

Computer simulations of lipid membranes and their interactions with proteins and nanoparticles

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Abstract

Membranes are multi-component lipid bilayers that are a crucial component of every cell. They act as a physical barrier protecting the cell, compartmentalize organelles, and control the movement of substances through them. Cells modulate local membrane properties by changing their lipid compositions and lateral organization. Lipids also localize and interact with proteins for many cellular processes like signaling, membrane remodeling, and trafficking.

Experimental techniques face inherent limitations in directly studying membranes at the nanoscale. Molecular dynamics (MD) simulations have emerged as a powerful computational tool that complements experimental studies by providing molecular-level insights into membrane behavior and interactions. MD simulations can elucidate how individual lipid molecules move, fluctuate, and interact with each other, and how proteins interact with lipid membranes at the atomic scale.

The research presented in this thesis leverages MD simulations to investigate several biologically relevant membrane systems. We use all-atom MD to investigate how the composition of membranes containing branched-tail lipids and cardiolipin affects properties of model bacterial membranes. Using all-atom MD, we identify how the dysferlin domain of Pex30 and the MreB extended N-terminal region interact with membranes of different compositions. We use coarse-grained MD to investigate the aggregation of curvature-inducing transmembrane proteins and of deformable nanoparticles anchored to the membrane with cholesterol.

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Chapter 1

Introduction

Membranes are a crucial component of every cell that act as physical barriers protecting the cell and compartmentalizing organelles [68, 38]. Cell membranes are composed of a bilayer of amphiphilic lipid molecules that have remarkable structural diversity [36]. The main classes of lipids that make up the membranes include glycerophospholipids, sphingolipids, and sterols. High-resolution mass spectrometry has revealed extensive chemical diversity among membrane lipids, not only between different membrane types, but also between opposing leaflets of the same membrane and even within the same leaflet [129, 10, 36, 75].

Glycerophospholipids make up the majority of lipids in cell membranes [134, 62]. They have a glycerol backbone with fatty acids at the sn-1 and sn-2 positions. The lipid headgroup consists of a phosphate group linked via an ester bond to various substituents that define the lipid's identity. Common headgroup substituents include choline, ethanolamine, and glycerol, which form phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG), respectively. Cardiolipin represents another abundant glycerophospholipid distinguished by its four acyl chains, synthesized through the condensation of two PG molecules by cardiolipin synthases [99, 116].

1.1 Effect of lipid composition on properties of cell membranes

Cells actively manipulate their lipid composition to regulate critical biological processes, including membrane protein insertion, signaling, endocytosis, and lipid droplet formation. This control over lipid composition is crucial as numerous genetic diseases like type 2 diabetes, cancer, Alzheimer disease, and cystic fibrosis are associated with mutations in specific lipid-related enzymes and lipid transporters that disrupt normal membrane composition [34, 35, 140, 115].

Lipids can organize into a liquid ordered phase (L_o) or a liquid disordered phase (L_d) at different temperatures [108]. The L_o phase is characterized by tight packing, increased membrane thickness, and restricted lateral diffusion compared to the L_d phase. The transition temperature between these phases is controlled by the chemical structure of the lipid. In cells, these phases can coexist as different lipid types organize to form nano or microdomains. In eukaryotes, interactions of sphingolipids, cholesterol, and saturated lipids form ordered nanodomains in the L_o phase called lipid rafts [36, 67, 87]. Lipid rafts are used to concentrate signaling proteins to enhance their efficiency [135].

The fatty acid composition serves as the primary determinant of membrane phase behavior and fluidity [108]. Cells actively regulate the fatty acid tails in the membranes under environmental stressors to maintain homeostasis [146, 99, 25]. The incorporation of unsaturated fatty acids is the most common mechanism for modulating membrane fluidity [85]. When a double bond is introduced into a fatty acid chain, it creates a 30° kink that disrupts orderly lipid packing. The location of the unsaturation in the fatty acid tail correlates with its disordering effect. Unsaturated bond closer to the head group have a higher disordering effect [41]. Polyunsaturated lipids, containing multiple double bonds, generate numerous kinks throughout the acyl tail, resulting in significantly increased membrane disorder [101].

Bacteria have evolved diverse strategies to modify fatty acid composition for membrane homeostasis [127, 146]. In some bacteria, unsaturated lipids can be converted to cyclic fatty acids through methylation of the double bond, forming cyclopropane rings in the lipid tails

[137]. These cyclic fatty acids retain the characteristic 30° kink but produce a smaller disordering effect compared to their unsaturated lipids. Increase in membrane thickness is seen when unsaturated lipids are converted to cyclic fatty acid lipids [15]. This modification is observed in *E. coli* during stationary growth phase [137].

Many bacteria lack the machinery required to synthesize unsaturated lipids and instead modulate the abundance of branched-tail lipids to fluidize membranes [22, 146, 28]. This adaptation is commonly seen in gram-positive bacteria, such as *B. subtilis*, *S. aureus*, and *B. cereus*, which produce branched-tail lipids to ensure membrane homeostasis when the environmental temperature is decreased [48]. Branched-chain lipids are also abundant in intracellular gram-negative bacteria with reduced genomes, such as *C. trachomatis* [142]. The predominant branched-chain fatty acids in these organisms contain iso- and anteiso-branched structures, featuring a single methyl group at either the second-to-last (iso) or third-to-last (anteiso) carbon position. Anteiso branching produces a greater disordering effect than iso branching because the methyl branch occurs higher up the acyl chain [86, 103, 66]. The effect of saturated and unsaturated lipids on membrane properties has been extensively studied. However, the effect of branched-tail lipids has received far less attention, and many open questions remain.

The relative size of the headgroup and the lipid tails affects the shape of lipids, which in turn influences the spontaneous curvature of membranes [25]. The shape of the lipid is characterized by a packing factor (P), which is the ratio of the lipid's volume to the product of the area occupied by its headgroup and the length of its fatty acid tails [71]. Lipids with packing factor equal to 1 are considered cylindrical lipids that preferentially localize to planar membrane regions. Lipids with packing factor less than or more than 1 are classified as conical or inverse conical lipids. These non-cylindrical lipids preferentially partition into regions of positive or negative membrane curvature and contribute to spontaneous membrane bending. The relationship between lipid shape and membrane curvature is particularly important during dynamic cellular processes that require extensive membrane remodelling like membrane fusion, fission, lipid droplet formation, and endocytosis [44, 148]. Lipids that can modulate spontaneous curvature facilitate these processes by reducing the energetic cost of membrane bending and stabilizing highly curved membrane regions.

In summary, lipid composition profoundly influences the fundamental properties of cell membranes, including fluidity, phase behavior, and curvature. These membrane properties, in turn, directly impact essential cellular functions. Significant gaps remain in our comprehensive understanding of how different lipid types quantitatively affect membrane properties. Quantitative understanding of how lipid composition affects membrane properties is crucial for understanding membrane biology and developing new therapeutic strategies.

1.2 Membranes regulate protein-mediated biological processes

Cell membranes mediate cellular functions by regulating protein-mediated biological processes. This is achieved either by direct interactions with lipids in the membranes or modulating membrane properties [36]. Many proteins have lipid binding domains that recruit them to regions of the membrane enriched in specific lipids. HIV glycoprotein gp41 has cholesterol-binding motifs which localize them to regions containing cholesterol [117]. Proteins with amphipathic helices insert into membranes regions with high packing disorder that exposes the hydrophobic interior of the membrane. Cardiolipin has been shown to bind to many amphipathic helices of fission-inducing proteins by electrostatic interactions[19, 102, 122]. Additionally, its inverse conical shape introduces packing disorder in the membrane, facilitating insertion. Lipid compositions that promote spontaneous curvature of the membrane can also influence the recruitment curvature sensing proteins to the membrane. Proteins containing BAR domains are recruited to regions of positive curvature [20].

The lipid composition in regions surrounding transmembrane proteins influences protein localization and oligomerization. It is favorable for transmembrane proteins to match their transmembrane hydrophobic regions to the hydrophobic thickness of the surrounding lipid bilayer, minimizing energetically unfavorable membrane deformation. When there is a mismatch, proteins either migrate to membrane regions with compatible thickness or

adjust their orientation through transmembrane domain tilting [50]. Hydrophobic mismatch-induced membrane deformation can also drive protein aggregation, as clustering multiple mismatched proteins can reduce the total membrane deformation compared to isolated proteins.

The interplay between direct lipid-protein interactions and membrane physical properties influences protein localization, aggregation, and functional organization within cellular membranes. Despite extensive research in the field, for many proteins the molecular basis of membrane-protein interactions and the dynamics of protein aggregation on membranes are not yet understood.

Experimental techniques face inherent limitations in directly studying properties of cell membranes and protein-membrane interactions at the nanoscale [75]. Molecular dynamics (MD) simulations have emerged as a powerful computational tool that complement experimental studies by providing molecular-level insights into membrane behavior and interactions [59, 43]. MD simulations solve Newton’s laws of motion to model the movement of atoms. MD simulations can elucidate how individual lipid molecules move, rotate, and interact with each other and proteins or nanoparticles at the atomic scale. Precise information of atomic positions of lipids can reveal local membrane properties. For example, fluctuations of splay angles of neighboring lipids from MD simulations can be used to estimate local membrane bending rigidity. MD simulations also allow for investigating how protein and nanoparticles interact with membranes.

MD simulations can be characterized as all-atom and coarse-grained simulations by the accuracy of atomic representation. All-atom simulations represent each atom explicitly and include precise representations of hydrogen bonding and electrostatic interactions [145]. Coarse-grained simulations reduce computational complexity by representing multiple groups of atoms as a single bead [78], allowing speed up in simulation time. However, coarse-grained simulations are less successful at representing hydrogen bonding, electrostatic interactions, and conformational entropy.

The first instances of MD simulations of lipids and surfactants comprised 76 surfactants simulated for 30 ps [24]. Exponential improvement in computational capabilities have enabled all-atom simulations of realistic membranes containing thousands of lipids and proteins

for microseconds [75]. Further, considerable developments in lipid and protein specific inter-atomic potentials have greatly improved accuracy in MD simulations. CHARMM36 is the most widely used all-atom force field and has been shown to accurately reproduce various membrane properties, including area per lipid, order parameters, and density profiles [145]. The MARTINI force field, the most popular force field used for coarse-grained lipid simulations, represents 4 heavy atoms and associated hydrogens as 1 bead, allowing for 10-100 fold speed up in simulation times [78].

The research presented in this thesis leverages MD simulations to investigate several biologically relevant membrane systems. We use all-atom MD to investigate how the composition of membranes containing branched-tail lipids and cardiolipin affects properties of model bacterial membranes. Using all-atom MD, we identify how the dysferlin domain of Pex30 and the MreB extended N-terminal region interact with membranes of different compositions. We use coarse-grained MD to investigate the aggregation of curvature-inducing transmembrane proteins and of deformable nanoparticles anchored to the membrane with cholesterol.

1.3 Chapter 2: Effect of branched-tail lipids and cardiolipin in model bacterial bilayers

Bacteria modulate the composition of their membranes to maintain membrane homeostasis [146, 99, 25]. Commonly, bacteria incorporate unsaturated lipids to maintain membrane fluidity [41]. Many bacteria however lack the machinery to produce unsaturations in their lipid tails and utilize branched-tail lipids instead [22, 146, 28]. Bacterial membranes also contain a large amount of cardiolipin (CL) [99, 116]. We use all-atom MD simulations to quantify the effect of branched-tail lipids and cardiolipin on the properties of membranes by systematically varying their concentration. The addition of branched-tail lipids to membranes decreases the bending rigidity, order parameter, and membrane thickness. The membranes transition from a gel phase to a liquid disordered phase as the percentage of branched tails in the bilayer increases. CL fine tunes the properties of membranes

by rigidifying membranes with branched tails and showing non-monotonic behavior in membranes with straight tails.

1.4 Chapter 3: Extended N-terminal domain of Chlamydial MreB is an amphipathic helix that localizes on membranes

Chlamydia trachomatis is an intracellular bacterium that causes sexually transmitted infections and trachoma. Due to its intracellular nature, Chlamydia has a significantly reduced genome that does not code for FtsZ, which is a highly conserved tubulin-like cell division coordinator of binary fission [5]. Despite the reduced genome, coccoid Chlamydia codes for the protein MreB, which is responsible for the rod shape of bacteria like *E. coli* [27, 128, 121]. Chlamydial MreB has an extended N-terminal region compared to its orthologs and localizes at the cell division site along with cardiolipin synthase, the precursor to cardiolipin [95]. However, the structure and molecular interaction of the extended N-terminal region with the membrane is not well characterized. We use all-atom MD simulations to show that the extended N-terminal region of MreB forms an amphipathic helix that spontaneously inserts into membranes lacking CL.

1.5 Chapter 4: Interaction of PEX30 with ER membranes

Lipid droplets (LDs) are energy storage organelles that form in the ER bilayer [136, 94]. Pex30, an ER membrane protein, is integral to the formation of these lipid droplets. Experiments show that the reticulon homology domain (RHD) and dysferlin domain of Pex30 are crucial for its membrane localization and directing it to the LD site [40], respectively. However, the molecular mechanisms of the interaction of these Pex30 domains with the ER membrane is not well understood. Using all-atom MD, we identify that DysF domain binds to membranes via two regions (residues 296–315 and 379–397) that consist of hydrophobic

insertion loops flanked by polar or charged residues. Contact analysis showed that DysF bound more strongly to bilayers containing PA lipids. Using coarse-grained MD simulations, we showed that the molecular wedge-like structure of RHDs causes them to induce curvature in the membrane and spontaneously aggregate.

1.6 Chapter 5: Particle deformability enables control of membrane-mediated forces

Nanoparticles adsorbed on a membrane can induce deformations of the membrane that give rise to effective interactions between the particles [106]. Previous studies have focused primarily on rigid nanoparticles with fixed shapes [107, 2, 3, 144]. However, DNA origami technology has enabled the creation of deformable nanostructures with controllable shapes and mechanical properties [139], presenting new opportunities to modulate interactions between particles adsorbed on deformable surfaces. We use coarse-grained molecular dynamics simulations to investigate deformable, hinge-like nanostructures anchored to lipid membranes via cholesterol anchors. We characterize deformations of the particles and membrane as a function of the hinge stiffness. Flexible particles adopt open configurations to conform to a flat membrane, whereas stiffer particles induce deformations of the membrane. We further show that particles spontaneously aggregate and that cooperative effects lead to changes in their shape when they are close together. Using umbrella sampling methods, we quantify the effective interaction between two particles and show that stiffer hinge-like particles experience stronger and longer-ranged attractions. Our results demonstrate that interactions between deformable, membrane-anchored nanoparticles can be controlled by modifying mechanical properties of the particles, suggesting new ways to modulate the self-assembly of particles on deformable surfaces.

Chapter 2

Effect of branched-tail lipids and cardiolipin in model bacterial bilayers

2.1 Introduction

Lipid membranes are a crucial component of every cell [68]. They act as a physical barrier protecting the cell and also orchestrate essential cellular processes including controlling the movement of substances through them, signal transduction, energy conversion, and compartmentalization [38]. Cells modulate properties of their membranes by changing their lipid compositions and lateral distribution [143, 36]. Bacteria have been shown to change composition of their membranes by modifying lipid tail length and incorporating unsaturations in the lipid tail, depending on their environmental conditions [146]. When environmental temperature is decreased, bacteria increase the proportion of unsaturated lipids in the membrane to maintain membrane fluidity [72]. However, many Gram-positive bacteria do not produce unsaturated fatty acids, and they regulate the ratio of branched-tail lipids to regulate membrane fluidity [22, 146, 28]. Additionally, lipidomic studies of some gram negative bacteria like *Chlamydia trachomatis* show that the majority of their lipids have branched-tails [142, 141]. Branched-tail lipid have been shown to have lower melting temperatures than the straight-tail lipids of the same length, indicating a similar behavior to unsaturated lipids [103]. Molecular dynamics studies of membranes containing branched-tail lipids have verified their fluidizing effect [86, 103, 66]. However, the effect of

branched-tail lipids on model bacterial lipids have not yet been fully explored. In this work, we systematically vary the concentration of lipids containing branched-tail lipids and study their effect on membrane properties using molecular dynamics simulations.

Along with modifications in lipid tails, bacteria also influence membrane properties by modulating the composition of the types of lipids that make up their membranes. Bacterial membranes are generally composed of phosphatidylglycerol (PG), cardiolipin (CL), and phosphatidylethanolamine (PE). CL is a 4-tailed lipid that has been shown to localize in areas of negative curvature due to its conical shape [105]. CL is also recruited in regions of cell division to induce curvature required for remodeling of membranes. CL-rich domains are also responsible in binding many membrane proteins in bacteria [23, 122]. Anionic CL influences protein binding due to electrostatic interactions with positively charged protein residues. The conical shape of CL also causes defects in lipid packing that proteins leverage to access the hydrophobic interior of the membrane. Despite the importance of CL in many biological processes in bacteria, there is a dearth of studies investigating how CL, especially branched tail CL, affects the properties of model bacterial membranes. In this work, we use all-atom molecular dynamics to systematically study how the properties of model bacterial membranes are influenced by different concentrations of straight-tail and branched-tail CL.

2.2 Methods

2.2.1 All-atom molecular dynamics

We used the CHARMM-GUI membrane builder to set up model bacterial bilayers consisting of 400 lipids (200 in each leaflet) [45, 46]. All simulations were performed in GROMACS 2021 using the CHARMM36 force field [133, 1, 60, 51]. The temperature was set at 310.15 K to resemble physiological conditions, and a concentration of 0.15 mM NaCl was used to neutralize the charge of the system.

The energy of the system was first minimized using steepest descent for 5,000 steps. Subsequently, the system was equilibrated in six stages using the standard equilibration procedure from CHARMM-GUI. During this procedure, the simulation time step was

increased from 1 to 2 fs while using the Berendsen thermostat and Berendsen barostat [8]. Subsequently, we simulated the systems for 200 ns using the Nosé-Hoover thermostat and Parrinello–Rahman barostat [90, 39, 98]. The final 100 ns was used for analysis.

2.2.2 Calculation of membrane properties

Trajectories generated from GROMACS were analyzed in Python using the MDAnalysis and lipypilic packages [81, 126].

The bending rigidity of the multi-component membranes was calculated by the analyzing the fluctuations of the splay angle α between neighboring lipid molecules as suggested by Khelashvili et al.[49]. We sample the probability distribution of splay angles of each pair of neighboring lipid types and plot the potential of mean force (PMF) of splaying these lipids,

$$PMF(\alpha) = -k_B T \ln \left[\frac{P(\alpha)}{\sin \alpha} \right] \quad (2.1)$$

The splay modulus between these lipids (χ_{12}) is then calculated by fitting a quadratic to the PMF at an interval of small α angles. The bending modulus of the bilayer (K_C) is then calculated by averaging of the splay moduli of all possible lipid pairs weighted by the total number of encounters off each lipid pair (φ_{ij}).

$$\frac{2}{K_C} = \frac{1}{\varphi_{\text{total}}} \sum_{\langle i,j \rangle} \frac{\varphi_j}{\chi_{12}^{ij}} \quad (2.2)$$

Order parameter and area per lipid are properties that describe the molecular packing of lipids in the bilayer. We calculate a coarse grained order parameter (S_{CC}) as,

$$S_{CC} = \frac{\langle 3 \cos^2 \theta - 1 \rangle}{2} \quad (2.3)$$

where θ is the angle between the membrane normal and the vector connecting two consecutive tail carbon atoms. S_{CC} quantifies how well the vector between two consecutive tail carbon atoms aligns with the membrane normal direction. For branched-tail lipids, the ante-iso branched carbon atom is excluded from the order parameter calculation. The area per lipid is

calculated by a 2D Voronoi tessellation on head group positions of each lipid. This technique divides the membrane surface into polygonal Voronoi regions around each lipid head group [70]. Each Voronoi region encompasses all the space that is closer to one particular lipid head group than to any neighboring lipid. The area of each Voronoi region is calculated as the area occupied by the lipid in that region. The area occupied by each type of lipid is then averaged to obtain the area per lipid of that lipid type.

To determine the membrane thickness, we measure the density profile of phosphate atoms in the z direction orthogonal to the membrane. The thickness of the membrane is calculated as the distance between the two peaks that represent the average head group positions of each leaflet.

2.3 Results and discussion

We utilized all-atom molecular dynamics simulations to probe the properties of model bacterial membranes consisting of phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and cardiolipin (CL). PG and PE were present in a ratio of 1:9 with varying amounts of cardiolipin. For the straight-tail lipid system, phospholipids containing dipalmitoyl (16:0-16:0) acyl chains were employed, resulting in DPPE, DPPG, and MDCL2. For the branched-tail lipid system, phospholipids containing asymmetric acyl chains composed of palmitoyl (16:0) and 12-methyltetradecanoyl (15:0 anteiso) were employed, resulting in PAIPE, PAIPG and PAICL2. All-atom simulations are needed as coarse-grained models do not adequately capture the properties of branched-tail lipids.

2.3.1 Branched-tail lipids fluidize membranes

We first sought to characterize the effects of branched-tail lipids in the absence of cardiolipin. To this end, we ran simulations of PE/PG bilayers with varying fractions of lipids containing branched tails. Figure 2.2 shows representative snapshots of the resulting bilayers. At low fractions of branched-tail lipids, the lipid tails are ordered and the bilayer adopts a gel-like state. At high fractions of branched-tail lipids, the lipid tails are disordered. At intermediate levels of branched-tail lipids, snapshots suggest the coexistence of gel and fluid domains.

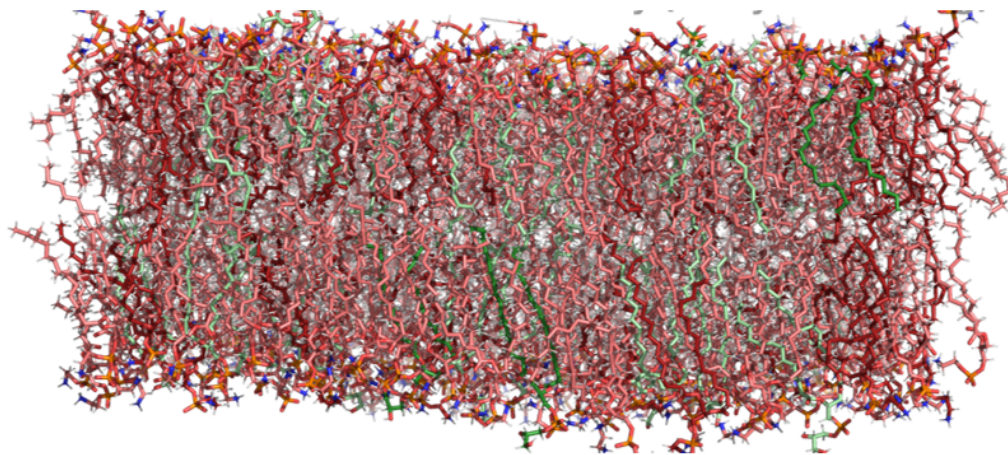
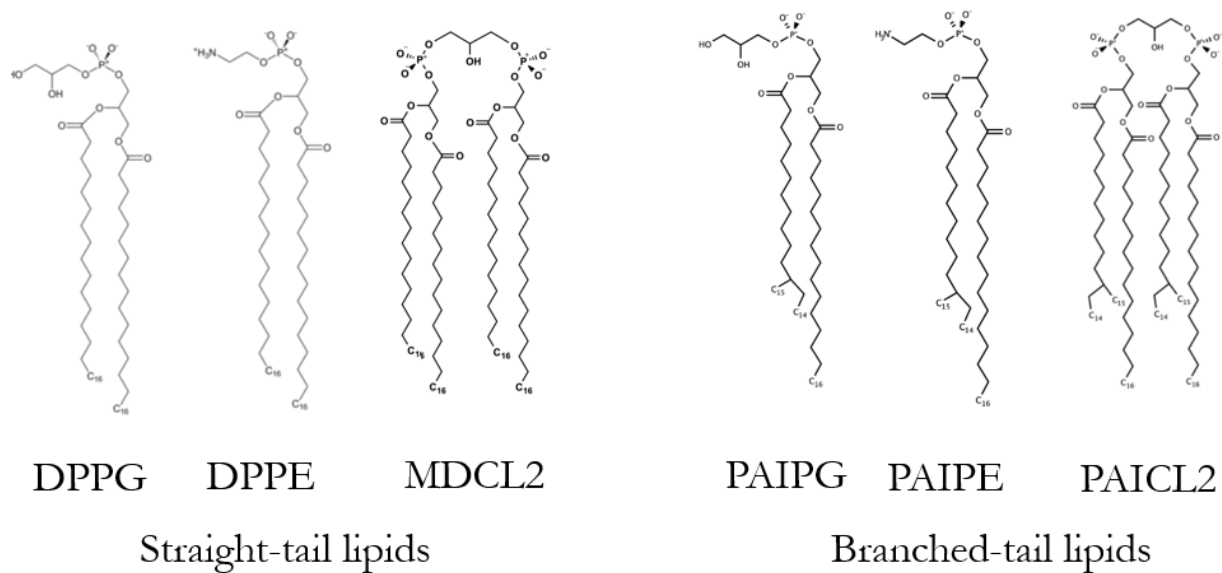


Figure 2.1: (A) Molecular representation of lipids that comprise the model membranes. (B) Snapshot of a simulated membrane.

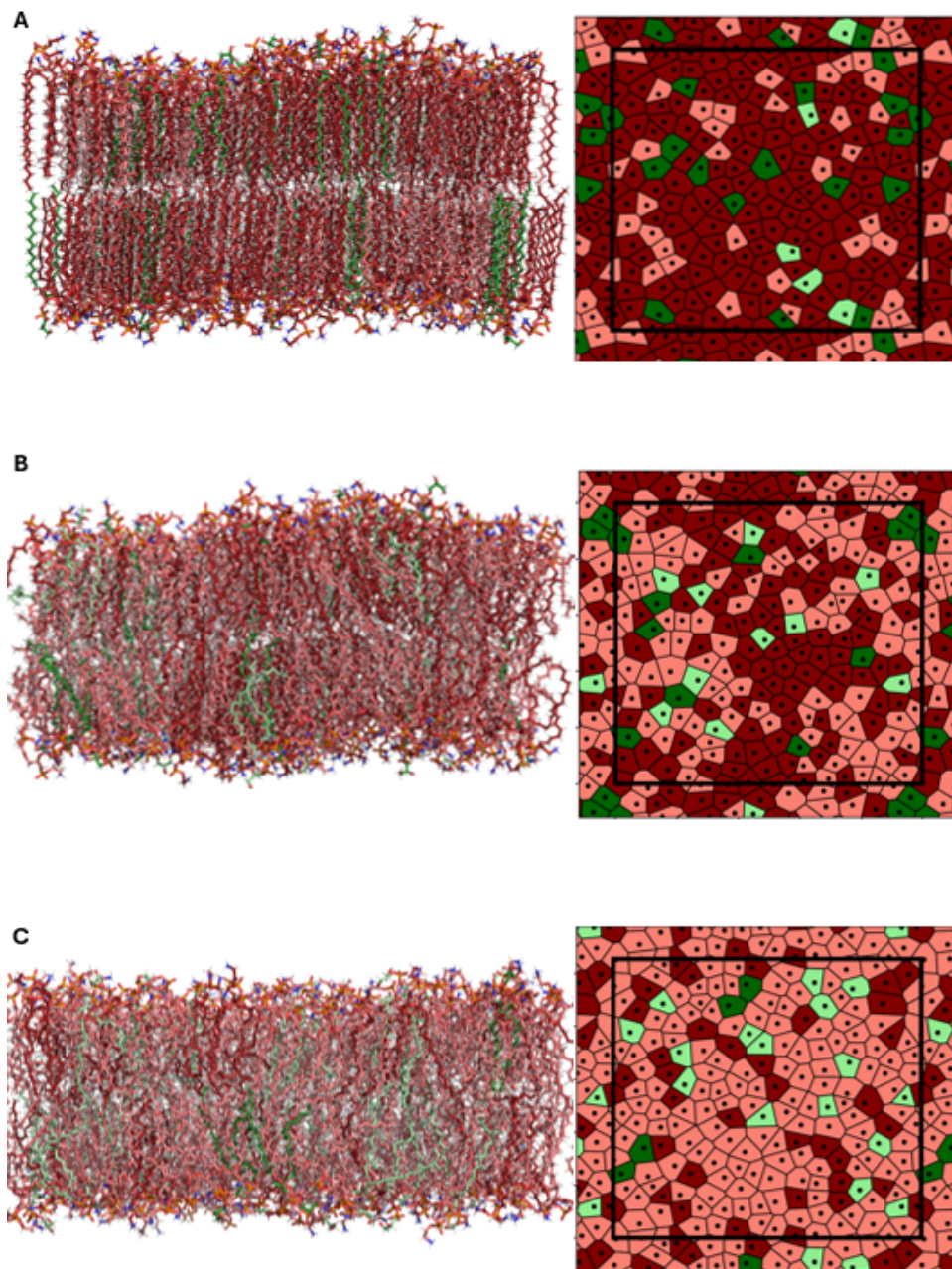


Figure 2.2: Snapshots of bilayers composed of straight-tail and branched-tail PE/PG lipids. (A), (B) and (C) shows bilayers with 20%, 50% and 80% branched-tail lipids. The snapshots are accompanied by Voronoi tessellations of the top leaflet of the bilayer showing the arrangement of lipid head groups. The black points denotes the position each lipid head group in the leaflet. Each lipid is enclosed in a Voronoi region which is the space where any point in this region is closer to the lipid inside it than to any neighboring lipid. The straight-tail PE and PG are represented in dark red and dark green respectively. The branched-tail PE and PG are represented in light red and light green respectively. As the percentage of branched-tail lipids are increased the snapshots suggest the lipid tail transitioning from an ordered phase to a disordered phase.

We further characterized the properties of the membrane to quantitatively analyze the transition seen in snapshots. As the fraction of branched-tail lipids increases, a decrease is observed in the bending rigidity of the membrane from $194 k_B T$ to $32 k_B T$ (Figure 2.3A). The order parameter is also observed to decrease for all lipids as branched-tail percentage increases (Figure 2.3B). The straight-tail lipids are considered to be in the gel phase when the order parameter is greater than 0.35 [119]. In our simulations, the straight-tail lipids maintain an order parameter consistent with the gel phase up to 50% branched tails and then show a sharp decrease in order parameter with further addition of branched-tail lipids. The order parameter of branched-tail lipids is always lower than that of straight-tail lipids. This can be attributed to the disorder in the tail caused at the ante-iso branching point.

Figures 2.3C and D show an increase in the area per lipid and a decrease in membrane thickness with increasing fraction of branched-tail lipids. These changes are consistent with the transition from an ordered gel state to a disordered liquid phase, where reduced lipid packing results in lateral membrane expansion and reduced bilayer thickness. Thus, branched-tail lipids in the lipid bilayer promote fluidization of the membrane, even when the temperature is well below the melting temperature of the saturated-chain lipids.

2.3.2 Cardiolipin can fine-tune properties of membranes

We next investigated the impact of cardiolipin (CL) on properties of bilayers comprised of either straight-tail (Figure 2.5) or branched-tail lipids (Figure 2.6). For straight-tail lipids, increasing the fraction of CL leads to non-monotonic behavior of the bending rigidity (Figure 2.5A). At low fractions of CL (0 - 20%), an increase in bending rigidity is seen with increasing % CL. This increase in bending rigidity is followed by a decrease as the fraction of CL is increased further (20 - 80%). Finally, rigidification is seen as the bilayer reaches 100% CL. In the branched-tail system, the bending rigidity increases monotonically as the fraction of CL increases. This rigidification seen in both systems at 100% CL could be attributed CL acting like two lipids joined together at the head group. Without PE/PG lipids, larger splay between CL molecules that occupy more area would be required. There is a marked difference in the bending rigidities of straight-tail lipids ($194 k_B T$ - $225 k_B T$) compared to branched-tail lipids ($31 k_B T$ - $46 k_B T$). This is accompanied by the straight-tail lipids having

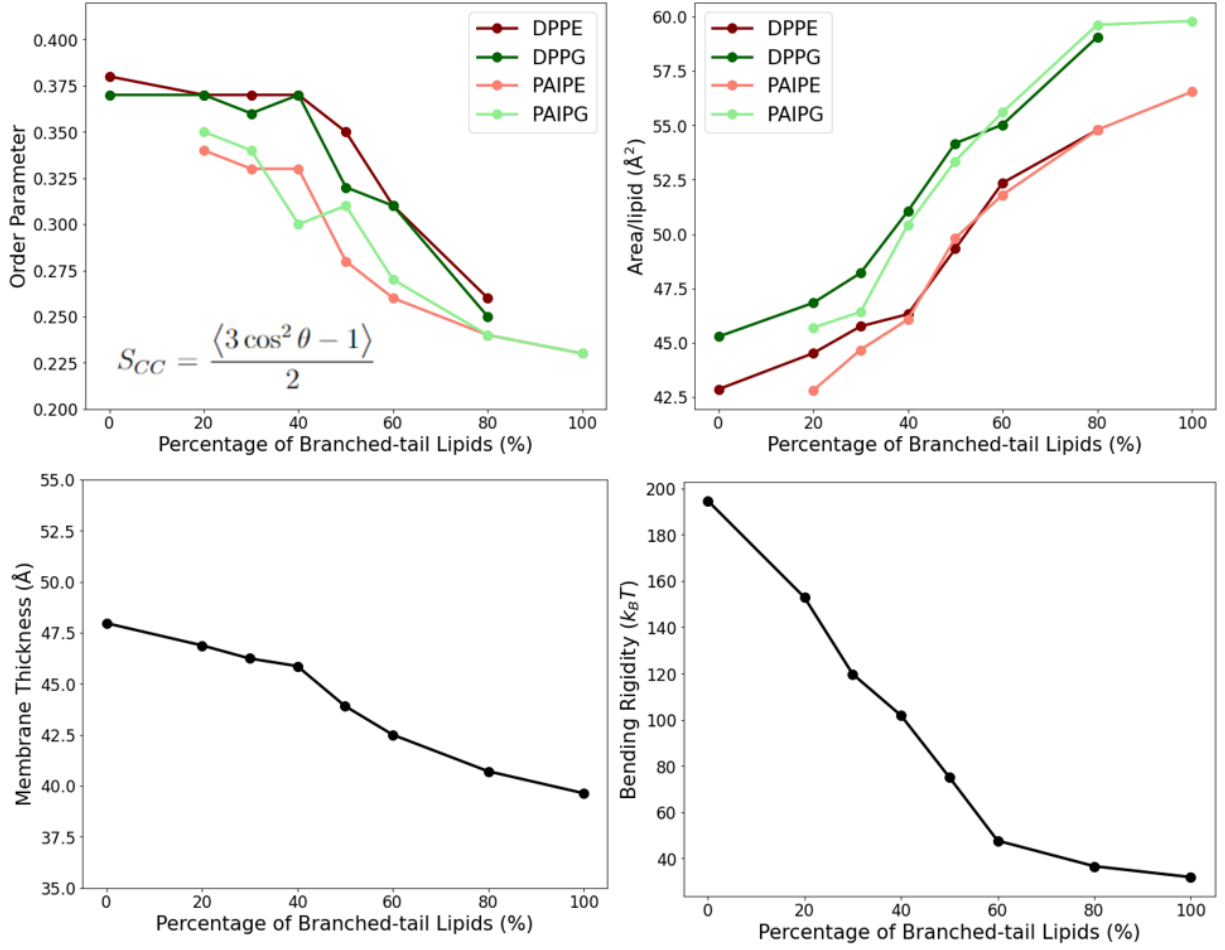


Figure 2.3: Properties of mixed PE/PG membranes as a function of percentage of lipids with branched tails. (A) Bending rigidity. (B) Lipid order parameter. (C) Area per lipid. (D) Bilayer thickness.

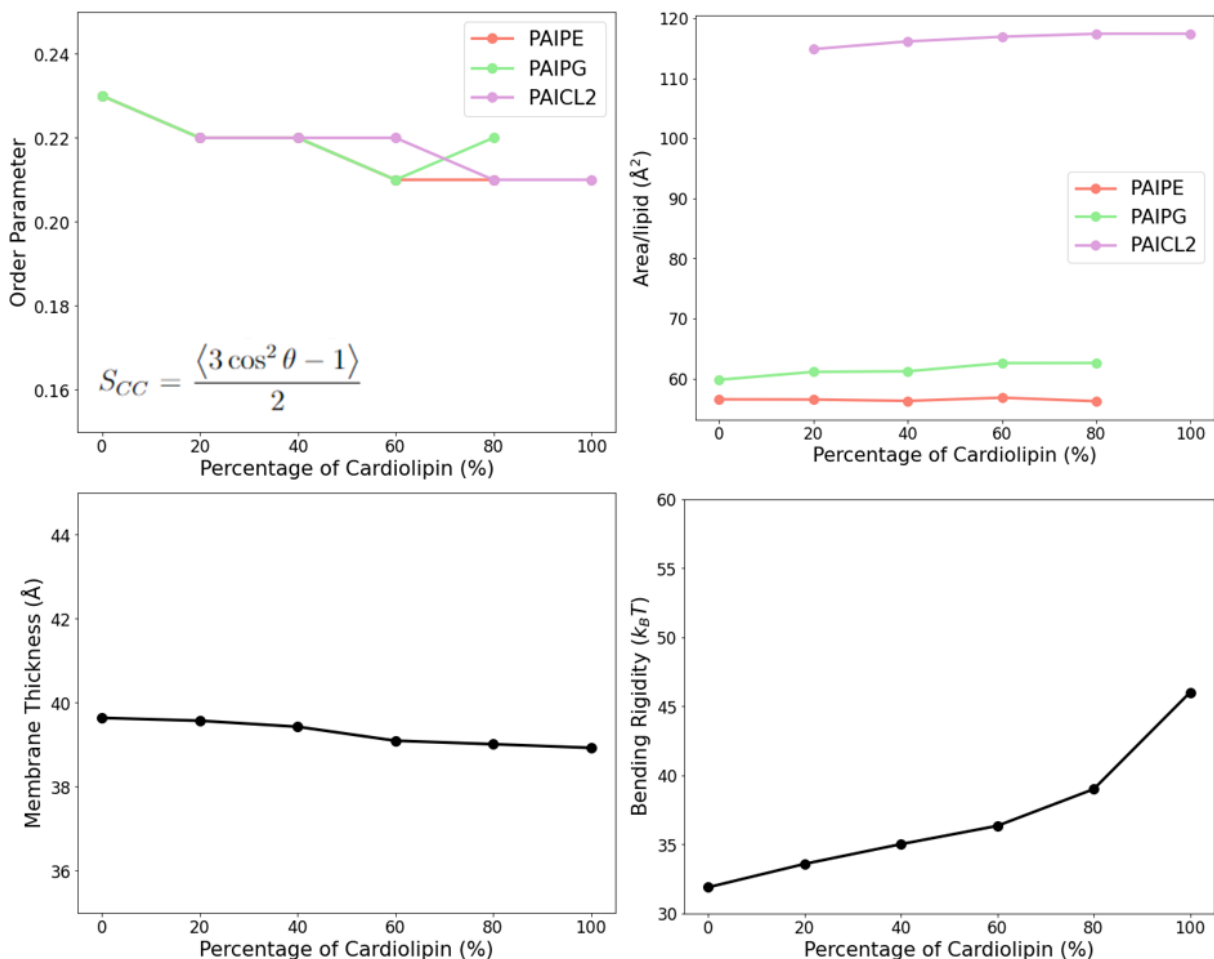


Figure 2.4: Properties of PE/PG/CL membranes branched-tail systems as a function of proportion of CL lipids (A) Bending rigidity (B) Order Parameter (C) Area/ lipid (D) Membrane thickness

higher lipid order parameters, larger membrane thickness, and smaller area-per-lipid than their branched-tail counterparts, indicating tighter and more ordered molecular packing in straight-tail lipids (Figures 2.5 and 2.6 B - D).

Along with the non-monotonic behavior seen in bending rigidity, the straight-tail system also exhibits non-monotonic behavior in order parameter, membrane thickness, and area per lipid. The trend in the order parameter and the membrane thickness, match each other, however they do not match the trend seen in bending rigidity. Tilting of lipids with respect to the membrane normal in the gel phase could be an explanation for this discrepancy in the order parameter and membrane thickness.

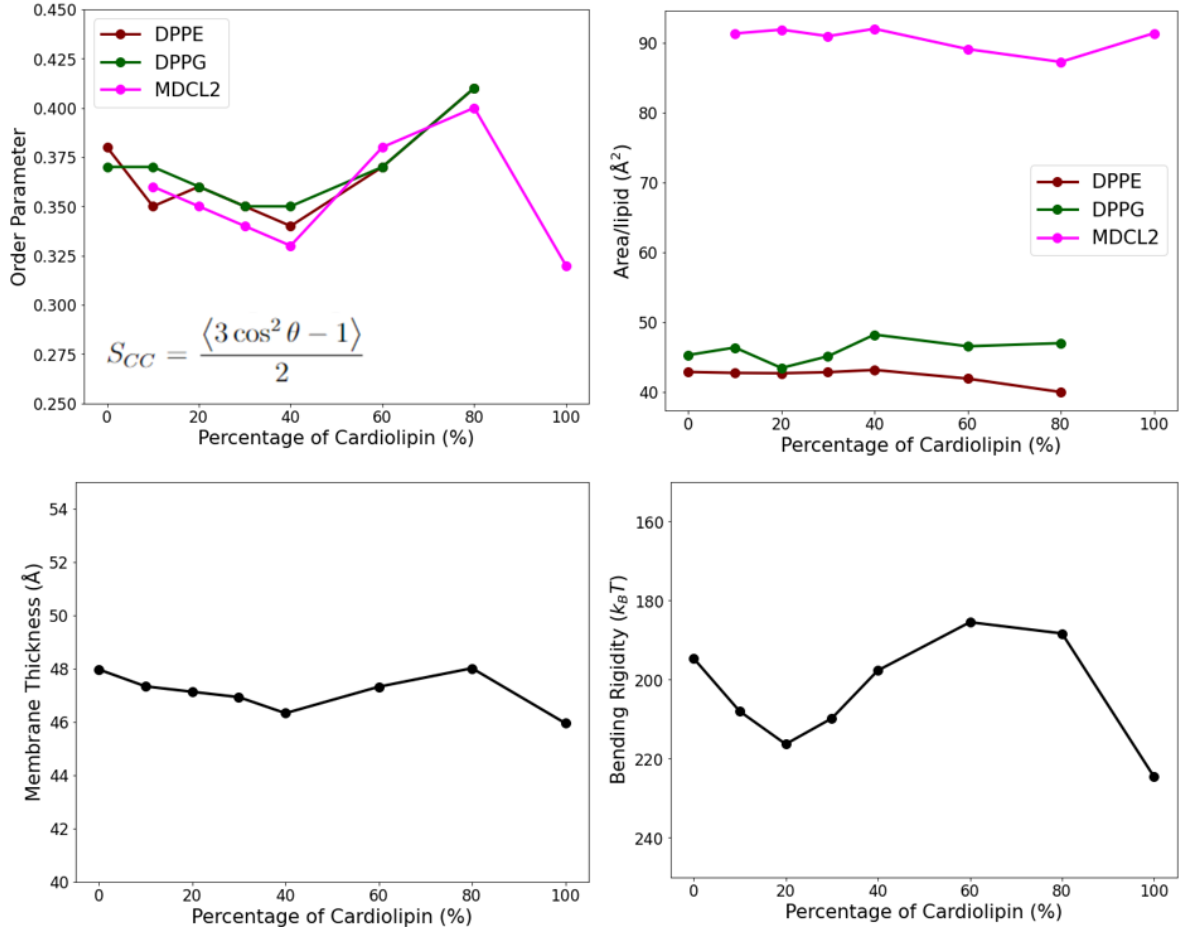


Figure 2.5: Properties of PE/PG/CL membranes straight-tail systems as a function of proportion of CL lipids (A) Bending rigidity (B) Order Parameter (C) Area/ lipid (D) Membrane thickness

2.3.3 Branched-tail cardiolipin leads to faster reduction in bending rigidity than branched-tail PE/PG

When branched-tail cardiolipin is introduced to the straight-tail PE/PG system, the bending rigidity decreases monotonically. A transition from an ordered to a disordered phase is observed, similar to when branched-tail PE/PG is introduced to a straight-tail PE/PG system.

We further compared the effect of adding branched-tail CL versus branched-tail PE/PG to straight-tail PE/PG bilayers. Figure 2.7 shows the change in bending rigidity of both systems. These systems are plotted against percentage of ante-iso tails for direct comparison. The systems containing branched-tail cardiolipin exhibit a larger decrease in bending rigidity until 30% anteiso tails, after which it levels out while the bending rigidity of the branched-tail PE/PG system continues to decrease. The initial faster decrease could be due to CL occupying a larger area compared to PE/PG lipids and causing defects in the lipid packing. At higher concentrations of CL, CL behaving like two PE/PG lipids joined at the head group could rigidify the bilayer compared to bilayers without CL.

2.4 Conclusions

In this work we analyzed the effect of branched-tail lipids in model bacterial membranes by systematically varying their concentration. The addition of branched-tail lipids to membranes was seen to decrease the bending rigidity, order parameter and membrane thickness. The membranes were shown to transition from a gel phase to disordered phase as the percentage of branched tails in the bilayer was increased. These results suggest that the abundance of branched-tail lipids in bacteria like chlamydia is likely to fluidize the membrane as an alternative to unsaturated lipids to facilitate cellular processes.

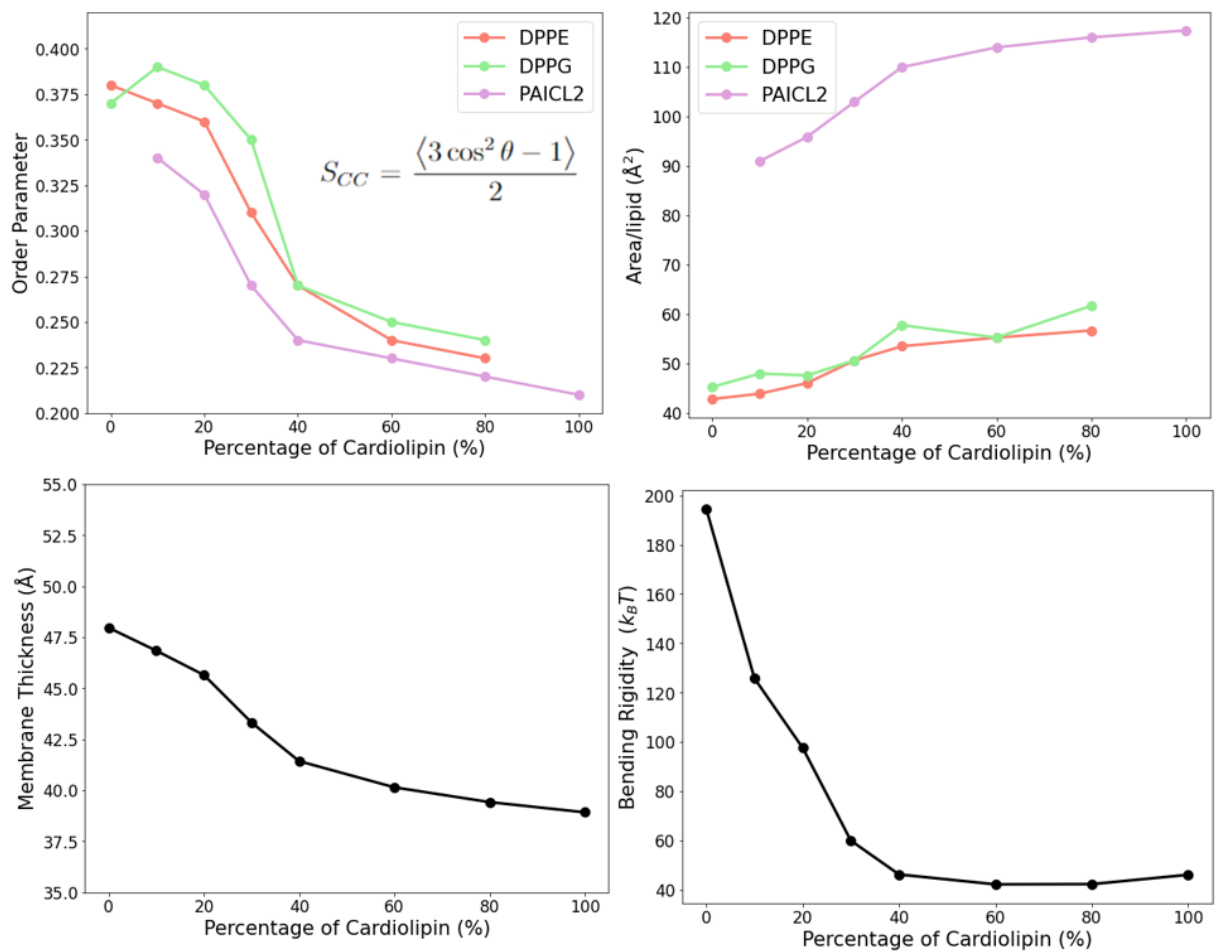


Figure 2.6: Properties of straight tail PE/PG with branched CL membranes as a function of proportion of CL lipids (A) Bending rigidity (B) Order Parameter (C) Area/ lipid (D) Membrane thickness

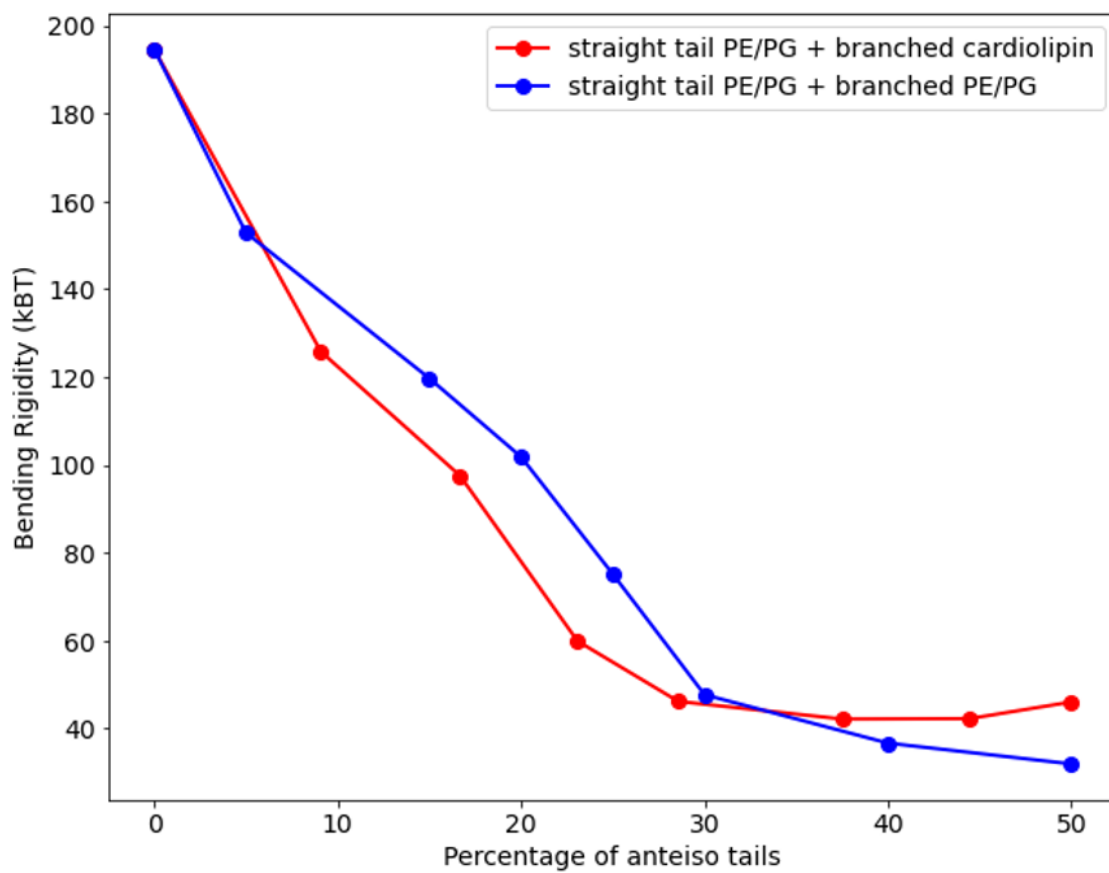


Figure 2.7: Bending rigidity CL branched vs PE/PG branched

Chapter 3

Extended N-terminal domain of Chlamydial MreB is an amphipathic helix that localizes on membranes

3.1 Introduction

Chlamydia trachomatis is an intracellular bacterium that causes sexually transmitted infections and trachoma. Due to its intracellular nature, Chlamydia has a significantly reduced genome that does not code for FtsZ, which is a highly conserved tubulin-like cell division coordinator of binary fission [5] in other bacteria. Chlamydia has been shown to divide via polarized budding process instead [97]. Despite the reduced genome, Chlamydia codes for the bacterial actin homologue MreB, which is most commonly associated with peptidoglycan synthesis in the lateral cell wall of bacilli to maintain their rod-like shape [27, 128, 121]. Since Chlamydia is a coccoid organism, the role of MreB is unclear. Ouellette et al. showed that MreB is essential for chlamydial growth and division using MreB-specific antibiotics [96]. Furthermore, they showed that chlamydial MreB interacts with other cell division proteins, such as FtsK. These results, along with MreB being observed to localize at the septum of a dividing cell [61], suggest that chlamydial MreB does not function in the same way as its orthologs in other bacteria and has been co-opted for cell division.

Our experimental collaborators showed that chlamydial MreB has an extended N-terminal region compared to its orthologs [61]. This extended N-terminus region shows a high amphipathic score on AMPHIPASEEK, and helical wheel analysis shows amphipathicity throughout the extended region. These results suggests that the extended N-terminal region could form an amphipathic helix. Amphipathic helices are a type of protein structure where one side of the helix is hydrophobic and the other is hydrophilic, which provides it with strong membrane-associating properties. The hydrophobic side of the helix inserts into the hydrophobic tail region of the membrane while the hydrophilic side sits on the surface of the membrane interacting with the lipid head groups and the aqueous environment. Our experimental collaborators showed that this extended N-terminal region of MreB is sufficient to localize green fluorescent protein (GFP) to the membrane in chlamydia and is necessary for interaction of MreB with other cell division proteins (RodZ, FtsK, FtsKN). Therefore, the extended N-terminus plays a key role in localizing MreB on the membrane and facilitating cell division by interacting with other chlamydial cell division proteins. However, the structure and molecular interaction of this extended N-terminal region with the membrane is not well characterized.

Cardiolipin (CL), a four-tailed lipid with a conical shape [105], was seen to accumulate at the leading edge of budding chlamydial cells [95]. Using antibiotics that target CL-containing membrane domains was seen to significantly alter the localization of MreB, which suggested that CL was important in directing the assembly of MreB [95]. In literature CL has been shown to interact and localize proteins containing amphipathic helices [19, 102, 122]. Anionic CL influences protein binding due to electrostatic interactions with positively charged protein residues. The conical shape of CL also causes defects in lipid packing that proteins leverage to access the hydrophobic interior of the membrane. The negative charge interacts with the positive residues of the amphipathic helices, and the inverse conical shape creates packing defects that could allow insertion of the hydrophobic residues into the interior of the membrane. In chlamydia, it is unclear if the extended n-terminal helix in MreB is responsible for CL directing its assembly. We use all-atom molecular dynamics to investigate how membranes with and without CL interacts with the extended N-terminal region of MreB.

In this work, we use ColabFold and PEPfold to obtain initial predictions of the structure of the extended N-terminal region of MreB. We then run unbiased all-atom MD simulations to refine the structure in contact with a membrane. We also investigate the membrane association of the extended N-terminal amphipathic helix in model chlamydial membranes and the effect of cardiolipin on membrane association of MreB.

3.2 Methods

Our model chlamydial membranes consisted of phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and cardiolipin (CL). PG and PE were present in a ratio of 1:9 with either 0% or 20% CL. We used asymmetric acyl chains composed of palmitoyl (16:0) and 12-methyltetradecanoyl (15:0 anteiso) resulting in PAIPE, PAIPG and PAICL2. We used CHARMM-GUI membrane builder to setup the two membranes [45, 46]. The structure of the extended N-terminus of MreB (residues 1-33) was predicted using ColabFold [82] and PEPFold [120, 79] on the full MreB sequence. The C terminus of the extended N-terminus from the predictions was patched with a methylamine group, to avoid end effects in simulations. The resulting extended N-terminus was then placed in the solvent phase and not in contact with the membrane using CHARMM-GUI. All simulations were performed in GROMACS 2018 using the CHARMM36 force field [133, 1, 60, 51]. The temperature was set at 310.15 K to resemble physiological conditions, and a concentration of 0.15 mM NaCl was used to neutralize the charge of the system.

The energy of the system was first minimized using steepest descent for 5,000 steps. Subsequently, the system was equilibrated in six stages using the standard equilibration procedure from CHARMM-GUI. During this procedure, the simulation time step was increased from 1 to 2 fs while using the Berendsen thermostat and Berendsen barostat [8]. Subsequently, we simulated the systems for 900 ns using the Nosé-Hoover thermostat and Parrinello–Rahman barostat [90, 39, 98]. 100 ns windows were used for analysis of lipid contacts.

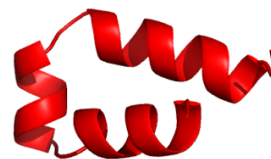
A**B**

Figure 3.1: Initial structure predictions of the extended N-terminus region used for simulations. (A) Collabfold prediction (B) PEPfold prediction

Trajectories generated from GROMACS were analyzed in Python using the MDAnalysis package [81, 32]. To analyze contacts, all lipids within a cutoff distance were counted around each residue. The cutoff for contact was set at 4.5 Å.

3.3 Results and discussion

We used Collabfold and PEPfold to obtain the initial predictions of the structure of the extended N-terminal region. PEPfold predicted 3 short helices that are joined together while Collabfold predicted a continuous single helix (Figure 3.1). We used unbiased all-atom MD simulations in the presence of model chlamydial bilayers to refine the initially predicted peptide structure and investigated the membrane targeting properties of the chlamydial extended N-terminal region.

In the simulations with Collabfold prediction as the initial configuration, the peptide started as a continuous helix and stayed as continuous helix throughout the simulation (Figure 3.2) for bilayers both without CL and with 20% CL. For the 0% CL case, the N-terminus region promptly inserted into the membrane showing strong membrane targeting activity. Residues with most pronounced lipid tail contact are predominantly hydrophobic residues that insert deeper into the membrane (Figure 3.3 B). The most pronounced lipid head group contact are positively charged residues that reside near the surface of the bilayer

(Figure 3.3 C). For the 20% CL case, the N-terminal region only partially inserted into the membrane (Figure 3.2 E). Hydrophobic residues 31 and 32 are the only residues with any substantial lipid tail contact. The positively charged residues maintain substantial lipid head group contact with the lipid head groups (Figure 3.3 F). The -2 charged CL lipids strongly interact with the positively charged residues of the amphipathic helix, however, the charged surface might lead to an energetic barrier for the hydrophobic residues to insert into the interior of the membrane.

In the simulations with the PEPfold prediction as the initial configuration, the peptide started as a 3 disjointed small helices reorganized to have a longer helix (Figure 3.4). In the 0% CL case, initial 3 helix structure reorganized into one longer helix and a disordered region, while in the 20% CL case, reorganized into one longer helix and a shorter helix with a disordered region. These changes in the structure of the N-terminus indicate that the PEPfold prediction is not as energetically favorable as the continuous helix predicted by Collabfold. These simulations need to be extended for longer time scales to obtain the equilibrium structure of the peptide. For the 0% CL case, simulations with PEPfold prediction as the initial configuration behave similar to simulations with Collabfold prediction as the initial configuration. Deep insertion into the membrane is seen with hydrophobic residues having the most pronounced lipid head group contact (Figure 3.5 B). In the 20% CL case, simulations with PEPfold prediction show more residues inserted into the membrane than simulations with Collabfold prediction (Figure 3.3D-F and Figure 3.5 D-F). Hydrophobic residues in the regions 1-10 and 28-32 of the PEPfold show significant contact with lipid tails compared to only residues 31 and 32 showing prominent lipid tail contact in Collabfold simulations.

Even though membranes with negatively charged CL should hypothetically have stronger membrane binding of amphipathic helices due to stronger charge-charge interactions, we observe only partial insertion of the peptide in CL-containing bilayers in our simulations. We hypothesize that the -2 charged head groups of CL lipids form an energetic barrier that thermally driven random motion is not able to overcome in our simulation time scale. Enhanced sampling techniques like umbrella sampling might be needed to overcome this barrier and observe complete insertion.

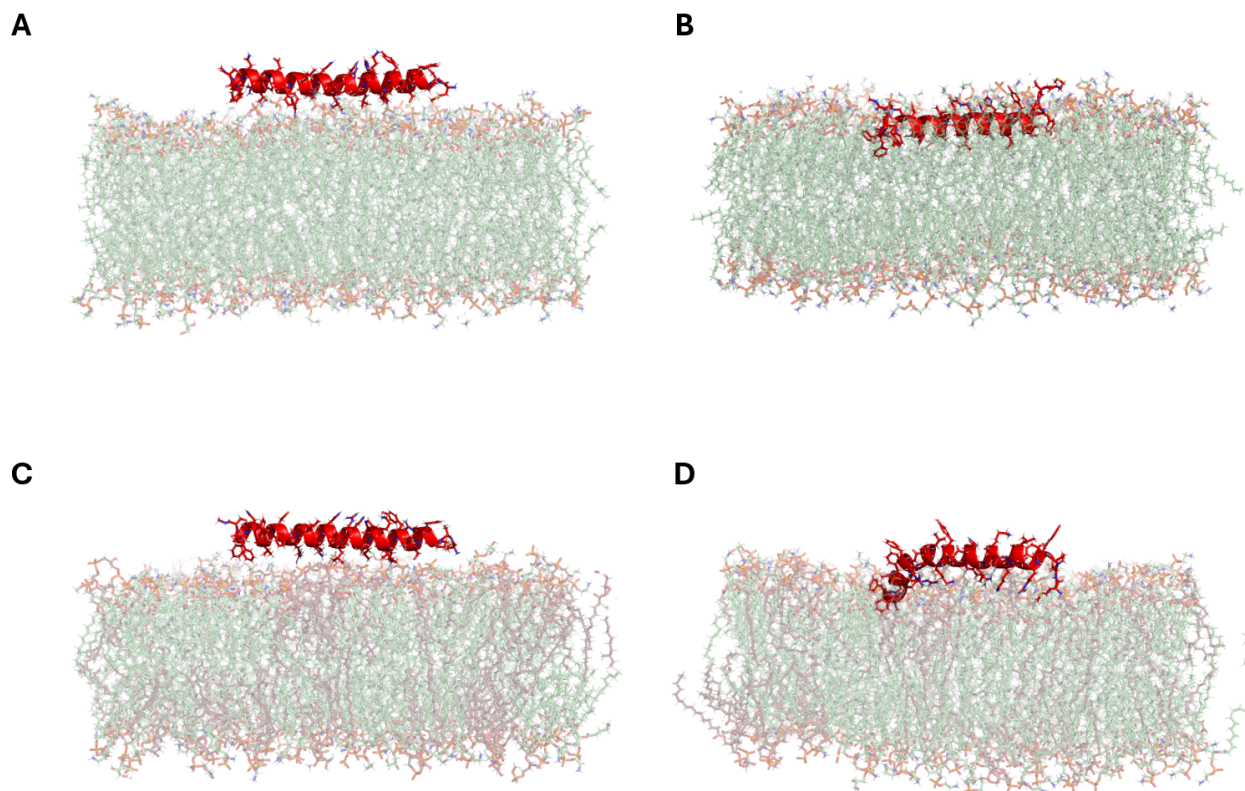


Figure 3.2: Snapshots of MreB extended N-terminus in simulations using the Collabfold structure as the initial configuration at 0 ns (A,C) and 900 ns (B,D). A and B show simulations with bilayers without CL and C and D shows are simulations with bilayers with 20% CL. PE and PG lipid tails are colored green and CL lipid tails are colored violet.

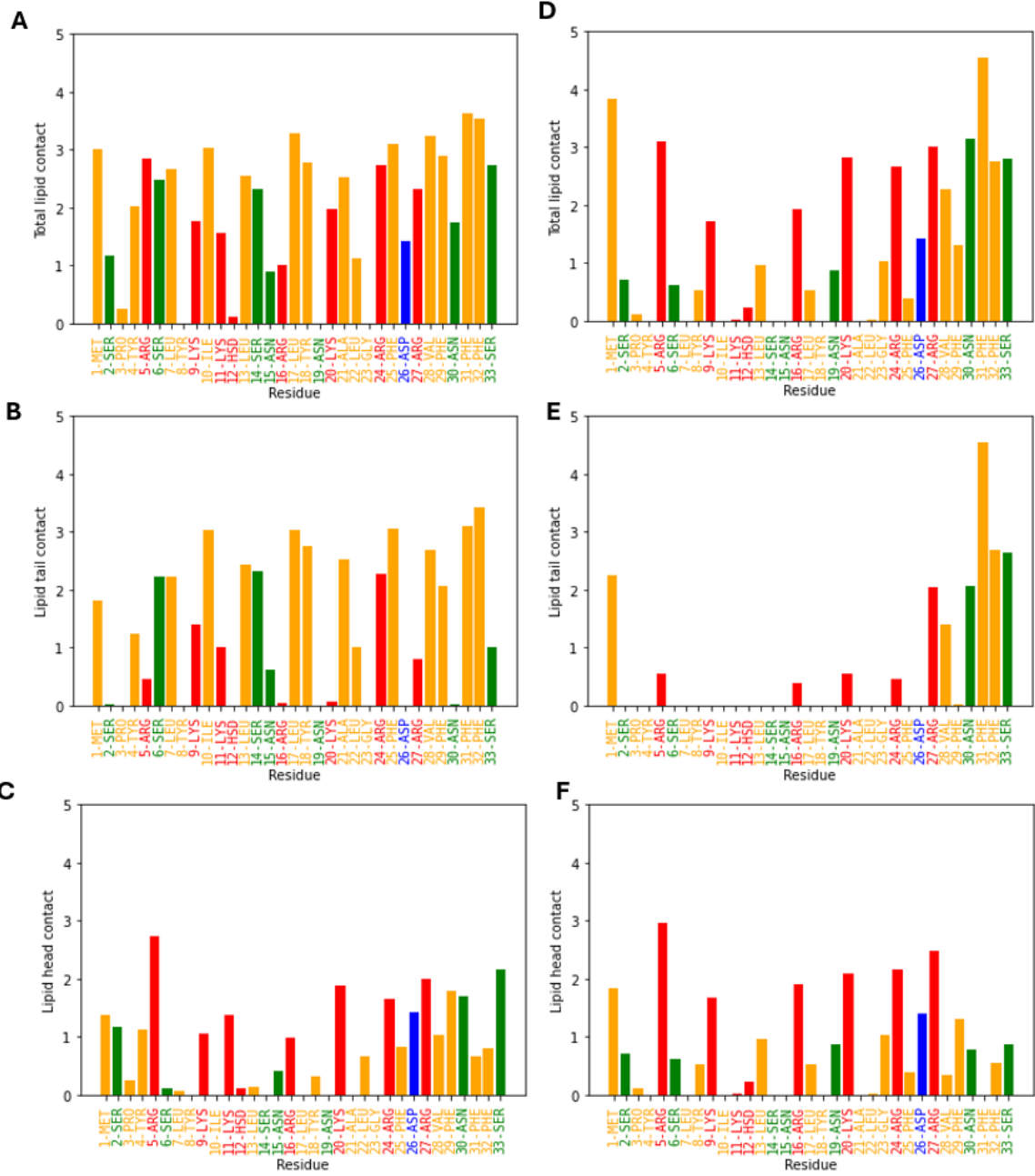


Figure 3.3: Average number of lipids in contact with each residue of the extended N-terminus in simulations using the Collabfold structure as the initial configuration. A-C shows contacts in bilayers without CL and D-F shows contacts in bilayers with 20% CL. A and D counts lipids when any part of the lipid is in contact with the residue, while B and E, and C and F, counts lipids only when atoms that are part of the tails or the head groups are in contact with the residue respectively. The residues on the x-axis and the bars are colored according to the polarity of the residue. Yellow - non-polar hydrophobic residue, green - uncharged polar residue, red - basic polar residue, blue - acidic polar residue. Contact defined as a lipid within 4.5 Å of the residue.

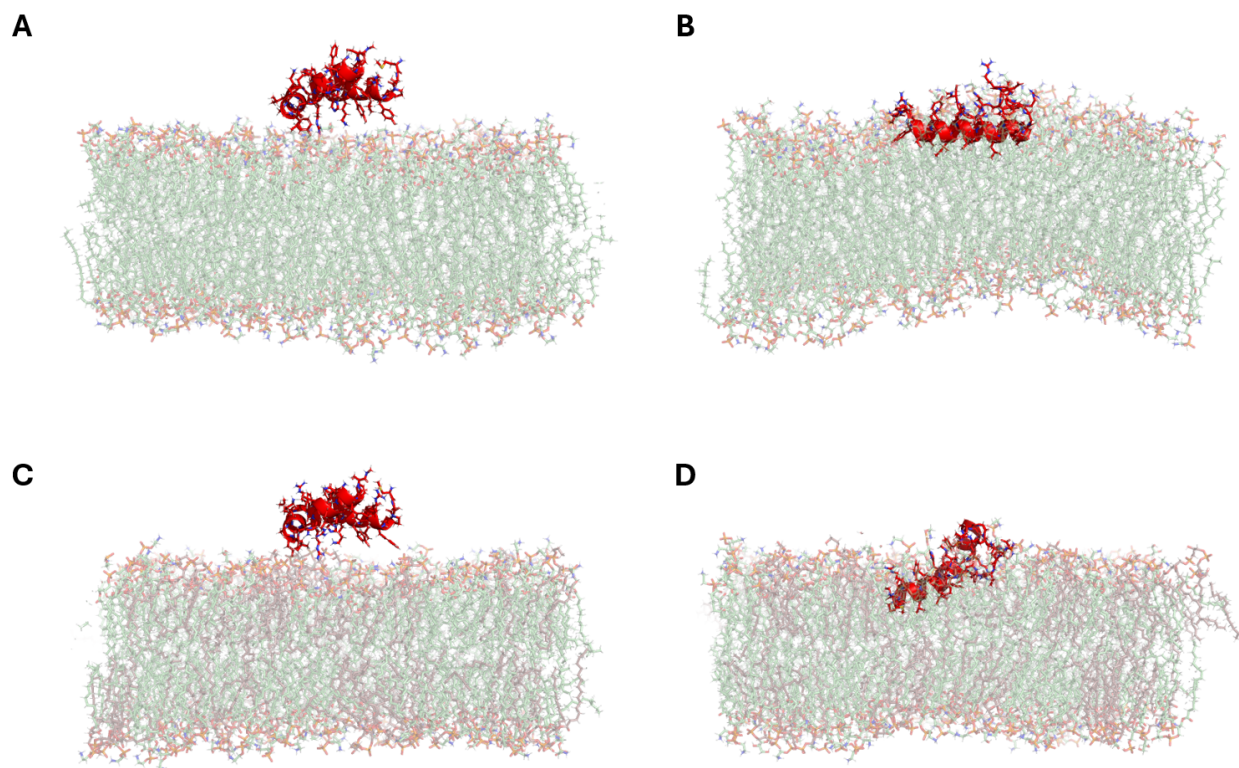


Figure 3.4: Snapshots of MreB extended N-terminus in simulations using the PEPfold structure as the initial configuration at 0 ns (A,C) and 900 ns (B,D). A and B show simulations with bilayers without CL and C and D shows are simulations with bilayers with 20% CL. PE and PG lipid tails are colored green and CL lipid tails are colored violet.

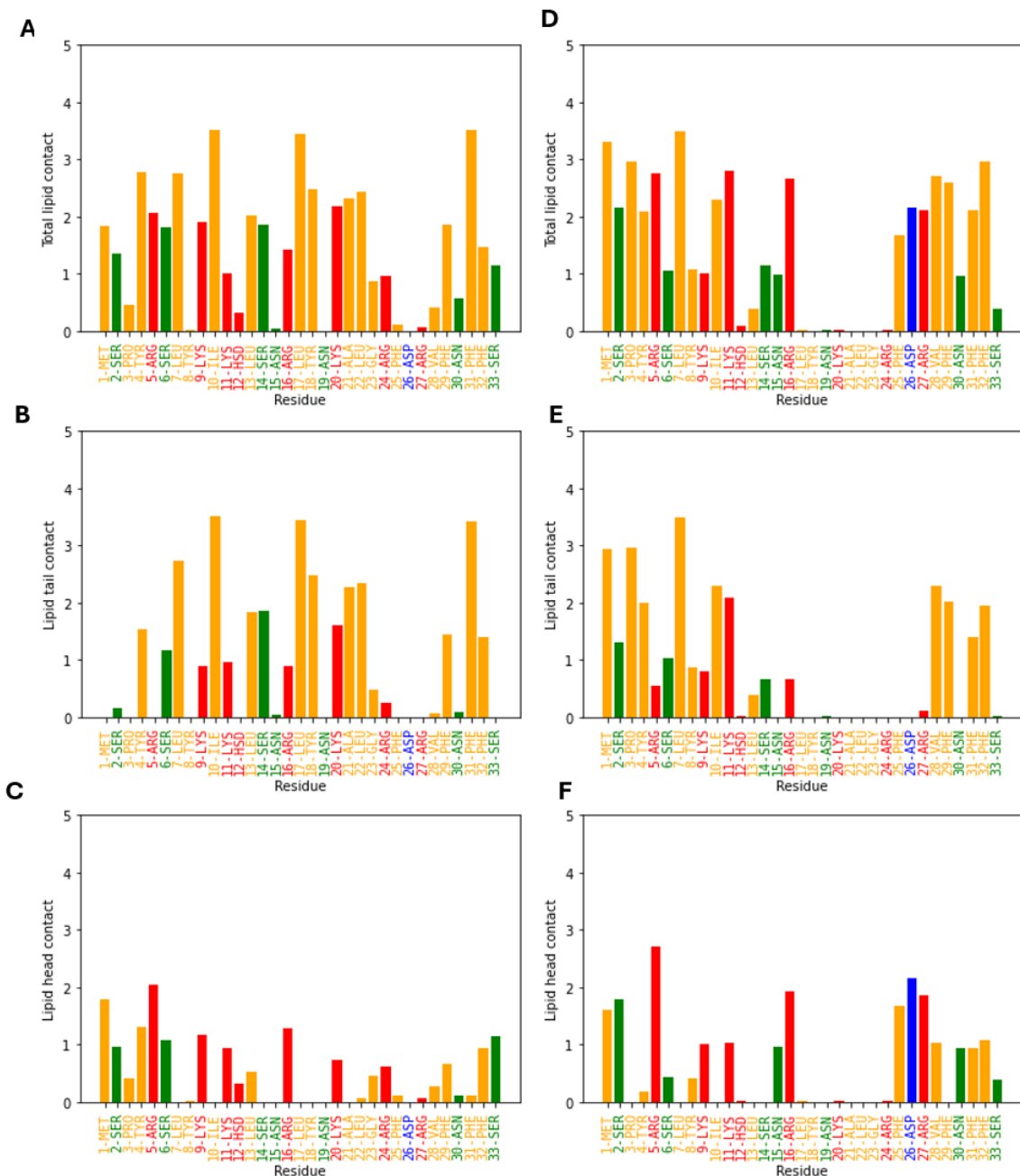


Figure 3.5: Average number of lipids in contact with each residue of the extended N-terminus in simulations using the PEPfold structure as the initial configuration. A-C shows contacts in bilayers without CL and D-F shows contacts in bilayers with 20% CL. A and D counts lipids when any part of the lipid is in contact with the residue, while B and E, and C and F, counts lipids only when atoms that are part of the aliphatic tails or the head groups are in contact with the residue respectively. The residues on the x-axis and the bars are colored according to the polarity of the residue. Yellow - non-polar hydrophobic residue, green - uncharged polar residue, red - basic polar residue, blue - acidic polar residue. Contact defined as a lipid within 4.5 Å of the residue.

3.4 Conclusion

We used all-atom MD simulations to investigate the structure and membrane interactions of the extended N-terminal region of chlamydial MreB with model chlamydial membranes. Using the initial structure predictions from Collabfold and PEPfold, we used unbiased MD simulation to refine the predicted structure. The continuous helix predicted by Collabfold was stable maintaining its secondary structure throughout the simulation. In contrast, the 3 disjointed helices predicted by PEPfold restructured to a longer helix and a disorganized region.

Further, we demonstrated that the extended N-terminal region inserts spontaneously into membranes lacking CL. In the timescales probed, it only partially inserted into membranes containing CL. The -2 negative charge of the head group of cardiolipin might be inducing an energy barrier for the hydrophobic residues to insert into the interior of the membrane. Future work using enhanced sampling techniques like umbrella sampling or metadynamics simulations could estimate the potential of mean force (PMF) as a function of z-distance from the membrane center and helicity of the peptide to provide quantitative assessment of the interaction strength between the extended N-terminal region and the membrane, thus providing additional insight into the equilibrium behavior of the protein.

Chapter 4

Interaction of PEX30 with ER membranes

Sections 4.2.1 and 4.3.1 in this chapter were adapted from:

House, M., Khadayat, K., Trybala, T.N., Nambiar, N., Jones, E., Abel, S.M., Baccile, J. and Joshi, A.S., 2025. Phosphatidic acid drives spatiotemporal distribution of Pex30 at ER-LD contact sites. *Journal of Cell Biology*, 224(7), p.e202405162. [40]

The simulation work described in this chapter was designed and performed by Nikhil Nambiar under the advisement of Steven Abel.

4.1 Introduction

Lipid droplets (LDs) are energy storage organelles that form in the ER bilayer [136, 94]. They are composed of neutral lipids in the core surrounded by a polar lipid monolayer. Formation of LDs can be influenced by several factors, such as ER membrane lipid composition, membrane curvature, surface tension, and proteins localized to ER subdomain [17, 130, 111, 80]. Pex30, an ER membrane-tubulating protein and seipin (Sei1), a highly conserved ER membrane protein localize at sites of LD formation. Seipin has been shown to play a crucial role in LD biogenesis [52]. Yeast cells devoid of seipin and Pex30 (*sei1pex30Δ*) exhibit severe growth defects whereas cells devoid of only seipin can be rescued from these defects by Pex30 [47, 138]. Pex30 in the *sei1Δ* mutant accumulates into a large punctum

associated with LD biogenesis sites [47]. These results suggest that the function of Pex30 is vital for LD biogenesis. However the mechanism of how Pex30 drives LD-biogenesis is not well understood.

Pex30 has three domains, a reticulon homology domain (RHD), a dysferlin (DysF) domain, and a Duf4196 domain. Our experimental collaborators, led by Dr. Amit Joshi, showed that the DysF domain is essential for the localization of Pex30 at LD biogenesis sites. Pex30 with deletions of the RHD (Pex30(DysF Δ)-GFP) did not rescue the growth defects of the *seilpex30 Δ* mutant. They measured the localization of Pex30(DysF Δ)-GFP and they were seen to localize to the ER membrane but do not accumulate at the LD biogenesis site [40]. Furthermore, they also showed that the DysF domain binds phosphatidic acid (PA) lipids using protein lipid overlay assays. However, the molecular mechanism of the interaction of DysF domain with PA lipids in the ER membrane is still not understood. We used all-atom and coarse-grained molecular dynamics to characterize the interactions between DysF domain and PC:PA lipid bilayers. Our results identify a hydrophobic insertion loop that anchors the DysF domain to the membrane. Through comparative analysis of PA-containing and PA-free membrane systems, we identify stronger binding of dysferlin with membranes that contain PA lipids.

The RHD in Pex30 has also been shown to be essential for LD biogenesis. Pex30 with deletions of the RHD (Pex30(RHD Δ)-GFP) did not rescue the growth defects of the *seilpex30 Δ* mutant. Pex30(RHD Δ)-GFP also did not localize on the ER membrane and was mostly cytosolic. RHD is a highly conserved transmembrane hairpin structure that has been shown to have membrane shaping properties [149]. The hairpin structure made of two short transmembrane domains, transmembrane domain 1 (TMD1) and transmembrane domain 2 (TMD2), that do not span the entire width of the membrane. This acts as a wedge that induces curvature in the membrane. The curvature caused by the RHD could be key factor in aggregation of the Pex30 into puncti. In literature, the RHD in FAM134B, a mammalian ER-phagy receptor, has been shown to sense positively curved regions of the membrane as well as induce membrane deformation [9]. FAM134B RHDs have also been shown to cluster spontaneously to amplify their membrane deformation, even causing spontaneous budding of asymmetric membranes [9, 123]. We used coarse-grained MD simulations to characterize

the deformations when a Pex30 RHD is present in the membrane. Furthermore, we also investigated aggregation of Pex30 RHDs when multiple RHDs are present in the bilayer.

4.2 Methods

4.2.1 All-atom molecular dynamics simulations of the DysF domain

We considered two membrane compositions (pure DOPC and 70:30 DOPC: DOPA) and used the CHARMM-GUI membrane builder to set up lipid bilayers consisting of 720 lipids [45, 46]. The structure of the DysF domain was predicted using ColabFold on the full Pex30 sequence [82]. The N terminus and C terminus of the DysF domain from the ColabFold prediction were patched with acetyl and methylamine groups, respectively, to avoid end effects in simulations. The resulting DysF domain was then inserted into the bilayer using CHARMM-GUI. The positioning of proteins in membranes server was used to determine the initial placement of the protein [69]. All simulations were performed in GROMACS 2018 using the CHARMM36 force field [133, 1, 60, 51]. The temperature was set at 303.15 K to ensure all lipids were in the liquid-disordered state, and a concentration of 0.15 mM KCl was used to neutralize the charge of the system.

The energy of the system was first minimized using steepest descent for 5,000 steps. Subsequently, the system was equilibrated in six stages using the standard equilibration procedure from CHARMM-GUI. During this procedure, the simulation time step was increased from 1 to 2 fs while using the Berendsen thermostat and Berendsen barostat [8]. Subsequently, we simulated the systems for 400 ns using the Nosé-Hoover thermostat and Parrinello–Rahman barostat [90, 39, 98]. The final 200 ns was used for analysis.

4.2.2 Coarse-grained molecular dynamics simulations of the DysF domain

For the coarse-grained molecular dynamics simulations, we used CHARMM-GUI Martini Maker to set up bilayers consisting of 1400 lipids with the same compositions as in the

all-atom simulations [45, 104]. The dysferlin domain was inserted into the solvent phase so that it was not in contact with the bilayer. The shape of the protein was preserved using a stiff elastic network (ElNeDyn) [84]. All simulations were performed in GROMACS 2018 using the Martini forcefield [133, 1, 77, 78]. The temperature was set at 303.15 K, and a concentration of 0.15 mM NaCl was used to neutralize the charge of the system. We used standard Martini parameters for all non-bonded interactions [7]. The energy of the system was first minimized using steepest descent for 5,000 steps. Subsequently, the system was equilibrated in five stages using the standard equilibration procedure from CHARMM-GUI. During this procedure, the simulation timestep was increased from 2 fs to 20 fs while using the velocity-rescaling thermostat and Berendsen barostat [8]. Subsequently, we simulated the systems for 7 μ s using the velocity-rescaling thermostat and the Parrinello-Rahman barostat [98]. The final 2.8 μ s, which was after the dysferlin domain associated with the bilayer, was used for analysis.

Trajectories generated from GROMACS were analyzed in Python using the MDAnalysis package [81, 32]. To analyze contacts, all lipids within a cutoff distance were counted around each residue. The cutoff for contact was set at 4.5 Å and 6 Å for all-atom and coarse-grained simulations respectively. The percentages of each type of lipid in contact were then calculated for residues with an average total lipid contact > 0.5 lipids.

4.2.3 Coarse-grained molecular dynamics simulations of RHD

We used the CHARMM-GUI Martini Maker [45, 104] to set up a model ER lipid bilayer consisting of 1200 lipids. The model lipid bilayer was composed of POPI, DOPS, POPS, DOPE, DOPC, ERGO and DOPA. The structure of the RHD was predicted using ColabFold on the full Pex30 sequence [82]. The resulting RHD was then inserted into the bilayer using CHARMM-GUI. The positioning of proteins in membranes server was used to determine the initial placement of the protein [69]. All simulations were performed in GROMACS 2018 using the MARTINI force field [133, 1, 77, 78]. The LINCS algorithm was used for bonded interactions [37]. Lennard-Jones interactions were cut off at 1.1 nm using the potential-shift-Verlet modifier. Coulombic interactions were also cut off at 1.1 nm using the reaction field method. The temperature was set at 303.15 K to ensure all lipids were in

the liquid-disordered state, and a concentration of 0.15 mM NaCl was used to neutralize the charge of the system. The bilayer was equilibrated using the standard multi-step equilibration procedure from CHARMM-GUI. The equilibration consisted of four stages, where the restraints on the bilayer head groups were relaxed and the simulation time step was increased from 2 fs to 20 fs. During equilibration, the temperature coupling was handled by the velocity-rescaling thermostat [12]. For all equilibration runs, we used isotropic Berendsen pressure coupling [8] with a reference pressure of 1 bar and compressibility of $3 \times 10^{-4} \text{ bar}^{-1}$. In production runs, we used the velocity-rescale thermostat and the Parrinello-Rahman barostat [98]. The production run was simulated for 6 μs .

For simulations with 4 RHDs in the membrane, the initial system with 1 RHD in membrane was replicated 4 times and combined. The resulting system was subsequently equilibrated again using the standard multi-step equilibration procedure from CHARMM-GUI. The production run was simulated for 20 μs .

Trajectories generated from GROMACS were analyzed in python using the MDanalysis package [81, 32]. PyMOL was used to create snapshots of simulations.

4.3 Results and discussion

4.3.1 PA recruits Pex30 by interactions with dysferlin domain

We conducted all-atom MD simulations to characterize interactions between the DysF domain and lipid bilayers with and without PA. We considered a pure DOPC bilayer and a mixed bilayer with 70% DOPC and 30% DOPA. The DysF domain was anchored to the lipid bilayer by two regions (4.1). The region with the deepest insertion into the bilayer and the most lipid contacts consisted of a sequence of four hydrophobic residues (299–302) flanked by tryptophan residues (298 and 303), which have propensity to reside near the lipid-water interface. The other residues in this region (residues 296–315) tended to reside closer to the bilayer surface and have less sustained lipid contact. The second region (residues 379–397) maintained the most contact around a hydrophobic phenylalanine residue, flanked by polar and charged residues (390–395). The membrane-anchoring regions were the same

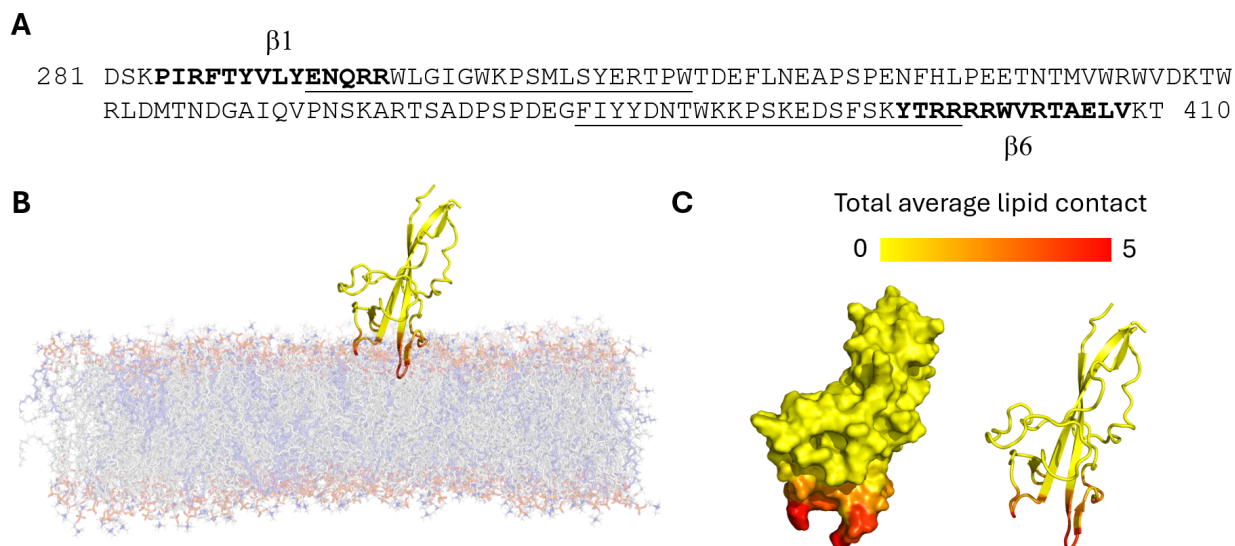


Figure 4.1: (A) Amino acid sequence of the DysF domain, with residues involved in membrane anchoring underlined (B) Snapshot from a simulation of the DysF domain with a 70:30 DOPC:DOPA bilayer. The tails of DOPC and DOPA are shown in grey and blue, respectively. (C) Surface and cartoon representation of the DysF domain. Each residue is colored based on its average number of lipid contacts, with a contact defined as a lipid within 4.5 Å of the residue.

for both membrane compositions, but there was an increase in the total number of lipid contacts with the protein when the bilayer contained PA (Figure 4.2, A and B). This was particularly evident for two positively charged arginine residues (296 and 297) in the first region. The second region also had elevated lipid contact in the PC:PA bilayer. For the bilayer containing PA, we further characterized the propensity of each residue to bind PA versus PC by determining the fraction of total lipid contacts with PA (Figure 4.2 C–E). Residues with contact percentage exceeding 30% exhibit more binding with PA than expected based on randomly distributed lipids. The contact of residues with the phosphate group of lipids, which is indicative of interactions with head groups, shows the most pronounced enhancement of PA interactions with DysF domain (Figure 4.2 D). Subsequent studies have corroborated these findings, identifying similar regions responsible for membrane binding of DysF [26].

From our all-atom MD simulations, we identified residues 296–315 and 379–397 as the regions responsible for membrane binding in DysF domain. To verify that our results were

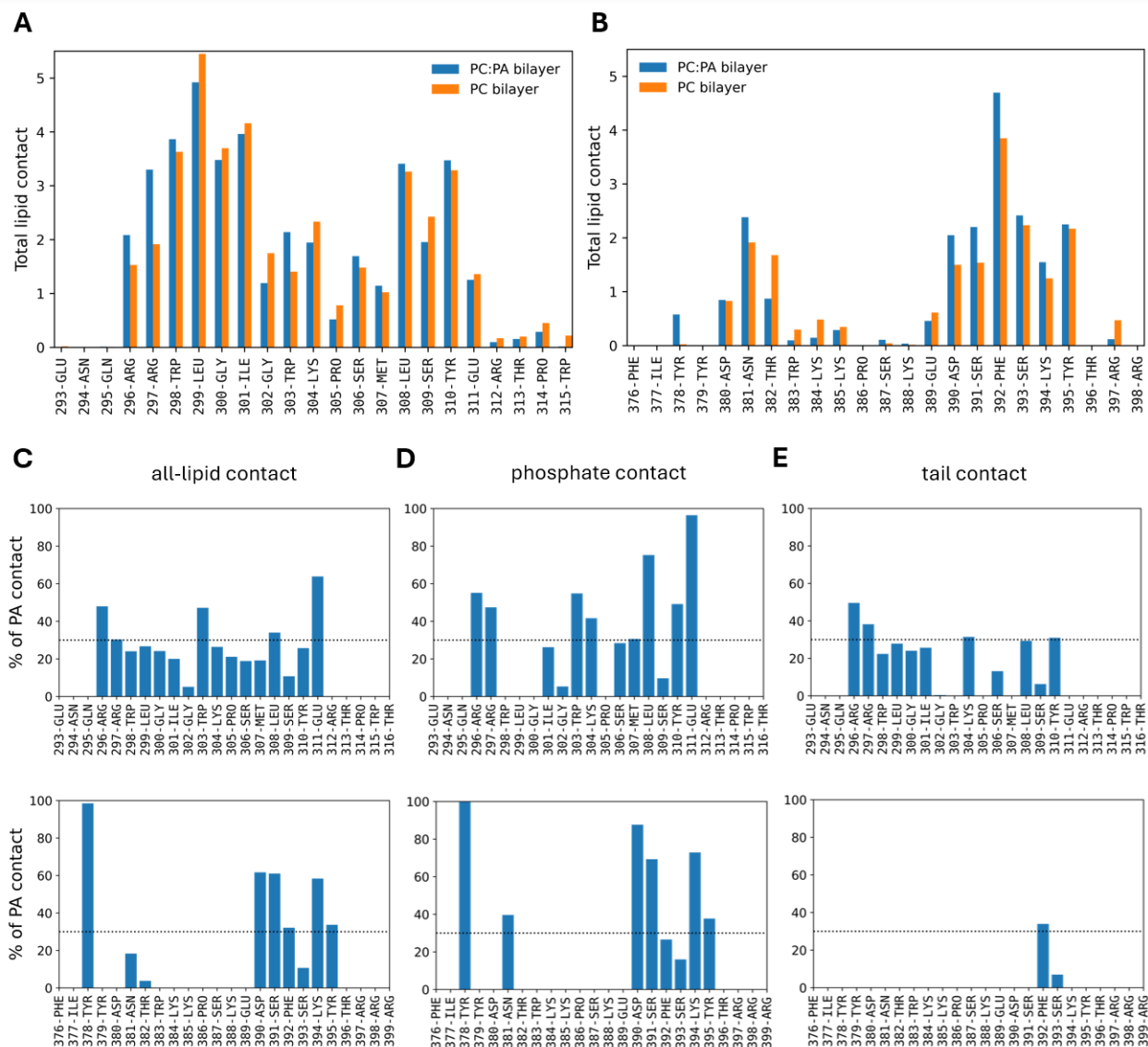


Figure 4.2: (A and B) Average number of lipids in contact with each residue of the DysF domain in all-atom simulations. Results for both pure DOPC and 70:30 DOPC:DOPA bilayers are shown. Only regions containing residues with lipid contacts are displayed. (C–E) Percentage of DOPA lipid contacts for each residue in the 70:30 DOPC:DOPA bilayer. Results are shown only for residues that have an average total lipid contact > 0.5 ; no bar is shown otherwise. The horizontal dashed line denotes the overall composition of DOPA in the membrane. The panels distinguish between contacts with all atoms of the lipid (C), only atoms of the phosphate group (D), and only atoms in the tail (E). All contact analysis was conducted over 200 ns of simulation.

not biased by the initial membrane-bound configuration, we performed additional unbiased coarse-grained MD simulations over extended timescales, starting with the DysF domain positioned outside the membrane. We considered a pure DOPC bilayer and a mixed bilayer with 70% DOPC and 30% DOPA to compare with our all-atom simulations. In both cases the DysF domain spontaneously associated with membrane and exhibited similar membrane anchoring regions to the all-atom simulations (Figure 4.3). The increase in the total number of lipid contacts with the protein when the bilayer contained PA is however not observed in our coarse-grained simulations (Figure 4.4 A and B). We hypothesized that the enhanced lipid contacts of DysF with PA containing bilayers in all-atom simulations result from electrostatic interactions between positively charged residues and negatively charged PA headgroups. The coarse-grained MARTINI model used has inherent limitations, including simplified non-polarizable water molecules and implicit screening interactions that poorly represent charge-based interactions [76]. These model limitations may explain why such enhanced contacts with PA containing bilayers were not observed in the coarse-grained simulations.

Using the results from our MD simulations, our experimental collaborators deleted the two regions that were membrane-associating to generate GFP-tagged Pex30 (296–315 Δ) and Pex30 (378–398 Δ). Both of these truncated Pex30 failed to aggregate at the ER-LD contact sites enriched with PA compared to wild type Pex30. Furthermore, these truncations do not rescue the growth defect of *seilpex30 Δ* mutant, suggesting these residues are essential for Pex30 function. These results together indicate that PA recruits Pex30 at the ER subdomain by interacting with these two regions in the dysferlin domain (residues 296–315 and 379–397).

4.3.2 Pex30 Reticulon homology domain induces curvature in ER membranes

The RHD in Pex30 has also been shown to be essential for LD biogenesis along with the DysF domain. The transmembrane loops in the Pex30 RHD, a short hairpin-like structure that does not fully span the thickness of the membrane. These hairpin structures function as molecular wedges, asymmetrically expanding one membrane leaflet relative to the other, thereby inducing local membrane curvature that is required for LD biogenesis.

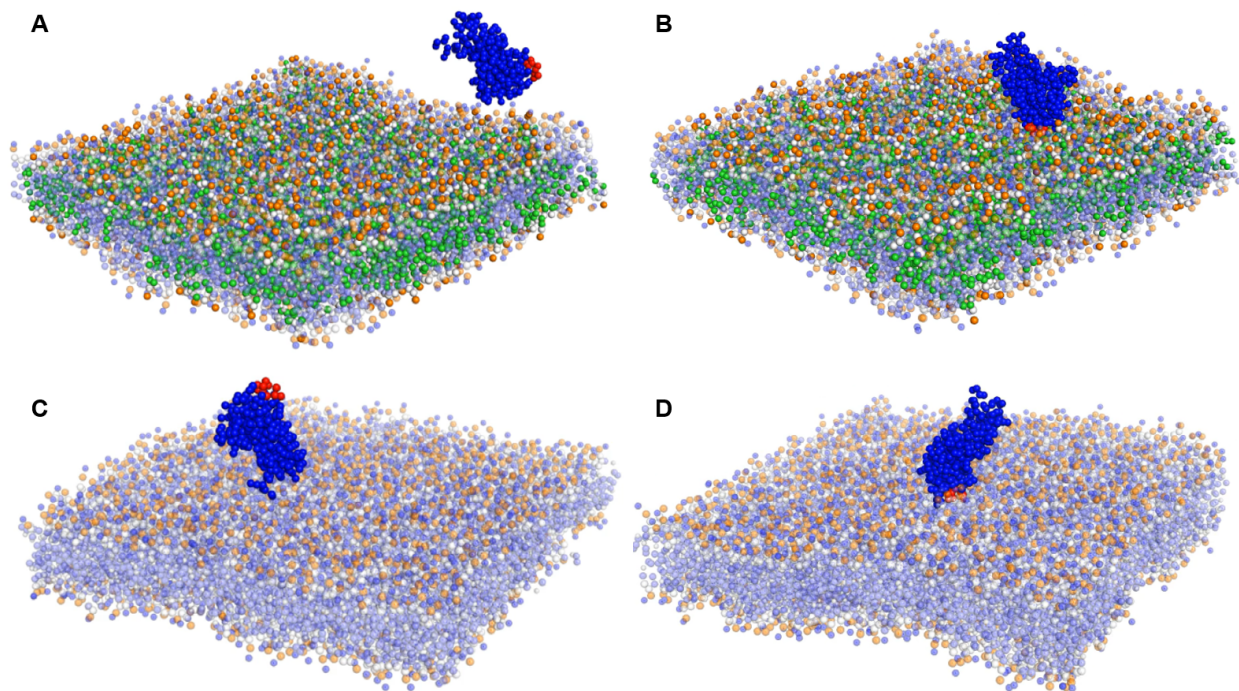


Figure 4.3: Spontaneous association of DysF domain to membranes in coarse-grained simulations. The DysF domain is shown as blue beads with residues 296–315 and 379–397 shown in red. The aliphatic tails of DOPC and DOPA are represented in blue and green respectively. (A and B) shows the DysF and a 70:30 DOPC:DOPA bilayer at 0 and 6 μ s respectively. (C and D) shows the DysF and a pure bilayer at 0 and 6 μ s respectively. In both cases, the DysF domain spontaneously associates into the membrane via the regions earlier identified in the all-atom simulations.

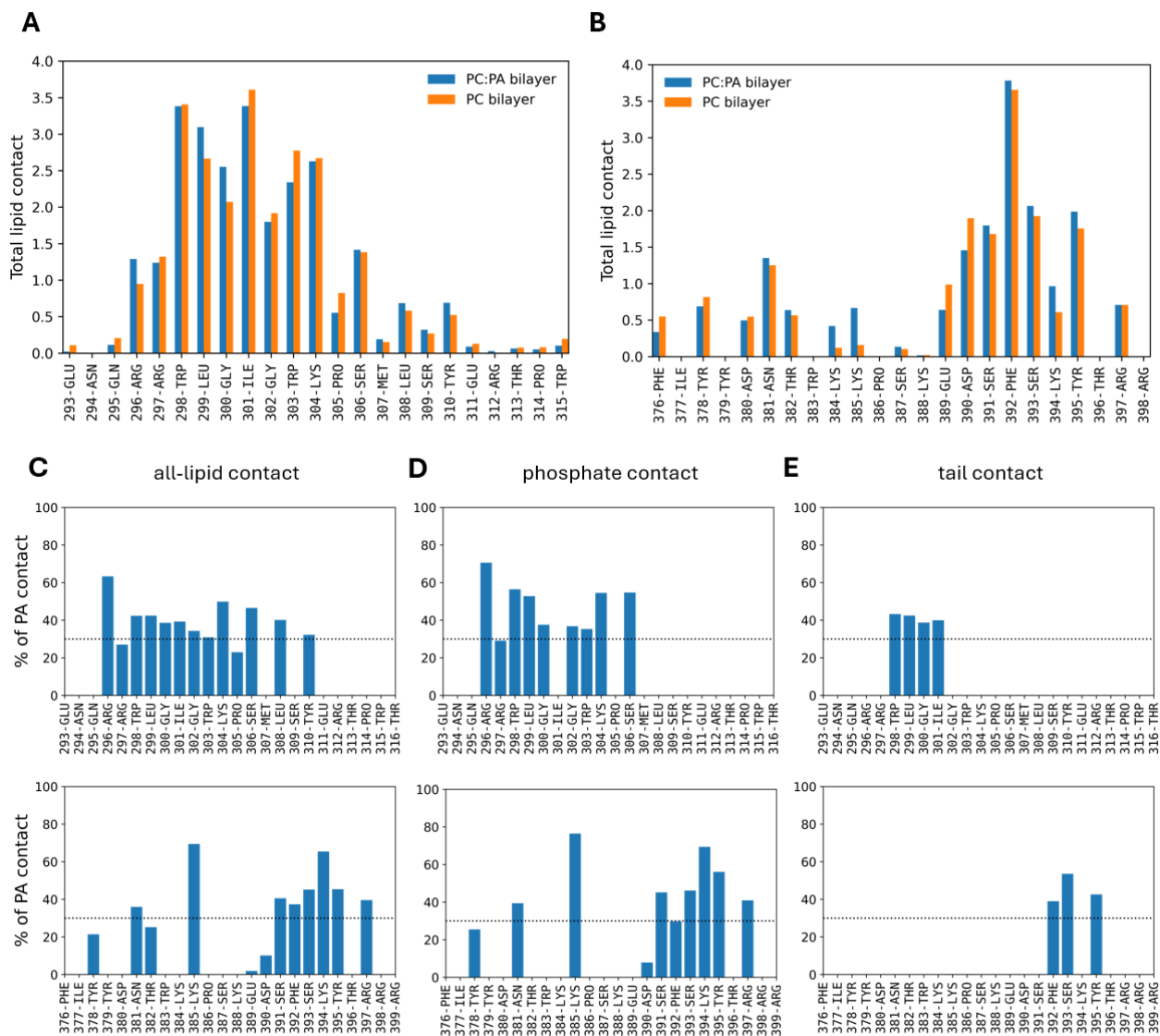


Figure 4.4: (A and B) Average number of lipids in contact with each residue of the DysF domain in CG simulations after insertion into the membrane. Results for both pure DOPC and 70:30 DOPC:DOPA bilayers are shown. Only regions containing residues with lipid contacts are displayed. (C–E) Percentage of DOPA lipid contacts for each residue in the 70:30 DOPC:DOPA bilayer. Results are shown only for residues that have an average total lipid contact >0.5 ; no bar is shown otherwise. The horizontal dashed line denotes the overall composition of DOPA in the membrane. The panels distinguish between contacts with all atoms of the lipid (C), only atoms of the phosphate group (D), and only atoms in the tail (E). All contact analysis was conducted over $2.8 \mu\text{s}$ of simulation.

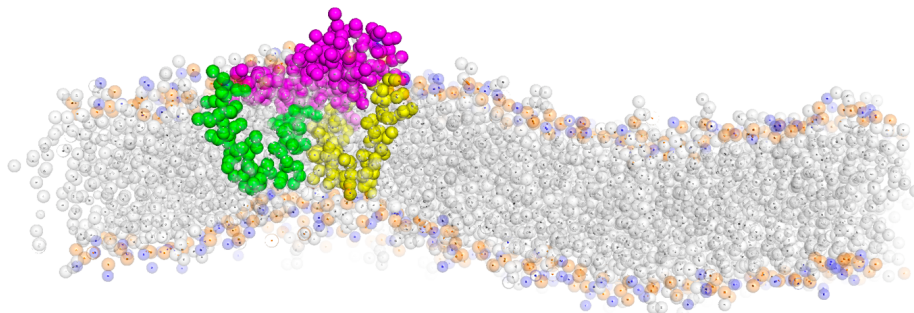


Figure 4.5: Snapshot of coarse-grained simulation of RHD in model ER bilayer. The transmembrane loops TMD1 and TMD2 of the RHD are shown in yellow and green respectively.

We first sought to characterize a single RHD in a model ER bilayer. Consistent with a wedge mechanism, our simulations demonstrate deformations in the membranes in regions surrounding the RHD. Figure 4.6 shows the locations of the RHD superimposed on a heatmap indicating the deviation of the membrane from its average position in the out-of-plane dimension (Δz). Positive height deviations of the membrane in the vicinity of RHD, coupled with negative height deviations at more distant locations, indicate the induction of positive membrane curvature around the RHD.

For large-scale membrane remodeling that is required for LD biogenesis, clustering of Pex30 proteins would be necessary to generate the membrane deformation required. To investigate the clustering of Pex30, we characterized the behavior of four RHDs embedded in an ER membrane. We initially positioned the RHDs at distant locations from one another and ran an unbiased MD simulation. We observed that two of the four RHDs (domains 0 and 3) spontaneously aggregated at approximately 7 μs and remained aggregated throughout the remainder of the simulation. This clustering likely results because it reduces the membrane deformation area and thus is energetically favorable. Figure 4.8 presents snapshots showing the clustered RHD configuration. The RHDs aggregate such that TMD1 of the first RHD interacts with TMD2 of the other RHD, while TMD2 of the first RHD

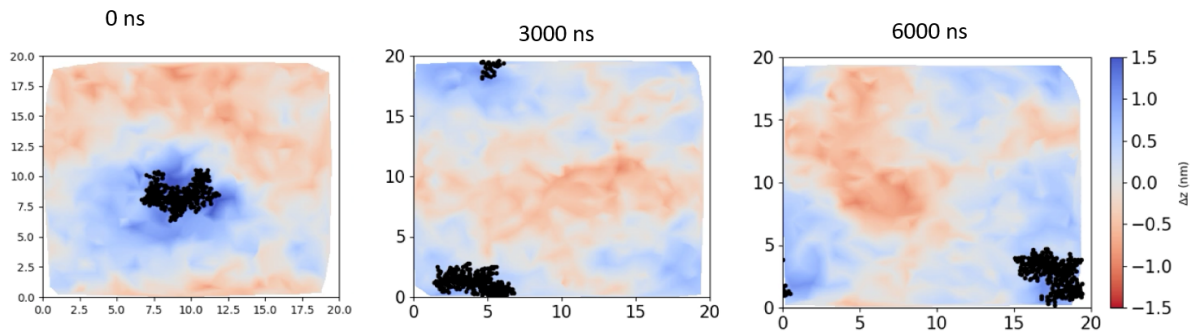


Figure 4.6: Top-down view of Pex30 RHD and deviations of the membrane height. Δz denotes the deviation of the membrane from its average position in the out-of-plane dimension. The black spheres represent the RHD. (A), (B) and (C) show snapshots at 0, 3 and 6 μs respectively.

correspondingly interacts with TMD1 of the second RHD. Figure 4.8 displays the time evolution of pairwise distances between the center of mass of each RHD. Following their initial association, RHDs 0 and 3 maintained a distance of approximately 4 nm throughout the simulation after association, which indicates an effective attraction between them. The lack of aggregation observed for the other two RHDs in our simulation can be explained by insufficient simulation time for thermally-driven random motion to bring the particles close enough for strong attractive interactions to occur.

4.4 Conclusions

Experimentally, both the DysF domain and the RHD in Pex30 have been shown to be essential in LD biogenesis. In this work, we used all-atom and coarse-grained molecular dynamics to investigate the roles of DysF and RHD at the molecular level.

Our simulations showed that the DysF domain binds to membranes via two regions (residues 296–315 and 379–397) that consist of hydrophobic insertion loops flanked by polar or charged residues. Lipid contact analysis of the DysF domain showed that DysF bound more strongly to bilayers containing PA lipids due to charge-based interactions of negatively charged PA lipids with polar and positively charged residues that flanked the hydrophobic insertion loops. These results support our experimental collaborator’s findings that show

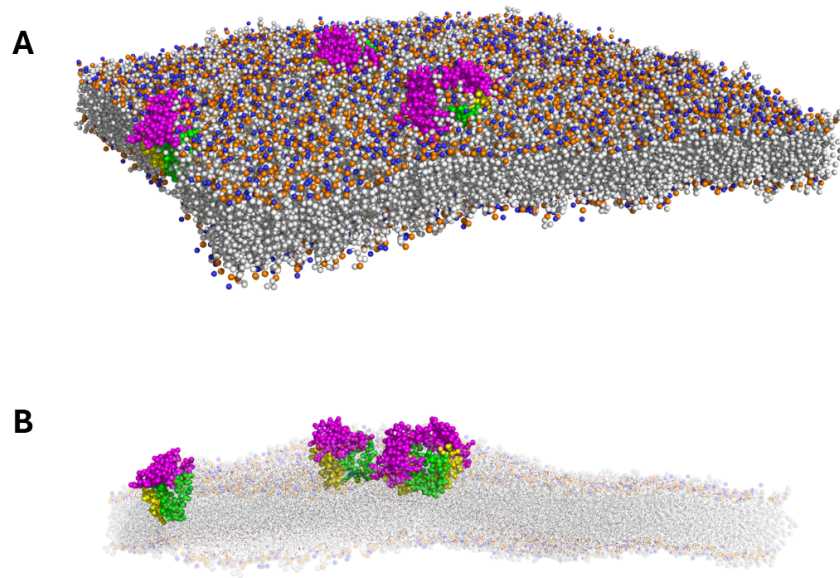


Figure 4.7: Clustering of RHDs in ER membrane. (A) and (B) shows shows two different views of the system where two RHDs have aggregated. The transmembrane loops TMD1 and TMD2 of the RHD are shown in yellow and green respectively. The RHDs aggregate such that TMD1 of the first RHD interacts with TMD2 of the other RHD, while TMD2 of the first RHD correspondingly interacts with TMD1 of the second RHD.

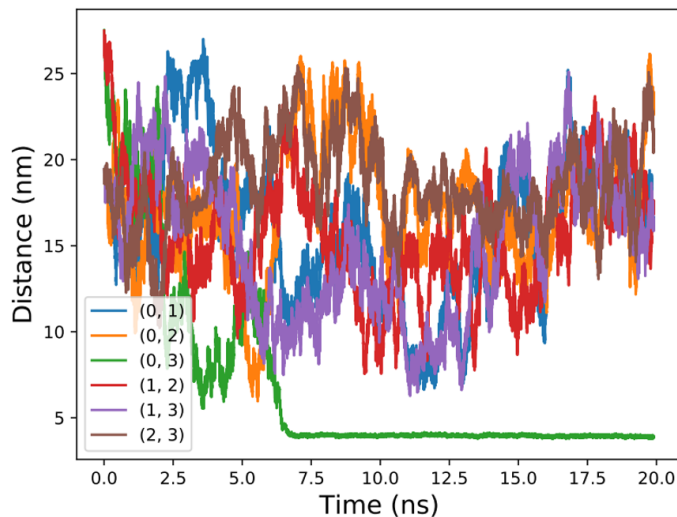


Figure 4.8: Pairwise distance between the center of masses of the 4 RHDs in the ER membrane as a function of time. RHDs 0 and 3 maintained a distance of approximately 4 nm throughout the simulation after association.

Pex30 localized at the LD-biogenesis site by interactions of the DysF domain with PA-enriched membranes.

Our simulations of the Pex30 RHD showed that their molecular wedge-like structure caused them to induce curvature in membranes. They also aggregate spontaneously when multiple RHDs are present in the membrane. Spontaneous aggregation of RHDs explain the accumulation of Pex30 as puncti at the LD biogenesis site. Future work to run longer simulations of multiple RHDs on a bilayer would shed light on the formation of large scale clusters and their impact on membrane shape. Further, using umbrella sampling, the effective membrane-mediated attractive force between RHDs could be estimated as a function of distance between them in the bilayer.

Chapter 5

Particle deformability enables control of membrane-mediated forces

Material in this chapter was adapted from:

Nambiar, N., Loyd, Z.A. and Abel, S.M., 2024. Particle deformability enables control of interactions between membrane-anchored nanoparticles. *Journal of Chemical Theory and Computation*, 20(4), pp.1732-1739. [88]

The simulation work described in this chapter was designed and performed by Nikhil Nambiar under the advisement of Steven Abel.

5.1 Introduction

The adsorption of nanoparticles onto a lipid bilayer can cause local deformations of the membrane. The local curvature induced by the particle causes an increase in the bending energy of the membrane, and the deformation field of one particle can interact with deformations caused by other nanoparticles in its vicinity [106]. These membrane-mediated interactions can lead to effective interactions between the nanoparticles. In some cases, the interaction of membrane deformations can lead to attractive interactions between particles. This can promote their self assembly [114], and in some cases, lead to large-scale deformations of the membrane [30, 112, 113, 125]. Physically, the attraction between particles arises largely due to the total bending energy of the membrane being lower when the particles aggregate.

Studies of membrane-mediated interactions between rigid nanoparticles have characterized how the membrane bending energy depends on the distance between particles, thus leading to a membrane-mediated force between particles that changes as a function of distance [107, 2, 3, 144]. For example, Reynwar et al. showed that spherical colloids experience an attractive force when sufficiently close due to changes in the bending energy of the membrane [107]. Membrane-mediated interactions can facilitate self-assembly of particles [4], and it has been shown that spherical particles adsorbed to planar and vesicular membranes can form linear aggregates and induce tubulation of the membrane [112, 113, 2]. For anisotropic particles, membrane-mediated interactions depend on the relative orientation of the particles [118, 30, 100], and changing the particle shape or properties of the membrane can lead to assembly in different configurations [125, 93]. Curved particles have been of particular interest, in part due to their similarity to BAR proteins, and can induce curvature that leads to mutual interactions, self-assembly, and membrane sculpting [89, 93, 124].

DNA origami nanoparticles have emerged as a valuable tool to study the interactions of membranes and nanoparticles [139]. DNA origami can be designed with a wide range of well-defined shapes, and they can be readily modified to incorporate cholesterol tags that anchor the particles to membranes. Rigid DNA origami structures assemble on membranes [53, 18], and curved DNA origami structures have been shown to induce morphological changes of membranes [33, 29]. DNA origami with cholesterol anchors can also produce synthetic pores in lipid membranes [31, 55, 6] and act as nanoscale sensors to measure membrane surface charges [92] and transmembrane potentials [91].

An exciting recent development is the design of deformable DNA origami nanostructures with controllable mechanical features [147]. DNA origami hinges are an example in which the stiffness of the hinge can be controlled by small changes in the design [147, 74]. Moreover, DNA origami hinges have been designed to be actuated by temperature, ions, and magnetic fields [73, 56, 132]. They also have applications as nanoscale devices, as demonstrated recently with cholesterol-anchored, flexible DNA hinges that act as nanoscale curvature sensors [11].

Deformable DNA origami nanoparticles open new avenues for studying particle-membrane interactions and resulting membrane-mediated interactions between particles. This is

because the particles and membrane can mutually deform one another, introducing new complexities into the problem. However, there have been few efforts to date that illuminate the impact of particle deformability [65]. Previous studies exploring interactions between semiflexible polymers and membranes revealed a complex interplay between deformations of the polymer and membrane [83, 63]. Additionally, aggregation of ligand-coated nanoparticles has been rationalized in terms of both shell deformability and membrane curvature [109, 58, 57]. We recently used continuum models and Monte Carlo simulations to study hinge-like particles adhered to an elastic membrane via a short-ranged attractive potential. We showed that strong adsorption promotes attractive, membrane-mediated interactions between particles, and that configurations of aggregates are influenced by hinge stiffness [64]. However, interactions of deformable, cholesterol-anchored particles in realistic membrane systems have not yet been explored.

We use coarse-grained molecular dynamics simulations to investigate hinge-like nanoparticles that interact with a realistic lipid bilayer by means of cholesterol anchors. The purpose of the study is to capture the key physical characteristics of a hinge-like particle and systematically understand the impact of the stiffness of the hinge on particle-membrane and particle-particle interactions. We first briefly introduce the model and investigate isolated particles, both in water and in contact with a lipid bilayer. We then characterize two particles on a membrane using unbiased simulations before using umbrella sampling to quantify the effective interaction between two particles. We conclude by discussing the cooperative behavior observed when particles aggregate and how a new degree of freedom — the mechanical properties of a deformable particle — can be used to control membrane-mediated interactions between particles. Simulation details can be found at the end.

5.2 Methods

5.2.1 Model of the hinge-like particle

We created a coarse-grained nanoparticle inspired by DNA origami hinges. The model was not intended to represent an actual DNA origami particle, but to capture key physical

characteristics in a manner that allowed us to systematically investigate its behavior when anchored to a membrane. The size of the particle was smaller than existing DNA origami hinge designs, which have longer arms, for computational tractability. The primary effect of size is to change the scale of the membrane deformations but not the underlying physics of the membrane-mediated interactions.

The nanoparticle consisted of two stiff arms connected at an angle to form a hinge as shown in Figure 5.1. Each of the arms consisted of 3 rows of 6 beads. The beads in each row were spaced 0.935 nm apart, which was sufficiently close to ensure that the hinges did not interpenetrate during simulations. The rows were spaced 2 nm apart, which is consistent with the diameter of a DNA double helix. All beads in the arms were connected in a rectangular lattice via harmonic bonds with force constant 12,500 kJ mol⁻¹ nm⁻². Cosine-based angle potentials were also imposed, with all 90° angles having a bending constant of 25,000 kJ/mol and all 180° angles having a bending constant of 250,000 kJ/mol.

Cholesterol anchors were included by incorporating the Martini SP1 bead (representing the portion of cholesterol containing the hydroxyl group) into the arms of the nanoparticle at the ends and center of rows 1 and 3. The rest of the beads in the arm were Martini beads of type Q0, which were used to mimic the phosphate linkages on the outside of a DNA double helix. A cosine-based angle potential was used to set the hinge stiffness: $V_a(\theta) = k(\cos(\theta) - \cos(\theta_0))^2/2$. The natural angle of the hinge (θ_0) was chosen to be 120°, and the force constant for the cosine potential was varied from 0 to 1000 kJ/mol. This potential was used for the angle defined at the middle bead along each of the three rows of beads.

The topology file for the hinge-like particle is included as Supporting Information.

5.2.2 Simulation details

In the MD simulations, we used standard Martini parameters for all non-bonded interactions [21]. The LINCS algorithm was used for bonded interactions [37]. Lennard-Jones interactions were cut off at 1.1 nm using the potential-shift-Verlet modifier. Coulombic interactions were also cut off at 1.1 nm using the reaction field method. All systems were solvated with standard Martini water, without utilizing anti-freeze beads. All MD simulations were performed in GROMACS 5.1.2 [133, 1]. The temperature was 303.15 K.

For simulations of the hinge-like particle in water without a lipid bilayer, we solvated the particle in a $24 \text{ nm} \times 24 \text{ nm} \times 24 \text{ nm}$ box. The system consisted of 109,221 beads. First, energy minimization using steepest descent was performed for 5,000 steps. Subsequently, the system was equilibrated in four stages while increasing the simulation timestep from 2 fs to 20 fs. During equilibration, the temperature coupling was handled by the velocity-rescaling thermostat [12]. For all equilibration runs, we used isotropic Berendsen pressure coupling [8] with a reference pressure of 1 bar and compressibility of $3 \times 10^{-4} \text{ bar}^{-1}$. In production runs, we used the velocity-rescale thermostat and the Parrinello-Rahman barostat [98]. The production runs were simulated for $2 \mu\text{s}$ and the final $0.5 \mu\text{s}$ was used for analysis.

For unbiased simulations with hinge-like particles and a lipid bilayer, we used the CHARMM-GUI Martini Maker [45, 104] to first set up a solvated bilayer with a composition of 1:1 DOPC:DPPC. The bilayer size was $25 \text{ nm} \times 25 \text{ nm}$ (2,000 lipids) and $50 \text{ nm} \times 50 \text{ nm}$ (7,536 lipids) for simulations with one and two hinges, respectively. The dimension orthogonal to the lipid bilayer in both cases was 24 nm. The bilayer was equilibrated using the standard multi-step equilibration procedure from CHARMM-GUI. The equilibration consisted of four stages, where the restraints on the bilayer head groups were relaxed and the simulation time step was increased from 2 fs to 20 fs.

One or more hinge particles were then inserted at random locations in the water phase by replacing water beads. The system with one hinge consisted of 132,283 beads; the system with two hinges consisted of 455,019 beads. The standard multi-step equilibration process from CHARMM-GUI was again used for energy minimization and equilibration. MD simulations were run to allow the hinge(s) to diffuse into contact with the lipid bilayer. Once the cholesterol anchors inserted into the lipid bilayer, the system was equilibrated for an additional $2.5 \mu\text{s}$.

Trajectories generated from GROMACS were analyzed in python using the MDanalysis package [81, 32]. PyMOL was used to create snapshots of simulations.

5.2.3 Umbrella sampling

We used umbrella sampling methods to determine the potential of mean force (PMF) as a function of the distance between the centers of mass of the two membrane-anchored particles

in the xy -plane. The final snapshot of the unbiased simulation with hinge stiffness 1000 kJ/mol was used as the initial input. For each hinge stiffness, this snapshot was used as the initial configuration and equilibrated for 1 μ s while using a harmonic potential of 1000 kJ mol⁻¹ nm⁻² centered at the prescribed distance between their centers of mass. The hinges were then pulled away from each other in the plane of the membrane (xy -plane) to generate initial snapshots for the umbrella windows. To do this, the natural distance of the harmonic potential was increased at a rate of 0.001 nm/ps. We used a window spacing of 0.45 nm to choose initial configurations and then performed simulations for each window with a force constant of 100 kJ mol⁻¹ nm⁻² for the harmonic umbrella potential. Each umbrella window was simulated for 1 μ s, and the last 0.5 μ s was used to calculate the PMFs. To sample configurations when the hinges were close together, we repeated the same procedure by creating snapshots while pushing the particles closer together. Data from each hinge stiffness was analyzed using the weighted histogram analysis method (WHAM) [54] implemented in GROMACS [42]. A correction term [131, 16] of $k_B T \ln(R)$, where R is the distance between centers of mass in the xy -plane, was added to the PMF obtained from WHAM to account for the size of state space being a function of R . Estimates of statistical errors for the PMFs were obtained using the Bayesian bootstrap method on complete histograms implemented in g_wham [42]. The method was used to generate 50 hypothetical PMFs for the error analysis.

5.3 Results and discussion

5.3.1 Isolated hinge-like particles

We first sought to characterize the deformable, hinge-like particle in water without a lipid bilayer present. To this end, we ran unbiased simulations to evaluate equilibrium behavior at different values of the hinge stiffness (k). Figure 5.2 shows the distributions of angles sampled by the hinge. At large values of k , the distributions are centered at the natural angle of 120°. The width of the distribution increases as the stiffness decreases, indicating that the hinge samples a broader distribution of angles when the angular potential is weaker, as expected.

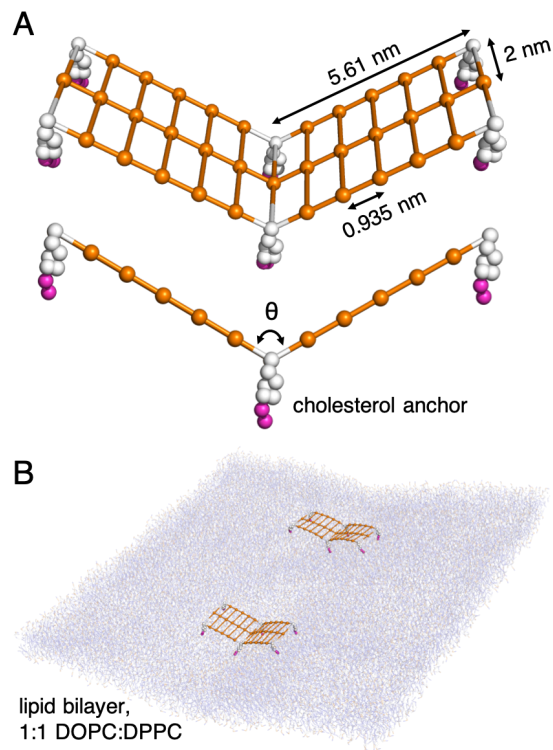


Figure 5.1: Schematic of the model. (A) Two views of the coarse-grained nanoparticle. Two “arms” are connected in a hinge-like configuration with a natural angle of 120° . The stiffness of the hinge is systematically varied. Six cholesterol anchors (white and fuchsia) serve to anchor the particle to a lipid bilayer. (B) Snapshot showing two particles anchored to a lipid bilayer.

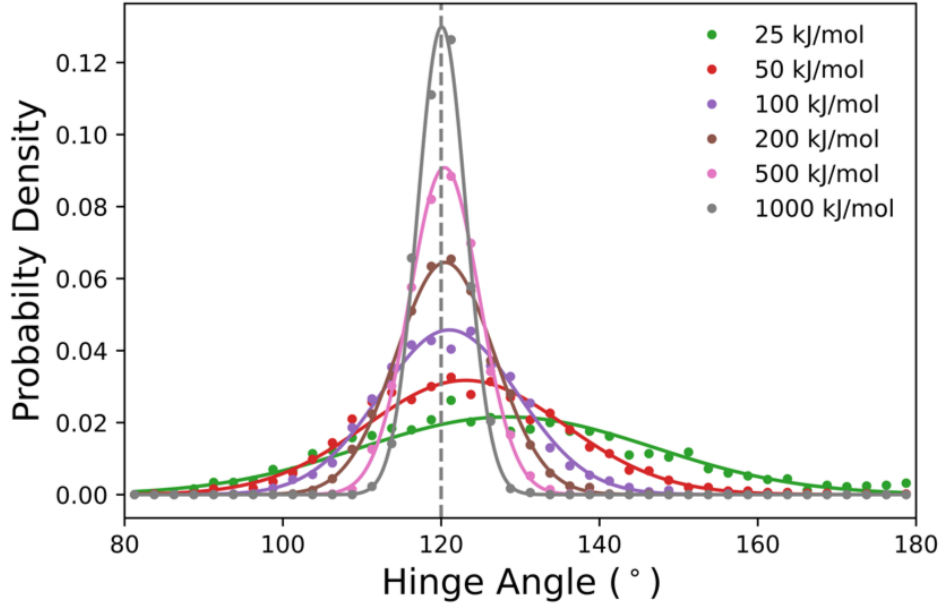


Figure 5.2: The probability density of the hinge angle (θ) at equilibrium for a hinge in water (no bilayer). Different values of the hinge stiffness (k) are shown. The vertical dashed line corresponds to the natural angle of the hinge (θ_0). Solid lines are Gaussian fits to the data (circles) meant to guide the eye.

For smaller values of k ($\lesssim 25$ kJ/mol), the distribution of angles shifts toward larger angles. This corresponds to more open configurations of the hinge. The effect is modest for $k = 25$ kJ/mol (Figure 5.2), and the effect is most pronounced when $k = 0$ (Figure S1). For this case, the maximum of the distribution is at 180° , which is likely due to electrostatic repulsion between charged beads constituting the arms of the hinge. The open configuration reduces unfavorable interactions, but the effect is sufficiently weak that only the most flexible hinges are substantively impacted. We also assessed whether the presence of cholesterol anchors impacted the distribution of hinge angles by conducting simulations in which the cholesterol anchors were removed. The removal of the anchors resulted in only small changes to the angles sampled by the hinge (Figure 5.3), indicating that they did not significantly perturb the behavior of the particle in water.

5.3.2 Stiff hinges deform membranes

To characterize the behavior of membrane-anchored particles, we first studied an isolated particle with its cholesterol anchors inserted in a lipid bilayer. We used unbiased simulations

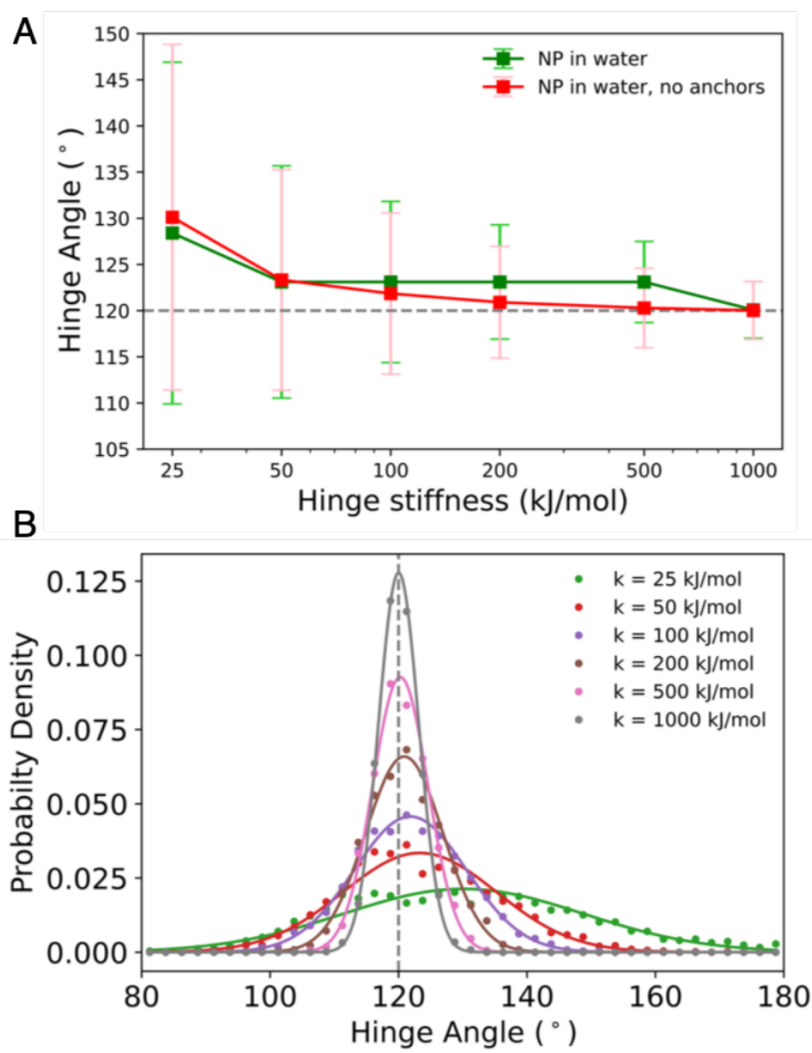


Figure 5.3: (A) Comparison of the angles sampled by isolated hinges in water with and without cholesterol anchors. (B) Probability density of the hinge angle (θ) at equilibrium for a hinge in water without cholesterol anchors. Different values of the hinge stiffness (k) are shown. Solid lines are Gaussian fits to the data (circles) meant to guide the eye. The dashed lines correspond to the natural angle of the hinge.

to assess how the membrane impacts configurations adopted by the hinge. Figure 5.4 shows sample configurations for hinges with different values of the bending stiffness. At a small value ($k = 25$ kJ/mol), the hinge appears nearly flat as it conforms to the flat lipid bilayer. For larger values, the hinge no longer appears flat, and it adopts configurations with angles closer to the natural angle of the hinge (120°). This coincides with a large-scale deformation of the lipid bilayer in the region near the hinge. The hinge is closest to its natural angle for the largest value of k . We also considered the effect of removing the cholesterol anchors from the particle. When they were removed, the particle did not remain associated with the lipid bilayer (Figure 5.5), indicating that the cholesterol anchors, and not the beads comprising the particle, are essential for promoting strong adhesion to the membrane.

Figure 5.6 shows the influence of the membrane on configurations of the hinge using the distribution of angles sampled by a membrane-anchored particle. Weaker hinges ($k \leq 100$ kJ/mol) are significantly deformed by the membrane, with an average angle of approximately 170° . In this regime, the hinge is flexible enough that it is energetically favorable for it to adopt a significantly deformed flat configuration while maintaining a relatively flat membrane shape. In contrast, stiffer hinges ($k \geq 200$ kJ/mol) adopt configurations that are closer to the natural angle of the hinge. The distribution of angles for the stiffest hinges ($k = 500$ and 1000 kJ/mol) are only slightly shifted compared with the distributions in water, while the intermediate stiffness ($k = 200$ kJ/mol) is intermediate between the natural angle and the fully open hinge. In this regime, the membrane conforms to the particle shape, resulting in particle-scale deformations of the membrane. Thus, membrane deformations can be controlled by the stiffness of the hinge.

5.3.3 Membrane-anchored hinges spontaneously aggregate

Studies of rigid nanoparticles have shown that membrane deformations induced by their adsorption can lead to effective interactions between the adsorbed particles. The results above demonstrate that the model hinge-like particle can induce deformations of a lipid bilayer, and that the extent of the deformation can be controlled by the stiffness of the hinge. Thus, we expect the hinges to experience deformation-mediated interactions. However, in

contrast with rigid particles, the presence of more than one hinge can lead to cooperative effects in which hinges mutually influence one another’s configurations.

To investigate membrane-mediated interactions between particles, we first conducted unbiased simulations of two hinges anchored to the lipid bilayer. Figure 5.7 shows the distance between the centers of mass of the two hinges as a function of time for various values of the hinge stiffness. We considered two sets of initial configurations: one in which the hinges started far apart, and one in which they started close together. When starting far apart (Figure 5.7A), the distance between particles fluctuates but, for most cases, eventually exhibits a rapid decrease to a stable plateau of ≈ 5 nm. This distance is consistent with them being side-by-side in close proximity. When starting close together (Figure 5.7B), the hinges stay in close proximity for all hinge stiffnesses, again indicating an effective attraction. These results suggest that, when sufficiently close, the two particles experience an effective attraction that causes them to aggregate. For particles started far apart, multiple independent trajectories (Figure 5.7C) exhibit substantial variability in the time at which the two particles come into close contact. This variability is consistent with thermally-driven, random motion leading to a stochastic waiting time until the two particles are sufficiently close to strongly attract.

Figure 5.8 shows snapshots taken from two simulation trajectories with weak and strong hinges, respectively. The locations of the hinges are superimposed on a heatmap indicating the deviation of the membrane from its average position in the out-of-plane dimension (Δz). This demonstrates the deformations induced by the hinges. The stiffer hinges induce larger deformations of the membrane, and the furrow-like nature of the deformation can be seen by the extension of the red color ($\Delta z < 0$) orthogonal to the direction of the arms of the hinge. For the stiff hinges, the deformation appears more localized around the particles when they aggregate.

The overall energy of the system is impacted by both the energy of deforming the hinges and the energy of deforming the membrane; the previous results suggest that it is energetically favorable for the two hinges to be in close proximity. To assess cooperative effects when two hinges are present, we analyzed the angles sampled by two membrane-anchored hinges when they started in close proximity (corresponding to Figure 5.7B). Figure

5.9 compares the behavior of two aggregated hinges to an isolated hinge anchored to the bilayer. When two hinges are in close proximity, the average angle of the hinge decreases, reflecting a configuration of the hinge that is closer to its natural angle of 120° . The cooperative effects are most pronounced at intermediate values of k . When k is small, the hinges remain almost completely flat and induce relatively little membrane deformation whether there are one or two hinges. When k is large, a hinge is sufficiently stiff to significantly deform the membrane, whether on its own or in conjunction with another hinge. At intermediate values of k , two hinges together allow the hinges to share the energetic cost of deforming the membrane, and each adopts a configuration closer to its natural hinge angle. Two hinges together thus cause a larger deformation than when only one isolated hinge is adsorbed to the membrane.

We have rationalized the aggregation of hinges in terms of lowering the energetic cost of deforming the membrane. It is possible that adhesion of the particles could promote aggregation through other changes to the membrane, such as lateral organization of lipids or a change in the phase of the bilayer [13]. However, we have not observed any notable changes in the membrane properties upon adhesion of the particles. Without anchored particles, the lipid bilayer is in the liquid disordered state [14]. Comparing simulations of bilayers with and without anchored particles revealed no substantial differences in the area per lipid or the average number of specific neighbors of DOPC and DPPC. Further, in the region of the bilayer near the particles, the composition is approximately that of the bulk, the average number of specific neighbors of a given lipid type does not change substantially, and visual inspection of snapshots (Figure 5.4) does not reveal ordering of the lipid tails. This evidence suggests that with anchored hinges, the bilayer remains in a liquid disordered state and there is no lateral domain formation. Further, the bilayer is symmetric with homogeneously distributed lipids, implying a spontaneous curvature of zero [110]. The marginal increase in lipid density in one leaflet due to the insertion of cholesterol anchors is not expected to significantly impact the the spontaneous curvature or lipid ordering and thereby the behavior of the hinges. Taken together, these observations indicate that the lipid bilayer can be regarded as a homogeneous, liquid disordered membrane both with and without anchored

particles, and that interactions of membrane deformations are likely the dominant physical mechanism underlying particle aggregation.

5.3.4 Hinge stiffness controls interactions between membrane-anchored particles

The spontaneous aggregation of two particles and the resulting stability of the aggregate demonstrate an effective attraction between two membrane-associated particles. To characterize the nature and magnitude of the effective interaction, we used umbrella sampling methods to calculate the potential of mean force (PMF) between the centers of mass of the two particles in the plane of the membrane.

Figures 5.10 and 5.11 show multiple PMFs, each of which was obtained for two hinges of a particular stiffness. A general feature is a minimum in the PMFs at a distance of ≈ 5 nm. This corresponds to the distance at which the hinges are in side-by-side contact, as demonstrated in Figure 5.8. Starting at larger distances, there is an effective attraction between the two hinges, and there are no appreciable energy barriers to prevent the spontaneous aggregation of the particles. A short-ranged (< 5 nm) repulsion is associated with short-ranged repulsive interactions of the beads comprising the hinges. These results confirm that it is energetically favorable for the hinges to aggregate spontaneously.

A key feature of Figure 5.10 is that stiffer hinges result in stronger attraction, as revealed by the increasing depth of the PMF. Further, stiff hinges experience an appreciable attractive force at larger distances than the weaker hinges. Physically, stiffer hinges cause larger deformations of the membrane, thus increasing the range of the bending and the total bending energy of the membrane.

The physics underlying the effective interaction between hinges is that the total deformation of the membrane is decreased when two hinges are close together, thus decreasing the bending energy of the membrane. This decrease in bending energy of the membrane is the driving factor for the aggregation of the hinge particles. We note that a modest attractive interaction is present even when the hinge is freely jointed ($k = 0$). This is likely because the particle becomes slightly distorted when the cholesterol anchors insert into the membrane

(Figure 2), thus impacting the local shape of the membrane. The results indicate that the overall energy is decreased when the particles are in close proximity, leading to an effective attraction. Our focus in this paper is on the effects of hinge stiffness, which clearly modulates the magnitude of membrane deformations and strength of attraction between particles, thus demonstrating that the degree of particle deformability can be used to tune membrane-mediated interactions between particles.

5.4 Conclusion

In this work, we studied deformable nanoparticles anchored by cholesterol to a realistic lipid membrane using coarse-grained computer simulations. Motivated by a new class of DNA origami designs [147, 74], we used a generic model of a hinge-like nanoparticle that allowed us to systematically change the stiffness of the hinge region. We showed that the particle and membrane could mutually deform one another: When the hinge was highly flexible, it adopted an open configuration and conformed to the flat shape of the membrane. As the hinge stiffness increased, it produced progressively larger deformations of the membrane. Mutual deformations were most pronounced at intermediate values of the stiffness, where both the particle and membrane were significantly deformed compared to their typical equilibrium configurations. We then studied membrane-mediated interactions between two particles, showing that two particles would spontaneously aggregate in a side-by-side configuration. When assembled this way, the deformations induced by the particles were aligned and formed a trough-like deformation perpendicular to the arms of the hinge. Aggregated particles also adopted configurations closer to their natural hinge angle and thus collectively induced larger deformations of the membrane. This effect was most pronounced at intermediate hinge stiffnesses.

A key result is shown in Figure 5.10, which demonstrates that the strength and range of attraction between two particles increases with increasing hinge stiffness. This is a consequence of stiffer particles inducing larger deformations of the membrane, which leads to a more pronounced decrease in the bending energy of the membrane when they are close together. This is consistent with previous work on rigid nanoparticles, in which particles that

induce larger curvature experience stronger membrane-mediated attraction [118]. However, in contrast with rigid nanoparticles, our work demonstrates that membrane-mediated forces acting on deformable particles can be controlled by changing mechanical properties of the particles. Thus, membrane-mediated self-assembly of particles can be influenced by mechanical properties of the particles alone. This further suggests that actuating the mechanical properties of the particles, for example by external perturbation [73, 56, 132], could provide a means to control particle-particle interactions in a time-dependent and externally controlled manner.

Our results further show cooperative effects when particles aggregate, whereby particles change their configurations because they can collectively deform the membrane. Such reconfigurability is not possible with rigid particles. FRET-based measurements may be useful for assessing changes in hinge configurations [11], and it will be interesting to study how self-assembly in large-scale systems is impacted by particle deformability and cooperative effects. It is also intriguing to speculate about whether deformable nanoparticles could alter properties of the membrane that in turn would modify the energetics of deformations. For example, curvature-sensitive lipids (if present) could be recruited to regions deformed by nanoparticles, and high densities of cholesterol anchors could possibly induce a phase transition near the nanoparticle. Such scenarios would change local properties of the membrane, thus altering the energetics of membrane deformations and providing another means to influence the interactions and self-assembly of deformable nanoparticles.

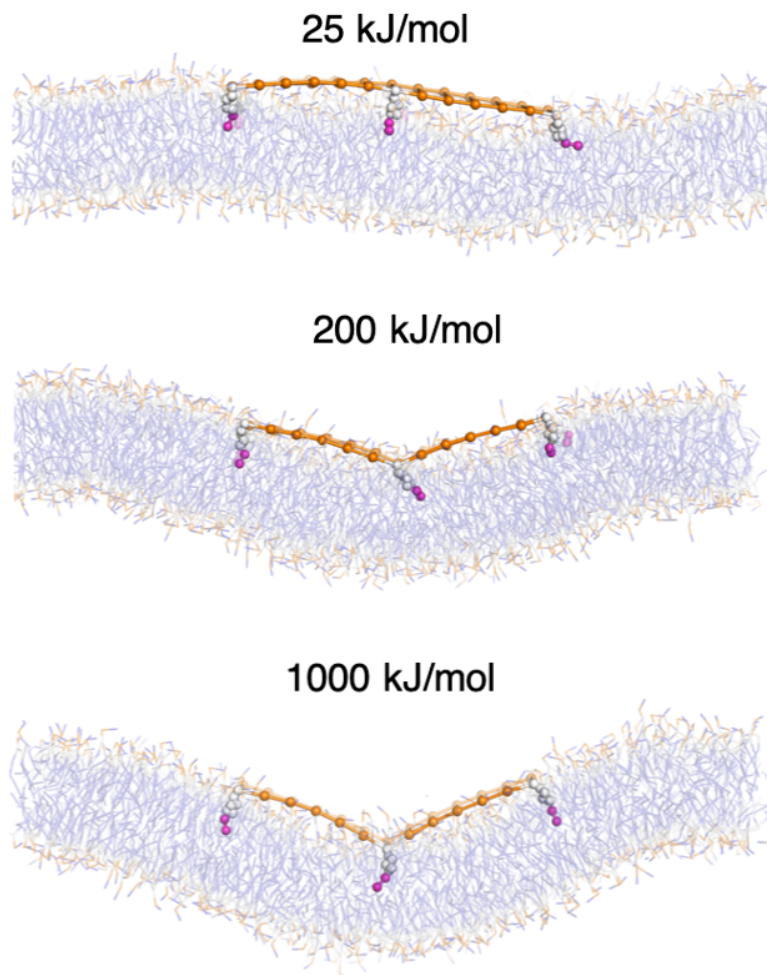


Figure 5.4: Typical equilibrium snapshots of a hinge-like particle anchored to a membrane. Three values of the hinge stiffness are shown ($k = 25, 200$, and 1000 kJ/mol).

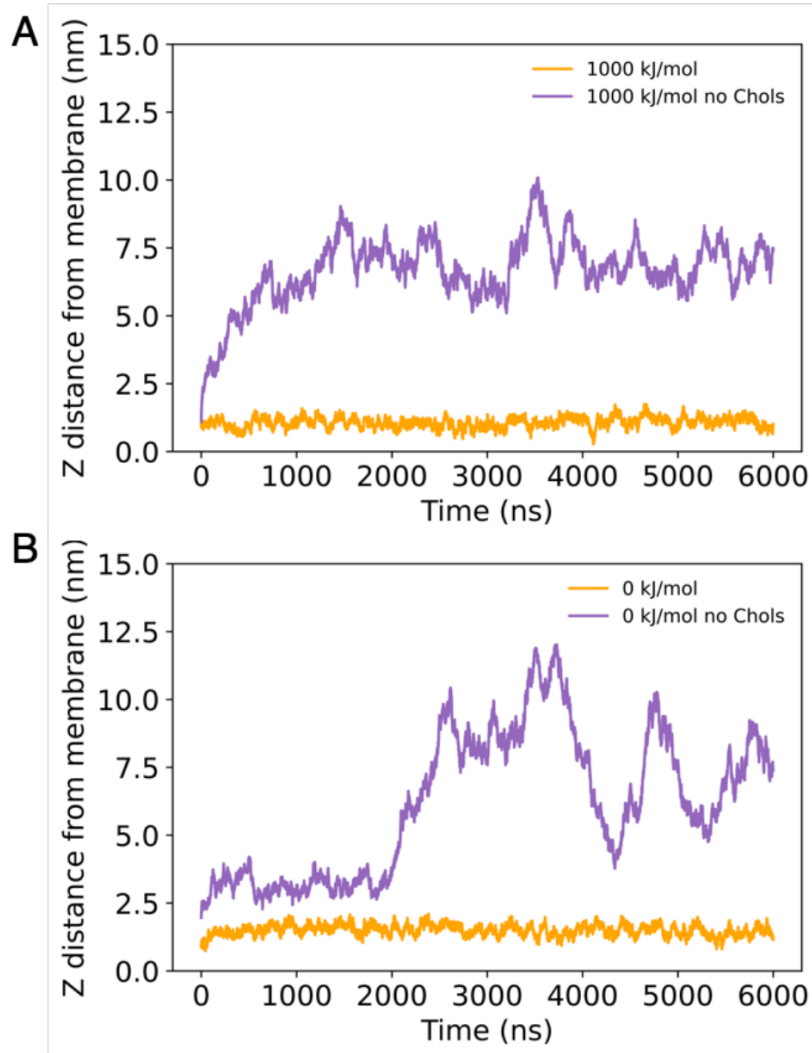


Figure 5.5: Distance from the center of mass of the hinge particle to the center of the bilayer with and without cholesterol anchors. Trajectories without cholesterol anchors were initially anchored to the membrane before the cholesterol anchors were removed at $t = 0$. Two hinge stiffnesses are shown (A, B).

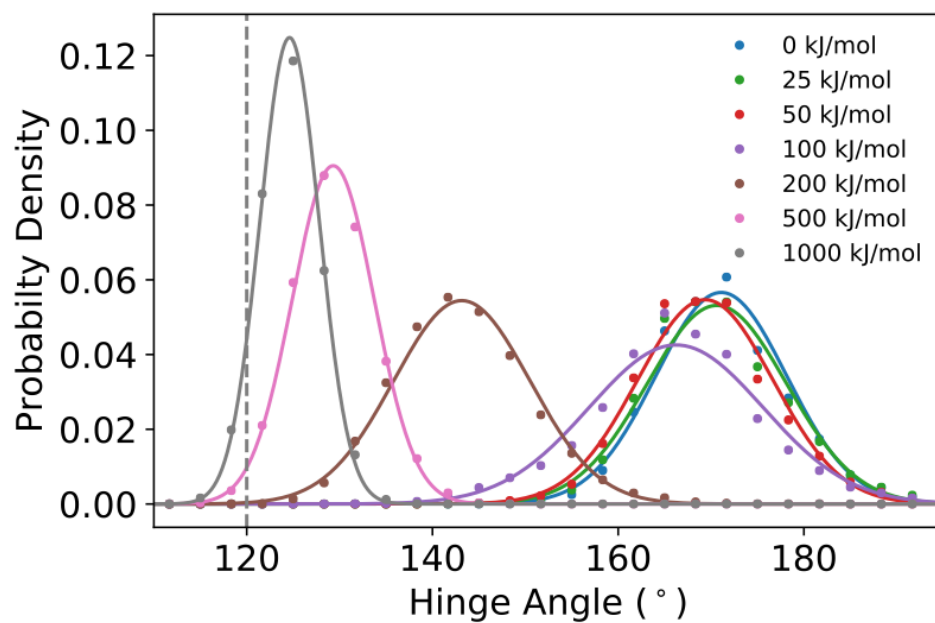


Figure 5.6: The probability density of the hinge angle (θ) at equilibrium for a hinge anchored to a lipid bilayer. Different values of the hinge stiffness (k) are shown. The vertical dashed line corresponds to the natural angle of the hinge (θ_0). Solid lines are Gaussian fits to the data (circles) meant to guide the eye.

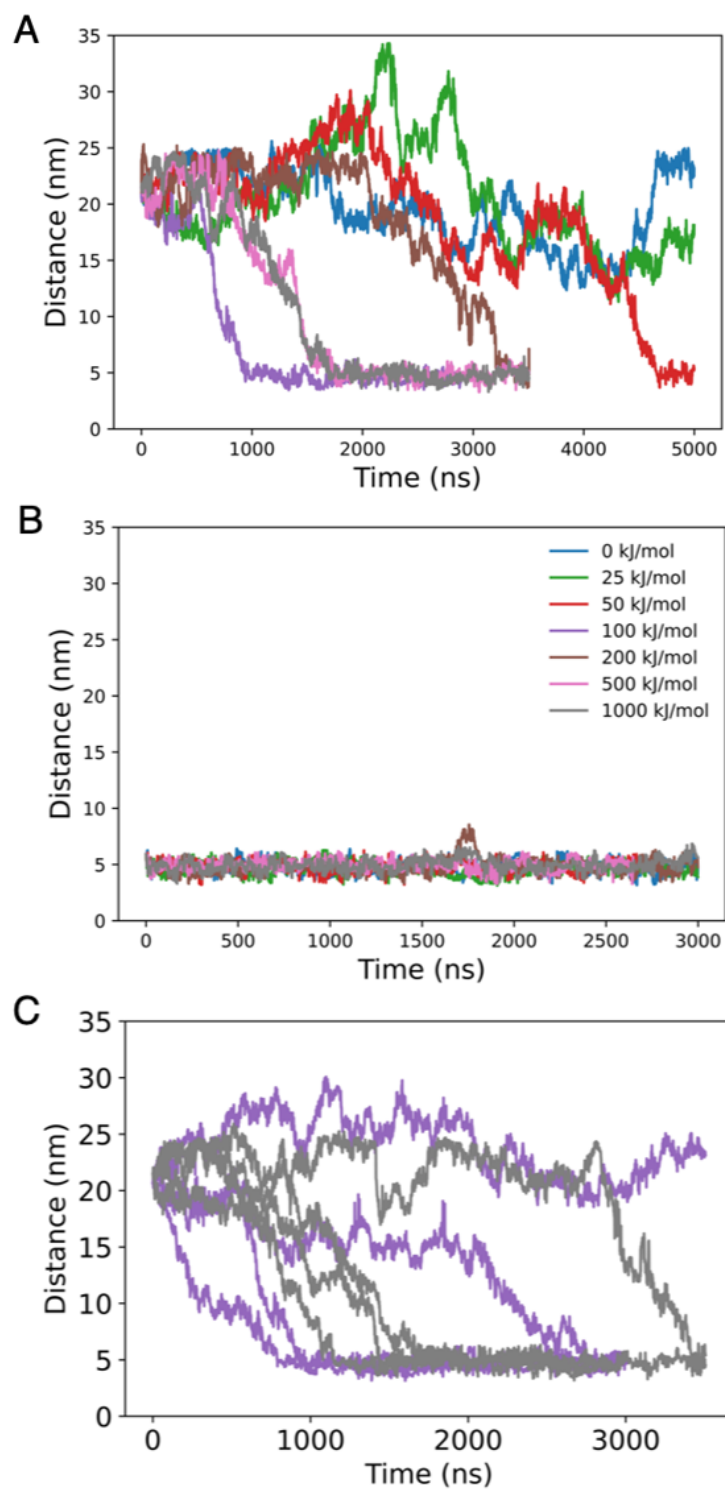


Figure 5.7: Distance between centers of mass of two particles anchored to a lipid bilayer as a function of time. (A) Hinges are started far apart. (B) Hinges are started close together, in side-to-side contact. (C) Comparison of multiple independent simulation trajectories with hinges started far apart, for $k = 100$ and 1000 kJ/mol.

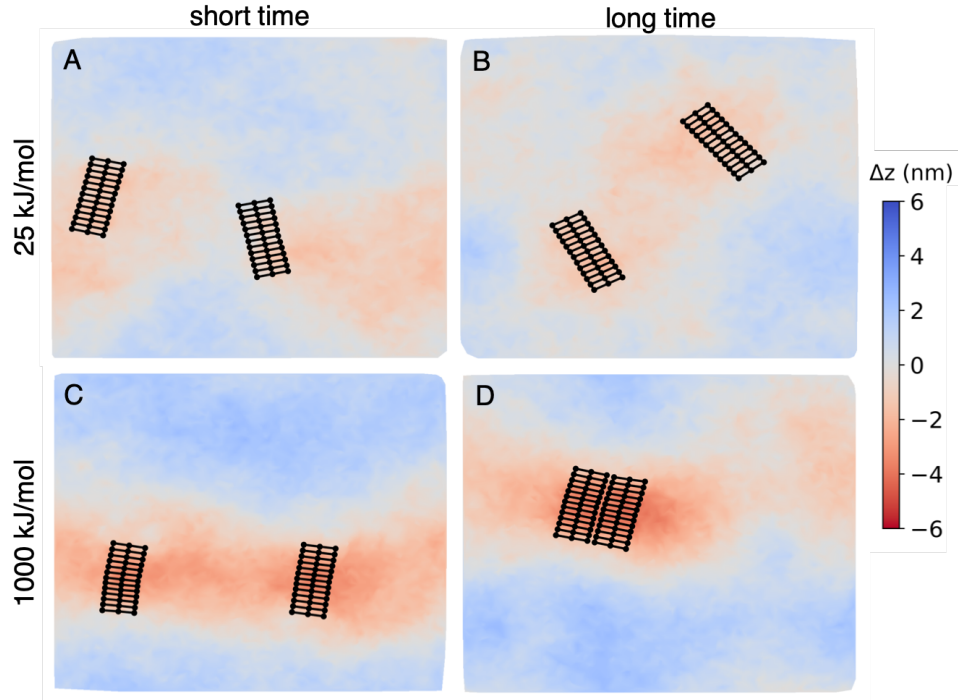


Figure 5.8: Top-down view of hinges and deviations of the membrane height. Δz denotes the deviation of the membrane from its average position in the out-of-plane dimension. (A), (B) Snapshots of weak hinges ($k = 25$ kJ/mol) at 50 ns and 3 μ s, respectively. (C), (D) Snapshots of strong hinges ($k = 1000$ kJ/mol) at 50 ns and 3 μ s, respectively.

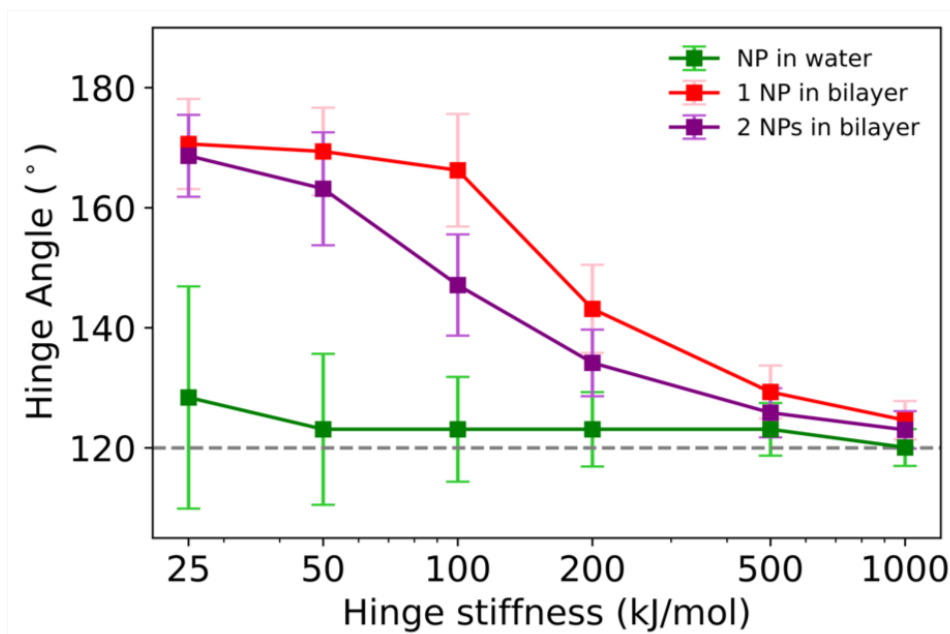


Figure 5.9: Comparison of the angles sampled by an isolated hinge in water, an isolated hinge anchored to a membrane, and two hinges anchored to a membrane when started in close proximity. The horizontal dashed line corresponds to the natural angle of the hinge (θ_0).

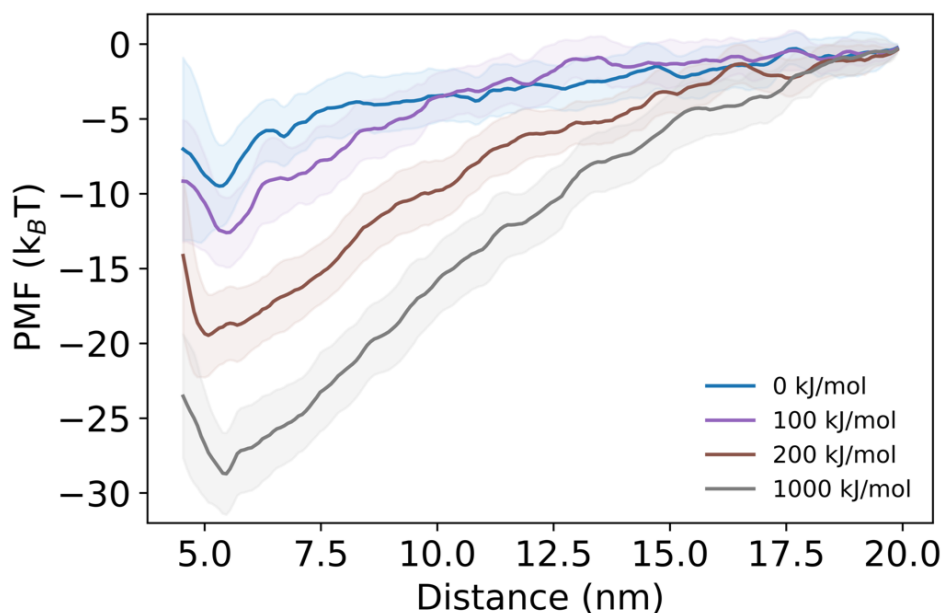


Figure 5.10: Potential of mean force as a function of the distance between the centers of mass of the two hinge-like particles in the plane of the membrane (xy -plane). The shaded region around each PMF represents the standard deviation of 50 hypothetical PMFs generated using a Bayesian bootstrap approach. Additional hinge stiffnesses are omitted for clarity and can be found in Figure 5.11.

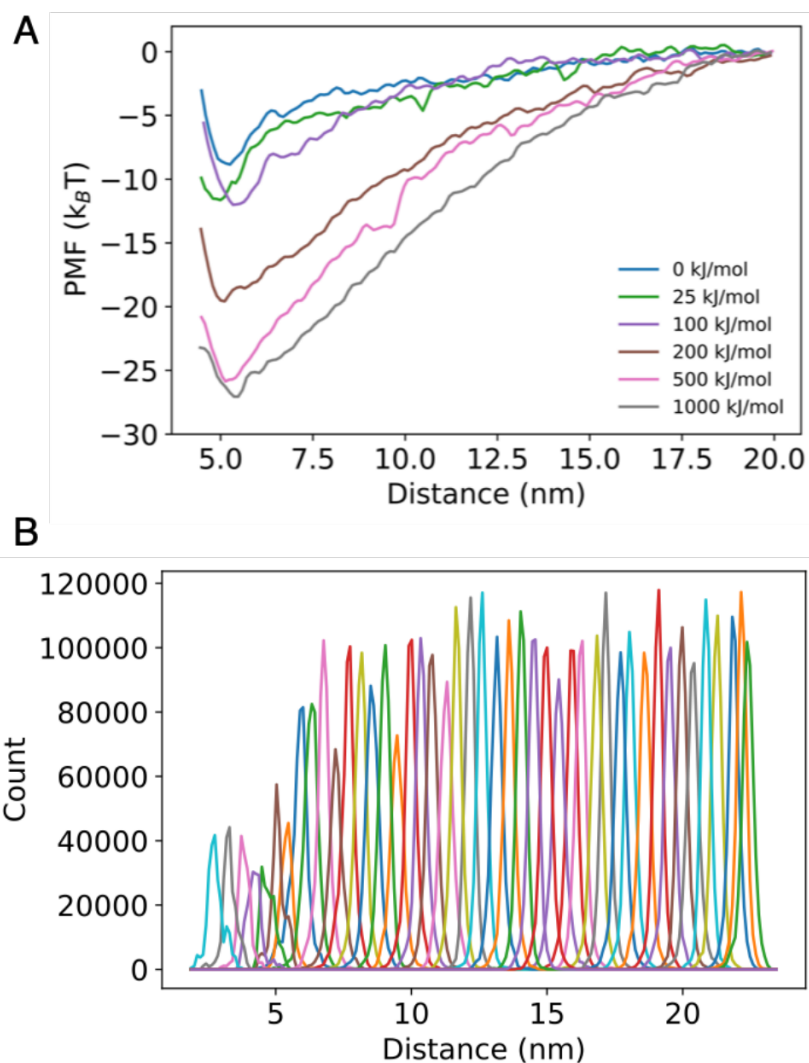


Figure 5.11: (A) Potential of mean force (PMF) as a function of the distance between the centers of mass of the two hinge-like particles in the plane of the membrane (xy-plane). (B) Histograms obtained for different windows during umbrella sampling. The case shown is for the stiffest hinge ($k = 1000$ kJ/mol).

Chapter 6

Conclusions

Membranes are a crucial component of every cell that act as physical barriers protecting the cell and compartmentalizing organelles. Cells dynamically manipulate the lipid composition of their membranes to regulate critical biological processes. To facilitate these biological processes, lipids control the physiochemical properties of the membranes as well as the ability of the membrane to modulate proteins. The research in this thesis employs MD simulations to investigate unresolved questions in membrane systems related to composition-dependent properties and the molecular mechanisms underlying protein-membrane interactions.

First, we analyzed the effect of cardiolipin and branched-tail lipids in model bacterial membranes by using all-atom MD simulations of membranes of varying compositions. The addition of branched-tail lipids to membranes decreased the bending rigidity, order parameter, and membrane thickness. The membranes transitioned from a gel phase to a disordered phase as the percentage of branched tails in the bilayer increased. These results suggest that the abundance of branched-tail lipids in bacteria like chlamydia is likely a means to fluidize the membrane as an alternative to unsaturated lipids. Cardiolipin fine tunes the properties of membranes by rigidifying membranes with branched tails and showing non-monotonic behavior in membranes with straight tails.

Next, we used all-atom MD simulations to investigate the structure and membrane interactions of the extended N-terminal region of chlamydial MreB with model chlamydial membranes. Using the initial structure predictions from Collabfold and PEPfold, we used unbiased MD simulation to refine the predicted structure. The continuous helix predicted

by Collabfold was stable, maintaining its secondary structure throughout the simulation. In contrast, the 3 disjointed helices predicted by PEPfold restructured to a longer helix and a disorganized region. Further, we demonstrated that the extended N-terminal region inserts spontaneously into membranes lacking CL. In the timescales probed, it only partially inserted into membranes containing CL. The negative charge of the head group of cardiolipin might induce an energy barrier, inhibiting the ability of hydrophobic residues to insert into the interior of the membrane.

Next, we used MD simulations to identify how ER membrane protein Pex30 interacts with the ER membrane. In this work, we used all-atom and coarse-grained molecular dynamics to investigate the roles of DysF and RHD at the molecular level. Our simulations showed that the DysF domain binds to membranes via two regions (residues 296–315 and 379–397) that consist of hydrophobic insertion loops flanked by polar or charged residues. Lipid contact analysis of the DysF domain showed that DysF bound more strongly to bilayers containing PA lipids due to charge-based interactions of negatively charge PA lipids with polar and positively charged residues that flanked the hydrophobic insertion loops. Our simulations of the Pex30 RHD showed that their molecular wedge-like structure caused them to induce curvature in membranes. They also aggregate spontaneously when multiple RHDs are present in the membrane. Spontaneous aggregation of RHDs explain the accumulation of Pex30 as puncti at the LD biogenesis site *in vitro*.

Finally, we used coarse-grained molecular dynamics simulations to investigate the dynamics of deformable nanoparticles on a membrane. We designed a hinge-like nanostructure inspired by DNA origami nanostructures anchored to lipid membranes via cholesterol anchors. We characterized deformations of the particles and membrane as a function of the hinge stiffness. Flexible particles adopted open configurations to conform to a flat membrane, whereas stiffer particles induced deformations of the membrane. We further showed that particles spontaneously aggregated and that cooperative effects lead to changes in their shape when they are close together. Using umbrella sampling methods, we quantified the effective interaction between two particles and showed that stiffer hinge-like particles experienced stronger and longer-ranged attractions. Our results demonstrate that interactions between deformable, membrane-anchored nanoparticles can be controlled by modifying mechanical

properties of the particles, suggesting new ways to modulate the self-assembly of particles on deformable surfaces.

Collectively, these projects advance our understanding of membrane properties and the interactions of proteins and nanoparticles with membranes across different biological phenomena. This enhanced understanding provides a foundation for designing therapeutic interventions and biotechnological applications.

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Appendices

A Appendix Figures

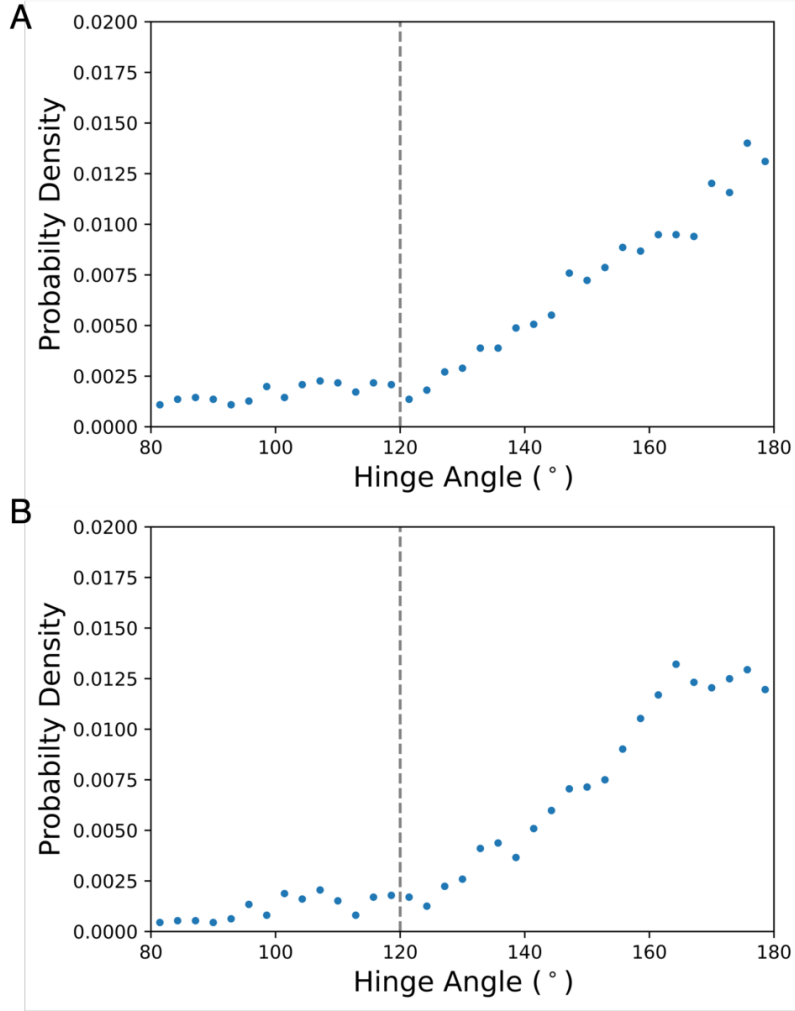


Figure 1: Probability density of the hinge angle (θ) at equilibrium for a hinge in water with $k = 0$. The vertical dashed line corresponds to the natural angle of the hinge. (A) With cholesterol anchors. (B) Without cholesterol anchors.

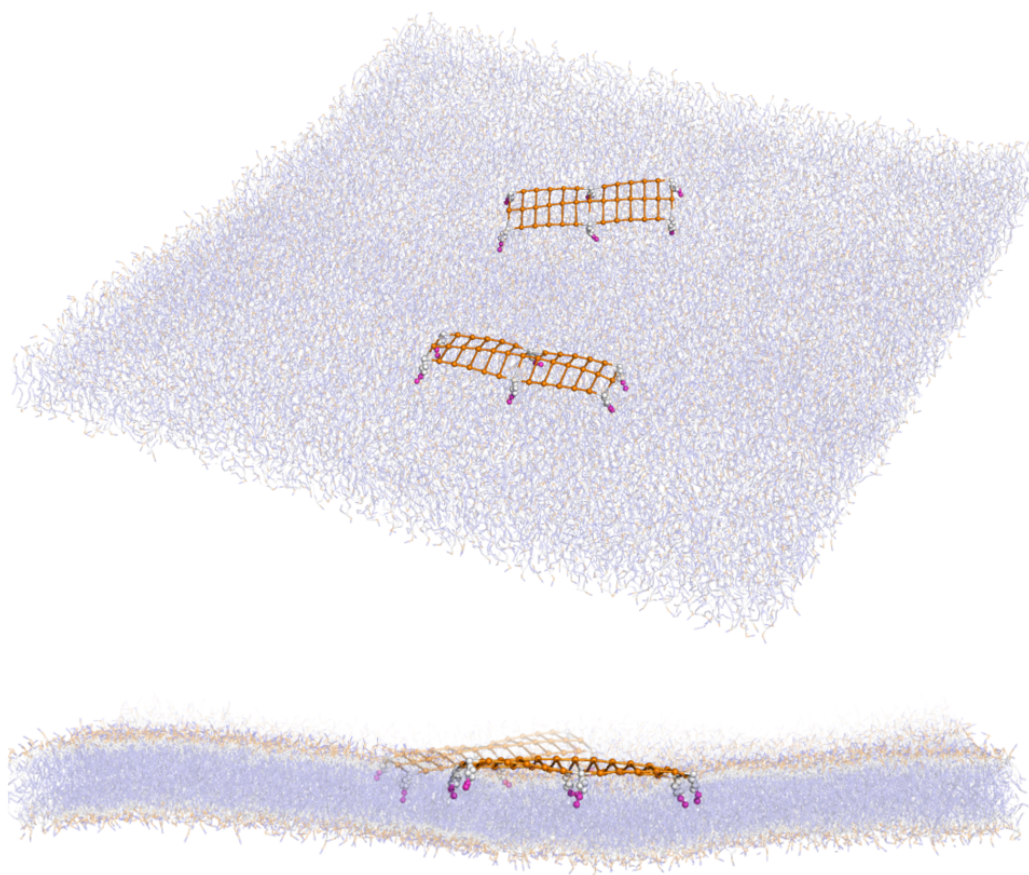


Figure 2: Simulation snapshot of two hinge-like particles anchored to a membrane with $k = 25$ kJ/mol, viewed from different angles. This demonstrates how the particles become slightly distorted when the cholesterol anchors insert into the membrane.

Vita

Nikhil Nambiar was born in Kozhikode, India on February 4, 1993. He received his Bachelor of Technology in Chemical Engineering from the Indian School of Mines – Indian Institute of Technology Dhanbad in 2015. After graduation, he spent six months as a research assistant at the Indian Institute of Science, followed by six months as a research intern at the Okinawa Institute of Science and Technology. He then pursued dual master’s degrees in Chemical Engineering and Colloids, Polymers and Surfaces at Carnegie Mellon University, graduating in 2017. After completing his master’s degrees, he continued as a research assistant at Carnegie Mellon University for eight months before beginning his doctoral studies in Chemical and Biomolecular Engineering at the University of Tennessee in January 2019. His doctoral research focused on using computer simulations to investigate the properties of lipid membranes and their interactions with proteins and nanoparticles.