8th Gateway NMR Conference

October 20th-21st, 2023

University of Notre Dame

Notre Dame, IN 46556

Mission

The 8th Gateway NMR Conference brings together researchers in the greater Midwest region to share their recent developments in the application and theory of Nuclear Magnetic Resonance (NMR) spectroscopy. The meeting has a relaxed character to promote a greater sense of community and collaboration across a considerable geographical region. In addition to the talks, an important component is the poster session, with awards. Together, they provide a vehicle for advancing the education and careers of graduate students and postdoctoral researchers.

Thank you to our sponsors

The 8th Gateway NMR Conference was made possible with a generous support from

- College of Science, University of Notre Dame
- Notre Dame Research, University of Notre Dame
- Department of Chemistry and Biochemistry, University of Notre Dame
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Organizing Committee

Local Organizing Committee

- Jeff Peng, University of Notre Dame (Chair)
- Evgenii Kovrigin, University of Notre Dame (Chair)

Gateway NMR Conference Secretary

- Rafael Brüschweiler, Ohio State University

Conference Schedule

All registration and conference events held in the **Jordan Hall of Science**

Friday, October 20th, 2023

By 5 pm - Arrive in South Bend and hotel check-in

5:00 pm - Registration and poster setup

5:00 - 8:00 pm - Social mixer

Saturday, October 21st, 2023

8:30am - 8:40pm -Welcome address

8:40 AM to 9:10 AM Keynote lecture:
"**Efficiently Driving Protein-based NMR for Generating Drug Leads**"
Andrew Namanja**,** *Abbvie, Inc.*

9:10 AM to 9:20 AM Q&A

9:20 AM to 9:40 AM
"**Solid-state NMR analysis of the *Pseudomonas aeruginosa* biofilm matrix**"
Courtney Reichhardt, *Washington University in St. Louis*

9:40 AM to 9:50 AM Q&A

9:50 AM to 10:10 AM
"**Automated NMR Spectral Processing by Deep Neural Networks**"
Dawei Li, *Ohio State University*

10:10 AM to 10:20 AM Q&A

10:20 AM to 10:30 AM Coffee break

10:30 AM to 10:50 AM
"***MA'AT* Analysis: Conformational Equilibria and Dynamics of Saccharides in Solution**", Anthony Serianni, *University of Notre Dame*

10:50 AM to 11:00 AM Q&A

11:00 AM to 11:10 AM Promoted Poster Talk
"**Solid-State NMR 13C Sensitivity at High Magnetic Field**"
Ruixian Han, *University of Wisconsin - Madison*

11:10 AM to 11:15 AM Short Q&A

11:15 AM to 11:25 AM Promoted Poster Talk
"**Characterization of the Biophysical Properties of a COG0523 Metallochaperone and a Mur Ligase from Acinetobacter baumannii**", Emma McRae, *Indiana University - Bloomington*

11:25 AM to 11:30 AM Short Q&A

11:30 AM to 11:50 AM
"**Selective Small Molecule Targeting of Conformational Protein Dynamics**"
Brittany Morgan, *University of Notre Dame*

11:50 AM to 12:00 PM Q&A

12:00 PM to 12:40 PM Lunch

12:40 PM to 1:45 PM Poster session

1:45 PM to 2:05 PM
"**NMR and MD characterization of membrane association and backbone dynamics of intrinsically disordered proteins**", Huan-Xiang Zhou, *University of Illinois Chicago*

2:05 PM to 2:15 PM Q&A

2:15 PM to 2:25 PM Promoted Poster Talk
"**Structural and Functional Studies of EPHA2 Using Solution NMR**"
Pravesh Shrestha, *Case Western Reserve University*

2:25 PM to 2:30 PM Short Q&A

2:30 PM to 2:50 PM
"**H3 tail dynamics in the histone language**", Emma A. Morrison, *Medical College of Wisconsin*

2:50 PM to 3:00 PM Q&A

3:00 PM to 3:15 PM Coffee break

3:15 PM to 3:25 PM Promoted Poster Talk
"**Role of the hairpin in HIV fusion through membrane position of the soluble ectodomain (SE) of HIV gp41**", Md. Rokonujjaman*, Michigan State University*

3:25 PM to 3:30 PM Short Q&A

3:30 PM to 3:50 PM
"**Applications of sPRE to study electrostatics and very weak cosolute-protein interactions**",
Yusuke Okuno, *Washington University in St. Louis*,

3:50 PM to 4:00 PM Q&A

4:00 PM to 4:20 PM
"**DNP Magic: Transforming Weak Signals into Powerful Insights**"
Bernie O'Hare, *Bruker Biospin*

4:20 PM to 4:30 PM Q&A

4:30 PM to 5:00 PM - Round-table discussion
"**Machine Learning and Artificial Intelligence in NMR**"

5:00 PM to 5:15 PM
"**Update on the Gateway 1.2 GHz NMR spectrometer at OSU**" and the next conference announcement, Rafael Brüschweiler, *The Ohio State University*

Farewell and Departure

Poster Abstracts

ARCHE-NOAH: NMR supersequence with five CEST type experiments for probing protein conformational dynamics

Rodrigo Cabrera Allpas[1], Alexandar L. Hansen[2], and Rafael Brüschweiler[1,2]

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An NMR NOAH-supersequence is presented consisting of five CEST experiments for studying protein backbone and side-chain dynamics by 15N-CEST, carbonyl-13CO-CEST, aromatic-13Car-CEST, 13Cα-CEST, and methyl-13Cmet-CEST. The new sequence acquires the data for these experiments in a fraction of the time required for the individual experiments, saving over four days of NMR time per sample.

Quantifying π bonding effects in bis(iminoxolene)ruthenium and osmium complexes through ultra-low temperature NMR

Patricia Rose H. Ayson, Seth N. Brown

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(Diso)2MCl2 (M = Ru or Os; Diso = 4,6-di-tert-butyl-2-(2,6 diisopropylphenylimino) benzoquinone) are prepared by reaction of the iminoquinone with the M(II) precursors {(cymene)MCl2}2. While the cis isomers can form two strong metal-iminoxolene π bonds and form symmetrical pseudo-octahedral structures, the trans isomers can only form a single π interaction in an octahedral geometry. Therefore, distortion of the molecule is observed to add another metal-ligand π bond and increase its stability. VT NMR (in 80:20 CF2Cl2:CDFCl2 down to 100 K) shows that the distorted structure is more stable than the symmetrical structure by the appearance of the decoalesced peaks observed at lower temperatures. (Diso)2MPPh3 are prepared by addition of cobaltocene and PPh3 to cis-(Diso)2MCl2. A distorted square pyramidal geometry of the metal phosphine complexes is observed. VT NMR (in CD2Cl2 down to 188 K) shows that the barrier of interconversion for the Ru phosphine complex is ~1.2 kcal mol^{-1} lower than the Os variant.

NMR-based structure determination of the tRNA trans-editing enzyme ProXp-al

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ProXp-ala is a key component of the translational machinery in all three Domains of life. This enzyme helps to maintain the fidelity of proline codon translation through aminoacyl-tRNAPro proofreading. In the first step of tRNA aminoacylation, the cognate aminoacyl-tRNA synthetase (aaRS) binds and activates an amino acid in the enzyme's synthetic active site. If a non-cognate amino acid passes this first selection step and is charged onto the tRNA, a distinct aaRS editing active site may recognize the mischarged tRNA and deacylate it. Alternatively, this editing reaction may be carried out by a separate enzyme that deacylates the mischarged tRNA in trans. ProXp-ala is responsible for editing Ala mischarged onto tRNAPro. Since trans-editing domains such as ProXp-ala bind their substrates after release from the synthetase, they must recognize not only the mischarged amino acid, but also the specific tRNA. Previous studies showed that *Caulobacter crescentus* ProXp-ala distinguishes tRNAPro from tRNAAla, in part, based on the unique tRNAPro acceptor stem base pair C1:G72. Previous crystallographic and NMR data revealed a role for conformational selection by the ProXp-ala α2 helix in Ala- versus Pro-tRNAPro substrate discrimination. The α2 helix makes lattice contacts in the crystal, which is a caveat of the crystallography data. NMR-based solution structure determination of the free ProXp-ala domain will allow a more accurate description of the position of the α2 helix in the absence of the substrate and will set the stage for structure determination of ProXp-ala bound to Ala-tRNAPro. Results obtained to date toward NMR structure determination of the free *Caulobacter crescentus* ProXp-ala domain will be presented.

Solid-State NMR 13C Sensitivity at High Magnetic Field

Ruixian Han, Collin G. Borcik, Songlin Wang, Owen A. Warmuth, Kevin Geohring, Charles Mullen, Mario Incitti, John A. Stringer, Chad M. Rienstra

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Sensitivity is the foundation of every NMR experiment, and the signal-to-noise ratio (SNR) should increase with static (B0) magnetic field, by a proportionality that primarily depends on the design of the NMR probe and receiver. In the low B0 field limit, where the coil geometry is much smaller than the wavelength of the NMR frequency, SNR can increase in proportion to B0 to the power 7/4. For modern magic-angle spinning (MAS) probes, this approximation holds for rotor sizes up to 3.2 mm at 14.1 Tesla (T), corresponding to 600 MHz 1H and 151 MHz 13C Larmor frequencies. To obtain the anticipated benefit of larger coils and/or higher B0 fields requires a quantitative understanding of the contributions to SNR, utilizing standard samples and protocols that reproduce SNR measurements with high accuracy and precision. Here, we present such a systematic and comprehensive study of 13C SNR under MAS over the range of 14.1 to 21.1 T. We evaluate a range of probe designs utilizing 1.6, 2.5 and 3.2 mm rotors, including 24 different sets of measurements on 17 probe configurations using five spectrometers. We utilizing N-acetyl valine as the primary standard and compare and contrast with other commonly used standard samples (adamantane, glycine, hexamethylbenzene, and 3-methylglutaric acid). These robust approaches and standard operating procedures provide an improved understanding of the contributions from probe efficiency, receiver noise figure, and B0 dependence in a range of custom-designed and commercially available probes. We find that the optimal raw SNR is obtained with balanced 3.2 mm design at 17.6 T, that the best mass-limited SNR is achieved with a balanced 1.6 mm design at 21.1 T, and that the raw SNR at 21.1 T reaches diminishing returns with rotors larger than 2.5 mm.

Excited-state observation of active K-Ras reveals differential structural dynamics of wild-type versus oncogenic G12D and G12C mutants

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Despite the prominent role of the K-Ras protein in many different types of human cancer, major gaps in atomic-level information severely limit our understanding of its functions in health and disease. Here, we report the quantitative backbone structural dynamics of K-Ras by solution nuclear magnetic resonance spectroscopy of the active state of wild-type K-Ras bound to guanosine triphosphate (GTP) nucleotide and two of its oncogenic P-loop mutants, G12D and G12C, using a new nanoparticle-assisted spin relaxation method, relaxation dispersion and chemical exchange saturation transfer experiments covering the entire range of timescales from picoseconds to milliseconds. Our combined experiments allow detection and analysis of the functionally critical Switch I and Switch II regions, which have previously remained largely unobservable by X-ray crystallography and nuclear magnetic resonance 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. These results advance our understanding of differential GTPase activities and signaling properties of the wild type versus mutants and may thus guide new strategies for the development of therapeutics.

Development of a supramolecular hydrogel for prime-boost vaccine delivery

Audrey Hansrisuk and Matthew J. Webber

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Vaccines greatly reduce disease, disability, and death from certain infectious diseases. Morbidity has been eliminated or reduced by 99% for diseases such as chickenpox and paralytic polio. Vaccines provide protection to individuals as well as to communities by reducing the spread of disease within a population. A full vaccination requires multiple doses to impart full long-term potential protection. With the high associated noncompliance rate of scheduled vaccinations, minimally invasive delivery methods could mitigate the need for follow-up visits for booster vaccinations. Biomaterial engineering and supramolecular host-guest chemistry are leveraged to develop a single-administered vaccine depot capable of a two-dose release. The supramolecular hydrogel design includes conjugating a host-guest molecule pair, cucurbit[7]uril (CB[7]) and p-Xylylenediamine (E9), onto polyethylene glycol (PEG) chains. Preliminary work includes synthesizing the PEG macromers and confirmation via ^{1}H-NMR.

Assembly of Anti-TRAP oligomers and role of trimeric AT in regulating tryptophan biosynthesis in Bacillus subtilis

Joseph Hazel, Craig McElroy, Elihu Ihms, Deepak Kumar Yadav, Melody Holmquist, Vibhuti Wadwha, Vicki Wysocki, Paul Gollnick, and Mark Foster

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Biosynthesis of tryptophan by Bacillus subtilis (Bsu) is regulated by complex protein machinery that is sensitive to intracellular cues that include pH, tryptophan concentration, and levels of uncharged tRNATrp. The trp RNA-binding attenuation protein (TRAP), is an 8 kDa monomer that forms homo-undecameric rings (TRAP11) in solution. Binding of free tryptophan to TRAP11 leads to increased affinity for the 5'-untranslated region of the nascent trp mRNA, resulting in transcription attenuation. The trp operon is further moderated by the TRAP-inhibiting protein anti-TRAP (AT), which is expressed in B. subtilis in the presence of uncharged tRNATrp. AT is a 5 kDa Zn-binding protein that has been shown to assemble into trimers (AT3) and dodecamers (AT12) in a pH-dependent equilibrium. AT3 can bind tryptophan-bound TRAP, preventing TRAP from binding to trp mRNA to attenuate tryptophan biosynthesis. However, both the relevance of the AT3 ⇌ AT12 equilibrium for TRAP binding and the mechanism of AT3 binding and inhibiting RNA binding by TRAP11 are still not well understood. Previous studies have produced conflicting models of the structure of the dodecameric form of AT and the nature of the interaction of AT with TRAP. Here, we examine the AT3 ⇌ AT12 equilibrium using biophysical tools to provide insight into the mechanism of oligomerization. Additionally, we determine the solution structure of AT3 using nuclear magnetic resonance (NMR) spectroscopy and use relaxation measurements to understand the dynamics of both oligomeric AT states. As both AT and trp RNA compete for tryptophan-bound TRAP, understanding the dynamic nature of the AT in solution helps to reveal the underlying mechanisms of the tryptophan regulation machinery.

Reference Protonated C-13 and Nonexchangeable H-1 NMR Chemical Shifts in Purine Residues of Unfolded RNA Hairpin Loops

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The use of chemical shift data for structural and dynamic analysis in structured RNA molecules is advancing rapidly. Progress is currently hindered, however, by the lack of a comprehensive reference chemical-shift database characteristic of RNA residues in minimally-structured or unfolded states. In this work, we focused on purine residues and adopted the procedure of incorporating the residue of interest in a terminal loop designed to minimize residual stacking interactions in such a way that only that single nucleotide carries isotope label, and used temperature dependence of observed peaks to identify resonances corresponding to a minimally-structured state. Interestingly, constructs with a variety of loop sequences converged to a tightly clustered set of resonances for all protonated 13C and nonexchangeable 1H nuclei, indicating a consistent minimally-structured state useful for reference in a variety of experimental situations. We propose a standard set of reference chemical shifts at 25 °C and (for 13C) appropriate temperature correction factors for adenosine and guanosine residues in unfolded RNA. Comparisons with commonly-observed shift values suggest that resonances including 13C8, 13C1′, 13C3′, and 13C4′ will be experimentally useful probes of local unfolding events.

Control of Bruker NMR Spectrometers from Python with KovriginNMR

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An object-oriented Python software package, KovriginNMR, has been designed to enable Bruker spectrometer control from Topspin Python interface. KovriginNMR is intended to help NMR users construct workflows incorporating multiple samples, experiments, as well as variable-temperature operation. A user is able to automate as much or as little of their workflow as desired, and the software may be started from user's own account (no need for NMR superuser privileges). KovriginNMR automatically logs events taking place during its operation and includes time stamps, sample and experiment names, data paths, thus assisting accurate record-keeping.

KovriginNMR Workflows enable fast development of complex experimental routines involving multiple samples and multiple experiments. The intended user of KovriginNMR Workflows is an NMR spectroscopist with a practical experience of operating Bruker spectrometers. KovriginNMR Workflows is a high-level programming environment aimed to extend Topspin and Spooler capabilities. The software does not shield an NMR user from a spectrometer or restricts any actions: entire range of functions of Topspin remains accessible. Any functionality of KovriginNMR may be bypassed by a user at any time. The simplest workflows may involve only several lines of Python code while others may be designed to run batteries of NMR tests on multiple samples in an unattended mode.

KovriginNMR VT enables automated NMR measurements in a broad temperature range. The software allows for recording and utilizing accurate temperature calibrations even if the probe sensor is highly non-linear. KovriginNMR VT directly controls all parameters of the Bruker variable-temperature unit and automatically sets temperature and gas flow appropriate to the temperature range, spinner material, and type of a chiller. One example is a temperature series of NMR spectra in which the temperature step may be varied according to the region of interest. In such a series, KovriginNMR VT automatically reaches each intended temperature, executes an equilibration delay, tuning, shimming, and acquisition. Experimental series may be followed by an automatic return of a spectrometer back to ambient conditions to make it ready for the next user. Fully unattended operation may effectively use the night time for recording of multi-point VT NMR or kinetic experiments.

KovriginNMR is free for academic users (http://lineshapekin.net/#KovriginNMR). Advanced users may create their own extensions for KovriginNMR, as long as they retain all copyright marks in the original Python code.

Predicting protein flexibility with AlphaFold

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AlphaFold2 has revolutionized protein structure prediction from amino-acid sequence. In addition to protein structures, high-resolution dynamics information about various protein regions is important for understanding protein function. Although AlphaFold2 has neither been designed nor trained to predict protein dynamics, it is shown here how the information returned by AlphaFold2 can be used to predict dynamic protein regions at the individual residue level. The approach, which is termed cdsAF2, uses the 3D protein structure returned by AlphaFold2 to predict backbone NMR N-H S2 order parameters using a local contact model that takes into account the contacts made by each peptide plane along the backbone with its environment. By combining for each residue AlphaFold2's pLDDT confidence score for the structure prediction accuracy with the predicted S2 value using the local contact model, an estimator is obtained that semi-quantitatively captures many of the dynamics features observed in experimental backbone NMR N-H S2 order parameter profiles. The method is demonstrated for a set nine proteins of different sizes and variable amounts of dynamics and disorder and its stability has been tested by leave-one-out cross validation. The information predicted by this method should prove useful for a direct initial assessment of protein flexibility in functional studies or protein ligand screening. We will show applications to various protein systems along with a public web server in development that will combine AlphaFold2 structures with contact-model-based dynamics prediction and process cdsAF2 calculations allowing the user to predict protein flexibility with a single AlphaFold2 PDB structure.

Characterization of the Biophysical Properties of a COG0523 Metallochaperone and a Mur Ligase from 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. 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 Acinetobacter baumannii: A1S_0934 and the client protein MurD, a Mur ligase used in peptidoglycan synthesis. Using various biophysical techniques, we demonstrate that A1S_0934 bind zinc with high affinity. Leveraging AlphaFold models of A1S_0934, structural differences in conserved motifs are compared to support alternative models for function. In addition, NMR spectroscopy and crosslinking experiments validate conformational changes in A1S_0934 structure as the result of nucleotide, zinc, and client protein binding as a further step to understand the role in Acinetobacter baumannii. Physical and functional features of the protein-protein interaction are defined for the first time and steps toward complete resonance assignments have been initiated to map the binding interface.

Integrative models of oncogenic mutants within the trans- and juxta-membrane (TMD/JMD) region of human epidermal growth factor receptors

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The trans- and juxtamembrane domains (TMD/JMD) of human epidermal growth factor receptors (HER/EGFR/ErbB) regulate the helix-helix dimerization interface to control receptor tyrosine kinase (RTK) activation. Patient-identified non-small cell lung cancer (NSCLC) mutations occur all along the EGFR (HER1) and HER2 TMD and JMD, but our structural understanding of how these patient-derived TMD/JMD mutations hijack wild-type RTK function to drive cancer is lacking. In this work, we integrate tools in experimental biophysics and computational modeling to evaluate the structural mechanism(s) driving these interactions in atomic detail. We use nuclear magnetic resonance (NMR) spectroscopy to assess how oncogenic mutations within the TMD/JMD perturb the architecture/interactions of the dimer interface. NMR-derived models are used to seed molecular dynamics (MD) simulations to probe how mutations affect TMD association and activation/stabilization via dimerization in a realistic membrane environment. We apply this strategy and focus our initial efforts on the EGFRA647T variant. We computed the free energy landscape from our simulations and show that the active state for the EGFRA647T□HER2WT heterodimer is stabilized by 10 kcal/mol. Conversely, the wild-type EGFR□HER2 heterodimer is stabilized by 5 kcal/mol for the inactive state suggesting the propensity of the EGFRA647T mutant favors the active state. These results are part of a multidisciplinary collaboration that share the common goal of constructing holistic models that detail how TMD/JMD variants are aberrantly activated in cancer.

Stability Prediction of Protein Systems Using SSNMR Spectroscopy

Eric Munson, Cole Tower, Rachana Sapkota

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Cryoprotecting proteins in solid matrices containing buffers, sugars, and surfactants is a common process to ensure protein stability in the solid state. Two main factors that influence the stability of a protein include the interaction of the protein with the stabilization matrix, and the mobility of the protein in the matrix. The protein must be intimately mixed with the stabilizing agent, usually a sugar, to maximize the ability of the protein to remain intact during storage. In addition, the sugar matrix should inhibit the mobility of the protein in the matrix to maximize stability. Solid-state NMR spectroscopy has been used to measure each of these parameters, with the SSNMR mobility and phase separation data compared to protein aggregation upon storage as determined by size exclusion data. The SSNMR data correlated with the aggregation data, where the best stability was achieved for homogeneous (no phase separation) formulations with the least mobility.

Combined use of Solid-State and Solution NMR to Understand Allosteric Transitions in a Ligand-Activated Oligomeric Protein

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Allosterically regulated proteins undergo changes in structure and dynamics upon binding to their activator ligands. The oligomeric ring-shaped protein TRAP is allosterically activated by tryptophan (Trp) to bind RNA, thereby regulating tryptophan biosynthesis in Bacilli2. We performed solution and solid-state NMR experiments on the 91 kDa TRAP protein from *Geobacillus stearothermophilus* to understand how binding of Trp to its 11 identical sites modulates its RNA binding function. We performed methyl CPMG relaxation dispersion experiments in solution on Thr γ2 and Ile δ1-13CH3, U-[12C, 2H 15N]-TRAP in the absence (apo) and presence of Trp to characterize chemical exchange in loops spanning the ligand binding sites1. These experiments showed strong dispersions indicative of μs-ms time scale exchange in apo-TRAP that were strongly dampened in Trp-bound TRAP. Complementary solid-state cross-polarization spectra of apo-TRAP yielded high quality spectra and side chain assignments for residues in the rigid core. A dynamic spectral editing (DYSE) approach using solid-state INEPT-based experiments identified residues invisible in solution experiments and solid-state CP-based experiments. 13C Ile chemical shifts were used to identify the rotameric states of methyl-bearing side chains for comparison to available crystal structures of Trp-bound TRAP. These experiments thus allow characterization of the structural and dynamic landscape of the apo-TRAP and new insights into the mechanisms of its regulation by Trp.

Determining critical structural residues in huPrP Y145Stop amyloids by single point mutations

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The conversion of certain alpha-helical monomers to beta-sheet-rich fibrils is determined to be the cause of many neurodegenerative diseases in humans and other animals. To understand how the structure of prion amyloids correspond to the spread and development of some of these diseases, we mutated the model prion protein Y145Stop at sites within the fibril core that correspond to differences between the human and mouse primary sequence. Once the structurally significant sites are identified, they can be analyzed alongside other data to produce a complete picture of how those residues contribute to the fibril structure. Solid-state NMR provides high-resolution data that can reveal small structural differences between samples and possible heterogeneity within one sample, while various forms of microscopy can identify quaternary fibril features as well as produce a high-resolution model of the fibril. This combination of techniques provides a new method of studying fibrils which could be expanded to other neurodegenerative diseases.

Extracting Changes in the Dynamic Responses of Protein Variants During Substrate Turnover by NMR Chemical Shifts

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The chemical shifts of protein backbone nuclei, such as amide 15N, vary with local structural variables such as torsion angles. Thus, chemical shift changes can give insight into structural dynamics. However, such structural information can become clouded by other phenomena that influence the local electron distribution, such as solvent accessibility, hydrogen-bonding, and substrate/ligand binding. Here, we attempt to extract the essential configurational changes of the ß-lactamase OXA 24/40 during active substrate turnover. We performed principal component analysis on the time-dependent 15N chemical shift changes from HSQC BEST-TROSY NMR experiments taken during active turnover of doripenem for WT and a mutant R261S, which use different mechanisms for lysing the drug. We observed a marked difference in the PC subspace for the two constructs, which may help explain their difference in activity. Furthermore, we observed further evidence that the R261S mutation affects the motion of the proximal active site loops that are thought to be responsible for mediating the drug-lysing mechanism in OXA 24/40 clinical mutants. This technique can be extended to other chemical shifts such as 13Ca and 13Cb to get a more comprehensive evaluation of the difference in dynamics of enzyme variants during active turnover.

Exploring the role of post-translational modification on β-lactamase activity of OXA24/40

Dharshika Rajalingam and Jeffrey W. Peng

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Acinetobacter baumannii is a multidrug-resistant bacterial pathogen that has become a threat to global health. *A baumannii* has become highly resistant to β-lactam antibiotics through their deactivation by various serine β-lactamases. An example is OXA24/40, the representative member of the carbapenem hydrolyzing class D β-lactamases (CHDLs), which can hydrolyze carbapenems, one of the most potent antibiotics available. Recently, it was shown that OXA24/40 can deactivate carbapenems via two mechanisms: hydrolysis and β-lactonization. This dual mechanism capability of OXA-24/40 and its clinical resistance mutations motivate towards a comprehensive understanding of the cellular processes affecting its β-lactamase activity. One of the crucial characterizations of prokaryotes to develop adaptation is post-translational modification (PTM), mainly phosphorylation. Post-translational phosphorylation of *A. baumannii* serine β-lactamases has recently been established but its functional impact remains unknown. We found that the OXA24/40 could be phosphorylated in vitro, and have used this to investigate the effects of post-translational phosphorylation on OXA-24/40 using NMR and MS. The Ser81 at the active STFK motif of OXA24/40 of catalytic pocket was identified as the site of phosphorylation using 1D 31P NMR experiment, whereas S81 is required to form an acyl-enzyme complex between enzyme and β-lactam antibiotics. The activity of completely phosphorylated OXA24/40 wild type against doripenem revealed that the phosphorylation of active Ser inactivates the □-lactamases activity of OXA24/40. The 1D 1H CPMG NMR-based activity assay of phosphorylated OXA24/40 against doripenem confirmed that both deactivating mechanisms are inhibited by phosphorylation. Carbamylated Lysine at the active STFK motif is one of the critical features of CHDL required for the acylation and deacylation reactions of the enzyme. The 1D 13C NMR experiment confirmed that the K84 of phosphorylated OXA24/40 is de-carbamylated. Phosphorylation of OXA24/40 affects both active S81 and carbamylated K84 of OXA24 that are required for the resistivity of β-lactamase. So, phosphorylation could be one of the reasons for the evolution of genetic mutation of OXA24/40 to broaden the antibiotic resistivity. Further research can lead to an understanding of the effect of phosphorylation on the clinical mutants of the OXA24-like β-lactamase family on the broadening of β-lactamase activity.

Role of the hairpin in HIV fusion through membrane position of the soluble ectodomain (SE) of HIV gp41

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The primary cause of AIDS is the human immunodeficiency virus (HIV), which infects target white blood cells by connecting (fusion) the membranes of the host cell and HIV, causing the viral nucleocapsid to deposit in the cytoplasm. HIV's Gp41, a single-pass integral viral membrane protein with a 150-residue soluble domain outside the virus, is involved in fusion catalysis [1,2]. This soluble ectodomain (SE) adopts a hairpin structure that contains N-helix-turn-C-helix, and two helices align themselves antiparallelly and there is van der Waals contact between them. The final fusion structure also consists of three hairpins joining together to form a six-helix bundle. The relationship with binding to the membrane suggests that the SE hairpin plays a significant role in fusion catalysis as opposed to playing no catalytic role at all. In some circumstances, the rapid membrane vesicle fusion caused by the hairpin [1] supports this occurrence. The quantitative similarity of HIV gp160 V513E-dominant reduction of fusion and infection with fusion by the gp41 ectodomain hairpin also supports an important fusion role for the final trimer-of-hairpins structure, as shown by a recent trans-dominance effect study using membrane vesicle fusion of V513E to wild-type gp41 ectodomain [3]. Since deep SE insertion indicates the ease of near membrane apposition and surface insertion predicts less ease of membrane apposition, the position of the SE hairpin may aid in a better understanding of membrane apposition (viral and host membrane) during fusion. As a result, this work aims to locate the SE hairpin in the membrane. We used either 13C-glucose or 13C-glycerol to express a large soluble ectodomain in a minimum medium, with a dilution of the 13C labeling accomplished by mixing with unlabeled glucose or by selectively labeled glycerol [4]. A protein that has been 13C-labeled and two 2H labels that are positioned in the middle of the membrane were measured to be as close as possible using the rotational-echo double-resonance (REDOR) solid-state NMR technique [5]. The manufacture of 13C proteins used a variety of carbon sources, and as a result, various labeled carbon peak assignments on the side chains of amino acids were observed in NMR. There was a significant REDOR dephasing for samples from different carbon sources, which shows that the soluble ectodomain is buried deep within the membrane.

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Structural and Functional Studies on Ephrin Receptor Yyrosine Kinase, EPHA2 Regulation Driven by Phosphorylation and Intra/intermolecular Interaction Using Solution NMR

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Eph receptor, the largest subfamily of single pass trans membrane receptor tyrosine kinase family play crucial role as a regulator during embryonic development, cell maturation, and adulthood. EphA2, a member of the Eph family receptor tyrosine kinases (RTK) is a key regulator of tumorigenesis and cancer progression. The importance of EphA2 in axon guidance and synaptogenesis is well established. While normally a repulsive signal, non-canonical cell migration promoting activity has been observed by unliganded EphA2 signaling mechanism. Here our focus was to dissect the effects of the sterile α motif (SAM) domain together with the phosphorylation on intracellular region (ICR) interactions of EphA2 in solution. Our study shows that the sterile α motif (SAM) domain acts as a regulator impeding kinase domains interaction. ICR oligomerization upon SAM domain deletion consequently increased kinase activity, compared to that of monomeric state of EphA2 ICR, which is comparatively slower. While the presence of the SAM domain hinders oligomerization of phosphorylated ICR, it appears that such protein-protein interactions are required, at least initially, for kinase activity regulation. Mutation studies of the linker region between kinase domain and SAM domain give insight into its regulatory role for EphA2 activity. Importantly, NMR shows when the 15N labeled SAM is titrated with unlabeled ICR, key residues on the SAM domain are perturbed indicating their role in binding to phosphorylated ICR in solution. Moreover, the SAM domain of SHIP2 (SH2-linked inositol phosphatase 2) has been associated with the co-localization of both proteins to the cellular membrane. We have mapped the residues on the SAM domain of SHIP2 upon interacting with EphA2-SAM using chemical shift perturbation. The activation of this receptor tyrosine kinase appears to involve an allosteric mechanism by disrupting SAM domain-kinase domain interactions. Furthermore, our structural insight and functional characterization of SAM domain-mediated EphA2-SHIP2 interaction could lead to a new direction in cancer therapeutics targeting EphA2.

Influence of Cu(II) binding on the assembly and core structure of Y145Stop human prion protein fibrils

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Neurodegenerative disorders such as Creutzfeldt-Jacob disease are characterized by accumulation of misfolded prion protein (PrP) aggregates1. Exploring the role of metal ions such as copper or zinc in the aggregation process is significant given their quintessential role in regulating some of the key physiological functions of cellular PrP and brain metal homeostasis2. Copper is known to bind to the octapeptide repeats (PHGGGWGQ) located in the N-terminal region of PrP in distinct binding modes dictated by the concentration of Cu2+ ions3. Here, we use solution NMR and EPR to investigate binding of Cu2+ and solid-state NMR to gain atomic level insights into structural changes in the amyloid core region of the Y145Stop human prion protein variant (huPrP23-144) caused by the binding of Cu2+ prior to fibril formation. Interestingly, we find that Cu2+ shows significant binding to histidine residues in both the octa-repeat as well as core regions and we find that amyloid fibrils formed by PrP23-144 monomers with bound Cu2+ adopt a core structure that is distinct from that found for wild-type huPrP23-144 fibrils but resembles that associated with one of the Gerstmann-Straussler Scheinker (GSS) disease associated prion protein mutants4. We also observe that this conformation reverts to that of native huPrP23-144 fibrils when the fibrillation is seeded with preformed fibrils of wild-type huPrP23-144. In addition, we investigated the role of Cu2+ binding on the fibril assembly kinetics and observed that protein aggregation is significantly slower with increasing amounts of bound Cu2+.

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Homogeneity and Mobility as Stability Predictors in Lyophilized Formulations

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The majority of new drugs approved by the U.S. Food and Drug Administration are biologics. Biologic drugs are typically formulated as a solution; however, solution formulations must be stored at refrigerated or frozen conditions to maintain stability. The 'cold-chain' is expensive, and prone to failures which can lead to destroyed doses. This became evident during the COVID-19 pandemic where 1900 doses of mRNA vaccines were destroyed when a freezer was accidentally unplugged in a Boston, MA hospital. Aside from accidents, cold-chain storage accounts for approximately 80% of the cost of vaccine programs in developing countries. Lyophilization (freeze-drying) is a technique that is often used to prepare protein formulations that are stable at room temperature. Stabilization of proteins by disaccharides in the lyophilized state often hinges on interactions between the protein and the disaccharide (system homogeneity) and sufficiently low mobility of the system. A method to predict the stability of a lyophilized protein was developed utilizing 1H T1 and 1H T1ρ relaxation times measured by solid state nuclear magnetic spectroscopy. 1H T1 and 1H T1ρ relaxation times were used to assess system homogeneity on 20 – 50 and 1 – 3 nm domains, as well as relative system mobility. Human serum albumin was lyophilized with disaccharides sucrose and trehalose in different relative concentrations. HSA/sucrose systems have longer 1H T1 relaxation times and are slightly more stable than trehalose systems in almost all cases shown. Ternary HSA/sucrose/trehalose systems have 1H T1 relaxation times between the binary HSA/sucrose and HSA/trehalose systems and do not result in a more stable system compared to binary systems. Under these stability conditions, a 1H T1 relaxation time below 1.5 s correlates with an unstable sample.

Molecular mechanism of PIN1-mediated regulation of the nuclear receptor PPARγ

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Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand sensitive nuclear receptor and master regulator of adipogenesis. There exist FDA approved drugs that bind the ligand binding domain (LBD) of PPARγ to induce a well characterized conformational change, which alters the activity of this transcription factor. However, the functionally critical N-terminal intrinsically disordered AF1 domain remains poorly understood from a structural standpoint due to the limited number of biophysical methods that can provide atomic level detail of binding interactions and AF1 conformational states. Peptidyl-prolyl cis/trans isomerase NIMA-interacting 1 (PIN1) is a known protein binding partner of PPARγ that exerts its catalytic activity specifically at pSer/pThr-Pro motifs, facilitating the cis/trans isomerization of proline bonds along the peptide backbone. Preliminary data from our lab and others suggest PIN1 binds the AF1 region of PPARγ with a much greater affinity than the LBD, an observation which presents the opportunity to better understand the role of AF1 function in PPARγ biology. This binding interaction suggests a cascade of post translational modifications (PTMs), including kinase-mediated phosphorylation and PIN1 enzyme-catalyzed proline cist/trans isomerization of the AF1 region, may be responsible for tuning the transcriptional activity of PPARγ and maintaining activation of adipogenic gene programs. To structurally characterize phosphorylation-dependent and isomerization-dependent conformational changes within the AF-1 region, nuclear magnetic resonance (NMR) spectroscopy will be extensively used alongside biolayer interferometry (BLI) and X-ray crystallography. To correlate the structural and molecular mechanisms described by NMR with functional effects observed in cells, this project will rely on structure-guided mutagenesis, transcriptional reporter assays, and knockdown experiments in pre-adipocytes to understand the role of PIN1 as a mediator of PPARγ function during adipogenesis. Gene expression analysis and assays that measure cellular endpoints associated with PPARγ function will further assess the roles of these PTMs within the AF1 domain in promoting adipogenic function.

HIV-1 reverse transcriptase monomer structure, the dimerization and the maturation

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The mature HIV-1 reverse transcriptase is a heterodimer that comprises 66 kDa (p66) and 51 kDa (p51) subunits. The latter is formed by HIV-1 protease-catalyzed removal of a C-terminal ribonuclease H (RNH) domain from a p66 subunit. The process is facilitated by forming a homodimer, p66/p66, and by nucleic acid binding that protects one RNH domain and destabilizes the other RNH domain. Recently, we have elucidated relative domain orientations in p66 monomer mutant p66, using small-angle X-ray scattering and NMR. Based on the results, the model for the p66/p66 and the maturation are discussed.

Molecular Basis of Nurr1-RXRα Activation

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Small molecule compounds that activate transcription of Nurr1-RXRalpha (NR4A2-NR2B1) nuclear receptor heterodimers are implicated in the treatment of neurodegenerative disorders, but function through poorly understood mechanisms. We show that RXRalpha ligands activate Nurr1-RXRalpha through a mechanism that involves ligand-binding domain (LBD) heterodimer protein-protein interaction (PPI) inhibition, a paradigm distinct from classical pharmacological mechanisms of ligand-dependent nuclear receptor modulation. NMR spectroscopy, PPI, cellular transcription assays show that Nurr1-RXRalpha transcriptional activation by RXRalpha ligands is not correlated with classical RXRalpha agonism but instead correlated with weakening Nurr1-RXRalpha LBD heterodimer affinity and heterodimer dissociation. Our data inform a model by which pharmacologically distinct RXRalpha ligands (agonists and Nurr1-RXRalpha selective agonists that function as RXRalpha antagonists) operate as allosteric PPI inhibitors that release a transcriptionally active Nurr1 monomer from a repressive Nurr1-RXRalpha heterodimeric complex. These findings provide a molecular blueprint for ligand activation of Nurr1 transcription via small molecule targeting of Nurr1- RXRalpha.