MOOT XXVI NMR Symposium, October 26-27, 2013, Queen's University, Kingston, Ontario
Final Program

Saturday, October 26th, 2013

8:00-8:50 AM — Registration
(Medical School Building, Atrium, 1st floor)

Morning Session-I, Chair: Seth Chitayat
(Medical School Building, Atrium, 1st floor, Room 132)

8:50-9:00 AM — Welcoming remarks

9:00-9:50 — Chasing Functional Dynamics in Biological Systems
R. Scott Prosser
University of Toronto

9:50-10:10 — Using Split Inteins to Reveal the Striking Modularity of Spider Wrapping Silk in Solution
Marie-Laurence Tremblay
Dalhousie University

10:10-10:30 — A Circularly Permuted Photoactive Yellow Protein as a Scaffold for Photoswitch Design
Anil Kumar, Darcy Burns, M. Sameer Al-Abdul-Wahid and G. Andrew Woolley
Department of Chemistry, University of Toronto

10:30-10:50 — **Coffee Break** (Medical School Building, Atrium, 1st floor)

Morning Session-II, Chair: Jan Rainey
(Medical School Building, Atrium, 1st floor, Room 132)

10:50-11:10 — Deciphering the Mechanism of E2 and E3 Enzymes in Ubiquitylation
Donald Spratt
Western University

11:10-11:30 — The Effect of Dilution on Isotropic Bicelles: Changes in Morphology and Miscibility of its Constituents
M. Beaugrand,[(1)] A.A. Arnold,[(1)] J. Henin,[(2)] D.E. Warschawski,[(2)] P.T. F. Williamson[(3)] and I. Marcotte[(1)]
[(1)]Department of Chemistry, Université du Québec à Montréal; [(2)]School of Biological Sciences, University of Southampton; [(3)]Institut de Biologie Physico-Chimique, CNRS, Université Paris Diderot

11:30-11:50 — Characterisation of PC/Tween-80 Model Membranes for the Study of Membrane Proteins and Peptides
Andrée Gravel, Alexandre A. Arnold and Isabelle Marcotte
Department of Chemistry, Université du Québec à Montréal

11:50-12:10 PM Micelle-Catalyzed Domain Swapping of the N-terminal Cytoplasmic Domain of Rhomboid Protease
Jason Ka-Cheong Kwok
Department of Chemistry, University of Ottawa

12:10-1:50 PM **Lunch** (University Club at 168 Stuart Street)

Afternoon Session, Chair: Glenn Facey
(Medical School Building, Atrium, 1st floor, Room 132)

1:50-2:10 PM Solid-State NMR Studies of Biomolecules under Ultra-Fast MAS and Amyloid Aggregates for Alzheimer's Beta
Yoshitaka Ishii
University of Illinois at Chicago

2:10-2:30 PM Application of ^{7}Li Nutation Curves to Differentiate Electrochemical Species,
Zoe E. M. Reeve, Alex. D. Bain and Gillian R. Goward
Department of Chemistry, McMaster University

2:30-2:50 PM The Peculiarities of Homonuclear Spin-Spin Coupling between Quadrupoles
Frédéric A. Perras and David L. Bryce
Department of Chemistry, University of Ottawa

2:50-3:10 PM Applications of ^{35}Cl SSNMR to Solid Pharmaceuticals: Detection of Polymorphs and Impurity Phases
Anthony R. Sandre,[1] Karen E. Johnston,[1] Andrew M. Namespetra,[1] Zhehong Gan,[2] Ivan Hung,[2] and Robert W. Schurko[1]
[1]Department of Chemistry and Biochemistry, University of Windsor, Windsor;
[2]National High Magnetic Field Laboratory

Poster Session, Chair: Gang Wu

3:10-5:00 PM **Cocktail and Poster Session**
(Medical School Building, Atrium, 1st floor)

4:30-5:00 PM **Collaborative PI Session**
(Medical School Building, Room 021)

6:00-9:00 PM **Dinner** (Sheraton Four Points Hotel, 285 King Street East)
After dinner speech: "Looking back on the MOOT meeting" **Alex Bain**

Sunday, October 27th, 2013

Morning Session-I, Chair: Isabelle Marcotte
(Medical School Building, Atrium, 1st floor, Room 132)

9:00-9:20 AM	NMR Studies of Homopolymers **Kalle Gehring** McGill University
9:20-9:40	Signaling through Dynamic Linkers as Revealed by PKA **Madoka Akimoto**, Rajeevan Selvaratnam, Eric T. McNicholl, Geeta Verma, Susan S. Taylor and Giuseppe Melacini McMaster University
9:40-10:00	Mapping the Interactions between the Alzheimer's Aβ-Peptide and Human Serum Albumin beyond Domain Resolution **Moustafa Algamal** McMaster University
10:00-10:20	Dipole-Dipole Coupling Investigation of Solid Acids and Composites for Proton Exchange Membrane Fuel Cells **Nicole De Almeida** McMaster University
10:20-10:40	**Coffee Break** (Medical School Building, Atrium, 1st floor)

Morning Session-II, Chair: Gillian Goward
(Medical School Building, Atrium, 1st floor, Room 132)

10:40-11:00	Slow and Steady Wins the Race - Multinuclear SSNMR of Unreceptive Nuclides Using Broadband Cross-Polarization Methods **Michael J. Jaroszewicz**, Kristopher J. Harris, Karen E. Johnston and Robert W. Schurko Department of Chemistry and Biochemistry, University of Windsor
11:00-11:20	^{2}H SSNMR Study of Metal-Organic Frameworks with Dynamic Interlocked Components **Christopher A. O'Keefe**, V. Nicholas Vukotic, Kelong Zhu, Stephen J. Loeb and Robert W. Schurko Department of Chemistry and Biochemistry, University of Windsor
11:20-11:40	Correlations Between Local Halogen Bonding Environments and Multinuclear Solid-State Magnetic Resonance Observables **Jasmine Viger-Gravel**, Julia Meyer, Sophie Leclerc, Ilia Korobkov, and David L. Bryce Department of Chemistry, University of Ottawa

11:40-12:00 PM	High Resolution Paramagnetically Enhanced Solid-State NMR Spectroscopy of Membrane Proteins at Fast Magic Angle Spinning **Meaghan Ward**, Shenlin Wang, Sridevi Krishnamurthy, Howard Hutchins, Michael Fey, Leonid Brown, Vladimir Ladizhansky University of Guelph
12:00-12:20	Characterization of Interactions between EPAC1 and the EPAC Selective Inhibitor ESI-09 by NMR Spectroscopy **Stephen Boulton** and G. Melacini McMaster University
12:20-12:35	Judges to hold their deliberations
12:35-12:45	Award Announcement
12:45	**Lunch** (Medical School Building, Atrium, 1st floor)

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Oral Presentations

O1. Chasing Functional Dynamics in Biological Systems

R. Scott Prosser
University of Toronto

Q. What do protein folding and misfolding pathways, G Protein Coupled Receptor (GPCR) activation mechanisms, and enzyme action have in common?
A. All are woefully described by two state processes. In NMR studies of biological systems, it often proves difficult to characterize states along reaction coordinate pathways or delineate conformers associated with signalling or activation. Quite simply, these states may exhibit subtle differences in structure and the entire process may take place so quickly that it is nearly impossible to spectroscopically characterize separate states. ^{19}F NMR has the distinct advantage that the chemical shift is exquisitely sensitive to van der Waals and electrostatic environments. At the same time, ^{19}F probes can be introduced to proteins either through covalent modifications or biosynthetic labelling, ultimately permitting the resolution of states. In this talk I will present examples of ^{19}F NMR studies of proteins, to provide an impression of what is possible and how this type of spectroscopy might benefit your NMR projects. I will briefly describe examples of studies of protein folding and the recent observation of a desolvated protein folding intermediate, enzyme kinetics and the reaction coordinate pathway associated with a 72 kDa complex, GPCR states and mechanisms of activation, and prion protein equilibria.

O2. Using Split Inteins to Reveal the Striking Modularity of Spider Wrapping Silk in Solution

Marie-Laurence Tremblay
Dalhousie University

The spider wrapping silk protein in Argiope trafisciata contains at least 14 tandem repeats of a 200 amino acid sequence flanked by non-repetitive N- and C-terminal domains. Wrapping silk self-assembles into the toughest type of silk fibre and, similar to all other types of spider silk, is an outstanding biomaterial with remarkable mechanical properties. We present the high-resolution solution-state NMR structure of a recombinantly produced 199 amino acid repeating unit (W1) of wrapping silk. W1 is composed of a solid helical globular core with a flexible unstructured C-terminal tail. With the use of split intein technology, in which two fragments of the intein protein self-associate and splice neighbouring "extein" protein fragments together by a series of nucleophilic displacement reactions, we were able to investigate the effect of repetitive domain tandemization on the dynamics of W2 (two covalently linked repeats) by selectively labeling each repeat with different NMR active isotopes. Our chemical shift assignments for W2 reveal that the globular core is identical from repeat to repeat and our backbone relaxation analysis reveals that the disorder in the ~xx amino acid linker and the stable helical core are conserved with tandemerization. In addition, we simulated a W2 protein structure using our NOE-derived distance restraints for the globular core of W1 with the addition of residual dipolar couplings and hydrodynamics data collected with segmentally labeled W2. Our structures of W1 and W2 with the addition of our dynamics data of wrapping silk reveal a beautiful "beads-on-a-string" architecture that we can extrapolate as the in vivo structure of the native wrapping silk protein.

O3. A Circularly Permuted Photoactive Yellow Protein as a Scaffold for Photoswitch Design

Kumar, Anil; Burns, Darcy; Al-Abdul-Wahid, M. Sameer; Woolley, G. Andrew
Department of Chemistry, University of Toronto

Photoswitchable proteins are powerful tools for external manipulation and probing of complex biochemical processes. In an effort to create new families of photoswitchable proteins that undergo novel types of conformational changes, we designed a circularly permuted PYP (c-PYP) variant of photoactive yellow protein (PYP), a relatively small blue light sensitive protein from Halorhodospira halophila. The c-PYP was created by connecting the N- and C- terminal of wt-PYP with a defined linker polypeptide and introducing new N-and C- termini at G115 and S114 respectively. The designed protein c-PYP is highly soluble and well folded when over expressed in E. coli, and undergoes PYP like photocycle upon exposure to blue light. UV-Vis absorption data indicate that c-PYP recovers more quickly than wt-PYP however. The lifetime of the light state (the partially unfolded state) is 100 ms, so that very bright light is required to produce significant quantities in the steady state. Targeted mutations viz. M121A and M121E have been made based on biophysical data from wt-PYP and were found to enhance the light sensitivity substantially by lengthening the lifetime of the light state to ~10 min. Nuclear magnetic resonance (NMR), circular dichroism, and UV–vis analysis indicated that the M121E-cPYP mutant also adopts a dark-state structure like that of wtPYP. Fluorine NMR studies with fluorotryptophan-labeled M121E-cPYP show that blue light drives large changes in conformational dynamics and leads to solvent exposure of Trp7 (Trp119 in wtPYP numbering), consistent with substantial rearrangement of the N-terminal cap structure. M121E-cPYP thus provides a scaffold that may allow a wider range of photoswitchable protein designs via replacement of the linker polypeptide with a target protein or peptide sequence.This work shows that new class of photo-controlled conformational change can be created by altering the backbone topology of a known photoswitchable protein.

Reference:

Kumar, Anil; Burns, Darcy; Al-Abdul-Wahid, M. Sameer; Woolley, G. Andrew, "A circularly permuted photoactive yellow protein as a scaffold for photoswitch design", Biochemistry, 2013, 52 (19), pp 3320-3331

O4. Deciphering the Mechanism of E2 and E3 Enzymes in Ubiquitylation

Donald Spratt
Western University

Cells grow and divide through a delicate balance between the synthesis and breakdown of proteins. Many of these proteins are involved in cell cycle progression and if they are not properly regulated by their degradation, cells can grow and proliferate in an uncontrollable fashion, a hallmark of many cancers. To better understand this process known as ubiquitylation, a group of specialized proteins called "E2" and "E3" enzymes are being examined in the presence and absence of the degradation marker protein "ubiquitin" to decipher how these enzymes function. For example, the E2 enzyme CDC34 and its interaction partner the E3 ligase Skp1/Cullin-1/F-box (SCF) are responsible for the degradation of key cell cycle regulator proteins in the cell. RING Box protein-1 (Rbx1) is a RING domain protein found in the SCF complex that recruits and facilitates the efficient transfer of ubiquitin from CDC34 to a substrate protein or an elongating ubiquitin chain. To better understand this key E2-E3 interacting pair, the structure of Rbx1 was determined using triple resonance assignments, ^{15}N and ^{13}C NOE assignments for distance restraints and CYANA to calculate three-dimensional structures. Protein-protein interactions were also examined by titrating Rbx1 with CDC34 to monitor chemical shift perturbations and to define the interaction surfaces used by these proteins within this E2-E3 complex.

By comparing the three-dimensional structures of different E2-E3 protein complexes with ubiquitin, the mechanism of these ubiquitylation proteins will be established at the atomic level.

O5. The Effect of Dilution on Isotropic Bicelles: Changes in Morphology and Miscibility of its Constituents

M. Beaugrand,(1) A.A. Arnold,(1) J. Henin,(2) D.E. Warschawski,(2) P.T. F. Williamson(3) and I. Marcotte(1)
(1) Department of Chemistry, Université du Québec à Montréal, Montréal (Québec), Canada
(2) School of Biological Sciences, University of Southampton, Southampton, UK
(3) Institut de Biologie Physico-Chimique, CNRS, Université Paris Diderot, Paris, France

Bicelles are model membranes made of long-chain dimyristoylphosphatidylcholine (DMPC) and short-chain dihexanoylPC (DHPC). They are widely used for structural studies as well as investigation of membrane interactions by both solution and solid-state NMR. However, experimental constraints may require their use at high dilutions at which their morphology is unclear. In this work, ^{31}P NMR has been employed to better understand the composition and morphology of isotropic bicelles at strong dilutions with q molar ratios (q = [DMPC]/[DHPC]) below 2. As the concentration is lowered, the concentration of free DHPC remains constant, in complete analogy with a critical micelle concentration (CMC), and the proportion of DHPC in the bicelles decreases, thus modifying their morphology. The critical bicellar concentration - or CBC analogous to CMC - was calculated by successive dilutions. It was shown to decrease with increasing q ratios between 0.15-1, and then to remain fairly constant at a value of 6 mM up to the maximum studied q ratio of 2. Taking into account the free DHPC molecules in solution, the effective q ratios (q*) were calculated. Our results show that q* values (and, thus, the bicelle morphology) are constant for total phospholipid concentrations above 100 mM. Below this concentration threshold, q* starts to increase significantly, especially for high q ratios. For the more diluted concentrations, between 2 and 25 mM and for q values above 0.75, samples become cloudy as DHPC-impoverished bicelles form large vesicles. FTIR spectroscopy results show that the miscibility is very low but not negligible below a q value of 2. This result is in good agreement with molecular dynamics calculations at a q of 0.25.

O6. Characterisation of PC/Tween-80 Model Membranes for the Study of Membrane Proteins and Peptides

Andrée Gravel, Alexandre A. Arnold and Isabelle Marcotte
Department of Chemistry, Université du Québec à Montréal, P.O. Box 8888, Downtown Station, Montréal, Canada, H3C 3P8

In this study, we provide a new membrane mimetic system made of a detergent commonly used to solubilise membrane protein, in order to facilitate the structural study of membrane proteins by NMR. Tween-80 is a fatty acid ester (oleate) of sorbitan polyethoxylate known to be a mild non-ionic surfactant. Phosphatidylcholine (PC)/Tween-80 (TW80) model membrane systems were characterized by solution- and solid-state NMR, by Atomic Force Microscopy (AFM), by Cryo-Electron Microscopy (EM) as well as Dynamic Light Scattering (DLS). DMPC (14:0) and DPPC (16:0) self assembled with TW80 to form oriented structures as demonstrated by ^{31}P and ^{2}H NMR. Solution-state NMR studies demonstrated the formation of smaller isotropic systems. Cryo-TEM studies allowed us to visualize the shape and size of PC/TW80 systems. These model membranes would allow for efficient membrane protein extraction by avoiding the detergent removal step during purification, thus minimizing protein aggregation or precipitation for the downstream NMR applications.

O7. Micelle-Catalyzed Domain Swapping of the N-terminal Cytoplasmic Domain of Rhomboid Protease

Jason Ka-Cheong Kwok
Department of Chemistry, University of Ottawa

Domain swapping is a mechanism for protein oligomerization that occurs through the exchange of identical structural elements, such as domains or secondary structural elements, between subunits. This type of oligomerization does not appear to be exclusive to any particular protein fold, as evidenced by the diversity of proteins that can undergo domain swapping. In addition, domain swapping is associated with a variety of protein functions, including receptor binding, allostery, and the regulation of enzyme activity.Moreover, there is great interest in understanding the mechanism of domain swapping, as it is involved in the formation of protein aggregates and fibrils associated with deposition diseases. Here, we find that the N-terminal cytoplasmic domain of E. coli rhomboid protease can undergo domain swapping and present the structure of its domain-swapped dimer solved by solution NMR. Interestingly, NGlpG dimerization can be accelerated by micelles composed of zwitterionic or anionic detergents, with the rate of monomer-dimer interconversion proportional to the size of the micelles. In particular, we find that hexadecyl-phosphocholine (Fos16) can enhance the rate of domain-swapping without altering the dissociation constant for the monomer-dimer equilibrium system, indicating that Fos16 can serve as a true catalyst for domain swapping. Fos16-catalyzed domain swapping was found to be first order with respect to the micelle concentration, and gives rise to a ~12 kcal/mol decrease in the transition state free energy, corresponding to a 108-fold increase in the domain-swapping rate under standard conditions. Given that micellar complexes and local lipid domains with irregularities can exist in vivo, the results from this study provide insight into physiological conditions that may nucleate and accelerate protein aggregation and fibril formation.

O8. Solid-State NMR Studies of Biomolecules under Ultra-Fast MAS and Amyloid Aggregates for Alzheimer's Beta

Yoshitaka Ishii
Dept. of Chemistry, University of Illinois at Chicago

In this presentation, we report two separate topics on biomolecular solid-state NMR (SSNMR) application and methodology. In the first topic, we discuss resolution and sensitivity enhancement in 1H and ^{13}C SSNMR by use of SAIL labeled samples under ultra fast magic angle spinning (MAS) conditions (~ 80 kHz) in a high magnetic field (1H frequency: ν_H = 750-800 MHz). Our data for SAIL labeled amino acid and protein samples show distinctive sensitivity enhancement by ^{1}H-detected 2D $^{1}H/^{13}C$ correlation over ^{13}C detected 1D SSNMR. The advantage of high-field SSNMR and effects of ^{2}H decoupling and other opportunities such as spectral editing under the ultra fast MAS condition are discussed. In the second topic, we characterize structures of amyloid fibril and spherical intermediate for 42-residue Alzheimer's β (Aβ(1-42)) by SSNMR. We present a new structural model on amyloid fibril of Aβ(1-42), which is distinct from the models that were previously studied for 40-residue Aβ(1-40). In addition, we will present NMR-based structural studies on spherical non-fibrillar assemblies of Aβ(1-42). Other topics will be also discussed.

O9. Application of ^{7}Li Nutation Curves to Differentiate Electrochemical Species

Zoe E. M. Reeve, Alex. D. Bain and Gillian R. Goward
McMaster University, Department of Chemistry, Hamilton ON, L8S 4L8.

With automotive applications in mind there has been a search for lighter and higher energy

density batteries. The Li-O2 battery is a promising energy storage candidate for electric vehicles as the energy density of the Li-O2 battery is equivalent to gasoline [1]. The high energy density results from the electrochemical formation of lithium peroxide from the reaction of molecular oxygen and metallic lithium. The reduced oxygen species at the cathode has been shown to consume the electrolyte resulting in electrolyte decomposition species [2]. A critical challenge during characterization of the discharge products is differentiating lithium peroxide from the electrolyte decomposition species. Here it is proposed that the discharge species may be identified from ^{7}Li nutation plots.

The small quadrupole moment of ^{7}Li allows for nutation plots to be collected where the Cq is equivalent to the applied r.f. power. Based on recent literature [3] it has been hypothesized that differences in the quadrupole coupling constants of the electrolyte decomposition species will result in observable differences in the nutation plots. The aim is to identify lithium peroxide formation in cathode samples using ^{7}Li nutation plots.

References:

[1] G. Girishkumar, McCloskey, B., Luntz, C., Swanson, S. and Wilcke, W. , Journal of Physical Chemistry Letters 2010, 1, 2193-2203.
[2] S. A. Freunberger, Y. Chen, Z. Peng, J. M. Griffin, L. J. Hardwick, F. Barde, P. Novak and P. G. Bruce, Journal of the American Chemical Society 2011, 133, 8040-8047.
[3] a) T. Leigh Spencer, G. R. Goward and A. D. Bain, Solid State Nuclear Magnetic Resonance 2013, 53, 20-26; b) T. L. Spencer, G. R. Goward and A. D. Bain, Canadian Journal of Chemistry 2011, 89, 764-769.

O10. The Peculiarities of Homonuclear Spin-Spin Coupling between Quadrupoles

Frédéric A. Perras and David L. Bryce
Department of Chemistry
University of Ottawa

Indirect nuclear spin-spin coupling (J coupling) provides useful information relating to the nature of chemical bonding in a system. J couplings have however almost exclusively been reported for spin-1/2 nuclei where splittings can be clearly observed in the NMR spectra. We show that the use of sophisticated sample rotation (i.e., DOR) can be used to average the quadrupolar broadening and reveal homonuclear J coupling between quadrupolar nuclei. Interestingly, unlike for spin-1/2 nuclei, the J coupling still affects the NMR spectra of magnetically equivalent (i.e., A2) quadrupolar nuclei. This is demonstrated experimentally with ^{11}B (I = 3/2) and ^{55}Mn (I = 5/2) DOR NMR experiments.[1] We also show that double-quantum filtered J-resolved experiments can be used to easily measure homonuclear J coupling involving quadrupolar nuclei.[2] In this case, simple doublets are obtained; however, the splittings are amplified when the spins are magnetically equivalent. The size of the splitting provides a stringent test of the symmetry of a molecule, as well as reporting on the nature of the chemical bond. This is demonstrated experimentally with the use of a series of diboron compounds for which the symmetry of the molecule can be broken through reaction with a ligand. This work provides a new and exciting approach to studying metal-metal bonding and diboron reagents, among other systems.

References:

1 Perras, F. A.; Bryce, D. L. J. Chem. Phys. 2013, 138, 174202.
2 Perras, F. A.; Bryce, D. L. J. Am. Chem. Soc. 2013, 135, 12596.

O11. Applications of ^{35}Cl SSNMR to Solid Pharmaceuticals: Detection of Polymorphs and Impurity Phases

Anthony R. Sandre,[1] Karen E. Johnston,[1] Andrew M. Namespetra,[1] Zhehong Gan,[2] Ivan Hung,[2] and Robert W. Schurko[1,*]
[1]Department of Chemistry and Biochemistry, University of Windsor, Windsor, ON, Canada, N9B 3P4
[2]National High Magnetic Field Laboratory, 1800 E. Paul Dirac Drive, Tallahassee, FL, USA, 32310-3706

The structure and integration of the active pharmaceutical ingredient (API) into the excipient matrix must be monitored at various stages of the manufacturing process. In recent years, increased emphasis has been placed upon product formulation and purification, since polymorphic forms of the same API may exhibit distinct properties, including bioavailability, stability, shelf-life and pharmacokinetics [1-3]. Many APIs are crystallized or solidified as hydrochloride (HCl) salts, where chloride anions serve to charge-balance positive organic moieties and stabilize the solid-state structures. Solid-state NMR (SSNMR) is a premier technique for probing the molecular structure of solid pharmaceuticals. ^{13}C and ^{1}H SSNMR experiments are most commonly used to study APIs; however, multinuclear NMR studies are becoming more common. Chlorine exists as two naturally occurring isotopes, ^{35}Cl and ^{37}Cl, both of which are spin-3/2 quadrupolar nuclei. The former is typically preferred for NMR experimentation due to a higher natural abundance, a larger gyromagnetic ratio and a smaller quadrupole moment. Herein, we present several applications of ^{35}Cl SSNMR for the study of HCl APIs in mixtures, including mixtures of: (i) distinct APIs and (ii) polymorphic forms of the same API. Acquisition of ^{35}Cl SSNMR spectra using the BRoadband Adiabatic INversion–Cross Polarization/WQCPMG (BRAIN-CP/WQCPMG) pulse sequence and several spectral editing techniques including effective T2 editing, T1 relaxation time constant measurements and spectral subtraction, allows us to demonstrate the viability of ^{35}Cl SSNMR for potential use in the high-throughput analysis of mixed solid phases of APIs.

References:

[1] Karpinski, P. H. Chem. Eng. Technol. 2006, 29, 233.
[2] Datta, S.; Grant, D. J. W. Nat. Rev. Drug Discov. 2004, 3, 43.
[3] Llinas, A.; Box, K. J.; Burley, J. C.; Glen, R. C.; and Goodman, J. M. J. Appl. Crystallogr. 2007, 40, 379.
[4] Harris, K. J.; Lupulescu, A.; Lucier, B. E. G.; Frydman, L.; Schurko, R. W. J. Magn. Reson., 2012, 224, 38-47.

O12. NMR Spectroscopy of NMR Homopolymers

Kalle Gehring
McGill University

Cytosine and adenine homopolymers can both form duplexes under acidic conditions. Nonetheless the structures are very different The NMR structure of the polycytidylic acid reveals it to be a four-stranded, intercalated structure. In contrast X-ray crystallography of polyadenylic acid reveals it is a two-stranded, parallel duplex which can form both at low pH and at neutral pH in the presence of ammonium ions. The talk will review strategies for the assignment of nucleic acid NMR spectra and the perspectives for the occurrence of homopolymer complexes in cells.

O13. Signaling through Dynamic Linkers as Revealed by PKA

Madoka Akimoto, Rajeevan Selvaratnam, Eric T. McNicholl, Geeta Verma, Susan S. Taylor and Giuseppe Melacini
McMaster University

Allosteric transitions have been the subject of extensive structural and dynamic investigations focusing mainly on folded domains. However, the current understanding of the allosteric role of partially unstructured linkers flanking globular domains is limited. Protein Kinase A (PKA) is a multi-domain signaling protein containing a regulatory subunit (R) and a kinase subunit (C). Here, we report that a dynamic N-terminal linker in R of PKA serves not only as a passive covalent thread, but also as an active allosteric element that controls activation of the kinase subunit by tuning the inhibitory pre-equilibrium of a minimally populated intermediate (apo R). Apo R samples both C-binding competent (inactive) and incompetent (active) conformations within a nearly degenerate free energy landscape and such degeneracy maximally amplifies the response to weak (~2RT), but conformation selective interactions elicited by the linker. Specifically, the R linker that in the R:C complex docks in the active site of C, in apo R preferentially interacts with the C-binding incompetent state of the adjacent cAMP-binding domain (CBD). These unanticipated findings imply that the formation of the inter-molecular R:C inhibitory interface occurs at the expense of destabilizing the intra-molecular linker/CBD interactions in R. A direct implication of this model, which was not predictable solely based on protein structure, is that the disruption of a linker/CBD salt bridge in the R:C complex unexpectedly leads to increased affinity of R for C. The linker includes therefore sites of R:C complex frustration and frustration-relieving mutations enhance the kinase inhibitory potency of R without compromising its specificity.

O14. Mapping the Interactions between the Alzheimer's Aβ-Peptide and Human Serum Albumin beyond Domain Resolution

Moustafa Algamal
Department of Chemistry and Chemical Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario, L8S 4M1, Canada.

Human Serum Albumin (HSA) is a potent inhibitor of Aβ self-association and this novel function of HSA is of potential therapeutic interest for the treatment of Alzheimer's disease. It is known that HSA interacts with Aβ oligomers through binding sites evenly partitioned across the three albumin domains and with comparable affinities. However, no information is currently available on the HSA – Aβ interactions beyond domain resolution. Here, we map the HSA – Aβ interactions at sub-domain and peptide resolution. We show that each separate sub-domain of HSA domain 3 inhibits Aβ selfassociation. We also show that fatty acids (FAs) compete with Aβ oligomers for binding to domain 3, but the determinant of the HSA / Aβ oligomer interactions are markedly distinct from those of FAs. While salt bridges with the FA carboxylate determine the FA binding affinities, hydrophobic contacts are pivotal for Aβ oligomer recognition. Specifically, we identified a site of Aβ oligomer recognition that spans the HSA (494- 515) region and aligns with the central hydrophobic core of Aβ. The HSA (495-515) segment includes residues affected by FA binding and is prone to self-associate into β-amyloids, suggesting that sites involved in fibrillization may provide a lead to develop inhibitors of Aβ self-association.

O15. Dipole-Dipole Coupling Investigation of Solid Acids and Composites for Proton Exchange Membrane Fuel Cells

Nicole De Almeida
McMaster University

Proton exchange membrane fuel cells (PEMFCs) require higher operation temperatures to prevent catalyst layers degrading due to CO poisoning. Currently, the most popular proton exchange membrane (PEM) is Nafion due to its high proton conductivity however does not conduct at high temperatures due to its dependence of water to conduct protons.[1] To achieve higher temperature ranges solid acids were investigated.[2] Cations with known dynamics were paired with anions to make an overall acidic salt.[3] Solid acids pairs investigated imidazole paired with trifluoromethanesulfuric acid as well as imidazole, benzimidazole and adenine paired with methanesulfonic acid. To be a prime candidate for fuel cell applications the acidic proton must be highly mobile to be able to possess high proton conductivity. Solid state NMR was utilized to show the relative mobility of the protons through double quantum filter (DQF) experiments. With DQF NMR dipole-dipole couplings are reintroduced on the timescale of rotor period. The DQF pulse sequence used was POST C7 coupled with DUMBO for ^{1}H homonuclear decoupling. With DUMBO-POST C7 buildup curves are obtain over various lengths of recoupling, where the maximum in the buildup curve indicates the strength of the dipole-dipole coupling.[4] Peaks at short recoupling times indicate strong dipole-dipole coupling and peals at long recoupling times indicating weak dipole-dipole couplings. The buildup curves can be simulated in spin evolution to extract the dipole-dipole coupling values. Using 2D DUMBO POST C7 all dipole-dipole coupling pairs can be determined to probe local mobility of all proton sites. Bulk proton conductivity measurements can be compared to DQF measurements to understand proton conduction within these materials.

References:

(1) Mauritz, K. A.; Moore, R. B. Chemical Reviews 2004, 104, 4535.
(2) Haile, S. M.; Boysen, D. A.; Chisholm, C. R. I.; Merle, R. B. Nature 2001, 410, 910.
(3) Traer, J. W.; Goward, G. R. Phys. Chem. Chem. Phys. 2010, 12, 263.
(4) JP, B.; C, T.; C, F.; SP, B. Phys Chem Chem Phys. 2009, 11, 6941.

O16. Slow and Steady Wins the Race - Multinuclear SSNMR of Unreceptive Nuclides Using Broadband Cross-Polarization Methods

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Many NMR-active nuclides are classified as unreceptive nuclei, due to their low gyromagnetic ratios (low Larmor frequencies), low natural abundances, large quadrupole moments and/or large chemical shift anisotropies (CSAs) leading to inhomogeneous broadening, and unfavorable relaxation characteristics (i.e., long longitudinal relaxation times and/or short transverse relaxation times), which make acquiring high-quality spectra of such nuclides difficult due to inherently low signal-to-noise (S/N).

Harris et al. recently presented a modified cross-polarization (CP) sequence, BRoadband Adiabatic INversion CP (BRAIN-CP), which is capable of transferring abundant spin polarization while simultaneously exciting broad frequency bandwidths using low B1 fields. The use of BRAIN-CP for the acquisition of CSA-dominated powder patterns of spin-1/2 nuclides has yielded spectra with increases in S/N of one to two orders of magnitude and drastically reduced experimental times compared to conventional direct excitation and/or CP/MAS methods.

We extend this work for the study of unreceptive quadrupolar nuclei such as ^{39}K, ^{25}Mg, ^{47,49}Ti,

^{93}Nb, and ^{14}N. Spectra acquired using the BRAIN-CP method are compared with those acquired using conventional methods, and the performance of each methodology is evaluated for each case. Additionally, the application of frequency-swept pulses for measuring longitudinal relaxation times of unreceptive spin-1/2 and quadrupolar nuclides, including ^{195}Pt, ^{199}Hg and ^{39}K, is presented. Finally, the potential for the widespread application of these methods to the routine study of unreceptive nuclei from across the periodic table is discussed.

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O17. ^{2}H SSNMR Study of Metal-Organic Frameworks with Dynamic Interlocked Components,

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Crown ethers, as isolated molecules or as intercalated or interlocked components of complexes, are known to be highly mobile in both solutions and the solid state [1]. The incorporation of crown ethers into metal-organic frameworks (MOFs) to produce dynamic molecules with mechanically interlocked components has recently garnered interest for the development of molecular level switches and machines [2]. X-ray crystallography and ^{13}C SSNMR are utilized for structural characterization of such systems, but provide little information on the modes and rates of the dynamic processes involving the crown ethers. ^{2}H SSNMR is a powerful technique for the study of such dynamic processes, due to the extreme sensitivity of the ^{2}H NMR powder patterns to different spatial and temporal changes at the molecular level. Simulations of ^{2}H SSNMR powder patterns can provide an accurate model of the various dynamic processes in these systems. In this lecture, I will present a study of several MOFs with interlocked components consisting of various framework structures and crown ether sizes. Variable-temperature ^{2}H SSNMR spectra were collected for these systems, and the EXPRESS software package was used to simulate powder patterns. From these data, several motional models are suggested. It is demonstrated that the dynamics in these systems can be drastically altered by small differences in either the framework structure or the size of the crown ether rings. ^{2}H SSNMR continues to reveal new motional modes in increasingly complex materials.

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O18. Correlations Between Local Halogen Bonding Environments and Multinuclear Solid-State Magnetic Resonance Observables

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Solid-state nuclear magnetic resonance (SSNMR) has proven to be a useful tool in the characterization of non-covalent interactions such as hydrogen bonding and cation-π interactions. Another non-covalent interaction which has drawn much attention in recent year is the halogen bonding (XB) interaction. XB is defined as an electrostatic interaction, R—X•••Y, between a halogen atom (X), and a nucleophilic region of another molecule (Y) such as a Lewis base, halide ion, or π-electrons [1]. We synthesized multiple types of XB compounds using 'iconic' halogen bond donors (perfluorobenzenes) and various ammonium/phosphonium halides (Cl^- or Br^-) or chalcogen-containing ($SePPh_3$, SCN^-, or $SeCN^-$) compounds. Multinuclear (^{13}C, $^{14/15}$N, ^{31}P, ^{77}Se, $^{35/37}$Cl, $^{79/81}$Br) magnetic

resonance experiments have been performed at applied magnetic fields strengths ranging from 4.7 to 21.1 T. From these experiments, significant changes in the chemical shift tensors for the ^{13}C [2] and ^{77}Se [3] nuclei directly involved in halogen bonding were observed in the presence of this non-covalent interaction. Also, the electric field gradient tensors of the halides involved in XB ($^{35/37}Cl$ and $^{79/81}Br$, I = 3/2) have demonstrated sensitivity to their local XB environment as evidenced by experiment and theoretical calculations (ZORA-DFT) of the natural localized molecular orbitals (NLMO). Furthermore, in the series of XB compounds containing chalcogens, the ^{31}P-^{77}Se J-coupling values for the spin pairs involved in halogen bonding are shown to be a sensitive probe of the interaction. Overall, these studies demonstrate how NMR spectroscopy is a very sensitive tool for detecting the changes in electronic structure which are caused by the non-covalent interaction.

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O19. High Resolution Paramagnetically Enhanced Solid-State NMR Spectroscopy of Membrane Proteins at Fast Magic Angle Spinning

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Magic angle spinning nuclear magnetic resonance (MAS NMR) is well suited for the study of membrane proteins in membrane mimetic and native membrane environments. These experiments often suffer from low sensitivity due in part to the long recycle delays required for magnetization and probe recovery, as well as detection of low gamma nuclei. Sensitivity can be increased through the use fast MAS, combined with the use of paramagnetically enhanced relaxation times to reduce recycle delays, as well as proton detected experiments. We demonstrate that spin diffusion is sufficient to uniformly distribute paramagnetic relaxation enhancement provided by either covalently bound or solvent CuEDTA over 7TM alpha helical membrane proteins. Using paramagnetic enhancement and low power decoupling in carbon detected experiments, we can recycle experiments ~13 times faster as compared to traditional methods. However, due to the small sample volume the overall sensitivity per unit time is still lower than that seen in the 3.2mm probe. However, the proton coil was found to show an increase in efficiency when compared to the 3.2mm probe. With the use of low power decoupling it was found that the 1.3mm probe could achieve sensitivity comparable to that of the 3.2mm in a given amount of time. This is an attractive prospect for mass limited samples, as this allows for a reduction in the amount of protein that needs to be produced, without the necessity for increased experimental time.

O20. Characterization of Interactions between EPAC1 and the EPAC Selective Inhibitor ESI-09 by NMR Spectroscopy

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Exchange proteins activated by cAMP (EPAC) are guanine exchange factors whose function have been linked to cell adhesion, cell junction formation, regulation of exocytosis, and cardiac function. Recently, overexpression of EPAC1 had been observed in cases of human pancreatic ductal adenocarcinoma (PDA), which has increased the search for EPAC selective inhibitors which may be

developed into drugs for treating types of pancreatic cancers. The largest obstacle for this is the identification of compounds which are selective enough to inhibit EPAC proteins without affecting the plethora of other cAMP binding proteins. In this study, we looked at a previously discovered EPAC selective inhibitor, 3-[5- (tert.-Butyl)isoxazol-3-yl]-2-[2-(3-chlorophenyl)hydrazono]-3-oxopropanenitrile (ESI-09) in order to characterize its interactions with the protein and identify the reason for its specificity over other cAMP binding proteins such as PKA. To do this we used an approach developed by our group known as Chemical Shift Projection Analysis (CHESPA) to identify residues which are shifted towards inhibition by the binding of ESI-09. We then attempted to determine the orientation of the drug in the active site of EPAC using a combination of STD epitope mapping, transfer NOEs and PRE experiments in coordination with some docking simulations.

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Poster Presentations

P1. Singularities in the Quadrupole Lineshape

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Many of us have spent hours looking at the second-order perturbed lineshape for half-integral quadrupolar nuclei. There are good programs that simulate this by doing some sort of a powder average of the frequencies. Since they are clearest in the spectra, it is the singularities that we concentrate on: the steps and the peaks. Their origins are clear. If we plot the frequency as a function of the angles, then the steps correspond to maxima and minima in the plot, and the peaks correspond to the saddle points. We have been working on deriving explicit formulae for the positions and the heights of these critical points, and for the simple case, it is quite straightforward. This means, perhaps, that we could use this to fit the singularities without the need for a powder average. This work is still in progress and we will report our progress.

P2. Probing the Determinants of PKA's High Affinity for Cyclic AMP by NMR Spectroscopy

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Protein Kinase A (PKA) is a critical enzyme in many signal transduction pathways. In particular, we are interested in the binding of cyclic adenosine monophosphate (cAMP) with the cAMP-binding domain A (CBD-A) of PKA. Misregulation of these biomolecules is highly correlated with ischemic stress and cardiac tumours. Similar CBDs found within other cAMP binding proteins, such as hyperpolarization-activated and cyclic nucleotide-gated ion channels (HCN), exchange protein activated by cAMP (EPAC), and protein kinase G (PKG) exhibit similar binding affinity for cAMP (sub μM range). Conversely, PKA displays nM affinity. The PKA vs. non-PKA comparative analyses of the crystal structures in their cAMP-bound state reveal only minor differences, providing little rationalization for the three orders of magnitude higher binding capabilities found in PKA. Deletion results suggest that the lid is not responsible for the tight binding of cAMP to the N-terminal domain of PKA, leaving the phosphate binding cassette (PBC) and/or the base binding region (BBR). By utilizing previously determined allosteric hotspots, along with the crystallographic structural data, we intend to mutate potential key residues. Thereafter, saturated transfer difference (STD) spectroscopy will allow us to quantitate the binding in terms of a dissociation constant (Kd). This is an excellent system to use STD, as it is suited for detecting binding between small ligand molecules and macromolecular receptors.

P3. Multinuclear Solid-State NMR Study of 43Ca and 87Sr Nuclei Present in Organic Molecular Environments

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Recently, we have been interested in the development of alkaline-earth metal (^{25}Mg, ^{43}Ca, and ^{87}Sr) solid-state NMR in order to establish meaningful correlations between their NMR parameters and crystallographic environments [1]. In this work, we have synthesized metal (Ca and Sr) complexes bearing aryl carboxylate and ethylenediaminetetracetic acid ligands. For the first time, ^{43}Ca solid-state NMR at multiple magnetic field strengths (from 9.4 to 21.1 T) is used to characterize metal-coordinated

nitrogen atoms. These are shown to affect both the ^{43}Ca chemical shift tensor and electric field gradient tensor parameters. With our contribution, it was then possible to establish, with a larger dataset of reliable NMR data, a correlation between the experimental and the gauge-including projector-augmented-wave (GIPAW) density functional theory calculated $|C_Q(^{43}Ca)|$ values. This correlation is then employed to investigate the much disputed structure of the vaterite polymorph of $CaCO_3$. In most cases, the WURST QCPMG method at B_0 = 21.1 T could be employed for the acquisition of high quality ^{87}Sr NMR spectra of the analogous strontium compounds. Large $|C_Q(^{87}Sr)|$ values are observed and are found to depend on the nature of the immediate Sr^{2+} coordination sphere. All 21.1 T experiments were acquired at the National Ultrahigh-Field NMR Facility for Solids.

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P4. CRAPT, An Improved Pulse Sequence for ^{13}C Spectral Editing

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The Attached Proton Test (APT) sequence had the advantage over DEPT of providing editing information for both protonated and non-protonated carbons. However, it had the disadvantage that it was very sensitive to variations in 1JCH. The edited HSQC sequence, which is another alternative to DEPT, had similar problems. However, several years ago, Krishnamurthy and co-workers showed that this weakness of HSQC could be largely overcome by replacing the hard ^{13}C 180 pulses by what they called CRISIS pulses. These were frequency-swept adiabatic ^{13}C pulses which compensated for variations in $^{1}J_{CH}$, based on the approximate linear relationship between these parameters and the chemical shifts of the corresponding carbons. We decided to see if similar improvements could be produced with APT by incorporating CRISIS pulses into this sequence, producing CRISIS-APT or CRAPT for short. This has been tested with four representative organic molecules (strychnine, menthol, cholcaliferol (vitamin d3) and isotachysterol. We find that CRAPT has an average signal/noise advantage over APT of ~ 40% but with greater advantages for carbons nearest the ends of the spectral windows. The average signal/noise for all carbons in the 4 test molecules is 85% of that for the corresponding carbons in standard ^{13}C spectra obtained in the same time. CRAPT also gives significantly better sensitivity than DEPTQ, particularly for non-protonated carbons. Thus it is possible to get an edited ^{13}C spectrum with CRAPT is less time than to get either a DEPTQ spectrum or a combination of a tandaed ^{13}C spectrum plus a DEPT!#% spectrum, making CRAPT a useful sequence for obtaining edited spectra of organic molecules

P5. In-Situ NMR Studies of the Alternative Lithium-Ion Battery Electrolytes

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Lithium ion batteries currently use a mixture of organic carbonates as the electrolyte of choice. They are suitable for this purpose due to a range of favorable properties including high lithium conductivity, favorable reactivity, and their wide electrochemical window. Their primary drawback however is significant safety concerns inherent to the high volatility and flammability of organic carbonates. Consequently, there has been a concentrated effort to find safer alternatives which do not compromise performance including viscous eutectic electrolytes (1) and ionic liquids (2). NMR can be used to evaluate candidate electrolytes on the basis of their chemical and diffusional properties.

Our research has focused on the implementation of an in-situ electrochemical cell based off a

design previously reported by Hallberg et al. (3) The advantages of this include the compatibility with standard 5mm NMR tubes and the relative ease of construction and customizability. Modifications to this design were made such that one of the lithium insertion electrodes was cycled in the actively measured area of the NMR coil. Diffusion ordered spectroscopy (DOSY) was used in combination with slice selective imaging to obtain information on diffusion coefficient and transference number of electrolyte species close to the electrode surface. Such measurements also allowed for observation of concentration gradients which can form as the result of low lithium transference numbers in some electrolytes.

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P6. SS-NMR Studies on Crystallization and Substitution in SAPO-5 Synthesized via Dry-Gel Conversion

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Molecular sieves have been scientifically and industrially important materials since their discovery. These porous, crystalline networks of tetrahedrally coordinated atoms bridged by oxygen are known as excellent sterically-selective catalysts. The framework of the aluminophosphate (AlPO4) family of molecular sieves is itself non-catalytic, but through heteroatomic substitution these materials can diversify into, among many others, the silicoaluminophosphate (SAPO) family of solid acid catalysts. Research into the methods by which these substitutions occur is important, as discoveries can lead to refined control over material property and function. SAPO-5 was chosen as model system of study. Powder X-ray diffraction and ^{27}Al, ^{29}Si and ^{31}P solid state NMR were used as the primary methods of investigation. 1D MAS, CP-MAS and Hahn-echo experiments were performed (9.4 T, spinning speed > 8 kHz) in order to monitor the electronic environment of these three nuclei as they progress from amorphous starting material to crystalline product. The SAPO-5 study determined that the dry gel conversion (DGC) methods of steam assisted conversion (SAC) and vapour phase transport (VPT) follow similar reaction pathways, resulting in two populations of silicon sites. One represents isolated $Si(OAl)_4$ species well incorporated into the framework (desirable for increased catalytic acidity), while the other represents silica islands within the framework. The initial core of the nuclei is $AlPO_4$ in nature and the Si is gradually incorporated into the lattice as the crystals grow. The vapour phase silicon uptake (VPSU) method provides a contrast to this data, inducing nucleation that occurs concurrently with Si incorporation and producing only isolated $Si(OAl)_4$ species.

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P7. Thermodynamics and Conformational NMR of G-rich Nucleic Acids

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G-quadruplexes are structures formed from guanine residues in G-rich nucleic acid sequences.[1] They are composed of three stacked G-tetrads, where four guanine residues are Hoogsteen hydrogen bonded to form a planar arrangement. G-quadruplexes exhibit extensive conformational heterogeneity, dependent on the salt content of the solution and nucleic acid sequence.[2] These cube-like structures are of interest in nucleic acid folding and dynamics due to the nature of their formation and high degree of structural variability. G-quadruplex containing sequences have been identified throughout the genome, though much of the study of G-quadruplexes has been focused on the G-rich telomere sequence and oncogene promoter sequences. When formed, these structures act as inhibitory elements to telomerase and the transcriptional machinery. The 27-basepair G-rich nuclease hypersensitivity element III (NHEIII) present in the c-myc promoter controls 80-90% of c-myc transcriptional activity.[3] With the cmyc G-quadruplex being so crucial in regulation of cell growth and division, it is important to understand its formation and structure. Obtaining a fundamental understanding of G-quadruplex folding and dynamics can apply to many other nucleic acid environments.

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P8. Solid-State ^{23}Na NMR Studies of Cathode Materials for Na-Ion Batteries

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Lithium ion batteries have received considerable attention for their use in portable electronics, and most recently, in hybrid electric vehicles and electric vehicles (1-3). Unfortunately, this overwhelming success has precipitated concerns over limited global lithium supply, and the subsequently increasing cost of lithium materials. This has motivated the reconsideration of sodium ion batteries, which were initially studied in the 1980s before the focus was shifted to the more popular lithium electrochemistry (4). While the larger, heavier, sodium ion decreases overall energy density for a given material vs. Li, the cost and abundance benefits are expected to outweigh this disadvantage. Essential to the success of the Na-ion battery, is the cathode (positive electrode) material, and development of materials suitable for this component is necessary. The use of solid-state NMR as a probe of structure and dynamics features in Na cathode materials is relatively new, and appropriate methods for studying properties such as Na-ion exchange have not yet been developed. ^{23}Na MAS NMR is applied here to provide insight into the mobile Na environments in the promising cathode Na_2FePO_4F. The two unique Na sites at 450 ppm and -180 ppm have been assigned based on the paramagnetic interactions of the Na nuclei with the nearby Fe centers. Structural constraints have been analyzed and a difference in observed Na-F/O-Fe bond angles appear to give rise to vastly different paramagnetic shifts for the two sites. These observations can be extended to other paramagnetic fluorophosphate systems (Li and Na) to characterize novel cathode materials. Furthermore, multinuclear studies are expected to yield important ion mobility information for this material.

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P9. Investigation of Co(I) NMR Parameters at Ultra High Field

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The only NMR active isotope for cobalt, ^{59}Co, is quadrupolar (I = 7/2) and has a large quadrupole moment (Q = 420 mbarn), which make the acquisition of its solid-state NMR spectra challenging. Only cobalt oxidation states of +3 and +1 are easily amenable to NMR studies since, in these cases, the cobalt centres are diamagnetic. Systematic solid-state NMR studies to date on Co(I) compounds are rare due to the paucity of the +1 oxidation state in cobalt complexes.[1] Five Co(I) complexes coordinated by cyclopentadienyl (Cp), carbonyl (CO), and fumarate ligands have been synthesized and characterized by single crystal X-ray diffraction. These complexes (CpCo(CO)(fumarate)) have been shown to catalyze [2+2+2] cycloaddition reactions. We report here the effects of changing the fumarate ligand as well as the Cp ring on the ^{59}Co chemical shift and electric field gradient tensors. Isotropic J-coupling constants ($^{1}J(^{13}C,^{59}Co)$) between the carbonyl ligand and cobalt are observed in natural abundance ^{13}C CP/MAS spectra. In addition to acquiring NQR spectra, high quality ^{59}Co solid-state spectra have been acquired at magnetic field strengths of 9.4 and 21.1 T (www.nmr900.ca). It is shown that the large quadrupolar interactions present in these compounds require the use of exact quadrupolar line shape simulations with the Quadrupolar Exact Software (QUEST) developed in our laboratory. The determined parameters are related to the structures of the compounds.

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P10. Cyclic-Nucleotide-Dependent Allostery in Protein Kinase G (PKG)

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Protein kinase G (PKG) is a major protein involved in eukaryotic cyclic GMP (cGMP) dependent intracellular signaling, playing a regulatory role in such processes as cell differentiation, platelet activation, memory formation and vasodilation [1-5]. However, the mechanism by which cGMP controls PKG activity is not fully understood. Therefore, the current research work sought to map key cGMP-controlled allosteric features of PKG. The current research was performed on a PKG Iβ fragment composed of the regulatory CNB-B domain of PKG Iβ, which was previously shown to play a key role in both PKG allostery and PKG selectivity for cGMP over cyclic AMP (cAMP) [1,6,7]. Using a combination of X-ray data and NMR-based analyses, notable features of PKG CNB-B allostery were revealed that distinguish it from the CNB domains of other proteins [8-12], and that may also help to explain the cGMP-versus-cAMP selectivity of the PKG CNB-B. These features will be further explored using mutations at key amino acid residues, in order to gain a more complete understanding of the relevance of these features to PKG CNB-B allostery and cGMP-versus-cAMP selectivity.

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P11. Dynamic Equilibria between Monomeric and Oligomeric Misfolded States of the Mammalian Prion Protein Measured by ^{19}F NMR

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The assembly of misfolded proteins is a critical step in the pathogenesis of amyloid and prion diseases, although the molecular mechanisms underlying this phenomenon are not completely understood. Here, we use ^{19}F NMR spectroscopy to examine the thermodynamic driving forces surrounding formation of β-sheet-rich oligomers early in the misfolding and aggregation pathway of the mammalian prion protein. We show that initial assembly of a small octameric intermediate is entropically driven, while further assembly to putative prefibrillar aggregates is driven by a favorable change in enthalpy. Kinetic data suggest that formation of the β-octamer represents a rate-limiting step in the assembly of prion aggregates. A disease-related mutation (F198S) known to destabilize the native state of PrP was also found to stabilize the β-octamer, suggesting that it can influence susceptibility to prion disease through two distinct mechanisms. This study provides new insight into the misfolding pathway leading to critical oligomers of the prion protein and suggests a physical basis for increased assembly of the F198S mutant.

P12. BRoadband Adiabatic INversion Cross Polarisation of Spin-1 Nuclei: Practical Considerations and Experimental Setups

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The recently developed BRoadband Adiabatic INversion Cross-Polarisation (BRAIN-CP) pulse sequence has been demonstrated to produce solid-state NMR (SSNMR) powder patterns that are of superior spectral quality compared to those acquired using conventional direct excitation methods (e.g., Hahn-echo, CPMG, etc.) [1]. The BRAIN-CP pulse sequence is a modified CP sequence that uses phase-modulated, frequency-swept Wideband, Uniform-Rate, Smooth-Truncation (WURST) [2] pulses on the X-channel (X = ^{2}D, ^{14}N, ^{35}Cl, etc.) during the contact period, which provides efficient polarisation transfer over broad frequency ranges. The BRAIN-CP pulse sequence can be combined with a series of broadband refocusing WURST pulses in the form of a CPMG echo train (i.e., BRAIN-CP/WURST-CPMG), yielding a CP experiment with the benefits of broadband excitation, S/N increases

from polarisation transfer and T2 enhancement, and reduced experimental times. In this presentation, the application of the BRAIN-CP pulse sequence for the routine study of spin-1 nuclides is explored. Particular attention is given to the experimental considerations for acquiring high-quality powder patterns of ^{14}N and ^{2}D in a variety of organic molecules. A systematic approach is suggested for parameterization of the BRAIN-CP pulse sequence in an attempt to make the acquisition of anisotropically broadened powder patterns rapid, efficient and routine.

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P13. Explaining the Serological Behavior of Streptococcus suis Serotypes 1 and 1/2 from Their Capsular Polysaccharide Structure and Biosynthesis

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Streptococcus suis is an important swine pathogen and one of the most important emerging zoonotic agents in humans. To date, 35 serotypes have been described based on capsular polysaccharide (CPS) antigenic diversity. Although most of them have been isolated from diseased pigs, serotype 2 and, to a lesser extent, serotype 14, are considered serious and high-risk zoonotic agents. So far, the CPS repeating unit structure has been determined only for these two serotypes. Important cross-reactions have been demonstrated for capsular serotype 1/2 with serotypes 1 and 2 antisera, as well as between serotypes 1 and 14. The cps loci, in particular the genes encoding putative glycosyltransferases and polymerases, are known for the four serotypes. Genetically, no sequence differences that may contribute to the antigenic differences observed between serotypes 2 and 1/2 have been found. To determine the structure of S. suis types 1 and 1/2 CPSs, reference strains were grown in Todd-Hewitt broth, cells were harvested, and the capsule was released by autoclaving. The purified CPS was obtained after extraction, precipitation, and gel filtration. Their repeating unit structure was determined by immunological, chemical, and chromatographic methods, as well as by high-resolution 1D and 2D 1H and 13C NMR spectroscopy. These structures were compared to those of types 2 and 14 CPSs in order to explain their serological behaviour. We confirmed our hypothesis that the structure of type 1 CPS is highly similar to that of type 14 CPS but somehow different from that of type 2 CPS, whereas type 1/2 CPS contains structural elements found mainly in type 2 CPS and, to a lesser extent, in type 1 CPS. Additionally, putative glycosyltransferase and polymerase genes in the cps loci of types 1 and 1/2 were tentatively assigned to the transfer of specific sugars in the repeating units.

P14. Insight into Spider Wrapping Silk Fiber Formation by Diffusion NMR

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Spider wrapping silk is composed of protein fibers. This is a marvelous biomaterial with strength and toughness surpassing most of today's synthetic products. However, the molecular-level structural features underlying its remarkable physical properties are not yet fully explored. Our recently determined atomic-level solution-state NMR structure indicates a strikingly compact, modular conformation for the 199 amino acid repeat unit (W1) of Argiope trafisciata aciniform spidroin 1 (to be published by Rainey et al.). Interestingly, while the recombinant W1 protein remains in solution in equilibrium between monomer units and nanoparticles (Xu et al., 2013, FEBS Letters), larger constructs consisting of two or more linked W1 units (i.e, W2, W3, and so on) spontaneously form fibers in vitro. In this work, we have performed diffusion NMR studies to correlate the global conformations of the W1, W2 and W3 proteins based upon their hydrodynamic behavior. Our results indicate that each multiunit protein retains the compact globular structure of the constituent W1 subunits with no major inter-subunit interactions needed to explain the hydrodynamic properties of the larger constructs. Thus, the silk fiber formation by aciniform spidroin 1 is likely instigated by intermolecular interactions between neighboring proteins directly facilitated by the strikingly modular "beads-on-a-string" architecture that it possesses.

P15. Investigations of Vapochromic Pt(bpy)(CN)2 and its Precursors Using 195Pt Solid-State NMR

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Much research has gone into investigating square-planar, d8 platinum complexes that have extensive applications as catalysts and sensors. Vapochromic materials fall in the latter category, as they are able to reversibly bind volatile organic compounds (VOCs) to produce a change in luminescence, which is usually manifested as a colour change. The basic structural motif for many of these systems is the compound $Pt(bpy)(CN)_2$, (bpy = bipyridine), which exhibits a red or yellow colour depending on the presence or absence of water vapour, respectively. This change is postulated to be a result of modifications in the intermolecular Pt—Pt distances along the direction of the long-range π-stacking structure, and concomitant change in the degree of Pt—Pt metallophilic interaction.(1)

^{195}Pt solid-state NMR provides a non-destructive method for investigating these interactions in vapochromic compounds, in order to better understand their molecular and electronic structures. The ^{195}Pt nucleus (spin = ½) is favourable for NMR experimental, due to its natural abundance of 33.3% and moderate Larmor frequency at even low field strengths. However, ^{195}Pt SSNMR spectra are challenging to acquire due to (i) inhomogeneous broadening arising from anisotropic chemical shift (CS) interactions and (ii) unfavourable longitudinal relaxation (T1) characteristics. With the advent of the WURST-CPMG(2) and BRAIN-CP/WURST-CPMG pulse sequences,(3) experimental times can be greatly reduced, giving rise to high quality 195Pt NMR spectra from which CS tensor parameters can be extracted. Further refinement of these parameters can be achieved using selective MAS and CP-Echo pulse sequences. The combination of CS parameters and crystallographic data provides insight into the relationships between the NMR parameters, luminescent properties and structures of the red and yellow forms of $Pt(bpy)(CN)_2$, as well as related systems.

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P16. Structural characterization of proapelin processing

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Apelin is a peptide hormone that binds to a cognate class A G-protein coupled receptor (the apelin receptor) to regulate fluid homeostasis, angiogenesis, the adipoinsular axis, and the cardiovascular and nervous systems. Apelin is expressed as a 77 amino acid preproprotein that is processed into bioactive isoforms of 13, 17, and 36 residues in length, all of which retain the C-terminus of preproapelin. Preproapelin contains dibasic amino acid motifs directly N-terminal to each isoform cleavage site, suggesting that proprotein convertase subtilisin kexin (PCSK)-dependent processing is necessary. In support of this, we recently showed that proapelin is preferentially cleaved into apelin-13 by PCSK3. We are now studying this interaction and further elucidating the processing pathway by studying the structure and dynamics of proapelin across the pH range of the secretory pathway. To do so, we have cloned human proapelin, the C-terminal 55-residue component of preproapelin produced by postulated N-terminal signal peptide removal. Proapelin was subsequently expressed in the E. coli C41(DE3) strain as a fusion protein with a hexahistidine tag and TEV protease cleavage site for Ni+ affinity purification. Following affinity chromatography and protease cleavage, the highly basic proapelin was obtained at very high purity by cation exchange chromatography. The structure of proapelin was analyzed by circular dichroism and fluorescence spectroscopy at pH 5, 6, and 7. 15N and 13C enriched proapelin is now being studied by NMR to determine its structure and dynamics as a function of environment. These findings will determine the relative accessibility of the dibasic sites in proapelin to PCSK enzymes, providing a structural basis for selective isoform production and facilitating identification of other enzymes involved in proapelin processing.

P17. Structural Characterization of the Apelin Receptor

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The apelin Receptor (AR) is a class A G-protein coupled receptor (GPCR) involved in regulation of fluid homeostasis, angiogenesis during tumour formation, adipoinsular axis function, and in regulation of the cardiovascular and central nervous systems. It is also an alternative co-receptor with CD4 for entry of human immunodeficiency virus type 1 (HIV-1) in cells. The apelin/apelin receptor system has also been shown as a potential target in treatment of type 2 diabetes. Until now, 23 out of ~800 human GPCRs have been structurally characterized. All of these structures were determined using x-ray crystallography and only one solid-state NMR structure for the backbone of a full-length GPCR is available. GPCRs are inherently dynamic molecules; hence, NMR spectroscopy is a well-suited technique to characterize the structural aspects as well as dynamics of ligand binding. It is very difficult to express a full length GPCR in an expression system suitable for producing uniformly 15N and 13C labeled proteins; hence, we are initially using the "divide and conquer" approach to study the full-length receptor in two parts. We have successfully expressed AR_TM1-3 (first three trans-membrane segments with N-terminal tail) using an AT-rich tag previously reported as enhancing yields in cell-free expression in C41 (DE3) strain of E. coli. We have successfully purified AR_TM1-3 and are working

towards its structural characterization using NMR spectroscopy. We are working on the expression of remaining part of the receptor using an E. coli based cell-free expression system. Moving beyond the divide and conquer approach, we will use split-intein mediated trans-splicing to covalently link the two parts of the receptor and produce the full-length receptor in segmentally isotope-labelled forms amenable to high-resolution NMR study.

P18. Structure, Dynamical Properties and Interspecies Comparison of Anchoring Threads from Bivalves

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In the intertidal zone, mussels anchor to solid surfaces using high-performance protein filaments called byssus. These fibers are composed of three collagen-rich copolymers called Pre-Cols which comprise silk-, elastin- and plant cell wall-like domains. Fibers are covered by a proteinaceous coating. Using high-resolution ^{13}C-^{13}C solid-state NMR and chemical shift predictions, we have evidenced various conformational domains which can be assigned to different regions of the fiber (Pre-Col domains or coating) in the threads of the blue mussel Mytilus edulis. The drastic changes in mechanical properties which occur upon hydration of the threads were studied at a molecular level. No major structural changes could be evidenced upon (de)hydration. A complete dynamical study from ns to ms timescales by high-resolution solid-state ^{13}C NMR enabled us to isolate the motional timescales which are responsible for the macroscopic mechanical changes. Our full structural and dynamical characterization of Mytilus edulis threads is now extended to the study of other species such as other mytlidae or competing bivalve species. Our interspecies comparison reveals differences in composition and crosslinking strategies which result in different mechanical properties of the threads.

P19. Monitoring Millisecond Folding Dynamics in Calmodulin Using ^{19}F NMR CPMG Relaxation Experiments

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Proteins are not static entities and often rearrange themselves into various conformations. These chemical exchange events can include the interconversion between a highly populated ground state and weakly populated higher energy states, which in the case of protein folding may involve both on- and off-pathway intermediates. Calmodulin (CaM), a ubiquitous calcium binding protein, has previously been shown to adopt a desolvated near-native intermediate state along its folding pathway. In this study, we explore the folding kinetics in CaM, biosynthetically enriched with 3-fluorophenylalanine, using ^{19}F CPMG-based relaxation dispersion NMR spectroscopy. ^{19}F CPMG experiments reveal that CaM undergoes two-site exchange, on a timescale of 3000 Hz, and also hint at the possibility of both on- and off-pathway folding/unfolding events.

P20. Capturing the Guest Dynamics in Metal-Organic Frameworks CPO-27-M by 2H Solid-State NMR

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Western University

Metal-organic framework (MOF) is a novel class of porous material, which has been shown to be suitable for a broad range of applications such as sensors with selective recognition. CPO-27-M MOFs

(M = Mg, Zn, Co, Ni, also known as M-MOF-74) are good candidates for this application due to the strong and site-specific interactions between open metal centers and guest molecules. Therefore, it is of fundamental importance to understand the interactions between guest molecules and the adsorption sites which are the open metal coordination sites. Unfortunately, directly probing the host-guest interactions by diffraction-based techniques such as XRD is challenging because guest molecules usually undergo rapid motions at ambient temperature. However, the information on host-guest interaction can be obtained by investigating the dynamics of the guest species with ^{2}H solid-state NMR: ^{2}H NMR lineshape of the guest molecule is very sensitive to the interaction with metal center. Simulation of the ^{2}H NMR lineshape can unravel the nature of the guest-metal interaction. In this work, we reported ^{2}H solid-state NMR studies of several typical guest molecules, including D_2O, acetone-d_6, CD_3CN, and C_6D_6, adsorbed on CPO-27-M. A clear trend of change of host-guest interaction has been observed by comparing the thermal motions of the same guest adsorbed on different metal centers and different guests on the same metal center, which provides valuable insights into how the guest molecule interacts with metal center.

P21. Investigating the Dynamic Behavior of CO_2 in Isostructural Metal-Organic Frameworks CPO-27-M

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Metal-organic frameworks (MOFs) are synthesized via self-assembly of metal ions with organic linkers to form three-dimensional networks. They offer reversible carbon dioxide adsorption and are promising materials for the selective capture of CO_2 from the atmosphere and flue gas. Among them, CPO-27-M (M = Mg and Zn) has an excellent capacity. Its framework is based on the interconnecting helical chains of edge-shared MO6 units coordinated by carboxylate, phenoxide and one water molecule, forming one-dimensional large honeycomb channels. Further mild dehydration to remove the water molecule can form the open metal sites. Investigating the dynamical behavior of adsorbed CO_2 will not only help to understand the mechanism of adsorption and desorption of CO_2, but also provide guidance for comparison and design of more efficient MOFs. Solid- state NMR is sensitive to local geometries and mobility of guest molecules. We use ^{13}C SSNMR to study the dynamical behavior of adsorbed $^{13}CO_2$ in CPO-27-Mg and CPO-27-Zn at a temperature range from 150 K to 403 K, and then compare the differences of adsorption dynamics. A stronger adsorption capacity on CPO-27-Mg is clearly shown from the breadth and trend of CSA patterns of adsorbed $^{13}CO_2$.

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P22. Solution NMR Structure of the eIF3g Zn-Binding Domain

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When we need proteins translated from mRNA, eukaryotic translation initiation factors (eiFs) interact with the ribosome, tRNAs, and other proteins, to bring together the necessary machinery for protein production. eIF3, the largest of these factors, is a 13-protein complex (~800 kD) which controls binding of a Met-tRNA complex to the 40S ribosomal subunit; eIF subunit 'g' is a 36 kD protein containing a Zn-binding domain and an RNA recognition motif. We expressed ^{13}C, ^{15}N-labelled protein corresponding to the Zn-binding domain of eIF3g, and determined its structure using solution state

NMR. This work will present the structure of the eIF3g Zn-binding domain, and how distance restraints (NOESY) and dihedral restraints (HNHA, HNHB) contributed to the structure refinement. A comparison to similar Zn-binding domains will also be presented.

P23. Allosteric Regulation of the Cardiac Protein SUR2A NBD1 by Phosphorylation
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ATP-sensitive potassium channels (KATP) channels are heteromultimeric complexes that are found in metabolically active tissues, including the heart, pancreas and smooth muscle. KATP channels couple the metabolic state of the cell to the membrane potential and therefore have critical roles in many biological processes. These channels are comprised of four copies of a pore forming subunit (Kir) that are surrounded by four copies of the sulfonylurea receptor (SUR). SUR proteins are members of the ABC transporter superfamily of proteins and contain the minimum ABC structure comprised of two membrane spanning domains and two nucleotide binding domains (NBDs). Gating of KATP channels is a complex process that involves multiple ligands and protein domains. ATP binding at the Kir subunit closes the pore, whereas ATP binding and hydrolysis at the SUR NBDs results in channel opening. Further, phosphorylation at any one of three sites in the SUR NBDs increases the open probability of the channel, with multiple phosphorylation events resulting in increased opening. We have investigated the structural, dynamic, and functional effects of phosphorylation on NBD1 of the cardiac-specific SUR protein, SUR2A. Studies from NMR spectroscopy indicate that phosphorylation occurs on an N-terminal disordered region of NBD1. Comparison of NMR spectra of non-phosphorylated and phosphorylated NBD1 with NBD1 lacking the N-terminal region indicate that phosphorylation disrupts interactions of the N-terminal phospho-regulatory region with the core of NBD1. Fluorescence ATP-binding and thermal stability studies suggest that phosphorylation or the removal of the N-terminal disordered region also increase the affinity for nucleotide. These data suggest that phosphorylation may regulate KATP channel gating by allosterically modulating the interaction of the phospho-regulatory region with the NBD1 core, thereby altering the equilibrium between active and inactive conformations.

P24. J-coupling in Various Selenium-Iodine Halogen Bonded Environments
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Halogen bonding (XB) is a non-covalent interaction, analogous to hydrogen bonding, between a halogen and a suitable electron donor (i.e., Lewis base or π electrons). This occurs due to the electrostatic interaction between the electron donor and an area of partial positive charge referred to as the sigma hole on the halogen atom [1]. XB is of particular interest due to its directionality and strength, with values between 5 and 180 kcal/mol [2]. In this study, solid-state NMR (SSNMR) spectroscopy and single-crystal X-ray diffraction (SCXRD) experiments are used to investigate the Se•••I halogen bonding environments in triphenylphosphine selenides co-crystallized with various iodosubstituted perfluorobenzene rings. The halogen bond resulted in differences in the ^{31}P-^{77}Se J-coupling values when compared to the starting material devoid of the XB interaction. Multinuclear magnetic resonance experiments (^{77}Se , ^{13}C , and ^{31}P (I = 1/2)) using slow or fast CP/MAS were used to acquire the chemical shift tensors of the nuclei involved in XB. Also, calculations of chemical shift tensors using atomic coordinates obtained from the SCXRD experiments were performed using density functional theory (DFT) with Gaussian 09 and CASTEP software in order to corroborate experimental findings.
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P25. Effect of Low Barrier Hydrogen Bonding on the Rotational Barrier of Carboxylate Groups in Dicarboxylate Monoanions

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Low-barrier hydrogen bonds (LBHBs) in some dicaroxylate monoanions were studied by a combination of ^{17}O NMR experiments and density functional theory (DFT) computations. Hindered rotation of the carboxylate group about the C-C bond was experimentally probed by variable-temperature ^{17}O NMR. The strength of the LBHBs was evaluated by examining the rotation barriers calculated in the presence and absence of LBHBs.

P26. Assessing the role of W260 in the binding of cAMP to PKA

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Protein kinase A (PKA) is a well studied system and its binding interactions with cyclic adenosine monophosphate (cAMP) have been well-established. Tryptophan 260 of PKA has been identified as a capping residue that forms a pi-stacking interaction with cAMP after it has entered the binding pocket. Two constructs were compared using NMR spectroscopy to assess the effect of W260 on the binding of cAMP. A 15N-1H HSQC spectrum of PKA 91-260 indicates that the C terminal branch is unstructured in solution and does not interact with cAMP in the binding pocket as expected when compared to PKA 91-244. In the context of the full-length protein, W260-cAMP interactions may be important for inter-domain orientation, but may not affect the binding affinity of cAMP.

P27. Integrated Study of $^{6,7}Li_2Mn_{1-y}Fe_yP_2O_7$ (y=0, 0.2, 0.5, 0.8, and 1) as Cathode Materials in Lithium Ion Batteries

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The $^{6,7}Li_2Mn_{1-y}Fe_yP_2O_7$ ($0 \le y \le 1$) pyrophosphates were synthesized and verified by powder x-ray diffraction (PXRD). Li-7 batteries with y=0.2, 0.8, and 1 as cathodes were characterized by galvanostatic cycling. In agreement with Wittingham[1] capacity increases with increasing iron content. Li-6 magic angle spinning (MAS) nuclear magnetic resonance (NMR) spectra of y=0, 0.2, and 0.8 demonstrate a decrease in resolution with increasing iron content.
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P28. Analysis of Electrolytes for Lithium Ion Batteries Using Solid State NMR Methods

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The development of novel electrolyte materials for lithium ion batteries has been the focus of much research in recent years.[1] Solid state electrolytes offer stability against attack by electrodes, and promise to sustain a higher operating voltage window, allowing the design of batteries with higher power output.[2]

Solid state NMR provides a unique and powerful tool to examine the mobility of lithium ions in these highly conductive electrolyte materials.[3] With the ability to focus on the mobile species alone, this tool facilitates the study of ionic hopping in a microscopic setting, avoiding grain boundary effects.

The solid state electrolyte $Li_6BaLa_2Ta_2O_{12}$ has a garnet-like structure, and is known to have one of the highest ionic conductivities of this class of materials, at 4×10^{-5} S/cm.[4] Previous studies focusing on conductivity and reactivity have been performed using bulk conductivity measurements, and powder X-ray diffraction.[5] In this work, these materials have been studied using solid state NMR techniques to assess ionic motion as a function of temperature, independent of grain boundary resistance.

Rotational Echo Double Resonance (REDOR) NMR is a heteronuclear technique capable of measuring the dipolar coupling between a pair of nuclei. This experiment is traditionally used to determine distances between an isolated pair of nuclei.[6] However, this technique can also been used to study ion mobility in materials, since dipolar coupling changes as a function of ion exchange.[7] We have utilized the fact that lithium has two NMR active isotopes, ^{6}Li and ^{7}Li, which are distributed throughout the crystallographic Li sites in lithium electrolyte materials. By measuring the changes in the dipolar coupling as a function of temperature we can assess the lithium dynamics in these materials.

Within this study we have used both ^{6}Li{^{7}Li}-REDOR and ^{7}Li{^{6}Li}-REDOR to study temperature dependent ionic motion in solid state electrolyte materials. Here the experiment produces a buildup curve, with a slope that is dependent on the dipolar coupling between ^{6}Li and ^{7}Li. Examination of this slope as a function of temperature allows us to study the temperature dependent dynamics in lithium ion conductors.

A comparison has been made between the electrolytes: $Li_6BaLa_2Ta_2O_{12}$, $Li_6BaLa_2Nb_2O_{12}$, and $Li_{0.5}La_{0.5}TiO_3$ to study the variation of ion mobility as a function of temperature. We have found that although similar ionic conductivities and activation energies have been reported for these materials in bulk conductivity measurements, the response of the NMR experiments to changes in temperature indicates different activation energy for lithium ion hopping in these materials. The deviation from previous studies is due to the effect of grain boundary resistance, and other bulk effects, which dominate the ionic conductivity.

This study highlights the overwhelming effect of bulk contributions. Moreover, these results point to the necessity to further understand the significance of bulk contributions, and cater material preparation to minimize these effects.

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P29. Carbohydrate recognition by a family 31 glycoside hydrolase from *Clostridium perfringens*
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Clostridium perfringens is a Gram-positive, anaerobic commensal member of the human gut microbiome, which secretes a battery of large multi-modular CAZymes that contribute to the natural turnover of mucosal layer of the gut by degrading associated complex glycans. *C. perfringens* is also an opportunistic pathogen and several of the secreted CAZymes have been identified as virulence factors. Among the most complex of these is a putative α-glucosidase from the GH31 superfamily, which contains a large number of non-catalytic ancillary modules, including three CBM32 modules that to date have not been characterized. Here, we present the crystal structures of *Cp*GH31 CBM32-1 and CBM32-3 in their apo forms, as well as the crystal structure of CBM32-3 in complex with galactose. NMR-based experiments were used to show that *Cp*GH31 CBM32-1 and CBM32-2 display a preference toward *N*-acetylgalactosamine. Together, these results heighten our understanding of the ligand-targeting specificities of *Cp*GH31and provide a framework for additional studies on coordinated sugar binding by this enzyme.

P30. Recognition of Activation Domain 1 from the Oncoprotein E2A-PBX1 by the Taz protein modules from the translation co-activation complex CBP/p300
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E2A is a transcription factor from the E-protein family that regulates B-lymphocyte development through interactions involving its activation domains 1 (AD1) and 2 (AD2) with CBP/p300. A chromosomal translocation t(1:19) creates an E2A variant, an oncoprotein termed E2A-PBX1 which comprises $E2A_{1-483}$ and most of the PBX1 transcription factor protein including its C-terminal DNA binding domain. E2A-PBX1 is responsible for the onset of pre-B-cell stage acute lymphoblastic leukemia (ALL) through the transcriptional activation of PBX-1 regulated genes via the recruitment of CBP/p300 by $E2A_{1-483}$. Previous work from our group determined the CBP-E2A interaction primarily requires the AD1 domain of E2A and the protein-protein interaction domain, KIX from CBP. However given the complex interplay of how other transcription factors associate with CBP such as the oncoprotein p53, we expanded our search to explore the role of CBP protein modules, Taz1 and Taz2, in E2A recognition. To this end, we pursued isothermal titration calorimetric (iTC) studies and high-resolution solution nuclear magnetic resonance (NMR) studies of Taz1 and Taz2 with $AD1_{1-37}$ from E2A. Dissociation constants of 50 μM and 6 μM were measured by iTC for the Taz1- and Taz2-$AD1_{1-37}$, respectively. This information guided our solution NMR studies, and enabled us to determine the high-resolution structure of the Taz2-$AD1_{1-37}$ complex (RMSD of 0.39 ± 0.04 Å) using NOE and Zn^{2+} coordination derived distance restraints. $AD1_{1-37}$-bound Taz2 comprises 4 distinct α-helices (α1: Gly1728-Gln1747, α2: Pro1756-Lys1769, α3: Pro1780-Lys1794, α4: Pro1804-Lys1810) with three conserved HCCC-type zinc (Zn^{2+}) coordination sites (Site1: His1744, Cys1746, Cys1753 and Cys1758; Site2: His1767, Cys1771, Cys1779 and Cys1782; Site3: His1792, Cys1796, Cys1810 and Cys1806). The $AD1_{1-37}$ peptide binds to the surface created by helices 1-3 of Taz2. The region comprising Thr12-

Met25 of $AD1_{1-37}$ is α-helical, which includes the hydrophobic LXXLL motif at the Taz2-$AD1_{1-37}$ interface. The structural disposition of amino acid residues within this homologous region is also common to the Taz2-p53 and KIX-AD1 complex structures, which highlights the complexity of E2A recognition by CBP due to the interplay between the Taz modules and KIX to regulate B-lymphocyte development.

P31. Québec/Eastern Canada High Field NMR Facility. Le Centre de RMN à Haut Champ du Québec et de l'Est du Canada

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The Québec/Eastern Canada High Field NMR Facility, a solution state NMR facility located at McGill University, in Montreal, Québec, will be presented. The centre provides academic, government and industrial researchers with access to high field NMR spectroscopy at 800 MHz and 500 MHz. Both NMR spectrometers are equipped with cryogenically cooled HCN probes, providing very high sensitivity. An overview of facility operation will be provided, along with highlights of recent user projects, demonstrating the range of experiments available to researchers.

P32. Synthesis and NMR Characterization of Myoglobin-HNO Adducts

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Nitroxyl, HNO, is known to mediate and regulate a variety of biological processes through its reactivity with various proteins. HNO has been used in pharmacology and therapeutic applications in treating alcoholism, heart failure, vasodilation platelet aggregation as well as cancer. As a result, it is important to obtain insights into the binding between HNO and its targets. Herein, we report synthesis and NMR characterization of adducts between myoglobin (Mb) and ^{17}O- and ^{15}N-labeled HNO, aiming to understand the structure and chemical bonding in this important system. This study will facilitate the establishment of an effective model for studying the ligand-protein interactions in the future.