Schedule for A Celebration of Magnetic Resonance at McMaster

65 Years of Magnetic Resonance at McMaster – from 1958 to 2023

Date: Friday, 13 OCT 2023
Time: 13:00 – 16:00 (1:00 PM – 4:00 PM) EDT
Location: MDCL-1105, McMaster University Main Campus

Schedule

- Welcome and Opening Remarks
- Remarks by **Dr. David Farrar**, President of McMaster University
- Remarks by **Dr. Gianni Parise**, AVP Research, McMaster University
- First invited speaker (Introduction by Gillian Goward)
  Magnetic resonance spectroscopy and imaging to study pregnancy and pregnancy complications, **Lindsay Cahill**, *Memorial University of Newfoundland*

- 14:15 – 14:45 BREAK

- 65 Years of Magnetic Resonance at McMaster (Bob Berno)
- Second invited speaker (Introduction by Giuseppe Melacini)
  Extreme Dynamics – A Critical Component of Biological Function – Through the Looking Glass of NMR Spectroscopy, **Lewis E. Kay,** *University of Toronto*

- 16:00 Closing Remarks

The 34th MOOT NMR Symposium

McMaster University
14-15 OCT 2023

Conference Schedule

Saturday, 14 OCT 2023: MDCL Building

8:15 – 9:00 Registration and Coffee/Pastries
9:00 – 9:10 Opening Remarks

Session A (Chair, Giuseppe Melacini), MDCL-1105

9:10 – 9:55 Plenary Lecture: Exploring conformational dynamics of the p97 molecular machine using solution NMR spectroscopy, **Rui Huang**, *University of Guelph*
9:55 – 10:15 1. Impact of Functional Groups on Lithium Salt Dispersion and Mobility in Polymer Electrolytes, **Gabrielle Foran,** *Université de Montréal*
10:15 – 10:35 9. Introducing Oxygen-17 labels onto amino acid side chains: L-serine and L-threonine, **Yuying Huang**, *Queen's University*

<BREAK>

Session B (Chair, Tara Sprules), MDCL-1105

10:55 – 11:15 2. Exploring host-guest interactions within a 600 kDa DegP protease cage complex by hydrodynamics and methyl-TROSY NMR, **Robert Harkness**, *University of Toronto*
11:15 – 11:35 5. Structure elucidation of bioactive compounds from Natura Health Products (NHP) using NMR Spectroscopy -A rapid authentication tool for quality assurance, **Vinayagam Varathan**, *University of Guelph*
11:35 – 11:55 13. Unravelling the local environments of halogen ions in MOFs with ^{35}Cl and ^{127}I solid-state NMR spectroscopy, **Wanli Zhang**, *Western University*

LUNCH: 12:00 – 13:30

Session C (Chair, Martine Monette), MDCL-1105

13:30 – 14:15 Plenary Lecture: Solid-State NMR Spectroscopy of the Periodic Table Enabled by Sensitivity-Enhanced Methods, **Aaron Rossini**, *Iowa State University*

14:15 – 14:35 12. Elucidating the activation mechanism of caspase-9 on the apoptosome using methyl-TROSY NMR, **Alexander Sever**, *University of Toronto*

14:35 – 14:55 17. Characterization of Conformational States of the Homodimeric Enzyme Fluoroacetate Dehalogenase by ^{19}F-^{13}C Two-Dimensional NMR, **Motasem Suleiman**, *University of Toronto (UTM)*

<BREAK>

Session D (Chair, Kevin Sanders), MDCL-1105

15:15 – 15:35 15. Early-Onset Parkinson Mutation Remodels Monomer - Fibril Interactions to Allosterically Amplify Synuclein's Amyloid Cascade, **Jinfeng Huang**, *McMaster University*

15:35 – 15:55 19. QUADRUPOLE-CENTRAL-TRANSITION NMR SPECTROSCOPY OF ALKALI METAL IONS, **Ziyao Peng**, *Queen's University*

15:55 – 16:15 34. Identifying the structural arrangement of co-factor p47 upon interaction with the AAA+ enzyme p97 using Paramagnetic Relaxation Enhancement NMR Spectroscopy, **Megan Black**, *University of Guelph*

Poster Session: 16:00 – 17:45

Banquet at the Buttery (ticket required)

Sunday, 15 OCT 2023: MDCL Building

8:30 – 9:10 Coffee/Pastries

Session E (Chair, Gillian Goward), MDCL-1105

9:10 – 9:55 Plenary Lecture: MR and MRI in the time domain with unusual magnets, **Bruce Balcom**, *University of New Brunswick*

9:55 – 10:15 29. Magic Angle Spinning Solid-State NMR Structural Study of a Light-Activated Potassium Channelrhodopsin, **Rajivan Raseekan**, *University of Guelph*

10:15 – 10:35 24. NMR Investigations of the Structural Role of Phosphorus in Aluminosilicate Glasses for Ion Exchange, **Zachary Booth**, *The Ohio State University*

<BREAK>

Session F (Chair, Rashik Ahmed), MDCL-1105

10:55 – 11:15 6. Flower species ingredient authentication using NMR methods, **Thirugnanasambandam Arunachalam**, *University of Guelph*

11:15 – 11:35 16. Operando NMR studies of diverse battery systems, **Kevin J. Sanders**, *McMaster University*

11:35 – 11:55 22. A Solid-State ^{13}C and ^{31}P NMR study of Metal Organic Framework Pesticide Complexes, **Chris Kirby**, *Agriculture and Agri-Food Canada*

Student Awards

Closing Remarks

<Box Lunches>

Oral Presentation Abstracts

Impact of Functional Groups on Lithium Salt Dispersion and Mobility in Polymer Electrolytes

Gabrielle Foran[1], Caroline St. Antoine[1], Mengyang Cui[2], Walker Zheng[2], David Lepage[1], Arnaud Prébé[1], David Aymé-Perrot[3], Gillian Goward[2], Mickael Dollé[1]

[1]Université de Montréal, [2]McMaster University, [3]TotalEnergies

Solid Polymer electrolytes are versatile, highly processible and electrochemically compatible with various solid electrode materials making them promising candidates for use in all solid-state batteries. This versatility results from the wide array of available ion-conducting polymer-salt combinations. However, most polymer electrolyte materials are made using lithium bis(trifluoromethanesulfonyl)imide (LiTFSI) due to its long history of achieving relatively high ionic conductivities in polymer electrolyte systems with the most famous being poly(ethylene oxide) (PEO). This project focuses on the possibility of obtaining better ionic conductivities with salts and/or in polymers matrices containing different functional groups. The conductivity of a polymer electrolyte is partially based on the ability of the polymer matrix to dissolve and bond to the salt. These interactions impact local-scale ion mobility which can be measured via NMR spectroscopy. In this work, polymer electrolytes were prepared using PEO, hydrogenated nitrile butadiene rubber and poly(propylene) carbonate. Ion mobility, lithium conductivity and salt-polymer interactions were investigated to compare the dissociation behaviour of LiTFSI and lithium cyano(trifluorosulfonyl)imide) in polymers with common salt-dissociating functional groups including ethyl, nitrile and carbonate to determine how salt-polymer interactions impact the ionic conductivity of these systems.

Exploring host-guest interactions within a 600 kDa DegP protease cage complex by hydrodynamics and methyl-TROSY NMR

Robert Harkness[1], Huaying Zhao[2], Yuki Toyama[1], Peter Schuck[2], Lewis Kay[1]

[1]University of Toronto, [2]National Institues of Health

The DegP protease-chaperone operates within the periplasm of Gram-negative bacteria where it assists in the regulation of protein homeostasis, promotes virulence, and is essential to bacterial survival under stress. To carry out these tasks, DegP captures substrates within cage-like complexes which form through the remodeling of an underlying network of pre-organized apo oligomers. Although the architectures of DegP cage complexes are well understood, little is known about the structures, dynamics, and interactions of client proteins within DegP cages, or whether these influence function. In this study, we establish the role of host-guest interactions throughout the DegP activation cycle using a model alpha helical client protein in combination with hydrodynamics, methyl transverse relaxation optimized spectroscopy (TROSY)-based solution NMR, and proteolytic activity assays. We find that, in the presence of the client, DegP cages assemble cooperatively with little intermediates. Our data further show that the N-terminal region of the bound client is flexible in solution and forms transient interactions with DegP which, in this case, have little influence on proteolysis. Finally, we show that a second layer of regulation, in the form of a cooperative structural transition of DegP's protease domains, occurs upon client engagement and leads to activation.

Authenticating Edible Oils and Post-biotic Fibers using NMR Spectroscopy and Multivariate Statistical Analysis

Arun Krishnamurthy[1]

[1]Purity-IQ, 150-Research Lane, Suite# 105, Guelph, ON, Canada N1G 4T2

Pre- and post-biotic products targeted towards improving gut microbiome are gaining popularity, and include dietary fibers, fermented products and their extracts, short-chain fatty acids, yeast, and bacterial extracts among others. Due to their health claims, it is prudent to develop methods to authenticate them and assess their consistency and purity, which can assist the manufacturers, suppliers, and vendors to meet the regulatory requirements, support label claims, and strategical placement of their product in the global market. Edible oils are a popular commodity and highly priced and sought-after products such as olive and avocado oils are prone to adulteration due to a disproportionate global supply and demand. Although gas chromatographic methods are popular, NMR-based methods are equally applicable and can deliver accurate and precise chemical information, which can aid in authenticating edible oils. Moreover, multivariate statistical methods can be embedded into NMR methodologies, which offer additional insights and variables to differentiate and assess the quality of oils and oil-based products. A brief account of how our methods provide advanced analytics and product insights that support the industry needs, and how Purity-IQ is helping companies ensure the authenticity of their products and their efforts to retrain customers' trust and satisfaction is presented.

Structure elucidation of bioactive compounds from Natura Health Products (NHP) using NMR Spectroscopy -A rapid authentication tool for quality assurance

Vinayagam Varathan[1], Thirugnanasambandam Arunachalam[1], Ragupathy Subramanyam[1], Steven Newmaster[1]

[1]University of Guelph

Plants have specific bioactive molecules that provide a basis for health claims and plausible explanations for observed efficacy from clinical trials. Quality assurance programs demand testing to verify these bioactive molecules are present in botanical ingredients. This provides a process for validating labels on products that are utilized by consumers who make informed purchases of food and natural health products. There are several targeted analytical methods that can test for specific molecules. Plants produce many metabolites of which only a few are targeted for testing; many other metabolites may be important for health and nutritional related benefits. Non-targeted NMR provides a spectral fingerprint for many metabolites in a test sample. This can be used species identity and metabolite consistency within botanical ingredients. Presently there is a gap in the published research concerning the identification of bioactive molecules within the NMR spectra of many botanicals. The objective of this research is to provide an NMR metabolite fingerprint analysis with structural elucidation of the bioactive molecules for several botanical species ingredients. This will provide a quality assurance method using NMR in which one sample analysis can provide i) botanical species identity, ii) product consistency, and iii) verification of bioactive molecules of interest.

Flower species ingredient authentication using NMR methods

Thiru Arunachalam[1], Vinayagam Varathan[1], Ragupathy Subrmanyam[1], Steven Newmaster[1]

[1]University of Guelph

Flowers have become increasingly popular as ingredients in food, beverages, cosmetics, and natural health products, and the supply chain for botanicals includes various forms of flowers. Fresh whole flowers are easier to identify than dried or processed flowers, which poses a problem for verifying flower species ingredients in the supply chain of multiple markets. The goal of this research is to develop methods for flower species ingredient verification through NMR metabolite fingerprint methods validated by DNA-based molecular diagnostics. These methods were used to identify 23 common flower species ingredients and to assign 22 bioactive compounds from the NMR spectra, which could serve as a tool for rapid quantification of flower ingredients. The NMR data analysis revealed significant information about the variation of metabolites present in different flower species, including color variants within species. This study provides a comparison of the benefits and limitations of alternative methods for flower species ingredient supply chain verification, necessary to support quality assurance. The metabolomic approach provides a basis for understanding the phytochemical structure of natural products, which may eventually be linked to efficacy in clinical trials and label claims regarding the health benefits of specific botanical formulations.

MR and MRI in the time domain with unusual magnets

Bruce Balcom[1]

[1]University of New Brunswick

The magnetic resonance phenomena appears in science, engineering and medicine in a bewildering range of implementations and methods. Unlike many other spectroscopies, magnetic resonance is a deep, subtle and elusive phenomena. But it is still usually based on a set of straightforward physical principles. Our own work is principally in the time domain. In this lecture I will outline time domain measurements (relaxation times) that we and others undertake to study physical systems. I will show implementation of these ideas on a range of unusual magnet systems and I will show the culmination of these ideas in a new style of variable field superconducting magnet.

Introducing Oxygen-17 labels onto amino acid side chains: L-serine and L-threonine

Yuying Huang[1], Gang Wu[1]

[1]Queen's University

The presence of oxygen element in many organic and biological molecules makes it a NMR target. The only NMR-active oxygen isotope, ^{17}O, is quadrupole (I = 5/2) and has 0.037% natural abundance, making ^{17}O NMR challenging [1]. The first step in many ^{17}O NMR studies will be to introduce the ^{17}O labelling into targeted molecule. It is readily achieved to labeled the amino acids' carboxylate group with ^{17}O-isotope [1]. Recently, Lin et al. demonstrated that ^{17}O-labeled amino acids can be incorporated into recombinant proteins [2]. However, ^{17}O-labeling of the hydroxyl group in amino acid sidechains such as L-serine and L-threonine has remained challenging. The sidechain hydroxyl group in proteins is known to play indispensable roles. For example, the hydroxyl group of Ser195 in chymotrypsin serves as the nucleophile at the active site to cleave a peptide bond [3]. In this presentation, we report a convenient synthesis of [3-^{17}O]-L-serine and [3-^{17}O]-L-threonine where the sidechain hydroxyl groups are ^{17}O-labeled.

References

[1] G. Wu, Prog. Nucl. Mag. Reson. Spectrosc. 52, 118-169 (2008).

[2] B. Lin, I. Hung, Z. Gan, P.-H. Chien, H. L. Spencer, S. P. Smith, G. Wu ChemBioChem 22, 826-829 (2021).

[3] L. Hedstrom, Chem. Rev. 102, 4501−4524 (2002).

Elucidating the activation mechanism of caspase-9 on the apoptosome using methyl-TROSY NMR

Alexander Sever[1, 2], Reid Alderson[1, 3, 4], Enrico Rennella[1, 3, 4], James Aramini[1, 3, 4], Zi Hao Liu[2, 4], Robert Harkness[1, 2, 3, 4], Lewis Kay[1, 2, 3, 4]

[1]Dept. of Chemistry, University of Toronto, [2]Molecular Medicine, The Hospital for Sick Children, [3]Dept. of Molecular Genetics, University of Toronto, [4]Dept. of Biochemistry, University of Toronto

The Caspase-9 (Casp9) protease plays an essential regulatory role in the intrinsic apoptotic pathway, with dysfunction leading to cancers and neurodegenerative disorders. Casp9 is composed of a Protease Domain (PD) and a Caspase Activation and Recruitment Domain (CARD), which are connected via a long-disordered linker. While it is well established that an interaction of the CARD with the apoptosome complex upregulates Casp9 cleavage activity, detailed mechanistic information regarding this process remains elusive. Previous biochemical studies of isolated Casp9 have demonstrated that dimerization of the PD is required for Casp9 proteolytic activity. Whether these findings can be translated to the biologically relevant Casp9-apoptsome complex is unclear, with contrasting models of activation being hypothesized. In this study, we use a combination of methyl-TROSY based NMR methods in tandem with biochemical assays to elucidate the activation mechanism of Casp9 upon apoptosome binding. Our data suggests that the apoptosome upregulates Casp9 activity via an induced proximity mechanism. In the absence of substrate the PD exhibits exceptionally weak dimerization affinity, remaining monomeric regardless of apoptosome binding, and only when substrate is present does dimerization occur. Thus, Casp9 activation is driven by crowding of multiple PDs, increasing the rate of dimerization and therefore substrate cleavage activity.

Unravelling the local environments of halogen ions in MOFs with 35Cl and 127I solid-state NMR spectroscopy

Wanli Zhang[1], Mathew Willans[1], Ivan Hung[2], Amrit Venkatesh[2], Zhehong Gan[2], Yining Huang[1]

[1]Western University, [2]National High Magnetic Field Laboratory

Metal-organic frameworks (MOFs) are emerging porous materials which have great potential for applications such as gas separation, catalysis, and drug delivery. The halide ions in MOFs exhibit versatile coordination chemistry, as important counterpart ions in the structure of MOFs. For example, halide can serve as μ_2, μ_3, μ_4 bridging ions and μ_1 terminal ions, leading to different properties and applications. In this work, ^{35}Cl SSNMR at 14.1 T is employed to characterize the halide ions with different local environments in MOFs. The inequivalent chlorin ions in MOFs are distinguished using the example of MOF YCM-22. The connection mode change of halide ions during the thermal treatment of [CuX(bpy)] (X = Cl-, I-) is monitored with ^{35}Cl SSNMR. ^{127}I SSNMR is practically challenging due to its large quadrupole moment. In this work, we report the ^{127}I NMR in MOFs at 35.2 T with field-step approach. The NMR parameters were calculated by plane-wave and model cluster DFT calculations to aid the structural analysis. We show that the combination of ^{35}Cl and ^{127}I SSNMR spectroscopy and theoretical calculations can provide valuable structure info on the local structure of MOFs.

Early-Onset Parkinson Mutation Remodels Monomer - Fibril Interac-tions to Allosterically Amplify Synuclein's Amyloid Cascade

Jinfeng Huang[1], Rashik Ahmed[1], Akimoto Madoka [1], Karla Martinez Pomier[1], Giuseppe Melacini[1]

[1]Department of Chemistry and Chemical Biology, McMaster University

Alpha synuclein (αS) aggregates are the main component of Lewy Bodies (LBs) associated with Parkinson's disease (PD). A long outstanding question about αS and PD pertains to the autosomal dominant E46K αS mutant, which leads to early PD onset and LB dementias. The E46K mutation promotes αS aggregation, but also stabilizes αS monomers in 'closed' conformers, which are compact and aggregation-incompetent. Hence, the mechanism of action of the E46K mutation is currently unclear. Here, we show that αS monomers harboring the E46K mutation exhibit more extensive interactions with fibrils compared to WT. Such monomer-fibril interactions are sufficient to allosterically drive transitions of αS monomers from closed to open conformations, enabling αS aggregation. We also show that E46K promotes head-to-tail monomer-monomer interactions in early self-association events. This multipronged mechanism provides a new framework to explain how the E46K mutation and possibly other αS variants trigger early-onset PD.

Operando NMR studies of diverse battery systems

Kevin J. Sanders[1], Amanda A. Ciezki[1], Breanna L. Pinto[1], Alexander Berno[1], Rohan Jadhav[1], Andres Ramirez Aguilera[2], Bruce J. Balcom[2], Ion C. Halalay[3], Gillian R. Goward[1]

[1]Department of Chemistry, McMaster University, [2]Department of Physics, University of New Brunswick, [3]General Motors Research and Development, Warren, Michigan

NMR is uniquely suited to monitor degradation and quantify products in batteries. The choice of RF probe is crucial to achieving high sensitivity in *operando* NMR experiments. The parallel-plate resonator (PPR) was proposed as a prime choice due to natural geometric matching to prismatic samples and production of uniform B_1 fields[1,2]. Here, optimized PPRs provide sensitive results in *operando* NMR studies of Li-ion batteries by ^{7}Li NMR, and studies of Zn-ion battery electrolytes by ^{1}H NMR.

Repeated cycling of silicon-based anodes at moderate/high rates yields accumulation of irreversible lithium metal and concentrated lithium silicides, identifying a key capacity fade mechanism by ^{7}Li NMR[3]. The response of plated Li-metal signal to increasing charging currents is particularly revealing and is quantified using ssNake[4] with a home-written intuitive sequential fitting tool[3]. Additionally, ^{1}H NMR identifies the accumulation of Mn(II) in the aqueous electrolyte of a MnO_2 half-cell upon repeated cycling. New insights into Si anode aging and cathode degradation are achieved with *in situ* and *operando* NMR measurements utilizing optimized RF probes. This methodology opens the door to routine NMR studies of electrochemical systems by way of high sensitivity measurements with ample temporal resolution.

[1]https://doi.org/10.1016/j.mrl.2023.01.002, [2]https://doi.org/10.1016/j.carbon.2021.12.082, [3]https://doi.org/10.1021/jacs.3c07339, [4]https://doi.org/10.1016/j.jmr.2019.02.006

Assessing Conformational States Analysis of 13C-5-fluoro-trp-enriched-Fluoroacetate Dehalogenase by 19F-13C Two-Dimensional NMR

Motasem Suleiman[1], Geordon A. Frere[1], Keith Taverner[1], Lauren Tabunar[1], Adnan Sljoka[2], Scott Prosser[1]

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Tryptophan plays a critical role in proteins by contributing to stability, allostery, and catalysis. Using fluorine (^{19}F) NMR, protein conformational dynamics and structure-activity relationships (SARs) can be studied via fluorotryptophan reporters. Tryptophan analogs such as 5-fluorotryptophan can be routinely incorporated into proteins during heterologous expression by halting endogenous tryptophan biosynthesis. Building upon the large ^{19}F chemical shift dispersion associated with 5-fluorotryptophan, we introduce an approach to the incorporation of ^{13}C-enriched 5-fluorotryptophan using a direct biosynthetic precursor, 5-fluoroanthranilic acid-(*phenyl*-$^{13}C_6$). The homodimeric enzyme fluoroacetate dehalogenase (FAcD), a thermophilic alpha/beta hydrolase responsible for the hydrolysis of a C-F bond in fluoroacetate, was expressed and biosynthetically labeled with ^{13}C-enriched 5-fluorotryptophan. The resulting two-dimensional ^{19}F-^{13}C heteronuclear correlation spectra provide complete resolution of all 9 tryptophan residues in the apo enzyme and FAcD saturated with the substrate analog bromoacetate. The spectra reveal a pronounced response with bromoacetate by two tryptophan residues which dynamically engage the substrate in the Michaelis-Menten intermediate. The role of each tryptophan residue in allosteric communication was validated with computational rigidity transmission allostery analysis.

QUADRUPOLE-CENTRAL-TRANSITION NMR SPECTROSCOPY OF ALKALI METAL IONS

Ziyao Peng[1], Gang Wu[1]

[1]Queen's University

Nuclear Magnetic Resonance (NMR) spectroscopy is pivotal in analytical chemistry, providing non-invasive, intricate insights into molecular structures and dynamics of compounds, predominantly applied to study spin-1/2 nuclei like ^{1}H, ^{13}C, and ^{15}N. However, alkali metal elements possess quadrupolar nuclei (spins >1/2), making NMR studies more challenging[1]. These ions are important to numerous biological and chemical processes. Quadrupole-Central-Transition (QCT) NMR spectroscopy has emerged as a powerful tool for analyzing quadrupolar nuclei in solutions[2]. This study delves into examining alkali metal-ion-cryptand complexes like Na[C222]Br and Rb[C222]Cl in glycerol, mimicking the slow tumbling motion of biological macromolecules due to glycerol's high viscosity. We analyzed ^{23}Na and ^{87}Rb QCT NMR signals in these complexes at varying temperatures and magnetic fields (11.7, 14.1 and 16.4 T), discovering that the QCT NMR signals in the slow-motion regime are significantly narrower than in aqueous solutions, highlighting this method's potential to decode intricate chemical transformations in biological macromolecules.

References

1. J. Zhu, G. Wu, Prog. Nucl. Magn. Reson. Spectrosco. 61 (2012) 1-70.

2. J. Zhu, G. Wu, J. Am. Chem. Soc. 133 (2011) 920-932.

Magnetic resonance spectroscopy and imaging to study pregnancy and pregnancy complications

Lindsay Cahill[1]

[1]Memorial University of Newfoundland

During pregnancy, appropriate placental metabolism is essential for fetuses to reach their growth potential. Altered placental metabolism may precede placental dysfunction, resulting in preterm birth, fetal growth restriction or stillbirth. NMR-based metabolomics is a growing field of research that provides insight into how biological systems function by studying low molecular weight metabolites. Our group has used magnetic resonance spectroscopy (MRS) and experimental mice to study the changes in placental metabolite profiles over gestation in healthy pregnancy and in mouse models of pregnancy complications. In this talk I will present studies using MRS to determine the impact of maternal exposure to micro- and nanoplastics on placental and fetal brain metabolism in mice. Magnetic resonance imaging was used to study the impact of plastics exposure on postnatal brain development. I will also present a study demonstrating alterations in placental metabolism associated with preterm birth in a human cohort. Finally, I will discuss next steps (opportunities and barriers) towards clinical translation.

A Solid-State 13C and 31P NMR study of Metal Organic Framework Pesticide Complexes

Chris Kirby[1], Hailey Hill[1], Craig MacKinnon[2]

[1]Charlottetown Research and Development Centre, Agriculture and Agri-Food Canada, [2]Department of Chemistry, Lakehead University

In recent years, metal organic frameworks (MOFs) have been gaining interest as a method for pesticide remediation. However, the structures of the MOF-pesticide complexes is not well understood. We have optimized the conditions to synthesize highly crystallized UiO-66 and UiO-67 using the line shapes observed in solid-state ^{13}C NMR. These crystalline MoFs were then loaded with organophosphate pesticides (glyphosate, glufosinate, ethephon) to their maximum capacity (as observed by the disappearance of the pesticides by UPLC-MS). Solid-state ^{13}C and ^{31}P NMR were used to gain insight into the pesticide-MoF complexes. Our ongoing work into the investigation of reactions of the MOFs and commercially available agri-products (e.g., glyphosate is the active ingredient in Roundup) will also be presented.

NMR Investigations of the Structural Role of Phosphorus in Aluminosilicate Glasses for Ion Exchange

Zackary Boothe[1]

[1]The Ohio State University

Chemically strengthened aluminosilicate glasses are used in various applications, from phone screens to airplane windshields, where cracks in the glass can have inconvenient and disastrous consequences. Adding small amounts of phosphorus to these glasses modifies the connectivity of the vitreous network creating stronger glasses in less time. An understanding of the structural role of phosphorus in these systems is therefore necessary for designing compositions that optimize the chemical strengthening process. Here, we present a comprehensive 31P NMR investigation on a series of aluminosilicate glasses with varying concentrations of P2O5 ranging from 0 mol% to 10 mol%. Initial MAS spectra revealed a significant difference in phosphorus site speciation, with higher isotropic chemical shifts in samples with lower aluminum-to-phosphorus ratios. To probe these lineshapes further, we performed Magic Angle Flipping (MAF) measurements that involve spinning the sample on and off the magic angle in two separate time domains. The result is a two-dimensional spectrum correlating an anisotropic lineshape to each isotropic frequency. With our recently developed MRInversion software, we inverted these MAF spectra to determine distributions of CSA parameters for each glass composition. The interpretation of these 31P shielding tensor parameters in terms of the structural role of phosphorus will be discussed.

Using 19F To Study Large Proteins

Elizabeth Connelly[1], Gary Shaw[1]

[1]Western University

Parkinson’s Disease (PD) is a neurodegenerative disease characterized by loss of dopaminergic neurons in the substantia nigra pars compacta. Population studies revealed 100 PD associated mutations in the *PARK2* gene encoding the RING-inBetween-RING E3 ligase parkin. This 52 kDa protein consists of five domains and 465 residues. Due to its size, traditional ^{13}C and ^{15}N structural studies are difficult to interpret. Selective amino acid methods, such as methyl-TROSY experiments, have improved NMR studies; however, overlapping peaks make discerning conformational changes difficult. Isotopes with low-background signals and high sensitivity, such as fluorine-19, could be the answer to simplifying spectra of large proteins while still discerning meaningful information. Preliminary data shows global rearrangements can be captured with ^{19}F-tryptophan and may be employed to address the transthiolation question.

Magic Angle Spinning Solid-State NMR Structural Study of a Light-Activated Potassium Channelrhodopsin

Rajivan Raseekan[1], Azam Askari Kermani[1], Leonid S. Brown[1], Vladimir Ladizhansky[1]

[1]University of Guelph

Channelrhodopsins are light-switchable ion channels that are actively used in optogenetics. Once introduced into neurons, their ion conductance can be selectively controlled by means of external light signals. The majority of known cation channelrhodopsins are selective for sodium or protons and can only be used as excitatory channels. Here, we report solid-state NMR investigations of a recently discovered potassium-selective channel HcKCR1 which can be used as an inhibitory tool. Like other rhodopsins, HcKCR1 is a seven-transmembrane α-helical protein. To perform a structural study of this protein, we reconstitute it in a lipid environment and perform multidimensional magic angle spinning (MAS) solid-state NMR. The result is spectra with high sensitivity and good resolution. We discuss the performance and the main features of these experiments which allowed for reliable ^{13}C and ^{15}N assignments of both the backbone and sidechains of 123 residues out of 264 residues, including part of the gate. These shifts are then used to analyze secondary structure and helical boundaries, determine protonation states of ionizable side chains and oxidation states of important cysteines. The analysis is still ongoing. The knowledge of shifts will form the basis of future studies regarding the gating mechanism and K^+ selectivity.

Identifying the structural arrangement of co-factor p47 upon interaction with the AAA+ enzyme p97 using Paramagnetic Relaxation Enhancement NMR Spectroscopy

Megan Black[1], Dr. Rui Huang[1]

[1]University of Guelph

The AAA+ (ATPases Associated with diverse cellular Activities) family of enzymes is essential for maintaining cellular homeostasis. One enzyme in this family is p97, also known as valosin-containing protein (VCP), which has many roles ranging from membrane fusion to proteasomal degradation. P97 works in conjunction with 30 known adaptors including the protein p47, the first of the adaptors to be recognized. P47 is a linear protein containing a UBA domain which binds ubiquitinated substrates for their delivery to p97 for unfolding. The UBA domain is separated from the rest of p47 by a long linker region, raising questions to its exact orientation within p47 and to p97 during their interaction. Using paramagnetic relaxation enhancement NMR spectroscopy, we have identified key residues on the N-lobe of p97-NTD and near the central pore of D1 that come in proximity to UBA, providing clues to p47-UBA's movement during its interaction with p97. Additionally, we were able to identify regions within p47 in proximity[ND1] with UBA such as in the linker and SEP domain. Together, this information provides insight to where UBA is located with respect to p97 and other p47 domains and thus assists in describing the mechanism of substrate delivery to p97.

Exploring conformational dynamics of the p97 molecular machine using solution NMR spectroscopy

Rui Huang[1]

[1]University of Guelph

Cellular activities reply on proper functioning of a myriad of large biomolecular complexes. One such molecular machines is p97, also known as VCP, that is a highly conserved and abundant cytosolic enzyme in the AAA+ (ATPases associated with diverse cellular activities) superfamily. It plays an indispensable role in protein homeostasis and has emerged as a promising therapeutic target for treatment of cancer, neurodegenerative disorders, and viral infection. p97 interacts with more than 30 adaptor proteins which recruit it to specific cellular tasks. We present here our previous and on-going characterization of the conformational dynamics of the p97 molecular machine using solution NMR spectroscopy, including the cooperative conformational interconversion of the p97 N-terminal domain and the substrate exchange process of the p97 complex. We also show our structural study on characterizing the dynamic complex formed between p97 and one of its adaptor proteins, p47, that directs p97 function to the remodelling of cellular membranes. We discovered three previously unidentified linear motifs residing at an intrinsically disordered linker region of p47 that play important roles in stabilizing and regulating the interactions within the complex. Our results highlight the important roles that IDRs can play in regulating the function of molecular machines.

Solid-State NMR Spectroscopy of the Periodic Table Enabled by Sensitivity-Enhanced Methods

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Solid-state NMR spectroscopy is a powerful technique for characterization of disordered materials and surface species because it provides precise information about local atomic environments and the connectivity of atoms. Furthermore, NMR spectroscopy is potentially applicable to nearly all the elements of the periodic table. However, many NMR-active nuclei are unreceptive and offer poor sensitivity and resolution for solid-state NMR experiments. Poor sensitivity typically arises because of some combination of nuclear properties (low-g, low natural abundance, large quadrupole moment, etc.), broadening of solids NMR signals due to anisotropic interactions, and unfavorable relaxation times. I will describe some methods to overcome these sensitivity challenges, and show how they enable high-resolution NMR experiments on inorganic materials such as 2D semiconductors, semiconductor nanoparticles and heterogeneous catalysts. I will show how proton detection can be applied for acquisition of wideline solid-state NMR spectra of both spin-1/2 and quadrupolar nuclei. I will demonstrate how ultra-high field (35 T) solid-state NMR spectroscopy can be applied to obtain homonuclear and heteronuclear 2D correlation NMR spectra of quadrupolar nuclei. Finally, I will illustrate how dynamic nuclear polarization (DNP) can be applied to obtain 2D heteronuclear correlation NMR spectra with dilute isotope pairs.

Poster Presentation Abstracts

1 - Impact of Functional Groups on Lithium Salt Dispersion and Mobility in Polymer Electrolytes

Gabrielle Foran[1], Caroline St. Antoine[1], Mengyang Cui[2], Walker Zheng[2], David Lepage[1], Arnaud Prébé[1], David Aymé-Perrot[3], Gillian Goward[2], Mickael Dollé[1]

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Solid Polymer electrolytes are versatile, highly processible and electrochemically compatible with various solid electrode materials making them promising candidates for use in all solid-state batteries. This versatility results from the wide array of available ion-conducting polymer-salt combinations. However, most polymer electrolyte materials are made using lithium bis(trifluoromethanesulfonyl)imide (LiTFSI) due to its long history of achieving relatively high ionic conductivities in polymer electrolyte systems with the most famous being poly(ethylene oxide) (PEO). This project focuses on the possibility of obtaining better ionic conductivities with salts and/or in polymers matrices containing different functional groups. The conductivity of a polymer electrolyte is partially based on the ability of the polymer matrix to dissolve and bond to the salt. These interactions impact local-scale ion mobility which can be measured via NMR spectroscopy. In this work, polymer electrolytes were prepared using PEO, hydrogenated nitrile butadiene rubber and poly(propylene) carbonate. Ion mobility, lithium conductivity and salt-polymer interactions were investigated to compare the dissociation behaviour of LiTFSI and lithium cyano(trifluorosulfonyl)imide) in polymers with common salt-dissociating functional groups including ethyl, nitrile and carbonate to determine how salt-polymer interactions impact the ionic conductivity of these systems.

2 - Exploring host-guest interactions within a 600 kDa DegP protease cage complex by hydrodynamics and methyl-TROSY NMR

Robert Harkness[1], Huaying Zhao[2], Yuki Toyama[1], Peter Schuck[2], Lewis Kay[1]

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The DegP protease-chaperone operates within the periplasm of Gram-negative bacteria where it assists in the regulation of protein homeostasis, promotes virulence, and is essential to bacterial survival under stress. To carry out these tasks, DegP captures substrates within cage-like complexes which form through the remodeling of an underlying network of pre-organized apo oligomers. Although the architectures of DegP cage complexes are well understood, little is known about the structures, dynamics, and interactions of client proteins within DegP cages, or whether these influence function. In this study, we establish the role of host-guest interactions throughout the DegP activation cycle using a model alpha helical client protein in combination with hydrodynamics, methyl transverse relaxation optimized spectroscopy (TROSY)-based solution NMR, and proteolytic activity assays. We find that, in the presence of the client, DegP cages assemble cooperatively with little intermediates. Our data further show that the N-terminal region of the bound client is flexible in solution and forms transient interactions with DegP which, in this case, have little influence on proteolysis. Finally, we show that a second layer of regulation, in the form of a cooperative structural transition of DegP's protease domains, occurs upon client engagement and leads to activation.

3 - Investigating the Gate-Opening Phenomenon in Elastic Layer-Structured Metal-Organic Framework-11 via CO2 Dynamic Study

Jiabin Xu[1], Wanli Zhang[1], Jun Zhong[2], Tsun-Kong Sham[1], Yining Huang[1]

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As a promising porous material for CO_2 adsorption and storage, Elastic Layer-Structured Metal-Organic Framework-11 (ELM-11) has attracted significant attention owing to its distinct gate-opening phenomenon. Rather than focusing solely on the material's structure, our investigation is centered around comprehending CO_2 dynamics and the host-guest interactions in order to better understand this behavior. In this work, we mainly employed variable-temperature (VT) ^{13}C solid-state nuclear magnetic resonance (SSNMR) spectroscopy to examine the host-guest interaction between $^{13}CO_2$ and MOF framework. This work aimed to shed light on the modes of CO_2 dynamics within both the gate-closed and gate-open configurations. Our findings from spectral analysis revealed the presence of two distinct adsorption sites, each characterized by different motion patterns in the gate-open form, as opposed to the single site observed in the gate-closed form. ^{11}B, ^{13}C, and ^{19}F Magic Angle Spinning (MAS) SSNMR, in conjunction with in-situ XANES experiments, were also conducted to probe and confirm the location of adsorption sites.

4 - Authenticating Edible Oils and Post-biotic Fibers using NMR Spectroscopy and Multivariate Statistical Analysis

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Pre- and post-biotic products targeted towards improving gut microbiome are gaining popularity, and include dietary fibers, fermented products and their extracts, short-chain fatty acids, yeast, and bacterial extracts among others. Due to their health claims, it is prudent to develop methods to authenticate them and assess their consistency and purity, which can assist the manufacturers, suppliers, and vendors to meet the regulatory requirements, support label claims, and strategical placement of their product in the global market. Edible oils are a popular commodity and highly priced and sought-after products such as olive and avocado oils are prone to adulteration due to a disproportionate global supply and demand. Although gas chromatographic methods are popular, NMR-based methods are equally applicable and can deliver accurate and precise chemical information, which can aid in authenticating edible oils. Moreover, multivariate statistical methods can be embedded into NMR methodologies, which offer additional insights and variables to differentiate and assess the quality of oils and oil-based products. A brief account of how our methods provide advanced analytics and product insights that support the industry needs, and how Purity-IQ is helping companies ensure the authenticity of their products and their efforts to retrain customers' trust and satisfaction is presented.

8 - Utilizing Solid-State 23Na NMR to Investigate the Effects of Gallium Doping in Sodium-Ion Battery Cathode Material NVPF.

Olivia Velenosi[1], Gillian Goward[1], Taiana Pereira[1], Kevin Sanders[1]

[1]McMaster University

Sodium-ion batteries (SIBs) are considered a complementary substitute for LIBs for several reasons; sodium behaves relatively similar to lithium, and has the benefit of lower cost and greater abundance. In this work, we investigate the effects of substituting gallium for vanadium in SIB cathode sodium vanadium fluorophosphate ($Na_3V_2(PO_4)_2F_3$, or NVPF). Ex-situ ssNMR is a valuable technique when investigating cathode materials, as the redox dynamics can be easily characterized due to our ability to 'watch' these reactions overtime. The NVPF cathodes were doped with gallium to achieve three different compositions: $Na_3V_{1.5}Ga_{0.5}(PO_4)_2F_3$, $Na_3V_{1.3}Ga_{0.7}(PO_4)_2F_3$, and $Na_3V_1Ga_1(PO_4)_2F_3$. The Ga-substituted structures were found to be similar but not identical to the parent NVPF phase, and the new phase NGPF is characterized for the first time. The three cathode compositions were cycled through several different C-rates and voltage domains to investigate the effect of substitution on the electrochemical performance. When cycled at a constant current, the $V_{1.5}Ga_{0.5}$ and $V_{1.3}Ga_{0.7}$ samples exhibited capacities comparable to NVPF, and we will continue to investigate the phases under different cycling parameters. We have selected these phases for further investigation with ex-situ ^{23}Na ssNMR spectroscopy acquired under magic angle spinning (MAS = 60kHz at 7.0T) at different states of charge.

10 - Impact of exposure to environmental pollutants on placental metabolism

Haley Adams[1], Katherine Steeves[1], Lindsay Cahill[1]

[1]Memorial University of Newfoundland

For a healthy pregnancy, the placenta needs to meet the metabolic demands of the fetus and supply it with sufficient oxygen and nutrients. Our group has recently shown using NMR-based metabolomics that genetic deficiency and environmental exposures can perturb the metabolic profile of the placenta, resulting in adverse pregnancy outcomes. This study aims to evaluate the effects of exposure to persistent organic pollutants, legacy and novel per- and polyfluoroalkyl substances (PFAS), on placental metabolism.

Placental tissue samples were collected from healthy control pregnant CD-1 mice and mice exposed to perfluorooctanoic acid and fluorotelomer ethoxylates throughout gestation. The tissue was flash frozen in liquid nitrogen and stored at -80°C. Metabolite profiles were determined using ^{1}H high-resolution magic angle spinning magnetic resonance spectroscopy on a Bruker 600 MHz spectrometer with a 3.2 mm MAS solid-state NMR probe. Data was analyzed using MestReNova and MetaboAnalyst.

The relative concentration of several metabolites that are essential nutrients for fetal development were found to be significantly altered in the PFAS-exposed groups. This study adds to the growing literature that has demonstrated the significant impact of environmental pollutants on placental function and emphasizes that efforts should be made to minimize exposure to pollutants during pregnancy.

11 - Metabolic biomarkers of neurodegeneration in a novel mouse model

Drew Locke[1], Lindsay Cahill[1], Grace Mercer[1], Sophie Kiefte[1]

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The brain is one of the most metabolically active organs and healthy brain metabolism is critical for normal memory and cognitive function. Recent evidence suggests a link between metabolic dysfunction and neurodegenerative diseases such as Alzheimer's disease (AD). While it is challenging to determine molecular and cellular mechanisms that precede the onset of AD in humans, animal models provide an opportunity to directly study the disease pathophysiology. This study aims to study brain metabolism throughout disease progression in a novel mouse model that reproduces several clinical features of AD.

Brain tissue samples were collected from the decrepit mouse model of neurodegeneration (a spontaneous mutation in a mitochondrial-associated gene) from 50 days (before disease onset) to 150 days (premature death). Healthy controls were included. Metabolite profiles were determined using 1H high-resolution magic angle spinning magnetic resonance spectroscopy on a Bruker 600 MHz spectrometer with a 3.2 mm MAS solid-state NMR probe. Data was analyzed using MestReNova.

We investigated whether metabolites differed between five anatomical regions of the brain. We will also present preliminary results demonstrating changes in the metabolite profiles in the decrepit and control mice over their lifetime. The study demonstrates the promise of NMR metabolomics to study brain health

12 - Elucidating the activation mechanism of caspase-9 on the apoptosome using methyl-TROSY NMR

Alexander Sever[1, 2], Reid Alderson[1, 3, 4], Enrico Rennella[1, 3, 4], James Aramini[1, 3, 4], Zi Hao Liu[2, 4], Robert Harkness[1, 2, 3, 4], Lewis Kay[1, 2, 3, 4]

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The Caspase-9 (Casp9) protease plays an essential regulatory role in the intrinsic apoptotic pathway, with dysfunction leading to cancers and neurodegenerative disorders. Casp9 is composed of a Protease Domain (PD) and a Caspase Activation and Recruitment Domain (CARD), which are connected via a long-disordered linker. While it is well established that an interaction of the CARD with the apoptosome complex upregulates Casp9 cleavage activity, detailed mechanistic information regarding this process remains elusive. Previous biochemical studies of isolated Casp9 have demonstrated that dimerization of the PD is required for Casp9 proteolytic activity. Whether these findings can be translated to the biologically relevant Casp9-apoptsome complex is unclear, with contrasting models of activation being hypothesized. In this study, we use a combination of methyl-TROSY based NMR methods in tandem with biochemical assays to elucidate the activation mechanism of Casp9 upon apoptosome binding. Our data suggests that the apoptosome upregulates Casp9 activity via an induced proximity mechanism. In the absence of substrate the PD exhibits exceptionally weak dimerization affinity, remaining monomeric regardless of apoptosome binding, and only when substrate is present does dimerization occur. Thus, Casp9 activation is driven by crowding of multiple PDs, increasing the rate of dimerization and therefore substrate cleavage activity.

13 - Unravelling the local environments of halogen ions in MOFs with 35Cl and 127I solid-state NMR spectroscopy

Wanli Zhang[1], Mathew Willans[1], Ivan Hung[2], Amrit Venkatesh[2], Zhehong Gan[2], Yining Huang[1]

[1]Western University, [2]National High Magnetic Field Laboratory

Metal-organic frameworks (MOFs) are emerging porous materials which have great potential for applications such as gas separation, catalysis, and drug delivery. The halide ions in MOFs exhibit versatile coordination chemistry, as important counterpart ions in the structure of MOFs. For example, halide can serve as μ_2, μ_3, μ_4 bridging ions and μ_1 terminal ions, leading to different properties and applications. In this work, ^{35}Cl SSNMR at 14.1 T is employed to characterize the halide ions with different local environments in MOFs. The inequivalent chlorin ions in MOFs are distinguished using the example of MOF YCM-22. The connection mode change of halide ions during the thermal treatment of [CuX(bpy)] (X = Cl-, I-) is monitored with ^{35}Cl SSNMR. ^{127}I SSNMR is practically challenging due to its large quadrupole moment. In this work, we report the ^{127}I NMR in MOFs at 35.2 T with field-step approach. The NMR parameters were calculated by plane-wave and model cluster DFT calculations to aid the structural analysis. We show that the combination of ^{35}Cl and ^{127}I SSNMR spectroscopy and theoretical calculations can provide valuable structure info on the local structure of MOFs.

14 - Investigation of the Effect of Mn (II) Contaminants on Lithium-Ion dynamics in Manganese-Rich Lithium-Ion Batteries (LIBs)

Runze Zheng[1], Mengyang Cui[1], Gillian Goward[1]

[1]McMaster University

Nickel-manganese-cobalt oxide cathode materials have been applied in most Li-ion batteries, but there are nevertheless some concerns regarding the stability of this material. Higher voltage has been shown to accelerate the dissolution of NMC 111 due to the release of more acidic components as a result of rapid electrolyte decomposition. Mn-contaminants are hypothesized to cause battery capacity fading and decrease the diffusion coefficient of Li^+ in the electrolyte due to the competing behavior Mn^{2+} will have with Li^+. For comparison, 0.05 M of $MnTFSI_2$ and 0.1 M LiTFSI solutions were prepared by combining the appropriate salts with 1 M $LiPF_6$ in EC/DMC (1:1 vt%) commercial electrolyte solution respectively to simulate the condition of a long-term used (manganese contaminated) NMC111 battery. With characterizations including ^7Li pulsed field-gradient nuclear magnetic resonance (PFG-NMR) spectroscopy, and galvanostatic charging and discharging cycling, we demonstrated the Mn (II)-contaminants effect on cycle performance and diffusion coefficient on Li+ dynamics. Under the influence of deliberate manganese salt-additive to the electrolyte, the coin cell shows a capacity fading and unstable charging behavior. The PFG-NMR measurements also validated our hypotheses, as the results showing that Mn-containment causes decrease 7% (0.12*10 -10 m^2/s) in the diffusion coefficient in Mn-contaminant electrolyte.

15 - Early-Onset Parkinson Mutation Remodels Monomer - Fibril Interac-tions to Allosterically Amplify Synuclein's Amyloid Cascade

Jinfeng Huang[1], Rashik Ahmed[1], Akimoto Madoka [1], Karla Martinez Pomier[1], Giuseppe Melacini[1]

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Alpha synuclein (αS) aggregates are the main component of Lewy Bodies (LBs) associated with Parkinson's disease (PD). A long outstanding question about αS and PD pertains to the autosomal dominant E46K αS mutant, which leads to early PD onset and LB dementias. The E46K mutation promotes αS aggregation, but also stabilizes αS monomers in 'closed' conformers, which are compact and aggregation-incompetent. Hence, the mechanism of action of the E46K mutation is currently unclear. Here, we show that αS monomers harboring the E46K mutation exhibit more extensive interactions with fibrils compared to WT. Such monomer-fibril interactions are sufficient to allosterically drive transitions of αS monomers from closed to open conformations, enabling αS aggregation. We also show that E46K promotes head-to-tail monomer-monomer interactions in early self-association events. This multipronged mechanism provides a new framework to explain how the E46K mutation and possibly other αS variants trigger early-onset PD.

16 - Operando NMR studies of diverse battery systems

Kevin J. Sanders[1], Amanda A. Ciezki[1], Breanna L. Pinto[1], Alexander Berno[1], Rohan Jadhav[1], Andres Ramirez Aguilera[2], Bruce J. Balcom[2], Ion C. Halalay[3], Gillian R. Goward[1]

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NMR is uniquely suited to monitor degradation and quantify products in batteries. The choice of RF probe is crucial to achieving high sensitivity in *operando* NMR experiments. The parallel-plate resonator (PPR) was proposed as a prime choice due to natural geometric matching to prismatic samples and production of uniform B_1 fields[1,2]. Here, optimized PPRs provide sensitive results in *operando* NMR studies of Li-ion batteries by ^{7}Li NMR, and studies of Zn-ion battery electrolytes by ^{1}H NMR.

Repeated cycling of silicon-based anodes at moderate/high rates yields accumulation of irreversible lithium metal and concentrated lithium silicides, identifying a key capacity fade mechanism by ^{7}Li NMR[3]. The response of plated Li-metal signal to increasing charging currents is particularly revealing and is quantified using ssNake[4] with a home-written intuitive sequential fitting tool[3]. Additionally, ^{1}H NMR identifies the accumulation of Mn(II) in the aqueous electrolyte of a MnO_2 half-cell upon repeated cycling. New insights into Si anode aging and cathode degradation are achieved with *in situ* and *operando* NMR measurements utilizing optimized RF probes. This methodology opens the door to routine NMR studies of electrochemical systems by way of high sensitivity measurements with ample temporal resolution.

[1]https://doi.org/10.1016/j.mrl.2023.01.002, [2]https://doi.org/10.1016/j.carbon.2021.12.082, [3]https://doi.org/10.1021/jacs.3c07339, [4]https://doi.org/10.1016/j.jmr.2019.02.006

17 - Assessing Conformational States Analysis of 13C-5-fluoro-trp-enriched-Fluoroacetate Dehalogenase by 19F-13C Two-Dimensional NMR

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Tryptophan plays a critical role in proteins by contributing to stability, allostery, and catalysis. Using fluorine (^{19}F) NMR, protein conformational dynamics and structure-activity relationships (SARs) can be studied via fluorotryptophan reporters. Tryptophan analogs such as 5-fluorotryptophan can be routinely incorporated into proteins during heterologous expression by halting endogenous tryptophan biosynthesis. Building upon the large ^{19}F chemical shift dispersion associated with 5-fluorotryptophan, we introduce an approach to the incorporation of ^{13}C-enriched 5-fluorotryptophan using a direct biosynthetic precursor, 5-fluoroanthranilic acid-(*phenyl*-$^{13}C_6$). The homodimeric enzyme fluoroacetate dehalogenase (FAcD), a thermophilic alpha/beta hydrolase responsible for the hydrolysis of a C-F bond in fluoroacetate, was expressed and biosynthetically labeled with ^{13}C-enriched 5-fluorotryptophan. The resulting two-dimensional ^{19}F-^{13}C heteronuclear correlation spectra provide complete resolution of all 9 tryptophan residues in the apo enzyme and FAcD saturated with the substrate analog bromoacetate. The spectra reveal a pronounced response with bromoacetate by two tryptophan residues which dynamically engage the substrate in the Michaelis-Menten intermediate. The role of each tryptophan residue in allosteric communication was validated with computational rigidity transmission allostery analysis.

18 - Characterizing the interaction between α-synuclein and stress-inducible phosphoprotein 1 in Parkinson's Disease

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The aggregation of alpha-synuclein (a-Syn), a presynaptic neuronal protein, is a hallmark of Parkinson's disease (PD). a-Syn lacks a well-defined structure and behaves as an intrinsically disordered protein (IDP), sampling an ensemble of conformations. Under pathological conditions, a-Syn forms toxic polymorphic oligomers and subsequently cellular inclusions comprised of fibrils, called Lewy bodies (LBs).

Molecular chaperones, such as Hsp90 and Hsp70, maintain the solubility of many neurodegeneration-associated IDPs, including a-Syn. Stress-inducible Phosphoprotein 1 (STI1), also known as Hsp-organizing protein (HOP) in humans, is a major co-chaperone to both Hsp90 and Hsp70. In two mouse models of a-Syn misfolding, STI1 co-immunoprecipitated a-Syn, and co-deposited with Hsp90 and Hsp70 in insoluble protein fractions. Overexpression of STI1 in PD mouse models exacerbates behavioural phenotypes when injected with a-Syn pre-formed fibrils. These findings reveal that STI1 modulates the generation and accumulation of toxic a-Syn conformers in mouse models of a-Syn misfolding.

Nuclear Magnetic Resonance (NMR) analyses revealed a dynamic binding mechanism between STI1 and a-Syn, mediated by the STI1 TPR2A domain and two independent binding motifs in the C-terminal of a-Syn. Both binding motifs of a-Syn independently interact with TPR2A and compete for a single binding interface, which can be effectively blocked by Hsp90 peptides.

21 - Identifying placental biomarkers of preterm birth using NMR-based metabolomics

Grace Mercer[1], Darcie Stapleton[1], Catherine Barrett[1], Rajshree Ghosh Biswas[2], William Wolff[2], Flavio Kock[2], Ronald Soong[2], André Simpson[2], Lindsay Cahill[1]

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Preterm birth (PTB) is one of the most common complications of pregnancy, affecting 8% of pregnancies in Canada. While there are no effective methods to prevent PTB, interventions exist to prevent the sequelae associated with premature birth. However, over 50% of preterm deliveries are unpredicted and many people who are identified as high-risk for PTB go on to deliver at term. There is therefore a need to develop reliable methods for the prediction of PTB.

Nine healthy term pregnancies and nine preterm pregnancies were recruited from Eastern Health (St. John's, Newfoundland). Following delivery, placental tissue samples were snap-frozen in liquid nitrogen. Comprehensive multiphase NMR spectroscopy experiments of intact tissue were performed on a 500 MHz Bruker Avance III spectrometer (MAS= 2.5kHz, temp=5°C). Data was analyzed using MestReNova and MetaboAnalyst.

Twenty-three low weight metabolites were identified in the placenta using ^{1}H and ^{13}C literature values and correlations from a 2D ^{1}H-^{1}H COSY and a ^{1}H-^{13}C HSQC. The relative concentrations of valine, glutamate, and creatine were decreased in the PTB placentas compared to controls while alanine and glucose were elevated. This study shows the promise of potential biomarkers in the placenta for the early detection of metabolic abnormalities that lead to PTB.

23 - Methodological Advances for the Characterisation of Human GPCRs by NMR Spectroscopy

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G protein-coupled receptors (GPCR) are a pharmacologically important class of transmembrane proteins. The functioning of GPCRs is governed by their highly dynamic nature, which can be studied by NMR spectroscopy. However, most standard NMR techniques cannot be directly applied to GPCRs due to their large size and the necessity to use higher expression hosts.

We present new methods for NMR studies of GPCRs. As such, we have established protocols for methyl and uniform ^{15}N labelling in mammalian cells grown in suspension. Highly sensitive data acquisition on the resulting fully protonated samples is feasible using the novel XL-ALSOFAST-HMQC with delayed decoupling. Pseudocontact shifts caused by Tm^{3+} labelled tool proteins were exploited for resonance assignment.

Using these methods, we characterise the conformational equilibrium of human β_1-adrenergic receptor constructs in presence of diverse effectors, thereby revealing novel aspects of receptor functioning.

24 - NMR Investigations of the Structural Role of Phosphorus in Aluminosilicate Glasses for Ion Exchange

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Chemically strengthened aluminosilicate glasses are used in various applications, from phone screens to airplane windshields, where cracks in the glass can have inconvenient and disastrous consequences. Adding small amounts of phosphorus to these glasses modifies the connectivity of the vitreous network creating stronger glasses in less time. An understanding of the structural role of phosphorus in these systems is therefore necessary for designing compositions that optimize the chemical strengthening process. Here, we present a comprehensive 31P NMR investigation on a series of aluminosilicate glasses with varying concentrations of P2O5 ranging from 0 mol% to 10 mol%. Initial MAS spectra revealed a significant difference in phosphorus site speciation, with higher isotropic chemical shifts in samples with lower aluminum-to-phosphorus ratios. To probe these lineshapes further, we performed Magic Angle Flipping (MAF) measurements that involve spinning the sample on and off the magic angle in two separate time domains. The result is a two-dimensional spectrum correlating an anisotropic lineshape to each isotropic frequency. With our recently developed MRInversion software, we inverted these MAF spectra to determine distributions of CSA parameters for each glass composition. The interpretation of these 31P shielding tensor parameters in terms of the structural role of phosphorus will be discussed.

25 - Fractionation Factors Reveal Hidden Frustration in an Ancient Allosteric Module

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Protein kinase G (PKG) is an essential regulator of eukaryotic cyclic GMP (cGMP) dependent intracellular signaling, controlling pathways that are often distinct from those regulated by cyclic AMP (cAMP). Specifically, the C-terminal cyclic-nucleotide-binding domain (CNB-B) of PKG has emerged as a critical module to control allostery and cGMP-selectivity in PKG. While key contributions to the cGMP-*versus*-cAMP selectivity of CNB-B were previously assessed, only limited knowledge is currently available on how cyclic nucleotide binding rewires the network of hydrogen bonds in CNB-B, and how such rewiring contributes to allostery and cGMP selectivity. To address this gap, we performed a comparative analysis of apo, cAMP- and cGMP-bound CNB-B using H/D fractionation factors. Comparative analyses of the bound states revealed mixed patterns of hydrogen-bond strengthening and weakening, pointing to inherent frustration, whereby not all hydrogen bonds can be simultaneously stabilized. Interestingly, contrary to expectations, these patterns include a weakening of hydrogen bonds not only within critical recognition and allosteric elements of CNB-B, but also within elements known to undergo rigid-body movement upon cyclic nucleotide binding. These results suggest that frustration may contribute to reversibility of allosteric conformational shifts by avoiding over-rigidification that may otherwise trap CNB-B in its active state.

26 - Using 19F To Study Large Proteins

Elizabeth Connelly[1], Gary Shaw[1]

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Parkinson’s Disease (PD) is a neurodegenerative disease characterized by loss of dopaminergic neurons in the substantia nigra pars compacta. Population studies revealed 100 PD associated mutations in the *PARK2* gene encoding the RING-inBetween-RING E3 ligase parkin. This 52 kDa protein consists of five domains and 465 residues. Due to its size, traditional ^{13}C and ^{15}N structural studies are difficult to interpret. Selective amino acid methods, such as methyl-TROSY experiments, have improved NMR studies; however, overlapping peaks make discerning conformational changes difficult. Isotopes with low-background signals and high sensitivity, such as fluorine-19, could be the answer to simplifying spectra of large proteins while still discerning meaningful information. Preliminary data shows global rearrangements can be captured with ^{19}F-tryptophan and may be employed to address the transthiolation question.

27 - Developing a Highly Integrated NMR Crystallography Approach for Determining Structures of Small Organic Molecular Solids

Owen Vander Meulen[1], Darren Brouwer[1]

[1]Redeemer University

NMR crystallography is an approach to structure determination that aims to integrate multiplecomplementary structural characterization techniques, with solid-state NMR spectroscopyplaying a prominent role alongside diffraction methods, quantum chemical calculations, andvarious modeling approaches. While NMR crystallography approaches have been shown to bequite successful, many strategies tend to be sequential rather than integrative. For example,crystal structure prediction (CSP) approaches may generate a set of possible structures for anorganic molecular solid (based on modeling of intermolecular forces in the solid-state) that arethen evaluated in a subsequent step by comparing quantum-chemical calculated NMRparameters for the candidate structures to experimentally measured values. But what if allinformation from solid-state NMR (chemical shifts, dipolar couplings), powder diffraction, andmodeling of intermolecular forces could be combined into a highly integrated approach that usedall available information at each stage of structure determination? This poster will describe someinitial work that explores several optimization algorithms, including particle swarmoptimization, genetic algorithms, and simulated annealing, that search for structures inbest agreement with (synthetic) solid-state NMR and powder diffraction data whilesimultaneously giving a low crystal lattice energy.

28 - A method to explore a potential protein-protein interaction region in human Aquaporin-1

Philip Drewniak[1], Sameer Al-Abdul Wahid[1], Elyse Roach[1], Vladimir Ladizhansky[1], Leonid Brown[1]

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Human Aquaporin-1 (AQP1) is a water-transporting, a-helical integral membrane protein, believed to be involved in several physiological processes. AQP1's function within a cell is governed largely by protein-protein interactions (PPI), in which the interactions can facilitate an association between proteins required for a reaction. One example is CO_2 shuffling in human erythrocytes, achieved through carbonic anhydrase II (CAII) and the band-3 protein. The occurrence of similar CAII binding motifs between AQP1 and band-3 may hint at a potential PPI that is yet unexplored. However, to investigate this, AQP1 must be solubilized in a native environment with conformational access to the C-terminal tail, the site at which the binding motifs reside. To accomplish this, we used the styrene-maleic acid lipid nanoparticle (SMALP) system. After successfully optimizing the solubilization protocol with SMA, the size and stability of these self-assembled nanodiscs were explored, using TEM, DLS, and Raman spectroscopy. We then explored this system using solution NMR, where we report a significant number of cross-peaks from the ^{1}H-^{15}N HSQC experiment, primarily in the intrinsically disordered region in the spectrum, likely originating from residues in the C-terminal tail region. This method establishes a strategy for studying protein-protein interactions on the C-terminal tail of AQP1.

29 - Magic Angle Spinning Solid-State NMR Structural Study of a Light-Activated Potassium Channelrhodopsin

Rajivan Raseekan[1], Azam Askari Kermani[1], Leonid S. Brown[1], Vladimir Ladizhansky[1]

[1]University of Guelph

Channelrhodopsins are light-switchable ion channels that are actively used in optogenetics. Once introduced into neurons, their ion conductance can be selectively controlled by means of external light signals. The majority of known cation channelrhodopsins are selective for sodium or protons and can only be used as excitatory channels. Here, we report solid-state NMR investigations of a recently discovered potassium-selective channel HcKCR1 which can be used as an inhibitory tool. Like other rhodopsins, HcKCR1 is a seven-transmembrane α-helical protein. To perform a structural study of this protein, we reconstitute it in a lipid environment and perform multidimensional magic angle spinning (MAS) solid-state NMR. The result is spectra with high sensitivity and good resolution. We discuss the performance and the main features of these experiments which allowed for reliable ^{13}C and ^{15}N assignments of both the backbone and sidechains of 123 residues out of 264 residues, including part of the gate. These shifts are then used to analyze secondary structure and helical boundaries, determine protonation states of ionizable side chains and oxidation states of important cysteines. The analysis is still ongoing. The knowledge of shifts will form the basis of future studies regarding the gating mechanism and K^+ selectivity.

30 - Rapid Simulations of Highly Distorted Multiple-Quantum Magic Angle Spinning NMR Spectra

Lexi McCarthy[1], Brendan Wilson[1], Deepansh Srivastava[1], Jay Baltisberger[2], Philip Grandinetti[1]

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Solid-state nuclear magnetic resonance (NMR) gives detailed information about the local structure around spin-active nuclei, but it is not easily accessible for quadrupolar (spin>1/2) nuclei. The primary way to measure these nuclei is the multiple-quantum magic-angle spinning (MQ-MAS) experiment, but measured lineshapes can be highly distorted. These distortions make lineshape analysis difficult and produce spectra without quantitative integrated intensities. Current methods for simulating these distortions are time-consuming, making least-squares fitting impractical. To address this, we have developed a simplified theoretical description of multiple-quantum excitation and mixing for NMR of half-integer quadrupolar nuclei. In this approach, multiple-quantum nutation behavior is recast in terms of reduced excitation and mixing curves. Second, 2D correlations of the static first-order anisotropic line shape to the second-order anisotropic line shape are used to transform the three-dimensional integral over three Euler angles into a single integral over the dimensionless first-order offset parameter. These transformations lead to a highly efficient algorithm for simulating MQ-MAS spectra for arbitrary rf field strengths, pulse durations, and MAS rates within the static-limit approximation, where pulse durations are less than 5% of a rotor period. By accounting for distortions, we can recover quantitative integrated intensities and more confidently measure quadrupolar NMR tensor parameters.

31 - Determining Hydrogel Mesh Size Using DOSY NMR:

Nils Nordstrom[1], Anuj Saini[1], Nahieli Preciado[2], Bob Berno[1], Todd Hoare[2]

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Diffusion Ordered Spectroscopy (DOSY) NMR is a powerful analytical tool that can be used to study diffusion processes in a mixture. A DOSY experiment consists of three components: a pulsed field gradient applied with increasing amplitude, an evolution period for the molecules to diffuse, and a second pulse to encode the effect of diffusion. Molecules that diffuse far show a large signal attenuation. Based on the attenuation of molecules the diffusion coefficient of a probe molecule can be determined.

By creating a gel in an NMR tube with dextran as a probe molecule, DOSY can be applied to determine the mesh size in poly (ethylene glycol) methacrylate (POEGMA) hydrogels. POEGMA is an In-situ gelling polymer with applications ranging from tissue engineering to drug delivery. Knowing the mesh size can give us valuable information such as how fast drug molecules can diffuse through the gel, and how cells are able to move through a scaffold. By varying different parameters such as the weight percent and the composition of the polymers, we can elucidate their effects on the mesh size.

32 - Investigating the location of the UBA domain of p47 relative to the p47-p97 complex using paramagnetic relaxation enhancement (PRE) NMR spectroscopy

Paige Kanters[1], Megan Black[1]

[1]University of Guelph

p97 is an essential AAA+ ATPase that plays a crucial role in protein homeostasis and several significant cellular processes. p97 is directed to different functions through the interaction with various adaptor proteins, such as p47, which recruits p97 to membrane remodeling. p47 consists of three structured domains connected by long, flexible linkers: ubiquitin-associated (UBA), shp1 eyc p47 (SEP), and ubiquitin regulatory X (UBX). The UBX domain and two linear motifs on the linker (SHP_N and SHP_C) interact with p97 directly, while UBA recruits ubiquitinated substrates to the p47-p97 complex. However, the mechanism by which UBA brings the substrate to the central channel of p97 for processing is currently not well understood. In this study, we aim to investigate the location of the UBA domain of p47 relative to the p47-p97 complex using paramagnetic relaxation enhancement (PRE) nuclear magnetic resonance (NMR) spectroscopy. Our study will provide a better understanding of the interaction that occurs between p47 and p97 and the functional mechanism of the complex.

33 - Characterization of the binding affinity between p47-UBA and Ubiquitin

Nicole Dcosta[1]

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P47 is the first discovered cofactor of the cytosolic p97 AAA+ (**A**TPases **A**ssociated with diverse cellular **A**ctivities) protease. The major role of the p97-p47 complex is in the regulation of membrane remodelling of the endoplasmic reticulum (ER), the nuclear envelope and the Golgi apparatus. The p47-UBA domain is composed of a three-helix bundle, that forms a stable complex with ubiquitin. The p97-p47-Ubiquitin complex acts as a regulatory signal for the recruitment of syntaxin 5 complex. Syntaxin 5 is a member of the Soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family and plays a major role in the maintenance of the Golgi structure. However, the binding affinity of ubiquitin to the p47-UBA domain has not been characterized. In this study, we aim to characterize the binding interaction between p47-UBA with wildtype and mutant ubiquitin. This will be accomplished using Nuclear Magnetic Resonance (NMR) and Isothermal Titration Calorimetry (ITC). The primary objective is to identify a ubiquitin variant that exhibits enhanced binding affinity to p47-UBA, serving as an effective inhibitor for disrupting the specific p97-mediated function.

34 - Identifying the structural arrangement of co-factor p47 upon interaction with the AAA+ enzyme p97 using Paramagnetic Relaxation Enhancement NMR Spectroscopy

Megan Black[1], Dr. Rui Huang[1]

[1]University of Guelph

The AAA+ (ATPases Associated with diverse cellular Activities) family of enzymes is essential for maintaining cellular homeostasis. One enzyme in this family is p97, also known as valosin-containing protein (VCP), which has many roles ranging from membrane fusion to proteasomal degradation. P97 works in conjunction with 30 known adaptors including the protein p47, the first of the adaptors to be recognized. P47 is a linear protein containing a UBA domain which binds ubiquitinated substrates for their delivery to p97 for unfolding. The UBA domain is separated from the rest of p47 by a long linker region, raising questions to its exact orientation within p47 and to p97 during their interaction. Using paramagnetic relaxation enhancement NMR spectroscopy, we have identified key residues on the N-lobe of p97-NTD and near the central pore of D1 that come in proximity to UBA, providing clues to p47-UBA's movement during its interaction with p97. Additionally, we were able to identify regions within p47 in proximity[ND1] with UBA such as in the linker and SEP domain. Together, this information provides insight to where UBA is located with respect to p97 and other p47 domains and thus assists in describing the mechanism of substrate delivery to p97.

35 - 1H NMR metabolomics study of pancreatic cancer cells

Fatema Abdullatif[1], Teklab Gebregiworgis[1]

[1]University of Western Ontario

Metabolic reprogramming is one of the hallmarks of cancer, allowing cancer cells to survive in a notorious tumor microenvironment characterized by limited nutrient supplies, oxygen scarcity, and acidic conditions. Cancer cells exhibit metabolic elasticity to adapt to this hostile environment. Understanding the metabolic adaptability of cancer cells is paramount for developing strategies for early cancer detection, unraveling the mechanisms of drug resistance, and identifying novel therapeutic opportunities.

Metabolomics is a comprehensive approach to studying small molecules, serving as a vital tool for analyzing variations in the identity and quantity of metabolites from biological samples. Nuclear Magnetic Resonance Spectroscopy (NMR) is one of the primary analytical tools used in metabolomics studies due to its non-destructive and quantitative nature, minimal sample preparation requirement, and reproducibility. Here, we present an NMR metabolomics-based approach to study pancreatic cancer cells metabolic reprogramming upon altered amino acid compositions of culture media. The metabolome from the cell-free extract and media were monitored using 1D ^{1}H NMR experiment. MestReNova, Chenomx, and MetaboAnalyst were utilized for metabolite annotation and data analysis, allowing us to study the effect of altered amino acid composition in the media on the endo- and exo-metabolome of pancreatic cancer cells.

36 - Integration of Benchtop NMR as a PAT tool for optimizing bioprocess monitoring and control

Gabriella Gerzon[1, 2], Yi Sheng[1], Marina Kirkitadze[2]

[1]York University, [2]Sanofi

Use of Low-field benchtop NMR to detect parameters of interest for upstream cell fermentation processes for biopharmaceutical manufacturing. Use of NMR to identify and predict parameters of interest that can be utilized to create predictive data models as a PAT tool for process information and feedback. Further application of these models could be implemented for future real-time process monitoring and control.

37 - Operando 7Li Nuclear Magnetic Resonance Integrated with a Novel Three-Electrode setup to study Lithium Metal Deposition on Anode

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[1]Department of Chemistry, [2]McMaster University

Lithium-ion batteries (LIB)s have made the storage of energy accessible and efficient for various portable electronic devices. However, LIBs are subject to capacity loss over time mainly due to lithium plating, which is partially reversible, and dendritic growth, which is irreversible, on the anode during charging.[1,2,3] Accurate determination of when plating starts and distinguishing it from the dendritic growth is crucial in figuring out how to control and/or stop these processes from occurring. While *operando* ^{7}Li Nuclear Magnetic Resonance (NMR) is an established method to monitor Li growth (plated and dendritic), all prior studies have used two-electrode cell designs, which do not permit independent measurement of cathode and anode potentials. Herein, we use *operando* ^{7}Li NMR and a novel three electrode setup to study full-cell lithium-ion batteries during fast charging experiments. For the first time, we correlate the development of these structures, as measured by NMR, with the anode potential, giving crucial insight into these Li growth phenomena. Our work demonstrates the potential of the three-electrode setup integrated with *operando* NMR as a powerful technique to investigate lithium growth in real-time which will eventually help improve the performance of lithium-ion batteries.

1. https://doi.org/10.1021/jacs.3c07339

2. https://doi.org/10.1016/j.jmr.2023.107527

3. https://doi.org/10.1016/j.carbon.2021.12.082

38 - Automated Fitting of NMR Spectra

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Fitting NMR spectra is essential, but fitting NMR spectra manually is time consuming, especially when hundreds or thousands of spectra need to be analyzed, as in *operando* NMR datasets[1]. My goal was to find a way to accurately fit NMR spectra without using large data models or machine learning. I designed a method called Multi-Fit, which is built into an existing NMR fitting tool, ssNake[2]. Using the fit parameters from a single spectrum, Multi-Fit deconvolves the adjacent spectrum, and continues this procedure for all subsequent spectra. This method saves days or weeks of manual fitting, because you need only one good manual fit to fit all data in the dataset. This approach has been used recently to achieve quantitative analysis of lithium speciation in silicon anodes[3].

[1] Operando (in operation) spectroscopy refers to continuous spectra collection from a system while it works under normal conditions.

[2] S.G.J. van Meerten, W.M.J. Franssen, A.P.M. Kentgens, ssNake: A cross-platform open-source NMR data processing and fitting application. (2019) doi: 10.1016/j.jmr.2019.02.006

[3] Kevin J. Sanders, Amanda A. Ciezki, Alexander Berno, Ion C. Halalay, Gillian R. Goward. Quantitative Operando ^{7}Li NMR Investigations of Silicon Anode Evolution During Fast Charging and Extended Cycling. (2023) doi: 10.1021/jacs.3c07339

39 - Quantification of Glycerol Electrooxidation Reaction Products via 1H-NMR

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For over a decade, the increase in biodiesel production has led to an over-production of its side product, glycerol, consequently decreasing its market value. Valorization of glycerol not only enhances the sustainability of the biodiesel industry but also yields economically advantageous products. The glycerol electrooxidation reaction (GOR) is recognized as promising approach due to its simplicity, eco-friendliness, and cost- effectiveness. Yet, its products with various functional groups; aldehyde, hydroxy-acids, hydroxy-diacids, making the product analysis challenging. Notably, High-Performance Liquid Chromatography (HPLC) remains the predominant method for product analysis due to its high sensitivity. However, Proton Nuclear Magnetic Resonance (1H-NMR) offers advantages over HPLC, being a non-destructive and highly sensitive technique. This work provides a road map for the quantitative analysis of GOR products by using an alternative method, 1H-NMR. The findings of this study reveal the impact of the chemical conversions in the alkaline medium and the pH of the electrolyte on the product quantification. As a proof-of-concept, a known concentration of a GOR product mixture was subjected to analysis. The result reveals a consistent correlation between quantified and actual product concentrations, underscoring the reliability of this technique for GOR product quantification and laying a foundation for quantifying similar complex mixtures.

40 - Atomic Resolution Mechanism for the PF4 Non-Antigenic to Antigenic State Transition Induced by Polyphosphates

Qiulin Ma[1], Jinfeng Huang[1], Ellen Mak[1], Giuseppe Melacini[1]

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Heparin-induced thrombocytopenia (HIT) is a potentially fatal complication of heparin treatment. Previous studies have shown that PF4 interactions with polyphosphates (polyPs), which are pro-coagulant inorganic polyanions released from activated platelets, can facilitate the formation of HIT antigenic complexes. However, the mechanism of polyPs-induced antigenic state is not fully understood. Here, we combine Nuclear Magnetic Resonance (NMR) and Dynamic Light Scattering (DLS) to elucidate how PF4 interacts with polyPs of different chain-lengths. First, DLS revealed that PF4/polyPs interactions exhibit a biphasic pattern. Second, polyPs exhibit a stronger binding affinity toward the closed end of asymmetric PF4 tetramers as compared to the open end. Third, polyPs induce a structural transformation in the dimeric PF4 mutant K50E, causing it to adopt a symmetric tetramer configuration. Overall, this study suggests that lengths of polyPs and the asymmetric tetrameric structure of PF4 play critical roles in determining the formation of antigenic complexes targeted by HIT antibodies. This study provides an unprecedented dynamic and allosteric picture of PF4/polyanion complexes and may also point out a potential direction on how to treat HIT-related diseases in the future. Moreover, the results reported here illustrate the effectiveness of NMR-based approaches in investigating the conformational change of asymmetric tetrameric proteins.

42 - Barry Shapiro’s NMR newsletters from 1958 to 2001: The story of NMR in 516 volumes

Clemens Anklin[1]

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Barry Shapiro published the NMR newsletters from 1958 to 2001. They started as the MELLONMR Newsletter turned into the IIT NMR Newsletter and for the longest period were known as the TAMU NMR Newsletters before ending their run as simply the NMR Newsletters after Barry's retirement. These Newsletters lived from the contributions of the subscribers. Each subscriber was required to submit a contribution at regular intervals. They consisted of important methodological developments, interesting applications, whimsical NMR stories and more. Many of the major names in the field provided regular contributions. Overall this collection is a history of NMR in the form of personal communications. The newsletters only existed in paper form and many collection have been forever lost in numerous purges of personal libraries. The digitized archives are available at https://ismar.org/barry-shapiros-nmr-newsletters/ A recording of a presentation from ENC 2023 is here: https://www.youtube.com/watch?v=17lO9dGTQag&t=102s

43 - Ammonia Quantification by NMR for Electrochemical Nitrate Reduction

Anja Schouten[1], Navid Noor[1], Clara Argentino[2], Madeline Lebreton[1], Drew Higgins[1]

[1]McMaster University, Department of Chemical Engineering, [2]McMaster University, Department of Chemistry and Chemical Biology

Ammonia today is critical for mass agriculture and plays a key role in the future for hydrogen storage. Currently, the Haber Bosch process produces ammonia which is notorious for producing over 1% of global CO2 emissions. Alternative production routes are therefore required, an electrochemical approach is advantageous because of the low costs, minimal pollutants, and scalability. Nitrates are a great candidate for electrochemical reduction as they are found abundantly in industrial wastewaters, contain low energy bonds, and are water soluble. A major concern in the electrochemical production of ammonia is accurate quantification of ammonia generated. There are two methods for ammonia quantification, quantitative ^{1}HNMR and UV-vis.

In developing a robust ^{1}HNMR method, the pulse sequence and preparation method were optimized. Water suppression mode was pivotal in generating clean and quantifiable peaks from the ammonium and the internal standard, maleic acid. Water suppression was found to be highly pH sensitive so the sample is carefully acidified post electrolysis. Further, the combination of solutions added to prepare the samples were optimized to allow for direct ammonium quantification. By optimizing both the pulse sequences and sample preparation procedure a robust method was developed with consistency between UV-vis and NMR results.

44 - Investigation of redox-dependent allosteric activation mechanisms of cGMP-dependent protein kinase Iα

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cGMP-dependent protein kinase Iα (PKG) is a key regulator of blood pressure and a target of antihypertensive drugs. PKG activity is tightly controlled by cGMP, a secondary messenger in the nitric oxide and natriuretic peptide signalling pathways. Oxidative stress affects the activity of PKG through the formation of a disulfide bridge in its regulatory domain (CNB-A). Oxidation-dependent regulation of PKG is a mechanism of action for a new class of antihypertensive drugs. However, the molecular mechanism behind this phenomenon is not fully understood. In this project, we are exploring the redox sensitivity of PKG with Nuclear Magnetic Resonance spectroscopy. We completed the NMR assignment of the backbone H^1/N^{15} of PKG CNB-A. Using comparative analysis of H^1/N^{15} HSQC spectra of reduced and oxidised CNB-A, we discovered that the formation of the disulfide triggers oxidation-dependent changes within the CNB-A domain. Allosteric perturbation analyses (CHESCA) of both reduced and oxidised states revealed that oxidation markedly rewires the allosteric network within CNB-A. Moreover, we show that oxidation impacts the dynamics within the regulatory domain of PKG, potentially destabilizing the inhibitory state of the kinase and enhancing its activity.

45 - Alpha-synuclein interactions with lipid membranes - the effect of lipid charge on the protein conformational dynamics

Mahdi Lavasani[1], Scott Ryan[2], Vlad Ladizhansky[1]

[1]university of guelph, [2]University of Calgary

Parkinson's disease (PD) is an age-related neurodegenerative system disorder that affects the neurons. PD is accompanied by the accumulation of toxic protein aggregates called Lewy bodies (LBs). α-synuclein (α-Syn) is the main constituent of LBs, and it plays a major role in PD pathogenesis. α-Syn is intrinsically disordered in solution, but adapts many conformations, depending on the environment. Biophysical studies suggest that lipid composition, and in particular, charge of the lipid membrane, may affect the conformation and dynamics of α-Syn. An alteration in the association of α-Syn with phospholipid membranes could change the balance between the cytosolic and membrane-bound α-Syn and result in α-Syn accumulation and aggregation. Here we use solid-state NMR to examine conformation of α-Syn bound to lipid large unilamellar vesicles (LUV) made of negatively charged DOPA and neutral DOPC. The C-terminus (residues 100-140) is flexible in all examined lipid compositions of varying charge (DOPA:DOPC of 40:60, 35:65 and 25:75 mol%). While the rest of the protein is rigid when bound to LUVs with higher (40% DOPA) negative charge, several other domains become considerably more flexible in LUVs with less negative charge. This structural information may help us identify suitable targets to reduce the formation of misfolded α-Syn.

46 - NMR at the Centre for Chemical Analysis and Training (C-CART)

celine schneider[1]

[1]Memorial University of Newfoundland

A quick presentation of the new location, as well as the current and incoming equipment of the NMR facility in C-CART at Memorial University.

47 - Investigating the Aberrant Inhibition of Protein Kinase A by Mutations Causing Hormonal Resistance

Leonardo Della Libera[1], Karla Martinez-Pomier[1], Madoka Akimoto[1], Katherine Van[2], Giuseppe Melacini[1, 2]

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Cyclic-AMP-dependent protein kinase A or PKA, is a downstream receptor essential for propagating and converting the cyclic-adenosine monophosphate (cAMP) signal into a cellular response. Several mutations in the *PRKAR1a* gene, encoding the PKA regulatory subunit isoform RIa, have been found to cause a disease known as acrodysostosis - a genetic disorder responsible for hormone resistance leading to congenital malformations. These gain of function mutations are known to alter PKA regulation; greatly limiting the release and activation of the catalytic subunit, and inhibiting PKA function. Although some of these mutants have already been investigated structurally, much about them is still unknown. As an example, static structures of the R366X mutant only exist in the inhibited catalytic subunit-bound form, and do not aptly reflect the conformational and dynamic changes that govern PKA regulation. Thus, we hypothesize that acrodysostosis mutations are responsible for perturbing the allosteric network of PKA, leading to a desensitized protein unable to properly respond to the cAMP signal. With the use of various solution NMR experiments (*e.g.* Saturation Transfer Difference, transfer NOESY, and T1 & T2 relaxation) we will examine the effects of these mutations on ligand binding and correlate this to their effects on PKA dynamics and allostery.

48 - Applications of NMR for Methodology Development

Nikki Ritchie[1], Lauren Irwin[1], Mathew Piotrowski[1], Meghan Fragis[1], Jarrod Johnson[1], Jake Magolan[1]

[1]McMaster University

Our lab has developed a method using Pummerer chemistry to synthesize novel alkylthioalkyl phosphonium chloride salts from sulfoxides. These salts can be utilized in Wittig chemistry producing vinyl sulfides in inseparable mixtures of E/Z isomers which were characterized using NMR.

Our lab discovered an alumina-templated ortho allylation that selectively installs prenyl chains ortho to a phenol. In our development of this chemistry, we found this reaction could produce several products that were difficult to separate. NMR was used to determine ratios of these products to optimize this reaction to favour our desired product.

This poster will outline these methodologies and the important role NMR played in the development of these methods.

49 - Effect of Fatty Acid Binding and Glycation on Human Serum Albumin Ability to Suppress Alpha-Synuclein Metal-Induced Toxicity

Karla Martinez Pomier[1], Rashik Ahmed[1], Jinfeng Huang[1], Giuseppe Melacini[1]

[1]McMaster University

Human serum albumin (HSA), the most abundant protein in blood plasma, is a potent inhibitor of several amyloidogenic proteins, including alpha synuclein (aSyn) and the Aβ peptide associated with Parkinson's and Alzheimer's disease, respectively. HSA decreases neurotoxicity through several mechanisms, which range from direct binding to monomeric and oligomeric species of amyloidogenic proteins to the sequestration of metal ions that promote aggregation. Cu(II) ions, for example, enhance aSyn fibrillization *in vitro* while also leading to neurotoxicity by generating reactive oxygen species (ROS). Using an integrated set of NMR experiments, we show that HSA is able to chelate Cu(II) ions from aSyn more efficiently than standard chelators such as EDTA, showing what is to our knowledge, the first evidence of cooperativity between HSA's two metal binding sites. We also found that fatty acid binding to HSA perturbs the cooperativity between its two metal binding sites, thus interfering with the chelation of Cu(II) ions from aSyn. On the other hand, glycation of HSA diminished Cu(II)-binding affinity but the cooperativity of both sites was conserved. Additionally, our results show that Cu(II)-bound HSA enhances binding to aSyn at both, the N- and C-termini. Our study not only emphasizes the importance of fatty acid binding and age-related posttranslational modifications such as glycation, for the neuroprotective mechanisms of HSA, but also highlights the potential of aSyn as a viable NMR-based sensor to investigate HSA-metal ions interactions.

51 - Balancing Promiscuity and Selectivity - Determining Factors Governing G-Protein Selectivity and Efficacy for the Adenosine A2A Receptor

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[1]University of Toronto

Biological processes are regulated by diverse mediators including hormones and neurotransmitters. These mediators must interact with the receptor allowing the transfer of information from the outside to the inside of the cell. GPCRs, targeted by 30% of FDA approved drugs, are the largest family of receptors which transduce signals across membranes. The adenosine A2A receptor (A2AR) is a prototypical G protein coupled receptor (GPCR) belonging to a superfamily of 7-transmembrane proteins. A2AR is promiscuous and couples to several G-proteins. In addition to its cognate G protein, Gs. 19F NMR identified facets of the receptor that dictated selectivity in agonist-stimulated A2AR-Gs and A2AR-Go complexes in phospholipid nanodiscs. TM6 adopted two activation states, whose differing outward displacements proved compatible with the larger and smaller volume H5 helices of Gs and Go, respectively. TM7 adopted an activation intermediate in addition to two activation states (A1 and A2) in the A2AR-G protein complex. While the nucleotide-free A2AR-Gs ensemble was biased toward the fully active A1 state, the A2AR-Go ensemble was characterized by a dynamic inactive fraction. TM7 activation states were correlated to distinct NPxxY configurations and an allosteric network connecting helix-8 (H8), known to influence G-protein coupling. Spectra of the H5-helix in both A2AR-Gs and A2AR-Go reveal extended and compact states of Gs/Go. The cognate Gs subunit is more biased toward the active (extended) state of the G-protein, corroborating differences in efficacy. Enhanced sampling MD simulations and allostery provide a mechanistic explanation for the observed promiscuity and enhanced selectivity of A2AR for Gs.