Abstract Booklet: Oral Presentations

Sept. 29 – Oct. 1, 2017

#	Start Time/Day	Presenter	Presentation Title	Page
1	9:20/Saturday	Lawrence McIntosh	An NMR Spectroscopist's Perspective of Transcriptional Regulation	1
2	10:05/Saturday	Danielle Smiley	Combining Ex Situ Na-23 Solid-State NMR and DFT to Study Polyanionic Cathode Materials for Sodium Ion Batteries	1
3	10:25/Saturday	Jinhui Zhu	S-Nitrosylation Suppresses Stromal Interaction Molecule-1 Activation by Stabilization of the Calcium Sensing Domains	1
4	11:10/Saturday	Andrew Rinald	Determination of ^{17}O NMR Tensors in Epoxides	2
5	11:30/Saturday	Rashik Ahmed	Molecular Mechanism for the (-)-Epigallocatechin Gallate-Induced Toxic to Nontoxic Remodeling of the Alzheimer's Disease Aβ Oligomers	2
6	11:50/Saturday	Austin Peach	^{35}Cl Solid-State NMR Spectroscopy of Fluoxetine HCl Cocrystals Synthesized Using Solvothermal and Mechanochemical Methods	3
7	12:10/Saturday	Karen Dunkerley	Structure of Phosphorylated UBL Domain and Insights into PINK1-Orchestrated Parkin Activation	3
8	14:10/Saturday	Blossom Yan	Understanding Proton Transport in Perfluorosulfonic Acid Membranes via Solid-State NMR	3
9	14:30/Saturday	Alison Upsdell	Solution Characterization of the Cohesin-Dockerin Dual Binding Mode: A High Affinity Protein-Protein Interaction Critical for Cellulosome	4
10	14:50/Saturday	Jason Zhang	Studying Metal-Organic Frameworks via Solid-State NMR: Structure Determination, Host-Guest interactions, and Gas Dynamics	4
11	15:10/Saturday	Daryl Good	Solid-State NMR Provides Evidence for Small-Amplitude Slow Domain Motions in a Multi-Spanning Transmembrane α-Helical Protein	5
12	9:30/Sunday	Robert Schurko	NMR Across the Periodic Table: Observing "Invisible" Nuclei in Organic, Inorganic and Organometallic Materials	5
13	10:15/Sunday	Teklab Gebregiworgis	Multiplexed Real-Time NMR GEF Assay to Measure Full Length GEFs Activity from Cells and Organoids	5
14	10:35/Sunday	Pierre Maisonneuve	Deciphering the Molecular Mechanism of RAF Family Dimerization and Regulation	6
15	11:20/Sunday	Chris Kirby	Non-Destructive Fatty Acid Profiling of Seeds by ^{1}H HRMAS	6
16	11:40/Sunday	Bryan VanSchouwen	Dynamical Basis of cGMP-vs.-cAMP Selectivity of Protein Kinase G	6
17	12:00/Sunday	Sean Holmes	NMR-Driven Refinements of Crystal Structures Using Dispersion-Corrected Density Functional Theory	7

(1) An NMR Spectroscopist's Perspective of Transcriptional Regulation

Lawrence McIntosh
University of British Columbia

Transcription factors, such as those of the ETS family, translate the genetic information of an organism by binding promoter/enhancer DNA, recruiting additional components of the transcriptional machinery, and responding to signaling pathways. The aberrant activities of these factors, or their upstream regulators, frequently leads to dysregulated gene expression and oncogenesis. Therefore, a major goal of our research program is to elucidate the molecular mechanisms underlying the control of ETS factors by DNA-binding auto-inhibition, protein partnerships and post-translational modifications. As will be exemplified in this talk, NMR spectroscopy is a powerful experimental tool for these studies, providing critical insights into the structures, dynamics, and interactions of the ETS factors and their DNA complexes..

(2) Combining Ex Situ Na-23 Solid-State NMR and DFT to Study Polyanionic Cathode Materials for Sodium Ion Batteries

Danielle L. Smiley, Dany Carlier, Gillian R. Goward
McMaster University

A combination of ^{23}Na NMR and DFT was implemented here in the investigation of Na_2FePO_4F, a potential Na ion battery cathode material.[2] With the help of DFT, the spectroscopically observed sites are assigned to the two crystallographic sites in the structure based on the degree and mechanism of transfer of unpaired electron spin density from the Fe d-orbitals to Na sites. Two-dimensional EXSY experiments reveal limited Na-Na exchange between the Na(1) and Na(2) sites, consistent with electrochemical observations.

Ex situ NMR measurements during the electrochemical charge and discharge cycles uncovers the coexistence of two distinct phases at all states of charge, indicative of an inherent biphasic desodiation method. These experimental results are corroborated with ab initio calculations of intermediate $Na^{1+}_xFePO_4F$ states, where this phase separation is energetically feasible. The persistence of this biphasic behaviour after 40 cycles implies a preservation of the framework structure and demonstrates the robust nature of the fluorophosphate network. This work demonstrates the effectiveness of a combined DFT and NMR approach in the study of paramagnetic cathode materials for Na ion battery applications.

(1) Ellis, B. L.; Makahnouk, W. R. M.; Rowan-Weetaluktuk, W. N.; Ryan, D. H.; Nazar, L. F. *Chem. Mater. 2010, 22 (3), 1059.*

(2) Smiley, D. L.; Goward, G. R. C*hem. Mater. 2016, 28 (21), 7645*.

(3) S-Nitrosylation Suppresses Stromal Interaction Molecule-1 Activation by Stabilization of the Calcium Sensing Domains

Jinhui Zhu, Xiangru Lu, Qingping Feng and Peter B. Stathopulos
Western University

Stromal interaction molecule 1 (STIM1) is an endo/sarcoplasmic reticulum (ER/SR) calcium (Ca^{2+}) sensor that activates store-operated Ca^{2+} entry (SOCE) following Ca^{2+} depletion. SOCE-induced elevation of cytosolic Ca^{2+} stimulates the calcineurin/nuclear factor of activated T cells (NFAT) pathway, which upregulates pro-hypertrophic gene transcription. Nitric oxide (NO) regulates diverse protein functions by S-nitrosylation, but the effects of NO on STIM1 activation are unknown. Here, we find that STIM1 S-nitrosylation inhibits SOCE and hypertrophy in cardiomyocytes. Further, we reveal that STIM1 S-nitrosylation by S-nitrosoglutathione (GSNO) results in luminal Ca^{2+} sensing domain stabilization, reduced oligomerization, enhanced rigidification, and suppressed hydrophobic exposure upon Ca^{2+}-depletion. Using solution nuclear magnetic resonance spectroscopy we demonstrate that the structural perturbations caused by S-nitrosylation are largely localized near the Cys49 and Cys56 modification sites. Further, paramagnetic relaxation enhancement (PRE) revealed that the STIM1 region containing these Cys residues transiently interacts with the core Ca2+ sensing EF-SAM domain of the protein. Remarkably, Cys-independent

mutations based on these PRE observations suppress Ca2+ sensitivity in vitro and inhibit SOCE in mammalian cells independent of NO exposure. Collectively, our data provide a structural and biophysical basis for S-nitrosylation-mediated SOCE inhibition.

(4) Determination of ^{17}O NMR Tensors in 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.

(5) Molecular Mechanism for the (-)-Epigallocatechin Gallate-Induced Toxic to Nontoxic Remodeling of the Alzheimer's Disease Aβ Oligomers

Rashik Ahmed, Bryan VanSchouwen, Naeimeh Jafari, Xiaodan Ni, Joaquin Ortega, Giuseppe Melacini
McMaster University

(−)-Epigallocatechin gallate (EGCG) effectively reduces the cytotoxicity of the Alzheimer's disease β-amyloid peptide (Aβ) by remodeling seeding-competent Aβ oligomers into off-pathway seeding-incompetent Aβ assemblies. However, the mechanism of EGCG-induced remodeling is not fully understood. Here we combine ^{15}N and ^{1}H dark-state exchange saturation transfer (DEST), relaxation, and chemical shift projection NMR analyses with fluorescence, dynamic light scattering, and electron microscopy to elucidate how EGCG remodels Aβ oligomers. We show that the remodeling adheres to a Hill–Scatchard model whereby the Aβ(1–40) self-association occurs cooperatively and generates Aβ(1–40) oligomers with multiple independent binding sites for EGCG with a Kd ~10-fold lower than that for the Aβ(1–40) monomers. Upon binding to EGCG, the Aβ(1–40) oligomers become less solvent exposed, and the β-regions, which are involved in direct monomer–protofibril contacts in the absence of EGCG, undergo a direct-to-tethered contact shift. This switch toward less engaged monomer–protofibril contacts explains the seeding incompetency observed upon EGCG remodeling and suggests that EGCG interferes with secondary nucleation events known to generate toxic Aβ assemblies. Unexpectedly, the N-terminal residues experience an opposite EGCG-induced shift from tethered to direct contacts, explaining why EGCG remodeling occurs without release of Aβ(1–40) monomers. We also show that upon binding Aβ(1–40) oligomers the relative positions of the EGCG B and D rings change with respect to that of ring A. These distinct structural changes occurring in both Aβ(1–40) oligomers and EGCG during remodeling offer a foundation for understanding the molecular mechanism of EGCG as a neurotoxicity inhibitor. Furthermore, the results reported here illustrate the effectiveness of DEST-based NMR approaches in investigating the mechanism of low-molecular-weight amyloid inhibitors.

(6) ^{35}Cl Solid-State NMR Spectroscopy of Fluoxetine HCl Cocrystals Synthesized Using Solvothermal and Mechanochemical Methods

Austin Peach, David Hirsh, Sean Holmes, Robert Schurko
University of Windsor

A significant concern for the pharmaceutical industry is the development of methods for improving the physicochemical properties (e.g., stability, solubility, bioavailability) of solid-state active pharmaceutical ingredients (APIs). Often, alternate solid forms of an API (e.g., salts, hydrates, and solvates) are synthesized to access the desired physicochemical properties. API cocrystals (i.e., new solid forms where the API is cocrystallized with a pharmaceutically acceptable coformer to produce a single solid phase) have gained interest in both the crystal engineering community and pharmaceutical industry. Childs et al. demonstrated that fluoxetine HCl, the active ingredient in the antidepressant Prozac, can cocrystallize via slow evaporation with three carboxylic acid coformers: benzoic acid, fumaric acid, and succinic acid.[1] Structural characterization of these cocrystals is crucial for understanding their mechanisms of formation, solid-state properties, and further development of other novel solid-state forms. As a proof-of-concept study, ^{35}Cl solid-state NMR (SSNMR) is used as a structural probe of the chloride anions in the fluoxetine HCl cocrystals. Chloride ions are crucial for the formation and stabilization of a complex network of hydrogen bonding interactions between the API and the three carboxylic acids. ^{35}Cl SSNMR is shown to be able to readily distinguish between the various cocrystals of the API. Further, quantum chemical calculations are used in tandem with the ^{35}Cl SSNMR measurements to elucidate the relationship between NMR observables and crystal structures. Additionally, we explore an alternative method for cocrystal formation via mechanochemical synthesis, and demonstrate the superiority of our new synthetic methods for fluoxetine HCl cocrystal formation over conventional recrystallization methods.

(1) Childs, S. L.; Chyall, L. J.; Dunlap, J. T.; Smolenskaya, V. N.; Stahly, B. C.; Stahly, G. P. *J. Am. Chem. Soc. 2004, 126 (41), 13335*.

(7) Structure of phosphorylated UBL domain and insights into PINK1-orchestrated parkin activation

Karen M. Dunkerley, Jake D. Aguirre, Pascal Mercier, Gary S. Shaw
Western University

The E3 ubiquitin (Ub) ligase parkin and serine/threonine kinase PINK1, are both are genetically linked to development of early onset Parkinson's disease. PINK1 phosphorylates both parkin's UBL (pUBL) and ubiquitin (pUb) to activate parkin's ubiquitination function through loss of autoinhibition and pUb binding, leading to mitochondrial turnover or mitophagy.

We hypothesized that phosphorylation of parkin's UBL domain at S65 causes a conformational change in the UBL structure that weakens the autoinhibitory interaction. The solution structure of pUBL was solved using multiple 2D and 3D NMR experiments, including amide exchange and ^{3}J HNHA couplings, to fully assign the structure of the phosphorylated UBL. Compared to UBL, pUBL had a conformational change directly upstream of pSer65 with the loss of a structural hydrogen bond and formation of a one-turn helix. The effect of phosphorylation was most pronounced in the surface electrostatics.

Since parkin activation is also achieved through binding of pUb, T_2 relaxation experiments were used to determine the importance of phosphorylation of UBL and binding of pUb in the context of full-length parkin. These studies revealed that phosphorylation of parkin alone is not sufficient for release of autoinhibition and it is the binding of pUb that fully activates parkin.

(8) Understanding Proton Transport in Perfluorosulfonic Acid Membranes via Solid-State NMR

Z. Blossom Yan, Alan P. Young, Gillian R. Goward
McMaster University

To understand the molecular physicochemical properties of perfluorosulfonic acid (PFSA) materials, local dynamics studies using double quantum recoupling ssNMR spectroscopy have been recently developed and validated.[1] The local dynamics information has been analyzed with respect to different temperatures and hydration

levels. The polymer side chain is proven to be more locally mobile which is reflected by the lower apparent dipolar coupling constant (Dapp) compared to the backbone. This observation agrees with the micro-phase separation morphology development. The dynamics investigation of different PFSA materials has been conducted at various conditions. In operando membrane performance analyses have been obtained at Ballard Power Systems. PFSA membranes have been tested in the membrane electrode assembly form. From the cyclic voltammetry measurements, the H_2 crossover values are extracted. There is a strong correlation between the H2 permeability and the PFSA side chain local dynamics from the NMR analysis. As the side chain mobility increases (lower Dapp), and increase in the H_2 permeability is observed. The link between the fundamental dynamics study and the PFSA performance analysis provides insight into gas transport mechanisms in this type of material.[2]

References:
[1] Yan, Z. B. et al. *Macromolecules, 2016, 49 (19), 7331-7339.*
[2] Schalenbach, M. et al. *J. Phys. Chem. C 2015, 119, 25145-25155.*

(9) Solution Characterization of the Cohesin-Dockerin Dual Binding Mode: A High Affinity Protein-Protein Interaction Critical for Cellulosome

Alison L. Upsdell, Holly L. Spencer, David N. Langelaan, Steven P. Smith
Queen's University

The anaerobic bacterium Clostridium thermocellum efficiently hydrolyzes lignocellulose via a multi-enzyme complex, termed the cellulosome. Various carbohydrate-active enzymes graft onto a scaffoldin through a high-affinity interaction between the enzyme-borne type-I dockerins and scaffoldin-borne type-I cohesins for complex assembly. The dockerins comprise a duplicated 22-residue sequence and a corresponding structural symmetry, which suggests the capability of contacting cohesin in two orientations 180° apart. X-ray crystallographic studies revealed both binding modes are possible but substituting the recognition determinants Ser45 and Thr46 with alanine residues was required. A dual binding mode has not been shown for wild-type dockerins in solution with our NMR data thus far demonstrating that both wild-type bound conformations occur simultaneously and equally. The mutant spectra of Ser11Ala/Thr12Ala and Ser45Ala/Thr46Ala dockerin select for the two orientations and display a subset of resonances influenced by cohesin, in which these residues are collectively affected for bound wild-type dockerin. Wild-type resonances are intermediate between those of the mutant dockerin modules, indicative of an average of the two populations and thus suggestive of a dual binding mode. The possibility of two binding orientations overcomes the steric hindrance inherent to assembly and this interaction can be utilized as a stable foundation for different catalytic nanomachines.

(10) Studying Metal-Organic Frameworks via Solid-State NMR: Structure Determination, Host-Guest interactions, and Gas Dynamics

Jason Zhang, Bryan E. G. Lucier, Sarah M. Mckenzie, Mihails Argangelskis, Tomislav Friščić, Joel Reid, Victor V. Terskikh, Yining Huang
Western University

Metal-organic frameworks (MOFs) are a class of microporous materials composed of metal centers or metal clusters connected by organic ligands. MOFs usually exhibit high surface areas and are well-suited for gas capture. Solid-state NMR (SSNMR) is a powerful characterization technique, since SSNMR experiments are sensitive to the short-range environment, and can provide element-specific atomic-resolution insight into materials. Therefore, SSNMR can be used to determine the formation of MOFs, prove MOF structures, study host-guest interactions, and reveal guest dynamics.

In this work, SSNMR experiments have been investigated to study the newly synthesized Ga- and In-fumarate MOFs (A520). 1H and 1H-13C CP MAS SSNMR experiments have been employed to study the formation of MOFs. Ultra-wideline 69/71Ga and 115In SSNMR experiments at 21.1 T were investigated to study the short-range environments of metal centers. 69/71Ga SSNMR spectra also provide great support to the Rietveld refined structure of Ga-A520. 13CO2 adsorption within A520 MOFs have been studied by acquiring static 13C and 1H-13C CP SSNMR experiments, which successfully revealed CO2 adsorption sites and CO2 dynamics within A520 MOFs. In addition to carbon dioxide, hydrogen and methane adsorbed in different MOF systems also have been studied by 2H SSNMR experiments. The information about gas dynamics and host-guest interactions have been successfully revealed in our

work.

(11) Solid-State NMR Provides Evidence for Small-Amplitude Slow Domain Motions in a Multi-Spanning Transmembrane α-Helical Protein

Daryl Good[1], Charlie Pham[1], Jacob Jagas[1], Józef R. Lewandowski[2], and Vladimir Ladizhansky[1]
University of Guelph(1), University of Warwick(2)

We use solid-state NMR relaxation rate and dipolar recoupling measurements to probe the backbone motions of the membrane protein Anabaena Sensory Rhodopsin (ASR). ASR is a seven transmembrane (7TM) alpha helical photosensor which interacts with its cytoplasmic transducer (ASRT) in a light-dependent manner. An absoprion of light results in the release of ASRT, which interacts with DNA, and likely regulates the expression of genes of several light-harvesting proteins. Here we report 15N R1 and R1ρ relaxation rates measured at two temperatures (7 °C and 30 °C) and at two magnetic fields (600MHz and 800MHz 1H frequency). We observed an order of magnitude variation in the R1 and R1ρ relaxation rates between the transmembrane (TM) regions and the interhelical loop regions of ASR. Qualitative analysis of the field and temperature dependences of the R1 and R1ρ relaxation times shows evidence of motions on two timescales, which we modeled as fast local motions and slower (nanosecond) collective motions. Combining relxation data sets with the order parameters from dipolar recoupling experiments we estimated the amplitudes and time scales of the two types of motions. Fast local motions occurring on the picosecond timescale were found to be of similar amplitude throughout the protein. In contrast, slow nanosecond collective motions were found to be of small amplitude in the center of the TM helices, and increasing towards the cytoplasmic end of the TM helices and in the interhelical loop regions. Larger amplitudes of motions on the cytoplasmic side of helices correlates with the ability of ASR to undergo large conformational changes in the process of binding/unbinding ASRT.

(12) NMR Across the Periodic Table: Observing "Invisible" Nuclei in Organic, Inorganic and Organometallic Materials

Robert W. Schurko
University of Windsor

Recent developments in pulse sequences and NMR hardware have opened up many "exotic" nuclides in the periodic table to experimentation by solid-state NMR. Many of these nuclides are classified as unreceptive, and have been avoided by NMR spectroscopists and chemists in general, due to factors such as low Larmor frequencies, low natural abundances, inconveniently short or long relaxation times, etc. In addition, there are numerous systems in which these nuclides have extremely broad NMR patterns resulting from large anisotropic chemical shielding or quadrupolar interactions. Such nuclei have long been classified as "invisible", since their NMR spectra cannot be observed using standard NMR pulse sequences. In this lecture, I will show that there are several robust strategies one can apply to acquire high quality solid-state NMR spectra of a variety of nuclei, including 10B, 14N, 27Al, 35/37Cl, 47/49Ti, 59Co, 63/65Cu, 69/71Ga, 91Zr, 93Nb, 139La, 195Pt, and 209Bi. Ultra-wideline NMR spectra, when coupled with X-ray crystallography, NQR spectroscopy, and ab initio methods, provide powerful probes of molecular structure in inorganic, organic and organometallic materials. New advances in dynamic nuclear polarization (DNP) NMR for the acquisition of ultra-wideline NMR spectra will also be discussed.

(13) Multiplexed Real-Time NMR GEF Assay to Measure Full Length GEFs Activity from Cells and Organoids

Teklab Gebregiworgis, Christopher B. Marshall, Tadateru Nishikawa, Nikolina Radulovich, Maria Jose Sandi, Rob Rottapel, Ming Tsao and Mitsuhiko Ikura
University Health Network – Princess Margaret Cancer Centre

Small GTPases are critical regulators that mediate several important cellular functions. These proteins act in a switch-like fashion, turning ‚Äòon‚Äô and ‚Äòoff‚Äô when bound with GTP and GDP respectively. The activation of these proteins is mediated by Guanine nucleotide Exchange Factors (GEFs), which catalyze exchange of GTP for GDP. A growing body of research indicates that the signaling that involves small GTPases is complex and multiplex;

however most of the commonly available GTPase activation assays do not reveal such complexity. Our presentation will introduce and demonstrate a unique real-time NMR-based multiplexed GTPase activation assay to simultaneously monitor full-length GEF activity for Kras, Rheb, RalB, RhoA, Cdc42 and Rac1 in a single system.

(14) Deciphering the Molecular Mechanism of RAF Family Dimerization and Regulation

Pierre Maisonneuve, Hugo Lavoie, Malha Sahmi, Sara Marullo, Neroshan Thevakumaran, Ting Jin, Marc Therrien, Frank Sicheri

Lunenfeld-Tanenbaum Research Institute

RAF kinases serve as functional relays within the RAS-RAF-MEK-ERK (RAS/ERK cascade). Unbridled signaling through the RAS/ERK pathway by activating mutations in RAS and RAF has emerged as a major driver of tumor formation (30% of all cancers). The RAF family includes three kinase-active isoforms ARAF, BRAF, CRAF and two pseudo-kinase isoforms KSR1 and KSR2. The active state of the RAF kinases is achieved by adoption of a dimer configuration of their kinase domains. RAF family members assemble into distinct homo- or heterodimers the biological relevance of which is newly emerging.

We have discovered a new allosteric mechanism driving RAF family dimerization leading to the activation of catalytic output. We found that in addition to the well-characterized ability of the kinase domains to dimerize, other domains also participate in the dimerization phenomenon. Using Nuclear Magnetic Resonance, we have determined the atomic basis of these new interactions and confirmed their functional relevance both in vitro and in cells.

(15) Non-Destructive Fatty Acid Profiling of Seeds by ^{1}H HRMAS

M.B. Fischer, Chris Kirby

Agriculture and Agri-Food Canada

The fatty acid profiles of intact flax seeds have been determined using ^{1}H HRMAS NMR. The viability of the seeds after spinning (up to 10 kHz) was the same as non-spun seeds determined by a germination test. An average of the individual seed results were compared to a rotor filled with seeds, showing no significant difference in the fatty acid profile determination. However, a comparison between the HRMAS results with those of the usual solution ^{1}H NMR method (oil extraction of seeds with a non-polar solvent) revealed minor discrepancies. Thus, the HRMAS experimental parameters (acquisition and processing) were investigated to determine the cause of these discrepancies.

(16) Dynamical Basis of cGMP-vs.-cAMP Selectivity of Protein Kinase G

Bryan VanSchouwen, Rajeevan Selvaratnam, Rajanish Giri, Robin Lorenz, Friedrich W. Herberg, Choel Kim, Giuseppe Melacini

McMaster University

Protein kinase G (PKG) is a major protein involved in eukaryotic cyclic GMP (cGMP) dependent intracellular signaling, playing a regulatory role in such processes as cell differentiation, platelet activation, memory formation and vasodilation. Notably, the signaling pathways controlled by PKG are often distinct from those regulated by cyclic AMP (cAMP), and so the selective activation of PKG by cGMP rather than cAMP is critical. However, the mechanism of cGMP-vs.-cAMP selectivity in PKG is only limitedly understood. Indeed, although the C-terminal cyclic-nucleotide-binding domain of PKG (CNB-B) binds cGMP with higher affinity than cAMP, the intracellular concentrations of cAMP are typically higher than those of cGMP, suggesting that the cGMP-vs.-cAMP selectivity of PKG is not controlled uniquely through affinities. Here, we show that cAMP is a partial agonist for PKG, and we elucidate the mechanism of cAMP partial agonism through comparative NMR analysis of the apo, cGMP- and cAMP-bound forms of PKG CNB-B. We show that although cGMP-activation is adequately explained by a two-state conformational selection model, the partial agonism of cAMP arises from the sampling of a third conformational state in which autoinhibition is partially still effective.

(17) NMR-Driven Refinements of Crystal Structures Using Dispersion-Corrected Density Functional Theory

Sean T. Holmes, Robert Schurko
University of Windsor

Solid-state nuclear magnetic resonance (SSNMR) spectroscopy is a leading technique for structure elucidation. The relationship between NMR observables and molecular-level structure can be interpreted through first-principles calculations, typically performed in the framework of density functional theory (DFT). When SSNMR spectroscopy and computational approaches are used together with diffraction methods, NMR crystallography can provide unrivaled insight into structure. When solids contain cations or anions, analysis of the NMR quadrupolar lineshape reveals essential insights into crystal packing.[1] This talk focuses on the use of quadrupolar SSNMR parameters in the refinement of crystal structures of organic solids. The role of dispersion forces on DFT-based structural refinements of crystals is assessed, especially as they relate to the prediction of nuclear electric field gradient (EFG) tensors. Quadrupolar NMR parameters obtained from experimental NMR studies are used to parameterize a common dispersion force field,[2] such that the resulting NMR-driven dispersion-corrected DFT refinements are able to yield consistently reliable predictions of EFG tensors in of organic solids.[3] For the prediction of ^{35}Cl EFG tensor parameters in particular, the optimization protocols described here lead to a substantial improvement in agreement with experiment relative to structures obtained by X-ray or neutron diffraction methods.

1. Hamaed, H.; Pawlowski, J.M.; Cooper, B.F.T.; Fu, R.; Eichhorn, S.H.; Schurko, R.W., *J. Am. Chem. Soc. 2008, 130, 11056-11065*.
2. Grimme, S., *J. Comput. Chem. 2006, 27, 1787-1799*.
3. Holmes, S.T.; Iuliucci, R.J.; Mueller, K.T.; Dybowski, C., *J. Chem. Phys. 2017, 146, 064201*.