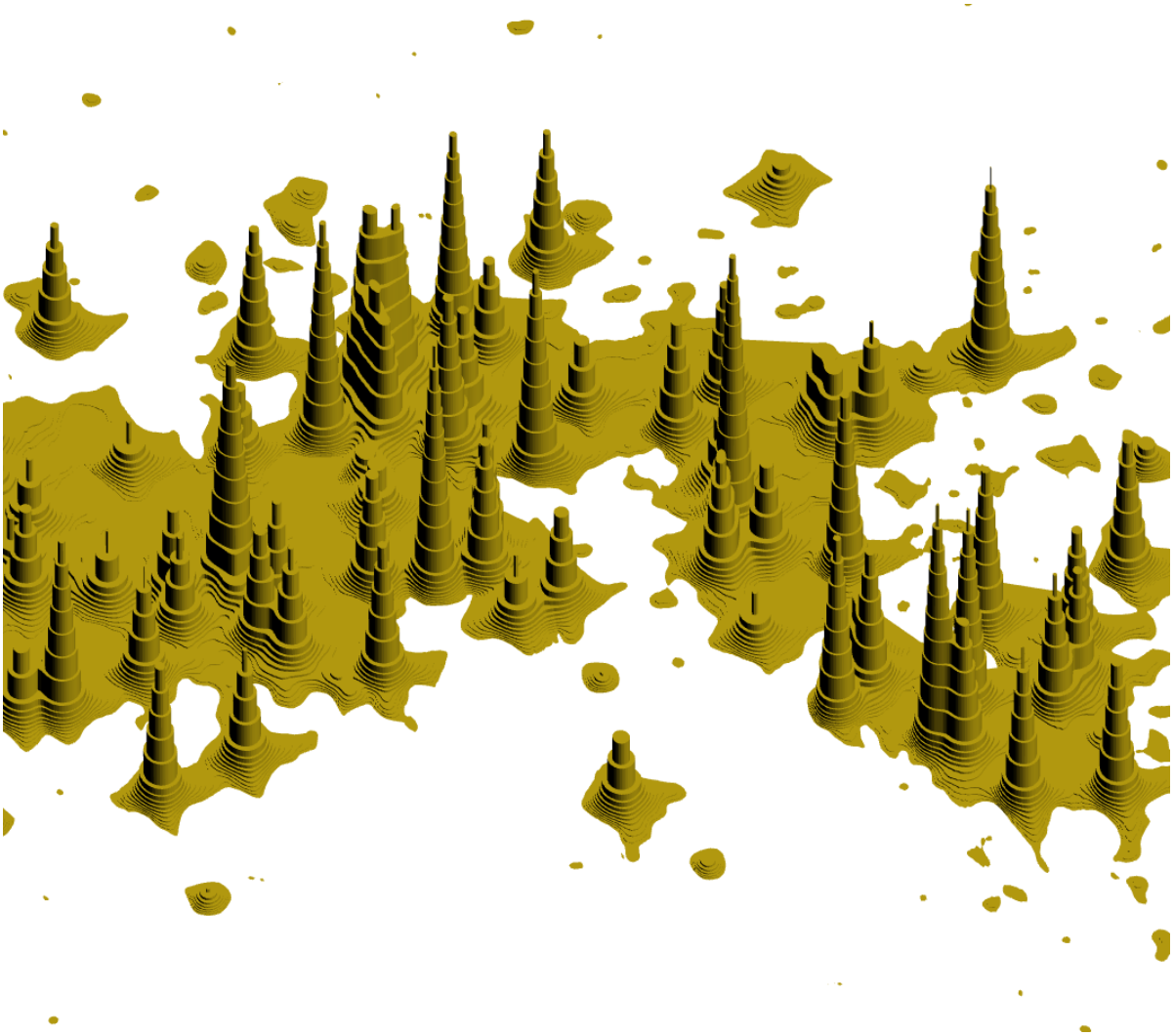
9th Gateway NMR Conference and Inauguration Symposium of the "National Gateway Ultrahigh Field NMR Center"



Ohio Union and CBEC Building

The Ohio State University, Columbus, Ohio

May 15 – 16, 2025

Organizing Committee

Rafael Brüschweiler (chair)

Lei Bruschweiler-Li

Dan Conroy

Mark Foster

Trent Franks

Philip Grandinetti

Alexandar L. Hansen

Chris Jaroniec

Kathy Johns

Dawei Li

Chunhua Yuan

Sponsorship

This conference is generously supported by

- The National Science Foundation (NSF)
- Bruker Biospin Corp.
- The Ohio State University
- Phoenix NMR

Special thanks

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The Gateway 1.2 GHz NMR project is supported by the NSF (grant RI-1 DBI-1935913). This conference is supported by the NSF (grant 2438365).

Occasion

The Gateway NMR Conference series is a one- to two-day meeting held each year in the eastern part of the Midwestern United States, which includes the states of Ohio, Michigan, Pennsylvania, Kentucky, Indiana, W. Virginia, and Tennessee. The Gateway NMR Conference brings together NMR practitioners to share their "latest and greatest" research, get to know each other, share news and know-how, initiate potential collaborations, and more. NMR Researchers from academia, national laboratories, and industry are encouraged to attend. The meeting also provides an opportunity to interact with vendors to learn about their products and services.

The 9th Gateway NMR Conference will be held at Ohio State on May 15 - 16, 2025. This conference, which is sponsored by the National Science Foundation (NSF), Bruker Biospin, and OSU will bring together scientists from North America and will be dedicated to the inauguration of the new *National Gateway Ultrahigh Field NMR Center* featuring the first *1.2 GHz NMR* spectrometer in the U.S.

View of the Bruker 1.2 GHz NMR spectrometer in the CBEC building at OSU, which is the centerpiece of the new National Gateway Ultrahigh Field NMR Center.

History of Gateway 1.2 GHz NMR spectrometer at Ohio State

The strong support by the Gateway NMR community, together with the US-NMR community at large, were essential in the lead-up to the NSF proposal for the acquisition of the 1.2 GHz NMR spectrometer. An RI-1 mid-scale research infrastructure pre-proposal was submitted to the NSF in early 2019, which was invited for a full proposal in the Spring of 2109 with R. Brüschweiler as PI and Mark Foster, Philip Grandinetti, Chris Jaroniec, and Blanton Tolbert as co-PI's. The award was made in the Fall of the same year. At that time, the 1.2 GHz (28 Tesla) NMR magnet technology, using a low-temperature superconducting magnet with a high-temperature superconducting insert, was still under development at Bruker. Less than a year later, the first 1.2 GHz NMR spectrometer was installed by Bruker in Europe (CERM, Florence, Italy). The 1.2 GHz NMR spectrometer at Ohio State was the 9th instrument installed world-wide and the first in North America. Three more 1.1 GHz ultrahigh-field NMR spectrometers have been installed in the U.S., at St. Jude Children's Hospital (Memphis), U. Wisconsin (Madison), and U. Georgia (Athens).

History of Gateway NMR Conference

- The 1st Gateway NMR Conferences was held at the University of Louisville, KY, on November 12, 2016, with Donghan Lee (chair), Mike Sabo, David Ban, Rieko Ishima, and Rafael Brüschweiler serving on the scientific organizing committee, which drew over 60 NMR scientists.
- The 2nd Gateway NMR Conference was held at Ohio State in Columbus, OH, with Rafael Brüschweiler (chair), Rieko Ishima, Justin Wu, and David Ban serving on the scientific organizing committee.
- The 3rd Gateway NMR Conference was held November 2 – 3, 2018, at the University of Pittsburgh with Rieko Ishima (chair), Rafael Brüschweiler, and David Weliky as the organizing committee, and with Drs. Andrew Hinck, and Damodaran Krishnan Achary serving on the scientific organizing committee.
- The 4th Gateway NMR was held September 21-22, 2019, at the University of Michigan with Ayyalusamy Ramamoorthy (co-chair), David Weliky (co-chair), Charles Hoogstraten, John McCracken, Sarah Keane, and Aaron Frank serving on the organizing committee.
- The 5th Gateway NMR Conference was held September 19-20, 2020 (virtually) from Vanderbilt University with Markus Voehler (co-chair), Donald Stec (co-chair), Michael Stone, Walter Chazin, and Chuck Sanders serving on the organizing committee.

- The 6th Gateway NMR Conference was held September 16-17, 2021 (virtually) from Miami University with Gary Lorigan (co-chair), Rick Page (co-chair), Anne Carroll, Michael Kennedy, Rob McCarrick, and Dave Tierney serving on the organizing committee.
- The 7th Gateway NMR Conference was held October 20-21, 2022, (virtually) was hosted by Andrew Lane (co-chair) and Teresa W-M. Fan (co-chair) at the University of Kentucky.
- The 8th Gateway NMR Conference was held at the University of Notre Dame, October 20-21, 2023 (in person) chaired by Jeffrey Peng and Evgenii Kovrigin.
- The 9th Gateway NMR Conference (this conference)
- The 10th Gateway NMR Conference is planned to be held in 2026 at Michigan State University (co-chairs: Charles Hoogstraten and Tuo Wang)

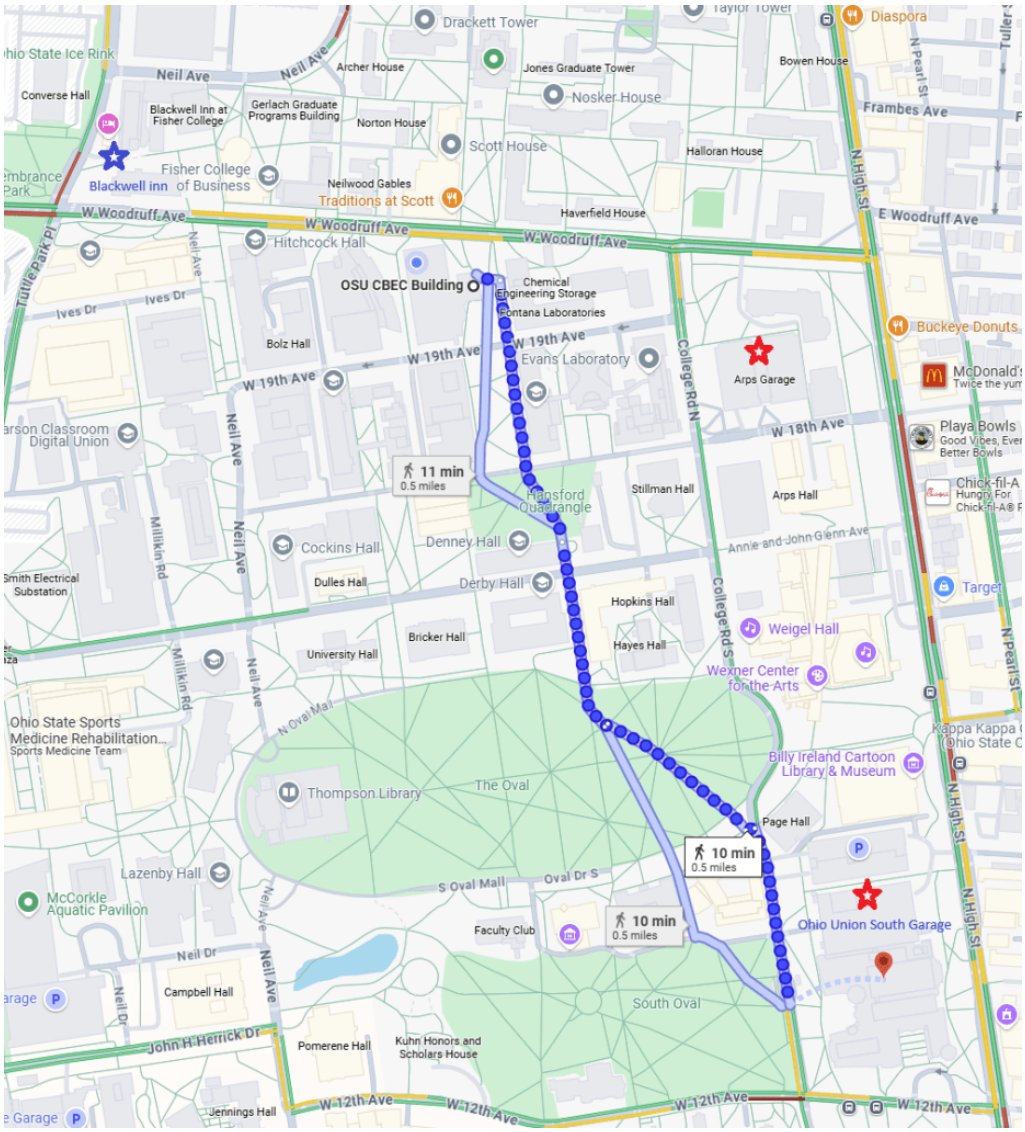
If you are interested in organizing a future Gateway NMR Conference, please contact the present conference secretary (E-mail: bruschweiler.1@osu.edu).

Venue and Map

The conference talks will take place at the **Ohio Union** on OSU campus.

The poster sessions will be in the basement of the **CBEC building** next to the 1.2 GHz NMR suite on North Campus about 10' walking distance from Ohio Union.

Please note that the Ohio Union North Garage will be under construction at the time of the conference, but the following parking garages are available: Union South Garage (which is next to the Ohio Union), ARPS, and Tuttle Garage are all available for visitors (parking fees can be paid by credit card at the exit gate). The Blackwell hotel is shown in the upper right corner of the map (blue star). More on-campus parking options can be found at: https://osu.campusparc.com/find-parking/academic-visitor-parking/



Conference program

Thursday, May 15, 2025

Location: Performance Hall, Ohio Union

8:00 – 8:30 Registration, coffee, tea and light pastry

8:30 – 9:00 **Welcome and Introductory Remarks**
Rafael Brüschweiler, David Horn (Dean), Krisztina Varga (NSF Program Director), Alain Belguise (Bruker)

Session 1 (Session chair: Rafael Brüschweiler)

Time	Speaker	Title
9:00 – 9:25	Bob Griffin (MIT)	Aducanumab binding to amyloid-β1-42 fibrils
9:25 – 9:50	Peter Wright (Scripps Research Institute, La Jolla)	Structure, dynamics, and interactions of proteins containing both disordered and structured domains
9:50 – 10:15		Coffee Break
10:15 – 10:35	Tatyana Polenova (U. Delaware)	MAS NMR, with and without DNP and integrative structural biology of viral protein assemblies
10:35 – 10:55	Kwaku Dayie (U. Maryland)	Advances in isotope labeling for RNA NMR
10:55 – 11:15	Jeff Peng (U. Notre Dame)	Studies of machine elements by NMR
11:15 – 11:35	Claudia Avalos (New York U.)	Correlating defect formation and photoluminescent properties in lanthanide doped cesium lead chloride using solid-state NMR
11:35 – 11:55	Pat Loria (Yale U.)	Allostery and substrate specificity in protein tyrosine phosphatases

Time	Speaker	Title
11:55 – 12:05	Radoslav Pavlovic (Northwestern U.)	NMR studies of synthetically designed biomaterials with neuronal and chondrogenic bioactivity
12:05 – 12:15	Lexi McCarthy-Carney (OSU)	Inverse methods for solid state NMR of quadrupolar nuclei

12:15 – 1:30 Lunch at Ohio Union

Session 2 (Session chair: Chris Jaroniec)

Time	Speaker	Title
1:30 – 1:50	Alexandar Hansen and Trent Franks (OSU)	The 1.2 GHz NMR spectrometer at OSU: capabilities, early results, and the user program
1:50 – 2:10	Dan Raftery (U. Washington)	Modeling blood metabolite homeostasis to reduce unexplained variance and reveal metabolic network relationships
2:10 – 2:30	Ann McDermott (Columbia U.)	Signaling in biological systems–Insights from NMR
2:30 – 2:40	Serge Smirnov (W. Washington U.)	Structure/dynamics and functional roles of sizable IDRs in dematin/villin cytoskeleton modulators
2:40 – 3:10		Coffee Break
3:10 – 3:30	Mei Hong (MIT)	Structures and mechanisms of virus ion channels from ^{19}F-enhanced solid-state NMR
3:30 – 3:50	Hashim Al-Hashimi (Columbia U.)	Bringing the structures of nucleic acids to life using NMR

Time	Speaker	Title
3:50 – 4:10	David Rovnyak (Bucknell U.)	NMR of small molecules: methods to metabolomics
4:10 – 4:20	Libin Ye (U. of South Florida)	Conformational dynamics of an Intermediate GPCR-G-protein complex

4:30 – 4:40 Walk from Ohio Union to CBEC

4:40 – 4:50 Poster setup in basement of CBEC (outside of NMR suite)

4:50 – 6:30 Poster session and NMR tours in CBEC. Official opening of National Gateway Ultrahigh Field NMR Center

6:30 – 8:00 Dinner in CBEC Lobby

Friday, May 16, 2025

Location: Performance Hall, Ohio Union

8:15 – 8:45 Coffee, tea and light pastry

Session 3 (Session chair: Mark Foster)

Time	Speaker	Title
8:45 – 9:05	Ad Bax (NIH)	Insights into the structures of metastable protein folding intermediates and Abeta oligomers from pressure-jump NMR
9:05 – 9:25	Lauren Marbella (Columbia U.)	Linking structure to function at electrochemical interfaces: Li-ion batteries and beyond
9:25 – 9:45	Art Edison (U. Georgia)	Small molecule analysis using ^{13}C NMR at 1.1 GHz
9:45 – 10:05	Haribabu Arthanari (Harvard Medical School)	The promised land of high-field NMR: potential, challenges, and opportunities

Time	Speaker	Title
10:05 – 10:15	Marvin Bayro (U. Puerto Rico)	Probing the bio-inorganic interface in polymers that regulate carbon mineralization
10:15 – 10:45		Coffee Break
10:45 – 11:05	Dorothee Kern (Scripps Research Institute)	The protein dance – from NMR experiments to AI predictions
11:05 – 11:25	Tuo Wang (Michigan State U.)	Solid-state NMR analysis of carbohydrate structure reveals the dynamic remodeling mechanism for fungal survival
11:25 – 11:45	Art Palmer (Columbia U.)	Conformational dynamics govern protein function: Insights from NMR spectroscopy and molecular dynamics simulations
11:45 – 12:05	Rob Tycko (NIH)	Time-resolved biomolecular solid state NMR
12:05 – 12:15	Irina Bezsonova (U. of Connecticut Health)*	Activation dynamics of ubiquitin-specific protease 7
12:15 – 12:25	Rodrigo Cabrera Allpas (OSU)*	COLMAR1d2d: Combining 1D with 2D NMR for the automated high-throughput identification and quantification of metabolites in complex mixtures

12:30 – 1:45 Lunch at Ohio Union

Session 4 (Session chair: Philip Grandinetti)

Time	Speaker	Title
1:45 – 2:05	Razvan Teodorescu (Bruker Inc.)	Pushing the boundaries of magnet technologies: The breakthrough behind ultra-High field NMR
2:05 – 2:25	Yi Zhang (Case Western Reserve U.)	NMR studies of structural mechanisms in chromatin biology
2:25 – 2:45	Chad Rienstra (U. Wisconsin)	Solid-state NMR at 1.1 GHz in NMRFAM
2:45 – 3:05	Lewis Kay (U. Toronto)	Solution NMR provides the missing link to understand function in large complexes
3:05 – 3:15	Andrea Poole (U. Louisville)	Functional site-distant mutations of hGMPK alter dynamics and kinetics
3:15 – 3:25	David Weliky (Michigan State U.)	REDOR NMR determination of quantitative populations of the very broad distribution of structures of the membrane-bound HIV fusion peptide – an evolutionary solution for chronic infection

3:25 – 4:00 Coffee Break

4:00 – 4:15 Walk from Union to CBEC

4:15 – 6:15 Poster session and NMR tours in CBEC

6:15 – 6:20 Removal of posters

6:45 – 8:30 Dinner Ballroom (2nd floor) of Blackwell Hotel (see map on p. 8)

Farewell

ABSTRACTS

Abstracts of invited talks:

Thursday, May 15, 2025

Session 1 (Session chair: Rafael Brüschweiler)

Aducanumab Binding to Amyloid-β1-42 Fibrils

Bob Griffin
Massachusetts Institute of Technology

Amyloid-β fibrils, implicated in Alzheimer's disease (AD), exhibit polymorphic structures sensitive to their formation conditions, with Aβ1-40 and Aβ1-42 as the primary isoforms. Previous structural studies using magic angle spinning nuclear magnetic resonance (MAS NMR) and dynamic nuclear polarization (DNP) have resolved high-resolution fibril structures and explored fibril polymorphism, including those seeded from AD brain plaques. Here, we extend these insights to investigate the binding interactions of Aducanumab, a monoclonal antibody, with recombinant Aβ1-42 fibrils.

We observe significant structural perturbations in the N-terminal region (residues 1–15) upon drug binding based on spectra obtained at 20 kHz MAS at 800 MHz. This study presented unique challenges due to the large size disparity between Aducanumab (~146 kDa) and Aβ1-42 fibrils (~5 kDa), compounded by the stoichiometry of only ~4.5 Aβ1-42 molecules per drug molecule, leaving limited labeled fibril sample for detection in the rotor. Despite this, site-specific changes were resolved, showcasing the sensitivity of modern MAS NMR techniques for challenging biological systems.

Additionally, we utilized adiabatic RFDR experiments to enhance sensitivity, enabling the detection of subtle interactions in these sparsely labeled systems. The characterization of adiabatic RFDR across spinning frequencies up to 100 kHz was also conducted, demonstrating its broader applicability and providing additional insights into the potential optimization of such experiments for future studies. While this serves as a digression, it highlights the relevance of high-speed MAS in addressing sample limitations and achieving detailed structural characterization.

Structure, dynamics, and interactions of proteins containing both disordered and structured domains

Peter E. Wright
Department of Integrative Structural and Computational Biology
The Scripps Research Institute, La Jolla, CA 92037

Intrinsically disordered proteins (IDPs) are highly abundant in eukaryotes and play a central role in key cellular regulatory pathways. Approximately half of the proteins in the human proteome and more than 80% of transcription factors contain extended regions that are intrinsically disordered. Molecular level characterization of proteins that contain both structured and disordered domains represents an enormous challenge to traditional methods of structural biology. Most structural studies to date have relied upon a reductionist, divide-and-conquer approach, in which the ordered and disordered regions are expressed independently and investigated in isolation using X-ray crystallography, cryo-EM, NMR and other biophysical tools. While this approach provides valuable information on the structure, dynamics, and interactions of the isolated domains, it can provide only limited insight into the interplay between the structured and disordered regions of the full-length protein. Within the cell, the ordered and disordered regions of a given protein act synergistically to allow it to perform its biological function; a detailed understanding of the underlying molecular mechanism can only be achieved through a hybrid approach that is focused on the properties of the intact, full-length protein. While NMR plays a major role, integration with data from other biophysical methods such as single molecule FRET, SAXS, and X-ray crystallography is required to derive molecular level insights into the dynamic intramolecular interactions that regulate biological function.

MAS NMR, with and without DNP and integrative structural biology of viral protein assemblies

Changmiao Guo[1,2]; Kumar Tekwani Movellan[1,2]; Roman Zadorozhnyi[1,2]; Somayeh Zeinalilathori[1,2]; Angela M. Gronenborn[1,2,3]; and Tatyana Polenova[1,2,3*]

(1) Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716;
(2) Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 Fifth Ave., Pittsburgh, PA 15261;
(3) Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA 15261

Our team's recent efforts to determine the structure and dynamics of protein assemblies from HIV-1, SARS-CoV-2, and P22 viruses will be presented. To investigate these assemblies and their complexes with small molecules at natural abundance, we integrate magic angle spinning (MAS) NMR, dynamic nuclear polarization (DNP), and computation. We will highlight the benefits of this approach for gaining atomic-level information that is otherwise inaccessible through other structural methods.

Advances in isotope labeling for RNA NMR

Theodore Kwaku Dayie* and Solomon Attionu
Department of Chemistry and Biochemistry
University of Maryland, College Park, MD 20742

RNA structural biology continues to play catch with that of proteins. NMR spectroscopy can bridge this gap, but formidable challenges of line broadening and signal overlap remain for RNAs with more than 35 nucleotides. Here, we showcase use of chemical enzymatic selective ^{2}H-, ^{15}N- and ^{19}F-^{13}C-labeling and NMR experiments designed to exploit these labels as potentially an exciting solution for RNAs as large as 200 nucleotides. Combined with other emerging technologies (e.g. ultrahigh fields), we think these advances hold promise for tackling large complexes with important biological functions.

Studies of machine elements by NMR

Jeff Peng
University of Notre Dame

TBA

Correlating Defect Formation and Photoluminescent properties in Lanthanide Doped Cesium Lead Chloride using Solid-State NMR

Thiago I. Rubio, Claudia E. Avalos*
Department of Chemistry
New York University, New York, NY 10003

Defects can be purposely introduced into the halide perovskites with the goal of tuning their optoelectronic properties. One such property is quantum cutting. Quantum cutting is defined as the emission of more than one low-energy photon subsequent to the absorption of one high-energy photon. Halide perovskites, when doped with lanthanide ions, are capable of exhibiting this process, particularly when Yb^{3+} is used as dopant, since only one electronic transition can occur, involving the ground state 2F_7/2 and the excited state 2F_5/2. Solid-state nuclear magnetic resonance spectroscopy (SSNMR) can provide information on how insertion of the Yb^{3+} ion can affect the local environments of the probed nuclei in the perovskite lattice. We investigate how increasing the number of Yb^{3+} dopants as well as mechanochemically induced point defects affects the local magnetic environment and the photoluminescence properties of the doped structure. We find that there is an optimum grinding time to maximize photoluminescence response and using paramagnetic Yb^{3+} and diamagnetic La^{3+} dopants combined with ^{139}La, ^{207}Pb and ^{133}Cs NMR we characterize the local symmetry of distinct vacancy sites that form upon doping.

Allostery and substrate specificity in protein tyrosine phosphatases

Emma Wang, Ryan Anderson, Jinchan Liu, Victor Batista, and Patrick Loria*
Department of Chemistry
Yale University, New Haven, CT 06520

Protein tyrosine phosphatases (PTP) are key regulatory enzymes in eukaryotes, prokaryotes, and archaea. The human PTP, PTP1B is a drug target due to its involvement in pathologies such as diabetes, obesity, and metastatic breast cancer. Because of its physiological importance there are numerous in vivo mechanisms for regulating PTP1B activity. Here, we explore by solution NMR and computation the role of allosteric regions in PTP1B and their impact on substrate specificity. We demonstrate that protein regions distant from the active site alter the substrate preference of PTP1B and suggest possible avenues to disease-specific ligands to modulate PTP1B function.

Session 2 (Session chair: Chris Jaroniec)

The 1.2 GHz NMR spectrometer at OSU: capabilities, early results, and the user program

Alexandar L. Hansen and Trent Franks
The Ohio State University

The Gateway 1.2 GHz instrument, the highest magnetic field instrument available in the Americas, is available for use and taking bookings. We will present the probes available for both solution and solid-state NMR and introduce the procedure for getting instrument time. We will present representative data collected with the 3mm solution NMR cryoprobe from protein, DNA, polymer, and metabolomics solutions and compare the 1.2 GHz data to experiments at lower field. We will present the ^{1}H spectra in biosolids using the Ultrafast MAS probes (0.7mm and 1.3mm) and demonstrate how this further enables the collection of structural restraints and dynamics information on complex biological systems such as fibrils and protein-DNA complexes. Finally, we will present the versatility of the slower spinning probes (3.2mm and 1.9mm) with state-of-the-art experiments on several nuclei and in a variety of materials such as zeolites and glasses.

Modeling blood metabolite homeostasis to reduce unexplained variance and reveal metabolic network relationships

Daniel Raftery* and G.A. Nagana Gowda
Department of Anesthesiology and Pain Medicine
University of Washington, Seattle WA 98109

Blood metabolite levels are affected by numerous factors, including demographic, clinical, genetic, as well as pre-analytical factors such as collection methods and geographical sites. This variance has caused deleterious consequences for many metabolomics studies, including the discovery and validation of potential metabolite biomarkers for various diseases, and thus represents a major challenge in metabolomics. It is important to understand the factors that cause the unwanted variance and develop methods and models that can reduce it. However, to date, the lack of suitable mathematical models for blood metabolite levels under homeostasis has hindered progress. We describe the development of quantitative models of blood metabolite levels in healthy adults based on multisite sample cohorts that reflect the current challenge. Four cohorts of samples obtained across three geographically distinct sites were investigated, focusing on approximately 50 metabolites that were quantified using ^{1}H NMR spectroscopy. A dramatic reduction in the site-to-site and sample to sample variation of metabolite levels (up to 96%) was achieved based on modeling each metabolite using demographic and clinical factors and especially other metabolites. Several metabolites that contributed disproportionately to such variation were associated with biosynthesis and degradation. The study demonstrates an intriguing, cross-pathway network effect of metabolites that can be utilized to better define basal homeostatic metabolite levels, which may have implications for improved health monitoring. In addition, recent results on sample quality biomarkers and metabolite profiling at 1.2 GHz will be discussed.

Signaling in biological systems - Insights from NMR

Yunyao Xu, Eric Keeler, Anish Shivamani, Chengming He and Ann McDermott*
Department of Chemistry, Columbia University, New York, NY 10027 and
New York Structural Biology Center, New York NY 10027

Ion channels are one of the largest cell-signaling superfamilies, underlying numerous critical health related phenomena. Dynamic allosteric processes in ion channels control key aspects of signaling, including inactivation and mean open time. Solid state NMR experiments on full length wild type channel in proteo-liposomes provide evidence for evacuation of ions from the selectivity filter during inactivation and thermodynamic coupling between channel opening and ion affinity. Furthermore, these experiments have identified residues that serve as “hotspots” for allostery. We examine an intermediate of the opening process and its conformational exchange processes.

Very high order oligomeric proteins including amyloids are increasingly seen to be involved in cell signaling in human health and disease. We examine structures of a number of oligomerized cell signaling factors, using NMR. For example, formation of the RIPK1:RIPK3 complex and the necrosome is a key commitment complex for TNF-induced necroptosis in the context of immune defense, cancer and neurodegenerative diseases. Using solid-state NMR, we determined the high-resolution structure of the necrosome core. RIPK1 and RIPK3 assume serpentine conformations, with short β-segments. Packing analogous to other amyloids results in a hydrophobic core with both hetero and homo protomer hydrophobic contacts, and unusual exposed “ladders” of interacting amino acids. This molecularly detailed structure of a hetero-oligomeric amyloid provides insights into the mechanisms of signal transduction and of inhibition of necroptosis. RIPK1:RIPK3 represents one member of a large family of analogous hetero-complexes involved in response to viral infection, and our recent work explores other examples.

Structures and mechanisms of virus ion channels from ^{19}F-enhanced solid-state NMR

Mei Hong*, Joao Medeiros-Silva, Iva Sucec, and Noah Somberg
Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

Many viruses encode ion channels that cause pathogenicity to cells. Coronaviruses encode an essential protein, the envelope (E) protein, whose ion channel activity is a virulence factor and a target of antiviral drugs. The small size and high hydrophobicity of E proteins made these protein resistant to characterization by X-ray crystallography and electron microscopy. In comparison, solid-state NMR spectroscopy is well suited for investigating the structure, dynamics and drug binding of these E proteins in lipid bilayers. I will present the high-resolution structures of the transmembrane domain of SARS and MERS E protein determined using solid-state NMR. Combining ^{13}C, ^{15}N-labeled proteins with sparsely fluorinated proteins, we have measured conformation-dependent chemical shifts, inter-atomic distances, helix orientations and oligomeric states of these proteins. These structures provide detailed insights into how the SARS E channel is activated to conduct protons and Ca2+, how small molecules bind SARS E to inhibit its channel activity, how MERS E structure differs from SARS E and how its structure suggests a novel ion conduction mechanism. I will also describe sensitivity-improved 19F-based REDOR techniques to measure long-range distances and to locate ligand binding sites in proteins with minimal resonance assignment.

NMR of small molecules: methods to metabolomics

David Rovnyak
Bucknell University

The adoption of ultra-high fields shines a spotlight on non-uniform sampling for improving evolution times with growing spectral windows. Yet, it is not trivial to generate sampling schedules of sufficient sparsity to yield the benefits of NUS, while also having sufficient fidelity in challenging small molecule experiments. To date, successful approaches have either been computationally intensive or required metric-based schedule curation by expert users. A set of theory-based tools is introduced that objectifies and automates the design of 1D-NUS schedules, taking the expert out of on-demand schedule design and making sparser NUS more accessible: a two-step filtering process enforces decoherence and artifact reduction while preserving broader schedule features. High fields also transform metabolomics, where the results of a recently completed multi-year study in characterizing the aqueous metabolites of steatotic liver disease will be shared, leveraging a standard-of-care biobank. A global epidemic, steatotic liver disease is likely the most widespread in the world, where accurate non-invasive tests of liver status are direly needed. A set of independent NMR studies, representing about 300 subjects in total, pursued protocol optimization, metabolite identification, reproducibility testing, detecting consensus biomarker of response, and final evaluation in a limited number of case studies. A metabolite panel for evaluating inflammation and fibrosis in steatohepatitis, considering both biomarker quality and biological grounding, will be presented and critically evaluated that ultimately highlights the potential for NMR metabolomics to detect changing liver status.

Conformational dynamics govern protein function: Insights from NMR spectroscopy and molecular dynamics simulations

Arthur G. Palmer III
Department of Biochemistry and Molecular Biophysics
Columbia University, New York, NY 10032

NMR spectroscopy is a powerful approach for probing conformational dynamics of biological macromolecules, uniquely providing both temporal and atomic resolution. Developments in instrumentation, and in experimental, theoretical, and computational methods continue to expand the role of NMR spin relaxation methods in elucidating biophysical concepts critical for specificity and affinity of molecular recognition by proteins and other macromolecules. Recent advances will be illustrated by applications to ribonuclease H enzymes, classical cadherin cell-cell adhesion molecules, and transcription factors. As a consequence of these advances, protein function increasingly can be understood in spatio-temporal detail.

Friday, May 16, 2025

Session 3 (Session chair: Mark Foster)

Insights into the structures of metastable protein folding intermediates and Abeta oligomers from pressure-jump NMR

Ad Bax*, Marty Gelenter and Elahe Masoumzadeh

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA

The equilibrium between a protein's folded and unfolded state is strongly impacted by hydrostatic pressure. Many proteins can be unfolded by applying a modest amount ($\leqslant$2.5 kbar) of pressure, compatible with measurements by NMR spectroscopy.

Rapidly and repeatedly dropping the pressure from denaturing conditions (i.e. 2.5 kbar) to 1 bar, synchronized with detection of the NMR spectrum, allows study of the actual protein folding process under native conditions. A device that allows such rapid (~3 ms) and repeated (100,000 times) switching, enables direct monitoring of the folding process by two- and three-dimensional NMR. NOE's and RDCs can be measured for metastable intermediates, allowing determination of their structures.

Hydrostatic pressure also can be used to resolubilize oligomerized or otherwise aggregated peptides and proteins. Such experiments are demonstrated for revealing structural information, including NOE contacts on the Alzheimer's related Aβ peptide in the oligomerized state, which differ substantially from the monomeric and the fibrillar states.

Linking structure to function at electrochemical interfaces: Li-ion batteries and beyond

Lauren Marbella
Columbia University

Despite the fact that the solid electrolyte interphase (SEI) on Li metal was described 45 years ago, it is still the only aspect of the battery that has ambiguity in function. As a community, we have struggled to establish structure-property-performance relationships for the SEI because it is a nanoscale composite that contains chemical compounds whose properties deviate from their bulk counterparts. In this talk, I will describe how we have used nuclear magnetic resonance (NMR) spectroscopy to characterize the structure and dynamics of interfacial phenomena in Li-ion and beyond Li-ion batteries and correlate these features with battery performance. In particular, I will focus on the use of NMR to quantify the source of Li inventory loss, the mechanism of transition metal dissolution, structural evolution at the electrode/electrolyte interface, and the function of the SEI. Insight from these methods, and the advent of GHz spectroscopy, will allow us to determine the precise mechanisms of failure that arise inside of functional devices as well as develop new approaches to mitigate performance decline.

Small molecule analysis using ^{13}C NMR at 1.1 GHz

Art Edison
University of Georgia

Direct detection of ^{13}C has several advantages for mixture analysis by NMR. Overlap in ^{13}C peaks is less than corresponding ^{1}H NMR spectra. Quaternary carbons can be detected, solvent peaks do not typically interfere with the spectrum, and chemical shifts are more stable with changes in pH. Even low concentrations of salt in aqueous samples can degrade ^{1}H sensitivity at high fields, while ^{13}C signals are less dependent on dielectric or salt effects owing to its lower frequency. Thus, ^{13}C NMR can be conducted in 5 mm tubes at high field, whereas ^{1}H NMR requires 3 mm or smaller tubes for adequate performance.

I will show some preliminary ^{13}C NMR results from various small molecule studies using the UGA/NAN 1.1 GHz spectrometer using a 5 mm TXO probe.

The promised land of high-field NMR: Potential, challenges, and opportunities

Haribabu Arthanari*, Ricarda Torner, Abhinav Dubey, Sebastian Schindler, Maxim Droemer, Thibault Viennet, Paul Coote and Gaurav Bhole
Department of BCMP, Harvard Medical School, Boston MA
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In an era of readily accessible structural data from de novo prediction, solution NMR remains indispensable for understanding protein structure, interactions, and dynamics under near-native conditions. It validates predicted models, reveals domain orientations and minor states, and illuminates allosteric mechanisms. NMR also clarifies how disordered regions modulate function. By adding richer structural insights, NMR complements existing knowledge and deepens our mechanistic understanding. Although high-field NMR offers superior resolution and sensitivity, fully harnessing its potential demands new approaches. This talk will highlight how NMR uniquely addresses mechanistic questions in biology and discuss strategies for leveraging high-field capabilities.

The Protein Dance – from NMR experiments to AI predictions

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Why can we not design efficient enzymes or highly selective drugs to date? While one can solve high resolution structures of ground states experimentally, and even now predict them with Alphafold; for biological function proteins need to traverse the entire energy landscape from the lowest energy state over the transition states into higher energy states. I will discuss the enormous power of NMR to experimentally determine high resolution structures of high-energy states that are often the biologically reactive species (1). Second, I will describe a first new method (AF-cluster) to predict multiple protein conformations using AI (2). Clustering a multiple-sequence alignment by sequence similarity enables AlphaFold2 to sample alternative states of known metamorphic proteins with high confidence (2). NMR played a crucial role in experimentally testing our new method. Finally I will highlight NMR at its best by providing experimental dynamics data for our first adventures to predict micro- to millisecond (µs-ms) motions by machine learning. Curated NMR data, combined with obtaining a large training data set by a bold call, were used as experimental input to train a classifier using several protein language models. Our resulting new dynamics model has predictive power for µs-ms dynamics, with the strongest predictive power for motions directly linked to biological function (unpublished).

1. J. B. Stiller et. al., Structure Determination of High-Energy States in a Dynamic Protein Ensemble Nature 2022, 603, 528–535

2. H.K. Wayment-Steele et al., Predicting multiple conformations via sequence clustering and AlphaFold2, Nature 2024, 625, 832–839

Solid-state NMR analysis of carbohydrate structure reveals the dynamic remodeling mechanism for fungal survival

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Invasive fungal infections pose a significant threat to human health, with high mortality rates persisting despite treatment. The rise of drug resistance has driven efforts to develop novel antifungal agents that target the fungal polysaccharides. Despite decades of molecular and biochemical research, the organization and interactions of polysaccharides in fungal cell walls remain poorly understood due to the complexity of analyzing these components within intact cells. Here, we highlight three recent advances enabled by $^{13}C/^{15}N/^{1}H$ solid-state NMR and Dynamic Nuclear Polarization (DNP). In Aspergillus, we identified structural modifications that reconfigure the carbohydrate network, enhancing resistance to the antifungal drugs named echinocandins, which target β-glucan synthesis. In Mucorales, major contributors to COVID-19-associated co-infections, we characterized a chitin- and chitosan-rich cell wall structure. Nikkomycin, a chitin synthesis inhibitor, selectively disrupted a specific chitin-chitosan-β-glucan complex while leaving other chitin structures intact, explaining the reduced susceptibility of these pathogens to this antifungal class. Finally, ^{1}H-detection of Candida albicans and the multidrug-resistant Candida auris revealed similar overall cell wall architectures but distinct remodeling responses to caspofungin treatment. These findings establish a structural framework for understanding fungal stress adaptation and antifungal resistance, offering crucial insights for the development of targeted therapies that disrupt cell wall biosynthesis.

Bringing the structures of nucleic acids to life using NMR

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A central goal of biomedical science is to develop a predictive understanding of living systems—from cells to tissues to entire organisms—based on the behavior of their constituent biomolecules. Achieving this goal would transform fields such as synthetic biology and drug discovery. For decades, structural biologists have pursued this vision by solving the static, three-dimensional structures of macromolecules at atomic resolution. Yet, despite these advances, we still lack a deep, predictive grasp of how even the simplest proteins and nucleic acids function in their native environments. Properties such as catalytic efficiency, specificity, fidelity, and stability often elude interpretation from static structures alone, even when multiple conformational states are known. In this talk, I will describe how solution state NMR spectroscopy is helping to reveal the dynamic conformational ensembles formed by DNA and RNA, and how these ensembles in turn are making it possible build quantitative and predictive models for cellular processes.

Time-resolved biomolecular solid state NMR

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National Institutes of Health
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NMR techniques are often used to characterize molecular structures and motions of biopolymers, but these are usually structures and motions that exist at thermal equilibrium or in a long-lived metastable state. Our lab has developed novel methods and equipment for NMR studies of time-dependent, nonequilibrium processes in which biopolymer structures in solution evolve in a unidirectional manner on the millisecond time scale. This time-resolved NMR approach involves rapid mixing or a rapid temperature drop to initiate a process, rapid freezing to trap intermediate states after a variable evolution time, and solid state NMR at very low temperatures with signal enhancements from dynamic nuclear polarization. This lecture will describe the methods and show results from several examples, including studies of calmodulin/peptide complex formation triggered by Ca^{2+}, protein folding triggered by a rapid temperature drop, and amyloid-beta peptide self-assembly triggered by a rapid pH drop.

Session 4 (Session chair: Philip Grandinetti)

Pushing the Boundaries of Magnet Technologies: The Breakthrough Behind Ultra-High Field NMR

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Innovations in magnet technology have propelled Ultra-High Field NMR to new heights, enabling unprecedented sensitivity and resolution for structural biology applications. Advancements in superconducting materials, magnet coil designs, and cryostat engineering have driven the evolution of NMR magnets over the past three decades. The state-of-the-art Ascend 1.2 GHz magnet is the highest field NMR magnet commercially available today operating at 28.2 Tesla. It represents a culmination of a 15-year development program overcoming significant challenges associated with the integration of High Temperature Superconductors (HTS), initially seen as unsolvable. Since its launch in 2019, the hybrid HTS/LTS technology based 1.2 GHz magnet has redefined the limits of NMR research in structural biology at more than a dozen laboratories worldwide, including the National Gateway Ultra-High Field NMR Center at Ohio State University.

NMR studies of structural mechanisms in chromatin biology

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Chromatin biology is governed by a complex network of protein interactions that shape gene expression. Post-translational modifications (PTMs) on histone tails act as key signaling hubs and are selectively recognized by reader proteins that modulate chromatin accessibility. Reader domains are rarely found as isolated modules; instead, they often function in tandem with other reader or writer domains, forming multifunctional units. This dynamic interplay among reader proteins, chromatin remodelers, and modifying enzymes gives rise to a finely tuned regulatory system that directs cellular processes ranging from development to disease. Using solution NMR spectroscopy, we have previously identified novel histone reader domains and uncovered autoregulatory mechanisms in chromatin-modifying complexes, revealing how epigenetic readers fine-tune the activity of associated catalytic modules. Here, we will discuss our recent NMR-based studies to understand the structural basis of new interactions in epigenetics.

1.1 GHz Solid-State NMR at NMRFAM

Chad Rienstra*,[1,2] Songlin Wang[1], Thirupathi Ravula[1,2], Lauren Price[1,2], Barry DeZonia[1,2], Collin Borcik[1], Alex Paterson[1], Marco Tonelli[1], Paulo Falco-Cobra[1], Peter Gor'kov[3,4], John Stringer[5], Sam Butcher[1], Katherine Henzler-Wildman[1], Leonard Mueller[6]

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(6) Department of Chemistry, University of California-Riverside, Riverside, CA

In this talk, I will share progress on installation and customization of the Bruker 1.1 GHz spectrometer housed at the University of Wisconsin-Madison National Magnetic Resonance Facility at Madison (NMRFAM) as part of the NSF-funded Network for Advanced NMR. The Bruker Ascend magnet reached field in November 2023 and was accepted in December 2023. Since then, we have optimized a series of third-party probe designs to achieve substantial improvements to the sensitivity and resolution for ^{13}C detection, enabling assignments of doubly-^{13}C,^{15}N-labeled proteins up to nearly 150 kDa. The best practices combine facility infrastructure upgrades, a low electric field resonator for ^{1}H decoupling, an efficient triple resonance tuning circuit for ^{13}C detection and ^{15}N decoupling, a ^{2}H lock channel, low noise preamplifiers, homonuclear LOW-BASHD decoupling during acquisition, and automated adjustment of shimming and cross polarization settings with OPTO software. For ^{1}H detection, we have developed a prototype probe design that doubles sensitivity for triple resonance protein experiments at 600-750 MHz and shows promise for additional improvements at 900 MHz and 1.1 GHz. Examples of scientific applications include large microcrystalline proteins, fibrils, membrane proteins, RNAs and complexes of antifungal drugs with sterols.

Solution NMR Provides the Missing Link to Understand Function in Large Complexes

Lewis E Kay
Departments of Molecular Genetics, Biochemistry, and Chemistry
University of Toronto

Over the past years there have been important advances in NMR methodology, enabling studies of molecular complexes. Such studies have provided valuable insights connecting dynamics with function – insights that are not available through other technologies. In this talk I will describe applications to a number of different systems that have been enabled by the NMR methodology.

Abstracts of promoted poster talks:

NMR studies of synthetically designed biomaterials with neuronal and chondrogenic bioactivity

Radoslav Pavlovic
Northwestern University

Supramolecular polymers of peptide amphiphiles (PAs) spontaneously assemble under near-physiological conditions, forming dynamic and internally ordered structures—key features for soft biomaterials that signal cells and drive regeneration. Understanding and optimizing these materials require atomic-to-supramolecular resolution, which we achieve using multinuclear and multiphase (M&M) NMR spectroscopy at high magnetic fields. Combined with microscopy and light scattering, this approach provides crucial insights into supramolecular species, signal stoichiometry, and dynamics under biologically relevant conditions.

We apply this methodology to supramolecular PA copolymers designed for spinal cord regeneration, which incorporate bioactive signals for β1-integrin and FGF2 receptor activation. M&M NMR revealed a dynamic equilibrium between small PA clusters and micron-long filaments silent in solution-phase NMR, with ^{1}H relaxometry, diffusometry, and NOESY characterizing their structure and cell-signaling relevance.

A second system leverages amphiphilic organic cations to fully ionize supramolecular nanostructures, creating a stable cloud of biologically relevant ions such as sodium and potassium. M&M NMR studies demonstrated enhanced PA ionization and stronger monovalent cation binding, providing a rationale for the observed neuronal bioactivity.

For cartilage regeneration, we investigated PA co-assemblies incorporating a cyclic peptide mimetic of TGF-β1 alongside a nonbioactive diluent PA. Solution-phase ^{1}H NMR relaxation times correlated supramolecular motion with intracellular signaling, while ^{1}H DOSY distinguished monomeric species from self-assembled structures, revealing distinct molecular interactions within TGF PA coassemblies.

I will present how key NMR insights advance our understanding of these supramolecular polymers' biological activity, guiding the design of functional nanostructures and soft materials while shedding light on their interactions with biological targets.

Inverse methods for solid-state NMR of quadrupolar nuclei

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Dynamic Angle Spinning (DAS) and Multiple-Quantum Magic Angle Spinning (MQ-MAS) are information-rich experiments that provide deep insight, especially for amorphous samples. However, analyzing the spectra of amorphous materials is difficult due to the statistical nature of the chemical environments surrounding observed nuclei. The typical way to analyze such datasets is to create a model, fit the spectrum for model parameters, and then use these parameters to understand the local environment. Recent work in the Grandinetti lab has shown that the spectrum inverse approach is often superior to this model-forward approach because it removes the need for a model and reveals the distributions inherent in the spectrum. In this work, we invert quadrupolar isotropic-anisotropic correlation spectra to underlying structure parameters. We apply a shear transformation to DAS datasets to achieve a purely anisotropic dimension and then invert using truncated singular value decomposition and smooth-LASSO regularization with hyperparameters determined with k-fold cross-validation. MQ-MAS datasets are more difficult to invert due to the significant lineshape distortions that occur. Recently, we have developed an improved algorithm to rapidly simulate MQ-MAS spectra that would allow inverse methods to be possible. We use this algorithm to generate a kernel and then invert to underlying structure parameters with the same algorithm. This approach allows for model-free analysis of 2D quadrupolar spectra and a deeper understanding of amorphous materials.

Structure/dynamics and functional roles of sizable IDRs in dematin/villin cytoskeleton modulators

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Human dematin is an F-actin bundling protein which helps regulate the shape and function of mature erythrocytes. The protein consists of a 315-resdiue N-terminal IDR fused with a folded, 68-residue villin-style headpiece. The IDR is hypothesized to form a non-covalent interface with the headpiece upon its phosphorylation at Ser381, a post-translational modification which abolishes F-actin bundling. The specific residues involved in this intramolecular regulatory interface and the mechanisms of binding remain unknown. To probe this, we are collecting solution NMR data for several segmentally labeled dematin derivatives. Presented here are the samples which share the 15N-labeled, 305-residue IDR segment fused via sortase-mediated ligation with either of the two versions of the headpiece at natural abundance: a wildtype and a phosphomimetic mutant (S381E). The mutant is hypothesized to display NMR spectral features consistent with non-covalent interaction between the IDR and the headpiece. Here we present our recent NMR data (15N-HSQC) for the two dematin variants recorded at 1200 MHz magnet strength. The excellent 1200 MHz instrument allows us to detect and resolve nearly 90% of the IDR backbone HN resonances. The spectra recorded at 7 ℃ and 25 ℃ indicates that the IDR conformation is sensitive to temperature. We also observe that although the S381E mutation of the headpiece alters the IDR chemical shifts only in a minor way, it selectively modifies signal volumes for all IDR's Trp side chains and possibly few backbone signals (at 7 ℃) indicating a key role of the aromatic residues in the intramolecular interface.

Conformational dynamics of an Intermediate GPCR-G protein complex

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Understanding the roles of intermediate states in signaling is pivotal to unraveling the activation processes of G protein-coupled receptors (GPCRs). However, the field is still struggling to define these conformational states with sufficient resolution to study their individual functions. Here, we demonstrate the feasibility of enriching the populations of discrete states via conformation-biased mutants, guided by a conformational landscape profiled by 19F-NMR. These mutants adopt distinct distributions among five states, from the fully inactive state S1 to the fully activated state S5, that lie along the activation pathway of adenosine A2A receptor (A2AR), a class A GPCR. Our study reveals a structurally conserved cation-π lock between transmembrane helix VI (TM6) and Helix8 that regulates cytoplasmic cavity opening as a "gatekeeper" for G protein penetration. A GPCR activation process based on the well-discerned conformational states is thus proposed, allosterically micro-modulated by the cation-π lock and a previously well-defined ionic interaction between TM3 and TM6. The function of these trapped intermediate states and their complexes were also studied, indicating that nucleotide exchange occurs at the stage of forming the intermediate GPCR-G protein complexes instead of the fully activated end-state complex. These advances fill up the structural and functional gaps of our understanding of GPCR activation and signaling complexity.

Probing the bio-inorganic interface in polymers that regulate carbon mineralization

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The molecular mechanisms of biomineralization are highly challenging to characterize at the atomic level due to the complexities of protein-inorganic-water interface. Likewise, bio-inspired polymers have been shown to control the nucleation and growth of minerals such calcium carbonate, but their mechanisms of action remain elusive. Our goal in this project is to probe the interactions between bio-inspired polymers that promote carbonate nucleation and the inorganic lattice. We show that, despite the low surface area to volume ratio of the crystals and the low concentrations of polymers needed for nucleation control, solid-state NMR spectroscopy at 700 MHz Larmor frequency provides novel information on the polymer conformation son the crystal surface. Also, we detect chemically distinct carbon sites on the calcite form of calcium carbonate that may play a role in polymer interactions that regulated nucleation and crystal growth. Relaxation measurements, water-filtered cross-polarization, paramagnetic relaxation enhancement, and variable temperature experiments provide additional insight on the structure and dynamics of the bio-inorganic interface.

Activation dynamics of ubiquitin-specific protease 7

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Ubiquitin-specific protease 7 (USP7) is a deubiquitinating enzyme that plays a crucial role in cellular processes, including the maintenance of genome stability and regulation of anti-viral and immune responses. Its dysfunction is linked to various cancers and neurodevelopmental disorders such as Hao-Fountain syndrome. Unlike other USP-family enzymes, the triad of catalytic residues in USP7 adopts an inactive conformation and undergoes rearrangement into the active state upon substrate binding. Despite its potential importance for regulating the enzyme's activity, the dynamics of USP7 have not been explored. In this study, we combine advanced CPMG NMR relaxation dispersion measurements with the analysis of enzyme kinetics to investigate the conformational dynamics of USP7 in solution and its role in enzyme activation. Our results suggest that apo-USP7 exists in a dynamic equilibrium, transiently switching between inactive and low-populated active conformations, indicating that enzyme activation can occur spontaneously, even in the absence of a substrate. Furthermore, we show that the Hao-Fountain syndrome-associated variant G392D enhances the conformational dynamics of the enzyme, leading to a significant increase in its catalytic activity. This study captures the sparsely populated, "invisible" active conformation of USP7 and demonstrates how changes in enzyme dynamics can contribute to activity, offering broader insights into enzyme function and disease mechanisms.

COLMAR1d2d: Combining 1D with 2D NMR for the automated high-throughput identification and quantification of metabolites in complex mixtures

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We present a protocol named COLMAR1d2d and its application by combining COLMAR1d with COLMARm allowing us to identify a maximal number of compounds by taking advantage of the added resolution of 2D experiments to obtain unambiguous and accurate ^{1}H chemical shifts for the molecules identified by COLMARm. The corrected ^{1}H chemical shifts are then used in COLMAR1d to directly quantify a much larger number of metabolites than is feasible without combining the two methodologies. We demonstrate the new approach for complex metabolomics systems, including mouse urine and P. aeruginosa biofilm. We show that the pool of fully analyzable and quantifiable metabolites becomes more reliable and substantially larger making the method ideal for large-scale high-throughput metabolomics studies in a rapid and user-independent manner.

Functional site-distant mutations of hGMPK alter dynamics and kinetics

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Human Guanylate Kinase (GUK1, hGMPK) is the only known enzyme that phosphorylates GMP to GDP. In cancer, GUK1 contains a variety of single, missense mutations, with nearly half occurring outside known functional sites (Tate et al., 2019, p.D941). While the impact of mutations in functional sites on enzyme activity is well understood, the effects of functional site-distant (FSD) mutations remain unclear. Previous [1H,15N]-HSQC spectra and kinetic experiments of hGMPK FSD mutants show little change in structure, but altered kinetic parameters such as kcat and Km, possibly due to changes in protein dynamics (Khan et al., 2019, p.11920). Currently, we are characterizing hGMPK-G3A and hGMPK-G3L to understand how the FSD mutations impact enzyme activity and protein dynamics. At 298K, hGMPK-WT exhibits a second-order forward reaction rate that is ~1.5-fold faster than G3A, yet ~3-fold slower than G3L. This suggests that G3A impedes catalytic activity while G3L enhances it. Comparison of picosecond-nanosecond dynamics and microsecond conformational exchange between WT and G3A reveals that apo-G3A is more rigid than apo-WT, but GMP binding partially 'rescues' G3A to a WT-like state. This is supported by a 2-fold decrease in the 2-state chemical exchange rate (kex) for apo-G3A compared to apo-WT with similar kex values during GMP binding. Our ongoing experiments aim to understand how G3L's protein dynamics compare to both WT and G3A, further elucidating the role of position 3 and FSD mutations in hGMPK. Understanding how single amino acid substitutions affect enzyme function is crucial for elucidating how FSD mutations modulate catalytic activity

REDOR NMR determination of quantitative populations of the very broad distribution of the structures of the membrane-bound HIV fusion peptide – an evolutionary solution for chronic infection

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The HIV fusion peptide (Fp) is a ~25-residue N-terminal segment of the larger Gp41 spike protein. Fp's bind membranes of target cells, which is needed for fusion (joining) HIV and cell membranes, a required step in infection. Fp's adopts intermolecular antiparallel beta sheet structure in membrane. Multidimensional NMR spectroscopy gave unclear results about the registry (alignment) of adjacent Fp's in the sheets. REDOR NMR of more selectively-labeled samples revealed there is a very broad distribution of registries. Global analysis of REDOR data from 18 differently-labeled samples resulted in quantitative fractional populations, with 12 different registries having populations 0.02. This very broad distribution is likely an evolutionary solution by HIV for chronic infection, because fusion function is maintained during the continual mutation that is required to evade the immune system. There is also a broad registry distribution for the engineered V2E mutation of Fp, which is correlated with total loss of HIV fusion and infection. However, the V2E distribution is very different and weighted to longer registries (beta sheets). Determination of molecular structural populations by NMR and other approaches has typically relied on the relative intensities of resolved signals. This work shows that REDOR NMR can be applied to determine broad structural distributions when there isn't such resolution among individual structures. It isn't yet known whether there are other functional proteins with similarly broad but defined structural distributions.

Abstracts of posters:

Phase separation modulates the conformational landscapes of proteins

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Biomolecules can be sequestered into membrane-less compartments, referred to as biomolecular condensates. Experimental and computational methods have helped define the physical-chemical properties of condensates. Less is known about how the high macromolecule concentrations in condensed phases contribute "solvent" interactions that can remodel the free-energy landscape of other condensate-resident proteins, altering thermally accessible conformations and, in turn, modulating function. Here, we use solution NMR spectroscopy to obtain atomic resolution insights into the interactions between the immature form of superoxide dismutase 1 (SOD1), which can mislocalize and aggregate in stress granules, and the RNA-binding protein CAPRIN1, a component of stress granules. NMR studies of CAPRIN1:SOD1 interactions, focused on both unfolded and folded SOD1 states in mixed phase and demixed CAPRIN1-based condensates, establish that CAPRIN1 shifts the SOD1 folding equilibrium toward the unfolded state through preferential interactions with the unfolded ensemble, with little change to the structure of the folded conformation. Key contacts between CAPRIN1 and the H80-H120 region of unfolded SOD1 are identified, as well as SOD1 interaction sites near both the arginine-rich and aromatic-rich regions of CAPRIN1. Unfolding of immature SOD1 in the CAPRIN1 condensed phase is shown to be coupled to aggregation, while a more stable zinc-bound, dimeric form of SOD1 is less susceptible to unfolding when solvated by CAPRIN1. Our work underscores the impact of the condensate solvent environment on the conformational states of resident proteins and supports the hypothesis that ALS mutations that decrease metal binding or dimerization function as drivers of aggregation in condensates.

Development of a rational ligand discovery method for dynamic proteins

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Only 20-40% of the human proteome is predicted to be ligandable with current noncovalent small molecule methodology, leading to a large array of biologically important and disease contributing proteins that are inaccessible with small molecules. Dynamic and disordered proteins, in particular, are often passed over as drug targets due to limited structural information and their scarcity of traditional binding pockets. In this work, MixMD simulations were utilized to identify cryptic pockets in the dynamic transcriptional coactivator Med25. MixMD uses organic solvents as probes with unique functional groups in molecular dynamics simulations to output occupancy maps which are then overlayed to identify pockets on a protein of interest. This technique has successfully identified known binding pockets and allosteric sites on enzymes but has not yet been applied to a highly dynamic protein such as Med25. Subsequent ligand library docking results predicted a diverse array of small molecules that could bind to the pockets, and ^{1}H, ^{15}N HSQC NMR and differential scanning fluorometry (DSF) have been employed to experimentally validate ligand binding. Utilizing these two orthogonal methods, different sets of ligands were identified as hits, meriting further controls and investigation. However, 40% and 35% hit rates were observed for ^{1}H, ^{15}N HSQC NMR and DSF, respectively, illustrating the success of MixMD pocket identification and ligand discovery. In the future, these methods can be used to further facilitate rational targeting of dynamic and/or disordered proteins, significantly expanding the ligandable human proteome.

Exploring H3 histone tail conformational ensembles in nucleosomes using NMR and MD simulations

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Biochemistry and Molecular Genetics

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Histone H3 N-terminal tails play a crucial role in chromatin organization and regulation. However, their dynamic nature makes the characterization of their conformational ensembles within the nucleosome difficult. This study integrates Nuclear Magnetic Resonance (NMR) spectroscopy and Molecular Dynamics (MD) simulations to elucidate the conformational ensemble of histone tails in a nucleosomal context. We performed a detailed analysis of experimental chemical shifts and those calculated from MD trajectories to gain deeper insights into this ensemble.

Structural dynamics of K-Ras by combining advanced NMR techniques

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The GTPase K-Ras is a signal transduction protein that cycles between the inactive guanosine diphosphate (GDP)-bound form and the active guanosine triphosphate (GTP)-bound form. Despite its importance in many types of human cancer, major gaps in atomic-level information severely limit our understanding of its function in health and disease. We recently determined quantitative backbone structural dynamics of K-Ras and two of its oncogenic mutants, G12C and G12D, by solution NMR experiments. The entire range of motional timescales from picoseconds to milliseconds was probed using nanoparticle-assisted spin relaxation (NASR), relaxation dispersion, and chemical exchange saturation transfer (CEST) experiments. These experiments allow the detection and analysis of functionally important Switch I and Switch II regions, which have previously remained elusive by both X-ray crystallography and NMR spectroscopy. Our data reveal cooperative transitions of K-Ras·GTP to a highly dynamic excited state that closely resembles the partially disordered K-Ras·GDP state. Quantitative residual dipolar couplings (RDCs) of K-Ras·GDP and K-Ras·GTP will be presented. Together with our NASR data, they underscore that existing X-ray crystal structures of K-Ras are inadequate representations of the structural-dynamic behavior of this challenging protein system.

Cre recombinase undergoes an α-β secondary fold switch

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Cre is a gene editing tool that has been critical in establishing transgenic animal models of human diseases. One advantage Cre possesses over similar gene editing tools is its conservative single-strand exchange activity. Cre accomplishes single-strand exchange via coupled conformational changes in tetrameric complexes, most notably in the trans-docking, C-terminal helix. Despite decades of study, comparatively less is known about the conformations Cre adopts prior to binding DNA.

We discovered via paramagnetic relaxation enhancement measurements that the C-terminal sequence docks in cis over the active site of the catalytic domain. These results are corroborated by backbone relaxation measurements, which exhibit rotational correlation times similar to residues known to be in the core of the enzyme. Using a combination of secondary chemical shift analysis, amide and methyl relaxation measurements, and NOE-guided structure refinement, we find that the apo Cre catalytic domain exhibits fold switching in its linker region and C-terminal sequence. The functional implications of this helix-to-strand transition have been shown to extend to DNA site discrimination. These findings provide necessary insights into the target search mechanism of Cre recombinase.

Surface electrostatic potential measurements of the nucleosome core particle reveal distinct histone tail structural dynamics

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The nucleus of a eukaryotic cell, approximately 10 microns in diameter, contains about 2 meters of DNA. To solve this compaction problem, chromatin is organized into structural units, the smallest of which is the Nucleosome Core Particle (NCP). The NCP consists of an octamer of histones with two copies of each of histones H2A, H2B, H3, and H4, wrapped by 147 base pairs of DNA. Each histone shares a common structural architecture with a folded domain that forms the core of the NCP and an N-terminal disordered tail (and a C-terminal tail for H2A) ranging between 10 and 35 residues and extending from the core. These lysine-rich tails are dynamic but also interact with the negatively charged DNA. The strength of the interactions is modulated by post-translational modifications (PTMs) to regulate gene expression, DNA condensation, and DNA repair, for example. We have used NMR spectroscopy to measure per-residue surface electrostatic potentials of the histones tails, both without and with acetylation or PARylation PTMs, to characterize how these modifications affect the tail structural dynamics through changes in local charges.

Structural plasticity and maturation of interleukin-18

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Proteins are dynamic molecules that interconvert between different conformations on a wide range of timescales. The often sparsely populated structures that result have been shown to play integral roles in protein function. We present here an example involving a small signaling protein, the cytokine interleukin-18 (IL-18), whose dynamics in the pro-form (inactive) may play a role in facilitating its maturation process. Recently published structures of pro-IL-18 and pro-IL-18 complexed with caspase-1 and caspase-4, proteases which cleave (activate) IL-18 reveal significant differences between the apo and complexed forms, particularly in the β1 and β* strands, though both forms adopt a β trefoil fold. To determine whether the structural changes accompanying caspase binding might be facilitated in part through the intrinsic dynamics of pro-IL-18, we performed a range of dynamics experiments that are sensitive to motions extending over twelve orders of magnitude, from ps-ns to seconds, including ^{15}N spin relaxation, CPMG, CEST, and HDX. We find that the β1 and β* strands, while rigid on the ps-ns timescale, undergo exchange on the μs-ms timescale involving at least three conformational states, in addition to potentially even slower motions which expose these strands to hydrogen exchange. Although our results indicate that the lowly populated states sampled by the apo protein characterized here do not recapitulate the bound form, the correlation between the residues showing dynamics and those participating in the structural changes accompanying caspase binding suggests that this plasticity likely plays an important role in the maturation of this cytokine.

NMR investigations of the structural role of phosphorus in aluminosilicate glasses for ion exchange

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Chemically strengthened aluminosilicate glasses are used in various applications, from phone screens to airplane windshields, where cracks in the glass can have inconvenient and disastrous consequences. Adding small amounts of phosphorus to these glasses modifies the connectivity of the vitreous network creating stronger glasses in less time. An understanding of the structural role of phosphorus in these systems is therefore necessary for designing compositions that optimize the chemical strengthening process. Here, we present a comprehensive 31P NMR investigation on a series of aluminosilicate glasses with varying concentrations of P2O5 ranging from 0 mol% to 10 mol%. Initial MAS spectra revealed a significant difference in phosphorus site speciation, with higher isotropic chemical shifts in samples with lower aluminum-to-phosphorus ratios. To probe these lineshapes further, we performed Magic Angle Flipping (MAF) measurements that involve spinning the sample on and off the magic angle in two separate time domains. The result is a two-dimensional spectrum correlating an anisotropic lineshape to each isotropic frequency. With our recently developed MRInversion software, we inverted these MAF spectra to determine distributions of CSA parameters for each glass composition. The interpretation of these 31P shielding tensor parameters in terms of the structural role of phosphorus will be discussed.

Metabolomic changes in biofilm of pseudomonas aeruginosa under tobramycin treatment identified by COLMARq

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Biofilms are linked to 80% of persistent microbial infections within the body and are responsible for 25% of patient deaths in hospitals. They are formed by pathogens, such as Pseudomonas aeruginosa, as a gel-like structure composed of extracellular polysaccharides, DNA, and proteins that surround and protect these bacteria. Biofilm exhibits much higher resistance to antibiotics than that of their planktonic growth form. Biofilms are difficult to eradicate and thereby significantly contribute to the ongoing surge of infectious diseases. To better understand the biochemical response of biofilm to antibiotic treatment, we treated biofilms by tobramycin, a widely used antibiotic, to identify and quantify metabolic changes. To identify metabolomic markers, we used the COLMARq method (Complex Mixture Analysis by NMR for quantification), which is available as a publicly accessible web server for the quantitative untargeted metabolomics analysis of cohorts of 2D NMR spectra enabling their streamlined semi-automated metabolite identification, quantification, and statistical analysis. Using COLMARq, we could identify statistically significant differences for more than 10 metabolites in dose dependent manner providing direct pharmacological insights into the behavior of biofilms when treated by antibiotics.

Insights into dynamically covalent and topologically constrained polymer through solid state NMR

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Dynamic covalent polymers represent a frontier in sustainable materials science due to their unique combination of mechanical robustness and recyclability. This work employs solid-state nuclear magnetic resonance (ssNMR) spectroscopy to elucidate the molecular mechanisms underlying vitrimer network rearrangement during thermal transitions. Using low-field ^{1}H multiple quantum (MQ) NMR techniques, we probe the relationship between bond exchange kinetics and macroscopic flow behavior. The temperature-dependent evolution of MQ coherences reveals distinct dynamic regimes corresponding to the glassy state, viscoelastic plateau, and terminal flow. Analysis of dipolar coupling distributions indicates heterogeneous mobility associated with different network topologies. Combining these measurements with rheological characterization and nano-IR AFM establishes direct correlations between molecular reorganization and viscoelastic properties. Our findings demonstrate that ssNMR, particularly low-field ^{1}H MQ methodologies, provides unique mechanistic insights into vitrimer transitions that complement traditional thermal and mechanical analyses, offering a powerful approach for rational design of next-generation recyclable network polymers.

Molecular insights into saline wetland soil carbon through sensitivity-enhanced solid-state NMR

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Saline wetlands serve as vital ecosystems for global carbon sequestration; however, the molecular composition and stabilization processes of soil organic matter (SOM) remain insufficiently understood. In this research, we utilized sensitivity-enhanced solid-state NMR techniques to achieve a high-resolution, molecular-level characterization of soil organic carbon (SOC) in coastal wetland environments. Through our analysis, various forms of glucan chains were identified using 2D ^{13}C-^{13}C and ^{1}H-^{13}C correlation experiments, confirming the presence of cellulose within surface soil layers. Additionally, the detection of both 2-fold and 3-fold xylan reinforced the structural integrity of plant-derived materials. Long-range ^{1}H-^{13}C correlation experiments further revealed molecular interactions between aromatic compounds and carbohydrates, supporting the preservation of plant structural cores within wetland soil. With decreasing salinity, compositional and packing variations in SOM were observed along the depth profile. Semi-quantitative multi-CP spectra demonstrated a progressive decline in polyethylene and carbohydrate components with increasing depth. These results emphasize the significance of saline wetlands as distinctive carbon reservoirs and offer molecular insights into their role in global carbon dynamics. This study highlights the transformative potential of DNP-enhanced solid-state NMR in soil science, providing a robust approach for refining predictive models of soil carbon cycling and guiding strategies to optimize carbon sequestration in wetland ecosystems.

Applications of paramagnetic relaxation enhancement NMR spectroscopy in solids and liquids

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In this contribution, we present recent efforts to characterize interactions in biomolecular interfaces using paramagnetic relaxation enhancement effects in NMR spectroscopy. In a first application, solvent PRE agents are used to distinguish surface from interior proton signals in calcium carbonate crystals. The goal of this project is to characterize the mechanism of carbonate crystal nucleation and growth regulation by adjuvant molecules. We find possible contact sites on the crystal surface for intermolecular interactions. In a second application, we use PRE effects to measure the solution interaction between HIV-1 capsid protein and potential anti-HIV drugs. The results and challenges encountered in these applications of PRE NMR are discussed.

Biological NMR at Indiana University

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The METACyt Biomolecular NMR Laboratory in the Department of Chemistry at Indiana University is part of the campus-wide NMR facility that houses 600 MHz and 800 MHz Bruker AVANCE NEO spectrometers equipped with 5 mm Triple Resonance TCI CryoProbes with cooled preamps for $^{1}H/^{19}F$, ^{13}C, ^{15}N, and ^{2}H, as well as a 1.9 mm Tri-Gamma solid-state MAS probe for the 800 MHz. This poster describes some recent and ongoing projects in protein NMR happening in this facility, including structure determination, backbone dynamics, methyl side-chain dynamics, mapping of binding sites, and monitoring ligand-induced conformational changes by ^{19}F NMR.

Solid state NMR spectroscopy; a valuable technique for deep structural insight into oil shale sediments

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Oil shale sediments, an unconventional energy resource, contain fossil fuels primarily kerogen which can be refined into an economically valuable fuel. The extraction and processing of these fuels are crucial to meeting the growing energy demand. Understanding the composition and properties of oil shale sediments is essential for optimizing the extraction processes and maximizing yields. Solid-state nuclear magnetic resonance (ssNMR) spectroscopy is a non-destructive analytical tool in characterizing organic components in oil shale sediments, where measurements are applied on samples in their unrefined state without further chemical or thermal treatment. Beyond conventional ssNMR techniques, such as ultrafast magic angle spinning, allow for precise identification of kerogen types, aromaticity, and the detection of various functional groups. An array of complimentary analytical techniques (powder XRD, FT-IR and SEM-EDS) were used to characterize the inorganic and organic components of oil shale sediments. The detailed structural and compositional data obtained from ssNMR analyses are invaluable for the energy sector as they enhance the economic potentiality of oil shale as an energy source.

Covalently targeting conserved cysteines in RNA binding domains

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RNA Binding Proteins (RBPs) control many cellular processes including transcription, translation, and alternative splicing. RBPs have the most disease annotated mutations of any protein class, but these proteins have historically been considered “unligandable” due to their i) highly dynamic and disordered nature and ii) high level of conservation within their RNA binding domains (RBDs). We hypothesized that differences in protein dynamics could be exploited to enable covalent ligands to selectively target conserved cysteines within RBDs. To investigate this, we screened a library of cysteine-reactive covalent ligands against homologous RBPs with differences in their protein dynamics: heterogeneous nuclear ribonucleoproteins H and F (hnRNP H and F). hnRNPs H and F are an excellent model system for RBP conservation because they have 85% sequence conservation, three parallel quasi-RNA recognition motifs (qRRMS), and three conserved cysteines (C22, C34, & C122) within their RBDs. Despite this conservation, we observed differences in the covalent labeling of hnRNP H and F during in vitro screenings. Notably, we found one small molecule with a ten-fold difference in selectivity for hnRNP H over hnRNP F and we consistently observed greater covalent labeling of cysteines in hnRNP H. This work demonstrates that covalent ligands can be used to differentially engage conserved cysteines. Moreover, our findings suggest that the unique dynamics of hnRNP H, specifically slower conformational exchange, may contribute to greater covalent labeling. By understanding how protein dynamics contribute to covalent selectivity, we can cultivate a powerful new approach for targeting RBPs and other dynamic and disordered proteins.

Diamond anvil cell high pressure NMR/DNP probe for protein-water dynamics studies

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Recent studies on the dynamics of AsLOV2, which belongs to a family of proteins found in all kingdoms of life and broadly utilized in bioengineering, found that the protein exiles some of its hydration water upon light activation. Further investigation of water-protein interactions under transient light activation events proved to be challenging for magnetic resonance. High pressure, on the other hand, has gained increasing interest over the past decade as a "clean" form of perturbation that drives protein into low-lying activation states which are likely related to the function of proteins. Our preliminary data suggests that AsLOV2 under pressure potentially enters the same state as light-activated AsLOV2. For detailed analysis of protein dynamics under pressure using NMR and Overhauser DNP (ODNP), we design a NMR/DNP probe incorporating a diamond anvil cell (DAC) as pressure source, which is built upon a static DNP probe we previously reported. DACs are broadly employed in high-pressure x-ray diffraction and optical spectroscopy due to its extraordinary pressure range (Mbar) and outstanding optical transparency and thermal properties. We anticipate using this piece of equipment for high-pressure NMR and ODNP studies of protein-water interactions, investigating water-ice phase transition, and exploring solid-state DNP at high pressure and diamond-based DNP.

Direct observation of water-directed pinning in tau-templated aggregation

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Tau assembles into fibrillar aggregates, which serve as pathological hallmarks of a group of neurodegenerative diseases collectively called tauopathies. Developing approaches to replicate disease-relevant fibril structures is crucial for developing therapeutic and diagnostic tools. Nevertheless, this requires an in-depth understanding of the templated aggregation mechanism, which informs the design criteria of seeding-competent fibrils. This study directly observes water-directed pinning during the templated aggregation of the jR2R3-P301L fibril, a 19-residue tau peptide that was reported to form prion-competent fibrils with a strand-loop-strand motif shared between 4R tauopathies. We found an in-register association of this tau peptide to fibrils by a series of ^{1}H-^{15}N correlation spectra, starting with pinning at site V300. Enhanced local water structuring around the pinning site, together with an enhanced release of structural water upon the peptide association, highlights the role of water in entropically driving this site-directed pinning, which serves as an initial step in tau amyloid aggregation. This study introduces a new concept of using hyper-localized structural water on the tau fibril surface as an indicator to identify the hot spot that assists templated aggregation.

Investigation of the residue-specific dynamics of 3CD protein, a key regulatory protein in poliovirus

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Poliovirus (PV), a positive-strand RNA virus, generates a polyprotein, which is later proteolyzed into component proteins. The PV proteolytic precursor, 3CD, has some different and emergent properties compared to its fully proteolyzed counterparts, the 3C protease and 3D RNA-dependent RNA polymerase. The 3CD protein is central to RNA and lipid interactions necessary for the viral infection Cycle. However, how these various functions are coordinated is poorly understood. We hypothesize that the internal conformational dynamics and the ability of viral 3CD proteins to transition into alternative conformations might serve as a mechanism to broaden the functional proteome. To explore this, we employed NMR spectroscopy to investigate the residue-specific dynamics of 3CD and its processed components. As 3CD is quite large by traditional NMR standards (i.e., 72 kDa), we initially use methyl probes (e.g. from Ala, Leu, Ile, Met, Thr, and Val), which have been found useful in the NMR investigations of other larger proteins. While our primarily assigned Ile and Met probes have been informative, we performed HMQC to assign additional $^{13}CH_3$ resonances, followed by using 3D NOESY experiments along with the crystal structure and established Ile/Met assignments. This assignment strategy takes advantage of expected $^{13}CH_3$ -$^{13}CH_3$ NOE patterns from the X-ray crystal structure. This assignment will help identify the residue-specific dynamics in the native environment, which is expected to unlock the functionally distinct protease (3C) and RNA-dependent RNA polymerase (3D) in 3CD and make it an outstanding allosteric platform for understanding the spatial and temporal regulation of the virus lifecycle.

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Solid-State NMR analysis of the schizosaccharomyces pombe cell wall reveals a critical role for alpha-amylase enzymes Aah1 and Aah3 in composition, structure, and function

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The fission yeast Schizosaccharomyces pombe serves as a widely employed model organism for understanding the eukaryotic cell cycle. Like other cell-walled organisms such as plants and bacteria, S. pombe must coordinate cell growth with cell wall expansion, but the mechanisms by which cell wall-synthesizing and remodeling enzymes mediate this process remain unclear. Among these enzymes, alpha-amylases represent an evolutionarily conserved family of cell wall-modifying proteins. Here we characterize the functions of Aah1 and Aah3, two putative alpha-amylase proteins in S. pombe, by analyzing polysaccharide structure using solid-state NMR. We found that unlike rod-shaped wildtype S. pombe cells, double mutant aah1Δ aah3Δ cells are nearly spherical, grow slowly, and have severe defects in cell separation following cytokinesis. NMR analyses revealed a significant reduction in the α-glucan matrix content, characterized by decreased structural polymorphism and reduced amounts of the major α-1,3-glucan domains across both rigid and mobile phases, along with the depletion of minor alpha-1,4-glucan structural domains. This reduction was compensated by upregulated beta-glucan biosynthesis, as evidenced by a two-fold increase in the levels of rigid beta-1,3-glucan and mobile beta-1,6-glucan in aah1Δ aah3Δ cells. The mutant cells also exhibited thicker, more rigid cell walls. The phenotype of aah1Δ aah3Δ cells resemble cells with impaired function of the transglycosylation domain of alpha-glucan synthase 1 (Ags1). Together with negative genetic interactions between mutants, our data suggest that Aah1 and Aah3 collaborate with Ags1 in the transglycosylation of alpha-glucan chains to generate fibers of appropriate dimensions to support proper cell morphology, growth, and division.

Influence of high-energy states on conformational dynamics of Nurr1 LBD

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The orphan nuclear receptor transcription factor Nurr1 is critical for regulating genes involved in dopamine production and neuronal survival. Previous work from our lab has shown that polyunsaturated fatty acids (PUFAs) and related lipid-like molecules can bind to the Nurr1 ligand-binding domain (LBD) to regulate Nurr1-mediated transcription. However, it remains unclear how the conformational ensemble of the Nurr1 LBD, including any high-energy states and functional protein dynamics, is regulated by ligand binding. Using protein folding studies and mutational analyses, along with NMR and MD simulations, we have identified a network of stable residues within the Nurr1 LBD that forms a scaffold for the conformational dynamics of connected regions, which serves as a folding nucleus. We also show that binding of PUFAs, docosahexaenoic acid (DHA) and arachidonic acid (AA), which are important for neuroprotection, shift the Nurr1 LBD conformational ensemble toward an intermediate state that is stabilized by this stable folding nucleus network. Mutation of residues within this stable network also promotes a shift toward the intermediate state. The network of stable residues may act as a conduit to transmit shifts in the Nurr1 LBD conformational ensemble to other domains in Nurr1 in response to ligand binding.

Opening-dissociation equilibrium of the E. coli β-clamp protein

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The ring-shaped E. coli β-clamp protein is an 81 kDa head-to-tail homodimer, which serves as a processivity factor for DNA polymerases and a binding platform that facilitates numerous protein transactions taking place on DNA during replication, repair, and damage response. The β-clamp dimer is opened and loaded onto DNA by the hetero-pentameric γ clamp-loader complex in an ATP-dependent process. One model of clamp loading implies an induced fit mechanism whereby the clamp-loader binds to and induces the opening transition of the closed clamp. An alternative model suggests a conformational selection mechanism whereby the clamp-loader selectively recognizes an open state of the clamp populated in solution. To elucidate the mechanisms of β-clamp recognition and opening by the γ clamp loader complex, we used a combination of biophysical methods, including amide and methyl TROSY NMR, AUC, and SAXS to probe opening-dissociation equilibrium of the β-clamp in solution, in which the clamp populates the closed-dimer, open-dimer, and monomer states.

Ultra high-resolution NMR and hybrid NMR/MS for exploring complex lipid systems in structural and functional studies

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Nuclear Magnetic Resonance (NMR) spectroscopy is an indispensable analytical tool for the structural elucidation of biomolecules in solution. However, chemical shift differentiation in conventional NMR experiments remains challenging in structurally similar molecular classes, such as saturated fatty acids (SFAs), due to significant signal overlap. To mitigate this issue, non-uniform sampling (NUS) approaches have been employed to improve resolution along the indirect ^{13}C-dimension within a given experimental time. Furthermore, as discussed earlier, spectral congestion in the direct proton dimension presents a more significant challenge than in the indirect dimension. To resolve this, pure shift J-coupling suppression techniques were incorporated. For this study, fish oil sample was analyzed using the real-time pure shift non-uniform sampling (rtPS-NUS) method in 2D HSQC experiments. The sample is well-known for containing high concentrations of docosahexaenoic acid (DHA, 22:6, n-3) and eicosapentaenoic acid (EPA, 20:5, n-3). Conventional HSQC NMR analysis often suffers from severe peak overlap, particularly in the olefinic carbon region of polyunsaturated fatty acids (PUFAs), making peak assignment challenging. The application of rtPS-NUS significantly improved spectral resolution, enabling the distinction of previously unresolved peaks. In this study, the application of rtPS-NUS NMR successfully resolved the unique peaks of DHA and EPA in fish oil, which consists of a complex mixture of saturated and unsaturated fatty acids. The rtPS-NUS approach demonstrated high resolution spectra, enabling the clear separation and assignment of individual peaks.

COLMARvista: an open source 2D and pseudo-3D NMR spectral processing, visualization, and analysis software in JavaScript

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COLMARvista is a versatile new software for intuitive processing and visual inspection of 2D and pseudo-3D NMR data, supporting both uniformly and non-uniformly sampled datasets. It offers fully automated spectral processing, including zero-filling, apodization, water suppression, Fourier transformation, and phase correction. Integrated with DEEP Picker and Voigt Fitter, it enables automated deconvolution and reconstruction for accurate quantitative analysis, including compound concentration and cross-peak- specific relaxation parameters, even in overlapping regions. Built in JavaScript, COLMARvista is platform-independent, runs in all modern web browsers, and requires no installation. It also serves as a model for sustainable software development in NMR, avoiding the obsolescence of legacy tools and supporting continued progress in the field.

Biomolecular NMR spectroscopy and other biophysical approaches to understand adaptation to metal starvation in Acinetobacter baumannii

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Vertebrate immune systems suppress bacterial growth at the host-pathogen interface through nutritional immunity, which employ neutrophils to starve bacteria of essential nutrients. Using nuclear magnetic resonance (NMR) spectroscopy as the primary biophysical technique, mechanisms of metal adaptation to infected host-mediated metal starvation in Acinetobacter baumannii can be probed. During these conditions of nutrient restriction, bacterial survival relies in part on COG0523 proteins, which are proposed to sustain metabolic pathways through the delivery of a metal cofactor to client enzymes. Here we characterize a putative COG0523 metallochaperones in A. baumannii: MigC and the client protein MurD, a Mur ligase used in peptidoglycan synthesis. Physical and functional features of the protein-protein interaction are defined for the first time with steps toward complete methyl resonance assignments to map the binding interface. This work exploits methyl NMR approaches to study these large proteins, allowing the dynamics of the systems and the cooperativity of ligand binding to be investigated.

Local structure determination of silicon, aluminum, and lithium in an oxide anode by nuclear magnetic resonance

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An amorphous oxide anode containing silicon and aluminum in equal atomic percent was prepared by solgel synthesis and used in as the active material in lithium ion battery anode. Magic angle spinning (MAS) solid state nuclear magnetic resonance and multiple quantum (MQ)-MAS were used to probe the local structure of silicon, aluminum, and lithium of the fresh and lithiated. Silicon and aluminum both appear in their respective oxide chemical shift region in both the fresh and lithiated samples. However, silicon shifts more positive indicating a disconnection of the network from primarily Q4 species to lower Q species. Through collection of MQ-MAS, aluminum is shown in three district environments, likely 4-, 5-, and 6-cordinate aluminum to oxygen in the fresh sample. Upon lithiation, only 4-cordinate aluminum remains. Currently, the delithiated anode is being investigated as well as lithium environment in lithiated anode. This work will shed light on the currently unknown lithium storage and removal mechanism of this amorphous oxide anode and those of similar structure.

Unravelling liquid-liquid phase separation by the interaction of the Picornavirus 3C(D) main protease, its mutants with genomic RNA elements utilising NMR and biophysics

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The poliovirus 3CD protein is a crucial protease that cleaves viral polyproteins and host cell defense proteins. The PV-3CD protein is a precursor of the 3C protease, which cleaves the viral polyprotein and host cell defense proteins, and 3D RNA-dependent RNA polymerase. The PV-3C domain interacts with cis-acting replication elements to regulate viral replication and translation, coordinating the virus's life cycle. The molecular mechanism of the interaction of PV-3C and RNA remained unclear. We have employed Nuclear Magnetic resonance (NMR) spectroscopy with advanced biophysical techniques like SAXS, ITC, AUC, and fluorescence microscopy to probe the sequence and structural prerequisite of RNA binding to PV-3C. NMR results detected the key binding residues from two binding sites of 3C with RNA; one binding site corresponds to E81-H89 residue containing loop and nearby alpha-terminal helix residues (G1-R13), and another binding site incorporating the catalytic triad residues H40, E71, and nearby residues T142. Other biophysical measurements have shown that PV-3C binds to a diverse variety of RNA with little to no sequence or structural dependence. Interestingly, PV-3C and RNA interactions induce liquid-liquid phase separation (LLPS). We were also interested in the single mutation on these amino acid residues involved in RNA binding to observe the effects on LLPS formation and are currently investigating it. LLPS has been identified in various viral infections and may be involved in virally driven apoptosis and virus propagation via stress granule formation/deformation. A similar study with 3CD suggests yet another function for this protein critical to the viral lifecycle.

Probing dynamics of Cre autoinhibition and its consequences on protein-DNA interactions

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Cre (Causes Recombination) is a site-specific DNA recombinase capable of manipulating genetic material at target loxP DNA sequences without producing cytotoxic double-strand DNA breaks (1). This makes it a compelling reagent for use in gene editing. Cre is used extensively in the study of human health and disease, but its use in gene therapy applications is hindered by a lack of understanding of the site-selection process; namely, how Cre locates and selectively binds to its target sequences in the context of genomic DNA (2). Recent solution-state nuclear magnetic resonance (NMR) data provided evidence that the C-terminal sequence of Cre interacts in cis with its interdomain linker, blocking the DNA binding interface of the catalytic domain, including active site residues (3). When bound to DNA, the C-terminus is extended and docks in trans with neighboring Cre protomers to aid in assembly and activation for single-strand DNA cleavage (4). While this conformational switch is thought to play a crucial role in activation of the catalytically active complex, its function in apo Cre is not well understood. In this work, we used solution state NMR to probe conformational dynamics of the C-terminus using amide chemical exchange saturation transfer (CEST) and methyl-CPMG relaxation dispersion. Complemented with other biophysical characterization of Cre-DNA complexes, these data yield compelling information on how this dynamic process effects interactions with DNA substrates to enable Cre to locate target its DNA sequences.

References:

1.Ghosh, K. & Van Duyne, G. D. Cre–loxP biochemistry. Methods 28, 374–383 (2002).

2.Foster, M. P., Benedek, M. J., Billings, T. D. & Montgomery, J. S. Dynamics in Cre-loxP site-specific recombination. Current Opinion in Structural Biology 88, 102878 (2024).

3.Unnikrishnan, A. et al. DNA binding induces a cis -to- trans switch in Cre recombinase to enable intasome assembly. Proc Natl Acad Sci USA 117, 24849–24858 (2020).

4.Stachowski, K., Norris, A. S., Potter, D., Wysocki, V. H. & Foster, M. P. Mechanisms of Cre recombinase synaptic complex assembly and activation illuminated by Cryo-EM. Nucleic Acids Research 50, 1753–1769 (2022).

Development of a proton spin network fingerprint library to support mass spectrometry-based identification of pharmacophore-bearing constituents in the botanical supplement Centella asiatica

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Centella asiatica (Apiaceae) is a perennial pennywort utilized for centuries in Eastern traditional medicine systems[1] that recently has been popularized as a botanical supplement for cognitive enhancement and neuroprotective effects.[2] C. asiatica shows preclinical evidence of its therapeutic potential in models of Alzheimer's Disease and other dementias,[3,4] but lack of consistency in Centella formulations limits validation of its medicinal benefits in human studies.[2] Many bioactive metabolites of C. asiatica, particularly caffeoylquinic acids (CQAs), are regio-isomers and can be difficult to differentiate with LC-MS methods alone. To complement standardization and quality control efforts, our lab has developed a novel detection methodology using NMR spectroscopy to identify and distinguish the pharmacophores of C. asiatica. Following a comprehensive isolation procedure on authenticated C. asiatica plant material provided by the BENFRA Center, compounds were examined using selective one-dimensional TOCSY (S1DT), resulting in the generation of characteristic proton Spin Network Fingerprints (pSNFs) that have been accumulated in our in-house library. This work has demonstrated that 17 CQAs and CQA analogues possess unique pSNFs which allow them to be distinguished from one another despite being regioisomers with similar fragmentation patterns. In addition, this strategy has shown that individual pSNFs can be generated from the ^{1}H NMR of the botanical extract to accurately confirm the presence and identity of various CQA isomers and be validated with library data. This project aims to integrate the pSNF library with mass spectrometric data from BENFRA for the development of a standardized C. asiatica formulation that is suitable for clinical studies.

References:

1) Brinkhaus, B.; Lindner, M.; Schuppan, D.; Hahn, E.G. Chemical, pharmacological and clinical profile of the East Asian medical plant Centella asiatica. Phytomedicine 2000, 7, 427-448.

2) Gray, N.E.; Alcazar Magana, A.; Lak, P.; Wright, K.M.; Quinn, J.; Stevens, J.F.; Maier, C.S.; Soumyanath, A. Centella asiatica: phytochemistry and mechanisms of neuroprotection and cognitive enhancement. Phytochem. Rev. 2018, 17, 161-194.

3) Gray, N.E.; Zweig, J.A.; Caruso, M.; Martin, M.D.; Zhu, J.Y.; Quinn, J.F.; Soumyanath, A. Centella asiatica increases hippocampal synaptic density and improves memory and executive function in aged mice. Brain Behav. 2018, 8, e01024.

4) Zweig, J.A.; Brandes, M.S.; Brumbach, B.H.; Caruso, M.; Wright, K.M.; Quinn, J.F.; Soumyanath, A.; Gray, N.E. Prolonged treatment with Centella asiatica improves memory, reduces amyloid-β pathology, and activates NRF2-regulated antioxidant response pathway in 5xFAD mice. J. Alzheimer's Dis. 2021, 81,1453-1468."

The Network for Advanced NMR at UW-Madison: 1.1 GHz solid-state NMR and knowledgebases

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The mission of NAN, the Network for Advanced NMR, is to provide access to state-of-the-art NMR instrumentation to investigators in the US and abroad. One 1.1 GHz NMR spectrometer has been installed at UW-Madison for SSNMR. NMRFAM is also developing two solid-state NMR knowledgebases focused on core biological SSNMR and materials SSNMR experiments. These knowledgebases demonstrate best practices, provide SOPs for consistent and reproducible data acquisition, and facilitate implementation of new experiments across facilities. Expert content includes optimized pulse sequences, parameter sets, sample data, and all other instrument files needed to recreate an experiment, along with processing protocols and processed data on a standard sample to support verification of pulse sequence performance. Additional knowledgebases for non-experts provide high-level introductions to demystify NMR for scientists in other fields, demonstrate the range of research questions that NMR can address and provide an overview of capabilities and practical considerations for those wondering whether NMR might be an appropriate approach for their research problem. The NAN institutions are seeking feedback on both existing protocols and community opinion into areas most in need of new knowledgebase content.

An enzymatic approach for Hα labeling of deuterated amino acids for NMR studies

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Isotropic labeling of larger proteins (30 kDa) with deuterium is a well- established technique for decreasing relaxation rates in a range suitable for Nuclear Magnetic Resonance (NMR) spectroscopy. Although uniform perdeuteration can be achieved to near completion, specific labeling of individual proton sites can be a challenge. Many different chemical approaches have been developed to make specifically protonated/deuterated labeled amino acids and use them in the bacterial cell cultures to produce specifically labeled proteins. Such an endeavor can be very expensive, time consuming and are typically only partially successful in labeling all the intended positions. For NMR relaxation measurements on larger proteins involving the HαCα bond, maintaining the proton at the α-position while the proximal protons are deuterated will greatly benefit the signal-to-noise of the experiment since it will reduce relaxation due to large dipolar interactions with proximal protons. Here, we describe a new approach to achieve this goal, which is an extension of an enzymatic approach published recently (1) wherein a Pyridoxal 5'-Phospahte-Dependent (PLP) Mannich Cyclase (LolT) was used to stereospecifically deuterate Hα positions of individual L-amino acids. Through this enzymatic reaction, almost all the amino acids are specifically deuterated at the α-position except for Ile. Instead of deuterating the α-position, we used LoIT to protonate the α-position in a mixture of deuterated amino acids (Silantes' ^{2}H, ^{13}C and ^{15}N media powder for E.coli). The resultant specifically labeled amino acid mixture exhibited different levels of protonation for the individual amino acid residues as measured by ^{13}C-^{1}H HSQC spectra.

Gating effect of hydrophobic bridge of OXA24/40 on activity against carbapenem using ^{19}F NMR.

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Gram-negative bacteria resist β-lactam antibiotics largely by deactivating with protein enzymes called β-lactamases, which are continually evolving in clinical settings to enhance their activity. Among these, carbapenem-hydrolyzing class D β-lactamases (CHDLs) are particularly concerning due to their ability to hydrolyze carbapenems, a critical class of broad-spectrum β-lactam antibiotic. Understanding the factors that regulate β-lactamase activity is crucial for developing effective therapies against multidrug-resistant pathogens like Acinetobacter baumannii.

Here, we use solution NMR to examine how structural fluctuations relate to carbapenemase activity in OXA-24/40, a representative CHDL. Its crystal structure suggests a conserved "hydrophobic bridge" (β5-β6 loop-M223 and P-loop-Y112), a tunnel-like structure occludes the active site, potentially impacting enzyme activity by a tight carbapenem binding. To probe the role of the hydrophobic bridge, we monitored M223 ε-CH_3 chemical shifts via ^{13}C SOFAST-HMQC upon substrate addition. Doripenem, an advanced carbapenem, induced a larger shift, suggesting local side chain reorganization. Given challenges in tracking Y112 shifts with ^{13}C NMR, we employed ^{19}F NMR using 3-^{19}F-tyrosine labeling. A downfield shift and increased Y112 ε-^{19}F intensity with doripenem indicate substrate interaction. Our findings enable us to track the potential interaction between M223 ε-CH_3 and Y112 ε-^{19}F and its influence on the carbapenemase activity of OXA24/40. This analysis could be extended to explore other CHDL subgroups, as well as specific class A and C β-lactamases that feature a conserved hydrophobic bridge within their active site.

Isotope labelling in mammalian cells for NMR studies of difficult to produce biomolecules

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Bacterial expression systems, most notably Escherichia coli, are widely used for the production of isotope-labelled proteins. Their prevalence in protein production is due to their simple culturing conditions and extensive metabolic versatility, which allows them to grow in minimal media using relatively inexpensive ^{13}C and ^{15}N sources. However, bacterial expression systems are limited in their ability to produce challenging protein targets and often struggle with large eukaryotic complexes, which are increasingly the focus of current biomolecular research. These protein targets require eukaryotic expression systems, where isotope labelling presents a more complex challenge.

Here, we present isotope-labelling protocols for producing isotope-labelled proteins in HEK293 cells grown in suspension. We have established cost-effective U-[^{15}N] labelling of proteins, as well as amino acid-specific labelling of the methyl groups in methionine, isoleucine, leucine, valine, and alanine. Furthermore, we also provide examples of difficult to produce protein targets and present NMR studies of these proteins to demonstrate the potential of these protocols in expanding the range of proteins accessible for biomolecular NMR studies.

First atomic resolution structure of an intramolecular higher-order five tetrad g-quadruplex

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ZEB1 (Zinc finger E-box binding homeobox 1) is a member of the homeobox transcription factor family and plays a key role in regulating the epithelial mesenchymal transition (EMT) in carcinoma cells. Direct inhibition of ZEB1 with small molecule therapeutics has not yet been achieved. Another avenue for regulating ZEB1 is through the stabilization of DNA G-quadruplexes (G4s) found within the ZEB1 promoter with small molecules. G4-small molecule binding can influence the transcription of adjacent genes, particularly cancer-related protein promoters like ZEB1, by repressing proteins that are difficult to target or considered “undruggable”. Here we have identified and structurally characterized with solution nuclear magnetic resonance spectroscopy (NMR) a unique and first of its kind, 5-tetrad, interlocked, intramolecular higher-order G4 fold using a thymidine scanning of the ZEB1 promoter. The two-stack and the three-stack G4 systems are essentially adopting a 5'-5' stacking interface, which is predicted as the most stable form of G4 dimerization. The high thermodynamic stability is confirmed using CD melting analysis. The interlocking of nucleotide G21 into the three stacked G4 domain is shown to be essential for its formation. The unique folding of the ZEB1 G4 offers a unique targetable landscape for the indirect inhibition of ZEB1 in carcinoma, specifically at the junction of the two G4 units that forms a pocket with the interlocking G21.

Probing DNAJB1 interactions with phosphorylated α-synuclein fibrils

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Amyloid fibrils formed by α-Synuclein (aSyn) protein are implicated in the pathogenesis of Parkinson's disease. aSyn fibrils, characterized by their rigid amyloid cores, also feature flexible intrinsically disordered regions that interact with various cellular components. Investigating the interactions of these flexible segments with other factors is crucial for diagnostic and therapeutic advancements. We employ dipolar and J-coupling based solid-state NMR experiments to probe the structural dynamics of these proteins within the fibrils. Molecular chaperones, particularly the J-protein DNAJB1, modulate aSyn aggregation, yet their binding mechanisms remain unclear. While DNAJB1 is known to interact with both monomeric and fibrillar aSyn, over 90% of aSyn fibrils in Lewy bodies are phosphorylated at S129 (pS129). Our findings show that DNAJB1 preferentially binds aSyn pS129 fibrils over wild-type fibrils. Solid-state NMR further reveals that DNAJB1 engages distinct interaction sites on pS129 versus WT fibrils, suggesting that phosphorylation at S129 may influence chaperone-mediated degradation efficiency.

Activation of caspase-9 on the apoptosome as studied by methyl-TROSY NMR

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The Caspase-9 (Casp9) protease plays an essential regulatory role in the intrinsic apoptotic pathway, with dysfunction leading to cancers and neurodegenerative disorders. Casp9 is composed of a Protease Domain (PD) and a Caspase Activation and Recruitment Domain (CARD), which are connected via a long-disordered linker. While it is well established that an interaction of the CARD with the apoptosome complex upregulates Casp9 cleavage activity, detailed mechanistic information regarding this process remains elusive. Previous biochemical studies of isolated Casp9 have demonstrated that dimerization of the PD is required for Casp9 proteolytic activity. Whether these findings can be translated to the biologically relevant Casp9-apoptsome complex is unclear, with contrasting models of activation being hypothesized. In this study, we use a combination of methyl-TROSY based NMR methods in tandem with biochemical assays to elucidate the activation mechanism of Casp9 upon apoptosome binding. Our data suggests that the apoptosome upregulates Casp9 activity via an induced proximity mechanism. In the absence of substrate the PD exhibits exceptionally weak dimerization affinity, remaining monomeric regardless of apoptosome binding, and only when substrate is present does dimerization occur. Thus, Casp9 activation is driven by crowding of multiple PDs, increasing the rate of dimerization and therefore substrate cleavage activity.

Probing the dissociation and reassembly pathway of an amyloidogenic transthyretin mutant

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Aggregation of transthyretin (TTR) is associated with devastating amyloid diseases. Amyloidosis begins with the dissociation of the native homotetramer (a dimer of dimers) to form a monomeric intermediate that assembles into pathogenic aggregates. This process is accelerated in vitro at low pH, but the process by which TTR dissociates and reassembles at neutral pH remains poorly characterized. Here we use ^{19}F NMR and a highly sensitive trifluoromethyl probe to determine the relative populations of the species formed by dissociation of a destabilized A25T variant. The A25T mutation perturbs both the strong dimer and weak dimer-dimer interfaces. A tetramer-dimer-monomer equilibrium model is proposed to account for concentration- and temperature-dependent population changes. All thermodynamic and kinetic parameters and activation energetics for dissociation of the native A25T tetramer, as well as a destabilized alternative tetramer (T*) with a mispacked F87 side chain, were extracted by van't Hoff and ^{19}F NMR line-shape analysis. The ^{19}F and methyl chemical shifts of probes close to the strong dimer interface in the dimer and T* species are degenerate, implicating interfacial perturbation as a common structural feature of these unstable species. All-atom molecular dynamics simulations further suggest more frequent F87 ring flipping on the nanoscale timescale in the A25T dimer than in the A25T tetramer. Our integrated approach offers quantitative insights into the energy landscape of the reversible dissociation and reassociation pathway of TTR at neutral pH.

Conformational dynamics of histone H4 tails in nucleosomes studied by NMR spectroscopy

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The nucleosome core particle, which is composed of nucleosomal DNA and histone octamer containing two copies each of histones H2A, H2B, H3 and H4. Dynamically disordered histone tails modulated by mutations, post-translational modifications (PTMs) and chromatin-associated proteins (CAPs) play critical roles in chromatin dynamics and regulation. Here, we study the conformational dynamics of the histone H4 tails in nucleosomes by NMR measurements of backbone amide ^{15}N spin relaxation rates. The data support that H4 tails engage in a fuzzy-complex interaction with DNA. Overall, the results show that histone H4 tails in nucleosomes are modulated by mutations.

NMR studies of chromatin compaction

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The sheer size of a eukaryotic genome (nearly 2 meters in length) necessitates its hierarchical compaction by histone proteins to fit within the nucleus (~6 μm in diameter). The organization of the genome into chromatin is crucial for in vivo gene expression. While the structural aspects of nucleosomal compaction are being studied (primarily through cryo-electron microscopy [1]), changes to the dynamics of the nucleosome due to compaction remain unknown. Here, we present an analysis of changes in the H3 histone protein in 16-mer nucleosome arrays (with and without compaction) using solid-state nuclear magnetic resonance (ssNMR). We employ high-speed magic angle spinning (MAS) rotors along with proton detection at ultrahigh field (1.2 GHz) to probe the changes in the globular core of the H3 histone protein upon compaction. We also highlight some key benefits of ultrahigh field ssNMR for studying large macromolecules.

References:

1. Boopathi, Ramachandran, et al. "Cryo-electron microscopy of the chromatin fiber." Current Opinion in Structural Biology 64 (2020): 97-103.

2. Quinn, Caitlin M., Mingzhang Wang, and Tatyana Polenova. ""NMR of macromolecular assemblies and machines at 1 GHz and beyond: new transformative opportunities for molecular structural biology."" Protein NMR: Methods and Protocols (2018): 1-35.

Structural characterization of organic molecules and water phases in biological solids using ^{17}O solid-state NMR spectroscopy

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Oxygen is ubiquitous in many organic molecules, inorganic materials, biological solids, and solvents, which can be found in a variety of different bonding environments (i.e., covalent, coordination, hydrogen bonds, etc.). Thus, ^{17}O (I = 5/2) solid-state NMR (SSNMR) spectroscopy is an attractive technique for elucidating key information about structures, properties, and reactivities. However, due to the extremely low natural abundance (ca. 0.04 %), it is required to work with isotopically enriched samples, which can be challenging to prepare due to experimental constraints and/or expensive reagents. Mechanochemistry has emerged as an attractive technique for isotopic labeling a variety of materials that is quick, efficient, cost-effective, and environmentally friendly.

Herein, we demonstrate the capabilities of multinuclear SSNMR with an emphasis on quadrupolar nuclei for structural characterization of small organic molecules featuring carboxylic acids and hydroxyl groups as well as probing water phases in alginate hydrogels and Rhizopus fungal cells. This poster will describe a synthetic protocol involving unlabeled, ^{18}O-labeled, and ^{17}O-labeled water for enriching organic molecules and their subsequent structural characterization using a combination of powder diffraction, infrared spectroscopy, mass spectrometry, and multinuclear SSNMR (^{1}H, ^{2}H, ^{13}C, and ^{17}O). Additionally, variable temperature NMR is used to probe various water phases in alginate hydrogels and Rhizopus cells, which have different resonances and relaxation properties, and could provide structural insight into polysaccharide packing. These methods will provide a foundation for developing 2D and 3D NMR techniques, which will be beneficial for elucidating structural information for increasingly complex biological solids.

The cysteine-rich domain of SEP15, an ER selenoprotein co-chaperone of UGGT, adopts a novel fold

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Proteins targeted to the secretory pathway mediate a myriad of biological processes but can only do so when properly folded. Within the endoplasmic reticulum (ER), folding and quality control of secretory proteins is facilitated by N-glycan modifications, which are recognized by a network of chaperones. The gateway to exit from the ER is governed by the enzyme UDP-glucose:glycoprotein glucosyltransferase (UGGT) and its oxidoreductase partner, the 15-kDa selenoprotein (SEP15 aka SELENOF). The interaction between these two chaperones is poorly understood, limiting understanding of their function. SEP15 is comprised of two domains, a C-terminal thioredoxin-like domain, the structure of which has been reported (PDB 2A4H), and an approximately 50-residue long N-terminal cysteine-rich domain (CRD) of unknown structure. Here, we use a combination of AlphaFold structural predictions and NMR spectroscopy to elucidate the structure of the SEP15 CRD, which mediates its interaction with UGGT. TALOS-N dihedral angles and a CS-Rosetta structure agree well with the AlphaFold prediction. Together, these data reveal that this domain forms a previously undescribed helical fold stabilized by three disulfide bonds between residues C10-C42, C21-C43, and C24-C39. Furthermore, our results validate a model of the UGGT/SEP15 complex (Williams et al., PNAS [2024]) and lay the foundation for future studies of its interaction with glycoprotein substrates.

Elastin recoil is driven by the hydrophobic effect

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Elastin is a robust matrix material found in all vertebrates. In a human lifetime, arterial elastin undergoes in excess of 2x109 stretching/contracting cycles without replacement. For over fifty years, the mechanism of entropic recoil has been controversial. Herein, we report a combined NMR and thermomechanical study that establishes the hydrophobic effect as the primary driver of elastin function. Using double quantum ^{2}H NMR, water ordering at the solvent:protein interface was observed as a function of stretch. In addition, the most extensive thermodynamic analysis performed to date was obtained by measuring elastin length and volume as a function of force and temperature in normal water, heavy water and with co-solvents. When stretched, elastin's heat capacity increases, water is ordered proportional to the degree of stretching, the internal energy decreases, and heat is released in large excess over the work performed. The combined results from NMR and thermodynamics show that recoil in elastin under physiological conditions is primarily driven by the hydrophobic effect rather than by configurational entropy as is the case for rubber. We propose that hydrophobic effect-driven recoil, as opposed to a configurational entropy mechanism, where hardening from crystallization can occur, is the origin of elastin's unusual resilience and its low elastic modulus.

Dynamics of globular proteins when interacting with zwitterionic silica nanoparticles by NMR spin relaxation

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NMR spin relaxation rates of proteins are highly sensitive to their interactions with nanoparticles and contain rich information about protein mobility and binding kinetics. Previously, we have shown that globular proteins interact with unmodified silica nanoparticles (SNPs) in a predominantly non-specific manner. The increase of backbone amide nitrogen transverse relaxation in the presence of SNPs quantitatively reports intramolecular protein dynamics from picosecond all the way up to the microsecond timescale. In contrast, coating SNPs with sulfobetaine siloxane (SBS) zwitterionic molecules profoundly alters their interactions with proteins. Here, we use ubiquitin and the Ras-binding domain (RBD) of B-Raf as model systems. Their characteristic amide transverse relaxation profiles reveal the axial reorientational motions about an axis perpendicular to the nanoparticle surface of the proteins in the NP-bound state, where the interactions involve the predominantly positively charged surface regions. This interaction mode closely resembles the one observed with POPG liposomes as demonstrated by Clore and co-workers. These findings point toward a global dynamics mechanism of proteins when interacting with organic or inorganic nanoparticles with densely charged soft surfaces.

Solid-State NMR visualizes the emergence of lignin-carbohydrate interactions during plant stem maturation

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Lignification is a crucial process during plant development but also imposes challenges in extracting carbohydrates for biofuel production. Understanding the lignocellulose organization can provide insights on lignin biosynthesis engineering and optimizing lignocellulose-based applications. Here we map the lignin-carbohydrate interactions by ^{13}C solid-state NMR in ^{13}C-labeled Arabidopsis inflorescence stems as secondary cell walls are being formed.

Analysis includes wild-type and two mutants that disrupt the biosynthesis of two lignin building blocks in Arabidopsis, guaiacyl (G) and syringyl (S) units, with the fah1 mutant deprived of S units and the and ref3 mutant having low overall lignin content but a higher S/G ratio. First, the lignin composition and lignin-carbohydrate interactions were examined for various segments of the stems during different growth stages. The G units were found to be deposited prior to the S units during early stages of cell development, whereas mature cell walls in the basal regions are enriched in S units and carbohydrate-lignin interactions. Second, we identified specific interaction patterns between different lignin units and their carbohydrate counterparts found at distinct locations of the cell wall. The acetylated xylan appears to be the dominant mediator of interactions with S-lignin, indicating the critical role of S-lignin in stabilizing carbohydrate-lignin interface in the secondary cell wall. Remarkably, methylated pectin, a key polymer of the middle lamella, displays interactions with G-lignin during early-stage lignification, suggesting the middle lamella pectin may serve as an initiation site for lignin deposition. These findings highlight the role of molecular mixing patterns in lignocellulose.

Hydrophobic interactions in disordered proteins

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The intrinsically disordered N-terminal transactivation domain (NTD) of estrogen receptor (ER or ERα) is a validated therapeutic target, yet its structural dynamics remain poorly understood, limiting direct therapeutic development. Using small-angle X-ray scattering and NMR spectroscopy, we identify specific amino acid clusters and hydrophobic interactions between aromatic-rich regions that drive residual structure formation in ER-NTD. While salt variations minimally affect distant interacting regions, disruption of hydrophobic interactions through Ser118 phosphorylation or mutations, rescues the impaired transcriptional activity, target-gene expression, and cell growth caused by phosphorylation-deficient S118A mutation. Further mechanistic studies reveal that hydrophobic clustering in ER-NTD modulates cofactor interactions while maintaining its overall disorder, with Ser118 phosphorylation fine-tuning these interactions through conformational changes.

Resolving the complex structure and dynamics of fungal polysaccharides with proton detection solid-state NMR

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Fungal infections are emerging as a significant medical threat, particularly among individuals with compromised immunity or metabolic abnormalities. However, our understanding of the pathophysiology of these infections, especially the role of fungal cell walls in growth and immune responses, remains limited. Fungal cell walls are complex, dynamic structures comprising a rigid crystalline core, hydrated semi-rigid regions, and highly mobile regions. These inherent dynamic heterogeneity poses significant challenges in determining cell wall structure. In this study. We employed ^{1}H-detected solid-state NMR at 60 kHz MAS to investigate intact cells of Rhizopus, Candida, and Aspergillus species. The rigid regions were studied using dipolar coupling-based methods, with selective detection of chitin and chitosan polysaccharides through 3D hcoCH3coNH and 3D hC2NH2 experiments. These techniques enabled magnetization transfer through the N-CO-CH_3 pathway for chitin and C2-NH2 pathway for chitosan. Spatial associations were mapped using 3D hCHH (^{1}H-^{1}H RFDR) correlations, while covalently bonded carbon networks were identified through 3D hCCH TOCSY. Mobile regions were probed using scalar coupling-based methods, including 2D J-INEPT-HSQC and 3D J-CCH (DIPSI-3). Semi-rigid regions were examined using spectral editing and relaxation filter methods. Dynamics were explored through ^{13}C R_1 and ^{13}C $R_{1\rho}$ relaxation data. The combination of these advanced NMR techniques provides detailed structural and dynamic insights into the fungal cell walls, offering a framework for investigating other complex cell wall systems, such as plant and bacterial cell walls.

4hJCHOCH Spin-coupling in a Lewis x trisaccharide as evidence of inter-residue C–H....O hydrogen bonding in aqueous solution

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Recent structural studies of the Lewis x (Lex) trisaccharide, aFuc-(1→3)[bGal-(1→4)]-bGlcNAc suggest that nonclassical inter-residue C–H···.O hydrogen bonding in aqueous solution contributes to the stabilization of its 3D structure and affects its biological properties. Key experimental evidence for the existence of this hydrogen bond in aqueous solution has been reported in the form of an inter-residue ^{4h}JCHOCH NMR spin-coupling constant between C5'Fuc and H1''Gal in Lex. Given the importance of this spin-coupling as experimental proof of persistent C–H....O hydrogen bonding in aqueous solutions of Lex, a methyl glycoside of Lex (MebLex) was prepared containing selective ^{13}C-labeling at C5'Fuc, and the well-resolved H1''Gal signal was examined in high-field ^{1}H NMR spectra (800 MHz) for evidence of splitting or line-broadening caused by the highly-abundant ^{13}C spin-1/2 nucleus at C5'Fuc. Despite the collection of high-resolution ^{1}H NMR spectra, neither discrete splitting of the H1''Gal signal nor evidence of line-broadening was observed, despite the reported value of ~1.1 Hz. Spectral simulation showed that this splitting and/or line-broadening would be observable if the reported J-value is correct. Subsequent DFT calculations on MebLex and a carbon analog gave very small and nearly identical calculated ^{4h}JC5',H1'' values, suggesting that the coupling is essentially zero. Based on the results of NMR analyses and DFT calculations, we found that ^{4h}JC5',H1'' in MebLex has an upper limit of ~0.4 Hz and that the value could be lower, calling into question its value as experimental proof of persistent nonclassical hydrogen bonding in aqueous solutions of MebLex and related structures. [Supported by NSF CHE 2002625]

Predicting protein dynamics with molecular dynamics trajectory emulators

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Molecular dynamics (MD) is an established method to simulate dynamics of biomolecules. However, the high computational cost of MD simulations limits their scope in real-world applications, such as drug discovery. Recently developed generative machine learning (ML) models, such as MDGen, offer the potential to model protein dynamics at significantly reduced computational cost and have been shown to generate thermodynamically accurate conformational ensembles of tetrapeptides and small proteins. Here, we apply MDGen to a set of five proteins of various sizes and degree of flexibility (i.e., ubiquitin, colicin E7 immunity protein, hen egg-white lysozyme, interleukin-4, and NCX1 two-helix bundle domain) for which nuclear magnetic resonance (NMR) ^{15}N-^{1}H S^2 order parameters have previously been measured. We generated a conformational ensemble for each protein with MDGen and then computed S^2 order parameters by using the isotropic reorientational eigenmode dynamics (iRED) method. We obtained an average Pearson correlation coefficient of 0.7 between the computed and NMR order parameters. The order parameters from MDGen ensemble are comparable with MD simulations but require significantly less computational resources. Nevertheless, limitations remain in MDGen's ability to model disordered protein regions and conformational changes occurring at slower time scales.