Graduate Research Plan

Molecular Dynamics of Dynamin Oligomers

***Motivation*:** Endocytosis is a cellular process used to internalize substances from outside of a cell by engulfing the substance with the cell membrane, and then bringing it inside the cell in a vesicle.

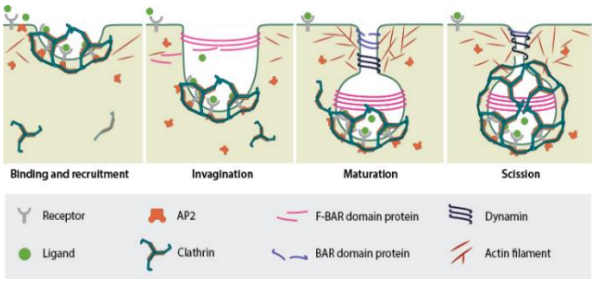

***Figure 1**. A summary of endocytosis. Here you can see the dynamin protein wrap around the neck of the vesicle, cutting the cell membrane in order to release the vesicle into the interior of the cell.*[5]

In the last step of endocytosis, the protein dynamin is recruited to the neck of the vesicle in order to cut the cell membrane and release the vesicle into the interior of the cell.[1] Dynamin, which is an enzyme, causes membrane scission by undergoing a large change in conformation and catalyzing the hydrolysis of guanine triphosphate (GTP).[2] Previous experimental work has tried to understand the mechanism for dynamin induced membrane scission, but there is no agreed upon mechanism reached as of now. Although fully atomistic, all-atom (AA) MD approaches can give an insight into the conformational changes that dynamin undergoes during membrane fission, this method can be limited to shorter length timescales and may not be able to consider the large-scale conformational changes that dynamin undergoes during the enzymatic hydrolysis of GTP.[3] **The problem I am trying to solve is to find a way to combine smaller scale all-atom (AA) MD simulations with larger scale, low resolution coarse-grained (CG) MD simulations, in which many atoms are grouped into single CG sites.**

Preliminary Work: I took various steps to understand how dynamin proteins oligomerize and move in solution. First, I modelled all the flexible loops that were missing from the available crystal structures for the human dynamin 1 isomer. I modeled loops for both the monomer and dimer to connect the GTPAse and Pleckstrin homology (PH) domains to the main stalk domain. These flexible loops act as hinges between the various protein domains and control how the domains move with respect to each other. Additionally, these loops are likely to be important for forming contacts that stabilize the protein oligomers in solution. To model the loops, I applied several methods. In my first attempt, I modelled the missing loops in the monomer and then used the monomer to form the dimer. Once the dimer was formed, I attempted to minimize and equilibrate the whole structure; however, close contacts between the modelled loops led to large steric clashes. This method also led to structures where I observed tangling of the loops. To solve this, I instead used the crystal structure of the monomer to form a dimer and modelled the loops on the whole dimer. With the loops modelled, I relaxed them in the gas phase before equilibrating the whole structure.

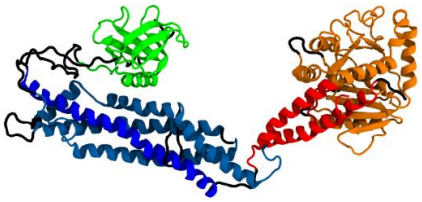

***Figure 2**. The crystal structure of the dynamin 1 monomer, with the loops filled in (black). These flexible loops were missing from the crystal structures.*

To study the conformational changes that dynamin undergoes during membrane fission, protein monomers and dimers were equilibrated in solution using molecular dynamics. After equilibrating different forms of the dimer, I was able to observe structures of dynamin that suggest important new inter-domain interactions. I observed some interaction between the PH/stalk domains and the GTPase domain, which have never been reported in the literature. This new interaction is especially exciting because it suggests that this protein may be able to auto-inhibit itself when in

solution and release its domains upon contact with the membrane. The interactions holding the domains together involve mostly electrostatic interactions (salt bridges and hydrogen bonds) which are likely to be affected by interactions with membranes that include a negative electrostatic potential.

Aim 1: ***Create New Simulations with Restraints on the Protein*****:** I will set up the dynamin monomer, dimer, and tetramer protein systems in order to catalyze their catalytic behaviors. When the helical coat forms, the protein arranges itself in a tetramer position, which is a dimer of dimers, and so it would be useful to observe all forms of the protein. Then, I will run simulations of the proteins to relax and equilibrate the systems. I will analyze these simulations by looking at trajectories of the proteins and plot distributions of the reaction coordinates to determine if there are any interesting conformation changes. Then, I will add restraints to the protein to highlight any interesting conformational changes that we have found in the trajectories.

Aim 2: ***Run Enhanced Sampling Simulations*****:** Afterwards, I will use the previous simulations as starting points to run enhanced sampling simulations, which involves changing certain collective-variables of the protein coordinates, in order to analyze the free energy changes that occur as the conformation of the protein changes.

Aim 3: ***Develop the Initial CG (coarse-grained) model*****:** Finally, I will develop a CG (coarse-grained) model of my protein by utilizing the information about the protein that I obtained from my initial tests; with the information I will have collected, I can decide which parts of the protein act like single collective units, and that will help me determine the CG sites. The interactions and forces of the protein will be determined from the AA-MD studies so that the CG model can reproduce the observed changes in free energy.

Intellectual Merit**:** The methods proposed within this work provide a deeper insight into the mechanisms behind endocytosis, something which has been sought for years via experimental techniques, and it was previously out-of-reach for standard simulations methods. **Previous experimental work has tried to understand the mechanism for dynamin induced membrane scission, but there is no agreed upon mechanism reached as of now.** Understanding how the various domains of dynamin move with respect to each other could help shed light on the mechanism of dynamin induced membrane fission, which would help us further understand how endocytosis works. Although fully atomistic, all-atom (AA) MD approaches can give an insight into the conformational changes that dynamin undergoes during membrane fission, this method can be limited to shorter length timescales and may not be able to consider the large-scale conformational changes that dynamin undergoes during the enzymatic hydrolysis of GTP.

Broader Impacts**:** By doing this, a multiscale model can be formed that considers large scale conformational changes and small-scale atomic level interactions. By using a multi-scale MD approach, we will have a way to study how small-scale molecular interactions lead to large changes in the cell.[4] Multi-scale modelling is a topic of computational chemistry that is continuously being studied and applied to other biological systems; understanding how to apply this to other systems can help further our understanding of the denaturing of proteins, such as alpha-synuclein, which degrades over time and can cause Parkinson's disease. Molecular Dynamics simulations allow us to study the molecular level interactions that occur in cellular systems, while being able to change parameters that can be difficult to control experimentally

References**:** [**1**]Mettlen, M.; Pucadyil, T.; Ramachandran, R.; Schmid, Sandra L. *Biochemical Society Transactions* 2009, *37* (5), 1022. [**2**] Daumke, O.; Praefcke, G. J. K. *Biopolymers* 2016, *105* (8), 580-593. [**3**] Kalia, R.; Talledge, N.; Frost, A. *Building a Cell from Its Component Parts*, Ross, J.; Marshall, W. F., Eds. 2015; Vol. 128, pp 165-200. [**4**] Low, H. H.; Loewe, J. *Current Opinion in Structural Biology* 2010, *20* (6), 791-798. [**5**] Pucadyil, T. J.; Schmid, S. L.*Cell 135* (7), 1263-1275.