Saturday, October 24th

8:00-9:00 **Registration and Continental Breakfast**
(John Hodgins Engineering Building, Lobby)

Morning Session I
Chair: Giuseppe Melacini
(John Hodgins Engineering Building, 2nd Floor, Rm. 264)

9:00-9:15 Opening Remarks

9:15-10:00 Plenary Lecture: Rams Ramamoorthy

10:00-10:20 Proton Detection for Signal Enhancement in Solid-State NMR Experiments on Mobile Species in Membrane Proteins
Meaghan E. Ward, Emily Ritz, Mumdooh A. M. Ahmed, Vladimir V. Bamm, George Harauz, Leonid S. Brown, Vladimir Ladizhansky
University of Guelph

10:20-10:40 Molecular Organization of Lipid-Bound and Amyloid Fibrils of Human Serum Amyloid A by Solid-State NMR
Jason Yau, Karen Simonetti, Sympascho Young, Quanh Huyhn, Simon Sharpe
Hospital for Sick Children

10:40-10:55 **Coffee Break**
(John Hodgins Engineering Building, Lobby)

10:55-11:15 Probing Modularity and Transitions of Spider Wrapping Silk Conformation by NMR
Muzaddid Sarker, Marie-Laurence Tremblay, Kathleen E. Orrell, Lingling Xu, Xiang-Qin Liu and Jan K. Rainey
Dalhousie University

11:15-11:35 Monitoring the Formation of a Zeolitic Imidazolate Framework Using ^{111}Cd Solid-State NMR
Christopher O'Keefe, C. Mottillo, T. Friščić and R. W. Schurko
University of Windsor

11:35-11:55 Detection of Sodium-Oxygen Battery Discharge Products with Solid-State NMR
Zoë E. M. Reeve, Kristopher J. Harris, Christopher J. Franko, Hossein Yadegari, Victor Terskikh, Xueliang Sun, and Gillian R. Goward
McMaster University

11:55-12:15 Understanding Guest Gas Dynamics Within Metal-Organic Frameworks
Bryan E.G. Lucier, Yue Zhang, Shoushun Chen, Yuanjun Lu, Hendrick Chan, Yining Huang
University of Western Ontario

12:30-14:00 Lunch at The Phoenix

Afternoon Session
Chair: Gillian Goward
(John Hodgins Engineering Building, 2nd Floor, Rm. 264)

14:00-14:20 ^{31}P CODEX NMR With Powder-Average Modelling for Measuring Lateral Diffusion in Multiplexed Lipid Bilayers
Angel Lai and Peter M. Macdonald
University of Toronto

14:20-14:40 The Effect of Unsaturated Lipids and Cholesterol on Bicelles: Liquid Disordered - Liquid Ordered Phase Coexistence
Miranda L. Schmidt and James H. Davis
University of Guelph

14:40-15:00 Characterization of Ln^{3+}-doped Nanoparticles Using Solid-State NMR
David A. Hirsh, B. Richard, B.E.G. Lucier, A.M. Ritcey, and R. W. Schurko
University of Windsor

15:00-15:15 Break

15:15-15:35 Solids NMR Characterization of Nanoparticles with Mixed Ligand Shells
Safiya Allie and **Linda Reven**
McGill University

15:35-15:55 Dynamic Nuclear Polarization Solid-State NMR of Membrane Proteins with Covalently Attached Cysteine-Specific Biradicals
Maxim A. Voinov, **Daryl B. Good**, Meaghan E. Ward, Sergey Milikisiyants, Marc Caporini, Melanie Rosay, Rachel A. Munro, Milena Ljumovic, Leonid S. Brown, Vladimir Ladizhansky, Alex I. Smirnov
University of Guelph

15:55-16:15 Investigating the Halogen Bond Donor by Covalent ^{35}Cl Solid-State NMR
Patrick M.J. Szell and David L. Bryce
University of Ottawa

16:30-18:30 Poster Session (University Club)
18:30-22:00 Banquet at University Club

Sunday, October 25th

8:00-9:00 **Coffee and Continental Breakfast** *(JHE Lobby)*

Morning Session II
Chair: Alex Bain
(John Hodgins Engineering Building, 2nd Floor, Rm. 264)

9:00-9:45 Plenary Lecture: Mike Noseworthy

9:45-10:05 Inhibition & Activation of Parkin
Jacob Aguirre, Condos T.E.C., Mercier, P., and Shaw, G.S.
University of Western Ontario

10:05-10:25 Intra-Ligand Allostery Induces Ligand Selectivity in the cAMP-Binding Domain of HCN
Stephen Boulton, Bryan VanSchouwen, and Giuseppe Melacini
McMaster University

10:25-10:45 Design and Characterization of a Disruptor Peptide to the E2A-PBX1:CBP/p300 Complex
David N. Langelaan, Marina R. Lochhead, David P. LeBrun and Steven P. Smith
Queen's University

10:45-11:00 **Coffee Break**
(John Hodgins Engineering Building, Lobby)

11:00-11:20 Structural Elucidation of Novel Antifungal Natural Products Isolated from an Endophyte Fungus in Raspberry Leaves
Kevin M. N. Burgess, Ashraf Ibrahim, Dan Sørensen and Mark W. Sumarah
NSERC Visiting Fellow

11:20-11:40 Congenital Hyperinsulinism-causing Mutations Cause Misfolding and Change Molecular Interactions in SUR1 NBD1
Claudia Alvarez, Marijana Stagljar, and Voula Kanelis
University of Toronto

11:40-12:00 Role of Dynamics in the Auto-Inhibition and Activation of the Hyperpolarization-Activated Cyclic-Nucleotide-Modulated (HCN) Ion Channels
Bryan VanSchouwen, Madoka Akimoto, Maryam Sayadi, Federico Fogolari and Giuseppe Melacini
McMaster University

12:00-12:10 **Poster Award Announcement and Closing Remarks**

Abstracts: Oral Presentations

O1: Proton detection for signal enhancement in solid-state NMR experiments on mobile species in membrane proteins

Meaghan E. Ward, Emily Ritz, Mumdooh A. M. Ahmed, Vladimir V. Bamm, George Harauz, Leonid S. Brown, Vladimir Ladizhansky
University of Guelph, Guelph, ON

The study of membrane proteins by solid-state nuclear magnetic resonance (ssNMR) spectroscopy is highly limited by the low sensitivity of these experiments. A popular method for increasing sensitivity is to detect protons directly, though these experiments generally require extensive deuteration of the protein, fast magic angle spinning, or a combination of both. Here we implement direct proton detection on a fully protonated sample to detect mobile entities selectively in membrane proteins. Through the use of insensitive nuclear enhancement of polarization transfer (INEPT) highly mobile residues with intrinsically narrow linewidths are selectively excited. We demonstrate these experiments on two proteins that exhibit different motional regimes. Myelin basic protein (MBP) is an intrinsically-disordered, peripherally membrane associated protein that is highly flexible while Anabaena sensory rhodopsin (ASR) is composed of seven rigid transmembrane α-helices connected by mobile loop regions. In both cases narrow spectral linewidths are maintained under direct proton detection and we see, on average, a 10 x increase in sensitivity in the proton detected 2D HSQC experiment when compared to carbon detection. We further show that these experiments can be easily extended to three dimensions and used to build complete amino acid systems, including side chain proton assignments which are not readily accessible in extensively deuterated samples and to obtain inter-residue correlations. Furthermore, we also detect additional systems which do not correspond to amino acids, but rather to lipids, sugar moieties, and/or carbohydrates which interact strongly with the protein.

O2: Molecular organization of lipid-bound and amyloid fibrils of human serum amyloid A by solid-state NMR

Jason Yau, Karen Simonetti, Sympascho Young, Quanh Huyhn, Simon Sharpe
Hospital for Sick Children, Toronto, ON

Serum amyloid A (SAA) is an acute-phase apolipoprotein evolved as part of the mammalian defense response to tissue injury, infection, and inflammation. It is primarily synthesized in the liver, and is known to associate to high-density lipoproteins (HDLs). During acute inflammation, SAA production is up-regulated 1000-fold to nearly mg/mL concentrations in the serum, and can replace apolipoprotein AI as the major apolipoprotein on HDLs. Thus the function of SAA has been postulated to play a role in cholesterol trafficking and uptake during inflammation. SAA have also been isolated in deposits of amyloid A (AA) amyloidosis – a systemic amyloidosis that occurs during chronic inflammation such as rheumatoid arthritis. Human SAA crystal structure showed the 104-residue protein adopting a 4-helix bundle oligomerized as a hexamer in solution (lipid-free). However, there are currently no models for SAA when it is associated to HDLs and in the amyloid state. Using

solid-state NMR (SSNMR), we are the first to study the structure of human SAA in its lipid- and amyloid-associated forms. Here, SAA remained predominantly α-helical when it was lipid-bound, but it underwent complete structural rearrangement into β-rich amyloids. Chemical shift assignments further suggested the C-terminal 30 residues of SAA, while disordered in lipid-free and in amyloid states, adopted an ordered structure upon lipid association. Thermal stability and Thioflavin T fibrillization kinetics of SAA were also different upon lipid association, suggesting difference in the molecular structure of lipid-bound and lipid-free SAA. Future studies are underway to elicit a high-resolution structure of both HDL- and amyloid SAA. This will help us understand the function of SAA in serum, and the molecular details of SAA conversion into the amyloid state in AA amyloidosis.

O3: Probing modularity and transitions of spider wrapping silk conformation by NMR

Muzaddid Sarker, Marie-Laurence Tremblay, Kathleen E. Orrell, Lingling Xu, Xiang-Qin Liu & Jan K. Rainey

Department of Biochemistry & Molecular Biology, Dalhousie University, Halifax, NS

Spider silk fibers are marvelous biomaterials surpassing their synthetic counterparts in mechanical properties. The wrapping silk, composed of the protein aciniform spidroin 1 (AcSp1), is the toughest of the spider silks. AcSp1 contains at least 14 repeats of a 200 amino acid core domain (W), flanked by a short C-terminal domain and likely a short N-terminal domain. We previously reported a strikingly compact, modular structure of the repeat unit W1 both in isolation and in the context of a paired two-unit W2 using solution NMR. Although W proteins in solution spontaneously self-assemble to form nanoparticles, stable monomeric solutions can also be formed – the longevity of the monomer appears to be inversely related to the size of the protein construct. Manual pulling of fibers from "dope" solutions containing W2, W3 or W4 is possible, with mechanical properties and morphology akin to the native form. We have determined translational diffusion coefficients using DOSY NMR and correlated the conformations of the W1, W2 and W3 proteins to their hydrodynamic behavior. Each multi-unit protein retains the compact globular structure of the constituent W subunits with no major inter-subunit interactions needed to explain the hydrodynamic properties of the larger constructs. A compactly folded "beads-on-a-string" architecture of W2 and W3, incorporating radius of gyration restraints derived from observed diffusion coefficients, perfectly matches the hydrodynamic measurements. To further our understanding of the fiber formation mechanism, which involves a major structural transition from an α-helix-rich state to a mixed α-helix/β-sheet state, we have also examined the changes in W1 conformation and dynamics under denaturing conditions. Unfortunately, the traditional mapping of structural transition employing heteronuclear ^{1}H, ^{13}C and/or ^{15}N correlation NMR experiments was proven to be of limited use due to spectral overlap exacerbated by loss of chemical shift dispersion during W1 denaturation. The ability of 19F-NMR to track these changes at the atomic-level was therefore tested. Four positions in W1, with different levels of solvent exposure, were mutated to tryptophan (R36W, F90W, F146W, Y169W) and 5-fluorotryptophan was incorporated at each site. The ^{19}F chemical shift, longitudinal relaxation time constant (T_1), and solvent isotope shift (SIS) reflective of local structural feature and level of exposure are investigated. Changes in local conformation and dynamics are reflected by the ^{19}F chemical shift and T1 perturbation upon titration with urea and dodecylphosphocholine (DPC). Taken together, these data demonstrate that each W domain behaves as an independent entity within a protein concatemer irrespective of its relative order.

This leads to the hypothesis that fiber formation by AcSp1 is instigated by intermolecular interactions between neighboring proteins directly facilitated by acinofrm silk's remarkable beads-on-a-string architecture.

O4: Monitoring the Formation of a Zeolitic Imidazolate Framework Using 111Cd Solid-State NMR

C. A. O'Keefe, C. Mottillo, T. Friščić and R. W. Schurko
University of Windsor, Windsor, ON

Mechanochemistry (the use of mechanical forces to provide the activation energy for a reaction) and accelerated aging (generating hybrid metal-organic materials under conditions of high humidity and slight heating) are two synthetic approaches that are consistent with the philosophy of green chemistry, as they use little to no solvent and non-toxic starting materials, and afford quantitative yields. Recently, these approaches have been applied to the synthesis of zeolitic imidazolate frameworks (ZIFs), which are a class of hybrid metal-organic compounds that have recently garnered great interest due to their uses in catalysis and gas storage. The mechanisms and factors affecting ZIF synthesis are largely unknown and are likely very different from their solvothermal analogues. Recent mechanistic studies utilized both in- and ex-situ X-ray diffraction (XRD) experiments to identify products and intermediate phases with known structures; however, the identification of short-lived intermediate phases in low concentrations was not possible as their signals are obscured by those of the starting materials. Herein, we describe the use of multinuclear SSNMR (^{111}Cd, ^{1}H, ^{13}C and ^{14}N) to characterize a series of cadmium-containing ZIFs with known structures. This information will then be used to elucidate the structure of a ZIF that can be isolated from both mechanochemical and accelerated aging reactions. Mechanochemical and accelerated aging reactions forming a cadmium-containing ZIF are then monitored ex situ using ^{111}Cd CP/MAS SSNMR. Using this technique, it is possible to observe signals corresponding to intermediates and products of the reactions, providing molecular-level structural information which could potentially be used to elucidate the mechanism of ZIF formation.

O5: Detection of Sodium-Oxygen Battery Discharge Products with Solid-State NMR

Zoë E. M. Reeve[1], Kristopher J. Harris[1], Christopher J. Franko[1], Hossein Yadegari[2], Victor Terskikh[3] Xueliang Sun[2] and Gillian R. Goward[1]*
[1]*Department of Chemistry and Chemical Biology, McMaster University, Hamilton, ON, Canada L8S 4M1*
[2]*Department of Mechanical and Materials Engineering, University of Western Ontario, London, Ontario N6A 5B9*
[3]*National Ultrahigh-Field NMR Facility for Solid, 1200 Montreal Road, Ottawa, ON, K1A 0R6*

^{23}Na NMR is shown to be a diagnostic tool to identify the reaction products formed within the sodium–oxygen (Na-O_2) battery. The Na-O_2 battery is an inexpensive, high energy density storage device; comprised of an air electrode, an electrolyte and a Na metal anode.[1] At the cathode during discharge, molecular oxygen is reduced to the superoxide radical, which further reacts producing Na_2O_2¬, and degrades the electrolyte. [1-3] The desired product Na_2O_2 has a unique ^{23}Na NMR signature from the main electrolyte decomposition species (Na_2CO_3) allowing Na_2O_2 to be clearly identified. With multiple quantum magic angle spinning (MQMAS)[4-5] the Na_2O_2 quadrupole lineshape is separated from the Na_2CO_3

lineshape in a mixture. ^{23}Na NMR of cycled cathodes is expected to provide valuable insight into the Na-O_2 battery chemistry.

[1] Q. Sun, Y. Yang, Z.-W. Fu, Electrochem. Commun. 2012, 16, 22.
[2] P. Hartmann, C. L. Bender, M. Vračar, A. K. Dürr, A. Garsuch, J. Janek, P. Adelhelm, Nat. Mater. 2013, 12, 228.
[3] H. Yadegari, Y. Li, M. N. Banis, X. Li, B. Wang, Q. Sun, R. Li, T.-K. Sham, X. Cui, X. Sun, Energy Eviron. Sci. 2014, 7, 3747.
[4] A. Medek, J. S. Harwood, L. Frydman, J. Am. Chem. Soc. 1995, 117, 12779.
[5] L. Frydman, J. S. Harwood, J. Am. Chem. Soc. 1995, 117, 5367.

O6: Understanding Guest Gas Dynamics Within Metal-Organic Frameworks

Bryan E.G. Lucier, Yue Zhang, Shoushun Chen, Yuanjun Lu, Hendrick Chan, Yining Huang
University of Western Ontario, London, ON

Microporous metal-organic frameworks (MOFs) have high surface areas and porosities, and are well-suited for gas capture. We have recently been studying the behaviour of gases adsorbed in MOFs via variable-temperature solid-state NMR (SSNMR) spectroscopy, which provides rich information on the dynamic motion of guest molecules as well as their binding strengths to the MOF host, and sheds light on the specific guest adsorption mechanisms. Combining the dynamic information available from SSNMR with complementary guest location data from X-ray/neutron diffraction or computational methods allows formulation of comprehensive guest motional models. This data is of particular importance for the design of MOFs featuring higher guest storage capacities and tunable adsorption strengths to address specific applications.

In this presentation, we will focus on our recent comprehensive study of CO_2 adsorption in the flexible MIL-53 MOF, demonstrating how ^{13}C SSNMR experiments reveal the type of CO_2 motions present, their rates, and rotational angles. ^{1}H-^{13}C CP SSNMR experiments, along with selective ^{2}H deuteration of ^{1}H sites, is used to examine the CO_2 adsorption site locations in MIL-53; a detailed motional model of CO_2 motion in MIL-53 is then proposed. We will also describe several of our other recent SSNMR studies regarding adsorption and dynamics of various gases with practical implications within MOFs such as UiO-66, MOF-74, and $Mg_3(COOH)_6$.

O7: ^{31}P CODEX NMR With Powder-Average Modelling for Measuring Lateral Diffusion in Multiplexed Lipid Bilayers

Angel Lai and Peter M. Macdonald
University of Toronto, Toronto, ON

Lateral diffusion of phospholipids is a process essential to membrane function, and its accurate determination can provide insights into kinetics of membrane-associated biochemical reactions. Here we describe the application of CODEX (Centerband-Only Detection of Exchange)[1] to measure lateral diffusion of phospholipids in lipid bilayers assembled into large unilamellar vesicles (LUV)[2]. CODEX is an ideal experiment for these systems because ^{31}P NMR can be measured in natural abundance, eliminating the need for synthetic labels. The ^{31}P CODEX spectrum for LUV composed of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) consists of a narrow resonance in both the liquid crystalline and gel

phases. With increasing mixing times, the resonance exhibits an exponential decay from which the correlation time for lateral diffusion can be extracted, provided the LUV size and size-distribution has been established, for example using dynamic light scattering. We also employ a simulation model based on the powder-average distribution of lipids on a sphere to obtain the lateral diffusion coefficient from experimental data. Lateral diffusion coefficients determined in this fashion agree with established literature values. Another advantage of ^{31}P CODEX is the ability to multiplex; since different phospholipid headgroups appear as separate resonances in an NMR spectrum, their individual decays can be monitored. Lateral diffusion coefficients have been measured in the gel phase, which would prove useful in studying membrane heterogeneities, such as those induced thermotropically, by various membrane-associating proteins or in lipid rafts.

(1) deAzevedo, E. R.; Hu, W.-G.; Bonagamba, T. J.; Schmidt-Rohr, K. Centerband-Only Detection of Exchange: Efficient Analysis of Dynamics in Solids by NMR. J. Am. Chem. Soc. 1999, 121, 8411–8412.
(2) Saleem, Q.; Lai, A.; Morales, H. H.; Macdonald, P. M. Lateral diffusion of bilayer lipids measured via 31P CODEX NMR. Chem. Phys. Lipids 2012, 165, 721–730.

O8: The Effect of Unsaturated Lipids and Cholesterol on Bicelles: Liquid Disordered - Liquid Ordered Phase Coexistence

Miranda L. Schmidt and James H. Davis
University of Guelph, Guelph, ON

Bicelles are magnetically oriented lipid mixtures typically made of long chain and short chain phospholipids in buffer and can provide a useful medium in which to study membrane bound peptides/proteins. 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dicaproyl-sn-glycero-3-phosphocholine (DCPC) are used as the bicelle mixture to which cholesterol and an unsaturated phospholipid, 1,2-dipalmitoleoyl-sn-glycero-3-phosphocholine (DPoPC), were added. The phase behaviour of DMPC/cholesterol/DCPC bicelles and DPoPC/DMPC/cholesterol/DCPC was investigated as a function of temperature using solid state deuterium NMR spectroscopy which is sensitive to the molecular motion and orientational molecular order of the sample. Cholesterol has an ordering effect on the long phospholipid chains and this is evident in the phase behaviour of the DMPC/cholesterol/DCPC bicelle mixtures. Liquid disordered - liquid ordered fluid phase coexistence was observed in DMPC/cholesterol/DCPC bicelles with cholesterol mole fractions of 0.13 and higher. DPoPC/DMPC/cholesterol mixtures exhibit two fluid phase coexistence over a broad range of temperatures and compositions analogous to DOPC/DPPC/cholesterol mixtures. Bicelles made with DPoPC/DMPC/cholesterol/DCPC align in the magnetic field of the spectrometer and also show liquid disordered - liquid ordered phase coexistence.

O9: Characterization of Ln^{3+}-doped Nanoparticles Using Solid-State NMR

D.A. Hirsh, B. Richard, B.E.G. Lucier, A.M. Ritcey, and R. W. Schurko
University of Windsor, Windsor, ON

Inorganic nanoparticles (NPs) containing lanthanide(III)-dopants have desirable optical properties (e.g., long luminescence lifetimes/slow emission rates) that make them

ideal for use in LEDs, lasers, and imaging. As these properties are highly sensitive to the local structure around the dopant ions, characterization of such NPs on a molecular level is vital to improve their rational design and preparation. Recently, we have used a combination of pXRD and SSNMR to identify crystalline NPs with the formula $(H_3O^+)Y_3F_{10}\cdot xH_2O$.[1] Surprisingly, these NPs adopt a zeolitic structure that is dramatically different from bulk YF_3 materials. These yttrium-containing NPs are a natural target for the incorporation of Ln^{3+} dopants, due to the similarity of the atomic radii of Y^{3+} and Ln^{3+} ions. Herein, we expand on our prior work with (H3O+)Y3F10•xH2O NPs to focus on similar particles that have been doped with diamagnetic (Sc^{3+}, Eu^{3+}) and paramagnetic (Er^{3+}) rare-earth elements. We present a structural characterization of these particles using multinuclear (^{89}Y, ^{45}Sc, ^{19}F, ^{1}H) SSNMR. These experiments reveal the core NP structures (and their similarities/differences with the undoped materials) as well as the dopant positions and uniformity within the NP. We also discuss the effect of dopant concentration on the NP structure. Our findings will aid in the design and synthesis of advanced rare-earth NPs with fine-tuned optical properties.

[1] Lucier, B. E. G., et al, J. Phys. Chem. C 2014, 118, 1213.

O10: Solids NMR Characterization of Nanoparticles with Mixed Ligand Shells

Safiya Allie and Linda Reven
McGill University, Montréal, QC

A model system consisting of metal oxide nanoparticles stabilized with aromatic and aliphatic ligands was studied by a combination of solids NMR experiments commonly used to detect phase separation in polymers. The goal was to determine whether solids NMR can be used to characterize the spatial distribution of the two ligands on the nanoparticle surface. Proton double quantum and HETCOR experiments were used to detect short range proximities of the two ligands. Longer length scales were probed by proton spin diffusion experiments. The domain sizes of the two components were estimated as the ratio of the two ligands was varied.

O11: Dynamic nuclear polarization solid-state NMR of membrane proteins with covalently attached cysteine-specific biradicals

Maxim A. Voinov, Daryl B. Good, Meaghan E. Ward, Sergey Milikisiyants, Marc Caporini, Melanie Rosay, Rachel A. Munro, Milena Ljumovic, Leonid S. Brown, Vladimir Ladizhansky, Alex I. Smirnov
University of Guelph, Guelph, ON

In the past 10 years Dynamic Nuclear Polarization (DNP) has become a powerful technique for enhancing the signal in Magic Angle Spinning (MAS) solid-state NMR experiments. By applying microwave irradiation (MW) at temperatures of approximately 100K the magnetic polarization is transferred from the highly polarized electronic spins to the nuclear spins of interest (e.g., ^{1}H nuclei) on the target molecule. Typically, both the molecule of interest and a molecule containing electronic spins such as a biradical are frozen in a glycerol/water matrix to achieve a homogeneous distribution of the biradical in the sample.

Recently, new approaches for obtaining DNP-enhanced NMR spectra of proteins without a glycerol/water matrix, which rely instead on the high indirect affinity between the radical and the protein, have been described and tested. Here we present an alternative matrix-free approach to DNP where we synthesis a biradical which can be directly covalently

attached to the protein through a disulfide bond. This new biradical ToSMTSL (Totapol Series MethaneThiosulfonate Spin Label) was synthesized by attaching a thiol-specific methanethiosulfonate group (-SSO2CH3) to a biradical that is based on a known DNP polarizing agent TOTAPOL.

We demonstrate the utility of the new biradical for DNP by specifically attaching it to a solvent exposed cysteine residue N148C on the ^{15}N-labelled protein Anabaena Sensory Rhodopsin(ASR). DNP experiments using a 400 MHz/263 GHz Avance III Bruker DNP-NMR spectrometer demonstrated a DNP enhancement of 15 (MW on/MW off) which is comparable to that obtained in a conventionally prepared ^{15}N ASR sample with TOTAPOL suspended in a d8-glycerol/D2O/H2O matrix (ASR-TOTAPOL). Using the knowledge of the location of the biradical we determined the paramagnetic quenching to cause a ~70 % reduction of the NMR signal intensity, which was comparable to that observed in the ASR-TOTAPOL sample.

O12: Investigating the Halogen Bond Donor by Covalent ^{35}Cl Solid-State NMR

Patrick M.J. Szell, David L. Bryce
University of Ottawa, Ottawa, ON

The halogen bond is a non-covalent interaction between the electrophilic region of a halogen (sigma-hole) and a nucleophile, which has gained popularity in the field of crystal engineering due to its strength and linearity. Despite recent advancements, the halogen bond donor remains completely uncharacterized by NMR due to the unfavourable spectroscopic properties of covalently bonded halogens. With the advent of increased field strengths and WURST pulse sequences, ^{35}Cl solid-state NMR spectroscopy of covalently-bonded chlorine atoms in organic molecules has recently been successfully demonstrated. Here, we present the first ^{35}Cl solid-state NMR study of chlorine atoms as halogen bond donors, with interpretation aided by crystallographic symmetry. In a series of chlorinated benzonitrile compounds, it was observed that the quadrupolar coupling constant (CQ) consistently increases and the asymmetry parameter (η) consistently decreases upon halogen bonding. Furthermore, the ^{35}Cl NMR parameters change according to the halogen bond distance and bond angle, which provides a sensitive tool to discriminate the halogen bond from other types of chlorine close contacts. A natural localized molecular orbital (NLMO) analysis attributes the changes to a decrease in the carbon-chlorine σ-bond orbital contribution, and an increase in the lone pair character. As a result, the CQ increases upon the formation of a chlorine halogen bond. This is distinguished from a short proton-chlorine contact, which causes an increase in the σ-bond contribution, thereby decreasing the CQ.

O13: Inhibition & Activation of Parkin

Aguirre, J.D., Condos T.E.C., Mercier, P., and Shaw, G.S.
University of Western Ontario, London, ON

Mutations in the gene encoding Parkin (PARK2) cause 50% of Autosomal Recessive Juvenile Parkinsonism cases resulting in early-onset Parkinson's Disease (PD). Parkin is an E3 ubiquitin ligase proposed to ubiquitinate a range of mitochondrial protein substrates, exerting substantial influence over mitochondrial dynamics such as fission, fusion and mitophagy. Dysfunction of this pathway and altered mitochondrial integrity is widely hypothesized as an origin of neurodegeneration in PD and several other neuropathies. Parkin

exists in an autoinhibited state maintained through an interaction between its N-terminal Ubiquitin-like (UBL) domain and C-terminal RBR motif. Available structures suggest a large conformational change must occur in order for the protein to be catalytically active. Using TROSY-NMR methods and other biophysical techniques, we performed a thorough structural characterization of parkin autoinhibition. Further, we show how phosphorylation, a proposed activator of parkin, disrupts the autoinhibited structure and primes parkin for catalysis. We find the role of the UBL domain is to prevent parkin activity in the absence of phosphorylation signals, and propose a model for parkin inhibition, optimization and catalysis. Our studies provide further insight into the complex regulatory mechanisms governing activation of Parkin and other RBR E3 ubiquitin ligases.

O14: Intra-Ligand Allostery Induces Ligand Selectivity in the cAMP-Binding Domain of HCN

Stephen Boulton, Bryan VanSchouwen & Giuseppe Melacini
McMaster University, Hamilton, ON

Allosteric signaling is generally observed from the viewpoint of the protein, in which changes in structure, dynamics and/or kinetics are probed to map long-range couplings between distal protein sites. However, allostery can be observed in ligands as well. The interaction of a substituent with the protein can often influence the interactions of other ligand substituents with protein counterparts. These types of intra-ligand allosteric couplings are relevant to optimize a drug's efficiency and structure-activity-relationships. Here, as a proof of principle, we develop a model for "intra-ligand allostery" in the hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channel, which binds cAMP selectively over cGMP. For this purpose, we developed a double mutant cycle using a small library of cyclic nucleotides that simulate the progressive conversion of cAMP to cGMP. Using chemical shift analyses we probed the shift in populations within the conformational equilibrium for each ligand and characterized their orientations and interactions within the binding site through a combination of transfer NOEs and mutations. We found that the C6 carbonyl in cGMP and cIMP clashed with R710 in HCN resulting in reduced protein activation, binding affinity and a base re-orientation from anti to syn. However, in cGMP the presence of an amino group at N2 permitted an additional hydrogen bond with T670, which drastically increased its affinity compared to cIMP. Interestingly, the presence of the amino group alone was insufficient in changing the affinity of 2NH2-cPuMP as the anti-to-syn transition caused by the C6 carbonyl was required to position the amino group close to T670. This intra-ligand cooperativity is further captured by the non-additivity of free energies from the respective functional group substitutions in the double mutant cycle. Overall, our findings illustrate the importance of identifying non-additivity in free energy changes, which can reflect conformational changes in the ligand bound state.

O15: Design and characterization of a disruptor peptide to the E2A-PBX1:CBP/p300 complex

David N. Langelaan, Marina R. Lochhead, David P. LeBrun and Steven P. Smith
Queen's University, Kingston, ON

E2A is a class I basic helix-loop-helix transcription factor that is essential for hematopoiesis. Not only is E2A needed for the proper regulation of B-lymphocyte development, disruption of E2A through a t(1;19) chromosomal translocation is associated with 4-12% of pediatric cases of acute lymphoblastic leukemia (ALL). The product of this translocation is the oncoprotein E2A-PBX1, which contains the activation domains of E2A and most of the PBX1 protein, including the DNA binding domain. Currently, E2A-PBX1 is thought to induce ALL through improper activation of PBX1 target genes through the E2A activation domains. Consistent with this model, previous work from our group has determined that leukemogenesis requires a direct interaction between E2A-PBX1 and the KIX domain of the histone acetyltransferase CBP/p300. Disruption of this protein-protein interaction would likely have therapeutic potential and to this end we have designed a peptide which disrupts the KIX:E2A-PBX1 complex. Using a variety of biophysical techniques such as nuclear magnetic resonance spectroscopy, X-ray crystallography, and isothermal titration calorimetry alongside functional assays such as pull-down and mammalian hybrid approaches we have characterized how this peptide disrupts the interaction between KIX and E2A-PBX1. This work outlines a potential therapeutic target for ALL and also provides a research tool to investigate transcription factor signaling networks.

O16: Structural Elucidation of Novel Antifungal Natural Products Isolated from an Endophyte Fungus in Raspberry Leaves

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An endophyte is a type of fungus that lives inside a plant without causing damage to the host. It is understood that many endophytic species produce secondary metabolites which are beneficial for the plants self defense from pests, bacteria and other invasive fungi. Thus, bioactive small molecules produced by endophytes are of great interest to the agricultural and food industries. From a survey of endophyte species present in Canadian crops, a strain of Hypoxylon submonticulosum was isolated from inside the leaf of a raspberry plant. When grown in liquid culture, the fungal extract showed both antifungal and antibacterial activity in disk diffusion assays. Using a liquid chromatography-mass spectrometry-solid phase extraction (LC-MS-SPE) system, the major component of the fungal extract was purified. From 1D and 2D NMR spectroscopy (^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC), it was a relatively straightforward task to elucidate the two-dimensional structure of a new triene-triol compound, (6E, 8E)-undeca-6,8,10-triene-2,4,5-triol. Other related derivatives have also been isolated and characterized from the extract. The challenge for this particular family of compounds remains in the determination of the relative stereochemistry between the three stereocentres in the linear molecule. To solve this structural problem, we have explored a multitude of chemical modifications available in the synthetic chemist's tool box. These reactions include the synthesis of an acetonide derivative as well as a ring-closing-metathesis in attempts to cyclize the linear molecule, thereby allowing for a NOE analysis and assignment of relative stereochemistry. Given the antifungal and antibacterial nature of the metabolites produced by this raspberry endophyte, further investigation is warranted for its potential use in raspberry growing and crop protection.

O17: Congenital hyperinsulinism-causing mutations cause misfolding and change molecular interactions in SUR1 NBD1

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The sulfonylurea receptor 1 (SUR1) is an ATP binding cassette (ABC) protein that forms the regulatory subunit in KATP channels found in the pancreas and the brain. MgATP binding and hydrolysis at the two cytosolic nucleotide binding domains (NBD1 and NBD2) in SUR1 control gating of the KATP channel pore.[1,2] Proper regulation of KATP channel gating by SUR1 is critical.[2] Over 100 mutations that lead to diabetes, hyperinsulinism, and developmental delay have been identified in different domains of SUR1, including the NBDs.[3] Therefore, molecular-level understanding of the structure and function of the NBDs is essential for designing improved treatments for SUR-related diseases.

Here we present biophysical and biochemical studies aimed at understanding the effect of disease-causing mutations on the conformation and nucleotide binding of SUR1 NBD1. Specifically, we are investigating SUR1 NBD1 mutations that cause congenital hyperinsulinism (C717Δ, G716V, R824G, R837Δ and K890T).[3] Our nuclear magnetic resonance (NMR) data shows that the hyperinsulinism mutation K890T causes chemical shift changes throughout the spectrum of NBD1, implying overall changes in protein conformation that may affect MgATP binding and inter-domain interactions in the SUR1 protein. Size-exclusion data show that the other hyperinsulinism mutations (C717Δ, G716V, R824G, R837Δ) produce mostly aggregated protein, likely as a result of misfolding of NBD1. Misfolding of NBD1 may be the underlying cause of reduced KATP trafficking seen with these mutations and hence decreased KATP channel gating observed in hyperinsulinism. Our fluorescence, circular dichroism, and microscale thermophoresis data corroborate the results that we have obtained by NMR spectroscopy. Our data provide molecular-level details on the effects of hyperinsulinism causing mutations in human SUR1.

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O18: Role of Dynamics in the Auto-Inhibition and Activation of the Hyperpolarization-Activated Cyclic-Nucleotide-Modulated (HCN) Ion Channels

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The hyperpolarization-activated cyclic-nucleotide-modulated (HCN) ion channels are cyclic AMP (cAMP) regulated proteins involved in nerve impulse transmission and heart rate modulation in neuronal and cardiac cells, respectively. cAMP modulates HCN activity through cAMP-dependent formation of a tetrameric gating ring spanning the intracellular region (IR) of HCN, to which cAMP binds. Although cAMP-associated conformational

changes in the IR cAMP-binding domain (CBD) were previously mapped, only limited information was available on HCN IR dynamics, which were hypothesized to play a critical role in the cAMP-dependent gating of HCN. Here, using MD simulations validated and complemented by experimental NMR and CD data, we comparatively analyze HCN IR dynamics in the four states of the thermodynamic cycle arising from the coupling between cAMP-binding and tetramerization equilibria. These MD simulations capture the active-to-inactive conformational transition that had remained elusive for other CBDs, and provide unprecedented insight on the role of IR dynamics in HCN auto-inhibition and its release by cAMP. Specifically, the IR tetramerization domain becomes more flexible in the monomeric states, removing steric clashes that the apo-state CBD would otherwise impose. Furthermore, the simulations reveal that the active/inactive conformational transition for the apo-monomeric CBD occurs through a manifold of pathways that are more divergent than previously anticipated. Upon cAMP binding, these pathways become disallowed, pre-confining the CBD conformational ensemble to a tetramer-compatible state. This conformational confinement primes the IR for tetramerization, and thus provides an explanation for how cAMP controls HCN channel gating.

Abstracts: Posters

P1: Use of ^{13}C methionine tags to study the effect of tetramerization in HCN channels

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, found throughout the nervous system and in the heart, sense the hyperpolarization phase of an action potential and function to regulate pacemaker activity and stabilize the resting state of the cell. Each HCN isoform (HCN1-4) has a 90 amino acid intracellular C-linker that acts as a bridge between two domains of the channel, and contains a newly discovered C-linker pocket (CLP). The discovery of the CLP is quite recent, meaning that its role in HCN channel gating is relatively unknown, with little information in the literature. However, it has been shown that a number of small molecules, specifically cyclic dinucleotides, are able to bind to the CLP and act as allosteric inhibitors of HCN4 channels. However, the effect of CLP binding on the oligomerization of the HCN4 channel has not been investigated. Therefore, we hypothesize that if the CLP is bound to a cyclic dinucleotide, or if mutated amino acid residues within the C-linker lead to a change in conformation, then the tetramerization of the intracellular region will be inhibited, leading to a shift in the equilibrium towards the monomeric state, and a disruption of the gating mechanism.

In order to test this hypothesis, specific residues within the C-linker of an HCN4 construct (residues 563-724) will be tagged with 13C methionine and visualized using NMR. Mutations of the tagged methionine residues will cause a peak in the NMR spectrum to disappear. Therefore, each missing peak will be assigned to a specific methionine tag. Once the peaks are assigned, disruptions to the C-linker can be studied. Using comparative analysis (i.e. comparing apo-CLP to holo-CLP, or comparing different mutations in the C-linker to the wildtype), we will be able to understand how the structure and assembly of the HCN4 construct is affected.

P2: Solid-state NMR Studies on Acid-Functionalized Graphene Oxides for Improved Fuel Cell Proton Conductivity

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This work aims to investigate the incorporation of acid-functionalized derivatives of graphene into constituent materials for polymer electrolyte membrane fuel cells (PEM-FCs). The electrolyte within these devices is most commonly made of Nafion™, a proprietary fluoropolymer, due to its high proton conductivity and chemical stability. Recently published literature provides evidence that graphene oxide (GO), a derivative of the super-material graphene, has intrinsic ion conductivity which is comparable to Nafion™, making GO a candidate for use in PEM-FCs.1 In addition, composites of functionalized GO and more common electrolyte materials show remarkable potential in terms of robustness and ionic conductivity.[2,3] Little work has been published on the mechanisms of ion conductivity or structural relationships within these materials. These mechanisms will be ideally studied through solid-state nuclear magnetic resonance (ssNMR) spectroscopy, thanks to its ability to probe structure and dynamics with high precision and sensitivity.

Multinuclear ssNMR was used to analyse the structure and dynamics of GO and a number of sulfonic acid derivatives of GO, both novel and previously reported. Upon functionalization or acidification, a collapse of interlayer spacing is seen in the materials through XRD. ^{13}C CP-MAS spectra showed the disappearance of surface-based oxygen groups upon GO functionalization, and identify functional group carbon sites. Dehydration of these samples allows the collection of ^{1}H spectra with resolved acid proton / water peaks. Proton dynamics of the bulk materials do not increase at high temperatures, and deuterium exchange is poor even under reflux. These results indicate that the increase in proton conductivity seen in PEM composites of these materials is likely only caused by functional groups on the surfaces of stacks of functionalized GO sheets. It is suggested that improved performance may be obtained where sheet stacking is minimal, allowing for an increase in the number of active proton conductive functional groups.

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P3: KimWipe Lithium-Ion Electrodes: A Brute Force Solution Towards ^{29}Si MAS-NMR of SiO anodes

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In the continued research effort towards higher capacity lithium ion batteries, higher capacity anode materials have been one of the primary focal points. Silicon monoxide, with a theoretical capacity of approximately 1500 mAh/g has over 3 times as much available specific capacity over traditional graphite anodes at 372mAh/g. While ^{7}Li MAS-NMR characterization of this material is relatively straightforward to achieve, ^{29}Si is substantially more difficult. Specifically, its relatively low abundance and gyromagnetic ratio, coupled with long T1 relaxation times on the order of several minutes make its analysis difficult. To compensate for this we propose the use of KimWipes as opposed to copper for the anode substrate. The most important advantage of this approach is that they can be stacked and coated with anode material on both sides, thus enabling significantly accelerated cycling of much larger amounts of material. Additional realized advantages include the ease with which the entire substrate can be packed into a rotor, and the enhanced performance achieved through the use of a porous substrate and aqueous binder solution. In the poster we present the cyclability of these rather non-traditional anode configurations in addition to the associated ^{7}Li and ^{29}Si NMR as a function of the state of charge. Furthermore, we extrapolate the utility of this electrode configuration to in-situ measurements of lithium ion batteries.

P4: Quantitative multispin distance information from homonuclear NMR recoupling experiments.

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High natural abundance nuclei usually occur as multiple spin networks in many materials. The multiple through-space dipolar coupling interactions and complicated geometry dependence of these spin networks render them complicated to analyze using

common solid-state NMR techniques. The distance information that can be extracted in a relatively straightforward manner from recoupling experiments of isolated spin pairs is much more challenging to obtain from those of multispin networks. Symmetry based 1D and 2D ^{31}P homonuclear recoupling experiments were carried out on a model compound, NaH_2PO_4, and the more complex $Cd_3(PO_4)_2$. The initial rises of the resulting double quantum recoupling curves were fit with various functions to extract an "apparent dipolar coupling," a quantity that has the potential to provide information on internuclear distances. Central to the fitting process is a normalization of the 1D and 2D recoupling data. This technique allows for the reliable acquisition of distance-dependant information from homonuclear solid-state NMR recoupling experiments on materials with multiple spin systems.

P5: Frequency-Swept Pulses for Acquiring Ultra-Wideline NMR Spectra Under Magic-Angle Spinning

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Many NMR-active nuclei are classified as unreceptive because of their low gyromagnetic ratios, low natural abundances, and/or unfavorable relaxation characteristics. Many of these nuclei give rise to very broad NMR powder patterns (i.e., ultra-wideline NMR powder patterns), which are affected by either large chemical shift anisotropies (CSAs) and/or large quadrupolar interactions (QIs). This makes acquiring high-quality ultra-wideline NMR (UW NMR) spectra difficult due to the (i) inherently low signal-to-noise (S/N) ratios, (ii) the reduced spectral resolution, and (iii) the limited excitation bandwidths associated with conventional, rectangular radio-frequency (rf) pulses.[1]

Magic-angle spinning (MAS) is a frequently used technique that reduces spectral breadths by spatially averaging all first-order NMR interactions, yielding NMR powder patterns that have higher S/N and increased spectral resolution. However, it is often not possible to achieve the required broad excitation bandwidth when using conventional rf pulses, which leads to distorted MAS NMR spectra. We demonstrate that the frequency-swept WURST[2,3] (wideband, uniform-rate, smooth truncation) pulse can be used to collect high-quality UW NMR powder patterns under MAS conditions. Specifically, we use the WURST-CPMG[4] and BRAIN-CP[5] (broadband adiabatic inversion cross polarization) pulse sequences to acquire high quality, high S/N spectra for both spin-1/2 and spin-1 quadrupolar nuclei using low-power rf field strengths. Additionally, we discuss methods for obtaining the isotropic chemical shifts, which are readily available from MAS spectra, when using CPMG-style acquisitions.

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P6: Uncovering the molecular basis of cardiovascular disease

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Kruppel-like factors are cardiogenic transcription factors involved in the proper function of cardiomyocytes and associated vascular smooth muscle cells (VSMCs). Deregulation of such transcription factors by mutation or altered expression is associated with the development of important risk factors for heart failure, including cardiac hypertrophy and VSMC inflammation. KLF proteins are hypothesized to carry out their functions by competing for binding to other transcription regulatory proteins. Through initial NMR titrations, we have shown that the Kruppel-like transcription factors bind to the TAZ2 domain of the transcriptional co-activator CBP/p300. The objectives of this study are to biophysically characterize the interactions of KLF proteins with TAZ2 and determine the three-dimensional structure of complexes involving these factors. Biochemical and biophysical methods, such as NMR spectroscopy, pull-down assays and isothermal titration calorimetry are being used to assess the importance of particular amino acid residues to the interactions between the Kruppel-like factors and CBP/p300.

P7: A Genetic Algorithm for NMR Crystallography of Materials with Multispin Networks

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Recently, solid state Nuclear Magnetic Resonance experiments have been developed to measure "apparent dipolar couplings" in materials with multispin networks of high natural abundance isotopes. This coupling information is inversely related to inter-atomic distances. This research explores an optimisation approach using "Genetic Algorithms" to solve crystal structures of materials from these experimental solid state NMR data.

P8: Quantitative Multispin Distance Information from Heteronuclear NMR Recoupling Experiments

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Multispin systems consist of isotopes with a high natural abundance of NMR active nuclei and are difficult to analyze using traditional solid state NMR (ssNMR) techniques due to the increased number of dipolar coupling interactions between nuclei and strong dependence on geometry. Consequently, it is difficult to extract meaningful distance information from ssNMR experiments carried out on systems with multispin networks. Recoupling curves resulting from ssNMR experiments that selectively recouple heteronuclear dipolar couplings can be fit and analyzed for isolated spin pair systems relatively easily, however, they become much more difficult to analyze for multispin systems. By testing various functions to fit the initial rise of recoupling curves produced from 1D and 2D symmetry-based $^{1}H\{^{31}P\}$ recoupling experiments on the model compound NaH_2PO_4, it was possible to extract an "apparent" dipolar coupling that could potentially be used to obtain distance information between nuclei. This new approach is made possible by a normalization procedure in the analysis of 1D and 2D recoupling data, from which the apparent dipolar

coupling between nuclei of various site types within the system could then be determined. These techniques allow for the extraction of meaningful distance information using ssNMR of heteronuclear multispin systems that was previously thought untenable.

P9: Fatty Acid Analysis of Fruits from Woody Plants by ^{1}H NMR

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AAFC

A diet high in polyunsaturated fatty acids has been associated with numerous health benefits (brain development, cardiovascular health, etc.). Fruit seed all contain different fatty acid (FA) profiles. In as much, we have analysed the FA composition of the fruit of several woody plants developed at the AAFC Agro-forestry Development Centre using 1H NMR. The FAs were extracted qualitatively from freeze dried fruit using a bench-top super-critical fluid extractor; a green chemistry method. A quantitative comparison of the FA profiles (saturated, monounsaturated, and polyunsaturated) as well as a comparison of the Omega-3 to Omega-6 ratios of the polyunsaturated FA was performed. These results will add secondary value to the plants.

P10: Solid-State NMR Studies of Fluorophosphate Materials for Na-Ion Batteries

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Sodium ion batteries have experienced a renaissance in recent years owing to the low cost and high abundance of sodium relative to lithium resources.[1] The fluorophosphate family of sodium cathode materials (Na_2MPO_4F, M=Fe, Co, Mg) is especially promising as the materials are both thermally and electrochemically stable, with the additional advantage of being relatively inexpensive depending on the redox active transition metal chosen. The paramagnetic nature of many of these materials makes their investigation by NMR non-trivial thereby necessitating the use of fast MAS and low external magnetic fields.[2] In particular, the Na_2FePO_4F phase was chosen for investigation as it has demonstrated potential as a useful cathode material for Na ion batteries. The as-synthesized Fe fluorophosphate was initially probed in an attempt to identify chemical exchange partners, for which both one and two-dimensional exchange spectroscopy techniques reveal an absence of such Na ion exchange on the timescale of the NMR experiments. In combination with structural results from established literature.[3,4] the NMR data imply that electrochemical activity of this class of materials is realized by Na mobility through ion exchange primarily between crystallographically equivalent sites.

Further, by incorporation of the material into an assembled Na ion cell and cycling to various states of charge, the desodiation mechanism for this material can be elucidated. Both ^{23}Na 1D and 2D MATPASS spectra establish that partially charged cathodes consist of both the original and oxidized phases, a result not realized by electrochemical or X-ray diffraction methods. Moreover, electrochemical tests in hybrid Na-Li cells corroborate the aforementioned results and open the door for Li-Na heteronuclear experiments in an effort to quantify the ion exchange time scale.

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P11: HIV-1 Vpu transmembrane protein oligomerization: combining atomic modeling with PFG diffusion NMR measurements.

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Vpu is an 81-residue transmembrane protein expressed by HIV-1 which enhances viral budding during infection. Vpu is reported to form ion channels in the membranes of HIV-1 infected host cells, but must form higher-order homo-oligomeric structures to do so. Vpu oligomeric complexes have been suggested to exist as tetrameric, pentameric, or hexameric arrangements, mediated by contacts within the N-terminal, single-pass transmembrane domain. We have expressed and purified recombinant full-length Vpu protein from bacterial cells in sufficient quantities for NMR study. Vpu forms a single oligomeric band in SDS-PAGE experiments. We have combined models of Vpu oligomers based on previous NMR studies with SDS micelles to predict translational diffusion parameters in solution, and by comparing these models against experimentally obtained PFG diffusion NMR data, we will determine the conformation of Vpu oligomers in aqueous SDS solution.

P12: Proton Detection in Magic Angle Spinning Solid-State NMR of Membrane Proteins

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Proton detection, in solid-state NMR is becoming an increasingly attractive and necessary method for the investigation of membrane proteins and larger protein complexes. Unfortunately, strong homonuclear couplings lead to broad, unresolved peaks in the ^{1}H spectrum. Although Magic Angle Spinning (MAS) alleviates some of this line broadening spinning frequencies in excess of 100 kHz would be required to completely remove the ^{1}H-^{1}H couplings. Until ultrafast spinning frequencies become readily achievable these couplings can be reduced via magnetic dilution with deuterons. This technique focuses on the reduction of the proton network thereby reducing ^{1}H-^{1}H couplings and resulting in increased resolution. This proton dilution paired with fast MAS (>50 kHz) has been shown to yield the sensitivity and resolution necessary to obtain ^{1}H assignments.

Though adequate resolution can be achieved at slower spinning, the use of fast MAS is attractive since the smaller rotors use much less sample. Signal loss from this can be compensated by the decrease in ^{1}H T1 resulting in faster recycling, thus allowing more scans to be run in the same experimental time.

We investigate the deuterium labeling techniques that allow for the incorporation of protons at various levels. Higher proton incorporation will provide more information as the number of protonated sites increases, at the expense of proton resolution. We aim to provide a compromise between resolution and protonation levels while maintaining reasonable experimental time. Through the use of the membrane proteins Proteorhodopsin and Anabaena sensory rhodopsin we investigate protonation levels and labeling techniques for the optimal sample for ^{1}H detected experiments in MAS solid-state NMR.

P13: The Development of a Deuterium Labeling Protocol of Yeast-expressed Membrane Protein for Structural Studies using Solid-state MAS NMR

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A number of obstacles exist in the study of eukaryotic membrane protein structure using solid-state NMR. Firstly, bacterial hosts are often used to produce proteins for studies due to their rapid cell growth, simple and cheap growth medium, and high expression. Bacterial expression systems, however, cannot be used to produce eukaryotic proteins, as bacteria lack the cellular machinery to make native-like post-translational modifications and achieve proper protein folding. Another difficulty that arises with studying membrane proteins is their size and the abundance of overlapping peaks as well as broadening of peaks from proton-proton coupling. To address the first roadblock, the yeast system Pichia pastoris is commonly used. This system maintains relatively high cell growth rate, high expression and low costs, while offering post-translational modifications and proper folding of eukaryotic membrane proteins. To address the second obstacle, deuterium labeling is used. Deuterons are introduced to replace many of the protons in the sample, reducing the number of overlapping peaks and increasing proton resolution. This in turn gives us a better understanding of the proteins through experiments like proton detection as well as advanced dynamics experiments. Although a number of previous studies have used deuterium labeling with soluble proteins, its use in studying membrane proteins in yeast systems like Pichia pastoris remains largely unexplored. Due to the cost of labeling samples with ^{2}H isotopes, it is important to grow cells in minimal media; this, coupled with the added stress of growing and producing proteins in D_2O pose challenges in optimizing to achieve high yields of protein. This study aims to develop a deuterium labeling protocol, using the previously mentioned yeast system, that addresses these challenges to further advance the use of solid-state NMR in membrane protein structural and dynamics studies.

P14: The Structural Effects of Hydration on Active Pharmaceutical Ingredients

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More than 50% of active pharmaceutical ingredients (APIs) are crystallized as simple salts, and of these, over 50% are HCl salts. In many instances, APIs can crystallize into pseudopolymorphic forms, such as hydrates or solvates, which have structures and molecular properties distinct from the non-hydrated or non-solvated solid phases. The polymorphic form of an API can influence its physicochemical properties, including bioavailability, shelf life, toxicity, and solubility. Additionally, each unique hydrate or solvate of an API represents unique intellectual property, and may be separately patented.

As such, it is very important to precisely structurally characterize all solid forms of APIs. Numerous methods, such as single crystal X-ray diffraction (scXRD), powder X-ray diffraction (pXRD), and ^{13}C NMR are often employed to accurately elucidate these structures. Previous work in our group has shown that ^{35}Cl SSNMR can be extremely valuable in the study of APIs in both bulk and dosage forms. In particular, we have shown that 35Cl powder patterns are extremely sensitive to slight modifications in the molecular structure of an API, and serve as unique spectral fingerprints for each compound.[1,2] The focus of this project is to use ^{35}Cl SSNMR, pXRD, as well as quantum-chemical calculations, to systematically study hydrates and anhydrous forms of HCl APIs. By analyzing their ^{35}Cl SSNMR spectra, we hope to study the hydrogen bonding interactions between chloride anions and water molecules, and the influence of water molecules on the molecular structures of the APIs. It is our hope that these proof of concept findings will be of interest to the pharmaceutical industry, for potential use in high throughput analysis of APIs, hydrate identification, and detection of impurities and disproportionation.

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P15: Determination of Proton-Proton Dipolar Coupling in Phosphate Solid Acids using the $R26_4^{11}$ Pulse Sequence

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The $R26_4^{11}$ pulse sequence has been extensively employed to determine homonuclear dipolar coupling in systems with isolated spin pairs but it has had limited use in more complex multi spin systems.[1] Phosphate solid acids, anhydrous proton conductors that have been investigated for use as membrane materials in PEM FCs, are comprised of a network of hydrogen bonded protons which surround phosphate oxyanions.[2] These systems, which have one or more equivalent proton environments, have been selected for analysis via the $R26_4^{11}$ pulse sequence. It was determined that in systems with a single proton environment, such as tetragonal KH_2PO_4, dipolar coupling decreases as a function of increasing temperature. This property, when coupled with increased proton conductivity, indicated increased proton mobility. In systems with multiple proton environments, such as monoclinic NaH_2PO_4, a decrease in dipolar coupling was observed as a function of increasing temperature when the sum of double quantum filtered intensity over all proton sites was considered. This correlated with an increase in proton conductivity over a similar temperature range. When individual proton sites were considered dipolar coupling did not decrease uniformly as a function of temperature which suggested that exchange and coalescence processes occurred and could not be adequately measured. For comparison purposes, dipolar coupling in $Ca_{10}(PO_4)_6(OH)_2$ was analyzed. The calcium deficient form contains multiple peaks and analysis of the sole hydrogen bonded proton yielded little change in dipolar coupling over the experimental temperature range. This correlated with the small increase in conductivity. It was therefore determined that the $R26_4^{11}$ pulse sequence was suitable for the determination of homonuclear dipolar coupling in multi spin systems with a single proton environment or systems with multiple proton environments that are not subject to exchange and coalescence processes.

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P16: Characterizing the Interaction of Amiloride Derivatives with Model Membranes and the HIV-1 Vpu Transmembrane Domain.

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During HIV-1 infection the accessory protein Vpu is responsible for the downregulation of several cell surface proteins. Tetherin, a Vpu target and host cell restriction factor, has been shown to inhibit the release of budding virions from the cell surface. The Vpu mediated downregulation of tetherin has been linked to a direct interaction between the transmembrane domains of the two proteins. Vpu activity has been shown to be inhibited by the presence amiloride-based drugs in vivo. The amiloride derivative 5-(N,N-hexamethylene) amiloride (HMA) functioned to restore Vpu mediated viral release- implying that the drug functions to directly inhibit the Vpu/tetherin association. We have carried out Förster resonance energy transfer (FRET) experiments that support a direct TM-TM association model, however the presence of HMA did not affect the association of the two proteins. Incidentally, we discovered the propensity of HMA to bind to phospholipid membranes. Using UV/vis spectroscopy as well as ^{31}P ssNMR under anisotropic and magic angle spinning conditions we have begun to characterize the role membrane/drug interactions play within our model system. Characterization of drug/lipid and drug/Vpu interactions will provide the necessary foundation for determining how amiloride derivatives prevent the Vpu-mediated antagonism of tetherin.

P17: Probing Lipid Involvement in Proprotein Processing: The Story of Proapelin and PCSKs

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Apelin is a peptide hormone that binds to a cognate G-protein coupled receptor to control angiogenesis, adipoinsular axis, and the cardiovascular system. Following signal peptide cleavage, a 55-residue proprotein, proapelin, is further enzymatically processed into 13-, 17-, and 36-residue C-terminal isoforms. We previously showed that proapelin can be processed into apelin-13 by one member of the proprotein convertase subtilisin kexin (PCSK) family. Processing to the longer isoforms has yet to be demonstrated. PCSKs are localized in various cellular compartments including the ER, Golgi, secretory vesicles, and cell membrane. Since our demonstration of apelin-13 production by PCSK3, proapelin has been observed extracellularly. Secretion prior to processing implies that this process may occur inside and/or outside the cell. Since the lipid composition of different cellular compartments and the cell surface are all distinct, location-specific conformational changes in proapelin may be expected upon lipid binding. By far-UV CD spectropolarimetry and ^{1}H, ^{15}N and ^{13}C chemical shift assignment, proapelin shows random coil characteristics. When incubated with anionic SDS and LPPG micelles, but not zwitterionic DPC micelles, increased α-helical properties are observed, suggesting preferential lipid interaction. Beyond simple chemical shift assignment, ^{15}N spin relaxation measurements suggest that proapelin is highly dynamic

in buffer, with significant reduction in dynamics in all micellar environments. Contrary to this, significant perturbations to ^{1}H-^{15}N HSQC spectra were observed only in the presence of LPPG and SDS, not in DPC, suggesting lipid-dependent conformational changes. We are currently carrying out a detailed analysis of conformation and dynamics as a function of proapelin cleavage site to test the potential involvement of membranes in its processing. This would represent the first demonstration that membranes influence proprotein processing and would facilitate the identification of new structural motifs and conformation changes that occur upon proprotein-membrane interactions.

P18: Solid-State NMR Spectroscopy of Metal-Metal Bonds in Organogallium Compounds: J-coupling Between Quadrupolar Nuclei

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Organometallic compounds containing metal-metal bonds are of great fundamental interest and generally offer enhanced catalytic activity in the fields of polymer and organic chemistry that are indispensable to modern life. In recent years, a new family of gallium catalysts has been used in a wide range of chemical processes. The crucial role of catalytic activity depends on the oxidation states of the metals as well as the nature of metal-metal bond. In addition, the role of multiple bonds between metals continues to attract attention and controversy in the scientific community, with many varying conclusions on the nature of the bonding.

We have prepared a series of gallium compounds which have been proposed to contain single, double, and triple Ga-Ga bonds. These have been characterized in detail by solid-state NMR techniques, including ^{1}H, ^{13}C, and ^{71}Ga SSNMR spectroscopy. However, the metal-metal bonds are difficult to characterize directly by SSNMR because of dominant dipole-dipole and quadrupole interactions, which leads to unresolved broad spectral lines. The advanced J-resolved NMR technique overcomes this problem and homonuclear J-coupling splittings may be easily extracted from 2D NMR spectra. The series of prepared samples, with different proposed bond orders, were investigated using a 2D ^{71}Ga two-pulse J/D resolved NMR experiment which allows for greater sensitivity, excitation bandwidth, and resolution. The dependence of the homonuclear J-couplings on the nature of the gallium-gallium bonds was analyzed. The resulting reduced homonuclear (Ga-Ga) K-coupling constants were directly compared with those known for carbon-carbon and boron-boron single, double, and triple bonds. The results confirm the presence of a triple bond in $Na_2[\{2,6\text{-}(2,6\text{-}iPr_2C_6H_2)_2C_6H_3\}Ga{\equiv}Ga\{2,6\text{-}(2,6\text{-}iPr_2C_6H_2)_2C_6H_3\}]$. Density functional theory calculations are also applied to interpret the experimental results. This combined approach provides new insights into multiple bonding between metals.

P19: PKA Inhibition and Activation: A Double-Conformational Selection Model for the Tandem cAMP-Binding Domains of PKA RIa

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cAMP is an essential second messenger involved in a variety of signal transduction pathways. The main receptor for cAMP in mammals is the ubiquitous regulatory subunit of

protein kinase A (PKA). PKA includes two components, a regulatory (R) subunit spanning two homologous cAMP binding domains, CBD-A and CBD–B, and a catalytic (C) subunit. cAMP binds CBD-B with high affinity making CBD-A accessible to a second molecule of cAMP, which in turn promotes the dissociation of the C subunit from the R subunit. Hence the cross-talk between CBD-A and CBD-B plays a central role in the activation of PKA. To further investigate the inter-CBD communication, we comparatively analyzed by NMR a two domain construct of the RIa subunit of PKA in its apo, cAMP and C- subunit bound states. Our investigation included both wt PKA RIa as well as a mutant that silences inter-domain interactions. The mutant vs. wt comparative NMR analysis revealed several inter-domain allosteric networks in PKA RIa. We also found several differences between the dynamic profiles of the structurally homologous CBD-A and B. For example, the base binding region (BBR) of CBD-B exhibits enhanced dynamics in the ps-ns time scale, whereas the BBR of CBD-A is devoid of significant ps-ns motions, which is supporting the notion that conservation of structure does not necessarily imply conservation of dynamics.

P20: Structural characterization of hematopoietic transcription factors

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B lymphopoiesis is a highly regulated process that orchestrates the commitment of hematopoietic stem cells to the B cell lineage. Various transcriptional regulators, including E2A and EBF1, play particularly critical roles in developmental progression towards mature B cells. Deregulation of this process can cause detrimental phenotypic changes, as seen in acute lymphoblastic leukemia, the most common form of pediatric cancer. Notably, expression of the oncogenic fusion protein E2A-PBX1, arising from a t{1;19} chromosomal translocation leads to the dysregulation of B cell hematopoietic transcription factors, inciting leukemogenesis. E2A, E2A-PBX1, and EBF1 impart their functions through recruitment of the transcriptional coactivator CBP/p300 via their intrinsically disordered transactivation domains. Here, we use solution NMR spectroscopy to characterize the E2A region (1-483) of E2A-PBX1, the transactivation domain of EBF1, and their interactions with various domains of CBP/p300. Our findings provide novel insights into lymphopoietic transcription factors and their role in B cell development and leukemogenesis.

P21: 7-Li NMR of Lithium-Ion Battery Cathodes

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This study evaluates changes in molecular environments of lithium-ion battery cathodes as a function of electrochemical cycling using $_7$Li Magic Angle Spinning (MAS) NMR. The commercially successful $Li(Ni_{1/3}Mn_{1/3}Co_{1/3})O_2$ (NMC) and the prospective $Li_2FeP_2O_7$ were de-lithiated/re-lithiated via galvanostatic cycling in coin cells to multiple states of charge. As established in literature, cycling of NMC gives rise to a sloped charge/discharge curve, indicative of a solid-solution type lithium extraction, and there is a 20-30 mAh/g capacity loss after the first charge.1 Likewise, as previously established, $Li_2FeP_2O_7$ demonstrates a sloped curve and the second lithium atom is not removable within the voltage range studied (2-4.5 V).2,3 The fast (60 kHz) MAS NMR spectra of cycled NMC show that with delithiation there is a gradual shift to lower frequency and peak narrowing

resulting in the resolution of two peaks. Peak resolution is most apparent in the fully charged (4.6 V) NMC spectra, and upon subsequent discharge to 2 V the spectrum obtained matches that of pristine NMC, indicative of structural reversibility. Similarly MAS NMR spectra of cycled $Li_2FeP_2O_7$ suggest structural reversibility in this cathode material. De-lithiation of $Li_2FeP_2O_7$ results in a change in line-shape and a shift to higher frequency which can be interpreted based on the selective removal of specific Li atoms from the crystal lattice.

P22: Quantification of Fatty Acids Bound to Human Serum Albumin by NMR

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Human serum albumin (HSA) is the most abundant protein in plasma and it transports drugs and endogenous ligands including fatty acids (FAs). HSA goes through conformational changes upon binding to FAs which affects albumin functions such as drug delivery or inhibiting potency for Aβ aggregation. In normal conditions HSA binds to 0.1 -2 FAs but some diseases are associated with the increase of this number. Hence, it is important to define the amount of free FAs. Cobalt-HSA binding assay (ACB) is the most commonly used method for measuring free FAs. However, there are some limitations with this method including the dependence on HSA concentration in plasma, the oxidation state of HAS, vulnerability of cobalt II to oxidation and the pH of plasma. The problem with extraction methods is the high affinity of defatting resin for long chain FAs which makes quantification very difficult. Therefore, a more robust and reproducible method is needed. In order to probe the amount of FAs' bound to HSA, we proposed to use ^{13}C-methyl-labelled oleic acid (OA) as a reporter molecule. Upon the addition of ^{13}C-OA, it binds sequentially to different FA binding sites on HSA. Therefore, we can observe peaks corresponding to the bound ^{13}C-OA that occupied the first three highest affinity binding sites on HSA by a good 1D- carbon NMR and the chemical shifts of the bound ^{13}C-OA changes in correlation with the amount of non-labelled FA bound to HSA. We examined all the drawbacks of the ACB assay for our proposed method and concluded that the method is independent of the concentration of albumin in the physiological range, the oxidation state of albumin, and the pH of plasma at the physiological pH range. Hence,^{13}C-OA assay can be an alternative way to confirm the quantification of long chain FAs bound to HSA.

P23: Molecular Dynamic Simulation of Protein Kinase G -cGMP -Binding Complex Domain B to Determine Binding Dynamics.

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PKG (Protien Kinase G) is a receptor for the secondary messenger cGMP (Cyclic guanosine monophosophate), and is responsible for some of the physiological effects associated with cGMP. PKG-cGMP binding regulates a number of different pathways such as cell memory formation, cell differentiation, platelet activation, and vasodilation. However, the mechanism of cGMP binding is not well understood and the thermodynamic cycle for this process has not been mapped. Previous work using protein NMR has provided information about the apo and holo states for PKG-cGMP binding. Molecular dynamic (MD) simulations will be used to provide unique insight on the metastable apo-active and holo-inactive intermediates and assess binding vs. purely allosteric contributions. Previous NMR results

will be used to validate the results of this simulation. The long term goal of this research will be to see how the holo-inactive form can be stabilized, that is how ligand binding can occur without activation, which is the basis of allosteric inhibition.

P24: Study of disease-related mutations of Protein Kinase A RIα affiliated with Carney Complex and Acrodysostosis

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Protein Kinase A (PKA) is responsible for regulating various important cellular processes. The importance of PKA activity is also supported by the presence of a great number of disease-related mutations of PKA. The studies of these disease-related proteins are important in enhancing our understanding of the structural basis of cyclic AMP (cAMP)-dependent allostery in the cAMP-binding domain (CBD) of PKA, and thus the underlying mechanisms of PKA activation, which is critical for the development of drug leads specific for eukaryotic CBDs. In this study, we have focused on two mutations of PKA regulatory subunit (RIα), Ala211Asp and Ala211Thr, which are associated with Carney Complex and Acrodysostosis, respectively. The diseases show opposed phenotypes, although both mutations are present in the same residue of PKA RIα. How each mutation affects the interactions of PKA RIα with cAMP and catalytic subunit, and thus the overall extent of PKA activation and the cAMP-dependent allosteric network of interactions are investigated.

P25: The Impact of Fluorine Substituents on A Model Pi-Conjugated Organic Polymer used in Solar Cells

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Organic pi-conjugated polymers are an exciting class of materials for harvesting solar energy, owing to the relatively low cost with which they can be synthesized and processed into films for use in solar cells. Charge transport in organic materials is inherently anisotropic and so, is very dependent on polymer packing. Therefore, understanding how the molecular morphology of these polymers in films affects their emergent performance is crucial for gleaning design rules to fabricate highly efficient solar cells. The incorporation of fluorine substituents in organic polymers has been shown to be a promising strategy towards increasing the performance of organic solar cells. In our study, we determined the molecular-scale effects of fluorine substituents in a model polymer using solid state NMR and computational methods. Using ^{13}C MAS and multiple quantum ^{1}H NMR spectroscopy, we found that fluorination induces changes in the polymer's main-chain conformations and intermolecular packing. We find that these positively influence the charge transport within the fluorinated polymer relative to its hydrogenated analogue, providing an explanation of the superior performance of that polymer in thin film solar cells.

P26: Probing the Interactions between Epigallocatechin gallate and Amyloid Beta Assemblies

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Alzheimer's disease (AD) is a neurodegenerative disorder that results in the deterioration of memory and cognitive abilities. The etiology of AD is associated with the abnormal aggregation of amyloid-beta (Aβ) peptides into fibrils. There is currently no cure for AD, however few medications have been able to address the symptoms of the disease. Drug development efforts for amyloid-based diseases have been largely hindered due to a poor understanding of the molecular basis of the structure-activity relationship of inhibitors. The successful creation of new inhibitors will require gaining a better understanding of the components necessary for activity as well as the key elements in the amyloid surface required for aggregation and toxicity. Currently a green tea extract, epigallocatechin gallate (EGCG), has gained clinical significance as a potential inhibitor of Aβ aggregation. Previous work using solid and solution state NMR has shown that EGCG interacts with the hydrophobic core of Aβ. In addition, MD simulations have identified that EGCG interacts with the main chain atoms of the C-terminal residues and the side-chain atoms of the hydrophobic core and N-terminal residues. Although previous research have given insight into the interactive surface between EGCG and Aβ peptides, MD simulations serve as a tool to create a hypothesis and further work using NMR is still required for validation. Therefore, we propose to use an NMR technique that generates atomic resolution data on the exchange reaction between Aβ monomers and NMR invisible protofibrils. The ^{15}N dark-state exchange saturation transfer (DEST) experiment reports on the relative probabilities of direct versus tethered contacts between monomer Aβ residues and protofibrils. Competition between monomeric Aβ and EGCG for the protofibril surface will generate residue-specific data on the probability of interactions between EGCG and protofibrils and give further insight on Aβ oligomerization.

P27: Elucidating the molecular mechanisms by which the HNH endonuclease gp74 activates the terminases in bacteriophage HK97.

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Bacteriophages are the most abundant entities in the biosphere (1). The last gene in the genome of the bacteriophage HK97 codes for gp74, an HNH motif-containing endonuclease (2). HNH motifs are characterized by two highly conserved His residues and an Asn residue (3). Gp74 is essential for phage head morphogenesis, as gp74 enhances the activity of the HK97 terminase enzymes toward the cos site(4). Notably, enhancement of the terminase-mediated cleavage of the phage cos site requires the presence of an intact HNH motif in gp74. Mutation of the canonical metal binding His in the HNH motif abrogates gp74 mediated-terminase activity. Although phages are widely studied, there is no structural or mechanistic evidence describing how the HNH motif within gp74 functionally interacts with the terminase enzymes to facilitate phage morphogenesis. For example, previous work on HNH-containing bacteriophage proteins does not address explicitly how divalent metal binding at the HNH motif of the endonuclease induces terminase activity, which is crucial for phage DNA packaging during morphogenesis (4, 5).

In addition, gp74 possesses no sequence similarity to HNH proteins for which the structure has been determined (3), making structural studies of gp74 necessary. We are using NMR spectroscopy to solve the solution structure of gp74. Toward this end, we have obtained assignments for 75 % of the backbone resonances of the protein. We are also using NMR spectroscopy to probe structural changes in gp74 bearing mutations that affect activity and to localize metal binding sites within the protein. Many metalloproteins contain multiple metal binding sites that include catalytic sites, such as the HNH motif, as well as structural sites. Correlation of the NMR data with planned in vivo experiments will define the role of specific gp74 residues in phage morphogenesis. Ultimately, this work will elucidate how metal binding in HNH endonucleases is crucial in the replication and morphogenesis of phages.

P28: Computational assessment and initial investigations of NMR trends in carbon tetrel bonds.

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Group IV tetrel elements may act as tetrel bond donors, whereby a region of positive electrostatic potential (σ-hole) interacts with a Lewis base. The results of calculations of interaction energies and NMR parameters are reported for a series of model compounds exhibiting tetrel bonding from a methyl carbon to the oxygen or nitrogen atoms in a range of functional groups. The ^{13}C chemical shift (δiso) and the $^{1c}J(^{13}C,^{17}O)$ coupling across the tetrel bond are recorded as a function of geometry. The sensitivity of the NMR parameters to the non-covalent interaction is demonstrated via an increase in δiso and in $|^{1c}J(^{13}C,^{17}O)|$ as the tetrel bond strengthens. There is no direct correlation between the NMR trends and the interaction energy curves; the energy minimum does not correspond to a maximum or minimum chemical shift or J-coupling value. Gauge-including projector-augmented wave density functional theory (DFT) calculations of δiso are reported for crystals which exhibit tetrel bonding in the solid state. Experimental δiso values for sarcosine, betaine, and caffeine and their tetrel-bonded salts generally corroborate the computational findings. This work offers new insights into tetrel bonding and facilitates the incorporation of tetrel bonds as restraints in NMR crystallographic structure refinement.

P29: In-situ NMR Diffusion Imaging: A Tool for Characterizing Ion Transport Properties in Li-Ion Battery Electrolytes

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Design and optimization of nonaqueous lithium ion battery electrolytes requires methods for measuring all relevant diffusional parameters, including ionic diffusivities and transference numbers. An accurate characterization of the transport parameters is not a trivial task due to the fact that the values of diffusion coefficients of ions in an electrolyte solution depend strongly on the salt concentration. At the same time, the salt concentration inside a battery is neither stationary nor homogeneous during application of electric potential; rather, it is a function of current density, time and distance from the electrodes.

Herein we demonstrate the application of in situ NMR diffusion imaging technique for real-time visualisation of concentration gradient formation and measurement of distribution of ionic diffusivities in the electrophoretic cell under applied electrical potential. Taking into

account that shape of concentration gradient profile depends on both salt diffusivity and transference number, one can also calculate the later parameter based on data obtained from the described experiment and equation of mass transport. The method was tested using home-made in situ cell with commercially available electrolyte consisted of 1 molar solution of $LiPF_6$ salt in 1:1 v/v mixture of ethylene carbonate (EC) and dimethyl carbonate (DMC); lithium metal and graphite served as anode and cathode in the cell.

P30: Parkin is Activated by Release of its Ubiquitin-Like Domain

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Autosomal recessive juvenile Parkinsonism is a hereditary form of Parkinson's disease directly linked to mutations in the E3 ubiquitin-ligase parkin. Parkin's protective features mediate mitochondrial control in dopaminergic neurons by attaching a small ubiquitin (Ub) molecule to damaged proteins in a process called ubiquitination, which then signals these proteins for degradation. Parkin exists in an autoinhibited state regulated by association of its N-terminal ubiquitin-like (Ubl) domain. Previous studies have shown that phosphorylation of parkin's Ubl domain (pUbl) and binding of phosphorylated-Ub (pUb) increases parkin ubiquitination activity. Until now, it was unknown how these events activate parkin on a molecular level. Here, we used isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) to show (1) parkin affinities for Ubl and Ub in phosphorylated and unmodified states, (2) a model complex for pUb and parkin binding and (3) that pUb binding displaces pUbl from parkin. ITC showed that parkin's affinity for its Ubl domain decreases when phosphorylated, while parkin's affinity for Ub is significantly tighter when phosphorylated. Differences in affinities suggest that parkin Ubl phosphorylation and pUb binding are activating events. NMR, chemical shift perturbation (CSP) analysis and paramagnetic relaxation enhancement experiments were used to determine the pUb binding interface. Ambiguous CSP restraints were used with HADDOCK software to determine a model complex of pUb and parkin binding. Finally, CSP analysis was used to show that the addition of a phosphorylation-mimetic Ub displaces parkin association with phosphorylation-mimetic Ubl. Results here detail how both phosphorylation of parkin's Ubl domain, and pUb binding events activate parkin by releasing association of parkin from its autoinhibitory Ubl domain.

P31: Critical interface residues necessary for PKA signal termination through the interaction between PKA RIα and PDE8A revealed by NMR.

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The specific binding of Protein Kinase A (PKA) and certain mammalian phosphodiesterases (PDE8A) has been proposed to terminate signal transduction. Unveiling the binding interface at the atomic level could lead to the design of translational therapeutics in the form of allosteric effectors for treatments involving aberrant PKA signaling. Amide hydrogen/deuterium exchange mass spectrometry (HDXMS) has been used to probe the interaction interface between PDE8A and RIα. The regions where binding occurs were

elucidated, but the interface was determined only at peptide resolution, not atomic. With the ability of two-dimensional nuclear magnetic resonance (2D-NMR) to monitor residues at the atomic level, the interface of PKA RIα and PDE8A can be elucidated. By istopically labelling PKA RIα and using the regions found by HDXMS, the first sites to probe through 2D-NMR would be in or near the two phosphate-binding cassettes (PBCs). Observing a shift in intensity could reveal specific residues involved in the interaction between PKA RIα and PDE8A, but only as a semi-descriptive indicator. Intensity losses and binding termination via point mutations on PKA RIα could be two avenues that, in combination, reveal the PKA RIα and PDE8A binding interface.

P32: Structural and biophysical aspects of disease causing mutations in the C2a domain of dysferlin

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Dysferlin mutations result in serious dystrophic conditions such as Limb Girdle Muscular Dystrophy type 2B (LGMD2B), due to severe defects in the process of sarcolemmal maintenance and membrane repair. Dysferlin contains seven conserved C2 domains which have universal roles in membrane binding and are often present in vesicle traffic-related proteins. The C2a domain of dysferlin contains one of the most commonly observed disease-causing mutations at residue V67. We show, using chemical shift perturbation (CSP) and spectropolarimetry (CD), that substitutions at this position have a severe destabilizing effect on the domain structure, in particular when a charged residue is substituted in. Several C2 domains have previously been shown to bind $Ca^{2+,}$ and to bind lipids in a Ca^{2+}-dependent manner. We show that Ca^{2+} has a small but consistent stabilizing effect on the native C2a protein under temperature stress, but not on the V67 mutants. Additionally, we look at the effect of Ca^{2+} ions on the structural aspects of C2a, and its capacity to bind lipids in a bilayer mimicking environment. Pure POPC nanodiscs co-migrate with wild type C2a on native PAGE in both Ca^{2+} saturated and Ca^{2+}-free conditions, but not with any of the V67 mutants. Our HSQC data of C2a with titrated nanodiscs also support this conclusion. Altogether, the data point at the structure destabilizing effects of mutations as the culprit to protein instability and loss of Ca2+ and membrane binding in the diseased state.

P33: ^{35}Cl Solid-State NMR Investigation of Pharmaceutical Cocrystals

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Active pharmaceutical ingredients (APIs) can be prepared in a variety of crystalline solid forms, including polymorphs, solvates, hydrates, and salts, or as amorphous materials. Their structural characterisation is important for identifying and discovering pure bulk APIs, as well as for the fingerprinting of APIs in solid dosage forms. Cocrystals, consisting of an API and a pharmaceutically acceptable coformer, can provide an alternative solid-state form. The rational design of cocrystals is a burgeoning area in crystal engineering, providing a powerful means of modifying physicochemical properties, such as solubility, stability, or bioavailability. Whilst X-ray diffraction is very useful for identifying phases and structure solution, it is limited when the patterns are not distinctive due to the presence of excipients, polymorphs with similar patterns, or amorphous materials. Solid-State NMR (SSNMR) is

well suited for identifying solid phases in both bulk and dosage forms, and is an indispensable tool for probing specific nuclides, particularly if the chosen nuclide is in the API and not in the excipients. For APIs that are formulated as hydrochloride (HCl) salts, ^{35}Cl is a logical choice, even though it is considered to be an unreceptive nucleus. 35Cl SSNMR can be easily applied for the differentiation of solid forms of HCl APIs, as the spectra are sensitive to subtle changes in the chlorine anion environment.[1] In HCl API cocrystals, the chloride anions typically have close contacts with both components. In this work, a series of samples prepared using a HCl salt of an API and different potential coformers is investigated using 13C and 35Cl SSNMR, to confirm cocrystal formation and examine the resulting structures. In addition, DFT calculations are used to predict NMR parameters.

[1] M. Hildebrand et al., CrystEngComm, 2014, 16, 7334; H. Hamaed et al., J. Am. Chem. Soc., 2008, 130, 11056.

P34: A Study of P=O···I-C Halogen Bonding via Solid-State Multinuclear Magnetic Resonance and Molecular Orbital Analysis

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Halogen bonding (XB) has been exploited in various applications, such as drug design, functional materials, and catalysis. Here, we have prepared five novel halogen-bonded co-crystals formed by two phosphine oxide (Ph_3PO and CH_3Ph_2PO) and three different iodofluorobenzene derivatives (p-$C_6F_4I_2$, o-$C_6F_4I_2$ and sym-$C_6F_3I_3$) using mechanochemical liquid-assisted grinding and slow evaporation. These halogen-bonded adducts exhibiting P=O···I-C motifs have been characterized by a combination of single-crystal X-ray diffraction, solid-state multinuclear (^{31}P, ^{17}O) magnetic resonance and quantum chemical calculations. Chemical shift (CS) tensors extracted from ^{31}P CP/MAS spectra offer an indirect view of XB on two phosphine oxide acceptors. ^{17}O-labelled Ph_3PO has been synthesized and used to obtain additional ^{17}O-labelled halogen-bonded compounds. ^{31}P and ^{17}O SSNMR have been performed to provide a direct insight into the correlation between XB and the resulting NMR observables: CS and electric field gradient (EFG) tensors, and J(^{31}P, ^{17}O) coupling. Several notable trends involving the XB strength (through a normalized distance parameter, RXB) have been produced using both GIPAW and ZORA DFT calculations on crystal structures. An additional natural localized molecular orbital (NLMO) analysis shows the main orbital contributions to J(^{31}P, ^{17}O) coupling and allows for the interpretation of these main contributions determine the overall linear trend between J(^{31}P, ^{17}O) coupling constants and XB strength. Another NLMO analysis applied to the ^{17}O quadrupolar coupling constant reveals a linear correlation between the oxygen pz lone pair orbital contribution and the value of RXB.

P35: ^{19}F-^{19}F Double Quantum NMR Dynamics Study in Proton-conducting Polymers

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SSNMR has been applied to reveal structure and local dynamics on a molecular level. 1H double quantum filter (DQF) NMR is a well-established strategy for probing local dynamics.[1-3] This concept has been applied here to characterize fluorinated ionomer

materials for the first time. ^{19}F DQ recoupling NMR experiments, where an R-symmetry sequence is applied, are used in this study to investigate the site-specific local dynamics of the polymeric electrolyte material, Nafion®, at various temperatures and humidity conditions. The initial build-up of the normalized double quantum (nDQ) curves generated are used as a measurement of local mobility. The apparent dipolar coupling constant (Dapp) can be extracted as a quantitative measurement of mobility. The side-chain and backbone local dynamics profiles can be distinguished. The side chain has more sensitive response towards the temperature and humidity changes, which indicates the side chain possesses higher local dynamics than the backbone. Alternative fluorinated electrolyte materials are also investigated in parallel. A link between material performance and dynamics properties can be established and monitored through this advanced NMR method.

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(3) Ye, G.; Janzen, N.; Goward, G. R. Macromolecules 2006, 39 (9), 3283-3290.

P36: Synthesis and NMR studies of site-specifically oxygen-17 labeled D-glucose

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Oxygen is among the most common elements found in organic and biological molecules, but remains the only one that has not yet been fully utilized in NMR studies. This is because the only NMR-active oxygen isotope, ^{17}O, has an exceedingly low natural abundance, 0.037%. Therefore, ^{17}O isotopic labeling is generally a prerequisite of ^{17}O NMR studies. In this work, we report synthesis and ^{1}H, ^{13}C, ^{17}O NMR characterization of three site-specifically ^{17}O-labeled D-glucose: D-[6-^{17}O]-glucose, D-[5-^{17}O]-glucose, and D-[3-^{17}O]-glucose. These compounds will be useful in the future development of new ^{17}O NMR techniques for improving spectral resolution.