeld[1,2,3], Dr. Kirill Sheberstov[1,2,3]
[1]Ecole Normale Supérieure, Paris, France, [2]Université PSL, Paris, France, [3]Sorbonne Université, Paris, France

In nuclear magnetic resonance (NMR), long-lived coherences (LLCs) constitute a class of zero-quantum (ZQ) coherences that have lifetimes that can be longer than the relaxation lifetimes T_2 of transverse magnetization. So far, such coherences have been observed in systems with two coupled spins with spin quantum numbers I = 1/2, where LLC corresponds to a coherent superposition between the singlet S_0 and the central triplet T_0 state [1,2]. Here we report on the excitation and detection of collective LLCs in AA'MM'XX' spin systems in molecules containing a chain of three methylene (-CH_2-) groups. In the resulting 2D spectra, the ω_2 domain shows SQ spectra with the chemical shifts of the CH_2 groups irradiated during the reconversion, while the ω_1 dimension shows ZQ signals in absorption mode with linewidths on the order of 0.1 Hz that are not affected by the inhomogeneity of the static magnetic field but can be broadened by chemical exchange as occurs in drug screening. The ZQ frequencies are primarily determined by differences between vicinal J-couplings [3].

[1] R. Sarkar, P. Ahuja, P. R. Vasos, G. Bodenhausen, Phys. Rev. Lett. 2010, 104, 053001.
[2] R. Sarkar, P. Ahuja, P. R. Vasos, A. Bornet, O. Wagnières, G. Bodenhausen, Prog. Nucl. Magn. Reson. Spectrosc. 2011, 59, 83–90.
[3] K. Sheberstov, A. Sonnefeld, G. Bodenhausen, J. Chem. Phys. 2024, 160, in press.

SLT-028: SpinCycle: a new tool for automatic pulse calibration and data collection, exemplified with a neurodegenerative IDP drug binding screen.

Dr Abi Turner[1], Dr Viliyana Lewis[1], Dr Virginia Casablancas-Antras[2], Mr Morgan Wright[2], Dr Robert Horne[3], Prof Andrew Baldwin[2]
[1]Rosalind Franklin Institute, Harwell, United Kingdom, [2]Department of Physical and Theoretical Chemistry , University of Oxford, UK, [3]Department of Chemistry, University of Cambridge, UK

In an idealised drug binding screen, a user will simply submit the samples for analysis and be provided with a report on any potential interactions with minimal input. For quantitative NMR-based screens, it is vital to maximise signal to noise, and so detailed attention to pulse lengths, water suppression and other bespoke requirements is essential.

To facilitate this, we have produced SpinCycle, an automation tool that calibrates user-selected parameters and collects NMR data on multiple samples and generates a report summarising the results. Specific calibration steps include sample injection, temperature calibration, locking, tuning/matching, shimming, user-specified parameter calibration, user-selected experimental set-up, data acquisition, then sample ejection. The software is open source, has a GUI written in WX-python, and is possible because of the TopSpin-python API.

We demonstrate the application of SpinCycle by acquiring Universal Saturation Transfer Analysis (uSTA) data on a range of pharmaceutically interesting ligands against intrinsically disordered proteins (IDPs) associated with neurodegenerative disease. Due to their inherent flexibility, these proteins are challenging to analyse by traditional structural techniques, increasing the difficulty to identify binding ligands. uSTA uses a protein subtraction step, which minimises the complexity of IDP proton spectra and enables direct analysis of the ligand interactions. We screened a broad range of compounds for potential binding to disease-linked IDPs, including alpha-synuclein and tau. The automatic spectral processing by SpinCycle, enabled easy identification of the top interactors, for which we obtained detailed structures showing the ligand pose when associated with the protein. Furthermore, we used uSTA to determine key interaction parameters, such as Kon, Koff and Kd.

We hope to build community support to enable collective development of a tool for automatic acquisition of NMR data, with the same accurate set-up to maximise sensitivity that would typically be manually provided by a skilled user.

SLT-029: Seedless: On-the-fly pulse calculation for NMR experiments

Dr Charles Buchanan[1,2], Dr Gaurav Bhole[3,4], Dr Gogulan Karunanithy[5], Dr Virgínia Casablancas Antràs[2], Professor Jonathan Jones[3], Professor Andrew Baldwin[2]
[1]Max Planck Institute For Terrestrial Microbiology, Marburg, Germany, [2]Kavli Institute for Nanoscience Discovery, University of Oxford, Oxford, United Kingdom, [3]Department of Physics, University of Oxford, Oxford, United Kingdom, [4]Harvard University, Boston, United States, [5]University College London, London, United Kingdom

Introduction:
I will present Seedless, a tool to generate NMR pulses 'on-the-fly' that enhance control of magnetisation and boost resulting signal in all scenarios. This control enables novel experiments, including effectively slowing down chemical shift evolution to allow CPMG experiments that typically span multiple fields to be acquired on a single spectrometer.

Aims:
All NMR experiments require sequences of RF ‘pulses’ to manipulate nuclear spins. Signal is lost due to non-uniform excitation of nuclear spins resonating at different chemical shifts and variations in homogeneity in the RF actually generated by hardware over the sample volume. To improve this, a highly optimised GRadient Ascent Pulse Engineering (GRAPE) implementation was designed.

Methods:
Each calculated pulse will perform one of 4 transforms on chosen chemical shift bands including: a universal rotation (e.g. 90° about the x axis), state-to-state transform (e.g. Z->Y), an XYcite (Z->XY plane), or a novel type, a suppression, that leaves spins minimally perturbed at all times during the pulse (perfect for water suppression).

Results:
First, using imaging experiments, I will demonstrate that Seedless pulses effectively increase the size of the coil volume and signal-to-noise ratio. Next, I will show experimental applications including ultra-broadband 19F pulses (300 ppm excitation bandwidth), a 15N HSQC with 58% increased S/N (950 MHz spectrometer + cryoprobe), triple resonance experiments such as HNCACO with 55% increased S/N (600 MHz spectrometer + RT probe), and a highly efficient pulse sequence for water suppression. Seedless-controlled CPMG spectra will show the power of precise pulsing beyond sensitivity enhancement.

Conclusion:
Seedless provides a means to enhance sensitivity in all pulse sequences in a manner that can be tailored to all samples/hardware being used. Further, the speed of this new rapid pulse design paradigm enables greater sophistication and capability within NMR experimental design.

SLT-031: Synthetic Chondroitin Sulfate E Multivalent Derivatives: Characterization and Interaction Studies with Langerin Receptor

Dr. M. Jose García-Jimenez[1], Dr. Sergi Gil[1], Dr. Pedro Domingez-Rodriguez[1], Dr Laura Roldan Revuelta[1], Prof. Javier Rojo[1], Dr. Jose L. de Paz[1], Dr Pedro M Nieto[1]
[1]CSIC, Sevilla, España

In this communication, we report our final studies on the structure of multivalent compounds displaying chondroitin sulphate sequences and their interaction with langerin. The disaccharides correspond to two alternative sequences of CS-E. We studied both structures in the free state and then in the presence of langerin. The structures in the bound state are equivalent to those in the free state, and compatible with the CS-E. The analysis of the STD-NMR experiments indicates many binding modes/sites depending on the nature of the core connecting the disaccharides. They are strongly dependent on the nature of the linker, for the monovalent ones that bind into the lectin site through Calcium chelation, the sequence GlcA-GalNAc binds via interaction of the terminal hydroxyl groups with the Ca2+ atom. As this is not available in the GalNAc-GlcA this does not bind. When buffer in the absence of Calcium was used, none of them was able to interact. The multivalent sequences were different as the binding site was other: the STD epitope mapping was different and they also showed binding interaction in the absence of calcium.
We propose that multivalency affects the binding sites and requirements.

SLT-032: From Rigid to Flexible: Impact of Macrocycle Loss on Tolaasin's Backbone Dynamics and Activity

Mr Durga Prasad[1], Dr Niels Geudens[1], Dr Benjamin Kovacs[1], Dr Jose C. Martins[1]
[1]Ghent University, Gent, Belgium

In this project, we investigate the impact of macrocycle loss on tolaasin activity. Tolaasin, a Cyclic Lipopeptide (CLiP) from Pseudomonas tolaasii, plays a critical role in causing brown blotch disease in mushrooms. Its 18-amino acid sequence features an N-terminal lipid tail and a macrocycle formed via an ester bond. Tolaasin exhibits inhibitory action against fungal and Gram-positive bacteria, underscoring its significance. Studies have shown that hydrolysing the ester bond, potentially opening the macrocycle, can detoxify tolaasin, highlighting the macrocycle's role in tolaasin's function. Understanding how specific structural changes alter membrane interactions is crucial for developing novel therapeutics and biocontrol agents.
Our approach involves studying hydrolysed tolaasin in parallel with the native molecule in SDS micelles using NMR spectroscopy. To enable advanced multidimensional structural analysis, we first produce 15N isotope-enriched tolaasin by cultivating the producing bacterium on a minimal medium supplemented with suitable labeled isotopically enriched precursors. Subsequently, isotopically enriched hydrolyzed form was synthesized through controlled alkaline hydrolysis.
A comprehensive analysis, including full resonance assignment and 15N R1, R2, and het-nOe experiments, allows us to investigate peptide backbone dynamics. The order parameters (S2) derived from model-free analysis of relaxation data provide insights into molecular motions occurring on a nanosecond to picosecond timescale. By employing reduced spectral density mapping at Jω0, JωN, and Jω0.87H, we distinguish residues with distinct rigidity and flexibility profiles in both forms of tolaasin. Furthermore, NH R1 rates are determined both in the absence and presence of a soluble paramagnetic relaxation agent. This facilitates mapping the PRE wave of tolaasin, extracting tilt and azimuth angles, and enabling the mapping of helix orientations. Our findings indicate that the opening up of the macrocycle results in a partial loss of peptide backbone rigidity, leading to involvement in microsecond dynamics by later exocyclic residues.

SLT-033: 2D ^{1}H,^{19}F STD-TOCSYreF NMR unravels the molecular recognition of a fluorinated high-mannose mimetic by the DC-SIGN receptor

Jonathan Ramírez-Cárdenas[1], Adrián Silva-Díaz[1], Juan Carlos Muñoz-García[1], María Carmen de la Fuente[1], Michel Thépaut[2], Franck Fieschi[2], Javier Ramos-Soriano[1], Jesús Angulo[1], Javier Rojo[1]
[1]Instituto de Investigaciones Químicas (IIQ), CSIC - Universidad de Sevilla, Seville, Spain, [2]Univ. Grenoble Alpes, CNRS, CEA, IBS, Grenoble, France

Dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) is a C-type lectin that has attracted the interest of the scientific community during the last two decades due to its key role in pathogen infection and immunomodulation processes. This lectin recognizes carbohydrates, mainly high-mannose ($Man_9GlcNAc_2$), and fucosylated oligosaccharides, in a Ca2+-dependent manner. $Man_9GlcNAc_2$ decorates several pathogen envelope glycoproteins, such as HIV gp120 or Ebola virus GP1. Determining the binding epitope of the sugar is fundamental to understanding this recognition event. Nuclear magnetic resonance (NMR) is a powerful tool to obtain this structural information in solution; however, when the sugar involved is a complex oligosaccharide, such as high-mannose, the signal overlap found in the NMR spectra precludes an accurate analysis of the interaction. The introduction of tags into these complex oligosaccharides can overcome these problems and facilitate NMR studies. Here, we show the study of the interaction of the Man_9 of high mannose with some fluorine tags with DC-SIGN. The strategic distribution of fluorine atoms along the ligand has enabled us to employ heteronuclear two-dimensional (2D) ^{1}H,^{19}F STD-TOCSYreF NMR experiments, applying for the first time the so-called initial slope approach. Our approach has facilitated the analysis of the Man_9/DC-SIGN interaction, unequivocally identifying the preferential recognition of D2 arm by the protein receptor.

SLT-034: Transcending resolution limits in diffusion NMR and HPLC

Mrs. Nouran Hamed[1], Prof. Gareth Morris[1], Prof. Mathais Nilsson[1]
[1]University of Manchester, Manchester, United Kingdom

NMR has been proven to be a powerful approach in analysis of mixtures. So far, NMR has succeeded in providing several ways to extract individual component spectra without physical separation. One of the most useful is DOSY; individual component signals are resolved in a pseudo-2D spectrum according to their diffusion coefficients. However, separation in DOSY is limited if species diffuse similarly and/or have overlap in their NMR spectra. Improvements to DOSY separation can be introduced, such as addition of an extra spectral dimension to offer enhanced resolution, e.g., HSQC-DOSY1. Another way is to combine bilinear DOSY data with a third dimension that encodes changes in the concentrations of mixture constituents, for example, acquiring diffusion NMR data as a function of time, during a chemical reaction, to generate a three-dimensional dataset2,3. These data can then be analysed with a multivariate statistical method such as Parallel Factor Analysis (PARAFAC). The independent variation in three dimensions is utilized by PARAFAC to extract the NMR spectrum of each component.

In this study, for a mixture that was only partially resolved by HPLC, changes in eluate concentration during a chromatographic run were used as the third dimension. Fractions were collected as the mixture eluted, and for each fraction a diffusion NMR data set was acquired. The DOSY timecourse was processed with PARAFAC using the General NMR Analysis Toolbox (GNAT)5, to extract the 1D subspectrum of each mixture component, together with the individual elution profiles and diffusion information.

This research complies with the conference theme of solution NMR methods and applications.

References

1 Dal Poggetto, G. et al. Anal Chem 90, 13695–13701(2018)
2 Khajeh, M. et al. Anal Chem 82, 2102–2108(2010)
3 Nilsson, M. et al. Chem Comm 1252–1254(2009)
4 Castañar, L. et al. Mag Res Chem 56, 546–558(2018)

SLT-035: RDCs and RCSA measured by Rheo-NMR of PBLG

Mr Fabian Hoffmann[1], Dr Karel Kouril[1], Dr Benno Meier[1], Prof. Dr. Burkhard Luy[1]
[1]KIT, Eggenstein-Leopoldshafen, Germany

Poly-γ-benzyl-L-glutamate (PBLG) is a widely known α-helical polymer in the field of rheology and NMR for its special use as a chiral liquid crystalline (LC) alignment medium. In certain nonpolar solvents (called helicoidal liquids) PBLG helices behave like rigid rods which uniformly align themselves at concentration above 10 % in magnetic fields[1]. The resulting anisotropy of the sample can be used to orient solute molecules inside the LC phase. Correspondingly, anisotropic parameters like residual dipolar couplings (RDCs) and residual chemical shift anisotropy (RCSA) of a molecule of interest can be measured.
Unfortunately, PBLG provides a minimum alignment strength that leads to very complex multiplets with widths easily exceeding 60 Hz in medium-sized organic molecules. As a result, long measurements are needed to gain a reduced set of RDCs, and the reliability of measured couplings can be compromised[2,3].
By combining controlled rheological conditions and NMR-spectroscopy (Rheo-NMR), the director of PBLG can be changed to point close to the x,y-plane by applying shear force inside the sample with a specifically designed rotary device. This turns the alignment vector from the z-direction ($\theta = 0°$) towards the x,y-plane ($\theta \approx 80°$) and thus the size of overall anisotropic interactions is significantly reduced due to the general scaling with $3\cos^2(\theta)-1$. Additionally, the shearing with its accompanying viscosity reduction provides generally higher signal-to-noise and even allows uniform alignment below the critical concentration of the LC phase.
In this work, we show the improvements through shearing for the measurements of RDCs and RCSA.

Literature:

[1] J. C. Horton, A. M. Donald, Polymer 32 (1991) 2418-2427.
[2] G. Kummerlöwe, B. Luy, TrAC 28, 4 (2009) 483-493.
[3] K. Czamiecka, E.T. Samulski, Mol. Cryst. Liq. Cryst. 63 (1981) 205-214.

SLT-036: Methodology for the facile NMR spectroscopy of low gamma spin-1/2 nuclei: Double polarisation transfer methods, and the 103Rh NMR spectroscopy and relaxometry of Rh(acac)3

Mr Harry Harbor-Collins[1], Dr Mohamed Sabba[1], Dr Markus Leutzsch[2], Prof Malcolm Levitt[1]
[1]University Of Southampton, Southampton, United Kingdom, [2]Max-Planck-Institut für Kohlenforschung, Mülheim, Germany

The NMR spectroscopy of spin-1/2 nuclides with low gyromagnetic ratio such as 103Rh, which has a gyromagnetic ratio ~32 times smaller than the proton, is onerous owing to weak NMR signal strength. Sensitivity can be improved through the application of polarisation transfer techniques; exploiting the greater sensitivity of a higher gamma, scalar coupled, nucleus. We discuss double polarisation transfer methods recently developed for the rapid indirect detection of 103Rh chemical shifts and relaxation times constants,1-3 and apply it here in the 103Rh NMR spectroscopy and relaxometry of the Rh(acac)3 transition metal complex. The 103Rh longitudinal T1 relaxation in Rh(acac)3 is shown to be dominated by spin-rotation with an additional, field dependent, contribution from the CSA. Double polarisation pulse sequences greatly improve the accessibility and sensitivity of the NMR technique for low gamma nuclei in complexes that exhibit prerequisite heteronuclear scalar couplings.

1. Caló, F. P.; Bistoni, G.; Auer, A. A.; Leutzsch, M.; Fürstner, A. Triple Resonance Experiments for the Rapid Detection of 103Rh NMR Shifts: A Combined Experimental and Theoretical Study into Dirhodium and Bismuth–Rhodium Paddlewheel Complexes. J. Am. Chem. Soc. 2021, 143 (32), 12473–12479. https://doi.org/10.1021/jacs.1c06414.
2. Harbor-Collins, H.; Sabba, M.; Moustafa, G.; Legrady, B.; Soundararajan, M.; Leutzsch, M.; Levitt, M. H. The 103Rh NMR Spectroscopy and Relaxometry of the Rhodium Formate Paddlewheel Complex. The Journal of Chemical Physics 2023, 159 (10), 104307. https://doi.org/10.1063/5.0165830.
3. Harbor-Collins, H.; Sabba, M.; Bengs, C.; Moustafa, G.; Leutzsch, M.; Levitt, M. H. NMR Spectroscopy of a 18O-Labeled Rhodium Paddlewheel Complex: Isotope Shifts, 103Rh– 103Rh Spin–Spin Coupling, and 103Rh Singlet NMR. The Journal of Chemical Physics 2024, 160 (1), 014305. https://doi.org/10.1063/5.0182233.

SLT-037: Pseudocontact shift NMR as tool for elucidating structure and dynamics of relevant biomolecules

Prof. Dr. Daniel Häussinger[1], MSc. Pascal Rieder[1], Dr. Feng_Jie Wu[2], Dr. Philip Rößler[3], Dr. Layara Akemi Abiko[2], Dr. Alvar Gossert[3], Prof. Dr. Stephan Grzesiek[2]
[1]Dept of Chemistry, University of Basel, Basel, Switzerland, [2]Biozentrum, University of Basel, Basel, Switzerland, [3]Dept. of Biology, ETH Zürich, Zürich, Switzerland

Introduction:

Pseudocontact shift (PCS) NMR has by now been established as a valuable tool for biomolecular NMR.[1] Specifically, the detailed three-dimensional information over a very long-distance range by sensitive HSQC-type experiments is particularly valuable. Our group designs and synthesises lanthanoid chelating tags (LCTs) that can be site-selectively conjugated to proteins, carbohydrates and nucleic acids in order to exploit PCSs for structural, dynamic and interaction studies.[2]

Aims:

While LCTs in most studies are conjugated to single-cysteine mutants of proteins, this approach can be difficult in complex proteins like G-protein coupled receptors (GPCRs). If the structure is very sensitive to the introduction or deletion of cysteines, a different strategy has to be applied. In addition, NMR assignment of proteins of higher organisms that require post-translational modifications and/or are more difficult to obtain in labelled form is frequently hampered. We wanted to develop a method that allows the use of LCTs without requiring single-cysteine mutants and yields assignment information from inexpensive 15N-labelled constructs.

Methods:

For cases that do not allow for direct tagging we have developed an indirect approach, where we used nanobodies (Nb60/Nb80) that mimic the G-protein in complex with β_1-adrenergic receptor (YY-β_1AR) and tagged the nanobodies in turn via thioether chemistry. The ternary complex YY-β_1AR – Nb80 – agonist was then characterised by PCS NMR.[3]

Results:

The new strategy yielded sizeable PCSs on the indirectly tagged YY-β_1AR-Nb80/Nb60 constructs and, thus, complete and unambiguous assignments of selectively 15N-Val or 15N-Tyr labelled ternary GPCR constructs. A combination of different attachment sites of the LCT on the Nb with various (ant-)agonists delivered a detailed structural picture.

Conclusion:

The new GPS-PCS method was successfully applied to a 100 kDa, micelle-bound membrane protein, but is not limited to GPCRs.

[1] Chem. Rev., 2022, 122(10), 9422-9467.
[2] Nature, 2022, 603, 528-535.
[3] JACS, 2022, 144(47), 21728-21740.

SLT-038: Detailed characterization of Broadband heteronuclear decoupling sequences: bandwidth, B1-tolerance, linewidth, and coupling-dependence of central and sideband signals

Prof. Burkhard Luy[1], Ms. Lisa Schröder[1]
[1]Karlsruhe Institute Of Technology (kit), Karlsruhe, Germany

Broadband heteronuclear decoupling sequences today are essentially characterized by their bandwidth over rf-amplitude ratio Ξ. While the bandwidth is certainly an important parameter, it is insufficient to decide on the applicability of a particular decoupling scheme in a certain pulse sequence scenario. In fact, compensation to B_1-miscalibrations and B_1-inhomogeneities of a probehead is certainly important information. Equally important is the target linewidth, which varies significantly between e.g. small molecule carbon-detected experiments with expected linewidths below 1 Hz as compared to proton-detected protein experiments with linewidths easily exceeding 10 Hz and correspondingly much lower requirements in terms of decoupling performance. Finally, efficient decoupling strongly depends on the size of heteronuclear J- or dipolar couplings to be decoupled. The larger such couplings get, the more difficult it is to achieve small linewidths, the full intensity of the decoupled central signal, or low decoupling sidebands. We look at all these properties for a variety of most popular and recently developed broadband heteronuclear decoupling sequences and derive certain relations and characteristic numbers to adequately address their performance. All numbers are calculated using the PulseDecoupler program package written in Julia.

SLT-040: Conformational dynamics and enzyme function of ribonuclease H from NMR spectroscopy and molecular dynamics simulation

Professor Arthur Palmer[1]
[1]Columbia University, New York, United States

Introduction: NMR spectroscopy and molecular dynamics (MD) simulation are powerful approaches for probing aspects of conformational dynamics in biological macromolecules. Methods that can be utilized to characterize dynamics on picosecond-nanosecond and on microsecond-millisecond time scales will be illustrated by application to the enzyme ribonuclease HI (RNaseH), which hydrolyzes the RNA strand of DNA:RNA hybrid molecules and is involved in diverse biological processes.

Aims: NMR spectroscopy and MD simulation are used to quantify the coupling between conformational dynamics and substrate recognition for RNaseH homologs from psychrotrophic, mesophilic, and thermophilic bacterial species.

Methods: NMR methods include chemical shift, spin-relaxation, scalar coupling, and residual dipolar coupling measurements. Spin-relaxation measurements include methyl deuterium relaxation recorded on a unique 475 MHz/950 MHz spectrometer pair. All-atom MD simulations include 100 nanosecond simulations over a range of temperatures and a 2 microsecond simulation at 300 K.

Results: Excellent agreement between NMR measurements and results from MD simulation justifies detailed mechanistic interpretation of NMR measurements based on the simulated trajectories. In particular, MD simulations predict an inhibitory mutation for E. coli RNaseH that has been verified experimentally. The results demonstrate that the populations of “closed” handle-loop conformations are correlated with the Michaelis constants for the RNaseH family members with an Arg residue at position 88 (in the E. coli numbering), but not family members with an Asn residue at this position. In addition, simulations motivate a simple model for side chain conformational dynamics based on backbone fluctuations, intra-rotamer fluctuations, and rotameric transitions.

Conclusions: Conformational dynamics of the substrate binding loops in RNaseH are closely coupled to substrate recognition by homologs adapted for enzymatic activity across a range of temperatures. The present work also demonstrates the continued advances in quantitative agreement between MD simulations and NMR spectroscopic measurements of protein dynamics.

SLT-041: STDock: Automating the "STD-NMR informed" docking pipelines for drug discovery.

Ernesto Alejandro Fajardo Díaz[1], Fabio Julián Delgado Alpízar[1], Julio Miguel Caballero Ruiz[3], Jochen Balbach[4], Celia González Moya[1,4], Roy González Alemán[2]
[1]University Of Havana, Havana, Cuba, [2]Laboratoire de Physique et Chimie Théoriques, , Francia, [3]Centro de Bioinformática, Simulación y Modelado, , Chile, [4]Institute of Physics, Biophysics, Halle, Germany

To create new drugs, researchers use various techniques to identify potential ligands that can interact with a target and regulate its functioning. The Saturation Transfer Difference (STD) NMR experiments and Docking simulations are two complementary methods. However, there are no existing tools that combine these methods effectively.
STD provides an epitope mapping of the ligand, in which the ligand's contacting atoms are ranked by their distance to the target. While ligand orientation is accessible by the technique, it is unable to localize where the binding event is taking place in the receptor. Docking, on the other hand, can generate millions of ligand conformations around the target, but deciding which is the most likely to be found experimentally is one of their known "Achilles heel". The main goal of this work is the seamless integration of Docking simulations with STD experiments to generate plausible binding modes for ligand-receptor complexes.
STDock is a Python package that automates several essential tasks like (i) the integration of NMR spectra to produce epitope mappings or calculate dissociation constants, (ii) launching exhaustive global Docking simulations, and (iii) filtering and ranking STD-compliant binding modes produced by Docking through a novel, unpublished STD-based scoring function.
STDock was applied in two case studies. In the first one, our package automated the STD data processing in less than a minute. In the second case, we highlight how wrong can be the "best" Docking pose and how easy it is to detect it and surpass it with our novel STD-based scoring function.
STDock provides flexible workflows that can speed up ligand-binding analysis and increase reproducibility, expanding the STD technique's use and enabling more STD-driven discoveries. The package also offers the structural bioinformatics community an integrated framework for obtaining atomic-level insights from STD data through computational modeling.

SLT-042: Domain-domain interactions and dimerization of the human λ-III immunoglobulin light chain FOR005 investigated by NMR spectroscopy

Olga Sieluzycka[1,2], Dr Tejaswini Pradhan[1,2], Dr Riddhiman Sarkar[1,2], Dr Georg Rottenaicher[3], Prof. Dr Johannes Buchner[3], Prof.Dr Bernd Reif[1,2]
[1]1,Department Chemistry, Bayerisches NMR Zentrum (BNMRZ), Technische Universität München (TUM), Garching, Germany, [2]Helmholtz-Zentrum München (HMGU), Neuherberg, Germany, [3]Department Chemistry, Center for Protein Assemblies (CPA), Technische Universität München (TUM), Garching, Germany

Misfolding of light chain (LC) immunoglobulin and deposition of amyloid fibrils gives rise to systematic amyloidosis. This study aims to characterize variable (VL) and constant (CL) domain-domain interactions in full length LC protein to better understand initial unfolding events. We aim to find out whether specific mutations influence domain-domain interactions in LC. To address this question, structure and misfolding of both patient and germline full length light chains are analysed via solution-state NMR. Patient mutations are found both in the backbone and the linker region. All proteins were expressed using 15N and 13C isotopically enriched media. We also prepared fibrils using ex-vivo or in-vitro patient seeds and characterize it using MAS solid state NMR. We obtained high quality solution-state NMR spectra of LC, VL and CL protein. Backbone assignment experiments have been performed for both LC and CL protein. To probe dimerization, concentration dependent HSQCs were recorded. We established a relationship between the concentration dependence of the NMR chemical shift and the oligomeric states of the respective protein. By comparison of the chemical shifts of VL, CL, and LC protein, we were able to identify the residues that are involved in domain interactions. To find out whether full length LC protein is able to form fibrils, we performed seeding experiments using VL fibril seeds and examined the resulting samples using MAS solid-state NMR. To probe fibril formation kinetics, we carried out Thioflavin T assays as a function of the protein concentration, and in presence and absence of fibril seed. Using NMR we were able to get molecular insight into the role of the mutations G136V and C214S for LC aggregation. We find that the LC protein is less likely to form aggregates on its own, but requires VL seeds. We hypothesize that protein unfolding is required for LC fibril formation.

SLT-043: Dynamic Properties of Water in Lipidic Cubic Phases by 2D NOE NMR Spectroscopy

Dr Thomas Meikle[2], Dr David Keizer[1], Professor Frances Separovic[1,3], Dr Shenggen Yao[1]
[1]Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne , Australia, [2]Baker Heart and Diabetes Institute, Melbourne, Australia, [3]School of Chemistry, The University of Melbourne, Melbourne, Australia

Introduction
In molecular self-assemblies, the dynamic properties of water molecules are considered critical in understanding their biological activity and behaviour. 1 Lipidic cubic phases (LCPs) are formed via the spontaneous self-assembly of certain lipids in an aqueous environment and have found applications in the crystallization of integral membrane proteins for structure determination by X-ray crystallography 2 and in drug/nutrient delivery/release in vivo. 3
Aims
To explore dynamic properties of water in LCPs.
Methods
2D 1H-1H NOESY and ROESY.
Results 4
Categorically different effective residence times of water molecules: (i) in proximity to the glycerol moiety of monoolein (MO), and (ii) adjacent to the hydrophobic chain towards the hydrocarbon tail of MO, as evidenced by the opposite signs of intermolecular NOE cross peaks between protons of water and those of MO in 2D 1H-1H NOESY spectra, are reported.
Changes in effective residence time of water molecules in proximity to the glycerol moiety of MO in LCPs upon storage at ambient temperature and in the presence of an additive lipid, cholesterol, are presented.
Atom-specific NOE build-up curves for protons of water and those of MO in LCPs formed at a hydration level of 35 wt%, are also reported.
Conclusions
The results provide new insight into the physicochemical properties and behaviour of water in LCPs, and demonstrate an additional avenue for experimental study of water-lipid interactions and hydration dynamics in model membranes and nanomaterials using 2D NOE NMR spectroscopy.
References
1 S. K. Pal, and A. H. Zewail, Chemical Reviews 104 (2004) 2099.
2 E. M. Landau, and J. P. Rosenbusch, Proceedings of the National Academy of Sciences of the United States of America 93 (1996) 14532.
3 D. J. McClements, Adv Colloid Interfac 219 (2015) 27.
4 T. G. Meikle et al., J Colloid Interface Sci 666 (2024) 659.

SLT-045: PHOSPHORUS GROUP ROTATION IN LANTHANIDE(III) COMPLEXES OF DOTA ANALOGUES STUDIED BY 17O NMR

Mr Adam Svítok[1], Mr Jan Blahut[2], Mr. Peter Urbanovský[1], Mr. Petr Hermann[1]
[1]Charles University, Faculty Of Science, Hlavova 2030/8, Prague 2, Czech Republic, [2]Institute of Organic Chemistry and Biochemistry, Czech Academy of Science, Flemingovo náměstí 2, Prague 6, Czech Republic

Introduction
Derivatives of DOTA form kinetically inert and thermodynamically stable LnIII complexes used in medicine, e.g., as MRI contrast agents, or PET/SPECT diagnostic and therapeutic agents. Properties of these complexes important for these applications depend on their solution dynamics. However, studies of solution dynamics of LnIII complexes of phosphorus acid derivatives of DOTA were limited and an intermediate with bidentate phosphonate/phosphinate was only predicted by DFT[1].
Aims
We wanted to experimentally confirm exchange between coordinated and non-coordinated oxygen atoms of the phosphorus groups, so-called “phosphonate/phosphinate rotation”, on LnIII complexes of model phosphonate/phosphinate ligands and to investigate mechanism of this process.
Methods and Results
Complexes of (paramagnetic) LnIII ions were studied by variable-temperature 17O/1H/31P NMR and the phosphonate/phosphinate rotation was verified by the determination of activation parameters of the process and by comparing the results with DFT data[2]. Furthermore, we determined that the process only changes the phosphorus configuration and not pendant arms orientation by 1H–1H EXSY on the EuIII complexes.
Conclusions
Our results confirm the phosphonate/phosphinate rotation in complexes of some LnIII ions and suggest the likely mechanism of this process. However, the rotation only occurs in twisted-square antiprismatic isomers, in complexes of large LnIII and significantly slows down with decreasing LnIII size. The reported data may be useful in all chemistry fields dealing with coordinated phosphorus acid derivatives in which the phosphorus group rotation is possible.
Acknowledgments
We thank the Grant Agency of the Charles University (No. 68122) for financial support. The experimental part of the project was also financially supported by Grant Agency of the Czech Republic (22-34083S).
References
1 Blahut, J., Hermann, P., Tošner, Z. & Platas-Iglesias, C. Phys. Chem. Chem. Phys. 19. 26662–26671 (2017).
2 Svítok, A., Blahut, J., Urbanovský P., & Hermann P. Chem. Eur. J. (2024). In press.

SLT-046: Aqueous Speciation Dynamics of Keplerate-type POMs

Ingrid Gregorovic[1], Dr. Nadiia Gumerova[1], Univ-Prof Dr Annette Rompel[1]
[1]University Of Vienna, Faculty Of Chemistry, Department Of Biophysical Chemsitry, Vienna, Austria

Chemical speciation, a process of identification and quantification of all chemical species present in a sample, has an essential role in understanding the solution behavior of different chemical compounds.[1] In this study, we focus on the speciation of two Keplerate-type polyoxometalates (POMs), $\{Mo_{72}V_{30}\}$ and $\{W_{72}V_{30}\}$, shown to potentially be applicable in various fields.[2]-[4] This study aims to understand their behavior under conditions common for catalytical and biological applications. Expanding our previous research[5], the detailed speciation of giant POMs has been mapped through extensive experimental investigation. Conditions of POMs 24-hour stability have been identified, along with various species that form during oxidation and decomposition processes across a range of pH values (pH 1-8) and buffer solutions (sodium phosphate, acetic acid-sodium acetate, TRIS-HCl, and HEPES buffers) in fresh solutions, after 24h incubation at room temperature and 37°C. The speciation map incorporates data from 369 different NMR spectra (including ^{51}V-NMR, ^{31}P-NMR, ^{13}C-NMR, and ^{1}H-NMR spectra), along with complementary spectroscopic techniques such as UV-vis-NIR, Resonance Raman, and ESI-MS across 78 different conditions. This comprehensive study provides valuable insights into the speciation of complex chemical species, paving the way for advancements in finding the various applications of currently existing POMs and laying the groundwork for the synthesis design of new POM archetypes.

1. N. I. Gumerova, A. Rompel, Chem. Soc. Rev. 2020, 49, 7568-7601.
2. M. Gong, W. Xin-Yu, M.-Q. Li, W.-X. Mu, Y.-D. Cao, H. Liu, Y.-G. Lv, X.-H. Qi, G.-G. Gao, Fuel 2023, 332, 126050.
3. B. Doughty, P. Yin, Y.-Z. Ma, Langmuir 2016, 32, 8116-8122.
4. G. Jin, S.-M. Wang, W.-L. Chen, C. Qin, Z.-M. Su, E.-B. Wang, J. Mater. Chem. A, 2013, 1, 6727-6730.
5. N. I. Gumerova, A. Rompel, Sci. Adv. 2023, 9, eadi0814.

Acknowledgment
We thank Austrian Science Fund FWF for financial support (projects P33089 (doi:10.55776/P33089) and P33927 (doi:10.55776/P33927)).

SLT-047: The data-driven AI approach to shimming in NMR.

MEng Sylwia Jopa[1], PhD Marek Bukowicki[1], PhD Alexandra Shchukina[3], Prof. Dr hab. Krzysztof Kazimierczuk[1,2], PhD Przemysław Olbratowski[2,4]
[1]Center of New Technologies, University Of Warsaw, Warsaw, Poland, [2]Faculty of Physics, University of Warsaw, Warsaw, Poland, [3]Faculty of Chemistry, University of Warsaw, Warsaw, Poland, [4]Warsaw School of Information Technology WIT, Warsaw, Poland

Conducting a long series of measurements, e.g., with an autosampler, comes at a risk of imperfections in magnetic field homogeneity, particularly when samples differ in volume or position in a spinner. Automatic shimming doesn't always give satisfactory results, especially when the same shim map is used for various samples. Our remedy to this problem is a post-acquisition lineshape correction via machine learning, i.e., using a convolutional autoencoder with attention. The approach is superior to the commonly used reference deconvolution. We train our network based on experimental calibration data – no assumption about specific magnetic field profiles is made. Thus, the method is general and can be used in any laboratory providing measurement services.

SLT-048: Paramagnetic NMR to study covalent ligand conformations in an enzyme

Dr Sivanandam Veeramuthu Natarajan[1,2], Mahin Saberi[2], Dr. Sybrin P. Schröder[2], Zirui Li[2], Prof Jeroen D. C. Codée[2], Prof Herman S. Overkleeft[2], Dr Hugo van Ingen[3], Prof Marcellus Ubbink[2]
[1]Svenskt NMR Centrum, University of Gothenburg, Gothenburg, Sweden, [2]Leiden Institute Chemistry, Leiden University, Leiden, The Netherlands, [3]NMR Group, Bijvoet Centre for Biomolecular Research, Utrecht University, Utrecht, The Netherlands

Use of paramagnetic tags [1] becomes integral part of NMR based structural biology, because restraints from paramagnetic NMR complement conventional NMR methods. Here, we investigate the possibilities to determine the conformation of a covalent ligand bound to an enzyme using paramagnetic NMR information. With the CLaNP-5 lanthanide tag [2] attached to the enzyme, we measured pseudo-contact shifts (PCS) and residual dipolar couplings (RDCs) for the protein as well as the ligand. These measurements were measured at three field strengths, 600 MHz, 850 MHz and 1.2 GHz, to evaluate the field-dependent parameters at the ultra-high-field strength. From the measured PCS we determined the metal tensors associated with the paramagnetic tags for the protein-ligand complex. Modelling the ligand conformation is currently in progress. Preliminary data suggest that the combination of PCS and RDC obtained with the CLaNP-5 is sufficient to obtain reliable models.

Acknowledgments:
This project was supported by the uNMR-NL Grid: A distributed, state-of-the-art Magnetic Resonance facility for the Netherlands (NWO grant 184.035.002).
[1] Miao et al. Chem. Rev. 2022, 122, 9571–9642;
[2] Keizers et al. J. Am. Chem. Soc. 2008, 130, 14802–14812

SLT-049: Spontaneous transfer of acetyl group between xylose and arabinose in arabinoxylan-derived oligosaccharide

Dr. Iveta Uhliariková[1], Dr. Vladimír Puchart[1], Dr. Monika Poláková[1], Dr. Maroš Bella[1]
[1]Institue Of Chemistry Slovak Academy Of Sciences, Bratislava, Slovakia

Introduction: Plant polysaccharides are a huge resource of annually renewable carbon. Some of them are esterified with organic acids, which results in a modification of their physico-chemical properties. The most common mode of esterification is acetylation. In graminaceae (grasses) and herbaceous plants the most abundant hemicellulose is arabinoxylan, backbone of which consists of 1,4-linked β-D-xylopyranosyl residues. Some of them are substituted at positions 3 and/or 2 by α-L-arabinofuranosyl moieties. Both positions may be also acetylated, while substitution with α-D-glucuronic acid or 4-O-methyl-α-D-glucuronic acid occurs at position 2 only.
Aims: Structure determination of the isomer formed during spontaneous acetyl group migration in the phenyl β-1,4-xylobioside carrying 3-O-arabinofuranosyl and 2-O-acetyl substituents on the non-reducing-end Xylp residue (Compound 1).
Methods: 1D and 2D NMR experiments were used to monitor acetyl group migration and to elucidate the structure of the product.
Results: It was found out that the spontaneous transesterification reaction was regioselective, involving the acetyl group transfer from position O-2 of the xylopyranosyl residue exclusively to position O-2 of the α-L-arabinofuranosyl moiety linked to position 3 of the same xylose. Finally, the acetyl group migration resulted in an equilibrium, where the arabinose 2-acetate predominated over the xylose 2-acetate.

Conclusions: This is the first report of acetyl group migration between different pentoses, which may have significant consequences on the structure of acetylated arabinoxylan fragments and the requirements on enzyme repertoire related to their biotechnological exploitation.
Acknowledgment: This work was supported by the Slovak Research and Development Agency under the contract no. APVV-20-0591, and by Scientific Grant Agency under the contract no. 2/0171/22.

SLT-050: Taking advantage of amide proton and nitrogen resonances in chitin oligomers

Piera Wiesinger[1], Gustav Nestor[1]
[1]Swedish University Of Agricultural Sciences, Uppsala, Sweden

Chitin is the second most abundant biopolymer in nature after cellulose and is composed of N-acetylglucosamine (GlcNAc) connected via β(1→4)-glycosidic bonds. Given its high abundance, interactions with other biomolecules and pharmaceutical applications are of high interest. Despite its prominence, there is still a need to develop methods to study structure and function of chitin and its corresponding oligomers. Efforts have been made to analyse chitin oligomers by NMR spectroscopy, but spectral overlap has prevented any differentiation between the interior residues. For glycosaminoglycans, such as hyaluronan, ^{15}N NMR has been utilized on amide and sulfamate groups to resolve individual residues in oligomers.(1-3) Herein, we present a similar approach on chitin oligomers.
Chitin oligomers up to hexaose with natural abundance of ^{15}N were analysed with NMR spectroscopy in H_2O:D_2O (9:1) solution. After comparison of three different ^{1}H,^{15}N-HSQC experiments, the CP-HISQC pulse sequence (4) was found to give best resolution and sensitivity for individual residues in the oligomer to be resolved in the nitrogen dimension. Detailed chemical shifts of amide proton and nitrogen are reported for the first time. Additionally, all oligomers were analysed for the presence and abundance of the amide cis form and its corresponding chemical shifts were assigned. According to previous studies on GlcNAc, the amide trans conformation is predominant, but about 1% of cis form is present.(5) The here gained information could contribute to determine, if the cis amide form plays a significant role in the conformation of polysaccharides containing N-acetyl groups.

References
(1) Blundell, C. D., et al., Glycobiology (2004) 14, 999.
(2) Pomin, V. H., Analytical and bioanalytical chemistry (2013) 405, 3035.
(3) Langeslay, D. J., et al., Analytical Chemistry (2013) 85, 1247.
(4) Yuwen, T.; Skrynnikov, N. R., Journal of biomolecular NMR (2014) 58, 175.
(5) Xue, Y.; Nestor, G., ChemBioChem (2022) 23, e202200338.

SLT-051: Molecular Dynamics of Enantiomers in an Electric Field

Mateusz Słowiński[1], dr Piotr Garbacz[1]
[1]Faculty of Chemistry, University of Warsaw, , Poland

We investigated the influence of an electric field on a quantum spin state of the 19-1H system in a light fluorinated chiral alcohol, namely 1,1,1-trifluoropropan-2-ol. Such an additional oscillating electric field used in a standard NMR experiment would potentially allow us to distinguish between enantiomers. In our studies, we used Gromacs to track the dynamics of molecules and SpinDynamica to compute the effect of the partial orientation of the molecules imposed by the electric field on the spin state. In particular, we employed molecular dynamics to track the rotational motion of molecules placed in an equilibrated box containing 831 molecules in an electric field oscillating at frequencies from 29 MHz to 2.9 GHz. The reliability of the molecular dynamics data was then confirmed by comparing the computed translational diffusion coefficient with the measured one by DOSY. Subsequently, we analyzed the rotational coefficients using molecular dynamics under different electric field amplitudes (up to 1 V/nm) and frequencies. By examining the frequencies at which molecules rotate as changes in the indirect spin-spin coupling tensor over time, we used SpinDynamica to calculate the NMR signal of the chiral molecule. The results of our calculations lead us to the conclusion that molecular spatial orientation in response to the electric field influences the NMR signal, permitting one to discriminate between the enantiomers of a chiral molecule in a direct way. PG and MS acknowledge the European Research Council for the financial support through the ERC Starting Grant (project acronym: NMER, agreement ID: 101040164).

SLT-052: Novel Lanthanoid Chelating Tags (LCTs) for pseudocontact shift (PCS) measurements on RNA

Leonie Bourgeois[1], Daniel Joss[1], Andreas Erharter[2], Prof. Dr. Daniel Häussinger[1], Christoph Kreutz[2]
[1]University Of Basel, Basel, Switzerland, [2]University of Innsbruck, Innsbruck, Austria

Non-coding RNAs and large RNA protein complexes are increasingly recognized as drug targets, and sophisticated nuclear magnetic resonance (NMR) methods are becoming a key technology in drug discovery. Pseudocontact shifts (PCS) in NMR spectroscopy offer a valuable tool to give long distance restraints up to 50 Å and beyond. Therefore, we gain information about functional dynamics of a wide range of biomolecules by attaching lanthanoid chelating tags (LCTs). The design of new LCTs applicable for PCS measurements in RNA is greatly desirable and opens new opportunities for structural characterization of RNA molecules. In this research work we present the design of new LCTs and a site-specific attachment to modified nucleic acids of RNA molecules. The LCTs generate sizeable PCSs and deliver precise structural restraints in RNA. They can, therefore, provide information about the structure and function of RNA and RNA-protein complexes and hence offer important data access, e.g. for drug screening processes.

SLT-053: Ln-M4B3T-DOTA – a new sterically overcrowded LCT for protein pseudocontact shift NMR spectroscopy

Annika Matt[1], Prof. Dr. Daniel Häussinger[1]
[1]University Of Basel, Basel, Switzerland

The development of new lanthanoid chelating tags (LCTs) is of great interest for paramagnetic NMR spectroscopy on proteins. M4-DOTA has proven to be an ideal scaffold for such applications. In addition, Joss et al. showed that substituting the methyl groups into isopropyl groups (P4T-DOTA) further decreases translation and rotational movement of the LCT and in turn allows an efficient transformation of the magnetic anisotropy onto the protein of interest. The main drawback of this scaffold was its dissipative synthesis.
Herein, we report a new LCT which is based on the already established M4-DOTA scaffold but modifies the lactic acid side arms. Surprisingly, it shows anisotropic susceptibility parameters exceeding those for P4T-DOTA.

SLT-054: Save while you produce: a simple, counter-intuitive method for improving the yield of isotope-labeled protein expression in flask-cultured Escherichia coli.

Dr. Miguel Ángel Treviño[1]
[1]Instituto de Química Física Blas Cabrera, CSIC, Madrid, España

Introduction and aim
An essential step in the study of proteins by NMR is to obtain them properly labelled. They are usually expressed in E. coli, using only labelled molecules as nutrients. In the most extended protocols, biomass is generated in rich media up to high optical density, the cells are centrifuged and switched to a minimal medium containing ^{13}C-glucose and ^{15}NH4Cl as carbon and nitrogen sources only. Bacteria are then cultured for 1-2 hours to allow isotope incorporation into precursors before the addition of the protein expression inducer. Although these methods improve direct culture in minimal medium, the price of ^{13}C-glucose has almost tripled in the last 2-3 years, so it is necessary to further optimise the yield of labelled protein per gram of ^{13}C-glucose.
Methods
BL21 star (DE3) bacteria transformed to express Cannabinoid Receptor Interacting Protein (CNRIP1) were used as a model to determine conditions for labelled expression. To evaluate each condition, dissolved glucose, optical density, protein yield by PAGE and band densitometry and percentage of labelling by mass spectrometry and by NMR were determined.
Results.
Established protocols were reviewed and compared with a new protocol using minimal medium for biomass production. In this way, we can eliminate the centrifugation steps by simply waiting until the unlabelled glucose is completely consumed and then adding the labelled glucose.
Contrary to what one might initially think,generating a large amount of biomass is not convenient because almost all of the added ^{13}C-glucose is consumed during the adaptation step and is not available for protein expression. Counter-intuitively, eliminating the incorporation step has no effect on the final label incorporation or protein yield.
Conclusions
A counter-intuitive unattended method that improves the yield of labelled protein in a 20-100% and reduces costs and active time has been optimised.

SLT-055: Influence of divalent cations on the extraction of organic acids in coffee determined by GC-MS and NMR

João Figueira[1], Malin L. Nording[2], Tove Bratthäll[2]
[1]Umeå University Swedish NMR Centre, ScilifeLab, Umeå, Sweden, [2]Umeå University Department of Chemistry, Umeå, Sweden

FINAL

Coffee flavor varies as a function not only of the coffee beans used, but also of the composition of the brewing water. It has been established that the perceived flavor of coffee is greatly affected by the water composition, and the matter has received substantial attention amongst professionals within the specialty coffee community [1]. A good cup of coffee is characterized by a balance between aroma, acidity, bitterness, and astringency, accompanied by a pleasant mouthfeel [2]. Perceived acidity is believed to be caused by organic acids such as chlorogenic, citric, malic, lactic, and quinic acid and to correlate to titratable acidity [3–5].
By adding $MgCl_2$ and $CaCl_2$ (at different concentrations), before and after brewing, the influence of these divalent cations on the extraction of organic acids (citric, malic, lactic and quinic acids) was studied by GC-MS and NMR.
At concentrations typically found in drinking water, the salts resulted in limited variation of the acid content, while ten-fold higher salt concentrations produced more pronounced variations. Comparisons between pre- and post-brew additions showed similar acid content in most cases, suggesting that extraction of acids proceeds independent of the water composition. Interactions taking place post-brew may, however, influence the perceived flavor.

[1] M. Wellinger et al., The Craft and Science of Coffee (2017), 381-398.
[2] A. N. Gloess et al., Eur. Food Res. Technol., 236 (2013), 607-627.
[3] A. Farah, Coffee: Emerging Health Effects and Disease Prevention (fourth ed., 2012), 21-58.
[4] L. Poisson et al., The Craft and Science of Coffee (2017), 273-309.
[5] W.B. Sunarharum et al., Food Res. Int., 62 (2014), 315-325.

SLT-056: Quantifying the passive permeability of clinically relevant compounds across a variety of biological membranes.

Mr Matthew Rice[1], Dr Jose Ortega Roldan[1]
[1]University Of Kent, Canterbury, United Kingdom

One of the greatest barriers to the development of novel antibiotics, anti-fungal and anti-cancer treatments is a very literal barrier in the form of biological membranes. Understanding the permeability of clinically relevant compounds across these different membrane types could help inform intelligent drug design to help improve drug delivery. Lipid environments have been shown to vary drastically among species and even within cancer types upon the development of drug resistance. Alternative lipid compositions also give rise to cases of antimicrobial resistance. Presented here is a novel method to quantify both the membrane adhesion and passive permeability of compounds across biological membranes. The membranes used can be formed using any available lipids extracted from the membranes of relevant species to closely mimic their native environments including lipids extracted from cancer cells. The method takes advantage of different solvent PRE effects on the intra and extracellular compartments of lipid vesicles. These alternative effects can be observed using 1H 1D CPMG spectra. We have used this method to assess the differences in compound permeability across several membranes including E. coli and Cisplatin resistant cancer cells and we are able to correlate these quantifications with other permeability metrics such as LogP values.

SLT-057: Optimized Standard Operating Procedures for 31P-NMR Metabolomics on Tissular Extracts

Dr. Jon Bilbao[1], Sara Martin-Ramos[1], Karen Alarcón[1], Dr. Tammo Diercks[1], Dr. Jose M Mato[1,2], Dr. Ganeko Bernardo Seisdedos[1,3], Dr. Oscar Millet[1,2,4]
[1]CIC bioGUNE, Derio, Spain, [2]CiBERehd, , Spain, [3]University of Deusto, Bilbao, Spain, [4]ATLAS Molecular Pharma, Derio, Spain

Phosphorylated metabolites (phosphometabolites) are key indicators of metabolic states and can be selectively analyzed by 31P NMR spectroscopy. To facilitate, improve, and promote their quantitative analysis we here propose optimized standard operating procedures that enhance 31P signal resolution, intensity, and accurate quantification of phosphometabolites in lipophilic and hydrophilic tissue extracts. For the latter, the most critical innovation is the use of basic glycine buffer in D2O at pD 9.5 which, in combination with EDTA, largely abolishes the detrimental effects of varying salt concentration (or osmolarity) by minimizing the troublesome variability of 31P signal frequencies and intensities of hydrophilic phosphometabolites. Adding only 0.5 mM Gadoteridol as paramagnetic relaxation enhancer further improves sensitivity and quantificability, by inducing faster and more uniform magnetisation recovery between scans. For lipophilic phosphometabolites we identified dimethyl sulfoxide (DMSO-d6) as most suitable solvent for best signal dispersion and stability, avoiding the alkaline hydrolysis commonly observed with other solvent systems such as CUBO. These methodological advances significantly enhance the coverage, accuracy, and reproducibility of phosphometabolite quantification, and have allowed us to analyze up to 50 phosphometabolites in extracts from mouse liver tissue. Thus, our study provides a robust recipe for quantitative phosphometabolite analysis by highly selective 31P NMR emphasizing, in particular, the critical role of solvent and buffer selection.

SLT-058: Benchmarking of 2D 15N-1H HSQC spectra; Comparison of signal-to-noise ratios across spectrometers, samples and pulse programs

Prof Göran Karlsson[1], Dr Ulrika Brath, Dr Cecilia Person
[1]Swedish NMR Centre at University of Gothenburg, Göteborg, Sweden

The quality of NMR spectra of 15N-labeled protein molecules depends on molecular size, protein dynamics, isotope labeling (e.g. deuteration), concentration, temperature and sample composition. It also relies on spectrometer hardware and software prerequisites such as magnetic field strength, probe specifics and choice of pulse program. For protein NMR samples in limited availability and/or with crowded spectral regions or low signal-to-noise ratio, it is important to consider optimization of the criteria above. We have investigated the performance of 2D 15N-1H HSQC and TROSY HSQC pulse programs on five different spectrometers (with magnetic fields stengths of 14.1 – 21.1 T, 600 – 900 MHz) equipped with six different cryogenic probes, based on spectral signal-to-noise ratios. With several probes available for a certain spectrometer, we were also able to benchmark the probe performance of a 3 vs. a 5 mm TCI probe at 21.1 T. Identical recordings of the pulse programs were acquired for the same sample solution in 3 and a 5 mm NMR tubes, thus enabling the comparison of the probe coil filling factor on the overall sensitivity for electronically conducting samples.
The results benchmark the outcome of the same experiment executed at various instruments, and will serve as a starting point to allow for a unified comparison between spectrometers at different sites, as described by the R-NMR project (https://r-nmr.eu). This study was conducted using three proteins with sizes 9 – 46 kDa with 2H labeling for the larger molecular weight proteins. To provide a guide to experimental selection depending on protein rotational correlation time, isotope labeling (including protein and solvent deuteration levels) etc. the set of pulse programs is suggested to be extented to include sequences with reduced interscan delay employing band-selective excitation or selectively optimized flip angle.

SLT-059: An NMR Study on the Enantioseparation of Licarbazepine by Electrokinetic Chromatography

Dr Antonio Salgado Serrano[1], Sandra Salido-Fortuna[2], Dr Maria Castro-Puyana[2], Prof Maria Luisa Marina[2]
[1]University of Alcala, CERMN, Alcalá de Henares, Spain, [2]University of Alcalá, Department of Analytical Chemistry, Alcalá de Henares, Spain

The enantiomers of the antiepileptic drug licarbazepine (lic-OH) were separated by electrokinetic chromatography (EKC) using two anionic cyclodextrins as the chiral selectors. These cyclodextrins form diastereomeric supramolecular aggregates (complexes) with each enantiomer in the EKC conducting buffer as a prerequisite for the enantiomeric separation. By changing the cyclodextrin a reversal of the enantiomer migration order (EMO) was observed. These two cyclodextrins, namely 2-carboxyethyl-β-cyclodextrin (CE-β-CD) and 2-carboxyethyl-γ-cyclodextrin (CE-γ-CD) were not pure isomers, but non-uniformly substituted compounds. The location of the 2-carboxyethyl substituents in each CD (regioisomery) was determined from HSQC experiments. Their degrees of substitution (DS) were calculated by comparing the integrals of the anomeric protons to those of the CH_2CH_2COOH residues. The stoichiometry of all complexes formed by each enantiomer with each cyclodextrin was determined from 1H NMR titrations [1] (Job plots) and was found to be 1:1 in all cases. The binding constants were obtained from another 1H NMR titrations series (Scott plots). With CE-γ-CD it was observed that two binding mechanisms could alternate upon increasing the CD concentration, while both maintained a 1:1 stoichiometry [2]. ROESY experiments corroborated the formation of inclusion complexes with both CD.

[1] - A. Salgado, B. Chankvetadze, J. Chromatogr. A, 1467 (2016) 95–144
[2] – S. Salido-Fortuna, A. Salgado, M. Castro-Puyana, M. L. Marina, Microchem. J. 196 (2024) 109621

SLT-060: Open-air green light ATRP monitored with in-situ Time-Resolved Diffusion NMR

Msc Marek Czarnota[1]
[1]Institute Of Physical Chemistry Polish Academy Of Sciences, Warszawa, Poland

Introduction
Atom Transfer Radical Polymerization (ATRP) with dual photoredox/copper catalysis is a powerful tool for obtaining good quality materials. This method provides high oxygen tolerance, so that the reaction can occur in the presence of the oxygen. Additionally, the reaction runs under green light irradiation in contrast to common ATRP which needs biocidal UV light. The product of ATRP reaction can be characterized using NMR spectroscopy. Diffusion Ordered Spectroscopy (DOSY) allows for measurement of molar mass of the polymer, and, in combination with Time-Resolved (TR) method, the measurements can be performed during the reaction.

Aims
We want to monitor in-situ ATRP with dual photoredox/copper catalysis reaction using NMR spectroscopy.

Methods
The illumination was delivered to the sample, in the spectrometer, through the fiber with etched tip put inside the coaxial insert, of the tube, immersed in the sample. We performed the TR-DOSY interleaved with proton experiments. From 1H spectra we knew the current monomer to polymer conversion. The illumination was turned off after the conversion reached a specific value. The light control was automated using Arduino module. When the reaction was finished, the Polydispersity Index (PDI) of the sample was measured using DOSY experiment in with number of scans was adjusted to keep the noise in the spectra at the same level. The results were validated using GPC/SEC measurement.

Results and conclusions
We obtained a series of spectra for samples of oligo(ethylene glycol) methyl ether methacrylate with final molar masses in range 25-400 kg/mol during reaction monitoring. We demonstrated that using the TR-DOSY the polymerization reaction can be monitored. We obtained the molar mass evolution with conversion and time.

SLT-061: In depth comparison of Fasted State Simulated Intestinal Fluid (FaSSIF) version 2 and 3 via NMR spectroscopy and complementary methods

Malte Mildner[1], Svetla Daskalova[1], Lena Scheller[2], Lorenz Meinel[2], Ann-Christin Pöppler[1]
[1]Institute of Organic Chemistry and Center for Nanosystems Chemistry, University of Würzburg, Würzburg, Germany, [2]Institute of Pharmacy and Food Chemistry, University of Würzburg, Würzburg, Germany

Efficient screening methods early in the drug development stage are key to quickly find promising drug candidates.[1] Solutions simulating conditions in the small intestine, the main absorption region for orally administered drugs, such as FaSSIF-V2 and V3 are essential.[2] Updated versions aim to better mimic the natural environment with modified concentrations of the main components taurocholate and lecithin or inclusion of oxidation products. NMR spectroscopy can be employed for all drugs[3] and to investigate interactions on a molecular level. It is therefore especially suited for a detailed comparison.

The aim is to identify how changes in composition of the FaSSIF versions affect the molecular level arrangement and evaluate the meaningfulness. The comparison was performed using the BCS class II drugs carbamazepine, danazol, efavirenz and ketoconazole.
Methods
NMR-Spectroscopy:1H,1H-DOSY,(selective)NOESY;HPLC,cryo-TEM

Carbamazepine was chosen as a negative control. Previously reported[4] similar dissolution behaviour indicated a non-interacting nature, which we experimentally identified. Danazol interacted weakly, but the very low solubility, signal broadening and overlap hampered the analysis. The signal appearances of ketoconazol and efavirenz changed significantly. For efavirenz one broad signal in FaSSIF-V2 and two broad signals in V3 were observed. Selective NOESY experiments showed interactions with lecithin in V2, but no visible cross-peaks with the oxidative products of V3. The concentration-dependent size and shape of the colloidal particles was investigated by DOSY and cryo-TEM.

FaSSIF-V2 seems to interact stronger with the investigated substances, resulting in one signal compared to two for efavirenz, less intense ones for ketoconazol and keeping the drugs dissolved over longer time periods. NOESY experiments indicate that interactions with lecithin are preferred over the degradation products. Knowledge about these preferences is essential to predict which version of FaSSIF is necessary to accurately describe the behavior in vivo to reduce surprises in clinical trials.

[1]G.Corti,F. Maestrelli, M. Cirri,S. Furlanetto,P.Mura,Eur.J.Pharm.Sci.2006,27,346-353.
[2]P.Augustijns,B.Wuyts,B.Hens,P.Annaert,J.Butler,J.Brouwers,Eur.J.Pharm.Sci.2014,57,322-332.
[3]G.K.Webster,S.Kumar,Anal.Chem.2014,86,11474-11480.
[4]A.Fuchs,M.Leigh,B.Kloefer,J.B.Dressman,Eur.J.Pharm.Biopharm.2015,94,229-240.

SLT-062: Where did the water and ethanol go? – Enhanced multiple solvent suppression with WEST

Ms Margot Sanchez[1], Ms Lisa Bourdel[1], Dr Thomas Paris[2], Mr Anthony Martinez[2], Dr Gaëtan Assemat[2], Pr Serge Akoka[1]
[1]Nantes Université, Nantes, France, [2]RS2D, Mundolsheim, France

Introduction: A World Health Organization estimation stated that at least 25% of all spirits are illicit. (1) Besides the health and economical issues, spirits authentication also represents an analytical challenge. NMR wise, the high concentration of water and ethanol in this type of sample makes the use of a solvent suppression technique unavoidable. Despite numerous pulse sequences, achieving sufficient suppression on both of these compounds is still challenging.
Aim: To address this problem, we developed a new pulse sequence that is robust, efficient and compatible with 2 channels spectrometer.
Methods: The core of the Water and Ethanol Suppression Technique (WEST) pulse sequence is WET. (2) Other solvent suppression blocks were added in order to encompass some limitations of it: (i) a selective inversion of the solvent signals to reduce the radiation damping ; (ii) a spatially selective 180° pulse and a NOESY section for faraway solvent suppression ; (iii) a zero-quanta filter to kill such coherences generated by the previous pulses ; (vi) a saturation block to suppress any residual water signal. We investigated this pulse sequence on a cohort of over 30 whiskies sample.
Results: The water frequency was measured using a standard proton acquisition and used as offset for the WEST spectrum. This was the only necessary frequency adjustment. The suppression efficiency was preserved even with a 5 Hz shift and 2 dB RF power deviation. Furthermore, compared to the WET sequence, as gold standard, we were able to reduce the solvent by at least 80%, lowering the residual signal to the level of the most concentrated metabolites.
Conclusion: The WEST sequence allows efficient water and ethanol suppression with minimal adjustments and using only 2 channels.

(1) Bryan, M. A. et al. J.Am.Soc.Brew.Chem. 2024, 1-17.
(2) Ogg, R.J. et al. J.Magn.Reson.Ser.B. 1994, 104, 1-10.

SLT-063: Fast access to protein dynamics using Hartmann-Hahn edited 15N-1H spectroscopy

Dr. Wiktor Adamski[1], Dr. François-Xavier Cantrelle[1], Dr. Davy Sinnaeve[1]
[1]Institut Pasteur de Lille; Université de Lille, U-1167; CNRS, EMR 9002, Lille, France

Introduction: Spectral congestion in protein NMR spectra is a never disappearing problem. Typically, this issue is solved by increasing the number of increments in the indirect dimension or dimensionality of experiments. This procedure is, however, bound by increase of acquisition times of spectra leading to greater costs of NMR experiments recorded, challenging their improvement. Especially for relaxation experiments, where sufficient sampling is required, or for zz-exchange experiments, where additional exchange cross peaks are present, this becomes an important limitation.

Aim: To reduce experimental time, an alternative approach is to use selective experiments that target particular ^{1}H-^{15}N correlations, allowing the use of simple 1D experiments. Selective Hartmann-Hahn coherence transfer has been previously proposed for this purpose (1, 2), but the selectivity of these experiments was limited. Here, we propose a scheme that provides clean 1D spectra with an improved selectivity of 15–20 Hz in ^{15}N with only a modest cost in sensitivity.

Results: Our approach combines an alternative and, to the best of our knowledge, neglected scheme for selective cross-polarization combined with an improved zeta-purge element (1, 3). We will show how our experiment allows to resolve otherwise overlapped in other approaches cross and diagonal peaks within a zz-exchange experiment, allowing to access the information on exchange in a fraction of the time required using non-selective (pseudo-3D) experiments (4).

Conclusions: The new experiment provides sufficiently selective and clean spectra to resolve information in acceptable measurement times, and will be very valuable for studying the dynamics of proteins featuring heavily congested spectra.

References:

1. D. Korzhnev et al., J. Am. Chem. Soc. 127, 713-721 (2005)
2. M. Nishizawa et al., J. Biomol. NMR 74, 205–211 (2020)
3. P. Pelupessy et al., Conc. Magn. Reson. 12, 103–124 (2000)
4. N. A. Farrow et al., J. Biomol. NMR 4, 727–734 (1994)

SLT-064: NMR monitoring of CO2 capture by threonine in various mixtures

Dr Astghik Shahkhatuni, Mrs Anahit Sakeyan, Mrs Tigranuhi Karapetyan, Mrs Sophie Petrosyan, Dr Arpine Harutyunyan[1]
[1]Scientific Technological Center of Organic and Pharmaceutical Chemistry, National Academy of Sciences, Yerevan, Armenia

Reliable assessment of CO_2 capture by various molecules and their mixtures and revealing their subsequent structural transformations is crucial for developing sustainable solutions to reduce carbon dioxide in atmosphere. Despite the demonstrated capability of NMR as a potent tool for estimating the CO_2 capture in solutions of amines, it is not widely used for these purposes.

Our research extensively utilizes NMR spectroscopy to track the CO_2 capture of threonine and its mixtures with various diamines. Diamines also capture CO_2, and in the mix they additionally serve as organic bases, enhancing the solubility of threonine in aqueous solutions. NMR spectroscopy not only can identify the product(s) that form as a result of CO_2 capture by amines and amino acids but also gives a quick and clear estimation of the relative concentration of reacted molecules. ^{13}C spectra are especially efficient in this endeavor, and they can detect unreacted CO_2 anions in the mixture. Additionally, NMR readily identifies and distinguishes carbamates with one or two CO_2 groups attached to the molecule.

The effects of several diamines on CO_2 capture of threonine at various concentrations were closely monitored by NMR, and the best practices for high levels of CO_2 capture were revealed.

This work was supported by the Science Committee of RA (Research projects 23LCG-1D019 and 20TTWS-1D049).

SLT-065: CT-ufb as a general scheme to enable H[C] editing in TROSY NMR: The 3D HN[CA]HA and HN[COCA]HA TROSY Experiments

PhD Tammo Diercks[1], PhD Andreas Schedlbauer[1], PhD Alain Ibañez de Opacua[1], PhD Oscar Millet[1], PhD Francisco Blanco[2]
[1]CiC BioGune, Derio, Spain, [2]CIB-CSIC, Madrid, Spain

Advanced protein NMR studies require prior signal assignment from a set of multiple resonance experiments that should employ the modern TROSY principle to exploit only the slower relaxing of two spin state selective (S^3) ^{15}N and HN coherences. By always yielding sharper signals and avoiding (^1H, ^{15}N) decoupling, TROSY has key benefits over conventional HSQC also for undeuterated proteins. There, HC frequency editing enables further spectral dispersion and critical information such as (i) sequential HA data (important for glycine rich or disordered regions), (ii) H-C anchor points for HCCH-TOCSY based sidechain assignment, (iii) ^{13}C assignment without deuterium isotope shifts, (iv) fold analysis via HA-HA' and HA-HN' NOE contacts, residual dipolar HA-CA couplings, HA chemical shift indices, etc.
Yet, indirect HC frequency sampling requires 90° ^1H pulses that also spoil the selected ^{15}N TROSY coherence (by scrambling HN spin states) and fast HN polarisation recovery (by saturating the Hm proton matrix and, thus, preventing constructive Hm→HN polarisation transfer). As a general solution to undo both adverse side effects (without need for selective ^1H pulses) we here introduce ^{13}C→HC Coherence Transfer with universal ^1H flip-back (CT-ufb) in the HN[CA]HA and HN[COCA]HA TROSY experiments, thus completing the TROSY toolbox for protein backbone assignment. While CT-ufb also enhances sensitivity for the HSQC versions (by minimising matrix Hm saturation), we furthermore show that TROSY may win even for undeuterated proteins when correctly exploiting several contributing polarisation pathways to balance losses from T_2(HN) with gains from faster T_1(HN) relaxation.

SLT-066: Ultraselective, Ultrahigh Resolution 1D TOCSY

Miss Emma Gates[1], Dr James R. D. Montgomery[1], Dr Marshall J. Smith[1], Dr Daniel A. Taylor[1], Dr Jonathan P. Bradley[2], Dr Daniel B. G. Berry[2], Dr Peter Kiraly[1], Prof Mathias Nilsson[1], Prof Gareth A. Morris[1], Dr Ralph W. Adams[1], Dr Laura Castanar[1,3]
[1]University Of Manchester, Manchester, United Kingdom, [2]Johnson Matthey, Sonning Common, United Kingdom, [3]Complutense University of Madrid, Madrid, Spain

While NMR spectroscopy is a powerful technique, ^{1}H NMR spectra often suffer from signal overlap because of both a narrow chemical shift range and wide multiplet structures, leading to difficulties in structural analysis. A common solution to this problem is to use 2D NMR methods to disperse signals over multiple dimensions, alleviating signal overlap at the expense of experiment time. Alternatively, selective 1D analogues of 2D ^{1}H experiments simplify spectra by extracting key information, typically in a fraction of the time required for a 2D experiment. Traditional selective excitation methods require chemical shift dispersion so multiplet overlap limits their effectiveness. GEMSTONE,[1] the recently developed ultraselective 1D NMR method, enables selective excitation of a single multiplet in an overlapped spectral region and can select a signal in a single scan unlike the earlier chemical shift selective filter (CSSF) method,[2] which requires multiple acquisitions to achieve the same selectivity. GEMSTONE-TOCSY[3] was developed to observe the entire spin system of a targeted multiplet; however, even with this development in ultraselective excitation, the resultant 1D correlation spectra can still show a high degree of signal overlap within the selected spin system. Here, we introduce a GEMSTONE-TOCSY-PSYCHE method which combines the ultraselective nature of GEMSTONE with the ultrahigh resolution of PSYCHE pure shift NMR,[4] yielding a 1D TOCSY spectrum with maximum resolution. The new method is demonstrated in the analysis of mixtures of Cinchona alkaloids, popular catalysts in asymmetric synthesis, and of glucocorticoids, used for treating conditions such as asthma.

References:

[1] P. Kiraly, et al., Angew. Chem. Int. Ed. 2021, 60, 666–669.
[2] L. D. Hall, T. J. Norwood, J. Magn. Reson. 1988, 78, 582–587.
[3] P. Kiraly, et al., Chem. Commun. 2021, 57, 2368–2371.
[4] M. Foroozandeh, et al., Angew. Chem. Int. Ed. 2014, 53, 6990–6992.

SLT-067: High Temperature 1H DOSY NMR reveals alteration of the molecular structure of water-extractable arabinoxylans during fermentation of wheat flour for sourdough production

Ir. Pasquinel Weckx[1,2], Ir. Victor Gonzalez Alonso[3], Prof. Dr. Ir. Luc De Vuyst[3], Dr. Ir. Eric Breynaert[1,2], Prof. Dr. Ir Johan Martens[1,2]
[1]Centre for Surface Chemistry and Catalysis – Characterization and Application Team (COK-KAT), KU Leuven, Heverlee, Belgium, [2]NMRCoRe - NMR/X-Ray Platform for Convergence Research, KU Leuven, Heverlee, Belgium, [3]Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Brussels, Belgium

Introduction

Arabinoxylans (AX) are important dietary fibers, predominantly encountered in wheat. They exist in water extractable and water unextractable versions and play a crucial role in human health. In food technology, especially the water-extractable arabinoxylans (WE-AX) are important due to their impact on viscosity and dough rheology. The production of sourdoughs is known to increase the WE-AX fraction, yet the underlying chemical mechanisms remain unclear. This study investigated the alteration of WE-AX during the fermentation of wheat flour for sourdough production using ^{1}H Diffusion Ordered SpectroscopY (DOSY) Nuclear Magnetic Resonance (NMR) at elevated temperature.

Aims

This research aims to fill this gap by employing ^{1}H DOSY NMR to analyze the structural changes of WE-AX during fermentation with different lactic acid bacteria (LAB) strains.

Methods

1D ^{1}H and ^{1}H DOSY NMR experiments were performed at elevated temperatures to increase the applicability of these techniques for large biomolecular compounds. Deconvolution of the spectra also revealed vital information about the substitution patterns of the WE-AX samples.

Results

The results reveal a size reduction of the WE-AX compounds, indicated by an increase in mobility of the molecules by analysis of the diffusion coefficient as well as the transverse relaxation times. Increases in the solid residuals also indicate that fermentation might render larger WE-AX compounds no longer soluble, explaining aforementioned results. Deviations from the general trend might be due to a creation of exopolysaccharides by some of the LAB strains, although further research is necessary to confirm this.

Conclusions

This study provides insights into the impact of fermentation on WE-AX during sourdough production, offering potential applications for improving sourdough bread quality and its health benefits. Simultaneously it demonstrates that the use of elevated temperatures can greatly improve the applicability of DOSY NMR for larger biomolecular populations.

SLT-068: Viral Origin of Immunity: Exploring the Disordered Domain of cGAS

Msc Hanna Kavaleuskaya[1,2], Dr. Dmitrii Blokhin[1], Dr. Suresh Vasa[1], Dr. Rasmus Linser[1,2]
[1]TU Dortmund, Dortmund, Germany, [2]Max Planck Institute for Molecular Physiology, Dortmund, Germany

Cyclic GMP-AMP synthase (cGAS) is a well-characterized cytoplasmic DNA sensor in humans and other vertebrates. Upon binding cytosolic dsDNA, cGAS synthesizes cGAMP from ATP and GTP, a secondary messenger that activates the STING-TBK1-IRF3 signaling pathway, leading to the production of type I interferons. Derivatives of this secondary messenger are highly valuable for treating inflammatory diseases and as immunotherapy against cancer.
cGAS comprises a catalytic structured core and a highly variable disordered N-terminal domain (NTD). The NTD interacts with centromeric DNA and histones in the nucleus, cytoplasmic double-stranded DNA of various lengths, and phosphatidylinositol-4,5-diphosphate to tether cGAS to the plasma membrane. However, the structural basis of NTD interactions is largely unknown.
Our research indicates that the NTD of cGAS has a polyphyletic origin from various herpesvirus proteins, including the large tegument protein UL36, tegument VP22, major viral transcription factor ICP4, and Epstein-Barr virus nuclear antigen EBNA-3B. This motivated us to elucidate the nature of DNA recognition in a site-specific manner using MD simulations and NMR spectroscopy. In the absence of DNA, simulations show that both the N- and C-terminus of the NTD fold into positively charged unstructured globules, separated by a loop comprising residues Gln24-Ala41. Following successful completion of backbone chemical shift assignments, first NMR-based interaction studies suggest that upon interaction with DNA, residues Ser26-Cys35 undergo chemical shift perturbations, and residue His31 disappears. Site-specific characterization of secondary-structural changes and relaxation properties will now help to evaluate the impact of DNA binding to the conformational ensemble as a function of the DNA context, which will further our understanding of cGAS target recognition.

SLT-069: Applying parahydrogen hyperpolarization to horticulture research: nhPHIP on affordable instrumentation

Ms Kärolin Kork[1], Dr Hedi Kaldmäe[2], Dr Kerti Ausmees[1], Dr Ulvi Moor[2], Dr Indrek Reile[1]
[1]National Institute Of Chemical Physics And Biophysics, Tallinn, Estonia, [2]Estonian University of Life Sciences, Tartu, Estonia

Non-hydrogenative PHIP (nhPHIP) is a parahydrogen hyperpolarization technique that offers three orders of magnitude sensitivity gains in NMR analysis of mixtures. Implementation of nhPHIP requires relatively simple accessories, without any changes to existing NMR instrumentation. We have demonstrated proof of concept applications for pharmacokinetics [1] and detection of N-heterocyclic metabolites [2]. The method has been recently extended to amino acids [3] and oligopeptides [4].

However, all mentioned examples have been demonstrated on high-end spectrometers (500 and 800 MHz) with cryoprobes. While such machinery is acceptable in fundamental bio(medical) research, it can be beyond reach for several applications wherein the added sensitivity of nhPHIP would otherwise be useful, e.g., agri- and horticulture.

For instance, floricane type raspberry plants have a two-year life cycle wherein new canes grow in the first year, followed by flowering and producing fruit in the second year. Horticultural research suggests the amino acid profiles of overwintering buds is correlated to their survival in the Northern European winter. Therefore, developing raspberry cultivars that can be cultivated economically in northern climates requires analytical support to evaluate buds’ amino acid content.

On lower end spectrometers, e.g. 300 MHz, amino acids are not spectrally resolved – even if enough buds can be extracted to raise them above the detection limit. Herein we demonstrate how nhPHIP hyperpolarization can help, resolving amino acid signals from small samples in fast 5-minute experiments on a 300 MHz spectrometer. We show quantification of raspberry buds’ amino acids and demonstrate the first instance of a practical analytical application of hyperpolarized NMR in horticulture.

References:
1. Reimets, et al. Anal. Chem. 2021, 93, 9480–9485.
2. Ausmees, et al. Chem. Commun. 2022, 58, 463–466.
3. Sellies, et al. Angew. Chemie Int. Ed. 2021, 60, 26954–26959.
4. Reimets, et al. Analyst 2023, 148, 5407–5415.

SLT-070: DOSY NMR Experiments at Variable Temperature. A thorough Analysis of Optimal Conditions for Reliable Results

Mr Israel Macho-Ávila[1], Dr. Gabriel González-Ferrer[1], Prof. Dr. Pablo Ballester[1,2]
[1]Institut Català d'Investigació Química (ICIQ-CERCA), The Barcelona Institute of Sciences and Techonology (BIST), Av. Països Catalans, 16, 43007 Tarragona, Spain, Tarragona, Spain, [2]ICREA, Passeig Lluís Companys, 23, 08010 Barcelona, Spain, Barcelona, Spain

1. Introduction:
In the year 2003 Pregosin and co-workers (Helvetica Chimica Acta, 2003, 86, 2364) established the necessary conditions for obtaining reliable results when performing DOSY NMR experiments at variable temperature. These conditions involved the use of NMR tubes with concentric insets, in order to minimize convection. As a result, the sensitivity of the experiments is substantially decreased, and the sample preparation is more cumbersome than usual.
The development of new pulse sequences with better convection compensation than the original ones, allowed measurements of DOSY NMR experiments at variable temperature, in samples with standard NMR tubes. However, a thorough analysis of the optimal experimental conditions has not yet been conducted.

2. Aims:
The aim of this work is to study and establish the optimal conditions to perform DOSY NMR experiments at variable temperature with the best possible results. We will focus on the use of the most advanced pulse sequences for convection suppression keeping the sample preparation process as simple as possible (e.g., using standard NMR tubes).

3. Methods:
For this study, we followed the Pregosin methodology (Helvetica Chimica Acta, 2003, 86, 2364). This involved acquiring the same DOSY experiment varying the diffusion time to determine the presence or absence of convection. We used different pulse sequences, conditions (spinning or non-spinning of the sample), instruments, and experiments (proton or heteronuclear with and without proton/heteronuclear decoupling).

4. Results and conclusions:
We describe and analyze the data obtained from the different measurements and establish the optimal conditions for acquiring DOSY at variable temperature in standard samples.

SLT-071: Lifetimes of long-lived states for fragment-based drug discovery

Anna Sonnefeld[1], Dr. Philippe Pelupessy[1], Prof. Dr. Geoffrey Bodenhausen[1]
[1]Ecole Normale Supérieure, Paris, France

Nuclear magnetic resonance (NMR) is an established technique in fragment-based drug discovery (FBDD) for the identification of small molecules that bind to macromolecules like proteins with weak affinity. Commonly used experiments include saturation transfer difference (STD), WaterLOGSY, and the measurement of transverse relaxation times T2 and T1rho. [1]

A recent addition to this toolbox of ligand-observed experiments is the characterization of binding via the monitoring of long-lived state lifetimes TLLS. Under certain conditions, this method can be more sensitive to binding events than T1, T2 and T1rho [2,3]. Here, we investigate the sensitivity and the accuracy of the determination of dissociation constants KD of protein-ligand complexes using TLLS-based methods [4] compared to WaterLOGSY, STD, T1, T2 and T1rho. In systems where conventional methods fail because they are not sufficiently sensitive to binding, TLLS-based methods could provide additional insight.

[1] M. J. Harner, L. Mueller, K. J. Robbins, M. D. Reily, Arch. Biochem. Biophys., 2017, 628, 132-147.
[2] N. Salvi, R. Buratto, A. Bornet, S. Ulzega, I. R. Rebollo, A. Angelini, C. Heinis, and G. Bodenhausen, J. Am. Chem. Soc., 2012, 134 (27), 11076-11079.
[3] R. Buratto, A. Bornet, J. Milani, D. Mammoli, B. Vuichoud, N. Salvi, M. Singh, A. Laguerre, S. Passemard, S. Gerber-Lemaire, S. Jannin and G. Bodenhausen, ChemMedChem, 2014, 9: 2509-2515.
[4] A. Sonnefeld, A. Razanahoera, P. Pelupessy, G. Bodenhausen, and K. Sheberstov, Science Advances, 2022, 8 (1-8), eade2113.

SLT-072: Combined photo-NMR and UV/Vis spectroscopyof In situ photo-induced transformations in Ruthenium nitrosyl complexes

Dr Axel Gansmüller[1], Dr Artem Mikhailov[1], Dr Nina Bukhtiiarova[1], Cyril Palin[1], Professor Theo Woike[1], Professor Dominik Schaniel[1]
[1]Université De Lorraine, Vandoeuvre Les Nancy, France

Nitrosyl linkage isomerism is an ability of nitrosyl ligands NO to bind a metal center in different ways – through nitrogen (M-NO), oxygen (M-ON) or by the side bond type (M-η2-(NO)). The metastable isomers (MS1 in case of O-coordinated NO and MS2 in case of side bonding of NO) can be formed after light irradiation of the ground state (GS). Depending on local molecular environment, these photoisomerization properties can be applied for data storage, for example, since these systems are molecular switches; on the other hand, these systems may as well play a crucial role in NO delivery applications such as photodynamic therapy (PDT).
The aim of our study is twofold: reveal the mechanisms of photoinduced linkage isomerization of the NO ligand in low temperature solutions, and characterize the complex ligand photo-release kinetics under room temperature irradiation. This can be achieved by directly linking the measured UV/vis optical properties to the structural evolution of the complexes.
To this end, we perform in-situ irradiation inside the NMR tube to follow the induced structural changes. The setup also includes simultaneous UV/vis absorbance measurements performed to follow the optical properties of the photoproducts. This is achieved using optical fibers as guides between the light sources, the sample and the spectrometers. All the irradiation and acquisition sequences are orchestrated by the NMR pulse programs via TTL pulses.

We here observe the evolution of several photoproducts at low temperature (including reversible photoswitching), along with the multiple photolysis products induced at room temperature. More than simply assigning the UV/vis absorbance bands to the species identified by NMR, our study shows that the techniques are complementary in the sense that some mechanisms can only be followed by one or the other; thus a combination of techniques is essential to obtain a complete picture of the photoreaction path.

SLT-073: Theoretical and practical aspects of b-matrix spatial distribution for diffusion tensor imaging

PhD Artur Krzyżak[1], Julia Lasek[1], Agnieszka Słowik[2,3]
[1]AGH University of Krakow, Krakow, Poland, [2]Collegium Medicum of Jagiellonian University , Krakow, Poland, [3]University Hospital in Krakow, Department of Neurology, Krakow, Poland

Introduction.
Commercial MRI systems provide b-matrices, constant throughout the measured volume, necessary to calculate the diffusion tensor. In reality, there is a spatial distribution of the b-matrix. The assumption of its constancy leads to large systematic errors that affect DTI metrics[1, 2] and tractography. The spatial distribution of the b-matrix generated by the gradient coils was introduced by Bammer[1]. Another approach is the B-matrix Spatial Distribution (BSD)-DTI solution, where the distribution is obtained from the study of phantom, a standard of a diffusion tensor[2]. In work we will discuss the recently published results for a large clinical study[3]. Moreover, we will demonstrate in practice the use of the BSD-DTI method to improve DTI metrics and fiber tracking on the example of volunteer brain scans.
Material and methods.
DTI data of 25 old female volunteer were acquired from a 3T Magnetom Vida scanner using SE-EPI with diffusion gradients in 20 directions; b-factor-(0,1000,2000s/mm2); TR-3900ms; TE-88ms; voxel-size-2.5×2.5×2.5mm3. The scanning took for 10 min. The DTI metrics and tractography were calculated with home – built software (NMR-LaTiS-Krakow), available for use at: nmrlab.agh.edu.pl/bsd.
Results and discussion.
The fractional anisotropy and mean diffusivity distributions differ statistically significantly ($p < 0.001$) between the standard and BSD approaches. Brain tractography after denoising and BSD-DTI is more anatomically realistic.
Conclusion.
Outcomes suggest the need to consider on a larger scale a solution based on the equation[4] for which the classical Stejskal-Tanner equation is a special case. The BSD-DTI technique effectively eliminates the influence of spatial systematic errors on DTI metrics and tractography. It can unify DTI measurements due to the independence (external diffusion tensor standard) on MR sequence parameters and the type of scanner.

References
1. Bammer et al.,MagnResonMed.2003,50(3),560–569
2. Krzyzak AT. Patent:USA.2014(2008);8643369B2
3. Krzyzak et al.,Neuroimage.2024; 33:286-295
4. Borkowski et al.,JMagnReson.2018;296:23-28.

Acknowledgments
Funding: Medical Research Agency contract no:2020/ABM/01/00006-00

SLT-075: High-resolution relaxometry for the characterization of the bound state of interacting ligand to macromolecules

Dr. Alessandro Ruda[1], Dr Duc-Duj Vu[1], Dr. Jorge Garibay[3], Prof. Kay E. Lewis[2], Prof. Fabien Ferrage[1]
[1]Ecole Normale Superieure, Paris, France, [2]University of Toronto, Toronto, Canada, [3]Bruker BioSpin, Wissembourg, France

INTRODUCTION

High-resolution relaxometry (HRR) is the approach that combines the possibility of fast field cycling with the advantages of high-resolution detection by shuttling the sample away from the magnetic center of a high-field magnet.

Recently, we developed a method to identify metabolites weakly binding to macromolecules in complex media using HRR.[1] Transient interactions are identified from their low-field signature in nuclear magnetic relaxation dispersion (NMRD) profiles. Can we determine the dynamics of a ligand in its bound state?

AIM

Here, we utilize HRR to extract site-specific information of the binding process of a medium-size ligand, chloroquine and its complex with a protein.

METHODS

Chloroquine weakly interacts with the 25 kDa α-monomer of the α7 subunit of the archaeal 20S proteasome from Thermoplasma acidophilium.[2] A 14.1 T Bruker spectrometer equipped with a newly developed sample-shuttle system was employed to record a set of longitudinal relaxation measurements over 100 magnetic fields between 40 mT to 14.1 T. Longitudinal relaxation rates were measured for different protein concentrations.

NMRD profiles for different protons of chloroquine were fitted with two states model where the ligand is assumed to be in fast-exchange between the bound and free form. Our approach was compared with other binding epitope mapping methods (STD[3] WaterLOGSY[4]).

RESULTS

The NMRD profiles of chloroquine show signal dependent dispersion elements attributable to different structural and dynamic behaviors for different moieties of the molecule. Our analysis suggests that High-Resolution relaxometry benefits from higher sensitivity and information content that conventional methods.

BIBLIOGRAPHY

1 - J. Am. Chem. Soc. 2021, 143, 9393−9404
2 - Biochemistry 2008, 47 (26), 6727–6734
3 - Angew. Chem. Int. Ed. 2003, 42 (8), 864–890
4 - J Biomol NMR 2000, 18 (1), 65-68.

SLT-076: A library of fluorinated prolines to study proline-rich motifs with ^{19}F NMR

Dr. Emile Ottoy[1], Prof. Bruno Linclau[1], Prof. Bruno Kieffer[2], Prof. Ilya Kuprov[3], Dr. Davy Sinnaeve[4,5]
[1]Department of Organic and Macromolecular Chemistry, Ghent University, Ghent, Belgium, [2]Université de Strasbourg, IGBMC, Strasbourg, France, [3]School of Chemistry, University of Southampton, Southampton, United Kingdom, [4]CNRS EMR 9002 – Integrative Structural Biology, Lille, France, [5]Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1167 – Risk Factors and Molecular Determinants of Aging-Related Diseases, Lille, France

Proline-rich motifs (PRMs) often occur in intrinsically disordered proteins, often forming sites for protein-protein interactions and thus heavily involved in organizing protein structures and key to many cell signaling processes and diseases. They are often challenging to study with NMR due to the lack of an amide proton and the limited signal dispersion of its aliphatic side-chain, especially in low-complexity sequences or in oligoproline stretches. We propose fluorinated prolines (FPros) as an attractive tool to question the role of individual proline residues.[1] Firstly, ^{19}F NMR provides easy spectroscopic access to specific residues, revealing for instance the cis-trans ratio. Secondly, fluorination alters proline's conformational dynamics, including the five-membered ring conformation of the side chain and the cis-trans ratio. This occurs in a way that depends on the precise stereospecific fluorine substitution. This form of conformational pre-organization has previously been very instructive to reveal the link between proline conformation and overall protein stability in folded proteins.

To make full use of both the ^{19}F NMR and pre-organizing abilities of FPros, we have characterized all 10 mono- and difluoroprolines substituted at the 3- and/or 4-positions using NMR on synthetic model compounds.[2,3] This includes an extensive full conformational landscape analysis of their five-membered rings using Density Functional Theory, allowing to fully map the ^{1}H and ^{19}F NMR properties as a function of conformation, such as ^{19}F CSA and J-couplings. We will present how these FPro residues can be put to good use as ^{19}F NMR reporters in PRMs on a number of examples.

1. Sinnaeve D., et al.. Magnetic Resonance, 2, 795-813 (2021).
2. Hofman G.J., et al. Chemical Communications, 54, 5118-5121 (2018).
3. Hofman G.J. , et al. Journal of Organic Chemistry, 84, 3100-3120 (2019).

SLT-077: The structure-property relationship in cryoprotective deep eutectic solvents.

Dr. Katarzyna Maria Dziubinska-Kuehn[1], Prof. Renaud Jolivet[1]
[1]Maastricht University, , Netherlands

Over the last years, the concept of designer solvents diversified into developing a new class of liquids, having (ultra-)low melting points, known as the deep eutectic solvents (DESs). The ease of predicting the DESs` properties is directly linked to their composition, based on specific molecular ratios of hydrogen bond acceptors (HBA) and donors (HBD), both contributing to the formation of an extensive hydrogen bond network. Moreover, the natural origin and benign character of the DES substrates are of high importance not only for fulfilling the green chemistry standards but also for broadening the medical and biological applications, e.g. if compared with another subclass of sustainable solvents, ionic liquids.
This contribution will focus on the structure-property relationship in the glycerol-based subfamily of DESs. Recent studies have shown that the oversaturation of HBA with HBD containing excess hydroxyl groups, such as glycerol, could possibly induce the cryoprotective properties of the overall DES solvent network. Herein, various HBAs will be paired with glycerol and/or ethylene glycerol to form DESs, and the structure of these solvents will be further assessed to understand the hydrogen bond pattern preferences, using one- and two-dimensional nuclear magnetic resonance (NMR) experiments, and via determination of the proton exchange rates at different water content. The selection of these methods allows to precisely determine the presence of water molecules in the close vicinity of each other or hydroxyl groups in the HBD network or the HBD self-aggregation into isolated voids.
Furthermore, the hydrogen bond network-cryoprotection relation is currently being screened using the cell viability essays, and the results will be presented in the context of (i) the DMSO golden standard for cryoprotection, and (ii) HBA:HBD molecular ratio in the selected DESs.

SML-001: Unraveling Enantiodiscrimination at the Molecular Level through NMR and Computational Insights

Tadeu Luiz Gomes Cabral[1,2], Matthias Stein[2], Claudio F. Tormena[1]
[1]University of Campinas, Campinas, Brazil, [2]Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany

The separation and identification of enantiomers are pivotal in the agricultural, pharmaceutical, and food fields. Matrix-assisted diffusion-ordered spectroscopy (DOSY) is an emerging approach that employs a chiral matrix for the evaluation of enantiomeric mixtures through Nuclear Magnetic Resonance (NMR) spectroscopy. However, this method lacks detailed insights into the chiral recognition modes and a detailed comprehension of the complexation process at the molecular level. In this study, we integrate experimental investigations with computational methods to elucidate the enantiodifferentiation of mandelic acid when (R)-BINOL is employed as a chiral matrix. ^{1}H and diffusion NMR measurements in $CDCl_3$ were conducted at 25 °C on a 600 MHz spectrometer using the Oneshot pulse sequence. Additionally, DFT and molecular dynamics (MD) studies were performed to support experimental findings. The experimental results reveal that the mandelic acid enantiomer exhibiting the highest shielding effect in the NMR spectrum shares the same chirality as the employed BINOL. Conversely, the enantiomer interacting more strongly (with a lower diffusion coefficient) has the opposite stereochemistry to the BINOL. DFT studies at the M06/def2-QZVP level confirm the preferred formation of enantioselective binding and emphasize the role of intermolecular hydrogen bonding to explain the observed shielding effect in the NMR spectrum. MD simulations can provide dynamic properties such as diffusion coefficients in good agreement with the experimental data. Furthermore, classical simulations offer valuable insights into the complexation process between (R)- and (S)-mandelic acid and BINOL over time, enhancing our understanding of experimental observations. The MD results indicate a higher concentration of (R)-BINOL molecules surrounding the (S)-enantiomer compared to the (R)-enantiomer, elucidating the lower diffusion coefficient observed in the (S)-enantiomer. Therefore, this integrated approach illustrates the feasibility of enantiodiscrimination through NMR and underscores the indispensable role of theoretical studies in unveiling molecular recognition processes.

SML-002: Dissecting the conformational stability of a glycan hairpin

Dr Ana Poveda[1], Nishu Yadav[5,6], Surusch Djalali[5,6], Manuel G. Ricardo[5], Peter H. Seeberger[5,6], Jesús Jiménez-Barbero[1,2,3,4], Martina Delbianco[5]
[1]CICbioGUNE-BRTA, Derio, Spain, [2]Ikerbasque, Basque Foundation for Science, Bilbao, Spain, [3]University of the Basque Country, EHU-UPV, Leioa, Spain, [4]Centro de Investigación Biomedica En Red de Enfermedades Respiratorias, Madrid, Spain, [5]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, [6]Freie Universität Berlin, Berlin, Germany

Introduction
Systematic structural studies of model oligo-peptides revealed important aspects of protein folding and offered design principles to access non-natural materials. Similarly, synthetic oligosaccharides could be valuable substrates to dissect the rules that regulates glycan folding, but their analysis is often limited due to synthetic and analytical complexity.

Aims
Taking a glycan capable of spontaneously folding into a hairpin conformation as model system, we analysed the factors that contribute to its conformational stability in water solution.

Results
Systematic chemical modifications of the glycan sequence, including the introduction of NMR labels and staples, revealed that conformational proclivity and multiple glycan-glycan interactions are the major determinants of folding stabilization.
Nuclear Magnetic Resonance studies assisted by molecular dynamics simulations revealed that minor modifications in the glycan primary sequence can be used to tune the rigidity of structural motifs remote to the modification sites.

Conclusions
These results could inspire the design of other glycan architectures with implications is glycobiology and material sciences

Yadav, N., et al., Dissecting the Conformational Stability of a Glycan Hairpin. J Am Chem Soc, 2024. 146(9): p. 6369-6376.

SML-003: DOSY and 2D NMR Characterization of Ag(I) Complexes Containing Enantiopure iPr-pybox and iPr-bopaH Ligands

Dra. Isabel Merino[1], Dra. Eire De Julián[2], Dra. Elena Lastra[2], Dra. Esther Bayón[2], Dr. Gustavo Manuel Borrajo[2], Dra. Pilar Gamasa[2]
[1]Unidad de RMN, Servicios Científico Técnicos, Universidad De Oviedo, Oviedo, , Spain,
[2]Departamento de Química Orgánica e Inorgánica, Instituto de Química Universitaria Enrique Moles (Unidad Asociada al CSIC), Universidad de Oviedo, Oviedo, , Spain

Transition metal complexes bearing enantiopure 2,6-bis(oxazolin-2'-yl)pyridine ligands (pybox ligands) have found great application as catalysts in asymmetric synthesis[1] due to their outstanding properties: presence of C2 symmetry axis, tridentate coordination ability and chirality provided by appropriate substitution on the oxazoline rings.
For the last years, we have centered our interest in the synthesis and study of the catalytic activity of several mono- and dinuclear Cu(I) complexes[2,3] bearing enantiopure pybox ligands. The efficiency or $[Cu_2\{(R,R)\text{-Ph-pybox}\}_2][X]_2$ (X= OSO_2CF_3, PF_6) on the enantioselective synthesis of propargylamines has been shown.[2]
Given the high interest of the Group 11 metals as catalysts, we considered the study of enantiopure Ag(I) metal complexes[4,5]. In this context, we have prepared new mono- and dinuclear Ag(I) complexes bearing (S,S)-iPr-pybox and (1R,2S)-indanepybox ligands[4], and bis[2-(oxazolin-2-yl)phenylamine (boapH) ligand.[5]

We present here the NMR characterization in the solution state of the mono- and dinuclear Ag(I) complexes $[Ag((S,S)\text{-iPr-bopaH})_2][OTf]$, $[Ag_2((S,S)\text{-iPr-bopaH})_2][OTf]_2$ and the dinuclear Ag(I) complex $[Ag_2((S,S)\text{-iPr-pybox})_2][OTf]_2$. As an initial step to evaluate their potential catalytic properties, an NMR study of their behavior in solution was carried out. The interactions between the cationic organic ligands and their counter ions were studied through ^1H and ^{19}F DOSY and $^1H^{19}F$ HOESY measurements, whereas the effect of the bopaH ligand featuring a distinctive NH moiety was evaluated through ^1H DOSY experiments and ^{15}N NMR spectroscopy.

[1] G. Desimoni, G. Faita, K. A. Jorgensen Chem. Rev. 2011, 111, 284.
[2] M. Panera, J. Díez, I. Merino, E. Rubio, M. P. Gamasa Inorg. Chem. 2009, 48, 11147.
[3] M. Panera, J. Díez, I. Merino, E. Rubio, M. P. Gamasa Eur. J. Inorg. Chem. 2011, 393.
[4] G.-M. Borrajo-Calleja, E. De Julián, E. Bayón, J. Díez, E. Lastra, I. Merino, M. P. Gamasa, Inorg. Chem. 2016, 55, 8794.
[5] E. De Julián. Doctoral Thesis, Universidad de Oviedo, 2016

SML-004: Teucrium montanum L. - Unrecognized Source ofPhenylethanoid Glycosides

Dr. sc. Ana Čikoš[1], mag. ing. Ana Mandura Jarić[2], mag. chem. Marijana Pocrnić[3]
[1]NMR Centre, Ruđer Bošković Institute, Zagreb, Croatia, [2]Department of Food Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia, [3]Department of Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia

Introduction

The market for functional foods has developed rapidly in recent years. This is due to growing consumer awareness of health and wellbeing, driven by the desire to prevent chronic diseases through diet, as well as advances in food science and technology that have enabled improvements in the nutritional profiles and bioactive components of everyday foods. The inclusion of herbal extracts as a rich source of bioactive compounds could be an effective means of expanding the range of high-quality functional foods.

Aims and methods

The aim of this study is to evaluate the bioactive potential of Teucrium montanum L., an understudied Mediterranean plant species, by in-depth structural elucidation of its polyphenolic profile using UHPLC-HR MS/MS and NMR spectroscopy. Heat-assisted (HAE), microwave-assisted (MAE) and subcritical water extraction (SWE) methods with water as the "green" solvent were chosen to maximise phenolic extraction yields from plants.

Results

A total of 12 phenolic compounds were identified in the samples of Teucrium montanum L. from six microlocations in Croatia. Nine of the phenols belong to the phenylethanoid glycosides. The structures of the three phenylethanoid glycosides PH5, PH6 and PH7, which turned out to be the dominant phenolic compounds in all extracts, were determined by NMR spectroscopy. The others were identified by UHPLC-HR MS/MS. Although the flavonoid subclass has been described as the largest group of phenolic compounds in Teucrium spp. only three flavonoid glycosides were detected here.

Conclusions

The results of this study, especially the maximum content of extracted polyphenolic glycosides (6.8 % dw) in HAE extract, indicate that T. montanum L. is an extremely valuable source of polyphenolic glycosides with potential applications in the biotechnological production of plant extracts, pharmaceuticals and the development of functional foods.

SML-005: J-compensated BIRDr and BIRDd,X elements for the measurement of RDCs

Yannik Woordes[1], Dr. Tony Reinsperger[2], Prof. Dr. Burkhard Luy[1]
[1]Karlsruhe Institute for Technology, Eggenstein-Leopoldshafen, Germany, [2]Bruker Biospin, Ettlingen, Germany

Bilinear rotation decoupling (BIRD) elements [1] are designed as isotope-selective spin echoes to refocus for example directly ^{13}C-bound protons, while remotely bound protons are left untouched. This element has been later called BIRD(d,X) to differentiate it from other elements, like the BIRD(r), which is essentially the inverse BIRD(d,X) element. [2] Such elements can be used to selectively decouple the two groups of protons, leading to reduced multiplets for e.g. the measurement of residual dipolar couplings (RDCs) in the indirect dimension. The conventional BIRD elements have the disadvantage that they only work well for a narrow range of one-bond couplings. For $^{1}J_CH$ couplings in isotropic samples this already can be an issue and in partially aligned samples experiencing a much wider range of couplings this leads to serious problems. We therefore designed BIRD elements which are less dependent on a specific $^{1}J_CH$ value and can accommodate a whole range of $^{1}T_CH$. In addition, the new J- (or T-) compensated BIRD elements are based on specifically designed universal rotation pulses that are compensated against B_1-inhomogeneity and offsets, following the COB-approach [3]. The elements are introduced in ^{13}C detected INEPT-type experiments. All experiments are applied to a partially aligned (-)-nicotine sample in PBLG experiencing a range of $^{1}T_CH$ values between 70 and 252 Hz.

[1] J.R. Garbow, A. Pines, “Bilinear rotation decoupling of homonuclear scalar interactions”. Chem. Phys. Let., 93 504-509 (1982)
[2] D. Uhrín, K.E. Kövér, “Modified BIRD pulses and design of heteronuclear pulse sequences”. J. Magn. Reson. A. 101 41-46 (1993)
[3] S. Ehni, B. Luy, “A systematic approach for optimizing the robustness of pulse sequence elements with respect to couplings, offsets, and B1-field inhomogeneities (COB)”, Magn. Reson. Chem. 50, S63-S72 (2012)

SML-006: Assignment of acyl-phosphine oxide conformations through 1D selective-NOESY, computational conformer fitting, and IR band analysis

Mr Alex W E Vaughan[1], Dr John N Moore[1], Prof Simon B Duckett[1], Dr Martin Thompson[2]
[1]University of York, York, United Kingdom, [2]Domino UK Ltd, Bar Hill, United Kingdom

Acyl-phosphine oxide photoinitiators induce polymerisation upon exposure to light via dissociation to form a radical pair. One of the most widely used is diphenyl(2,4,6-trimethylbenzoyl)-phosphine oxide (TPO).
DFT calculations predict the presence of two main conformers of TPO, trans- and synclinal-, with similar energies. Further analysis by symmetry adapted perturbation theory reveals how dispersion interactions between the phenyl and mesityl rings stabilise the synclinal-conformer. The conformational search algorithm CREST predicts a single trans-conformer, and a large number of synclinal-conformers with carbonyl-phosphinoyl dihedral angles varying between 42.8°-87.8° that lie close in energy.
Variable temperature quantitative 1D-selective NOESY experiments to investigate the nOe between protons on the phenyl and mesityl rings provide key information because the magnitudes of the nOe effects differ between conformers. The observed integrals are calibrated using the DFT calculated distance between the ortho-methyl and meta-protons on the mesityl ring. A least squares fitting algorithm is used to fit the calibrated nOe data to the predicted nOe interactions of the CREST calculated conformers to deduce the conformer composition as a function of solvent and temperature. This quantitative nOe study of TPO demonstrates an approach to analyse structure in flexible species where the ^{3}JHNH and ^{3}JHH coupling data that are routinely available for conformational analysis of proteins are not available.
Steady-state IR spectra in various solvents unlock further understanding. Single carbonyl and phosphinoyl bands found in acetonitrile broaden in less polar solvents such as chloroform. Band fitting reveals the presence of at least two peaks within the phosphinoyl and carbonyl bands, where the relative magnitudes of the peaks vary with solvent polarity. DFT calculated IR stretching frequencies for the trans- and synclinal-conformers enable the peaks to be assigned, and provide support for the conformer compositions deduced by nOe studies.

SML-007: 31P NMR parameters for stereochemical analysis of DOPO derivatives

Anna Hruzíková[1,2], Aneta Ešnerová[2], Vahid Barati[2], Lucie Tučková[1], Ivana Císařová[2], Aleš Růžička[3], Ondřej Baszczyňski[2], Eliška Procházková[1]
[1]Institute Of Organic Chemistry And Biochemistry Of The Czech Academy Of Sciences, Prague, Czech Republic, [2]Faculty of Science, Charles University, Prague, Czech Republic, [3]Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic

Molecules possessing a stereogenic center on a phosphorus atom are classified as P-chirogenic. These compounds find important applications in the fields of metal-free catalysis, asymmetric synthesis, coordination chemistry, and medicinal chemistry. Therefore, the knowledge of their exact stereochemistry is necessary. X-ray crystallography is commonly used for this purpose, however, obtaining a suitable single crystal can be a challenging process. In contrast, NMR spectroscopy offers valuable insights into phosphorus-based structures. 31P NMR spectra can be easily recorded on commonly used NMR probes due to 100% natural abundance and spin ½. Despite these advantages, the utilization of 31P NMR in structural analysis remains an underexplored area that holds potential for enhancing the accuracy of stereochemical analysis. In this work, we explored utilization of 31P NMR parameters complemented by quantum-chemical calculations to determine relative configuration of diastereomers based on the DOPO structure (9,10-dihydro-9-oxa-10-phosphaphenanthrene-10-oxide) substituted by a variety of amino acids. We compared results obtained using different solvation models, and we further employed molecular docking within an alignment medium (PBLG) to enhance efficacy of RDC analysis.

SML-008: Pushing the Limits of NOAH Supersequences for Routine Spectroscopy

Dr. Choon Boon Cheong[1]
[1]Institute Of Sustainability For Chemicals, Energy And Environment (isce2, A*star), Singapore, Singapore

NOAH supersequences provide significant conveniences and time savings for 2D NMR analysis, particularly for structural elucidation of small molecules. However, currently NOAH supersequences can only run up to 5 2D correlation experiments. Other necessary 2D experiments are incorporated either by interleaved acquisition or performed independently, thus compromising F1 resolution, sensitivity and/or experimental speed.

To design NOAH supersequences that include multiple 1H-1H NOAH modules and expand the number of sequentially-acquired 2D experiments, pulse sequences were generated, spliced and adapted from the GENESIS NOAH pulse programme generator and the Bruker pulse sequence library.

A NOAH pulse sequence with 9 modules containing 10 unique 2D experiments was successfully prepared, incorporating a new DOSY module for molecular weight estimates and affording an overall time savings of >75%. DOSY experiments need fractionally fewer gradient strength increments compared to 2D t1 increments, thus providing more scans per increment. The DOSY module is best used in NOAH supersequences with minimal/no decoupling or long pulses to avoid heating effects that reduce the accuracy of size estimations. Longer repetition times also help reduce amplifier duty cycle, avoid probe damage and mitigate heating effects.

Where multiple 2D 1H-nX correlation is desired, 1H-1H modules can be divided over two or more sets of NOAH experiments. These shorter supersequences suitable for workhorse spectrometers with 2-channel BBFO probes, and are less taxing on hardware thus allowing shorter repetitions times, with a more modest 56% time savings.

To conclude, the scope of NOAH experiments has been further expanded to include a terminal DOSY module and the sequential collection of up to 10 sequential experiments without interleaving, providing the full range of information in a single sequential acquisition for the structural elucidation of small molecules. Using several shorter NOAH supersequences for multiple 1H-nX and 1H-1H correlations is also an effective strategy for efficient routine experimentation.

SML-009: Optimal NMR measurements for Automated Structure Verification

Dr Emine Sager[1], Dr Markus Blatter[1], Dr Andreas Lingel[1]
[1]Global Discovery Chemistry, Novartis Biomedical Research, Basel, Switzerland

Structural analysis of compounds is an essential part of drug discovery, without complete structural characterization, no new drug could become available on the pharmaceutical market. Therefore, chemical structure elucidation and verification is a critical activity for synthetic chemists and Nuclear magnetic resonance (NMR) spectroscopy is one of the preferred techniques used for structure elucidation. However, collecting optimal NMR spectra comes with several challenges. Data interpretation is often time-consuming and sample properties are very inhomogeneous regarding concentration, molecular size, complexity, scaffolds, and functional groups which makes the collection of the right NMR data with the optimal quality in the shortest time challenging.

Having consistently the right signal intensity regardless of sample amount or spectrometer sensitivities, hence, avoiding to over- or underestimate the number of scans, is fundamental for performing efficient acquisition as well as fast and accurate structure elucidation or verification. In addition, constant quality greatly facilitates unattended processing routines such as the Automated Structure Verification (ASV) where accurate peak picking is crucial and can be obtained only using high quality NMR data.

In our laboratories, we previously established an in-house solution to ensure high quality spectra by adjusting parameters of NMR experiments according to the sample concentration and quality, but the maintenance of theses scripts over time is cumbersome as instrumentation and experiments are changing. Therefore, we tested the commercially available solution SmartDriveNMR by Bruker which can be centrally maintained.

On the poster, a comparison of the two solutions will be presented with an emphasis on adaptations to enable ASV, which allows accelerated NMR data analysis, working paperless and creating machine readable data.

SST-003: Proton detected fast MAS solid-state NMR studies of the hepatitis D virus S and L proteins

Yang Yang[1], Marie-Laure Fogeron[1], Lauriane Lecoq[1], Beat H. Meier[2], Anja Bockmann[1], Morgane Callon[1]
[1]MMSB - UMR 5086 CNRS-Université de Lyon, Lyon, France, [2]Physical Chemistry - ETH Zurich, Zurich, Switzerland

The Hepatitis D virus (HDV) small antigen (S), as well as its larger variant (L) are the only proteins of this smallest virus infecting humans. Of their structure, only their isolated assembly domain was characterized, while the organization of the full-length protein remained in the dark.
We here use proton detected fast magic-angle-spinning (MAS) solid-state NMR to show that the structure of the assembly domain is for the largest part conserved in full-length S and L, and that it represents the only rigid part in the protein. First data on the protein in presence and absence of viral RNA, mimicking the ribonucleoprotein (RNP) complex, highlight several residues that change chemical shifts.
In view to study RNP formation in detail, we set out to sequentially assign the protein, including its side chains, using 110 kHz MAS and 1.2 GHz. For our analyses, we synthesized the proteins using cell-free protein synthesis. This helped to achieve higher assignment completeness, since it allows for straightforward selective labeling. We thereby succeeded in assigning de novo 91 % of the backbone resonances and 71 % of the side chain resonances.

SST-004: Probing the active sites of molybdenum-based catalysts by 95Mo solid-state NMR spectroscopy at high magnetic fields

Phd Student Celine Moussa[1], Professeur des Universités Olivier Lafon[1], Research Engineer Julien Trebosc[2], Research Engineer Andrew Rankin[2], HDR Mostafa Taoufic[3], Director of Research Régis Gauvin[4], Director of Research Laurent Delevoye[1]
[1]Univ. Lille, CNRS, Centrale Lille, Univ. Artois, UMR 8181-UCCS-Unité de Catalyse et Chimie du Solide, 59000 Lille, France, [2]Univ. Lille, CNRS, INRAE, Centrale Lille, Univ. Artois FR 2638 – IMEC – Institut Michel-Eugène Chevreul, 59000 Lille, France , [3] Univ. Lyon 1, CPE Lyon, CNRS UMR 5265, Laboratoire de Chimie Catalyse Polymères et Procédés (C2P2), Université de Lyon, F-69616 Villeurbanne, France , [4]PSL Research University, Chimie ParisTech - CNRS, Institut de Recherche de Chimie Paris, 75005 Paris, France

Olefin metathesis is a catalytic reaction widely used at industrial level for the production of a broad variety of compounds and materials. This process relies on the utilization of heterogeneous catalysts, with some of the most efficient being based on Mo(VI) complexes derived from Schrock-type homogeneous catalysts. However, their heterogenization can alter the assembly of ligands and metal hence, understanding their catalytic behavior requires determining their atomic-level structure. Solid-state NMR is a unique technique to provide structural information about the local environment of the catalytic center, namely molybdenum.
In addition to classical 1H, 13C, 15N or 17O NMR studies, 95Mo solid-state NMR could offer such valuable insights into the local structure of active sites. However, its low sensitivity poses a challenge in accurately detecting and characterizing molybdenum-containing compounds. Additionally, the quadrupolar interaction of the 95Mo nucleus with its surrounding electric field gradient results in significant spectral broadening, complicating the interpretation of the NMR data. Therefore, acquiring high-quality spectra with sufficient resolution and sensitivity requires advanced techniques and equipment, such as high-field NMR instruments and sophisticated pulse sequences.
In the present work, we investigated molecular complexes, relevant to catalytic reactions, focusing on systems with mono-oxo and mono/bis-imido functionalities. Using a 28.2 T NMR spectrometer, and an optimized QCPMG pulse sequence, high quality spectra could be obtained and analyzed. NMR parameters, including CQ and δCSA, complement isotropic chemical shift to probe the 95Mo local environment. Experimental data reveal significant variations in NMR parameters, encompassing chemical shifts from −300 to 1000 ppm, CQ ranging from 3 to 8 MHz, and δCSA spanning 500 to 1500 ppm, thus paving the way for further exploration into more complex grafted systems. Theoretical calculations confirm the local structure of the complexes, aiding further investigation of surface Mo catalysts.

SST-005: Stereo-Array Labelled Protein 1H NMR at 100 kHz Magic Angle Spinning

Dr. Edward Saliba[1], Dr. Morgane Callon[2], CNRS Director Anja Böckmann[2], Professor Doctor Alexander Barnes[1], Professor Doctor Beat Meier[1]
[1]Department of Chemistry and Applied Biosciences, ETH-Zürich, Zürich, Switzerland, [2]Molecular Microbiology and Structural Chemistry, Université de Lyon, Lyon, France

Introduction

Stereo-array isotope labelling (SAIL) is a strategy for selective deuteration of -CH2- and -CH3 groups in amino acids[1]. This has major implications for solid-state nuclear magnetic resonance (NMR) spectroscopy, as most of the 1H linewidth of homogeneous protein systems under magic angle spinning (MAS) arises from the large network of 1H dipolar couplings[2].

Aims

In this work we have aimed to further understand the contribution of -CH2- groups to the linewidth of 1H NMR spectra, and the extent to which deuteration of one of the 1H spins affects the linewidths of the remaining 1H's in amino acid and protein systems.

Methods

To further understand the effect of SAIL, we have collected NMR data on the first transmembrane and cytosolic domains of glycoprotein N of the CCHF virus that has protonated, U-[13C,15N] phenylalanine and cysteine residues and SAIL, U-[13C,15N] glycine residues at 100 kHz MAS. We compare this data with numerical simulations using SPINEVOLUTION to estimate the degree of narrowing obtained with SAIL.

Results

Our work suggests that SAIL can have a dramatic impact on the linewidth of protein 1H resonances. The replacement of one of the 1H spins in a -CH2- group removes a 1H dipolar coupling of ~21 kHz. This, in effect, also removes all higher-order terms associated with it, which are the dominant terms for homogeneous resonance broadening.

Conclusion

We show that the selective deuteration of -CH2- groups in amino acids drastically narrows their 1H NMR linewidths.

References

(1) Doerr, A. SAILing toward Larger Protein Structures. Nature Methods 2006, 3 (5), 340–340. https://doi.org/10.1038/nmeth0506-340.
(2) Callon, M.; Luder, D.; Malär, A. A.; Wiegand, T.; Římal, V.; Lecoq, L.; Böckmann, A.; Samoson, A.; Meier, B. H. High and Fast: NMR Protein–Proton Side-Chain Assignments at 160 kHz and 1.2 GHz. Chem. Sci. 2023, 14 (39), 10824–10834. https://doi.org/10.1039/D3SC03539E.

SST-006: New Methods for Signal Enhancement in Wideline Solid-State NMR

James Kimball[1,2], Dr. Adam Altenhof[1,2], Dr. Michael Jaroszewicz[3], Max Bußkamp[4], Philipp Keill[4], Dr. Michael R. Hansen[4], Dr. Lucio Frydman[4], Dr. Robert Schurko[1,2]
[1]Department of Chemistry & Biochemistry, Florida State University, Tallahassee, United States, [2]National High Magnetic Field Laboratory, Tallahassee, United States, [3]Department of Chemical & Biological Physics, Weizmann Institute of Science, Rehovet, Israel, [4]Institute for Physical Chemistry, Westfälische Wilhelms-Universität, Münster, Germany

Ever since the inception of the NMR experiment, low signal-to-noise ratios (SNRs) have hindered its remarkable capabilities. Developments in pulse sequences, probes, and hardware, along with the increasing availability of high magnetic field strengths, have all served to spur the advancement of the field; however, low SNRs continue to plague those pushing the limits of modern day NMR. The ability to probe the most unreceptive nuclei in the periodic table, such as ^{103}Rh and ^{57}Fe, is next to impossible without the aid of sophisticated techniques aimed at enhancing the NMR signal. [Schurko, Acc. Chem. Res., 2013] At the heart of these methods is cross polarization (CP), [Pines et al., J. Chem. Phys., 1972] which has been used since the 1970's for signal enhancement, and still serves as a ubiquitous building block of pulse sequences and NMR methodologies to this day.

In this work, I will describe our recent efforts on improving and optimizing CP experiments involving transfer of polarization from protons to unreceptive nuclei. This is accomplished by the exploitation of both Zeeman [Harris et al., JMR, 2013; Kimball et al., J. Phys. Chem. A, 2023] and dipolar [Jaroszewicz et al., JACS, 2021] proton reservoirs as sources of polarization. Transfer of Zeeman order is mediated by frequency-swept pulses under both static and MAS conditions and wideline powder patterns are directly detected. Transfer of dipolar order is accomplished using low-power single-channel matching conditions, allowing for the indirect mapping of wideline static NMR powder patterns via observation of ^{1}H signal depletion. Efficient CP across frequency bandwidths of up to 1 MHz is demonstrated using both types of methods. The NMR spectra presented herein are unprecedented and have rich implications for addressing the serious problems which are presented by the reliance of our society on platinum-group elements. [Holmes et al., Chem. Sci. 2024]

SST-007: Artefact-free 13C-13C double-quantum spectroscopy at natural isotopic abundance using DNP in the 30 to 100 K range

Mr Quentin Reynard-feytis[1], Mr Eric Bouleau[2], Dr Subhradip Paul[1], Dr Sabine Hediger[1], Dr Gaël De Paëpe[1]
[1]Univ. Grenoble. Alpes, CEA, CNRS, IRIG, MEM, Grenoble 38000, France, [2]Univ. Grenoble Alpes, CEA, IRIG, DSBT, Grenoble 38000, France

Introduction:

Advances in MAS-DNP have improved the inherently low sensitivity of NMR by several orders of magnitude, enabling increasingly challenging applications. This includes, for instance, the ability to record diverse correlation experiments (^{13}C-^{13}C, ^{13}C-^{15}N, ^{29}Si-^{29}Si) on powdered solids at natural isotopic abundance (NA) (Smith, JPCP 2019). In addition to circumventing the need for isotopic labelling, working with low NA nuclei also enables the measurement of long-distance polarization transfers, since it significantly decreases the dipolar truncation phenomenon encountered in labeled materials. However, such powerful 2D experiments are often hindered by the presence of severe artifacts, such as t_1-noise, which comes from inconsistencies among the indirect dimension. Due to its multiplicative nature, it poses significant challenges as cross-peaks may be obscured amidst the t_1-noise generated by the stronger signal from uncorrelated spins, resulting in unclear or even unusable NMR spectra.

Aims & Methods:

In this work, we first introduce t_1-noise in NA DQ-SQ correlation spectroscopy and demonstrate that it originates from the fluctuations of abundant uncoupled nuclei during the t_1 evolution. Then, we introduce a novel strategy to reduce t_1-noise significantly, which makes use of the conversion of DQ Coherences into dipolar order terms (zz-terms) before the application of a z-filter block that destroys the magnetization of uncoupled nuclei. We derive the theoretical framework to explain that it can be applied to various dipolar recoupling sequences and notably to the SR26 supercycle.

Results & Conclusion:

The approach is then illustrated on two different setups by comparing DQ-SQ spectra of powdered solids recorded at 100 K using cAsymPol-POK and N2 spinning and at 30 K using AsymPol-POK and He spinning (Paul, JMR 2023). Finally, the attenuation in cross-peak intensity and t_1-noise arising from the application of the method is discussed, as well as its impact on the “effective signal-to-noise” in the indirect dimension.

SST-008: How MAS NMR of amorphous calcium carbonate provides proof for the pre-nucleation cluster pathway

Sanjay Vinod Kumar[1], Dr Maxim B Gindele[2], Dr Venkata Subbrao Redrouthu[1], Prof Denis Gebauer[2], Dr Guinevere Mathies[1]
[1]Universität Konstanz, Konstanz, Germany, [2]Leibniz Universität Hannover, Hannover, Germany

Non-crystalline intermediates, such as amorphous calcium carbonate (ACC), play a crucial role in biomineralization. Obtaining insight into the structures of these intermediates is notoriously difficult - there is no such thing as a unit cell. MAS NMR, however, goes a long way.
A series of one- and two-dimensional experiments at 9.4 T of ACC nanoparticles pointed to the presence of two chemically distinct environments. Numerical simulations, for which the magnetic properties of monohydrocalcite, a crystalline form of calcium carbonate with the same stoichiometry as ACC, served as a starting point, provided further specifics about these environments. We found that the first environment consists of immobile calcium and carbonate ions with embedded structural water molecules, which undergo 180° flips. The second consists of water molecules, which undergo slow, but isotropic motion, and dissolved hydroxide ions.
Meanwhile, investigations by conductive atomic force microscopy (C-AFM) revealed that ACC nanoparticles conduct electricity. Since solid salts are insulators, this remarkable observation can only be reconciled with the properties of the two environments by assuming that the mobile water molecules form a network through the ACC nanoparticles. The dissolved hydroxide ions carry the charge.
The networked structure is a consequence of the formation pathway of ACC. In aqueous solution, calcium and carbonate ions form dynamic assemblies termed pre-nucleation clusters.[1] The clusters can undergo phase separation and form dense nanodroplets.[2] When the solution is quenched to prepare solid ACC, the nanodroplets merge into larger aggregations, giving rise to the rigid, less mobile environment in the ACC nanoparticles. The network of mobile water molecules remains from imperfect coalescence of the droplet surfaces during dehydration.[3]

[1] Gebauer et al., Science 2008, 322 (5909), 1819–1822.
[2] Wallace et al., Science 2013, 341 (6148), 885–889.
[3] Gindele et al., Nat. Commun. 2024, 15 (1), 80.

SST-009: NMR Signatures and Coordination Environments of Pt Single Atoms

Dr. Alexander Yakimov[1], Dr. Jonas Koppe[2], Mr. Domenico Gioffrè[1], Mr. Marc-Eduard Usteri[1], Dr. Sharon Mitchell[1], Dr. Anne Lesage[2], Prof. Andrew Pell[2], Prof. Dr. Javier Pérez-Ramirez[1], Prof. Dr. Christophe Copéret[1]
[1]ETH Zurich, Zürich, Switzerland, [2]CRMN Lyon, Lyon, France

Single-atom catalysts (SACs) are an alternative to traditional nanoparticle-based catalysts, attracting great attention in the field of heterogeneous catalysis. The unique feature of SACs – exposure of every metal atom to the substrate – results in unprecedented metal utilization and catalytic activity. Despite these great advantages, rational design of SACs is challenging: while single atoms are detected by microscopy, a molecular-level understanding of the metal-site structure is currently lacking due to the absence of appropriate spectroscopic techniques providing local information about the chemical environment and the electronic structure.

Pt single atoms supported on various carbon nitride materials are an excellent representative of SACs. For studying Pt sites, 195Pt solid-state NMR is selected due to the exceptional sensitivity of the lineshape regarding specific Pt coordination environments. However, most 195Pt NMR signals exceed the excitation bandwidth of conventional radio-frequency pulses. Furthermore, the low Pt loading in SACs severely reduces the sensitivity, which prevented its application to SACs as well as interpretation of observed NMR lines so far.

Here we show that ultra-wideline NMR methodology, using frequency-swept pulses and CPMG (Carr-Purcell-Meiboom-Gill) acquisition, enables the acquisition of high-quality spectra. This is achieved by combining the latest advances in pulse-design strategies to maximize signal-to-noise and enable broadband magic-angle spinning (MAS) with low temperatures (100 K) and fast repetition rates for additional sensitivity enhancement.

Under these conditions, the 195Pt NMR signatures of SACs with Pt loadings down to 2 wt% across ranges of carbon nitride supports under static and MAS conditions can be recorded. The analysis of the signatures by state-of-the-art DFT calculations provides a detailed, atomic-scale description of the present Pt sites, including the direct chemical environment, and the local bonding geometries, and, finally allows us to assess the homogeneity of the metal sites, a first step towards rational design.

SST-010: Magic angle spinning NMR with in-situ irradiation for examining light sensitive samples

Dr Thomas Hooper[1], Dr Bapi Pradhan[1], Dr Rodrigo de Oliveira-Silva[1], Professor Dimitrios Sakellariou[1]
[1]KU Leuven, Leuven, Belgium

Light plays a pivotal role in many solid-state materials technologies such as: UV cured polymers for additive manufacturing; heterogeneous photocatalysis of chemical reactions; and photo-voltaic semiconductors to harness solar energy. Solid-state nuclear magnetic resonance (NMR) is a key tool for characterizing these materials, but opaque NMR rotors limit 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 magic angle spinning (MAS) at frequencies up to 12 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. Experimental optimization of the in-situ irradiation MAS NMR probe-head is presented and initial results are demonstrated. We have utilized the methodology to: i) track the polymerization of a UV initiated gel; and (ii) study the photo-degradation of organic lead halide perovskite photovoltaic materials.

SST-011: In situ crystallization studies of zeolites and MOFs using static and MAS NMR

Dr Sambhu Radhakrishnan[1,2], Mr. Dries Vandenabeele[1], Mr. Ahmet Dok[3], Dr. Vinod C Chandran[1,2], Dr. Nikolaus Doppelhammer[2], Prof. stijn Van Cleuvenbergen[3], Prof. Christine Kirschhock[1], Dr Eric Breynaert[1,2]
[1]KU Leuven - Center for Surface Chemistry and Catalysis, Leuven (3001), Belgium, [2]NMRCoRe - NMR/X-Ray platform for Convergence Research, Leuven (3001), Belgium, [3]KU Leuven - Department of Chemistry, Kortrijk, Belgium

Transformation of hydrated silicate ionic liquids (HSIL) to pollucite was studied with in situ 27Al NMR spectroscopy in both static and MAS conditions. Room temperature ZIF-8 formation was investigated statically using direct excitation 1H NMR. Static experiments were performed using a dedicated high pressure cell design, while the MAS experiments employed commercial 5mm high-pressure MAS rotors.
In situ 27Al NMR, detecting Al speciation and transformation within a Hydrated Silicate Ionic Liquid medium and its removal from solution during its conversion into crystalline Pollucite zeolite. In combination with ex situ synchrotron X-ray diffraction experiments and MEEIS data this allowed to detect that the initial growth occurred from Al-rich prenucleation clusters, leading to zoned crystals with increased Si/Al ration in their outer regions.
In situ 1H NMR combined with harmonic light scattering, inherently sensitive to structural changes, with NMR spectroscopy, which reveals molecular exchanges between particles and solution, we were able to capture the crystallization mechanism of ZIF-8 in unprecedented detail. Initially, oligomerization forms small, prenucleation clusters with an excess of protonated ligands in a pre-equilibrium state. When these clusters aggregate to form amorphous precursor particles, protonated ligands are released, leading to an amorphous charge neutral structure that subsequently transforms into crystalline ZIF-8 through intraparticle reorganization. Later stages involve solution-mediated Ostwald ripening, where the growth mechanism changes to incorporation of monomers from solution.

SST-012: Probing chalcogen bonds - solid-state NMR to the rescue!

Ms Tamali Nag[1], Ms Carina Almario[1], Dr. David L. Bryce[1]
[1]Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ottawa, Canada

Chalcogen bonds1 (ChB) are an emerging class of noncovalent interactions which find importance in the fields of crystal engineering, catalytic synthesis, materials science, pharmaceuticals, and biomaterials. The local environment, structure, and geometry of a ChB are affected due to the presence of different substituents and can be modelled to obtain a wide array of compounds featuring a significant chalcogen-bonded interactions.

Here, we report our studies of various novel and previously synthesized ChB cocrystals using solid-state NMR (SSNMR) as a probe of the ChB donor, acceptor, and the ChB itself. Single-crystal X-ray diffraction and powder X-ray diffraction studies of the ChB cocrystals provide essential preliminary structural analyses of the ChB cocrystals. Selenodiazoles and telluradiazoles are used as the potential ChB donors and a variety of ChB acceptors were chosen to yield reproducible and higher quality ChB cocrystals.

NMR observables such as chemical shift tensors, J-coupling tensors and EFG tensors were measured and trends have been elucidated with respect to the pure ChB donor/acceptor NMR response upon ChB formation.2,3 J-coupling between the quadrupolar bromine isotopes of ChB acceptor tetraphenylphosphonium bromide interacting with tellurium of the ChB donor, telluradiazole, led to evidence of non-Fermi contact coupling across the ChB as a result of large J-anisotropies (125Te and 79/81Br).4 NMR observables were computed using periodic and non-periodic calculations to support the experimental evidence obtained from SSNMR.

References:

1. Aakeroy, C. B.; Bryce, D. L.; Desiraju, G. R.; Frontera, A.; Legon, A. C.; Nicotra, F.; Rissanen, K.; Scheiner, S.; Terraneo, G.; Metrangolo, P.; Resnati, G. Pure Appl. Chem. 2019, 91, 1889-1892.
2. Nag, T.; Ovens, J. S.; Bryce, D. L. Acta. Cryst. C. 2022, 78, 517-523.
3. Almario, C.; Nag, T.; Bryce, D. L. Facets. 2023, 8, 1-14.
4. Nag, T.; Terskikh, V. V.; Bryce, D. L. Angew. Chem. Int. Ed. 2024, in press.
https://doi.org/10.1002/anie.202402441

SST-013: Resolving structures of paramagnetic systems in chemistry and materials science by ultra-fast solid-state MAS NMR

Dr. Jonas Koppe[1], Mr. Thomas C. Robinson[1], PhD Kevin J. Sanders[2], PhD David Proriol[3], Prof. Clare P. Grey[4], Prof. Andrew J. Pell[1], PhD Guido Pintacuda[1]
[1]Centre de RMN Très Hauts Champs de Lyon (UMR5082 - CNRS, ENS Lyon, UCB Lyon 1), Université de Lyon,, 69100 Villeurbanne, France, [2]Department of Chemistry, McMaster University, , Canada, [3]IFP Energies Nouvelles, 69360 Solaize, France, [4]Department of Chemistry, University of Cambridge, Cambridge, United Kingdom

Probing NMR-active nuclei in close proximity to paramagnetic centers remains as a great experimental challenge. Large hyperfine couplings between the electronic and nuclear magnetic dipoles cause fast decaying NMR signals and extremely broad resonances, often preventing the acquisition of meaningful NMR data. Enabled by recent technological advances, the application of ultra-fast magic-angle spinning (MAS) at 100 kHz and beyond has emerged as a promising experimental approach, as it allows for efficient averaging of the strong hyperfine couplings. Yet, its successful application to paramagnetic organic and inorganic materials remains limited.
Here we show that one of the potential difficulties of ultra-fast MAS, the reduction in sensitivity associated with the small-diameter rotors (≤ 0.7 mm), is more than compensated by the unprecedented improvements in spectral resolution achieved for highly paramagnetic solids. Furthermore, we highlight that specifically tuning frequency-swept pulses that are required for broadband excitation and adiabatic inversion at 100+ kHz MAS allows us to minimize the sensitivity penalty.
The combination ultra-fast MAS and our latest advances in pulse-design strategies pushes the limit of detection of paramagnetic solid-state NMR, and establishes a new avenue to characterize the geometry and electronic structures of functional paramagnetic systems in chemistry and material sciences, which we have here showcased for paramagnetic organometallic catalysts and battery materials.

SST-014: Exploring CO2 sorption mechanisms in green cellulose and chitosan aerogels using solid state NMR and DFT calculations

Daniel Pereira[1], Mirtha Lourenço[1], Mariana Sardo[1], Armando Silvestre[1], Ildefonso Montesinos[1], Luis Mafra[1]
[1]University Of Aveiro, Aveiro, Portugal

Carbon-dioxide (CO_2) is the most greenhouse gas emitted by human activities and one of the biggest responsible for climate changes. CO_2-emissions must be dramatically reduced to mitigate global-warming. Several technologies have been proposed to remove CO_2 from the air or flue gases, but amine-scrubbing is the benchmark method to remove CO_2 at industrial-scale applications. Amine-based liquid absorbents suffer from limitations such as poor chemical stability, toxicity, and demand high-energy regeneration stages. Alternatively, adsorption technology uses solid adsorbents, which are promising candidates for large-scale carbon capture due to their lower-energy regeneration requirements and durability over many cycles, two of the main cost drivers in CO_2 capture technologies.
The practical implementation of large-scale carbon capture technologies requires the availability of CO_2 selective and low-cost materials that are renewable. Polysaccharide-based adsorbents are abundant, renewable and biodegradable, making them a promising candidate for this use. However, the CO_2 capture mechanisms in these materials remain largely unknown.
In this work, CO_2 sorption mechanisms on cellulose and chitosan aerogels were studied by solid-state NMR spectroscopy and DFT calculations. A combination of 1D 13C cross-polarization (CP), 2D 13C-{1H} LG-CP HETCOR and CP kinetics NMR experiments were performed to characterize confined chemisorbed and physisorbed CO_2 adsorbed species. First-principle DFT calculations were used to aid the NMR assignments of the different types of adsorbed CO_2 species. We show, for the first time, that while cellulose aerogels adsorb CO_2 purely via physisorption processes, chitosan aerogels instead capture CO_2 via chemisorption and physisorption processes. The chemisorbed species in chitosan aerogels were assigned as ammonium carbamate and carbamic acid species. These results contribute towards a better understanding of the adsorption processes in these materials, and it is a steppingstone for future improvements in the performance of these sustainable solid sorbents.

SST-015: Multi-diagnostic dielectric+NMR spectroscopy and the properties of water under nano-confinement

Dr Alysson F. Morais[1,2], Dr Sambhu Radhakrishnan[1,2], Gavriel Arbiv[1,2,3], Dr Vinod Chandrasekharan Nair[1,2], Johan Martens[1,2], Eric Breynaert[1,2,3]
[1]Centre for Surface Chemistry and Catalysis (COK-KAT), Celestijnenlaan 200F Box 2461, 3001-Heverlee, Belgium, , , [2]NMR for Convergence Research (NMRCoRe), KU Leuven, Celestijnenlaan 200F Box 2461, 3001-Heverlee, Belgium, , , [3]Center for Molecular Water Science (CMWS), Notkestraße 85, 22607 Hamburg, Germany, ,

Introduction of a sample in an NMR coil modifies the frequency response of the probe circuit, a phenomenon revealed by the detuning of the probe – the dielectric shift – seen from the wobble curve. Prior to NMR measurements, this detuning is corrected for by tuning and matching the probe and, in general, not much importance is given to its magnitude. Nevertheless, it provides access to the dielectric properties of the sample, transforming NMR probe heads into multi-diagnostic characterization tools able to simultaneously perform NMR and dielectric spectroscopy measurements. In this presentation, a method to measure dielectric permittivity using NMR probe heads will be shown [1]. This method was evaluated on a series of standard samples using commercial CPMAS probe heads. The results accurately match literature data collected by standard dielectric spectroscopy techniques. As an application of the method, the properties of water confined in the pores of a series of microporous and mesoporous silicate materials with tuned surface chemistry were investigated. In these materials, water adsorbs to defects and Brønsted acid sites, which is directly probed by NMR spectroscopy. The nanoconfinement also affects the hydrogen bonding properties of water, which in turn causes a drastic decrease in its dielectric permittivity and is reflected in the dielectric data recorded using the NMR probe head. Asides confined water systems, in situ multi-diagnostic dielectric/NMR spectroscopy will also benefit the fields of battery research, food quality control and sensing, where both NMR and dielectric/impedance spectroscopy are common characterization techniques. During this presentation, a theoretical background for the new methodology will be provided using an effective electric circuit model of a CPMAS probe head with solenoid coil to describe the detuning resulting from the insertion of dielectric samples in the coil space. [1] Anal. Chem. 2024, 96, 13, 5071–5077.

SST-016: Stratification of Solid-State NMR Spectra of Proteins Based on the Amino-acid Side-chains

Mr MOPIDEVI MOHANA VENKATA SUBBA RAO[1], Mr Sahil Ahlawat[1], Dr Sreejith Raran-Kurussi[1], Prof Vipin Agarwal[1]
[1]Tata Institute Of Fundamental Research Hyderabad, Hyderabad, India

Solid-state Nuclear Magnetic Resonance (ssNMR) is a powerful tool for studying the structure and dynamics of solids, including polymers, proteins, and other complex systems. Peak assignment in ssNMR can be challenging due to limited resolution resulting from several factors such as broad peaks, anisotropic interactions, misalignment of spinning-angle from magic-angle and multiple other interactions. Multi-dimensional NMR experiments are often employed to overcome spectral overlap and improve resolution. However, these experiments require time-intensive sophisticated pulse sequences and data processing techniques, adding complexity to the assignment process. Spectral simplification has often been addressed by employing various isotope labeling schemes (2-13C-Glycerol, 1,3-13C-Glycerol, SAIL, ILV, etc…). In solution-state NMR, sequences like MUSIC (MUltiplicity Selective In-phase Coherence transfer) were developed for filtering peaks based on the amino-acid type. This study demonstrates that side-chain differences amongst the different amino acids can be exploited to distinguish peaks in 2D CH/NH solid-state HETCOR spectra. Although sequential resonance assignment is still required, we expect these experiments to reduce ambiguities and thus, accelerate the resonance assignment approaches. We will showcase our novel methodology on GB1 (6.2 kDa) and MBP (40.7 kDa) proteins.

SST-017: 2H NMR unravel a new understanding of the lipid dynamics in the skin barrier

Dr. Oskar Engberg[1], Dr. Ferdinand Fandrei[2], Tomáš Havrišák[2], Dr. Lukáš Opálka[2], Petra Pullmannová[2], Dr. Andrej Kováčik[2], Martin Juhaščik[2], Professor Kateřina Vávrová[2], Professor Daniel Huster[1]
[1]Institute of Medical Physics and Biophysics, University of Leipzig, Leipzig, Germany, [2]Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic

The skin barrier (stratum corneum) is a complex structure of equimolar ratios of the lipids: ceramides, free fatty acids, and cholesterol. Traditionally it has been thought to form a very rigid crystalline barrier, to prevent water dehydration and protect against pathogens. The aim was to study if the skin barrier consists of a rigid structure, a fluid structure, or a combination. By utilizing solid-state 2H NMR spectroscopy of a skin barrier model membrane system consisting of differently deuterated lipids, we can acquire anisotropic information about the motions of the individual lipids species or lipid chains. By line shape simulations of the 2H NMR spectra, we can infer the lipid phase and order parameters of the individual lipids or lipid chains present. 2H NMR is sensitive to detect the crystalline structure, but also fluid and isotropic motions. We show that certain lipids (cholesterol, sphingosine chain of ceramide[NS], the omega linked unsaturated lineolate chain of Cer[EOS]) show more fluid and isotropic motions while certain lipids (Lignoceric acid) and lipid chains (the acyl chain of Ceramide[NS]) exist mostly in the crystalline phase. We have further probed different derivatives of the ultralong Cermide[EOS] with different omega-linked chains (saturated stearate, branched phytanate, unsaturated oleate) to understand how the different lipid structures modify the dynamics and structure of the skin barrier. In summary, we suggest improved models of the lipid structure of the skin barrier based on the detailed analysis of 2H NMR spectra. These new models highlight that the skin barrier has both fluid and rigid compartments.

SST-018: Evaluation of 13C-13C TOCSY as a viable option for sensitivity-enhanced solid-state NMR experiments: -Application of triple sensitivity-enhanced 4D experiments for assignments of the 30 kDa membrane protein GlpG

Dr Carl Öster[1], Dr Veniamin Shevelkov[1], Dr Sascha Lange[1], Prof. Adam Lange[1]
[1]Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Berlin, Germany

Chemical shift assignments of large membrane proteins by solid-state NMR experiments are challenging. Recent advancements in sensitivity-enhanced pulse sequences, using TROP and/or SPEPS for magnetization transfers, have made it feasible to acquire ^{1}H-detected 4D spectra of these complex proteins within reasonable timeframes. However, obtaining unambiguous assignments remains difficult without access to side-chain chemical shifts. Drawing inspiration from sensitivity-enhanced TOCSY experiments in solution NMR, we have explored the potential of ^{13}C-^{13}C TOCSY mixing as a viable option for triple sensitivity-enhanced 4D experiments aimed at side-chain assignments in solid-state NMR.
Through simulations and experimental trials, we have identified optimal conditions to achieve uniform transfer efficiency for both transverse components and to minimize undesired cross-transfers. Our experiments, conducted on the 30 kDa membrane protein GlpG (with varying protonation levels) embedded in E. coli lipids, have demonstrated enhanced sensitivity compared to the most effective dipolar and J-coupling-based ^{13}C-^{13}C mixing sequences. Notably, non-uniformly sampled 4D hCXCANH spectra with exceptionally high sensitivity were obtained in just a few days using a 600 MHz spectrometer equipped with a 1.3 mm probe operating at MAS rates of 55-60 kHz. The assignments facilitated by this methodology have allowed us to analyze the interactions between GlpG's cytosolic and transmembrane domains in the full-length protein, revealing a distinct conformation compared to our previous observations of a truncated version lacking the cytosolic domain. We anticipate that this approach will prove valuable for investigating other challenging proteins and hope it will stimulate the development of new and improved ^{13}C-^{13}C mixing sequences tailored for sensitivity-enhanced solid-state NMR experiments.

SST-019: Proteins in native membranes reveals by solid state NMR

Dr. Morgane Adélaïde[1], Dr. Florian Lindemann[1], Dr. Edward P. Saliba[2], Dr. Matthias Herrera-Glomm[1], Dr. Barth Van Rossum[1], Nils Creemer[1], Dr. Anne Diehl[1], Pr. Alexander Barnes[2], Pr. Hartmut Oschkinat[1]
[1]Leibniz-forschungsinstitut Für Molekulare Pharmakologie, Berlin, Germany, [2]ETH Zurich, Zurich, Switzerland

The 34kDa outer membrane protein G (OmpG) of Escherichia coli, is a porin, which allows the uptake and secretion of nutrients, ions, and proteins in Gram-negative bacteria. That beta barrel has short intracellular turns and long extracellular loops. Different roles of the extracellular loops were proposed as their implication in the opening and closing of the pore thanks to histidine from loops 6 and 7. In order to understand the molecular mechanism of OmpG, its structure has been studied in lipidics environments with increasing complexity. However, it is still critical to study it in a native functional context. The solid-state Nuclear Magnetic Resonance (ssNMR) is a suitable technique to access this kind of information at an atomic level. This kind of study faces challenges such as poor resolution and poor sensitivity, which increases difficulty to access complete resonance assignment. However, it is possible to use stronger magnets, ultrafast magic angels spinning which accelerate acquisition, while reducing sample requirement to improve resolution. In addition to ssNMR hardware improvement, the external loops of OmpG are composed of tryptophan which can be used as reporters as their side chain NH signal can be easily observed in 1H–15N correlation spectra. By the use of tricks (selective labeling…) and higher ssNMR condition, we access new information of OmpG inside native outer membrane of Escherichia coli.

SST-020: Assessing CO2 capture via solid-state NMR adsorption techniques

Dr Ricardo Vieira[1], Dr Marina Ilkaeva[1], Mr Daniel Pereira[1], Dr Mirtha Lourenço[1], Dr Mariana Sardo[1], Dr Ildefonso Marin-Montesinos[1], Dr Luis Mafra[1]
[1]Universidade de Aveiro, Albergaria-a-Velha, Portugal

Growing concern about rising levels of atmospheric CO2, a green-house gas, due to the excessive exploitation of fossil fuels demands immediate actions to be taken. The decades-old industrial technology based on liquid amine absorbents suffers from several limitations such as poor chemical stability and high regeneration energy requirements. Hence, the use of solid sorbents able to selectively capture CO2 is a desirable alternative. To improve the performance of solid sorbents and make them feasible at the industrial level, it is crucial to elucidate CO2 capture mechanisms. In addition to conventional techniques, such as volumetric and gravimetric gas adsorption, the use of spectroscopic techniques, namely solid-state (ss) NMR, can be a great asset, as it unveils extremely useful information on the CO2 adsorption mechanisms.
ssNMR spectroscopy has already been used previously by our group to discriminate the nature of different types of chemi- and physisorbed CO2 species formed upon adsorption, which is an undeniable advantage of using this technique. [1-2]
We have now developed a quantitative ssNMR based CO2 adsorption protocol, comparing the obtained data with volumetric adsorption techniques. The results have revealed to be equivalent in assessing the total CO2 uptake as well as the adsorption values at different pressures proving that this spectroscopic technique may be applied for the CO2 capture capacity assessment. Moreover, ssNMR, due to its discriminate power, is able to provide individual isotherms for each CO2 species formed upon adsorption, which is impossible to obtain through conventional adsorption techniques, highlighting the potential of ssNMR in this field. [1-2]
References

[1] Daniel Pereira, et al. ACS Omega Article ASAP
[2] Marina Ilkaeva, et al. Journal of the American Chemical Society 2023 145 (16), 8764-8769

SST-021: Deciphering adsorption mechanisms of confined guests using NMR methods

Luís Mafra[1]
[1]CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

The most recent advancements in understanding CO2 speciation and trimethylphosphine oxide (TMPO) molecules confined in a range of porous materials using solid-state (ss) NMR spectroscopy, will be addressed.[1,2] Adsorbate speciation, encompassing type, location, and interaction of guest molecules with the host framework, critically influences many properties in catalytic and CO2-adsorbent materials such as gas sorption capacity/kinetics, selectivity, acid strength and cyclic stability. However, due to limitations in probing confined guest structures, an atomic-level picture of adsorption remains elusive, hindering the design of improved materials.
This talk showcases various examples on the use of ssNMR spectroscopy as a unique site-selective technique to: 1) investigate the structure and dynamics of CO2 species adsorbed at porous materials under dry and moist conditions;[3-5] 2) obtain CO2 adsorption isotherms, showcasing that ssNMR is the only way to quantify physisorbed and chemisorbed CO2 fractions (up to six distinct types of confined CO2), where other conventional techniques (volumetric or gravimetric adsorption) fail to distinguish distinct CO2 species;[6] 3) obtain unprecedented atomistic description of the host–guest and guest–guest interactions of TMPO probe molecules confined within HZSM-5 molecular-sized voids combining ssNMR and ab initio molecular dynamics (AIMD)-based computational modelling. This approach provides evidence of TMPO dimerization in confinement, challenging established acid site assignments with significant implications for probe molecule selection in porous material characterization.[2]

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.

References
[1] Sardo,M.,Morais,T.,Soares,M.,Vieira,R.,Ilkaeva,M.,Lourenço,M. Marin-Montesinos,I.,&Mafra,L.Chem. Comm.,2024,60,4015.
[2] Bornes,C.,Fischer,M.,Amelse,J.A.,Geraldes,C.F.G.C.,Rocha,J.,&Mafra,L.,J.Amer.Chem.Soc.,143,2021,13616–13623.
[3] Mafra,L.,Čendak,T.,Schneider,S.,Wiper,P.V.,Pires,J.,Gomes,J.R.B.,&Pinto,M.L.J.Amer.Chem.Soc.,139,2017,389–408.
[4] Vieira,R.,Marin-Montesinos,I.,Pereira,J.,Fonseca,R.,Ilkaeva,M.,Sardo,M.,&Mafra,L.J.Phys.Chem.C,125,2021,14797–14806.
[5] Sardo,M.,Afonso,R.,Juźków,J.,Pacheco,M.,Bordonhos,M.,Pinto,M.L.,Gomes,J.R.B.,&Mafra,L.J.Mater.Chem.A,9,2021,5542–5555.
[6] Ilkaeva,M.,Vieira,R.,Pereira,J.M.P.,Sardo,M.,Marin-Montesinos,I.,&Mafra,L.,J.Amer.Chem.Soc.,145,2023,8764–8769.

SST-022: PSi-chic f-block bonding: CSA analysis of tris-phosphide Lanthanide(III) complexes

Mr David V Bonham[1], Mr Jack Baldwin[2], Dr Gemma K Gransbury[2], Dr Toby Thompson[2], Dr George F S Whitehead[2], Dr Iñigo J Vitorica-Yrezabal[2], Prof Nicholas F Chilton[2,3], Prof David P Mills[2], Dr Daniel Lee[1]
[1]Department of Chemical Engineering, University Of Manchester, Manchester, United Kingdom, [2]Department of Chemistry, University Of Manchester, Manchester, United Kingdom, [3]Australian National University, Canberra, Australia

The participation of f-orbitals in bonding can lead to complex chemistry. This complexity results in diverse coordination geometries and bonding modes. With applications of f-block elements ranging from catalysis to nuclear energy, it is thus crucial to understand their bonding behaviour to help in designing and optimising their use. The understanding of f-block chemistry is currently limited for Lanthanide- Phosphorus (Ln-P) bonds [1] and there is relatively little literature on the tris(trimethylsilyl)phosphide ligand, $\{P(SiMe_3)_2\}$, for Ln-complexation, which presents it as a good candidate for study. To understand the ligand's electronic behaviour in bonding Ln elements, and thus Ln-P chemistry, the ^{31}P Chemical Shift Anisotropy (CSA) can be determined from solid-state NMR spectroscopy; for compounds containing Ln-P bonding, the CSA will not only report on chemical shielding (and as such the nature of bonding f-electrons) but also any influence from the quadrupolar and/or paramagnetic nature of the Ln isotope.
In this presentation, a structurally-analogous series of tris-phosphide Ln(III) complexes and their associated NMR spectra at 9.4 and 16.5 T will be discussed for Ln = (La, Ce, Pr, Nd, Sm). The CSA and related parameters will be highlighted and contrasted to silicon analogues, $\{Si(SiMe_3)_3\}$. Importantly, the ^{31}P CSA can be determined for all Ln, which is not the case for the ^{29}Si of Ln-Si in the silicon analogues.

[1]: J. Du, P. J. Cobb, J. Ding, and S. T. Liddle, Chem. Sci., 2024, 15, 13–45. doi: 10.1039/D3SC05056D

SST-023: Using 0.7 mm rotors at 1.2 GHz to investigate membrane proteins in liposomes

Spyridon Kosteletos[1], Dr. Evgeny Nimerovsky[2], Dr. Carl Öster[1], Dr. Sascha Lange[1], Dr. Loren Andreas[2], Prof. Dr. Adam Lange[1]
[1]Department of Molecular Biophysics, Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Buch, Germany, [2]Department of NMR based Structural Biology, Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany

Rhomboid proteases are a family of intramembrane enzymes, capable of cleaving substrates embedded within lipid bilayers. Their malfunction has been correlated to diseases such as Alzheimer's and Parkinson's. Structural properties can reveal information important to drug development. Expressing mammalian proteins has several limitations, though. By comparing evolutionarily related proteins, we can gain valuable insight and thereby avoid such limitations.
E.coli's GlpG is one of the best-studied rhomboid proteases. Our group has been studying this system extensively via solid state (ss) NMR [1, 2, 3], using prior deuteration and proton-back exchange of the sample. This labelling pattern has limited the observation of residues embedded within the lipid bilayer. Here, using ultrafast MAS, we were able to observe part of these regions, as well as the cytosolic domain of GlpG. For this purpose, [^{13}C, ^{15}N]-labelled GlpG reconstituted into proteoliposomes was packed into a 0.7 mm rotor and spun up at 100 kHz. NMR spectra were recorded using a 1.2 GHz magnet.
The high spectral quality allows the assignment of residues via a backbone walk, previously not accessible from deuterated samples. Being able to identify intramembrane sections will delineate the structural properties of GlpG. This method allowed us to study GlpG (276 residues) using only ~250 µg of protein, which bodes well for the investigation of eukaryotic rhomboid proteases in the future.

1. Shi et al. "Structure and dynamics of the rhomboid protease GlpG in liposomes studied by solid-state NMR." JACS 141.43 (2019): 17314-17321.
2. Bohg et al. "A combination of solid-state NMR and MD simulations reveals the binding mode of a rhomboid protease inhibitor." Chemical Science 12.38 (2021): 12754-12762.
3. Bohg et al. "The opening dynamics of the lateral gate regulates the activity of rhomboid proteases." Science Advances 9.29 (2023): eadh3858.

SST-024: Insights into Na insertion in organic carboxylate anodes using solid-state NMR spectroscopy and DFT calculations

Mr Heitor Seleghini[1], Dr Kieran Griffiths[2,3], Dr Aamod V. Desai[1,3], Dr Valerie R. Seymour[2,3], Dr Robert Armstrong[1,3], Prof Russell E. Morris[1,3], Prof John M. Griffin[2,3], Prof Sharon E. Ashbrook[1]
[1]University Of St Andrews, St Andrews, United Kingdom, [2]Lancaster University, Lancaster, United Kingdom, [3]The Faraday Institution, Didcot, United Kingdom

The growing demand and cost increase of lithium-ion batteries (LIBs) has prompted intense research on the development of similar technologies based on more abundant and cheaper elements, with one of the alternatives being the use of Na as a replacement for Li.[1] Many of the materials used in LIBs are not suitable for sodium-ion batteries (NIBs), posing a particularly significant problem for anode materials, as Na intercalation is often unfavourable and materials can suffer from significant volume changes and mechanical stress as a result.[1]

One class of possible anode materials for NIBs are conjugated carboxylate-based coordination compounds, which do not exhibit large volume changes or significant modifications to the long-range structure upon Na intercalation, but still require improvement concerning their conductivity, cycling stability and energy density.[1] Such materials are usually synthesised in a single step from the reaction of an organic carboxylic acid with sodium hydroxide, and upon cycling, the electrochemical insertion of Na occurs together with the reduction of the organic backbone.[1,2]

Aiming to understand the Na insertion, together with the structural changes resulting from multiple charge and discharge cycles of the sodium benzenedicarboxylate (Na_2BDC) NIB anode, an ex-situ study of the electrochemical charging and discharging process has been performed. This has been combined with a study of the chemically sodiated anode material synthesised using a radical mediator. This work was carried out using multinuclear and multidimensional solid-state NMR spectroscopy, alongside ab initio random structure searching (AIRSS) and DFT calculations to help understand the structural changes taking place. From such techniques, it was possible to study the dynamic of sodium ions in the material, the identification of different sodiated polymorphs, and the structural changes caused by the insertion and removal of sodium upon cycling on the Na_2BDC anode.

References
[1] ChemSusChem, 2020, 13, 4866.
[2] Magn.Reson.Chem., 2022, 60, 489.

SST-025: NASICON-based electrode electrochemical degradation studied by ex-situ solid state NMR

Dr Vytautas Klimavicius[1], Dr. Jurgis Pilipavicius[2,3], Ms. Nadezda Traskina[2,3], Mr. Aurimas Dubauskas[1], Mr. Matas Manionis[1], Dr. Linas Vilciauskas[2,3]
[1]Vilnius University, Institute of Chemical Physics, Vilnius, Lithuania, [2]Center for Physical Sciences and Technology, Vilnius, Lithuania, [3]Vilnius University, Institute of Chemistry, Vilnius, Lithuania

The shift towards sustainable energy necessitates the development of energy storage technologies that rely on non-critical materials. Aqueous Na-ion batteries, offering enhanced safety, lower costs, and greater sustainability, present a promising alternative to Li-based technologies. Although Na-ion batteries typically have a lower energy density, this trade-off is less significant for applications such as stationary power grid stabilization.

This study highlights recent advancements in the development of NASICON-structured (Na Super Ionic CONductor) materials for use as battery electrode materials. NaTi2(PO4)3 (NTP) was selected as the starting material and was coated with a carbon nanolayer to enhance electronic conductivity. The resulting composite, combined with a binder and carbon filler, was applied to current collectors using the doctor blading method. These electrodes were then used to assemble an electrochemical cell, which underwent cycling. Post-cycling, the NASICON-based electrode materials were prepared for solid-state NMR measurements.

31P MAS NMR measurements revealed the formation of amorphous phosphate phases during the cycling of the electrochemical device. Over time, the amount of these amorphous phosphates decreases due to interactions with the electrolyte, which effectively dissolves the degradation products. 23Na MAS and MQMAS NMR measurements highlighted the role of sodium sulfate (Na2SO4) used as an electrolyte in the formation of sodium carbonates throughout the device's lifetime. 13C CP MAS data confirmed the formation of carbonates. Additionally, 47/49Ti MAS NMR spectra indicated a reduction in the amount of NTP after electrochemical cycling.

Funding by the Research Council of Lithuania under grant S-MIP-23-47 is gratefully acknowledged.

SST-026: New correlation methods in solid-state NMR

Pr Jean Paul Amoureux[1], Dr Yury Kolyagin, Dr Julien Trebosc, Dr Hiroki Nagashima, Pr Olivier Lafon
[1]Lille University France, Lille, France, [2]AIST, Tsukuba, Japan

We present four innovative 2D techniques for solid-state NMR.
1) through-bond homonuclear correlation (J-HOMCOR). We present (i) how a Composite Refocusing (CR) block can improve the 1D resolution for crowded spin-systems (e.g. 13C nuclei in fully labeled proteins), and (ii) a new robust 2D refocused INADEQUATE experiment incorporating a z-filter and a CR block, offering improved resolution along F2 dimension;
2) through-space heteronuclear correlation (D-HETCOR). We present 2D several T-HMQC correlation methods to probe proximities between distinct half-integer quadrupolar isotopes. These methods are easy to set up and very robust with respect to the offset and spinning speed fluctuations, which limits the amplitude of t1-noise. In particular, we propose the T-HMQC� variant, where two additional �-pulses selective of the central transition (CT) allow the selective observation of the CT single-quantum coherences along the indirect dimension;
3) high-resolution D-HETCOR using multiple-quantum filter. We present a new D-HMQC-MQ sequence to observe through-space correlations between spin-1/2 and -3/2 nuclei with isotropic resolution along the indirectly detected quadrupolar channel.
4) DNP-enhanced MQMAS (see Nagashima et. al, J. Phys. Chem. Lett., 15, 18 (2024) 4858). We present several pulse sequences to transfer the DNP-enhanced 1H polarization to quadrupolar nuclei and subsequently acquire their 2D MQMAS spectra. By varying the duration of the transfer, we can separate the signals of the quadrupolar nuclei in the surface, subsurface and bulk regions of nanoparticles.

SST-027: Satellite transition NMR at 225 kHz MAS

Dr Ago Samoson[1], Ats Kalldma, Mirtel Tomiing, Caspar Koidla, Mai-Liis Org, DR Kalju Vanatalu
[1]Tallinn University Of Technology, Tallinn, Estonia

Faster spinning offers several new perspectives for NMR. While evolution of H-MAS applications in structural biology is well established, domain of other, in particular quadrupole nuclei, is much less explored. Nuclei with spins of 5/2 (like 17O) and 9/2 are of particular interest due to smaller powder spread of the second order quadrupolar interaction on satellite transitions as compared to the central transition. We show how increased spinning rates fight the first order quadrupolar interaction and allow to concentrate more intensity in the spinning centerband, also how magic angle tuning will affect the spectra.

Reference: Samoson, A. (1985) Satellite transition high-resolution NMR of quadrupolar nuclei in powders. Chem. Phys. Lett, 119, 29–32

SST-028: Characterisation of membrane proteins with 19F NMR at 100 kHz MAS using fluorinated amino acids

Mr Samuel Seidl[1], Ms Anouk Ebenezer[1], Ms Jennifer Anne Winzer[1], Dr. Johanna Becker-Baldus[1], Prof. Dr. Clemens Glaubitz[1]
[1]Goethe University Frankfurt, Frankfurt am Main, Germany

Introduction
Membrane proteins are an essential interface between cells and their surroundings. Despite advancement in their structural elucidation, a biophysical characterisation especially in a membrane environment remains challenging due to their preparation, yield or size. In NMR spectroscopy, the dynamic nature of membrane proteins can complicate a spectrum when conventional extensive 13C/15N labelling is used (Verardi et al. 2012). Subsequently, site-selective fluorine labelling offers an alternative to simplify spectra and its responsiveness to environmental changes (Kitevski-LeBlanc and Prosser 2012).
Methods
Here, we explore 19F-MAS-NMR recorded at 100 kHz sample spinning rate using green light-absorbing proteorhodopsin (GPR) embedded in liposomes as a model system. GPR was labelled with 5-fluorotryptophan. The ultrafast MAS NMR approach was chosen to take advantage of efficient averaging of 19F-1H dipolar couplings and CSA at high field (850 MHz).
Results
We incorporated 5-fluorotryptophan into GPR in order to evaluate the spectral quality for this approach for membrane proteins in order to characterise the side-chain dynamics by simple 1D spectra and T2 measurements.
All of the 10 19F-Trp can be resolved in 1D spectra and have been partially assigned. Sidechain dynamics were explored by temperature and pH-dependent measurements. Individual lineshapes were characterised by spectral deconvolution and T1/T2 measurements. Mutations have been used for assignment and characterisation of the functional impact of 19F labelling of individual tryptophans at the oligomer interfaces and within the retinal binding pocket.
Conclusions
19F-MAS NMR at 100 kHz MAS yields well-resolved spectra for large anisotropic systems which allows to resolve the role of tryptophans at functionally important sites within GPR (Maciejko et al. 2019).

SST-029: Analysis of Oregano Adulteration with Olive Leaves Using Solid-State NMR Spectroscopy

Dr David Pantoja-Uceda
[1]Instituto Química Física "Blas Cabrera", IQF. CSIC, Madrid, Spaian

The adulteration of spices is an increasing problem in the food industry, with significant implications for product safety and quality. According to a recent European Union report, oregano is among the most adulterated spices, often mixed with olive leaves. This study aims to address this issue using solid-state Nuclear Magnetic Resonance (ssNMR) spectroscopy to identify and differentiate the spectral patterns of natural oregano and olive leaves.
First, pure samples of oregano and olive leaves were collected, dried, and prepared for spectroscopic analysis. SsNMR spectra were obtained for each type of leaf, allowing for the identification of distinctive spectral features. Subsequently, commercial oregano samples were analyzed to detect the presence of adulterants.
Preliminary results show unique spectral patterns for oregano and olive leaves, facilitating the identification of adulteration in commercial samples. The solid-state NMR technique has proven effective in distinguishing between pure leaves and detecting the presence of olive leaves in products labeled as pure oregano.
This study highlights the importance of implementing solid-state NMR, to ensure the authenticity and quality of commercialized spices. The ability to accurately identify adulteration not only protects consumers but also strengthens confidence in the food supply chain.

NMR experiments were performed in the "Manuel Rico" NMR Laboratory (LMR) of the Spanish National Research Council (CSIC), a node of the Spanish Large-Scale National Facility for Biomolecular NMR (ICTS R-LRB)

SST-030: Cryogenic 13C and 3He NMR of novel endofullerenes and endofullerides

Murari Soundararajan[1], George R Bacanu[1], Vijyesh K Vyas[1], Francesco Giustiniano[1], Mark Walkey[1], Gabriela Hoffman[1], Sally Bloodworth[1], Richard J Whitby[1], Malcolm H Levitt[1]
[1]University Of Southampton, Southampton, United Kingdom

The fullerene C_{60} is a truncated icosahedral molecule with an internal cavity large enough to accommodate guest molecules. Through molecular surgery [1], it is possibly to reliably insert small molecules into C_{60} with high yield, which exhibit interesting properties due to the confinement and structure of the cages.
We present here ^{13}C NMR of $^{13}CO@C_{60}$ indicating a change in the rotation of the endohedral molecule at cryogenic temperatures from isotropic to hindered, and a preliminary investigation of this behaviour.

Alkali metals are known to react with C_{60} to produce intercalation compounds known as fullerides, with unconventional electronic properties [2]. Endohedral molecules inserted into the cages of such fullerides serve as sensitive probes of the electronic environment of the cages. We present ^{3}He NMR of the endohedral species in the fulleride $Rb_3(^{3}He@C_{60})$ above and below its superconducting transition temperature of 30 K, displaying the characteristic Knight shift and Korringa behaviour, along with T_2 and exchange correlated NMR measurements to investigate superconducting vortex dynamics.

[1] Bloodworth and Whitby, Commun. Chem. (2022) 5 121
[2] Takabayashi and Prassides, Philos. Trans. Royal Soc. A (2016) 374, 20150320

SST-031: From polydopamine precipitate to nanometer coating layers – a structural comparison using solid-state NMR spectroscopy

PhD student Andrea Simion[1,2], Dr Xenia Filip[1], Dr. Ioana-Georgeta Grosu[1], Dr. Ana-Maria Raluca Gherman[1], Dr. Claudia Lar[1], Dr. Claudiu Filip[1]
[1] National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania, [2]Babes Bolyai University, Cluj-Napoca, Romania

Abstract: Despite many investigations, the atomic-scale description of the adhesion mechanism of polydopamine (PDA) is not fully elucidated. In this work, a new experimental approach is proposed to understand the mechanism that occurs at the interface between PDA thin films with different thicknesses deposited onto SiO2 nanoparticles. For this purpose, the dopamine's catechol ring was deuterated, and the two positions of the carbon atoms of the aliphatic chain were isotopically labeled. 2H solid echo and 13C/15N cross-polarization solid-state NMR experiments combined with electronic microscopy were used for the characterization of the prepared compounds. Nano-sized PDA coating layers were found to be structurally equivalent to bulk PDA (precipitate). A large number of strong and rigid packed monomeric units carrying aliphatic carbons and -NH3+ were found to have a quite high contribution to the PDA surface for films with a thickness of less than 5 nm, compared to that of the inner oligomer layers. Based on these findings, the PDA adhesion mechanism, as well as further possible correlations that could be explored at the PDA-substrate interface using this approach are discussed.

Acknowledgments: This study was funded by the MCID through the "Nucleu" Program within the National Plan for Research, Development, and Innovation 2022–2027, Project PN 23 24 01 05, and through the Installations and Special Objectives of National Interest (IOSIN), IZOSTAB. A.S. acknowledges the Doctoral Fellowship from the Babes Bolyai University, Cluj-Napoca, Romania.

References:
[1] X. Filip, A. Simion et all., Applied Surface Science 649(2007):159190, DOI: 10.1016/j.apsusc.2023.159190

SST-032: Solid-state NMR of quadrupolar nuclei in magnetically oriented microcrystals

Tomoya Kamide[1], Yasuto Noda[1], Kazuyuki Takeda[1]
[1]Graduate School of Science, Kyoto University, Kyoto City, Japan

Microcrystals suspending in viscous medium can be aligned three-dimensionally (3D) by a modulated rotating magnetic field, forming a 3D Magnetically Oriented Microcrystal Suspension (MOMS) [1], and leading to a spectrum akin to that would be obtained from a single crystal. Here, we extend NMR of MOMS, previously demonstrated for ^{13}C NMR [2], to quadrupolar nuclei with a spin quantum number greater than one-half. Through the MOMS approach, we aim at high-resolution measurements of otherwise extremely broad resonance line of the quadrupolar nuclei.
In this work, we demonstrate ^{14}N (spin1) NMR of a MOMS of L-alanine [3]. Using a home-built ^{1}H-^{13}C-^{14}N triple resonance probe equipped with a stepping-motor-driven, sample rotation functionality, microcrystals of L-alanine dispersed in insoluble liquid with viscosity of 12 Pa s were aligned to a common orientation in 7 T by repeating a cycle of 180-degrees turn within 2 s followed by a 1 s pause. By varying the tilt angle of the sample relative to the field at the moment of ^{14}N signal acquisition, a rotation pattern similar to, but not exactly identical to, that for the single crystal was obtained. The origin of the slight discrepancy and the resonance linewidth are discussed in terms of potential misalignment and distribution of the orientations of the microcrystals.

[1] T. Kimura, T. Tanaka, et al., Crystal Growth and Design 13 (5) (2013) 1815–1819.
[2] R. Kusumi, H. Kadoma, et al., Journal of Magnetic Resonance 309 (2019) 106618.
[3] T. Kamide, Y. Noda, K. Takeda, Solid State Nuclear Magnetic Resonance 131 (2024) 101924.

SST-033: Investigation of the mechanosensitive channel MscLby solid-state NMR

Alexandra Kovinko[1], Prof. Dr. Chaowei Shi[2], Dr. Carl Öster[1], Dr. Sascha Lange[1], Natalja Erdmann[1], Prof. Dr. Adam Lange[1]
[1]Leibniz-Forschungsinstitut Für Molekulare Pharmakologie (FMP), Berlin, Germany, [2]University of Science and Technology of China, Hefei,, China

MscL is a mechanosensitive channel of large conductance with a homopentameric structure and the largest gated pore. It acts as an emergency valve, releasing cytoplasmic solutes during osmotic changes, and is found in most bacterial species. This feature makes it a promising target for a new class of antibiotics. Although extensive research has been conducted to solve the structure of the closed state of MscL using methods such as X-ray crystallography and molecular dynamics simulations, the molecular structure of the open state remains unknown.

Traditional methods like patch-clamp electrophysiology or FTIR spectroscopy have provided significant insights into the MscL channel, but they lack the precision necessary for studying molecular structure in detail.

Magic angle spinning solid-state NMR (MAS ssNMR) is a crucial technique that enables the highest possible resolution for studying small, insoluble proteins, allowing exploration under various pH and temperature conditions. Therefore, we use MAS ssNMR to investigate the MscL protein from Escherichia coli (Ec-MscL) and aim to elucidate the structure of its open state in the future.

Previously, MscL from Methanosarcina acetivorans (Ma-MscL) has been investigated using carbon-detection ssNMR experiments. In our project, we used a deuterated sample of Ec-MscL embedded in azolectin lipids. By applying a high external magnetic field (900 MHz) and ultrafast MAS spinning (55 kHz), we performed proton-detection experiments and recorded high-resolution spectra for the backbone assignments.

Assuming that the channel remains inactive in the rotor during spinning, we can assign the protein in its closed state. Our long-term aim is to acquire NMR spectra of MscL in its open state, which will provide new insights into the functionality of the protein at atomic resolution.

SST-034: MAS NMR investigation of the ubiquitin-like modifier FAT10 and its interaction with the proteasome

Charlotte Weiss[1], Nicola Catone[2], Leon Schöwe[1], Dr. Venkata SubbaRao Redrouthu[1], Dr. Annette Aichem[3], Prof. Marcus Groettrup[3], Dr. Guinevere Mathies[1]
[1]Department of Chemistry, University of Konstanz, Konstanz, Germany, [2]Biotechnology Institute Thurgau, Kreuzlingen, Switzerland, [3]Department of Biology, University of Konstanz, Konstanz, Germany

FAT10 (human leukocyte antigen-F adjacent transcript 10) is an alternative signal (besides ubiquitin) for fast and irreversible protein degradation by the 26S proteasome. It plays a role in cellular reactions against incoming pathogens, but much remains unknown about its function(s). FAT10 is weakly folded and for this reason only structures of stabilized mutants are available.[1,2] These stabilized forms of FAT10 are well conjugated, but degradation is hampered, suggesting that the loose folding is biologically relevant.

We first investigated a microcrystalline sample of the stabilized N-domain of FAT10. Based on 13C-13C, 15N-13C, and 15N-13C-13C correlation experiments at 800 MHz, we could assign 62 of the 83 amino acid residues. Subsequent torsion angle analysis showed good agreement with the X-ray structure.[2] This showed that investigation of FAT10 by MAS NMR is feasible.

Attempts to prepare isolated wild-type N-domain of FAT10 in a regular structure were not successful. We therefore invoked an alternative strategy: we prepared complexes of stabilized N-FAT10 and of wild-type N-FAT10 with the adapter protein NUB1L (NEDD8 ultimate buster 1 long). NUB1L binds N-FAT10 via ubiquitin-associated domains of class 3 and speeds up proteasomal degradation. 13C-13C, 15N-13C, and 15N-13C-13C correlation spectra have been recorded. Resonance assignments will be a first step towards determining the structure of wild-type FAT10. Chemical shift perturbations will give insight into conformational changes upon binding to NUB1L.

To date, no interaction motifs of the FAT10 targeting mechanism for proteasomal degradation have been identified. Careful reading of the literature, however, suggests a motif for the interaction between the N-domain of FAT10 and ubiquitin-associated domains of class 3. A calculation with AlphaFold in multimer mode has tentatively confirmed this hypothesis. We expect that our investigations will deliver the experimental evidence.

1. Theng et al. PNAS 2014
2. Aichem et al. Nat. Commun. 2018

SST-035: A TLR4/MD-2 directed mechanism of Polymyxin antibiotics to restrain LPS mediated proinflammatory immune responses

Mr Ali Javed[1], Mr Roy van Beekveld[1], Mr Charalampos Ntallis[1], Mr Markus Weingarth[1]
[1]Utrecht University Netherlands, Utrecht, Netherlands

Introduction: Antimicrobial peptides (AMPs) have emerged as a promising alternative to conventional antibiotics not only because of their novel bactericidal mechanisms but also for their additional immunomodulatory potential and the ability to enhance host defense. TLR4 is an important therapeutic target to control LPS-induced dysregulated immune response i.e. sepsis from gram negative bacterial infection. LPS neutralizing AMPs have been shown to antagonize TLR4 signaling by interfering with LPS-TLR4 complex formation directly or indirectly.
Aim: We aimed to investigate the function of Polymyxin E (PME), Polymyxin B (PMB) and its derivative Chimeric peptide antibiotic (CPA) to antagonize TLR4 signaling by directly interacting with the signaling components of the host cell.
Methods: Flow cytometry and confocal microscopy were used to assess PMB and LPS attachment on mammalian cells. LPS neutralization was assessed by determining TLR4 mediated cell activation. Molecular docking and Molecular Dynamics (MD) simulations were used to assess the binding mode of Polymyxin and the TLR4/MD2 complex. Lastly, cross-polarization in-cell solid-state NMR spectroscopy was used to determine binding affinity of 15N labelled Polymyxin for HEK cells.
Results: Experiments showed that the peptides' pre-treatment prevented the binding of LPS to cell surface in a concentration and time dependent manner. Also, the LPS internalization of TLR4 was shown to be inhibited by peptides' pre-treatment, indicating a competition between peptide and LPS for the TLR4/MD2 binding site. Polymyxin was shown in silico to reside in the LPS binding pocket of TLR4/MD-2. Furthermore, Polymyxin showed comparatively more affinity for TLR4 expressing HEK cells.
Conclusion: Polymyxin antibiotics were shown to interact with host-cell membranes with targeting specifically TLR4 mediated signaling. Polymyxin competes with LPS for TLR4/MD2 binding thus has an extra anti-inflammatory tool. Further investigation could provide important insights to develop AMPs with optimized TLR4 targeting properties.

SST-036: Cristallisation kinetics of ITQ-13, a solid-state NMR study.

Matteo Tarchi[1], Dr. J. Alejandro Vidal Moya[1], Dr. Fernando Rey[1], Dr. Teresa Blasco[1]
[1]Instituto de Tecnología Química, Universitat Politècnica de València – Agencia Estatal del Consejo Superior de Investigaciones Científicas, Avda. de los Naranjos s.n., 46022, VALENCIA, España

Zeolite ITQ-13 exhibits an ITH structure, featuring three intersecting medium-pore channels (9x10x10) within an orthorhombic unit cell, housing d4r, stf, lau, and mel cages.[1] When synthesized using HF-containing gels, fluoride is typically incorporated into the smallest cages, compensating OSDA cations. Moreover, Ge incorporation stabilizes d4r cages by replacing Si.

This study investigates the crystallization of pure silica and Ge-containing ITQ-13 zeolite, synthesized in a fluoride medium with hexametonium as the OSDA. The focus is on understanding the roles of fluoride anions, OSDA cations, and Ge incorporation during crystallization, primarily using solid-state NMR and XRD.

Samples were synthesized heating at 175 ºC during different times (1,2,4,6,10 and 15 days) gels of the following composition:
(1-x) SiO_2 : x GeO_2 : 0.28 $HM(OH)_2$: 0,56HF : 7H_2O.
29Si and 19FNMR spectra of the resulting materials were recorded with Bruker Avance III HD 400 MHz spectrometer.
The XRD patterns of pure silica and Ge-containing (Si/Ge = 6) ITQ-13 samples are characteristic of ITQ-13 with some peaks of ITQ-34 zeolite, especially in the first stages of the synthesis [2]. The 19F NMR spectra show two signals at -38 and -66 ppm corresponding to fluoride ions in the d4r cage and mel cages respectively[3,4] The intensity of both signals increases with the crystallisation time. As for pure silica, the 19F NMR spectra of the Ge-containing samples show two signal at -8 and -20 ppm of fluoride within the d4r and the mel cages, respectively, with Ge atoms incorporated in it.[5] Results allow to conclude that as the zeolite crystallization proceeds, more fluoride anions are incorporated in ITQ-13 zeolite, and that the the cristallisation process could pass through a rearrangement of one zeolite into the other.

1. Corma, A (2003).
2. Corma, A. (2009).
3. doi:10.26434/chemrxiv-2021-3n170.
4. Pulido, A. (2006).
5. Vidal-Moya, J. A (2003).

SST-037: Sensitivity Enhancement for Half-Integer Quadrupolar Nuclei.DFS, QCPMG and SSFP revisited

Prof.dr. Arno Kentgens[1], Angel Wong[1], Dr. Mattia Negroni[1]
[1]Magnetic Resonance Research Center, Radboud University, Nijmegen, Netherlands

Quadrupolar nuclei (I > ½) (> 74 % of nuclides in the periodic table) are relevant in material science. Unfortunately, quadrupolar nuclei are often insensitive owing to a low gyromagnetic ratio, natural abundance, and/or large quadrupole moment. As such, sensitivity enhancement techniques are often needed for practicality. For half-integer quadrupolar nuclei, the sensitivity of the central transition can be enhanced via double frequency sweeps (DFS).[1] In DFS, satellite transition population is inverted into the central transition, resulting in a higher population difference than thermal equilibrium. The enhancement can be further boosted if multiple population inversions are performed. This can be achieved via rDFS, where the DFS and detection block is repeated several times before allowing the spin system to relax back to thermal equilibrium.[2] Despite its benefits, studies on rDFS have been surprisingly limited since its introduction. In particular, investigations have only been accomplished using a repetition time (τ_rep) significantly less than T1 (e.g. 0.001T1), while the behavior of rDFS using other τ_rep regimes (i.e., 0.001T1 < τ_rep < 5T1) has yet to be examined. Furthermore, previous rDFS studies were performed using one-pulse as detection, and the combination of rDFS with sensitivity-enhancing detection techniques has yet to be explored. Here, we investigate the effect of τ_rep on rDFS and show that rDFS can reach a steady state when τ_rep is increased. By combining rDFS with techniques such as QCPMG, a drastic boost in sensitivity was observed. We explored this for isotopes with different spin quantum numbers such as 39K (I=3/2), 17O (I=5/2) and 49Ti (I=7/2). Enhancements up to 46x were achieved under steady-state conditions, translating to a 20x increase in sensitivity enhancement per unit time.

[1] A.P.M. Kentgens and R. Verhagen, Chem. Phys. Lett. 300 (1999) 435.
[2] A. Brinkmann and A.P.M. Kentgens, J. Phys. Chem. B 110 (2006) 16089.

SST-038: Integrating molecular dynamics simulations and solid-state NMR experiments to uncover the mechanisms of membrane-active antibiotics against Gram-negative bacteria.

MSc Charalampos Ntallis[1], MSc Roy van Beekveld[1], Dr Vlad Cojocaru[2,3], Dr Markus Weingarth[1]
[1]NMR Spectroscopy, Bijvoet Centre for Biomolecular Research, Department of Chemistry, Faculty of Science, Utrecht University, Utrecht, Netherlands, [2]STAR-UBB Institute, Babeș-Bolyai University, , Cluj-Napoca, Romania, [3]Computational Structural Biology Group, Utrecht University, Utrecht, Netherlands

The rapid emergence worldwide of Gram-negative 'superbugs' adds significantly to the antimicrobial resistance (AMR) crisis and has an overwhelming impact on human health. The urgent need for novel drugs that can overcome AMR underlines the importance of understanding the mode of action of membrane targeting antibiotics. The cell envelope of Gram-negative bacteria consists of an inner membrane, surrounded by a thin peptidoglycan cell wall and an outer membrane (OM), with the latter being the target of lipopeptide antibiotics of last resort for treating multi-drug resistant infections. The OM is a unique asymmetric bilayer and the complex lipopolysaccharides (LPS) make it a daunting task to study its structure and dynamics. In the past molecular modelling approaches neglected the asymmetric nature of OM and/or utilized coarse-grained force fields, mainly due to computational cost. In this study, we present a computational approach to build a realistic and asymmetric OM model of the Gram-negative bacterium Escherichia coli using all-atom Molecular Dynamics (MD) simulations and high-performance computing. In the future, the integration of the experimental ssNMR data with the MD simulations will serve for the understanding of the killing mechanism of antibiotics that target the OM. Highlighting the molecular mechanisms that lead to AMR in pathogenic Gram-negative bacteria can provide the rational for designing new therapeutic strategies.

SST-039: Elucidation of ECM dynamics through solid-state INEPT enabled by phase-modulated dipolar decoupling

Ms Annika J. Wegner-Repke[1], Dr Uliana Bashtanova[1], Professor Melinda J. Duer[1]
[1]Department of Chemistry, University of Cambridge, Cambridge, United Kingdom

The extracellular matrix (ECM) consists of various proteins, lipids and sugars, with collagen being one of the major components. Its relative content in the matrix influences the mobility of surrounding ECM constituents and overall matrix mechanical properties, which affect cell behaviour. Solid-state NMR is a unique tool to study these dynamics on timescales relevant to cell signalling. ^{13}C CP/MAS and INEPT are polarisation transfer experiments used complementarily to elucidate molecular dynamics. Although INEPT is usually only efficient in the fast motional regime, Elena et al. reported a method for through-bond coherence transfer in solids by employing decoupling using mind-boggling optimisation (DUMBO).

We aim to characterise differences in the molecular dynamics between two hydrated U-^{13}C proline labelled, in vitro-grown ECM samples with different collagen contents by applying the DUMBO INEPT experiment, comparing results with CP/MAS and INEPT spectra. At short polarisation transfer times (τ), DUMBO INEPT selectively yielded spectra of rigid protein components, primarily collagen for sample 1 and a random coil protein – like elastin – for sample 2. Polarisation transfer efficiency decreased with increasing τ for more immobile proline ^{13}C, due to faster loss of transverse magnetisation of directly bonded protons, indicating different proline ring-pucker motions in the two samples. The emergence of signals from (phospho)lipids at longer τ delays in the collagen-rich matrix (sample 1) indicates their high mobility. In contrast, in sample 2 the presumed phospholipid headgroup ^{13}C signals appear at lower DUMBO INEPT τ times and are absent in the INEPT spectrum, suggesting lower flexibility.

Overall, we demonstrated that DUMBO INEPT performs efficiently on fully hydrated, frozen ECM samples, and captures ^{13}C signals from rigid as well as mobile components in one spectrum. Variation of DUMBO INEPT τ values allows spectral editing, providing valuable information about biomolecular dynamics without the need for specific relaxation time measurements.

SST-040: Solid-state NMR for methyl group resonance assignment

Dorothea Dierks[1], Dr. Alexander Klein[1], Prof. Dr. Rasmus Linser[1]
[1]Tu Dortmund, Dortmund, Germany

Understanding protein dynamics is essential for unravelling biological processes, such as enzymatic catalysis and regulatory cues. Nuclear magnetic resonance (NMR) spectroscopy provides a powerful tool to investigate these dynamics in detail. However, traditional solution-state NMR has limitations, especially for large proteins. To overcome these limitations, we are developing fast-MAS solid-state NMR methods, in combination with different labelling schemes, for resonance assignments, structure elucidation, and additional relaxation parameters.
For assessing NMR structures and dynamics, side-chains protons represent crucial reporters. Especially methyl protons represent valuable reporters of protein dynamics, aided by the variety of available precursors for their selective labelling. Unambiguous NMR resonance assignment, however, represents a major bottleneck. Here we present a new pulse sequence combining higher-dimensional amide-to-amide (HNcacoNH) with sidechain-to-backbone (HCccaNH) correlations in a time-shared manner. While the direct linking of the amide correlation will enable an unambiguous backbone walk, the methyl groups can be assigned to the primary structure in a straightforward way using the simultaneous sidechain-to-backbone correlations. We introduce the outlined strategy, which we expect to be paramount for interrogating various aspects of protein functionality, presenting preliminary results on two protein targets, the SH3 domain of α-spectrin as well as human carbonic anhydrase II (hCAII), an enzyme and drug target of 29 kDa molecular weight.