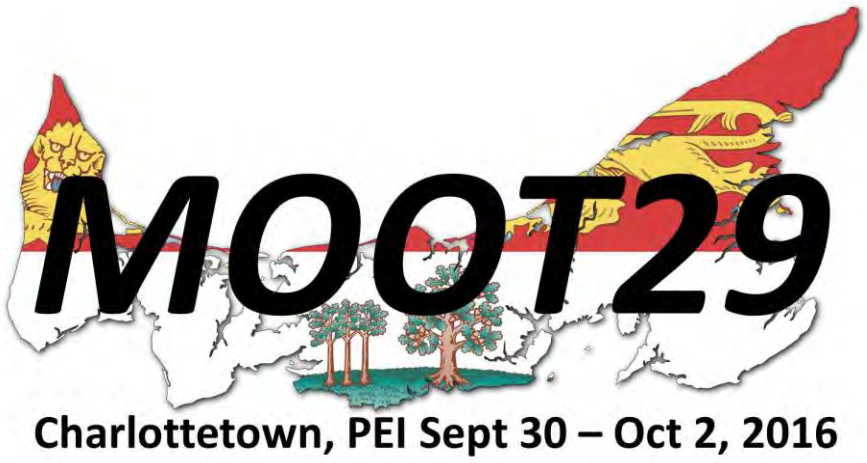


Friday, September 30th

19:00 – 21:00 **Mixer & Pre-Registration: Upstreet Craft Brewing (41 Allen Street)**

Saturday, October 1st

8:00 – 9:00 **Registration and Poster Setup: Best Western Cavendish and Brackley Rooms**

Session I: Best Western Stanhope Rooms A&B
Chair: Gillian Goward

9:00 – 9:15 **Opening Remarks**

9:15 – 10:00 **Plenary Lecture**

The Wide World of Magnetic Resonance

Bruce J. Balcom
University of New Brunswick

10:00 – 10:25 **Wideline NMR Spectroscopy of Unconventional Nuclei in Oxide Glasses**
A.L. Paterson, J. Sinclair, U. Werner-Zwanziger, J.W. Zwanziger
Dalhousie University

10:25 – 10:45 **Refreshment Break**

10:45 – 11:10 **Isoform-dependent membrane binding preferences differ between apelin and apela, cognate peptide ligands for the same GPCR**
Kyungsoo Shin, Muzaddid Sarker, Shuya K. Huang, and Jan K. Rainey
Dalhousie University

11:10 – 11:35 **Deuteration of *Vibrio splendidus* lipid membranes and initial characterisation by *in vivo* ^{2}H solid-state NMR**
Zeineb Bouhlel[1,2], Dror E. Warschawski[3], Alexandre A. Arnold[1], Réjean Tremblay[2] and Isabelle Marcotte[1]
[1]Université du Québec à Montréal, [2]Université du Québec à Rimouski, [3]Centre National de la Recherche Scientifique (Paris)

11:35 – 12:00 **Isolation and Structural Characterization of New Oximidine Compounds**
Mark H. Nabuurs, Susan Boyetchko, Jason L. McCallum and Christopher W. Kirby
University of Prince Edward Island and *Agriculture and Agri-Food Canada*

12:00 – 13:30 **Lunch Break: Best Western Stanhope Rooms A&B**

Session 2: Best Western Stanhope Rooms A&B
Chair: Isabelle Marcott

13:30 – 13:55 **Quadrupole Central Transition NMR Spectroscopy of Quadrupolar Nuclei in Solution**
Jiahui Shen, Victor Terskikh, Xianqi Kong, Binyang Lin, and Gang Wu
Queens University

13:55 – 14:20 **A solid-state NMR study of tellurite-based glass materials**
Mounesha Garaga, Ulrike Werner-Zwanziger, Josef Zwanziger
Dalhousie University

14:20 – 14:45 **Recorder Velocimetry using MRI**
Amy-Rae Gauthier and Ben Newling
University of New Brunswick

14:45 – 15:00 **Stretch Break**

15:00 – 15:25 ***In vivo* study of marine microalgae membranes by solid-state NMR**
Jean-Philippe Bourgouin[1], Alexandre Poulhaza[1,2], Francesca Zito[2], Alexandre A. Arnol[1], Dror E. Warschawski[1,2] & Isabelle Marcott[1]
[1]Université du Québec à Montréal, [2]Centre National de la Recherche Scientifique (Paris)

15:25 – 15:50 **Determination of Ethanol Content Using Modern Low Field Direct Injection NMR**
Jennifer N.D. Vacon and Christopher W. Kirby
University of Prince Edward Island and *Agriculture and Agri-Food Canada*

16:00 –18:00 **Poster Session with Refreshments: Best Western Cavendish and Brackley Rooms**

18:30 – 22:00 **Banquet: Lobster on the Wharf (2 Prince Street)**

Sunday, October 2nd

Session 3: Best Western Stanhope Rooms A&B
Chair: Chris Kirby

9:00 – 9:25 **A ^{2}H NMR Portrait of the Interaction of Antimicrobial Peptides with Intact Bacteria**
Marwa Laadhari, Andrée E. Gravel, Alexandre A. Arnold & Isabelle Marcotte
Université du Québec à Montréal

9:25 – 9:50 **Visualization of ion transfer dynamics in Li-ion batteries by *in situ* MRI**
Sergey A. Krachkovskiy, J. David Bazak, Bruce J. Balcom and Gillian R. Goward
McMaster University and University of New Brunswick

9:50 – 10:15 **^{17}O and ^{29}Si NMR in Mixed Ion Silicate Glasses: Correlating Structure with Macroscopic Glass Properties**
U. Werner-Zwanziger, C. Calahoo, and J. Zwanziger
Dalhousie University

10:15 – 10:35 **Refreshment Break**

10:35 – 11:00 **Recoupling experiments tailored for studying lipids**
Dror E. Warschawski, Alexandre A. Arnold and Isabelle Marcotte
Centre National de la Recherche Scientifique (Paris) et Université du Québec à Montréal

11:00 – 11:25 **Amphiphilicity is a key determinant in the membrane interactions of synthetic 14-mer cationic peptide analogs**
Michèle Auger, Matthieu Fillion, Burkhard Bechinger and Normand Voyer
Université Laval

11:25 – 11:50 **Characterization of Metal-organic Frameworks: What can we learn from solid-state NMR Spectroscopy?**
Yining Huang
Western University

11:50 – 12:00 **Student Prizes (Sponsored by the CanJChem) & Closing Remarks**

Posters

1) **Solid-State ^{17}O NMR and Computational Studies of Halogen Bonding in Iodosylbenzene**
Andrew Rinald and Gang Wu
Queen's University

2) **Structural characterization of novel fungal hydrophobins**
David N. Langelaan, Holly L. Spencer, Paul Jeronimo, Julie M. Grondin, Julie-Anne Gandier, Aaron Fountain, Emma R. Master and Steven P. Smith
Dalhousie University and Queen's University

3) **Solid-state ^{61}Ni NMR of diamagnetic nickel(0) complexes**
Peter Werhun and David L. Bryce
University of Ottawa

4) **NMR Structural Studies of Fusarium Secondary Metabolites**
Barbara Blackwell[1], Adilah Bahadoor[1], Whynn Bosnich[1], Danielle Schneiderman[1], Yves Aubin[2], and Linda Harris[1].
[1]Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, [2]Centre for Biologics Evaluation, Biologics and Genetic Therapies Directorate, Health Canada

5) **Structural Investigations of Supercontracted Spider Dragline Silk**
Justine Dionne, Thierry Lefèvre and Michèle Auger
Université Laval

6) **Validation of *in Vitro* Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy for the Clinical Measurement of Urinary Galactitol**
Vesnaver M., Chen Z., Larroque AL., Das SK., Jean-Claude B. and Parente F.
McGill University

7) **Spectroscopic investigation of α-synuclein 71-82, a peptide derived from a protein involved in Parkinson's disease**
B. Martial, É. Bruneau, T. Lefévre & M. Auger
Université Laval

8) **Magnetic Resonance Imaging of Core Flooding in a Metal Core Holder**
Ming Li, Dan Xiao, Mojtaba Shakerian, Armin Afrough, Fred Goora, Florin Marica, Laura Romero-Zerón, Bruce Balcom
University of New Brunswick

9) **Variable-Temperature NMR Imaging Studies of Li-Ion Battery Electrolytes Under Polarization**
J. David Bazak, Sergey A. Krachkovskiy, Gillian R. Goward
McMaster University

10) **Gene cloning and expression analysis of mitochondrial glutathione reductase from the Arabian camel (*Camelus dromedarius*) liver in *Escherichia coli***
Mona Shujaa Alharbi, Abdulrahman Mohammad Alsenaidy, Sooad Khalaf Aldaihan, Anwar Ahmed
King Saud University, Saudi Arabia

11) **NMR & Computational Study of Paramagnetic Compounds**
Yizhe Dai and Gang Wu
Queen's University

12) **NMR of Vpu transmembrane peptides solubilized in detergent, lipid, and SMA copolymer environments**
David Davidson and Simon Sharpe
SickKids and University of Toronto

13) **^{23}Na Solid-State NMR Studies of Stability and Reversibility in Sodium Battery Materials**
Zoë E. M. Reeve, Christopher J. Franko, Kristopher J. Harris, Hossein Yadegari, Xueliang Sun and Gillian R. Goward
McMaster University

14) **Towards understanding Bcl-xL BH4 domain mediated Bax inhibition through segmental isotopic labeling and NMR spectroscopy**
Qinyan Song, Jan K. Rainey and Paul X-Q Liu
Dalhousie University

15) **Characterization of porous media by employing two-dimensional NMR: D-T_2 and T_1-T_2**
Sarah Vashaee, Ben Newling, Bruce J. Balcom
University of New Brunswick

16) **A Convenient Synthesis of [3-^{17}O]-L-Serine and [3-^{17}O]-L-Threonine by Mitsunobo Reactions**
Betty Lin and Gang Wu
Queen's University

17) **^{19}F NMR studies of STAT3/STAT5 proteins for assay development of small molecule inhibitors**
Elvin D. de Araujo and Patrick T. Gunning
University of Toronto at Mississauga

18) **Identifying ideal ^{19}F-tryptophan labeling schemes to characterize GPCR conformation and dynamics using NMR spectroscopy**
Calem Kenward, Kyungsoo Shin, Muzaddid Sarker, Jan K. Rainey
Dalhousie University

19) ^{1}H NMR Fatty Acid Profiles: Fruits from Woody Plants as Potential Polyunsaturated Fatty Acid Sources

M.B. Fischer, A.T. Quilty, J.N.D. Vacon, J.L. McCallum, R. Soolanayakanahally, W. Schroeder, C.W. Kirby
Agriculture and Agri-Food Canada and University of Prince Edward Island

20) Confirmation Methods for Phytosome formation of Ginsenosides with Phospholipids

M.B. Fischer, M. Richard, D.G. Kay, C.W. Kirby
Agriculture and Agri-Food Canada and Neurodyn Inc.

21) Characterizing the interaction between Kruppple-like Factor 4 and CBP/p300

Brigid Conroy, David Langelaan, James Omichinski and Steven Smith
Queen's University

22) Ligated Peptide Sequences vs. Native Hexapeptides of *Tau* Protein

Anamika Sulekha, Jiji A. C., and Vinesh Vijayan
Indian Institute of Science Education and Research, Thiruvananthapuram, Kerala, India-695016.

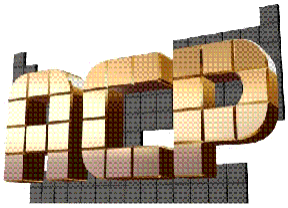
MOOT29 Map

Distances and ~Walking Time

BW to Upstreet: 1.3 km ~16 min
BW to Lobster on the Wharf: 750 m ~ 9 min

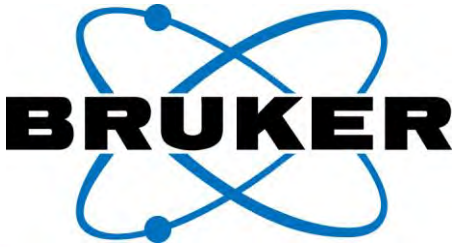
There are numerous eating and/or drinking establishments in the downtown core, all within a 10 minute walk from the BW. Approximate walking times to the conference venues are given here as a guide (from google maps). With the exception of Upstreet (which is north of downtown), parking can be busy in downtown Charlottetown thus walking from the BW is the best solution in general.

THANK YOU MOOT29 Sponsors!!



Agriculture and Agri-Food Canada

Agriculture et Agroalimentaire Canada



Cambridge Isotope Laboratories, Inc.
isotope.com

UNIVERSITY of Prince Edward ISLAND

Abstract Booklet

The Wide World of Magnetic Resonance

Bruce J. Balcom

Magnetic resonance is without doubt the world's most versatile and informative spectroscopic technique. The range of physical systems which may be interrogated, and the range of information which can be extracted, is simply breathtaking. In this lecture I will attempt to bridge NMR spectroscopy and Magnetic Resonance Imaging through simple examples that show the similarities between the methodologies, and instances where developments in one area have benefited the other. I will also attempt to show through our own work in MRI simple methodologies that permit one to measure or understand MR instrument performance.

Wideline NMR Spectroscopy of Unconventional Nuclei in Oxide Glasses

A.L. Paterson, J. Sinclair, U. Werner-Zwanziger, J.W. Zwanziger

Ultra-wideline WCPMG NMR spectroscopy is investigated as a probe of typically inaccessible nuclei in oxide glasses. ^{139}La WCPMG NMR has been recently used to study less-ordered systems [1]. We present ^{139}La WCPMG ssNMR spectra of binary and ternary lanthanum-containing glasses, and draw comparisons to recent work on crystalline lanthanum structures [2]. A structural model is developed to estimate the oxygen coordination number of lanthanum in the glass phase, as compared to diffraction techniques.

[1] Dithmer, L.; Lipton, A. S.; Reitzel, K.; Warner, T. E.; Lundberg, D.; Nielsen, U. G. Environ. Sci. Technol. 2015, 49 (7), 4559–4566.

[2] Paterson, A. L.; Hanson, M. A.; Werner-Zwanziger, U.; Zwanziger, J. W. J. Phys. Chem. C 2015, 119 (45), 25508–25517.

Isoform-dependent membrane binding preferences differ between apelin and apela, cognate peptide ligands for the same GPCR

Kyungsoo Shin, Muzaddid Sarker, Shuya K. Huang, and Jan K. Rainey

The membrane catalysis theory states that a ligand must interact with the membrane prior to its cell surface receptor. The initial membrane-ligand association step enhances the rate of receptor binding by: increasing local concentration of ligand; reducing diffusion from a 3D to a 2D process; and/or, inducing conformational change for receptor recognition. Variations in membrane composition mean that ligands may encounter a variety of environments, with potential for lipid-dependent preferences in both binding and conformation. We tested for evidence of membrane catalysis with two peptide hormones: apelin and apela. Both hormones can be processed into a variety of isoforms. While apelin exists as 55, 36, 17, or 13-residue isoforms, apela may be 32, 22, or 11 residues long. All isoforms retain the C-terminus and bind to and activate the same class A GPCR (the apelin receptor). This, in turn, regulates a variety of physiological systems, particularly in the cardiovascular system. Far-UV CD and solution-state NMR spectroscopy showed that all apelin isoforms exhibits β-turn characteristics in the presence of anionic but not zwitterionic micelles, suggestive of a preferential lipid interaction. Conversely, apela-32 showed a similar level of conformational change with both zwitterionic and anionic micelles, but removal of the N-terminal region restored anionic micelle preference, as observed in apela-11. Thus, membrane-associated apelin and apela isoforms differ in response to membrane composition even though they are ligands of a single GPCR. Given that composition of membranes can vary between cell and organelle types, preferential membrane-ligand association may regulate the rate of apelin/apela-GPCR complex formation (potency & efficacy), signaling pathways (Gαi vs. β-arrestin), and signaling mechanism (endocrine vs. autocrine). Characterizing ligands of the apelin receptor presents a rare opportunity to demonstrate membrane catalysis as a method of controlling and diversifying hormonal signaling methods both under physiological conditions and for therapeutic targeting.

Deuteration of *Vibrio splendidus* lipid membranes and initial characterisation by *in vivo* ^{2}H solid-state NMR

Zeineb Bouhlel[1,2], Dror E. Warschawski[3], Alexandre A. Arnold[1], Réjean Tremblay[2] and Isabelle Marcotte[1]

^{2}H solid-state NMR is a useful tool to probe the organisation and dynamics of the membrane phospholipids. The application of this technique to intact bacteria is further advantageous as it allows studying the lipids in their natural environment. To do so, the first challenge is to selectively ^{2}H-label the lipids of these complex microorganisms. In this work, we developed a protocol to deuterate the membrane phospholipids of the aquatic pathogenic bacteria, *Vibrio splendidus*. The labelling was optimised by growing bacteria in culture medium enriched with deutrated palmitic acid PA-d_{31} and selecting an appropriate detergent to ensure an efficient incorporation of PA-d_{31} in the membrane without affecting the bacterial growth. This protocol allowed carrying out ^{2}H NMR experiments on fresh bacteria sampled at their maximum growth performances. Samples were studied in the static mode as well as with magic angle spinning (MAS). Our results showed that PA-d_{31} was successfully incorporated, allowing an initial characterisation of the lipid phases and dynamics of *V. splendidus* membranes by measuring deuterium spectral moments. The acyl chain fluidity will be discussed with respect to different temperatures as well as to supplementing unsaturated fatty acids in combination to PA-d_{31} in the growth media. Biochemical analysis showed that bacteria viability rates were maintained as high as 95% after MAS experiments.

(1) Department of Chemistry, Université du Québec à Montréal, P.O. Box 8888, Downtown Station, Montréal, Canada H3C 3P8

(2) Institut des Sciences de la Mer, Université du Québec à Rimouski, P.O. Box 3300, Rimouski, Québec, Canada G5L 3A1

(3) Institut de Biologie Physico-Chimique, CNRS - Université Paris Diderot, 13 rue Pierre et Marie Curie, F-75005 Paris, France

Isolation and Structural Characterization of New Oximidine Compounds

Mark H. Nabuurs, Susan Boyetchko, Jason L. McCallum and Christopher W. Kirby

Recently we have identified and isolated a series of new macrolactone natural products known as oximidines from soil microbial cultures. The oximidines belong to a broader benzolactone enamide class of compounds which possess anti-cancer activity. Oximidines contain a functionally diverse twelve membered macrolactone core, in addition to an uncommon O-methyl oxime functionality. We have thoroughly studied these structures using a full range of 1D and 2D NMR experiments, including NOE experiments for stereochemical assignment at various sites. Herein, we discuss the results of these experiments and the structural assignment of these compounds.

Quadrupole Central Transition NMR Spectroscopy of Quadrupolar Nuclei in Solution

Jiahui Shen, Victor Terskikh, Xianqi Kong, Binyang Lin, and Gang Wu

While nearly 70% of the elements in the periodic table have their nuclear spins greater than 1/2 (known as quadrupolar nuclei), NMR studies of these quadrupolar nuclei are far less common than for spin-1/2 nuclei such as ^{1}H and ^{13}C. This is largely because quadrupolar nuclei often give rise to broad NMR signals. Recently, a new technique known as Quadrupolar Central Transition (QCT) NMR has been shown to be able to produce relatively sharp NMR signals for quadrupolar nuclei in solution. In this presentation, we will show several examples of ^{17}O (spin-5/2) and ^{59}Co (spin-7/2) QCT NMR studies of biologically important molecules and demonstrate the general utility of QCT NMR.

A solid-state NMR study of tellurite-based glass materials

Mounesha Garaga, Ulrike Werner-Zwanziger, Josef Zwanziger

Tellurite-based glasses have attracted much attention in view of application to optical devices, especially for fibre technology. Complementary to quantum chemical calculations and diffraction techniques,[1, 2] solid-state NMR is a powerful tool to study the local structure around Te atoms in a glassy or crystalline framework,[3, 4] which would vary with the modifier concentration. Here we use static ^{125}Te solid-state NMR to establish the structure of potassium tellurite glasses, $(K_2O)x\ (TeO_2)(1-x)$ at chemical composition $5\% \leq x \leq 20\%$. In particular, NMR parameters such as the isotropic chemical shift, the chemical shift anisotropy and the asymmetry parameter that could be extracted from ^{125}Te NMR spectra confirms the existence of different structural units, TeO_4 and TeO_3 polyhedra. Such a deep understanding at molecular level is crucial in order to build glass materials for real applications.

References:

[1] J. C. McLaughlin, S. L. Tagg, J. W. Zwanziger, D. R. Haeffner, S. D. Shastri, Journal of Non-Crystalline Solids 2000, 274, 1.

[2] J. C. McLaughlin, S. L. Tagg, J. W. Zwanziger, Journal of Physical Chemistry B 2001, 105, 67.

[3] D. Holland, J. Bailey, G. Ward, B. Turner, P. Tierney, R. Dupree, Solid State Nuclear Magnetic Resonance 2005, 27, 16.

[4] D. Larink, M. T. Rinke, H. Eckert, Journal of Physical Chemistry C 2015, 119, 17539.

Recorder Velocimetry using MRI

Amy-Rae Gauthier and Ben Newling

Spatially-resolved NMR, that is, MRI, is a highly versatile tool used for a wide variety of applications in chemistry, geology, physics, and of course medicine. This study aims to demonstrate the application of MRI to the measurement of gas flow through a recorder. In the field of musical acoustics, the recorder is used as a representative for a whole family of instruments. Also, several outstanding questions remain about the sound-production mechanisms in these instruments, which means that new tools and techniques are in demand. For these measurements, the 3D motion-encoded SPRITE pulse sequence developed at UNB was employed. Velocities were mapped in a real and unaltered recorder played with sulfur hexafluoride gas. Maps of the mean gas velocity were produced alongside maps indicative of the uncertainty in mean velocity (R-squared maps). Maps of the mean-squared displacement were also produced to indicate regions of turbulent fluctuation in the gas velocity. These measurements, when compared with simulations and added to the library of other acoustical techniques, help to provide a more complete picture of gas motion in a wind-instrument

In vivo study of marine microalgae membranes by solid-state NMR

Jean-Philippe Bourgouin(1), Alexandre Poulhaza(1,2), Francesca Zito(2), Alexandre A. Arnol(1), Dror E. Warschawski(1,2) & Isabelle Marcott(1)

(1)Département de Chimie, Université du Québec à Montréal, P.O. Box 8888, Downtown Station, Montreal, H3C 3P8, Canada (2)UMR 7099, CNRS - Université Paris Diderot, IBPC, 13 rue Pierre et Marie Curie, F-75005 Paris, France

In addition to providing structural integrity to cells and organelles, lipid membranes are the hub of important processes such as ion transport, signalling and trafficking, and constitute one of the first barriers to contaminants. Our laboratory has thus developed a range of experiments designed to study biological cells *in vivo* by solid-state NMR (1). We have investigated *in vivo* the green unicellular microalgae *Chlamydomonas reinhardtii* by ^{13}C solid-state (SS-)NMR using magic-angle spinning (MAS) (2). We have also successfully applied ^{2}H labelling and MAS SS-NMR to study the lipid membrane of bacteria under various growth conditions (3). Pursuing on this *in vivo* high-resolution trajectory, the objective of this work was to establish the feasibility of studying the lipid membranes of intact *Chlamydomonas reinhardti* by ^{2}H SS-NMR with MAS. Since spectra obtained with non-selective labelling suffer from severe spectral overlap, new selective labelling strategies had to be tested, and their efficiency was probed and quantified. Since sensitivity is also improved by using MAS, we have also addressed the effect of spinning on microalgae viability. We will present a comparative assessment of the membrane rigidity of two different strains of *Chlamydomonas reinhardtii*, under various growth conditions and temperatures, by in vivo ^{2}H SS-NMR. This opens the way for using ^{2}H MAS NMR as a diagnostic tool for the health of microalgae and other microorganisms.

(1) Warnet, Arnold, Marcotte and Warschawski (2015) "In-Cell Solid-State NMR: An Emerging Technique for the Study of Biological Membranes" Biophys. J. 109:2461–2466.

(2) Arnold, Genard, Zito, Tremblay, Warschawski and Marcotte (2015) "Identification of lipid and saccharide constituents of whole microalgal cells by ^{13}C solid-state NMR" Biochim. Biophys. Acta 1848:369-377.

(3) Warnet, Laadhari, Arnold, Marcotte and Warschawski (2016) "A ^{2}H magic-angle spinning solid-state NMR characterisation of lipid membranes in intact bacteria" Biochim. Biophys. Acta. 1858:146-152.

Determination of Ethanol Content Using Modern Low Field Direct Injection NMR

Jennifer N.D. Vacon and Christopher W. Kirby

NMR spectroscopy is an invaluable analytical technique for structural elucidation of small and macro-molecules. Hence the continual importance of teaching the theory and application of NMR spectroscopy for structure elucidation (mainly ^{1}H and ^{13}C) to undergraduate students. The quantitative nature of NMR spectroscopy, particularly ^{1}H NMR, allows it to be an excellent analytical technique and thus an excellent technique to teach in technical collages. However, high field NMR spectrometers are not financially feasible for many small institutions due to their large price tag and ongoing cryogenic costs or due to the lack of a dedicated space to house the instrument. A number of manufacturers have been developing low field, cost-effective, cryogen-free, benchtop NMR spectrometers that can measure multi-nuclear and multi-dimensional NMR spectra for molecular analysis, as well as QA/QC applications. With this in mind, we are developing an efficient QA/QC method for the determination of ethanol content in solutions (including pure ethanoic standards, beer, wine, spirits and other fermented beverages such as kombucha) using a direct injection low field NMR spectrometer (picoSpin-45). These methods will be used in our local college and/or undergraduate curricula in the near future.

A ^{2}H NMR Portrait of the Interaction of Antimicrobial Peptides with Intact Bacteria

Marwa Laadhari, Andrée E. Gravel, Alexandre A. Arnold & Isabelle Marcotte

The emergence of drug-resistant pathogens has prompted the search of new antimicrobial molecules with novel action mechanisms. Antimicrobial peptides (AMPs) are promising candidates to fight against infectious diseases since they can disrupt the bacterial lipid barrier. In this work, we focus on aurein 1.2 (GLFDIIKKIAESF-NH2) and caerin 1.1 (GLLSVLGSVAKHVLPHVVPVIAEHL-NH2) isolated from the skin secretions of the Litoria genus of Australian tree frog. These cationic peptides have an alpha-helical secondary structure in a membrane environment and are active against Gram(-) and Gram(+) bacteria. Previous studies using model phospholipid membranes suggest that aurein 1.2 and caerin 1.1 act via a carpet and transmembrane mechanism, respectively. To take into account the bacterial cell wall complexity, especially the presence of lipopolysaccharides and peptidoglycan, AMP-membrane interactions should ideally be studied with intact microorganisms. We have thus developed a protocol to ^{2}H-label phospholipids in *Escherichia coli* and *Bacillus subtilis* membranes, and investigated how aurein 1.2 and caerin 1.1 interact with these bacteria in vivo by ^{2}H solid-state NMR. Static and MAS NMR spectra combined with spectral moment analysis confirmed the effect of both AMPs on the hydrophobic core of the bacterial membranes, i.e., a decrease in the lipid acyl chain order, but at higher peptide concentrations for *B. subtilis*. Interaction of the AMPs with other cell wall components of the Gram(+) bacteria, such as teichoic acids in the peptidoglycan, would decrease their actual concentration on the membrane surface. This work thus shows that the action mechanism of the AMPs depends on their local concentration as well as the membrane environment, and stresses the importance of studying intact bacteria.

Visualization of ion transfer dynamics in Li-ion batteries by *in situ* MRI

Sergey A. Krachkovskiy, J. David Bazak, Bruce J. Balcom and Gillian R. Goward

The design and optimization of nonaqueous lithium ion battery electrolytes require methods for measuring all relevant diffusional parameters, including diffusion constants and ionic transference numbers. An accurate characterization of the transport parameters is not a trivial task due to the fact that the values of ionic diffusion coefficients 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.

Most recently, MRI was used for the *in situ* characterization of materials used in electrochemical energy devices. The main advantage of MRI over NMR and electrochemical techniques is its ability to provide spatially resolved details about chemical and dynamic features of species in solution. Therefore, such data represent a more accurate representation of processes in an electrolyte solution under an applied electrical potential, instead of values averaged over the entire volume of a sample cell.

Using *in situ* MRI, we have unambiguously demonstrated that electrolyte solutions can experience large concentration polarizations during battery operation. Because of that, the difference in ionic diffusivities at opposite ends of the cell reaches up to 60% and obviously has to be taking into account for the accurate description of mass-transport. The evolution of ionic concentration profile in electrolyte can be described by Nernst-Plank equation and distributions of the transport parameters can be calculated by inverse modeling of the MR images. Moreover, carrying out experiment under conditions of stabilized concentration gradient and combining the PFG NMR diffusion measurement with *in situ* MRI techniques into single experiment, one can directly extract all necessary transport parameters. In that case the experimental uncertainties in the data for the regions in the immediate vicinity of the electrodes, which create difficulties for the inverse modelling analysis of MRI data will be effectively avoided. Furthermore, implementation of pure phase-encoding MRI methods allows us to visualize not just transport of ions through the electrolyte, but their intercalation into electrodes as well, leading to the next level of battery performance characterization.

^{17}O and ^{29}Si NMR in Mixed Ion Silicate Glasses: Correlating Structure with Macroscopic Glass Properties

U. Werner-Zwanziger, C. Calahoo, and J. Zwanziger

In glass chemistry, the mixed-ion effect occurs when physical properties of systems with mixed network modifier ions deviate significantly from the rule of mixtures. Understanding this effect is desirable in order to predict the properties of the many industrially relevant glasses whose properties are tailored by the addition of a large number of modifiers. The origin of the mixed-ion effect observed in ion conduction arises from lowered mobility due to competition within ion channels. However, the deviation from linearity observed in static and mechanical properties is not explainable in terms of ion mobility alone. Two mixed-ion series, xLi_2O - $(30-x)K_2O$ - $70SiO_2$ and xLi_2O - $(50-x)MgO$ - $50SiO_2$, were synthesized and characterized. ^{17}O 3Q-MAS and ^{29}Si MAS NMR experiments were used to study the mixed-ion effect at the structural level in these glass systems. Insights into the local structure were then correlated with macroscopic properties such as density, hardness, Young's modulus, refractive index, and ion conductivity. The maxima in activation energy observed in ion conductivity measurements correlated well with maximum deviation in other macroscopic properties.

Recoupling experiments tailored for studying lipids

Dror E. Warschawski, Alexandre A. Arnold and Isabelle Marcotte

Recoupling experiments in solid-state NMR have become popular in the late 1980s for measuring coupling constants and deducing very accurate distances to help determine biomacromolecules structures. Creative spectroscopists then designed a variety of pulse sequences for various applications, mostly playing with magic-angle spinning and synchronized pulses of different shapes, colours and flavors.

Lipids are considered semi-solids because they experience fast translation and very fast rotation, and therefore weaker couplings than in “real” solids such as proteins for example. In addition, coupling values in lipids do not translate into structures, but into more vague concepts such as “order parameters” or “phases”, usually probed by static ^{2}H or ^{31}P NMR.

Recoupling experiments therefore had to be adapted to lipids, and the first such experiment in 1997 was the DROSS experiment, derived from Rob Tycko’s “4-p pulses” experiment. Whereas Tycko’s experiment aimed at measuring ^{13}C CSA with the help of cross-polarization and high-power decoupling, DROSS was adapted to measuring ^{13}C-^{1}H dipolar couplings with the help of INEPT polarization transfer and low-power decoupling. DROSS later helped to determine “order profiles” in unsaturated lipids, which was impossible with ^{2}H NMR.

Today, we present an improved such sequence for measuring ^{31}P CSA, based on Tycko’s ROCSA pulse sequence, without any polarization transfer or decoupling. This allows the determination of individual lipids’ CSA in a lipid mixture, and is aimed at disentangling coexisting lipid phases in complex systems. The goal of such a project is to be able to adapt it to the study of biological membranes in entire and living cells, as an additional tool for in cell NMR.

Amphiphilicity is a key determinant in the membrane interactions of synthetic 14-mer cationic peptide analogs

Michèle Auger, Matthieu Fillion, Burkhard Bechinger and Normand Voyer

A wide variety of organisms produce antimicrobial peptides as part of their first line of defense. Among them, the naturally occurring cationic antimicrobial peptides represent a promising alternative to fight against multiresistant bacteria which are an important clinical problem. Contrary to conventional antibiotics which alter a specific target, the main target of cationic antimicrobial peptides is the membrane(s) of pathogens. We have previously shown that a non-natural peptide composed of 14 residues (10 leucines and 4 phenylalanines modified with a crown ether) has an α-helical conformation, and is able to disrupt lipid bilayers but is not selective towards bacterial membranes. To gain selectivity against negatively charged membranes, leucines of this 14-mer have been substituted by positively charged residues (lysine and arginine). An important feature of the mechanism of action of antimicrobial peptides is the membrane topology, i.e. the location of the peptide in the membrane. Membrane topology of the α-helical peptides has been investigated by performing ^{15}N solid-state NMR experiments in oriented samples and the results suggest that the peptides do not adopt a well-defined orientation in the membrane. Furthermore, we have determined the relative distance between the peptides and the phospholipids by measuring the $^{15}N\{^{31}P\}$ dipolar coupling with the REDOR technique. In order to better characterized the defects induced by the cationic analogs, ^{31}P solid-state NMR experiments have been performed on both unoriented and oriented samples (glass plates and bicelles). Analysis of the spectral patterns underlines that the peptides do not compromise the membrane integrity and that the mechanism of action involves the formation of pores in a similar fashion as the toroidal and sinking-raft mechanisms.

Characterization of Metal-organic Frameworks: What can we learn from solid-state NMR Spectroscopy?

Yining Huang

Metal-organic frameworks (MOFs) are a novel type of porous materials. They can be prepared by self-assembly of metal ions/clusters with organic linkers. These materials have many current and potential applications. Solid-state NMR (SSNMR) spectroscopy is a key technique for MOF characterization. It provides nuclide-specific information on both structure and dynamics, which is complementary to that obtained from diffraction based methods. In this presentation, I will give a brief overview of our recent work on MOF characterization by multinuclear SSNMR. The examples include directly probing the local environment of a variety of metal centers; identifying chemically different species; resolving crystallographically non-equivalent sites in the unit cell; characterizing the defects in the framework; locating the binding sites of gaseous molecules (CO_2, H_2) and obtaining dynamic info of guest species adsorbed in the MOF frameworks.

Solid-State ^{17}O NMR and Computational Studies of Halogen Bonding in Iodosylbenzene

Andrew Rinald and Gang Wu

Iodosylbenzene (PhIO) is a commonly used oxygen transfer agent. Due to its insolubility in organic solvents, no single crystal structure has been obtained for the molecule, thus its physical parameters are largely unknown. A previous extended X-ray absorption fine structure (EXAFS) study suggests that iodosylbenzene forms a halogen bond linked linear polymer in the solid state, thus denoted as (PhIO)n. To date computational methods have been unsuccessful in modeling the polymeric structure of (PhIO)n, as conflicting structures have been produced by different groups. By synthesizing this molecule with a ^{17}O label, the molecule could then be studied using solid-state ^{17}O NMR spectroscopy. Through the use of NMR spectroscopy, crucial structural (and energetic) information can be deduced. As well, by isotopically labelling the compound, the ^{17}O atom can be readily transferred to other molecules, allowing previously unstudied molecules to be investigated using solid-state ^{17}O NMR techniques. In addition, we have performed extensive quantum chemical computations to gain new insights into the halogen bonding and polymeric structure of solid iodosylbenzene. The computational results were compared to experimental structural and energetic parameters found in literature as well as results obtained through solid-state ^{17}O NMR spectroscopy.

Structural characterization of novel fungal hydrophobins

David N. Langelaan, Holly L. Spencer, Paul Jeronimo, Julie M. Grondin, Julie-Anne Gandier, Aaron Fountain, Emma R. Master and Steven P. Smith

Perhaps due to their complex life cycles and biological niches, fungi produce many proteins with unusual functions and biochemical properties, such as the hydrophobins. Hydrophobins are small globular proteins ubiquitously secreted by filamentous fungi that act as a functional amyloid to form a water repellent coating over aerial hyphae and spores. Also, secreted hydrophobins are extremely surface active and greatly reduce the surface tension of water. The interesting biochemical properties of hydrophobins give them many potential biomedical and industrial uses as alternative foam stabilizers, emulsifiers or in surface modification. Despite this potential, a comprehensive molecular-based understanding of the hydrophobin structure/function relationship is lacking.

We have identified a subset of hydrophobin genes that are unusual and as such, it is unclear what their structural and biophysical properties are.

With this in mind, we have cloned these novel hydrophobin genes into *E. coli* expression vectors and assessed their potential for biophysical characterization. We are now using a combination of X-ray crystallography, nuclear magnetic resonance spectroscopy, and biophysical techniques to characterize the properties of these unusual proteins.

Solid-state ^{61}Ni NMR of diamagnetic nickel(0) complexes

Peter Werhun and David L. Bryce

Organonickel complexes are widely used in preparative chemistry and as homogeneous catalysts; yet despite the chemical and industrial significance of nickel compounds, characterization by NMR spectroscopy of the active nickel isotope, ^{61}Ni, remains a largely unexplored field. This is especially true in the solid state, where studies have thus far been limited to nickel alloys. The inherent properties of the spin 3/2 ^{61}Ni nucleus do not themselves impede investigation by NMR: the quadrupole moment is manageable and its gyromagnetic ratio (with Larmor frequency 8.936% of ^{1}H) is low but not appalling. Rather, ^{61}Ni NMR is limited primarily by the low natural abundance of ^{61}Ni (1.19%) and by the peculiarities of nickel chemistry. The most common oxidation state of nickel is nickel(II), but high symmetry nickel(II) complexes (those with octahedral or tetrahedral coordination) are mostly paramagnetic, which significantly limits the utility of ^{61}Ni NMR as a probe for these compounds. Further, diamagnetic tetracoordinate nickel(II) compounds are usually of square planar geometry, which results in significant quadrupolar broadening. While they tend to be rather unstable, nickel(0) compounds appear to be the best suited for observation by NMR as they are both high-symmetry and diamagnetic. For example, the primary chemical shift reference for ^{61}Ni, nickel tetracarbonyl, is a tetrahedral nickel(0) compound. With this in mind, ^{61}Ni solid-state NMR was performed on a series of compounds: bis(cyclooctadiene)nickel(0), tetrakis(triphenylphosphine)nickel(0), and tetrakis(triphenylphosphite)nickel(0). Due to the highly toxic nature and volatility of nickel tetracarbonyl, chemical shifts were calibrated using the absolute frequency of the ^{1}H resonance of tetramethylsilane, and pulse parameters were optimized on ^{35}Cl or ^{37}Cl resonances. The experimental spectra that will be presented are the first solid-state ^{61}Ni spectra of organometallic nickel. Further, computational results are assessed relative to experimental findings.

NMR Structural Studies of Fusarium Secondary Metabolites

Barbara Blackwell[1], Adilah Bahadoor[1], Whynn Bosnich[1], Danielle Schneiderman[1], Yves Aubin[2], and Linda Harris[1].

[1]Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada. [2]Centre for Biologics Evaluation, Biologics and Genetic Therapies Directorate, Health Canada, Ottawa, ON, Canada

The genome of *Fusarium graminearum* contains a significant number of both clustered and singular genes involved in secondary metabolism, many of which are expressed during plant infection and may play a role in pathogenicity. With the exception of the trichothecene gene cluster which is well characterized, many of the products of these genes remain undetermined. At ORDC, there is the capability of producing a variety of selective gene disruptants of *Fusarium* which permits the characterization of novel secondary metabolites associated with the target gene when cultured in liquid media. NMR is extensively used in the structural determination of these gene products, the elucidation of biosynthetic pathways and the characterization of metabolite profiles. This is illustrated by three examples of recent projects: a) the elucidation of the details of the culmorin biosynthetic pathway, b) discovery of a novel cyclopeptide associated with a nonribosomal peptide synthase (NRPS) gene, and c) metabolite profiling of naturally occurring Canadian *F. graminearum* isolates that lack the ability to hydroxylate the trichothecene skeleton at the C-8 position.

Structural Investigations of Supercontracted Spider Dragline Silk

Justine Dionne, Thierry Lefèvre and Michèle Auger

Spider dragline silk is a semicrystalline polymer of great interest in materials science due to its unique mechanical properties and biocompatibility. Spun from the major ampullate glands, this type of silk possesses an exceptional combination of elasticity, strength and toughness. At the molecular level, spider silk is composed of fibrous proteins organized into crystalline nanodomains embedded in an amorphous matrix.

In the presence of liquid water or high humidity, the amorphous phase is plasticized and its hydrogen bonding network disrupted, which results in the shrinking of the fiber up to 50% of its initial length. This phenomenon, known as supercontraction, is triggered by the entropic folding of the polypeptide chains resulting from the water-induced increase in chain mobility.

Our previous NMR studies have shown that different types of silk (major and minor ampullate silk and cocoon silk) exhibit similar secondary structures while having differences in their relaxation dynamics for certain residues (Ala, Gly and Gln). Moreover, dragline silks reeled at different speed have been analyzed. These result that higher is the reeling speed, lower is the organization of the fiber.

However, these interesting results only concern one spider species in the native state. The aim of the present study is therefore in one hand to reproduce the previous experiments with other species, and on the other hand, to characterize the relaxation dynamics for both the native and the supercontracted fibers by solid-state NMR.

Finally, those findings will be correlated to anterior structural and orientational results obtained by Raman spectromicroscop

Validation of *in Vitro* Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy for the Clinical Measurement of Urinary Galactitol

Vesnaver M., Chen Z., Larroque AL., Das SK., Jean-Claude B. and Parente F.

BACKGROUND: Classical Galactosemia (galactose-1-phosphate uridyltransferase (GALT) deficiency, OMIM # 230400) is an inherited disorder presenting in the first few weeks of life as a life-threatening illness with hepatic, renal and cerebral involvement. A fast and accurate diagnosis is needed to initiate specific treatment and avoid long term sequels. Individuals with GALT deficiency accumulate galactitol in body tissues and fluids. Therefore, the detection of galactitol in urine is a key finding for the diagnosis of classical galactosemia.

OBJECTIVES: The aim of this study was to clinically validate (^{1}H-NMRS) for the quantification urinary galactitol by assessing precision, linearity, repeatability and accuracy.

METHODS: Normal urines (n=50), urine spiked with galactitol and urine from affected individuals were treated with phosphate buffer and analyzed on an AVANCE III HD 600 MHz NMR Bruker spectrometer equipped with a cryo-probe CPQCI ^{1}H-^{31}P/^{13}C/^{15}N. All spectra were processed with TOPSPIN 3.5pl2. Quantification was performed using 5 mM benzoic acid solution as an external standard.

RESULTS: At pH 7.4, galactitol gives a specific signal at 3.99 ppm (triplet) and 3.70-3.65 ppm (multiplet) in both D_20 and urine. The linear range is 1 to 12 mM. The limit of detection is 0.2 mM. Using the multiplet signal for quantification, the within run imprecision (Coefficient of Variation, CV) is 0.25 % and 0.66 % at concentrations of 14 mM and 3 mM respectively (n=10). Galactitol signal is undetectable in normal urines.

CONCLUSIONS: ^{1}H-NMR is a fast and accurate method to quantify galactitol in urine.

Spectroscopic investigation of α-synuclein 71-82, a peptide derived from a protein involved in Parkinson's disease

B. Martial, É. Bruneau, T. Lefévre & M. Auger

Our research project focuses on a specific peptide of α-synuclein which is an amyloid protein known to be involved in the neurodegenerative Parkinson's disease. Aggregates of this protein are found in Parkinson's disease patients' brain, and more specifically, in the nerve tissues. This protein can be found in several forms, comprising monomers, oligomers and protofibrils. The secondary structure of these compounds has been described as a parallel β-sheet structure. All along this 140 amino acids protein, a specific portion plays an important role in the aggregation process, namely the non-amyloid-β component (NAC, sequence 61-95). In the core of this NAC, the amino acid sequence 71-82 appears to be crucial in the fibril formation process.

In the present study, we have investigated the secondary structure and thermal stability of the peptide fragment 71−82, α-syn 71−82, as a function of concentration, ionic strength and temperature, as well as its interactions with phospholipid model membranes using various spectroscopic techniques. The data show that α-syn 71−82 is mainly disordered in solution with the presence of a few β-sheet structure elements. The peptide reversibly forms intermolecular β-sheets with increasing concentration and ionic strength, and decreasing temperature, suggesting that it is subjected to a thermodynamic equilibrium between a monomeric and an oligomeric form. This equilibrium seems to be slightly affected by the presence of zwitterionic membranes. Conversely, the influence of the peptide on zwitterionic lipid bilayers is small and concentration-dependent. By contrast, α-syn 71−82 is strongly affected by anionic vesicles. The peptide indeed exhibits a dramatic conformational change, reflecting an extensive and irreversible self-aggregation, every amino acid being involved in a parallel β-sheet conformation. The aggregates appear to be located near the membrane surface but do not perturb significantly the membrane order. Comparing these results with the literature, it appears that α-syn 71−82 shares several general properties and structural similarities with its parent protein. These common points suggest that the sequence 71−82 may overall contribute to the behavior and properties of α-syn.

Magnetic Resonance Imaging of Core Flooding in a Metal Core Holder

Ming Li, Dan Xiao, Mojtaba Shakerian, Armin Afrough, Fred Goora, Florin Marica, Laura Romero-Zerón, Bruce Balcom

Magnetic Resonance (MR) is widely employed in the petroleum industry for down-hole logging and for laboratory core analysis. Sensitivity of the magnetic resonance experiment to fluid type and fluid environment makes it uniquely well suited to these applications. The same advantages should accrue to Magnetic Resonance Imaging (MRI) measurements of core flooding experiments. The ability of MRI to directly measure fluid saturation and fluid environment in three dimensions as a function of time has, to this point, not been fully realized for core flood applications. In this poster we describe the development of a series of technologies, which have overcome the traditional limitations.

We have developed a new generation of non-magnetic metallic core holders, (Hastelloy based) which permit MRI measurements at 5000 psi and temperatures above 100 °C. The RF probe is located inside the metal case to increase the experimental SNR. MRI requires the application of precise rapidly switched magnetic field gradients to encode position and motion in the MR signal. The presence of substantial metal in the sample space ordinarily degrades magnetic field gradient performance. However, the high resistivity of Hastelloy and related alloys results in short lived eddy currents, which do not significantly impair gradient switching. We have developed experimental methods to measure and correct magnetic field gradient waveforms to ensure near ideal gradient performance, even in the presence of a metal core holder.

Fast and accurately switched magnetic field gradients permit the introduction of fast frequency encoding MRI methods that are quantitative even for short T_2 populations (a few msec). These new methods permit 3D MRI of core plug samples in low field MRI instruments that are common in the industry. 3D imaging is possible with imaging times on the order of 10 minutes. This is sufficiently fast to permit time resolution of core flooding measurements.

Variable-Temperature NMR Imaging Studies of Li-Ion Battery Electrolytes Under Polarization

J. David Bazak, Sergey A. Krachkovskiy, Gillian R. Goward

Central challenges in the widespread deployment of lithium ion batteries are the state of charge (SOC) and state of health (SOH) estimation problems, which both require elaborate electrochemical modelling approaches. The microscopic models necessitated by the electrolytes used in these battery systems, which derive from concentrated solution theory, contain many free parameters. In order to obtain an accurate description of the dynamics in the battery system, it is therefore necessary to directly assign a large subset of these parameters and determine how they behave over a range of operational regimes.

The dynamics of the electrolyte domain in conventional electrochemical battery models are often overlooked, relative to those of the electrodes and interfaces. To this end, our research involves the combined experimental approaches of chemical shift imaging and PFG-NMR for an *in situ* battery mimic on a 300 MHz wide-bore spectrometer, for ^{19}F signals from the PF_6- anions in the electrolyte, to investigate the mass transport properties of the anions in the electrolyte as a function of both operating temperature and driving current.[1] Results obtained thus far include a quantitative demonstration of the ion concentration polarization under the influence of a driving current and a demonstration of how temperature alters the obtained steady-state anion concentration gradients, along with a spatially-resolved determination of the anion diffusion coefficient across the inter-electrode axis of the polarized cell at several temperatures. In conjunction with a suite of electrochemical techniques for studies of the electrode materials, the transport parameter values obtained from these experiments and the associated uncertainties will be used to perform a sensitivity analysis of the full-order, microscopic electrochemical model, with the aim of eventually developing an improved reduced-order model, enhanced with electrolyte dynamics, which is suitably compact and efficient for online SOC and SOH estimation.

1. Krachkovskiy, S. A.; Bazak, J.D.; Werhun, P.; Balcom, B.J.; Halalay, I.C.; Goward, G.R. Visualization of Steady-State Ionic Concentration Profiles Formed in Electrolytes During Li-Ion Battery Operation and Determination of Mass Transport Properties by In-Situ Magnetic Resonance Imaging. J. Am. Chem. Soc. 2016, 138 (25), 7992

Gene cloning and expression analysis of mitochondrial glutathione reductase from the Arabian camel (*Camelus dromedarius*) liver in *Escherichia coli*

Mona Shujaa Alharbi, Abdulrahman Mohammad Alsenaidy, Sooad Khalaf Aldaihan, Anwar Ahme

Glutathione reductase (GR) is highly conserved among diverse taxa and has important biochemical functions. These functions may facilitate survival in harsh conditions, but the role of GR from the liver of the Arabian camel (*Camelus dromedarius*) is unknown. In this study, the mitochondrial glutathione reductase gene (Gsr) from the *C. dromedarius* liver was cloned and highly expressed in *Escherichia coli* to gain insight into GR functions in the liver. After amplification of the cDNA encoding the functional unit of Gsr (1.2 kb), the products were cloned into the PGEM-T Easy and PET28a vectors. Gsr expression was confirmed using immunoblotting technique (45 kDa). Recombinant GR was purified to homogeneity using Ni-NTA resins. The optimum pH was 7 and optimum temperature was 35°C in 50 mM K_3PO_4 buffer, pH 7.5. The Michaelis constants, Km, for the substrates glutathione disulfide (GSSG) and NADPH were 45.6 µM and 63.5 µM, respectively; moreover, maximal velocity (Vmax) values were 3.969 × 10^{-2} Units/mg and 1.497 × 10^{-1} Units/mg.

NMR & Computational Study of Paramagnetic Compounds

Yizhe Dai and Gang Wu

Unlike diamagnetic compounds, paramagnetic samples usually have wide chemical shift ranges and broadened signals in NMR experiments. It is mainly due to the strong hyperfine interactions between the magnetic dipoles of unpaired electrons and nuclei. In order to find a rough range for some signal, quantum chemical calculations were always done first to estimate the positions. The results were summarized in terms of different types of atoms and chemical groups. By comparing the calculation results with experimental data and some literature values, the validity of the calculation and experiment methods was confirmed. This research focused on NMR study of paramagnetic compounds containing oxygen atoms directly bonded to vanadium. In the research, visible NMR spectra of different compounds of ^{1}H, ^{13}C and ^{17}O were recorded. Some of the spectra could be verified by the literature works, which shows the reliability of the experiment results. In this case, we know the rest of the discoveries are reliable.

NMR of Vpu transmembrane peptides solubilized in detergent, lipid, and SMA copolymer environments

David Davidson and Simon Sharpe

The structural study of membrane proteins by NMR spectroscopy can be limited in scope due to the challenges presented by the membrane mimetic systems used for solubilization. Common detergent systems amenable to solution NMR may result in non-native protein structural features, while lipid bilayer liposome systems are nearly native environments, but often suffer poor linewidths and low spectral resolution relative to solution NMR of small soluble proteins. We have used a selectively labeled peptide of the HIV-1 Vpu transmembrane domain to compare detergent, lipid, and lipid/styrene maleic acid copolymer systems for suitability in membrane protein structural characterization using conventional solution and solid-state NMR experiments.

^{23}Na Solid-State NMR Studies of Stability and Reversibility in Sodium Battery Materials

Zoë E. M. Reeve, Christopher J. Franko, Kristopher J. Harris, Hossein Yadegari, Xueliang Sun and Gillian R. Goward

For the first time solid-state ^{23}Na NMR is demonstrated to be a diagnostic tool for screening 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, a non-aqueous electrolyte and a Na metal anode. At the cathode during discharge, molecular oxygen is reduced to the superoxide radical, which further reacts producing either one or both of the desirable products; sodium superoxide (NaO_2) and sodium peroxide (Na_2O_2). In addition to the anticipated electrochemistry, the superoxide radical also attacks the electrolyte resulting in electrolyte decomposition species, where sodium carbonate (Na_2CO_3) is the main undesirable product.[1] Currently the underlying battery chemistry is still unclear but that can be revealed through the careful characterization of electrochemically-cycled electrodes. The reaction products have unique ^{23}Na NMR signatures allowing the species to be distinguished with 1D NMR. NaO_2 is paramagnetic and thus is identified by a short T_1 relaxation time and temperature dependent chemical shift. The Na_2O_2 and Na_2CO_3 spectra are dominated by the quadruple interaction and for a mixed sample the Na_2O_2 quadruple lineshape can be isolated with triple quantum magic angle spinning. This investigation includes electrochemically-cycled cathodes, where the electrochemical product is identified via solid-state ^{23}Na NMR.

Towards understanding Bcl-xL BH4 domain mediated Bax inhibition through segmental isotopic labeling and NMR spectroscopy

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

Bcl-xL is an anti-apoptotic Bcl-2 family protein that regulates homeostasis and cell survival. The canonical mechanism of apoptosis inhibition is achieved through direct protein-protein interaction with pro-apoptotic proteins Bax and Bak that prevents their hetero-/homo-oligomerization and mitochondrial outer-membrane permeabilization. The BH4 domain of Bcl-xL also confers anti-apoptotic property but the mechanism is unknown. Bcl-2, another member of the anti-apoptotic Bcl-2 family protein, has a similar BH4 domain that was shown to inhibit BAX activation. In order to characterize the potential structural interaction of Bcl-xL BH4 domain with Bax, we applied segmental isotope labeling aim to label only the BH4 domain of Bcl-xL in contact of full-length Bcl-xL protein and determine the structure & structural transition of Bcl-xL BH4 domain upon Bax binding through protein NMR spectroscopy. Here we achieved protein trans-splicing of BH4 domain of Bcl-xL onto rest Bcl-xL fragment, the key step towards segmental isotope labeling, and successfully purified the reconstituted Bcl-xL protein. The next step will be to produce the segmental isotope labeled Bcl-xL and perform NMR experiments on the protein.

Characterization of porous media by employing two-dimensional NMR: D-T_2 and T_1-T_2

Sarah Vashaee, Ben Newling, Bruce J. Balcom

One of the most important ideas in nuclear magnetic resonance (NMR) spectroscopy is the introduction of an additional NMR dimension that depend on molecular properties such as size, shape, mass, and charge. The dispersion based on such properties yields new information about the NMR spectra.

Diffusion ordered NMR spectroscopy (DOSY) has been employed to perform two-dimensional NMR measurements with chemical shifts on one axis and the distribution of diffusion coefficients on the other to recognize the components in complex mixtures and their interactions.

Three-dimensional DOSY experiments has also been performed in which a diffusion dimension is added to conventional two-dimensional NMR (COSY-DOSY, HMQC-DOSY, NOESY-DOSY, TOCSY-DOSY).The initial motivation for the development of three-dimensional DOSY was to obtain additional dispersion of NMR peaks in order to avoid overlap.

Oil companies are also employing NMR measurements for a large range of applications, such as characterizing formation fluids during reservoir evaluation and assessing formation. In partially saturated rocks the brine and oil T_2 distributions typically overlap so it is important to be able to distinguish the different liquids which are present in reservoir rock samples. Two-dimensional diffusion–relaxation(D-T_2) and T_1-T_2 distribution functions can be implemented for fluid identification of porous media. A spin echo pulse field gradient-CPMG sequence and an inversion recovery-CPMG sequence can be employed to measure bulk D-T_2 and T_1-T_2 distribution for porous media saturated with oil and brine. The successful extraction of both bulk D–T_2 and T_1–T_2 distribution functions for applications of fluid typing is presented in this work. We also utilized band selective adiabatic inversion radio frequency pulse to measure D-T_2 for regions of interest across the sample.

A Convenient Synthesis of [3-^{17}O]-L-Serine and [3-^{17}O]-L-Threonine by Mitsunobo Reactions

Betty Lin and Gang Wu

Oxygen is one of the most abundant elements in biological molecules. Oxygen has only one naturally occurring NMR isotope, ^{17}O, with 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 the smallest of molecules. However, recent studies have shown ^{17}O NMR to be a valuable tool for studying biological molecules. Because ^{17}O has low natural abundance, ^{17}O-isotopic labeling is a prerequisite to any ^{17}O NMR study. In this work, we show that [3-^{17}O]-L-serine and [3-^{17}O]-L-threonine can be readily prepared by Mitsunonbo reactions. These ^{17}O-labeled amino acids will be used in the future to be incorporated into proteins.

^{19}F NMR studies of STAT3/STAT5 proteins for assay development of small molecule inhibitors

Elvin D. de Araujo and Patrick T. Gunning

The Signal Transducer and Activator of Transcription (STAT) proteins play a pivotal role in the onset and progression of a number of cancers where various isoforms, most notably STAT3 and STAT5, are constitutively active. The molecular mechanism of STAT protein activation involves phosphorylation, which allows for association of the SH2 domains of the STAT proteins. This effectively results in the formation of a STAT protein homodimer, which is capable of translocating to the nucleus and inducing transcription of various target genes. Therefore, inhibition of STAT activity/dimerization through small molecule drugs has a large therapeutic potential, with several low micromolar inhibitors already identified. The development of rapid screening strategies for in vitro drug binding to STAT3 would facilitate advancement of potent small molecule inhibitors. To this end, we have optimized growth, expression and purification of full length STAT3 and STAT5 from recombinant E. coli that has allowed for several fold increases in protein yield over conventional methods. ^{19}F NMR studies of small molecule binding to STAT3 and STAT5 have allowed for the first time, a direct evaluation of the covalent mechanism of action of these inhibitors. We have employed temperature and time-dependent ^{19}F NMR studies to examine the electrophilicity of different inhibitors through their reactivity profile with reducing agents such as glutathione. 2D ^{19}F-^{1}H HOESY experiments have explored the conformational dynamics of the different molecules that contribute to their potency in targeting STAT3 and STAT5. These studies, in conjunction with our other screening platforms, have been invaluable for developing a mechanistic and potency profile of newly developed small molecule inhibitors targeting the STAT protein family.

Identifying ideal ^{19}F-tryptophan labeling schemes to characterize GPCR conformation and dynamics using NMR spectroscopy

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

The apelin receptor (AR) is a class A G-protein-coupled receptor (GPCR) with wide distribution throughout the body. Two peptide hormones, apelin and apela, bind to the AR to regulate the cardiovascular system, central nervous system, and adipoinsular axis. Similar to other class A GPCRs, AR is likely to be inherently dynamic in the cell membrane. ^{19}F is not naturally occurring within proteins; however, introduction of ^{19}F-labelled residues provides an outstanding means to characterize conformational changes in response to membrane mimetic, ligands, and other environmental factors. In recent work, GPCR dynamics have been very effectively probed by labeling of specific residues, with incorporation of ^{19}F-labelled amino acids providing particularly valuable insights into GPCR behaviour. The conformational and dynamic properties of AR are being probed using solution-state NMR spectroscopy for two fragments 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). Specifically, ^{19}F is being biosynthetically incorporated into tryptophan residues, selected due to low abundance in AR, allowing for discrete ^{19}F NMR signals. ^{19}F-labelling of tryptophan residues was achieved by addition of ^{19}F-substituted indole precursors during expression in minimal medium in *Escherichia coli*. AR55 and TM1-3 samples were prepared with 4-, 5-, or 6-fluoroindole to identify the optimal ^{19}F probe position to characterizing AR conformation and dynamics under our micellar NMR conditions. The proteins were also uniformly ^{15}N-enriched, allowing for use of ^{1}H-^{15}N HSQC experiments as a diagnostic of protein conformation. Our results demonstrate that the observed ^{19}F NMR peak pattern can be highly variable depending upon both the NMR conditions and ^{19}F position on the indole ring. Further examination also revealed slight perturbations in the ^{1}H-^{15}N HSQC spectra in both SDS and DPC micelles in ^{19}F configuration dependent manner, primarily localized to cross-peaks corresponding to residues close in sequence to the Trp positions. Tantalizing differences are seen in behaviour between the various ^{19}F label positions, with the implication that an optimal strategy may involve incorporation of more than one type of fluorotryptophan label into a single protein sample to efficiently and comprehensively characterize structure, topology, dynamics, and ligand binding.

^{1}H NMR Fatty Acid Profiles: Fruits from Woody Plants as Potential Polyunsaturated Fatty Acid Sources

M.B. Fischer, A.T. Quilty, J.N.D. Vacon, J.L. McCallum, R. Soolanayakanahally, W. Schroeder, C.W. Kirby

A diet high in polyunsaturated fatty acids (PUFA) has been associated with numerous health benefits and the relative amount of its components, omega-6 (18:2 FA) and omega-3 (18:3 FA), affects its overall impact. Fruit seed contain different FA profiles; therefore, the fruit of several woody plants developed at the AAFC Agroforestry Development Centre were explored as potential PUFA sources, which would add secondary value to these plants. The FA profile (saturated, 18:1, 18:2, and 18:3 FAs) was determined using ^{1}H NMR. For quantification, the individual bisallylic ^{1}H areas of 18:2 and 18:3 were used and the results were compared to those obtained via the conventional approach of using the methyl ^{1}H areas. Differences were large if significant interferences occurred in the methyl ^{1}H signal region. Of the 19 fruit types quantified, 13 had >40% PUFA. The dogwood and red elder fruit yielded the most FA with high PUFA content, containing 18:2/18:3 ratios close to that of an optimal diet. The sea buckthorn also had high FA yields but its major component was 18:1 and its 18:2/18:3 ratio was not optimal.

Confirmation Methods for Phytosome formation of Ginsenosides with Phospholipids

M.B. Fischer, M. Richard, D.G. Kay, C.W. Kirby

To improve the bioavailability of water-soluble natural plant products, phytosome and liposome complexes have been developed. These complexes contain polyphenolics associated with phospholipids which act as hosts and, in the case of the phytosome, the lipophilic host directly interacts with the hydrophilic guest via hydrogen bonds, making the guest an integral part of the inverse-multilamellar-vesicle-membrane. ^{1}H NMR provides evidence of complex formation via chemical shift changes of protons near the site of host-guest interaction, decreased diffusion values (DOSY), and changes in through space interactions (ROESY) relative to the solutions of the individual components as well as their mechanical mixture. However, when the spectral changes are not distinct by these conventional NMR techniques, we have incorporated another confirmation method of adding a protic solvent which breaks up the complex and allows monitoring of the ^{1}H signals of the freed guest.

Characterizing the interaction between Kruppple-like Factor 4 and CBP/p300

Brigid Conroy, David Langelaan, James Omichinski and Steven Smith

Vascular inflammation is central to the development of several cardiovascular diseases, which collectively are the largest cause of death worldwide. Transcriptional regulation of the inflammatory response in vascular smooth muscle cells (VSMCs) remains incompletely understood. Krüppel-like Factor (KLF) 4 and KLF15 are members of a family of zinc-finger transcription factors that play opposing roles in regulation of the VSMC inflammatory response, with KLF4 being proinflammatory and KLF15 anti-inflammatory. Both KLF4 and KLF15 are known to bind to the transcriptional coactivator CBP/p300. In addition, KLF4 is acetylated by CBP/p300, which is important for its transcriptional activity. KLF4 and KLF15 contain acidic transactivation domains (TADs) thought to mediate their interactions with the TAZ2 domain of CBP/p300. We propose a model for the KLF4-mediated VSMC inflammatory response in which the TADs of KLF4 and KLF15 compete for binding to TAZ2. Here, we characterize the interaction between KLF4-TAD and the TAZ2 domain of CBP/p300 using NMR spectroscopy. KLF4-TAD was disordered in solution and bound to the TAZ2 domain in the same region as the first TAD of KLF15. These results support a model in which KLF4-TAD and KLF15-TAD1 compete for binding to the TAZ2 domain of CBP/p300 during the VSMC inflammatory response.

Ligated Peptide Sequences vs. Native Hexapeptides of *Tau* Protein

Anamika Sulekha, Jiji A. C., and Vinesh Vijayan,

Indian Institute of Science Education and Research, Thiruvananthapuram, Kerala, India-695016.

Tau is one of the many microtubule-associated proteins, which in its native form is highly water-soluble. Abnormal hyper phosphorylation of the *Tau* protein can result in the depolymerisation of microtubular assembly. The detached *Tau* proteins self-assembly to form paired helical filaments further aggregation of which results in the formation of neurofibrillary tangles. Six isoforms of *Tau* has been reported in human body of which the longest one, *Htau* contains 441 amino acids. Towards the C-terminal of *Htau* are 4 repeat domains (R1, R2, R3 and R4) with the help of which it binds to the microtubular assembly. Of the four repeat domains, R2 and R3 are reported to form the core of the fibrils. Recent reports showed that specific hexapeptide sequences VQIINK of R2 (VQ-6*) and VQIVYK of R3 (VQ-6) serve as the core for aggregate formation.

In this project we attempted to ligate specific hexapeptide sequences of R2 and R3 domains that triggers the aggregation of *Tau*. Our aim was to understand the changes in conformation, aggregation pattern, kinetics and stacking of the ligated product, if any, in relation to the native hexapeptides. Chemistry of Staudinger ligation was equipped to ligate the two hexapeptides. The aggregation of the coupled hexapeptides was observed to be faster with respect to the individual hexapeptides. There was also an indication that sufficient β-strand propensity existed for the linked hexapeptides before initiation of aggregation.