Membranes, motors, and molecular modeling

In 1953 Stanley Miller mixed methane, ammonia, and hydrogen in glass tubes. A flask with heated water and one with electrodes connected the tubes, and when a spark of electricity bolted across the electrodes, the apparatus produced amino acids, the building blocks of all proteins. When I first heard about this experiment in high school, I was attracted to its simplicity and elegance. Now, I recognize that it’s explanatory power originates in the novelty of looking at a seemingly impenetrable question—the origin of life—from a new perspective. I am looking for a research environment where disciplines are combined to tackle problems on the boundary of biological and medical science.

My research interests center on uncovering the physical principles that underlie biological and chemical phenomena. I try to create more accurate molecular simulations by improving physics-based force fields and combining new simulation procedures with nonequilibrium analytical tools. I will extend my previous work on biological membranes to elucidate driving forces behind the formation of membrane domains featuring phosphoinositides. I will also run simulations of synthetic molecular motors, such as those designed by Ben Feringa, to characterize their mechanical properties. Finally, I will participate in a systematic overhaul of force field science, changing the way force fields are built and how they are distributed, by focusing on transparent and rigorous optimization criteria, in an open setting. These investigations will provide a detailed accounting of the physical principles that are at work in biological systems. My undergraduate degree in physics combined with my graduate work in biochemistry and biophysics, together with my postdoctoral training in computational chemistry, makes me well suited to this research plan.

# Summary of previous research

## Improving free energy calculations

Accurate binding thermodynamics calculations remain one of the central goals of biomolecular simulations. While in the Gilson group, I contributed to an overall evaluation of more than ten different methods, from research groups around the world, used to compute host-guest binding free energies and enthalpies (Yin, Henriksen, Slochower, Shirts, et al. [2016](#ref-BGsUYQln)). As part of that work, I extended our own method (attach-pull-release (Yin, Henriksen, Slochower, and Gilson [2016](#ref-uzHaEv9Z))) by completely rewriting the code in Python and adding several advanced features enabling larger and more challenging systems to be used (this code is available on [GitHub](https://www.github.com/slochower/pAPRika)). More recently, I have also worked on assessing the efficiency and reproducibility of how different algorithms converge toward free energy estimates (forthcoming publication).

## The evolution of molecular motors

Biological motors are enzymes that use chemical energy to drive directional motion. Although motor proteins must have arisen through random mutation and natural selection, it is not clear how the evolutionary leap from non-motor enzymes to molecular motors could have occurred. I showed that any chiral molecule driven out of equilibrium should undergo cycles of conformational change (Slochower and Gilson [2018](#ref-1BfYw0gk2)). This work was highlighted by [UCSD](http://ucsdhealthsciences.tumblr.com/post/173707350285/its-not-intelligent-design-so-how-did) and [“New and Notable”](https://www.cell.com/biophysj/fulltext/S0006-3495(18)30444-2) in Biophysical Journal.

## The design of new force fields

As part of the open force field consortium—a collaboration with Dr. David Mobley, Dr. John Chodera, Dr. Michael Shirts, Dr. Lee-Ping Wang, and Dr. Chris Bayly— I participated in the effort to build new molecular mechanics force fields based on direct chemical perception instead of indirect, atom-type based approaches (forthcoming publication). I have also contributed to the design, coding, and implementation of a new force field format—SMIRKS Native Open Force Field (SMIRNOFF)—based on a hierarchical structure that supports customized combining rules, partial bond orders, polarizability, and other advanced features. The tools and data are open source and available on [GitHub](https://github.com/openforcefield).

## Simulations of complex membranes

Continuing the work that I started during graduate school at the University of Pennsylvania (Slochower et al. [2013](#ref-1G0A01ZNq), [2014](#ref-SdO7fVnR), [2015](#ref-1E1rz4J4o), **???**; Wang, Slochower, and Janmey [2014](#ref-Ag1rh6TA)), I’m interested in simulating increasingly complex, and thus more physiologically relevant, models of biological membranes. We recently reported (forthcoming publication) how the interplay between lipid species, cholesterol, counterions, and water near the membrane interface can lead to the formation of nano-scale lipid clusters. A natural next step is to understand the role proteins play in stabilizing these clusters, as discussed below.

# Detailed research plan

## How are highly enriched clusters of negatively charged phospholipids stabilized?

Phosphoinositides are a minor class of lipids located on the inner leaflet of the plasma membrane, found at about 0.5 mole percent of all phospholipids in mammalian cells (10-100 μM), that have an outsized biological role. This class of lipids participates in myriad cell processes including attachment of the cytoskeleton, membrane fusion, vesiculation, and both activation and inhibition of enzymes (Di Paolo and De Camilli [2006](#ref-GGlssBvj)). Disruption in signaling pathways associated with the phosphoinositides have been implicated in several cancers as well as Charcot-Marie-Tooth disease along with other neurodegenerative disorders (Faiderbe, Chagnaud, and Geffard [1992](#ref-8Xw2kuUO); Lin et al. [1999](#ref-12CAxA8dE); Katso et al. [2001](#ref-l2gqdgv); Engelman [2007](#ref-izLqFTEH); Miled et al. [2007](#ref-1HRoQaadQ); Bunney and Katan [2010](#ref-1DCzqvykg)). Among all phospholipids, PtdIns(4,5)*P*2 may be the most common regulatory lipid: hundreds of cytosolic proteins have been found to bind PtdIns(4,5)*P*2 in vitro (Lemmon [2008](#ref-uyKE7bWV)) including proteins that cause or sense membrane curvature (McLaughlin et al. [2002](#ref-3EmJ4esY); Bradley and Radhakrishnan [2017](#ref-5PUA7pLD)).

PtdIns(4,5)*P*2 is one of the most highly charged molecules in the cell, carrying a net charge from -3 to -5 at physiologically pH, owing to a phosphodinoester and two phosphomonoester groups (Kooijman et al. [2009](#ref-8pFCG7HG)). Despite their high charge, through the use of super-resolution microscopy, there is mounting evidence for nano-scale clusters of PtdIns(4,5)*P*2 within cells. In neuronal cells, clusters of PtdIns(4,5)*P*2 have been visualized using fluorescent phosphoinositide-binding domains and phosphoinositide-specific antibodies (van den Bogaart et al. [2011](#ref-U2YHSNKE).; Honigmann et al. [2013](#ref-Gw4f4Ayu)). These clusters are circular, roughly 70–90 nm in diameter, and composed of greater than 80% PtdIns(4,5)*P*2. A separate study found clusters of around 60 nm for PtdIns(4,5)*P*2, and larger clusters for PtdIns(3,4,5)*P*3, in the plasma membrane (J. Wang and Richards [2012](#ref-rvpDeSHJ)). Even in the absence of proteins, *in vitro* assays in the Janmey group have demonstrated PtdIns(4,5)*P*2 clusters of 40–50 nm, by AFM and TEM imaging, after the addition of certain divalent cations (Wang et al. [2012](#ref-LhOwGz4k)).

### How do divalent cations grow and stabilize clusters?

The finite and well-defined size of PtdIns(4,5)*P*2 clusters suggests there is a balance between the mutual electrostatic and steric repulsion of the negatively charged lipids and the attraction mediated by Ca2+. A key piece of evidence is the observation that a single Ca2+ ion is able to bring together groupings of up to three lipids and lead to changes in lipid packing (as detailed in a publication currently under review). The ability to nucleate clusters

This ability is found with other divalent ions, such as Mg2+, or the monovalent ions Na+ and K+ and agrees with experiment. Building upon an automated toolchain for constructing heterogeneous membrane compositions for molecular simulations (“BioPhysCode,” [n.d.](#ref-14RTQvTQS); “BioPhysCode Portal” [2018](#ref-aHkuGDrS)), I will construct and run simulations of PtdIns(4,5)*P*2 clusters. That the clusters are round suggests the domains are fluid, rather than gel or crystalline. As in my previous work, I will test several variables independently by varying the counterion species, counterion concentration, and presence or absence of proteins.

### What are the physical properties of the clusters?

### How does the ability of PtdIns(4,5)*P*2 to bind proteins change when it is present in a cluster?

I am going to test the hypothesis that PtdIns(4,5)*P*2,

## Determine the mechanical and physical characteristics of synthetic molecular motors.

### Background

* Synthetic motors are a triumph.
* Molecular motors have been synthesized, but the design principles are not clear. (Sometimes they are, but not always.)
* These motors operate out of equilibrium.
* Consider introducing information ratchets.
* Standard statistical techniques are unsuitable for this type of analysis.

### Details

* What is the torque of molecular motors?
* What is the theoretical maximum speed?
* What determines the speed? The efficiency?
* Can we design better molecular motors?
* Can we suggest synthetic changes to make better molecular motors?
* Can we understand something about the *mesoscopic* properties of synthetic molecular motors? (Thinking about Feringa’s liquid crystal alignment paper.)
* In what ways are synthetic molecular motors like biological motors: processivity, directionality, energy requirements.

## Use host-guest binding thermodynamics to drive automated force field optimization.

### Background

## Determine the mechanism by which highly enriched clusters of negatively charged phospholipids are stabilized.

### Background

* Current simulations do not capture the richness of biological membrane.
* It may not be necessary to capture it all, but we need something more than we have.
* We know that relatively rare phospholipids play an important role in signaling.
* Evidence that PPI deficiency causes problems and *in vivo* clusters are enriched.

### Details

* Make better models, parameterizing new molecules where necessary, for both lipid and non-lipid constituents, to replicate the rich milieu of the plasma membrane.
* Simulations with proteins. Does actin stabilize clusters? Does curvature stabilize or destabilize clusters? Look at Markus’ papers and Tobias’ papers.

### Funding

* PPI diseases, nano-bio, Mark…

## Motors

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## Force fields

### Background

* Existing force fields have been created in a haphazard way.
* Parameterized against liquid state data that does not accurately reflect the kind of intermolecular interactions at play for receptor and ligand binding.
* Add figure showing APR calculation.

### Details

* SMIRNOFF makes it easy to change parameters, although this method could be done with an existing force field (such as GAFF, GAFF2, or even something coarse-grained, potentially).
* Run benchmark set with force balance
* Sensitivity analysis could be used to determine which parameters ought to be changed
* Incorporate more advanced host molecules to test specific functional groups

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