Houston Area Molecular Biophysics Program (HAMBP) Training Grant

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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 2019 – Fall 2020

David Boragine
Biochemistry and Molecular Biology, Baylor College of Medicine
Primary Mentor: Dr. Timothy Palzkill, Pharmacology (BCM)
Optimization of the BLIP-II Interaction with PBP2a as a Protein Based Therapeutic Option for MRSA
Methicillin-resistant Staphylococcus aureus (MRSA) confers resistance to the commonly prescribed β-lactam antibiotics through acquiring the novel penicillin-binding-protein (PBP2a), thus causing a rapidly growing global health crisis. Previously, our lab has discovered that β-lactamase inhibitory protein II (BLIP-II) can bind and inhibit PBP2a, albeit weakly. However, the binding affinity between BLIP-II/PBP2a can be enhanced through a directed evolution approach to find a tighter binding BLIP-II variant in efforts to solve the first BLIP-II/PBP2a co-crystal structure. My project will focus on optimizing the BLIP-II/PBP2a binding interaction in order to further elucidate the binding mechanism via X-ray crystallography and to determine the effect of BLIP-II on β-lactam efficacy versus MRSA bacteria. Overall, this work has the potential to aid drug discovery by serving as a promising foundation for developing a potential protein based therapeutic option, or a diagnostic tool, for clinical use.

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.

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.

Jonathan Mercado
Molecular and Cellular Biology, Baylor College of Medicine
Primary Mentor: Dr. Francis T.F. Tsai, Biochemistry and Molecular Biology (BCM)
Targeting Leishmania Hsp100 to abolish infective parasite stage differentiation
Leishmania is the causative parasite of the vector-borne neglected tropical disease, Leishmaniasis. The activity of the *Leishmania* Hsp100 chaperone protein allows for the parasite to differentiate into its infective stage in humans. My project will provide insight into the potential use of small molecules for manipulating chaperone function. Identifying small molecules that interact with *L.mexicana* Hsp100 could then be used as chemical probes for future studies to understand Hsp100 activity (or lack thereof) during mammalian infections. The results of my work will be used to combat Leishmaniasis and other human infections caused by pathogenic microbes.

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.

Matthew Ykema
BioSciences, Rice University
Primary Mentor: Dr. Yizhi (Jane) Tao, BioSciences (Rice Univ)
Characterizing Astrovirus Particles To Investigate The Mechanism Of Proteolytically-Mediated Viral Maturation
Understanding the structure of viral capsid proteins allows for the determination of key infectious domains and targets for vaccines and anti-viral compounds. One target for structural studies is the human Astrovirus, a single-stranded RNA virus that causes gastroenteritis in children and immunocompromised individuals. The virus has a unique maturation process, in which the capsid must be proteolytically cleaved in order to induced host cell entry and exit. I aim to identify the structural changes of the capsid that occur during the proteolytically-mediated maturation using X-ray crystallography and cryo-electron microscopy.