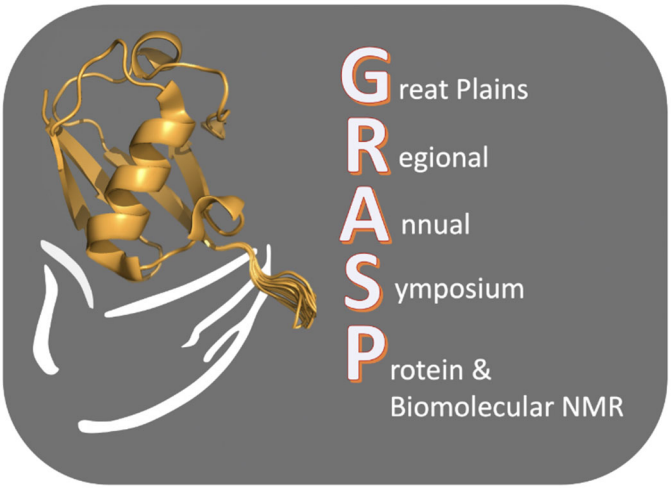

2024 GRASP NMR Symposium

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Symposium Information

The 15th annual GRASP NMR Symposium was held at Iowa State University on October 25 – 26, 2024.

Plenary Speakers

Dr. Haribabu Arthanari, Ph. D., Department of Biological Chemistry and Molecular Pharmacology, Harvard University
Emerging Methods in Solution NMR: Illuminating Blind Spots in Mechanistic Biology

Charles D. Schwieter, Director of the Computational Biomolecular Magnetic Resonance Core, NIDDK, NIH
Toward an atomic model of Huntingtin Exon 1 fibrils & the Workshop: *Structure Determination with XPLOR-NIH*

Invited Speakers

Dr. Ernesto Fuentes – University of Iowa
Ph.D., Department of Biochemistry and Molecular Biology
Insights into Pseudomonas aeruginosa directed motility by integrative structural biology

Dr. Katherine Henzler-Wildman – University of Wisconsin - Madison

Ph.D., Department of Biochemistry
The Network for Advanced NMR

Dr. Robert Powers – University of Nebraska - Lincoln
Ph.D., Charles Bessey Professor of Chemistry at the University of Nebraska – Lincoln, Director of the Systems Biology core facility with in the Nebraska Center for Integrated Biomolecular Communication
Metabolomics: the Good, the Bad, and the Ugly

Dr. Beat Vogeli – University of Colorado, Anschutz Medical Campus
Ph.D., Associate Professor of Biochemistry
Where NMR is better than AI: Allostery within and between protein domains viewed by eNOEs

Dr. Nicholas Levinson – University of Minnesota – Minneapolis
Ph.D., Department of Pharmacology
Mechanistic basis for the allosteric suppression of c-Myc ubiquitination by Aurora kinase A

Dr. Smita Mohanty – Oklahoma State University
Ph.D., Department of Chemistry,
Olfaction: Structure and Function Studies of a Pheromone-Binding Protein

Promoted Speakers

Aayushi Singh (Venditti Lab. – Iowa State University)
Structure-based discovery of a non-competitive FTO inhibitor bound to a cryptic site at the domain interface

Stephan Azatian (Latham Lab. – University of Minnesota)
Structure and Dynamics of the Zn Hook Domain in the Mre11-Rad50 Complex

Steven Siang (Roche Lab. – Iowa State University)
DNA binding activity and conformation of ATF4 are influenced by a secondary basic region unique to the ATFx subfamily of bZip transcription factors

Jeffrey Krall (Vögeli Lab. – University of Colorado)
Biophysical Characterization of the Nucleic Acid Binding Domains of Z-DNA Binding Protein 1 (ZBP1)

Manu Veliparambil Subrahmanian (Veglia Lab – University of Minnesota)
Rapid Pulsing Broadband NMR Experiments for Fast Acquisition of Protein Spectra at High and Ultra-High Magnetic Fields

Baboucarr Faal (Venditti Lab – Iowa State University)
The C-terminal tail of human Alkbh7 controls its nucleic acid demethylase activity

Om Prakash (Kansas State University)
Simultaneous Inhibition of two Serine Proteases: NMR Insights into the Molecular Function

Workshop

A workshop on: Structure Determination with XPLOR-NIH was hosted in the afternoon on October 26th.

POSTER PRESENTATIONS (*Best poster competition)

POSTER PRESENTATIONS (*Best poster competition)

Poster 1*

Structure and Dynamics of the Zn Hook Domain in the Mre11-Rad50 Complex

Stephan Azatian (Latham Lab.)
University of Minnesota

The Mre11-Rad50 complex (MR) is important for DNA double-strand break repair in all organisms. Previous research has suggested that conformational changes in the globular, enzymatic region of Rad50 propagate long-range allosteric changes through the coiled- coil region to the apical zinc hook domain (ZHk), a distance of >500 Å. We show that mutation of residues in the ZHk show significant effects on MR catalytic activity. Crystal structures by other groups of the isolated ZHk suggest multiple conformations in solution, possibly emerging from interactions of the globular domain with DNA and ATP substrates. These conformations of the ZHk have not been well characterized. To study the ZHk by NMR spectroscopy as part of the full MR complex, we purified three segments of Rad50 consisting of the N-terminal coiled-coil region, methyl-labeled ZHk, and C-terminal coiled- coil region, and ligated the segments using two enzymes. Using methyl-based NMR, we have observed reduced 1H R2 relaxation rates upon the addition of a non-hydrolysable analog of ATP and double-stranded DNA in methyl ZHk-labeled MR but not in isolated ZHk. In addition, we observe a field dependence in 1H R2 relaxation rates in methyl ZHk-labeled MR upon addition of substrates. Relaxation rates derived from Forbidden methyl 1H triple-quantum coherence transfer experiments suggest a change in the flexibility of the ZHk methyl groups upon addition of substrates. Measurements from zero, double, and quadruple quantum relaxation experiments help uncover conformational exchange rates in the ZHk. Methyl group RDCs for substrate-bound ZHk-labeled MR may help validate models of conformational populations. In the future, heterogeneous populations of structures will be generated and confirmed with RDC data of ZHk-labeled MR.

Poster 2*

Targeting ATF4-C/EBPÎ² Heterodimerization: An Integrative Strategy for Oncogenic Activity Suppression

Manuela Chaves-Mejia (Roche Lab.) Iowa State University

The ATF4-C/EBPÎ² heterodimer is a crucial transcriptional regulatory complex involved in diverse cellular processes, particularly in the Integrated Stress Response (ISR) pathway and tissue-specific gene regulation in mammals. As members of the bZip family, ATF4 and C/EBPÎ² exhibit structural complementarity that governs their interaction with DNA and dimerization with other partners.

Notably, the overexpression of both ATF4 and C/EBPÎ² under cellular stress conditions has been associated with the regulation of cell growth, apoptosis, and metastasis in various cancer types, suggesting a dual role for ATF4-C/EBPÎ² in oncogenesis promotion and suppression. Despite the significant impact of cancer diseases, particularly in humans, therapeutic interventions targeting this pathway face major challenges due to multigenic networks, the development of chemoresistance, and tissue-specific responses. This project aims to address these challenges by employing ProteinMPNN for the in-silico design of a dominant-negative peptide, such as a modified C/EBPÎ², to disrupt the heterodimerization of ATF4 with its natural binding partners. Subsequent in-vitro Fluorescent Polarization assays will assess the ability of this peptide to inhibit DNA binding and transcriptional activity of ATF4, with the potential to shed light on new therapeutic strategies for suppressing oncogenic activity.

Poster 3*

Oxidative Activation S. aureus SrrA

Nathan Clack (Fuentes Lab.) University of Iowa

The S. aureus TCS SrrAB regulates the cell's response to redox stress. Canonically, TCS response regulators are activated by phosphorylation by the sensor kinase. However, we find that oxidation of SrrA in the absence of SrrB is sufficient to induce dimerization and stimulate DNA binding.

Poster 4*

Phosphorylation induced changes in protein Conformational Dynamics modulate mRNA Binding in UP1

Sayan Das (Venditti Lab.) Iowa State University

Human hnRNP-A1 is a multifunctional RNA binding protein which is responsible for various aspects of nucleic acid processing. The N terminal region named Unwinding protein 1 (UP1) comprises of two RNA Recognition Motifs (RRMs) connected by a flexible linker. In-vivo studies have demonstrated that double phosphorylation at serines 4 and 6 induces binding of UP1 with mRNAs of anti-apoptotic proteins like BCL-XL and XIAP. Here, we combine solution NMR and MD simulations to investigate the effect of phosphorylations on the structure and dynamics of the UP1. We show that formation of a cluster of negative charges on the UP1 N-terminus causes its tail to transiently dock on a positive patch present in one of the two UP1 RNA binding sites. We speculate that this transient interaction modulates the thermodynamics and selectivity of RNA binding to the RRM2 of UP1, therefore providing a structural interpretation for the phosphoregulation of RNA recognition and binding.

Poster 5*

Towards Functional Characterization of AlkBH2 Using NMR Spectroscopy

Paige Davis (Venditti Lab.) Iowa State University

AlkBH2 is a member of the AlkB family of Fe2+ and Î±-ketoglutarate-dependent dioxygenases, which play a key role in DNA repair through oxidative demethylation of alkylated nucleotides. Specifically, AlkBH2 removes methylated lesions such as m3C and m1A from double-stranded

DNA, preserving genomic integrity. Overexpression of AlkBH2 has been linked to higher incidences of cancers, including glioblastoma, bladder, and lung cancers, and is associated with resistance to certain cancer therapies. By using NMR spectroscopy to investigate the solution conformational dynamics of AlkBH2, we aim to gain insight into its substrate specificity and binding mechanisms. This knowledge could be pivotal in developing selective inhibitors that may improve cancer treatment outcomes.

Poster 6*

Towards the characterization of functional dynamics in the Human Alkbh1 enzyme

Dilini Dissanayake (Venditti Lab.) Iowa State University

Human Alkbh1 is a member of the AlkB family of proteins. Like other AlkB family proteins, hAlkbh1 is a Fe2+ and alpha-ketoglutarate-dependent dioxygenase that catalyzes oxidative demethylation of modified nucleotides and amino acids. hAlkbh1 shows activity against six different substrates such the m6A in ssDNA/ bulge or bubble DNA, m3C in DNA/RNA, methylated lysine in histone H2A, m1A in mitochondrial tRNA for the demethylation, oxidation of m5C in mitochondrial tRNA and lyase cleavage of AP sites in DNA. Also, hAlkbh1 has significant involvement in many cancers, metastasis, developmental defects, hypothermia, etc. Since it is established that protein conformational dynamics play an important role in regulation of AlkB enzymes, my work focus on the characterization of hAlkbh1 functional dynamics. In this conference, I will present my work in (i) the optimization of a protein expression and purification protocol for hAlkbh1, (ii) the establishment of protocols to assay the demethylase and AP lyase activities of hAlkbh1, and (iii) the investigation of hAlkbh1 conformational dynamics by solution NMR

Poster 7*

The terminal tails of human Alkbh7 control its nucleic acid demethylase activity

Baboucarr Faal (Venditti Lab.) Iowa State University

The AlkBh7 protein, a member of the AlkB family of dioxygenases, plays a pivotal role in epigenetic regulation through its involvement in demethylation processes. This presentation focuses on the search for active substrates of AlkBh7 we investigated the demethylation activity of AlkBh7 for both the full-length and truncated variants and identified only the FL to be active towards m3C substrate. The result is couple to binding based on the CSP data. Additionally, we performed the backbone assignment of AlkBh7, a key step in understanding its three-dimensional structure and dynamic behavior at the atomic level. We report the backbone 1H, 15N, and 13C chemical shift assignments for both the full-length protein and a double construct of human ALKBH7. From these chemical shifts, we derived information about AlkBh7's secondary structural elements, such as alpha helices and beta strands, providing insights into its overall fold. Using the TALOS+ program, we generated a secondary structure prediction based on the assigned backbone resonances, which aligns with the previously reported X-ray structure of the enzyme. These findings, along with the activity assay results, will support future investigations into the protein’s structural dynamics, offering crucial insights into substrate recognition and enzyme regulation processes.

Poster 8*

Biophysical Analysis of an Interaction Between a PilZ Domain Protein and the Response Regulator PilH in Pseudomonas aeruginosa

Nicholas Hammons (Fuentes Lab.) University of Iowa

Pseudomonas aeruginosa and Staphylococcus aureus commonly co-infect the lungs of cystic fibrosis patients. Time-lapsed microscopy shows that P. aeruginosa directs motility towards S. aureus through action of the type IV pilus leading to mixed colony formation. Comprehensive screening of potential cyclic-di-GMP (cdG) effector proteins revealed that the putative cdG receptor, PA0012, is important for the directed motility phenotype in P. aeruginosa. Furthermore, bacterial two-hybrid assays suggest that PA0012 regulates type IV pili dynamics through the PilChp chemosensory system by direct interactions with the response regulator PilH in a cdG dependent manner. To understand how PA0012 regulates the type IV pili, we characterized PA0012â€™s interaction with cdG and PilH. We solved the structure of PA0012 through X-ray crystallography and revealed that it is a standalone PilZ domain that binds an intercalated dimer of cdG through a flexible N- terminal arm. 1H-15N HSQC NMR titration experiments and analysis of 15N T2 relaxation rates demonstrated how interactions with cdG lead to an ordering of the N-terminus and rigidification of the Î²-barrel core. We show through kinetic analysis of PilH phosphorylation states that PilH phosphorylation has a t1/2 ~ 45 minutes. We treated PilH with BeF3- in an attempt to mimic a persistent phosphorylated conformation. 1H-15N HSQC NMR analysis of BeF3- treated PilH showed significant chemical shift perturbations which suggested a BeF3- bound state. With this in hand, we conducted quantitative pull-down assays using apo and cdG bound PA0012 as prey and PilH in the unphosphorylated state and treated with BeF3- as bait. Results from this imply that interactions between both proteins are transient. We are beginning to probe these transient interactions through 1H-15N HSQC NMR titration experiments. These studies will be the first to establish a direct link between the Pil-Chp pathway through PA0012â€™s interaction with PilH to regulate type IV pili dynamics.

Poster 9*

Characterization of 7-fluorotryptophan as a Protein-Observed Fluorine NMR Amino Acid Probe for Biomolecular Interactions

Ruoqi Jiang (Pomerantz Lab.) University of Minnesota

Protein-observed fluorine (PrOF) NMR is a powerful technique for studying biomolecular interactions, requiring fluorine incorporation into proteins. Fluorine chemical shift perturbations during titration of a binding partner can quantify binding affinity and reveal binding site information. 4, 5, and 6-fluorotryptophan (4, 5 and 6FW) have been incorporated into proteins biosynthetically with 5-fluorotryptophan being routinely used in the field and its NMR properties well-characterized, whereas 7-fluorotryptophan (7FW) has remained greatly understudied. Recent discovery of 7FW crosslinking to a nearby phenylalanine upon UV irradiation also makes it an intriguing candidate for fluorine labeling. Here, we provide an analysis on the utility of 7FW as an amino acid probe for biomolecular interactions. We expressed 7FW labeled BRD4 BD1 and performed PrOF NMR titration experiments with DNA and small molecules. 7FW BRD4 BD1 exhibited distinct resonances and similar chemical shift dispersion to 5FW BRD4 BD1 and gave comparable binding affinities of the interactions studied. Binding affinities of (+)-JQ1, a ligand of BRD4 BD1 with known affinity, to 7FW, 5FW and unlabeled BRD4 BD1 being similar also

indicates that there is minimal functional perturbation of 7FW incorporation to the protein, which was further supported by complementary fluorescence polarization experiments. Additionally, 7FW was found to be more responsive to DNA than 5FW in these experiments. The NMR chemical shift responsiveness of 4, 5, 6 and 7FW to changes in solvent polarity was also examined using their corresponding fluoroindoles as mimics of their sidechains and 7-fluoroindole was found to be the most responsive in polar solvents. We also probed the utility of 7FW for PrOF NMR by measuring its NMR relaxation properties and found that it has comparable T1 and T2* to 5FW. Overall, our work demonstrates that 7FW is a suitable alternative amino acid probe for PrOF NMR to characterize biomolecular interactions.

Poster 10*

Biophysical Characterization of the Nucleic Acid Binding Domains of Z-DNA Binding Protein 1 (ZBP1)

Jeffrey Krall (Vögeli Lab.) University of Colorado

Z-DNA Binding Protein 1 (ZBP1) is a critical pattern recognition receptor within the innate immune response to viral infection. ZBP1 senses foreign nucleic acids in the unusual, left-handed Z-conformation via binding through its N-terminal ZÎ±1 and ZÎ±2 domains and activates downstream pro-necroptotic and -apoptotic pathways to initiate cell death and allow for viral clearance. Both dsDNA and dsRNA can adopt the Z-conformation, however, the conformational change is energetically expensive, especially for dsRNA, and requires chemical modification or protein-induced right-to-left-handed conversion and stabilization. ZBP1 has been previously shown to bind and convert B-DNA to the Z- conformation and was assumed to be able to convert A-RNA as well, despite the lack of experimental validation. Here, we use a variety of NMR and other biophysical experiments to characterize the Z-DNA and Z-RNA binding properties of ZBP1â€™s ZÎ±1 and ZÎ±2 domains. While ZBP1â€™s ZÎ± domains are able to convert and stabilize unmodified dsDNA in the Z-conformation, both are incapable of flipping unmodified A-conformation dsRNA. We show that ZBP1â€™s ZÎ± domains require dsRNAs with Z- promoting chemical modification in order for them to bind and stabilize the Z- conformation. This works highlights the functional variability of ZÎ± domains and narrows down the potential physiological substrates of ZBP1 in infection and disease.

Poster 11*

Investigating the Mechanism and Dynamics of Single-Stranded DNA Binding to the Mre11-Rad50 Complex from Pyrococcus furiosusa

Olivia Krise (Latham Lab.) University of Minnesota

A necessary responder to DNA damage is the complex Mre11-Rad50 (MR) which is highly conserved between all kingdoms of life. MR helps initiate the repair process for both double-stranded and single-stranded DNA breaks. However, most is known for the binding and repair of double-stranded DNA (dsDNA) whereas little known on how MR recognizes single-stranded DNA (ssDNA) as no experimental models exist. Methyl-based heteronuclear multiple quantum coherence (HMQC) data indicate that there are different chemical shift perturbations (CSPs) upon ssDNA binding compared to the CSPs seen for dsDNA binding with large CSPs within the capping domain of Mre11. Paramagnetic relaxation enhancement (PRE) data also shows ssDNA binding in

the nucleotide binding region of Rad50. Thus, our preliminary data suggests that ssDNA binds to MR differently than dsDNA with the capping domain of Mre11 being an important factor for this binding.

Poster 12*

Dissecting the Molecular Determinants of ATF5 Liquid-Liquid Phase Separation

Urval Patel (Roche Lab.) Iowa State University

Activating Transcription Factor 5 (ATF5) is a bZip transcription factor critical for maintaining cellular homeostasis under stress. It plays a pro-survival role in tumorigenesis and cancer progression. The transactivation domain of ATF5 contains two proline-rich motifs (PRMs) and several post-translational modification sites, whose contributions to protein-DNA and protein-protein interactions, as well as gene regulation, remain poorly understood due to purification challenges. A novel protocol developed in Dr. Roche's lab has successfully overexpressed and purified functional ATF5 using an N-terminal GB1 solubility tag. Upon isolation, ATF5 was found to form dynamic droplets, suggesting the occurrence of liquid-liquid phase separation (LLPS). Emerging evidence connects LLPS to oncogenesis and metastasis, underscoring the importance of ATF5 in these processes. Preliminary sequence analysis has identified potential phase-separating regions in the disordered N-terminal segment and in the linker between the disordered and structured domains of ATF5. However, the precise molecular mechanisms governing ATF5 LLPs are still unclear. To address this, we aim to define the conditions that influence ATF5 phase separation by systematically examining the effects of pH, salt concentration, crowding agents, and protein concentration on phase formation. The ultimate goal of this research is to identify the specific regions or motifs within ATF5 responsible for LLPs and to understand how this phenomenon regulates ATF5's function, particularly in relation to cancer and other pathological conditions

Poster 13*

Characterization of an insect Pheromone Binding Protein: Implications for Environmentally Friendly Pest Management

Pratikshya Paudel (Mohanty Lab.) Oklahoma State University

The Asian corn borer (ACB), Ostrinia furnacalis (Lepidoptera: Crambidae) is a polyphagous agricultural pest of many crops, widely distributed across Asia, Australia, and Oceania. The current approach for controlling ACB infestation primarily relies on pesticides. Nevertheless, the adverse outcomes from pesticide use cannot be disregarded, including issues such as environmental pollution, risks to human well-being, the emergence of pest resistance, and the possibility of secondary pest outbreaks. Thus, there is an urgent need for controlling this invasive pest in an environmentally friendly and species-specific manner. The mating process in the moth, Ostrinia furnacalis is facilitated through the sex pheromones emitted by the females and detected by the males. Pheromone Binding Proteins (PBPs), present in male moth antennae bind and transport the hydrophobic pheromones to the olfactory receptors initiating olfaction. Ostrinia furnacalis Pheromone Binding Protein 3 (OfurPBP3) is preferentially expressed in male moth antennae. A detailed structural characterization and mechanistic study of the OfurPBP3 is needed to design the pheromone mimetic for effective pest management. The cloning, expression of the recombinant

OfurPBP3 in E. coli, refolding from the inclusion bodies (IBs), and the characterization of the protein by fluorescence spectroscopy, and high-resolution solution NMR are presented.

Poster 14*

Understanding regulation of ATF4 by multisite phosphorylation

Austin Petfalski (Roche Lab.) Iowa State University

ATF4 is a key regulator of the Integrated Stress Response, coordinating pro-survival and pro-apoptotic signals in response to a variety of cellular stresses. Overexpression of ATF4 has been linked to numerous human cancers, where it contributes to tumor cell proliferation, stress adaptation, and metastasis. Additionally, misregulation of ATF4 is associated with resistance to proteasome inhibitors and chemotherapeutic agents, underscoring its potential as a therapeutic target. ATF4 contains two phosphorylation cassettes: a threonine-rich cassette involved in transcriptional specificity and a serine- rich cassette regulating its cellular degradation. Using in vitro phosphorylation assays combined with intact mass spectrometry, we identified a functional crosstalk between the two cassettes, potentially mediated by long-range intramolecular interactions. Fluorescence polarization experiments further revealed that phosphorylation of the threonine cassette significantly reduces ATF4's affinity for its cognate DNA sequence, while phosphorylation of the serine cassette had no discernible effect on DNA binding. Our findings illuminate a tightly regulated phosphorylation network that fine-tunes the transcriptional activity of ATF4.

Poster 15*

Elucidation of the Mechanisms of Inter-domain Coupling in the ~130 kDa Enzyme I via AlphaFold Modeling, Coarse Grained Simulations, and NMR

Sergey Sedinkin (Venditti Lab.) Iowa State University

Large-scale interdomain rearrangements are critical for the function of proteins like Enzyme I (EI), a ~130 kDa multidomain oligomer involved in converting phosphoenolpyruvate (PEP) into pyruvate during its catalytic cycle. Understanding how external stimuli affect the spatial domain organization and conformational transitions of such enzymes is challenging. Here, we combine AlphaFold2 structural modeling, coarse-grained molecular dynamics simulations, NMR residual dipolar coupling data, and high- pressure solution NMR techniques to explore the thermodynamics and conformational dynamics of EI across different states. We demonstrate that at lower temperatures, EI preferentially samples its catalytically competent closed state, while high-pressure NMR reveals complete dissociation into a stable monomeric form above 1.5 kbar. The conformational ensembles of both monomeric and dimeric states, generated using our advanced simulation protocol, provide detailed insights into the reduction of inter- and intra-domain conformational entropy throughout EI's catalytic cycle. Our study not only uncovers the role of entropy in EI activation but also highlights the utility of our ensemble refinement protocol in investigating the structure and dynamics of other complex multidomain proteins. We have also made our methodology accessible via a Google Colab page (https://potoyangroup.github.io/Seq2Ensemble/) to facilitate its broader application.

Poster 16*

Structure-based discovery of a non-competitive FTO inhibitor bound to a cryptic site at the domain interface

Aayushi Singh (Venditti Lab.) Iowa State University

The fat mass and obesity-associated fatso (FTO) protein is a member of the AlkB family of dioxygenases whose overexpression links to several metabolic diseases, including obesity, type 2 diabetes, Alzheimerâ€™s, and various types of cancer. FTO is an important target for pharmaceutical research, and several selective and non-selective competitive inhibitors have been developed against the enzyme. However, given the competitive nature of the available inhibitors, obtaining complete subfamily selectivity still presents an unresolved challenge. Here, we describe the discovery of C6, a selective inhibitor of FTO resulted from high throughput virtual screening targeted to FTO cryptic pockets. Analysis of the FTO-C6 interaction by solution NMR, molecular dynamics simulations, and enzyme kinetic assays shows that, differently from the FTO inhibitors developed so far, C6 binds to a cryptic pocket between the FTO structural domains, and affects the enzyme function non-competitively by perturbing the off-line/in-line conformational equilibrium of the Î±-ketoglutarate co-substrate. Since FTO is the only member of the of the AlkB family that presents multiple structural domains, we expect further development of C6 to result in a new family of highly selective FTO inhibitors that can be used alone or in combination to pre-existing inhibitors to improve their potency and selectivity

Poster 17*

AI-designed RF pulses for biological NMR applications: from struc-ture determination to metabolomic analysis.

Gianluigi Veglia University of Minnesota

The GENETICS-AI, or Generator of Triply Compensated RF Pulses via Artificial Intelligence, is a software based on AI for designing highly inhomogeneity-compensated RF pulses. With GE-NETICS-AI, we have created new RF shapes for excitation, inversion, and refocusing operations that significantly enhance the sensitivity of widely used NMR experiments in biological applica-tions. We demonstrate that these new RF pulses greatly improve the sensitivity of NOESY ex-periments for protein, RNA, and DNA samples, as well as for metabolomic analysis of biological fluids. Compared to commonly used shaped pulses, AI-generated pulses have a broader band-width and better inhomogeneity compensation, offering the potential to increase the efficiency of NMR experiments across a range of magnetic fields, from low to ultra-high.

Poster 18*

Rapid Pulsing Broadband NMR Experiments for Fast Acquisition of Protein Spectra at High and Ultra-High Magnetic Fields

Manu Veliparambil Subrahmanian (Veglia Lab.) University of Minnesota

Longitudinal Relaxation Enhanced (LRE) experiments, such as SOFAST and BEST sequences, allow for faster data collection without sacrificing sensitivity. However, the narrow irradiation bandwidth and poor homogeneity of their RF pulses limit their application at ultra-high magnetic

fields. To address this, we introduce a new class of experiments known as RAPID (RApid Pulsing broadbanD). These experiments utilize band-selective RF pulses designed using our proprietary GENETICS-AI software, which combines evolutionary algorithms and artificial intelligence to create RF pulses with significantly broader bandwidths and uniform excitation over a wider range of chemical shifts. We have incorporated these RF shapes into various pulse sequences, including 2D 1H, 15N HMQC, and 1H,15N TROSY-HSQC sequences, as well as prototypical 1H,15N, and 13C triple-resonance experiments. Applying the RAPID versions of these pulse sequences to proteins ranging from 22 to 180 kDa has demonstrated a noteworthy increase in sensitivity, particularly for larger proteins. These new experiments have potential applications in high-throughput and real-time NMR studies, such as dynamic process analysis and in vivo studies at ultra-high magnetic fields. Furthermore, the RAPID versions of the TROSY pulse sequences for double and triple-resonance experiments will expedite biomolecular spectroscopy at high- and ultra-high magnetic fields.