# *2015 MBL Physiology Group Projects*

**Session 1: June 22-July 4**
Joe Howard, Jennifer Lippincott-Schwartz, Rob Phillips, and Orion Weiner

**Howard Group Projects: Scaling laws in development**
**TAs**: Shirin Bahmanyar, Hugo Bowne-Anderson, Akatsuki (Aki) Kimura, Jonathan Rodenfels

The Howard group has a set of projects centered around changes in organelle size during embryogenesis.

Development inside an egg shell poses interesting cell biological challenges. Because total volume is constrained, large cells divide into smaller cells, yet each cell must have the right number and size of organelles and other materials. This leads to interesting scaling properties. For example, if the cell division time is a constant, then the total amount of DNA will increase exponentially. If the cells fill a fixed volume, then the amount of plasma membrane that encloses the cell will also increase exponentially, but at a slower pace with a power-law exponent 1/3 instead of 1.

In addition to geometric constraints, biological constraints, which are still very poorly understood, lead to interesting scaling properties of organelles during development. For example, nuclear size decreases with cell size during development, even though the absolute amount of DNA per cell is fixed. Is the DNA exerting different pressures on the nucleus, perhaps due to variation in transcriptional activity or nuclear-cytoplasmic transport? And how are changes in the concentrations of nuclear proteins and the area of the nuclear lamina coordinated with the changing diameters of nuclei?

Another open question is the demand that cell proliferation puts on overall metabolism As the complexity of the embryo increases, do the size and surface area of mitochondria adapt? And to what extent does the diffusion of metabolites into and out of the interior of large embryos limit growth?

Project 1: Nuclear size during *C. elegans* development (S. Bahmanyar, A. Kimura)

*C. elegans* is a wonderful model systems for studying early development with excellent microscopy combined with an abundance of genetic probes for visualizing molecules such as chromatin and membranes. Nuclei decrease in diameter from 10 microns down to 2 microns during development, and we will combine in vivo and in vitro approaches for trying to understand what controls nuclear size. By labeling the nuclear envelope we will precise quantify nuclear size to determine whether there is scaling power law, and use genetic, chemical and mechanical perturbations to probe the underlying determinants of size such as the amount of DNA, the activity of the nuclear import machinery and the influence of chromatin condensation. These experiments will be complemented with mechanical experiments on isolated purified nuclei. Do the mechanical properties such as compressibility or elasticity change with size or DNA content? What roles do the lamins have on nuclear size and mechanics?

Project 2: Metabolic scale during zebrafish development (J. Rodenfels, H. Bowne-Anderson)

How do metabolic demands of organisms change during embryogenesis? Using the zebrafish as a model organism, we will image mitochondrially targeted GFP transgenic lines to determine how the amount and location of mitochondria change during development. We will use the advanced imaging facilities available at the MBL to determine whether there are changes in mitochondrial morphology. In parallel experiments we will use highly sensitive calorimetry and oxygen consumption measurements to quantitate changes in metabolic activity during the cell cycle, and from one cell cycle to the next. Can we correlate changes in metabolism with the overall size of the embryo, the amount of membrane or DNA?

**Lippincott-Schwartz Group Projects: Probing cell and organelle dynamics using live cell imaging**

TAs: Tim Petrie, Chris Obara, Dan Feliciano, Sabine Petry and Ray Alfaro-Aco

Project 1: Global to Local: Linking Cell Adhesion to Bioenergetics in Stem Cells (T. Petrie)

Regenerative medicine largely relies on the use of in-vivo delivered pluripotent stem cell populations to repair tissue damaged by injury or disease. Various signals from the microenvironment can greatly affect stem cell fate decisions. Human mesenchymal stem cells (hMSCs) are a clinically relevant population of bone marrow-derived progenitor cells capable of robust differentiation into mesodermal lineages such as bone, cartilage, fat, muscle, and tendon. These cells undergo differential fate transitions on substrates of varying mechanical and chemical surface properties, including extracellular matrix moieties and substrate stiffness. These substrate properties can, in turn, modulate cell shape, polarization, adhesive receptor binding, and cell volume to direct lineage-specific differentiation and directly modulate therapeutic efficacy. Although it is well understood that directing global hMSC physical properties, such as cell shape, can "push" these cells down a specific lineage specification, it is not well-understood what intracellular processes are reorganized to affect this change. In particular, the idea that adhesive alterations in receptor binding and cytoskeletal arrangement due to substrate property changes may affect critical metabolic changes has been relatively unexplored. We hypothesize that global adhesive and physical cell changes directly modulate "signature" bioenergetic processes to permit differentiation toward a specific lineage.

This project will explore how manipulating physical cell properties, including shape, volume, polarization, and adhesion receptor engagement and localization, modulates mitochondrial dynamics, lipid metabolism, and cell membrane potential. We will use bioengineering tools, including micro and nano-contact printing and biomaterial functionalization schemes, to design substrates that modulate these different physical hMSC states. HMSCs will be labeled with markers for different organelles (i.e., ER, lipid droplets, autophagosomes and mitochondria), integrins and cytoskeletal elements, and these markers tracked through live imaging. Moreover, we will use pharmacological agents to disrupt cytoskeletal elements and metabolic processes to assess how these factors contribute to lineage specification on substrates of various properties. Along with stem cell culture, students will learn stem cell differentiation protocols and transfection techniques. Students will also learn multi-spectral live imaging techniques, quantification analyses, and microscope operation on spinning disk confocal, structured illumination, and light sheet machinery. The goal of this broad-ranging project is to unite adhesion and metabolism processes while providing the student with hands-on experience using robust bioengineering and imaging techniques to explore varied cell biology questions.

Project 2: Making microtubules from the Golgi – when, how and why (S. Petry and R. Alfaro-Aco)

Features critical for Golgi function, such as polarized layering of its cisternae, are maintained by the microtubule (MT) cytoskeleton. Surprisingly, the Golgi itself is able to form, or nucleate, MTs from its membrane, as recently uncovered. This challenged the long-held dogma of centrosomes as the sole source of microtubule formation. Further investigation of Golgi-derived MTs revealed that approximately 50% of the MT network is derived from the Golgi in non-dividing retinal pigment epithelial cells. These findings have spurred the investigation of additional roles of Golgi-derived MTs, adding direction of cellular trafficking, polarized cell migration, and dendrite formation to its growing list. Yet, mechanistic details of how MTs are generated from the Golgi, how they are arranged into a local MT architecture, and how this network supports specific Golgi functions remains to be determined.

Our approach for investigating these questions will be to fractionate the Golgi from tissue and define its MT nucleation capacity *in vitro* by various microscopy techniques, including TIRF microscopy. Candidate proteins, which have been suggested to mediate MT formation from the Golgi, will be investigated. This can be performed at three levels: in cells, in *Xenopus laevis* egg extract, and with purified proteins. This project merges research on the MT cytoskeleton with the Golgi via microscopy and biochemical methods.

Project 3: Cytoplasmic and organelle intermixing during cell-to-cell fusion (D. Feliciano and C. Obara)

Cellular membranes are lipid bilayers that define the boundaries of cells and intracellular organelles. They serve as barriers to isolate and compartmentalize intracellular processes and chemical reactions but also undergo selective fusion to allow mixing of specific compartments. Because membrane fusion is energetically unfavorable, it requires specific protein fusion machinery (i.e., fusogens) to allow precise modulation of the efficiency, frequency, and location of fusion events. The activity of many fusogens is tightly regulated to facilitate rapid cellular processes such as release of neurotransmitters from axons, restructuring of the peripheral endoplasmic reticulum, and formation of multinucleated cells after cell-to-cell fusion during tissue differentiation and development. Cell-to-cell fusion provides an interesting example, since it may require a number of independent plasma membrane (PM) and organelle fusion events and is required for bone, muscle and placenta development in all mammals.

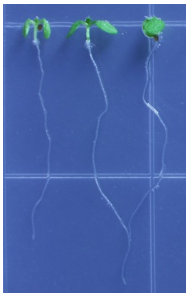
Despite many years of study and characterization of the proteins involved in cell-cell fusion, there are still many outstanding questions for how this process is regulated and leads to cytoplasmic mixing between two cells. In this project, we will investigate two aspects of cell-cell fusion: 1) how fusogens and their receptors organize at the cell surface to create fusion pore(s) and the spatio-temporal dynamics of this process; and 2) how intracellular organelles mix once two cells have fused. Cells will be transfected with different fusogens of human or viral origin (Syn-1, Syn-2, VSV-G, etc.) and their co-receptors to induce cell-cell fusion. The sites and behavior of fusion events will be analyzed using live-cell multicolor imaging. In addition, cytoplasmic and organelle mixing will be studied in real time. We will address how quickly cytoskeletal systems (i.e., microtubules and actin) of fused cells intermix, and whether ER, nucleus, Golgi and mitochondria remain cell-specific or merge into single compartments. We will also look for changes in cell volume and PM membrane voltage during cell-cell fusion, examining the roles of endocytosis and exocytosis and the effects of pharmacological perturbants. Students should expect to learn basic cell biology and membrane biophysics techniques while studying a fundamental aspect organismal biology at the cellular level, as well as develop a substantial exposure to live-cell imaging techniques both at the diffraction limit of light as well as superresolution microscopy (STED, SIM, and Airyscan technologies). If desired, students will also be exposed to wavelength space imaging techniques (linear unmixing and lambda scan approaches).

**Phillips Group Projects: Physiology of the Bacterial Cell**
TAs: Liz Haswell, Gary Borisy, Blair Rosetti, Rob Brewster, Griffin Chure

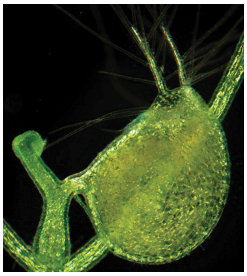
The Phillips group is bringing a diverse collection of problems on subjects ranging from the biophysics of how genes are transferred and regulated to single-cell studies of the oral microbiome. We are interested in both theoretical and experimental approaches and are always open to students finding their own exciting directions to pursue.

Project 1: Exploring membrane forces in plants and bacteria

Using a variety of genetically encoded biosensors, membrane markers, and voltage-sensitive dyes, we will explore the ways in which organisms sense and response to mechanical stimuli.

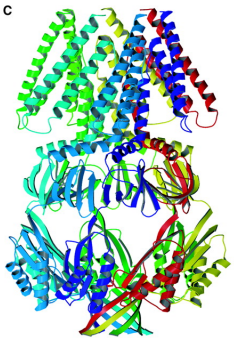


*A. Land plants.* Land plants must address a broad array of mechanical challenges during their lifespan, including gravity and touch perception, osmoregulation, and developmental events. *Arabidopsis thaliana*, the primary model system for flowering plants, has small seedling roots that are ideal for fluorescent imaging. We will investigate membrane depolarization, $Ca^{2+}$ influx, and subcellular rearrangements in response to changes in the gravity vector, growth into obstacles, or lateral root emergence. We will also have some pollen from tobacco ready to be transformed with ion flux sensors to measure changes in ion flux dynamics during tube growth into and around barriers.



Ibarra-Laclette & Perez-Torres

*B. Aquatic carnivorous plants*. The aquatic carnivorous bladderwort *Utricularia gibba* captures and digests small prey inside of a bladder (see image at left). When prey animals come into contact with trigger hairs at the periphery of the bladder, which is normally maintained under negative water pressure, it quickly inverts, sucking prey along with water into the chamber. Whether this is due to a strictly mechanical displacement of the trigger hairs, or if there is amplification of the signal through bioelectric signaling is a matter of recent debate. We will attempt to address these hypotheses with voltage sensitive dyes and time-lapse imaging.



Bass et al., 2002

*C. Bacteria.* Though they are often considered to be the most basic of organisms, single-celled bacteria still have much to teach us about mechanotransduction. The genome of *Escherichia coli*, the workhorse of the molecular biology lab, encodes 6 mechanosensitive ion channels thought to serve as osmotic release valves, preventing cellular lysis under hypoosmotic shock (see the crystal structure of one of these, MscS, to the right). It has been proposed that having multiple channels with slightly different tension sensitivities and conductances serves to provide a graded response to membrane tension—the cell need only release as many osmolytes as needed for the cell to survive a given osmotic challenge. We will use genetically encoded membrane voltage and membrane tension sensors to quantitatively determine the relationship between membrane tension and ion flux in a series of MS ion channel mutant strains.

Project 2: Biogeography of Microbial Communities at the Micron Scale

Microbes frequently live in communities of substantial complexity both in the physical environment and in symbiotic or pathogenic relationships with other organisms. Increasingly, it is recognized that the organization of microbial communities in unique micro-niches may have to be explored at high resolution to provide the basis for elucidating the rules of community assembly and function and to understand their role in health and disease. Although next-generation DNA sequencing technology and metagenomics have revolutionized the analysis of microbial communities, a major gap in our understanding is the lack of spatial information at the micron scale,--information

necessary to characterize the interrelationships between members of a microbial community and with host tissue. The proposed projects on the oral microbiome are designed to help fill this gap. They are presented below in order of increasing risk.

Project 3: The Oral Microbiome

The Human Oral Microbiome will be used as a test microbial population. This is a good choice because it is complex, accessible, and biologically important. The human mouth is colonized by a microbial community estimated to consist of approximately 700 species living in oral biofilms. However, little is known about the micron-scale architecture of these oral biofilms. To understand how oral biofilms develop and how they transition from commensal to pathogenic, we need to determine which microbes physically contact one another and host tissues, which are often in close proximity, and which avoid close proximity. A major impediment to understanding these spatial relationships has been the inability to simultaneously obtain position and molecular identity for the majority of cells in a microbial community. We have developed a visualization strategy, Combinatorial Labeling And Spectral Imaging - Fluorescence *In Situ* Hybridization (**CLASI-FISH**), and have used it to identify up to 15 fluorescent labels simultaneously in a field of view. We will use this strategy to analyze and quantify the organization of microbes from distinct niches in the healthy mouth. We will use DNA-RNA hybridization with fluorescently-tagged, oligonucletotide probes, spectral imaging with a laser-scanning, confocal microscope equipped with a 32 anode array spectral detector and linear unmixing with a custom MATLab designed algorithm. The Human Microbiome Project has identified 9 distinct habitats in the human mouth but there may well be more. We have results on plaque and have just begun to study the tongue but have barely scratched the surface, which means there should be plenty of opportunity for discovery.

*Biogeography of Plaque*

Last year's class looked at plaque. They found amazing, multi-genus communities that we called "hedgehogs" because of their overall hemispherical appearance with spiky filaments at their perimeter decorated with cocci. One line of investigation could build on these observations by trying to understand the pathway of community assembly. Another line of investigation could search for additional communities as we don't think that hedgehogs are the only communities in plaque.

*Biogeography of the Tongue*

We have encouraging initial results but, really, this is a brand new line of study. The tongue dorsum carries the heaviest load of microbes in the mouth and, from metagenomic analysis, is very different from plaque. So, we expect to see novel and interesting structures. For both tongue and plaque, students in the course will have the option to sample their own microbiomes or to use samples obtained by non-student volunteers.

*Reconstruction of "Hedgehogs" in vitro*

Now that we have identified most of the microbial players in hedgehogs, it is possible to conceive of reconstituting the community in vitro. This won't be easy but success in such an endeavor would be an important breakthrough as it would enable the experimental dissection of each microbial member's contribution to the community. It would establish a platform for understanding community assembly, dynamics and maintenance. We will explore microfluidic approaches allowing control over oxygen gradients, nutrient gradients and shear flow.

Project 4: Quantifying transcription through the cell-cycle in bacteria under diverse growth conditions

The level of transcription from a given gene is coupled to the exterior environment in which it grows in a way that can appear quite complex. Detailed molecular models of transcription are unable to account for changes to important parameters like growth rate, whose primary purpose is to modulate the relative abundances of the key genetic elements involved in gene expression such as polymerases, the molecular machines that actually transcribe DNA to RNA, to transcription factors which are responsible for regulating the rate of transcription, to the actual number of copies of the

gene. Furthermore, the bacterial cell must double the concentration of each of these components over the course of its lifetime in preparation for division with each component having its own characteristic doubling rate. This picture points to a complicated time-dependence of transcription as a function of growth rate and age of a cell.

Using a simple genetic architecture as a starting point, we will measure how the genetic machinery necessary for transcription couples to the environment using quantitative single-cell mRNA FISH. This single-cell measurement technique will allow us to compare our results to quantitative predictions of transcription from simple transcription models. These theories make strict predictions for both the mean level of expression and the variability in transcription as a function of the internal factors being controlled by the environment and (dis)agreements can inform us of the viability of such mechanistic models.

Project 5: Single-molecule studies of horizontal gene transfer

Bacterial populations live in a fertile cocktail of foreign genetic material. Whether by viral infection, conjugative transfer, or direct uptake of DNA through transformation, bacteria frequently share their genetic information with their neighbors, including those of different species, strongly influencing bacterial evolution. Horizontal Gene Transfer (HGT) has been typically studied in bulk requiring the growth of saturated cultures and counting surviving colonies after being plated on selection. As naturally occurring populations are not near saturation and typically grow in non-homogenous mixtures (such as biofilms and colonies), understanding the dynamics and frequency of HGT at a single-cell level will provide insight into how frequently it occurs in nature.

We have established a genetically-encoded fluorescent system that allows for the sequence-specific visualization of DNA *in vivo* in *Bacillus subtilis* and *E. coli.* We will use single-cell microscopy to observe and measure the frequency of HGT via transformation and phage transduction. We will also tune various parameters of the transferred DNA such as concentration, linearity vs. circularity, and homology with the accepting cell's chromosome to understand what barriers DNA must overcome in natural environments to be successfully transformed.

**Weiner Group Projects: Dissecting the molecular logic of cellular decisions with optogenetics**
TAs: Alba Diz-Muñoz, Brian Graziano, Doug Tischer (UCSF), Jared Toettcher (Princeton)

While fluorescent proteins like Green Fluorescence Protein (GFP) have become essential *observational* tools for mapping the location, abundance and activity of gene products within living organisms, we lack robust complementary molecular tools to *manipulate* biological systems with similar precision. Light-gated protein modules (proteins that change activity upon exposure to light) provide a possible solution to this problem. An early advance was the discovery of naturally light-gated ion channels that can be used to manipulate the excitability of neuronal cells. More recently, we and others have extended the optogenetics toolkit by adapting additional genetically-encoded light-responsive proteins to control activities of a wide array of other cellular currencies and cascades.

Our generic and genetically encoded light-control module is based on plant phytochromes. PhytochromeB (PhyB) is a red/infrared responsive protein that normally controls seedling stem elongation in *Arabidopsis thaliana*. Upon exposure to red (650nm) light, PhyB changes conformation and, within seconds, binds a PIF (Phytochrome Interacting Factor) protein. This association is reversed within seconds with infrared (750nm) light or is stable for hours in the dark. The Phy/PIF system has several significant advantages over the other optogenetic platforms, including its superior speed, reversibility, spatial control, and ability to be multiplexed with multiple conventional fluorescent proteins. By fusing PhyB to membrane anchors and PIF to signaling proteins of interest, we have achieved unprecedented spatial and temporal control of a wide range of intracellular signaling currencies in a wide range of cellular contexts. In this module, we use our optogenetics system both inside and outside of cells to probe open questions in cell biology, such as cell scaling and T cell activation.

<u>Project 1: Optogenetic-based protein inactivation to investigate cell scaling</u>
In addition to activating signaling cascades by optogenetic-based recruitment of proteins *to* their normal site of action, we've recently modified our system to optogenetically sequester proteins *away* from their normal site of action for rapid, reversible, titratable inhibition of protein function. This system provides a general strategy for acutely and reversibly inhibiting proteins of interest by controlling their localization and therefore their availability to partners and substrates. We have used the temporal and spatial precision of the system to identify when and where a given protein's activity is necessary for function. Acute sequestration can also reveal the functional importance of a given protein in a manner that is normally masked by compensation following slower loss-of-function perturbations such as genetic knockout or knockdowns. For example, the Bem1 protein was previously thought to play a redundant role in budding yeast polarity based on the knockout phenotype, but through acute light-dependent sequestration we find that this protein is essential for polarity. Following rapid loss of Bem1 function, cells lose polarity and grow uniformly until they burst. Because we have reversible, light-based control over Bem1, we can *transiently* block its activity to generate cells that are upwards of 20-fold larger in volume vs. unperturbed cells. Cells have many aspects of their physiology (organelle size and number, organization of the actin and microtubule cytoskeletons) that must be scaled to cell size to support proper growth and division. By pairing our optogenetic control of cell growth with a variety of live cell readouts for cytoskeletal and organellar markers, students in this module will investigate how cells scale these components relative to their total volume.

<u>Project 2: Optogenetic interrogation of T cell activation.</u> T cells are organizers of the adaptive immune response, for which ligand discrimination is paramount. Peptides, either from pathogens or the host, are loaded onto major histocompatibility complexes (pMHC) and presented on the cell's surface to the T cell receptor (TCR). Whatever ligand a T cell recognizes instructs the rest of the adaptive immune system to attack. If the T cell receptor responds to self-antigens, autoimmunity can result. But if the receptor ignores foreign antigens, infections can linger. TCR ligand discrimination is so specific that single amino acid changes can separate a strongly recognized ligand from one that is ignored. How

the TCR discriminates between these different ligands is a fundamental unanswered question in immunology. The simplest way for cells to discriminate between TCR ligands would be to read the net occupancy of the receptors, with higher affinity ligands engaging more receptors at equilibrium, thus sending a larger "activation signal" to the cell. However, this occupancy model is inconsistent with the finding that low affinity ligands do not mimic degree of cell activation seen for high affinity ligands when their concentrations are adjusted to promote equal TCR occupancy.

A major model of TCR activation is the kinetic proofreading model, which postulates a temporal delay made up of multiple irreversible steps between ligand engagement and TCR activation. The model assumes that the TCR can only progress from one step to the next while the pMHC is engaged, and the TCR instantly resets to the initial state when the pMHC dissociates. This mechanism is based on the kinetic proofreading that gives ribosomes a specificity far beyond what would be predicted from tRNA equilibrium binding alone. Consistent with this hypothesis, 3D kinetic measurements show that stimulatory pMHCs have low off-rates while non-stimulatory pMHCs have high off-rates. If kinetics, not occupancy determines TCR activation, this would explain why even high doses of non-stimulatory pMHCs do not mimic stimulatory pMHCs. However, there is currently no good method to specifically change individual ligand properties to test how they affect receptor activation. Traditional methods of making point mutations to change pMHC kinetics not only affect the dissociation rates but also alter other important biophysical parameters, such as ligand binding geometry and ligand on-rates. Experimentally varying the length of time the pMHC engages the receptor while keeping potential confounding variables constant is the only clean way to assess the specific contribution of ligand kinetics to TCR activation. Towards this goal, we have adapted our optogenetic system to directly manipulate the kinetics of TCR engagement by ligand. Students in this module will use this optogenetic system to help us investigate how T cells monitors when, where, how long, and how many TCRs are engaged with ligand for regulating the decision to activate.

<u>Project 3: Open-end optogenetics</u>

In addition to the more defined projects above, students can also pursue more open-ended optogenetics projects. By taking advantage of the cutting edge microscope systems at the Physiology Course, we'd love to see how much we can push the boundaries of spatial control (3D and sub-resolution), temporal control, and automated control of light patterns for sophisticated quantitative biology experiments.

We have optogenetic control over fundamental cellular currencies such as Ras and PI3K that are used in a wide range of cellular processes. We've investigated these signals in only a subset of cascades and cell types and would be excited to work with adventurous students to expand these into other contexts this summer.

**Session 2: July 6-July 18**
Dan Fletcher, Bob Goldstein and Wallace Marshall

**Fletcher Group Projects: Organization at the cytoskeleton-membrane interface**
TAs: Mike Vahey, Sungmin Son, Andrew Harris, and Brian Belardi

The cell membrane, a flexible lipid bilayer packed with proteins that surrounds cells, plays a fundamental biochemical and biophysical role in numerous biological processes. It interacts closely with the actin cortex, a dynamic network of filaments and associated binding proteins located just beneath the plasma membrane. Both the cortical actin network and the cell membrane can be rapidly and precisely reorganized, such as during formation of intercellular junctions, where the clustering of transmembrane proteins is followed by their connection with contractile actin bundles. The fundamental mechanisms that give rise to spatial organization of the membrane and cortical cytoskeleton are active topics of research that have benefited from recent advances in fluorescence microscopy methods and in vitro reconstitution techniques. This summer the Fletcher group will use a combination of bottom-up and top-down approaches to study organization at the cytoskeleton-membrane interface. We will use model membranes, reconstituted actin filaments, and live cells to investigate several key questions:

- How does the length of a transmembrane domain influence its spatial organization in membranes?
- How do interactions between intracellular domains of transmembrane proteins and actin filaments drive spatial confinement in the membrane?
- How do mechanical constraints on cortical actin structures alter their organization and composition?

Tools and Techniques

Students will learn a range of basic and advanced methods for creating and studying cell membranes and the actin cytoskeleton:

- Membranes: techniques for making giant unilamellar vesicles (GUVs) and supported lipid bilayers (SLBs)
- Microscopy: confocal, TIRF, and wide-field fluorescence techniques
- Microfabrication: techniques from soft lithography and micro-contact printing

Project 1: Organizing the membrane, through thick and thin

Far more sophisticated than just stacked sheets of lipid molecules, the plasma membrane exemplifies the cell's capacity for multitasking – forming a signaling cluster in one place, initiating endocytosis someplace else, and all of this while maintaining polarity, probing the environment, and withstanding assault from extracellular and intracellular pathogens. Balancing all of these functions requires precise control over the spatial and temporal organization of the membrane, but a detailed understanding of the tools that are at the cell's disposal for accomplishing this task has been elusive. Recently, hydrophobic matching – the hypothesis that variations in membrane thickness help to organize proteins according to their transmembrane domain length – has been suggested to play a role in processes ranging from vesicle fusion to viral budding. In this project, we will use engineered and natural membrane proteins, together with traditional fluorescence microscopy, FRET, and super-resolution techniques, to put this hypothesis to the test. In both cells and synthetic membranes, we will investigate the influence of transmembrane domain length on protein organization. By comparing the behavior of engineered membrane proteins to their natural counterparts – focusing on examples from membrane fusion and viral budding – this project will seek to characterize one of the tools the cell might use to keep its complex membrane in order.

Project 2: Entrapment at the membrane

Cell membranes present many obstacles to unimpeded, random diffusion of lipids and proteins. Lateral diffusion barriers are paramount in diverse biological processes, ranging from organismal development to nutrient uptake. These physiological activities rely on forming distinct identities in an otherwise continuous membrane environment. For instance, dividing yeast must differentiate between a mother and a bud membrane and neurons must maintain axonal and somatodendritic plasma membrane domains necessary for neuronal polarity. It has been proposed that barriers in the membrane involving actin filaments restrict lateral trajectories of lipids and proteins, the so-called “picket fence” model. However, the exact nature of these barriers, and how they lead to membrane corrals, remains oblique. In this project, we will explore whether (i) adaptor proteins that link actin filaments to the membrane and (ii) transmembrane protein clusters are sufficient to confine diffusion of lipids and membrane proteins. We will use several techniques, including in vitro reconstitution of actin-membrane complexes and patterned substrates coupled to adherent cells, to probe the role of adaptor proteins in restricting lateral diffusion. By leveraging these techniques, we will investigate how kinetic parameters of adaptor protein binding impact the barrier function of the actin cytoskeleton and whether dense protein complexes can block the thermally-driven movement of membrane-bound components. Insights derived from these experiments will shed light on the cell’s ability to harness the cortical actin cytoskeleton to organize and sort the highly complex and heterogeneous plasma membrane.

Project 3: Structure and mechanics of actin networks

Actin filaments form a diverse range of distinct structures that share a common cytoplasm. Our current perspective views actin as a structural element that is assembled into higher order structures through its interaction with a vast set of actin binding proteins (ABPs). These ABPs have been shown to assemble, disassemble, remodel, polymerize, and crosslink filaments in a context dependent manner, such as the formation of the leading edge in migrating cells. Mutations leading to abnormal ABP function are abundant in the literature and often associated with pathologies such as Muscular Dystrophy, X-linked Neutropenia, and cancer. Intriguingly many of these pathologies present with symptoms associated with cellular mechanical defects, suggesting a potential link between ABP activity, actin network structure, cell and tissue mechanics. In this project we will examine the role of differential ABP activation in the formation of actin networks, including their structural organization and mechanical properties. Using a top-down approach, we will investigate these characteristics at the cellular level and compare to reconstituted actin networks under similar mechanical conditions. We will utilize methods from soft-lithography, such as microcontact printing and flexible substrates, to expose cells and reconstituted networks to mechanical stresses. These techniques will be combined with overexpression of fluorescently tagged ABPs, constitutively active ABPs and gene silencing to examine the role of ABP activation in setting actin network architecture and mechanical characteristics. The results obtained from these experiments will bridge a gap in our understanding of how the regulation of actin network architecture by ABPs determines network mechanical properties in normal physiology and how ABP mis-regulation leads to mechanically associated pathologies.

**Goldstein Group Projects: *C. elegans* cells, biochemistry on a coverslip, water bears**
TAs: Jenny Heppert, Dan Dickinson, Frank Smith, Thomas Boothby

Project 1: Manipulating cells by hand and inventing artificial eggshells for *C. elegans*

Have you ever wanted to move cells around by hand? Moving cells into contact by hand is a powerfully direct technique for addressing questions about the necessity and sufficiency of signaling between cells. We'll try two things. (1) We'll teach you how to remove envelopes from *C. elegans* embryos and manipulate individual cells by low-tech methods. We'll bring *C. elegans* strains with a variety of fluorescent protein tags for you to work with (think membranes, cytoskeleton proteins, signaling pathway components etc.) Using your own interests and creativity as a guide, we'll help you develop interesting questions that can be addressed by combining cells from the labeled strains. Our secret goal is that someday you'll make use of what you learn to apply these low-tech tools to other systems. (2) Our methods for manipulating *C. elegans* cells suffer from some uncertainty about how well the *in vitro* system mimics *in vivo* biology. We will try to put *C. elegans* embryonic cells back inside artificial eggshells that we'll attempt to build from PDMS. If embryos can continue normal development inside artificial eggshells, it could open a world of interesting questions that are currently unanswerable.

Project 2: Single-molecule biochemistry of cell polarity

Cell behavior results fundamentally from physical interactions between biological macromolecules. Biochemical experiments can be used to study these interactions, but traditional biochemistry discards spatial and temporal information by homogenizing large numbers of cells. We are working to obtain spatially and temporally resolved information about protein-protein interactions. We gain temporal resolution by using microfluidic devices to prepare lysates from single, staged *C. elegans* embryos. Protein-protein interactions in these lysates are then interrogated by single molecule pull-down (SiMPull; Jain et al. *Nature* 2011, 473: 484-88). We will use these tools to explore the interplay between cell adhesion and cell polarity. Embryonic cells often polarize along an axis defined by contacts with neighboring cells, but the molecular interactions that drive this behavior are for the most part unknown. We'll manipulate cell contacts both physically (manipulating cells by hand) and genetically (using RNAi), and use SiMPull and live imaging to study resulting interactions between adhesion and polarity complexes. These experiments should shed light on how cells integrate information from their neighbors to generate a reproducible axis of polarity.

Project 3: Attempting to make transgenic water bears

Water bears are microscopic, 8-legged animals, comprising thousands of species that make up an entire phylum (the Tardigrades). Water bears belong to a clade of molting animals (Ecdysozoa) that also includes both *C. elegans* and *Drosophila*. This position on the tree of life—near two excellent model systems—makes water bears attractive for investigating the evolution of many biological mechanisms. For this reason, we have adopted a water bear species, *Hypsibius dujardini*, as a new lab model. We currently have methods to disrupt gene functions (by RNAi) and to detect gene expression in this species. Developing gene transfection and genome editing techniques in *H. dujardini* would be valuable. In this project, you will design and perform experiments to try chemical and physical transfection techniques on water bears. Will you be the first person on earth to express GFP in a water bear? If that sounds too easy, you will be presented with an additional challenge of incorporating GFP into the genome of *H. dujardini* by CRISPR.

Project 4: Investigating how certain animals and cells can survive remarkable extremes

Water bears are probably the toughest animals on earth, with many species being able to survive boiling temperatures, being frozen, dried, irradiated, and even sent into the vacuum of outer space. But marine water bear species are reported to be killed by such harsh conditions. What do extremophile water bears make that non-extremophile water bears don't? We will hunt for, sequence,

and compare the transcriptomes of non-extremophile marine water bears with extremophile species. We have some ideas already about differences we might find, so we'll come with some water bear genes already cloned and ready to be transfected into mammalian cells. We hope to learn if specific water bear genes are sufficient for conferring any water bear superpowers to mammalian cells, and to learn more about the localization and function of extremophile gene products during different stresses. In our downtime we'll try imaging and establishing cultures from wild water bears as well as attempt to generate water bear cell lines.

**Marshall Group Projects: Cell Geometry and Behavior**
TAs: Hiro Ishikawa, Mark Slabodnick, Tatyana Makushok, and Sisi Chen

Cells are not just bags of enzymes, rather they are geometrically complex, self-organizing structures capable of a surprising level of complex behaviors. This summer we will explore the dynamic geometry and behavior of cells at three different levels: dynamic regulation of transport inside cilia, propagation of spatial information from mother to daughter cells during mitosis, and the mechanics of cytoplasm during cellular wound healing in giant cells.

Project 1: Complex dynamics of intracellular transport**.** Active transport is a key facet of cell physiology. The intraflagellar transport (IFT) system is a motile process occurring in cilia. IFT is mediated by a kinesin-2 which pulls an array of protein complexes known as IFT particles, which in turn are thought to capture cargo and bring the cargo protein to the tip of the cilium, where assembly occurs. Regulation of ciliary length is, at least in part, mediated by regulation of IFT, but the pathways the control IFT are not known. Our previous studies have found evidence that injection of IFT particles into the cilium shows avalanche-like behavior and that the rate of injection is regulated by ciliary length. This summer we will probe the dynamics of IFT in a novel way using photo-switchable and split GFP constructs, combined with new image analysis methods that we will develop during the course. This project will combine live-cell TIRF imaging, algal genetics, image analysis, and quantitative time-series analysis.

Project 2: Symmetry relation between sister cells during cell division. One of the big unanswered questions in cell biology is what mechanisms determine cell shape. In the late 1970's Albrecht-Buehler published a series of papers suggesting that sister cells tend to resemble mirror images of each other. This symmetry relation suggested that the process of cell division, and/or the propagation of spatial information from the mother cell, should play important roles in cell shape determination. However, Albrecht-Buehler's observations were based on subjective visual assessment of shape similarity and could not therefore be applied in a statistically rigorous way. Our goal is to investigate the symmetry relation between sister cells using quantitative metrics for cell shape. Based on past attempts to investigate this question. we hypothesize that mechanical interactions between the sister cells, as well as with the substrate, may influence the symmetry in shape by affecting force balance between sisters. We will test this idea by exploring how shape similarity and symmetry is affected by variation in substrate adhesion. This project will combine computational image analysis with fixed and live cell imaging.

Project 3: Flow and mechanics of cytoplasm during Stentor regeneration. The giant ciliate Stentor coeruleus is a classical model system for studying regeneration and morphogenesis of single cells. In a classical experiment done by Thomas Hunt Morgan and published in the MBL Biological Bulletin, a single cell can be cut in half, and each half will regenerate a normally proportioned cell. This dramatically illustrates the ability of cells to enforce defined scaling relations among the size of their components, but virtually nothing is known about how this works. Two physical questions arise when one considers this experiment. First, if scaling requires long-range communication between distant parts of the cell, how is this achieved for such large cell, which can be more than 1 millimeter long? Second, how is it even possible to perform surgery - why doesn't the cell contents simply bleed out when these large cuts are made? The answer to both questions will involve the rheological properties of the cytoplasm. If cytoplasm is relatively gel-like, this could explain why it does not leak out during surgery, but then it would make it more difficult for long range diffusion of macromolecules to play a role in coordinating growth and regeneration. Our own observations have suggested that cytoplasm undergoes considerable flow, and can even flow back into a cell after wounding. To explore this phenomenon, we will quantify cytoplasmic flow in intact and wounded cells and explore the molecular origins of the motile force that drives these flows.

**Session 3: July 20-Aug 1**

Ethan Garner, Nicole King, Jane Kondev/Rob Phillips, and Alison Sweeney

**Garner Group Projects**
TAs: Matt Paszek, Jessica Polka, Ye-Jin (Jenna) Eun

Project 1: Growth, shape, and internal organization of prokaryotic cells: probing in the Z- dimension *(Ethan Garner and Matt Paszek)*

Despite the long-held idea that prokaryotes are bags of enzymes, bacteria contain a variety of filamentous systems to define their cell shape. They then use other systems to arrange their cellular contents within these defined volumes.

Our group has found that, unlike eukaryotes, prokaryotic build their cells using local, short length scale rules: A) the filaments that define rod shape can sense and move around the curvature of the bacterium, and B) the filaments powering division pinch cell in two at one point. Both of these cell shape defining filaments are highly bent, suggesting that they interact with, and deform the membrane at a local scale. To probe how these filaments sense, interact with, and deform membranes will be using a novel form of microscopy developed by Matt Paszek, Scanning Angle Interference Microscopy (SAIM), which gives nanometer resolution in the Z-direction with fluorescence time lapse imaging. These studies will be combined with A) observations of interacting components and B) live perturbations to test if bacteria can sense and repair local points of damage to see how the filaments affect the local membranes during this process. We will also test if there are lipid domains in bacteria by combining SIAM with super resolution imaging and single molecule tracking.

Project 2: Bacterial acid-actuated pistons (Jessica Polka)

R bodies are giant, acid-actuated pistons in bacteria that function to disrupt eukaryotic membranes. Their extension is fully reversible and is ATP-independent. Their large size (500nm in diameter when compact, up to 20um long when extended) is achieved by polymerization of small proteins. I will bring purified R bodies (both wild type and mutants) as well as high-throughput assays for measuring their state (compact vs extended).

With these tools in hand, we will examine the force of R body extension (working with techniques developed with Dan Fletcher). We will also examine how the velocity of extension correlates with the size and width of these structures. The pH-driven extension of R bodies appears to be a highly cooperative process, and with mutants we will probe the mechanisms underlying the cooperatively of extension. Exploiting the natural function of R bodies as machines for delivering toxins to eukaryotes, we will attempt to use R bodies to deliver foreign cargoes to eukaryotic cells. This project also offers ample opportunity to collaborate on computational and physical models.

Project 3. Growth, Division, and shape of archaeal cells, hailing from the 3rd domain of Life *(Ye-Jin (Jenna) Eun)*

We will examine how archaeal cells can use packed arrays of proteins to define cell shape using the model archaeon *Halobacterium salinarum*. *H. salinarum* is an extreme halophile, favoring saturating levels of salt in its environment. Archaea do not have a rigid peptidoglycan cell wall. Yet *H. salinarum* cells can still form geometric shapes, such as triangles, rectangles, and other polygons, shapes reminiscent of inorganic materials using the S-layer, a packed array of proteins on the cell surface. We will explore how cells form these shapes by 1) quantitative observation, and speckle tracking of *H. salinarum* cells growing and dividing, 2) perturbations of cell shape by physical confinement and osmotic shifts. With this data, we will attempt to build a computational model of *H. salinarum* cell wall growth. The experiments will involve chemical labeling of cells, manipulation of cells using microfluidics and microchambers, and quantiative image analysis. We will also examine how *H. salinarum* cells divide, by imaging ftsZ, a tubulin homolog, tracking its it dynamics in respect to cell shape and proteins that regulate its localization. These experiments will

involve fluorescence polarization microscopy, structured illumination microscopy, high precision particle tracking, microfluidics, and fluorescence recovery after photobleaching.

**King Group Projects: Elucidating morphogenesis and host-microbe interactions in choanoflagellates, the closest living relatives of animals**
TAs: David Booth, Kayley Hake, Laura Wetzel, and Arielle Woznica

The evolution of cell adhesion and signaling mechanisms was essential for the origin of animal multicellularity. Indeed, modern animal development and physiology (including in humans) rests upon a regulatory foundation nucleated by ancient adhesion and signaling mechanisms. Moreover, because the first animals evolved in oceans teeming with bacteria, interactions with bacteria are likely to have exerted important influences on animal origins. Although animals first evolved over 650 million years ago, we can reconstruct imporant aspects of their biology through the study of choanoflagellates, the closest living relatives of animals.

Participants in this module will join a relatively small cadre of people with experience studying choanoflagellates in the laboratory. In addition, we will collect and study "wild" choanoflagellates and bacteria from diverse natural settings near the MBL.

Project 1: Investigate the cell biology of multicellular development in diverse choano species. The mechanisms by which choanoflagellates form colonies, establish cell polarity, and reproduce can provide crucial insights into the transition to animal multicellularity. Rosette formation has begun to be characterized in the model species *S. rosetta*. However, choanoflagellates are as diverse as animals and little is known about colony development in other species. We will investigate colony development in diverse choanoflagellates, including species isolated at the MBL, using fluorescent and live-microscopy techniques. Additionally, we will test whether environmental cues (particularly those from bacteria) influence multicellular life history stages in these species.

Project 2: How do choanos sense and response to environmental cues? Choanoflagellates are surrounded by chemical signals produced by environmental bacteria and other microbial eukaryotes. However, we understand very little regarding the mechanisms by which choanos navigate their environment to find friends, foes, and food. This project investigates how the model species *S. rosetta*, senses its environment through chemical, light, and mechanical cues. We will employ live imaging to investigate the responses of *S. rosetta* and other choanoflagellate species to diverse environmental stimuli, including pH, light, metabolites, bacterial prey, and bacterially produced small molecules.

Project 3: Genetic engineering of choanos**.** While genomes and transcriptomes from 21 species have been sequenced, and forward genetics has recently allowed us to link phenotype to genotype in choanofalgellates, unlocking the full potential of choanoflagellates will require the establishment of reverse genetics and genome editing. This project aims to establish methods for introducing exogenous genes into different choano species using electroporation, transfection, and other available technologies. Luminescence assays and fluorescence microscopy will report successful transformation and may further be developed for other projects.

Project 4: Phagocytosis of bacteria by choanos – influence of fluid flow, size selectivity, and food vacuole dynamics**.** The choanoflagellate *S. rosetta* captures bacteria using an apical flagellum surrounded by a feeding collar composed of actin-filled microvilli. Flow produced by the apical flagellum drives prey bacteria to the feeding collar for phagocytosis. In this project we will first investigate what in general triggers phagocytosis. Different bacteria expressing fluorescent markers and different sized fluorescent labelled beads will be used to monitor ingestion and egestion of particles. In addition, we will investigate if bacteria and beads are transported to the base of the collar by motor-driven transport or by fluid flow? Finally, as phagocytized particles get transported to food vacuoles we will investigate what happens to these particles in food vacuoles and study the dynamics of food vacuoles to get a broader understanding of choanoflagellate vacuolar biology.

Project 5: Cell differentiation in response to bacterial and environmental cues**.** Choanoflagellates have a diverse and complex life history consisting of morphologically distinct cell types including multicellular forms, free swimmers, adherent cells, and gametes. We have found that bacterial signals regulate rosette development and gametogenesis; however, we know very little about how other differentiation events are regulated. In this project, we will use live imaging, fluorescent microscopy, microfluidics, and morphometrics to characterize the morphology and behavior of different cell types. In addition, we will collaborate with members of the Microbial Diversity class to test the potential influences of diverse environmental bacteria on cell differentiation processes throughout the *S. rosetta* life cycle.

**Kondev and Phillips Group Projects: Physical Biology X**

TAs: Lishibanya Mohapatra, Tal Einav

Mathematics can be used as a different kind of microscope to reveal aspects of living matter that are otherwise hidden from view. The projects we will do will have a common goal, to develop mathematical models of structures and processes within the cell, which can be tested experimentally. The key is to turn a mechanistic understanding of a biological process into math. That's the" Physical Biology" aspect of this rotation. The "X" part is an invitation to you to come to us with a biological problem that you've worked on in previous rotations, that you are working on at your home institution, or simply something you've heard a lecture on or read about, and we will help you translate it into mathematics. Think of it as a BYOB (bring your own biology) party where we will supply the physics and math! We will analyze the mathematical models we construct using paper-and-pencil theory and simple computer simulations with an eye toward making experimentally testable predictions.

We also have specific problems that you're welcome to explore, which we are amused by, in case the BYOB formula doesn't work for you. These include:

1. Size of cytoskeleton structures: Cytoskeleton structures in cells are used for a number of different functions, including intracellular transport and cell motility. One of the puzzles posed by cytoskeleton structures is how multiple structures that differ in size and shape coexist in the same cytoplasm and use the same set of building blocks (e.g., actin and tubulin proteins) from which they self-assemble. We will consider simple models of actin and microtubule polymerization that take into account the finite pool of monomers present in the cytoplasm as well as the presence of nucleator molecules that help initiate the formation of large cytoskeleton structures (e.g., yeast formins that nucleate actin cables). Using these models we will first consider different scenarios of size control based on the presence of a finite monomer pool. Then we will consider different kinds of feedback mechanisms mediated by proteins that bind to F-actin and microtubules (e.g., myosin and kinesin motors) that lead to size-dependent rates of assembly and disassembly. Using a chemical master equation we will attempt to map out a phase diagram of filamentous structures that can co-exist in the same cytoplasm.

2. Functional organization of chromosomes: The spatial organization of chromosomes in cells is not random and there are numerous experiments that suggest that this organization plays a role in determining the function of chromosomes. For example, when the DNA suffers a break it can be repaired by homologous recombination whereby the same sequence on a different chromosome (or somewhere else on the same chromosomes) is used as a template to repair the break. For this repair reaction to initiate the two DNA sequences have to come in close proximity, to within a few nanometers of each other. This raises the question of homology search (how do the two pieces of DNA find each other), and how the spatial organization of chromosomes affects it. To address this question we will use random-walk polymer models of chromosomes in budding yeast. We will focus on coming up with predictions for how the genomic location of the homologous sequences affects their recombination frequency. We will also consider competition experiments where two or more templates can be used to make the repair, and compute the probability of using the different templates.

Another example of the functional organization of chromosomes is the problem of how distant enhancers affect gene expression. Using simple polymer models of chromosomes we will investigate the effect of enhancer position with respect to the promoter on the amount of gene expression.

3. Allostery and biology's greatest model: In the 1960s when famous French biologist Jacques Monod discovered allostery, the idea that macromolecules in the cell have two different conformational states that can be regulated by signaling molecules, he referred to it as the "second secret of life". Using the tools of statistical mechanics, we will use the Monod-Wyman-Changeux model to predict the properties of important macromolecules of the cell such as ion channels, transcription factors and membrane receptors. This model formalizes the idea of allostery and shows how the binding of a regulatory ligand can alter the free energy balance between the active and inactive states of the molecule. The goal of this project is to explore the hidden unity of a variety of seemingly unrelated problems in biology and to make testable predictions.

**Sweeney Group Projects**
TAs: Asja Radja, James Townsend, and Analisa Hill

We study the evolution of novel soft materials. Evolutionary biology can now mechanistically address the evolution of genes and enzyme function, and also begin to understand large-scale patterns of morphological evolution. However, the mechanistic understanding of how new materials and pathways to self-assembly have emerged over evolutionary time is relatively rare. We're a biophysics group located in a top soft matter physics department, and our business is to find new and creative ways to fuse these two perspectives. In the process, we gain insight into unprecedentedly rich mechanisms for assembling soft materials, and can also address the appearance of much of the "stuff" of life that isn't visible to canonical evolution methods. We'll be bringing soft-matter characterization methods such as dynamic and static light scattering, rheology, super-resolution microscopy together with genetic manipulation and genomic insight to understand how novel materials appear in life's history. We're also very excited by the opportunity to tap the rich organismal diversity available at MBL for new ideas, and eager to support any student-generated projects on local creatures.

Project 1: Self-assembly of gradient index optics in squid

Squid lenses generate a highly sensitive eye with acute vision by making a gradient index sphere. This gradient index is generated by S-crystallin proteins, which manage the feat of assembling a stable, transparent gel at all packing fractions. We have recently explained this capability using "patchy colloid" theory. In this project we continue to probe the material evolution of the squid lens by using static and dynamic light scattering techniques, and microrheology, to better understand the equilibrium state of these gels and how they are built. We'd also like to understand the growth of the lens. Larval squid are "born" with ~100 μm lenses that must grow to a size of ~1 cm in the adult, a million-fold volume change, but maintain vision adequate for hunting the entire time. Does the gradient grow throughout the life span of the animal, and if so, how? Or, does the larval squid simple have a single-index lens and poor vision, with layers of continuously lower density added throughout life? Our soft matter toolbox will also be brought to bear on this question.

Project 2: Mechanical origins of micro-architectures in pollen

Flowering plant pollen grains are famous for their gorgeously intricate, exquisitely controlled microarchitectures in their external protective "exine"- a single plant makes billions of near-identical pollen cells in its lifetime, yet these architectures are conserved and identifiable over geological time. Until recently, many proposed mechanisms of exine pattern formation were purely biochemical. We propose a novel view in which bottom-up mechanical processes akin to thermodynamic phase transitions may cause the final, decorated exine structure. Pollen development is relatively well-studied, but few papers are comparable between species or pollen geometry classes, so it is difficult from the current view to ask questions about mechanical origins of microstructure. To test our hypothesis, we plan to study the correlation of cytoplasmic and cytoskeletal factors to final exine structure in *Passiflora incarnata* using various microscopic techniques. We also plan to physically manipulate the pollen with mechanical force and observe the development of pollen microstructures.

Project 3: The slimes that bind us: Ctenophore gels and early animal bodies

Recent analyses of basal metazoan genomes support a new picture of early animal evolution, one in which ctenophores, a type of non-stinging jellyfish, a very-early branching group of animals. These animals now have well-characterized genomes, their most obvious macroscopic feature, their transparent, gelatinous body, isn't readily understandable from this perspective, and soft matter physics may have something to say. Ctenophore bodies are composed mostly of a voluminous hydrogel, the enigmatic tissue that puts the "jelly" in these jellyfish. Does this tissue represent an ancient trait shared by the earliest animal ancestors, or is it merely a readily evolvable physical adaptation to the problem of creating a body in seawater? Is the ability to build a hydrogel body a way

to increase the length scales available to evolution to exploit, while not requiring much additional information about tissue organization or complexity? To assess this question, a more detailed knowledge of the structural properties and chemical makeup mesoglea will be required. We will obtain ctenophores (*M. leidyi*) from local waters and investigate the viscoelastic and biochemical properties of their mesoglea. As a very sparse hydrogel of structural proteins and sugars, it is difficult to study by traditional biochemical means, but amenable to techniques drawn from condensed matter physics and materials science. In this project, we will combine microrheology, Raman spectroscopy, and visible light scattering with the known natural history and biology of ctenophores to probe the structure of mesogleal gels in adult ctenophores, as well as the developmental progression of this structure in developing ctenophore embryos that we will spawn on site. We will also simulate various environmental conditions (altered salinity, contaminants, etc.) to investigate how ctenophore mesoglea reacts to the ever-changing world of coastal waters and infer new insights about the chemical properties of the tissue from these environmental responses.