Abstract Booklet: Poster Presentations

Sept. 29 – Oct. 1, 2017

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(1) Synthesis of ^{17}O-Labeled Glucose-6-Phosphate via Enzymatic Reactions

Neil Sengupta, Jiahui Shen, Gang Wu
Queens University

Glycolysis is an important pathway for carbohydrate metabolism in biological systems. Oxygen is one of the most common elements found in carbohydrates but is the only element that has not yet been used in NMR studies of glucose metabolism, due to its exceedingly low natural abundance of 0.037%. In this work, we synthesized [6-17O]-glucose and incorporated this into the hexokinase catalyzed reaction of glycolysis to form [6-17O]-glucose-6-phosphate. This work marks the first important step in using 17O NMR to monitor the glycolytic pathway.

(2) The NMR and DNP Centre at the University of Guelph

M. Sameer Al-Abdul-Wahid, Andy Y.H. Low
University of Guelph

The University of Guelph NMR Centre supports 20 local research groups, as well as external academic and industrial researchers, in the disciplines of biophysics, chemistry, food and environmental science, and biology. The NMR Centre comprises six spectrometers spanning 400 MHz to 800 MHz, including a 600 MHz Dynamic Nuclear Polarization spectrometer, to study the structure and function of food, chemicals, proteins, carbohydrates, and biological membranes. The NMR Centre also provides access to a wide range of probes, including 800 MHz bio-MAS probes from 1.3 mm to 4 mm rotor diameter, 5mm solution cryoprobes for enhanced sensitivity, and an F/C/H probe for ^{19}F NMR studies with optional ^{1}H decoupling. An overview of the facility, along with recent experiments of interest to the NMR community in the fields of chemistry, protein biochemistry, and metabolomics, will be presented.

(3) Probing the Metal Centres in Calcium Based Metal-Organic Frameworks by Natural Abundance ^{43}Ca Solid State NMR

Shoushun Chen, Bryan E.G. Lucier, Victor V. Terskikh, Yining Huang
Western University

Metal-organic frameworks (MOFs) are porous materials constructed from metal centres joined by organic linkers.[1] MOFs have many applications, including gas storage, gas separation, catalysis and chemical sensing.[2] MOFs often feature toxic metals like Co, Cu, Al or In, but “green” and cheap metals such as calcium can be incorporated instead. Thorough characterization is critical to understand the origins of MOF properties, and solid state NMR (SSNMR) has proven to be an important structural investigation technique, especially when a suitable single crystal for X-ray diffraction is not available.

^{43}Ca SSNMR experiments on MOFs are extremely challenging and quite rare due to the intrinsic insensitivity and very low natural abundance (0.135 %) of the quadrupolar ^{43}Ca nucleus. We describe a natural abundance ^{43}Ca SSNMR study on four calcium based MOFs (CaSDB, CaBDC, CaPDC and CaBTC) at an ultrahigh magnetic field of 21.1 T. It will be shown that ultrahigh-field ^{43}Ca SSNMR is a very sensitive probe of local structure about calcium in MOFs, and can be used to investigate changes in the calcium environment triggered by processes such as activation and guest adsorption.

[1] Z. R. Herm, J. A. Swisher, B. Smit, R. Krishna, J. R. Long, J. Am. Chem. Soc. 2011, 133, 5664-5667.
[2] Y. He, W. Zhou, G. Qian, B. Chen, Chem. Soc. Rev. 2014, 43, 5657-5678.

(4) Optimizing Fluorotryptophan-Based ^{19}F NMR to Probe Ligand-GPCR Interactions

Calem Kenward, Kyungsoo Shin, Muzaddid Sarker, Jan K. Rainey
Dalhousie University

The apelin receptor (AR) is a class A G-protein-coupled receptor (GPCR) activated by two peptide hormones, apelin and apela. A high gyromagnetic ratio and broad chemical shift range, alongside a lack of naturally occurring ^{19}F in proteins, allows for use of ^{19}F-labels to characterize response to folding, ligands, and other environmental factors. We have biosynthetically incorporated tryptophan 19F-labelled at the 4-, 5-, 6-, and 7-positions into AR. Solution-state NMR spectroscopy was used to probe conformation and dynamics of two portions of AR: the N-terminus and first transmembrane (TM) segment (residues 1-55, AR55) and the first three TM segments of AR (residues 1-137, AR

TM1-3). Our results demonstrate that ^{19}F NMR chemical shift, peak pattern and dynamics are highly variable, depending upon both the NMR conditions and the ^{19}F position on the indole ring. Perturbations in the ^{1}H-^{15}N HSQC spectra of AR segments were also apparent in a ^{19}F configuration-dependent manner, primarily localized to cross-peaks corresponding to residues proximal to the Trp. Addition of AR ligand peptides resulted in perturbations to the ^{1}H-^{15}N HSQC spectra, with corresponding ligand concentration-dependent changes in ^{19}F peak intensity. From this study, strategies are suggested for effective fluorotryptophan incorporation and application for ^{19}F NMR studies of membrane protein topology, structure, dynamics, and ligand binding.

(5) ^{27}Al Solid State NMR of Calcium-Aluminum Rich Inclusions (CAIs) from the Solar Nebula

Victoria Houde, Roberta Flemming, Viktor Terskikh, Audrey Bouvier
Western University

CAIs are some of the oldest materials in the solar system, thought to have condensed directly from the solar nebula. Carbonaceous chondrites (CCs) are some of the least altered solar system materials; these contain CAIs which can be relatively unchanged since their formation in the solar nebula. The composition of CAIs extracted from CCs, and the degree of cation order-disorder, can be used to study formation conditions in the early solar nebula. The minerals spinel ($MgAl_2O_4$), gehlenite ($Ca_2(Al,Mg)(Al,Si)O_7$), and fassaite ($Ca(Mg,Al)(Al,Si)_2O_6$) in CAIs from carbonaceous chondrites NWA 2364 and NWA 6991 are examined using ^{27}Al MAS NMR and 3QMAS NMR to look at the environment around the Al atoms, with the aim of deriving temperature information.

(6) Dissecting the Molecular Basis of Nrf2-p21 Interaction

Nadun Chanaka Karunatilleke, Anne Brickenden, Wing-Yiu Choy
Western University

Nuclear factor erythroid 2 - related factor 2 (Nrf2) is the major transcription factor that coordinates the cellular responses to oxidative and environmental stress. It also plays a critical role in tumorigenesis and chemo-resistance, making Nrf2 an attractive therapeutic target for cancer. p21 plays critical roles in cell cycle control, DNA replication and repair, and cell proliferation and apoptosis. Under oxidative stress, overexpressed p21 interacts with Nrf2, and protects it from ubiquitination and the subsequent proteasomal degradation, leading to the up-regulation of Nrf2-mediated cytoprotective gene transcription. The molecular basis of the p21-Nrf2 interaction remains unknown to date.

Initial nuclear magnetic resonance (NMR) titration experiments were performed to locate the p21 binding site in the Neh2 domain of Nrf2 by chemical shift mapping. Further NMR experiments will be conducted to identify residue-specific conformational changes in the Neh2 domain and p21 upon binding. Isothermal titration calorimetry experiments will then be performed to determine the stoichiometry and affinity of p21-Neh2 binding. The results will provide insights into identifying or designing molecules that selectively modulate Nrf2-p21 interactions.

(7) Methods of Structural Solution of Mixed-Linker Cadmium Imidazolate Frameworks

Jacqueline E. Gemus, Chris A. O'Keefe, and Robert W. Schurko
University of Windsor

Zeolitic Imidazole Frameworks (ZIFs) are a class of porous molecular frameworks consisting of divalent metal nodes that are joined together by imidazolate linkers,[1] and can be made using either one type of imidazolate or more than one type (i.e., a mixed-linker ZIF). The latter case has been shown to improve flexibility and selectivity in the molecular sorting properties of the ZIFs.[2] Mechanochemical synthesis (MS), a technique that offers quantitative yields with little or no solvent, is an established technique for generating single-linker ZIFs and may lead to novel mixed-linker ZIFs. A combination of powder X-ray diffraction (pXRD) and solid-state NMR (SSNMR) has been previously used to solve the structures of the products of MS, including single-linker frameworks. However, in the case of mixed-linker ZIFs, some of the SSNMR data is of diminished use due to peak overlap; the most promising and least ambiguous SSNMR data arises from observation of the metal node. I will present the results of a series of MS reactions intended to generate mixed-linker cadmium ZIFs, including the corresponding multinuclear SSNMR spectra (^{1}H, ^{13}C, ^{111}Cd) and pXRD data, as well as preliminary first-principles calculations of $^{111/113}Cd$ magnetic shielding tensors that may be used to develop a computational method for future structural solution.

[1] A. Phan et al. *Acc. Chem. Res. 2010, 43, 58*

[2] K. Eum et al. *J. Am. Chem. Soc. 2015, 137, 12*

(8) Epigenetic, structural, and functional characterization of the E2A-PBX1 oncogenic transcriptional network

Marina R. Lochhead, David N. Langelaan, Kyster Nanan, Holly L. Spencer, Steven P. Smith, David P. LeBrun
Queen's University

Acute lymphoblastic leukemia (ALL) is a cancerous disorder originating in B-lymphoid progenitor cells. In 5% of ALL cases, the oncogenic transcription factor E2A-PBX1 is expressed as a result of the somatic chromosomal translocation 1;19. E2A-PBX1 comprises three disordered N-terminal E2A transcriptional activation domains (AD) and the DNA-binding homeodomain of PBX1. These structural features suggest E2A-PBX1 recruits transcriptional co-activators by means of the AD and induces ALL through deregulation of PBX1 target genes. We have determined using NMR spectroscopy that the E2A AD1 interacts directly with the transcriptional co-activator p300 required for leukemogenesis. We have found that E2A-PBX1 does not localize to PBX1 recognition sequences and instead localizes to genomic sites bound directly by the master B-lymphopoietic transcription factors, E2A and EBF1. To this end, we have investigated the mechanism by which E2A-PBX1 associates physically with B-lymphopoietic enhancers. We have characterized interactions involving E2A/EBF1 and E2A-PBX1 using sequential-ChIP and NMR spectroscopy. We are discerning which genes are deregulated by E2A-PBX1 and the mechanisms by which this happens using knockdown studies. Our results illustrate a revised model of the role of E2A-PBX1 in ALL suggesting the formation of a multi-protein complex at lymphopoietic enhancers comprising E2A-PBX1, E2A/EBF1, and p300, which perturbs enhancer function and contributes to the development of ALL.

(9) Zeolite Structure Determination Using Solid-State NMR Experiments through the Application of Combinatorial Tiling Theory

Chelsey L. Hurst, Janelle G. Vander Hout, Darren H. Brouwer
McMaster University

A subset of zeolite materials that has been increasingly investigated for their diverse functionality are layered silicates, or 2D layered zeolites, which consist of discrete zeolite-like layers. When the layers do not periodically align structure determination for these 2D materials can be very difficult due to broadened x-ray diffraction peaks.

We are developing an approach to solve the structures of difficult-to-characterize network materials such as layered silicates that combines solid-state NMR (ssNMR) experiments with combinatorial tiling theory. We have applied combinatorial tiling theory, which involves tiling a 2D plane or 3D space with geometric shapes, to explore zeolite-like structures in the 2D plane toward its eventual application in 3D space. The relative intensities and connectivities of atoms in these network materials determined by 1D and 2D ssNMR experiments can be used to generate possible "D-symbols" which describe the connections between tiles, and thus atoms, in the plane. Each D-symbol uniquely encodes for a possible crystal structure. After systematically determining all possible crystal structures, the most likely structure can then be identified from x-ray diffraction and additional calculations. This combinatorial tiling approach to structure determination holds promise for directly solving crystal structures from ssNMR experiments in cases where diffraction data is quite limited.

(10) Conformational Arrangement of the E2-Ubiquitin Conjugate for Parkin Loading

Tara EC Condos, Gary S. Shaw
Western University

Autosomal recessive juvenile Parkinsonism (ARJP) is a hereditary form of Parkinson's Disease directly linked to mutations in the E3 ubiquitin-ligase parkin. Parkin mediates mitochondrial control in dopaminergic neurons by attaching a small ubiquitin (Ub) molecule to damaged proteins in a process called ubiquitination. Parkin activity is dependent on the binding of an E2-Ub conjugate, but it is currently unknown which conformational arrangement E2-Ub uses for binding. Conformations of the E2-Ub conjugate can be either open, closed or backbent. Recent studies by our group and others suggest that a phosphorylated-Ub (pUb) molecule activates parkin by allosterically revealing a pocket for E2-Ub to bind. The goal of this work is to determine which E2-Ub conformational arrangement pUb-

activated parkin recognizes.

We have used NMR with deuteration and selective-labelling to determine a mechanism for the 63 kDa complex of E2-Ub binding to pUb-activated parkin. CSP analysis was done from reciprocal titration experiments of activated parkin and E2-Ub samples to determine surfaces of interaction. Ubiquitination assays were performed to assess the effects of known ARJP mutations on parkin activity. Mutations that abolished parkin activity were proposed to be at the parkin and E2-Ub binding interface. Data will be processed with HADDOCK software to create a model of binding. We hypothesize that the UbcH7-Ub conjugate binds to activated parkin in an open conformation.

(11) Lipid Interactions with Anabaena Sensory Rhodopsin

Jeff de Vlugt, Meaghan Ward, David Bolton, Markus Weingarth, Vladimir Ladizhansky
University of Guelph

Anabaena sensory rhodopsin (ASR) is an integral membrane protein that inhabits the phospholipid bilayer of the freshwater cyanobacterium Anabaena. The utility of solid-state NMR spectroscopy for investigating the complex relationship between ASR and lipids is explored. Different motional regimes of lipid molecules are selectively excited using insensitive nuclear enhancement of polarization transfer (INEPT) and cross-polarization (CP). Spin systems in INEPT spectra are identified as lipid head groups and backbones, while peaks from CP spectra represent interactions between lipids and transmembrane helices. The super cycled post C-5 double quantum experiment was also conducted to resolve the degenerate chemical shifts of the lipid acyl tail. Solid-state NMR provides evidence that lipids are tightly bound to ASR, serving as a unique method for probing protein-lipid interactions at the atomic level.!

(12) Solid-State ^{17}O NMR and Computational Study of Epoxides

Andrew Rinald, Gang Wu
Queen's University

Epoxides are a biologically and synthetically important class of molecules. They are present in numerous biological hormones, notably in many juvenoids and insect sex pheromones, and are also important building blocks in organic synthesis, typically used in ring opening mechanisms to make regioselective ethers and alcohols. To date, no solid-state ^{17}O NMR investigations have been performed on epoxides leaving much unknown about the NMR spectroscopic properties of this unique ring structure. Using solid-state ^{17}O NMR spectroscopy, much can be learned about this unusual bonding situation, specifically information on the magnitude and orientation of the chemical shielding (CS) and quadrupole coupling (QC) tensors. Over the course of this investigation the sensitivity of the orientation of the CS and QC tensors to changes in the orientation of the substituents attached to epoxides was studied. This was done by synthesizing two symmetric epoxide-containing regioisomers and using computational as well as solid-state ^{17}O NMR methods to compare the orientations of their CS and QC tensors. In addition, extensive calculations were performed to computationally determine the angular dependence of ethereal ^{17}O CS and QC tensors using the simplest model ether and epoxide.

(13) Structural Characterization of the Interaction Between p53 and Plakoglobin

Qinyan Song, Jan K. Rainey, Paul X-Q Liu
Dalhousie University

The tumor suppressor p53 maintains genome intergrity and prevents oncogenesis. The core domain of p53 interacts with p53-specific promoters and promotes transcription of downstream genes. In addition to its DNA binding activity, the core domain also binds to numerous nuclear and cytoplasmic proteins. Plakoglobin, a catenin family protein, activates p53 through direct interaction between C-terminus of plakoglobin (plakoCTD) and the core domain of p53 (p53DBD). We successfully assigned the backbone chemical shift of plakoCTD and we demonstrated that a methione-rich motif of plakoCTD is involved in the interaction with p53DBD. Interestingly, plakoCTD cannot bind to near full-length p53, suggesting that N-terminus of p53 has a negative effect on plakoCTD binding. plakoCTD phosphor-mimic mutants showed enhanced binding affinity towards p53 DBD, suggesting post-translational modifications may play a role in this interaction. Further experiments are underway to fully define the binding surface of plakoCTD on p53DBD and to evaluate the structural transitions upon binding to p53DBD.

(14) Optimal Control of Frequency-Swept Pulses for the Acquisition of Ultra-Wideline Solid-State NMR Spectra

Adam R. Altenhof, Austin W. Lindquist, Lucas D.D. Foster, Robert W. Schurko
University of Windsor

Solid-state NMR (SSNMR) spectra often feature very broad patterns, which can range from hundreds of kHz to several MHz in breadth; those that exceed ca. 250 kHz are considered to be ultra-wideline (UWNMR) patterns. Since high-power rectangular pulses are generally insufficient for the excitation of UWNMR patterns, special techniques must be used for their acquisition.[1] Frequency-swept pulses are utilized for broadband excitation and refocusing, and are therefore useful in the acquisition of UWNMR spectra.[1] In particular, Wideband Uniform Rate Smooth Truncation (WURST) pulses have been widely used for acquiring UWNMR spectra of both spin-1/2 and quadrupolar nuclides; however, these pulses have limitations in terms of their excitation bandwidths and ability to produce distortion-free spectra.[2] In this work, we explore two new facets of pulses used in UWNMR spectroscopy: (i) the use of other frequency-swept pulses such as hyperbolic secant (HS) and tanh/tan (THT) for broadband excitation and refocusing, and (ii) the design of new broadband pulses via the use of optimal control theory (OCT).[3] In the first case, HS and THT pulses are tested both theoretically and experimentally on spin-1/2 and quadrupolar nuclides, and results are compared to those obtained from analogous WURST pulses. In the second case, new OCT Optimized Broadband Excitation and Refocusing (OCTOBER) pulses, are generated from WURST, HS, and THT pulses as starting points using OCT as implemented in the SIMPSON software package.[4] Different levels of performance, in terms of excitation and pattern uniformity, are explored.

(1) Schurko, R. W. *Acc. Chem. Res. 2013, 46, 1985.*
(2) O'Dell, L. A.; Schurko, R. W. *Chem. Phys. Lett. 2008, 464, 97.*
(3) Garwood, M.; Lance D. *J. Magn. Reson. 2001, 155-177.*
(4) Tosner, Z. et al. *J. Magn. Reson. 2009, 197, 120.*

(15) Stable Isotopic Labeling of Glycine using ^{17}O and its Incorporation into Yeast Ubiquitin

Binyang Lin, Holly L. Spencer, Steven P. Smith, Gang Wu
Queen's University

Oxygen is among the most abundant elements in biological molecules. It has only one naturally occurring NMR isotope, ^{17}O, at a very low abundance (0.037%). ^{17}O has a nuclear spin of 5/2 (known as quadrupolar), which usually results in broad ^{17}O NMR signals even for small organic molecules. However, recent studies have shown that either solid-state ^{17}O NMR or solution ^{17}O NMR for slowly tumbling large macromolecules is feasible for studying biological molecules. The objective of our research is to prepare ^{17}O-labeled proteins to be used for ^{17}O NMR studies. In this project, ^{17}O labels were first introduced into the –COOH groups of [15N]-glycine and [1-^{13}C]-glycine by acid-catalyzed hydrolysis using 40% enriched $H_2{}^{17}O$. The synthetic [^{13}C, ^{17}O]- and [^{15}N, ^{17}O]- glycines were then incorporated into yeast ubiquitin!

(16) Pores, Channels, and Cages: CO2 Locations and Dynamics in MOFs and Zeolites

Bryan E.G. Lucier,[1] Bowei Wu,[1] Paul D. Boyle,[1] Victor V. Terskikh,[2] and Yining Huang[1]
Western University(1), University of Ottawa(2)

Metal-organic frameworks (MOFs) and zeolites are porous materials capable of gas adsorption, however, their compositions are quite different. Both MOFs and zeolites have demonstrated the ability to adsorb the greenhouse gas CO_2 and are promising materials for applications in carbon capture. Variable-temperature ^{13}C solid-state NMR (SSNMR) spectroscopy provides rich information on the dynamics of guest CO_2 molecules, as well as their binding strengths to the MOF and zeolite hosts. SSNMR can also shed light on the specific CO_2 adsorption site locations. Combining the dynamic information available from SSNMR with complementary data from techniques such as X-ray diffraction (XRD) permits the formulation of comprehensive CO_2 motional models. This data is of particular importance for the design of MOFs and zeolites featuring higher CO_2 storage capacities and tunable adsorption strengths to address specific applications.

We describe our recent insights obtained using variable-temperature (VT) ^{13}C SSNMR spectroscopy and single-crystal XRD to investigate CO_2 adsorption locations, CO2 dynamics, and the effect of CO_2 adsorption on host structure in the small-pore microporous MOF α-zinc formate [$Zn_3(HCOO)_6$], the $M_2(bdc)_2DABCO$ (M = Cu, Ni, Zn,

Co) MOF, zeolite 13X exchanged with cations such as Li and K, the NaY zeolite, and zeolites 3A, 4A, and 5A. ^{67}Zn SSNMR results are also included.

(17) Calcium Binding to the Dysferlin C2A Domain

Yuning Wang
Western University

Dysferlin is a membrane repair protein involved in the trafficking of proteins and vesicles around injured membranes. It is composed of seven, intermittently spaced C2 domains (C2A-C2G) and a C-terminal trans-membrane helix. Mutations in any one of five C2 domains (C2A, B, D, E, G) can cause muscular dystrophy including limb girdle muscular dystrophy type 2B (LGMD 2B) and Miyoshi myopathy (MM). The calcium-binding property of C2A domain plays a regulatory role in dysferlin interaction with phospholipids and other proteins in membrane repair. In this project, the calcium-binding mode of dysferlin C2A is studied. NMR titration and ITC experiments show that C2A has at least three calcium-binding sites at the top loops of the domain, including two tight binding sites and one with lower affinity. Through site-directed mutagenesis, D16 and D18 are shown to be important for calcium coordination, and mutation of any of them can completely eliminate calcium binding to C2A, implying a cooperative relationship of the binding sites. With more studies to be done, calcium-binding to dysferlin C2A will be detailed to provide mechanistic understanding of calcium signaling in dysferlin-mediated membrane repair.

(18) The Acquisition of ^{2}H Solid-State NMR Spectra with Frequency-Swept Pulses

Lucas D. D. Foster, Austin W. Lindquist, Stanislav L. Veinberg, Robert W. Schurko
University of Windsor

Solid-state NMR (SSNMR) experiments on integer spin nuclei (i.e., I = 1) yield powder patterns that often appear as "Pake doublets". This appearance is due to (i) the dominant contributions of the first-order quadrupolar interaction, and (ii) overlapping patterns from the two single-quantum ($+1 \leftrightarrow 0$ and $0 \leftrightarrow -1$) transitions. ^{2}H SSNMR powder patterns can be as broad as 350 kHz, resulting in: (1) low signal-to-noise (S/N), (2) poor spectral resolution, and (3) spectral distortions when using conventional rectangular pulses to acquire these spectra. (1) and (2) have been addressed, in part, by using either quadrupolar-echo or CPMG pulse sequences with magic-angle spinning (MAS); however, these issues have not been soundly addressed for experiments on static samples. Frequency-swept (FS) pulses that can uniformly excite and refocus magnetization have permitted the acquisition of ultra-wideline (UW) NMR spectra[1,2] (i.e., spectra with breadths in excess of 250 kHz) of a variety of nuclides. Such pulses should be able to address all the aforementioned issues. I will report on the use of several techniques to directly and indirectly provide uniform excitation of ^{2}H SSNMR powder patterns, including explorations of WURST-CPMG and BRAIN-CP[3,4] experiments. Additionally, I will provide an analysis of the behaviour of the two single-quantum ($+1 \leftrightarrow 0$ and $0 \leftrightarrow -1$) transitions throughout the aforementioned pulse sequences and an explanation of how they influence the appearance of ^{2}H SSNMR powder patterns.

[1] L.A. O'Dell, Solid State NMR 2013, 55–56 (2013) 28–41.
[2] Schurko, R. W. Acc. Chem. Res. 2013, 46, 1985.
[3] O'Dell, L. A.; Schurko, R. W. Chem. Phys. Lett. 2008, 464, 97.
[4] Harris, K. J. et al. J. Magn. Reson. 2012, 224, 38.

(19) Comparison of HDX Protocols to Probe the Dynamics of Parkin

E. Aisha Freeman, Gary S. Shaw
Western University

Parkinson's disease (PD) is a neurodegenerative disorder that affects approximately 1 in 500 Canadians. Parkin encoded by PARK2 has been implicated in both sporadic and familial forms of PD. Parkin is an E3 ubiquitin ligase, catalyzing the final step of ubiquitin transfer to the protein designated for degradation. However, the series of conformational changes required for this ligase functionality have yet to be fully characterized. NMR Hydrogen Deuterium Exchange (HDX) can be used to probe a protein's conformational changes with residue specific resolution. A set of protocols needed to be compared for HDX in native conditions using a flexible yet stable domain of the

parkin protein; the RCat or required for catalysis of ubiquitin transfer domain. RCat was subjected to different methods of deuterated solvent introduction and NMR running temperatures. Results show that direct dilution at room temperature was the most optimal condition for parkin, however the best method will vary for other proteins.

(20) Examining the Adsorption of Small Molecules in the Cu(INAIP) MOF via Solid-State NMR

Mansheng Chen, Shoushun Chen, Yue Zhang, Bryan E.G. Lucier, Yining Huang
Western University

Metal-organic frameworks (MOFs) with high porosities, tunable properties, and customizable interior surfaces have shown great promise for applications in catalysis, chemical sensing, gas adsorption, and gas storage. The excellent gas adsorption performance of MOFs is typically attributed to host-gas attractive interactions arising from the incorporation of open metal sites or functional groups with strong binding sites. The amide group (-OCNH-) is of particular interest for MOF linkers because it possesses both the -CO- and -NH- functional sites, at which host-guest hydrogen bonding interactions can be established.

The Cu(INAIP) MOF lacks open metal sites, yet still exhibits excellent guest adsorption abilities due to the presence of uncoordinated amide groups. The framework is a three-dimensional four-fold interpenetrated network containing one-dimensional rhombic-shaped tunnels. The CH_4, C_2H_2 and CO_2 guest adsorption sites have been accurately located by single-crystal XRD methods. The types of adsorbed gas dynamics in Cu(INAIP), along with their associated motional rates and angles, have been investigated using in situ variable-temperature SSNMR experiments ranging from 133 to 393 K. Our combined SCXRD and SSNMR experimental data has proven that the amide group within this MOF can directly interact with gas molecules, and more specifically, that the carbonyl moiety of the amide group acts as the primary guest binding site in this system.

(21) An Investigation of Proton Conducting Sites in Solid State Proton Conductors

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Recent fuel cell development has targeted intermediate temperature fuel cells. These devices have fast start up times and are resistant to CO poisoning.[1] Solid electrolytes are more reliable than their aqueous counterparts as they can be employed over a broad temperature range (typically 100-400 °C) without flooding or drying.[1] Tin pyrophosphates are interesting electrolyte candidates because they contain no native protons. However, protons can be added synthetically through either excess H_3PO_4 or doping with lower valence cations.[2] Phosphate solid acids are another class of solid state proton conductors that have previously been used in the construction of a laboratory-scale fuel cell.[3] These materials are known to undergo superprotonic phase transitions resulting in significant increases in proton conductivity.[3] Site specific proton dipolar coupling was quantified in solid acid samples using dipolar recoupling NMR techniques. This technique and ^{31}P and ^{1}H-^{31}P NMR were used to investigate the dynamics of proton bearing phosphorous species in indium-doped tin pyrophosphate samples.

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2. Anfimova et al. Solid State Ionics, 2015, 278, 209-216
3. Haile, S.M. et al. Faraday Disc., 2007, 134, 17-39.

(22) Probing Nitrite Ion Dynamics in $NaNO_2$ Crystals by Solid-State ^{17}O NMR

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We report a solid-state ^{17}O (I = 5/2) NMR study of the nitrite ion dynamics in crystalline NaNO2. Variable temperature (VT) ^{17}O NMR spectra were recorded at three magnetic fields, 11.7, 14.1 and 21.1 T. The VT ^{17}O NMR data suggest that the NO_2^- ion in the ferroelectric phase of $NaNO_2$ undergoes two-fold flip motion about the crystallographic *b*-axis and the corresponding rotational barrier is 68 +/- 5 kJ mol^{-1}. We also obtained a 2D ^{17}O EXSY spectrum for a stationary sample of $NaNO_2$ at 250 K, which, in combination with 1D ^{17}O NMR spectral analyses, allowed precise determination of the relative orientation between the ^{17}O quadrupolar coupling and chemical shift tensors in the molecular frame of reference. The experimentally determined ^{17}O NMR tensors for $NaNO_2$ were in

agreement with quantum chemical calculations produced by a periodic DFT code BAND.

(23) Characterization of the MITF Transcription Factor and its Interaction with CBP/p300

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Transcription factors control gene expression and often coordinate fundamental processes such as cell growth and differentiation. The microphthalmia-associated transcription factor (MITF) is a melanocyte-specific protein essential for melanocyte development. MITF has also been identified as a lineage-specific oncoprotein in melanoma, where knockdown of MITF function results in cell senescence. MITF carries out its function by recruiting transcriptional co-regulators to gene promoters to modify gene transcription, including the homologous histone acetyltransferases CBP/p300. MITF contains N-terminal and C-terminal activation domains as well as a central basic helix-loop-helix DNA binding motif. Using a combination of pull-down experiments, NMR spectroscopy, biophysical studies, and functional assays we have determined that the N-terminal activation domain of MITF is intrinsically disordered in solution and interacts with the TAZ2 domain of CBP/p300 with high affinity. NMR-based titrations indicate that MITF also interacts with the KIX and TAZ1 domains of CBP/p300. These results support a model in which MITF may interact with multiple domains of CBP/p300 to activate transcription of MITF-target genes and provides insight as to how MITF may control gene expression in melanoma.

(24) Characterization of a Class IB Hydrophobin Structure by NMR Spectroscopy

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Hydrophobins are low molecular weight (5-20 kDa) self-assembling proteins secreted by fungi that accumulate at hydrophobic-hydrophilic interfaces and are extremely surface-active. Hydrophobins may undergo structural rearrangement and oligomerize to form rodlets, which are an insoluble functional amyloid that coats fungal spores to act as a water repellent, facilitate dispersal into the air, and prevent immune recognition. Due to their biochemical properties hydrophobins are a target for commercial applications, where they could be incorporated as biodegradable foam stabilizers or emulsifiers. To better understand what sequence characteristics determine hydrophobin properties, we are characterizing the structure and properties of a class IB hydrophobin from Serpula lacrymans (SlaHyd1). SlaHyd1 has only one charged amino acid, meaning it may have unusual properties compared to other hydrophobins. We expressed uniformly C/ N-labeled SlaHyd1 in E. coli and then purified it to homogeneity using Ni affinity and ion exchange chromatography. We then determined the high-resolution structure of SlaHyd1 using NMR spectroscopy. SlaHyd1 contains a four strand anti-parallel β-sheet that is connected by three loop sequences (L1-L3). The β-sheet folds upon itself to form a β-barrel-like structure, L1 contains an α-helix, L2 is a dynamic loop, and L3 is a three residue β-hairpin. Overall, the structure of SlaHyd1 is consistent with SC16, which is another class IB hydrophobin. This data indicates that class IB hydrophobins have a consistent three-dimensional structure despite having a variety of sequence properties and will form the basis of future mutagenesis experiments and examination of rodlet properties to further characterize these proteins.

(25) Deducing Adsorbed Gas Behaviour in Ultramicroporous Metal-Organic Frameworks using Solid-State NMR Spectroscopy

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Metal-organic frameworks (MOFs) are porous, crystalline materials that are of interest due to their demonstrated applications in the adsorption of gases. Ultramicroporous MOFs feature pores measuring less than 7 Å in diameter, and are known to possess strong selectivity towards the adsorption of CO_2 against the adsorption of nitrogen, hydrogen, and methane, despite the absence of open metal sites in many of these materials. To develop a comprehensive understanding of the adsorbed guest molecule locations, dynamics, and interactions with the framework, we have performed multinuclear solid-state nuclear magnetic resonance (SSNMR) studies of CO_2 and

D_2O adsorption within the ultramicroporous MOFs ZnAtzOx1 and SIFSIX-3-Zn.[2] Through the use of ^{13}C and ^{2}H SSNMR experiments, the types of motion, the motional rates, and the motional angles of $^{13}CO_2$ and D_2O adsorbed within these frameworks can be determined. The guests are shown to be nearly immobilized within the channels of both MOFs, and there is a relatively small change in motional parameters with temperature. Cross polarization, REDOR, and ^{67}Zn SSNMR experiments offer insight into the location of adsorbed CO_2 and its interactions with the framework atoms.

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(26) NMR Studies to Elucidate Unique Structural Elements in the HK97 gp74 HNH Endonuclease and Define Key Metal Binding Residues

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The last gene in the genome of the bacteriophage HK97 codes for gp74, an HNH endonuclease. HNH endonucleases digest DNA in the presence of metals and are characterized by two highly conserved His residues and an Asn residue. Gp74 is essential for phage head morphogenesis, as an intact HNH motif in gp74 is required for enhancement of terminase-mediated cleavage of the phage cos site. Mutation of the canonical metal binding His in the HNH motif abrogates gp74 mediated-enhancement of terminase activity. Notably, bioinformatic and biochemical studies indicate that involvement of HNH endonuclease in terminate-mediated packing of DNA into the phage head is common amongst tailed bacteriophages, such as HK97.

Although phages are widely studied, there is no definitive structural or mechanistic evidence as to how gp74 and gp74-related HNH proteins enhance activity of the phage terminate complex. Here report data from NMR spectroscopy showing that gp74 contains an insertion of two a-helices within the HNH motif. This insertion is seen in gp74-like proteins, but not other HNH endonuclease for which there is structural information. In addition, NMR studies have elucidated residues within gp74 required for metal binding and terminase activity. This work will identify how metal binding to gp74 is crucial in the replication and morphogenesis of phages.

(27) Multinuclear Solid-State NMR Spectroscopy of Halide Salt Cocrystals

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The rational design of multi-component single-phase materials known as cocrystals is a flourishing area in crystal engineering. Cocrystals have distinct properties in comparison to their pure constituent components (e.g., solubility, stability, etc.)[1,2] providing a means of tailoring the physicochemical properties of a crystalline solid form. As such, there has been considerable recent interest in the synthesis of cocrystalline forms of active pharmaceutical ingredients (APIs), for use in dosage formulations. While there have been numerous reports of the synthesis of API cocrystals, there are relatively few papers describing rational syntheses or mechanisms of cocrystal formation. Solid-state NMR (SSNMR) is well suited for studying the formation of cocrystals, since it is sensitive to the local structural changes that result from the intermolecular interactions in cocrystals (e.g., hydrogen bonding).[3-5] In this poster, we present a multinuclear (^{35}Cl, ^{23}Na, ^{13}C, ^{2}H, and ^{1}H) SSNMR study of NaCl:Urea:Hydrate cocrystals made via mechanochemical syntheses. SSNMR, in tandem with pXRD, thermogravimetric methods and synthetic optimization, allowing for the identification of distinct cocrystalline phases, the detection of impurities, and the potential monitoring of mechanisms of cocrystal formation. Characterization of these simple model systems, accompanied by NMR crystallographic characterization via quantum chemical computations,[6] will develop a methodological framework for future studies of increasingly complex cocrystals of APIs and pharmaceutically acceptable coformers.

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[6] Chierotti, M.R.; Gobetto, R. *CrystEngComm, 2013, 15, 8599.*

(28) The Drug Discovery Platform (DDP) of the Research Institute of the McGill University Health Center (RIMUHC): Discover and Develop Next-Generation Diagnosis, Prognosis and Therapeutic Approaches

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Drug Discovery Platform – Research Institute of the McGill University Health Centre

The DDP is a state-of-the-art facility based in Montréal, QC providing structure-based drug design, and metabolomics analysis by solution and High Resolution Magic Angle Spinning (HR-MAS) NMR spectroscopy, together with liquid-chromatography mass spectrometry (LC-MS). Our services are offered to local and international academic and industrial researchers. With drug discovery expertise and analytical instrument facilities, the platform identifies and validates promising new biological and chemical entities (including biomarkers) for life-threatening diseases, and provides access and training to users on a broad range of cutting-edge technologies such as NMR spectroscopy (600 and 400 MHz) equipped with HR-MAS and cryoprobes, along with LC-MS and MALDI-TOF/TOF MS. These technologies which are available at the platform will be presented in this poster.