National Institute of General Medical Sciences

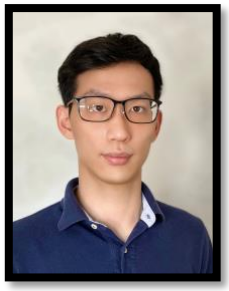
Houston Area Molecular Biophysics Program (HAMBP) Training Grant

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

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

Meet the Trainees

Currently appointed: Fall 2020 – Fall 2021

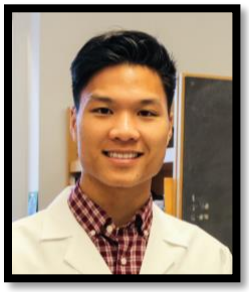


Caleb Chang
BioSciences, Rice University
Primary Mentor: Dr. Yang Gao, BioSciences (Rice Univ)
Adapting polymerase η with X-ray crystallographic techniques for drug design
Many current-day cancer treatments, including nucleoside-analog drugs remain ineffective, and frequently, patients end up developing 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
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
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.

Miriam Gavriliuc
Biology and Biochemistry, University of Houston
Primary Mentor: Dr. Yuhong Wang, Biology and Biochemistry (Univ of Houston)
Measuring the Mechanical Forces During Ribosome Translocation via Elongation Factor G Crosslinking
Elongation factor G (EF-G) is a bacterial protein that catalyzes the movement of the ribosome down the mRNA, however, this mechanism is not well understood. My project will focus on determining the role of EF-G in keeping the ribosome on the correct position of the mRNA by studying the force EF-G produces. Using internally crosslinked EF-G, I will be able to trap the protein in different stages of the translocation reaction and measure the force. To measure the force, I will use Force Induced Remnant Magnetization Spectroscopy (FIRMS), a technique developed by our lab.



Anthony Hoang
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.

Nabina Paudyal
Biochemistry and Molecular Biology, University of Texas Health Science Center - Houston
Primary Mentor: Dr. Vasanthi Jayaraman, Biochemistry and Molecular Biology (UT Health)
Understanding the allosteric mechanism in Kainate receptors by probing single molecule FRET and molecular dynamics simulation.
Kainate receptors are ionotropic glutamate receptors involved in both pre-synaptic and post-synaptic neurotransmission. Very limited structural data is available for the full-length structure of kainate receptors and no structures are available for its auxiliary protein NETO that alters the biophysical property of the receptor. This resulted in the limited understanding of the mechanism underlying the extent of agonism on kainate receptor and also acquired gap of knowledge regarding modulation of kainate receptor by auxiliary subunits. We propose to address this using single molecule FRET and molecular dynamics simulation with the goal of gaining a comprehensive understanding of the modulation of the receptor gating mechanism and allosteric communication between the extracellular domains, transmembrane segments and intracellular domains due to agonists of varying efficacy and auxiliary proteins.

Seth Scott
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.

Joshua Rosario-Sepulveda
Quantitative and Computational Biosciences, Baylor College of Medicine
Primary Mentor: Dr. Theodore Wensel, Biochemistry and Molecular Biology (BCM)
Secondary Mentor: Dr. Zhao Wang, Biochemistry and Molecular Biology (BCM)
Structure and Activation Mechanisms in a Group III Metabotropic Glutamate Receptor
Mutations in the metabotropic glutamate receptor type 6 (mGluR6) result in defective signal transmission from photoreceptors in the retina to ON bipolar cells, causing congenital stationary night blindness and other retinal and refractive eye pathologies. To understand its mechanism of action and of disease, I will study mGluR6 by solving high-resolution structures of the receptor in apo and ligand-bound states, using electron cryo-microscopy single particle reconstruction (cryo-EM SPR). A complex with mGluR6 and the heterotrimeric G protein will be solved to sub-nanometer resolution as well. Ligand binding and G protein specificity assays will help determine the parameters that confer most plausibility and structural stability to this protein complex. Single molecule Förster resonance energy transfer (smFRET) will measure the distribution of a range of conformational states and the kinetics of transitions between them, for mGluR6 and other group III mGluRs.

Jessica Symons

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.