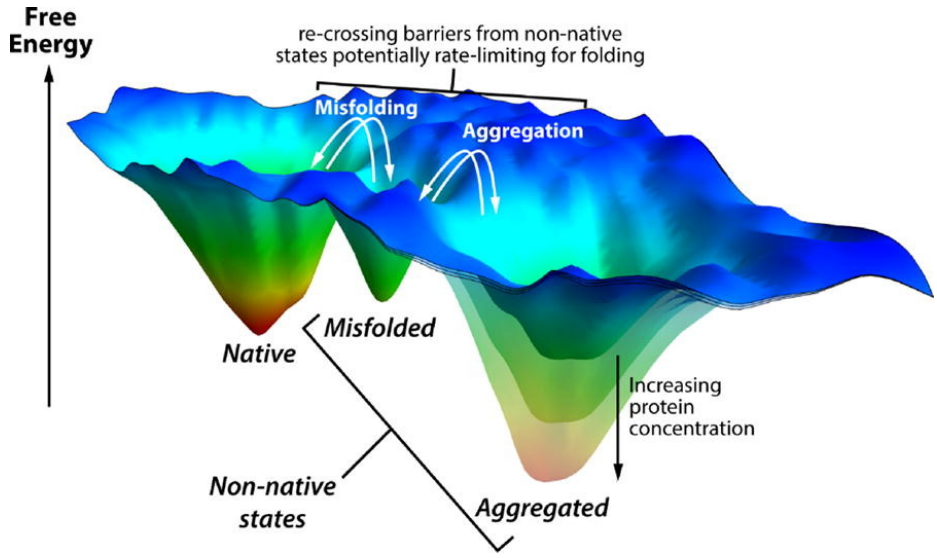
https://mbnmeeting.org

# Molecular Biophysics in the Northeast (MBN2024)

at UMass Amherst on April 13, 2024

# Program




(Powers and Gierasch, 2021)

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# Event Description

The second *Molecular Biophysics in the Northeast* (MBN2024) meeting will provide an opportunity for members of the experimental and theoretical molecular biophysics community in the northeastern region to discuss current research topics. This event will emphasize the engagement of trainees (from undergraduates to post-docs) through oral presentations (selected from abstracts) and posters. The majority of the oral presentations will be given by students and post-docs, and there will be more than 80 posters presented throughout the day. This year, we have over 170 registered participants, representing over 20 colleges, universities and research institutes. It is our hope that, by bringing together a diverse group of researchers, this event will help the field bridge our understanding of the relationships between physical principles, molecular structure and biological function.

We would like to acknowledge very generous supports from the **Department of Chemistry** and **the Model to Medicine (M2M) Center** of the Institute for Applied Life Sciences (IALS) at the University of Massachusetts Amherst, which made this event possible. We also acknowledge IT support from Northeastern University.

**Organizing Committee:**

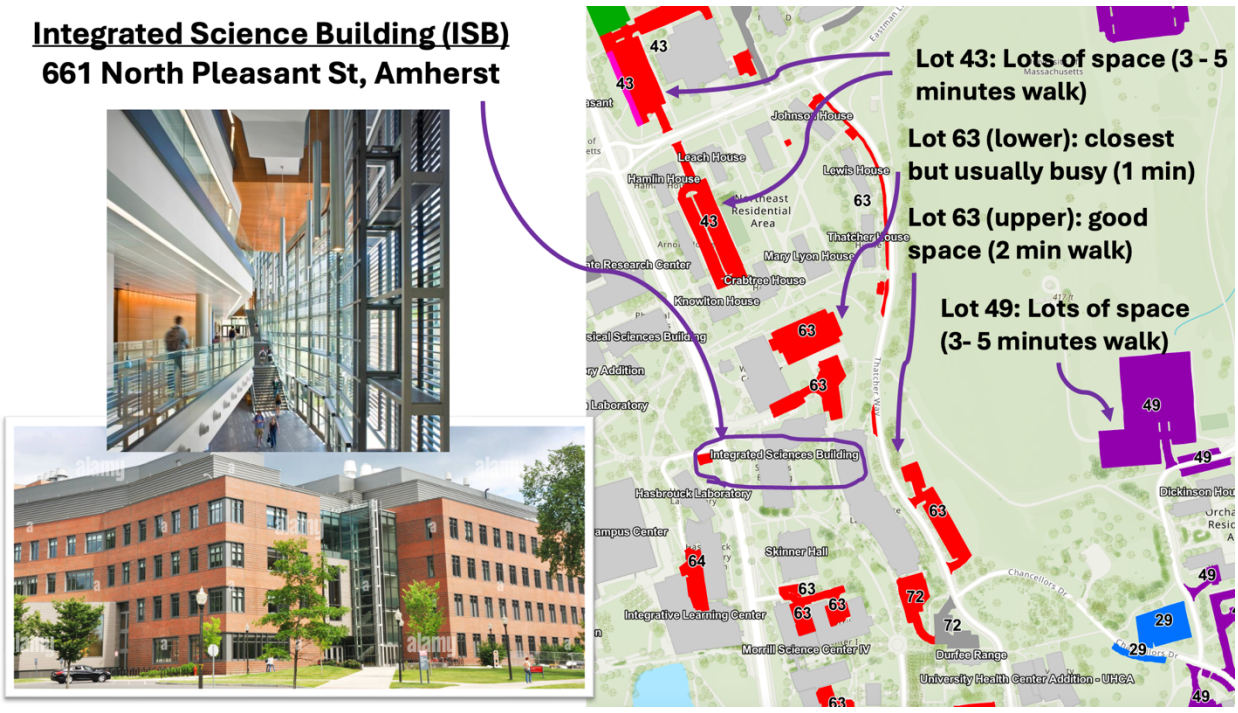
Jianhan Chen (Chair) - University of Massachusetts Amherst
Sijia Dong - Northeastern University
Eric May - University of Connecticut
Krisztina Varga - University of New Hampshire
Paul Whitford - Northeastern University

# Arrival Information:
# Event Location and Parking Details

**Venue**: The conference will be held in the **Integrated Science Building (ISB)** on UMass Amherst campus. All talks will be in **ISB 135** (our gen chem auditorium) and posters will be set up in the Atrium (first floor) as well as corridor spaces on the second and third floor. Additional information about the building can be found at: https://www.umass.edu/cp/integrated-sciences-building

**Driving direction**: you can enter "Integrated Science Building, UMass" in Google Map to navigate directly to ISB. The street address is: 661 N Pleasant St, Amherst, MA 01003

**Parking**: *Free on campus during weekends*! Isn't that awesome? You can park in any space that does NOT have additional restrictions. Please pay attention to the signs at the entrance to the parking lot and in front of the parking space. The closest lots to ISB are marked above. **Lot 43 will probably be the best one** with a lot of space and is 3-5 minutes of easy walk from ISB.



# Molecular Biophysics in the Northeast (MBN2024)

at UMass Amherst on April 13, 2024

## Opening Session

**9:00 - 9:30** Check in, coffee and poster setup
**9:30 - 9:35** Welcome (Jianhan Chen, UMass Amherst)
**9:35 - 9:45** Introduction of IALS and Keynote Speaker (Peter Chien, UMass IALS M2M Director)
**9:45 - 10:15** Opening Keynote Speaker : Lila Gierasch (UMass Amherst)
*How Hsp70 molecular chaperones recognize substrates that need their help: Selective promiscuity*

## Topical Session 1: Dynamic interactions of proteins (Chair: Xinqiang Ding, Tufts)

**10:20 - 10:40** Haribabu Arthanari (Harvard University)
*A tale of tails: The functional role of disordered regions in the human proteome*
**10:45 - 10:55** Nabin Kandel (RPI)
*Tau and Lipid Bilayer Interactions Are Driven by Electrostatics and Inhibited by Cholesterol*
**10:58 - 11:08** Yeseul Lee (UMass Amherst)
*Push-and-pull mechanism of single-molecule movement of alpha-Synuclein under nano-confinement*

**11:10 - 11:30: Coffee Break**

## Topical Session 2: Biomolecular folding and assembly (Chair: Sweta Vangaveti, SUNY Albany)

**11:30 - 11:50** Ashley Carter (Amherst College)
*DNA folding with protamine*
**11:55 - 12:05** Anna Bock (Brown University)
*Characterizing the conserved pH sensor in the yeast prion protein Sup35*
**12:08 - 12:18** George Makhatadze (RPI)
*Experimental and computational studies of the effects of high hydrostatic pressure on biomolecular interactions*

## Lunch and Poster Viewing (12:20 - 2:30)

We ask that presenters be available at their posters from **1:00-1:45 (odd #)** or **1:45-2:30 (even #).**

## Topical Session 3: Drug and biologics design (Chair: Sean Edington, UNH)

**2:30 - 2:50** Paul Robustelli (Dartmouth College)
*Targeting Intrinsically Disordered Proteins and Biomolecular Condensates with Small Molecule Drugs*
**2:55 - 3:05** Jeffrey D. Tamucci (UConn)
*Powering the powerhouse: MD simulations illustrate how mitochondria-targeted tetrapeptides alter membrane electrostatics and have the potential to improve mitochondrial bioenergetics*
**3:08 - 3:18** Jeanne Hardy (UMass Amherst)
*Harnessing protease reactions for detection and treatment of viral infection*

## Topical Session 4: Enzyme function and engineering (Chair: Constance J Jeffery, UIC)

**3:20 - 3:40** Erika Taylor (Wesleyan College)
*Aminoglycosides Revisited: Evidence for Inhibition of Heptosyltransferase I from Escherichia coli*
**3:45 - 3:55** Bhumika Jayee (Northeastern University)
*Computational study of a red light-driven reaction in a flavin-dependent photoenzyme*
**3:58 - 4:08** Bao Nguyen (UMass Amherst)
*Mechanistic insights into Ca2+ sensitivity regulation of CaMKII*

**4:10 - 4:30: Coffee Break**

## Closing Session

**4:30 - 4:35** Keynote Introduction (M. Muthukumar, UMass Amherst)
**4:35 - 5:05** Closing Keynote Speaker : Ken Dill (SUNY Stony Brook)
*The Origins of Life: An uncanny resemblance to the old Protein Folding Problem*

**5:10** Closing remarks/poster removal

# Keynote Speakers

Ken A. Dill is the Laufer Family Endowed Chair of Physical and Quantitative Biology, and Distinguished Professor of Physics, Chemistry and Applied Math at Stony Brook. He is interested in the physics of how proteins fold; the microscopic origins of the unusual physical properties of water; the foundations and applications of variational entropy-based principles in statistical physics; and how the laws of physics constrain and enable the biological properties and evolution of cells. A graduate of MIT ’71, Dill received his PhD from UCSD under the guidance of BH Zimm and postdoctoral training at Stanford with PJ Flory. Dill was a faculty member at University of Florida, University of Utah, and UCSF prior to his move to Stony Brook as the founding director of Laufer Center for Physical & Quantitative Biology in 2011. He is a member of National Academy of Science and a fellow of American Academy of Arts and Sciences.

Lila M. Gierasch is a Distinguish Professor Emeritus of Chemistry, Biochemistry and Molecular Biology at the University of Massachusetts Amherst. She is interested in understanding how protein folds in vitro and in vivo; how molecular chaperones facilitate the folding, trafficking, assembly and disassembly, and degradation of proteins and protein complexes; and the origins and mechanisms of protein aggregation in vivo and their roles in misfolding-based diseases. Her research employs a wide range of biochemical and biophysical approaches, particularly, nuclear magnetic resonance, as well as computer modeling and simulation. A graduate of Mnt Holyoke ’70, Gierasch received her PhD from Harvard under ER Blout in 1975. She was a faculty member at Amherst College, University of Delaware, and UT Southwestern prior to her move to UMass Amherst as the Head of Chemistry in 1994. She is a member of National Academy of Science and a fellow of American Academy of Arts and Sciences.

# Presenter Instructions

**Posters**

Pushpins will be provided to all poster presenters. Each poster will have an area of at least 36" x 48" (landscape, or portrait). If you need a larger display area, please let us know. We have some boards that are larger, and they will be available on a first-come, first-serve basis. Just let us know via email if you need a larger board, so that we can accommodate your needs.

Posters will be on display in the Integrated Science Building. When you arrive in the morning, you will be assigned a specific board. It is recommended that you arrive promptly at 9am, to ensure you have enough time to check in and hang up your poster. Posters should be removed after the Closing Session.

**Speakers**

With the full schedule that we have planned, it is important that we smoothly transition between talks and minimize technical delays. Unfortunately, if it takes too long to begin your presentation, we may have to skip your talk (we want to avoid this). If you are speaking, you may use a shared machine (preferred), or your own computer.

*If you would like to use a shared machine:* Please send your talk via email, Google Drive, or OneDrive, to Paul Whitford (p.whitford@northeastern.edu), no later than 12pm on Friday, May 12th.

*If you plan to use your own machine:* Please be prepared to connect via VGA *and* HDMI. We ask that you are prepared for both, in case of any technical issues (we all know they happen at the worst times!).

Regardless of how you plan to present, it is recommended that you have a back-up with you on a thumb drive, so that you can promptly change devices, should that be necessary. In addition, in order to remain on schedule, it is very important that you test your presentation in the auditorium at the designated time window below:

Speakers in the Opening Session or Topical Session 1 - 9:00-9:30 am
Speakers in Topical Session 2 - 11:10-11:30 (morning coffee break)
Speakers in Topical Sessions 3 or 4 - 2:00-2:30 (end of lunch/poster period)
Speakers in the Closing Session - 4:10-4:30 (afternoon coffee break)

# Poster Titles

Below is the list of presenters and poster titles, ordered alphabetically by the first author name. Full author lists and abstracts may be found at the end of this document.

| Poster # | First Author | Affiliation | TItle |
|---|---|---|---|
| 1 | Amanda Ferrante | Northeastern University | Elucidating the Effects of Cholesterol on the Function of Surfactant Protein C Using Atomistic Molecular Dynamics |
| 2 | Amin Abek | UMass Amherst | Unraveling Pausing and Termination in RNA Synthesis: Mechanistic Model and Practical Applications |
| 3 | Amit Shenoy | Northeastern University | Extracting the Binding Affinities Between Various Viral Glycans and MBL via Molecular Docking |
| 4 | Anjali Dhar | Dartmouth College | Ensemble-based molecular docking of small molecule ligands on the disordered protein a-synuclein |
| 5 | Ann Titus | Northeastern University | Modeling the Vapor-Liquid Equilibration Curve of Pentadecanoic Acid Using Gibbs Ensemble Monte Carlo Simulations |
| 6 | Aparajita Chakraborty | Dartmouth College | Identifying ligand binding modes of small molecules binding to $\alpha$-Synuclein |
| 7 | Aryaman Tapal | UMass Amherst | Exploring the Fusion of Quantum Computing and Chemistry with TeraChem's Exciton Model |
| 8 | Atif Shafique | Northeastern University | How do catalytic lysines and glutamates in enzymes gain their catalytic properties? |
| 9 | Chengeng Yang | University of Connecticut | Coarse-grained models of elastin assemblies |
| 10 | Eugenia M. Clerico | UMass Amherst | The basis of substrate binding by Hsp70 and its impact on cellular functions |
| 11 | Constance (Connie) Jeffery | University of Illinois at Chicago, Northeastern University (Sabbatical) | Metabolic Enzymes Moonlighting as RNA Binding Proteins |
| 12 | Deng LI | Northeastern University | Computational modeling of the binding mechanisms of surfactant protein D (SP-D) with glycans |
| 13 | Aaron Gomez Feinstein | University of Connecticut | Explicating auto-phosphorylation and high frequency dynamics of human PKR |
| 14 | Elika Shams | University of Connecticut | Investigating the Structural Consequences of a Tropoelastin Mutation through Classical Atomistic Molecular Dynamics Simulation |
| 15 | Eric D. Sakkas | Wesleyan University | Fingerprinting Base Stacking Systems |
| 16 | Felipe Curtolo | Northeastern University | Probing substrate binding in a light-activated enzyme: A bias-exchange metadynamics study of Caulobacter segnis ene-reductase |
| 17 | George Wanes | Northeastern University | Quantifying the effects of ionic environment on the dynamics of biomolecular assemblies |

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| **18** | Gustavo Mondragón-Solórzano | Northeastern University | Effect of aromatic amino acids in the stabilization of the photoactive electron donor-acceptor complex in the Gluconobacter-ene oxidoreductase. |
| **19** | Hana Zeghal | SUNY Albany | The Switchback DNA: Structural Insights and Implications in Trinucleotide Repeat Disorders through Molecular Dynamics Simulations |
| **20** | Hayley Cline | Mount Holyoke College | Characterization of heat shock protein expression from mouse brain tissue |
| **21** | Heather Omoruyi | Northeastern University | Investigating Cancer Association of ERK2 Mutations: Computational Analysis of ERK2 Catalytic Pathway |
| **22** | Helen Danielson | Brown University | Insights into the Structural Characteristics of TDP-43's Disordered C-terminal Domain within a Condensed Phase |
| **23** | Isabella Jankowski | UMass Amherst | Hydrogen Deuterium Exchange Mass Spectrometry of the CheW Coupling Protein in E.coli Chemoreceptor Complexes |
| **24** | Jamuna K. Vaishnav | UMass Amherst | Synthesis and quality assessment of in vitro RNA – novel synthesis and analytical approaches |
| **25** | Janis Dong | Northeastern University | EnCoPhAC-DB and PDB-LIKE: Identifying photoenzyme candidates through database creation and analysis |
| **26** | Jason Kantorow | Northeastern University | An Algorithm for the Detection and Correction of Buried Glycan Structures Toward Improved in Silico Viral Immunogen Design |
| **27** | Jessica Allen | UMassAmherst | Solid-State NMR Reveals Signaling-Related Changes in Functional Chemotaxis Receptor Complexes |
| **28** | Jian Huang | UMass Amherst | PIP2 Regulation of the TRPV4 Channel: Binding Sites and Dynamic Coupling |
| **29** | Jiaqi Zhu | Dartmouth College | A small molecule drug stabilizes the intermolecular association of a phase separating fragment of the disordered androgen receptor transactivation domain |
| **30** | José F. Mercado Ortiz | Brown University | Impact of ALS-associated mutations in a C-terminal "hot-spot" on TDP-43 phase separation and aggregation |
| **31** | Julia Zaborowsky | Brown University | Probing the sequence determinants of phase separation with engineered synthetic FUS. |
| **32** | Jyoti Verma | University of New Hampshire | Molecular Basis for Allosteric Inhibitor Selectivity in IGF-1R kinase |
| **33** | Kairong Dong | UMass Amherst | Linker Effects on CaMKII Dodecamer Dynamics |
| **34** | Katherine Wahlbeck | UMass Amherst | Regulation of ATP affinity to the bacterial chemotaxis kinase CheA |
| **35** | Korey M. Reid | Dartmouth College | Investigation into the influence of 1,6-hexanediol on protein dynamics and solvation, insight into phase-separation propensity of disordered proteins via solvation theory |

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| **36** | Kuo Chen | UMass Amherst | Kinetic Analysis of Thermally Induced Aggregation of Native Human Gamma-D Crystallin |
| **37** | Leila Sharifi | University of Connecticut | Molecular Modeling of AAV Capsid-capsid Interaction Under Different Salt Concentrations, and Surfactants to investigate the aggregation |
| **38** | Lev Levintov | University of New Hampshire | Conformational Effects Associated with Chemical Modifications in RNA |
| **39** | Logan Brown | University of New Hampshire | Characterization of an Antifreeze Protein, ApAFP752 |
| **40** | Madeline Clark | Brown University | Defining the Molecular Mechanisms of HuR- and MiR29a-Mediated VEGF-A mRNA Stabilization |
| **41** | Mahmoud Sharawy | University of Connecticut | Elucidating the pH-Dependent Dynamics of Enterovirus D68 Capsid Using Continuous Constant pH Molecular Dynamics |
| **42** | Mary Jo Ondrechen | Northeastern University | AI for prediction of protein function and for elucidation of enzyme function and effects of mutations |
| **43** | Maxwell He | Northeastern University | Enhancing Antibody-Antigen Interaction Sampling with Molecular Dynamics and Docking Simulations |
| **44** | Michael Weir | Wesleyan University | GNN codon adjacency regulates protein translation |
| **45** | Minglun Li | UMass Amherst | RNA Translocation through Protein Nanopores: Unfolding of Secondary and Tertiary Structures |
| **46** | (cancelled) | | |
| **47** | Natesan Mani | Northeastern University | Investigating the Impact of Furin Cleavage on SARS CoV-2 Spike Structure with Molecular Dynamics Simulations |
| **48** | Ned Debold | UMass Amherst | Myosin's affinity for phosphate depends on both concentration and strain |
| **49** | Nicole Voce | Northeastern University | Experimentally probing the effect of confinement geometry on lipid diffusion |
| **50** | Paloma Figueroa | Northeastern University | Deciphering MBL-MANLAM Interactions: Computational Insight into MBL Binding to Tuberculosis |
| **51** | Patrick Corrigan | University of Connecticut | Shedding light on Surfactant Mediated Endosomal Escape of Nucleic Acid Therapeutics through Coase-Grained Molecular Dynamics Simulations |
| **52** | Rey Carten | University of Connecticut | Exploring the Structure and Assembly of Janus-base Nanotubes through Multiscale Molecular Dynamics Simulations |
| **53** | Ron Hills | University of New England | Accounting for Ion Polarization to Stabilize Lipid Bilayers in CgProt 3 |
| **54** | Roy Planalp | University of New Hampshire | Probe of Labile Iron in the Mitochondrion and Relation to Ferroptosis |
| **55** | Ruolan Cheng | UMass Amherst | Structural determinants of electrostatically-guided assembly of protein/polyanion complexes |

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| **56** | Ryan Pennington | University of Georgia | High Temperature Analysis of the two Dimers of Beta-lactoglobulin |
| **57** | Samantha Biaesch | SUNY Albany | Parameterization and molecular modeling of wobble uridine in bacterial tRNA |
| **58** | Sandra Byju | Northeastern University | Dependence of P/E tRNA Hybrid Formation on Subunit Rotation in the Ribosome |
| **59** | Sarah Tobia | UMass Amherst | DNA-mediated Assembly of Functional Chemoreceptor Complexes in E.coli |
| **60** | Sean Edington | University of New Hampshire | Probing ion binding structure and dynamics with infrared spectroscopy |
| **61** | Seyed Mostafa Hosseini Nasab | University of Connecticut | An Image-Based Coarse-Grained Model for Biological Polymeric Networks |
| **62** | Shrishti Barethiya | UMass Amherst | Overcoming Extrapolation Challenges of Deep Learning by Incorporating Physics in Protein Sequence-Function Modeling |
| **63** | Shuyi Chen | UMass Amherst | Erratic Electrons: Uncovering the Energy Output of Singlet Fission in Dimer Chromophores |
| **64** | Simran Pandey | Northeastern University | Exploring the interaction between MUC2 and intestinal signaling molecules using molecular dynamics simulations |
| **65** | Son Nguyen | University of New Hampshire | Simulation Studies of a Mitomembrane Targeting Bioconjugated Peptide |
| **66** | Sparsh Makhaik | UMass Amherst | Exploring the Druggability of Chikungunya Virus Protease nsP2 using Biomolecular NMR |
| **67** | Sweta Vangaveti | SUNY Albany | The Unfolding Story of tRNA: Insights from molecular dynamics |
| **68** | Thomas Bregnard | UMass Amherst | Identification of Exosites in Caspase-6 for Development of Substrate-Selective Inhibitors |
| **69** | Thomas Tran | UMass Amherst | Bacterial chemoreceptor signaling complexes control kinase activity by stabilizing the catalytic domain of CheA |
| **70** | Tongyin Zheng | Brown University | Structure and position-specific interactions of prion-like domains in transcription factor Efg1 phase separation |
| **71** | Trisha Brady | UMass Amherst | Investigating Conformational Heterogeneity of Various Caspase-9 Maturation States |
| **72** | Tristan Melfi | SUNY Albany | Computing The Performance of Nucleic Acid Force Fields on Non-canonical Structures |
| **73** | Vaishnavi Katragadda | Dartmouth College | Experimentally characterizing the binding mechanism of a prospective castration-resistant prostate cancer therapeutic to the disordered transactivation domain of the human androgen receptor |
| **74** | Vinnie Widjaja | Brown University | Modulation of MIF-2 structure and function via cysteine residues and a small molecule Ebselen |
| **75** | Walid M. Fahssi | SUNY Albany | Insights into the structural consequences of inosine modified G-quadruplexes using Molecular Dynamics |

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| **76** | Xinqiang Ding | Tufts University | Bayesian Multistate Bennett Acceptance Ratio Methods |
| **77** | Yang Wang | Boston College | Interplay of steric and diffuse ion effects regulates the final stages of aminoacyl-tRNA accommodation |
| **78** | Yu-Bai Xiao | University of Connecticut | The direct influence on mechanical properties of tropocollagen by AGE adducts |
| **79** | Yuri Kwon | University of New Hampshire | Investigating protein robustness in Bacillus subtilis |
| **80** | Zheyu Zhang | Northeastern University | Annotating small molecules in the Protein Data Bank with ChatGPT |

**9:45 – 10:15 am Opening Keynote**

## How Hsp70 molecular chaperones recognize substrates that need their help: Selective promiscuity

**Lila M. Gierasch**

Departments of Biochemistry & Molecular Biology and Chemistry
UMass Amherst

Successful folding of proteins in the cell and their delivery to appropriate destinations are challenging processes. Incompletely folded proteins are sticky, causing them to be prone to inappropriate interactions and aggregation, problems now recognized to underlie many pathologies, especially neurodegenerative diseases. A complex system of molecular chaperones and degradation machines operates in the cell to mitigate against protein misfolding diseases. The maintenance of protein homeostasis, or proteostasis, relies on the ability of chaperones and degradation machines to bind to proteins that need their help to fold to their native states or are irrevocably misfolded. We have sought to understand the basis of the recognition of substrates by the highly prevalent and multifunctional molecular chaperones, the Hsp70s. The most amazing aspect of the protein homeostasis network is that it acts on the entire proteome. Hence, chaperones must have promiscuous binding. However, it is crucial that chaperones only bind to those proteins that are incompletely folded. In this talk, I will describe how the use of peptides as models for regions of proteins that act as recognition sites has provided insight into the basis of selective promiscuity in Hsp70 chaperone/substrate binding.

**4:35 – 5:05 pm Closing Keynote**

**The Origins of Life: An uncanny resemblance to the old Protein Folding Problem**

**Ken A. Dill**

Departments of Chemistry, Physics & Astronomy, Applied Math & Statistics
SUNY Stony Brook

How did the first living cells come into being from the earth's molecular soup 3.5 billion years ago? Despite much speculation - maybe RNA came first, or proteins, or chemical networks - there's not yet a consensus origins story. New insights are coming from thinking not just about life's molecules, but about biology's process of adaptation. The Darwinian evolution process must have preceded the origins of life. This, and the apparent needle-in-a-haystack nature of sequence space, indicate a key role of proteins in life's origins.

**10:20 - 10:40 Haribabu Arthanari (Harvard)**

***A tale of tails: The functional role of disordered regions in the human proteome***

Haribabu Arthanari
Harvard University

A third of the human proteome is predicted to be disordered, yet these sections of the protein harbor functional relevance. The molecular mechanism of how certain enzymes and transcription factors are regulated by their own disordered section has largely remained a mystery because of limitations in our experimental methods. Nature, however, cleverly uses these disorder segments sometimes as on/off switched and in other instances as a molecular rheostat to fine-tune the enzymatic activity, primarily through post-translation modification. The investigation proposed here demands the latest in NMR technology including those we are developing in our lab and combining them with crafty biochemistry including novel labeling schemes, and protein semi-synthesis. shed light on the molecular determinants that regulate the function. The talk will provide several examples of the "structural" aspects of how disordered proteins regulate function.

**10:45 - 10:55 Nabin Kandel (RPI)**

***Tau and Lipid Bilayer Interactions Are Driven by Electrostatics and Inhibited by Cholesterol***

Kandel, Nabin, Department of Biological Sciences, Center for Biotechnology and Interdisciplinary Studies
Rensselaer Polytechnic Institute, Troy, NY, USA
Li, Shanlong, Department of Chemistry, University of Massachusetts, Amherst, MA, USA
Enoki, Thais A., Department of Molecular Biology and Genetics, Cornell University, Ithaca, Cornell, NY, USA
Palumbo, Jacob, Department of Biological Sciences, Center for Biotechnology and Interdisciplinary Studies
Rensselaer Polytechnic Institute, Troy, NY, USA
Chen, Jianhan, Department of Chemistry, University of Massachusetts, Amherst, MA, USA
Forth, Scott, Department of Biological Sciences, Center for Biotechnology and Interdisciplinary Studies
Rensselaer Polytechnic Institute, Troy, NY, USA
Wang, Chunyu, Department of Biological Sciences, Center for Biotechnology and Interdisciplinary Studies
Rensselaer Polytechnic Institute, Troy, NY, USA

The molecular mechanism of tau interaction with lipid bilayer, a key step in tau secretion and aggregation, is still elusive. This study focuses on understanding how membrane components, such as cholesterol and membrane charge, influence the interactions between tau protein and membranes. The role of electrostatic interactions is explored, particularly involving anionic membrane components like POPG, and the effects of various salts and heparin. The tau-bilayer binding is robustly inhibited either by the removal of negative membrane component or from electrostatic screening effects coming from various salts. Notably, increasing cholesterol also significantly diminished tau binding. Our data underscores the importance of charge density mismatch between tau and bilayer with increasing cholesterol. The charge density is low on tau, due to its more extended conformation as an intrinsically disordered protein (IDP). Increased charged density and order parameter due to increasing cholesterol in the bilayer leads to a charge density and dynamical mismatch, decreasing tau-bilayer binding. Our results have potential therapeutic significance in terms of the role of cholesterol in tau-membrane interactions and in neurodegenerative diseases like Alzheimer's disease.

**10:58 - 11:08 Yeseul Lee (UMass Amherst)**

***Push-and-pull mechanism of single-molecule movement of alpha-Synuclein under nano-confinement***

Lee, Yeseul, Polymer Science and Engineering, University of Massachusetts, Amherst
Muthukumar, Murugappan, Polymer Science and Engineering, University of Massachusetts, Amherst

Intrinsically disordered proteins (IDPs) and their assemblies with other biomolecules constitute a plethora of biomolecular condensates (and membrane-less organelles). Central to the behavior of such condensates is how the IDPs move around in crowded electrolyte conditions. Towards unraveling the underlying mechanism of mobility of IDPs, we have investigated the movement of single molecules of alpha-Synuclein through a nanopore under an external electric field, using a combination of single-molecule electrophysiology, statistical mechanics theory with the Fokker-Planck formalism, and computer simulations. We find that alpha-Synuclein moves like a composite of two engines with opposing velocities and exhibits a paradigm shift in the current understanding of electrophoretic translocation of proteins and polynucleotides. In addition, our combined three-prong approach of experiments, theory, and Langevin Dynamics simulations, enables a mapping of energy-movement correlation with protein sequence. Our work shows that the specific charge sequences of IDPS can be uniquely determined from the voltage dependence of their electrophoretic translocation times.

**11:30 - 11:50 Ashley Carter (Amherst College)**

***DNA folding with protamine***

Carter, Ashley, Amherst College

DNA may be the most important molecule on the planet. It is found in every cell of every organism. It is used in nanotechnology to engineer nanostructures, in material science to create active hydrogels, in medicine as a therapeutic or anti-inflammatory, in sustainability research as a method for carbon capture, in computer science for data storage, and in biosensing as a way to detect harmful chemicals. In many of these applications, folding of the DNA is important. This folding can regulate gene activation, can change the material properties, or can affect the stability of the DNA. Here we are going to look at the physics of how one particular protein—protamine—folds and loops DNA.

**11:55 - 12:05 Anna Bock (Brown)**

***Characterizing the conserved pH sensor in the yeast prion protein Sup35***

Bock, Anna, Brown University
Fawzi, Nicolas, Brown University

The yeast protein Sup35 is widely known for its ability to form heritable aggregates, also known as prions, in Saccharomyces cerevisiae. Recent insight into this protein's ability to undergo liquid-liquid phase separation implicates the negatively charged residues in the lesser studied M domain as a pH sensor, however, insight into the mechanism was limited. Sup35 is conserved across millions of years of evolution within yeast, and the pH sensor is also conserved in the Schizosaccharomyces pombe M domain. This work characterizes this conserved pH sensitivity in both S. cerevisiae and S. pombe M domains with atomic detail by using solution nuclear magnetic resonance (NMR) spectroscopy. We examine the impact of glutamic and aspartic acid in the M domain by calculating the residue-specific pKa of these amino acids, finding an upshift of both glutamic and aspartic acid pKa in the M domain. We also uncovered a C-terminal helical region with the highest proportion of pKa upshift, indicating that this region is important to M domain sensing. Cellular chemical environment sensing occurs through different pathways, and disordered regions, such as the M domain of Sup35, are prime candidates because they are entirely solvent exposed. Through this work, we aimed to characterize acidic environment sensing through a potentially conserved mechanism, which could also translate to other prion-like domains.

**12:08 - 12:18 George Makhatadze (RPI)**

***Experimental and computational studies of the effects of high hydrostatic pressure on biomolecular interactions***

George Makhatadze, RPI

Understanding the thermodynamic mechanisms of adaptation of biomacromolecules to high hydrostatic pressure can help shed light on how piezophilic organisms can survive at pressures reaching over 1,000 atmospheres at the bottom of the oceans. Here I will summarize our recent experimental and computational work on various aspects of the effects of high hydrostatic pressure on biomacromolecules. Particular emphasis will be placed on the volume changes, $\Delta V$, that accompany conformational transitions in biomacromolecules such as proteins, protein-ssDNA complexes, and dsDNA duplexes. The importance of $\Delta V$ comes from the fact that it defines the pressure dependence of stability $\Delta V=(d\Delta G/dP)T$. Thus, the response of the system to changes in pressure is driven by Le Chatelier's principle: if volume changes upon unfolding are positive, an increase in hydrostatic pressure will lead to an increase in stability, whereas if the changes are negative, the stability will decrease with the increase in hydrostatic pressure. The results will be discussed in terms of the relative contributions of volume changes associated with voids and hydration to the net volume changes that accompany conformational transitions in biomacromolecules.

Acknowledgments: This work was supported by a grant NSF CHEM/CLP-1803045.

**2:30 - 2:50 Paul Robustelli (Dartmouth)**

***Targeting Intrinsically Disordered Proteins and Biomolecular Condensates with Small Molecule Drugs***

Paul Robustelli, Dartmouth College

Intrinsically disordered proteins (IDPs), which represent ~40% of the human proteome, play crucial roles in a variety of biological pathways and biomolecular assemblies and have been implicated in many human diseases. IDPs do not fold into a well-defined three-dimensional structure under physiological conditions. Instead, they populate a dynamic conformational ensemble of rapidly interconverting structures. As a result, IDPs are extremely difficult to experimentally characterize and are largely considered “undruggable” by conventional structure-based drug design methods. Our laboratory utilizes a combination of computational and biophysical methods to characterize the molecular recognition mechanisms of intrinsically disordered proteins in atomic detail. Here I will discuss recent progress in our efforts to characterize the interactions of IDPs with small molecule drugs, understand molecular mechanisms that drive the formation of biomolecular condensate, and understand how small molecule drugs modulate biomolecular condensate stability.

**2:55 - 3:05 Jeffrey D. Tamucci (UConn)**

***Powering the powerhouse: MD simulations illustrate how mitochondria-targeted tetrapeptides alter membrane electrostatics and have the potential to improve mitochondrial bioenergetics***

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Mitochondrial dysfunction is implicated in nine of the ten leading causes of death in the US, yet there are no FDA-approved therapeutics to treat it. Synthetic mitochondria-targeted peptides (MTPs) offer promise as they have been shown to restore healthy mitochondrial function and treat many common diseases. At the cellular level, MTPs accumulate strongly at the inner mitochondrial membrane (IMM), reduce proton leak, and improve ATP production. Modulation of electrostatic fields around the IMM is a key aspect of their mechanism of action (MoA); however, molecular mechanistic details have remained elusive. In this study, we employed all-atom molecular dynamics (MD) simulations to investigate the interactions of four MTPs with lipid bilayers and calculate their effect on membrane structural and electrostatic properties. Building on previous experimental findings, the simulations reveal that MTPs achieve a reduction in the dipole potential by disordering both lipid head groups and water layers proximal to the bilayer surface. We also find that MTPs decrease the bilayer thickness and increase the membrane's capacitance. Double-bilayer simulations further reveal that MTPs allow more potential energy to be stored across the IMM at a given transmembrane potential difference, making membranes more resilient to hyperpolarization-induced electroporation and potentially explaining how MTPs both increase ATP production and reduce proton leak. Lastly, we reveal a passive transmembrane transport pathway for MTPs, potentially shedding light on a previously unanswered question, how MTPs get to the IMM. In conclusion, this study underlines how MTPs' interactions with lipid bilayers serve as a fundamental component of their MoA.

**3:08 - 3:18 Jeanne Hardy (UMass Amherst)**

***Harnessing protease reactions for detection and treatment of viral infection***

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Viral infections have catastrophic impacts on the public. Though there have been notable advancements in detection methods, there remains need for rapid point-of-care detection strategies for viral infections that are accurate, require minimal instrumentation, and distinguish active from resolved infection. We present a colorimetric approach for virus sensing by direct color generation on a cotton swab. By appending a chromophore to the peptide recognition sequence for SARS-CoV-2 main protease, we created a reporter that produces a color change upon protease hydrolysis. The reporter is selective towards the SARS-CoV-2 $M^{pro}$, is readily observable by eye or smartphone.

Flaviviruses, continue to threaten public health. The associated NS2B-NS3pro proteases are essential to the flaviviral life cycles and can interfere with host responses, making the proteases good drug targets. While the proteases are highly identical, the catalytic efficiency and the substrate selection are divergent, so the unique properties of viral proteases may contribute the disparate viral pathologies. NS2B-NS3 proteases transition between two widely different conformational states: an *'open'* (inactive) conformation and a *'closed'* (active) conformation. We developed versions of NS2B-NS3pro that allow us to trap the enzyme in distinct conformations. Enzymatic activity is dependent on the movement of NS2B and the flexibility of the protease core. Using unbiased *N*-terminomics to identify 31 human proteins cleaved by the NS2B-NS3 protease we observed that the NS2B is essential for recognition of host cell substrates. Replacing the NS2B region suggests that the co-factor is the principal determinant in ZVP substrate selection. Based on this observation, we report a 440 nM inhibitor of ZVP that functions by blocking interactions between the NS3 core and NS2B cofactor.

**3:20 - 3:40 Erika Taylor (Wesleyan)**

***Aminoglycosides Revisited: Evidence for Inhibition of Heptosyltransferase I from Escherichia coli***

Milicaj, Jozafina - Wesleyan University
Hassan, Bakar A. - Wesleyan University
Sham, Yuk Y. - University of Minnesota
Taylor, Erika A. - Wesleyan University

Inhibitors of the Heptosyltransferase I (HepI) from E. coli have been sought for many years because of disruption of the gene encoding HepI prevents maturation of the lipopolysaccharide on the surface of Gram-negative bacteria, which results in reduction of virulence, cell motility, adhesion and nutrient uptake. Additionally, disruption of the gene for HepI also results in increased membrane permeability and a deep rough cellular phenotype.1-4 Coincidentally, aminoglycosides have long been known be more effective in treating Gram-negative bacterial infections than for Gram-positive infections, while also causing the creation of pores in bacterial cell membranes upon cell treatments.5 While aminoglycosides have been shown to bind to ribosomes, recent studies have shown that they only result in minimal changes in protein synthesis in live cells.6 Recent studies in our lab suggest that aminoglycosides are potent inhibitors of HepI, and result in changes to the LPS biosynthesis on the bacterial cell surface. Detailed kinetic and computational studies demonstrate that aminoglycosides can act as competitive, and non-competitive inhibitors of HepI. These studies may lead to the reassignment of the mechanism of action of aminoglycoside antibiotics.

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**3:45 - 3:55 Bhumika Jayee (Northeastern)**

***Computational study of a red light-driven reaction in a flavin-dependent photoenzyme***

Bhumika Jayee, Gustavo Mondragon, Nithin Chintala, Nathaniel White, Sijia S. Dong
Northeastern University

Light-driven biocatalysis has emerged as a powerful approach to unlock new enzymatic reactivity modes. Flavoenzymes owe their effectiveness to the presence of a redox-active cofactor that can act as a photoredox catalyst. The irradiation with visible light on these flavin-dependent “ene”-reductases (EREDs) enables new catalytic functions, which complement the natural reactivity repertoire of flavoenzymes. One of the properties of these flavoenzymes is the catalysis of asymmetric radical cyclization of α-chloroamides via intramolecular hydroalkylation. Herein, a comprehensive computational study of these flavoenzymes is presented with high level multireference calculations to study the absorption spectra of these excited species in comparison to the experimental spectra. We studied the interactions of the ligand and substrate docked with different mutations of the protein and its effect on the absorption spectra.

**3:58 - 4:08 Bao Nguyen (UMass Amherst)**

***Mechanistic insights into Ca2+ sensitivity regulation of CaMKII***

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Ana Torres Ocampo, UMass Amherst
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Ca2+ plays a key role in physiological processes such as memory formation and cardiac pacemaking. Ca2+/calmodulin-dependent protein kinase II (CaMKII) is responsible for decoding the Ca2+ oscillation in these Ca2+-sensitive cells. In this study, we aim to characterize the structure of CaMKII with the hope of providing a molecular basis for CaMKII activation. There are four CaMKII genes (α, b, d, g) that are expressed differentially throughout the body. These paralogs share the same domain organization: an N-terminal kinase domain, a regulatory segment, a variable linker region, and a C-terminal hub domain (oligomerization domain). We show here that the variable linker region regulates CaMKII activation allosterically through a charge-dependent manner, regardless of the length of the linker. Using small angle x-ray scattering, we show that a negatively charged linker shifts the holoenzyme to a more compacted state compared to a positively charged linker. We present here a new crystallographic structure of CaMKIId holoenzyme showing a domain-swap conformation of dimeric subunits in the holoenzyme, where the kinase of one subunit is docked on the hub domain of another subunit. We generated monomeric and dimeric CaMKII via interfacial mutations and show that the oligomeric state of CaMKII also influences Ca2+/CaM sensitivity. Using molecular dynamics simulation with a tetrameric CaMKII that is either in a swapped conformation or non-swapped conformation, we show that domain swapping facilitates the linker-dependent regulation. Our data shed new light on how the linker region affects CaMKII structure and allosteric regulation for Ca2+ sensitivity.

# Poster Abstracts

**Poster #1**

## Elucidating the Effects of Cholesterol on the Function of Surfactant Protein C Using Atomistic Molecular Dynamics

Ferrante, Amanda, Northeastern University
Minkara, Mona, Northeastern University
Javanainen, Matti, University of Helsinki
Li, Deng, Northeastern University

The pulmonary surfactant (PS) system is indispensable for allowing all of the biophysical interactions within our quantitative physiology. Within this complex lipoprotein assembly where gas exchange occurs, four primary proteins function in pathogen defense and facilitate efficient gas exchange during respiration. Of these proteins, surfactant protein C (SP-C) plays a pivotal role in modulating crucial biophysical properties including surface tension and interfacial adsorption of lung surfactant to the air-water interface; mechanisms needed for respiration and preventing alveolar collapse. Previous studies suggest that SP-C has a specific interaction with cholesterol, where thermodynamic properties of the interface are controlled for functions including oxygen diffusion, surfactant recycling, and maintaining membrane integrity, yet, the physical and chemical mechanism of this interaction remains unknown. There is evidence that cholesterol may inhibit the surface pressure-decreasing ability of pulmonary surfactant which is alleviated by the hydrophobic surfactant proteins, however the extent of this is also unknown. Using Atomistic Molecular Dynamics, we embed SP-C in four component lipid monolayers with different concentrations of cholesterol, to analyze membrane behavior, lipid sorting and diffusion, and to understand the biophysics needed for homeostasis within the pulmonary surfactant system for transport, dynamics, and control. This study also seeks to develop improvements to the accuracy and replicability of the current surfactant model. The findings of this work yield pervasive applications for human health and improved understanding of the pulmonary surfactant system.

**Poster #2**

## Unraveling Pausing and Termination in RNA Synthesis: Mechanistic Model and Practical Applications

Abek, Amin, UMass Amherst
Martin, Craig, UMass Amherst

While an ideal model system for RNA polymerases broadly, T7 RNA polymerase has emerged as a cornerstone in the biomedical realm. Nonetheless, the efficiency of RNA synthesis can be impeded by sequences inducing pausing and termination during elongation. Our research delves into addressing this challenge through the development of a functional SELEX (Systematic Evolution of Ligands by EXponential enrichment) method tailored for the discovery and validation of sequences that lead to pausing and termination, which in applications can impact efficacy and quality. Leveraging RNA-seq analysis on RNA samples derived from a diverse pool of DNA sequences encompassing potential pause/termination sites, we are identifying sequences associated with diminished RNA yield. We will then interpret and refine these results in the context of different models for elongation complex stability and transcription termination. This work promises to empower both academic researchers and industry professionals with a novel toolset for optimizing RNA manufacturing processes.

**Poster #3**

## Extracting the Binding Affinities Between Various Viral Glycans and MBL via Molecular Docking

Shenoy, Amit, Northeastern University
Li, Deng, Northeastern University
Minkara, Mona S, Northeastern University

Airborne pathogens attack the body by entering through the lungs, which have several lines of defense. The first defense line is made up of proteins known as lectin family proteins, which contain a carbohydrate recognition domain (CRD) that can recognize and bind glycans. Glycans are carbohydrate add-ons found on common viruses such as Sars-Cov2 and Influenza-A. One such protein, Mannose-Binding-Lectin (MBL), still requires more research to understand its specific binding mechanism against different pathogens. Further research is needed to understand MBL's structural and chemical binding properties to provide insight into a successful immune response and serve as a reference model for synthetic biologists to improve natural human immunity. In this study, a suite of software tools is used to model the interaction between an MBL model from the Protein Data Base (PDB) and an in-house library of viral glycan models to understand this binding mechanism better and determine the specific binding affinities of MBL to each candidate glycan through a series of computational modeling techniques. The goal is to identify patterns among the modeled MBL-glycan complexes to uncover the optimal binding positions and publish the findings.

**Poster #4**

## Ensemble-based molecular docking of small molecule ligands on the disordered protein a-synuclein

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Intrinsically disorder proteins (IDPs), which lack well-defined three-dimensional structure under physiological conditions, are implicated in many human diseases. Conventional structure-based drug discovery techniques aren't well suited for IDPs, as their dynamic structures make ligand-protein interactions difficult to predict. All-atom molecular dynamics (MD) computer simulations validated by NMR spectroscopy have provided an avenue to probe ligand interactions with IDPs. However, MD simulations of IDPs are computationally expensive and not practical for screening large libraries of small compounds against disordered systems. We aim to provide a less computationally expensive method to gauge IDP-ligand interactions by using molecular docking, a technique that predicts bound ligand conformation using force-field based scoring functions. Here, we develop and benchmark an ensemble molecular docking protocol to screen small molecule compounds against a large, realistic structural ensemble of the disordered C-terminal region of a-synuclein, an IDP whose aggregation is associated with Parkinson's disease. We compare results of binding mechanisms predicted from our ensemble docking protocol with those observed in long timescale MD simulations and observe surprisingly good agreement. Our results suggest that ensemble-based docking strategies show potential for predicting the relative affinities of small molecule drugs to IDPs.

**Poster #5**

## Modeling the Vapor-Liquid Equilibration Curve of Pentadecanoic Acid Using Gibbs Ensemble Monte Carlo Simulations

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Potoff, Jeff
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Northeastern University

Key Words: Langmuir Monolayers, Monte Carlo Simulations, Coexistence Curve

This project focuses on utilizing Gibbs Ensemble Monte Carlo simulations to analyze the vapor-liquid equilibration curve of Pentadecanoic Acid (PDA). Pentadecanoic Acid is a commonly employed substance for modeling Langmuir monolayers, presenting a significant computational challenge. The successful computational modeling of monolayers, particularly with PDA, is a crucial milestone with implications for more accurate simulations of Langmuir monolayers which are found in the respiratory system.

Langmuir monolayers play a pivotal role in this study, as they are prevalent in the lungs. PDA specifically is similar in structure to palmitic acid which is a monolayer of lipids found in the lungs. By producing an accurate model of pentadecanoic acid's structure, effective Gibbs Ensemble Monte Carlo simulations can be run to test properties such as density at various temperatures. The simulation will contain 2 boxes, a vapor phase box and liquid phase box between which the PDA particles will switch. The data of the particle movements will be collected over thousands of time steps to build a coexistence curve with density and temperature. With each breath, the intricate interplay of vapor and liquid phases in these monolayers occurs. The computational success in modeling PDA monolayers not only contributes to advancing our understanding of basic physiological functions but also holds promise for enhancing simulations relevant to drug development.

Ultimately, this project stands as a significant step toward unraveling the complexities of monolayer behavior, especially in the context of PDA, and has broader implications for the refinement of simulations critical to the pharmaceutical industry's pursuit of novel therapeutic compounds.

**Poster #6**

## Identifying ligand binding modes of small molecules binding to $\alpha$-Synuclein

A. Chakraborty, T. Sisk & P. Robustelli
Dartmouth College

Intrinsically disordered proteins (IDPs), characterised by their lack of rigid three-dimensional structure, play important roles in a large number of physiological pathways. Several IDPs and proteins with large-disordered regions have been known to play important roles in many human diseases, however drugging IDPs remain challenging as a result of their highly conformationally dynamic nature. Previously, NMR spectroscopy was utilized to identify the binding sites of several small molecules to the disordered protein α-synuclein, and unbiased molecular dynamics (MD) computer simulations were shown to reproduce the experimentally determined binding sites (Robustelli et. al., JACS, 2022). Here, we seek to obtain deeper insight into the variability of binding mechanisms observed in long timescale MD simulations of the previously identified ligands using statistical modelling techniques such as Markov state modelling (MSMs) and the variational approach for Markov processes. We observe substantial challenges in identifying distinct kinetically separable states, suggesting that low affinity small molecule disordered protein interactions may be largely diffusive in nature. We further analyse the networks of intermolecular ligand protein interactions that influence the residence times of disordered protein binding events.

**Poster #7**

## Exploring the Fusion of Quantum Computing and Chemistry with TeraChem's Exciton Model

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Duong, Keith, University of Massachusetts Amherst
Lalwani, Anish, University of Massachusetts Amherst
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Deciphering molecular behavior at the quantum level is crucial for advancements in quantum computing and chemistry. Spin-orbit coupling (SOC), a phenomenon in which an electron's nuclear orbit influences its spin, significantly impacts a molecule's electronic properties. Excitons, electron-hole pairs which are bound together by electrostatic forces, provide a powerful means of examining SOC due to their sensitivity to electronic interactions. This study leverages TeraChem's established excitonic model to examine SOC in multi-molecule systems such as electronic dynamics. By constructing and diagonalizing the resulting Hamiltonian matrix, which captures the electronic interactions within the system, we can unravel the intricate dance of electrons and SOC's influence. This research aims to not only illuminate SOC's role in molecules but also inform the design of materials with precisely tailored electronic properties for future quantum technologies. This convergence of advanced computational chemistry and quantum physics paves the way for breakthroughs in understanding and manipulating matter at the atomic level, accelerating our ability to understand and control the building blocks of our universe.

**Poster #8**

## How do catalytic lysines and glutamates in enzymes gain their catalytic properties?

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Mirabelli, Michelle, Northeastern University
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The lysine side chain, a primary amine, is a weak base and is fully protonated at neutral pH in the free amino acid. How does it become a strong base or nucleophile in enzyme active sites? Catalytic potency may be enhanced through elongation of the buffer range of the catalytic residues. This is achieved through strong electrostatic coupling of the protonation equilibria of active ionizable residues with those of other ionizable residues. Computational analysis using Partial Order Optimum Likelihood (POOL) was performed on 150 enzymes that represent all six major enzyme classifications and a variety of different folds. All contain one or more lysine or glutamate residues that have been found to be catalytically active in previously reported experiments. The catalytic lysine and glutamate residues reported here have strong coupling interactions to other residues that obey one or both inequality expressions reported by Coulther, Ko and Ondrechen in 2021. Specifically, the catalytic lysines are strongly coupled with tyrosine or cysteine residues wherein these anion-forming residues have an intrinsic pKa higher than that of the lysine or else are strongly coupled to at least one other lysine residue with intrinsic pKa difference within 1 pH unit. The catalytic glutamates studied here are strongly coupled to at least one other aspartate or glutamate residue with intrinsic pKa difference within 1 pH unit or else are strongly coupled to a histidine residue, wherein the intrinsic pKa of the acidic residue is higher than that of the histidine. The interactions help us in identifying roles of these supporting residues and also in identifying arrays of interacting residues that are characteristic of specific biochemical functions, thus supplying functional information that can be used to annotate protein structures of unknown function. This analysis provides insight into how catalytic lysines and glutamates achieve their catalytic power. Acknowledgement: NSF MCB-2147498 & US-Pakistan Knowledge Corridor; MM and HE supported by NSF DBI-2031778.

**Poster #9**

## Coarse-grained models of elastin assemblies

Chengeng Yang and Anna Tarakanova
University of Connecticut

Elastin is a highly flexible structural protein of the extracellular matrix that imparts elasticity to connective tissues and organs. Elastin assembles into elastic fibers through its soluble precursor, tropoelastin, a process called elastogenesis. Tropoelastin is secreted by cells, undergoing a self-association phase where tropoelastin monomers assemble into multimers. Multimeric elastin forms intra- and inter-molecular crosslinks and deposits into microfibrillar networks, ultimately forming native elastic fibers. Recently, atomistic models have been established for monomeric elastin. Models at higher scales, representative of native multimeric elastin assembly, however, are yet to be developed. Here, we report coarse-grained multimeric elastin models with accurate compactness and lower critical solution temperature (LCST). We coarse-grain the system based on the derived atomistic structure of tropoelastin, with four different parameter sets describing short-range pairwise interactions, and compare results with the radius of gyration (Rg) of tropoelastin structures inferred from small-angle X-ray scattering data. We then build multimeric tropoelastin models and run co-existence simulations to compare LCST with measurements from experiments. Overall, our multimeric elastin models provide a comprehensive understanding on elastin assemblies, help reveal the influence by mutation and by the changes of crosslinks on the downstream process in elastogenesis, as well as enable future potential applications to engineer elastin-based biomaterials.

**Poster #10**

## The basis of substrate binding by Hsp70 and its impact on cellular functions

Eugenia M. Clerico[1], Alexandra K. Pozhidaeva[2], Lea M. Dorries[1], Lila M. Gierasch[1,3]

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Heat shock protein 70 (Hsp70) chaperones play a wide array of essential roles in the cell. All Hsp70 functions depend on their ability to bind their client proteins, discriminating between incompletely and properly folded substrates. The study of the Hsp70s bound to short peptides mimicking substrates revealed the details of the direct interaction between five hydrophobic "core" residues in the substrate and five "pockets" in the canonical binding site in the substrate-binding domain (SBD) of the chaperone. Still, this information is not enough to explain the molecular origin of the Hsp70 selectivity for available sites on clients in concert with their promiscuity in binding different sequences. We studied how the *E. coli* Hsp70 (DnaK) binds to alkaline phosphatase and found that several sequences often bind to the chaperone in the opposite orientation (C- to N-) from what was previously believed to be preferred (N- to C-). Because substrates in both binding orientations establish similar hydrogen bonds with DnaK, we postulate that the preferred binding mode is a result of the optimal positioning of the substrate side-chain in the SBD pockets regardless the backbone orientation. To test our hypothesis, we have studied the binding orientation preference of complexes between DnaK and peptides with identical amino acid composition but with their sequences in opposite order ("flipped"), and to peptides with "palindromic sequences" around the residue that occupies the "central pocket" of the chaperone.To test the preferred orientation of the bound peptides we employ disulfide-mediated crosslinking, and $^{13}$Cd-methyl NMR. We find that some peptides retain the same binding orientation despite having their sequence reversed, and sometimes a mixture of binding modes were sampled. Our results paint a picture of chaperone binding that is based on small energy differences between different sites and the ability of the chaperone to optimize side chain fit regardless of backbone direction. In the presence of physiological nucleotides and co-chaperones, this binding will be transient and site preference dynamic. Work is underway to follow up on the role of Hsp70 binding preferences in specific functions and the impact of binding orientation on the fate of the substrate.

**Poster #11**

## Metabolic Enzymes Moonlighting as RNA Binding Proteins

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Jeffery, Constance, University of Illinois at Chicago and Northeastern University (sabbatical)

RNA binding proteins play key roles in many aspects of RNA metabolism and function, including splicing, translation, localization, stability and degradation. Within the past few years, proteomics studies have identified dozens of enzymes in intermediary metabolism that bind to RNA. The wide occurrence and conservation of RNA binding ability across distant branches of the evolutionary tree suggest that these moonlighting enzymes are involved in connections between intermediary metabolism and gene expression that comprise far more extensive regulatory networks than previously thought. The effects on RNA function are likely to be wider than regulation of translation, and some enzyme-RNA interactions have been found to regulate the enzyme's catalytic activity. Several moonlighting enzyme-RNA interactions have been shown to be affected by cellular factors that change under different intracellular and environmental conditions, including concentrations of substrates and cofactors. We are using a combination of biochemical, computational and structural biology methods to study the molecular structures and mechanisms involved, the effects of these interactions on the catalytic and RNA functions, and the factors that regulate the interactions. Understanding the molecular mechanisms involved in the interactions between the moonlighting enzymes and RNA and their regulation, as well as the effects of the enzyme-RNA interactions on both the enzyme and RNA functions, will lead to a better understanding of the role of the many newly identified enzyme-RNA interactions in connecting intermediary metabolism and gene expression.

Acknowledgements: This research is funded by NSF grant number 2321442.

**Poster #12**

## Computational modeling of the binding mechanisms of surfactant protein D (SP-D) with glycans

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The innate immune response is the first line of human immune function against pathogenic invasion. Surfactant protein D (SP-D) acts against glycan-rich pathogens such as Influenza A viruses (IAV) and SARS-CoV-2 in the lung. Despite its importance, the molecular-level binding mechanisms of SP-D to the terminal saccharides on pathogens' surface glycan remain poorly understood. In this study, we employed several computational chemistry methods, including induced-fit docking (IFD), binding pose metadynamics (BPMD), and alchemical absolute binding free energy calculations, to investigate the dynamics of SP-D interaction with various monosaccharides, disaccharides, and oligosaccharides. Our findings revealed a core binding site centered around the calcium ion, critical for primary glycan binding. As saccharide size increased, we identified specific adjacent residues that further stabilized the binding. Notably, we observed a consistent calcium chelation mode in the carbohydrate recognition domain (CRD) of SP-D during glycan binding. We found that the stable chelation geometry correlates with the enhanced binding affinity. This insight into SP-D binding mechanisms helps to interpret previous experimental results of a double mutant SP-D with heightened trimannose binding and provides a foundation for designing more precise SP-D based strategies for targeting specific pathogens.

**Poster #13**

## Explicating auto-phosphorylation and high frequency dynamics of human PKR

Feinstein, Aaron Gomez, University of Connecticut

Protein kinase R (PKR) operates as a first-line defense in the eukaryotic innate immune system, binding to viral dsRNA and initiating a cascade of autophosphorylation and activation. Once active, PKR phosphorylates its primary substrate, eIF2α, thereby inhibiting translation initiation in the infected cell. While it is acknowledged that back-to-back dimerization of the kinase domain is a precursor to PKR activation, the detailed mechanism remains largely uncharted. Our investigation elucidates the structural mechanistic basis and energy landscape for PKR activation, with a special emphasis on the αC helix — a critical hub in kinase activation and signal integration — explored through extensive all-atom molecular dynamics simulations. Leveraging window-exchange umbrella sampling, we constructed a free energy profile illustrating the stabilizing role of back-to-back dimerization in fostering a catalytically competent conformation of PKR. This process is facilitated by hydrophobic packing of residues in the αC helix at the homodimer interface, accentuating the central role of the αC helix in the activation mechanism. Additionally, we introduce SeQuential Radial-propagation Localization (SQRL), an analytical tool crafted to quantify the dynamic "bandwidth" through specific paths of atoms in a macromolecule. SQRL aims to trace radial successive atomic displacements from a kinetic energy source to distal molecular domains, identifying new allosteric signaling pathways. While still in its developmental stage, we envision SQRL as a potent instrument for finding molecular loci that are amenable to mechanical perturbation.

**Poster #14**

## Investigating the Structural Consequences of a Tropoelastin Mutation through Classical Atomistic Molecular Dynamics Simulation

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Elastin, a vital protein in the extracellular matrix of connective tissues, plays a pivotal role in providing elasticity and resilience to various tissues such as skin, blood vessels and lungs. Tropoelastin, the building block of elastin, may undergo several genetic mutations that can lead to various structural alterations, impacting its functionality and contributing to a number of connective tissue disorders. According to a clinical study, a missense variant in the elastin (ELN) gene that substitutes Serine for Glycine (G162S) has been reported twice in ClinVar as a variant of uncertain significance. In this study, we apply classical atomistic molecular dynamics simulations to explore the structural consequences of this mutation. Our investigation focuses on elucidating the effects of this mutation at the atomic level, providing insights into the altered conformational structure and stability of the mutant protein compared to the wild type of counterpart. We simulate microsecond trajectories and compare the behavior of the wild type and mutant tropoelastin proteins in explicit solvent environments over extended time scales. Our results reveal that the mutant type is more dynamic, fluctuating more, and less likely to assume a compact shape as it has a larger radius of gyration and more solvent accessible surface area. These changes are attributed to a local structural disturbance caused by the mutation. These findings advance our understanding of tropoelastin's structure-function relationship and clear the path for future therapeutic approaches aimed at connective tissue illnesses caused by tropoelastin mutations.

**Poster #15**

## Fingerprinting Base Stacking Systems

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Scopino, Kristen, Biology
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Weir, Michael P., Biology, College of Integrative Sciences
Wesleyan University

The central dogma envisions protein translation as the ribosome reading one mRNA codon in its aminoacyl (A) site at a time as it adds to its growing peptide chain. However, our recent studies suggest that ribosomes also perceive the identity of the +1 codon, the codon next in line to enter the A-site. This activity is likely mediated by a subdomain of the ribosome named the CAR surface, which interacts through hydrogen bonding and pi stacking with the +1 codon. Minor changes in +1 codon identity can lead to widespread allosteric movement throughout the A-site region; here, we explore whether this movement can be characterized by changes in inter-nucleotide pi-stacking throughout the structure. We introduce StACKER, a robust Python package for observing pi-stacking interactions in a molecule. Using molecular dynamics (MD) simulations of the CAR interaction surface neighborhood with varying +1 codons, A-site codons, and A-site tRNA anticodons, we show that StACKER's "Stacking Fingerprints" allow for qualitative comparison of minor conformational adjustments between two similar molecules. Additionally, polar plots created in StACKER can distinguish the type of pi-stacking occurring in an identical residue pairing across two different structures. In conclusion, StACKER provides an accurate diagnostic for allosteric shifts throughout a molecule and demonstrates specific changes in inter-nucleotide interactions.

**Poster #16**

## Probing substrate binding in a light-activated enzyme: A bias-exchange metadynamics study of Caulobacter segnis ene-reductase

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Enzymes typically bind substrates in their active sites with high affinity, rendering catalysis stereo- and chemoselective. Recent studies have revealed that flavin-containing enzymes can be repurposed for photoactivated catalysis, allowing them to perform alternative reactions and use non-native substrates. These photoenzymes utilize light to form reactive electron donor-acceptor (EDA) complexes, bypassing the thermal requirement for accessing the transition state, as seen in conventional catalysis. To explore the mechanisms governing substrate binding and catalysis in photoenzymes, we employed bias-exchange metadynamics simulations on the ene-reductase enzyme from Caulobacter segnis (CsER). Our investigations revealed that non-native substrates exhibit moderate to low binding affinities, adopting multiple metastable states rather than a single binding mode. Notably, arginines and aromatic residues play crucial roles in substrate binding, dictating CsER's selectivity based on substrate orientation within the active site. Moreover, these results suggest that light absorption enables even imperfectly bound substrates to participate in catalysis through EDA complex formation upon illumination. These findings not only help understand how non-native substrates bind in photoenzymes, but also provide valuable insights to inform the rational design and optimization of novel photoenzymes.

**Poster #17**

**Quantifying the effects of ionic environment on the dynamics of biomolecular assemblies**

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It is known that ions play a crucial role in biomolecular assemblies and catalytic reactions. In particular, the concentration of monovalent and divalent ions, such as magnesium ions (Mg2+), stabilize tertiary structure of RNA. Here, we aim to understand the relative contributions of diffuse (hydrated) and chelated (partially dehydrated) Mg2+ ions on the dynamics of RNA. For this, we are extending an all-atom structure-based models (SMOG models) that have explicit diffuse monovalent and divalent ions. Our extensions require that one consider the coordination distances and geometries between ions and phosphate groups, and the relative energetics of inner-shell and outer-shell interactions. Calibration of the model involves comparison with results from explicit-solvent models, as well as from titration experiments. As an example, the accuracy of the model is assessed by comparing the predicted number of excess Mg2+ ions for RNA molecules with experimental values. With these benchmarks, we are now positioned to study the effects of the ionic environment on large-scale rearrangements and functional dynamics of RNP assemblies, such as the ribosome.

**Poster #18**

## Effect of aromatic amino acids in the stabilization of the photoactive electron donor-acceptor complex in the Gluconobacter-ene oxidoreductase

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Gluconobacter-ene oxidoreductase (GluER) is a flavin mononucleotide (FMN) based enzyme present in Gluconobacter oxydans that catalyzes chemical reactions in the ground state. Its chemical reactivity has been repurposed for producing molecules of pharmaceutical interests using visible light sources within the cyan, or red regions of the spectrum, starting the chemical reaction with a photoinduced charge transfer (CT) state after forming an electron donor-acceptor (EDA) complex. The presence of two Tryptophan residues (66, and 100) and the Tyrosine-177 residue within the active site can stabilize the photoinduced CT state, as they interact with the substrate via π stacking. Herein, we study the role of these three amino acids in the CT state stabilization in the EDA complex using multiconfiguration pair-density functional theory in 67 substrate and FMN conformations from two GluER types: the wild-type and a variant. The results show that the amino acids in the wild-type GluER can stabilize the EDA complex in its CT state in dark states within the cyan region of the spectrum, while for the red region the amino acids do not play any role in the stabilization of the CT state., In contrast, the T36E variant where the CT state in the EDA complex is not stabilized by the presence of these aromatic amino acids.

**Poster #19**

## The Switchback DNA: Structural Insights and Implications in Trinucleotide Repeat Disorders through Molecular Dynamics Simulations

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Recent research has highlighted the unique capabilities of DNA nanostructures, demonstrating their potential in specific biological applications such as biosensing and improved drug delivery. For the development of these technologies, structural characterization studies to determine the thermal stability, architecture robustness, and configuration parameters of these motifs are often prioritized. In this study, we focused on the recently discovered DNA motif referred as the switchback DNA, by undertaking a set of detailed characterization analyses. The motif has the intrinsic ability to assemble, similar to several existing nanostructures. Its uniqueness lies in the structure's distinctive parallel strand orientation. Through molecular dynamic simulations, we have contrasted the conformations of the switchback DNA with the conventional B-form duplex. Molecular docking studies of two categories of small molecules provided insights into drug binding modes and further confirmed the structure ability as potential delivery vehicles. Lastly, we also showed that these DNA motifs can form within the short tandem repeats common to several repeat-expansion diseases. We have characterized the structure’s role with these diseases by modeling a trinucleotide CTG/CAG repeat associated with Myotonic Dystrophy I and Huntington's disease, in the switchback and duplex conformations. Our simulations and calculated thermodynamic properties using the Molecular Mechanics/Poisson Boltzmann Surface Area (MMPBSA) method, show that both conformations can exist, though the duplex is slightly preferred over the switchback conformation.

**Poster #20**

## Characterization of heat shock protein expression from mouse brain tissue

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Heat shock proteins (Hsps), a class of stress-response chaperone proteins, are an integral part of the maintenance of proteostasis. Hsps prevent aberrant protein misfolding, aggregation, and degradation, which are common disease pathologies. The proteostasis network, built of Hsp and co-chaperone proteins, responds differentially depending on the type of stress condition or disease, yet a clear understanding of the specific proteins impacted by various conditions remains to be determined. Therefore, evaluating protein expression changes in response to multiple conditions will lead to better understanding of how different components of the proteostasis network are altered in response to stress.

To study the stress response of Hsps under various conditions, our group is developing an RT-qPCR methodology to capture and characterize the expression of multiple heat shock proteins in a murine model. We are working to optimize sample processing of frozen brain punches from various regions of the mouse brain. Current work looks at improving RNA yields from samples using various methods with Trizol reagent and increasing cDNA purity in subsequent cDNA synthesis. Previous work has confirmed acceptable efficiency of various RT-qPCR primers for Hsp 70, Hsp 27, Hsf1, and GAPDH. Successful optimization and development of this methodology will allow us to generate baseline Hsp expression patterns in mouse brain samples, and eventually study their response to various stress conditions. Ultimately, this work will contribute to the study of proteostasis and our understanding of how the chaperone network responds to stress and prevents protein aggregation diseases.

**Poster #21**

## Investigating Cancer Association of ERK2 Mutations: Computational Analysis of ERK2 Catalytic Pathway

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Extracellular signal-regulated kinase ½ (ERK2), a vital component of mitogen-activated protein kinase signaling, remains incompletely understood despite available X-ray crystal structures. This research employs computational techniques, including molecular mechanics, coupling analysis, and QM/MM simulations, to elucidate the mechanisms governing ERK2 activation and deactivation. The evaluation of both wild-type ERK2 and cancer-associated variants is directed to uncover novel insights into its catalytic properties and role in cancer pathways. Furthermore, a comparative analysis of ERK2's catalytic efficiency is conducted between the protein substrate, myelin basic protein (MBP), and peptide substrate, ERKtide, to highlight key differences in substrate specificity. By identifying these differences, my research aims to shed light on ERK2's substrate recognition mechanisms and its potential implications in disease pathogenesis. Ultimately, this project is goal-oriented to advance our understanding of ERK2 function, providing valuable insights into its role in cancer progression and the impact of mutations on catalytic activity and cellular signaling dynamics.

**Poster #22**

## Insights into the Structural Characteristics of TDP-43's Disordered C-terminal Domain within a Condensed Phase

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TAR DNA-binding Protein 43 (TDP-43) is an intrinsically disordered protein involved in RNA metabolism. TDP-43 protein aggregates have been well-documented to contribute to neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS) and Alzheimer's Disease. The liquid-liquid phase separation (LLPS) of TDP-43 is thought to be essential to its function in physiological and pathogenic conditions. Previous work has illustrated that the disordered C-terminal domain (CTD) heavily contributes to the phase separation of TDP-43, and that an $\alpha$-helix in the conserved sequence region (CR) of the CTD is an important initiator of TDP-43 LLPS. We are now interested in the structural details of LLPS: if the CR retains a helical structure, and what parts of the CTD mediate contacts within the phase. An aggregation-resistant variant of TDP-43 CTD was constructed to allow for the creation of a homogenous phase-separated TDP-43 CTD sample for analysis via NMR spectroscopy. Solution-state NMR data indicated the CTD of TDP-43 largely retains its disordered structure in the condensed phase. However, the broadening and disappearance of peaks corresponding to the CR suggests the CR adopts a more structured state within the phase. Solid-state NMR data of the condensed phase sample has also identified a structured motif in the CR, with helical characteristics, suggesting that the $\alpha$-helix is conserved in the phase-separated state. Understanding atomistic details of TDP-43 LLPS will give more insight to the transition from LLPS to toxic protein aggregates, aiding our comprehension of neurodegenerative diseases.

**Poster #23**

## Hydrogen Deuterium Exchange Mass Spectrometry of the CheW Coupling Protein in E.coli Chemoreceptor Complexes

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Chemotaxis is a biological process that enables bacteria to swim towards nutrients and away from repellents. Chemotaxis occurs through the work of core signaling units bound together through protein-protein interactions. Each core signaling unit contains the transmembrane receptor, the histidine kinase CheA, and the coupling protein CheW. The key question we aim to answer is whether CheW plays only a structural role in the arrays, or whether it is also involved in propagation of the signal from the receptor to CheA. To answer this question, I perform hydrogen-deuterium exchange mass spectrometry (HDX-MS) on CheW in solution and in kinase-on and kinase-off complexes.

The HDX of CheW in solution suggests the beta barrel core of the protein is stable while the CheA-CheW binding surfaces fully exchange with deuterium in 3 minutes. Overall, 61% of the peptides in CheW are fully exchanged with deuterium in 3 minutes which is similar to its structural homolog, the P5 domain of CheA. Less deuterium exchange was observed in the kinase-on state of CheW compared to CheW in solution. All three protein-protein interfaces in CheW exhibit less HD-exchange in kinase-on complexes compared to solution. This indicates CheW is being incorporated into the in-vitro vesicle-bound assemblies. We hypothesize CheW has weaker protein-protein interactions in the kinase-off state and thus will have more deuterium exchange in kinase-off complexes. It remains to be seen whether the uptake differences between signaling states is similar to the CheA-P5 domain, which suggests CheW plays a role in the chemotaxis signal propagation pathway.

**Poster #24**

## Synthesis and quality assessment of in vitro RNA – novel synthesis and analytical approaches

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Messenger RNA (mRNA) holds a pivotal role in the field of therapeutics, representing a groundbreaking approach in medicine. At the same time, CRISPR (guide) RNA and long noncoding RNA are on track to have significant impact in medicine and in our understanding of biology. Ensuring high purity is crucial for all RNA-based fundamental and therapeutic applications. A significant impurity in the manufacturing of in vitro-transcribed (IVT) mRNA is double-stranded RNA (dsRNA), which has the potential to trigger robust anti-viral immune responses. Existing detection methods for impurity lack high sensitivity and/or are time-consuming. To address these obstacles, we have developed a fluorescence-based assay that can be carried out off-line or can be incorporated on-line in a continuous RNA manufacturing process to quantify both mRNA yield and quality. In this fluorescence-based assay, we utilize molecular beacons to identify the ratio of double-stranded vs. single-stranded RNAs in an mRNA pool. To test the system, we analyse 1 kb mRNA synthesized under conditions known to generate (titratable amounts of) dsRNA impurities. The results obtained are in agreement with slower off-line methods such as dot blots and cell-based assays. The molecular beacon assay allows the rapid and sensitive monitoring of dsRNA impurities and promises to be inexpensively incorporated into a chip-based continuous flow synthesis pipeline).

**Poster #25**

## EnCoPhAC-DB and PDB-LIKE: Identifying photoenzyme candidates through database creation and analysis

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Natural enzymes containing certain cofactors have the potential to be repurposed into photoenzymes. These enzymes catalyze reactions under visible light irradiation and hold potential in fields such as organic synthesis, biomedical research, and green chemistry. However, the characteristics that existing natural enzymes should possess to have the potential to become photoenzymes are still unclear. In this work, we analyzed all existing enzymes in the Protein Data Bank (PDB) that have photoactivatable cofactors and substrates co-crystalized to understand the properties of photoenzymes and identify photoenzyme candidates. However, since the availability of relevant small-molecule functional annotations on Protein Data Bank (PDB) is inconsistent, extracting enzyme information manually becomes time-consuming and vulnerable to human error. To analyze the data in a high-throughput manner, we developed the Protein Data Bank Ligand of Interest Likelihood of Enzymes (PDB-LIKE) method to identify the substrate or inhibitor of interest from an enzyme's PDB metadata and associated literature using a confidence-based scoring algorithm. Using this method to identify ligands of interest, we then built a database, Enzymes Containing Photoactivatable Cofactors Database (EnCoPhAC-DB), to store both the reaction and structural data for enzymes that contain potentially photoactivatable cofactors. Aided by the EnCoPhAC-DB framework, we conducted cofactor-substrate distance analysis, phylogenetic analysis, and sequence similarity analysis on thousands of enzymes to identify viable photoenzyme candidates.

**Poster #26**

## An Algorithm for the Detection and Correction of Buried Glycan Structures Toward Improved in Silico Viral Immunogen Design

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Using the principles of molecular dynamics (MD) and atomistic molecular force fields such as CHARMM36, we have previously designed a pipeline of tools to effectively model dynamic glycan topologies atop spike proteins of viruses like HIV, human influenza, and SARS CoV2; however, due to the erratic dynamics of glycans, currently developed force fields often erroneously predict glycan positions to be buried within the protein scaffolds to which they are bound. This causes an underestimate of the resultant glycan shielding's full glycoprotein surface coverage where experimental models have confirmed that no such glycan burial exists and therefore provides an inaccurate model for use in designing complementary immunogenic therapies like antibodies. Here, we propose a simple algorithm to detect buried glycan positions and then modify the physical position of their respective glycosylation sites using GROMACS to generate a more realistic trajectory upon recalculation. This algorithm is intended to be provided to users as a webserver and eventually an MD-integrated mechanism to recalculate glycan positions as needed at each generated trajectory frame. This will allow researchers to ensure that designed therapies contact epitope sites unprotected by glycan shielding with greater confidence.

**Poster #27**

## Solid-State NMR Reveals Signaling-Related Changes in Functional Chemotaxis Receptor Complexes

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Bacterial chemotaxis receptor complexes form signaling arrays that sense the environment during chemotaxis. These complexes are unique to chemotactic bacteria, making them ideal targets for new antibiotics to block cell mobility. The complexes include a transmembrane trimer-of-dimers of the chemoreceptor associated with a histidine kinase, CheA, and a coupling protein, CheW. The mechanism of signal transduction through these complexes is unknown. NMR and HDX mass spectroscopy data collected by our lab shows that only a small fraction of the chemoreceptor is rigid, this is most likely the protein interaction region (PIR). Resonance assignments so far in 2D and 3D 13C-15N spectra of the chemoreceptor in functional complexes are from the PIR plus a few residues on either side of it. Preliminary comparisons of the spectra of different signaling states show significant changes. Further assignment of these spectra will identify residues involved in changes at the protein interfaces that propagate the signal to the other proteins in the complex. NMR studies have also shown that the majority of residues in the coupling protein, CheW, are rigid. 2D spectra of CheW in complexes with different signaling states has shown chemical shift and intensity changes for multiple peaks. Further assignments of the spectra as well as interface-focused experiments will be used to probe the chemoreceptor-CheW interface to understand what changes occur to control the activity of the kinase CheA. This work will help to understand the overall mechanism of signaling in chemoreceptor complexes.

**Poster #28**

## PIP2 Regulation of the TRPV4 Channel: Binding Sites and Dynamic Coupling

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Transient Receptor Potential subfamily V4 (TRPV4) is a non-selective cation channel that plays important roles in thermos-sensing, osmoregulation, nociception and bone homeostasis. The activation and regulation mechanisms of TRPV4 remains poorly understood at present, even with recent determination of cryo-EM structures in both activated and inactivated states. PIP2, a highly negatively charged lipid, has been shown to regulate TRPV4 activities. However, existing studies show controversial or even contradictory effects of PIP2 on TRPV4 as well as the potential binding sites of PIP2. Analysis of available cryo-EM structures suggests that the previously proposed sites on the N-terminal domain and the ARD domain are either too energetically costly for binding or too far from the membrane interface and are thus unlikely the primary sites for PIP2 regulation. Instead, we identified two possible PIP2 binding sites near the cytosolic membrane interface using structural analysis and molecular docking. Free energy simulations are performed to resolve the relative importance of these two sites. Furthermore, using atomistic simulations followed by network analysis, we determine the impacts of PIP2 binding on the dynamic coupling among various domains. The results provide important new details on the possible molecular basis of PIP2 regulation of TRPV4 activities

**Poster #29**

## A small molecule drug stabilizes the intermolecular association of a phase separating fragment of the disordered androgen receptor transactivation domain

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Intrinsically disordered proteins, which do not adopt well-defined tertiary structures under physiological conditions, are implicated in numerous human diseases and represent a large pool of potential drug targets. Small molecules that target the disordered transactivation domain of the androgen receptor have entered human trials for the treatment of castration-resistant prostate cancer (CRPC). Experimental studies have demonstrated that the androgen receptor forms transcriptional condensates upon activation by androgens in cells, and that small molecules that inhibit androgen receptor transcriptional activity increase the propensity of androgen receptor to phase separate in vitro. NMR studies have identified Tau-5 R2, a partially helical 24-residue disordered fragment of androgen receptor (L391-G414) as an oligomerization interface that drives condensate formation. Here, we utilize long time-scale all-atom molecular dynamics computer simulations to simulate the dimerization of two monomers of Tau-5 R2 in the presence and absence of the small molecule EPI-002. We observe that Tau-5 R2 monomers form highly dynamic and heterogenous dimers that rapidly associate and dissociate in an unbiased 100us simulation, and that dimer formation is predominantly mediated by interactions between aromatic residues. We find that the addition of EPI-002 increases the kinetic and thermodynamic stability of Tau-5 R2 dimers, and that EPI-002 mediated dimers are substantially more helical and collapsed than dimers formed in the absence of the molecule. These results provide an atomic resolution mechanistic description of how a small molecule can stabilize the intermolecular association of a phase separating disordered protein.

**Poster #30**

## Impact of ALS-associated mutations in a C-terminal “hot-spot” on TDP-43 phase separation and aggregation

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Fused in sarcoma (FUS) is a nuclear RNA-binding protein whose aggregation is linked to neurodegenerative diseases. Certain disordered proteins including FUS form liquid-like condensates via liquid-liquid phase separation (LLPS). The low complexity domain of FUS (FUS LC) is a key mediator of LLPS. FUS LC forms multivalent interactions with many residue types and contains SYGQ-rich sequences that stabilize the protein’s liquid phase. The details underlying these weak interactions and the importance of specific amino acid proximity are not well understood. Creating and modifying a synthetic FUS LC allows us to probe specific biomolecular interactions that cause disordered protein LLPS. Here, we engineered a repeated 14 mer sequence to mimic the general patterns and bulk composition of amino acids in FUS LC. Preliminary data suggest the synthetic FUS 14mer forms aggregates while WT FUS remains liquid-like. Many differences between synthetic FUS and WT could be responsible for this aggregation, including sequence charge and amino acid sequence features. We aim to modify engineered FUS by (1) adding aspartic acid to learn the impact of charged residues on FUS LC LLPS and (2) altering the level of disorder to learn how the organization of repeats may dictate the LLPS of FUS LC. Results suggest the addition of aspartic acids improves phase separation in 14mer FUS, and the sequence order of amino acids has a profound impact on 14mer phase separation. In improving our understanding of FUS LLPS thermodynamics, we can better understand the mechanisms at play, and make informed decisions on NDD treatments.

**Poster #31**

**Probing the sequence determinants of phase separation with engineered synthetic FUS**

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Fused in sarcoma (FUS) is a nuclear RNA-binding protein whose aggregation is linked to neurodegenerative diseases. Certain disordered proteins including FUS form liquid-like condensates via liquid-liquid phase separation (LLPS). The low complexity domain of FUS (FUS LC) is a key mediator of LLPS. FUS LC forms multivalent interactions with many residue types and contains SYGQ-rich sequences that stabilize the protein's liquid phase. The details underlying these weak interactions and the importance of specific amino acid proximity are not well understood. Creating and modifying a synthetic FUS LC allows us to probe specific biomolecular interactions that cause disordered protein LLPS. Here, we engineered a repeated 14 mer sequence to mimic the general patterns and bulk composition of amino acids in FUS LC. Preliminary data suggest the synthetic FUS 14mer forms aggregates while WT FUS remains liquid-like. Many differences between synthetic FUS and WT could be responsible for this aggregation, including sequence charge and amino acid sequence features. We aim to modify engineered FUS by (1) adding aspartic acid to learn the impact of charged residues on FUS LC LLPS and (2) altering the level of disorder to learn how the organization of repeats may dictate the LLPS of FUS LC. Results suggest the addition of aspartic acids improves phase separation in 14mer FUS, and the sequence order of amino acids has a profound impact on 14mer phase separation. In improving our understanding of FUS LLPS thermodynamics, we can better understand the mechanisms at play, and make informed decisions on NDD treatments.

**Poster #32**

## Molecular Basis for Allosteric Inhibitor Selectivity in IGF-1R kinase

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Kinases are crucial mediators in signaling pathways and have been identified as prospective therapeutic targets. However, achieving inhibitor selectivity among kinases is a significant challenge. We investigated molecular basis of the selectivity of an allosteric inhibitor (MSC1609119A-1) of the insulin-like growth factor-I receptor kinase (IGF1RK), reported to be ineffective for the homologous insulin receptor kinase (IRK). Specifically, we investigated the structural and energetic basis of allosteric binding of this inhibitor to each kinase by combining molecular modeling, molecular dynamics (MD) simulations, and thermodynamic calculations. Our findings suggest that the conformations of the residues M1054 and M1079 coupled with the outward movement of the αC-helix and the steady DFG (D-in and F-out) motif conformation, favor the selectivity of MSC1609119A-1 toward IGF1RK. Furthermore, we postulate that the selectivity attained is a result of the differences in the electrostatic interaction energy and the formation of a unique hydrogen bond in the IGF1RK pocket. The hydrogen bond between the indole ring of the inhibitor and Val1063 of IGF1RK, which is absent with the corresponding Val1060 residue of IRK, provides directionality and specificity to the allosteric inhibitor, thereby making it selective for IGF1RK over IRK. Our study suggests that the conformations of the allosteric pocket residues that lead to inherent differences in the binding affinity are responsible for the selectivity of the allosteric inhibitor for IGF1RK.

**Poster #33**

## Linker Effects on CaMKII Dodecamer Dynamics

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CAMKII (calcium/calmodulin-dependent kinase II) belongs to a family of multifunctional serine and threonine protein kinase encoded by four genes and has 4 different isoforms CAMKIIα, CAMKIIβ, CAMKIIγ and CAMKIIδ. CAMKII can form a dodecamer and each monomer contains a kinase, a regulatory segment, a linker and a hub domain. The available atomic structures of CAMKII show a compact, autoinhibited state that is inaccessible to calmodulin due to kinase docking against the central hub. Activation of CAMKII presumably involves an equilibrium between the compact and open states of CAMKII. Previous studies from the Stratton lab have found not only the kinase and hub domains, but also the linker between the regulatory segment and the hub domain can affect the activity of CAMKII. In particular, alternative splicing leads to numerous linker variants, and these variants can influence the activation of CAMKII by calmodulin as reflected by the measured EC50 values. Here, we construct atomistic models of selected linkers based on the newly solved cryo-EM structure of CAMKIIδ dodecamer, followed by atomistic simulations using the CHARMM36m explicit solvent protein force field. The results suggest that dynamic interactions of charges on the linker and regulatory helix play a key role in modulating the stability of the close state of CAMKII complex and thus its activation. The simulations also reveal the roles of specific charge residues that are consistent with mutagenesis experiments. Taken together, our simulation and experimental studies provide key new insights into the regulation of CAMKII activation through its variable linker region.

**Poster #34**

## Regulation of ATP affinity to the bacterial chemotaxis kinase CheA

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Bacterial chemotaxis receptor complexes are responsible for biasing swimming behavior of bacteria towards favorable environments. Receptors bind ligands such as aspartate and start transmitting signal to the flagellar motor by inactivating the associated kinase CheA. CheA is a histidine kinase with 5 domains. ATP binds to the P4 catalytic domain, autophosphorylates the substrate P1 domain, then transfers the phosphoryl group to a response regulator CheY that binds on the P2 domain of CheA. The receptors inactivate the kinase CheA by decreasing the autophosphorylation activity. This could be regulated by modifying the ATP binding affinity of the P4 domain or by modifying the interaction of the P4 catalytic site and the P1 substrate site. This study aims to determine whether interaction of the P1 domain and P4 domain affects the binding affinity of the catalytic site of P4 for ATP. Using TNP-ATP fluorescence binding assays, I aim to determine whether the substrate P1 domain has a role in the regulation of ATP-P4 binding affinities in a CheA dimer. I compare the binding affinity of ATP in WT CheA and compare it to a construct of CheA without P1 present (CheA P3P4P5). This study also aims to determine the role of a previously undiscovered weak binding affinity of P1 and ATP, which we discovered through chemical shift perturbation NMR experiments. Through these experiments, we will clarify the factors that contribute to ATP binding to the catalytic site of CheA, and thus the activation mechanism of CheA.

**Poster #35**

## Investigation into the influence of 1,6-hexanediol on protein dynamics and solvation, insight into phase-separation propensity of disordered proteins via solvation theory

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Biomolecular condensates have been implicated in many human diseases. The molecule 1,6-hexanediol has been shown to dissolve condensates with varied chemical compositions. The effect of alkanediols on condensate stability has been well studied in the fused in sarcoma (FUS) protein, a disordered protein with transient helical character. Condensate formation of FUS has been linked to a variety of sarcomas and neurodegenerative diseases. Experimental measurements have shown that 1,6-hexanediol dissolves FUS condensates in solution, while other alkanediols do not1. Here, we employ all-atom molecular dynamics computer simulations to investigate the effects of 1,6-hexanediol on the conformational ensemble and solvation properties of a 43-residue partially helical fragment of the FUS protein (FUS1-43). We observe that 1,6-hexanediol destabilizes compact helical conformations of FUS1-43 and biases its conformational ensemble towards less helical and more extended conformations. Interestingly, these effects do not appear to result from direct interactions between FUS and 1,6-hexanediol. We quantify the influence of 1,6-hexanediol on FUS solvation using Kirkwood-Buff integrals. We observe that 1,6-hexanediol acts as an excluded osmolyte that increases the average local solvation density of FUS1-43 when compared to simulations run in neat water. Based on these results, we hypothesize that increased hydration of disordered proteins in the presence of 1,6-hexanediol thermodynamically disfavors the hydrophobic association of disordered proteins required to form biomolecular condensates. We compare the effects of 1,6-hexanediol to those observed in simulations of FUS1-43 in the presence of 2,5-hexanediol and trimethylamine N-oxide (TMAO).

Perdikari, Murthy, Fawzi, biorxiv, 2022

**Poster #36**

## Kinetic Analysis of Thermally Induced Aggregation of Native Human Gamma-D Crystallin

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Aggregation of Human Gamma-D Crystallin ($H\gamma D$) results in opacity of human lens, called cataract. Towards a fundamental molecular understanding of the kinetic pathways of cataract formation, we have performed in vitro laser light scattering experiments on $H\gamma D$ solutions by varying temperature and ionic strength. We have examined the effects of different heating methods and variations in salt concentration on the aggregation process, particularly in terms of the time-dependent changes in scattering intensity and aggregate size. Our findings show that gradual heating can initiate aggregation at temperatures below those required for thermal unfolding, whereas rapid quenching lowers the critical temperature needed for aggregation compared to gradual heating. Notably, the aggregation of $H\gamma D$ observed here does not involve protein unfolding. The aggregation is reversible for shorter duration of temperature jump, but is irreversible for longer durations. Additionally, presence of sodium chloride in the protein solution significantly lowers the temperature threshold for aggregation compared to a standard PBS buffer solution alone. By applying crystal growth theories, we elucidated the intriguing aggregation behavior of $H\gamma D$. This investigation sheds light on the complex pathways of protein aggregation, offering new perspectives for understanding lens protein behavior under stress conditions.

**Poster #37**

## Molecular Modeling of AAV Capsid-capsid Interaction Under Different Salt Concentrations, and Surfactants to investigate the aggregation

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**Purpose:**
Adeno-associated virus (AAV) is a non-enveloped virus with approximately 26 nm diameter, which exhibits great potential as a gene delivery tool. However, its manufacturing has challenges such as lower yields, and moderate purity, attributed to degradation during various stages of production. This degradation occurs potentially via aggregation at low ionic strength and insufficient surfactant concentration. These mechanisms of degradation remain poorly understood, and there is a lack of a systematic approach to formulation development. To address this, a coarse-grained molecular dynamics (CG-MD) simulations approach was utilized to understand the molecular level of capsid-capsid interaction under different types and concentrations of salts and surfactants. The model was validated with experimental data.

**Methods:**
CG-MD simulations were performed using the GROMACS package with MARTINI force fields. AAV8 (PDB: 6v12) with 2.2kb single-strand DNA was selected as the model viral capsid. Energy minimization used the steepest descent algorithm, followed by a 10 ns equilibration step. Production runs were performed using the Nosé−Hoover thermostat and the Parrinello−Rahman barostat at 1 bar pressure for each 20-fs time step in an isothermal-isobaric ensemble.

**Results:**
We investigated the effect of different types and concentrations of salts on AAV vector aggregation. Our results showed that NaCl and MgCl2 salts at various concentrations could prevent aggregation to some extent. AAV vector aggregation was influenced by the ionic strength of the solution, and NaCl salts were effective at preventing aggregation when present at a concentration above 0.05 M. Additionally, surfactants such as poloxamer 188 at concentrations ranging from 0.001% to 0.01% (w/v) were tested, and our findings suggest that the type and concentration of surfactants are critical factors that affect AAV vector aggregation.

**Conclusion**
Our developed computational model, with a microsecond timescale, evaluated the behavior of different salts and surfactants with varying concentrations to understand the factors preventing AAV8 aggregation. This analysis provided insights that would be difficult to obtain from experimental studies. The CG-MD simulations proved powerful in simulating macromolecular complexes and guiding the interpretation, and direction of experiments. Our simulations provided important insights into AAV vector formulation development, which could lead to the development of more efficient and stable gene delivery systems.

**Keywords:**
Adeno-associated viruses, molecular dynamics, capsid aggregation, protein interaction, ssDNA, salt, surfactant.

**Poster #38**

## Conformational Effects Associated with Chemical Modifications in RNA

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Post-transcriptional modifications in RNA play an essential role in various cellular processes, including gene regulation, transcription, and translation. Currently, hundreds of chemical modifications have been identified in RNA molecules ranging from methylation to more complex chemical modifications. Despite recent advancements in the mass-spectrometry and sequencing technologies which enabled identification and characterization of post-transcriptional modifications in RNA, a full understanding of the effects of modifications on the RNA structure, folding, and dynamics remains elusive. In this work, atomistic simulations were utilized to better understand structural and conformational effects of methylation, a common modification to RNA molecules. At first, the energetics and dynamics of syn/anti configurations adopted by the methyl group in the methylated adenine mononucleotide were characterized, thus resolving the effects of methylation on one of the basic building blocks of RNA. Then, an RNA hairpin capped by a methylated tetraloop motif was studied, a commonly observed structural motif in RNA consisting of four nucleotides which serves as a recognition site for proteins. Methylation of the tetraloop was identified to enhance its flexibility, thereby revealing conformations accessible to the reader protein.

**Poster #39**

## Characterization of an Antifreeze Protein, ApAFP752

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Antifreeze proteins are found in a wide range of cold adapted organisms, and they contribute to their freeze resistance. Antifreeze proteins adsorb to the ice surface and inhibit the growth of ice crystals. The goal of this project is to investigate the mechanism by which antifreeze proteins protect against the damage typically inflicted by the cold, including the underlying molecular mechanism of ice-binding. Here we are presenting structural and functional characterization of an antifreeze protein, ApAFP752 from the desert beetle Anatolica polita utilizing nuclear magnetic resonance (NMR) spectroscopy and other biophysical methods. Current work focuses on the application of this protein in cryopreservation protocols, and on engineering a version of the antifreeze protein with enhanced activity.

**Poster #40**

## Defining the Molecular Mechanisms of HuR- and MiR29a-Mediated VEGF-A mRNA Stabilization

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Human antigen R (HuR) is a ubiquitous RNA-binding protein that plays a role in the post-transcriptional regulation of several mRNAs. In the context of peripheral artery disease (PAD), HuR binds and stabilizes vascular endothelial growth factor A (VEGF-A) mRNA, promoting its expression and subsequent increased arteriogenesis in response to cardiac stress. In aged mice, the HuR binding and half-life of VEGF-A mRNA is reduced, as is arteriogenesis following artery ligation. A microRNA, miR-29a, has recently been implicated in binding and stabilizing VEGF-A mRNA at a site adjacent to the HuR binding site. The goal of this project is to define the interactions between HuR, the 3' UTR of VEGF-A mRNA, and miR-29a via the use of nuclear magnetic resonance (NMR) spectroscopy, microscale thermophoresis (MST), and other biophysical techniques. These methods will be used to evaluate the impact of ligand binding (i.e. mRNA or miRNAs) on the conformation and dynamics of HuR to further define the molecular details of the interaction between HuR, VEGF-A mRNA, and miR-29a.

**Poster #41**

## Elucidating the pH-Dependent Dynamics of Enterovirus D68 Capsid Using Continuous Constant pH Molecular Dynamics

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Enterovirus D68 (EV-D68) is increasingly recognized as a public health challenge due to its potential to cause severe respiratory disease. Our investigation uses continuous constant pH molecular dynamics (CpHMD) capabilities within the Amber suite, using the Gbneck2 implicit solvent model, to investigate the pH-responsive structural dynamics of EV-D68. This advanced computational approach enables us to calculate precise residue-specific pKa values, shedding light on protonation state fluctuations across a spectrum of pH levels. Preliminary results from our simulations have revealed considerable shifts in the pKa values of key residues of Asp, Glu, and His located near the symmetry axes of the capsid. These shifts are hypothesized to be instrumental in the capsid uncoating process and the subsequent release of the viral genome. Our efforts provide a granular view of the structural changes that occur during the critical phase of host cell entry, where acidic conditions prevail. By mapping these conformational alterations, our research aims to pinpoint strategic intervention points for the design of antiviral drugs. Focusing on EV-D68, our work aims to delineate the molecular events that underpin its infective journey, improving our broader understanding of viral mechanics, and guiding the genesis of new therapeutic avenues.

**Poster #42**

## AI for prediction of protein function and for elucidation of enzyme function and effects of mutations

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Our Machine Learning methodology, Partial Order Optimum Likelihood (POOL) is used to predict biochemically active amino acids in the three-dimensional structures of proteins. Computed electrostatic and chemical properties of individual amino acids serve as input features. Our most recent applications of POOL are described. From predicted local sites of biochemical activity, the biochemical functions of Structural Genomics proteins of unknown function are predicted by local structure matching of predicted spatial arrays of active amino acids with those of proteins of known function. Examples from the Haloacid Dehalogenase superfamily, with experimental testing of function by direct biochemical assay, are featured. POOL analysis also uncovers the types of interactions that enable protein structures to transform the weakly acidic or basic side chains of amino acids into strong acids, strong bases, or nucleophiles in the active site. Finally, we predict the effects of mutation at specific sites on biochemical activity. Predictions correlate well with experimental kinetics data for wild type and disease-associated mutations of human DNA polymerase kappa. Supported by NSF MCB-2147498 and CHE-1905214.

**Poster #43**

## Enhancing Antibody-Antigen Interaction Sampling with Molecular Dynamics and Docking Simulations

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Deep learning techniques are currently employed to analyze protein-protein interactions for classification and prediction purposes. However, the flexibility and unique structures present in antibody-antigen (Ab-Ag) interactions pose a challenge, limiting experimental observations to docked states only. To improve Ab-Ag model specificity, we are using molecular dynamics and docking tools in a high-performance computing environment to generate training data in the form of negative samples. This complements observed successful Ab-Ag docking and will aid the creation of a more scientifically robust explainable AI model. Successful development of Ab-Ag analysis methods holds the potential to improve protein engineering and vaccine design.

**Poster #44**

## GNN codon adjacency regulates protein translation

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The central dogma treats the ribosome as a molecular machine that reads one mRNA codon at a time as it adds each amino acid to its growing peptide chain. However, this and previous studies suggest that ribosomes actually perceive pairs of adjacent codons as they take three-nucleotide steps along the mRNA. We examined GNN codons which we find are surprisingly overrepresented in eukaryote protein-coding open reading frames (ORFs), especially immediately after NNU codons. Ribosome profiling experiments in yeast revealed that ribosomes with NNU at their aminoacyl (A) site have particularly elevated densities when NNU is immediately followed (3') by a GNN codon, indicating slower mRNA threading of the NNU codon from the ribosome's A to peptidyl (P) sites. Moreover, if the assessment was limited to ribosomes that have only recently arrived at the next codon, by examining 21-nucleotide ribosome footprints (21-nt RFPs), elevated densities were observed for multiple codon classes when followed by GNN. This striking translation slowdown at adjacent 5'-NNN GNN codon pairs is likely mediated in part by the ribosome's CAR surface which acts as an extension of the A-site tRNA anticodon during ribosome translocation and interacts through hydrogen bonding and pi stacking with the GNN codon. The functional consequences of 5'-NNN GNN codon adjacency are expected to influence the evolution of protein coding sequences.

**Poster #45**

## RNA Translocation through Protein Nanopores: Unfolding of Secondary and Tertiary Structures

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The translocation of RNA is greatly influenced by secondary and tertiary structures, making it challenging to track the structural changes during translation. We open up a new simulation framework that can capture the properties of the multi-level structures and the dynamical translocation of RNA. Our method is based on a combination of oxRNA model for RNA and Poisson–Nernst–Plank calculation for the electric field in protein nanopores. Through systemtic studies of the translocation behavior in three protein nanopores: α-hemolysin, CsgG, and MspA, three featured stages (pseudoknot, melting, and molten globule) have been explicitly identified based on the contact map and current traces. Further classification of the two translocation modes (fast and slow modes) based on the translocation time leads to the discovery of the following new physical phenomena: (1) In the fast mode, the average translocation speed is found to be independent of the nanopore geometry. Instead, it is primarily controlled by the electrical potential difference between the trans and cis sides of the nanopore. (2) In the slow mode, the molten globule stage is the key factor in slowing down the translocation of the hairpin RNA instead of the melting of the base pairs. Finally, we find that the electric field distribution is mainly responsible for the molten globule and not the geometry of the nanopore. These results provide a fundamental understanding of role of the secondary and tertiary structures on the translocation of RNA in direct RNA sequencing platform based on single-molecule electrophoresis. The framework for RNA translocation is promising for the exploration of advanced translocation problems and the design of the new protein nanopore.

**Poster #47**

## Investigating the Impact of Furin Cleavage on SARS CoV-2 Spike Structure with Molecular Dynamics Simulations

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The Coronavirus Spike glycoprotein (S) plays a key role in viral attachment, fusion and immune response; hence it is necessary to have a thorough understanding of how its dynamics drive function. One major difference between SARS CoV-2 and its predecessors is the presence of a furin cleavage site (FCS), which creates two subunits (S1, S2) of the Spike. The cleavage of this furin-responsive RRAR motif accelerates the post receptor-binding cascade of events by enhancing S1 shedding from S2 and is responsible for increased infectivity and transmissibility of the virus. However, structural studies on the spike protein have generally been done with the FCS mutated to prevent furin action, and hence the post-cleavage dynamics of the actual spike protein remain elusive. To address this, we have performed large-scale atomistic Molecular Dynamics simulations of this protein in the receptor-accessible (RBD-up) and the receptor-inaccessible (RBD-down) conformations, with a complete glycosylation profile. To extract the essential dynamics that may drive the cleavage-triggered transitions, we performed a combined Principal Component Analysis (PCA) on the furin-cleaved and uncleaved systems. Our findings indicate that both these systems have different directions along which they exhibit their extreme motions. We also found that the furin-cleaved conformation exhibits a larger number of highly correlated motions than the uncleaved conformation These findings provide valuable insight into the Spike S1-S2 shedding transition, which can be used to design therapeutics that implement perturbation strategies.

**Poster #48**

## Myosin's affinity for phosphate depends on both concentration and strain

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Intensely contracting skeletal muscle rapidly loses the ability to generate force, due in large part to phosphate (Pi) inhibiting myosin's force-generating capacity, in a process that is strain dependent. Crucial aspects of the mechanism underlying this inhibition remain unclear. Therefore, we directly determined the effects of increasing [Pi] on myosin's ability to generate force against progressively higher resistive loads in a laser trap assay with the requisite spatial and temporal resolution to discern the mechanism of inhibition. Myosin's force-generating capacity decreased inversely with increasing [Pi], an effect that became more pronounced at higher resistive loads. The decrease in force resulted from myosin's accelerated detachment from actin, which was also load-dependent. Contrary to widely accepted hypotheses the rebinding of Pi to actomyosin did not appear to reduce force through either preventing, or reversing, myosin's powerstroke. These data therefore point toward a mechanism in which the rebinding of Pi to actomyosin reduces force during fatigue solely by accelerating myosin's detachment from actin.

**Poster #49**

## Experimentally probing the effect of confinement geometry on lipid diffusion

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The lateral mobility of membrane components has been shown to play a key role in the kinetics and processes associated with membranes. The influence that membrane inhomogeneities have on the lateral mobility of lipids, proteins, and cholesterol is not yet well understood. We investigate the effect of confinement on the two-dimensional diffusion of DLPC lipids in supported lipid bilayers. Specifically, we explore how changing the geometry of escape routes affects the diffusion of confined lipids. We compare lipid diffusion through narrow necks formed by periodic arrays of circles to diffusion into semi-circles with openings equal in size to the narrow necks between circles. To further probe the effect of geometry on diffusion, we study how diffusive behavior changes when semi-circles have four openings—whose lengths are equal to the size of the narrow necks between circles—as opposed to one opening, whose length is equal to the sum of the narrow neck lengths between circles. We demonstrate a platform that can steer the long-range diffusion of lipids using simple oxide deposition approaches, enabling us to systematically explore how confinement size and shape impact diffusion over multiple length scales. We probe diffusion and its length scale dependence through optical microscopy techniques, namely Fluorescence Recovery After Photobleaching (FRAP) and Fluorescence Correlation Spectroscopy (FCS). It has been shown that obstructed diffusion can be described by multiple different theoretical models, and we directly compare our systems to these models, finding that certain descriptions capture underlying trends while, in some cases, others unexpectedly breakdown.

**Poster #50**

## Deciphering MBL-MANLAM Interactions: Computational Insight into MBL Binding to Tuberculosis

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Understanding interactions in the lungs is crucial to combat infections and design effective interventions. That's why at the Minkara COMBINE Lab, we use computational techniques to study these interactions at a molecular level. Mannose-binding lectin (MBL) is a protein that plays a vital role in the immune system's fight against lung infections. MBL attaches to invaders such as bacteria, viruses, or yeast, activating the complement system that is crucial for the immune response. One common infection that affects the lungs is tuberculosis, which is caused by a bacterium called Mycobacterium tuberculosis. Tuberculosis contains glycans, including mannose-capped lipoarabinomannan (MANLAM), which are essential for mycobacterial survival during infection. MBL effectively combats infection by binding to mannose within these glycans. Our study focuses on understanding MBL's interaction with MANLAM in tuberculosis. MANLAM contains several mannose caps to which MBL binds. We are currently generating 3D computational models for each of the mannose caps. This effort aims to provide visual clarity and understanding of the components of the mannose caps. To explore how MBL binds to the mannose caps, we employ induced fit docking. This method facilitates the calculation of binding free energies between the mannose caps and MBL, along with Molecular Dynamics simulations to deepen our understanding of their interactions. In summary, leveraging computational methods to study MBL's interactions with tuberculosis offers bioengineers a powerful toolkit to unravel the complexities of immune responses, accelerate research progress, and ultimately contribute to developing strategies for combating tuberculosis and other diseases.

Keywords: MBL, Tuberculosis, MANLAM, mannose-caps, Computational

**Poster #51**

## Shedding light on Surfactant Mediated Endosomal Escape of Nucleic Acid Therapeutics through Coase-Grained Molecular Dynamics Simulations

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Nucleic acids are both programmable and biosafe, making them ideal therapeutics. However, delivery of nucleic acids to the cell poses several challenges, including degradation prior to reaching their intended target. To overcome these obstacles, we have designed a nucleic acid delivery system, composed of a degradable surfactant micelle functionalized with oligonucleotides. These Nucleic Acid Nanocapsules (NANs) are designed to be endocytosed and disassemble in the endosome, releasing oligonucleotides conjugated to a surfactant. Experimental assays have shown the inclusion of the surfactant increases the ability of the oligonucleotide to cross the endosomal membrane barrier and enter the cytosol, which is a major bottleneck in nucleic acid delivery. To increase the endosomal escape capabilities of our delivery system, we have designed surfactants that, upon degradation, contain between one and three hydrophobic tails.

We have employed molecular dynamics (MD) simulations to evaluate the hypothesis that the three tailed and two tailed surfactants are better able to induce endosomal escape of the nucleic acid cargo due to their improved lipid disruption capabilities. We have parameterized the degraded surfactant in the coarse-grained Martini 3 forcefield against all-atom MD data. In a simulated lipid bilayer, we have tested how each surfactant changes the bilayer structural properties and alters the energetics of translocation of particles across the membrane.

From these studies, we hope to gain insights into the role that the additional surfactant tails may play in the lipid disruption capabilities of these molecules, and ultimately develop design rules for constructing the most effective nucleic acid delivery system.

**Poster #52**

## Exploring the Structure and Assembly of Janus-base Nanotubes through Multiscale Molecular Dynamics Simulations

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In the field of biomedical engineering, nanomaterials have proven to be a promising area of study, with functionalized nanoparticles being used for drug and gene delivery as well as various other purposes. Janus-base nanotubes (JBNts) are a relatively new type of nanotube with particular promise due to their high biocompatibility and ability to conduct electrons. JBNTs (also known as helical rosette nanotubes) are made up of monomers which spontaneously self-assemble in aqueous solution via π-π stacking and complementary hydrogen bonding to form a series of six-membered rings (rosettes) stacked into a nanotube shape, with functionalized sidechains oriented outwards We present stable all-atom models of native-state JBNts with four amino acid sidechains (Lys, Gly, Asp, Arg) in GROMACS, structural details such as sidechain conformation and inner channel solvation structure. In addition, we present elastic-network coarse-grained model for these JBNts, as well as characterizing basic interactions with elements of the cell membrane. Finally, we propose that the mechanism of self-assembly is entropically driven and analogous to protein folding: hydrophobic interactions (π-π stacking) drive monomer association, while hydrogen bonding interactions impart specificity which are refined as the reaction mixture samples conformational space until native-state nanotubes are formed.

**Poster #53**

## Accounting for Ion Polarization to Stabilize Lipid Bilayers in CgProt 3

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CgProt is a coarse-grained force field for simulations of membrane-protein systems, originally developed by force matching atomistic reference simulations through a method known as multiscale coarse-graining (MS-CG). To stabilize large protruding non-cylindrical proteins for microsecond simulation in prokaryotic membranes, explicit salt counterions were added to the implicit solvent model. Scaled ion interaction potentials were developed to capture electronic polarization as a mean field correction. Consistent with atomistic simulations using electronic continuum correction (ECC), calcium but not sodium or chloride are attractive for the zwitterionic bilayer interface. Stabilization by counterions enabled constant surface tensions simulations at 0 to 25 mN/m that reproduce the area per lipid in POPE and POPG bilayers. A tension of 50 mN/m was still needed to equilibrate the POPC bilayer.

**Poster #54**

## Probe of Labile Iron in the Mitochondrion and Relation to Ferroptosis

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We report bifunctional iron chelating peptides with subcellular localization properties and fluorescent reporting of iron binding. Dysregulation of iron has been linked to a variety of disorders including neurodegeneration and cancer, and one underlying process is thought to be a programmed cell death pathway, ferroptosis, which may be related to lipid peroxidation caused by weakly bound intracellular iron. Iron chelators shown to modulate and monitor free iron concentration in cells include derivatives of the tachpyr, hydroxypyridinone, and pyridoxal isonicotinoyl hydrazone families. Iron chelators are also shown to arrest the ferroptotic pathway, though further understanding of cellular iron metabolism is essential prior to medicinal use of synthetic and natural iron chelators. We have prepared bioconjugated peptides by orthogonal Fmoc-based solid-phase peptide synthesis (SPPS). Fluorophores and iron chelators are attached to monitor local iron concentrations through fluorescence quenching. High-performance liquid chromatography (HPLC) and mass spectrometry are employed to assess purity of synthesized species. Epimerization of the diaminopropionic acid residue upon resin loading was discovered via HPLC, informing several adjustments to SPPS methods. Sensors are being prepared with varying iron binding capabilities and different fluorophores to optimize relevant photophysics. Mechanism of subcellular targeting is characterized via coarse-grained molecular dynamics. Simulations are run on GROMACS using the system generated by CHARMM-GUI with the MARTINI force field.

**Poster #55**

## Structural determinants of electrostatically-guided assembly of protein/polyanion complexes

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Understanding the molecule mechanism of interaction between lactoferrin and glycosaminoglycans (GAGs) is critical, as lactoferrin achieves its multifunctional activities through electrostatic interaction with cellular GAGs, highly sulfated biological polysaccharides. However, the structural heterogeneity of such polyanions provides challenges for understanding the molecular mechanism of lactoferrin and GAGs bindings. We use unfractionated heparin (UFH), belonging to GAGs, to study non-covalent interaction with human lactoferrin (hLF) by native mass spectrometry (native MS) coupled with limited charge reduction (LCR). We found that UFH chains are saturated by hLF molecules in a hLF: UFH=3:1 molar ratio. Up to five lactoferrins can be accommodated on a heparin chain, consistent with the molecule modeling studies indicating that eight saccharide units are required to bind with a single lactoferrin. This study provides further insight into molecular mechanisms of hLF/heparin interactions, contributing to a better understanding and modulation of the multifunctional activities of lactoferrin.

**Poster #56**

## High Temperature Analysis of the two Dimers of Beta-lactoglobulin

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Protein folding dictates protein function. Understanding and visualizing the misfolding of proteins is crucial to understanding diseases such as Alzheimer's, Parkinson's, Huntington's, etc. To visualize this protein folding we combine the GROMACS program with pyMOL, allowing these visualizations to be done without human error or expensive equipment while maintaining accurate results. The simulation is run in a vacuum to mimic the conditions of mass spectrometry. Because heat causes protein denaturation, we heat proteins to high temperatures and simulate the results, from which protein misfolding can be studied and understood. In this project, the beta-lactoglobulin protein is used as a model, believed to be responsible for enhancing immune response. The open and closed dimers of beta-lactoglobulin resulted in different collision-induced unfolding at extremely high temperatures (300-900K). To study this unfolding reaction further, we increase these temperatures to 1200 K where the rate of collision-induced unfolding and protein denaturation can be analyzed in relation to lower temperatures. By furthering biological understanding of protein misfolding, we are one step closer to being able to prevent the biological dysfunction of protein misfolding.

**Poster #57**

## Parameterization and molecular modeling of wobble uridine in bacterial tRNA

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Over 140 naturally occurring modifications have been discovered in RNA to date. Many of these modifications are found within transfer RNA (tRNA) where they regulate important cellular functions such as translation by modulating base-pairing preferences, structure, and stability of RNA. We focus on the modifications that occur on uridine at the 34th wobble position within bacterial tRNAs. These modifications have been linked to stress pathways in bacteria and can be exploited for antibiotic development. In this project, our goal is to use all-atom molecular dynamics simulations (MDS) to parameterize and model 12 uridine modifications and characterize their base-pairing specificities. This set of uridines have either been modified with a sulfur, selenium, or geranyl group at the second position with an aminomethyl (nm), methylaminomethyl (mnm), or carboxy-aminomethyl (cmnm) at the fifth position. The first step in running a MDS with RNA modifications is to obtain simulation parameters: bonded (strength of bonds, angles and dihedrals) and non-bonded (size and charges) parameters, for each of the atoms in the modified nucleotide. For calculating partial charges, we generated the structures of the modified nucleotides, followed by geometry optimization (WEBMO) and subsequently charge calculations (R.E.D.D server). All other parameters were adapted from the AMBER99 Chen-Garcia forcefield. In MOE, the completed uridine modifications were introduced into a duplex and to prepare the system for running MDS in GROMACS. We used the results from our simulations for in silico characterization of base-pairing preferences of these modifications.

**Poster #58**

## Dependence of P/E tRNA Hybrid Formation on Subunit Rotation in the Ribosome

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The ribosome is a massive two subunit nucleoprotein assembly responsible for protein synthesis in all living cells. The ribosome and the tRNA molecules undergo several large-scale functional rearrangements during the protein elongation cycle. Here we explore two such rearrangements in the ribosome and tRNA molecules during the translocation step in bacteria, namely, P/E tRNA hybrid formation and inter-subunit rotation. We utilize multi-basin all-atom structure-based (SMOG) models and the Ribosome Angle Decomposition (RAD) method to study the dependence of P/E hybrid formation on rotation of the small subunit body with respect to the large subunit. Structure-based models provide a simplified energetic description, where experimental structures are defined to be the potential energy minima. We employ all-atom structure-based models to simulate spontaneous P/E hybrid formation. By assessing the tRNA dynamics for different degrees of small subunit body rotation, we are identifying the precise physical relationship between these two large-scale collective processes. Mean first passage time (mfpt) for P/E hybrid formation as a function of subunit rotation revealed a non-monotonic dependence. The mfpt for P/E hybrid formation decreases as body rotation increases to a threshold value beyond which it increases. The all-atom SMOG model provides insights into the structural factors that govern the dynamics, including revealing individual proteins that introduce sterically-induced barriers that lead to non-monotonic behavior.

**Poster #59**

## DNA-mediated Assembly of Functional Chemoreceptor Complexes in *E. coli*

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Chemotaxis is the process by which an organism moves in response to a chemical stimulus. The chemotaxis signaling pathway in *E. coli* relies on a complex array of transmembrane chemoreceptors with cytoplasmic binding partners to transmit a signal and produce a response. The specific mechanisms and dynamics of signaling are not fully understood, and experimentation is limited by the ability to reconstitute the array's quaternary structure in vitro. Current assembly methods of these complexes achieve homogenous native assembly and allow for activity; however, they lack the precise control necessary to determine how the array and receptors control kinase activity and signal transmission. Using a bi-tetrahedron DNA-nanotechnology scaffold, precise assembly of complexes can be achieved. DNA oligonucleotides extended from the tetrahedron are covalently linked to an N-terminal, cys-modified receptor protein. The DNA tetrahedron with oligonucleotide extensions has been functionally assembled, and successful linkage between the oligonucleotide extensions and the protein has been achieved. Optimizing this product formation will allow for the formation of functional chemoreceptor complexes with the ability to direct assembly and control the architecture of the array. While these techniques are currently being tested for in vitro assembly to study structure and function, modifications can be made to the system to allow for in vivo applications of sensing and delivery.

**Poster #60**

## Probing ion binding structure and dynamics with infrared spectroscopy

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We use infrared spectroscopy as a direct probe of structure and dynamics in signaling metalloproteins and chelating compounds that serve as models of biological ion binding. These measurements provide a window into the biophysics of ion-mediated signaling that supplies both picometer-scale structural sensitivity and sub-microsecond temporal resolution. In combination with molecular dynamics and electronic structure calculations, this experimental approach extends the snapshots offered by complementary methods in structural biology to provide a new view of ion binding and signaling events.

Biological ion binding structures and signaling pathways mediate nearly all physiological processes. These elements of the cellular machinery are essential to human life and sensitive not only to the equilibrium structural changes that accompany ion binding, but also to the dynamics of these transitions: the speed with which they occur, the stability that they confer, and the flexibility of the ion-induced local and global structures. Modifications to these dynamic properties that are not readily discerned by conventional structural tools may hold the key to understanding cancer, cardiac arrhythmia, and other diseases associated with mutations within or nearby ion binding sites in signaling proteins.

**Poster #61**

## An Image-Based Coarse-Grained Model for Biological Polymeric Networks

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Polymeric networks, ubiquitous in nature, exist in diverse shapes and forms. Notably, they constitute the building blocks of human tissues. Collagen and elastin fiber networks, large fibrous networks within the extracellular matrix (ECM), exemplify these structures. Traditionally, polymeric networks and their constituents have been studied using either atomistic or large-scale models. However, atomistic simulations of large molecular constituents like elastin lead to significant computational costs. Conversely, while large-scale models often represent fibers as beams for finite element analysis, this approach can overlook crucial intrinsic polymeric properties. This study proposes a bead-spring model for large polymeric networks based on quick-freeze/deep-etch electron microscopy images of the polymeric ECM structure. This approach significantly reduces computational costs while capturing the inherent polymeric nature of the system. We present a comprehensive parametric study to explore the mechanical response of the proposed model. In particular, we investigate the variability in the network's mechanical response forconstitutive laws of the fiber elements. We propose that the model's inherent customizability allows for adaptation to various imaging modalities, diverse polymeric networks and the incorporation of different material properties therein.

**Poster #62**

## Overcoming Extrapolation Challenges of Deep Learning by Incorporating Physics in Protein Sequence-Function Modeling

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Predictive protein sequence-to-function mapping is crucial to understanding genetic diseases, protein evolution, and protein engineering. The sequence-to-function relationship of proteins is very complex. Deep learning algorithms such as neural networks and convolutional networks have become very popular for leveraging deep mutational scan data to construct predictive models of protein function. These networks, trained with large data sets, can capture complex mapping between the input and output via hidden layers with non-linear activation function, as demonstrated in recent NN4dms models. However, it remains very challenging for these models when extrapolated to variants with positions or mutation types not seen in training data. We propose to use physics-based modeling to quantify the energetic effects of all training and test mutant data sets. We show that incorporating these physical energetics within the graph convolution networks (GCN) could significantly improve the performance of positional and mutational extrapolation compared to the original NN4dms models. Additional improvement may be achieved by calculation of more accurate physical energies and including additional physics-based properties such as dynamic fluctuation and coupling.

**Poster #63**

## Erratic Electrons: Uncovering the Energy Output of Singlet Fission in Dimer Chromophores

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Light-harvesting molecules (chromophores) are the catalytic foundation of the light-dependent process of photosynthesis. In the excitation process of a chromophore, the promotion of electrons to higher energy levels can allow for their binding to the resulting holes, forming a unit called an exciton. The exciton will move across several chromophores, becoming delocalized before the recombination of electrons and holes to emit light. The movement of these delocalized electrons across molecules over time is critical to the understanding of photosynthetic processes; chromophores like zeaxanthin, notable in its role of photoprotection from UV radiation, exhibit singlet fission within their electronic states. Singlet fission promotes a second electron to an excited state of higher spin, which doubles the output of electric charge from a single photon of absorbed light.

We present a computational exciton model aimed at deciphering how the interaction of excitons in dimer molecules--underpinning the singlet-fission process--affects overall molecular dynamics. We find that the biological chromophores native to photosynthetic light-harvesting complexes possess the energetic requirements to undergo singlet fission through the correlated triplet pair supported by dimers of carotenoid pigments.

**Poster #64**

## Exploring the interaction between MUC2 and intestinal signaling molecules using molecular dynamics simulations

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Intestinal mucus, a natural hydrogel, modulates interactions between our underlying tissues and the contents of our gastrointestinal track. Understanding the molecular interactions of mucin glycoprotein, a critical building block of mucus, with signaling molecules such as bile salts, phospholipids, and fatty acids, is important to comprehend their significance in intestinal inflammation and associated diseases and facilitate the development of related therapeutics. In this study, we performed all-atom (AA)and coarse-grained (CG)classical molecular dynamics simulations of simplistic molecular models of the intestinal mucin MUC2 and its interaction with bile salts (sodium taurocholate and sodium taurodeoxycholate) and phospholipids (DOPC- 1,2-dioleoyl-sn-glycero-3-phosphocholine). The complete atomistic structure of MUC2 was assembled using AlphaFold2, homology modeling, and cryoEM maps, and used the model to study interaction patterns of the signaling molecules throughout the length of MUC2. In order to understand the aggregation properties of these glycoproteins which lead to their hydrogel behavior, we have constructed a 3D prototype peptide-glycan network, based on consensus MUC2 Variable Number of Tandem Repeats (VNTR)sequence, and native glycoform distribution. Intestinal signaling molecules have been introduced at physiological concentration to the mesh to elucidate the structural determinants that mediate binding and signaling. Key drivers of intermolecular associations such as hydrogen bonds, salt bridges, hydrophobic packing, as well as effects on rheological properties of mucin with the signaling molecules have been determined. CG simulations are also reported to study bile micelles (formed by bile salts, phospholipids, and fatty acids) with the mucin. The outcomes of this double-pronged approach of combined AA and CG simulations will be presented and discussed.

**Poster #65**

## Simulation Studies of a Mitomembrane Targeting Bioconjugated Peptide

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Iron is an important trace metal that is essential for the functioning of various biological systems and processes. Iron dysregulation has been linked to various disorders including neurodegeneration and cancer. A unique, iron-dependent type of regulated cell death called ferroptosis is believed to involve iron-catalyzed lipid peroxidation that occurs in the mitochondrion or endoplasmic reticulum. To probe labile iron in the mitochondrion, we have studied a Szeto-Schiller (SS) peptide conjugated to an iron chelator, 8-hydroxyquinoline (8HQ), and a fluorophore (dansyl). In this work, we will describe atomistic molecular dynamics (MD) simulation studies of the interactions between the model SS peptide and a mitochondrial-mimicking membrane. Non-equilibrium MD simulations were also used to elucidate the mechanism of peptide translocation across the membrane at different velocities. The findings from these simulation studies reveal the mechanism of peptide entry and transport across the mitomembrane as well as associated energetic barriers.

**Poster #66**

## Exploring the Druggability of Chikungunya Virus Protease nsP2 using Biomolecular NMR

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Chikungunya virus is an arboviral infection which has infected more than 2 million people across 60 countries in the past 10 years alone. CDC suspects that it may result in a widespread outbreak across the globe in the coming years owing to the absence of any drug or preventive vaccine. Pathogens causing these infections are enveloped positive-sense single-stranded RNA viruses which encode a protease that plays a critical role in viral replication and maturation. Since proteases are essential to the viral life cycle, they have been successfully exploited as drug targets for treating viral diseases such as Hepatitis-C and HIV. These findings strengthen the motivation for exploring non-structural protein 2 (nsP2) as a significant antiviral drug target. In this work, we have screened a 500 compound fragment library against chikungunya virus protease nsP2 to validate binding using biomolecular NMR 19F, 1H and STD-NMR experiments. $^{1}$H- $^{15}$N HSQC (2D)- NMR experiments were further carried out on the identified hits and labelled protein to study specific ligand binding and then determine dissociation constant (Kd) using chemical shift perturbation experiments. Our next steps involve identifying the regions of compound binding in the protein through a combination of triple resonance (3D)-NMR experiments for backbone assignments. We will use the knowledge thus gained to synthesize molecules with higher binding affinity and ultimately develop an effective inhibitor against this disease.

**Poster #67**

## The Unfolding Story of tRNA: Insights from molecular dynamics

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RNA undergoes post-transcriptional modifications that can significantly alter its structure and function. Transfer RNAs (tRNAs), the RNAs that decode the mRNA to produce proteins, in particular, are subject to extensive modification. Over 100 distinct naturally occurring nucleotide modifications have been discovered in tRNAs. Though the experimentally resolved structures of the tRNAs with and without modifications show very little structural differences, yet the stability, codon bias and translational efficiency of tRNAs is significantly impacted by the absence of modifications. Magnesium ions also play an important role in stabilizing RNA structure. Using temperature replica exchange MD (T-REMD) we investigated the unfolding pathway and conformational dynamics of tRNA Phenylalanine from S. Cerevisiae, both with and without modifications, and under different magnesium ($Mg^{2+}$) ion concentrations. Our observations indicate that modified nucleotides in crucial areas of the tRNA form a complex network of hydrogen bonds and stacking interactions, which are vital for the tRNA's tertiary structure stability. Additionally, our simulations reveal that these modifications aid in creating ion binding sites on the tRNA. Interestingly, we found that high concentrations of $Mg^{2+}$ ions can stabilize the tRNA's tertiary structure even in the absence of modifications. These insights shed light on the complex interplay between modifications, magnesium ions, and RNA structural stability.

**Poster #68**

## Identification of Exosites in Caspase-6 for Development of Substrate-Selective Inhibitors

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Caspase-6 is an aspartic acid-specific cysteine protease with roles in both apoptosis and neurodegeneration. Caspase-6 contributes to Alzheimer's and Huntington's diseases through cleavage of tau and huntingtin, respectively. Conversely, cleavage of the substrate DJ-1/PARK7 by caspase-6 plays a protective role against development of Parkinson's disease. While caspase-6 presents a tempting target for drug design, its capacity to both cause and prevent neurodegenerative disease highlights the need to selectively block cleavage of only desired targets. At the heart of this endeavor is the need to understand how caspase-6 engages each of its various disease-related substrates. We hypothesize that, in addition to binding substrates at its active site, caspase-6 uses discrete binding interfaces, or exosites, to increase the specificity of target engagement. Our focus is on identifying exosites on caspase-6 that contribute to DJ-1 binding and mapping the corresponding binding sites on DJ-1. We have been unable to observe the interaction of wild-type DJ-1 with caspase-6 due to low binding affinity. However, we have generated a novel DJ-1 mutant (mutDJ-1) that forms a complex with caspase-6 as seen by size exclusion chromatography. mutDJ-1 exhibits a less folded structure than WT DJ-1, which suggests that the enhanced binding of this mutant is due in part to a more accessible cleavage site. We are currently using NMR spectroscopy to resolve the binding interfaces between these two proteins at the atomic level. The discovery of exosites that mediate this and other caspase-6-substrate interactions will empower efforts to design substrate-selective caspase-6 inhibitors.

**Poster #69**

## Bacterial chemoreceptor signaling complexes control kinase activity by stabilizing the catalytic domain of CheA

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Although bacterial chemotaxis is one of the best-understood signaling systems, it remains unclear how chemoreceptors regulate the activity of the associated histidine kinase CheA. A signaling complex comprises of two trimer-of-dimers of membrane-bound chemoreceptors, two CheW coupling proteins, and dimeric CheA. We have used hydrogen deuterium exchange mass spectrometry (HDX-MS) to reveal how *E. coli* CheA domain structure and dynamics change within signaling complexes during kinase activation and inhibition. CheA is a five-domain homodimer: P1 bears a phospho-accepting His48, P2 binds downstream effector proteins, P3 mediates dimerization, P4 is the catalytic domain, and P5 binds CheW and the chemoreceptor. We assemble native-like complexes of CheA with CheW and the cytoplasmic fragment (CF) of the aspartate receptor bound to vesicles. Kinase-off states (free CheA and CheA in complexes with unmethylated CF) exhibit rapid deuterium uptake in P4. In contrast the kinase-on state (CheA in complex with methylated CF) exhibits significantly slower deuterium uptake in P4, suggesting that stabilization and destabilization of P4 is key to kinase activation and inhibition. P3 exhibits faster deuterium uptake in complexes with the unmethylated receptor, suggesting that the mechanism of adaptation involves destabilization of P3. We propose this destabilization is due to increased interactions between P3 and unmethylated CF. The very slow exchange of two helices in P1 in all states identifies the dimerization interface of the P1/P1'. HDX-MS results will guide single-molecule Förster resonance energy transfer (smFRET) experiments to determine whether P1 forms a parallel or antiparallel dimer.

**Poster #70**

## Structure and position-specific interactions of prion-like domains in transcription factor Efg1 phase separation

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Candida albicans, a prominent member of the human microbiome, can make an opportunistic switch from commensal coexistence to pathogenicity accompanied by an epigenetic shift between the white and opaque cell states. This switch is under precise regulation by transcription factors (TFs), with Enhanced Filamentous Growth Protein 1 (Efg1) playing a central role. Previous research has emphasized the importance of Egf1's prion-like domain (PrLD) and the protein's ability to undergo phase separation for the white-to-opaque transition of C. albicans. However, the underlying molecular mechanisms of Efg1 phase separation have remained underexplored. In this study, we delved into the biophysical basis of Efg1 phase separation, revealing the significant contribution of both N-terminal (N) and C-terminal (C) PrLDs. We found that Efg1 N-PrLD and C-PrLD are mostly disordered though have prominent partial α-helical secondary structures. NMR titration experiments suggest that the partially helical structures in N-PrLD act as hubs for self-interaction as well as Efg1 interaction with RNA. Using condensed-phase NMR spectroscopy, we uncovered diverse amino acid interactions underlying Efg1 phase separation. Particularly, we showed that the transient α-helical structure is present in the phase-separated state and highlighted the importance of aromatic residues within these structures for phase separation. Together, these results enhance the understanding of C. albicans TF interactions that lead to virulence and provide a foundation for potential antifungal therapies targeting the transcriptional switch.

**Poster #71**

## Investigating Conformational Heterogeneity of Various Caspase-9 Maturation States

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Uncontrolled cell death underpins neurodegeneration and cancers, necessitating tight regulation. Caspase-9 (casp-9), a key apoptosis protease, lacks a full-length, high-resolution structure, leaving domain interactions and their influence on activity enigmatic. Using native ion mobility-mass spectrometry (IM-MS) and structural modeling, we probe casp-9's conformations in various maturation states. We are using collision cross sections (CCSs), coupled with structural modeling, to filter casp-9 models. Casp-9 has a pro-domain, called CARD, a large subunit, and a small subunit, that assemble to form a monomer. WT casp-9 displays several charge state distributions (CSDs), indicating conformational heterogeneity. Three CSDs are associated with the monomeric state, one with the dimeric state, and one each for CARD+large and small. Measured CCS values vary for the three CSDs of casp-9 monomer, with the lowest monomer charge state (12+) having a CCS of 3320 ± 10 Å and the highest (32+) having a CCS of 8230 ± 70 Å. Native MS measurements of ΔCARD, show only one monomer CSD, suggesting that the flexible CARD pro-domain is the primary cause of the conformational heterogeneity of the casp-9 monomer.

Interestingly, while the monomer shows three CSDs under native nanospray conditions, only one is observed for dimer, suggesting stabilization. Possibly, structural changes during dimerization prevent further alterations in CARD orientation. More data is required to better understand the role of the CARD in casp-9 structural heterogeneity. We are also investigating casp-9 dimer formed in the presence of z-VAD-fmk, a substrate mimic which inactivates the complex while forcing dimerization. The results from these experiments will be presented.

**Poster #72**

## Computing The Performance of Nucleic Acid Force Fields on Non-canonical Structures

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Modern molecular dynamics have provided accurate, insightful, and cost-effective simulations of a variety of chemical systems. However, they are not without their limitations. Specifically, many nucleic acid force fields fail to capture non-canonical constructs. We see a clear preference in parameters towards standard duplexes. Advancements in structural biochemistry and nanotechnology promote the desire for atomistic descriptions of structures that contain a variety of non-standard motifs. As these aforementioned force fields are certainly capable of accurately simulating duplexes and a limited catalog of non-canonical motifs. We aim to derive new parameters from these force fields that will be able to capture structures that contain a diverse set of non-canonical motifs.

**Poster #73**

## Experimentally characterizing the binding mechanism of a prospective castration-resistant prostate cancer therapeutic to the disordered transactivation domain of the human androgen receptor

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Castration-resistant prostate cancer (CRPC) is a condition diagnosed in 30-40% of prostate cancer patients. CRPC arises when the androgen receptor (AR) becomes drug-resistant to small molecule drugs that target its structured ligand binding domain. Common drug candidates only extend patient survival by 3-4 months due to mutations in the ligand binding domain of the AR, the production of alternative splicing targets, or androgens generated by the tumor itself, resulting in active AR and tumor growth despite treatment. Targeting the intrinsically disordered N-terminal transactivation domain of AR (AR-NTD), represents a promising strategy for treating CRPC, and several small molecule AR-NTD inhibitors have entered clinical trials. Our laboratory has previously characterized the binding of CRPC drug candidates with all-atom molecular dynamics (MD) computer simulations, finding that these ligands drive the formation of collapsed helical conformations of the AR-NTD by stabilizing dynamic hydrophobic cores of aromatic residues. Here, we experimentally investigate the effects of small molecule binding to WT AR-NTD and two mutants (W397A/W433A, W397G/W433G) designed to probe the importance of helical content, aromatic residues, and chain dimensions for conferring ligand affinity with NMR spectroscopy, circular dichroism, and SAXS. Our results suggest a surprising malleability of IDP:small molecule binding modes in response to mutations.

**Poster #74**

## Modulation of MIF-2 structure and function via cysteine residues and a small molecule Ebselen

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The macrophage migration inhibitory factor (MIF) family of cytokines, comprised of MIF and D-dopachrome tautomerase (DDT, MIF-2), are homologs that share ~35% sequence identity and a nearly identical structure. This structural similarity is suggested to contribute to their overlapping enzymatic activities and activation of the cluster of differentiation 74 (CD74) receptor. MIF and MIF-2 are thought to be controlled allosterically via crosstalk between their N-and C-termini, using homologous, but not identical, residues. Prior studies show that while some aspects of this "allosteric pathway" are preserved in the MIF superfamily, structure alone does not preserve the allosteric mechanism of the canonical MIF species. Thus, there is a need to characterize additional residues in MIF-2 that modulate allosteric function. Recent work has identified cysteines in MIF as allosteric switches for the same enzymatic activity. Small molecules targeting the enzymatic site of MIF also affect the structure of nearby cysteines. Ebselen is a small molecule that forms a selenylsulfide bond with MIF cysteine residues. MIF contains three cysteines, C56 and C59 (enzymatic oxidoreductase site) and C80, while MIF-2 contains two cysteines, C23, C56. Ebselen is hypothesized to disrupt the MIF trimer structure, leading to nonfunctional monomeric subunits. However, it remains unclear whether ebselen has similar effects on MIF-2. To investigate the role of cysteines as allosteric modulators of MIF-2, we will use site-directed mutagenesis, nuclear magnetic resonance (NMR), molecular simulations, in vitro and in vivo biochemistry to understand the mechanism of ebselen binding to MIF-2 and its effect on homotrimer disruption through cysteine modification. Preliminary studies suggest that ebselen partially disrupts the MIF-2 homotrimer, but the structural population of such species is crucial to defining mechanistic similarities within the MIF superfamily.

**Poster #75**

## Insights into the structural consequences of inosine modified G-quadruplexes using Molecular Dynamics

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G-quadruplexes represent distinctive secondary structures resulting from guanine-rich sequences within DNA or RNA. These structures are characterized by stacked guanine (G) tetrads, bonded via Hoogsteen hydrogen bonds. Widely distributed across genome regions, G-quadruplexes play crucial roles in regulating gene expression, DNA replication, telomere maintenance, and genomic stability. Their capacity to influence gene expression in conditions such as cancer and neurological disorders has positioned G-quadruplexes as promising targets for drug development. In RNA and DNA, inosine (I) is a prevalent nucleotide modification akin to guanine. UV-melting and Circular dichroism spectra indicate that substituting G with I alters the conformation and stability of G4 quadruplexes, consequently affecting their interaction with small molecules. To gain deeper insights into the impact of inosine substitutions on G-quadruplex-ligand complexes, we conducted molecular docking on G-quadruplex configurations with varying G-I substitution frequency and location within a telomeric sequence (TTAGGG). Comprehending the factors underlying the structural diversity of modified G-quadruplexes and their interactions with small molecules unveils new pathways for exploring their functional significance and potential applications in drug development and therapeutic targeting.

**Poster #76**

## Bayesian Multistate Bennett Acceptance Ratio Methods

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The multistate Bennett acceptance ratio (MBAR) method is a prevalent approach for computing the free energies of thermodynamic states. In this work, we introduce BayesMBAR, a Bayesian generalization of the MBAR method. By integration of configurations sampled from thermodynamic states with a prior distribution, BayesMBAR computes a posterior distribution of free energies. Using the posterior distribution, we derive free energy estimations and compute their associated uncertainties. Notably, when a uniform prior distribution is used, BayesMBAR recovers the MBAR's result but provides more accurate uncertainty estimates. Additionally, when prior knowledge about free energies is available, BayesMBAR can incorporate this information into the estimation procedure by using nonuniform prior distributions. As an example, we show that by incorporating the prior knowledge about the smoothness of free energy surfaces, BayesMBAR provides more accurate estimates than the MBAR method. Given MBAR's widespread use in free energy calculations, we anticipate BayesMBAR to be an essential tool in various applications of free energy calculations.

**Poster #77**

## Interplay of steric and diffuse ion effects regulates the final stages of aminoacyl-tRNA accommodation

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Protein synthesis involves an elaborate series of large-scale conformational rearrangements in the ribosome. Although long-lived intermediate states of this process can be characterized by experiment, understanding the kinetics of ribosomal conformational dynamics, which are governed by associated free energy barriers, requires the aid of computational study. To this end, we investigate the dynamics of aminoacyl-tRNA tail accommodation, whereby the amino acid-loaded tail of tRNA enters the peptidyl transferase center (PTC) immediately prior to peptide bond formation. We find that there exists a pronounced steric barrier to tail accommodation, originating from the presence of a pocket region formed from Helix 89, 90, and 92 along the accommodation pathway. Moreover, two pathways for tail accommodation can be distinguished, upper and lower, depending on whether the tail moves through or bypasses the pocket region. Finally, we introduce electrostatics and explicit ions into our energetic model in order to probe the role of ion interactions in mediating the physical mechanisms of tail accommodation.

**Poster #78**

## The direct influence on mechanical properties of tropocollagen by AGE adducts

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A protein with a long lifespan, collagen is prone to accumulate advanced glycation end-products (AGEs) with age. These AGEs are not only considered markers for indicating the severity of senescence but also influence tissue mechanics, contributing to fragile bones and hardened skin. While many crosslinking AGEs have been studied as direct stiffeners of the bio-tissues by contributing strong covalent bonds, the non-crosslinking AGEs (AGE adducts) are merely studied as the indices of aging or the signaling factors of pathology. However, some recent experimental results have shown that the magnitude of the number of AGE adducts is in the same order as enzymatic crosslinks, which are orders of magnitude more abundant than crosslinking AGEs. Based on this evidence, we consider one of the AGE adducts – carboxymethyllysine (CML) – and investigate its direct impact on the conformational and mechanical properties of tropocollagen molecules by molecular dynamics simulations. We observe that, for some specific sequences, the CML modification eliminates critical intramolecular hydrogen bonding and thus straightens the peptide, eventually, decreasing the energy absorption capacity to mitigate external tension. This study shows that non-crosslinking AGEs directly affect the behavior of collagen molecules and provides likely mechanisms by which these modifications take place. Understanding the role of AGE adducts and their contribution to aging effects may support solutions to anti-senescence in the future.

**Poster #79**

## Investigating protein robustness in *Bacillus subtilis*

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Proper protein folding is of the greatest importance to biological function. When a protein is denatured or misfolded, its normal functionality is compromised, often leading to serious illnesses. Understanding the evolutionary rates of proteins is crucial for understanding their normal functions and regulation of biological processes involving local conformational changes in molecular interactions. Due to the limitations of simulations in considering the relative importance of other biological and functional effects of proteins, it is imperative to address this gap in knowledge with empirical research datasets. In this study, we investigated the thermodynamic properties of various *Bacillus subtilis* proteins, of those where we observe the two-state model of protein unfolding against increasing temperature. We examined how protein abundance relates to their stability, in correlation with the translational robustness theory. We anticipate that our research will contribute to a better fundamental understanding of protein stability, as well as the conformational changes proteins may undergo in varying environments.

**Poster #80**

## Annotating small molecules in the Protein Data Bank with ChatGPT

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The Protein Data Bank (PDB) has been an important resource for enzyme research for decades. The small molecules section in the PDB entry lists the non-polymer molecules that bind with the macromolecule. These small molecules can be solvents, cofactors, substrates, products, and inhibitors in enzymes. However, there are no annotations of each small molecules' role in each PDB entry. Large language models (LLM) such as ChatGPT, developed by OpenAI, have been used to solve scientific problems by providing background knowledge and proper prompts. Here, we demonstrate the possibility of annotating the PDB small molecules section to assign each small molecules' role using ChatGPT. We validated the results by comparing the GPT results with 500 manually annotated PDB entries, and we achieved around 70% to 80% consistency between the GPT results and the manual results. This work demonstrates that GPT could be a promising tool for annotating scientific data.