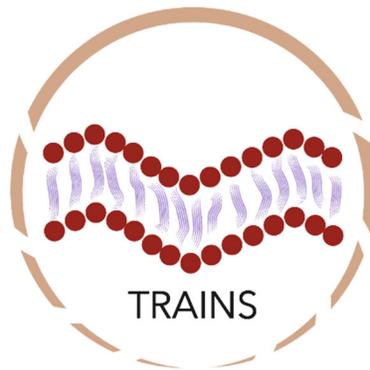


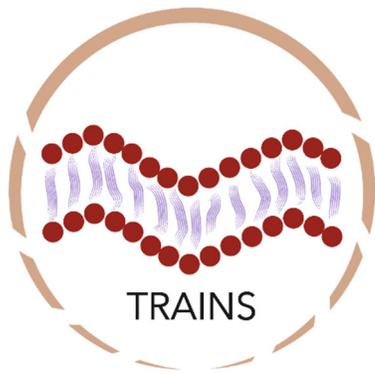
Transitions and Research Across INterfaceS

Research Project Information Packet: Spring 2022¹

A National Science Foundation Funded Program, Award #2150298



1. The information in this packet was last updated on 2/24/2022 and is subject to change.



Transitions and Research

Across INterfaceS

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What is TRAINS?

TRAINS is an intense, 15-month research program to transition Rio Hondo Students to earning STEM degrees at baccalaureate-granting institutions. The students selected to participate in the program will begin June 2022 and will end in August 2023. ***The application period for the program will open in Late March 2022 and selections will be made by Late April.***

TRAINS students will conduct paid research (\$10k per student, to be paid throughout the program) in the cutting-edge fields of biophysics and biological engineering under faculty mentors at USC. **TRAINS** Students will have their own graduate student/post-doctoral mentors at USC dedicated to advancing their STEM careers.

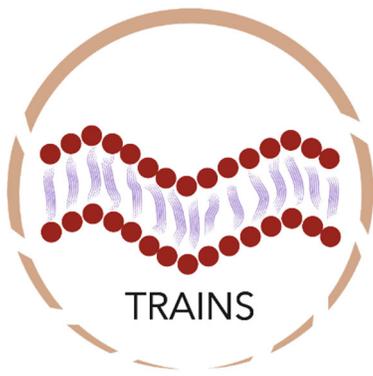
TRAINS students are assumed to be completely new to STEM research (and applications from such candidates are highly encouraged!)

TRAINS is intended to assist Rio Hondo students transfer to highly competitive baccalaureate-granting institutions by providing them access to courses and research opportunities normally not found at the community college and *is meant for students approximately 1 year from transferring to a four-year institution in a STEM degree program.*

TRAINS students will work with one of the six faculty members at USC on the projects described on the attached documents.

TRAINS is a joint project between Rio Hondo College, High Point University, the University of Southern California, and the National Science Foundation's *Improving Undergraduate STEM Education (IUSE)* initiative office. This program seeks to bring recent advances in STEM knowledge into undergraduate education and improve the overall quality of STEM undergraduate education in the United States.





Fabien Pinaud

Associate Professor of Biological Sciences and Physics and Astronomy



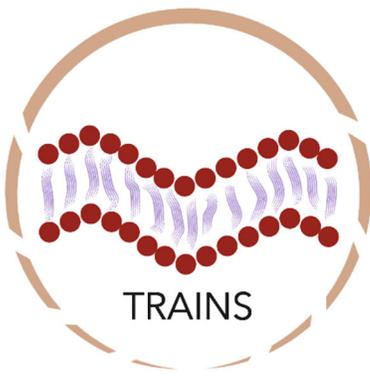
Proposed Research

The laboratory of PI Fabien Pinaud has the fundamental aim of understanding how protein diffusion, location and interactions with nanoscale structures of the plasma and nuclear membranes of cells are capable of finely modulating normal and pathological cellular signaling. Using highly interdisciplinary approaches at the interface between cell biology, biological chemistry, biophysics, and super-resolution optical imaging, we seek to grasp the fundamental molecular mechanisms that govern membrane nanostructures/protein function relationships during cellular mechanosignal processing, integration and regulation. In line with the scientific theme of "Physical Biology at the Membrane Interface," our research will involve: (i) characterizing the nanoscale organization of caveolin-1, a key plasma protein that participates to the dynamic formation of caveolae membrane reservoirs and regulates plasma membrane tensions in response to forces in human cells, and (ii) defining the molecular functions of emerin, a mechanotransducing protein of the nuclear envelope, that is involved in nuclear shape maintenance against forces and is directly implicated in a variety of human laminopathies. These projects have been selected for their accessibility to young, community college scholars and their potential to be developed into long-term projects.

Cell biomechanics and plasma membrane caveolin nanodomains

Mechanical forces exerted on cells direct many important cellular and tissue processes, including cell adhesion, migration and invasiveness. The cell plasma membrane (PM), as the first organelle exposed to these cues, plays a pivotal role for cell adaptations and responses to these forces, notably via homeostatic control of membrane tension and PM plasticity. In these processes, the membrane curving protein caveolin 1 (cav1) forms invaginated (caveolae) and flat nanodomains (cav1-scaffolds), which are emerging as unique and critical mechanotransducing hubs and membrane tension buffering structures of mammalian cells. Caveolae and cav1-scaffolds are implicated in normal cell functions but also in numerous pathologies that involve changes in the mechanical properties of cells. Yet, how and why these two types of cav1 assemblies modulate the biophysical properties of the PM remains largely undefined. Indeed, despite extensive study and theorization of the mechanistic roles played by cav1 at the PM many questions related to its plastic organization and physical functions remain unanswered. What is the nanoscale structural organization and the plasticity of different cav1 assemblies across the PM? How do these assemblies participate to mechanosignal integration across different length scale at the cell surface? Is the plasticity of cav1 assemblies inherently coupled to the local mechanical and tension states of the PM? How do intra and extracellular forces in the range of piconewtons influence this plasticity?

The PI proposes to correlate the 3-dimensional organization of cav1 nanodomains with local forces exerted across the PM, using super-resolution optical imaging of cav-1 in combination with quantitative cellular force measurements with optical force sensors based on Förster resonance energy transfer (FRET). These measurements will be performed for cells subjected to various adhesion landscapes using custom-designed cell micropatterning techniques. As such, the project will provide new and quantitative understanding of how the plasticity of cav1 nanodomains participates in homeostatic cell responses to specific adhesion cues and local forces by remodeling the architecture of the PM.



Fabien Pinaud

Nuclear envelope organization of emerin and implications for Emery-Dreifuss muscular dystrophy

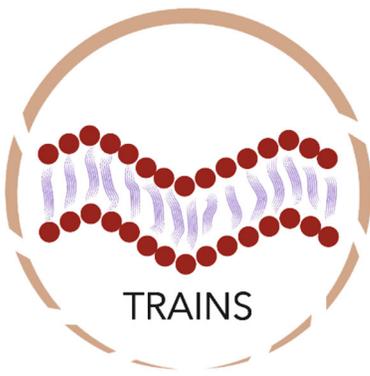
Emerin is an integral membrane protein of the inner nuclear envelope (INE) that binds to various nucleoskeletal partners and is a critical actor for the maintenance of the nucleus architecture and for nuclear mechanotransductions in response to forces. When mutated or absent, emerin causes Emery-Dreifuss muscular dystrophy (EDMD), an envelopathy whose underlying mechanisms and muscle specific effects are not fully understood. In particular, how emerin participates in molecular scaffolding at the INE and helps protect the nucleus against mechanical strains has remained largely elusive. As the PI recently showed using state-of-the-art optical microscopy, this is because the spatial organization of emerin and its mechanotransducing functions are modulated on distances of just a few nanometers at the INE, a length-scale inaccessible by conventional microscopy imaging techniques. Defining the pathogenesis of EDMD and other envelopathies, therefore demand new approaches that can establish the nanoscale structural organization of the INE while simultaneously modulating the mechanical landscape of nuclei in intact cells.

The PI proposes an innovative integration of super-resolution microscopy and single molecule tracking, nuclear biomechanics, biochemistry and quantitative biophysical analyses: (i) to establish the structural organization and the mechanotransducing functions of emerin at the nanoscale in human cells and (ii) to uncover the mechanisms by which its mutation results in abnormal nuclear mechanics in EDMD.

Students participating in this project will receive training: in (i) molecular biology with the design of emerin mutants to be expressed in cells, (ii) super-resolution and single molecule microscopy, (iii) cell micropattern designs to impose force on nuclei in intact cells and (iv) a variety of biochemical and physical characterization techniques to analyze nuclear envelope extracts. Beyond the hands-on training, students will also acquire fundamental notions of fluorophore photophysics, which are central to single molecule optical imaging and notions of statistical physics with the calculation and the analyses of mean square displacements. This project is currently funded under NIH NIAMS-AR076514.

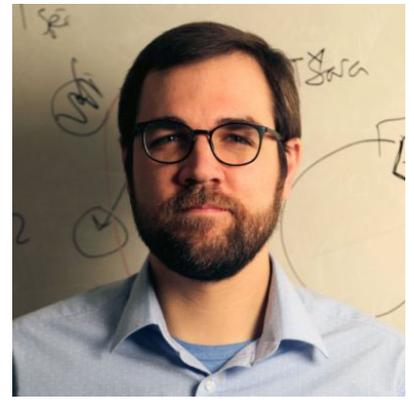
Conclusion

The technical and methodological elements of the proposed research projects will provide approachable routes for participating students to rapidly acquire laboratory and analytical skills that fall well within the scientific theme of "Physical Biology at the Membrane Interface", with a focus on cellular membranes. The PI anticipates that these exciting and highly multidisciplinary projects will also entice students to pursue long-term research interests in membrane biophysics.



James Boedicker

Associate Professor of Physics and Astronomy



Proposed Research

We tackle research questions related to cellular networks, with focus on cell-cell interactions between microbes and the resultant emergent behaviors within larger populations. Many of the current research directions are well-suited for undergraduate students with little prior research experience. Most projects can also incorporate theoretical work and biophysical simulations. In the past, theoretical projects have been successfully adapted for undergraduate researchers. Here, three projects are proposed, the first on large-scale pattern formation and collective behavior of bacteria, the second on molecular mechanisms of bacterial vesicle production and uptake, and the third on bacterial communication pathways. As students supported by this program come into the lab, they will have the opportunity to explore these research options and tailor a unique project that best match their interests and long-term research goals. Mentored students will meet with the PI either weekly or biweekly for one hour, depending on the student's schedule and the status of the project. Students will also participate in weekly laboratory meetings and give a presentation of their research progress once a semester.

Large-scale pattern formation and collective behavior of bacteria

We have been working for many years on the collective behavior and pattern formation of bacteria. Past projects have involved comparing pattern formation in different strains of wild bacterial isolates, quantifying the entropy changes associated with bacterial aggregation patterns, examining the collective motility within bacterial cocultures, and discovering the collective movement of *Enterobacter cloacae* aggregates (Figure 1). The lab has built custom imaging setups to collect time-lapse data of pattern formation, image analysis tools to process the data, and developed both continuum and agent-based models of bacterial pattern formation. We have isolated and characterized several strains of *Enterobacter* and *Aeromonas* that have demonstrated novel collective behaviors. This existing infrastructure helps new students get up and running with projects focused on bacterial motility and pattern formation.

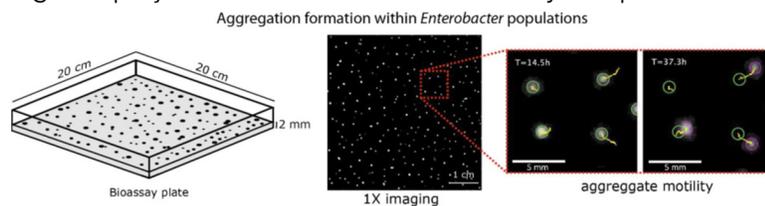
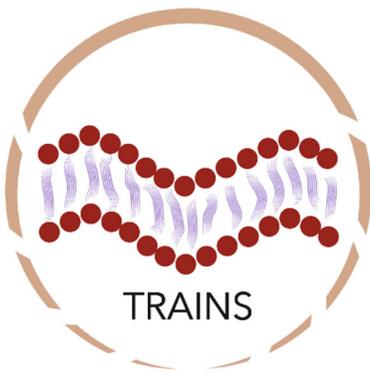


Figure 1: Formation of aggregates by *Enterobacter cloacae* on agar surfaces has been captured using a DLSR camera. After formation, aggregates movement was observed on plates, the result of the collective motility of millions of cells.

Students coming into the lab interested in projects on pattern formation and bacterial motility will start by reproducing results of aggregate formation within low weight percent agarose. This basic experiment will introduce them to the lab and familiarize them with related equipment and analytical techniques. This training also includes an introduction to the simulations that reproduce collective behaviors, including the formation of aggregates and swarm bands. Next, the students will develop their own project ideas with help from the PI and senior lab members.



James Boedicker

There remain unsolved experimental questions on the role of gel height and headspace oxygenation level on aggregate formation and collective movement. Additionally, pattern formations can be compared in closely related natural isolates and mutant strains. The lab has sequenced the genomes of many of the strains in our collective and have the genetic capabilities for transposon mutagenesis screens or more directed gene deletions.

Molecular mechanisms of bacterial vesicle production and uptake

A second major project area of the lab is centered on the exchange of extracellular vesicles between bacteria. All bacterial species likely produce and take up extracellular vesicles under standard culturing conditions, and many of these vesicles naturally contain biomolecules from the cell membranes, periplasm, and cytoplasm. The biomolecules packaged within vesicles includes double-stranded DNA, including chromosomal fragments and plasmids. Our major focus is the biophysics of vesicle exchange in the context of horizontal gene transfer. Prior work in the lab has examined how biological parameters including plasmid characteristics, the host and donor strain, and molecules that modulate membrane structure influence the production and uptake of vesicles as well as the packaging of DNA cargo into vesicles. Modeling work has even examined how vesicle-mediated gene transfer, as a fourth major pathway of horizontal gene transfer, might modulate patterns of gene-flow within diverse bacterial populations.

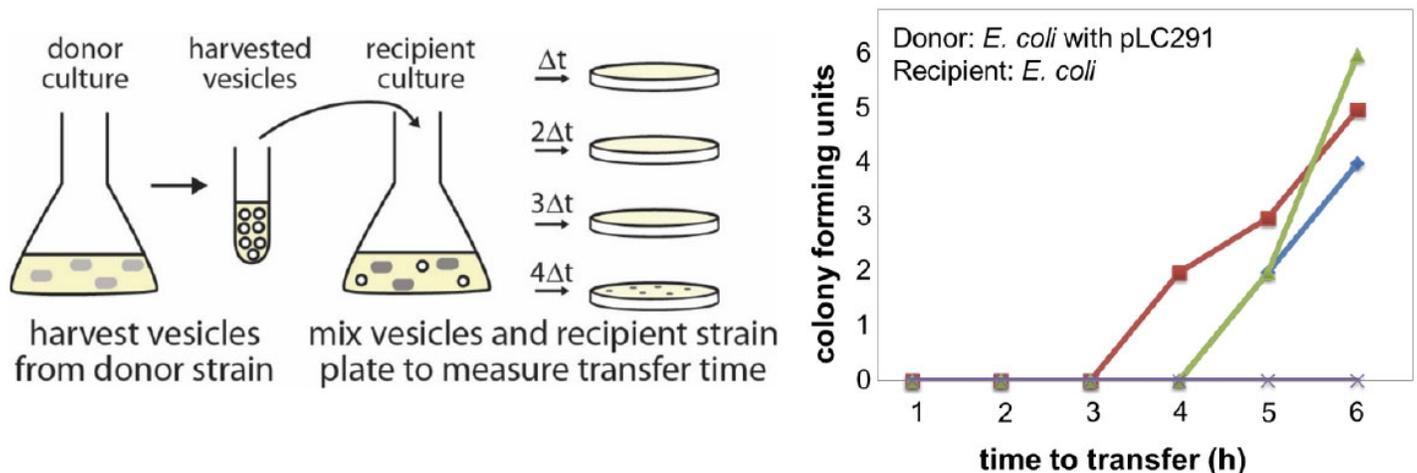
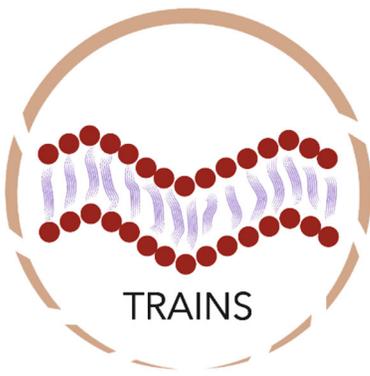


Figure 2: An assay for vesicle-mediated transfer of genes. Vesicles containing a plasmid were harvested from a donor culture of *Escherichia coli*. Purified vesicles to a recipient culture. After several hours, the uptake of vesicles resulted in colonies of antibiotic resistant recipient cells.



James Boedicker

New students can leverage the lab's extensive prior work in this area. The lab has equipment and expertise to isolate vesicles and characterize them using techniques including TEM, AFM, protein gels, nanoparticle counting, and qPCR. The lab has examined vesicle exchange in *Escherichia coli* as well as several other laboratory strains and wild isolates. The lab has also established assays to quantify gene transfer mediated by vesicle exchange, and we are currently in the process of benchmarking a high throughput assay for vesicle-mediated gene transfer using 96 well plates and bacterial cocultures. There are many open questions regarding the mechanisms that modulate the rate and specificity of vesicle exchange between bacteria. After initial training in the basics of vesicle isolation and gene transfer measurements, students can develop a project related to molecular mechanisms that regulate vesicle exchange or even identify mutants or wild isolates with unusual capacity for gene transfer via vesicle exchange. Students will work with the PI and senior members of the lab for help designing and initiating their own project.

Bacterial communication pathways

A third major research area is related to bacterial communication and quorum sensing gene regulation. Past projects have included studies of signal crosstalk in bacterial strains and communities that utilize multiple chemically similar signaling molecules and the influence of spatial structure on signal exchange. We have created and validated a variety of strains with fluorescent reporters for the exchange of several acyl-homoserine lactone and auto-inducing peptide signals. We have also developed both microfluidic and more macroscale approaches to analyze the role of spatial distribution and signal exchange/activity. This includes several models of signal exchange in both well-mixed and spatially explicit contexts that can be used to explore the influence of spatial structure and crosstalk on signal exchange.

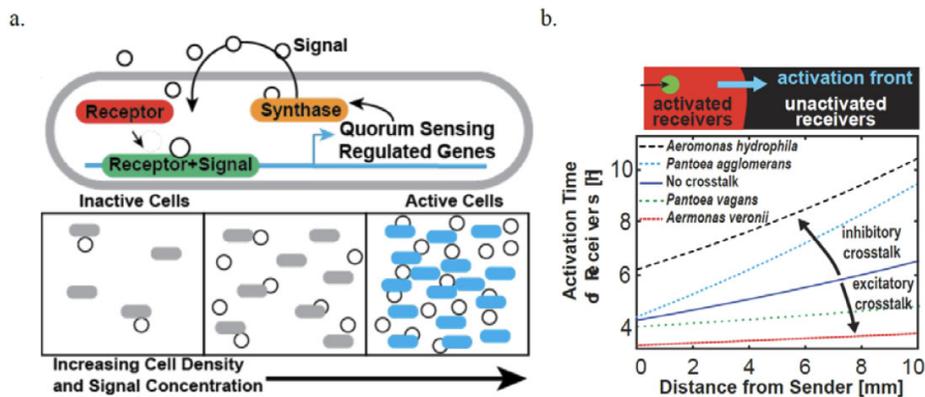
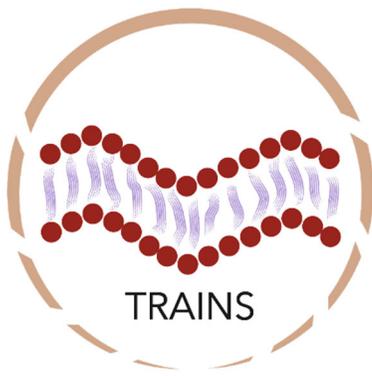
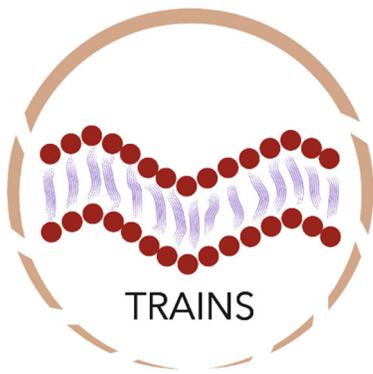


Figure 3: (a) Illustration depicts the quorum sensing pathway in bacteria. (b) An assay to quantify crosstalk between different quorum sensing signals has been depicted. Analysis shows that crosstalk either inhibits or excites quorum sensing in bacteria.



James Boedicker

Students can leverage this infrastructure to receive training in experiment and theoretical approaches to examine bacterial signal exchange and design novel research directions. Experimental training includes running plate-based assays on quorum sensing interference using developed approaches and strains. Training will also include image analysis and Matlab-based simulations of signal exchange. Next students will work with the PI and senior laboratory members to design and plan projects related to signal exchange. Potential areas to explore include the ability of crosstalk and spatial structure to modulate the community-level activity state of a multi-strain community of bacteria utilizing multiple signal variants. This work could include quantifying the type and strength of crosstalk between multiple natural isolates of *Aeromonas* that can utilize multiple signaling molecules (11). There is also interest in exploring and understanding the role of single-cell heterogeneity in quorum sensing regulation within small populations of cells. Although many reporter strains have already been created, projects in this area may include learning and implementing modern gene construction to work with new signaling systems and bacterial strains.



Noah Malmstadt
**Professor of Chemical
Engineering and
Materials Science and
Chemistry**

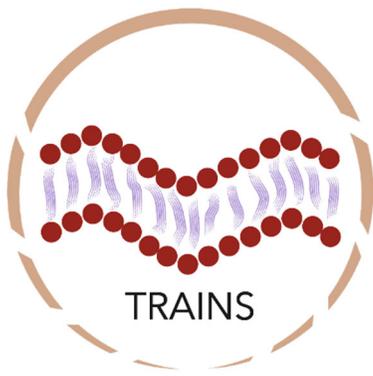


Proposed Research

The Malmstadt lab researches the relationship between lipid composition and bulk membrane properties in model biomembranes. This work includes investigations of how lipid composition is related to membrane permeability to small molecules and dissolved gasses, how membrane composition can alter the function of integral membrane proteins (particularly G protein-coupled receptors), and how lipids and proteins interact to determine the bulk mechanical properties of cell membranes. This research is based on engineering tools for building model cell membranes with functional incorporated proteins and analyzing the behavior of these model membranes in microstructured environments. The projects that will be available to participants as part of the proposed program include the following:

How do intrinsic membrane proteins alter the bulk mechanical properties of lipidic membranes? The cell plasma membrane is built from lipids and proteins. Traditionally, lipids have been thought to establish the mechanical structure of the membrane while proteins control its active functions. There are proteins which specifically act to bend, deform, and exert force on the membrane. However, recent experimental and theoretical studies have hinted towards the idea that intrinsic membrane proteins in general also contribute to the structural and mechanical properties of the membrane. These mechanical properties in turn control membrane behavior. The goal of this proposal is to determine how bulk membrane mechanical properties are affected by the presence of intrinsic membrane proteins the functions of which are not specifically to deform the membrane. These bulk mechanical properties have profound implications for how we understand some of the most important processes in biology, including signaling, viral infection, and transport in and out of cells.

The goal of this project is to quantify the effects of integral membrane proteins on two key mechanical parameters that determine how easily biomembranes can bend—the intrinsic curvature and bending modulus. These parameters describe how much energy is required to membranes them in essential physiological processes such as exocytosis and cell division. To accomplish these, we will first develop a minimal model system that we can use to induce curvature in a lipid bilayer. This system is based on a set of shaped peptides that insert spontaneously into lipid bilayers and curve them in a predictable manner (Fig. 1). We will use this system to tune and optimize our measurement techniques of micropipette aspiration (MPA) and vesicle fluctuation analysis (VFA). Then, we will apply these measurement techniques to the study of full transmembrane proteins.



Noah Malmstadt

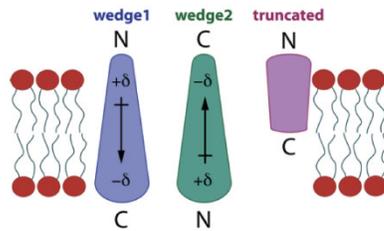


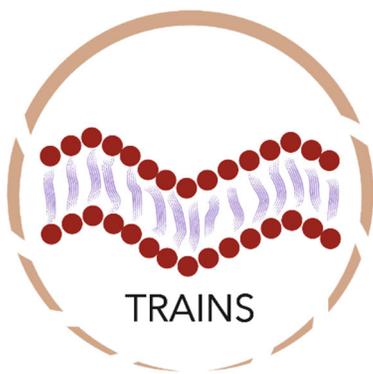
Figure 1: Schematic of shaped peptides designed to interact with the membrane curvature. Wedge-shaped peptides are designed to induce membrane curvature by lipid packing through the transmembrane region. Truncated peptide is designed to insert only partly into the membrane. N- and C-termini are marked on all peptides; α -helical dipoles are shown on wedge peptides, which are designed with dipoles in opposite directions to control for potential dipole-dipole interactions. A schematic of a lipid bilayer is shown for scale.

We will work with three proteins: 5-HT1AR, SoPIP2;1, and OmpF. These three proteins were chosen because their transmembrane domains represent a conceptually important range of structures and dynamics: 5-HT1AR is an α -helical bundle with high conformational flexibility, SoPIP2;1 is an α -helical bundle with low conformational flexibility, and OmpF is a β barrel. By determining how each of these proteins alters membrane intrinsic curvature and bending modulus, we will establish a basic framework for the relationship between protein structural rigidity and bulk membrane mechanical properties. This project is currently funded under NSF PHY-1915017. Students working on this project will learn fundamental soft matter mechanical principles and develop conceptual connections between chemical structures and material properties.

Quantifying gas transport across lipid bilayers.

Diffusion of dissolved gasses such as carbon dioxide through cell membranes is an important step in physiological processes, especially for the ability of erythrocytes to clear carbon dioxide. Several proteins, such as aquaporins, the RhAG complex, and AmtB, have been proposed to be gas transporters. There is currently a debate about the extent to which carbon dioxide transport is dominated by protein transporters or by passive transport through the lipid bilayer itself. This debate hinges on disagreements about the quantitative measurement of membrane permeability to carbon dioxide. This is compounded by disagreements about the correct theoretical framework for predicting gas transport.

In this project, we will measure the diffusion of carbon dioxide across membranes composed of POPC and varying amounts of cholesterol. We use an assay combining microfluidics and fluorescence microscopy schematically represented in Fig. 2. We use a microfluidic device to rapidly and controllably combine a CO₂-rich buffer with giant unilamellar vesicles (GUVs). The GUVs contain the pH-sensitive fluorophore HPTS, which decreases in fluorescence as CO₂ permeates the membrane and acidifies the lumen. This scheme provides for high-throughput measurement while maintaining single-vesicle information. GUVs are an attractive model system for permeability measurements because they are defect and solvent free, and have a large size. Because the characteristic time scale is proportional to the surface to volume ratio, permeation across a 10 μ m GUV will take a hundred times longer to equilibrate than the same permeation across a 100 nm large unilamellar vesicle, making fast kinetics significantly easier to measure.



Noah Malmstadt

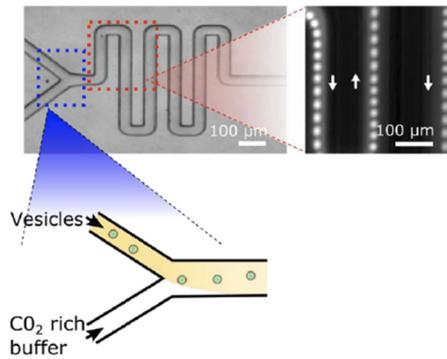


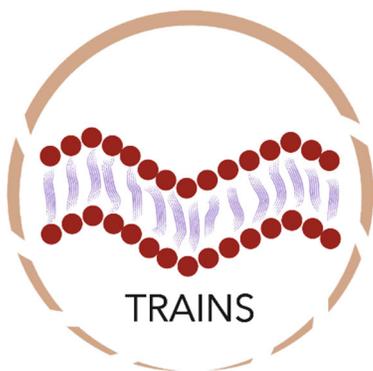
Figure 2: Schematic representation of the experiment. Buffers enriched in CO₂ and buffer containing vesicles encapsulating pH-sensitive fluorophore mix in a Y-channel. As the solutions mix, CO₂ diffuses across the lipid membrane, lowering the pH and the fluorescence level. This is imaged as the vesicle traverses the serpentine channel (bright-field micrograph), allowing the same vesicle to be imaged over a longer period of time without immobilizing it. The fluorescence micrograph shows a pseudo-strobe time lapse formed by superimposing micrographs of the same vesicle taken 20 ms apart.

After developing relationships between lipid composition and CO₂ transport, we will integrate putative gas transport protein into model GUVs and measure how membrane permeability changes with these proteins. This project will introduce students to fundamental concepts in transport processes as well as microfluidic device design, fabrication, and analysis. This project is funded by the Office of Naval Research as part of a Multidisciplinary University Research Initiative (MURI subcontract FP00209340).

Determining how lipid composition alter the functionality of G protein-coupled receptors.

G protein-coupled receptors (GPCRs) facilitate signal transduction in all eukaryotic cells. GPCRs play central roles in many processes related to human health: they represent perhaps the most important class of protein drug targets. The structure and function of integral membrane proteins including GPCRs are influenced by the local properties of the lipid bilayer in the plasma membrane. There are reasons to expect that this would be especially true for GPCRs, which exhibit unusually high conformational flexibility in their transmembrane domains. In addition, there is evidence from structural studies that certain GPCRs may directly bind to lipids at the interface of receptor dimers or between the transmembrane helices of a single protein.

Our recent work on the human serotonin receptor 5-HT_{1A}R has highlighted how bulk properties can alter GPCR function. Our motivation for exploring this protein was the central role it plays in several disease states in mental health, including depression and anxiety. We recently published a study showing how varying lipid composition changes the functional activity of 5-HT_{1A}R. We used an assay that measured downstream signaling catalyzed by 5-HT_{1A}R. Using a ligand exchange format in which the receptor was preloaded with antagonist and its activity upon addition of agonist was observed, we measured a significant dependence of receptor activity on the composition of the lipid bilayer. **Fig. 3** summarizes of how 5-HT_{1A}R activity changes with the concentration of membrane ordering lipid species. We will use a technique for measuring GPCR activity based on this study to determine how other GPCRs change activity as a function of lipid bilayer composition.



Noah Malmstadt



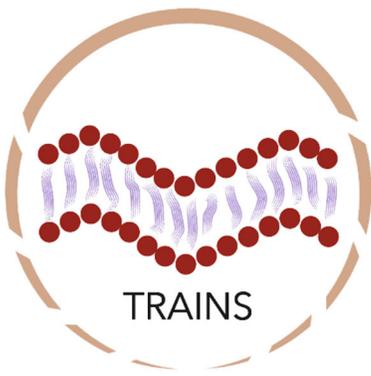
Figure 3: Dependence of serotonin receptor activity on lipid composition. As the concentration of ordering lipids such as cholesterol (Chol) or sphingomyelin (BSM) increases, the rate at which the serotonin receptor 5-HT_{1A}R exchanges antagonist for agonist also increases.

In particular, we will focus on the adenosine 1A receptor A1AR, which plays a key role in the neurobiology of Parkinson's Disease. Our work will focus on how changes to cell membrane composition that can be mediated by simple therapeutic interventions such as diet or statin drugs can alter the behavior of this protein. Students will be introduced to fundamental concepts of protein/membrane interaction biophysics, as well as learning methods of quantifying and modeling enzymatic reactions. This work is funded by the National Institutes of Health (1R21CA204708).

Conclusion

The proposed research will introduce students to key intersections between chemical engineering principles (diffusional transport, chemical rate processes) and model biological systems, which fit well under scientific theme of "Physical Biology at the Membrane Interface." Together with this fundamental training, students will be equipped with core laboratory skills including microscopy and handling of fragile protein and lipid materials.





Peter Chung
**Assistant Professor of
Physics and Astronomy**



Proposed Research

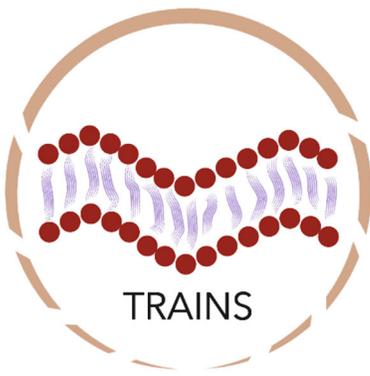
The laboratory of PI Peter Chung has the fundamental aim of understanding the biophysical function of intrinsically disordered proteins (IDPs). Although associated with nearly every major neurodegenerative disease, the physiological function and diseased dysfunction of IDPs remain not well-understood due to their persistence as unfolded biopolymers. However, this property makes them uniquely amenable to characterization under the paradigm of polymer physics. In line with the scientific theme of “Physical Biology at the Membrane Interface,” our research priorities will be biophysically characterizing IDPs that interact with subcellular membranes. These projects have been selected for both their accessibility to community college students and ability to develop into long term projects.

Reprogramming organelle sequestration via phosphorylation of synapsin-rich liquid droplets

Although IDPs can persist as non-interacting biopolymers in solution, at physiologically-relevant concentrations select IDPs can interact and form the basis for subcellular biomaterials within the cell. In the neuron, the IDP synapsin phase separates into protein-rich liquid droplets, akin to polymer coacervation. This structure can serve a variety of functions, including the sequestration of organelles before they are needed within the neuron. However, biology allows for an additional layer of sophistication; IDPs can be “reprogrammed” via post-translational modifications to control their ability to sequester organelles.

Perhaps the most consequential of these modifications is phosphorylation, or the addition of a divalently-charged phosphate group to a specific site in an IDP. As phosphorylation can occur at multiple and distinct sites, an IDP (like synapsin) can be reprogrammed with a unique “phosphorylation code.” Each code can correspond to a variety of changes in bulk materials properties, from structural dissolution to increased robustness of phase behavior. Critically, this code can also modify the IDP motifs that directly interact with organelles, thereby regulating their diffusive behavior within an IDP-based biomaterial. While it is clear that each phosphorylation code has the ability to effect biomaterials function at multiple length scales (from bulk to molecular transport), the difficulty in assessing the dynamic phosphorylation state of proteins within a cell makes understanding that relationship experimentally intractable.

The PI proposes to bypass dynamic phosphorylation states of protein within the cell by applying recent advances in synthetic biology to produce phosphoproteins with specific phosphorylation codes. By utilizing purpose-designed cells to express proteins with non-canonical amino acids, we can express protein sequences that code for non-canonical phosphoserine, thus producing bulk quantities of protein nearly identical to canonically phosphorylated protein. Once confirming that these proteins retain their ability to form protein-rich liquid droplets, the dynamics of organelles inside protein-rich liquid droplets will be measured thru advanced microscopy methods and X-ray scattering techniques.



Peter Chung

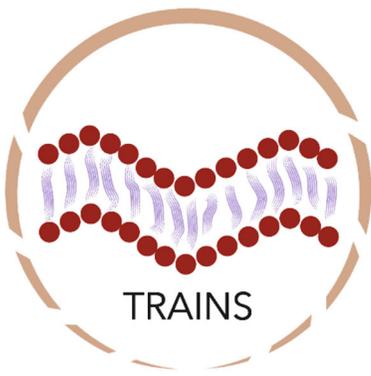
Students participating in this project will receive training in advanced protein expression techniques, especially with cell lines that express proteins with non-canonical amino acids. Additionally, students will learn how to produce liposomes that mimic the compositional and curvature properties of organelles that are putatively sequestered within the synapsin-rich liquid droplet. After having purified these proteins and produced organelle-mimicking liposomes, students will be trained on high-resolution confocal microscopy and X-ray photon correlation spectroscopy, two complementary techniques to measure the diffusion of organelles within protein-rich liquid droplets. This project is currently funded under NSF DMR-2104854.

Elucidating membrane-tension as a key parameter controlling IDP-membrane interactions Synaptic vesicles (SVs) are membrane-bound organelles that fill and store neurotransmitters for later release and exist in functionally heterogeneous populations. While IDPs have been known to probe for various physical parameters of SVs (including lipid composition and membrane curvature), there has been increasing evidence that IDPs can detect membrane tension associated with the filling of SVs. As the functionally heterogeneous populations of SVs exhibit differential protein binding of cytosolic IDPs, membrane tension may be a key parameter in functionally differentiating SVs.

While a cell-free system of purified proteins and SV-mimicking liposomes could be used to demonstrate osmotic pressure as a parameter controlling the association of proteins to SV, quantitatively measuring the changing binding affinity as a function of membrane tension presents a challenge. Not only must measurements be taken for various protein-to-lipid ratios to obtain a complete binding curve, the changing response to membrane tension dictates that a binding curve must be taken for each osmotic condition. If the dynamic parameters of protein post-translational modifications and changing lipid composition of SVs were to be also included, assessment of this phenomena becomes experimentally intractable.

The PI proposes to instead use high-throughput techniques to rapidly obtain complete binding curves for proteins on osmotically-stressed SV-mimicking liposomes. We will indirectly measure protein binding by fluorophore-tagging proteins and measuring protein binding via fluorescence anisotropy. When a fluorophore-tagged protein is excited with polarized light it emits fluorescence with the same polarization, but they tumble rapidly (relative to their fluorescence lifetime) and lose their initial polarization. When bound to a much larger liposome, the rotational diffusion of a fluorophore-tagged protein decreases and thus preserves its initial polarization and indicates binding. Due to the higher quantum yield for these artificial fluorophores, sample requirements are much lower and allow for the use higher throughput platforms such as a microplate reader in concert with an automated pipetting platform for sample production.

Students participating in this project will receive training in advanced protein expression techniques and production of SV-mimicking liposomes in various membrane tension states. Furthermore, students will learn to program automated pipetting platforms via Python and reduction of large data sets acquired from the microplate readers by using MATLAB. From a learning perspective, the overall goal of this project would be to familiarize students with the handling and analysis of large data sets. This project is currently funded under startup funds associated with PI Peter Chung.



Peter Chung

Detecting membrane-templated IDP aggregation

Nearly all neurodegenerative diseases exhibit late-stage protein aggregates, which have been found to be predominantly composed of IDPs. However, most IDPs associated with these late-stage protein aggregates are considered stable at physiological concentrations and solution conditions, including alpha-synuclein (forming Lewy bodies in Parkinson's disease). While efforts have sought to identify small molecule cofactors that initiate aggregation, dysfunctional organelles have been suggested as potential cofactors instead [5]. By binding to an organelle membrane, not only does the local protein concentration increase but the membrane-bound state may offer access to protein conformations more likely to undergo aggregation.

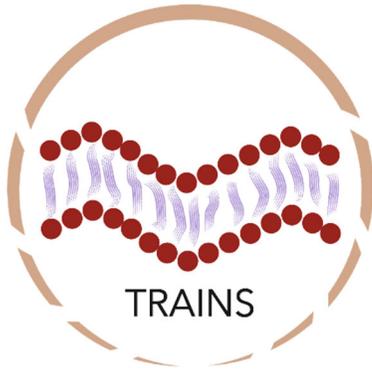
Demonstrating membrane-templated IDP aggregation by organelles could be key in linking organelle dysfunction to IDP aggregation within the cell. Dysfunctional organelles are known to undergo a variety of changes, including alterations to lipid composition (via increased peroxidation, lipid scramblase dysfunction, etc.) and membrane tension. By identifying the membrane conditions by which organelles can promote aggregation, it may be possible to correlate these changes to possible neuronal disease states.

The PI proposes to measure IDP aggregation on liposomes mimicking compositions corresponding to dysfunctional organelle states using complementary chemical and physical techniques. Initially, membrane-bound IDPs can be glutaraldehyde cross-linked to preserve any potential IDP aggregates. After dissolving liposomes using detergents, the remaining chemically cross-linked protein aggregates can be size identified through SDS-PAGE (a technique that has already been validated for alpha-synuclein on SV-mimicking liposomes). To uncover the kinetics of membrane-templated aggregation, the diffusion of nanoparticle-tagged membrane-bound IDPs can be measured on membrane surfaces using X-ray photon correlation spectroscopy under conditions identified by chemical cross-linking assays.

Students participating in this project will receive training in advanced protein expression techniques and production of liposomes mimicking organelles in various diseased states. Moreover, students will learn how biochemical and physical characterization techniques can complement each other, with the overall goal of elucidating a possibly novel pathway in neurodegenerative disease. This project is currently funded under startup funds associated with PI Peter Chung.

Conclusion

The proposed research, while containing common elements in technique and methods, present accessible pathways for students to effectively onboard into the scientific theme of "Physical Biology at the Membrane Interface." Given the long-term relationship this program is seeking to build, these projects will hopefully be the springboard by which students can commit to a lifelong appreciation and commitment towards science



Wade Zeno

Assistant Professor of Chemical Engineering and Materials Science



Proposed Research

Surfaces are inherently catalytic due to their propensity to concentrate reactants. Cellular membranes, which are composed of a heterogeneous mixture of lipids and proteins, are fascinating examples of reactive surfaces. They are critical interfaces at which many biochemical reactions and processes occur, including cell signaling, exocytosis, and endocytosis (Fig. 1). These functions are relevant to understanding cellular physiology as well as the mechanisms behind diseases that include neurodegenerative disease and cancer. PI Wade Zeno's research laboratory uses biophysical methods to elucidate cellular mechanisms that occur between proteins and lipids at membrane interfaces. In addition, the PI's laboratory develops membrane-inspired biomaterials for therapeutic applications. Specifically, the lab explores (i) the role of intrinsically disordered proteins (IDPs) in biological systems toward discovering physical mechanisms in biological pathways, (ii) the use of membrane-derived nanomaterials toward development of therapeutic technologies.

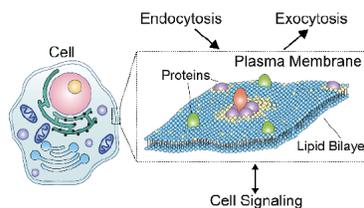
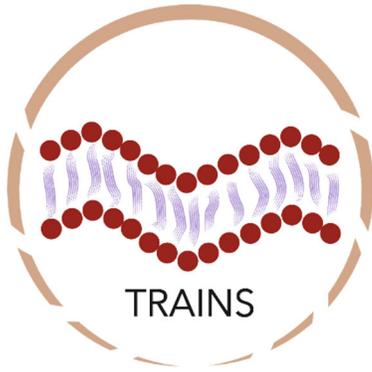


Fig. 1: The protein-lipid interface

Understanding Disorder at the Protein-Lipid Interface to Drive Solutions for Disease Treatment

Curved membrane structures are essential for proper cellular physiology. These structures are the result of interactions between lipids and proteins. Endocytic and synaptic vesicles are examples of curved membrane structures that are involved in various cell signaling processes. Disruption to these processes results in a variety of diseases, including Parkinson's disease, Alzheimer's, and cancer.¹ Therefore, developing a mechanistic understanding of the protein-lipid interactions involved in these cellular processes is essential for combating these illnesses. Our lab will use biophysical methods to elucidate the mechanisms of protein-lipid interactions that occur at curved membrane surfaces. Specifically, their lab will explore biophysical interactions between intrinsically disordered proteins (IDPs) and curved biological membranes. This proposed work will fill the gap between fundamental protein-lipid biophysics and our current understanding of how membranes function in cells.

IDPs are a class of proteins that have garnered considerable interest in biological research over the last decade due to their implications in neurodegenerative disease and cancer. IDPs are proteins that do not fold and therefore lack structure. Water-soluble IDPs have dynamic properties and are well described using polymer theory.² One IDP that the applicant will examine is α -synuclein (α Syn), which is found on the surface of synaptic vesicles. α Syn is disordered in solution but adopts a partial amphipathic helix upon binding to the membrane surface (Fig. 2).



Wade Zeno

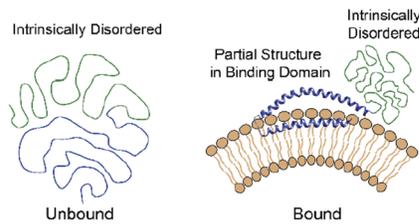


Fig. 2: Structure of α -synuclein

α Syn is of immense biological significance due to its pathological state, where it forms insoluble fibrils that are implicated in Parkinson's disease.

This protein, as well as many others, exist in a dynamic equilibrium between their membrane-bound and -unbound states. In the disease state, this delicately balanced equilibrium is disrupted by the presence of aggregates in solution or by changes to the protein-lipid affinity on the membrane surface.²⁻⁴ To date, no techniques exist that allow for direct measurement of the binding kinetics between IDPs and curved membranes. This lack of critical information arises from the difficulty associated with performing the necessary experiments. Therefore, our laboratory will develop the tools to explore these protein-lipid interactions and elucidate the biophysical mechanisms of protein exchange on the membrane surface. This innovative work will develop tools to characterize relevant, yet poorly-understood phenomena and provide valuable, mechanistic insight to processes that are essential to understanding and combating disease.

Engineering Protein-Lipid Nanodiscs for Therapeutic Applications

Nanodiscs (NDs) are biomimetic structures that are capable of solubilizing integral membrane proteins (Fig. 3). GPCRs are integral membrane proteins that represent a large class of drug targets. Therefore, NDs show great promise as a therapeutic technology. However, a limitation of NDs is the incorporation of membrane proteins into the disk. There are two main mechanisms by which membrane proteins are incorporated - cell-free expression and detergent solubilization/reconstitution. Most of the commercially available cell-free systems are unable to perform essential post-translational modification, while detergent solubilization protocols are protein-specific and can often result in protein destabilization. Therefore, the proper insertion of physiological transmembrane proteins remains a major challenge.

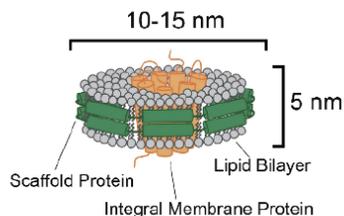
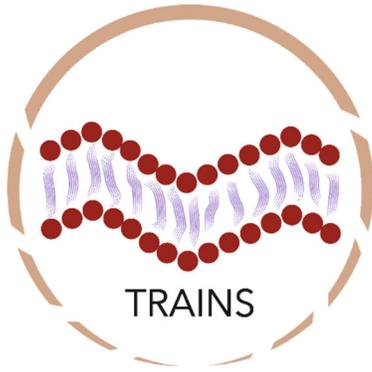


Fig. 3: Structure of nanodisc

To meet this challenge, our laboratory will develop a technique that harvests transmembrane proteins directly from the plasma membrane of mammalian cells and efficiently incorporates them into NDs. NDs will be synthesized via the schematic displayed in Fig. 4. Mammalian donor cells will be edited to express the membrane protein of interest. Afterward, donor cells will shed their plasma membrane to form Giant Plasma Membrane Vesicles (GMPVs) via a chemically-induced vesiculation process referred to as "blebbing".⁵ Once the GPMVs are isolated, they can be directly exposed to amphipathic membrane scaffold proteins, which will spontaneously interact with the membrane to self-assemble into NDs.



Wade Zeno

ND structures will be characterized using electron microscopy, while functional binding studies can be performed using fluorescence correlation spectroscopy (FCS). Our lab will first utilize proof-of-concept, synthetic integral membrane proteins before eventually moving on to biologically relevant GPCRs.

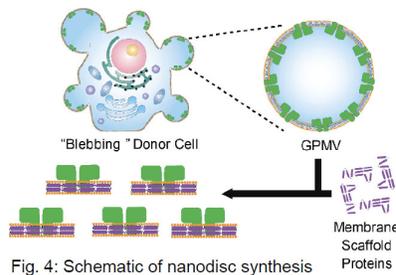
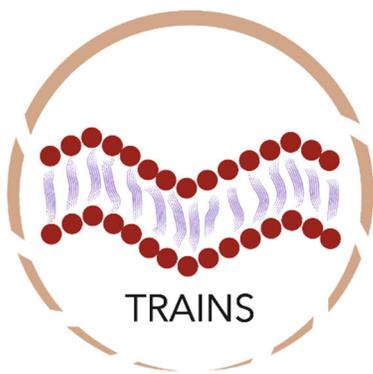


Fig. 4: Schematic of nanodisc synthesis

This technology will then be used for developing therapeutic platforms for screening GPCRs that are implicated in disease. Specifically, the GPCR-NDs will be immobilized in a porous substrate through which small ligands can flow freely. This cell-free system will allow for the monitoring of binding between GPCR-NDs and cocktails of small ligands. Similarly, libraries of GPCRs can be immobilized in the porous substrate while a single ligand is passed through. This technology can also be utilized to incorporate major histocompatibility complexes (MHCs), which are peptide-binding membrane proteins that are involved in the activation of T Cells.⁶ This specific type of porous substrate can be used to improve cancer treatment by providing an efficient means of performing adoptive T Cell therapy, where T cells are taken from the patient's own immunosuppressed tumor tissue and activated *ex vivo*.

Conclusion

The proposed research, while containing common elements in technique and methods, present accessible pathways for students to effectively onboard into the scientific theme of "Biological Physics at the Membrane Interface." Given the long-term relationship this program is seeking to build, these projects will hopefully inspire a younger generation of URM students to pursue rewarding careers in science and engineering.



Moh El-Naggar

Divisional Dean for the Physical Sciences and Mathematics at USC



Proposed Research

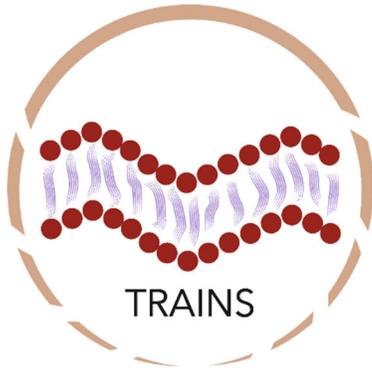
The El-Naggar laboratory has the fundamental aim of understanding and harnessing biotic-abiotic electron transfer at the interface of living cells and solid-state electrodes. Much of these interactions are mediated by membrane-associated metalloproteins and bacterial membrane nanowires, and are therefore firmly within the scope of the proposed theme 'Physical Biology at the Membrane Interface'.

Background

Life cannot exist without Electron Transfer (ET). The stepwise movement of electrons within and between molecules dictates all biological energy conversion strategies, including respiration and photosynthesis. Respiratory organisms acquire energy by controlling the flow of electrons from electron donors (food) to electron acceptors (oxidants) along a 'highway' of reduction-oxidation (redox) cofactors. This electron flow can also drive proton transfer to charge a biological membrane that powers the synthesis of life's energy currency in the form of ATP molecules. ET mechanisms are at play in bacteria, archaea, the mitochondria of eukaryotes (including animals), and are also critical for the function of chloroplasts in photosynthesis. With such a universal role across all three domains of life, bioenergetics and the biophysical basis of ET have been the subject of intense experimental and theoretical studies. Indeed, broad mechanisms of ET over small length scales in biomolecules are now well appreciated; coherent tunneling describes ET between nanometer-spaced cofactors and incoherent hopping may describe transport across long cofactor chains distributed within membranes.

In contrast to the intracellular reactions described above, our understanding of biological ET has been upended, during the last decade, by remarkable observations of fast long-distance extracellular electron transport (EET) to minerals and direct inter-cellular electron transport (DIET) within microbial communities. These remarkable organisms have evolved charge transfer mechanisms to solid surfaces outside the cells, allowing them to use abundant minerals as electron acceptors for respiration, instead of oxygen or other soluble oxidants that would normally diffuse inside cells. By performing EET to or from electrodes, such microbes can also be used as biocatalysts for converting the energy stored in diverse chemical fuels to electricity (biological fuel cells), or vice versa (microbial electrosynthesis), in technologies that impact human health (water treatment), renewable energy recovery, and CO₂ capture. In addition, the protein electron conduits that these organisms evolved to interact with the abiotic world may offer both new concepts and inspiration for (bio)electronic interfaces between cells and traditional electronics (e.g. human-machine interfaces).

On the basic science side, the El-Naggar group contributed to this area by (i) demonstrating multistep electron hopping in outer membrane associated cytochromes that allow biotic-abiotic electron exchange; (ii) uncovering the structural basis of cytochrome-containing bacterial nanowires in an important environmental organism; (iii) demonstrating multi-cell electron conduction; and (iv) discovering spin selective electron transport in cell surface electron conduits. In fact, with the discovery of bacterial nanowires, conductive bacterial biofilms, and now multicellular cable bacteria, the length scales of microbial ET observations have jumped by 7 orders of magnitude, from nanometers to centimeters, during the last 15 years! Our mechanistic understanding of these processes, however, lags behind these observations.



Moh El-Naggar

To bridge this knowledge-gap, the El-Naggar group is heavily focused on biophysical studies to discover the identity, structure, and charge transport physics in these unusual biological conductors that bridge cellular membranes to abiotic surfaces. The following specific projects are highlighted here because of their educational value and potential to engage community college students in different phases of the discovery process.

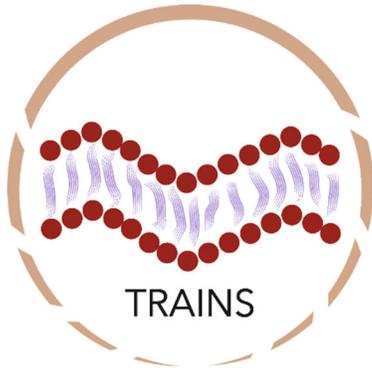
Unraveling the Mechanisms of Centimeter Scale Electronic Conduction in Cable Bacteria.

There is now mounting evidence of centimeter-scale electron transport in multicellular cable bacteria within marine and freshwater sediments worldwide. These multicellular filaments, composed of thousands of end-to-end cells, gain energy from the long-distance electron conduction process by coupling sulfide oxidation in deeper sediment layers to oxygen reduction near the sediment-water interface. Coordinated charge transport over these distances was previously thought impossible in biological materials, and may necessitate coherent or higher efficiency electron transmission channels that go beyond the performance of redox hopping mechanisms typically encountered in biology. The overarching goal of this Keck Foundation funded project is to unravel the electron transport physics in this still-mysterious molecular pathway, and understand how it impacts energy distribution over unprecedented length scales. Specifically, we are: (1) Identifying the physics-based mechanism(s) of electron conduction in cable bacteria and their networks of cell envelope conductive protein nanofibers; (2) Observing and quantifying the energy distribution along conducting cable bacterial cells by monitoring cellular membrane potentials *in vivo*; and (3) Revealing the identity of the charge carriers through cell fractionation and proteomic techniques.

This project can involve undergraduate trainees who interact with El-Naggar, postdoctoral scholars, and graduate student mentors. Community college students interested in this effort will receive training while assisting in environmental sampling and incubation of bacteria, *in vivo* microscopy, electronic measurements, and cell fractionation strategies necessary for downstream omics analyses. A unique aspect of this project is that it demonstrates a 'complete journey of discovery' with an experimental pipeline that connects environmental sampling in the Los Angeles area, laboratory culturing, and biophysical measurements. This 2021-2024 project is currently funded by the W.M. Keck Foundation (Award 8626).

The Mechanisms and Implications of Chiral Induced Spin Selectivity in Biological Systems.

In early 2020, the El-Naggar group reported evidence that the bacterial membrane cytochromes responsible for electron transfer to external surfaces exploit not only the electron's charge but also its spin through the Chiral Induced Spin Selectivity (CISS) effect. This effect couples the electron's spin to its linear momentum in a chiral potential and has been previously demonstrated in many organic materials, but the hypothesis that it is also central to biomolecules and physiological functions is just now coming into focus.



Moh El-Naggar

An important goal of this AFOSR funded project is to discover the structural determinants (protein secondary structure and organization of redox centers) of this spin selectivity in membrane cytochromes and biological nanowires. Preliminary data suggest that the magnitude of spin selectivity associated with electron transport through multiheme cytochromes correlates with the percentage of helical secondary structure. The project will systematically test the impact of secondary structure and heme chain configuration by quantifying spin polarization in a library of structurally related membrane cytochromes with increasing helical content. Going beyond spin polarization in single molecules, we will test whether this property is preserved in nanowires composed of cytochrome chains produced by bacteria (bacterial nanowires). In addition to the biological significance, spin filtering protein wires would have implications for a new generation of biomolecular spintronic devices.

This project may present excellent training opportunities for community college students who will assist graduate student and postdoctoral mentors while receiving training in cloning, protein (cytochrome expression), and protein purification using a combination of immobilized metal affinity chromatography and anion exchange or gel filtration. Opportunities are also available for student trainees to be involved in electronic (conductive scanning probe) and spintronic (Hall voltage) measurements of the purified cytochromes. This 2021-2026 project is currently funded by the Air Force Office of Scientific Research (AFOSR Award FA9550-21-1-0418).



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