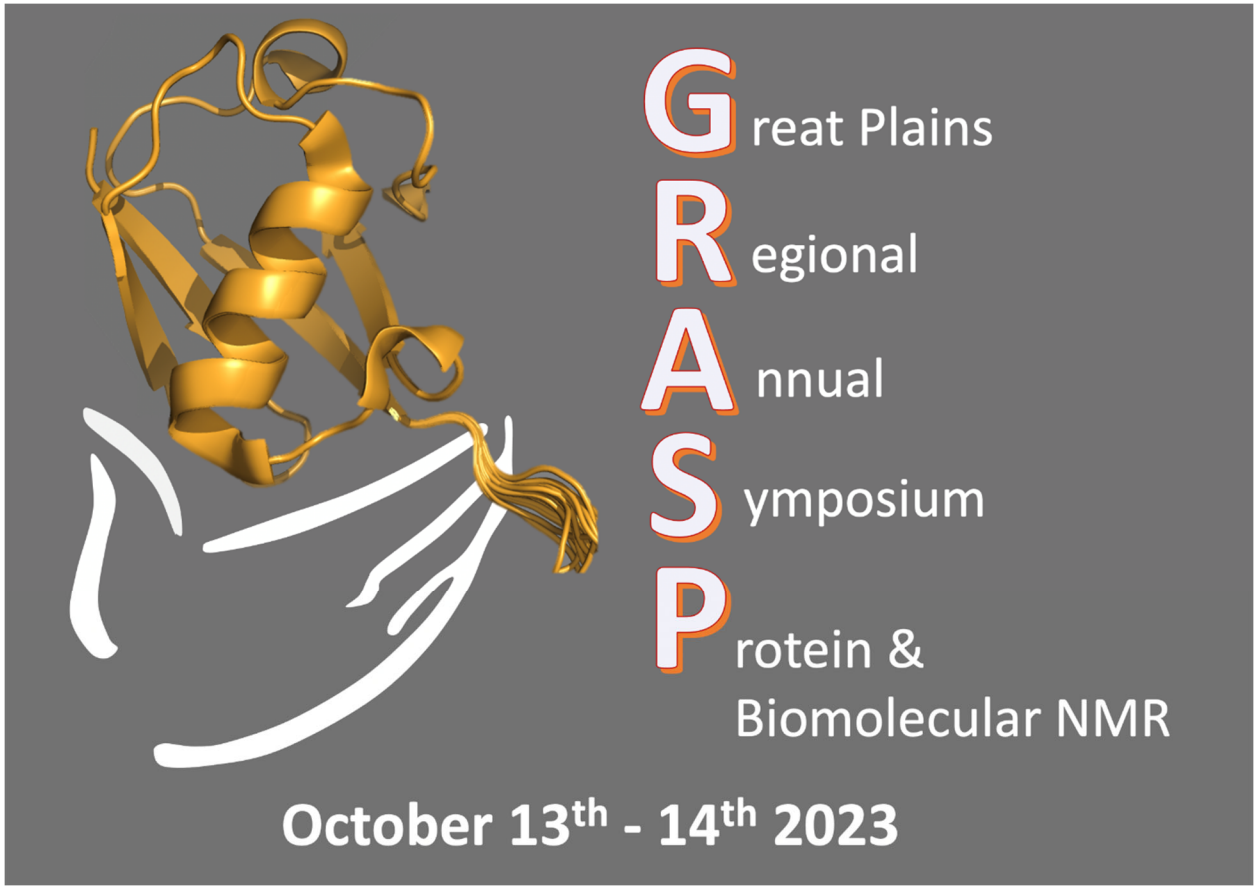
BOOK OF ABSTRACTS

13th Great Plains Regional Annual Symposium on Protein and Biomolecular Nuclear Magnetic Resonance (GRASP NMR)

October 13-14, 2023

Iowa State University



ORAL PRESENTATIONS

Oral Presentation 1

Biochemical and Biophysical Approaches to Characterize the Molecular Basis of Abnormal Cell Signaling Function Involving Ras-Related Proteins

Paul D. Adams, Ph.D

Professor of Chemistry and Biochemistry
Professor of Cellular and Molecular Biology
Distinguished Faculty Member, The Honors College
University of Arkansas, Fayetteville

Ras-related proteins are often mutated in several human cancers, making them excellent protein models to probe structure-function relationships of cell-signaling processes mediating cell transformation. Target-based approaches to avert Ras-stimulated abnormal signal transduction should be enhanced by a better understanding of the structural biology of Ras-related Protein-Protein Interactions (PPIs), as well as protein function. Cell division cycle 42 (Cdc42) is the model Ras protein being studied by this laboratory. Cdc42 is a Rho subfamily member of Ras-related proteins. This subfamily was among the first oncogenic proteins identified with significant roles in a variety of cellular events. Moreover, mutations and PPIs involving Cdc42 have highlighted the involvement of these proteins' roles in Ras-stimulated hyperactivity. As such, our goal is to understand key factors, using biophysical and biochemical techniques and approaches, which underlie the molecular basis of Cdc42 activity. Our central hypothesis is that there are unique structural and dynamic features of Cdc42 that can be exploited to modulate protein interactions and influence abnormal cell signaling activity. We are also attempting to exploit these structural and dynamics features to characterize small molecule targets that may interact directly with Ras-related proteins, such as Cdc42, and potentially disturb PPIs that have roles leading to abnormal cell-signaling.

Dr. Paul D. Adams is from Baton Rouge, LA. He received a B.S. (Biochemistry) from Louisiana State University, a Ph.D. (Biochemistry) from Case Western Reserve University and was a Postdoctoral fellow in Molecular Medicine at Cornell University. At the University of Arkansas-Fayetteville (UAF), Adams's research uses biochemical and biophysical techniques to study proteins with roles in oncogenesis, and his work has been funded by the NIH, NSF and the state of Arkansas. Adams is also the PI of a $1,100,000 NSF S-STEM Grant focused on providing pathways for students from Arkansas rural areas to pursue STEM degrees at UAF.

Oral Presentation 2

Structural heterogeneity in a central domain of the transcription-regulating 7SK RNA

Catherine D. Eichhorn, Ph.D

Assistant Professor of Chemistry
University of Nebraska, Lincoln

RNA is a structurally adaptive biopolymer that partners with proteins to play critical roles in cellular regulation. In metazoa, 7SK ribonucleoprotein (RNP) negatively regulates RNA Polymerase II transcription elongation by sequestering and inactivating the kinase activity of the positive transcription elongation factor b (P-TEFb). Release of P-TEFb from the 7SK RNP requires assembly of additional proteins that bind to the central stem-loop 3 (SL3) of 7SK RNA; however, little high-resolution information is known regarding 7SK SL3 structure or protein recognition. To characterize 7SK SL3 structural features we integrated solution NMR spectroscopy, optical melting, and chemical probing approaches. We found that the distal region of SL3 undergoes exchange between two structurally distinct states. The equilibrium between the two states can be modulated by point substitutions, temperature, and ionic strength. Importantly, residues involved in conformational exchange correlate to sites of protein recognition and post-transcriptional modifications. These results provide new insights into the structural dynamics of a 7SK RNA domain involved in transcription regulation.

Catherine is an Assistant Professor of Chemistry at the University of Nebraska - Lincoln. She received a BA in Chemistry at Bryn Mawr College and PhD in Chemical Biology at the University of Michigan Ann Arbor with Hashim Al-Hashimi using NMR to study RNA dynamics. Her postdoctoral research, with Juli Feigon, focused on structure and assembly of the core 7SK ribonucleoprotein complex. At UNL, her research group is centered around the folding and function of regulatory RNAs and RNPs using integrated structural biology, biophysical, and chemical biology tools.

Oral Presentation 3

Novel Class of specific FTO inhibitors that binds to transient allosteric pockets

Aayushi Singh

Graduate Student – Department of Chemistry
Iowa State University

The fat mass and obesity-associated FTO protein belongs to Fe^{+2} and alpha-ketoglutarate (αKG)-dependent AlkB dioxygenase family and catalyzes the demethylation of the N6-methyladenosine (m6A), an epigenetic marker that controls several metabolic pathways by modulating transcription, translation, and cellular localization of nucleic acids. Since the discovery that its overexpression links to the development of obesity and cancer, FTO was the target of structure-based drug design (SBDD) efforts. Several FTO inhibitors were revealed in the past decade, which either compete with the αKG co-substrate or with the m6A primary substrate to bind to the enzyme. Consequently, most of the inhibitors lack selectivity for FTO over the other members of the Alkb family. Only four selective FTO inhibitors were reported so far. However, the binding modes and mechanisms of action of such molecules are under investigated. Herein, by using in-silico and NMR-based drug screening, we discover two novel FTO inhibitors, namely C4 and C6, which bind in two transient surface pockets located at the domain interface and inhibit FTO activity. Furthermore, we found that C6 selectively inhibits FTO over Alkbh5, the member of the Alkb family that presents the closest similarity to FTO. Kinetic studies show that both inhibitors C4 and C6 affects the enzyme activity competitively but with moderate to weak binding affinity, respectively. Our results indicate that structural ensembles encompassing protein structural flexibility reveals potential druggable sites which may not be captured by the X-ray crystal structure alone. In general, our work demonstrates that protein conformational dynamics can be exploited for the structure-based design of inhibitors with subfamily selectivity.

Aayushi Singh graduated from University of Delhi, India with a Master's of Science in physical chemistry. She joined IIT Delhi in 2016 as a Junior Research Fellow focusing on studying the effects of small natural compounds on the stability and aggregation of human alpha-synuclein. Also, she characterized various transcription regulatory proteins of Mycobacterium tuberculosis. Aayushi joined the Venditti lab at Iowa State University in Fall 2019. She is currently investigating allosteric regulation of bacterial Enzyme I and allosteric drug design for human cancer related protein FTO.

Oral Presentation 4

Solution structure of poly(UG) RNA

Riley Petersen

Graduate Student – Department of Biochemistry
University of Wisconsin, Madison

Poly(UG) or “pUG” RNAs are UG or GU dinucleotide repeat sequences that are highly abundant in eukaryotes. Post-transcriptional addition of pUGs to RNA 3¢ ends marks mRNAs as vectors for gene silencing in C. elegans. We have determined the solution structure of the free pUG RNA (GU)12 by nuclear magnetic resonance spectroscopy and small and wide-angle x-ray scattering (NMR-SAXS-WAXS). The low complexity sequence results in overlapped NMR signals that complicate assignment of chemical shifts. To overcome this problem we utilized single, site-specific deoxyribose modifications which did not perturb the structure and introduced well-resolved methylene signals that could be used to validate chemical shift assignments. Additionally, we developed optimized pulse sequences for RNA to assign all 1H, 13C, 15N, and 31P resonances as well as efficiently measure low j-coupling magnetization transfer across the tetrad hydrogen bonds. In total, 549 NOEs, 21 residual dipolar couplings, 72 hydrogen bonds and 143 dihedral angle restraints as well as the SAX-WAX data were used to refine the structure. The solution structure ensemble has a root mean squared deviation (RMSD) of 0.62 Å and is a compact, left-handed quadruplex with a Z-form backbone, or “pUG fold.” Hydrogen-deuterium exchange experiments indicate the structure is highly kinetically stable with quartet opening rates on the several days timescale. Overall, the structure agrees with our previously determined crystal structure of (GU)12 bound to the ligand N-methyl mesoporphyrin IX (NMM), indicating the pUG fold is unaltered by docking of the NMM ligand. The solution structure reveals conformational details that could not be resolved by the crystal structure and explains how the pUG fold can form within longer RNAs.

Riley Petersen is a senior PhD candidate in Sam Butcher’s laboratory at the University of Wisconsin-Madison and National Magnetic Resonance Facility at Madison (NMRFAM). Riley received her B.A. in Chemistry from Central College (Pella, IA) in 2019 and her M.S. in Physical Chemistry as part of her PhD work in 2021. Her research focuses on using solution NMR to study RNA structure and dynamics. She has taught RNA NMR at the Solution Structural Biology Workshop with NMRFAM and the Network for Advanced NMR (NAN).

Oral Presentation 5

Plenary Lecture

A look at the past and a glimpse at the future of biological NMR

Ad Bax, Ph.D

Distinguished Investigator
Laboratory of Chemical Physics
National Institute of Diabetes and Digestive and Kidney Diseases

In the early 1980s, a magnificent series of developments and half a century of research culminated in the ability to reliably derive three-dimensional, atomic resolution structures of small proteins from the NMR signals of nuclear spins. However, the many hundreds or even thousands of unique ^{1}H signals in any given protein made the analysis of their NOE interaction network a formidable task. The development of molecular cloning and bacterial overexpression came to the rescue by offering a straightforward avenue to enriching the ^{13}C and ^{15}N isotopic composition of proteins, thereby making it possible to access the plethora of heteronuclear NMR techniques originally developed for small organic molecules. By dispersing the interaction between ^{1}H, ^{13}C and ^{15}N spins in three- or even four-dimensional NMR spectra, resonance overlap was greatly diminished, thereby tremendously simplifying spectral analysis and greatly extending the size limit of proteins amenable to NMR in subsequent decades. As an additional benefit, the relaxation properties of individual ^{15}N and ^{13}C spins, which are well-defined reporters for motions within their structural framework, enabled the characterization of internal protein dynamics in exquisite detail.
Currently, much of biological NMR is focused on unraveling the mechanisms behind protein function, revealing the critical importance of protein and nucleic acid dynamics and the details of pivotal transiently populated states. One area that has remained largely intractable to biophysical methods concerns the structure and dynamics of rapidly forming oligomeric aggregates of proteins linked to amyloid disease. By exploiting the long-recognized sensitivity of such systems to hydrostatic pressure, we demonstrate that pressure-jump NMR experiments now start to reveal atomic details of such species.

Ad Bax received his Ph.D. in 1981 from the Delft University of Technology, The Netherlands, for work related to development of two-dimensional nuclear magnetic resonance (NMR) techniques, which he carried out at Delft and Oxford Universities. After post-doctoral work in solid-state NMR with Gary Maciel at Colorado State University, he joined NIH where he has been working on the development and application of a wide variety of advanced multi-dimensional NMR techniques. Dr. Bax has received numerous international awards, and he is a corresponding member of the Dutch Royal Academy of Sciences, a Fellow of the American Academy of Arts and Sciences, and a Member of the National Academy of Sciences.

Oral Presentation 6

Quantitative NMR analysis of the mechanism and kinetics of chaperone Hsp104 action on amyloid-β42 aggregation and fibril formation

Shreya Ghosh, Ph.D.

Post-doctoral Student – Laboratory of Chemical Physics
National Institutes of Health

Hsp104 is a disaggregase chaperone that separates polypeptides from protein aggregates and then refolds them to their native state. Hsp104 is unique because it has been shown to both prevent aggregation as well as disaggregate mature amyloid fibrils. While such distinctive functions of Hsp104 is common knowledge, the pathway, mechanism, and kinetics associated with such function remains unanswered.
To this end, I investigated the mechanism involved in preventing amyloid beta (Aβ)-42 fibril formation by Hsp104 by using a combination of spectroscopic and imaging techniques. We performed a series of SOFAST-HMQC experiments, in presence of varying concentrations of Hsp104 compared to Aβ-42 monomers. The NMR signal comes only from ^{15}N-labeled Aβ-42 while the 600 kDa Hsp104 is NMR invisible. In absence of any chaperone, Aβ-42 completely fibrilizes after ~30 hours as indicated by the complete loss of NMR signal intensity. When Hsp104 is added, fibrilization either gets significantly delayed or completely abrogated, depending on how much Hsp104 is added. Such results provide strong evidence towards Hsp104's power in preventing fibrilization. The absence of fibrils in presence of Hsp104 was further verified by electron microscopy and atomic force microscopy. Finally, using the NMR data, we performed global fitting that sheds light into the kinetics and mechanism of Hsp104's activity. At high Aβ-42 concentrations, aggregation occurs via a branched mechanism: (1) an on-pathway irreversible fibrillization process which leads to mature fibrils, and (2) an off-pathway reversible oligomerization process which leads to non-fibrillar oligomers that are too large to be observed directly by NMR but too small to be imaged. Hsp104 binds reversibly to sparsely populated Aβ-42 nuclei that are present in nanomolar concentrations with very high affinity, thereby completely inhibiting the on-pathway process at substoichiometric ratios of Hsp104 to Aβ-42 monomers.

Shreya Ghosh received her B.Sc and M.Sc degrees in Chemistry at Visva Bharati University in India. She then joined the lab of Dr. Sunil Saxena at University of Pittsburgh for her Ph.D. Her doctoral work involved developing copper-based spin labels for proteins and DNA to be used for electron paramagnetic resonance (EPR) spectroscopy. Using these labels and EPR-based distance measurements, she studied interactions between protein and DNA and associated conformational changes. She is currently pursuing her postdoctoral research in the lab of Dr. G. Marius Clore at the National Institutes of Health in Maryland. Her work there involves probing chaperone mechanisms and kinetics involving aggregating client proteins using NMR spectroscopy as the primary technique

Oral Presentation 7

Conformational Dynamics and the Substrate Selectivity Mechanism of hFTO

Dan Burns

Graduate Student – Department of Biochemistry, Biophysics, and Molecular Biology
Iowa State University

The fat-mass and obesity associated FTO protein is a eukaryotic Fe^{2+} and alpha-ketoglutarate dependent Alkb dioxygenase that catalyzes the demethylation of various N-methylated nucleobases. N6-methyladenosine (m6A) is an important epigenetic marker, regulating various processes such as transcription, translation, and cell differentiation and its mis-regulation is associated with diseases such as obesity and cancer. The molecular mechanism that explains how FTO differentiates between its preferred substrate, m6A, and other methylated bases is a clinically important question particularly for therapeutic development. In this work we investigate the role of FTO's conformational dynamics in substrate selectivity. NMR and molecular dynamics simulations indicate that alternative bases and methylations propagate instability from the binding pocket through a network of residues while m6A promotes a subtle reorientation of the co-substrates to the catalytically competent state.

Daniel Burns received his bachelor's degree in Marine Biology from the University of Hawaii in 2009. The fascinating details of biology at the microscopic level continued to pique his curiosity and ultimately drove him to pursue a PhD in biochemistry at Iowa State University. Today Daniel utilizes computational methods to investigate protein dynamics and their relationship to function.

Oral Presentation 8

Structure Determination of Stannous Fluoride Solutions Used as Components of Commercial Toothpaste Products

Joseph Bequette

Graduate Student – Department of Chemistry
Iowa State University

Stannous fluoride solubilized with tetrasodium pyrophosphate (TSPP) is used as an effective antimicrobial agent in commercial toothpaste products. However, oxidation of Sn^{2+} to Sn^{4+} results in lowered antimicrobial activity. Potassium nitrate prevents the oxditation of stannous ions, although the mechanisms are not understood. We have applied a variety of multinuclear solution and solid-state NMR experiments in order to better understand the structure of SnF_2 based toothpastes. ^{19}F NMR spectra show most stannous fluoride is hydrolyzed in solution. ^{119}Sn and ^{31}P NMR spectra reveal several different Sn-TSPP complexes are present, which appear unaffected by the addition of nitrate. 2D ^{31}P EXSY NMR spectra show uncoordinated TSPP exchanges with two Sn-TSPP complexes when nitrate is present. ^{17}O and ^{15}N NMR spectra suggest most nitrate remains uncoordinated, but careful titration of stannous fluoride into a solution of nitrate shows a slight change in chemical shift. Dynamic Nuclear Polarization enhanced ^{119}Sn and ^{17}O solid-state NMR experiments corroborate SnF_2 hydrolysis and preferential Sn-TSSP complexation when TSPP is present.

Joe Bequette is a second-year graduate student in Professor Aaron Rossini's solid-state NMR group. Joe was a non-commissioned officer in the United States Marine Corps before obtaining his B.S. in chemistry from Minnesota State University, Mankato and his Master's degree in chemistry at the University of North Dakota. Prior to joining the Rossini group, Joe worked as a full-time chemistry lecturer and lab manager at Eastern Kentucky University.

Oral Presentation 9

AI-designed RF Pulses and Pulse Sequences for NMR experiments at High- and Ultra-High Magnetic Fields

Manu Veliparambil Subrahmanian, Ph.D.

Researcher – Department of Biochemistry, Molecular Biology & Biophysics
University of Minnesota, Minneapolis

The advent of the high and ultra-high magnetic fields has propelled NMR spectroscopy of biomacromolecules, increasing sensitivity and resolution. However, the increased Zeeman splitting of the energy levels requires RF pulses with broader bandwidth to excite all nuclear spins uniformly. Current RF pulses are inadequate to cover the bandwidths of ultra-high magnetic fields. To solve this problem, we have developed a new software, GENETICS-AI, which utilizes a combination of an evolutionary algorithm and artificial intelligence to design novel RF pulses with high fidelity and tunable inhomogeneity compensation levels that irradiate significantly larger bandwidths, improving the performance of inversion, refocusing, and excitation operations[1]. Here, we demonstrate the sensitivity improvements with these new RF pulses for homonuclear and heteronuclear experiments at high fields. We will show newly developed Water irradiation devoid (WADE) pulses and their performance for homo and heteronuclear NOESY, HMQC, and HSQC experiments. Additionally, we introduce two new experiments, RAPID-TROSY and RAPID-HMQC, for fast acquisition of NMR spectra[2-4]. These longitudinal ^{1}H relaxation-enhanced experiments promise to speed up the characterization of the structural and dynamic analysis of biomolecules at high- and ultra-high fields.

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Manu Veliparambil Subrahmanian is a Senior Researcher at Prof. Gianluigi Veglia's lab at the University of Minnesota. He earned his Masters in Physics from Cochin University of Science and Technology, India, and later pursued his Ph.D. at the Indian Institute of Science, Bangalore, specializing in Quantum Computing using NMR (2014). During his postdoctoral research with Professor Gianluigi Veglia, he co-authored a patent on Radiofrequency Pulse design in Magnetic Resonance. His research interests include RF pulse design for NMR and MRI, allosteric networks in proteins, NMR data processing, and quantum information processing using NMR.

Oral Presentation 10

Lessons Learned From Metabolomics Studies of Mitochondrial Metabolism and Function

Liping Yu, Ph.D.

Director and Adjunct Professor – Department of Biochemistry and Molecular Biology
Carver College of Medicine – University of Iowa

Interpreting mitochondrial function as affected by comparative physiologic conditions is confounding because individual functional parameters are interdependent. Here, we have studied mitochondrial function of muscle and interscapular brown adipose tissue (IBAT) from wild-type (WT) or diabetic animals by using NMR spectroscopy at the clamped levels of inner mitochondrial membrane potential ($\Delta\Psi m$) to determine the metabolic profiles of the key intermediates in the TCA cycle. We have correlated these metabolic profiles to the measured mitochondrial functional parameters such as $\Delta\Psi m$, O_2 flux, ATP production, reactive oxygen species (ROS) production, etc. Our results show that there is divergent control in skeletal muscle and IBAT for oxaloacetate and mitochondrial complex II respiration. In this presentation, we will discuss some of the lessons learned from these metabolomics studies, particularly, related to the role of oxaloacetate in mitochondrial complex II respiration.

Liping Yu obtained his B.S. in Chemical Engineering in 1982, his M.S. in Food Science in 1985, and his Ph.D. in Chemistry in 1989. After one-year postdoctoral, Liping joined the NMR research group under Steve Fesik at Abbott Laboratories in 1990 where he worked on a variety of drug targets. Then, Liping joined the University of Iowa in 2007 as an NMR facility director of Carver College of Medicine. Liping is interested in molecular structures and functions of glycans, in protein-ligand interactions, and in metabolic profiling of mitochondria, particularly on the role of oxaloacetate in mitochondrial complex II respiration.

Oral Presentation 11

NMR as a tool to decipher the mechanistic links between aging-associated Pigmentation Phenotypes

Morkos Henen, Ph.D.

Assistant research professor – School of Medicine
University of Colorado, Denver

Aging presents intriguing dichotomies in pigmentation: hyperpigmentation (aging spots) and hypopigmentation (hair graying), seemingly opposing yet interconnected. Our study investigates these phenomena via an inherited pigmentation disorder within a family. Employing cellular assays, we've unveiled SASH1's pivotal role in inducing stem-like traits in human melanocytes. Notably, the SASH1 S519N variant exhibits impaired function. Family members carrying this variant display accelerated hair graying and distinct dyspigmentation. Our comprehensive assays reveal the disordered SPIDER domain in SASH1's interaction with TNKS2, crucial for instilling stem-like traits. The SPIDER S519N variant impacts their association kinetics and affinity. NMR analyses shed light on SPIDER domain modifications underlying the disorder. SASH1 emerges as a regulator maintaining melanocyte stem cell (McSC) and mature melanocyte (MC) balance. The SPIDER S519N variant disrupts this equilibrium, suggesting its role in diverse aging-associated pigmentation phenotypes.

Morkos Henen, Ph.D. in molecular biology from Vienna University, Austria (group of Robert Konrat). Following a prestigious Marie Curie Postdoctoral Fellowship at CNRS, Lille, France, he conducted further postdoctoral research at the University of Pittsburgh, USA. Currently an assistant research professor at the University of Colorado, Denver, USA (group of Beat Vögeli). PI on NIH-NIAID grant focusing on small molecules targeting COVID-19. His research interests are NMR and biophysics, focusing on IDPs, drug discovery, and innovative method development.

Oral Presentation 12

Fusion Peptide Sculpting of a Membrane with Insertion of Charged and Polar Groups

Steve Van Doren, Ph.D.

Professor of Chemistry
University of Missouri

The fusion peptide of SARS-CoV-2 Spike is essential for infection. How this charged and hydrophobic domain occupies and affects membranes needs clarification. There has been a need for measurements of the depth of fusion peptide insertion and simulations that can account for the sustained insertion of fusion peptide measured by membrane fusion assays, NMR, and neutron reflectivity. The depth of the fusion peptide in zwitterionic, bilayered micelles at pH 5 (resembling late endosomes) was measured by paramagnetic NMR relaxation enhancements used to bias molecular dynamics simulations. Asp830 inserted deeply, along with Lys825 or Lys835. Protonation of Asp830 appeared to enhance agreement of simulated and NMR-measured depths. While the fusion peptide occupied a leaflet of the DMPC bilayer, the opposite leaflet invaginated with influx of water and choline headgroups in around Asp830 and bilayer-inserted polar sidechains. NMR-detected hydrogen exchange found corroborating hydration of the backbone of Thr827 and Phe833 inserted deeply in bicelles. Pinching of the membrane at the inserted charge and the intramembrane hydration of polar groups agree with theory. Formation of corridors of hydrated, inward-turned headgroups was accompanied by flip-flop of headgroups. Analogous distortions of membranes by influenza hemagglutinin were previously hypothesized to promote viral entry.

Steve Van Doren did his PhD in Biophysics at the University of Illinois under the mentorship of Tony Crofts and Bob Gennis. His PhD research with them concerned membrane-associated enzymes of electron-transport. At Illinois he became interested in magnetic resonance via EPR of metalloenzymes. He pursued postdoctoral training in protein NMR with Erik Zuiderweg at the University of Michigan. He joined the faculty of the University of Missouri in 1995. Mechanisms of molecular recognition a long-term interest of his. He has undertaken protein interactions with membranes by NMR since 2010.

Oral Presentation 13

H3 tail dynamics in the histone language

Emma Morrison, Ph.D.

Assistant Professor of Biochemistry
Medical College of Wisconsin

Chromatin is dynamically reorganized during development and in response to stimuli, and the post-translational modification of histones is a key component of this regulation. The basic subunit of chromatin is the nucleosome core particle, comprising two copies each of the histones H2A, H2B, H3, and H4 wrapped by 147 base pairs of DNA. The intrinsically disordered histone termini, or tails, protrude from the core and are heavily post-translationally modified. Previous studies have shown that the histone tails exist in dynamic ensembles of DNA-bound states within the nucleosome. Histone tail interactions with DNA are involved in nucleosome conformation and chromatin organization and inhibit interactions with a subset of histone-binding proteins. Arginine and lysine side chains form favorable interactions with DNA and are sites of charge-modulating post-translational modifications (PTMs) such as citrullination and acetylation, respectively. Our current focus is on the longest histone tail, the H3 tail. Four arginines and six lysines are relatively evenly spaced along the H3 tail sequence, suggesting multivalent interactions with DNA poised for regulation by PTMs. Here, we use NMR spin relaxation experiments to investigate the contribution of these basic residues to H3 tail dynamics within the nucleosome core particle. By mutating arginine or lysine to glutamine as citrulline or acetylation mimetics, we begin to probe the site-specific effect of these PTMs on H3 tail dynamics. We find that these mimetics result in an increase in the regional mobility of the H3 tails with implications for tail accessibility and histone PTM crosstalk. These studies support a role for dynamics within the histone language and speak to the importance of charge-modulating histone PTMs in regulating chromatin dynamics, starting at the level of the basic subunit of chromatin.

Dr. Morrison received her B.A. in Chemistry from Johns Hopkins University where she trained with Dr. Joel Tolman. She continued on to study mechanisms of broad ligand specificity in a multi-drug resistance transporter with Dr. Katherine Henzler-Wildman, receiving her Ph.D. in Molecular Biophysics from Washington University in St. Louis. Dr. Morrison transitioned into the field of chromatin regulation as a postdoctoral fellow with Dr. Catherine Musselman at the University of Iowa. Dr. Morrison joined the faculty at the Medical College of Wisconsin in 2019 where her lab investigates the role of histone tail conformation and dynamics in chromatin regulation.

Oral Presentation 14

Structure and dynamics of the CSTF2 RNA recognition motif drive mRNA cleavage and polyadenylation

Michael Latham, Ph.D.

Associate Professor – Department of Biochemistry Molecular Biology and Biophysics
University of Minnesota, Minneapolis

Nascent pre-mRNA 3'-end cleavage and polyadenylation (C/P) involves numerous proteins that recognize multiple RNA elements. Human CSTF2 binds to downstream U- or G/U-rich sequences through its RNA recognition motif (RRM) to regulate C/P. Here, I will describe our efforts to characterize the structural landscape and protein dynamics that underlie CSTF2 RRM binding to a naturally occurring U-rich RNA. Using NMR spectroscopy, isothermal titration calorimetry (ITC), and in vivo C/P assays, we have examined the role of electrostatic interactions in the RNA binding reaction and highlight a disease-causing mutant – D50A – which leads to greater affinity for RNA, due to a faster on-rate, resulting in alternative C/P in the brains of mice carrying mutation. These studies also illuminated an enthalpy-entropy compensating binding mechanism that is supported by pico-to-nanosecond timescale dynamics. Subsequently, we have used amide proton temperature coefficients and NMR-observed titration experiments to uncover a multi-step binding mechanism where the RRM likely scans the target RNA looking for the U- or G/U-rich sequence. Our combined biophysical results highlight the importance of CSTF2 RRM structure and dynamics in tuning the balance between nascent mRNA binding and C/P in vivo.

Prof. Latham obtained his B.S. in Chemistry from Hampden-Sydney College in 1999. After a period in industry, he completed his Ph.D. in Biochemistry in 2008 from the University of Colorado, Boulder with Prof. Arthur Pardi. Latham performed post-doctoral research training with Prof. Lewis Kay at the University of Toronto. He started his research group at Texas Tech University in 2015 and moved to the University of Minnesota in 2022. His research program focuses on determining functionally important structures; understanding the roles of protein motion, cooperativity, and allostery; and characterizing cancer-associated mutations within macromolecular assemblies.

Oral Presentation 15

Merging NMR, biochemistry, and cell biology tools to explore the penumbra of dynamic ncRNA-protein interactions

Nicholas Reiter, Ph.D.

Assistant Professor – Department of Chemistry
Marquette University

This talk examines the interdependence of RNAs and proteins during molecular recognition events at the chromatin interface, with a focus on interactions within RNA tertiary structures and intrinsically disordered protein regions. The histone lysine specific demethylase-1 (LSD1) is a multi-domain chromatin remodeling enzyme complex with structured and unstructured regions and catalyzes H3K4me1/2 and H3K9me1/2 demethylation. LSD1 can interact with a myriad of proteins, metabolites, and nucleic acids, including the TERRA 'UUAGGG' repeat RNA. LSD1 is often mis-regulated in many cancers, including glioblastoma, colorectal, acute myeloid leukemia (AML), and Ewing's Sarcoma (EWS). It is critical to better understand LSD1's molecular mechanisms, yet we are only beginning to understand how this enzyme engages with large nucleic acid-protein assemblies and cancer-associated protein IDRs. Here, we present biophysical and cell biology experiments that 1) identify the location of a G-rich RNA-LSD1 interaction, 2) define an auto-regulatory role for LSD1's IDR, and 3) propose a molecular mechanism for how G-rich RNAs influence LSD1's catalyzed demethylation reaction on nucleosomes. NMR studies of TERRA reveal unique hydrogen bonding patterns across the G-quartet tiers as well as a distinct ribose sugar pucker pattern, both of which serve to stabilize a stacked GQ RNA structure that is preferentially recognized by LSD1. We propose that these specific RNA-LSD1 interactions may serve to mask nucleosome binding interactions. In addition, cell biology data shows that mutual enrichment of LSD1 and TERRA at telomeres can trigger condensate clustering and that condensate formation at telomeres may function to regulate the alternative lengthening of telomeres (ALT) mechanism, which is present in 10% of all cancers. Taken together, this work suggests an integrated model for how the biophysical properties of a histone modification enzyme-RNA interaction can impact the ALT molecular mechanism.

Nick received his B.A. in chemistry from Carleton College, and subsequently performed biochemistry research at the Mayo Clinic (MN). He was introduced to the RNA world and applied NMR spectroscopy and biophysical tools in Sam Butcher's lab (UW-Madison) and was an NRSA post-doctoral fellow in Alfonso Mondragon's lab (Northwestern), where he determined the structure of a ribonuclease P holoenzyme-tRNA complex. As a PI, he joined the Biochemistry Department at Vanderbilt where he began his NIH funded program, and subsequently joined Marquette's Chemistry department, where his lab continues to focus on how large RNA structures interact with dynamic protein assemblies.

POSTER PRESENTATIONS (*Best poster competition)

Poster 1*

Quantitative NMR analysis of the mechanism and kinetics of chaperone Hsp104 action on amyloid-β42 aggregation and fibril formation

Shreya Ghosh (Clore Lab.)
National Institutes of Health

Hsp104 is a disaggregase chaperone that separates polypeptides from protein aggregates and then refolds them to their native state. Hsp104 is unique because it has been shown to both prevent aggregation as well as disaggregate mature amyloid fibrils. While such distinctive functions of Hsp104 is common knowledge, the pathway, mechanism, and kinetics associated with such function remains unanswered.
To this end, I investigated the mechanism involved in preventing amyloid beta (Aβ)-42 fibril formation by Hsp104 by using a combination of spectroscopic and imaging techniques. We performed a series of SOFAST-HMQC experiments, in presence of varying concentrations of Hsp104 compared to Aβ-42 monomers. The NMR signal comes only from ^{15}N-labeled Aβ-42 while the 600 kDa Hsp104 is NMR invisible. In absence of any chaperone, Aβ-42 completely fibrilizes after ~30 hours as indicated by the complete loss of NMR signal intensity. When Hsp104 is added, fibrilization either gets significantly delayed or completely abrogated, depending on how much Hsp104 is added. Such results provide strong evidence towards Hsp104's power in preventing fibrilization. The absence of fibrils in presence of Hsp104 was further verified by electron microscopy and atomic force microscopy. Finally, using the NMR data, we performed global fitting that sheds light into the kinetics and mechanism of Hsp104's activity. At high Aβ-42 concentrations, aggregation occurs via a branched mechanism: (1) an on-pathway irreversible fibrillization process which leads to mature fibrils, and (2) an off-pathway reversible oligomerization process which leads to non-fibrillar oligomers that are too large to be observed directly by NMR but too small to be imaged. Hsp104 binds reversibly to sparsely populated Aβ-42 nuclei that are present in nanomolar concentrations with very high affinity, thereby completely inhibiting the on-pathway process at substoichiometric ratios of Hsp104 to Aβ-42 monomers.

Poster 2*

Toward the Solution Structure and Conformational Dynamics of Human Alkbh7

Baboucarr Faal (Venditti Lab.)
Iowa State University

The ALKBH7 protein, a member of the AlkB family of dioxygenases, plays a crucial role in epigenetic regulation through its involvement in demethylation processes. This poster presentation focuses on the nuclear magnetic resonance (NMR) backbone assignment

of ALKBH7, a fundamental step in understanding its three-dimensional structure and dynamic behavior at the atomic level. Herein, we report the backbone 1H, 15N, 13C chemical shift assignment of 22kDa construct of human Alkbh7in the apo and holo forms. Experiments were acquired at 25Â°C by heteronuclear multidimensional NMR spectroscopy. Through chemical shift analysis we derive information about ALKBH7's secondary structure elements, such as alpha helices and beta strands, providing insights into its overall fold. Using, the program TALOS+, a secondary structure prediction was generated from the assigned backbone resonance that is consistent with the previously reported X-ray structure of the enzyme. M1A is one of the substrates active towards Alkbh7, the result of this activity will be discussed. The reported assignment and activity assay will allow investigations of the protein structural dynamics anticipated to provide crucial insight regarding fundamental aspects in the recognition and enzyme regulation processes.

Poster 3*

Determining the molecular mechanisms of behavior of the small multidrug resistance transporter PAsmr

Andrea Wegrzynowicz (Henzler-Wildman Lab.)
University of Wisconsin, Madison

The Small Multidrug Resistance transporters are among the smallest known multidrug efflux pumps and remove toxic compounds, including many antiseptics, from multidrug-resistant pathogens. SMRs were thought to confer only resistance to drugs and antiseptics via tightly-coupled 2H+:1 drug antiport, but recent evidence demonstrates that EmrE, an SMR from E. coli and a model for understanding transport, can perform antiport, symport, and/or uniport based on a â€œfree-exchangeâ€ model. This model suggests that SMRs may induce susceptibility to some compounds rather than resistance, either through direct influx/symport or by rundown of the proton-motive force (PMF) through uncontrolled proton uniport. PAsmr, an EmrE homolog from P. aeruginosa, has now been shown to confer both resistance and susceptibility, depending upon the substrate, but the molecular determinants of these opposing biological outcomes are not fully understood. Here, PAsmr was expressed and purified at the scale required for NMR experiments. Solution NMR 2D and 3D experiments were performed to gain initial understanding of the dynamics and characteristic amide chemical shifts of PAsmr, and chemical shift perturbations from different substrates will help identify binding interactions responsible for opposing biological outcomes.

Poster 4

Structural analysis of a Pseudomonas aeruginosa second messenger binding protein

Christopher Ptak
University of Iowa

In Pseudomonas aeruginosa, exploratory motility driven by interspecies interactions is regulated by a chemosensory pathway involving the intracellular second messenger, cyclic di-GMP (cdG). Within this signaling pathway, the PilZ domain protein, PA0012, is a cytoplasmic cdG receptor that interacts with other proteins in a cdG-dependent manner. Here, we assign backbone chemical shifts for both the apo and cdG-bound states of PA0012. Insights derived from NMR experiments are further examined in the context of the cdG-bound PA0012 crystal structure.

Poster 5

Targeting the KIF15-TPX2 PPI to Overcome KIF11 Inhibitor Resistance in Epithelial Ovarian Cancer

Justin Douglas
University of Kansas

Despite extensive efforts and successes in developing targeted drugs and immunotherapies for other cancers, cytotoxic chemotherapies continue to be the most used treatment of epithelial ovarian cancer (EOC). Identification of drugs targeting cellular machinery independent of genomic and genetic status continues to be a strong clinical need. An RNAi-based screen of the druggable genome across a diverse histological panel of EOC cell lines elucidated KIF11 as essential in maintaining EOC cell viability. KIF11, a mitotic spindle assembly motor protein, has been targeted clinically. Although pharmaceuticals are well tolerated, potent, and specific, the objective response rates to KIF11 inhibitors in clinical trials were commonly less than 10%. The efficacy of KIF11 inhibitors is blunted via a compensatory motor kinesin, KIF15. The overexpression of KIF15 has been shown to fully compensate for KIF11 in the formation of the bipolar spindle apparatus. Silencing KIF15 significantly sensitizes cells to KIF11 inhibitors and resensitizes resistant cells to KIF11 inhibitors. A screening approach using Alpha technology was developed to identify compounds that inhibit the protein-protein interaction (PPI) between KIF15 and TPX2, a unique approach to inhibiting KIF15 from previous efforts. Of the nearly 200,000 compounds screened, 177 compounds were selected to be further characterized based on assay performance and chemical properties. These compounds were screened for TPX2 or KIF15 binding by STD-NMR and waterLOGSY. Three compounds with two chemotypes were confirmed to bind KIF15. No compounds were found to bind TPX2. The three confirmed KIF15 binders by STD-NMR and waterLOGSY were all classified as strongly synergistic in vitro using viability

assays in EOC cell lines. To date, two chemotypes have been identified as putative KIF15 inhibitors uniquely targeting the KIF15-TPX2 PPI. The data indicating KIF15 inhibition in combination with KIF11 inhibition is synergistic, thus demonstrate a potential novel treatment approach for women with EOCs.

Poster 6*

Stability and Solubility of RBM7 RRM protein in vitro

Jennie Nguyen (Eichhorn Lab.)
University of Nebraska, Lincoln

P-TEFb (positive transcription elongation factor P-TEFb) plays an essential regulatory role in RNA Polymerase II transcriptional elongation. P-TEFb is sequestered in an inactive state by the 7SK ribonucleoprotein (RNP) and must be released to have catalytic activity. The RNA-binding motif 7 (RBM7) protein was recently found to promote P-TEFb release from the 7SK snRNP complex in response to DNA damage. The release of P-TEFb activates RNA polymerase II, enabling effective transcription elongation to repair DNA damage. However, detailed research on the impact of RBM7 on P-TEFb regulation is still restricted because recombinant RBM7 constructs have poor solubility in vitro. Here we show our two-fold approach to improve the solubility of the RBM7 RNA recognition motif (RRM) domain for in vitro characterization. First, we take advantage of existing interactions between the RBM7 RRM and the ZCCHC8 zinc knuckle domain. ZCCHC8 constructs were cloned, recombinantly expressed, and purified using column affinity chromatography. Proteins were either co-purified or purified separately and combined to evaluate binding. Second, to directly improve RBM7 RRM stability we used the Rosetta â€œStabilize Protein: PMâ€ program to predict the stability of point mutations of the RBM7 RRM amino acid sequence. After in silico screening, constructs were selected for recombinant expression and purification. Construct folding and stability were evaluated using solution-state NMR spectroscopy and solubility assays. These results provide insights into RBM7 RRM folding and interactions with ZCCHC8 protein. Improving the stability of recombinantly expressed RBM7 RRM will enhance our ability to conduct in-depth investigations into the role of RBM7 on the release of P-TEFb from the 7SK snRNP.

Poster 7*

Solution structure of poly(UG) RNA

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University of Wisconsin, Madison

Poly(UG) or “pUG” RNAs are UG or GU dinucleotide repeat sequences that are highly abundant in eukaryotes. Post-transcriptional addition of pUGs to RNA 3¢ ends marks mRNAs as vectors for gene silencing in C. elegans. We have determined the solution

structure of the free pUG RNA (GU)12 by nuclear magnetic resonance spectroscopy and small and wide-angle x-ray scattering (NMR-SAXS-WAXS). The low complexity sequence results in overlapped NMR signals that complicate assignment of chemical shifts. To overcome this problem we utilized single, site-specific deoxyribose modifications which did not perturb the structure and introduced well-resolved methylene signals that could be used to validate chemical shift assignments. Additionally, we developed optimized pulse sequences for RNA to assign all 1H, 13C, 15N, and 31P resonances as well as efficiently measure low j-coupling magnetization transfer across the tetrad hydrogen bonds. In total, 549 NOEs, 21 residual dipolar couplings, 72 hydrogen bonds and 143 dihedral angle restraints as well as the SAX-WAX data were used to refine the structure. The solution structure ensemble has a root mean squared deviation (RMSD) of 0.62 Å and is a compact, left-handed quadruplex with a Z-form backbone, or “pUG fold.” Hydrogen-deuterium exchange experiments indicate the structure is highly kinetically stable with quartet opening rates on the several days timescale. Overall, the structure agrees with our previously determined crystal structure of (GU)12 bound to the ligand N-methyl mesoporphyrin IX (NMM), indicating the pUG fold is unaltered by docking of the NMM ligand. The solution structure reveals conformational details that could not be resolved by the crystal structure and explains how the pUG fold can form within longer RNAs.

Poster 8*

Novel Class of specific FTO inhibitors that binds to transient allosteric pockets

Aayushi Singh (Venditti Lab.)
Iowa State University

The fat mass and obesity-associated FTO protein belongs to Fe^{+2} and alpha-ketoglutarate (αKG)-dependent AlkB dioxygenase family and catalyzes the demethylation of the N6-methyladenosine (m6A), an epigenetic marker that controls several metabolic pathways by modulating transcription, translation, and cellular localization of nucleic acids. Since the discovery that its overexpression links to the development of obesity and cancer, FTO was the target of structure-based drug design (SBDD) efforts. Several FTO inhibitors were revealed in the past decade, which either compete with the αKG co-substrate or with the m6A primary substrate to bind to the enzyme. Consequently, most of the inhibitors lack selectivity for FTO over the other members of the Alkb family. Only four selective FTO inhibitors were reported so far. However, the binding modes and mechanisms of action of such molecules are under investigated. Herein, by using in-silico and NMR-based drug screening, we discover two novel FTO inhibitors, namely C4 and C6, which bind in two transient surface pockets located at the domain interface and inhibit FTO activity. Furthermore, we found that C6 selectively inhibits FTO over Alkbh5, the member of the Alkb family that presents the closest similarity to FTO. Kinetic studies show that both inhibitors C4 and C6 affects the enzyme activity competitively but with moderate to weak binding affinity, respectively. Our results indicate that structural ensembles encompassing

protein structural flexibility reveals potential druggable sites which may not be captured by the X-ray crystal structure alone. In general, our work demonstrates that protein conformational dynamics can be exploited for the structure-based design of inhibitors with subfamily selectivity.

Poster 9*

Structural and dynamic studies of the role of dsDNA binding to the functional mouse CRES amyloid

Ritika Kukreja (Latham Lab.)
University of Minnesota, Minneapolis

The epididymal lumen contains a complex cystatin-rich nonpathological amyloid matrix with putative roles in sperm maturation, sperm protection and host-defense. One of the components of this amyloid matrix is the CRES (cystatin-related epididymal spermatogenic) sub-group of proteins. A typical aggregation pathway involves assembly from monomer to intermediate oligomers to advanced amyloids. With the use of NMR and X-ray crystallography, we discovered that CRES amyloid formation is complex and likely utilizes several mechanisms, including a unique interaction driven by structural changes in the CRES loop from a flexible linker in the monomer to a Î²-strand conformation in the advanced amyloid. This is in addition to traditional domain swapping typical of other cystatins. Additionally, the epididymal lumen contains extracellular DNA (eDNA) which can be released by apoptotic or necrotic cells and is found to be derived from somatic cells in the epididymis. Web-like DNA structures have been observed contaminating and often ensnaring multiple sperm cells, but the role of this eDNA in the epididymis is still not known. Several other host defense amyloids, such as those found in bacterial biofilms, contain eDNA which can act as a template to initiate amyloid assembly. Thus, there arises a possibility of CRES monomers templating off DNA to form amyloid, which might lead to a different assembly mechanism. Early binding studies revealed that CRES exhibits moderate dsDNA binding affinity. With the contributions of solution-state NMR and other biophysical techniques we further studied the structures and dynamics associated with DNA-bound CRES. We also studied the effect of DNA binding on CRES N-terminal processing, typical of other cystatins, and our studies reveal that it is a crucial initiation step towards amyloid assembly. These results help to better understand how this functional amyloid assembles and how dsDNA binding alters the formation of these amyloid assemblies.

Poster 10*

Role of SRSF2 RRM C-terminal residues in 7SK snRNA recognition

Linh Hua (Eichhorn Lab.)
University of Nebraska, Lincoln

Transcription is a tightly regulated process that occurs in the nucleus of the cell and involves the production of RNA from DNA using RNA Polymerases. The 7SK small nuclear ribonucleoprotein (snRNP) regulates transcription elongation by controlling the activity of positive transcription elongation factor b (P-TEFb). Studies have shown that the Serine-Arginine Splicing Factor 2 (SRSF2) protein interacts with 7SK snRNP to promote P-TEFb release and upregulation of RNA Polymerase II during transcription. SRSF2 has an RNA recognition motif (RRM) at the N-terminus and a Serine-Arginine (SR) rich domain at the C-terminus. The goal of my research is to identify key amino acids in the SRSF2 RRM in order to understand how SRSF2 interacts with 7SK snRNA. In this project, we focus on C-terminal boundaries of SRSF2 to evaluate the impact on construct stability, expression efficiency, and RNA recognition. We cloned, recombinantly expressed, and purified SRSF2 RRM constructs with various C-terminal boundaries. Next, we performed NMR 1H-15N HSQC experiments to evaluate protein folding and identify chemical shift changes. Finally, Electrophoretic Mobility Shift Assay (EMSA) experiments were performed to preliminary assess if SRSF2 RRM C-terminal constructs have differences in binding to 7SK snRNA constructs. These data provide insight into SRSF2 residues important for 7SK snRNA binding, setting a foundation for understanding the role of SRSF2-mediated 7SK snRNP function.

Poster 11*

Structural Studies of Stem Loop 3 of 7SK RNA

Shilla Owusu Ansah (Eichhorn Lab.)
University of Nebraska, Lincoln

Internal loops significantly impact the flexibility, folding, and overall 3D structure of RNA. Recently, the Eichhorn lab has discovered that the distal region of stem loop 3 (SL3) of 7SK RNA, a regulator of RNA polymerase II transcription, undergoes exchange between two conformational states. While one state has two symmetric internal loops, the second state has three asymmetric internal loops. These differences are due to significant base-pair rearrangements in which several residues are unpaired in one state and base-paired in the other state. We hypothesize that these differences may result in structural differences between the two states. Here, we engineered SL3 RNA constructs that stabilize the secondary structures of these two states and investigated the structural dynamics of these constructs using solution state NMR spectroscopy. We performed line shape analysis and analyzed chemical shift perturbations as a preliminary comparison of the two states. We measured residual dipolar couplings to determine interhelical bending

about the internal loops. We find that there are significant differences between the two states, particularly for internal loop residues. These findings provide initial insights into the impact of internal loops on the structural characteristics of RNA, laying the foundation for further exploration of SL3 3D structure and dynamics.

Poster 12*

Understanding Conformational Differences between UP1-WT and its phosphomimetic double mutant S4D/S6D

Sayan Das (Venditti Lab.)
Iowa State University

Human hnRNP A1 is a multi-functional protein involved in many aspects of nucleic-acid processing such as alternative splicing, micro-RNA biogenesis, nucleocytoplasmic mRNA transport and telomere biogenesis and maintenance. The N-terminal region of hnRNP A1, also named unwinding protein 1 (UP1), is composed of two closely related RNA recognition motifs (RRM), and is followed by a C-terminal glycine rich region. Fibroblast growth factor 2 (FGF-2) induced S6 kinase 2 (S6K2) binds and phosphorylates hnRNPA1 on the Ser4/6 sites, increasing its association with BCL-XL and XIAP mRNAs to promote their nuclear export. In the cytoplasm, phosphorylated S4/6-hnRNPA1 dissociates from these mRNAs de-repressing their Internal Ribosome Entry Site (IRES) -mediated translation. The overexpression WT-hnRNPA1 reduced basal levels of BCL-XL and XIAP and prevents further induction by FGF-2. However, the S4D-S6D phosphomimetic hnRNPA1 mutant increased the basal BCL-XL and XIAP levels by over 2-fold and did not prevent their further induction by FGF-2. Previous in-vivo studies could not explain the conformational changes that could occur in the S4D/S6D double-mutant that led to such a difference from the wild type. Here, by using solution NMR, we highlight the phosphorylation-induced changes in UP1 conformational dynamics that that could explain the dramatic differences reported in protein function.

Poster 13*

Short Linear Motifs within the ZαZβ unit modulate domain interactions and substrate specificity

Jeffrey Krall (Vogeli Lab.)
University of Colorado

All isoforms of Adenosine Deaminase Acting on RNA 1 (ADAR1) contain a Zβ domain, while the interferon inducible ADAR1p150 isoform also contains an N-terminal Zα domain connected to Zβ via a ~100 residue intrinsically disordered region (IDR). Both domains have been characterized through biophysical and structural techniques individually, however, few studies have investigated the domains in the context of a larger protein

construct. Schwartz et. al (1999) proposed that ZαZβ could act as a single bipartite domain, however, further characterization of the two domains in tandem have not been undertaken. Here, we use a variety of NMR experiments to investigate the structure, dynamics, and potential roles of the domains as it is found within full length ADAR1. Our results reveal two previously unreported Short Linear Motifs (SLiMs) of ~9 residues in the IDR, which exhibit higher-than-average tumbling times. Both SLiMs bind to the Z-RNA binding interface on Zα and the structurally homologous region on Zβ and mutation of the SLiMs lead to drastically reduced tumbling times of the entire construct. The SLiMs compete with Z-RNA for binding to the Zα domain, presenting a potential role for the autoregulation of Zα by increasing RNA-binding specificity.

Poster 14*

Z-form Adoption of Nucleic Acid is a Multi-Step Process Which Proceeds Through a Melted Intermediate

Parker Nichols (Vogeli Lab.)
University of Colorado

The left-handed Z-conformation of nucleic acids can be adopted by both DNA and RNA when bound by Zα domains found within a variety of innate immune response proteins. Zα domains stabilize this higher-energy conformation by making spe-cific interactions with the unique geometry of Z-DNA/Z-RNA. However, the mechanism by which a right-handed helix con-torts to become left-handed in the presence of proteins, including the intermediate steps involved, is poorly understood. Through a combination of Nuclear Magnetic Resonance and other biophysical measurements, we have determined that in the absence of Zα, under low salt conditions, d(CpG) and r(CpG) constructs show no observable evidence of transient Z-conformations greater than 0.5% on either the intermediate or slow NMR timescales. At higher temperatures, we observe a transient unfolded intermediate. The ease of melting a nucleic acid duplex correlates to Z-form adoption rates in the presence of Zα. The largest contributing factor to the activation energies of Z-form adoption as measured by Arrhenius plots is the ease of flipping the sugar pucker, as required for Z-DNA and Z-RNA. Together, these data validate the previously proposed zipper model for Z-form adoption in the presence of Zα. Overall, Z-conformations are more likely to be adopted by double-stranded DNA and RNA regions flanked by less stable regions, and by RNAs experiencing torsional/mechanical stress.

Poster 15*

^{19}F-NMR studies to understand pH induced structural changes in protective antigen anthrax toxin and in comparison, with a dominant-negative mutant

Srinivas Gonti (Bann Lab.)
Wichita State University

T he anthrax toxin protective antigen (PA) forms a membrane spanning pore at acidic pH that is required for translocation of the two enzymatic components of the anthrax toxin, edema factor (EF) and lethal factor (LF). The anthrax toxin protective antigen (PA) has been labelled site specifically with p-fluoro-phenylalanine at Phe427, a residue known to form the phi-clamp in the pore structure. The phi-clamp forms a narrow 6Å iris that is required for translocation of edema factor (EF) and lethal factor (LF) into cells. We have shown recently that in the prepore state the ^{19}F-F427 resonances are dynamic and exhibit two distinct resolvable resonances despite the heptameric 444 kDa structure. The resonances occur at~-37 and -37.2 ppm, with the latter exhibiting a much lower intensity than the former in the prepore state. However, upon transition to the pore, the -37.2 resonance predominates and becomes sharp, suggesting that the transition to the pore state becomes more dynamic. We hypothesize that the -37.2 resonance in the prepore state represents the pore local structure, while the -37 ppm resonance is reflective of the prepore state. The two resonances may explain the dominant-negative mutations at D425, which, when mutated to Asn, Glu, Ala or Gln block the ability to form the pore state. Mutation at D425 to any of these amino acids would stabilize the prepore state, and as such little to no pore conformation would be accessible to these proteins. To verify this, we have investigated the ^{19}F-NMR of the prepore in the context of a dominant-negative mutantD425A. We show that labeling a D425Amutantsite-specifically at F427 PA with p-fluorophenylalanine exhibits a single broad resonance, with little evidence of the minor pore resonance, suggesting that dominant negative mutants act by changing the local structure around F427, versus disruption of hydrogen bonding

Poster 16*

Solution NMR Study of E. Coli Alkb Substrate Specificity

Kyle Malcolm (Venditti Lab.)
Iowa State University

Solution NMR experiments including chemical shift perturbation, relaxation dispersion, and various types of exchange spectroscopy report on protein interactions and conformational dynamics and tell a detailed story about the solution behavior of a protein. Our ongoing research involves characterization of the conformational dynamics of the E. Coli Alkb, a 25kDa Fe^{2+} and αKG-dependent dioxygenase. AlkB is particularly interesting due to its promiscuity. It catalyzes nucleotide demethylation of several different methylated substrates with a range of activity, while also having appreciable binding to methylated nucleotides for which it is not catalytically active. This property provides a spectrum of complexes that can be investigated in their varied degrees of conformational change and solution dynamics. We have developed a method for interrogating the different complexes by replacing the catalytically active Fe^{2+} with Zn^{2+} which renders the complex catalytically inactive while retaining the geometry of the holoenzyme with nucleotide bound. Current work includes backbone assignment of the enzyme bound to

Zn^{2+} and αKG; relaxation dispersion measurements, and determination of enzyme kinetics.
This project is part of a larger project of the Venditti Lab which is studying the mammalian AlkB homolog family (AlkBH1-7 and FTO) in addition to E. Coli Alkb. Some members of the AlkB homologs retain similar functions to E. Coli Alkb, while others have developed novel functions. The goal of the project is to characterize the structure and conformational dynamics of each protein of interest to make hypotheses about the differences that are responsible for the divergence of functions in the Alkb homologs. This includes a general aim of describing how changes in structure and conformational dynamics of similar proteins can modify substrate specificity.

Poster 17*

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

Stephan Azatian (Latham Lab.)
University of Minnesota, Minneapolis

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. Crystal structures 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 ^{1}H R_2 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. Relaxation rates derived from CPMG and Forbidden experiments help uncover the dynamics of the coiled-coils and ZHk during MR-substrate interactions. In addition, mutation of residues involved in stabilizing the hydrophobic cleft and hydrogen bond interactions between the two strands in the “open” model showed significant effects on MR catalytic activity. Methyl group RDCs for substrate-bound ZHk-labeled MR may help validate models of conformational populations. In the future, heterogenous populations of structures will be generated and confirmed with RDC data of ZHk-labeled MR.

Poster 18*

Conformational Dynamics and the Substrate Selectivity Mechanism of hFTO

Dan Burns (Venditti Lab.)
Iowa State University

The fat-mass and obesity associated FTO protein is a eukaryotic Fe^{2+} and alpha-ketoglutarate dependent Alkb dioxygenase that catalyzes the demethylation of various N-methylated nucleobases. N6-methyladenosine (m6A) is an important epigenetic marker, regulating various processes such as transcription, translation, and cell differentiation and its mis-regulation is associated with diseases such as obesity and cancer. The molecular mechanism that explains how FTO differentiates between its preferred substrate, m6A, and other methylated bases is a clinically important question particularly for therapeutic development. In this work we investigate the role of FTO's conformational dynamics in substrate selectivity. NMR and molecular dynamics simulations indicate that alternative bases and methylations propagate instability from the binding pocket through a network of residues while m6A promotes a subtle reorientation of the co-substrates to the catalytically competent state.

Poster 19

Tailoring RF pulses for NMR experiments using an evolutionary algorithm and artificial intelligence

Manu Veliparambil Subrahmanian (Veglia Lab.)
University of Minnesota, Minneapolis

We present our new software for designing RF pulses tailored to specific NMR problems. GENETICS-AI, or Generator of Triply Compensated RF Pulses via Artificial Intelligence, is comprised of an evolutionary algorithm that creates a library of ~200,000 RF shapes and an artificial intelligence module from Python that learns from the RF library and generates optimal solutions to a given NMR problem [1]. GENETICS-AI can be used for excitation, inversion, refocusing, and selective excitation operations for several pulse schemes, spanning from high-resolution NMR to imaging [2]. Here, we show several applications for custom-design new water suppression schemes and accelerated acquisition of heteronuclear correlated spectra (HMQC and TROSY) for biopolymers [3,4].

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Poster 20*

Toward the understanding of the role of conformational dynamics in the regulation of the human Alkbh1 protein

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 also a Fe^{2+} and alpha-ketoglutarate-dependent dioxygenase that catalyze oxidative demethylation of modified nucleotides and amino acids. In particular, hAlkbh1 protein shows activity against six different substrates such as 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 been connected to many cancers, metastasis, developmental defects, hypothermia, etc. Since there is lack of information about how conformational dynamics mediate the function and substrate specificity of hAlkbh1, we have developed protocols to obtain mg amount of enzymatically active hAlkbh1 protein from recombinant expression in minimal media. Optimization of these protocols will enable solution NMR studies aimed at understanding the role of protein conformational dynamics in the regulation of the hAlkbh1.

Poster 21*

Investigating the structural features of tandem pseudouridine residues in an RNA duplex

Sebastian Arteaga (Znosko Lab.)
Saint Luis University

Pseudouridine is the most frequently occurring modified ribonucleotide in nature, found in many organisms. These organisms have numerous enzymes that catalyze the conversion of uridine into pseudouridine. The pseudouridine modification occurs in many types of RNA, including tRNA, rRNA, and small nuclear RNAs (snRNAs) that form parts of the spliceosome involved in pre-mRNA processing. A conserved region of the Homo sapiens U1 snRNA that interacts with pre-mRNA contains tandem pseudouridine residues near the 5'-splice site. When these residues are not converted to pseudouridine, there is

decreased splicing activity. Two mimics of this splice site, one containing tandem pseudouridine residues and one containing tandem uridine residues, were studied by optical melting experiments and NMR to determine stability and structural differences between the two mimics. The NOESY walk for each oligonucleotide mimic has been completed, along with optical melting experiments. The cross-peaks from the 2D NOESY experiments will be used to generate distance restraints and run MD simulations to derive 3D structures of the two mimics. The work here should result in a better understanding of pseudouridine stability and structure and provide potential explanations for the reduced splicing activity in the absence of pseudouridine in U1 snRNA.

Poster 22*

An Innovative Approach for Assigning Side-Chain Methyl Group 2D Correlation Spectra and Screening for Best Fitting AlphaFold2 Structure

Ajeak Vigneswaran (Latham Lab.)
University of Minnesota, Minneapolis

The first step toward NMR structural and dynamics studies of biomolecules is to assign the resonances in 2D correlation spectra to specific atoms in the molecular structure. In the case of large protein complexes (>100 kDa), side-chain methyl group labeling is used to overcome the NMR size limit. The assignment of the 2D methyl correlation spectra often relies on comparing NOESY data and a high-resolution 3D structure. Here, we show a way to assign a 2D spectra when a high-resolution crystal structure is not available by generating many structures from AlphaFold 2 and filtering them using NOEs and RDCs to find the most accurate structure which satisfies the side-chain methyl group assignments. The protein Xrs2 holds a crucial role in DNA double-strand repair, but many of the structural and dynamic features that contribute to that role remain unresolved. There are no crystal structures for Xrs2, so we used AlphaFold2 and Rosetta structure prediction tools to computationally derive models for Xrs2. Various strategies such as selective point mutations, labeled growth using various precursors, 3D-$^{13}C,^{13}C,^{1}H$ NOESY, and RDCs were utilized for the assignment of the Xrs2 2D methyl correlation spectra. We show that for the proteins that do not have an experimentally determined structure, AlphaFold2 can be used to generate a multitude of potential structures. Methyl group NOESY and RDCs can be used to filter for the best structure from among them while resulting in the assignment of the spectra.

Poster 23

Elucidation of the mechanisms of inter-domain coupling in the monomeric state of Enzyme I by high-pressure NMR

Sergey L. Sedinkin (Venditti Lab.)
Iowa State University

Enzyme I (EI) initiates the carbohydrate phosphotransferase system (PTS), a signal transduction pathway that controls the central carbon metabolism in bacteria. The transfer cascade is initiated by binding of phosphoenol pyruvate (PEP), which induces a series of intra- and interdomain conformational rearrangements and results in phosphorylation of EI. Prior to the binding, EI undergoes another key large-scale conformational rearrangement in the form of a monomer–to-dimer transition. The monomeric state of EI is inactive and unable to bind PEP. Since both oligomeric states are present at physiological concentrations of EI (1 - 10 μM), the monomer–dimer equilibrium of the enzyme has been often suggested to be a major regulatory element for PTS.
Expanding on the recently published structural characterization of the isolated EIC monomeric state using a combination of high-pressure NMR and molecular simulations here we report a structural analysis of full-length EI monomeric state which was conducted by collecting backbone residual dipolar couplings (RDC) at 2 kbar. A conformational ensemble was generated using a recently described refinement protocol that takes advantage of the high computational efficiency of coarse-grained molecular dynamics (cgMD) to produce an ensemble of structures that is consistent with experimental RDC. The results of the structural analysis revealed that the dimer-to-monomer transition does not result in any significant local unfolding of the EIN domain but is associated with a significant increase in interdomain flexibility. Altogether, this study highlights the critical role played by conformational fluctuations in EI enzymatic process and suggests that the catalytical cycle of EI is driven by a general decrease in conformational entropy, at both an intra- and interdomain level.

Poster 24*

Dissecting the Interplay between Transactivation and DNA Binding Domains of the ISR effector ATF4

Steven Siang (Roche Lab.)
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Transcription factors (TFs) play the first and fundamental role in gene expressions. Most transcription factors possess two main functional domains: transactivation domain (TAD) and DNA binding domain (DBD). Most TADs are largely disordered and play a key role in modulating the transcriptional activity by binding to various coactivators and possessing

multiple post-translational modification sites. Despite their crucial importance, the structural and functional basis of TADs remains poorly understood, which is one of the main reasons TFs are considered “undruggable.” Here, we focus on activating transcription factor 4 (ATF4), an essential Integrated Stress Response (ISR) effector with multiple binding partners that regulate the expression of more than 200 genes in mammalian cells. Using solution nuclear magnetic resonance spectroscopy complemented by a range of biophysical methods, we first characterize the structural features of the ATF4-TAD domain. We found that the isolated ATF4-TAD is predominantly yet not fully disordered in solution. Importantly, we found that the N-terminal region of TAD is involved in transient long-range interactions with its DBD, also known as the basic-leucine zipper (bZip) domain. Finally, as well documented in many other transcription factors, our biochemical assays show that ATF4-TAD modulates the DNA-binding specificity of the ATF4-bZip domain. However, our results also show this type of fine regulation is reciprocal. In vitro phosphorylation assays with the CKII kinase show that the presence of bZip domain is required for phosphorylation of the TAD domain. This study uncovers the structural features of the TAD domain of ATF4 and its coupling with the bZip domain. These findings provide a basis for further research into exploiting ATF4 as a potential therapeutic target in cancer biology and ISR-implicated diseases.

Poster 25*

Towards solving conformational dynamics of ALKBH2 for understanding regulation and substrate preference

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ALKBH2 is a member of the ALKB family of proteins, which are Fe^{2+} and alpha-ketoglutarate-dependent dioxygenase which catalyze oxidative demethylation of modified nucleotides and amino acids. ALKBH2 shows specific preference for demethylating base positions m1A and m3C, and is especially active on double stranded DNA. Overexpression of ALKBH2 is associated with higher incidence of glioblastoma, bladder, lung, and other cancers, and may be particularly implicated in cancer treatment resistance. NMR spectroscopy can be used to study its conformational dynamics to discover more information about substrate preference and specificity, which could be used to find selective inhibitors and learn more about ALKBH2 regulation.