MOOT35
McGill University

Montreal QC
October 19-20, 2024

Program and Abstracts

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All of the talks, breaks, lunches and poster session will take place in the Bronfman Building, 1001 Sherbrooke St. W. Entrance via the rear door.

Saturday, 19 October 2024

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Bronfman Lobby

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Why We Need High Field Solid-State NMR

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Robert Schurko
National High Magnetic Field Laboratory

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09:55 - 10:15
Towards a High-Performance EPR Facility at University of Toronto

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Joerg Reichenwallner
University of Toronto, Biochemistry

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Zeeman-Perturbed Nuclear Quadrupole Resonance Spectroscopy: a Powerful Technique for Characterizing Halogen Bonding in Cocrystals

ID 9
Alireza Nari
University of Ottawa

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Second Floor Lounge

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	11:15 - 11:35 Characterization of ARL15 as an atypical GTPase regulating magnesium homeostasis	ID 22 Luba Mahbub Department of Biochemistry and Centre de recherche en biologie structurale, McGill University, Montréal, Canada
	11:35 - 11:55 Unveiling the Substrate Delivery Mechanism in the p97-p47 Complex	ID 24 Megan Black University of Guelph
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	13:50 - 14:10 Exploring a non-conventional method : nitrogen-14 overtone solid-state nuclear magnetic resonance	ID 7 Audrey-Anne Lafrance University of Ottawa
	14:10 - 14:30 Determining Ion Dynamics Within Fast Ion Conducting Solid State Sodium Electrolytes Using 23Na Magic-Angle-Spinning (MAS) NMR and Simulated 1D Spectra	ID 21 Cameron A. Gurwell McMaster University
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	16:10 - 16:30 Polymeric and Nonpolymeric Per- and Polyfluoroalkyl Substances (PFAS) in Building Materials from Canada	ID 25 Min Liu Department of Earth Sciences, University of Toronto; Department of Chemistry, Université de Montréal

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	11:30 - 11:50 Atomic-level observation of RAS GTPase activity in live cells using NMR spectroscopy	ID 8 Regina Strakhova IRIC, University of Montreal
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Please join Elena Baglietto from Quantum Technology for a tour of the McGill Magnetic Resonance Facilities Helium Recovery System. The labs are a few minutes walk away from the conference site, and the tour will leave after box lunches have been picked up.

Oral Presentation Abstracts

Why We Need High Field Solid-State NMR

Robert Schurko[1, 2]

[1]National High Magnetic Field Laboratory, [2]The Florida State University

NMR spectroscopy is undoubtedly one of the most important techniques for the characterization of molecular-level structure. The low-energy nature of NMR allows for unmatched detail in atom-by-atom assignments and monitoring of reactions and dynamical processes; however, this also renders the NMR signal one of the weakest among all of the spectroscopies, which presents great challenges for NMR spectroscopists. There are numerous strategies to address this issue, including better probes, advanced pulse sequences, low-temperature operation, and higher magnetic fields - but among these, experimentation at high magnetic fields yields the most benefits.

Solid-state NMR (ssNMR) spectroscopy particularly benefits from high magnetic fields, not only for signal enhancement, but for increased resolution, reduction of peak broadening (especially for half-integer quadrupolar nuclides), expanding the range of observable nuclides, improving quantitative analysis, and providing increasingly detailed information on dynamics. Hence, in my presentation, I will discuss the significant advantages of high-field SSNMR spectroscopy for studying all manner of chemical, biological, and materials systems, along with strategies for further improvements, including spin polarization transfer techniques and GHz-class NMR probes. I will show a variety of examples for different nuclides across the Periodic Table, both from my research group, as well as from the NMR User Program at the National High Magnetic Field Laboratory (MagLab).

Towards a High-Performance EPR Facility at University of Toronto

Joerg Reichenwallner[1], Oliver P. Ernst[1, 2]

[1]University of Toronto, Biochemistry, [2]University of Toronto, Molecular Genetics

Electron paramagnetic resonance (EPR) spectroscopy has proven itself to be an exceptionally powerful magnetic resonance tool in a broad variety of scientific disciplines. Especially, in Biochemistry, Biophysics, Inorganic and Physical Chemistry, pulsed dipolar spectroscopy (PDS) and hyperfine spectroscopic techniques have emerged as inevitable tools to screen complex functional dynamics and processes in paramagnetic catalytic centers or synthetic or biological macromolecules such as proteins. Employing EPR spectroscopy usually requires site-specific modification via covalently attaching paramagnetic species to the substrate in order to obtain EPR signals. This so-called site-directed spin-labeling (SDSL) approach facilitates topologically targeted investigations that reveal intrinsic (spin) dynamics, conformational changes, presence of conformational ensembles, or formation of supramolecular assemblies. Most significantly, this allows us to detect and analyze paramagnetic species, as well as investigate intricate nanoscale processes with high spatial resolution that remain rather obscured in most other spectroscopic techniques. Whereas most model systems do not require high instrumental sensitivity, investigating e.g. G-protein-coupled receptors (GPCRs) via EPR bears several intrinsic challenges that can be only overcome via appropriate purification strategies and state of the art high-performance equipment. Although EPR in Toronto was so far mainly employed as such an integrative structural biological tool, the project portfolio is currently expanding with steadily increasing numbers of collaborations from across Canada. We highlight recent progress and enhanced performance capabilities of this EPR facility at University of Toronto which is unique in Canada.

Zeeman-Perturbed Nuclear Quadrupole Resonance Spectroscopy: a Powerful Technique for Characterizing Halogen Bonding in Cocrystals

Alireza Nari[1], David Bryce[1], Mubassira Rahman[1], Patrick Szell[1]

[1]University of Ottawa

The spectroscopy of quadrupolar nuclei is influenced by the relative magnitudes of the Zeeman and quadrupolar interactions. In the regime of negligible or very small magnetic field, we observe pure nuclear quadrupole resonance (NQR) or Zeeman-perturbed NQR (zp-NQR), while the dominance of the Zeeman interaction leads to quadrupole-perturbed NMR.

Here, we explore the application of zp-NQR to nuclei with a spin of 5/2 (^{127}I) and spin of 3/2 ($^{79/81}$Br), focusing on transitions between the energy levels $m_I = \pm 1/2$ and $m_I = \pm 3/2$ (the lower frequency line for $I = 5/2$ and the sole transition for spin $I = 3/2$ nuclei). This approach is particularly advantageous for nuclei with $I \geq 5/2$, where the higher frequency transitions exceed 600 MHz, surpassing the capabilities of conventional instrumentation.

We first determine the NQR resonance frequencies in halogen-bonded cocrystals across the expected frequency range of 250 to 360 MHz. Following this, we implement zp-NQR using an EPR electromagnetic field, which generates a high-quality, uniform, adjustable weak magnetic field suitable to perturb the pure NQR transitions. The zp-NQR spectra were recorded for several halogen-bonded cocrystals with I-N, I-O, Br-N, and Br-O interactions to ascertain the asymmetry parameter (η) and C_Q values utilizing exact simulations.

ZORA density functional theory (DFT) simulations yield quadrupolar parameters that effectively corroborate our experimental findings. Our findings reveal a significant correlation between the quadrupolar parameters and the structural characteristics of the cocrystals, enhancing our understanding of halogen bonding and its impact on the local electronic environments.

pH-sulfate synergy regulates processing and mechanics of mussel byssus protein condensates

Hamideh. Rezvani Alanagh[1], Tara Sprules[1], Anthony Mittermaier[1], Matthew Harrington[1], Adam Hendricks[1], Magda G. Sánchez-Sánchez[1]

[1]McGill

Fluid protein condensates are used as precursor phases for fabricating extracellular protein-based materials including elastin, spider silk, and mussel byssus. The byssus, utilized by mussels for anchoring in marine environments, consists of tough, self-healing adhesive fibers. Byssus formation involves the secretion of protein condensate droplets under acidic conditions that subsequently solidify under basic seawater conditions. We currently have a poor understanding of the physicochemical triggers and molecular-level interactions at play, in particular the role of pH and sulfate anions previously identified during native fabrication. Here, we investigated the pH and sulfate-dependent structural and mechanical response of condensates made from a recombinant byssus protein (mfp-1) using optical tweezers microrheology, FRAP, confocal Raman spectroscopy, and NMR. We found that the protein concentration in condensates increased, and the viscoelastic response became more rigid under basic conditions in the presence of sulfate ions compared with chloride ions, consistent with spectroscopic analysis indicating different molecular interactions under these different chemical conditions. These studies highlight the crucial interplay between sulfate anions and pH in tuning condensate viscoelasticity via control of intermolecular interactions, providing insights into the natural byssus formation process with relevance for bio-inspired materials processing of sustainable plastics and materials for tissue engineering.

Characterization of ARL15 as an atypical GTPase regulating magnesium homeostasis

Luba Mahbub[1], Guennadi Kozlov[1], Emma Lee[1], Thushara Nethramangalath[2], Caroline Knorn[1], Loren W Runnels[2], Kalle Gehring[1]

[1]Department of Biochemistry and Centre de recherche en biologie structurale, McGill University, Montréal, Canada, [2]Rutgers-Robert Wood Johnson Medical School, Piscataway, New Jersey, USA

CBS-pair domain divalent cation transport mediators (CNNMs) are a family of four membrane proteins involved in magnesium transport and homeostasis. CNNMs mediate efflux of Mg^{2+} ions on their own and regulate influx of divalent cations when coupled to the transient receptor potential ion channel subfamily M member 7 (*TRPM7*). Phosphatases of regenerating liver (PRL or PTP4A) bind and inhibit CNNM Mg^{2+} efflux and can promote TRPM7-mediated ion influx. The small GTPase ADP-ribosylation factor like GTPase 15 (ARL15) was identified as a CNNM-binding partner and an inhibitor of divalent cation uptake by TRPM7 ion channel. In this study, we characterize ARL15 as an atypical GTPase. Using isothermal titration calorimetry (ITC) and Phosphorus-31 NMR spectroscopy, we show that ARL15 has low GTP-binding affinity and low intrinsic GTP hydrolysis rate as compared to other conventional GTPases. ARL15 binds to the cytosolic CBS-pair domains of CNNMs with low micromolar affinity and this binding abrogates CNNM Mg^{2+} efflux activity. The crystal structure of the complex between ARL15 GTPase domain and CNNM2 CBS-pair domain reveals the molecular determinants of the interaction. Loss of CNNM-binding leads to a loss in the ability of ARL15 to suppress CNNM2 and TRPM7. Our structural analysis and ^{1}H-^{15}N HSQC NMR experiments indicate that ARL15 competes with PRL phosphatases for CNNM binding. As PRL phosphatases are oncogenic proteins that promote tumor metastases by increasing intracellular magnesium, our study provides insights into a potential tumor suppressor role of ARL15 through an antagonistic effect on divalent cation transport by the CNNM-TRPM7 complex.

Unveiling the Substrate Delivery Mechanism in the p97-p47 Complex

Megan Black[1], Peter Kim[1], Paige Kanters[1], Rui Huang[1]

[1]University of Guelph

The AAA+ (ATPases Associated with diverse cellular Activities) family of enzymes is essential for maintaining cellular homeostasis. The AAA+ ATPase p97, also known as Valosin-containing protein (VCP), has an array of functions ranging from membrane fusion to proteasomal degradation. p97 works in conjunction with 30 known adaptors to carry out these functions. p47, the first of the adaptors to be recognized, has three structured domains connected by flexible linkers. Its UBA domain binds ubiquitinated substrates for their delivery to p97 for processing. A long, intrinsically disordered linker follows the UBA domain, raising questions to its exact orientation within p47 and the p97-p47 complex. Using chemical shift perturbations, we have identified two novel binding sites between the p47 linker and p97 N-terminal domain (NTD) that may aid in directing the UBA domain toward the p97 central pore. Using Paramagnetic Relaxation Enhancement (PRE) NMR spectroscopy, we have extensively probed p47 to identify regions of proximity both within p47 alone and in complex with p97. We have translated these results into distance constraints for molecular simulations. Further work will include probing p97 at key locations in the NTD and D1 ring to find close contacts in p47 when in complex. NMR derived constraints will guide the use of additional techniques such as crosslinking mass spectrometry, fluorogenic assays with mEos3.2, and ATPase activity assays. 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.

Unveiling the Kinetic Tango: Exploring G-Quadruplex Ligand Binding Kinetics and Transfer Mechanisms for Enhanced Therapeutic Strategies

Hariz Nizal[1], Anthony Mittermaier[1]

[1]McGill University

Guanine quadruplexes (G4s) are non-canonical nucleic acid secondary structures which are thought to play regulatory roles in biological processes such as DNA replication, transcription, and telomere maintenance. This is significant as G4-containing genes have been implicated in oncogenesis, highlighting their potential as valuable targets for therapeutics. Small molecules, such as porphyrin derivatives, have been shown to interact with G4s modulating their stability and function. In therapeutics, the kinetics of G4-ligand interactions are important as they determine the bound lifetimes of these complexes. These in turn govern how the ligand finds its in-cell target and the extent to which it becomes trapped in off-target complexes.

In this study, we utilise NMR relaxation and SPR experiments to measure the binding kinetics of the cMYC G4 with the porphyrin TMPyP4. Our results provide insights into the binding mechanisms and dynamics of G4-ligand complexes. We found that the dissociation of the studied ligand from their G4 partners occurs so slowly, that the ligand becomes effectively trapped onto the first G4 structure it encounters. However, we discovered that this dissociation rate increased dramatically as the concentration of G4 increased. Further analyses indicated a novel mechanism in which ligands are transferred directly between G4s via collisions in solution. We suggest a pathway mimicking facilitated diffusion in protein-DNA interactions through which ligands can reach intended quadruplex targets by utilizing much more readily available neighbouring duplex strands. This has potential implications for the development of new therapeutic strategies targeting G4s.

MAPPING THE INTERACTION OF ANTIMICROBIAL PEPTIDES WITH THE CELL WALL OF GRAM-POSITIVE BACTERIA USING SOLID-STATE NMR

Arunima Sandeep[1], Laila Zaatouf[2], Alexandre A. Arnold[1], Dror E. Warschawski[1, 2], Isabelle Marcotte[1]

[1]Université du Québec à Montréal (UQAM), [2]Sorbonne Université

The interaction of cationic antimicrobial peptides (cAMPs) with bacterial lipid membranes is well studied, but there is limited knowledge on how they interact with the cell wall. In Gram(+) bacteria, the cell wall consists in a large, complex and insoluble polymer, mainly composed of polysaccharides, peptidoglycan (PGN) and negatively charged, phosphate-rich wall teichoic acids (WTAs). This envelope is crucial for cell survival and is the first crossed by cAMPs en route to the lipid membrane. In this work, we studied the interaction of cAMPs with Gram(+) bacterial cell walls. More specifically, solid-state NMR (ss-NMR) was employed to investigate the interaction sites of three cAMPs: aurein 1.2, caerin 1.1 and DMS-DA6-NH_2, with the cell wall of *Bacillus subtilis*. Using ^{31}P ss-NMR, we monitored interactions between cAMPs and WTAs in extracted cell walls. We compared ^{31}P spectral line shapes and relaxation times (T_1 and T_2) in the presence and absence of cAMPs, revealing peptide-specific interactions with the glycerol-phosphate groups of the WTAs, which alter cell wall dynamics. Additionally, ^{13}C ss-NMR also revealed changes in the dynamics of different cell wall carbohydrate atoms. We further extended the study to *Staphylococcus aureus,* known for its pathogenicity, and similar dynamic changes in ^{31}P and ^{13}C ss-NMR were observed with DA6. Our results suggest that cAMPs significantly interact with the cell walls of Gram(+) bacteria, thus expanding our understanding of cAMP's action mechanisms. This is essential for addressing the global issue of antibiotic resistance and propose alternative therapeutic strategies.

Exploring a non-conventional method : nitrogen-14 overtone solid-state nuclear magnetic resonance

Audrey-Anne Lafrance[1], David Bryce[1]

[1]University of Ottawa

Nitrogen is one of the main elements found on earth. Its most common isotope, nitrogen-14, with a natural abundance of 99.636%, is not commonly studied by NMR spectroscopy because it is a spin-1 nucleus lacking a central transition. The large quadrupole moment gives rise to broad signals extending over several megahertz wide. This leads to complications in acquiring high-quality spectra. Instead, the nitrogen-15 isotope is often the isotope of choice because it is a spin-1/2 nucleus. Unfortunately, it has a low natural abundance of 0.364%, which makes it time-consuming to get a good NMR spectrum without expensive isotopic enrichment.

^{14}N overtone solid-state NMR spectroscopy is an unconventional method that can be used to study nitrogen-14 directly. By doubling the Larmor frequency at which nitrogen-14 is irradiated, the double quantum transition *m* = +1«-1 becomes observable. This forbidden transition is possible because of the perturbation of the Zeeman interaction by the large quadrupole interaction mentioned earlier. An interesting phenomenon that was discovered in previous literature is that the overtone peak position is dependent on the direction and the speed at which the sample is spinning. In this work, we report a ^{14}N overtone solid-state NMR study of a variety of compounds, with an emphasis on developing a novel tool to characterize dynamic processes.

Determining Ion Dynamics Within Fast Ion Conducting Solid State Sodium Electrolytes Using 23Na Magic-Angle-Spinning (MAS) NMR and Simulated 1D Spectra

Mengyang Cui[1], Cameron A. Gurwell[1], Darren H. Brouwer[2], Kevin J. Sanders[1], Gillian R. Goward[1]

[1]McMaster University, [2]Redeemer University

Solid state batteries are gaining increasing attention due to their intrinsic safety compared to liquid electrolyte batteries, as well as their potential for higher energy density. Sodium-based batteries present a lower cost solution to the increasing price of lithium metal in batteries, while reducing our environmental impact. For sodium batteries, the study of ^{23}Na NMR nuclei gives a non-destructive description of the local structure within the solid electrolyte ion conductor between the anode and the cathode. For $Na_{4-x}Sn_{2-x}Sb_xGe_5O_{16}$[1], there are three Na sites, two which are coalesced. Through antimony doping, the highest ionic conductivity within the series (at x = 0.2) reached 0.11 mS cm^{-1} at room temperature via EIS[1]. The Na1 site (8d) is considered relatively immobile based on its standard quadrupolar line shape. Na2 (4c) and Na3 (4c) sites are considered more dynamic due to their unresolved peaks, which suggests that the two sodium sites are rapidly exchanging. 1D and Multiple Quantum (MQ) MAS ^{23}Na NMR were run on a series of NMR spectrometers (7, 11.7, and 20 T) to obtain experimental isotropic shifts and the quadrupolar product (PQ) between C_q and η for the Na2-3 coalesced peak. These values were then used to ratify a set of parameters found theoretically using the EXPRESS program[2] for MATLAB. Here, we present our findings using ^{23}Na NMR to determine the ion dynamics within a solid-state sodium electrolyte.

1. Novikov, S., et al., *Inorganic Chemistry* **2023**, *62*, 16068-16076.

2. Vold, R. L.; Hoatson, G. L., *J Magn Reson* **2009,** *198* (1), 57-72.

Understanding Lithium Salt Distribution in Doped Thermoplastic Vulcanizate Electrolytes via an Analysis of Salt-Solvent-Polymer Interactions

Gabrielle Foran[1], Joseph Chidiac[1], Caroline St-Antoine[1], Paul Nicolle[1], Arnaud Prébé[1], Mickael Dollé[1]

[1]Universite de Montreal

Lithium salt distribution in polymer electrolytes depends strongly on interactions between the lithium salt and various components of the electrolyte. These may include one or more polymers and absorbed water or other solvents. These interactions, along with information on relevant dynamic processes can be characterized using solid-state NMR spectroscopy. Thermoplastic vulcanizate electrolytes are complex systems comprised of a thermoplastic phase, an elastomeric phase and a lithium salt. These electrolytes have been shown to possess both the ionic conductivity of the thermoplastic phase and the mechanical strength of the crosslinked elastomeric phase. Lithium salt can be distributed between these phases, impacting the overall ionic conductivity of the system. The mechanical strength of these electrolytes is sufficient to allow for the incorporation of liquid dopants without significantly compromising their resistance to compressive forces. Although these dopants have been shown to increase ionic conductivity, they also alter salt-polymer interactions in the material. Lithium NMR spectroscopy was used in conjunction with infrared spectroscopy to show that solvent addition affects both lithium salt distribution between the polymeric phases in the electrolyte and the relative strength of interactions between the salt and the polymer phase. These changes resulted in increased lithium ion mobility as demonstrated by measuring lithium ion diffusion and ionic conductivity. Ionic conductivities on the order of those observed in the dopants themselves were not obtained in these systems. This phenomenon was attributed to dopant addition producing a plastified polymeric phase through which ionic conductivity occurred.

NMR Metabolomics of Mosquito Samples using Artificial Neural Networks

Olivia Bianco[1], Aisha Abdi[1], Megan Meuti[1], Matthias Klein[2]

[1]The Ohio State University, [2]McGill University

NMR spectroscopy, besides mass spectrometry, is one of the main techniques employed in *metabolomics*, the comprehensive analysis of small molecule metabolites in living systems. Despite NMR's simplicity and molecular coverage, some sample types challenge NMR's intrinsic limitations, especially regarding sensitivity. We here present recent developments in NMR metabolomics of challenging sample types, namely the analysis of mosquitoes, with average body weights of about 2.5mg. This study focused on changes related to *diapause*, an adaptation to survive the cold season, which is notable due to mosquitoes ceasing their blood-sucking behavior, and the potential to induce diapause to prevent disease transmission. Northern house mosquitoes (*Culex pipiens*) were reared under long-day (summer) and short-day (winter) conditions to induce diapause, and then exposed to Major Royal Jelly Protein 1 (MRJP1) to assess impact on diapause. Single mosquitoes were collected, and whole-body extracts prepared for 1D ^{1}H NOESY measurements on an 850 MHz Avance III HD Ascend spectrometer with an inverse cryoprobe and z-gradients (Bruker, Billerica, MA). The R package *mrbin* was used for untargeted analysis of the obtained spectra. Data were analyzed using linear models and artificial neural networks (ANN). Results showed that optimized sample preparation and data analysis yielded clear and consistent NMR spectra of the mosquito metabolome. Strong metabolic differences between short day and control groups, and between feeding regimes were observed. We also present updated strategies for data analysis of metabolomics datasets, including a new metric score to identify features of interest in ANN models.

Untargeted 1D 1H NMR metabolic profiling of Pin1 inhibition in pancreatic cancer

Fatema Abdullatif[1], Xiao Zhen Zhou[1, 2], Kun Ping Lu[1, 2], Teklab Gebregiworgis[1, 2]

[1]Department of Biochemistry, Schulich School of Medicine & Dentistry, Western University, London ON, [2]Department of Oncology, Schulich School of Medicine & Dentistry, Western University, London ON

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive solid malignancy with mortality rates almost equal to its incidence. One of the main challenges in treating PDAC is its ability to develop resistance to chemotherapy by rewiring its metabolism, underscoring the urgent need for new therapeutic agents and novel target identification. Pin1, a peptidyl-prolyl isomerase often overexpressed in cancers, including PDAC, plays key role in regulating multiple oncogenes and tumor suppressors, impacting many hallmarks of cancer, making it a promising target for therapeutics intervention. In vitro studies show that Pin1 inhibitors decrease pancreatic cancer cell viability, and in vivo studies further demonstrate that Pin1 inhibition enhances the efficacy of both chemotherapeutic and immunotropic agents.

The potential role of Pin1 in regulating cancer cell metabolism, however, remains largely unexplored. To understand the molecular mechanism of action of Pin1 inhibition, we first conducted sequence motif searches and structural analysis to assess surface-accessibility, leading us to hypothesize that Pin1 may regulate multiple metabolic enzymes, and thus may rewire cancer cells metabolism. To explore this, we utilize Nuclear Magnetic Resonance (NMR)-based metabolomics to study both the exo- and endo-metabolome of PDAC cells following Pin1 inhibition. We collected ^{1}D ^{1}H NMR spectra of cell lysates and conditioned media of Pin1 inhibitor- treated and untreated cells, the spectra were further analyzed using multivariate and univariate analysis. Our findings show that Pin1 inhibition results in a distinctive metabolic phenotype, specifically those involved in glycolysis, including glucose, and lactate, and some amino acids such as branched chain amino acids.

Development and characterization of a noncovalent 19F-containing NTA-based probe for protein NMR spectroscopy

Tran Thanh Tam Pham[1], Jayatee Ray[1], Krishna Desireddy[1], Stephen Bearne[1, 2], Jan Rainey[1, 2, 3]

[1]Department of Biochemistry & Molecular Biology, Dalhousie University, [2]Department of Chemistry, Dalhousie University, [3]School of Biomedical Engineering, Dalhousie University

This study explores the development and application of a novel noncovalent ^{19}F-NMR probe designed to bind to His-tagged proteins via a metal-chelated interaction. This probe offers advantages over traditional covalent labeling methods, including potential applications in on-cell NMR and for biophysical study of proteins with reduced interference with protein function. The probe, consisting of a trifluoromethyl group for ^{19}F-NMR detection and a nitrilotriacetic acid (NTA) group for metal ion chelation, was synthesized and its metal, peptide, and protein binding properties and suitability for application were characterized using ^{19}F and ^{1}H NMR spectroscopy. Metal binding studies demonstrated specific interactions with Ni^{2+}, Co^{2+}, and Zn^{2+} ions, evidenced by distinct chemical shifts in the ^{19}F-NMR spectra. Further investigation with imidazole to mimic histidine residues confirmed the probe's suitability to bind to His-tagged proteins. Application of the probe to His-tagged SUMO protease and to biotinylated peptides both in unbound form and complexed with avidin showed varying degrees of probe-His-tag binding, elucidated through ^{19}F-DOSY experiments. This study establishes a versatile approach for noncovalent ^{19}F-labeling in protein NMR spectroscopy, offering insights into protein-protein interactions and potential future applications in structural biology.

Polymeric and Nonpolymeric Per- and Polyfluoroalkyl Substances (PFAS) in Building Materials from Canada

Min Liu[1], Chunjie Xia[2], Hui Peng[3], Elizaveta Zvereva[3], Sébastien Sauvé[4], Marta Venier[2], Miriam Diamond[5]

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We hypothesize that outdoor building materials are a significant source of PFAS to the environment. To test this hypothesis, three categories of outdoor products sold on the Canadian market (145 in total), namely outdoor paints, sealers, and textiles, were characterized for total organic fluorine (TOF) using 1D- and two-dimensional Diffusion-Ordered Spectroscopy (2D-DOSY) fluorine nuclear magnetic resonance (^{19}F NMR), followed by PFAS analysis with liquid chromatography-high-resolution mass spectrometry (LC-HRMS) and gas chromatography-mass spectrometry (GC-MS). TOF was detected in 22, 13, and 21 of the tested paints, sealers, and textiles, at concentrations of 0.1-16.1, 0.4-2490, and 0.3-74.0 μmol/g, respectively. 2D DOSY revealed that 6:2 fluorotelomer (FT) nonpolymers were the major PFAS in paints, while those in sealers comprised both side-chain fluorinated polymers (SCFPs) and nonpolymers. Three types of precursors (most likely SCFPs), including 6:2 FT, 8:2 FT, and perfluorobutane sulfonyl fluoride (PBSF)-based, were identified in outdoor textiles. Targeted, suspect, and nontargeted analysis by LC-HRMS and targeted GC-MS analysis further revealed that paints and sealers with 6:2 FT nonpolymers mainly contained nonvolatile 6:2 FT mono-, di-, tri- phosphate esters (6:2 monoPAP, 6:2 diPAP, 6:2 triPAP) and 6:2 FT di-pyrophosphate, followed by volatile 6:2 fluorotelomer alcohol (6:2 FTOH). In contrast, outdoor textiles primarily contained volatile n:2 FTOH (n=8 or 6) or N-methyl perfluorobutane sulfonamide alcohol (MeFBSE). These results support the hypothesis that some, but not all, outdoor paints, sealers, and textiles could be significant sources of polymeric and nonpolymeric PFAS to the environment and biota.

Interesting Experiences in using NMR for Drug Discovery- In Pharma, CRO/P, Biotech and Academia

Steven LaPlante[1]

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NMR Crystallography of Cannabinoids

Yishu Shi[1], Tristan LaCasse[1], Douglas MacKenzie[1], Victor Terskikh[1], Andreas Brinkmann[1]

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The Metrology Research Centre at the National Research Council Canada develops and provides certified reference materials to laboratories performing cannabinoid analysis of cannabis and hemp. in this contribution we present our recent progress in characterizing selected cannabinoids by NMR crystallography, the combined approach of solid-state NMR, powder X-ray diffraction, and density functional theory (DFT) calculations. We performed natural abundance ^{13}C cross-polarization magic-angle spinning (CP-MAS) and ^{13}C-^{13}C single-quantum double-quantum (SQ-DQ) correlation experiments. Furthermore, the parameters for the ^{13}C chemical shift anisotropies (CSAs) were determined by CSA recoupling experiments. First results on our efforts to label cannabinoids with ^{18}O and ^{17}O and subsequent ^{17}O NMR experiments will be presented. The experimental results are complemented by calculations of the NMR parameters by periodic DFT using geometry-optimized structures and structures generated by molecular dynamics (MD) and path-integral MD calculations.

We will also use the opportunity to advertise the upcoming opening for a postdoc in the Metrology group for the project "Parahydrogen-Induced Hyperpolarization: Applications to Quantitative NMR and Quantum Information Processing", in which the successful candidate will setup and exploit parahydrogen enhanced NMR spectroscopy to push the sensitivity of quantitative NMR to unprecedented limits, enabling orders of magnitudes lower limits of detection and quantification.

Site-specific isotope analysis by isotopic NMR and GC-IRMS spectrometry with a numerical model for Food Authentication and Environmental Forensic Studies

Phuong Mai Le[1]

[1]National Research Coucil Canada

The stable isotope analysis extremely valuable for the identification of food origins and environment forensics. Isotope ratio mass spectrometry (IRMS) determinate the bulk isotope of the whole molecule, while isotope NMR is currently the only method to determinate isotope ratios at individual positions, providing more intramolecular information for food authentication and traceability of pollutants. In this work, we implemented ^{13}C- and ^{2}H-qNMR pulse sequences and developed a new deconvolution model for the measurement of the NMR peak areas to analyze carbon and hydrogen isotope composition of Vanillin, one of the most popular flavor used in food industry and Methyl tert-butyl ether (MTBE), a gasoline additive with the highest production volume worldwide, frequently detected as soil and groundwater contaminant. The set of carbon and hydrogen isotope findings greatly contribute to identify compounds from different sources and overcome the limitations of existing method. To validate our results obtained, inter-laboratory comparison studies was conducted using two independant GC-IRMS and qNMR methods. We obtained remarkably consistent values between two methods for different samples [1; 2; 3].

This qNMR approach would also be expanded to investigate in other research such as food and pharmaceutical fields.

Reference:
[1] Markus Greule, Phuong Mai Le, Juris Meija, Zoltan Mester, Frank Keppler. *Journal of the American Society for Mass Spectrometry,* 35,100-105, (2024).
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[3] Phuong Mai Le, Markus Greule and al. Manuscript on ^{2}H-NMR measurements in preparation

Conformation and dynamics of monomeric, phase separated, and cross-linked resilin biomaterials

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Resilin is an elastomeric protein found in insects that provides flexibility and locomotive function in numerous biological contexts. Recombinant resilin and resilin-derived proteins share resilin's capacity for liquid-liquid phase separation (LLPS) and formation of materials with high elasticity and biocompatibility, making it a promising biomaterial for regenerative medicine and tissue engineering. While most prior research has focused on domain 1 of pro-resilin, the role of resilin domain 3 (D3) in self-assembly and material properties is not well understood. Here, we used nuclear magnetic resonance, electron paramagnetic resonance, and small-angle X-ray scattering to study the conformation, dynamics, and intermolecular interactions of resilin D3 as a monomer, in the phase separated state, and as a crosslinked gel. We show that D3 remains unusually dynamic and is primarily disordered in all three states. In elucidating the mechanism of D3 LLPS, we find a complex array of electrostatic and π-cation interactions, and also provide evidence of a short sequence motif that drives local hydrophobic collapse to facilitate LLPS, even in the absence of discrete structural elements. Overall, these results highlight the complex mechanisms governing resilin LLPS, with implications for utilizing resilin-derived sequence features in rational design of self-assembling biomaterials.

The Velvet Worm Slime: Unique Adhesive Fibers with Peculiar Phosphonated Glycoproteins

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Velvet worm (Onychophorans) have a unique way to hunt. Indeed, velvet worm can expel a fluid, named slime, that will instantly stick and trap the prey. Once excreted, the slime transitions mid-air from a fluid protein suspension to solid fibers extremely adhesive and tough. Interestingly, the resulting material can be resolubilized in water and subsequently form new fully functional fibers. Therefore, understand the molecular interactions driving slime fiber formation and recycling is extremely inspiring to design new bioinspired functional materials.

First, using ^{31}P NMR, we unambiguously detected phosphonate in the slime, a rare molecule in terrestrial organisms. Phosphonates consist of a carbon atom directly bound to phosphorous. In nature, such molecular motif is extremely stable, but rare because it comes with energetic cost. Nevertheless, transcriptomic data further confirmed the presence of phosphonate synthesis capability in the slime glands. Then, using MAS-DNP and a unique ^{1}H-^{13}C-^{31}P triple resonance probe, we proved that phosphonates were found as 2-aminoethyl phosphonate (2-AEP) attached to glycans decorating large slime proteins. This was also confirmed by mass spectrometry of these proteins. We also showed that this modification is conserved across distantly related species, pointing to its critical functional role in the slime properties. Overall, this study is the first report of terrestrial organisms forming functional material from phosphonated glycoproteins. Also, this work offers new perspectives on the role of phosphonates in biological materials and opens pathways for future research into bioinspired materials that could mimic the slime's recyclable fiber formation.

Atomic-level observation of RAS GTPase activity in live cells using NMR spectroscopy

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RAS small GTPases orchestrate multiple signaling pathways. Binding to GTP allows canonical GTPases to adopt an active conformation and interact with various downstream effectors; thereby determining signalling output (proliferation/survival, gene transcription, apoptosis, etc). RAS mutations are found in 1/3 of human cancers, with KRAS mutated in a remarkable 22% of solid tumours. To date, all biophysical data of RAS activity comes from *in vitro* assays. This is a major limitation considering the complexity of RAS-effector signaling in living cells. Thus, we require innovative approaches to study these proteins in their native environment.In-cell NMR spectroscopy (IC-NMR) has the potential to move classical NMR experiments into living cells, allowing us to monitor GTPase structure and activity in real time in their native environment. This is achieved by transducing uniformly (^{15}N) or selectively (^{13}C-Ile) labelled proteins purified from *E. coli* into living mammalian cells through electroporation. We optimized the sample preparation for different cell lines (HEK-293T, HeLa, THP-1, Nalm-6). Our current results show that IC-NMR can discern active-vs-inactive states of GTPases, as transduced active mutants exchanged GDP to GTP post-electroporation. Moreover, we can detect and quantify RAS interactions with small molecules, binding partners and visualize multiple GTPases at the same time.IC-NMR is a powerful technique that allows us to elucidate how GTPases cycle between active/inactive states, how oncogenic mutations perturb cycling and the mechanisms that dictate signaling output in cells. Detailed biophysical characterization of these key proteins will help us to directly target RAS and ultimately improve outcomes for cancer patients.

Antimicrobial peptide mechanism of action on bacterial membranes, determined by in vivo solid-state NMR

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Staphylococcus aureus is a Gram-positive pathogenic bacterium that is resistant to a wide range of antibiotics. DMS-DA6-NH2 (DA6) is a novel antimicrobial peptide (AMP) that has high efficacy on various bacterial strains (1). *In vivo* ^{2}H solid state Nuclear Magnetic Resonance (NMR) is used to study AMP mode of action that disrupt bacterial membranes (2-3). We studied the bacterial membrane lipid profile (head groups and fatty acids) and then optimised the bacterial culture conditions with deuterated palmitic acid (PA-d_{31}), in order to label the lipids. The *in vivo* ^{2}H solid state NMR spectrum is then characterised by a central peak surrounded by spinning side bands on both sides, whose spectral moment M_2 can be measured and is related to membrane rigidity (2). It is then observed that the membrane rigidity decreases progressively when DA6 concentration is increased. These results were compared with those of AMPs whose mode of action are already known (3). It is deduced that DA6 has a pore-forming effect on the membrane of *S. aureus.* This is a template protocol for the determination of the mechanism of action of other peptides, a prerequisite for the patenting of such drugs for human disease treatments.

References :

Cardon et al., PLoS ONE 13:e0205727 (2018)

Warnet et al., Biochimica et Biophysica Acta, 1858:146-152 (2016)

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Poster Abstracts

5 - Understanding Lithium Salt Distribution in Doped Thermoplastic Vulcanizate Electrolytes via an Analysis of Salt-Solvent-Polymer Interactions

Gabrielle Foran[1], Joseph Chidiac[1], Caroline St-Antoine[1], Paul Nicolle[1], Arnaud Prébé[1], Mickael Dollé[1]

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Lithium salt distribution in polymer electrolytes depends strongly on interactions between the lithium salt and various components of the electrolyte. These may include one or more polymers and absorbed water or other solvents. These interactions, along with information on relevant dynamic processes can be characterized using solid-state NMR spectroscopy. Thermoplastic vulcanizate electrolytes are complex systems comprised of a thermoplastic phase, an elastomeric phase and a lithium salt. These electrolytes have been shown to possess both the ionic conductivity of the thermoplastic phase and the mechanical strength of the crosslinked elastomeric phase. Lithium salt can be distributed between these phases, impacting the overall ionic conductivity of the system. The mechanical strength of these electrolytes is sufficient to allow for the incorporation of liquid dopants without significantly compromising their resistance to compressive forces. Although these dopants have been shown to increase ionic conductivity, they also alter salt-polymer interactions in the material. Lithium NMR spectroscopy was used in conjunction with infrared spectroscopy to show that solvent addition affects both lithium salt distribution between the polymeric phases in the electrolyte and the relative strength of interactions between the salt and the polymer phase. These changes resulted in increased lithium ion mobility as demonstrated by measuring lithium ion diffusion and ionic conductivity. Ionic conductivities on the order of those observed in the dopants themselves were not obtained in these systems. This phenomenon was attributed to dopant addition producing a plastified polymeric phase through which ionic conductivity occurred.

10 - Unraveling Lanthipeptide Biosynthesis: High-Resolution Insights via Nuclear Magnetic Resonance

George Hanna[1]

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HalM2 is a model class II lanthipeptide biosynthetic enzyme, catalyzing post-translational modifications of the HalA2 precursor peptide. It recognizes the *N*-terminal leader peptide portion of the precursor and facilitates the dehydration of seven serine/threonine residues and the formation of four thioether rings in the *C*-terminal core peptide with high fidelity, yielding a single poly-macrocyclic isomer. Despite extensive study, the fidelity source remains unknown, necessitating high-resolution NMR techniques. The enzyme kinetics will be studied at an atomic level via a time-resolved HSQC-NMR experiment where a ^{13}C labeled HalA2 is incubated with HalM2 and $^{13}C/^{1}H$ correlation spectra are collected over 24 hours. Changes in peak intensities will provide kinetic information on each HalA2 dehydration and cyclization event. This approach offers details about timing of the post-translational modifications and high-resolution insight into the regio- and stereochemistry of the thioether bridges. We will also employ saturation transfer difference (STD)-NMR to identify HalA2 peptide residues crucial for enzyme recognition and probe the putative extended peptide binding site using a variety of HalM2 variant enzymes. These NMR approaches will provide the first atomic-level details to the complex, multistep maturation of an antimicrobial lanthipeptide opening the door for rational manipulation of the system through targeted binding interactions.

12 - Modulating human angiogenin subcellular localization using small molecule screening: assessing biological impact and biophysical properties

Trung Hieu Bui[1], Myriam Létourneau[1], Marie-Aude Pinoteau[1], Olga Lucia Saavedra Sanabria[1], Yarileny Castellanos Villamizar[1], Steven R. LaPlante[1], Yves St-Pierre[1], Nicolas Doucet[1, 2]

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Human angiogenin (hANG), also known as ribonuclease 5, is a multifunctional growth factor that plays a vital role in developing blood vessels and various complex biological processes associated with diseases such as cancer and neurological disorders. Recent research has shown that the functional expression of hANG depends greatly on its ability to translocate into the cell nucleus and relocalize within different subcellular compartments under changing cellular conditions. Evidence also suggests that hANG relocalization is associated with several neurological pathologies. This promptsconsideration of the impact of hANG subcellular relocalization on its diverse molecular activities. In this study, we aimed to develop allosteric modulators of hANG that can inhibit some of its specific roles in tumor progression. Using NMR, we screened a small chemical library of fragments and identified two potential small-molecule modulators, 5P and 5F. Our results show that both compounds inhibit hANG-associated HeLa cell proliferation, while fluorescence-based confocal microscopy and Western blotting analyses illustrate that 5P can enhance nuclear translocation. In parallel, we found that the aminoglycoside antibiotic neomycin (Neo) can also inhibit nuclear translocation bydirectly interacting with hANG. Finally, we show that hANG activity is altered either through enhanced nuclear translocation (facilitated by 5P) or upon suppressed nuclear translocation (induced by Neo). Our findings may provide evidence supporting novel strategies for the development of protein-targeting modulators in various pathologies.

13 - Structural characterization of the interaction between the UBA domain of p47 and ubiquitin using NMR spectroscopy

Nicole Dcosta[1]

[1]University of Guelph

The p97-p47 complex, consisting of the p97 AAA+ (**A**TPases **A**ssociated with diverse cellular **A**ctivities) protein and the p47 cofactor, plays a crucial role in membrane remodelling of the endoplasmic reticulum (ER), the nuclear envelope and the Golgi apparatus. The complex recruits monoubiquitinated syntaxin 5, a member of the Soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family, as a substrate, which is a critical step in mediating post-mitotic reassembly of the Golgi apparatus. Substrate recruitment relies on the interaction between the ubiquitin association (UBA) domain of p47 and ubiquitin. However, this interaction has not been characterized in detail. In our study, we investigated the interaction between $p47_{UBA}$and ubiquitin using Nuclear Magnetic Resonance (NMR) spectroscopy. The dissociation constant (Kd) of the complex was determined to be 32.7 ±13.7uM by NMR titration. Using chemical shift perturbation (CSP), we mapped out the interaction sites on both proteins and constructed a structural model using NMR CSP constraints-guided rigid-body docking. The structural model of the complex reveals a conserved binding mode between the UBA domain and ubiquitin with key residues and interactions identified at the binding interface. Ubiquitin variants were then selected and tested guided by phage-display and AlphaFold prediction-based virtual screening in search for higher affinity binders for potential therapeutic applications in the future.

14 - Advancing Structural Biology: Tools at the Université de Montréal

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Structural biology aims to understand the spatial organization of biological molecules and their relationship to function, providing critical insights into fundamental biological processes and the development of therapeutic strategies. The Structural Biology Platform at the Université de Montréal, established in 2017 with funding from the Canada Foundation for Innovation, provides state-of-the-art tools for academic and industrial researchers to explore these questions.

The platform houses a variety of advanced instruments. It includes three high-field NMR spectrometers (500, 600, and 700 MHz) with Bruker NEO consoles, which are ideal for studying protein-protein, protein-nucleic acid interactions, and screening RNA-small molecule interactions for drug design. These systems also enable sequential biomolecular assignments and molecular structure calculations.

Additionally, the platform supports biological small-angle X-ray scattering (BioSAXS) with a liquid-metal-jet X-ray source, offering rapid and high-throughput analysis of macromolecular shapes and dynamics.

Thanks to recent philanthropic funding, two cryo-electron microscopes (Cryo-EM) will soon be available: a JEOL CryoARM 200 and a 300 kV cryo-EM system (model TBD), both capable of single-particle analysis and atomic-resolution structure determination.

The platform also offers a TwoMP mass photometer from Refeyn for measuring single-molecule masses in solution, and a size-exclusion chromatography system with multi-angle light scattering (SEC-MALS) for absolute molar mass determination. Other available tools include an isothermal titration calorimeter (ITC) and robotics for crystal tray preparation.

15 - Binding of PINK1 mitochondrial targeting sequences to TOM20

Simon Veyron[1], Tara Shomali[1], Andrew Bayne[1], Jean-François Trempe[1]

[1]McGill University

Mutations in PINK1 or PARKIN cause early-onset Parkinson's disease (EOPD). These two proteins are involved in mitochondrial quality control, a process critical for neurons. Mitochondrial damage results in PINK1 import arrest on the Translocase of the Outer Mitochondrial Membrane (TOM) complex, which leads to PINK1's accumulation and activation of its ubiquitin kinase activity, which in turn triggers mitochondrial turnover. However, how PINK1 is imported into mitochondria and which molecular signal triggers its activation remain to be elucidated. Recently, we discovered that PINK1's N-terminal and C-terminal extensions (NTE-CTE), which chaperone the kinase domain, bind to the TOM20 subunit, a process which is essential for its activation (Eldeeb et al 2024, *PNAS*). TOM20 typically binds mitochondrial targeting sequences (MTS) of precursor mitochondrial proteins and channels them to the TOM complex. In addition to the kinase domain and the NTE-CTE, PINK1 harbors two MTS at the N-terminus, but their relative roles remain unclear.

Here, our goal is to characterize the interaction of TOM20 with the two MTS and the NTE-CTE of PINK1. We have purified the ^{15}N-labeled cytosolic domain of human TOM20 and assigned its ^{15}N-^{1}H HSQC spectra using triple resonance NMR methods. Titration with peptides corresponding to PINK1 MTS-1 or MTS-2 reveal a binding preference for MTS-2, and little interactions with the isolated NTE. Our findings suggest a model for PINK1's import with implications for its processing, folding, and transport in neurons.

18 - Government of Canada Ultrahigh Field NMR Collaboration Platform

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The NRC Metrology NMR Facility (Ottawa, Ontario) is a state-of-the-art laboratory dedicated to advancing the precision and accuracy of nuclear magnetic resonance (NMR) spectroscopy for both liquid and solid-state applications. We specialize in developing and validating NMR-based methodologies across various scientific fields, from molecular structure determination to advanced materials characterization. Our facility is equipped with cutting-edge instruments, including a one-of-a-kind 900 MHz high-field NMR spectrometer capable of both liquid and solid-state NMR, alongside a wide range of specialized NMR probes.

Our mission is to foster collaboration among academic, industrial, and governmental researchers by sharing our expertise in metrological NMR. We provide a variety of services, including custom NMR experiments, calibration standards, and training for users. Whether your research is fundamental or applied, our team is here to optimize your NMR data collection and interpretation.

The NRC Metrology NMR Facility is open to external users seeking access to high-field NMR instrumentation. We encourage researchers from universities, national labs, and industries to leverage our resources. Through collaboration, you can access the latest advancements in NMR technology and expert guidance to accelerate your research.

For more information about facility access, project collaborations, or specific NMR services, please visit our poster, or contact us directly. Be sure to inquire about our recently advertised postdoctoral position in parahydrogen NMR.

19 - Molecular View of Lignin-Carbohydrate Interactions in Arabidopsis thaliana by Solid-State NMR

Peng Xiao[1], Wancheng Zhao[2], Sarah Pfaff[3], Daniel Cosgrove[3], Tuo Wang[1]

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The evolution of lignin is a crucial event in the secondary cell wall during plant development. Monitoring the lignin deposition and their interactions with carbohydrates during the lignification process is important for understanding the structure of lignocellulose and to optimize their material-based applications. Here we present a variety of solid-state NMR (ssNMR) based research to probe the molecular-level organization of lignocellulose in intact cell walls. First, we monitor the lignification process in ^{13}C-labeled inflorescence of *Arabidopsis thaliana,* where the lignin composition and lignin-carbohydrate interactions of different segments of the inflorescence stems were examined via series of 1D and 2D ssNMR spectroscopy during different growth stages. These series of experiments paint a picture of ssNMR structural snapshots of the lignification process *in vitro*, allowing us to track the changes of lignin composition and lignin-carbohydrate interactions in intact cell walls. We further compared the wild-type plant with the F5H and C4H mutants, with the former deprived of the syringyl (S) lignin unit and the latter altering the guaiacyl (G) unit to S unit ratio. The NMR results revealed a complicated pattern of lignin-carbohydrate interactions affected by both the developmental stage and the chemical nature of the lignin involved in such interactions.

23 - RNA-binding specificity of the LARP1 LaM Domain

Guennadi Kozlov[1], Jianning Jiang[1], Anne Noronha[2], Chris Wilds[2], Kalle Gehring[1]

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La-related proteins (LARPs) comprise a family of RNA-binding proteins involved in a wide range of posttranscriptional regulatory activities. LARPs share a unique tandem of two RNA-binding domains, La motif (LaM) and RNA recognition motif (RRM), together referred to as a La-module, but vary in member-specific regions. Recently, LARP1 was shown to bind and stabilize mRNA 3' poly(A), but the molecular basis of that is unclear. LARP1 has been well characterized functionally but no structural information exists for its La-module.

In the present study, we used NMR to investigate the nature of the LARP1 La-module. Remarkably, we find that unlike other LARPs, the module does not contain a RRM domain. Furthermore, NMR and ITC studies demonstrated the stand-alone LaM domain binds RNA with submicromolar affinity and preference for A-rich sequences. Multiple high-resolution structures of LARP1 LaM in complex with poly(A) sequences of different length revealed the molecular basis for specificity for the RNA 3'-end and identify LaM residues Q333, Y336 and F348 as the most critical for RNA binding. Moreover, NMR and ITC experiments uncovered an unexpected bias for poly(A) sequences with single guanine substitutions. The selectivity for singly substituted poly(A) sequences suggests LARP1 plays a role in the stabilizing effect of poly(A) tail guanylation, while the plasticity in base recognition underlines the importance of the ribose 3´-terminus for RNA binding. The study provides novel insights into poly(A) 3' protection activity by LARP1.

26 - Characterization of Neural Cell Growth Polymer Materials using Solution and Solid-State NMR

Amanpreet Parihar[1], Sarah Collins[1], Violeta Toader[1], Timothy Kennedy[2], Christopher Barrett[1], Linda Reven[1]

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As part of a long-standing collaboration between McGill Chemistry and the Montreal Neurological Institute (MNI), new water-soluble polymers have been developed to self-assemble onto surfaces as layers and multilayers, for improved neural cell growth coatings. Some of these coatings are also active in optical sensing and signaling to neural cells, via incorporation of light-responsive azo dyes.

dPGA, a dendritic polymer, has recently been shown to be an effective and improved next-generation surface coating for neural cell growth. However, little work has been done to understand its properties, to help guide optimization for neuroengineering. It is hypothesized that mobility contributes to its success as a coating. We used solid-state magic-angle spinning (MAS) NMR to perform proton relaxation measurements, characterizing its mobility upon immobilization on a surface.

Finally, we investigated the tautomeric forms of light-responsive azobenzene dyes also relevant to this neuro-engineering project. Determining whether they exist in the azo form (enol) or the hydrazone form (keto) is vital for understanding their structural properties for potential applications. This was achieved through variable temperature 2D 1H - 15N HMBC NMR experiments.

27 - Untargeted 1D 1H NMR metabolic profiling of Pin1 inhibition in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is an aggressive solid malignancy with mortality rates almost equal to its incidence. One of the main challenges in treating PDAC is its ability to develop resistance to chemotherapy by rewiring its metabolism, underscoring the urgent need for new therapeutic agents and novel target identification. Pin1, a peptidyl-prolyl isomerase often overexpressed in cancers, including PDAC, plays key role in regulating multiple oncogenes and tumor suppressors, impacting many hallmarks of cancer, making it a promising target for therapeutics intervention. In vitro studies show that Pin1 inhibitors decrease pancreatic cancer cell viability, and in vivo studies further demonstrate that Pin1 inhibition enhances the efficacy of both chemotherapeutic and immunotropic agents.

The potential role of Pin1 in regulating cancer cell metabolism, however, remains largely unexplored. To understand the molecular mechanism of action of Pin1 inhibition, we first conducted sequence motif searches and structural analysis to assess surface-accessibility, leading us to hypothesize that Pin1 may regulate multiple metabolic enzymes, and thus may rewire cancer cells metabolism. To explore this, we utilize Nuclear Magnetic Resonance (NMR)-based metabolomics to study both the exo- and endo-metabolome of PDAC cells following Pin1 inhibition. We collected ^{1}D ^{1}H NMR spectra of cell lysates and conditioned media of Pin1 inhibitor- treated and untreated cells, the spectra were further analyzed using multivariate and univariate analysis. Our findings show that Pin1 inhibition results in a distinctive metabolic phenotype, specifically those involved in glycolysis, including glucose, and lactate, and some amino acids such as branched chain amino acids.

28 - Nuclear Magnetic Resonance (NMR) Metabolomic Profiles Provide Verification of Botanical Ingredient Species Identity, Metabolite Consistency and the Presence of Key Bioactive Plant Compounds in Food and Natural Health Products

Thiru Arunachalam[1], Vinay Varathan[1], Ragu Subramanyam[1], Steve 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 bioactive 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 Nuclear Magnetic Resonance (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 elucidation of bioactive molecules within the NMR spectral fingerprints of many botanicals. The objective of this research is to provide an NMR metabolite fingerprint analysis with structural elucidation of the bioactive molecules for 50 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 (metabolite fingerprint), and iii) verification of bioactive molecules of interest.

29 - Investigating localized backbone dynamics and hydrodynamics of the globular domain of a spider pyriform silk repetitive unit

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Spider silks are natural protein-based biomaterials with exceptional mechanical properties and hold great promise for applications ranging from high-performance textiles to regenerative medicine. Among seven different types of silks, pyriform silk attaches web silks to substrates and connects different silk types together. The pyriform silk of *Argiope argentata* has a central repetitive region consisting of a 234-amino acid unit (the “Py unit”) repeated 21 times. In the solution state, the Py unit has a structured core of 6 alpha-helices that retains its structuring upon truncation of N- and C-terminal intrinsically disordered segments. Meanwhile, in the fibrous state, the pyriform protein undergoes a structural transformation with a loss of alpha-helical content and a gain of beta-sheet content. To understand the underpinnings of this transformation, the structured core of the Py unit is characterized by solution-state nuclear magnetic resonance (NMR) spectroscopy. Temperature-dependent chemical shift mapping for a ^{15}N-labelled Py unit indicates minimal temperature-induced structural perturbation in its alpha-helical core. ^{15}N spin relaxation measurements reveal that the alpha-helical core exhibits minimal local backbone motion, tumbling as a globular unit, except for one interhelical linker that exhibits a notably higher degree of dynamics. Evaluation of rotational and translational diffusion demonstrates that the core of the Py unit behaves as a compact structure, with hydrodynamic behaviour consistent with a well-packed protein. These results suggest that the more dynamic segment we observe midway through the structured core of the Py core would provide an initiation point for decompaction, structural transformation, and/or intermolecular interactions.

30 - Characterizing the in vitro reaction catalyzed by rhodoquinone biosynthesis enzyme

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Rhodoquinone (RQ) is a close analogue of electron carrier ubiquinone (UQ), which is essential to the bioenergetics of diverse bacteria and selected eukaryotes that survive in low-oxygen environments. RQ allows the electron transport chain to function with fumarate as a final electron acceptor instead of oxygen. Rhodoquinone biosynthesis enzyme (RquA), identified in a *Rhodospirillum rubrum* RQ-deficient mutant, is homologous to *S*-adenosyl-*L*-methionine (SAM)-dependent methyltransferases and is required for RQ biosynthesis in bacteria by catalyzing the conversion of UQ to RQ. Although being a methyltransferase, the RquA reaction is not a canonical methyl transfer reaction. In this study, we characterized the mechanism of *in vitro* RQ biosynthesis using a combination of mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy. Recombinant RquA was over-expressed in *Escherichia coli,* solubilized in a detergent Brij-35, and purified by nickel affinity and size-exclusion chromatography. RquA requires UQ_3 (3 indicating the number of isoprenyl units) and SAM as substrates and Mn^{2+} as a metal cofactor to produce RQ_3, where the alpha-amino group of SAM is transferred during RQ biosynthesis. An NMR-based *in vitro* functional assay using isotopically labeled substrates was used to identify additional reaction products, including methylthioadenosine, bicarbonate, methanol, and an aldehyde hydrate. These findings reveal that RquA is a non-canonical SAM-dependent enzyme that does not catalyze methyl transfer, but instead transfers amino group from SAM.

31 - Exploring the bonding configurations in tellurium oxide: a comparative XPS and NMR spectroscopic analysis

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Discovering to which, if any, of the known crystal phases of TeO_2 the glass is most similar, is important both to model the glass and to understand why it is moderately difficult to form from the melt. Garaga *et al.* showed with ^{125}Te NMR that the Te has predominantly gamma-TeO_2 character. [1] However, neutron diffraction and Raman spectra have been interpreted as suggesting that Te=O bonds are present.

In this work, high resolution x-ray photoelectron spectroscopy results on the various TeO_2 structures are presented, and correlated where possible with the NMR-derived chemical shielding. The O 1s lines are overall congruent with the findings of Garaga *et al.*, with the glass being the most deshielded agreeing with its lower O 1s binding energy indicating a shorter Te-O bond}. [1] The similarities of the gamma-TeO_2 and the glass XPS spectral deconvolutions are presented, with explanations of the differences arising from contributions from alpha-TeO_2-like motifs. The narrow range of O 1s binding energies suggests that the oxygen environments are consistent with similar bridging oxygen configurations and do not indicate the presence of Te=O bonds, but resolution is insufficient to definitively disprove their presence. Finally, utility of using high resolution XPS in concert with NMR is described.

[1] M. N. Garaga, U. Werner-Zwanziger, J. W. Zwanziger, A. DeCeanne, B. Hauke, K. Bozer, and S. Feller. Short-range structure of TeO2 glass. J. Phys. Chem. C, 121(50):28117-28124, 2017.

34 - Operando 7Li Nuclear Magnetic Resonance Integrated with an In-Situ Electrochemical setup to study Lithium Metal Deposition on the Anode of Lithium-ion Batteries

Emma Magee[1], Kevin Sanders[1], Zoya Sadighi[1], Gillian Goward[1]

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Lithium-ion batteries (LIBs) have long been the favoured rechargeable battery in industry due to their high energy density.[1] When a LIB is charged, ions can build up on the surface of the anode and form “plated” or metallic lithium. Metallic lithium can become disconnected from a cell’s circuit and remain stuck on the anode during the cell’s discharge, decreasing capacity. Metallic lithium can grow in a form similar to dendrites decreasing a batteries’ overall safety.[1] ^{7}Li NMR can be used to investigate lithium plating and dendrite formation.[3]Herein, we discuss using both a two-electrode cell assembly, simultaneously with *operando* ^{7}Li NMR, to observe lithium movement in a LIB and compare the relative amount of metallic lithium between different anode materials at different charging rates.

1. Zhang, H., et al., *Energy Storage Materials.* 36, 2021, 147-170.

2. Sanders, K., et al., *JACS*. 145, 39, 2023, 21502-21513.

35 - A Quantitative Study by 7Li Operando NMR on Composite Anode Materials for Lithium-Ion Batteries

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Lithium-ion batteries (LIBs) have revolutionized the world with their diverse applications in energy storage.[1] Although, LIBs are widely used in various fields, they still require further improvements, in energy density, lifespan, and faster charging.[2] However, fast charging can lead to lithium plating on the anode, which causes safety concerns and capacity loss. *Operando* ^{7}Li Nuclear Magnetic Resonance (NMR) offers powerful quantitative tools that allows not only the detection of the onset of Li plating but also differentiates the reversibly plated and irreversible dendritic forms of lithium metal on the anode surface.[3] Here, we used our recently reported parallel-plate resonator radio frequency (RF) probe and cartridge-type cell to analyze the pattern of lithium plating/dendritic growth on a carbon and silicon composite anode material.[4] At lower charging current rates, the plated lithium is reversible whereas when charged at higher current rates (1C-3C) it is irreversible and forms dead lithium that is not electrochemically active. The incorporation of silicon into the anode material decreases lithium plating compared to pristine graphite anode material. Thus, a technique that enables the investigation of different lithium species in real-time will help improve the fast-charging protocol and thus the performance of lithium-ion batteries.

References:
1. Nzereogu, P. U., et al. *Applied Surface Science Advances* vol. 9 100233 (2022).
2. McDowell, M. T., et al. *Adv. Mater.* 25, 4966-4985 (2013).
3. Sanders, K. J., et al. *J. Am. Chem. Soc.* 145, 21502-21513 (2023).
4. Sanders, K. J. *et al. Carbon N. Y.* 189, 377-385 (2022).

36 - Interaction of bacteriophages with bacterial membranes by solid-state NMR

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Furunculosis, caused by *Aeromonas salmonicida* subspecies *salmonicida* (Ass), poses a significant challenge for the aquaculture industry due to its economic impact. Current treatment for Salmonidae relies heavily on antibiotics, but increasing bacterial resistance is a growing concern. This project investigates the use of bacteriophages as a sustainable and effective alternative. Phages are viruses that specifically target and destroy bacteria, offering a potential solution to reduce antibiotic overuse. The project's goal is to elucidate the mechanism by which phages interact with Ass membranes, using advanced in vivo solid-state nuclear magnetic resonance (SS-NMR) techniques.

The first step of the project involves studying phage interactions with synthetic membranes mimicking Ass. Growth curves were established to harvest bacteria at the end of the exponential phase. The lipid composition of the bacteria membranes was determined using Phosphorus-31 (31P) solution NMR, revealing phospholipids PE (86%), CL (1%), and PA (13%). Fatty acid chain profiles were obtained via GC-MS.

Based on this data, multilamellar vesicles were prepared using POPE, POPG, and Palmitic acid-d31 in a molar ratio of 65:13:22. 31P SS-NMR was used to study the effects on the phospholipid head groups, while deuterium (2H) SS-NMR with deuterated lipids probed the non-polar bilayer region.

These results will advance our understanding of how phages target Ass and guide the next step, an SS-NMR study of phage interactions with intact Ass, aiding in the development of phage-based strategies for furunculosis control in aquaculture.

38 - Towards solution NMR studies of the of the small RNA binding protein p19

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[1]University of Ottawa

p19 is a viral RNA silencing suppressor protein that sequesters short-interfering RNA (siRNA) from the post-transcriptional gene silencing (PTGS) pathway in plants to prevent viral genomic degradation. As p19 uniquely binds to double stranded RNA with size selectivity and minimal sequence dependence, it is an attractive candidate for use as a biotechnology tool. Previous attempts to increase the affinity of p19 for therapeutically relevant small double stranded RNAs, such as microRNAs (miRNAs), and the use of unnatural amino acid incorporation to confer new functionalities has been successful. However, despite considerable efforts, x-ray crystallography structures have only been resolved for p19 in complex with siRNA. Here we describe our efforts towards the study of p19 structure through solution-state NMR. Optimization of ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) spectra gave ~85% of expected backbone peaks for a functional, covalently linked dimer variant of p19 with three cysteine residues mutated to isoleucine (2XIII). This variant is the most promising candidate for obtaining backbone chemical shift assignments which lays the groundwork for chemical shift perturbation studies to gain insight into the ability of p19 to accommodate non-canonical structural features characteristic of miRNA.

40 - Elucidating Salt-Solvent-Polymer Interactions to Better Understand Lithium Salt Distribution in Thermoplastic Vulcanizate Electrolytes

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[1]Unversité de Montréal

Lithium ion mobility in polymer electrolytes depends strongly on interactions between the lithium salt and various components of the electrolyte. These may include one or more polymers and absorbed water or other solvents. Thermoplastic vulcanizate electrolytes comprised of poly(caprolactone) (PCL) and hydrogenated nitrile butadiene rubber (HNBR) were prepared. Advantages of this type of electrolyte include the retention of both the mechanical and electrochemical stability of the elastomeric HNBR phase in addition to the mobility and processability of the thermoplastic PCL phase. Although modest ionic conductivities were obtained for the as-prepared electrolytes, the addition of 20 wt% lithium salt-containing solvents (either triethyl phosphate or ethylene carbonate) improved conductivity by about two orders of magnitude. The presence of these solvents along with two polymers that are capable of lithium salt dissociation raises questions regarding the distribution of lithium salt in the electrolyte and the participation of each phase in the observed ionic conductivity. Namely, whether the observed enhancement in ionic conductivity is a result of ion mobility through the added liquid phase. Ionic conductivity, NMR measurements of salt distribution and ion mobility and IR spectroscopy of lithium-coordinating functional groups were acquired. It was found that the lithium salt exists in a solvent-swelled polymer phase and that the overall ionic conductivity of a given electrolyte depends on a combination of lithium salt distribution, interactions with the conductive thermoplastic phase and local-scale polymer mobility.

43 - Nucleotide Binding and the Cell Division Regulator MinD

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The three proteins of the Min system are crucial for proper septum localization in bacterial cells, with the absence of the Min system leading to the formation of minicells in bacilli cells like *Escherichia coli* (Ec) and the loss of viability due to abnormal division in coccal cells like *Neisseria gonorrhoeae* (Ng). While the Ec MinD protein has been extensively characterized, less is known about the Ng variant. In addition, x-ray crystal structures of nucleotide bound Ec MinD have not revealed the conformational changes that are induced by nucleotide binding. Solution NMR would provide a complementary approach that may provide insight into these conformational changes in a dynamic solution system. This poster will present the initial progress towards the generation of NMR samples for Ng MinD required triple resonance spectroscopy experiments.

44 - Structural characterization of the interaction between the UBA domain of p47 and ubiquitin using NMR spectroscopy and computational prediction tools

Emilie Carson[1]

[1]University of Guelph

p97 is an AAA+ protein that works with various adaptor proteins to carry out a diverse array of cellular functions. One of the adaptors, named p47, directs p97 to assist the reassembly of the Golgi body from membrane fragments after mitosis. p47 recruits the ubiquitinated syntaxin-5 (a t-SNARE) via its ubiquitin-associated (UBA) domain to the p97-p47 complex, which leads to further deubiquitinating of the substrate as well as priming of the SNARE complexes that are essential to rebuild the Golgi body. The purpose of this study is to screen for ubiquitin variants with varied binding affinities to p47-UBA, which could lead to insights on disrupting this p47-p97 function. Our study uses nuclear magnetic resonance spectroscopy (NMR) titrations to investigate the binding interactions and to determine the binding affinities. In addition, we evaluate the effectiveness and accuracy of computational approaches, such as AlphaFold and RosettaDDGPrediction, in prediction of dissociation constant using our benchmark experimental data. Our results suggest a good correlation between AlphaFold2-Prodigy computation approach and experimental data with a R2 value of 0.70, but no correlation is identified between prediction using AlphaFold3-Prodigy or RosettaDDGPrediction and the experimental data.

45 - Investigating the location of the UBA domain of p47 in the p47-p97 complex using paramagnetic relaxation enhancement (PRE) NMR spectroscopy

Paige Kanters[1], Megan Black[1], Rui Huang[1]

[1]University of Guelph

p97 is an essential AAA+ ATPase that plays a crucial role in protein homeostasis and participates in several significant cellular processes. p97 is directed to different functions by interacting with adaptor proteins, such as p47, which recruits p97 to membrane remodeling. p47 consists of three structured domains connected by long, flexible linkers: UBA, SEP, and UBX. The UBX domain and two linear motifs on the linker (SHPN and SHPC) interact with p97 directly. Meanwhile, the UBA recruits ubiquitinated substrates to the central pore of the complex where the substrate is subjected to the translocation mechanism of p97. How the UBA brings substrates to the central channel of p97, and the translocation mechanism of the p47-p97 complex are both not well understood. In this study, we aim to investigate the location of the UBA domain of p47 in the p47-p97 complex using paramagnetic relaxation enhancement (PRE) NMR spectroscopy. An iodoacetamido-proxyl spin label (IPSL) was attached to a residue in the UBA and linker domain of p47 to develop distance constraints between these domains and p97 when in complex. The results suggest that these two regions on p47 are in proximity to the N-terminal domain and central pore of p97. The NMR spectroscopy results will be supported with a substrate unfolding assay utilizing the photoconvertible fluorescent protein, mEos3.2, to examine the translocation mechanism of the p47-p97 complex. Our study will provide a better understanding of the interaction that occurs between p47 and p97 and the functional mechanism of the complex.

46 - Advancing Quantitative NMR for High-Precision Isotopic Analysis with rnmrfit

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Quantitative NMR (qNMR) plays a pivotal role in high precision isotopic analysis, spanning applications from quality control to forensics. A key challenge lies in the ability to differentiate signal from background noise, which is essential for achieving accurate peak areas. In addition, robust high-throughput data processing tools are needed to meet the growing demands of modern analytical workflows. To that end, we present an improved NMR peak fitting framework to enhance precise quantification by accurately separating signals from baseline noise. The developed framework was implemented as a new version of rnmrfit in the more convenient and stable Rust programming language as a standalone, user-friendly NMR data processing and peak-fitting tool. This tool employs a novel semi-global peak fitting approach, while simultaneously applying phase and baseline corrections, resulting in significantly improved signal clarity. We considered the impact of common processing parameters on peak fitting performance and found that line broadening improved precision when restricted to approximately 2 to 5 times the peak width, while mild zero filling of up to a factor of 1.75 was found to improve precision. We validated rnmrfit using ^{2}H vanillin data, comparing its performance against widely used commercial software such as TopSpin and MestReNova. rnmrfit consistently demonstrated superior precision across nearly all spectral regions, both in absolute and relative terms. Our tool not only enhances the accuracy of qNMR but also provides an accessible solution for high-throughput data processing, promising broader adoption and ease of use for researchers and analysts.

47 - Investigating the Autoinhibitory Mechanism of Ubiquitin Specific Protease 8 (USP8)

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[1]University of Guelph

Ubiquitination is vital to eukaryotic biology. By varying the site of attachment and degree of polyubiquitination, a “ubiquitin code” is created. This code oversees a wide range of cellular activities such as signalling, trafficking, gene expression and protein degradation. The “erasers” of this code are the deubiquitinases (DUBs), which facilitate the removal of ubiquitin. The ubiquitin-specific protease (USP) family are cysteine proteases that make up the majority of the DUBs. USP8, specifically, is responsible for the removal of ubiquitin on the endosomal growth factor receptor (EGFR), allowing for receptor recycling. Described as a hand with curled fingers, the catalytic domain (CD) forms a pocket where ubiquitin binds and is cleaved from a tagged protein. Located proximally to the CD is a small WW-like domain that is tethered via a linker harbouring a 14-3-3 binding motif. It has recently been shown that the WW domain can also bind the pocket of CD and prevent ubiquitin binding - leading to the autoinhibition of the enzyme. It is hypothesized that the removal of this inhibition is an underlying cause of Cushing’s disease. 3-D solution NMR was used to obtain a full backbone assignment of the WW domain. Peak assignments were then used to analyze NMR titration data leading to the identification of residues forming the binding interface, as well as the dissociation constant (Kd) of the interaction. Future work will aim to use NMR to identify the mechanism by which 14-3-3 proteins are able to potentiate the autoinhibitory effect of the WW domain.

48 - Comparison between NMR relaxometry and conventional methods to study the effect of transportation and storage on set-style yogurts properties

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The impact of vibration or altitude post-production treatment on the quality of set-style yogurts was assessed on a technology platform simulating the conditions encountered during road and air transportation. Rheological (elastic and viscous moduli, viscosity, firmness, stress relaxation) and physicochemical (pH, titratable acidity, syneresis) properties of yogurts were evaluated for 22 days, in parallel with NMR relaxometry. In a first analysis, NMR transverse relaxation decay signals were fitted to a sum of three exponentials, giving the following significant results: T_{21} and A_1, referring to protein concentration and gel heterogeneity, were significantly (P = 0.0034 and P = 0.0408, respectively) affected by the interaction between post-production treatment and storage; T_{22}, representing the bulk water mobility highly sensitive to acidification, was significantly (P < 0.0001) affected by storage; a significant (P = 0.0178) decrease of T_{23}, associated with spontaneous syneresis and referring to water represented by the protons exuded from the gel, was observed over time. However, extracting relaxation time constant and amplitude values gives only a fraction of the information compared to the NMR signal. In a second time, a partial least squares discriminant analysis showed that NMR relaxometry was able to significantly (P < 0.001) differentiate yogurts with post-production treatments from control (87.0% accuracy). Thirdly, correlations (R^2 = 0.83 and R^2 = 0.79, respectively) were observed between NMR signals and stress relaxation or firmness by partial least squares regression. The better sensitivity of NMR relaxometry compared to conventional methods offers potential for quality defect detection of yogurts during transportation.

49 - Optimization and Derivation of Aptamers Targeting Immune Checkpoint Modulator PD-1, for Therapeutic and Diagnostic Applications in Immune-Oncology

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The PD-1/PD-L1 axis represents an essential immune checkpoint pathway that regulates immune responses and maintains self-tolerance. Programmed Cell Death Protein 1 (PD-1) is an inhibitory receptor expressed on the surface of activated T-cells, B cells, and other immune cells. Its ligand, Programmed Death Ligand 1 (PD-L1), is expressed on various cell types, but notably on the surface of tumor cells. When PD-1 binds to PD-L1, it transmits an inhibitory signal that reduces T-cell proliferation, cytokine production, and cytolytic activity. This interaction serves as a mechanism exploited by tumor cells to evade immune detection and destruction. The goal is to derive and optimize an aptamer that can effectively disrupt the PD-1/PD-L1 interaction, offering a promising alternative to current antibody-based therapies for cancer treatment. We have successfully obtained ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) spectra for both ^{15}N-labeled PD-1 and PD-L1. Utilizing ^{15}N-^{1}H HSQC, we are in the process of evaluating aptamers to characterize their binding interfaces with these proteins. Concurrently, competition assays have been conducted to determine the aptamers' affinity for PD-1 relative to PD-L1. These preliminary results will indicate if certain aptamers exhibit higher affinity for PD-1, potentially enabling them to outcompete PD-L1 and effectively mitigate the T-cell inhibitory effect. These findings may pave the way for novel therapeutic strategies to enhance anti-tumor immunity.

52 - The effect of weathering on the release of per- and polyfluoroalkyl substances (PFAS) from outdoor building materials

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To assess the effect of weathering on per- and polyfluoroalkyl substances (PFAS) released from outdoor building materials, one paint, one sealer, and three textiles were exposed to elevated ultraviolet (UV) radiation for two months and then analyzed for total organic fluorine (TOF) by ^{19}F-NMR, and individual PFAS by LC-HRMS and GC-HRMS. Results showed that weathering can influence the products' TOF contents and individual PFAS concentration profiles. Under UV exposure, the TOF content showed a 2.9-58.2% reduction for the paint and three textiles, whereas a 1500% increase for the sealer. A common increase in PFAA concentrations was observed for the paint, sealer, and textiles, which may result from the hydrolysis of 6:2 fluorotelomer side chain fluorinated polymers (6:2 FT SCFPs), or degradation of 6:2 nonpolymers. A variety of polyfluoroalkyl acids with different chain lengths were also formed. These products together could explain 0.02-4.5% of TOF content loss in the paint and textiles, while 26.6% of TOF content increase in the sealer. The declined volatile PFAS concentrations from GC-HRMS analysis can explain an additional 1.4-26.5% of TOF content loss in textiles. The ^{19}F-NMR results indicate the formation of smaller-size oligomeric or nonpolymeric PFAS from 6:2 FT SCFPs in the sealer under UV exposure. Overall, the UV light caused photolytic transformations of PFAS in the paint and textiles, while increasing the presence of oligomeric and non-polymeric PFAS consistent with releases from 6:2 FT SCFPs in the sealer. Therefore, the weathering could result in the release of more mobile PFAS from outdoor building materials.

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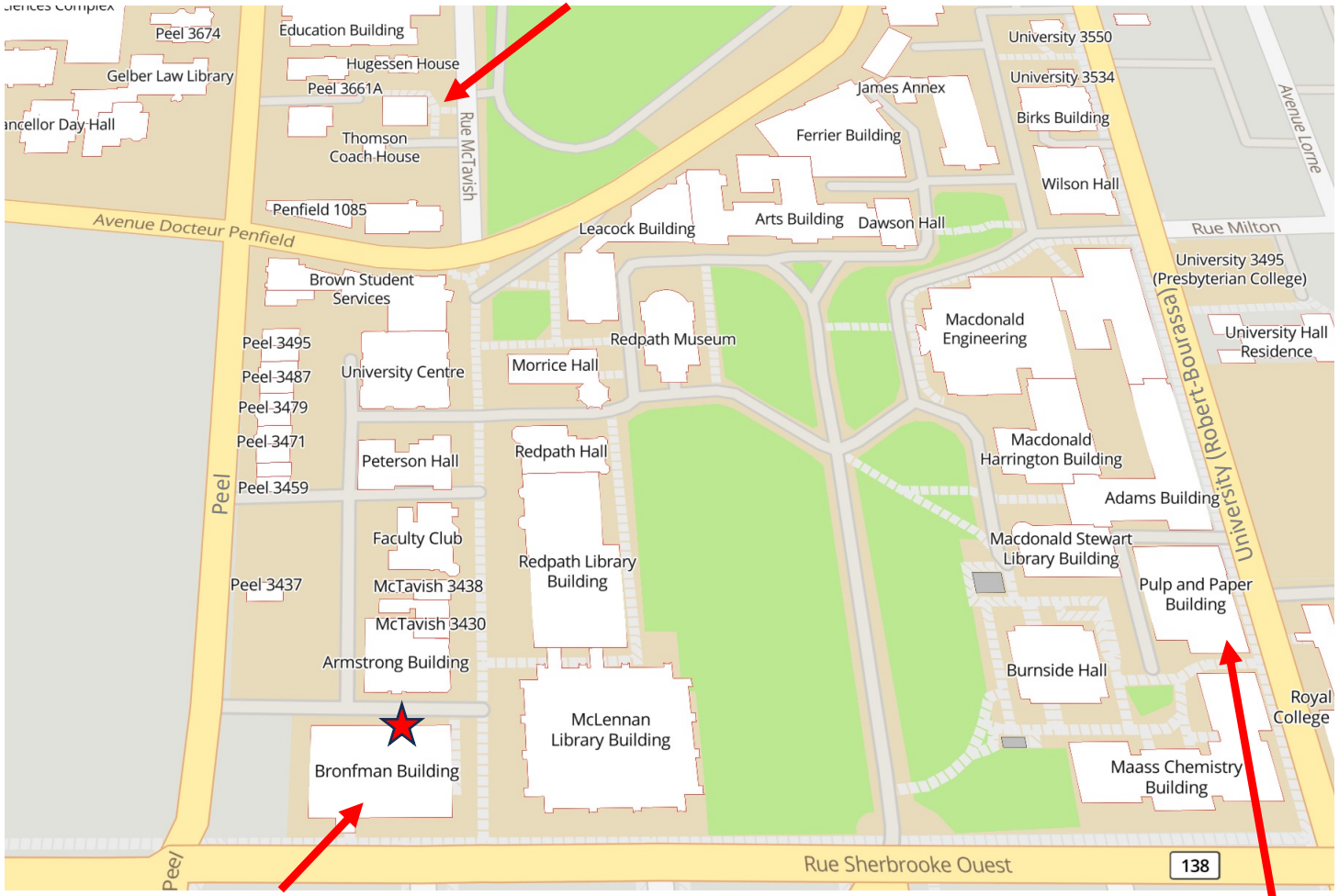

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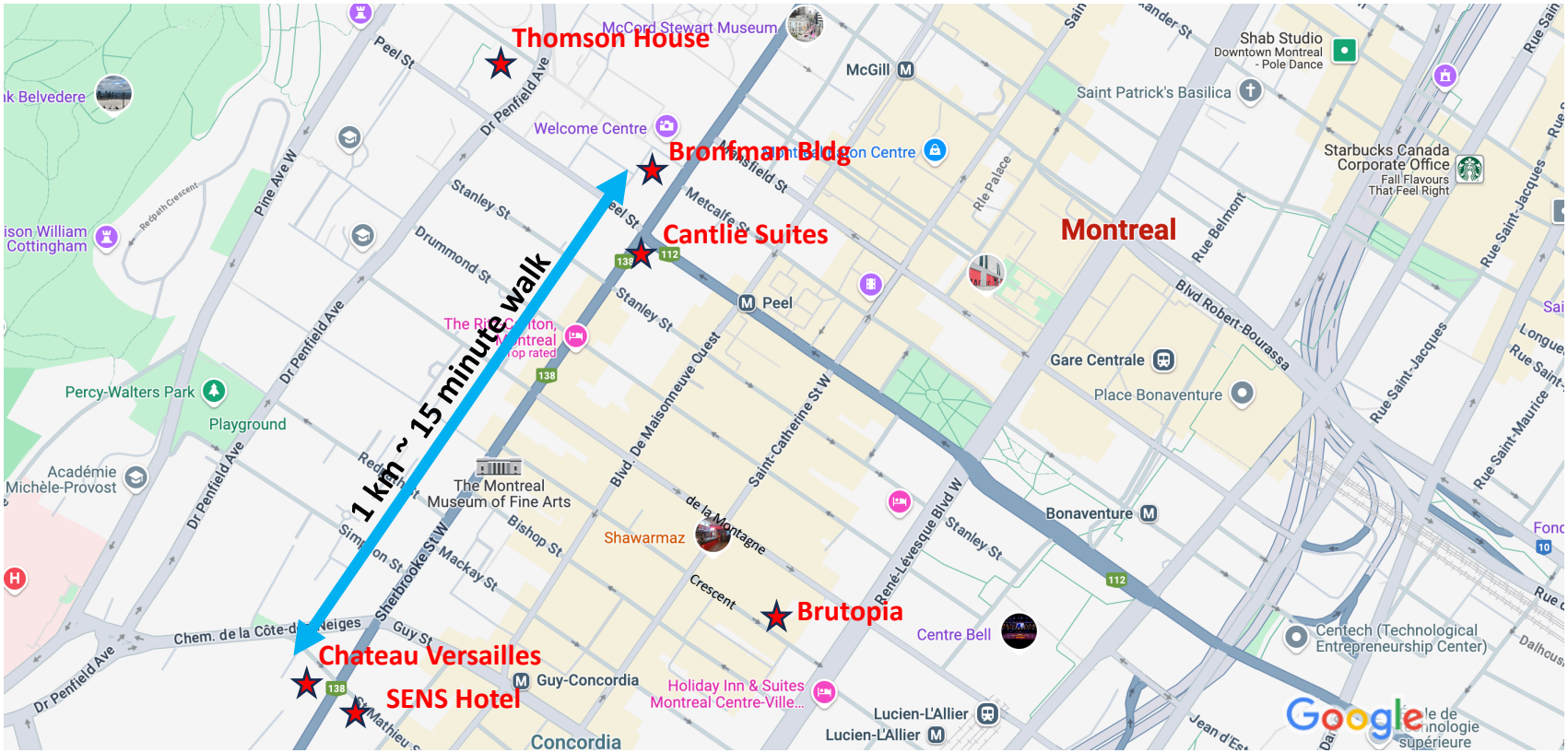

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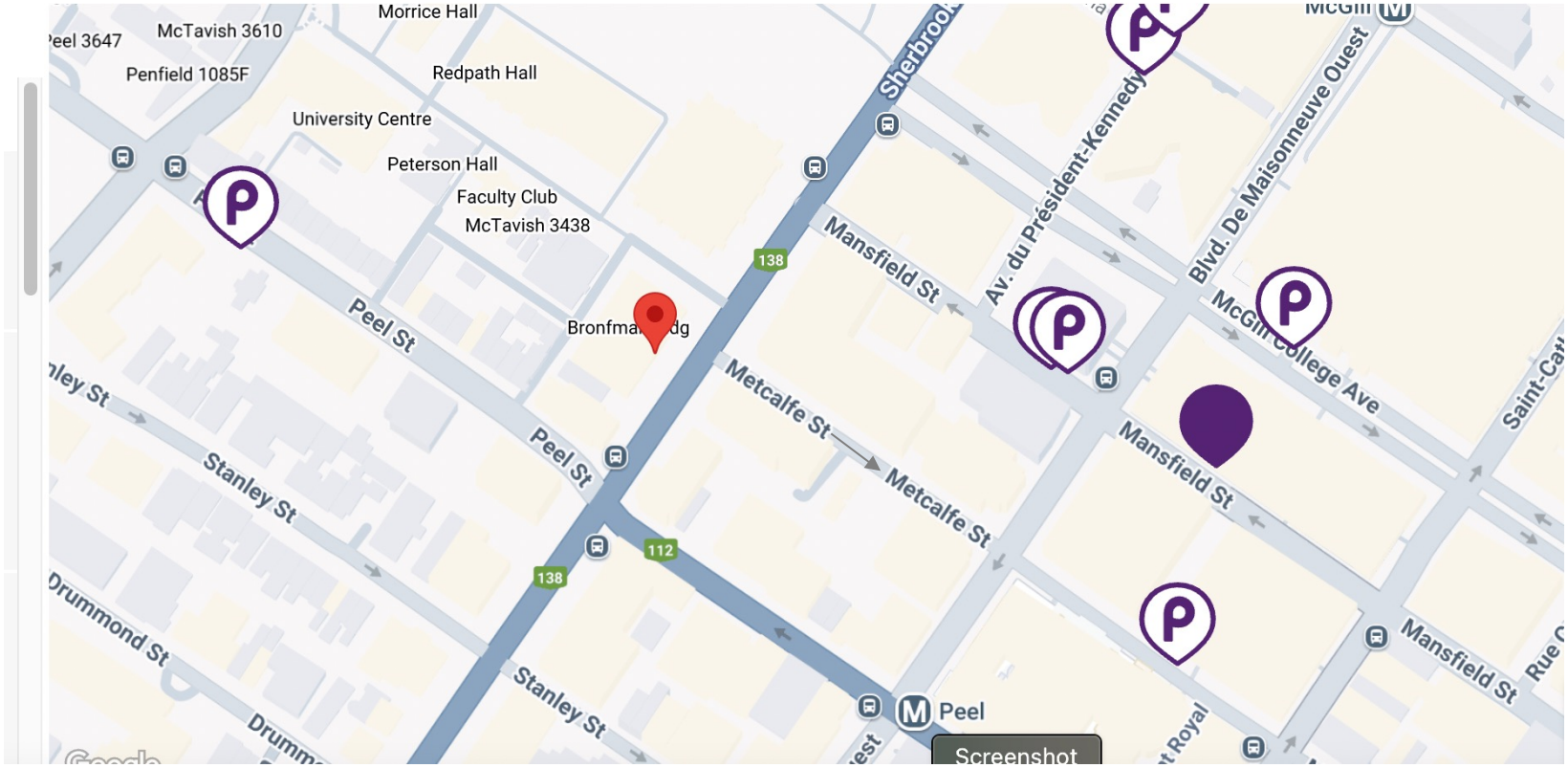
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