16th ANNUAL BIOPHYSICS SYMPOSIUM

12TH OF SEPTEMBER

9:00 AM - 5:30 PM
at Pancoe Auditorium and Pancoe Cafe
Evanston Campus

BIOPHYSICS AND BIOCHEMISTRY CAREER PANEL

11:45 PM - 12:25 PM

NU STUDENT POSTER SESSION

4:00 PM - 5:30 PM

Learn more here

Register here

SPEAKERS

Rebecca Berlow, PhD
University of North Carolina

Josefina del Marmol, PhD
Harvard Medical School and
Howard Hughes Medical Institute

Anum Glasgow, PhD
Columbia University

Allison Williams, PhD
University of California,
San Francisco

Stacy Malaker, PhD
Yale University

Katie Hatstat, PhD
University of California,
San Francisco, DeGrado Lab

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Northwestern University

THE MOLECULAR BIOPHYSICS TRAINING PROGRAM AND STRUCTURAL BIOLOGY & BIOPHYSICS CLUSTER OF NULABS

Table of Contents

Session 1

Chair:	**Nickolas Fisher**, *Co-Chair of the Symposium Organizing Committee*
8:15 – 8:45	**Light breakfast and refreshments**
8:50 – 9:00	**Welcome Address by Heather Pinkett. Ph.D.,** *Director of the Molecular Biophysics Training Program*
9:00 – 9:05	**Schedule introduction by Nickolas Fisher,** *Co-Chair of the Symposium Organizing Committee*
9:05 – 9:45	**Distinguished Speaker: Stacy Malaker, Ph.D.,** Chemistry Dept., Yale University "Pioneering Biomarker Discovery through Exploration of Mucin Glycoproteins"
9:45 – 10:00	**NU Predoctoral Research Presentation by Nickolas P. Fisher** "Probing Human Oocyte Heterogeneity by Single-Cell Proteoform Imaging Mass Spectrometry"
10:00 – 10:40	**Distinguished Speaker: Rebecca Berlow, Ph.D.,** Biochemistry and Biophysics Dept., University of North Carolina at Chapel Hill "Tuning the Conformational Ensembles of IDPs through Sequence-Encoded Multivalent Interactions"

Session 2

Chair:	**Gracie Siffer**, *Co-Chair of the Symposium Organizing Committee*
11:00 – 11:40	**Distinguished Speaker: Josefina del Mármol, Ph.D.,** Biological Chemistry and Molecular Pharmacology Dept., Harvard Medical School "Structural Basis of Odor Sensing in Insects"
11:40– 12:25	**MBTP Career Panel**: Beyond Academia, Panelists will include members from industry, government, & academia
12:25 – 1:30	**Lunch in Pancoe Café**

Session 3

Chair:	**Chloe Jackson,** *Co-Chair of the Symposium Organizing Committee*
1:35 – 1:40	**Welcome Back by Chloe Jackson,** *Co-Chair of the Symposium Organizing Committee*
1:40 – 2:00	**Emerging Post-Doctoral Speaker: Katie Hatstat, Ph.D.,** Pharmaceutical Chemistry Dept., University of California, San Francisco "Engineering Bacterial Signal Transduction"
2:00 – 2:40	**Distinguished Speaker: Allison Williams, Ph.D.,** Biochemistry and Biophysics Dept., University of California, San Francisco "Penetrating Bacterial Defenses by Targeting Cell Wall-Degrading Enzyme Machine"
2:40 – 3:00	**NU Postdoctoral Candidate Research Presentation by Divyansh Prakash, Ph.D.**, "Characterization of a Small Metal-Binding Protein from Methane-Oxidizing Bacteria"
3:00 – 3:40	**Distinguished Speaker: Anum Glasgow, Ph.D.,** Biochemistry and Molecular Biophysics Dept., Columbia University "Distinct Energetic Blueprints Diversify Function of Conserved Protein Folds"
3:40 – 3:50	**Closing Remarks by Heather Pinkett, Ph.D.**, *MBTP Director*
4:00 – 5:30	**Graduate Student and Post-Doctoral Candidate Poster Session & Faculty Networking**

Distinguished Speaker Biography

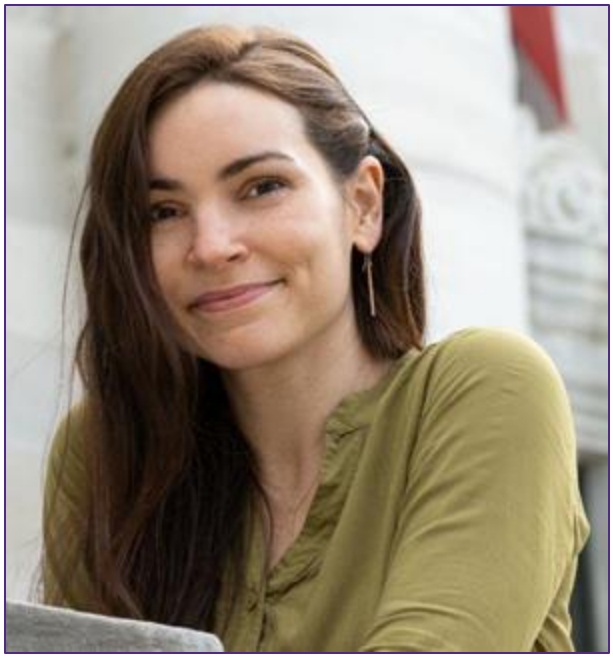
Rebecca Berlow, Ph.D.
University of North Carolina,
Chapel Hill

Dr. Rebecca Berlow is an Assistant Professor in the Department of Biochemistry and Biophysics at the University of North Carolina at Chapel Hill and a member of the Cancer Cell Biology program at the Lineberger Comprehensive Cancer Center. She received her B.A. in Chemistry from The Johns Hopkins University and her Ph.D. in Molecular Biophysics and Biochemistry from Yale University in the laboratory of Dr. J. Patrick Loria. She then worked as an American Cancer Society postdoctoral fellow and staff scientist in Dr. Peter Wright's lab at Scripps Research.

In 2022, Dr. Berlow started her independent research group at UNC, where her laboratory utilizes a wide range of experimental approaches to integrate biophysical studies of intrinsically disordered and dynamic proteins with functional studies of proteins and peptides in vivo. Dr. Berlow is active in teaching and mentoring as part of multiple training programs at UNC, including the NIH T32-funded Molecular and Cellular Biophysics Training Program, and she currently mentors trainees from the Biological and Biomedical Sciences and Chemistry graduate programs. Additionally, Dr. Berlow is an Associate Editor of Biophysical Reports and the Social Media Editor for the Journal of Molecular Biology, and she has held numerous leadership roles in international scientific societies, including her current role on the Committee for Professional Opportunities for Women of the Biophysical Society.

Distinguished Speaker Biography

Josefina del Marmol, Ph.D.
Harvard University

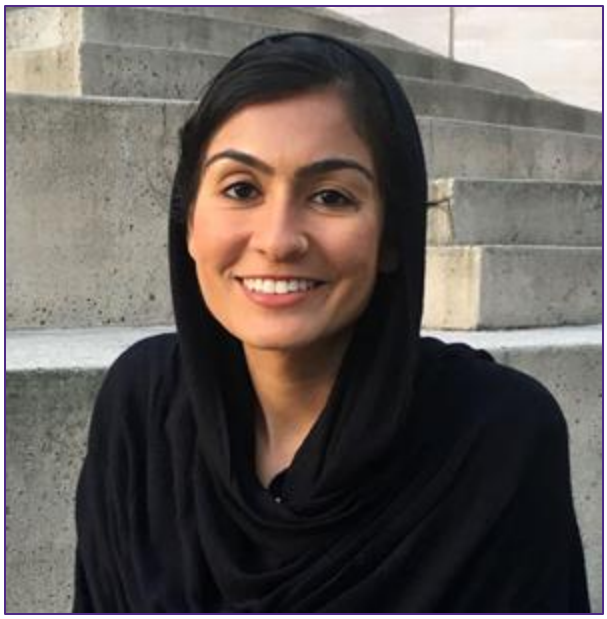


Dr. Josefina del Mármol is an Assistant Professor of Biological Chemistry and Molecular Pharmacology at Harvard Medical School and a Freeman Hrabowski Scholar at the Howard Hughes Medical Institute. Dr. del Mármol was born and raised in Argentina and graduated from the Universidad de Buenos Aires with a Licenciatura degree. She then emigrated to the US to join the laboratory of Roderick MacKinnon at the Rockefeller University for her graduate studies. There, she received a PhD in Molecular Neurobiology and Biophysics with a predoctoral fellowship from the Howard Hughes Medical Institute studying the molecular basis of the sense of touch. After a short postdoctoral stay at UC Berkeley, she joined the laboratory of Vanessa Ruta at the Rockefeller University to study the structural basis of odor recognition.

Her lab investigates the molecular mechanisms of sensory transduction, with special emphasis in understanding how mosquitoes sense humans, which leads to the transmission of pathogens that cause deadly diseases like malaria, dengue and yellow fever. Dr. del Mármol is the recipient of a NIH MOSAIC K99/R00 Pathway to Independence award, the AChems Polak Young Investigator Award, and is a Pew Biomedical Scholar since 2023, among other honors.

Distinguished Speaker Biography

Anum Glasgow Ph.D.
Columbia University

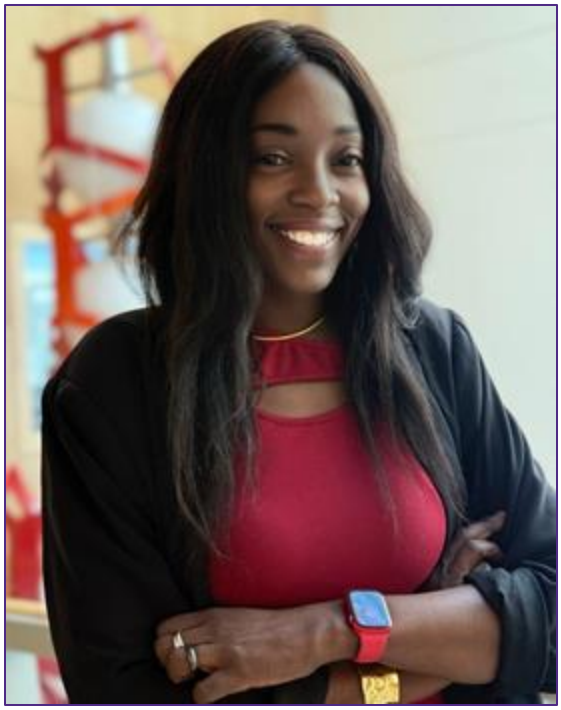


Dr. Anum Glasgow is an assistant professor in the Department of Biochemistry and Molecular Biophysics at Columbia University. She received a PhD from UC Berkeley, where she engineered a bacterial protein secretion system for applications in biotech in Dr. Danielle Tullman-Ercek's group. Anum pivoted from her experimental background to work in computational biophysics to build biological sensors and antiviral therapeutics as a postdoctoral fellow in Dr. Tanja Kortemme's group at UC San Francisco. Her postdoctoral work includes the first example of a de novo-designed chemically inducible dimerization system, in which two proteins bind a small molecule to generate programmable cellular responses. During the COVID-19 pandemic, Anum co-led a team to rapidly develop a receptor trap therapeutic for treating SARS-CoV-2 infections.

Anum continues to build and apply experimental and computational approaches to probe how protein systems evolve, undergo conformational changes, and interact with other biomolecules, while maintaining a focus on biotherapeutics development. Dr. Anum Glasgow is the recipient of American Cancer Society Catalyst Award and Paul Marks Scholar Award in 2024.

Distinguished Speaker Biography

Allison Williams Ph.D.
University of California, San Francisco



Dr. Allison Williams is an Assistant Professor and Chan Zuckerberg Investigator in the Department of Cellular and Molecular Pharmacology at UCSF, tackling the molecular machinery that makes bacterial pathogens so formidable. Her lab dissects bacterial cell wall biogenesis and stress response activation—two pillars of microbial survival—using a powerhouse toolkit of structural biology, including X-ray crystallography, cryo-electron microscopy, and single-molecule reconstruction. With a BS in Biochemistry from UMass Boston and a PhD from Duke University, Dr. Williams has built a research program that goes beyond individual molecular machines, uncovering the hidden conversations between bacterial survival networks. Rather than targeting single proteins—an approach bacteria rapidly outmaneuver—her lab disrupts entire pathways, cutting off bacterial adaptability at its source. Committed to shaping the next wave of scientific innovators, Dr. Williams fosters a lab culture rooted in scientific rigor, fearless curiosity, and collaboration. Her leadership spans graduate education, research ethics, and institutional initiatives driving computational and structural biology innovation. A firm believer in the power of collaboration, Williams maintains strong ties across UCSF, UC Berkeley, and beyond. By bridging structural biology, microbiology, and host-pathogen interactions, her work is redefining how pathogens can be defeated.

Distinguished Speaker Biography

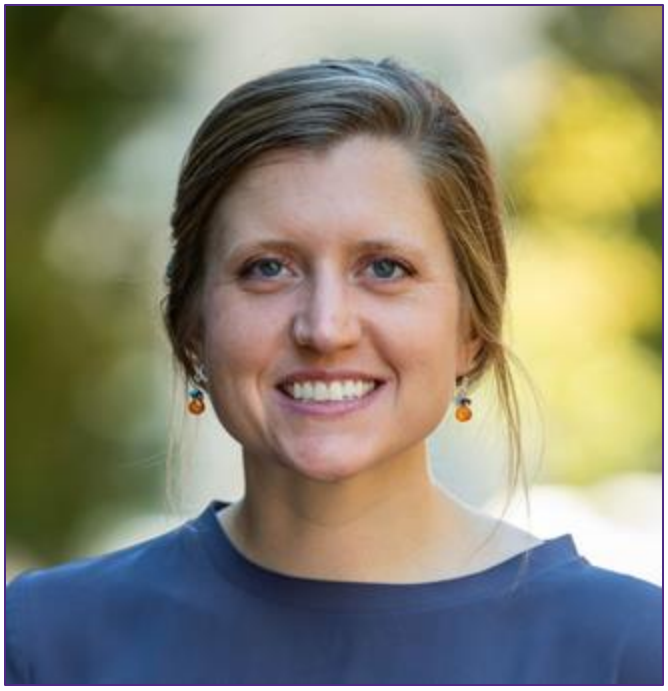
Stacy Malaker, Ph.D.
Yale University

Dr. Stacy D. Malaker is an Assistant Professor of Chemistry at Yale University, where she devises mass-spectrometry and glycoproteomic tools to decode mucins—densely O-glycosylated extracellular proteins that play pivotal yet poorly understood roles in human disease. Her laboratory integrates new enzymatic digestion strategies, advanced MS fragmentation, and custom bioinformatics to map mucin glycans at site resolution and probe how aberrant glycosylation influences cell signaling and immune evasion. She earned dual B.S. degrees in Biochemistry and Anthropology-Zoology from the University of Michigan, then completed a Ph.D. in Chemistry at the University of Virginia under Donald Hunt, profiling cancer glycopeptides with electron-transfer dissociation.

As an NIH postdoctoral fellow in Carolyn Bertozzi's laboratory at Stanford, she pioneered chemoproteomic approaches to track mucin-domain glycoproteins in living cells, work that galvanized her independent research program when she joined Yale in 2021. Malaker's contributions have been recognized with the Royal Society of Chemistry's Chemistry-Biology Interface Horizon Prize, the ASBMB Early Career Faculty Award, and inclusion in the *Journal of Proteome Research* *40 Under 40* list, among other honors. Committed to mentoring scientists at the chemistry–biology interface, she aims to translate analytical innovations into new diagnostics and therapeutics for cancer and mucosal diseases.

Emerging Speaker Biography

Katie Hatstat, Ph.D.
University of California,
San Francisco



Dr. A. Katherine Hatstat is a UC President's Postdoctoral Fellow in Bill DeGrado's laboratory at UC San Francisco, where she merges structural biology with de novo protein design to discover how bacterial histidine-kinase receptors sense their surroundings and relay signals across membranes. Her current work centers on a Zn^{2+}-responsive sensor domain that can be grafted onto diverse two-component systems, clarifying the thermodynamic and conformational principles of signal transduction while enabling programmable, orthogonal signaling circuits.

Katie completed her Ph.D. in Chemistry in Chris McCafferty's group at Duke University, using biochemical, biophysical, and chemoproteomic approaches to reveal how an N-arylbenzimidazole compound rescues α-synuclein-induced trafficking defects via a novel NAB2–Rab1a pathway (published in Cell Chemical Biology and RSC Chemical Biology). Her expertise spans recombinant protein expression, proteomics, and small-molecule synthesis. She is passionate about mentoring and aims to launch an interdisciplinary lab that rewires protein signaling pathways for therapeutic innovation.

Distinguished Speaker Abstract

Tuning the Conformational Ensembles of IDPs through Sequence-Encoded Multivalent Interactions

Rebecca B. Berlow, Ph.D.

Department of Biochemistry and Biophysics at the University of North Carolina at Chapel Hill, Chapel Hill, NC

The intrinsically disordered protein CITED2 is essential in regulating the transcriptional response to oxygen deficiency and other key cellular processes that are important for human health and are often disrupted in disease. CITED2 is a fully disordered protein with interaction motifs positioned throughout the protein sequence that engage a diverse set of molecular partners through dynamic, multivalent interactions. While the interactions of the C-terminal transcriptional activation domain of CITED2 are well-characterized, little is known about the functional roles of the CITED2 N-terminus and its potential impacts on interactions of CITED2 with other transcriptional regulators. Using solution NMR spectroscopy in combination with complementary biochemical and biophysical techniques, we have identified that intramolecular interactions between the N- and C-terminal regions of CITED2 bias the conformational ensemble towards more compact states. These intramolecular interactions regulate the accessibility of conserved interaction motifs and alter the intermolecular interactions of CITED2 in diverse contexts. We anticipate that long-range interactions in CITED2 are essential for fine-tuning the CITED2 interaction landscape and controlling its functional specificity in transcriptional regulation.

Distinguished Speaker Abstract

Structural Basis of Odor Sensing in Insects

Josefina de Marmol, Ph.D.

Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY

A large diversity of odorant molecules reaches the olfactory sensory organs of animals at any given time. The key role of odorant receptors (ORs) is to translate the chemical complexity of the environment into interpretable neuronal signals. To this goal, ORs exhibit a wide range of ligand specificities: while many ORs are promiscuous, responding to dozens of chemically diverse odorants, some highly selective and activated by a single compound. This diversity in chemical specificity of ORs underpins the extraordinary discriminatory power of the olfactory system. In this talk, I will present our work towards understanding the structural basis of chemical specificity of ORs. Using cryogenic electron microscopy (cryoEM) and functional assays, we characterized ORs from various insect species, shedding light on the structural strategies that allow ORs to encode the vast chemical space of odorants.

Distinguished Speaker Abstract

Distinct Energetic Blueprints Diversify Function of Conserved Protein Folds

Anum Glasgow, Ph.D.

Department of Biochemistry and Biophysics at the University of North Carolina at Chapel Hill, Chapel Hill, NC

How do protein domains with highly conserved three-dimensional folds perform radically disparate biochemical functions? To understand this, we mapped the energetic landscapes of a family of bacterial transcription factors and their anciently diverged structural homologs, the periplasmic binding proteins. Using hydrogen exchange/mass spectrometry, bioinformatics, X-ray crystallography, and molecular dynamics, we uncovered an unexpected contrast: despite binding the same sugars, the two families evolved unique "energetic blueprints" to support their distinct functional requirements. To test if differences in energetic ensembles have functional consequences, we rationally redesigned the protein fold for tunable ligand-driven transcriptional responses. We found that energy-driven protein engineering produced synthetic transcription factors with the theoretically anticipated ligand-induced transcriptional outputs. Thus, decoding energetic blueprints among conserved protein folds provides an explanation for diverse functional adaptations, paves an alternative roadmap for protein design, and offers a new approach for engineering challenging drug targets.

Distinguished Speaker Abstract

Penetrating Bacterial Defenses by Targeting Cell Wall-Degrading Enzyme Machine

Fahimeh Hajiahmadi[1], Maryam Alehashem[1], Mariya Lobanovska[1], Ivanna Lopez[1], Berliza Soriano[1], Monita Muralidharan[1],

Allison Williams, Ph.D.

[1]Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA

Bacterial survival depends on a dynamic balance between peptidoglycan synthesis and degradation/remodeling, processes mediated by coordinated enzyme machines. Recent structural and biochemical studies from our lab reveal that lytic transglycosylases (LTs) such as LtgA can assemble into higher-order complexes that expand their functional reach. The LtgA–LtgD complex highlights how redundancy among LTs can be harnessed through direct interaction to enhance glycan strand cleavage, while the LtgA–Ape1 complex demonstrates functional crosstalk between glycosidic bond cleavage and removal of peptide-linked anhydromuropeptides. Here, we present the first structural and mechanistic framework for cooperative PG degradation enzymes in action, resolving a long-elusive process that defines how bacteria orchestrate multi-enzyme degradation of their cell wall. This work establishes that PG hydrolases are not always solitary actors in gram negative bacteria but components of integrated molecular machines, whose coupled activities accelerate cell wall turnover during growth and division. At the same time, these complexes expose vulnerabilities that can be therapeutically exploited. By uncovering a first-in-class structural paradigm for hydrolase cooperation, our findings open new avenues to penetrate bacterial defenses and restore antibiotic efficacy.

Distinguished Speaker Abstract

Pioneering Biomarker Discovery through Exploration of Mucin Glycoproteins

Stacey Malaker, Ph.D.

Department of Chemistry at Yale University, New Haven, CT

Mucin-domain glycoproteins are densely O-glycosylated and play key roles in a host of biological functions. However, their dense O-glycosylation remains enigmatic both in glycoproteomic landscape and structural dynamics, primarily due to the challenges associated with studying mucin domains. Here, we present advances in the mass spectrometric analysis of mucins, including the characterization of mucinases, enrichment techniques, and complete mucinomic mapping of translationally relevant mucin proteins.

Emerging Speaker Abstract

Engineering Bacterial Signal Transduction

Katie Hatstat, Ph.D., William Degrado, Ph.D.

Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA

Nature has evolved dynamic proteins that act as sensors, detecting changes in the environment and producing signals that allow cells to adapt and respond accordingly. For example, bacterial histidine kinases (HKs) detect environmental stimuli and transduce a signal across the membrane through multiple modular subdomains, initiating a phosphorelay and transcriptional response. Despite their ubiquity, HKs remain structurally elusive, HK specificity is still largely unknown, and our mechanistic understanding of HK signal transduction is still evolving. My research combines protein design and protein engineering to test and expand our fundamental knowledge of signal transduction from first principles. Here, I explore the modularity of HKs by generating large chimera libraries and correlating biophysical features of subdomains with receptor function. I also test the ability of emerging generative protein design tools to build functional HK subdomains de novo. Together, the combination of protein design and engineering expands our understanding of natural signaling mechanisms and enables us to build new-to-nature signaling systems.

Post Doc Podium Presenter Abstract

Characterization of a Small Metal-Binding Protein from Methane-Oxidizing Bacteria

Divyansh Prakash[1], Jose Ayala[1], Josephine Gan[2], Caitlin Palmer[1], Brian Hoffman[2], Calvin Henard[3], Amy Rosenzweig[1]

[1] Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208
[2]Department of Chemistry, Northwestern University, Evanston, IL 60208
[3]Department of Biological Sciences and the BioDiscovery Institute, University of North Texas (UNT), Denton, TX 76203-5017

Methane gas is the second most potent greenhouse gas after carbon dioxide and contributes significantly to the Earth's rising temperature. Methane-oxidizing bacteria (MOB), known as methanotrophs, use methane as their only carbon and energy source, acting as a methane sink. The first step in the methanotroph metabolic pathway is the conversion of methane to methanol by the particulate methane monooxygenase enzyme (pMMO), which requires copper. However, a complete understanding of pMMO has been hindered by the low enzymatic activity observed during its isolation and purification. This reduced activity may result from the absence of partner proteins that are lost during purification. Such proteins may be crucial for the function of pMMO, assisting in metal acquisition, maintaining a specific redox state, protecting against oxygen radicals, and delivering electrons or protons to the active site. One uncharacterized protein that may fulfill these roles is the soluble small metal-binding protein (SmbP), which is abundant in MOB. Previous studies on SmbP from ammonia-oxidizing bacteria (AOB) have suggested a role in the cellular management of copper[1] although this hypothesis lacks experimental support. We have expressed SmbP from *Methylococcus capsulatus (Bath)* in *E. coli*. ICP-MS analysis indicates that SmbP selectively binds one equivalent of copper, also evidenced by the presence of an optical d-d* transition band at ~630 nm. The EPR spectrum of Cu-SmbP is consistent with a type 2 Cu site, and ^{2}H and ^{15}N ENDOR provide additional insight into the primary coordination sphere of Cu bound to SmbP. Progress toward establishing the role of SmbP in *M. capsulatus* (Bath) physiology will also be reported.

Reference:
Barney, B. M.; LoBrutto, R.; Francisco, W. A. Characterization of a Small Metal Binding Protein from *Nitrosomonas Europaea*. *Biochemistry* **2004**, *43* (35), 11206–11213. https://doi.org/10.1021/bi049318k.

Pre Doc Podium Presenter Abstract

Probing Human Oocyte Heterogeneity by Single-Cell Proteoform Imaging Mass Spectrometry

Nickolas P. Fisher[1,2], Vijaya Lakshmi Kanchustabahm[3], Elizabeth L. Tsui[4,5], Chelsea Lock[1], Tian Xu[3], Hannah B. McDowell[4,5], Indira Pla Parada[3], Diane C. Saunders[4,5], Jared O. Kafader[1,3], Monica M. Laronda[4,5,6*], Neil L. Kelleher[1,2,3,7,8,9*]

[1]Department of Chemistry, Northwestern University, Evanston, IL, USA; [2]Department of Molecular Biosciences, Northwestern University, Evanston, IL, USA; [3]Proteomics Center of Excellence, Chemistry of Life Processes Institute, Northwestern University, Evanston, IL, USA; [4]Stanley Manne Children's Research Institute, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA; [5]Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; [6]Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; [7]Department of Biochemistry and Molecular Genetics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; [8]Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL, USA; [9]Chan Zuckerberg Biohub Chicago, Chicago, IL, USA

Ovarian tissue cryopreservation (OTC) is utilized by clinics to preserve oocytes for pediatric patients who are at increased risk of becoming infertile due to a diagnosis or treatment. Maturing oocytes are often released during OTC. We are interested in identifying proteoforms that may improve in vitro maturation of oocytes into eggs for these patients as an additional fertility preservation strategy. We are utilizing single cell proteoform imaging mass spectrometry (scPiMS), which is a nanoDESI direct sampling approach coupled to individual ion mass spectrometry (I^2MS) detection, to profile human oocytes to identify and assess the intact proteoform population between single oocytes. We have used this scPiMS approach to analyze 24 denuded oocytes from 6 different participants/donors aged 1.71 years old to 33. We have also utilized the high spatial resolution afforded by the scPiMS probe (~100 μM) to selectively sample oocytes and cumulus granulosa cells within 4 cumulus oocyte complexes (COCs) from two participants to identify proteoforms that are cell-type specific. We have identified ~78 proteoforms on average across the 28 oocytes sampled, with an average identification rate of THRASH proteoform features of ~55%. We have identified several proteoforms that are highly specific to oocytes rather than cumulus granulosa cells, including oocyte expressing protein homolog (OOEP), KH-domain containing protein 3 (KHDC3), and ferritin light chain. We utilized this data to further understand the proteomic heterogeneity of single oocytes between patients and between multiple oocytes from the same donor. Finally, we demonstrate the utility of scPiMS for analyzing patient specific proteoform landscapes by identifying OOEP and KHDC3 proteoforms with SNPs and variable modifications across the cohort. We ultimately aim to expand our analysis to quantify the abundance of these proteoforms and understand how proteoform landscapes change in the context of age.

Poster Presenter Abstract

Investigating the Role of Dynactin Composition on Microtubule Dynamics

Kristen Dominique Amarillo[1], Jenna R. Christensen, Ph.D.[1]

[1]Interdisciplinary Biological Science Program, Northwestern University, Evanston, IL 60208

The dynein motor complex is a large multi-subunit complex that walks on microtubules to orchestrate vital cellular processes such as cell division, cargo transport, and organelle distribution. The dynein motor complex is composed of the motor protein dynein, the 11subunit co-factor dynactin, and a coiled-coil adaptor. Dynactin and the coiled-coil adaptor serve to stabilize the dynein motor for processive long-distance movement along a microtubule. Current studies highlight the transport roles of the dynein motor complex. However, how dynein and dynactin regulate microtubule dynamics is understudied. Proper microtubule dynamics are critical for cellular processes such as cargo transport and mitosis. Previous studies in budding yeast have shown that knocking out different dynactin components result in opposite effects on microtubule stability. We hypothesize that variation in dynactin composition affects microtubule dynamics. Using the genetically tractable filamentous fungi, Aspergillus nidulans, I will generate single knockouts of dynactin components and use live cell imaging to track changes in microtubule dynamics using EB1-mCherry, a protein that tracks the plus ends of microtubules. In parallel, I will use single molecule total internal reflection fluorescence (TIRF) microscopy to examine how purified dynactin subunits, sub-complexes, and finally, a full dynactin complex, affect the stability and dynamics of microtubules as well as the association/dissociation rates of dynactin components on microtubules. Overall, we aim to characterize how functional diversity in the dynactin complex affects microtubule dynamics through in vitro and in vivo methods.

Poster Presenter Abstract

The Effect of Trinucleotide Extrusions on DNA Looping

Jackson Anderson, PhD[1], Brian Cannon, PhD[1]

[1]Physics Department, Loyola University Chicago, Chicago, IL 60660

Tandem repeat motifs in DNA are associated with numerous genetic disorders and cancers. Single-stranded domains containing these repeat motifs can arise during DNA processing and adopt numerous types of non-helical structure that alter the local geometry of the DNA and increase genetic instability. Here, we report on the effect of these domains on DNA looping - an important DNA structural transition in DNA organization and in protein-DNA interactions. DNA looping was monitored by single-molecule FRET to track real-time looping events in individual, immobilized dsDNA molecules. The DNA constructs contained single-stranded, trinucleotide repeat domains of the sequence - (CAG)n. These repeat domains exist as three-way junctions and induce local bends that alter the geometry of the flanking dsDNA without increasing the overall size of the looping DNA. The local bend angles were measured for different repeat numbers and using coarse-grained models. Discrete looping and unlooping events via changes in FRET were observable for DNA containing the single-stranded repeat domains. The size of the single-stranded domains (as determined by the number of repeats) altered the looping behavior of the DNA in a size-dependent manner consistent with the bend angles attributed to the different domain sizes. The changes in looping behavior due to the presence of these single-stranded domains are sensitive to the environmental conditions, such as monovalent ion concentration, indicating that changes in non-helical structure in the single-stranded domains for a given number of repeats can alter DNA looping.

Poster Presenter Abstract

Engineering Local Chromatin Accessibility Using Pioneer Factor NF-Y

Michael M. Anderson[1], Joshua N. Leonard[1]

[1]Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL 60208

Synthetic gene circuits harnessing transcription factors (TFs) and synthetic transcription factors (synTFs) have emerged as powerful tools for potent and user-defined gene activity across a diverse range of contexts and applications. However, many challenges remain with the potency and stability of such systems, especially in contexts where epigenetic silencing poses a major barrier to the activation of these systems. Current approaches to address such concerns are highly limited insofar as they lack the ability to potently enhance TF activity without imparting additional leakiness to the promoter. To address this limitation, we appeal to an underexplored category of transcription factors called pioneer factors, which possess the innate capacity to bind nucleosome-rich regions of chromatin and prime neighboring regions for activation. By harnessing the chromatin opening potential of the ubiquitous pioneer factor Nuclear Factor Y (NF-Y) we demonstrate the robust capacity of a pioneer factor to consistently improve activity of synthetic gene circuits irrespective of factor identity or strength across diverse contexts using the COMET toolkit. This study thus represents the first major step towards the inclusion of pioneer factors into the mammalian synthetic biology toolkit, providing new opportunities for increased potency and stability for improved scientific and therapeutic outcomes.

Poster Presenter Abstract

Dynein Light Chains Regulate Dynein Motor Function and Cargo Movement

Alexander Bemben[1], Jenna R. Christensen, PhD[1]

[1]Department of Molecular Biosciences, Interdisciplinary Biological Sciences Graduate Program, Northwestern University, Evanston, IL 60208

Molecular motors are essential facilitators of intracellular transport, meeting the diverse transport needs of different organisms and cell types. The cytoplasmic dynein complex ('dynein' here) is a mega-Dalton motor protein complex composed of 6 subunits that is critical for the intracellular transport of numerous cargoes. Dynein moves processively towards the minus end of polarized microtubules, either transporting cargoes over long distances or exerting pulling forces on microtubules, depending on the biological context. While subunits such as the dynein heavy chain are well characterized, others have less clear roles, such as the three dynein light chains LC8, Roadblock, and Tctex. We found that the dynein light chains encoded in the genome vary across the fungal kingdom; specifically, many fungi lack genes for Roadblock and/or Tctex. Interestingly, we found that loss of Roadblock or Tctex only occurs in fungi that have no known instances of dynein involvement in long distance 'walking' transport but that still use dynein for 'pulling' transport. Therefore, we hypothesize that Roadblock and Tctex are critical for dynein 'walking' but not for dynein 'pulling'. To test this, we knocked out light chains in Aspergillus nidulans, a filamentous fungus which uses dynein for both walking and pulling. We fluorescently tagged and imaged transport of endosomes (a 'walking' cargo) and nuclei (a 'pulling' cargo). We found that Roadblock is critical for proper endosome movement, supporting the idea that Roadblock is involved in long-distance 'walking' transport. To understand the mechanisms behind this, we tested the effects of light chain knockouts on localization of dynein and its co-factor dynactin and their involvement in the formation of active dynein complexes. Overall, deciphering the importance of the dynein light chains in the transport of different cargos will enhance our understanding of the differences in molecular and biophysical requirements for dynein's 'walking' and 'pulling' processes.

Poster Presenter Abstract

Statistical Physics Centered Machine Learning Approaches to Design Intrinsically Disordered Proteins for Molecular Function

Jackson Boodry[1], Neha Tyagi[1], Krishna Shrinivas, Ph.D.[1]

[1]Chemical and Biological Engineering, Northwestern University, Evanston, IL 60208

Biological condensates are membrane-less domains of cellular organization that play key roles in biological information processing. Condensate formation is often driven by phase transitions and are commonly induced by the presence of intrinsically disordered proteins or regions (IDPs/IDRs). In contrast to the well-established sequence-structure-function paradigm for folded proteins, IDPs derive their function from an ensemble of interconverting conformations rather than an established structure. The highly dynamic nature of IDPs means their ensemble characteristics are not amenable to analysis with data-driven machine learning approaches to protein structure like AlphaFold or RosettaFold, and the lack of a standardized database of experimental IDP properties makes development of novel data-driven approaches a challenge. Molecular dynamics simulations are one typical physics-based method to interrogate IDP characteristics, but the large computational demand makes design of de-novo IDPs a challenge. Recent efforts in the field have developed analytical methods to predict IDP ensemble properties, allowing efficient exploration of the IDP sequence space at a comparatively low computational cost. Here we present a methodology to invert these theoretical relationships between IDP sequences and observable properties. IDP sequences are tailored rationally in a physics-based differentiable framework with specified single-chain, phase behavior, and molecular recruitment properties. Accuracy of predicted ensemble properties for optimized IDPs is assessed through single-chain and slab molecular dynamics simulations. Future work on this subject includes collaboration with experimentalists to test recruitment of designed IDPs, as well as the development of topologically diverse condensate-like architectures to mimic biological structures.

Poster Presenter Abstract

Functional Role of Aps Proteins on Dynein Activation and Coordinated Nuclear Movement.

Miranda Flores Escobar[1], Jenna R. Christensen, Ph.D.[1]

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Nuclear rotation and/or movement over large distances is essential for various cellular processes. Studies suggest that cytoplasmic dynein-1 is the primary motor that drives nuclear movement, although the exact mechanisms behind this activity are not well understood. Dynein is a motor that walks toward the minus ends of microtubules and transports cargo through interactions bridged by activating adaptors. Certain adaptors can alternatively anchor dynein to the cell membrane, allowing dynein to move cargo by "pulling" on microtubules. In my studies I use the filamentous fungus, *Aspergillus nidulans*, to understand how multiple sets of dynein-dynactin-adaptor complexes work together in different areas of the cell to coordinate the synchronous movement of multiple nuclei in this organism. We hypothesize that two proteins ApsA and ApsC are dynein adaptors that affect nuclear movement through the regulation of dynein's biophysical properties. Through live cell imaging and nuclear tracking, we've observed that both ApsA and ApsC affect nuclear speed and distribution. We have also identified dynein binding motifs in both ApsA and ApsC through multiple sequence alignments, suggesting that they are dynein adaptors. In our next step, we will use single-molecule TIRF microscopy to determine how ApsA and ApsC affect dynein's landing rate, run length, and velocity on microtubules. Together, I aim to characterize ApsA and ApsC as dynein adaptors and uncover how dynein activation coordinates nuclear movement at the cellular and molecular level.

Poster Presenter Abstract

Biophysical Investigation of the Nucleolus Reassembly

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The nucleolus is a notable multilayered molecular condensate that plays a critical role in ribosome biogenesis, which is essential for protein synthesis and cell growth. Therefore, cells must ensure the proper distribution of this organelle to daughter cells during mitosis. Unlike membrane-bound organelles, which undergo fragmentation to allow equal distribution, the nucleolus is a membraneless organelle that undergoes dynamic disassembly and reassembly driven by cell cycle and signaling cues. Despite recent progress in characterizing the nucleolar proteome and its role in mitotic disassembly, the mechanisms ensuring accurate nucleolar inheritance and the biophysical principles underlying its reassembly remain unclear. Therefore, we plan to combine a CRISPR/Cas9-based imaging technique with nucleolar proteins tagged with fluorescent markers to achieve specific *in vivo* visualization of rDNA, the DNA sequences that encode ribosomal RNA, located within Nucleolar Organizer Regions (NORs), the chromosomal loci where the nucleolus assembles. Time-lapse microscopy in HeLa cells expressing this system will allow us to track rDNA movement and diffusion rates, as well as quantify the sequential assembly kinetics of distinct nucleolar layers by monitoring the temporal recruitment of layer-specific proteins. In parallel, we will measure changes in the droplet size of these proteins and analyze the dynamics of prenucleolar body fusion at NORs during assembly under both normal conditions and biochemical perturbations. Together, these analyses will enable us to model nucleolar reassembly dynamics in eukaryotic cells.

Poster Presenter Abstract

High-Throughput Hydrogen-Deuterium Exchange to Measure Protein Energy Landscapes

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Protein biophysical properties are determined by their energy landscapes. Understanding the sequence and structural determinants of protein energy landscapes can advance protein engineering by designing proteins with optimal energy landscapes. To accurately capture a proteins energy landscape involves quantitatively assessing both protein stability and dynamics. Moreover, to learn sequence and structural determinants of protein energy landscapes not limited to one protein context involves experimentally probing a wide sequence space. In this work, we have applied a high throughput approach leveraging HDX mass spectrometry to measure opening free energy distributions for thousands of proteins. We have obtained over 5,000 opening free energy distributions for a variety of small domain proteins (e.g., SH3, WW, LysM domains) and ααα, ßßαßß, and ßαßß de novo designs. Our results show a wide range of energy landscape profiles with varying global stabilities and opening energy distributions. Additionally, we have derived a cooperativity metric that reflects the variation in the opening free energy distribution across the protein sequence allowing us to compare proteins from different topologies. Our analysis reveals that within the ααα protein domain, cooperativity is correlated with the amount of side chain contacts and compactness of a protein whereas global stability is correlated with the type of contacts formed within the protein. For the ßßαßß domain, proline count was shown to be important for cooperativity and AlphaFold pLDDT was important for global stability. These initial results can be used as starting points to engineer proteins with ideal stability and cooperativity.

Poster Presenter Abstract

Biophysical Approaches to Selectively Modulating NTSR1 for Substance Use Disorder Treatment

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Neurotensin receptor 1 (NTSR1) is a G protein-coupled receptor (GPCR) implicated in addiction and neurological disorders. Traditional NTSR1-targeted therapies often lead to significant side effects due to their non-specific activation of various signaling pathways. Recent studies have identified arrestin-biased allosteric ligands as promising candidates for selective modulation of NTSR1, offering potential therapeutic benefits with reduced side effects. This research aims to elucidate the molecular mechanisms underlying selective G protein subtype inhibition and inherent arrestin agonist activity by these allosteric ligands. Specifically, we focus on the novel compound SBI-553, which has shown efficacy in reducing addictive behaviors in rodent models without adverse side effects. Our approach investigates how SBI-553 promotes arrestin-biased signaling by altering the timing, strength, and preassembly of transducer recruitment to NTSR1 using kinetic BRET assays, fluorescence polarization, and complementation-based interaction assays. Building on this, we will implement an NMR fragment-based discovery workflow to identify and optimize novel allosteric ligands that tune arrestin and G protein subtype selectivity. Ligands will be prioritized using pharmacophore modeling, virtual screening, and biophysical and functional assays to guide the rational design of safer, transducer-selective therapeutics. This work advances the understanding of NTSR1 allosteric modulation and contributes to the broader field of GPCR-targeted drug discovery.

Poster Presenter Abstract

Understanding the Homo- and Heterodimerization of Transcription Factors Regulating Pleiotropic Drug Resistance

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Saccharomyces cerevisiae adapts to exposure to structurally diverse compounds through its pleiotropic drug resistance (PDR). This response is characterized by the expression of multiple ABC transporters and is controlled by zinc cluster transcription factors (TFs) Pdr1, Pdr3, and Stb5. These TFs bind to pleiotropic drug response elements (PDREs) in the promoters of their target genes. Our lab has shown that Pdr1 and Pdr3 have overlapping, but non-redundant transcriptional regulation, and both proteins are necessary for maximal transcriptional activation of PDRE-regulated genes. While the exact mechanism of PDR regulation is not fully understood, both homo- and heterodimerization of these TFs are thought to contribute to the ability of a few TFs to regulate the expression of several exporters in response to different compounds. Analytical ultracentrifugation in the presence and absence of PDRE sequences revealed that the DNA-binding domains of Pdr3 and Stb5, but not Pdr1, require PDREs to homodimerize at physiologically relevant concentrations. Because Pdr3 and Stb5 do not heterodimerize with each other, but do heterodimerize with Pdr1, this suggests a mechanism by which these TFs are able to differentially form homo- and heterodimers by interacting with the more abundant Pdr1 directly on target PDREs. Because DNA appears to drive dimer formation, future studies will investigate how variations in PDRE sequences affect TF assembly. The structure of Pdr1's dimerization domains has revealed the residues involved in homodimerization, and structures of Pdr3 and Stb5's dimerization domains, in combination with structures of their zinc cluster domains bound to PDREs will reveal their molecular basis of dimerization and DNA binding. These studies represent the first structural insights into zinc cluster TFs capable of heterodimerization. Modelling and interaction predictions of heterodimers based on our homodimer structures will reveal how heterodimers form and provide a platform for modulation of the PDR response through alteration in the interaction interfaces of these TFs.

Poster Presenter Abstract

Cryo-EM Studies of the Type II ABC Molybdate Uptake Importer, MolB_2C_2-A from *Haemophilus influenzae*

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Haemophilus influenzae (Hi) is a gram-negative commensal pathogen of the human nasopharynx that can cause opportunistic ear infections and respiratory disease complications in young children and immunocompromised adults. To persist in the host, Hi utilizes ATP Binding Cassette (ABC) importers to facilitate the uptake of vital nutrients and metabolites, including molybdate. As a redox-active enzyme cofactor, molybdate has vital implications in several downstream metabolic processes of the pathogen. For that reason, it has been proposed as a promising target for combating Hi virulence. Among the two molybdate transport systems in Hi, we focused on resolving key transport states of the Type II importer, MolB_2C_2-A via cryo-EM. Our current understanding of Type II mediated substrate transport stems from large substrate transport systems that do not include MolB_2C_2-A, a small substrate importer. With limited structural data available, our work on Hi MolB_2C_2-A aims to shed light on the mechanism of small substrate delivery by Type II importers. Our cryo-EM structures of MolB_2C_2-A in different transport states reveal unique conformational changes that have not been reported for other well characterized Type II importers. In the absence of nucleotide, each lobe of the binding protein MolA interacts tightly with the membrane-embedded complex at each side, and with the top of the periplasmic gate via residues in its substrate binding pocket. The result of this docking event leads to subtle MolA conformational changes with one lobe remaining rigid and the other rotating slightly outward near the membrane interface. Upon nucleotide binding, however, these conformational changes become more pronounced leading to the complete loss of interactions with one of the MolA lobes. In this half-associated conformation, MolA can still bind substrate, albeit more weakly, as shown by molecular dynamics (MD) simulations. This is the first structural evidence obtained for the nucleotide-driven loss of affinity between the binding protein and the membrane complex, whose dynamics we are currently further investigating. Together, these cryo-EM structures offer the first snapshots in the small substrate transport mechanism of Hi MolB_2C_2-A. With this body of work, we hope to inform our current understanding of the role of MolB_2C_2-A in Hi virulence and help aid the development of new therapeutics.

Poster Presenter Abstract

Structural and Functional Characterization of the ProVWX Osmoregulatory ATP-Binding Cassette (ABC) Transporter

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The ProVWX (ProU) ABC importer from *Yersinia pestis* enables the bacteria to adapt to different osmoenvironments as it moves between host species. The ProU transporter is a canonical Type I ABC importer in which the substrate-binding protein (SBP: ProX) delivers substrate to the core transporter, which comprises two transmembrane domains (TMDs: ProW) and two nucleotide-binding domains (NBDs: ProV). ATP binding and hydrolysis in the NBDs drive conformational changes that result in open or closed TMDs, which enable the transport of substrate. Within the NBDs are C-terminal accessory domains classified as cystathionine-ß-synthase (CBS) domains, which are predicted to play a regulatory role in the protein. The CBS domains are predicted to change conformation in response to different environmental conditions, including variations in salt concentration and the lipid composition of the membrane. Additionally, CBS domains of other transporters have been shown to inhibit transport activity through the binding of inhibitors. These inhibitors lock the CBS domains in a closed conformation, preventing the movement of the NBDs and TMDs that is required for the transport of substrate. However, the regulation mechanism is not yet known for the ProU osmoregulatory transporter. To elucidate the regulatory mechanism, typical structural techniques such as cryogenic electron microscopy (cryo-EM) and x-ray crystallography are insufficient due to their static nature. Therefore, electron paramagnetic resonance (EPR) spectroscopy will be used to capture the conformational dynamics of these protein domains and elucidate the regulatory mechanism. To accomplish this, 5 different cysteine mutations used for MTSL spin-labeling were introduced into the NBDs, including within the CBS domains. Preliminary continuous wave (CW) and double electron-electron resonance (DEER) EPR spectroscopy data with the isolated NBDs and CBS domains have revealed a change in CBS domain conformation upon addition of 10mM ADP nucleotide. Further experiments will be conducted to determine conformational changes in the presence of different nucleotides, varying salt concentrations, and in different lipid environments. Elucidating the inhibition cycle and regulation mechanism of this importer will reveal key insights into how pathogenic bacteria survive in different host environments and inform the development of novel antibiotics.

Poster Presenter Abstract

Activity of SMC Complex ClsN at Varied Acidity and Temperature

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Structural Maintenance of Chromosome (SMC) proteins are essential in the regulation of chromosome structure and gene expression in eukaryotic cells. Some Archaea, like those of the Sulfolobus family, contain chromosomal features that show similarity to those of eukaryotic cells. SMC-like proteins help regulate the Sulfolobus chromosome, including Coalescin, or ClsN. Coalescin has been observed to form loops and condense Chromatin, helping produce A and B compartments. It has also been shown that Sulfolobus uses ClsN to achieve this. This series of experiments seeks to better understand how ClsN is activated to regulate chromosomal structure within Sulfolobus. Being a thermophilic and acidophilic organism, Sulfolobus, and therefore ClsN, is predicted to behave optimally at low pH and high temperature. The objective is to determine the critical force - the force that the protein produces - under acidic (pH 5.2) and high temperature (60 C) conditions. This is accomplished through the use of magnetic tweezers. A magnetic bead is attached to one end of a DNA strand, and the other end of the DNA strand is attached to the cover slip of a flow cell. A variable magnetic field is applied, and the extension of the DNA strand is observed through a microscope. Variance in DNA extension when introduced to ClsN and a ClsN-ATP mixture help determine the force with which ClsN compresses and organizes DNA. The findings of this experiment are that Acidic conditions can somewhat regularly aid in the compression of DNA by as much as 10% at 0.7pN, and that high-temperature conditions without lowered pH do not allow ClsN to compress DNA.

Poster Presenter Abstract

Shedding Light on the Dark Channel via Large-Scale Ion Channel Classification

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Ion channels are pore-forming membrane proteins that control the flow of ions across cellular membranes, enabling processes such as electrical signaling, sensory perception, and maintenance of cellular homeostasis. Because they are central to physiology and implicated in numerous diseases, ion channels represent one of the largest and most important classes of drug targets. Among them, Calcium Homeostasis Modulator (CALHM) channels are poorly understood voltage-gated ion channels implicated in neuronal signaling, taste perception, and neurological disease, yet their molecular mechanisms and evolutionary diversity remain largely unexplored. Ion channel genes in the human genome are essential for cellular function and disease, comprising one of the largest classes of druggable proteins, yet limited understanding of their diverse roles hampers development of selective chemical probes. Vast ion channel sequence data across organisms offer untapped potential to reveal unique regulatory mechanisms and functional specializations, but extensive sequence diversification and the lack of a unified resource make these data difficult to use effectively. Here, we integrate sequence, structural, and functional data on 419 human ion channels from disparate sources, combining large language model-assisted literature mining to curate a comprehensive "channelome," and use orthology inference to expand ion channel identification to over 48,000 orthologs across diverse taxa. We show that conservation depth and taxonomic distribution can be translated into functional similarity by clustering ion channels into functionally relevant groups, enabling predictions for understudied members. As proof of concept, we identify co-conserved sequence patterns in the CALHM family and demonstrate, through mutational and electrophysiological analyses, that evolutionarily constrained residues altered in human disease significantly affect channel gating. This work provides new tools and resources for large-scale comparative ion channel analysis, laying the groundwork for functional characterization of "dark" channels and advancing therapeutic discovery.

Poster Presenter Abstract

Converging Pathways of Chemical and Thermal Activation in Nociceptor TRPM3

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Sensing noxious heat is crucial for survival, as it triggers pain responses that help prevent tissue damage. The TRPM3 ion channel plays a key role in detecting noxious heat and is an emerging target for treating pain and neurological conditions like epilepsy. In this study, we combined functional and structural analyses to investigate TRPM3 activation by various stimuli, including heat, the synthetic superagonist CIM0216, and the anticonvulsant antagonist primidone. Our results demonstrate that TRPM3 is inherently dynamic, with its intracellular domain (ICD) sampling both resting and activated conformations, though it prefers the resting state under unstimulated conditions. The superagonist CIM0216 binds to the S1–S4 transmembrane region, causing conformational changes in the ICD that shifts the equilibrium towards channel activation. Notably, heat triggers similar ICD rearrangements, pointing to a shared activation mechanism between thermal and chemical stimuli. Functional data further support this mechanism, showing that mutations enhancing ICD mobility significantly increase TRPM3's responsiveness to both heat and chemical agonists. These findings highlight the ICD as a critical regulator of thermal sensitivity in TRPM3 and suggest this mechanism may be conserved across other TRPM channels. Additionally, we show that primidone competes with CIM0216 at the same binding site but produces an opposite effect and acts as an inhibitor, blocking channel activation. Together, these insights reveal how distinct chemical, and thermal stimuli converge on a common activation pathway in TRPM3 and lay the groundwork for designing targeted therapies. This study not only advances our understanding of temperature sensing in ion channels but also provides a structural basis for the development of TRPM3-specific treatments for pain and neurological disorders.

Poster Presenter Abstract

Development of Novel Isotope Labeling Method for Rapid NMR Resonance Assignment

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10-25% of the human proteome is predicted to comprise of intrinsically disordered proteins (IDPs) but rational drug discovery efforts and high-resolution structural studies are impeded by their dynamic nature. Nuclear magnetic resonance (NMR) spectroscopy is the premier tool for studying IDPs, as it captures their conformational ensembles and dynamic behavior in solution, but spectral overlap and limited resolution pose challenges for large or disordered proteins. An additional complication is the enrichment of IDPs in hydrophilic "disorder-promoting" residues (e.g., SGADEPQ), that greatly increases the number of coincident resonances. This overlap makes it difficult to assign each NMR signal to its corresponding residue, a necessary step for subsequent studies to determine structure, dynamics, ligand binding interfaces, etc. My research focuses on developing a new method, called codon-specific isotope labeling, to solve this problem. This approach employs cell free protein synthesis systems to selectively assign unique isotopologues (e.g. ^{15}N, $^{15}N/^{13}C$, etc.) to each codon of a given amino acid. Each isotopologue produces a unique NMR spectral pattern (e.g. singlet, doublet, etc.) that facilitates rapid assignment. I will undertake proof-of-principle studies using green fluorescent protein (GFP) as a model system to selectively label leucine, serine and glycine residues. I will then apply it to EWS-FLI1, a structurally-understudied IDP that is the oncogenic driver for the childhood cancer Ewing Sarcoma. My approach will drastically reduce the number of required samples and the complexity/acquisition time of NMR spectra. It will be applicable to any biomolecule compatible with cell free expression systems and is hypothetically expandable to all 20 common amino acids –fundamentally expanding NMR resonance assignment. Additionally, this project aims to make these tools more accessible to scientists worldwide, fostering broader involvement in cutting-edge research.

Poster Presenter Abstract

Synthetic Rewiring of Virus-Like Particles via Circular Permutation Enables Modular Peptide Display and Protein Encapsulation

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Virus-like particles (VLPs) are self-assembling nanoparticles derived from viruses with potential as scaffolds for myriad applications. They are also excellent testbeds for engineering protein superstructures. Engineers often employ techniques such as amino acid substitutions and insertions/deletions. Yet evolution also utilizes circular permutation, a powerful natural strategy that has not been fully explored in engineering self-assembling protein nanoparticles. Here, we demonstrate this technique using the MS2 VLP as a model self-assembling, proteinaceous nanoparticle. We constructed, for the first time, a comprehensive circular permutation library of the fused MS2 coat protein dimer construct. The strategy revealed new terminal locations, validated via cryo-electron microscopy, that enabled C-terminal peptide tagging and led to a stable protein encapsulation strategy via covalent bonding – a feature the native coat protein does not permit. Our systematic study demonstrates the power of circular permutation for engineering new features as well as quantitatively and systematically exploring VLP structural determinants..

Poster Presenter Abstract

Optimization of a Multinuclear Nonheme Iron-Dependent Oxidase (MNIO) for Structural Determination

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Nontypeable *Haemophilus influenzae* (NTHi) is a human pathogen implicated in infections and exacerbations of chronic obstructive pulmonary disease (COPD). Recent genomic mining efforts revealed a novel virulence factor, designated HvfA. Our laboratory established that HvfA is a ribosomally synthesized, posttranslationally modified peptide natural product (RiPP). The HvfA precursor peptide is expressed from the *hvf* biosynthetic gene cluster, which also encodes HvfB, a multinuclear nonheme iron-dependent oxidase (MNIO), and HvfC, a RiPP recognition element (RRE)-containing partner protein. The MNIO and its partner protein form a heterodimer that binds the precursor peptide and performs six posttranslational modifications of cysteine residues within HvfA to oxazolone and thioamide groups, yielding the mature virulence factor. Spectroscopic characterization has shown that HvfB contains a trinuclear iron cofactor that is likely responsible for cysteine oxidation, but the structure of the MNIO and its unusual metal cofactor remains unknown. X-ray crystallographic studies have proven intractable due to persistent issues with protein crystallization, so alternative measures have been taken to optimize the MNIO for structural determination by cryogenic electron microscopy (cryoEM). We engineered a terminus-to-terminus genetic fusion (HvfBCfusion) of the MNIO and the partner protein that replicates the native HvfBC heterodimer, enhances catalytic activity, and, importantly, increases the molecular weight to within practical range for cryoEM. Current work focuses on further optimization of HvfBCfusion for cryoEM analysis by integrating a rigid, nanobody-based LegoBody protein scaffold. Employing this macromolecule "carrier" scaffolding system overcomes the molecular weight barrier that excludes small proteins from cryoEM while also circumventing the prototypical structural biology bottleneck of protein crystallization. Determining the structure of HvfBCfusion will offer new insights into the emerging MNIO family of enzymes involved in the production of diverse RiPP natural products.

Poster Presenter Abstract

Transcription-based Affinity Biosensors for Electrochemical Analysis of Steroid Hormones

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Biomolecular sensing systems have enabled quantification of health-related biomarkers, facilitating early disease diagnosis, treatment, and management. However, direct measurement of small molecule targets, such as hormones, neurotransmitters, and metabolites, remains a challenge due to their small size and lack of compatible receptors. In this work, the programmability of transcription factors (TFs) was leveraged with an electrochemical sensing approach that enabled detection of small molecules (steroids). To achieve this, monolayer functionalization was adapted to integrate TFs as affinity receptors into the previously reported molecular pendulum platform. In this way, a target molecule allosterically binds to a TF receptor, induces TF dissociation from its DNA operator, and produces a "signal-off" detection mechanism. Decreasing faradaic current due to TF dissociation allows for small molecule measurement with chronoamperometry. Using this strategy, the steroid-responsive transcription factor (SRTF1) receptor enabled electrochemical detection of progesterone at concentrations as low as 100 picograms per milliliter. Moreover, SRTF1 variants with expanded target specificity towards cortisol were generated by directed evolution, demonstrating the scalability of this platform. Future work is underway to further evolve TFs with programmable specificities for desired small molecules, introduce a linker to tether the TF to the surface for reagentless sensing, and validate detection in complex biofluids such as human saliva and serum.

Poster Presenter Abstract

Measuring Protein Stabilities in High Throughput Using cDNA Display and Pulse Proteolysis

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Protein folding stability is a challenge facing many biological nanotechnologies, as engineered proteins need to remain folded and active across a wide range of conditions, including temperature, pH, and mechanical stresses that can occur in manufacturing or at the application site. But it is difficult to engineer proteins for stability due to the lack of a deep understanding of how specific amino acids contribute to protein folding and stability. There is a need for large amounts of protein stability data so we can build better machine learning models to predict protein folding stability based on sequence. To overcome the low throughput of traditional methods of measuring protein stability, we are designing a novel method using cDNA display and pulse proteolysis to measure the global folding stabilities of thousands of proteins at a time. Our goal is that with this new method we will curate a large dataset of protein folding stabilities and use machine learning to uncover useful sequence and structural properties of stability that will aid in future protein design.

Poster Presenter Abstract

A Facilitated, Proton-Driven Pathway for Copper Import in Prokaryotes: Functional and Spectroscopic Analysis of the Mst OB3b Copper Transporter, CopD

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Copper is an essential cofactor for enzymes involved in respiration and redox homeostasis/defense, and thus organisms have developed elaborate copper-handling pathways to ensure proper transport and delivery to cuproenzymes while preventing cytotoxicity. Recent studies of copper transport have focused primarily on export and copper trafficking in the periplasm of prokaryotes. In contrast, the mechanisms for copper import into the cytoplasm remain uninvestigated: this process has been viewed as unnecessary since most cuproenzymes are extracytoplasmic. Increasing evidence suggests that periplasmic cuproenzymes are loaded with copper that is first trafficked into the cytoplasm, followed by metalation via transport ATPases in the inner membrane. The CopD protein family, comprising of hypothesized inner membrane copper transporters, has been implicated along with CopC proteins in bacterial copper homeostasis and resistance. Here we show that overexpression of CopD from the methanotroph Methylosinus trichosporium OB3b leads to overaccumulation of copper in vivo, with a 2-3x selectivity for copper over other metals. Furthermore, two highly conserved histidine residues and one tryptophan residue located along the proposed ion conduction pathway are critical for import, and an additional soluble domain linked to the transport domain is required for full functionality. In vitro studies of CopD in proteoliposomes show that the kinetics of metal transport are very fast and specific to copper ($K = 2.13 \pm 1.25$ μM), and that proton concentrations in the liposome change upon translocation, suggesting a facilitated ion symporter mechanism. These results are consistent with the CopD family playing a role in bacterial copper import, and further characterizing this transport pathway will fill an important gap in bacterial copper homeostasis.

Poster Presenter Abstract

Gentlest Ascent Dynamics for Enhanced Sampling and Rapid Energy Landscapes Exploration

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The exploration of molecular configuration space is often hindered by thermally activated barriers on complex, high-dimensional potential energy surfaces. These barriers make conventional molecular dynamics (MD) simulations inefficient for accessing rare but important transitions. Enhanced sampling techniques based on collective variables (CVs) are commonly used to overcome this limitation. However, they typically require prior knowledge of the system's slow modes, which can be difficult to determine for large or disordered systems. In this work, we present a method based upon gentlest ascent dynamics (GAD), a technique originally developed to locate index-1 saddle points. GAD computes the Hessian matrix to identify the eigenvector corresponding to the lowest eigenvalue—the direction of minimum curvature. It then projects this direction out of the force vector, effectively steering the system away from local minima and toward saddle points. We repurpose this idea to create a finite-temperature framework called gentlest ascent dynamics for enhanced sampling (GADES). Instead of searching for saddle points, GADES flattens the energy landscape along the minimum curvature eigenfunctions to eliminate energy barriers and promote efficient diffusive dynamics between system critical points (i.e., minima and saddles). Importantly, GADES does not require predefined CVs or prior knowledge of the energy surface. The first phase of GADES promotes rapid sampling of the important metastable states of the system, but, since the dynamics do not evolve according to a gradient potential, they do not admit reweighting to the Boltzmann distribution (i.e., Gibbs measure). In the second phase of GADES, the trajectories are used to uncover high-variance or slow CVs via data-driven nonlinear dimensionality reduction methods such as diffusion maps, autoencoders, or deep time-lagged independent component analysis, and learned CVs are then implemented within efficient off-the-shelf CV-biasing enhanced sampling techniques such as metadynamics to recover a low-dimensional, system-specific embedding of the molecular system and the Boltzmann distribution over this projection. We validate GADES in molecular dynamics simulations of a number of biomolecular systems of varying size and complexity to demonstrate its capacity to efficiently navigate and sample molecular energy without requiring any a priori knowledge of CVs or critical points.

Poster Presenter Abstract

The Effects of Cleavage Deficiency on Adhesion GPCR Dynamics

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Survival of cells depends on receiving information from their environment and responding accordingly. In eukaryotes, one of the main pathways for cell signaling is through G-protein coupled receptors (GPCRs), which constitute the largest family of cell surface receptors in humans. Due to their widespread expression and involvement in almost every physiological process, GPCRs have become the largest family of drug targets, accounting for over 30% of FDA approved drugs. Therefore, understanding their mechanism of activation and signaling modulation is important for the design of therapeutics. My research is focused on adhesion GPCRs (adGPCRs), an understudied family of GPCRs involved in a unique form of mechanosensing. Canonical activation relies on an autoproteolytic cleavage event that enables the force-induced shedding of the extracellular region, resulting in constitutive activity. Attempts to resolve structures of full-length aGPCRs often utilize a cleavage deficient mutation that prevents autoproteolytic cleavage, assuming no additional structural perturbation on these proteins. Unfortunately, these structures consistently fail to capture the orientations of the ECR with respect to the 7TM and have yet to achieve atomic resolution. There is ample evidence that ECR conformations allosterically regulate the signaling of these receptors, but little understanding of how specific conformations and dynamics translate to signaling levels or if specific conformations bias receptors to specific pathways. My approach is uniquely suited to investigate the fascinating dynamic relationship between the ECR and the 7TM domain and elucidate the effects of cleavage deficiency on these dynamics. Using a combination of single-molecule FRET (smFRET), protein engineering, and functional assays, I have uncovered significant differences between the conformational dynamics and range of motion in these cleavage-deficient receptors compared to their wild-type counterparts. Therefore, treating these mutations as simple covalent linkages should not be considered a reliable strategy.

Poster Presenter Abstract

A Single-Molecule Approach to Observe Domain Movement Dynamics in Type IA Topoisomerases

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DNA topoisomerases are responsible for maintaining the integrity of genomes by relaxing supercoiled DNA. For Type IA topoisomerases, this is done by using a strand-passage mechanism. While much about the mechanism has been discovered, the conformational changes that allow for these actions are still under investigation. There are two current hypotheses regarding these dynamics: in one model, Domain III of the enzyme (which contains the active site) moves far away (~6 nm) from the other domains to allow for strand passage; in another model, there is a smaller conformational change that brings domain III closer to the other domains to push the strand through. Here I report the results of single-molecule experiments to help validate these models. We use Single-molecule Fluorescence Resonance Energy Transfer (smFRET) experiments observe the dynamics of a fluorescently tagged enzyme in the presence and absence of DNA. Since smFRET is highly sensitive to changes in distance, we are able to directly observe changes in conformation, giving more insight into the dynamics of Type IA topoisomerases.

Poster Presenter Abstract

Comprehensive Structural and Functional Characterization of a Key Virulence-Regulatory Transcription Factor in *Streptococcus pneumoniae*

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Streptococcus pneumoniae is a gram-positive, opportunistic pathogen, well known as the major cause of bacterial pneumonia worldwide. It asymptomatically colonizes the nasopharyngeal region of the upper respiratory tract and spreads to the lungs, initiating the hallmark infection associated with pneumonia by exploiting weakness in the hosts' immune system. Beyond this primary infection of lung epithelial tissue, *S. pneumoniae* then enters the blood stream to colonize other tissues, such as the heart or central nervous system, causing sepsis or meningitis, respectively. Previous studies demonstrate the switch from commensal to pathogenic infection, and the severity of said infection, as controlled through the regulation of bacterial capsule synthesis. Glanville et al. has previously identified a broadly conserved transcription factor, SpxR, that regulates capsule synthesis through a distal cis-regulatory element containing a 12 base-pair inverted repeat motif, termed 37-CE. However, the biophysical basis by which SpxR regulates pneumococcal capsule formation remains unknown; therefore, elucidation of this would provide valuable insights into future antibacterial drug development that targets this essential virulence mechanism. Current efforts utilize mass photometry and single-particle cryo-electron microscopy (cryo-EM) to elucidate the mechanism by which DNA binding and capsule transcription regulation occurs. Recent mass photometric data have led to the discovery of a stable, tetrameric conformation of the *Staphylococcus aureus* SpxR homologue bound to the 37-CE cis-regulatory element. This has prompted a redoubled cryo-EM grid optimization effort to ultimately solve the structure of SpxR bound to the cis-regulatory element. Subsequent aims seek to unveil the relationship between SpxR transcription regulation and cellular energetics, namely through biophysical interrogation of the interactions between SpxR and 5'-Phosphoadenylyl-(3'à5')-adenosine (pApA). pApA is hypothesized to be the downstream signaling molecule responsible for the dissociation of SpxR from the cis-regulatory element, and understanding its role as a ligand for SpxR has many broader implications regarding pneumococcal capsule regulation and the *in vivo* stimuli associated with it.

Poster Presenter Abstract

Ezrin Activation Requires Membrane Binding via PI(4,5)P_2 to Release the Phosphorylated C-Terminus

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Ezrin is a peripheral membrane protein that regulates tension at the plasma membrane via reversible linkage to the actin cortex. This reversible linkage is regulated by a phosphorylation site in the ezrin C-terminal domain (CTD), inducing a conformational change that releases the CTD from an autoinhibited state where it is bound to the FERM domain. However, the molecular details of this process, including the purported role of the phosphoinositide lipid PI(4,5)P_2, remain obscure. Here, we examine the mechanistic steps of ezrin activation and thermodynamic free energy profiles of FERM-CTD dissociation via atomistic molecular dynamics. We find that upon ezrin binding to a model lipid membrane, the presence of PI(4,5)P_2 stabilizes attachment of the FERM F1 and F3 subdomains but destabilizes the F2-CTD interaction. Further, through well-tempered contact-map metadynamics simulations we find that the barrier to CTD domain dissociation from the FERM domain comes from CTD-F3 interactions, and that the barrier for reassociation is much higher when the CTD is phosphorylated; we also show this mechanism of association is similar for EBP50-ezrin interactions. A revised mechanism of ezrin activation is proposed which suggests that upon binding to a membrane containing PI(4,5)P_2, the CTD is transiently associated with the FERM domain until T567 phosphorylation by the LOK kinase, after which it is no longer able to associate with the FERM domain and is enabled to bind to filamentous actin.

Poster Presenter Abstract

Assessing Protein-Lipid Interactions with a Low-Cost, Accessible Centrifugation Assay

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Assessing protein insertion and association with membranes is often a critical step that follows protein synthesis for both fundamental studies on protein folding and structure as well as translational applications that harness proteins for their activity. Traditionally, membrane protein association with membranes involves ultracentrifugation, which can be time-consuming and inaccessible in low-resource scientific environments. In this study, we develop an accessible method to purify vesicle-integrated cell-free expressed proteins from unincorporated protein or lysed membranes. We use a table-top microcentrifuge, capable of reaching speeds up to 21,130 × g, and a sucrose gradient to effectively separate the bulk of the cell-free expression components from proteoliposomes. We validate our approach can be used to measure membrane association of a variety of proteins, such as peripheral and transmembrane proteins as well as lipid-specific proteins, and our method can be extended to membrane proteins derived from cellular membranes. Our approach provides a more accessible, cost-effective, and low-volume alternative for isolating proteoliposomes from misfolded and unassociated membrane proteins that should be applicable for fundamental biophysical studies and applications involving cell-free expressed membrane proteins.

Reference: Villaseñor, Citlayi G., et al. “Assessing protein-lipid interactions with a low-cost, accessible centrifugation assay.” Biophysical Reports, vol. 5, no. 3, Sept. 2025, p. 100224, https://doi.org/10.1016/j.bpr.2025.100224.

Poster Presenter Abstract

Measuring Spycatcher Reactivity In High-Throughput

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Understanding how a protein's amino acid sequence controls biophysical properties like stability, folding, and reactivity is key to designing better enzymes. However, this task remains challenging due to the complexity of protein conformational ensembles and the scarcity of functional data across sequence space. Large-scale experiments can help uncover how specific residues and higher-order interactions affect function, offering the basis for improved computational models and better design strategies. Combining high-throughput functional mapping with de novo design of new enzymes has been out of reach due to the size and complexity of enzymes typically targeted. Therefore, we aim to map the sequence-function landscape of SpyCatcher, a small reactive protein that forms a covalent bond with its substrate SpyTag, investigating both natural and de novo sequences. While not a true enzyme, SpyCatcher's small size (81 residues) and simple mechanism make it a tractable minimal model for enzyme-like reactivity. We developed a cDNA-display-based assay to assess SpyCatcher reactivity and applied it to measure reaction rates for a deep mutational scanning library of approximately 1,800 SpyCatcher variants. We resolve reaction rates across ~5 orders of magnitude. We also show that reaction rates depend on SpyTag concentration, a key factor in translating observed rates into kinetic parameters, and we measure folding stability. Efforts to design de novo SpyCatchers and to verify rate measurements in low-throughput are ongoing. In the future, we will use cDNA display-based assays to measure the reactivity, substrate binding kinetics, and stability of ~ 1 million SpyCatcher variants, both natural and de novo designed. By producing interpretable data that links sequence to biophysical properties, we aim to enhance our ability to design proteins with tailored functions.

Poster Presenter Abstract

Insights into GapR Protein-DNA Interactions: Supercoiling and Stiffening Effects Revealed by Magnetic Tweezers

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Understanding how proteins interact with DNA is central to explaining chromosome structure during key processes such as replication and transcription. Here we examine the bacterial protein GapR, noted for its preference for overtwisted DNA. Although in vivo work has shown GapR in stabilizing the positive supercoils generated during these processes, the underlying biophysical features of GapR–DNA interactions have been poorly characterized. Using magnetic tweezers, we uncover how GapR reshapes DNA topology. Binding of GapR to a 10-kb pFOS1 fragment drives overwinding, shifting the DNA's superhelical density by ~1.5%~1.5%. The effect is strongly cooperative: a titration model fits the data with an apparent Kd of 450 nM and a Hill coefficient of 3.2. GapR also stiffens DNA. Force–extension measurements show the persistence length increasing from ~40 nm to ~75 nm upon binding, indicating a substantial mechanical reinforcement. Further more, GapR also increases the twist persistence length of dsDNA making the protein more resistant to tortional stress. These results suggest a robust structural role for GapR in stabilizing overtwisted DNA in safeguarding chromosome integrity during replication and transcription. Together, these findings clarify how GapR modulates both DNA topology and mechanics. They set the stage for broader investigations into GapR's contributions to bacterial chromosome organization and its crosstalk with other DNA-active enzymes, including type II topoisomerases.

Poster Presenter Abstract

Intracellular Sodium Dynamics and Ion Translocation Kinetics in Na^+/K^+-ATPase Mutant Neurons

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The Na^+/K^+-ATPase is an ion pump essential for maintaining electrical stability by coupling the hydrolysis of ATP with the transport of sodium ions out and potassium ions into cells. Specific mutations in the catalytic subunit of the neuronal Na^+/K^+-ATPase gene (*ATP1A3*) cause a rare condition called alternating hemiplegia of childhood (AHC), a neurological disorder marked by episodes of transient paralysis, seizures, abnormal brain development, and severe long-term consequences. It is not clear how *ATP1A3* mutations affect intracellular Na^+ (Na^+_i) dynamics or ion translocation kinetics, but it has been shown that ATP1A3 is essential to return $[Na^+]_i$ to basal levels after periods of high activity and that mutations can impair the ability of neurons to compensate for activity-dependent rises in $[Na^+]_i$. *We hypothesize* that AHC mutations in *ATP1A3* render neurons unable to recover from high intracellular Na^+ loads, predisposing the cells to apoptosis through secondary calcium overload and neurotoxicity. Through the study of AHC mouse model cerebellar Purkinje neurons, a cell type highly dependent on ATP1A3, we will examine Na^+/K^+-ATPase pump current and neuronal activity using whole cell patch clamp recording. We will also investigate changes in Na^+/K^+-ATPase enzymatic activity through assays of ATP hydrolysis and assess the kinetics of $[Na^+]_i$ overload and restoration to basal levels using a Na^+ sensitive dye in tandem with GCaMP measurement of intracellular calcium concentration. Because Na^+ dyes are incompatible with intact systems (e.g. brain slices), we will develop a genetically-encoded $[Na^+]_i$ biosensor based on the Na^+-binding KCNT1 ion channel. This will improve on available Na^+ dyes by allowing for investigation of the consequences of ion translocation defects and activity-dependent increases in $[Na^+]_I$ *in vivo*. Through this work, we will better understand the pathophysiology of ATP1A3-related disorders and create a novel molecular tool to study Na^+ in a variety of systems.

Poster Presenter Abstract

Predictive Modeling of Riboswitch Function through Integrating Biophysical Mechanism into Machine Learning Models

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RNA regulates gene expression through dynamic folding into structures that guide molecular recognition and cellular decisions. Predicting how sequence encodes these dynamic structures and functions remains a central challenge in molecular biophysics. Riboswitches, structured noncoding RNAs that sense small molecules and undergo conformational changes, provide a powerful model system to address this challenge. Their regulation depends on energy landscapes, ligand interactions, and transcriptional timing, underscoring the fundamentally biophysical basis of their function. Advances in massively parallel assays such as Sort-Seq and TECdisplay, combined with high-throughput RNA structure probing (DMS, SHAPE), now enable comprehensive mapping of sequence–structure–function relationships. Yet, unified predictive models that incorporate these mechanisms remain lacking. This project seeks to integrate biophysical principles into machine learning frameworks to achieve predictive modeling of riboswitch function. Models will be developed to predict ZTP riboswitch activity from sequence while incorporating folding energetics, co-transcriptional kinetics, and base-pair stability to capture how intermediates and ligand-guided folding shape function. This framework will then be expanded with high-throughput structure probing data to explicitly encode folding landscapes, improving predictive accuracy and interpretability. Finally, transfer learning across diverse riboswitch classes will assess model generalizability and uncover conserved regulatory principles.By embedding biophysical mechanisms into graph neural networks trained on high-throughput functional datasets, this work aims to uncover physical principles of RNA regulation and build interpretable predictive tools for rational riboswitch design, with broad implications for diagnostics and therapeutics.