1 Activities Planned Under This Fellowship

% of Time Spent on Each Aim			
Year	*Xenopus Oocyte Lipid Concentration*	*Source of Partitioning*	*Elasticity Package*
1	60	20	20
2	40	35	25
3	10	45	45
4	0	10	90

I have completed my required courses already for the CCIB PhD; from now on I need to only take dissertation credits. I am being funded through a teaching assistantship but if awarded this fellowship, I would put the entirety of my time toward the projects in this proposal. My research would continue to be carried out at the CCIB on the Rutgers-Camden Campus. CCIB requires one local seminar and two poster presentations per year to show progress in the program. I will also give at least one yearly external presentation at the Biophysical Society Annual Meeting.

2 Master's Research and Transition into Doctoral Research

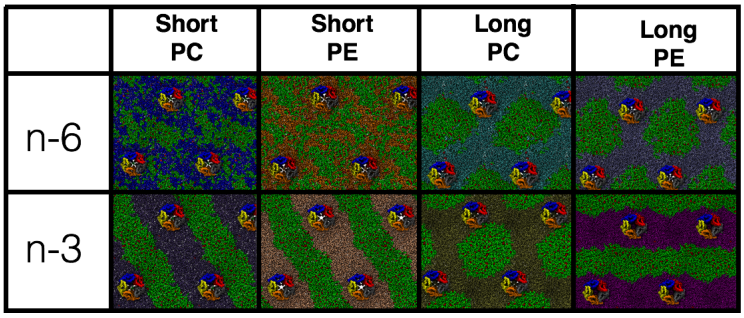

Figure 1: Domain Partitioning: Imaged above are repeated periodic images of 23x23 nm^2 systems. Green represents the saturated lipid DPPC, red represents cholesterol. The third color in all images represent a PUFA of various saturation and length. Short $n-6$ are di-16:2 and long $n-6$ are di-22:5. Short $n-3$ are di-16:3, and long $n-6$ are di-22:6. Short chained n-6 PUFA are not observed to fully de-mix. From these ternary mixtures, we can easily observe PUFAs as the dominant boundary lipids around nAChR.

I started my graduate career in Dr. Brannigan's laboratory working on my Master of Science in Computational and Integrative Biology. My primary goal was to use coarse-grained molecular dynamics simulations to determine how the concentration of cholesterol around nAChR depends on the overall amount of cholesterol in the bulk membrane.

Dr. Brannigan's research had previously investigated interactions of cholesterol with nAChR and other receptors in its family via atomistic simulations [Brannigan et al, 2008 pnas, Hénin et al, 2014 bpj]. In atomistic simulations, lipids cannot diffuse more than a few lipid widths from the protein during the simulation, so the local lipid concentration needs to be realistic from the initial simulation frame. The concentration of cholesterol around the nAChR was hypothesized to be higher than the bulk membrane, but the actual local concentration was also unknown in experiments, making it difficult to draw direct comparisons.

To overcome this limitation, I carried out coarse-grained MD simulations using the MARTINI Force Field (version 2.2) on the nicotinic acetylcholine receptor (nACHR) embedded in mixed membranes, which allowed us to test for a difference between local concentration and bulk concentration.

Membrane composition was chosen based on the most abundant lipids in the native membrane of the nAChR from the *Torpedo* electric ray [Unwin et al. 2005], which happened to include an abundance of lipids with $n-3$ polyunsaturated fatty acids (PUFA) chains. Previously, it was hypothesized, based on evidence that cholesterol binds directly with nAChR, that

the local cholesterol concentration would be high.

To our surprise, lipids with long-tailed $n-3$ PUFA chains (rather than cholesterol) showed the highest affinity for annular sites on the nAChR. Moreover, nAChR had a preference for cholesterol poor, liquid-disordered domains (we did see cholesterol bind directly to deeper, non-annular nAChR sites, however), see Figure 1. I tested this result in many different membranes and found that nAChR had a consistent preference for the liquid disordered domain and for n-3 PUFA acyl chains, regardless of headgroup.

My proposed research builds off our initial findings. We are going to move away from ternary mixtures to significantly more realistic compositions allowing us to better assess native boundary lipids and to have a glimpse of which domain nAChR partitions into. This provides us an opportunity to characterize and understand what causes nAChR (and potentially other pentameric ligand gated ion channels) to partition; is it caused by direct lipid interaction, or an indirect elastic effect from the bulk membrane?

3 Training and Goals

For me this means I am capable of contributing tools to computational chemists and biophysicists. I should be able to understand the different components of membrane theory when applied to both biology and soft-mater material science. Most importantly, I should able to discuss not only my results, but also my research plans, and how my work has evolved and changed based on my results.

First, I need to enhance my understanding of membrane physics that was theorized by Helfrich and expanded over the past 45 years. These theories will be the foundation of our proposed work allowing us to better characterize and measure nAChR partitioning, and its boundary lipids.

Secondly, I have a foundation developed for constructing analysis programs, but I do not have the skill to build an integrated package for VMD. I need to learn how to of combine the strengths of multiple scripting languages to build intuitive applications for myself and others to use. I also must improve my programming etiquette, allowing other researchers to use and or customize programs with ease to fit their own needs. Time spent learning and trouble shooting the fundamentals above will improve my scientific reasoning. Honing this reasoning is paramount to becoming an independent researcher, and being able to trouble shoot issues that arise.

Finally, whether accepted or rejected, applying to this fellowship begins building, to me, the most important skill, scientific communication. Under this fellowship, it my goal to not only publish the work I plan to do, but to present it to the scientific community. I must learn to write both theory and evidence succinctly; this will not only allow me to publish and make a name for myself, but also obtain funding to expand my research. Presenting data I must learn be able to clearly explain and sell our tools.

After finishing my graduate education I aim to work in a university setting as a tenured faculty member, where I start a computational laboratory for graduate level biophysics, computational chemist and theoretical material scientists (geared towards biomedical). Ideally I want to provide a setting where incoming scientists can foster an interest in studying computational science applied biomedical topics (such as lipid based disease, neurological signaling). The hope is working with experimentalists working in neurology and pharmaceuticals, to improve our computational models.

I believe, with the help of this fellowship, that the research we have proposed will supply me with the opportunities to accumulate the skills I need to make this lab a reality. The paramount skills I need are a more complete understanding of elastic membrane theory, building both standalone applications and plugins, predicting data trends to independently build hypotheses and experiments, and scientific communication.