Construction and Moecular Dynamics of a Nanodisc for Membrane Protein Simulation

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Abstract

Phospholipid nanodiscs are used to stabilize membrane proteins when the protein is removed from its native biological environment by providing interaction with a lipid bilayer, allowing the protein to be isolated without denaturing. An amphipathic polymer (Poly(styrene-co-maleic acid);SMA) has been used to encircle a discoidal lipid bilayer, keeping the bilayer from dispersing. Using small-angle neutron scattering (SANS), a structural mapping of the protein may be obtained from this stabilized structure. To confirm experimental results, computer modeling is commonly used to predict the SANS profile of proteins. If the experimentally determined neutron scattering profile matches the theoretical computer generated profile, experimental researchers are reassured their results are valid. Predictive modeling of SANS profiles for membrane proteins was not possible since no suitable nanodisc model had been created, leading to uncertainty in the structure of the membrane proteins being studied. Also, the interaction and orientation between the bilayer and the SMA polymer had not yet been precisely determined through experimental studies. In our research, we used computer modeling and molecular dynamics to construct and simulate a nanodisc. In the process of creating this nanodisc for membrane protein modeling, we simulated the behavior of the SMA polymer belt, providing insight into this interaction. The lipid used was 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) since this lipid was also used in research studies. Parameters for the SMA polymer were developed through CGENFF by analogy to known structures.

1. Introduction

Membrane proteins are vital components of biological systems. These proteins exist in a lipid bilayer membrane, such as cell walls. There are many different types of membrane proteins, each with a unique biological purpose. As proper cellular function has a dramatic impact on human health, any defects in membrane proteins can cause devastating diseases. The structure of proteins is closely linked to their function, so if the protein undergoes a structural change, it will likely have reduced efficiency or harmful biological effects. One example of

proteins with modified structure becoming harmful is prions. These are proteins that have misfolded to become toxic and cause disease. Prion diseases, such as mad cow disease, are generally fatal. Since we do not understand prion structure, we do not understand how or why prions form. Various diseases and disorders have been attributed to misfolded proteins. Although not all are as deadly as prions, investigation into misfolded membrane protein structure is necessary. Perhaps once the relation between the misfolded structure and the harmful function is understood, these diseases may be alleviated. However, prior to directly studying the misfolded structure, the proper structure must be determined as a baseline.

One method used to determine the structure of membrane proteins is small-angle neutron scattering (SANS). However, many membrane proteins, specifically transmembrane proteins, tend to denature when removed from their native cell membrane environment. Due to their non-polar nature, these proteins become inherently unstable when exposed to experimental conditions, such as a water solvent. Synthetic lipid bilayers, such as nanodiscs, have been successfully used to replicate the membrane protein's native environment. Nanodiscs are a lipid bilayer with the hydrophobic lipid tails of the bilayer encircled by a belt to protect them from the water. This belt is often composed of membrane scaffold protein (MSP).

Contrast variation is a powerful technique used in SANS which varies the concentration of D_2O in water solvent to match the scattering length density of specific parts of the sample. By matching the scattering length density of various parts of the sample, the unmatched portion may be clearly observed and studied. This technique requires the sample to have different scattering lengths densities at different parts. However, the traditional nanodisc design, wrapped in MSP, suffers from very similar scattering length densities between the MSP belt and the protein sample being studied. This raises difficulties in differentiating between the belt and the protein sample in MSP nanodiscs.

Our work has been with a modified nanodisc design in which the lipid bilayer is wrapped by an polymer (Poly(styrene-co-maleic acid);SMA) composed of styrene and maleic acid residues instead of amino acid residues. The composition of the polymer has been experimentally determined to be 35% SSS, 54% MSS/SSM, 11% MSM, and 0% MM.

This dramatically changes the scattering length density of the polymer belt, allowing the protein to be clearly studied. The SMA nanodisc also opens a new area of study in which receptor proteins are bound to the nanodisc, allowing G-coupled proteins to interact with the receptors. This will be likely be difficult since the entire SMA nanodisc needs to be matched out so the G-coupled protein may be studied, but this would not even be theoretically possible using an MSP nanodisc.

There is very little research on SMA nanodiscs. Experimental data has confirmed the SMA polymer wraps the lipid bilayer, but the number of polymer strands and the orientation is unknown.

As the behavior and characterization of these nanodiscs is extremely difficult to determine through experimental research, we created an all-atom nanodisc model to simulate SMA nanodiscs. This nanodisc model can be used in molecular dynamics simulations to predict and confirm experimental data when membrane proteins are bound to the SMA nanodisc.

2. Methods

2.1. *Polymer Parameterization*

We needed to create force field parameters for the SMA polymer. Molecular dynamics simulation programs use these parameters for each atom to properly predict the behavior of the system. We created five trimers models using Maestro, which was the shortest polymer chain we could simulate to study the central residue. We used CGENFF to generate our starting parameters. CGENFF is a program that calculates force field parameters based on analogy to known structures. Styrene was already in their database, so we had ideal parameters for the styrene residue. There was no maleic acid residue in the CGENFF database, but there was carboxylate, which is very similar. We created deprotonated maleic acid residues with a full negative charge shared by resonance between the oxygens for our polymer. By varying what the central residue in the trimer was bonded to, we studied any changes in the parameterization. CGENFF also provided a penalty score for assessing the similarities to know structures, which was well within an acceptable range for our structures. Our parameters will later be validated through quantum mechanical calculations, but these low penalty scores and close similarities to known structures give us confidence in our parameters.

2.2 *Infinite Bilayer Rod*

Prior to constructing our full nanodisc model, we needed to test our parameters and study the lipid to polymer interaction. Water solvent and ions (0.2 M NaCl) were added to an equilibrated DMPC patch in VMD to match experimental conditions. Excess water was removed from the bilayer and the periodic boundary conditions (PBC) were set such that the bilayer would be effectively infinite. The PBC allowed sufficient solvent in the x and z dimensions that the lipids on $+x/+z$ would have no interaction with the lipids on $-x/-z$. The y PBC dimension was precisely measured to allow the lipids on $+y$ to directly interact with the lipids on $-y$ as though they were adjacent (see figure 1). This method created an infinity long DMPC rod to replicate the interaction of the lipid bilayer with the SMA polymer, while avoiding the lengthy computation time required for simulation of larger systems. Due to this reduced computational load, we were able to run various MD simulations in a short period of time. SMA polymers of three different compositions were placed different distances and orientations from the lipid rod, providing insight into their interaction. These experiments are ongoing, so our results are merely preliminary. We studied a polymer composed entirely of styrene residues with no maleic acid (Poly SSS), a polymer of alternating styrene residues and maleic acid residues (Poly MSM), and a polymer of random composition such that it matched experimentally determined polymer ratios.

2.3 *Full Nanodisc Model*

The original DMPC bilayer patch was duplicated three times to create a 2x2 grid of bilayer. The gaps in this grid were closed through a series of constrained simulations to create an equilibrated and homogenous box, as seen in figure 2. These simulations are summarized in figure 3. A DMPC bilayer cylinder was trimmed using VMD from this equilibrated box to match the experimentally determined dimensions of the SMA nanodisc (radius 38 ± 2 Å).

2.4 *Wrapping of the Bilayer*

Dynamics were run (2 ns, vacuum) on the trimers used in the parameterization. Internal coordinates (ICs) were defined based on the random orientation of these timers at the last frame of the run. We wrote ICs for the linking patches connecting residues to define the first three atoms of the next residue based on the position of atoms in the previous residue. Our ICs for the residues themselves were defined such that they could construct the full residue based on only the first three atoms. We also defined ICs for our capping patches at the end of the polymer strands.

We wrote a script in Python to wrap the SMA polymer around the cylindrical DMPC bilayer. The atomic positions within each residue were defined through our ICs, but the script defined the positions of the overall residues. The dihedral angles from bonds between residues in the trimers were recorded at each time step during the dynamics run and used as a sampling base for the Python script. The Python script then chose suitable dihedrals from this range such that the polymer was closely wrapped to the bilayer. This script also checked for steric overlap and rotated the residues accordingly. We also were able to easily vary the composition of the polymer through the python script, enabling us to modify our nanodisc to experiment with different S/M ratios.

3. Results

3.1 *SMA Polymer Interaction with DMPC*

Our results in these experiments are only preliminary, so we are hesitant to conclude anything definitive at this time. However, we observed strong interaction between the purely styrene residue composed polymer and the non-polar tails in the bilayer. In a 0.2 ns NPT ensemble, this polymer rapidly moved its center of mass 4 Å due to the attractive force. The alternating residue polymer and the random composition polymer showed significantly less attraction to the bilayer than the purely styrene residue based polymer. Both of maleic acid containing polymers showed the charged oxygens on the carboxylate groups of the maleic acid orienting away from the lipid tails. This orientation appeared more clearly in the random

composition polymer than in the alternating composition polymer, but we do not yet have enough computational results to make a definitive conclusion.

4. Discussion and Ongoing Work

The behavior of the purely styrene residue composition polymer matches our expectations based on the non-polar nature of both styrene and the hydrocarbon lipid tails. The performance of this polymer supports our parameterization as the polymer acted as predicted. Although our maleic acid containing polymers did not show as much attraction as we had hoped, further computational experiments are required. The charged carboxylates oriented away from the lipid tails, but this effect could only be observed when the polymer was placed very close to the bilayer as a starting condition. Since maleic acid never bonds directly to maleic acid in the SMA polymers, the alternating residue polymer has the most polar composition possible. Due to this strong polarity, the alternating residue polymer is expected to have the weakest attractive interaction with the bilayer, perhaps even repulsive. The negatively charged DMPC head groups are also expected to repel the negatively charged carboxylate groups on the maleic acid residue, further weakening the attractive effect from the styrene residues. We know these nanodiscs self-assemble based on experimental results, so we predict our random composition polymer will have an attractive force to the bilayer in our computational experiments. Substantially longer simulations and more orientations of the polymer are required to observe this effect in our simulations.

Our parameterization through CGENFF needs to be refined through quantum mechanical calculations. Although the low penalty scores and close similarities to known structures in the CGENFF database support our parameters, quantum mechanics is required for proper validation.

We have constructed an SMA wrapped nanodisc, but calculations still need to be performed to ensure it is experimentally relevant. The surface area per lipid head group and the thickness of the bilayer have yet to be calculated and compared to experimental results. The flexibility of the DMPC bilayer in the nanodisc also has to be determined to confirm it is a valid replication of native cell membrane. If the flexibility of the nanodisc bilayer does not match, the membrane protein could change from its native conformation upon binding, rendering the structure determination of protein meaningless.

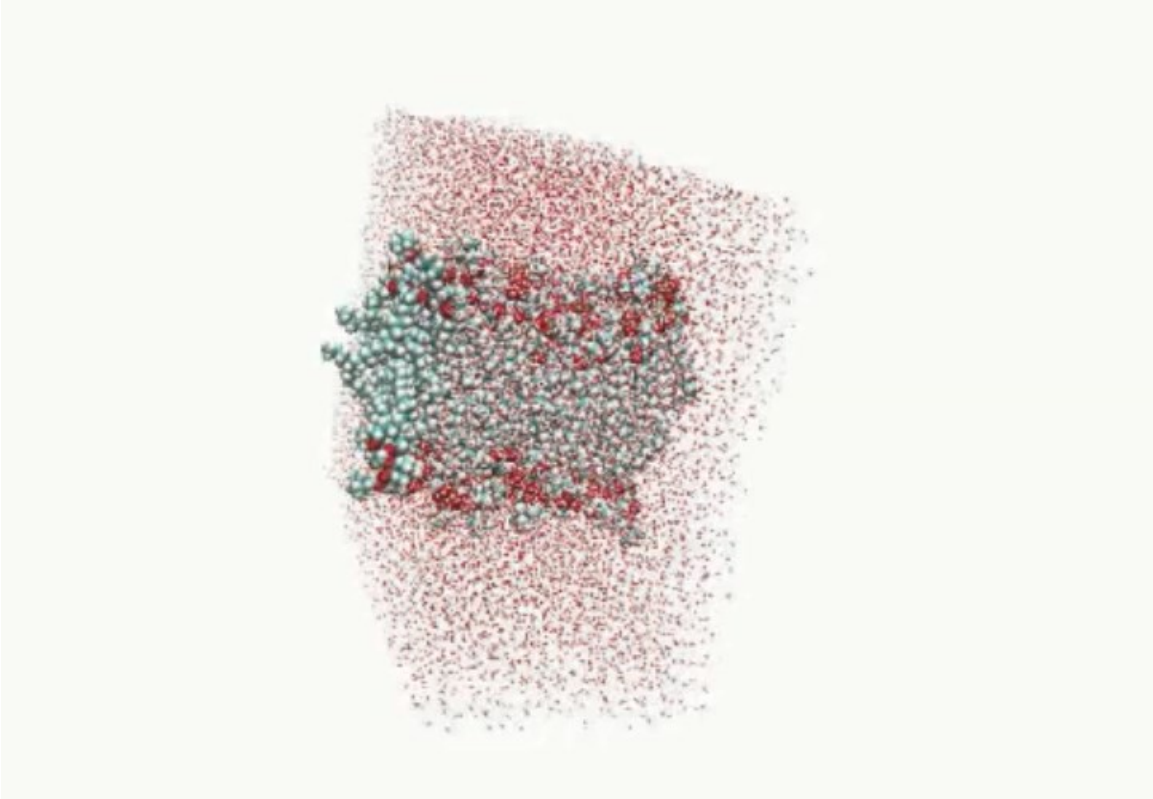

Figure 1. Figure 1 is an infinitely long DMPC bilayer rod in water solvent and 0.2000 M NaCl.

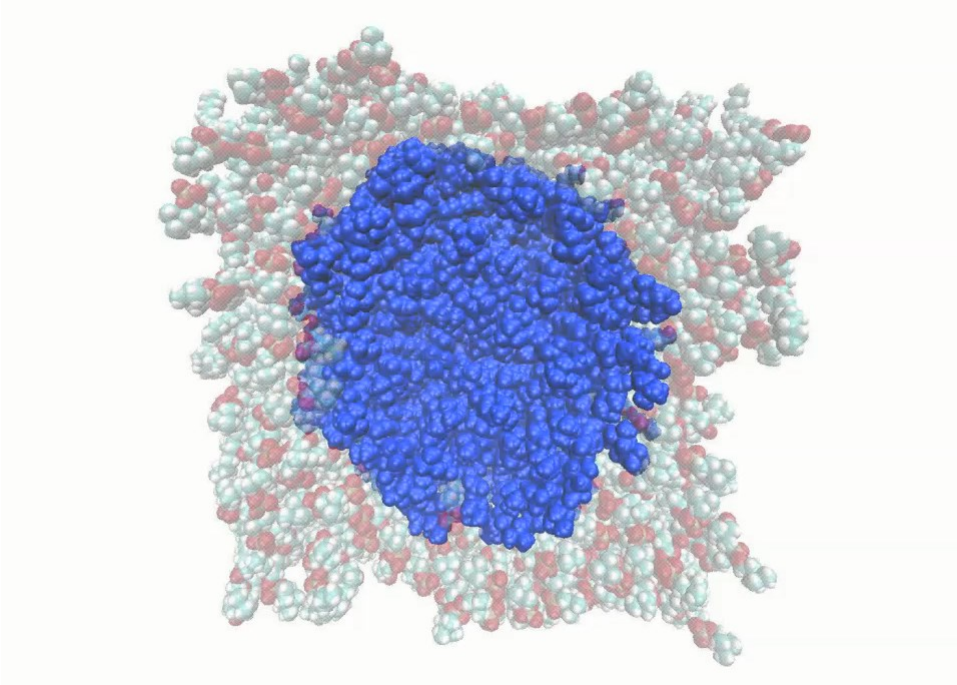

Figure 2. Figure 2 is an equilibrated DMPC bilayer with the nanodisc cylindrical trim highlighted in blue. The radius of this cylinder is 38 ± 2 Å.

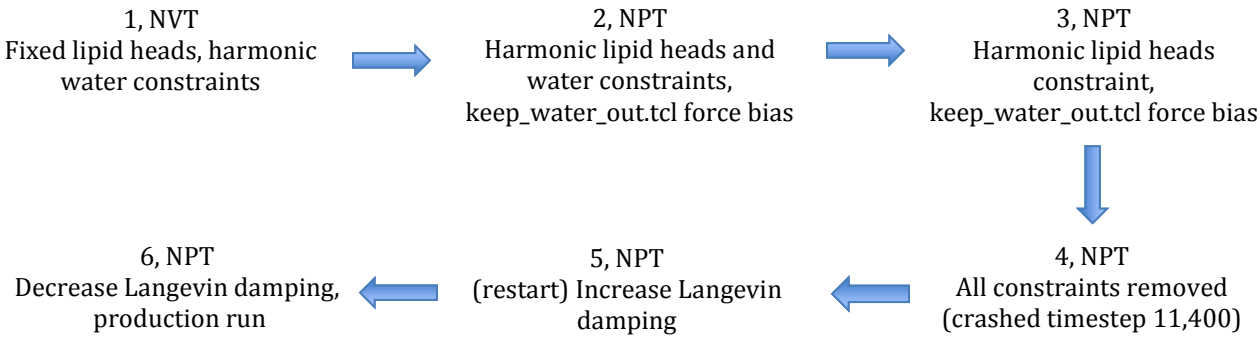

Figure 3. Figure 3 is a summary of the DMPC patch equilibration path.