

National Ultrahigh-Field NMR Facility for Solids

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Second Annual Solid-State NMR Workshop

May 26, 2007, Delta Winnipeg, Albert Room

Session 1 Chair I. Moudrakovski (Steacie Institute for Molecular Sciences NRC)

- 13:00-13:05** **Welcome** (Albert Room)
- 13:05-13:20** V. Terskikh (National Ultrahigh-Field NMR Facility for Solids) National Solid-State NMR Network
- 13:20-13:55** D. Bryce (University of Ottawa) Solid-state $^{35/37}\text{Cl}$ NMR Spectroscopy as a Probe of Inorganic Chloride Pseudopolymorphs
- 13:55-14:30** E. Chekmenev (Huntington Medical Research Institutes and California Institute of Technology) Towards ^{17}O Solid State NMR Spectroscopy of Ion-selective Channels at Ultra-high Magnetic Fields
- 14:30-15:05** M. Auger (Université Laval) Study of Biological Solids at High Field: Perspectives and Applications
- 15:05-15:20** **Coffee Break** sponsored by **CortecNet** (www.cortecnet.com)
- Session 2 Chair** G. Penner (University of Guelph)
- 15:20-15:55** W. Maas (Bruker BioSpin Corp.) Topics in Solid State NMR: RF Heating and Statistical Methods for NMR analysis of polymorphism
- 15:55-16:30** T. Polenova (University of Delaware) High and Low Resolution Solid-State NMR Spectroscopy of Proteins: Studies of Structure and Enzymatic Reactivity
- 16:30-17:05** D. Brouwer (Steacie Institute for Molecular Sciences NRC) Solid-State Proton NMR at 900 MHz
- 17:10-18:30** **Reception** (Campaign B Room) sponsored by **Bruker Canada** (www.bruker-biospin.com)

Solid-state ^{35/37}Cl NMR Spectroscopy as a Probe of Inorganic Chloride Pseudopolymorphs

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As part of our ongoing development of chlorine solid-state NMR spectroscopy in inorganic and bioinorganic systems, a series of alkaline earth chloride hydrates has been studied in order to characterize the chlorine electric field gradient (EFG) and chemical shift (CS) tensors and to relate these observables to the structure around the chloride ions. Chlorine-35/37 NMR spectra of solid powdered samples of pseudopolymorphs (hydrates) of magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), strontium chloride (SrCl_2 , $\text{SrCl}_2 \cdot 2\text{H}_2\text{O}$, and $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$), and barium chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) have been acquired under stationary and magic-angle spinning conditions in magnetic fields of 11.75 and 21.1 T. Chlorine-35 quadrupolar coupling constants (C_Q) range from essentially zero in cubic anhydrous SrCl_2 to 4.26 ± 0.03 MHz in calcium chloride dihydrate. CS tensor spans, Ω , are between 40 and 72 ppm, e.g. $\Omega = 45 \pm 20$ ppm for $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$. Quantum chemical calculations were employed in an attempt to reproduce and interpret the experimental data. Several calculations using traditional orbital-based basis sets, as well as plane wave-pseudopotential density functional theory, were used to model the extended solid lattices of these materials. Overall, this work builds upon our current understanding of the relationship between chlorine NMR interaction tensors and the local molecular and electronic structure, and highlights the particular sensitivity of quadrupolar nucleus solid-state NMR spectroscopy to the differences between pseudopolymorphic structures.

Towards ^{17}O Solid State NMR Spectroscopy of Ion-selective Channels at Ultra-high Magnetic Fields

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^{17}O , spin 5/2 quadrupole nuclei have recently been extensively employed to study ion-binding in proteins because of the high sensitivity of its quadrupolar coupling (QC) and chemical shift (CS) to the intermolecular interactions. While ^{17}O spectroscopy is considered difficult, the advent of high magnetic fields potentially allows for functional studies of large ion channels, the Holy Grail for many biochemists today. Much of the ^{17}O results to date were largely obtained from relatively small molecules such as individual amino acids. Recently we were able to demonstrate that ion binding significantly affects both the CS and QC of carbonyl oxygens in polycrystalline **Gly-Gly-Gly**. Moreover, it was found that ^{17}O is a significantly more sensitive probe for ion binding than the more typically used ^{15}N nuclei of the peptide backbone. We also studied ion binding by ^{17}O anisotropic CS in the cation conductive pore of **gramicidin A**, the binding site of which has similar intermolecular interactions that contribute to the biologically important function of high selectivity and high conductance rate in ion selective channels. While the sensitivity is always a challenge for NMR spectroscopy, we take advantage of high fields (19.6T and 21.1T) to aid the sensitivity in addition to high ^{17}O isotopic enrichment (~60%), favorable relaxation ($T_1 \sim 0.6$ ms, $T_2 \sim 0.25$ ms) and orienting the channels with respect to B_0 , which resulted in reducing the line width from >500 ppm to ~25 ppm, a 20 fold reduction. The insights gained from ion binding effects on CS in the relatively small gramicidin A pore helps potentially to approach the **KcsA potassium channel**. The preliminary results suggest that librational motions have negligible effects, CS tensor span and CS distribution at various amino acid positions are similar to those observed in crystalline solids.

Study of Biological Solids at High Field: Perspectives and Applications

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Recent statistical analyses of the complete or partial genomes from several organisms identified as many as 30% of all open reading frames encoding membrane proteins. In view of these statistics, the development of NMR spectroscopy for membrane protein structure determination is of the utmost importance. Several solid-state NMR methods have been developed in the last two decades to obtain structural information on these protein systems for which structural information is often difficult to obtain by techniques such as X-ray diffraction and multidimensional solution NMR. One of the main advantages of using ultrahigh field NMR spectrometers for these experiments is the increase in sensitivity, which is particularly useful for the determination of the structure of labelled peptides and proteins incorporated at low molar ratios in biological membranes since these experiments are often very difficult and compromised due to the small quantities of labelled materials. In addition, the increase in resolution obtained with an ultra high-field instrument is very beneficial for the complete resonance assignment and the structure determination of uniformly $^{15}\text{N}/^{13}\text{C}$ membrane proteins. Examples of such applications will be presented in this talk, as well as preliminary results obtained on very small amounts of silk fibres. In addition, an overview on the upcoming developments for the study of biological solids at the National Ultrahigh-Field NMR Facility for Solids in Ottawa will be presented.

Topics in Solid State NMR: RF Heating and Statistical Methods for NMR analysis of polymorphism

Werner Maas, Bruker BioSpin

In this talk I will present two current issues in solid state NMR.

First I will discuss RF sample heating, which results from the interaction between the electric field and the sample. In the case of wet and salty samples (such as for biological samples) the use of high amplitude proton decoupling can lead to excessive sample heating. This situation can be alleviated by reducing the E-field at the location of the sample. A low inductance proton RF coil can achieve this and this approach is used in Bruker BioSpin's *Efree* probes.

The second topic relates to the quantification of polymorphism and a statistical approach to analyzing such samples. Polymorphism is an important topic in drug development, and solid state NMR is uniquely positioned to provide insight in the state of the pharmaceutical ingredient. We are exploring the use of statistical approaches with the aim of potentially developing a quality control method for the pharmaceutical industry.

High and Low Resolution Solid-State NMR Spectroscopy of Proteins: Studies of Structure and Enzymatic Reactivity

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General approaches for resonance assignments and structural analysis in uniformly and differentially enriched proteins and protein assemblies by magic angle spinning NMR spectroscopy are discussed using a 108-amino acid residue E.coli thioredoxin as an example. It will be demonstrated that a combination of multidimensional homo- and heteronuclear correlation experiments yield nearly complete resonance assignments for the uniformly enriched full-length protein as well as for two differentially enriched non-covalent complexes formed by its complementary fragments. Secondary and tertiary structure analysis is presented. The practical considerations for high-resolution solid-state NMR studies of uniformly enriched polypeptides are outlined, including sample preparation protocol, instrumentation and pulse sequences. The potential of employing differential labeling strategies for high-resolution structural studies of protein interfaces and protein assemblies by solid-state NMR spectroscopy is addressed.

In the second part of the lecture, ^{51}V solid-state MAS NMR spectroscopy will be introduced as a direct probe of the geometric and electronic environments of vanadium sites in proteins. Experimental results will be presented for a 67.5 kDa vanadium chloro- and bromoperoxidases.

Solid-State Proton NMR at 900 MHz

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Solid-state ^1H NMR spectroscopy generally suffers from poor spectral resolution due to the narrow ^1H chemical shift range and the dominant ^1H - ^1H homonuclear dipolar interactions present in most materials. However, new opportunities are emerging as these challenges are being met by advances in magic-angle spinning technology, the development of advanced pulse sequences (and the hardware to implement them) and the availability of high magnetic fields. This workshop will describe the capabilities for solid-state ^1H NMR on the 900 MHz instrument at the Canadian National Ultrahigh-Field NMR Centre for Solids, including fast magic-angle spinning (up to 35 kHz), combined rotation and multiple pulse (CRAMPS) homonuclear decoupling sequences, various two-dimensional homonuclear and heteronuclear correlation experiments, and symmetry-designed proton CSA recoupling experiments.