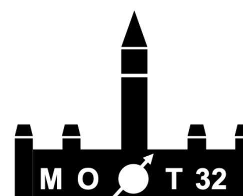
32nd MOOT NMR MiniSymposium



Abstracts: Oral Presentations

[1] **Keynote Lecture: Balancing protein stability and strain: (Mis)folding and function defined by NMR.** Elizabeth Meiering, *University of Waterloo* (page 2)

[2] **Mechanisms of Specific versus Nonspecific Interactions of Aggregation-Prone Inhibitors and Attenuators.** Stephen Boulton, *McMaster University* (page 2)

[3] **Linking Structure and Mechanics in Pyriform Silk Proteins** Jeffrey R Simmons*, Dalhousie University* (page 3)

[4] **Examining the Mechanism of EPAC1 Inhibition by NMR Spectroscopy.** Hongzhao Shao, *McMaster University* (page 3)

[5] **Membrane Protein Unfolding and Stability Investigated by Solid-State NMR.** Peng Xiao, *University of Guelph* (page 4)

[6] **First in cell 2D ^{13}C solid-state NMR assignments of *Chlamydomonas reinhardtii*'s cell wall and starch.** Alexandre Poulhazan, *Université du Québec à Montréal* (page 4)

[7] **Non-Destructive Methods in the Analysis of Soil.** Will McCombs, *Charlottetown Research and Development Center, Agriculture and Agri-Food Canada and University of Prince Edward Island* (page 5)

[8] **NMR Characterization of the origins of exceptional proton mobility in protic ionic liquid-based fuel cell electrolytes.** Mohammad Hasani, *Chalmers University of Technology* (page 5)

[9] **Observing Effects of Cardiolipin on α-Synuclein using Solid-State Nuclear Magnetic Resonance.** Justin Medeiros, *University of Guelph* (page 6)

[10] **Characterization of nucleotide binding and dimerization in the bacterial cell division regulator MinD.** Adam Cloutier, *University of Ottawa* (page 7)

[11] **Investigating apelin receptor C-terminal tail interactions with membrane-mimetic micelles.** Tran Thanh Tam Pham, *Dalhousie University* (page 7)

[12] **Using NMR to investigate the dynamics and calcium-binding mechanisms of dysferlin C2A domain.** Yuning Wang, *Western University* (page 7)

[13] **Keynote Lecture: NMR Tips Tricks and Techniques.** Glenn Facey, *University of Ottawa* (page 9)

[14] **Structural Basis of Alpha Synuclein Assembly Toxicity Inhibition by Human Serum Albumin.** Rashik Ahmed, *McMaster University* (page 9)

[15] **NMR study of molecular hydrogel system based on bile acids.** Puzhen Li, *Université de Montréal* (page 10)

[16] **Tetrel bonds: Evidence and insights obtained from solid state NMR crystallographic investigations of theophylline- and caffeine-based cocrystals.** Scott A. Southern, *University of Ottawa* (page 10)

[17] ***In situ* Magnetic Resonance Spectroscopy and Imaging of Li-plating onto and Diffusion within Anodes of Li-Ion Batteries** Kevin J. Sanders, *McMaster University* (page 11)

[18] **Probing carboxylate flipping dynamics in hydrogen-bonded systems by solid-state** Yizhe Dai[1], *Queen's University* (page 11)

[1] Keynote Lecture: Balancing protein stability and strain: (Mis)folding and function defined by NMR

Elizabeth Meiering

University of Waterloo

In nature, proteins evolve to fold and function sufficiently well to fulfil their biological roles; yet, stable folding and function are often at odds, and delicately balanced. Changes in protein structure with time, i.e. dynamics, are central to stability and function and can be easily perturbed by mutations, such as those occurring in disease or protein engineering. Despite intensive study, the balance between protein folding and function remains difficult to predict or control with precision. NMR in combination with complementary biophysical and biochemical techniques can provide a deep understanding of the molecular mechanisms governing proteins. This talk will explore stability and strain in two cases: hisactophilin, a histidine-rich pH-dependent myristoyl-switching protein; and Cu,Zn superoxide dismutase (SOD1) in which many mutations have been found to cause the devastating neurodegenerative disease, amyotrophic lateral sclerosis (ALS). We find that distributed native state strain in hisactophilin is critical for its switching function. SOD1 also exists in a fine balance, with mutations tipping its folding towards distinct misfolding and aggregation that may be linked to neurotoxicity. Mutations in both proteins give rise to wide-ranging effects, defined at high resolution using a variety of NMR methods, illuminating mechanisms that may apply to many proteins.

[2] Mechanisms of Specific versus Nonspecific Interactions of Aggregation-Prone Inhibitors and Attenuators

Stephen Boulton[1], Rajeevan Selvaratnam[1], Rashik Ahmed[1], Katherine Donovan[1], Xiaodong Cheng[2], Giuseppe Melacini[1]

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A common source of false positives in drug discovery is ligand self-association into large colloidal assemblies that nonspecifically inhibit target proteins. However, the mechanisms of aggregation-based inhibition (ABI) and ABI-attenuation by additives, such as Triton X-100 (TX) and human serum albumin (HSA), are not fully understood. Here, we investigate the molecular basis of ABI and ABI-attenuation through the lens of NMR and coupled thermodynamic cycles. We unexpectedly discover a new class of aggregating ligands that exhibit negligible interactions with proteins but act as competitive sinks for the free inhibitor, resulting in bell-shaped dose–response curves. TX attenuates ABI by converting inhibitory, protein-binding aggregates into nonbinding coaggregates, whereas HSA minimizes nonspecific ligand interactions by functioning as a reservoir for free inhibitor and preventing self-association. Hence, both TX and HSA are useful tools to minimize false positives arising from nonspecific binding but at the cost of potentially introducing false negatives due to suppression of specific interactions.

[3] Linking Structure and Mechanics in Pyriform Silk Proteins

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Spider silks are biomaterials mechanically comparable to high strength steel and Kevlar, used for many diverse adaptations by spiders. The orb-weaving spiders produce up to seven distinct types of silk, including pyriform silk. In nature, pyriform silk is spun as part of a glue-coated attachment disc, connecting web silks to disparate materials and to each other. Even though this silk fills a critical role in web formation, prior to our work neither the structural nor mechanical properties of pyriform silk had been widely investigated. Based on the central pyriform silk repetitive domain from *Argiope argentata*, we have successfully engineered recombinant pyriform silk-based proteins. In contrast to mechanical extremes of strength at the expense of extensibility or vice versa seen in most silks, we show that recombinant pyriform silk is both strong and extensible. To develop a detailed understanding of the structure-function relationship for this silk, we are characterizing its structure in both the solution- and fibre-states. Far-UV circular dichroism spectroscopy over a range of temperatures implies a predominantly α-helical structure in solution that is independent of the number of repetitive units connected together. Distinct regions of order and disorder in the repetitive unit are implied based upon degrees of NMR chemical shift dispersion correlated to the sign of heteronuclear ^{1}H-^{15}N nuclear Overhauser effect enhancements, consistent with a sequence that has segregated structured and disordered/polyproline-II regions. Using triple-resonance (^{1}H, ^{13}C, ^{15}N) NMR-based to allow backbone and side chain chemical shift assignment, we are now gaining insight into which regions of the protein appear to be more folded and could potentially play a role in the fibrilogenesis process. In the fibrous state, Raman spectral amide I bands exhibit an anticipated shift from predominantly a-helix in solution to b-sheet in fibres. Moving forward, we will be localizing the regions in which pyriform silk structural changes occur between the soluble and fibrous states, allowing direct linkage back to the protein elements which underpin fiber mechanical behaviour.

[4] Examining the Mechanism of EPAC1 Inhibition by NMR Spectroscopy

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The Exchange Protein Activated by cAMP (EPAC) is a guanine exchange factor for Rap1 GTPase.[1] Two distinct isoforms of EPAC, EPAC1 and EPAC2, are distributed differently in human tissues.[2] In the Melacini laboratory we are interested in examining EPAC1 inhibition through NMR spectroscopy. The mechanism of action of several EPAC1 inhibitors were characterized using NMR Spectroscopy[3-5]. The EPAC1 - ligand binding interface is mapped through intermolecular NOEs measured by ^{15}N and ^{13}C isotope filtered NOESY-HSQC experiment. Combining intermolecular NOE mapping experiment with other protein NMR methods, such as saturation transfer difference, transfer Nuclear Overhauser Effect spectroscopy and chemical shift mapping, we reveal how ligands interact with the EPAC1 cyclic nucleotide binding (CNB) domain. Specifically, we determine how EPAC1 ligands allosterically shift the hinge region between its active/inactive states and stabilize mixed intermediates within the allosteric thermodynamic cycle arising from the coupling of auto-inhibitory and binding equilibria[6].

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[5] Membrane Protein Unfolding and Stability Investigated by Solid-State NMR

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[1]*University of Guelph*

In the past 60 years, significant progress has been made in the field of protein folding. However, comparing to the vast knowledge gained for soluble proteins, a comprehensive understanding of membrane protein folding in its native environment still remains a formidable challenge in protein biophysics. Our research aims to investigate the driving forces behind membrane protein folding, and to provide insight into the molecular nature of the unfolding/folding pathway. In our research, we combine hydrogen-deuterium (H/D) exchange and solid-state NMR (ssNMR) detection to site-specifically follow the sequence of the thermal unfolding events in a lipid-embedded transmembrane protein of seven-helical (7TM) architecture, Anabaena Sensory Rhodopsin (ASR). The observed reversible part of the unfolding pathway in ASR provides a glimpse into the possible folding events in the early stages of folding. With the results on ASR, we have established a successful methodology of NMR detected H/D exchange experiment and demonstrated that it is amenable to probing the thermal unfolding of membrane-embedded protein.

[6] First in cell 2D ^{13}C solid-state NMR assignments of *Chlamydomonas reinhardtii*'s cell wall and starch.

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Sugar is an important building block for life. In microalgae such as *Chlamydomonas reinhardtii*, saccharides play an important energetic role and are stored in starch grains for example. They are also involved in the glycosidic bonds found in the glycoproteins of the cell wall.
The first objective of this work was to characterize starch in *C. reinhardtii*. This glucose polymer is made of amorphous and crystalline domains, the latter being mainly found into two different crystalline structures, *i.e.*, A and B-types. These structures have specific physicochemical properties and are valued differently in drug formulation, for example. Using a high-resolution solid-state NMR approach on ^{13}C-labelled starch, we were able to identify starch constituents in the two crystalline forms and in the amorphous state, and assign the starch observed *in vivo* to the A-type. The 2D-INADEQUATE experiments enabled the assignment of non-reducing end groups that had never been reported, as well as the assessment of starch chain length, crystallinity, hydration and conformational disorder. The second objective of this work was to investigate the structure of the proteins scaffolding *C. reinhardtii*'s cell wall, with a special focus on glycans of this assembly mainly made of hydroxyproline-rich glycoproteins. Using high-resolution 2D ^{13}C solid-state NMR experiments such as TOBSY or INADEQUATE on ^{13}C labelled cell-wall extracts, we identified several amino acids, saccharides, as well as links between them, thus evidencing the molecular and dynamic complexity of the cell wall constituents. Moreover, highly crystalline, amorphous and hydrated regions could be differentiated. Finally, solid-state NMR experiments after chemical modifications of the cell wall showed that glycans are essential for the cell wall structure and rigidity.
Altogether, this work demonstrates how high-resolution ^{13}C solid-state NMR can be used to detect and identify key components of intact microalgal cells, thus eliminating time consuming and potentially altering purification steps. These results show how these NMR methods provide atomic resolution details on organic disordered solids, even in living microorganisms, making *in situ* solid-state NMR a powerful tool to study molecules directly in their native environment.

[7] Non-Destructive Methods in the Analysis of Soil

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The distinctive red colour of Prince Edward Island (PEI) soil is due to large iron content (hematite), which can be problematic in the acquisition of NMR spectra. Phosphorus, one of the three main plant macronutrients, can mineralize through interactions with iron and more notably, aluminium, in PEI soil. Default analytical methods for the analysis of phosphorus speciation within soil, including solution NMR, typically includes extraction protocols and analyses, which only partially represent interactions within a soil matrix in the natural environment. Thus, we are taking a non-destructive, solid-state chemistry approach in order to analyze soil. We have acquired a variety of ^{31}P and ^{27}Al solid-state NMR experiments of PEI agricultural soils. The ^{27}Al NMR spectra displayed at least three distinct aluminium environments; two tetrahedral (~70 ppm, ~60 ppm) and one octahedral (~5 ppm), varying in relative intensities by soil sampled across the province over 20 years. Techniques also incorporated into solid-state analysis include Powder X-Ray Diffraction (PXRD), K- and L-edge X-ray Absorption of Near Edge Structure (XANES), in order to investigate the mineral component of PEI soil. Understanding the mineral profile of PEI soil is crucial in understanding how phosphorus can interact in the solid state, potentially affecting its bioavailability. Our ongoing efforts into characterizing the ^{31}P-^{27}Al interactions and micro-scale structure of PEI soils will be presented.

[8] NMR Characterization of the origins of exceptional proton mobility in protic ionic liquid-based fuel cell electrolytes.

Mohammad Hasani[1]

[1]Chalmers University of Technology

One of the key factors in the performance of electrolytes, as far as charge transport is concerned, is their conductivity. Even in the simplest cases of charge transport in the liquid state, proton, the smallest ion, shows exceptional/anomalous transport behavior, something of great consequence in technologically relevant systems such as fuel cell electrolytes. As an alternative class of such electrolytes, protic ionic liquids (PILs) are proposed as alternative anhydrous proton conducting electrolytes for medium temperature fuel cells. Noting the success of water-containing electrolytes and recognizing faster proton mobility than structural relaxation (via e.g. Grotthuss mechanism) as their advantage, such advantage is envisaged for PILs and in some cases deduced. A detailed characterization of the mechanism(s) resulting in such expectational proton mobility remains a challenge. NMR spectroscopy, in the author's opinion, has much to offer as a powerful technique for the study of microscopic dynamic processes in terms of the wide range of timescales of events, from nanosecond to second, it is capable of probing.
PILs roughly follow the general electrolyte behavior of classical aqueous electrolytes in some senses. The strong-weak classification of electrolytes is recently translated by the author to PIL literature as described by a degree of ionization, a, measured from ^{15}N NMR chemical shifts of two representative PIL systems [1]. The effect of incomplete ionization has been recognized before on the charge transport properties of PILs yet described in terms of a rather ambiguous/controversial measure, ionicity [2]. NMR spectroscopy has also been shown useful in the study of the energetics of ionization (to result in a basicity scale of PIL anions) [3] and charge transport properties (through self-diffusion coefficient measurements) [4].
Dynamic NMR spectroscopy is particularly useful when probing the more challenging cases of anomalous proton mobility as it is believed to always involve proton exchange. A number of NMR techniques can be used to probe proton dynamics including an array of relaxometry methods. We report in a recent study [5] that lineshape analysis and exchange spectroscopy (EXSY) can be used to quantify the proton exchange rate in a selected system. The temperature dependence of this exchange rate is analyzed to obtain an activation energy to obtain a value of 58.3±0.9 kJ×mol^{-1}for proton exchange between the PIL cation and a cosolvent,

water. The larger activation energy needed for proton transfer in this case indicates a higher energy barrier compared to that of proton exchange in water (~10 kJ×mol^{-1}) [6] and makes fast proton motion more difficult. The obtained exchange rates were used in the Karger equation to explain the diffusion behavior of the exchangeable proton as measured by PFG-STE method and no appreciable anomaly in translational dynamics with respect to the exchangeable proton is observed.
In this conference contribution, the authors previous work is reported to frame the scientific problem and present the results of recent efforts toward a solution.

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[9] Observing Effects of Cardiolipin on α-Synuclein using Solid-State Nuclear Magnetic Resonance

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[1]*University of Guelph*

α-Synuclein is the main component of protein aggregates termed Lewy bodies, a hallmark of neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and other synulceopathies. α-Synuclein naturally exists in an equilibrium between its intrinsically disordered form in solution and alpha-helical membrane bound form; disturbing this equilibrium can initiate a formation of amyloid fibrils which are toxic in their early stages, leading to neuronal death. α-Synuclein has been shown to cluster to mitochondria in response to Cardiolipin exposure from the inner to outer mitochondrial membrane, which then facilitates the binding and refolding of α-Synuclein fibrils back into their non-toxic membrane-bound monomer form, acting as a buffer to synucleopathy. We utilize Solid-State Nuclear Magnetic Resonance to provide a clear structural view of membrane bound and fibrillar α-Synuclein in the presence of Cardiolipin in order to characterize the dissolution and refolding of fibrils. By observing the effects of anionic DOPA lipids on fibrils we wish to determine whether this effect is Cardiolipin specific or a general characteristic of anionic membranes. Initial experiments were focused on the mobile region of α-Synuclein and a full assignment of residues 106-140 has been completed. Recent results have shown clear refolding of fibrils in the case of DOPA while the Cardiolipin case shows less mobility, suggesting that refolding in the presence of Cardiolipin results in a slightly different bound conformation.

[10] Characterization of nucleotide binding and dimerization in the bacterial cell division regulator MinD

Adam Cloutier[1], Natalie K. Goto[1]

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Bacterial cell division relies on the cell division septum to form at the mid-cell position. In gram negative bacteria, this is mediated by three proteins, MinC, MinD and MinE. These proteins interact with each other and the membrane in a dynamic, oscillating process which prevents cell division septum formation at the cell poles. Many *in silico* models have been constructed of the Min system in an attempt to describe its self-organizing behaviour. A common constraint applied in all these models is that, in order to prevent rapid re-binding of MinD to the membrane, the rate of exchange of bound ADP for ATP must be relatively slow (on the order of ~1 s^{-1}). To obtain an experimental measure of this rate, a partial assignment of backbone resonances was completed for MinD, requiring deuterium labelling and amino acid-specific selective unlabelling. ATP binding did not give rise to a dimeric state that had previously been proposed, although a disulfide-linked dimer in the absence of nucleotide was observed, likely as an artifact of the purification process. Binding of both ADP and ATP to monomeric MinD was studied using NMR, and a upper limit to exchange rates between nucleotide-bound and apo states determined. Our results support models requiring a slow nucleotide binding step, and help enhance understanding of how Min proteins sustain oscillations required for normal cell division.

[11] Investigating apelin receptor C-terminal tail interactions with membrane-mimetic micelles

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G-protein coupled receptors (GPCR) typically have an amphipathic helix (“helix 8”) followed by the C-terminal tail. The 71 residue C-terminal tail of AR (“AR tail”), that is intrinsically disordered, yet increases in helicity under membrane-mimetic condition. Using diffusion ordered spectroscopy (DOSY), the study investigates the interaction between the C-terminal tail and micelles through salt screening. The diffusion constant (D_c) of the AR-tail increases in response to high salt concentration indicated that salt screens out any electrostatic interactions of the AR-tail and micelles, with minimal changes in overall viscosity. The presence of micelles induces a greater level of helicity and stability in helix 8 as more favourable electrostatic interactions form between AR-tail and micelles. The study expands our understandings of such interaction and how it results in the amphipathy and helicity of helix 8 domain.

[12] Using NMR to investigate the dynamics and calcium-binding mechanisms of dysferlin C2A domain

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Background:
Failure to repair injured sarcolemmal membranes leads to muscular dystrophy, a degenerative disorder that results in increasing weakness and gradual wasting of skeletal muscles. Dysferlin is a membrane repair protein involved in trafficking of proteins and vesicles around injured membranes in skeletal muscle cells.

Nearly 600 mutations have been identified in the dysferlin gene (DYSF) causative for limb girdle muscular dystrophy type 2B (LGMD2B), Miyoshi myopathy (MM) and distal myopathy (DMAT) forms of the disease. Dyferlin is a 230 KD cytosolic-facing, membrane bound protein composed of seven, intermittently spaced C2 domains (C2A-C2G). The N-terminal C2A domain appears to be the most important in membrane repair and is also the location of several substitutions and that cause LGMD2B or MM. The C2A domain demonstrates the strong interaction with phosphoinositide and phosphotidylserine membrane surfaces in a calcium-dependent manner. It is not clear how calcium-binding to the dysferlin C2A domain alters the structure of the protein and how this might affect its interaction with the inner membrane surface or accessory proteins.

Objectives:

We aimed to characterize the structures of dysferlin C2A domain in both apo and calcium bound state and how calcium-binding alters the structure and affects the dynamics of the protein by elucidating the detailed calcium binding mechanisms.

Results

1. The apo-C2A shows increased flexibility that can be rigidified by calcium binding

The structures of dysferlin C2A domain in both apo and calcium bound state were solved by NMR spectroscopy and X-ray crystallography. The apo structure shows remarkable flexibility in the loop region. To assess this, the peak intensity ratios of each residue at pH 4.5 and 7.5 were analyzed. Residues from different regions of the protein showed striking differences: the loops L1-L3 and the regions near them had the smallest ratios, while most β-sheet regions showed a ratio near unity. This indicates that residues in loops L1-L3 are exposed to solvent and are subject to amide exchange with the bulk H2O solvent. To examine possible differences in flexibility we completed heteronuclear ^{1}H-^{15}N NOE and T2 relaxation experiments for the apo- and Ca-C2A domains. The NOE data showed little difference for the β1-β5 and β7-β8 regions between the apo- and Ca-C2A domains. In contrast, several residues in loops L1-L3 and β6 had smaller nOe values in apo-C2A compared to Ca-C2A. In addition, several loop residues exhibited significantly larger T2 values than the average, indicative of faster molecular tumbling of these residues. All these observations suggest that the loop region of C2A is flexible in the absence of calcium and calcium binding dampens the flexibility.

2. Ca^{2+} binding is affected by multiple factors

Several residues affecting calcium binding were mutated to investigate the detailed calcium binding mechanisms. Mutations of two residues gave surprising findings, D16 and D71. Residue D16 does not coordinate calcium ions in the crystal structure. However, $C2A^{D16N}$ was shown to bind to calcium about 10 times weaker than the wild type C2A domain and the binding in $C2A^{D16K}$ protein was undetectable suggesting that loss of negative charge of any aspartate in the loops will change the electrostatic potential, making calcium recruitment and coordination less favorable. D71 coordinates a single calcium ion using its carboxyl group. Calcium binding to the $C2A^{D71K}$ protein was found to be abolished. Examination of the calcium-free $C2A^{D71K}$ ^{1}H-^{15}N HSQC spectrum showed many of the new signals were located very close to signals in the calcium-bound C2A spectrum. Further, the calcium-free $C2A^{D71K}$ domain had a melting temperature that was very similar to Ca-C2A by about 10˚C higher than apo-C2A. We attribute these observations to the ability of the K71 side chain to occupy a similar position as a calcium ion observed in the crystal structure putting the ε-NH_3^+ group near the negatively charged side chains for calcium-binding residues. This proposal indicates that $C2A^{D71K}$ may mimic the calcium-bound state and have increased stability compared to the wild-type C2A domain.

Conclusions:

In this work, we assessed the dynamics of the C2A domain and confirmed the flexibility change upon calcium binding. We also found multiple factors contribute to the calcium binding property including binding ligands, electrostatic potential, and flexibility. Our work provides important new insights into the mechanism of calcium binding to the dysferlin C2A domain as a link for dysferlin-mediated membrane repair.

[13] Keynote Lecture: NMR Tips Tricks and Techniques

Glenn Facey

University of Ottawa

The field of NMR spectroscopy has changed dramatically over the last 35 years. Hardware and software developments have enabled measurements today not even dreamed about decades ago. Students entering the field of NMR today face a different world compared to those entering the field in the 1980's. I will reflect of some of the changes and highlight some of the techniques that have maintained my interest in the field.

[14] Structural Basis of Alpha Synuclein Assembly Toxicity Inhibition by Human Serum Albumin

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The generation of oligomeric intermediates arising during the aggregation of alpha synuclein (αS) is a central feature of Lewy body diseases such as Parkinson's disease (PD) (*1*). Recent findings suggest that trace amounts of αS are released into the extracellular environment by unconventional exocytosis from neurons (*2*), and that these extracellular species contribute to the pathology observed in PD (*3*). Extracellular chaperones, such as Human Serum Albumin (HSA), have been shown to inhibit the self-association of αS. However, the mechanism underlying αS aggregation inhibition by the endogenous fatty acid (FA)-transporter HSA remains poorly understood. By combining solution NMR with biolayer interferometry, dynamic light scattering, electron microscopy, wide-angle X-ray diffraction, size exclusion chromatography coupled with multiple angle light scattering, fluorescence spectroscopy and cell viability assays, we provide an unprecedented mechanism of αS assembly (αS_n) toxicity inhibition by HSA. Here we show that HSA binds αS_{n-} with sub μM affinity through hydrophobic interactions that are independent of FA binding to the two high-affinity sites in HSA. HSA-binding of αS_n shifts the populations of low molecular weight (MW) oligomers and high MW fibrils into "worm-like" intermediates with reduced toxicity and, concurrently, perturbs the conversion of NMR-visible monomers into NMR-invisible αS_n. Notably, we show that HSA inhibits the interaction of αS_n with membranes and that the effects of HSA and membrane addition to αS_n are non-additive, arising possibly also from the direct interactions of HSA with membranes. These results reveal that extracellular chaperones help maintain protein homeostasis not only by assisting the folding and assembly of proteins, but unexpectedly, also suppressing aberrant interactions with membranes, which promote the formation of toxic intermediates and enhance neuronal dysfunction.

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[15] NMR study of molecular hydrogel system based on bile acids

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Gelation can take place in aqueous or organic media by the self-assembly of small molecules or polymers, producing gels with networks structure. Low molecular weight gelator (MW < 2000 g/mol) can self-assemble to form a network and immobilize water inside, creating molecular hydrogels. However, the mechanism and condition of gelation for the molecular gel are not clear.
Bile acids are a series of steroidal compounds existing in the digestion system of human and animals to facilitate the digestion of fat and hydrophobic nutrients. They and their derivatives may form molecular gels. To better understand the gelation mechanism and the gelation condition, we did a systematic study on the self-assembling behavior of different bile acids. We also use NMR techniques to have a better understanding of the gelation process on the molecular level.

[16] Tetrel Bonds: Evidence and Insights Obtained from Solid State NMR Crystallographic Investigations of Theophylline- and Caffeine-based Cocrystals

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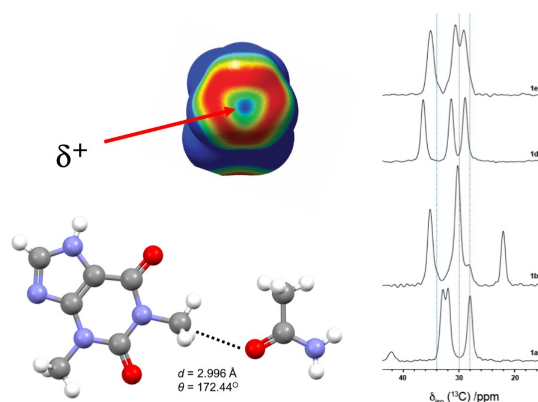
In recent years, the concept of noncovalent bonding has expanded to include a specific and interesting class of interactions analogous to hydrogen bonding, called σ-hole interactions.[1] These interactions result from the depletion of electrostatic density on the opposite end of a covalent bond between an electron-withdrawing substituent and a 'donor' atom from groups 14-17 in the periodic table, for which the specific interaction is named.
The tetrel bond (TB) is an example of a σ-hole interaction where the bond donor is one of the group 14 elements (T=C, Si, Ge, Sn, Pb).[2] While the tetrel elements, particularly carbon, are well known to form TBs, their solid state NMR (SSNMR) responses can often be overwhelmed by other effects including those of crystal packing. Furthermore, tetrel atoms in organic cocrystals often bond with four substituents, giving rise to steric problems which may impact the strength of these interactions.
This work expands on a previous SSNMR study of organic cocrystals exhibiting carbon TBs.[3] Both SSNMR and computational approaches are used to examine a series of cocrystals formed from either caffeine or theophylline and a number of other small acceptor molecules. Quantum chemical calculations consistently show an increase of the ^{13}C chemical shift by about 3-5 ppm upon TB-induced cocrystalization. However, the experimental NMR responses are not always straightforward, and can be conflated with other crystallographic effects. For example, weak hydrogen bonding can compete with the sometimes-subtle NMR responses due to TBs. Experimentally, we observe examples of both situations. Combined use of ^{13}C SSNMR and quantum chemical calculations help to understand the contributions of these effects to the NMR response and is thereby a valuable tool for the proper interpretation of cocrystallization induced shifts.



[17] In situ Magnetic Resonance Spectroscopy and Imaging of Li-plating onto and Diffusion within Anodes of Li-Ion Batteries

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Li-ion batteries are nowadays considered as the main source of energy for an electric vehicle (EV) application; however, the significantly longer "refueling" time compared to standard internal combustion engine vehicles is a substantial disadvantage from the perspective of the end-user. It is speculated that increasing the charging rate by a factor of three should significantly reduce the gap between EVs and conventional automobiles, promoting further penetration of EVs on the mass market. However, a significant drawback of very rapid charging is lithium plating on the negative electrode, which arises if the current exceeds the intercalation rate at which lithium diffuses into the negative electrode. In this case, a film of metallic Li will deposit on the surface, which subsequently reacts with electrolyte, leading to an increase of the cell internal resistance, a lower capacity, and potential short-circuiting in extreme cases.

Here we report an application of the parallel-plate RF probe to monitor *in situ* deposition of Li metal on a graphite anode during charging of a single layer prismatic cell, assembled with electrodes extracted from a commercial battery. We have demonstrated that part of the plated lithium was able to intercalate into the graphite after the current was turned off. Moreover, the signal of deposited Li metal consists of two resonances corresponding to (1) a "Li film" on the surface of the electrode, and (2) to dendrites orthogonal to electrodes' planes. Our data demonstrate that the Li metal intercalation into the graphite is primarily happening from the former type of deposited metal, while the lithium stored in dendrites can partially dissolve into the electrolyte during the consecutive discharge of the cell. Importantly, we were able to quantify the amount of reversibly and irreversibly deposited lithium. Finally, a coexistence of three stages of intercalated into graphite Li (2L, 2 and 1) is demonstrated during a fast charge cycle, suggesting a non-uniform lithiation of the electrode in that case.

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[18] Probing carboxylate flipping dynamics in hydrogen-bonded systems by solid-state

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Molecular motion/dynamics is an important aspect of solid materials. The most commonly used solid-state NMR technique for probing molecular dynamics is ^{2}H NMR. However, in many cases, the functional groups of interest contain only non-hydrogen atoms, thus limiting the use of ^{2}H NMR. In this talk, we will show how solid-state ^{17}O NMR can be used to detect carboxylate flipping dynamics in hydrogen-bonded systems. We will demonstrate that information obtained from solid-state ^{17}O NMR studies about molecular motion can be directly related to hydrogen-bonding energetics.