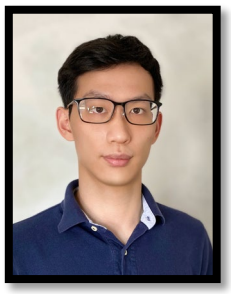
Houston Area Molecular Biophysics Program (HAMBP) Training Program

Grant No. T32 GM008280
Program Director: **Theodore Wensel**, PhD
Professor and Chairman, Department of Biochemistry and Molecular Biology
Baylor College of Medicine

http://www.gulfcoastconsortia.org/home/training/molecular-biophysics-hambp/

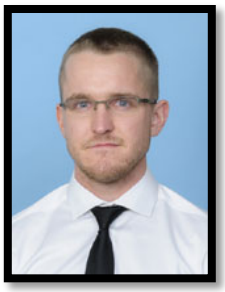
Meet the Trainees

2021 – 2022



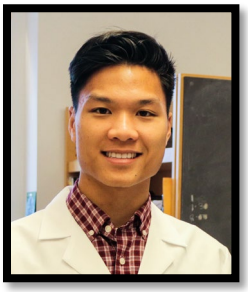
Caleb Chang
Appointed August 1, 2020 – July 31, 2022
Department of BioSciences, Rice University
Primary Mentor: Dr. Yang Gao, BioSciences (Rice Univ)
Adapting polymerase η with X-ray crystallographic techniques for drug design
Many current-day cancer treatments, including nucleoside-analog drugs remain ineffective, and frequently, patients end up developing resistance. The improvement of such therapy is severely limited by the inadequate knowledge of how polymerases differentiate between correct and incorrect DNA bases. The non-replicative translesion polymerase, polymerase η is unregulated in many cancers; thus, this protein remains an essential drug target for combined chemotherapy. I plan to use polymerase η as a model to investigate the molecular mechanisms behind polymerase fidelity and nucleoside-analog drug resistance with time-resolved X-ray crystallography. I also aim to find novel, allosteric inhibitors against polymerase η with fragment-based drug design screenings in collaboration with Xpose Therapeutics.

Alexander Ditzel
Appointed July 1, 2019 – June 30, 2022
Department of BioSciences, Rice University
Primary Mentor: Dr. George Phillips, BioSciences (Rice Univ)
Use of a Cell-free Protein Synthesis System and Coupled Enzyme Reactions to Synthesize Natural Products
My project focuses on using a cell-free system and coupled enzyme reactions to assemble natural products *in vitro*. I utilize a cell-free protein synthesis system to perform *in vitro* transcription and translation and then have the enzymes build the natural products in the same system. I utilize LC-MS to ensure that the correct small molecules are produced and analyze the progression of the reactions. Cell-free systems have a number of advantages in the synthesis of natural products, including speed, automation capability, reduced metabolic engineering needs, and the ability to utilize toxic compounds as substrates to make novel products.



Nolan Dvorak
Appointed September 1, 2020 – August 31, 2022
Department of Pharmacology and Toxicology, University of Texas Medical Branch at Galveston
Primary Mentor: Dr. Fernanda Laezza, Pharamacology and Toxicology (UTMB)
Elucidating the Structural Constituents of Fibroblast Growth Factor 14 that Confer Its Modulatory Effects on the Voltage-Gated Sodium Channel 1.6
Disruption of protein:protein interactions (PPI) between voltage-gated Na^+ (Na_v) channels and their regulatory accessory proteins causes channelopathies. Despite restoration of these perturbed PPIs serving as a novel therapetuic approach, efforts to develop small molecule modulators of these surfaces is hindered by an incomplete understanding of the structural motifs of auxilary proteins that confer functional modulation of the pore-forming α subunit of Na_v channels. Focusing on the PPI between $Na_v1.6$ and its auxilay protein fibroblast growth factor 14 (FGF14), we employ whole-cell patch-clamp electrophysiology in conjuction with the application of pharmacological mimics of the β12 sheet and β8-β9 loop of FGF14 to interrogate the specific roles of these structural motifs in modulating the kinetics of $Na_v1.6$ channels. These functional studies are additionally coupled with advanced imaging techniques to validate the primacy of these structural motifs in regulating FGF14:$Na_v1.6$ complex assembly and channel trafficking. This approach that employs whole-cell patch-clamp electrophysiology, pharmacological probes, and advanced imaging techniques will enable the elucidation of crucial domains of auxilary proteins that confer functional modulation of Na_v channels through PPIs, strucutral insights that are crucial in developing efficacious small molecule modulators of perturbed PPIs within the Na_v channel macromolecular complex.

Mandi Feinberg
Appointed July 1, 2021 – June 30, 2022
Department of Biochemistry, Cellular, and Molecular Biology - Molecular Biophysics Educational Track, University of Texas Medical Branch at Galveston
Primary Mentor: Dr. Kyung Choi, Biochemistry and Molecular Biology (UTMB)
Structure of West Nile virus RNA promoter, stem-loop A, and its interaction with viral polymerase NS5
West Nile virus (WNV) is the causative agent of West Nile fever in humans, an emerging infectious disease, and the most common mosquito-borne disease in the United States. WNV replication is dependent upon the presence of the stem loop A (SLA) structure in the 5' untranslated region (UTR) of the viral genomic RNA. The viral polymerase, non-structural protein 5 (NS5), interacts with the 5' SLA and initiates synthesis of the negative strand. My project aims to identify and understand how WNV NS5 interacts with SLA to initiate RNA synthesis by determining the structure of the SLA in its native state and bound to the viral polymerase.



Anthony Hoang
Appointed July 1, 2020 – June 30, 2022
Department of Chemical, Physical, and Structural Biology, Baylor College of Medicine
Primary Mentor: Dr. Ming Zhou, Biochemistry and Molecular Biology (BCM)
Mechanism of iodide and bicarbonate transport and inhibition in mammalian pendrins
Pendrin is a crucial anion exchange transporter with iodide and bicarbonate transport capabilities. Mutations in iodide transport are known to lead to Pendred syndrome, a genetic disorder leading to congenital deafness and goiter, while overexpression of its bicarbonate transport may lead to hypertension. Drug discovery and therapeutics would benefit from understanding the mechanisms of transport, including details on ion binding sites, conformational changes during transport, and the role of a highly conserved region. I aim to use cryo-electron microscopy single particle reconstruction (cryo-EM SPR) to solve structures for pendrin during transport, and to verify its function and binding with proteoliposome transport assays and surface plasmon resonance (SPR). I also propose to analyze inhibition by performing virtual screens, and test binding affinity and thermodynamics with isothermal titration calorimetry (ITC) and a liposome flux assay.

Jordan Johnson
Appointed September 1, 2021 – August 31, 2022
Department of Biology and Biochemistry, University of Houston
Primary Mentor: Dr. Yuhong Wang, Biology and Biochemistry (UH)
Force Generation and Mechanism Elucidation of EF-Tu
Elongation factor thermal unstable (EF-Tu) is a bacterial enzyme that delivers aminoacyl-tRNAs to the ribosome during translation, however its mechanism is not well understood. My project will be observing the conformational changes of EF-Tu using FRET and determining if a power stroke is generated, similarly to other elongation factors, using Force Induced Remnant Magnetization Spectroscopy (FIRMS). I will also observe how mutations in the GTP binding pocket will affect EF-Tu's mechanism and function as mutations in EF-Tu have been linked to a variety of health issues and it is unknown how mutations contribute to the occurrence of these health issues

Seth Scott
Appointed August 1, 2019 – July 31, 2022
Department of Molecular Biophysics Educational Track, University of Texas Medical Branch at Galveston
Primary Mentor: Dr. Kyung Choi, Biochemistry and Molecular Biology (UTMB)
The Role of miR-122 and PCBP2 in Promoting Hepatitis C Virus Replication
Hepatitis C Virus (HCV) remains an important human pathogen, known for its persistent infections of the liver, which uses its RNA genome as a template for both protein translation and RNA synthesis. The viral genome can only be used as a template for one process at any given moment and requires precise regulation and balance between protein and RNA production to maintain a persistent infection. The host factors, microRNA 122 (miR-122) and Poly-C Binding Protein 2 (PCBP2) have both been identified as having a role in HCV protein and RNA synthesis. My project aims to understand the mechanism by which these factors promote HCV replication and elucidate how the interplay between these factors balances the relative rates of viral protein translation versus viral RNA synthesis.

Savannah Seely
Appointed August 1, 2021 – July 31, 2022
Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch
Primary Mentor: Dr. Matthieu Gagnon, Microbiology and Immunology (UTMB)
Investigating the Molecular Mechanism of Ribosome Recycling
In all organisms, the ribosome decodes mRNA and synthesizes proteins through four essential steps: initiation, elongation, termination, and recycling. Currently, more than 50% of clinically relevant antibiotics target the ribosome. Remarkably, the final step, recycling, is the least characterized and has not been exploited for structure-based drug design. Recycling is the necessary bridge between termination and initiation, and a better understanding of the molecular aspects of this step could lead to development of new therapeutics. I propose to determine the molecular mechanism of recycling in the human pathogen *Pseudomonas aeruginosa*, which employs a specialized Elongation Factor-G that functions exclusively in recycling. I will also investigate features within the ribosome that are involved in its disassembly by determining the structure of amikacin bound to the ribosome; an antibiotic that has been reported to cause recycling deficits. Taken together, I will determine at high resolution the mechanism of ribosome recycling by elucidating what ribosome features are altered as well as what molecular interactions occur between the ribosome and recycling factors. The anticipated results of these studies will improve our knowledge of this step in translation and may open new doors for the development of antibiotics.

Jessica Symons
Appointed September 1, 2019 – August 31, 2022
Department of Biochemistry and Cell Biology, University of Texas Health Science Center - Houston
Primary Mentor: Dr. Ilya Levental, Biochemistry and Cell Biology (UT Health)
Molecular determinants and biophysical consequences of lipid asymmetry in mammalian plasma membranes
A fundamental and broadly conserved feature of eukaryotic cells is an unequal distribution of lipids between the two leaflets of the plasma membrane bilayer. Maintaining lipid asymmetry is energetically costly, implying an essential, though as yet poorly understood, physiological role.

While the broad features of phospholipid distribution between plasma membrane leaflets have been defined for decades, the asymmetric distribution of cholesterol, the most abundant component of the plasma membrane, remains a major open question. In my project, I will measure cholesterol distribution and define the phospholipidomic redistribution during plasma membrane scrambling. Further, I will deduce biophysical consequences (e.g. fluidity, thickness, permeability, and lateral organization) of changes in lipid asymmetry using biomimetic and mammalian plasma membranes.

Questions: Contact Jessica Poli
jessica.poli@rice.edu , (713)348-4752