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Physical Considerations of the Organization of Inclusions in Lipid Bilayer Systems

by

Shachi Katira

A dissertation submitted in partial satisfaction of the requirements for the degree of

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in

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of the

University of California, Berkeley

Committee in charge:

Professor Mohammad Mofrad, Co-chair
Professor Berend Smit, Co-chair
Professor George Oster
Professor Francis Szoka

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Physical Considerations of the Organization of Inclusions in Lipid Bilayer Systems

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Shachi Katira
Abstract

Physical Considerations of the Organization of Inclusions in Lipid Bilayer Systems

by

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Doctor of Philosophy in Bioengineering
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Professor Mohammad Mofrad, Co-chair
Professor Berend Smit, Co-chair

Lipid bilayers, along with embedded inclusions such as cholesterol and proteins, constitute a biological membrane—the interface between a cell or organelle and its environment. Understanding the structure of a biological membrane and the physical principles responsible for the organization of membrane inclusions is crucial to understanding the processes that occur on the surface of a cell or organelle such as inter-cellular signaling, immune synapse processes, exo- and endocytosis, and membrane fusion. In this work, we study the organization and effect of inclusions in a lipid bilayer using statistical mechanics principles and large-scale molecular simulations.

In the first part of this work, we propose a generic physical force for the assembly of inclusions in lipid bilayers—the orderphobic effect. Inspired by modern theories of the hydrophobic effect, this force is governed by the physics of the first-order phase transition between the ordered (i.e., solid-like) and disordered (i.e., fluid) phases of lipid bilayers. We show the existence and nature of this force using coarse-grained molecular dynamics simulations, and demonstrate the lateral assembly of two model proteins, or ‘orderphobes’, within a lipid bilayer. The effect is powerful and operates at nano- to mesoscopic scales, and could be a potential explanation for the clustering of proteins in a biological membrane.

In the second part of this work, we focus on hydrophobic inclusions within the lipid bilayer that give rise to organelles known as lipid droplets. These droplets, earlier thought to be passive stores of hydrophobic material in an otherwise aqueous cytosol, have now been implicated in various metabolic diseases. Since the growth and behavior of nascent droplets is sub-microscopic, little is known about the details of this process. We construct a computational framework that allows for a lipid reservoir to study asymmetric inclusions in a lipid bilayer such as the lipid droplet. Further, we identify key stages in the growth and budding of this organelle.
To Mona, Tushar and Divyank Katira
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Chapter 1

Introduction

Biological membranes are more than simple boundaries that separate the inside of a cell from its environment. They are involved in a range of processes such as cell–cell signaling, exocytosis and endocytosis, formation and functioning of immune synapses, and membrane fusion. Biological membranes are constituted by a variety of bilayer-forming lipid species, smaller molecules such as cholesterol, and a variety of transmembrane and peripherally bound proteins (Figure 1.1). Historically, several models were proposed for the structure and organization of the membrane and its proteins. Gorter and Grendel proposed that the cells they studied were covered by “a layer of fatty substances two molecules thick” [1], while Danielli and Davson proposed a “very thin lipoid film with a protein film adsorbed upon it” [2]. Modern notions of the structure and organization of biological membranes are derived from Singer and Nicolson who proposed the “fluid mosaic model” in 1972 [3]. This model suggests that membranes are composed of lipid bilayers in their fluid state, with embedded inclusions such as proteins that are free to diffuse around and show no long-range order or organization.

In the intervening years since these models have been proposed, a plethora of studies have probed the structural and functional properties of membranes, both in the context of biological membranes, as well as synthetic preparations such as liposomes. Studies of membranes can be considered to stem from three broad perspectives—a biological perspective where functional aspects of the membrane are discovered, a biochemical perspective where the different molecular components residing in and acting on the membrane are identified, and a biophysical perspective which places emphasis on the physical properties of the membrane and its relation to biological structure and function. In this dissertation, we approach biological membranes from the latter perspective, i.e. the role of physical properties of the membrane on the behavior of its inclusions, as well as the role of inclusions in changing the properties of the membrane. We focus on two kinds of inclusions—a generic transmembrane protein, and a generic hydrophobic inclusion.

In this chapter, we motivate our chosen problems, review the methods that have been used to address them, and provide an overview of the results presented in this work.
1.1 Motivation

The fluid mosaic view of biological membranes was challenged by the lipid “raft” hypothesis put forth by Simons and Ikonen in 1997 \cite{4,5}. This hypothesis proposed the existence of short and long range order brought about by the preferential packing of cholesterol and sphingolipids into micro-domains in the plane of the membrane. These micro-domains could have specific proteins embedded in them and would form a solid-like raft floating laterally in a fluid sea of lipids. This hypothesis was attractive because it exploited the properties of bilayer components to provide a way for proteins that are related to a single process, to come together in a non-random fashion to perform their functions in a concerted manner. However, the hypothesis was met with skepticism because the most cited proof for the existence rafts was the detergent resistance of its components, i.e., certain lipids such as sphingomyelin and cholesterol separate out from the rest of the membrane when it is acted upon by detergents \cite{6}. This separation does not guarantee that detergent resistant fractions form their own
distinct domains in the membrane. Additionally, no raft-like micro-domains were observed under the microscope, further contributing to skepticism related to the lipid raft hypothesis.

The invention of super-resolution microscopy techniques in the last decade, such as stimulated emission depletion microscopy [7], has led to some advances in the visualization of the sub-microscopic structure of a cell membrane. Strikingly, images captured with these novel imaging techniques show that nanoscopic clusters of proteins do indeed exist in a cell membrane (see Figure 1.2), contrary to the lack of organization postulated by the fluid mosaic model [8]. However, the physical principles responsible for the formation of these clusters are still unclear, as is the relationship between these observed clusters and the lipid raft hypothesis. In fact, the question of whether or not lipid rafts exist is considered one of the mysteries of the cell [9]. One of the central results of this work proposes a generic physical mechanism by which proteins may cluster in biological membranes.

In addition to transmembrane proteins, we also study hydrophobic inclusions within a membrane, a structure referred to as a lipid droplet. The interior of a cell is an aqueous solution. However, in addition to water-soluble species, hydrophobic or oily species are also a part of a cell’s metabolism. A cell stores this oily phase by surrounding it with a surfactant. The resulting structure is referred to as a lipid droplet [10]. Lipid droplets were thought to be passive stores of oil until the last decade when they have been increasingly implicated in metabolic disorders such as obesity, diabetes and atherosclerosis. However, little is known of the formation of this organelle since a nascent droplet is a sub-microscopic entity. The latter part of this work is concerned with developing a picture for the growth and formation of these organelles.

In this context, we introduce the tools used in this work to study lipid bilayers and their inclusions—we employ large-scale molecular simulations based on the theoretical framework of statistical mechanics. We attempt to first understand the physical properties of a single component bilayer, and apply the results of that understanding to solutes in context of a biological membrane. We begin with an introduction to molecular modeling and simulations which have been widely used to gain insight into the structure of lipid bilayers and their solutes. Molecular simulation, guided by the principles of statistical mechanics, has been widely used to (i) provide insights at a molecular scale for sub-microscopic phenomena, for example, the role of water in the functioning of certain types of ion channels [11], and (ii) to test fundamental physical principles that may underlie the structure and function of membranes, for example, the distribution of tilt angles of dimerized transmembrane helices was found to mostly depend on simply the difference in length of the hydrophobic regions of the membrane and the helices [12].

1.2 Mesoscopic molecular modeling

One of the first molecular simulation studies on surfactants was performed twenty five years ago and helped gain insight into micelle structure and formation in surfactant–water systems, and monolayers at oil–water interfaces [13]. A search of the literature reveals several studies
Figure 1.2: Stimulated emission depletion micrograph of stable protein assemblies in plasma membrane sheets extracted from rat neuroendocrine cells: The red spots indicate the presence of membrane proteins which are aggregated into stable assemblies in the membrane. The scale bar is 2 $\mu$m and 500 nm on the left and right respectively. Adapted from “Multi-protein assemblies underlie the mesoscale organization of the plasma membrane”, SK Saka et al., Nature Communications 2014 licensed under CC BY 4.0 http://creativecommons.org/licenses/by/4.0/
CHAPTER 1. INTRODUCTION

bilayer, such as the phase behavior, requires access to much longer time and length scales. Furthermore, several processes in a biological membrane occur over much longer length and time scales. One strategy to access higher length and time scales is to integrate over the faster atomic degrees of freedom, and approximate several atoms into one coarse-grained particle. At the cost of atomic resolution, but with an accompanied gain in computational efficiency, several coarse-grained models have been developed for lipid bilayers, some with explicit representations of solvent molecules [20, 21, 22, 23] and others with implicit solvent fields [24, 25, 26].

Several different strategies have been used to construct these models of which we describe a few here:

• Boltzmann inversion: The idea here is that distributions of structural properties $p(x)$ such as bond angle or bond length distributions exhibited by the atomistic model should be reproduced by the coarse-grained model $27, 28$. The coarse-grained potential, $U_{\text{coarse-grained}}$, is a sum of functionals of distribution functions obtained from the sampling of an atomistic model, $U_{\text{coarse-grained}} = \sum -k_B T \ln p(x)$. A multidimensional free energy surface cannot be accurately reproduced by simply summing over the distributions of a few free degrees of freedom. To improve upon the potentials obtained using this method, the distributions $p_{\text{coarse-grained}}(x_{\text{coarse-grained}})$ can be compared to the distributions $p(x)$ to add iterative corrections to $U_{\text{coarse-grained}}$ [29]. Coarse-grained potentials obtained via this technique are highly specific to the particular molecular system for which the atomistic sampling was performed. This makes the potentials difficult to transfer to other systems.

• Force matching: The original force matching technique was proposed to derive classical potentials from $ab$ initio calculations [30]. Izvekov and Voth used this method to derive coarse-grained potentials from atomistic calculations [31]. The premise of this method is that the forces on a coarse-grained particle or ‘bead’ should be similar to the forces acting on individual atoms comprising the bead in atomistic simulations. The forces on individual atoms from an atomistic simulation are averaged over and used to create a coarse-grained potential acting on each coarse-grained bead. A least-squares minimization is performed to ‘match’ the forces on a coarse-grained bead with those on its atomistic counterparts.

• Reproduction of thermodynamic properties: A third strategy used for several different models, two of which we will use in this work, is to choose a functional form for the potential energy and parameterize it to reproduce thermodynamic quantities such as isothermal compressibility, free energies of solvation and vaporization, and/or phase behavior [20, 23, 22, 21]. For example, the MARTINI model parameterizes the Lennard-Jones potential for a lipid bilayer to reproduce experimental free energies of partitioning polar and apolar phases for several different molecules. A second model used in this work employs parameters derived from Flory–Huggins theory for mixing of polymer solutions. These two models are described in greater detail in Chapter [2].
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At the other end of the spectrum of resolution lie lattice models. These have been extensively used to gain insight into the behavior of substances such as water \cite{32, 33}. These models have received renewed interest in studies of pure and multi-component bilayer systems since Honerkamp-Smith \textit{et al.} showed that the phase behavior of a wide variety of lipid mixtures belongs to the two-dimensional Ising universality class \cite{34}. These models have been used to understand the properties of biological membranes from the perspective of phase transitions. For example, it has been shown that a cytoskeleton-like structure interferes with the phase separation of membranes near a miscibility critical point \cite{35}. In another such example, Mouritsen \textit{et al.} have used a Potts-like model to describe the inclusions of different hydrophobic thicknesses in a membrane \cite{36}. This model demonstrates wetting-driven assembly, wherein a binary mixture of lipids contains proteins that have preferentially favorable interactions with one of the lipids due to their similar hydrophobic thickness. Each of these proteins stabilizes a few solvation shells of the favored lipid around itself. This protein-induced local phase separation of the lipids leads to an interface with a line tension. Assembly is driven by the tendency of the system to reduce energy due to the line tension by reducing the perimeter of this interface by bringing the proteins together. We revisit lattice models in the concluding chapter to discuss their impact in light of the results presented in this work.

At a scale where fluctuations no longer play a role, continuum theories have been extensively used to study the elastic properties of the membrane and curvature effects \cite{37}. A system of lipids bears several similarities to a liquid crystal system including phase behavior. Liquid crystal theory consists of an extensive body of work much of which is being applied to study lipid bilayers \cite{38}.

1.3 Overview of this work

This work can be divided into three parts—in the first we study the phase transition between the ordered and disordered phases of a lipid bilayer (Chapter 3), in the second we demonstrate the existence of a lateral assembly force in the plane of a lipid bilayer engendered by proximity to this order–disorder transition (Chapter 4), and finally in the third we study the growth and formation of a lipid droplet (Chapter 5).

1.3.1 Phase transitions in lipid bilayers

Lipid bilayers primarily exhibit two phases—a solid-like ordered phase where the lipid chains are uniformly organized, and a liquid-like disordered phase where the lipid chains are scrambled. Depending on the lipid type, temperature, surface pressure and bilayer hydration levels, several different phases can be seen, some of which are shown in Figure 3.1 \cite{39}. Several solid-like phases have been observed—the $L_c$ phase is a highly ordered structure with tilted chains, the $L_{\beta}$ phase exhibits highly ordered erect chains, the $L_{\beta'}$ phase is also ordered and tilted but the chains are more flexible, and the ripple or $P_{\beta'}$ in which the surface of
the bilayer takes on a corrugated appearance. Additionally, a liquid-like or \( L_\alpha \) phase with disorganized chains has also observed. This phase is deemed by Singer and Nicolson as the sole biologically-important phase in their fluid mosaic model. The solid-like structures \( L_c, L_\beta \) and \( L_\beta' \) occur at lower temperatures and higher surface pressures, while the liquid phase occurs at higher temperatures and lower surface pressures \([39, 40]\). The \( P_\beta' \) phase is a spatially modulated structure that occurs in between the solid-like and liquid-like phases while heating and cooling in the phosphatidylcholine species of lipids. The surface of a \( P_\beta' \) bilayer appears to have a rippled structure. The molecular origins of this structure remain unknown. It is possible that the undulations are caused by a buckling of the bilayer as it is heated and cooled at finite rates \([41]\). It has also been speculated that \( P_\beta' \) is a modulated phase with alternate liquid-like and solid-like regions \([42]\). Apart from the structure, the different phases differ in diffusivity, with the solid-like phases being more rigid than their liquid counterpart, and mechanical properties such as bending rigidity with the solid-like phases having larger bending moduli than the liquid phase.

It is interesting to note that several different phases of the bilayer can be obtained by a minimalist coarse-grained model that uses a bare minimum of design principles—a water-like particle, a hydrophilic particle, and a hydrophobic particle. Using simple systems with these three ingredients, Kranenburg et al. create model bilayers that exhibit a solid-like, liquid-like, and even a corrugated ripple-like phase \([42]\), depending on the temperature and surface pressure (or tension). They find that the phase behavior of different types of lipid species is dependent on the relative interaction energies between the lipid head groups and water. When lipid head groups have a preference for each other compared to water, their sizes are effectively smaller (compared to head groups with solvation shells) and they pack with each other better, for example phosphatidylethanolamine. These model lipids do not form the rippled phase, consistent with experiments on phosphatidylethanolamine \([43]\). For lipid head groups which have a stronger preference for water relative to each other and have effectively larger sizes, such as phosphatidylcholines, a rippled phase appears as does a tilted one, consistent with experiments on phosphatidylcholines \([44]\). Such simple models can therefore capture the physical essence of the effect of head group chemical compositions on the phase behavior of the bilayer. Other models with varying levels of coarse-graining also exhibit the presence of ordered and disordered phases \([20, 21, 45]\).

However, in addition to the structures of the different phases, we find that the order of the transition between the two phases can also play a vast role in the properties of the bilayer, and therefore the properties of its embedded solutes. Differential scanning calorimetry experiments have shown that the transition between the ordered and disordered phases of a bilayer show a large, sharp peak in the heat capacity \([46]\). This indicates that it is a first-order transition. On further examination of the minimalist model, which reproduces the structures of several different phases, we find that the transitions between those phases appear to be continuous and not first-order. Signatures of the lack of a first-order transition have also been observed for this model by Rodgers et al. \([47]\). Other models such as the MARTINI model \([23]\), however, have shown signatures of a first-order phase transition \([45, 47]\).
Figure 1.3: Some of the different structures observed in lipid bilayers: (A) \( L_c \): a solid, highly ordered structure with tilted chains, (B) \( L_\beta \): a structure with ordered, erect chains, (C) \( L_{\beta'} \): an ordered, tilted solid-like phase, but more disordered than the \( L_c \) structure, (D) \( P_{\beta'} \) or ripple phase that exhibits a corrugated structure with possibly alternating patches of solid and liquid-like lipids, and (E) \( L_\alpha \) or fluid phase where the lipid chains are completely disordered. Adapted from “Phase Transitions of Lipid Bilayers”, a dissertation by Marieke Kranenburg, 2004.
Figure 1.4: Illustration of a pre-transition layer: A and B are two phases separated by a first-order phase transition. At a temperature below, but proximal to, the phase transition temperature, a solute with preferential interactions with B compared to A (i.e. a B-philic solute) stabilizes a pre-transition layer in its vicinity. This pre-transition layer resembles the phase B and is therefore associated with an A–B interface.

Why is the order of this transition important? Consider two phases A and B separated by a first-order phase transition. When the temperature is below, but close to, the transition temperature, there can exist at the surface of A a thermodynamically stable region resembling B referred to as a pre-transition layer [48]. Additionally, if a solute is embedded in A (see Figure 1.4), and has a preference for the phase B over the phase A, a pre-transition layer that resembles the phase B is stabilized in the vicinity of the solute. This pre-transition layer is associated with an A–B interface between the induced B region in the solute vicinity, and the bulk A. Now consider two solutes embedded in A, each stabilizing their individual A–B interfaces. It is thermodynamically favorable to assemble the two solutes into one cluster, thus minimizing effective interfacial area (interfacial length in two-dimensions) and the energetic cost of the interface. This pre-transition effect brought about by proximity to a first-order phase transition can thus bring about solute assembly in a bulk medium. An everyday example where this principle causes large-scale assembly is the hydrophobic effect in water. The phase transition in this case is the liquid–vapor transition. Even though this transition occurs at 373 K for atmospheric pressure, the free energy difference between
the two phases at room temperature is small enough for a pre-transition effect. According to statistical mechanics theories of the hydrophobic effect, an ideal hydrophobe—simply, a volume-excluding solute—when immersed in liquid water, stabilizes a vapor region and hence a liquid–vapor interface in its vicinity. Two hydrophobes self-assemble to reduce the effective vapor region surrounding them, hence minimizing energy due to the interfacial tension.

Since heat capacity measurements have indicated that the order–disorder transition in bilayers is first-order, it is possible that pre-transition effects occur laterally in (quasi-) two-dimensions in the plane of the membrane. It therefore becomes important that, in addition to observing the different phases in a molecular model, the order of the transition is also correctly reproduced. As mentioned above, the MARTINI coarse grained molecular model shows signatures of a first-order transition between the ordered and disordered phases, such as hysteresis \[45, 47\]. However, hysteresis is merely a signature of the existence of an energy barrier, and not necessarily an energy barrier that scales with system size, as in a first-order phase transition \[49\]. In Chapter 3 of this work we attempt to confirm that the transition is of first-order in the MARTINI model.

A study of the transition in the MARTINI model shows a large hysteresis loop consistent with the hysteresis observed by Marrink \textit{et al.} \[45\]. However, further analysis of the solid-like ordered phase in the MARTINI model reveals the existence of several dislocations in the lattice; the lattice is far from perfect. Let us now examine the structure of a perfect lattice, a solid. In three dimensional systems, the structure of solids differs from liquids in two respects, broken translational symmetry, as well as broken orientational symmetry with respect to reference crystallographic axes. Figure 1.5 shows how the two broken symmetries are related. Rotating a perfectly crystalline set of atoms not only breaks orientational order, but also breaks translational order. On the other hand, translating a perfectly crystalline set of atoms only breaks translational order while preserving orientational order with respect to reference axes.

Could there be a third phase distinct from solid and liquid which exhibits long range orientational order but not long range translational order? Such a phase, referred to as the hexatic phase, results from theories of two-dimensional melting and has been subsequently shown in experiments in two-dimensional and some quasi-two-dimensional systems \[50\]. This phase is relevant to the question about the order of the transition in the MARTINI model because the KTHNY theory of two-dimensional melting predicts that the transition between the hexatic and liquid phases is continuous \[50\]. If the MARTINI model exhibits the hexatic phase, the KTHNY theory predicts that the transition is continuous and we would therefore have to find another model, or tune the existing model to reproduce the experimentally-observed first-order transition.

Analysis of the ordered phase of the MARTINI model in Chapter 3 reveals that it is indeed hexatic, implying that the transition in the MARTINI model is also continuous like in the minimalist model as per the KTHNY theory. Interestingly, it has been shown very recently that the KTHNY theory does not hold for some classes of particle interaction potentials \[51, 52\]. Specifically, potentials with repulsive interactions of \(r^{-n}\) where \(n > 6\) show a first-order transition between the hexatic and liquid phases. The MARTINI model employs the
Figure 1.5: The hexagonal arrangement of atoms on the left side of both the top and bottom pictures has perfect translational and orientational order. This perfect arrangement is rotated in the top picture, and translated in the bottom picture. Rotating the hexagonal arrangement in the top figure destroys orientational order with respect to a reference axis, and also destroys translational order. Translating the hexagonal arrangement in the bottom figure destroys the translational order but maintains the orientational order [50].
Lennard-Jones potential with \( n = 12 \). In other words, even though the MARTINI model exhibits the hexatic phase, it is likely that the transition is first-order.

In Chapter 3, we stabilize a free interface between the ordered and disordered phases and establish coexistence—strong evidence that the transition in the MARTINI model is first-order. This brings us back to our original motivation for reproducing the order of the transition in a molecular model—the existence of a pre-transition effect that can affect the assembly of solutes in a lipid bilayer, and possibly a biological membrane. Additionally, it is interesting to see the existence of the novel hexatic phase in a model bilayer, and it is possible that this phase also occurs in experiments of the ordered phase, although the experimental data so far are inconclusive on whether or not the translational correlations in bilayers are long-ranged [53]. The hexatic phase and the theory of dislocations can have interesting implications on the mechanism of melting, the mechanical properties of the material and the behavior of the material on a substrate [50].

### 1.3.2 The orderphobic effect

In Chapter 4, we show the existence of a generic physical force engendered by the statistical mechanics of a first-order phase transition between the ordered (i.e., solid-like) and disordered (i.e., liquid-like) phases of a lipid bilayer—the orderphobic effect. This effect is a pre-transition effect inspired by the modern theoretical framework of the hydrophobic effect and operates at the nano- to mesoscopic length scale. Similar to the hydrophobic effect, where a sufficiently large ideal hydrophobe such as a hard sphere breaks the hydrogen bond network of water and stabilizes a vapor-like region in its vicinity, we hypothesize that inclusions such as proteins can act as ‘orderphobes’ and stabilize a disordered region in their surroundings. Using large-scale molecular simulations, we show that forces similar to hydrophobic assembly can play out in quasi-two-dimensions in the plane of the membrane, to bring about assembly of membrane inclusions. Just as two large hydrophobes, each stabilizing a vapor-like region in their vicinity, assemble to minimize the effective energy due to vapor–liquid surface tension, two orderphobes would assemble to minimize the effective energy due to order–disorder line tension.

Given the likely existence of a first-order phase transition in the MARTINI model system, we are now in a position to test the existence of the orderphobic effect. In Chapter 4, an ideal orderphobe, i.e. a cylinder with hydrophobic thickness that resembles the disordered phase, is embedded in the ordered phase. We show that an orderphobe is able to induce a disordered region in its vicinity, and therefore an order–disorder interface. The fluctuations of this induced interface are very similar to the fluctuations of the free order–disorder interface in Chapter 3. Two orderphobes embedded in the bilayer, each stabilizing disorder in its surroundings, should therefore assemble into a single structure that minimizes the cumulative disordered region and the associated energy due to the interfacial tension. Indeed, we observe that two orderphobes assemble in an ordered bilayer. The orderphobic effect gives rise to a powerful assembling force which acts over the nano- to mesoscopic scale, and could potentially explain the clustering of proteins on the surface of the cell.
Figure 1.6: Enrichment of SNARE proteins during vacuole fusion: the top panel shows clustering of the SNARE protein, Vti1p (tagged with green fluorescent protein (GFP)), during vacuole fusion (see white arrows). The bottom panel shows an absence of protein clustering due to the addition of a fusion inhibitor, ENTH. The red dye labels the membrane while the green dye specifically labels the SNARE protein. Bright green clusters of SNARE proteins are visible in (H). Adapted from Fratti et al. 2004, Journal of Cell Biology, copyright of Rockefeller University.
Clustering is a widespread phenomenon that occurs during fusion of synaptic vesicles and vacuoles, formation of immune synapses, and cell signaling among other processes. Figure 1.6 shows the clustering of proteins at the junction where two vacuoles are beginning the fusion process, the specific physical mechanisms for which are still unknown. It is possible that mechanisms similar to the orderphobic effect are responsible for clustering during these processes. A variety of theories have been proposed to explain the assembly of proteins. These include elasticity, electrostatic forces and Casimir forces. Several forms of membrane-mediated elastic forces have been proposed to act between proteins. However, potentials of mean force calculated for proteins interacting elastically via the membrane have shown only weak, short-ranged attractions. The orderphobic force proposed is distinctly stronger and longer-ranged than elastic effects. Charged lipids such as phospho-inositides play an important role in clustering, however the molecular mechanism by which this occurs is as yet unknown. Additionally, weak but long-ranged Casimir forces have been proposed to act between membrane inclusions on the basis of recent experiments that suggest that the cell membrane composition is close to a miscibility critical point. However, it is not clear whether a membrane very close to a critical point can give rise to large and stable aggregates such as those seen via stimulated emission depletion microscopy experiments (Figure 1.2).

The hydrophobic effect is a very powerful force of assembly that gives rise to a large range of structures including folded proteins, micelles, and therefore possibly even bilayers. It is possible that since an analogous effect driven by the existence of a first-order phase transition exists laterally in the plane of the membrane, it can be similarly powerful in clustering the solutes occurring in the membrane.

1.3.3 Lipid droplet formation

In Chapter 5, we study the effect of another kind of inclusion within a lipid bilayer—an oily phase. Cells, both prokaryotic and eukaryotic, synthesize hydrophobic substances such as triglycerides, neutral lipids, and sterol esters. In the aqueous cytosol, the strategy that cells use to store and mobilize these oily molecules is the creation of an organelle known as the lipid droplet. The lipid droplet is an agglomeration of hydrophobic molecules bounded by a lipid monolayer. These droplets diffuse around the cell, transporting their hydrophobic cargo between organelles.

A few different models have been proposed for the growth and budding of this organelle. There is general consensus that hydrophobic molecules are synthesized and secreted between the two leaflets of a bilayer by a transmembrane enzyme. This process continues until the nascent droplet buds off, taking part of the membrane with it. The models differ on whether one or both the leaflets are involved in the process. Genetic studies have revealed that there are no single gene products found to be responsible for droplet budding from a bilayer. It is therefore possible that the process is purely physical. We attempt to study the budding process by inserting oil-like molecules into a lipid bilayer. We find that nascent droplets spontaneously become asymmetric and, for a sufficiently high concentration of oil,
Figure 1.7: Two slices of a 3D tomographic reconstruction of a lipid droplet in a yeast cell. The first slice shows a gap between the large, centrally located lipid droplet and the parent membrane (dark outline). The second slice is another section of the same organelle at a different depth. This slice shows an association of the droplet with the parent bilayer adapted from Wolinski et al. 2014, Journal of Cell Science by Company of Biologists, reproduced with permission of Company of Biologists, Ltd. in the format “Republish in a thesis/dissertation” via Copyright Clearance Center.
spontaneously bud off, taking the top leaflet with them. To sample and characterize the various stages involved in the budding process, we perform umbrella sampling on a single droplet from the nascent stage to the budded stage. Doing so, in a molecular simulation, poses the technical difficulty that the number of membrane lipids in the system is constantly increasing to accommodate the growing droplet. We therefore require a lipid reservoir that adds or removes lipids from the system as required. Sampling the grand canonical ensemble, however is practically untenable for a dense system such as lipid bilayer. To this end, we construct an efficient parallel implementation of grand canonical configurational bias Monte Carlo to make the process viable (Chapter 2). This computational framework can also be used to study other asymmetric inclusions or processes that have more lipids on one leaf of the membrane than the other, or that involve flow of lipids into or out of the system.

Our results support the model of a lens-shaped nascent oil droplet bulging outwards and finally budding off taking only the outer leaflet with it. The results are also consistent with a morphological transition between a droplet partially enveloped by the surrounding monolayer to nearly completely wrapped by it, similar to the shapes seen in vesicles enclosing two immiscible fluids. Once the completely-enveloped stage is reached, budding occurs spontaneously. The completely wrapped configuration is characterized by a neck where lipids are simultaneously part of both the parent bilayer as well as the droplet. Budding occurs when these neck lipids commit to either the droplet or the parent bilayer.
Chapter 2
Molecular Models and Simulation Methods

We begin this chapter with a description of the models we use in this work. An appropriate model is chosen based on the problem being addressed. We use two lipid bilayer models—the MARTINI coarse-grained force field simulated using molecular dynamics, and a minimalist, soft–repulsive model simulated using hybrid dissipative particle dynamics–Monte Carlo. The first model is used for our studies on phase transitions in lipid bilayers since this model reproduces the correct order of the transition as discussed in Chapter 3. The second model is used for our studies on budding and formation of oil droplets from membranes in Chapter 5. In addition, we discuss the methods used to investigate the behavior of these models—molecular dynamics, dissipative particle dynamics and Monte Carlo to sample different ensembles.

2.1 The MARTINI model

We begin with a description of one of the coarse-grained models used in this work—the MARTINI model. The MARTINI coarse-grained model approximates four carbon-like atoms to one coarse-grained bead \[45\] \[23\]. This model has been extensively used to study the properties of not only lipid bilayers and transmembrane proteins, but a wide variety of structures as shown in Figure 2.1 \[15\]. The model consists of several different kinds of particles grouped into five broad categories—polar, non polar, apolar and charged. Within these categories, the particles differ based on hydrogen bonding capability and/or degree of polarity. It has an explicit description of solvent, with an average of four water molecules corresponding to the size of one solvent particle. Recently, a polarizable solvent particle has also been introduced into this model \[66\], however, we shall see that for our purposes the standard solvent particle will suffice.

The parameterization of each of the different kinds of beads, each representing a small molecule such as propane or acetic acid, was based on the reproduction of experimental values of (i) the free energy of hydration, (ii) the free energy of vaporization, and (iii) the
Figure 2.1: Illustration of the MARTINI mapping of coarse-grained particles (cyan) overlaid onto atomistic structures for (A) a solvent particle representing four water molecules, (B) a polarizable water particle with embedded charges, (C) a dimyristoyl phosphatidylcholine molecule, (D) a polysaccharide fragment, (E) a peptide, (F) a DNA fragment, (G) a polystyrene fragment and (H) a fullerene molecule. Adapted from md.chem.rug.nl/cgmartini/index.php/about.
partitioning free energies between water and a number of organic phases.

Here, we focus on the model and its parameters for a bilayer-forming lipid with saturated aliphatic chains such as dipalmitoyl phosphatidylcholine (DPPC). A graphical representation of the model, along with its atomistic counterpart are shown in Figure 2.1C. The hydrophilic head group of DPPC is composed of four beads with varying degrees of polarity to represent the (i) choline, (ii) phosphate, and (iii) two ester linkages. The two terminal head group beads carry one positive (choline) and one negative (phosphate) charge respectively. The aliphatic chains of the lipid are modeled as a series of identical apolar beads. Typically, DPPC has four beads in each tail with the tail length varying as the number of carbon atoms in the chain. The interaction potential between particles includes bonded interactions, non-bonded interactions and electrostatic interactions.

2.1.1 Non-bonded interactions

A shifted Lennard-Jones 12–6 potential is used to model non-bonded interactions given by

\[
U_{\text{LJ}}(r_{ij}) = 4\epsilon_{ij} \left[ \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right],
\]

(2.1)

where \( r_{ij} \) is the distance between two particles \( i \) and \( j \), \( \sigma_{ij} \) is a parameter that describes the smallest approachable distance between the two particles and \( \epsilon_{ij} \) is a parameter that describes the strength of their interaction. \( \sigma_{ij} \) is chosen to be 0.47nm for most interaction pairs, setting a length scale for this model.

In addition to this Lennard-Jones interaction energy, charged groups (such as the head groups of DPPC) interact through a shifted Coulombic potential,

\[
U_{\text{electrostatic}}(r_{ij}) = \frac{q_i q_j}{4\pi\epsilon_0\epsilon_r r_{ij}},
\]

(2.2)

where \( q_i \) and \( q_j \) are the magnitudes of charge on beads \( i \) and \( j \). The charged head group beads each bear a full charge. A relative dielectric constant \( \epsilon_r \) is used for screening the potential.

2.1.2 Bonded interactions

Bonds between beads are described by a weak harmonic potential,

\[
U_{\text{bond}}(r_{ij}) = \frac{1}{2}K_{\text{bond}}(r_{ij} - R_{\text{bond}})^2,
\]

(2.3)
where $R_{\text{bond}}$ is the equilibrium bond distance and is set to $\sigma_{ij} = 0.47\,\text{nm}$ with the force constant $K_{\text{bond}} = 1250\,\text{kJ}\,\text{mol}^{-1}\,\text{nm}^{-2}$. The Lennard-Jones interaction is not calculated between bonded particles. Chain stiffness is represented via a weak harmonic potential for bond angles,

$$U_{\text{angle}}(\theta) = \frac{1}{2} K_{\text{angle}} [\cos(\theta) - \cos(\theta_0)]^2. \quad (2.4)$$

For saturated lipid tails, $K_{\text{angle}} = 25\,\text{kJ}\,\text{mol}^{-1}\,\text{rad}^{-2}$ with an equilibrium angle $\theta_0 = 180^\circ$. Distributions of bond lengths and angles with these parameters compare well with corresponding distributions from atomic simulations. For cis-unsaturated chains, $K_{\text{angle}} = 45\,\text{kJ}\,\text{mol}^{-1}\,\text{rad}^{-2}$ and $\theta_0 = 120^\circ$, while for trans-unsaturated chains $K_{\text{angle}} = 45\,\text{kJ}\,\text{mol}^{-1}\,\text{rad}^{-2}$ and $\theta_0 = 180^\circ$. For particles such as cholesterol which possess ringed structures, additional dihedral constraints are added to prevent out-of-plane distortion of the rings.

### 2.1.3 Antifreeze particles

A peculiar problem with the solvent in this model is that it crystallizes at unrealistically high temperatures, typically between 280 and 300 K. Extended exposed surfaces, such as the model proteins we will encounter in a future chapter, act as nucleation sites and lead to rapid freezing of the coarse-grained water. A straightforward, practical solution is to introduce ‘antifreeze’ particles which are similar to the ordinary solvent particles but have a larger size. Ordinary solvent particles are slightly more attracted to the antifreeze particles than to each other. These large particles disrupt solvent lattice formation in small concentrations (10%). However, in larger concentrations both particles nucleate and freeze independently.

As reported by other studies of this model, these antifreeze particles have no effect on some measured properties of a bilayer, such as area per lipid, transition temperature into the ordered phase, and the lipid self-diffusion constant\[23\]. Certain properties of the bilayer, such as the formation of tilt and ripple phases, are dependent on the interaction between water and the lipid head groups. Both the tilt and the ripple phase disappear when the water–head group interaction is weakened\[42\]. The effect of anti-freeze particles on such solvent-dependent properties is as yet unknown. However, in the liquid $L_\alpha$ phase the use of antifreeze particles presents straightforward solution that in all likelihood does not tangibly affect in-plane bilayer structure.

This model is able to reproduce experimental results for key quantities such as area per lipid, bending modulus and area compressibility modulus for DPPC and several other lipids. In addition, it reproduces the lateral stress profiles of a bilayer calculated from all atom simulations. In summary, The MARTINI model is a very versatile molecular model that can be easily extended to different kinds of molecules. We employ this model in Chapter 3 and Chapter 4 of this work to study the order–disorder transition in DPPC and consequences of that transition on solute assembly.
2.2 Molecular dynamics simulations

We perform molecular dynamics simulations on the MARTINI model using the GROMACS molecular dynamics package \[67\] using similar algorithms and parameters as described by Marrink et al. in \[23\] for the original model. We use a leapfrog integrator with a time step of 30 fs to propagate the system. This time step is an order of magnitude higher than an atomistic molecular dynamics time step since the fast degrees of freedom of atomic motion are coarse-grained. The recommended range of time step for this model is 20–40 fs. We use periodic boundary conditions in the \(x\), \(y\) and \(z\) directions. A constant temperature is maintained in the simulations using the Berendsen thermostat \[68\]. When required, a constant surface pressure is maintained using the Berendsen barostat as described below.

2.2.1 Berendsen thermostat

The Berendsen thermostat is analogous to coupling the system to an external heat reservoir at a constant temperature \(T_r\). Any deviation of system temperature from \(T_r\) is corrected by rescaling the particle velocities and hence the effective temperature of the system as determined by the kinetic energies. The rescaling of velocities is performed at every time-step \(\Delta t\), or every few time-steps. The velocities are rescaled by a factor \(\lambda\) determined as

\[
\lambda = \left(1 + \frac{\Delta t}{\tau_T} \left(\frac{T_r}{T} - 1\right)\right)^{1/2}.
\]

\(\tau_T\) is a coupling time constant that determines the time scale over which the current system temperature \(T\) reaches the reservoir temperature. The rate of change of temperature at each instant is proportional to the difference in temperature between the system and reservoir, i.e.,

\[
\frac{dT}{dt} = \frac{1}{\tau_T} (T_r - T).
\]

The Berendsen thermostat does not generate Boltzmann-distributed configurations because it suppresses fluctuations in the kinetic energy. This error scales as \(1/N\) where \(N\) is the number of particles in the system, and is therefore small for a system with several thousands of particles. However, a thermostat that correctly samples the canonical ensemble, such as the Nosé–Hoover thermostat, \[69, 70, 71\] should be used to measure fluctuation-driven quantities such as the diffusion constant and heat capacity.

2.2.2 Berendsen barostat

To maintain a constant pressure and constant surface tension, the volume (or area) of the simulation box is considered a dynamic variable that fluctuates during the course of the
.simulation. We use the Berendsen barostat to maintain a constant pressure and surface tension \(68\). Similar to the Berendsen thermostat, the system is coupled to a constant pressure reservoir with pressure \(P_r\). At each time-step \(\Delta t\) the size of the simulation box and the coordinates of the particles are rescaled to correct any deviations from this reference pressure. This rescaling is performed by a matrix \(\mu\), with each element \(\mu_{ij}\) defined for an isotropic system as

\[
\mu_{ij} = \delta_{ij} - \frac{\Delta t}{3\tau_P} \beta_{ij} (P_{rij} - P_{ij}),
\]

where \(\beta\) is the isothermal compressibility of the system and \(\tau_P\) is a constant that determines the time scale over which the current system pressure \(P\) decays to \(P_r\). This decay rate is given by

\[
\frac{dP}{dt} = \frac{1}{\tau_P} (P_r - P).
\]

This pressure correction can also be used to maintain a constant surface tension \(\gamma\). \(\gamma\) is defined as

\[
\gamma = \int_{z_1}^{z_2} (P_N(z) - P_T(z)) \, dz.
\]

\(P_N\) and \(P_T\) are the normal and tangential components of pressure as calculated from the stress tensor. \(P_N = P_{zz}\) and \(P_T = \frac{P_{xx} + P_{yy}}{2}\) for a system with an interface, such as a lipid bilayer, with the normal to the interface in the \(z\) direction. The \(P_{xx}\), \(P_{yy}\) and \(P_{zz}\) components of the stress tensor can be individually controlled to set the surface tension \(\gamma\). To impose a constant surface tension of zero on a lipid bilayer, while maintaining a bulk pressure of 1 bar, we set \(P_{xx} = P_{yy} = P_{zz} = 1\) bar. This has been referred to as the ‘semi-isotropic’ coupling of pressure.

The rescaling used in the Berendsen barostat does not guarantee correct sampling of the isobaric ensemble, and therefore also the isotension ensemble. However, it has been used as a standard barostat with the MARTINI model. Rodgers et al. showed that a barostat that correctly samples the isobaric ensemble is required to correctly sample the isotension ensemble, for the semi-isotropic method of pressure coupling \(72\). An algorithm proposed by Martyna et al. correctly generates the isobaric ensemble \(73\).

2.3 A minimalist coarse-grained model

We employ a second coarse-grained model in this work which employs soft and purely repulsive potentials for lipid bilayer systems, and is simulated using dissipative particle dynamics
as described in the next section. This model has been developed by Venturoli et al. and Kranenburg et al. for simple bilayer systems, and extended by de Meyer et al. and Benjamini et al. for bilayer inclusions such as cholesterol and transmembrane proteins. This is a minimalist model which uses three different types of spheres, all of which repel each other to varying degrees. This simple model is able to reproduce micelle and bilayer self-assembly, as well as the phase behavior of cylindrical lipids.

A schematic of the minimalist model is shown in Figure 2.2. Similar to the MARTINI model, an average of three carbon-like particles are approximated into one coarse-grained particle or bead. This model also has an explicit description of solvent, with an average of three water molecules approximated to one solvent particle. There are two kinds of beads that constitute lipids or surfactants—a hydrophilic bead that has relatively favorable interactions with solvent, and a hydrophobic bead that has relatively unfavorable interactions with solvent. This model represents the simplest of design principles required to assemble a lipid bilayer and other surfactant assemblies. A matrix specifying the magnitudes of repulsive interactions between the different kinds of particles is shown in Figure 2.2D. These parameters are derived for water to reproduce its compressibility and radial distribution function, while for surfactants they can be related to the Flory–Huggins theory for polymer mixing. In addition to solvent particles and lipids, we introduce a hydrophobic ‘oily’ bead also shown in Figure 2.2 to model hydrophobic molecules, such as cholesteryl esters and neutral lipids, which accumulate in a lipid bilayer forming lipid droplets (see Chapter 5).

### 2.3.1 Non-bonded interactions

The non-bonded interactions between two particles $i$ and $j$ in this model are soft, purely repulsive, conservative forces described by

$$f_{ij}^C = \begin{cases} a_{ij} (1 - |r_{ij}|/R_c) \hat{r}_{ij} \quad (|r_{ij}| < R_c) \\ 0 \quad (|r_{ij}| \geq R_c) \end{cases},$$

(2.10)

where $r_{ij}$ is the distance between two particles $i$ and $j$, $\hat{r}_{ij}$ is the unit vector connecting $i$ and $j$, and $R_c$ is the cut-off distance for the conservative force. $a_{ij}$ is the magnitude of repulsion as determined from the interaction matrix in Figure 2.2D in units of $\epsilon_0$. $R_c$ is set to $1.0d_0$ where $d_0$ is the reduced unit for length for this model.

Groot et al. obtained a value of $a_{ij} = 25\epsilon_0$ for water–water interactions by reproducing the radial distribution functions and isothermal compressibility of water at room temperature. The interaction parameters for the remaining beads have been connected to mixing and demixing of polymer solutions on a Flory–Huggins lattice model, and further parameterized by Venturoli et al. and others to reproduce quantities such as the area per lipid of a DMPC bilayer. Kranenburg et al. show that the phase behavior of different kinds of lipids, such as phosphatidylcholines and phosphatidylethanolamines, can be obtained simply by changing the relative interactions between lipid heads and solvent particles. The model DPPC molecules obtained in this fashion showed the tilted gel ($L_{\beta'}$)
Figure 2.2: A minimalist model for lipid bilayers: (a) A coarse-grained water bead representing three water molecules. (b) A single-tailed lipid with a hydrophilic head (blue) and a hydrophobic tail (red). Bonds are shown as solid, black lines, and constrained angles are shown as dashed lines. (c) A hydrophobic bead representing an oil inclusion within the membrane. (d) An interaction matrix showing the strength of relative repulsive interactions between the different pairs of particles in units of $\epsilon_0$. 
phase as well as, surprisingly, the ripple \( P_{β'} \) phase observed in X-ray scattering experiments of lecithins \[39\] at temperatures below the melting point. On the other hand, the model for DPPE molecules, did not exhibit rippled structures, consistent with the phase diagram for phosphatidylethanolamines \[43\].

### 2.3.2 Bonded interactions

Similar to the MARTINI model, the bonded interactions are described using a harmonic force to constrain the bond lengths described by

\[
F_{\text{bond}} = -K_{\text{bond}} (r_{ij} - R_{\text{bond}}),
\]

where the force constant \( K_{\text{bond}} = 100 \epsilon_0 \) and the equilibrium bond distance \( R_{\text{bond}} = 0.7 d_0 \). Similarly, a harmonic force \( F_{\text{angle}} \) is defined as

\[
F_{\text{angle}} = -\nabla \left( \frac{1}{2} K_{\text{angle}} (\theta_{ij} - \theta_0)^2 \right).
\]

The force constant \( K_{\text{angle}} = 6 \epsilon_0 \text{rad}^{-2} \) and the equilibrium angle \( \theta_0 = \pi \text{ rad} \) for the hydrophobic bonded beads in the system. In the case of lipids with two tails, the equilibrium angle between the two tails was set to \( \theta_0 = \pi/2 \text{ rad} \) with a force constant of \( 3 \epsilon_0 \text{ rad}^{-2} \). The parameters for equilibrium bond lengths and bond angles were obtained by Venturoli et al. from all-atom simulations \[74\]. Dihedral bonded interactions were added by Benjamini et al. to model transmembrane proteins with \( \alpha \)-helical structures \[78\]. We use dissipative particle dynamics to simulate this model.

### 2.4 Dissipative particle dynamics

Dissipative particle dynamics (DPD) is a numerical technique proposed by Hoogerbrugge and Koelman for simulation at mesoscopic length scales and larger time scales compared to molecular dynamics simulations \[79\]. Each particle in DPD is considered as a group of several individual atoms or even molecules. In addition to a conservative force between particles, there are also pairwise dissipative and random forces between particles to account for the omitted degrees of freedom. In the limit of a small integration time step, the DPD method conserves momentum and provides correct hydrodynamic behavior, i.e. the Navier–Stokes equation is obeyed. In this regard the DPD method is an improvement over similar mesoscale techniques, such as Brownian dynamics, where momentum is not conserved. The relationship between the magnitude of the dissipative and random forces was derived by Español and Warren to ensure that the fluctuation dissipation theorem is satisfied, and the system evolves...
to an equilibrium distribution \[80\]. Soft interactions between the particles, such as the ones described in the previous section, allow a larger time step while integrating the equations of motion, as compared to molecular dynamics simulations.

The force on a particle \(i\) at any instant is given by

\[
F_i = \sum_{i \neq j} f^{C}_{ij} + \sum_{i \neq j} f^{D}_{ij} + \sum_{i \neq j} f^{R}_{ij}.
\]

(2.13)

The conservative force \(f^{C}_{ij}\) is usually chosen to be a pair potential between particles for non-bonded and bonded interactions that only depends on the position of atoms relative to each other and not their velocities. The dissipative force \(f^{D}_{ij}\) represents a viscous force that depends on both the positions and velocities, while the random force \(f^{R}_{ij}\) adds thermal fluctuations to the system and depends solely on particle positions. These forces are defined as

\[
f^{D}_{ij} = -\gamma \omega_D(r_{ij})(\mathbf{r}_{ij} \cdot \mathbf{v}_{ij})\hat{r}_{ij},
\]

(2.14)

\[
f^{R}_{ij} = \sigma \omega_R(r_{ij})\zeta_{ij}\hat{r}_{ij},
\]

(2.15)

where \(r_{ij}\) is the vector between two particles \(i\) and \(j\) while \(\mathbf{v}_{ij} = \mathbf{v}_i - \mathbf{v}_j\). \(\hat{r}_{ij}\) is the unit vector in the direction of \(r_{ij}\). \(\gamma\) and \(\sigma\) determine the strength of the dissipative force and random forces between the particles respectively. \(\omega_D(r_{ij})\) and \(\omega_R(r_{ij})\) describe the variation with distance of the dissipative and random forces respectively. \(\zeta_{ij}\) is a random variable normally distributed with mean 0 and variance 1. The conservation of momentum required to produce correct hydrodynamic behavior requires that \(F_i = -F_j\) and therefore \(\zeta_{ij} = -\zeta_{ji}\).

Español and Warren showed that for the algorithm to satisfy the fluctuation-dissipation theorem and generate Boltzmann-weighted configurations, the dissipative and random forces are related as

\[
\omega_D(r_{ij}) = [\omega_D(r_{ij})]^2,
\]

(2.16)

while \(\sigma\) and \(\gamma\) are related as

\[
\sigma^2 = 2k_BT\gamma.
\]

(2.17)

In our dissipative particle dynamics simulations, we choose \(\omega_R(r_{ij})\) to have the same dependence on \(r_{ij}\) as the conservative forces described in the previous section, for computational convenience:

\[
\omega_R = \begin{cases} 
1 - |r_{ij}|/R_c & (|r_{ij}| < R_c) \\
0 & (|r_{ij}| \geq R_c)
\end{cases},
\]

(2.18)
and $\omega_{D}(r_{ij})$ is therefore set as

$$\omega_{D} = \begin{cases} 
(1 - |r_{ij}|/R_{c})^{2} \hat{r}_{ij} & (|r_{ij}| < R_{c}) \\
0 & (|r_{ij}| \geq R_{c})
\end{cases}.$$ (2.19)

We set the amplitude of the random force $\sigma = 3$ with $\gamma = \sigma^{2}/2k_{B}T$.

### 2.4.1 Integration scheme

The integration of Newton’s equations of motion to advance the system in time is performed using the velocity Verlet algorithm [81]. In this algorithm, the positions and velocities are not concurrently computed. This is not a hindrance in molecular dynamics simulations as the forces depend only on the particle positions and not their velocities. In DPD, however, since the dissipative force depends on particle positions as well as velocities, the velocities need to be calculated concurrently with the particle positions. A predicted velocity $\tilde{v}_{i}$ is used to calculate the velocity-dependent force. The velocity Verlet equations are given by:

$$r_{i}(t + \Delta t) = r_{i}(t) + \Delta t v_{i}(t) + \frac{1}{2} (\Delta t)^{2} f_{i}(t),$$ (2.20)

$$\tilde{v}_{i}(t + \Delta t) = v_{i}(t) + \lambda \Delta tf_{i}(t),$$ (2.21)

$$f_{i}(t + \Delta t) = f_{i}(r(t + \Delta t), \tilde{v}(t + \Delta t)), $$ (2.22)

$$v_{i}(t + \Delta t) = v_{i}(t) + \frac{1}{2} \Delta t (f_{i}(t) + f_{i}(t + \Delta t)).$$ (2.23)

These equations reduce to the velocity Verlet algorithm for $\lambda = 0.5$ with forces independent of velocities.

### 2.5 Reduced units

In simulations, it is convenient to represent physical quantities in terms of basic units related to the mass, length, time and energy scales of the system concerned. These units, called reduced units, serve the practical purpose of avoiding having to keep track of the very large or very small absolute numerical values of the quantities being calculated. In coarse-grained simulations, converting reduced units to physical units is not straightforward because of the inexact mapping between coarse-grained beads and their atomistic counterparts. For example, both the MARTINI model and the minimalist soft–repulsive model map three or four non-hydrogen atoms to one coarse-grained bead. Depending on the atoms mapped, the sizes and masses of the resulting beads could be very different. In this section, we note the basic units used in our dissipative particle dynamics simulations:
• Mass: It is assumed that all beads in the system have unit mass.

• Length: The cut-off diameter $R_c$ is used as the basic length unit ($d_0$). This length can be related to a physical length by comparing a coarse-grained solvent particle to three water molecules. A unit volume $R_c^3$ holds an average of three beads, and therefore approximately nine water molecules. If the volume of the water molecule is taken to be approximately $30 \, \text{Å}^3$, then $R_c = 6.46 \, \text{Å}$.

• Time: The dissipative particle dynamics time cannot be exactly connected to a real time, because of coarse-graining. The simulation time, however, can be related to real time by comparing transport coefficients in the system to those in atomistic simulations or experiments. Comparing the diffusion coefficient of water yields a time step of 5 ps \[82\].

• Temperature: We obtain a temperature scale relating the reduced temperature to real temperature by comparing the melting temperatures of lipids in experiments that study bilayer phase transitions \[82\].

### 2.6 Monte Carlo methods

By construction, dissipative particle dynamics allows us to sample the $NVT$ ensemble where $N$ is the total number of particles in the system, $V$ is the volume of the system and $T$ is the temperature maintained by the dissipative and random forces. Lipid bilayers change their properties in response to changes in their environment including temperature, surface tension, and lipid concentration. For example, a liposome can expand or contract due to application of surface pressures because lipids allow changes in their area per lipid. To allow the volume of the bilayer system to fluctuate under an imposed pressure, we sample the $NPT$ or isobaric ensemble, where $P$ is the pressure of the system. To allow the area of a bilayer to fluctuate independently of the system volume, we need to sample the $NP_{\perp}T$ or isotension ensemble, where $P_{\perp}$ is the component of the stress tensor in the direction normal to the bilayer, and $\gamma$ is the surface tension of the membrane. Similarly, to allow for changes in the number of lipid particles in the system, we sample the $\mu VT$ or grand canonical ensemble where $\mu$ is the chemical potential of lipids in the system. In this section, we discuss Monte Carlo sampling in the isotension and grand canonical ensembles.

#### 2.6.1 Isotension ensemble

The isotension ensemble allows us to impose a constant surface tension on the bilayer while sampling fluctuations in its area. Several attempts have been made to sample the isotension ensemble using Monte Carlo and molecular dynamics simulations. Here, we describe the approach derived by Rodgers et al. to propose Monte Carlo moves consistent with the
isotension ensemble \cite{72}. The free energy differential describing this ensemble is given by a Legendre transform,

\[
dF = -T \, dS + V \, dP - \gamma \, dA + \mu_{\text{lipid}} N_{\text{lipid}} + \mu_{\text{water}} N_{\text{water}},
\]

(2.24)

where $\mu_{\text{lipid}}$ and $\mu_{\text{water}}$ are the chemical potentials of the lipid and water phases respectively, conjugate to $N_{\text{lipid}}$ and $N_{\text{water}}$, the number of lipids and number of water molecules in the system. The Gibbs Phase Rule allows us to specify three intensive variables in a lipid bilayer system, $P$, $\gamma$ and $T$, which is not possible in an oil–water system. In an oil–water system, the surface tension cannot be specified independently of the bulk pressure and there are only two independent intensive degrees of freedom, $P$ and $T$. The addition of a surface phase, such as a lipid bilayer, is required to specify the interfacial tension independently of the bulk pressure. This is possible since the surface phase can change the number of lipids per unit area, and hence the surface tension. Adding a surface phase increases the number of components, while not changing the number of bulk phases, giving a total number of three intensive degrees of freedom.

Rodgers et al. showed that two types of moves need to be proposed to correctly sample the isotension ensemble—changes in box size in the plane of the membrane $L_{||}$ keeping volume $V$ constant, and changes in box size perpendicular to the plane of the membrane $L_{\perp}$ keeping $L_{||}$ constant (see Figure 2.3) \cite{72}.

**Attempts to change $L_{||}$ keeping $V$ constant**

This move involves the following steps:

- **Choosing a change in $L_{||}$**, $\Delta L_{||} \in [-L_{||}\text{max}/2, L_{||}\text{max}/2]$, and calculating the new, proposed box length $L_{||}\text{new} = L_{||}\text{old}$,

- **Calculating a new value for $L_{\perp}$** to hold volume constant, $L_{\perp}\text{new} = \frac{V}{L_{||}\text{new}^2}$,

- **Rescaling the centers of mass of the particles in the plane of the membrane by** $\frac{L_{||}\text{new}}{L_{||}\text{old}}$ and along the bilayer normal by $\frac{L_{\perp}\text{new}}{L_{\perp}\text{old}}$ to calculate the energy difference $\Delta U$ between the old and new states and,

- **Accepting this $L_{||}$ change move with acceptance probability**

\[
\text{acc} \left( L_{||}\text{old} \rightarrow L_{||}\text{new} \mid V \right) = \min \left[ 1, \frac{L_{||}\text{new}}{L_{||}\text{old}} \exp \left( -\beta \gamma \Delta A - \beta \Delta U \right) \right].
\]

(2.25)
Figure 2.3: An illustration of a system containing a surface-acting component, such as a lipid bilayer, that can change its surface tension independent of the pressure in the system by changing its area per lipid. The length in the plane of the membrane is defined as $L_{\parallel}$ while the length perpendicular to the membrane is defined as $L_{\perp}$.

**Attempts to change $V$ keeping $L_{\parallel}$ constant**

This move involves the following steps:

- Choosing a volume change $\Delta V \in [-V_{\text{max}}/2, V_{\text{max}}/2]$, and calculating a proposed new volume, $V_{\text{new}} = V_{\text{old}} + \Delta V$,

- Calculating a value for $L_{\perp}$ holding $L_{\parallel}$ and hence the area in the plane of the membrane constant, $L_{\perp,\text{new}} = \frac{V_{\text{new}}}{L_{\parallel}^2}$,

- Rescaling the centers of mass of the particles along the bilayer normal by the ratio $\frac{L_{\perp,\text{new}}}{L_{\perp,\text{old}}}$ to calculate the energy difference $\Delta U$ between the old and new states and,
Accepting this volume change move with acceptance probability

\[
\text{acc } (V_{\text{old}} \rightarrow V_{\text{new}} \mid L_{\parallel}) = \min \left[ 1, \frac{(V_{\text{new}})}{(V_{\text{old}})}^{N-1} \exp \left( -\beta P \Delta V - \beta \Delta U \right) \right].
\]

(2.26)

\(V_{\text{max}}\) and \(L_{\parallel \text{max}}\) are constants defining the span of volume and length changes that are feasible given the type of system. For lipids and other molecules with bonds we rescale only the first beads of each molecule and translate the rest of the beads to avoid changing bond lengths drastically (this would almost always result in move rejection), i.e. only non-bonded energies are altered.

### 2.6.2 Grand canonical ensemble

Lipid bilayer simulations have been restricted to systems with constant number of lipids \(N_{\text{lipid}}\). While this serves the purpose of computing ensemble properties of a bilayer and its behavior around various solutes, certain structures and processes require the number of lipids in the system to change. One example is the lipid droplet, a structure that we study in Chapter 5, where hydrophobic molecules accumulate between the two leaflets of the membrane and subsequently bud out as a droplet. Studying this growth process involves not only a change in the number of ‘oily’ species accumulating in the membrane, but also a change in the number of lipid molecules in the bilayer to accommodate the budding droplet. For such cases, we require a reservoir of molecular species at constant chemical potential in contact with the system under observation, which can add or remove molecules from the system to keep its chemical potential constant. In other words, we need to sample from the grand canonical or \(\mu VT\) ensemble, where \(\mu\) is the chemical potential of the relevant species.

Sampling the \(\mu VT\) ensemble involves attempts to insert and delete molecules in the system. In our model, the hydrophobic contents of the droplet are modeled simply as hydrophobic beads that prefer to be surrounded by lipid tails rather than head groups or solvent particles. It is relatively straightforward to insert and delete a single-particle molecule. In the case of insertion, a location for an additional particle is chosen at random and accepted with the following probability

\[
\text{acc } (N \rightarrow N + 1) = \min \left[ 1, \frac{V}{\Lambda^3 (N + 1)} \exp \left( \beta \mu - \beta U(N + 1) + \beta U(N) \right) \right].
\]

(2.27)

\(N\) is the number of particles of the species under consideration and \(U(N)\) and \(U(N + 1)\) are the energies of the system before and after the insertion attempt. \(\mu\) is the chemical potential of the species under consideration and \(\Lambda\) is defined as a thermal de Broglie wavelength equal to \(h/\sqrt{2\pi m k_B T}\). On the other hand, for deletion, an existing particle is chosen at random and removed with probability
Figure 2.4: An illustration of the grand canonical ensemble with constant chemical potential $\mu$, constant volume $V$ and constant temperature $T$ that permits the flow of energy as well as particles between the system and a reservoir.

\[
\text{acc} \left( N \rightarrow N - 1 \right) = \min \left[ 1, \frac{A^3 N}{V} \exp \left[ -\beta \mu + \beta U(N - 1) - \beta U(N) \right] \right]. \tag{2.28}
\]

The number of successful insertion and deletion attempts for a single-bead molecule are high enough even for very dense systems, such as a lipid bilayer, to achieve good sampling of the $\mu VT$ ensemble. However, for the case of multi-particle chain such as a lipid, the probabilities of successful insertions and deletions are extremely low for a dense system such as a lipid bilayer. It is very rare to find a lipid-shaped cavity in the system to insert a new molecule into. Most of the moves are rejected because they would result in high energies
from overlapping neighboring particles. To improve sampling of the $\mu VT$ ensemble for chain molecules, we employ several biased Monte Carlo techniques that bias the insertion and deletion attempts. The biased attempts are performed such that they have a better chance of being accepted compared to random insertion, and the bias is subsequently accounted for in the acceptance probabilities. The first scheme we use is referred to as configurational bias Monte Carlo [83].

### 2.6.3 Configurational bias Monte Carlo

![Diagram of bond-by-bond chain growth in configurational bias Monte Carlo](image)

Figure 2.5: An illustration of the bond-by-bond chain growth in configurational bias Monte Carlo for a single-tailed lipid with hydrophilic groups in cyan, and hydrophobic groups in red. At every step in the chain, $k = 3$ independent bond orientations are proposed for every bead that is to be placed (dashed lines). Out of the three trials, one is chosen based on its Boltzmann weight (solid lines). This continues until the entire chain is grown. The resulting configuration is then accepted or rejected based on the acceptance criteria.
Configurational bias Monte Carlo greatly improves sampling of chain molecules in dense systems. This method involves the generation of a chain molecule one bond at a time with several trial configurations at each step. An illustration of this method is shown in Figure 2.5. It biases the insertion of molecules towards configurations of lower energy, and hence higher probability. Out of the multiple trials at each step in the chain growth, the trial with the lowest energy, and hence the highest Boltzmann weight, is selected. The probability of acceptance of this biased insertion is subsequently unbiased by calculation of a Rosenbluth factor $W$ which represents the partition function of all the attempted configurations. The Rosenbluth factor can be related to excess chemical potential of the system and reflects the improbability of insertion or deletion of molecules in the system. The Rosenbluth weight is computed as follows:

- A random position is chosen for insertion of the first bead. The Boltzmann weight of this insertion is determined by its non-bonded interactions $u^{(i)}_{\text{non-bond}}$, and is given by $\exp[-\beta u^{(i)}_{\text{non-bond}}]$.

- For the placement of the next bead $i$, $k$ trials are generated as shown in Figure 2.5. The orientations of these $k$ trials are chosen from a Boltzmann distribution associated with the bonded energies of that bead, $u^{(i)}_{\text{bond}}$ which includes bond length and, wherever applicable, bond angle energies. In other words, the probability of generating a trial segment $t$ is proportional to $\exp[-\beta u^{(i)}_{\text{bond}}(t)]$.

- Now that $k$ trials have been generated, we calculate the non-bonded interaction energies $u^{(i)}_{\text{non-bond}}$ for each of the trials. One of these $k$ trials is selected with a probability

$$p^{(i)}_{\text{non-bond}}(t) = \frac{\exp[-\beta u^{(i)}_{\text{non-bond}}(t)]}{u^{(i)}_{\text{non-bond}}}, \quad (2.29)$$

where

$$u^{(i)}_{\text{non-bond}} = \sum_{b=1}^{k} \exp[-\beta u^{(i)}_{\text{non-bond}}(t_b)]. \quad (2.30)$$

- This systematic placement of beads continues until the chain of length $n$ has been completely grown. The Rosenbluth weight of this trial chain molecule is now defined as

$$W_{\text{non-bond}} \equiv \prod_{i=1}^{n} \frac{u^{(i)}_{\text{non-bond}}}{k}. \quad (2.31)$$
CHAPTER 2. MOLECULAR MODELS AND SIMULATION METHODS

Insertion of a molecule

Once a non-random trial configuration, biased towards lower energy configurations is generated by the procedure described above, it is accepted for insertion with the following probability, unbiased by the Rosenbluth weight $W$:

$$\text{acc}(N \rightarrow N + 1) = \min \left( 1, \frac{q(T) \exp(\beta \mu) V}{N + 1} W_{\text{non-bond}} \right),$$

(2.32)

where $\mu$ is the chemical potential of the chain species and $q(T)$ is the kinetic contribution to the partition function related to the thermal de Broglie wavelength for atoms as $q(T) = 1/\Lambda^3$.

Deletion of a molecule

For molecule deletion, an existing molecule is chosen at random and its Rosenbluth weight is calculated. This is done by using the existing configuration as the first ‘trial’, generating the remaining $k-1$ trials, and computing the probabilities at each stage, as described above. The existing configuration is accepted for deletion with the following probability, again unbiased by the Rosenbluth weight,

$$\text{acc}(N \rightarrow N - 1) = \min \left( 1, \frac{N}{q(T) \exp(\beta \mu) V M} \right).$$

(2.33)

2.6.4 Parallelization of chain growth

Configuration bias Monte Carlo easily lends itself to parallelization on a multi-core CPU or GPU where the growth of several chains can be attempted concurrently per thread to greatly increase the speed of sampling [84]. In the parallel case, each of the $M$ trial chains that are grown in parallel has its own Rosenbluth factor $W_p$. A combined Rosenbluth factor $Z$ is calculated as the sum of all the individual Rosenbluth factors. One of the trial chains is selected as the proposed insertion to be accepted or rejected with the following probabilities:

$$\text{acc}(N \rightarrow N + 1) = \min \left( 1, \frac{q(T) \exp(\beta \mu) V Z}{(N + 1) M} \right),$$

(2.34)

and the proposed deletion accepted or rejected with the probability

$$\text{acc}(N \rightarrow N - 1) = \min \left( 1, \frac{N}{q(T) \exp(\beta \mu) V Z M} \right).$$

(2.35)
The probability of successful moves in a dense system such as a lipid bilayer is greatly increased by parallelization. In addition to parallelization of configurational bias Monte Carlo, we use two sampling tricks—density bias Monte Carlo and orientation bias Monte Carlo—which exploit the anisotropies of our system.

### 2.6.5 Density bias Monte Carlo

In configurational bias Monte Carlo, the first bead is placed randomly and the decision to accept or reject a trial insertion is made after the entire chain is grown. In a system with a lipid bilayer, most of the system is composed of the bulk solvent phase. In such a scenario, most of the trial attempts would begin and continue in the bulk phase with almost no chance of acceptance. However, we can utilize the information we possess about the location of the head groups in the bilayer system, and preferentially begin the configurational bias Monte Carlo sampling in those regions of the system. Rodgers et al. showed that the insertion of molecules with a strong preference for the bilayer can be made much more efficient by biasing the locations of insertion based on where the bilayer is most likely to be found \[85\]. This was referred to as density bias Monte Carlo, with the acceptance probabilities unbiased to account for the initial bias in position.

The position of the insertion of the first bead is now no longer random but chosen according to a probability \( p(z) \). A sample distribution \( p(z) \) is shown in Figure 2.6 and represents the probability of finding a head group bead in a box while traveling along the direction of the bilayer normal (\( z \) direction). The acceptance probabilities for insertion are modified as

\[
\text{acc}(N \rightarrow N + 1) = \min \left( 1, \frac{q(T) \exp(\beta \mu) V W_{\text{non-bond}}}{(N + 1) p(s)} \right),
\]

while for deletion, the acceptance probabilities are modified as

\[
\text{acc}(N \rightarrow N - 1) = \min \left( 1, \frac{N}{q(T) \exp(\beta \mu) V W_{\text{non-bond}}} \frac{p(s)}{p(s)} \right).
\]

Here \( p(s) \) is the probability of the position \( s \) as chosen from the distribution function \( p(z) \). This ensures that insertion attempts are made within or close to the bilayer, and greatly improves the sampling efficiency.

### 2.6.6 Orientation bias Monte Carlo

After using configuration and density bias Monte Carlo, we observe that although the first lipid bead was placed near other lipid heads according to \( p(z) \), a significant fraction of the attempts to add the second bead were made outside the lipid phase. According to electron density profiles of lipid bilayers as shown in experiments \[86\], and in our model, the head
Figure 2.6: Density biasing: Probability distribution function $p(z)$ according to which the insertion of lipid heads is attempted. It corresponds to the equilibrium distribution of positions of lipid heads in a lipid bilayer in the $L_\alpha$ phase.

The group region is more dense than the water phase or the tail region. Therefore, it is likely that a bead grown in the water phase would encounter fewer overlaps than one grown in the head region, and thus be more energetically favorable. However, once the second bead
is added outside the lipid phase, the remaining hydrophobic lipid beads continue to grow in the water phase or along the interface. Such attempts are almost always rejected because of their high overall energy.

Figure 2.7: Orientation biasing: Probability distribution function $p(\theta)$ according to which the first bond angle is sampled. This distribution is the equilibrium distribution of bond angles in a lipid bilayer in the $L_{\alpha}$ phase.

We develop acceptance rules to bias the generation of the first bond in the lipid to aid
sampling of relevant orientations of the lipid. Specifically, the orientation of the second bead
is not chosen randomly on the surface of a sphere around the second bead, but according
to a probability distribution of angles \( p(\theta) \). The angle under consideration is that between
the first bond in our lipid model (Figure 3), and the \( z \) axis. We choose \( p(\theta) \) to be the
distribution of this angle in a lipid bilayer at equilibrium (Figure 5). This results in an
increase in acceptance by at least 50%. To account for this bias, the acceptance probabilities
for insertion are modified as

\[
\text{acc}(N \rightarrow N + 1) = \min \left( 1, \frac{q(T) \exp(\beta \mu) V W_{\text{non-bond}} \sin \theta}{2p(\phi)p(s)} \right),
\]

while for deletion, the acceptance probabilities are modified as

\[
\text{acc}(N \rightarrow N - 1) = \min \left( 1, \frac{N}{q(T) \exp(\beta \mu) V W_{\text{non-bond}} \sin \theta} \right),
\]

where \( p(\phi) \) is the probability of the chosen angle from according to the distribution \( p(\theta) \).
The \( \frac{\sin \theta}{2} \) factor accounts for the fact that there are more points on the circumference of a
circle than at the poles.

### 2.6.7 Lipid reservoir

Using the techniques described above, we simulate a patch of membrane in the \( \mu_{\text{lipid}} VT \)
ensemble with lipids being added and removed from the patch as necessary to keep \( \mu_{\text{lipid}} \)
constant. A rectangular frame at the periphery of the membrane patch is treated as a lipid
reservoir, as shown in Figure 2.8. Grand canonical Monte Carlo attempts are performed
within this peripheral frame. As the lipids can diffuse between the membrane patch and
the membrane reservoir, they reach an equilibrium where both the patch and the reservoir
have the same chemical potential. A similar approach has been used by Heffelfinger et al.
who developed a dual control volume grand canonical molecular dynamics method for spatial
control of chemical potential [87]. If the membrane contains an asymmetric inclusion, such as
a budding droplet, lipids will be added to the system to accommodate its growth. Similarly,
if a droplet is fusing back into the membrane, lipids will be removed from the bilayer to keep
\( \mu_{\text{lipid}} \) constant.

### 2.7 Hybrid dissipative particle dynamics–Monte Carlo

In our simulations of the soft–repulsive model, we combine dissipative particle dynamics with
an appropriate Monte Carlo algorithm to extend sampling to different ensembles [42, 85].
Figure 2.8: Construction of a membrane reservoir (top view along the membrane normal): the lipid bilayer patch to be studied is surrounded by lipid reservoir maintained at constant lipid chemical potential $\mu_{\text{lipid}}$.

The simulation is treated as a Markov chain of states each of which is defined by the number of particles in the grand canonical ensemble or the size of the box in the isotension/isobaric ensemble. For example, in the isotension or $NP_{\perp}\gamma T$ ensemble, at each step in this chain, we can choose to perform either (i) a random number of dissipative particle dynamics steps that maintains constant temperature, (ii) a volume change move holding area constant that maintains constant $P_{\perp}$ or (iii) an area change move holding volume constant to maintain constant $\gamma$. These moves are usually chosen with probability 0.6, 0.2 and 0.2 respectively. The number of dissipative particle dynamics steps is chosen at random in order to maintain detailed balance.

Depending on the efficiency of sampling, certain moves may have to be attempted more than others. For example, in the grand canonical ensemble for lipids, successful chain growths can be sufficiently rare. For such simulations, we choose the probability of grand canonical moves to be 0.8, while the probability of a dissipative particle dynamics trajectory of random
length is 0.2.
Chapter 3

The Order–Disorder Transition is a First-order Hexatic to Liquid Transition in Model Bilayers

In this chapter, we characterize the order–disorder transition in model lipid bilayers using molecular dynamics simulations. We calculate the surface tension–temperature phase diagram to chart out the regions where a lipid bilayer exists in the ordered or disordered phase. We use the Halperin–Nelson order parameter to distinguish between the two phases. The model bilayers show short-ranged translational order, while the orientational order is long-ranged. This shows that these model bilayers exhibit the hexatic phase as found in two-dimensional melting. In addition, we calculate the fluctuation spectrum for the interface between the ordered and disordered phases to obtain the interfacial tension. We find that the interface exhibits capillary scaling and has a line tension of $11 \pm 1$ pN. Coexistence between the two phases with a stable free interface between them leads us to the conclusion that the transition is first order. These observations help us lay a foundation for a pre-melting layer-like effect arising from proximity to a first-order phase transition described in Chapter 4.

3.1 Introduction

Pure and multi-component lipid bilayers are of much interest in the biophysics community as they serve as model systems for biological membranes. The physical and chemical properties of lipids, including their phase behavior, could play a significant role in the structure and

function of these membranes [88, 89, 90]. Lipid bilayers primarily exhibit two phases, an ordered solid-like phase often referred to as the ‘gel’ phase ($L_{\beta}$ or $L_{\beta}'$ phase), and a more disordered liquid-like phase ($L_{\alpha}$ phase) [39]. A schematic of the ordered and disordered phases is shown in Figure 3.1. This transition is believed to be a first-order transition, as suggested by differential scanning calorimetry experiments that show a sharp peak in heat capacity as a function of temperature for several different lipid species [46].

![Ordered and disordered phases of a lipid bilayer](image)

**Figure 3.1:** A schematic of the ordered and disordered phases of a lipid bilayer with an ordered packing and a random arrangement of the tails respectively.

A molecular model that faithfully reproduces the phase behavior of a lipid bilayer would lend itself to a better understanding of how lipids behave around solutes such as proteins and cholesterol. Atomistic-level models, while providing structural details and better accuracy, are far too computationally expensive to be put to use to rigorously study the phase behavior of a bilayer system. To enable access to larger length and time scales, but at the cost of reduced accuracy, several molecular-scale models have been constructed for lipid bilayers and studied using molecular dynamics or Monte Carlo simulations [20, 45, 22, 21, 14]. Many of these models exhibit an ordered, solid-like phase and a disordered liquid-like phase. These models also exhibit signatures of the transition being first-order. However, proof of a first-order transition in these systems has not yet been shown.

Some studies of these coarse-grained lipid bilayers have reported hysteresis while transitioning from one phase to the other [20, 45]. While hysteresis is a signature of an energy barrier, it is not clear if the free energy barriers in these model systems scale with system size as in a first-order phase transition [49]. Rodgers et al. calculated the heat capacity from simulations of lipid bilayers using two different model systems to mimic a differential scanning calorimetry (DSC) experiment [47]. They find that the MARTINI coarse-grained model [23] shows a peak in heat capacity similar to DSC experiments on phosphatidylcholine bi-
CHAPTER 3. THE ORDER–DISORDER TRANSITION IS A FIRST-ORDER HEXATIC TO LIQUID TRANSITION IN MODEL BILAYERS

layers. This is another promising signature that the order of the transition is indeed one, however, demonstration of the free energy barrier’s dependence on system size is required to unambiguously show dependence of the free energy barrier on system size. Since free energy calculations are technically challenging for such systems, we stabilize a free interface between the two phases for two different system sizes to show that the transition is first-order.

In this work, we investigate the nature of the transition between the ordered and disordered phases in a coarse-grained model using molecular dynamics simulations. We determine that the transition is of first order for a model dipalmitoyl phosphatidylcholine bilayer by stabilizing a free interface between the two phases. Interestingly, we find that the ordered phase in this coarse-grained model is a hexatic phase, as is common in melting of two-dimensional and some quasi-two-dimensional systems [50]. The nature of this transition allows us to postulate pre-melting layer effects in lipid bilayers and similar consequences that are engendered by proximity to a first-order phase transition (see Chapter 4).

3.2 Order–disorder transition in a model lipid bilayer

We use the MARTINI coarse grained force field to model a lipid bilayer system [23] in which four carbon atoms (or equivalent) are approximated as one coarse-grained bead as described in Chapter 2. This model has an explicit solvent with approximately four water molecules scaled to one solvent particle. Dipalmitoyl phosphatidylcholine (DPPC) is chosen as the model lipid species. This model of DPPC exhibits two distinct phases—an ordered phase and a disordered phase. The ordered phase is analogous to the ‘gel’ or $L_{\beta}$ phase, while the disordered phase is analogous to the liquid or $L_{\alpha}$ phase.

The ordered phase has a regular tail packing compared to the disordered phase in which the tail arrangement is disorganized. Representative configurations from the ordered and disordered phase are shown in Figure 3.2. Figure 3.2 also shows the surface tension–temperature phase diagram for this model for a system with 1152 lipids and 18000 solvent particles. The bilayer is in the ordered phase for all points below the tie line and in the disordered phase for all points above it. Starting with the ordered phase, as the surface tension decreases (i.e., as the bilayer is compressed) a larger temperature is required to melt the bilayer. This explains the negative slope of the tie line. The model exhibits a hysteresis region that spans almost 30 K for this system size as shown in Figure 3.3. The points in the phase diagram have been shown for the heating curve.

Area per lipid molecule is often used as an order parameter to distinguish between the two phases. Figure 3.3A shows the change in area per lipid with temperature while heating and cooling a bilayer at zero surface tension. This value was chosen because unconstrained, free-standing lipid bilayers are believed to be tensionless [91]. Both heating and cooling is performed at a rate of 1.2 $\mu$s every 3 K. There are finite jumps in area per molecule as the system transitions between the two phases, and a significant hysteresis. A convenient visual representation to distinguish between the two phases is to view the end particles of each lipid chain in any one monolayer. This representation is shown in Figure 3.4 for the ordered
CHAPTER 3. THE ORDER–DISORDER TRANSITION IS A FIRST-ORDER HEXATIC TO LIQUID TRANSITION IN MODEL BILAYERS

Figure 3.2: Phase Diagram: The surface tension–temperature ($\lambda - T$) phase diagram showing configurations from the ordered (left) and disordered (right) phases from the MARTINI model. The hydrophilic head groups of the lipid are shown in gray while the hydrophobic tail groups of the lipid are shown in pink. The tails of the ordered phase are regularly packed while the tails of the disordered phase are randomly arranged. Starting from the ordered phase, the disordered phase can be reached either by increasing the temperature, or by increasing the surface tension.

and disordered phases. These tail particles appear hexagonally-packed in the ordered phase and randomly arranged in the disordered phase. To quantify the order in the packing, we use a rotationally invariant version of the Halperin–Nelson order parameter, $\phi_6^{(i)}$ defined as
Figure 3.3: Order parameter variation with temperature: variation in the order parameters (A) area per lipid, and (B) $\phi_6$, with temperature, during heating (red) and cooling (blue). Both order parameters show a finite jump as a function of temperature when crossing the melting or freezing temperatures while heating and cooling. (B-inset) For calculation of $\phi_6$, the six nearest neighbors of a particle $i$ are used. The angle $\theta_{ij}$ is the angle made by a bond vector connecting a neighbor $j$ to the central particle $i$ with an arbitrary reference axis (the $y$-axis in this case).
CHAPTER 3. THE ORDER–DISORDER TRANSITION IS A FIRST-ORDER HEXATIC TO LIQUID TRANSITION IN MODEL BILAYERS

\[ \phi_6^{(i)} = \frac{1}{36} \left( \sum_{j=1}^{n=6} e^{6i\theta_{ij}} \right) \left( \sum_{j=1}^{n=6} e^{-6i\theta_{ij}} \right), \]  

where \( \theta_{ij} \) is the angle between a vector connecting particle \( i \) to \( j \) with respect to any reference axis, and the summation is performed over the six nearest neighbors the particle \( i \) (Figure 3.3B-inset). In a perfect hexagonal arrangement, \( \phi_6^{(i)} \) takes a value of 1, while for a random arrangement it is closer to 0.

The ensemble average of \( \phi_6^{(i)} \) denoted as \( \langle \phi_6^{(i)} \rangle \) varies with temperature as shown in Figure 3.3B. The finite jump as a function of temperature, as well as the hysteresis, are in agreement with previous work on this model by Marrink et al. \[45\].

3.3 Interface between the ordered and disordered phases

If the transition between two phases is first order, it should be possible to stabilize a flat interface between the two bulk phases. We first establish coexistence of the ordered and disordered phases for different system sizes to show that the transition is first order. Figure 3.5 shows this coexistence for a system size of \( N = 3900 \) lipids with a flat interface stabilized between the two phases. This was done by juxtaposing an ordered bilayer equilibrated at 285 K and zero surface tension with a disordered bilayer equilibrated at the same conditions corresponding to the cooling and heating curves of the hysteresis loop in Figure 3.3. This prepared system is then equilibrated in the \( NP_{\perp}AT \) ensemble to maintain an area per lipid intermediate between the two phases, thus stabilizing the interface. The line tension \( \gamma_{od} \) can then be calculated from the power spectrum of the interfacial fluctuations.

To calculate the fluctuations of the interface, we first identify the location of the interface at each instant by following the procedure in \[92\] as described below. The interface can be defined as a contour line tracing all points in space where the value of the order parameter \( \phi_6 \) is exactly in between that of the ordered and disordered phases. The particle-level \( \phi_6^{(i)} \) field, however, is discontinuous and varies sharply in space precluding the identification of a continuous contour. To obtain a smooth and continuous field, we coarse-grain the particle-level order parameter \( \phi_6^{(i)} \) yielding

\[ \bar{\phi}_6(r, t) = \sum_i g(|r - r_i|, \xi) \phi_6^{(i)}, \]  

where \( g \) is a Gaussian coarse-graining function given by

\[ g(r, \xi) = \frac{1}{\sqrt{2\pi\xi^2}} \exp\left(-\frac{r^2}{2\xi^2}\right), \]  

and \( \xi \) is chosen to be the correlation length of the ordered phase, computed from the correlation function plotted in Figure 3.6B. To compute the coarse-grained field, we consider
Figure 3.4: Snapshots from (A) the ordered phase, and (B) the disordered phase displaying the tail ends of each lipid in one leaflet of the bilayer. This is a top view of the membrane, looking along the membrane normal. The tail end beads are regularly packed in the ordered phase and randomly arranged in the disordered phase.
Figure 3.5: The interface, at one instant, between a bulk ordered region (left) and a bulk disordered region (right) is marked by a gray contour line.
the tail end particles of one monolayer projected onto a two-dimensional surface, as shown in Figure 3.5. A square lattice is superposed on the surface, and the coarse-grained field \( \phi_6 \) is evaluated at each lattice node. For numerical convenience, the coarse-graining function is truncated and shifted to zero at 3\( \xi \). We then identify all regions on the lattice where \( \phi_6 \) values on adjacent lattice nodes correspond to the two different phases. Finally, the instantaneous order–disorder interface is identified by interpolating between these adjacent lattice nodes to find the set of points \( \bar{s} \) satisfying \( \bar{\phi}_6(\bar{s}, t) = \frac{\langle \phi_6^{(i)} \rangle_{\text{dis}} + \langle \phi_6^{(i)} \rangle_{\text{ord}}}{2} \). Here \( \langle \phi_6^{(i)} \rangle_{\text{ord}} \) and \( \langle \phi_6^{(i)} \rangle_{\text{dis}} \) are the ensemble averages of the ordered and disordered \( \phi_6^{(i)} \) values, respectively.

A snapshot of the identified instantaneous interface is shown in Figure 3.5. This free interface is stable for the length of simulations performed, 1.8 \( \mu \)s in this case. In Figure 3.6A we plot the Fourier spectrum of the height fluctuations of this interface for two different system sizes with the larger system containing approximately double the length of the interface as the smaller one. For wave vectors \( k \) with magnitude smaller than 0.1 nm\(^{-1} \) we find that the associated height fluctuations \( \langle |\delta R_k|^2 \rangle \) are proportional to \( \frac{1}{k^2} \). This is consistent with the scaling expected from the theory of capillarity \[93\], where

\[
\langle |\delta R_k|^2 \rangle \sim \frac{L k_B T}{\gamma_{\text{od}} k^2}.
\] (3.4)

\( L \) here is the length of the interface. The interfacial line tension is found to be \( \gamma_{\text{od}} \) is 11\( \pm \)1 pN.

As can be seen in Figure 3.6B, the height fluctuations are consistent with capillarity theory for two different system sizes, for wave vectors of magnitude smaller than 0.1 nm\(^{-1} \), with additional modes in the larger system. The stable interface along with the capillary scaling data shows that the transition between the ordered and disordered phases is indeed first order.

### 3.4 The ordered phase exhibits properties of the hexatic phase

The hexatic phase, a phase that exists in between the solid and liquid phases in a two-dimensional system of particles, is characterized by short-ranged translational order and long-ranged orientational order. According to the Kosterlitz, Thouless, Halperin, Nelson and Young (KTHNY) theory \[94, 95, 96, 97, 98\], the melting of a two-dimensional solid is a two-step process—a continuous transition into the hexatic phase followed by a continuous transition into a liquid. The ordered phase in the MARTINI model exhibits large numbers of free dislocations as shown in Figure 3.7. The dislocations are identified as pairs of neighboring particles, one with five neighbors and another with seven neighbors, i.e. 5–7 pairs. While dislocations are observed in both the solid and hexatic phases, pairs of dislocations are bound in the solid phase and free in the hexatic phase.
Figure 3.6: Capillary fluctuations of the instantaneous interface: (A) Fourier spectrum of the fluctuations of the instantaneous interface yielding the line tension $\gamma_{od}$ between the two phases (B) $\phi_6$ correlation function for an ordered bilayer at 279 K used to determine the Gaussian width $\xi$ for the coarse-graining function $g$. 
Figure 3.7: Dislocations in the ordered phase of the MARTINI model identified as 5–7 pairs: a pair of particles, one with five neighbors (fuchsia) and another with seven neighbors (yellow). The black beads are the tail end particles of each lipid belonging to one monolayer.
CHAPTER 3. THE ORDER–DISORDER TRANSITION IS A FIRST-ORDER HEXATIC TO LIQUID TRANSITION IN MODEL BILAYERS

To test whether the ordered phase in the MARTINI model exhibits the hexatic phase, we treat a bilayer as a quasi-two-dimensional surface using the final beads of each lipid of one leaflet as shown in Figure 3.4. Using these particles, we calculate the translational correlation function $g_G$ and the orientational correlation function $g_6$.

The translational correlation function $g_G$ is defined as,

$$ g_G = \langle \rho_G(r) \rho^*_G(0) \rangle. $$

Here, $\rho_G$ is defined as,

$$ \rho_G(r_i) = \exp[iG \cdot (r_i)], $$

and $G$ is a reciprocal lattice vector. A two-dimensional solid has long-ranged translational order that decays as a power law. According to the KTHNY scenario, the hexatic phase is characterized by a short-ranged translational correlation function with a decay faster than $r^{-1/3}$. In Figure 3.8A, we calculate the translational correlation function $g_G$ for a system of 41472 lipids. It exhibits a decay of $g_G$ faster than $r^{-1/3}$. For these calculations, we use the final beads of each lipid tail in one leaflet of the model bilayer, projected onto a two-dimensional surface, as shown in Figure 3.4.

The orientational correlation function is defined as,

$$ g_6 = \langle \psi_6(r) \psi^*_6(0) \rangle. $$

Here, $\psi_6$ is the orientational bond order parameter proposed by Halperin and Nelson \[96\] defined as,

$$ \psi_6(r_i) = \frac{1}{6} \sum_{j=1}^{6} \exp[6i\theta_{ij}(r_i)], $$

where $\theta_{ij}(r_i)$ is the angle between the bond vector connecting a particle $i$ and one of its six nearest neighbors $j$ and the X-axis. The summation is performed over the six nearest neighbors of each phase. Figure 3.8B shows that the orientational correlation function $g_6$ for a system of 41472 lipids, is indeed long-ranged.

The relatively short-ranged translational order and the long-ranged orientational order show that the model lipid bilayer exhibits a hexatic phase. The KTHNY theory predicts that the hexatic to liquid transition in a two-dimensional system is a continuous transition. However, recent results from simulations of a hard-disk system have revealed this transition to be first order \[51\]. Our results in the previous section are consistent with a first-order hexatic to liquid phase transition. There have been suggestions in the experimental literature that gel phases of phospholipids can be hexatic but the data are not of sufficiently high resolution to
determine the range of translational order\cite{53}. Marrink et al. speculated that the MARTINI model might exhibit a hexatic phase\cite{45}. The hexatic phase, although first discovered in a purely two-dimensional system (the hard disk model), has also been observed in other quasi-two-dimensional systems such as colloidal suspensions\cite{99,100}.

3.5 Discussion

We show that the order–disorder transition for DPPC in the MARTINI model is a first-order hexatic to liquid transition. The ordered phase shows a long-ranged orientational correlation but short-range translational correlation, suggesting that it is hexatic. Cooling or pressurizing this hexatic phase further would continuously transform it to a solid, with both orientational and translational long-range order. Observations of a solid phase, however, are hindered by the propensity of the solvent in the MARTINI model to crystallize at or near 294 K, thus affecting dynamics of the bilayer. This crystallization can be prevented by replacing a small fraction of the solvent with larger, ‘antifreeze’ particles that deter crystal formation\cite{23}. However, the process of transitioning from the hexatic phase to the solid phase requires simulation over long time scales that are difficult to access. The adequacy of the length of our hexatic phase simulations is verified in Appendix C.

Recent work by Kapfer and Krauth on two-dimensional systems has shown that the order of the hexatic to liquid transition depends on the steepness of the repulsive interactions between particles\cite{52}. They find that for soft disks with repulsive potentials \( r^{-n} \), the transition is continuous as predicted by the KTHNY theories for \( n \leq 6 \) while for harder disks with \( n > 6 \) the transition is first order. We note that the MARTINI model employs the Lennard-Jones potential which might have an effect on the order of the hexatic to liquid transition. Our results for the order of the transition in the MARTINI model are consistent with the findings of Rodgers et al. who found signatures of a first-order phase transition in the same model system\cite{47}. Interestingly, a different model with soft, repulsive interactions was found by them to have a continuous phase transition that we speculate will be closer to the KTHNY scenario. This dependence of the order of the phase transition on the interaction potentials could be a potentially important guideline in choosing or constructing a coarse-grained model for lipid bilayer simulations. In Appendix A we show signatures of a continuous transition for the model with soft, repulsive interactions (i.e. \( n << 6 \)) consistent with the results of Kapfer and Krauth in\cite{52}.

Although the MARTINI model is able to reproduce the order of the transition in DPPC bilayers, the structure of the ordered phase has one significant difference in that the model bilayers do not exhibit a tilted phase\cite{45}. It is speculated that DPPC bilayers tilt because the head groups are large and cannot pack as closely as the tails. The tilted configuration allows tails to pack closely while accommodating the head groups. Tilt has been induced in the MARTINI model by decreasing the size of tail particles relative to head particles\cite{45} and in a soft-sphere model by increasing repulsion between heads\cite{42}. Using either strategy to induce tilt, it is likely that the nature of this transition continues to be first-order in the
Figure 3.8: Correlation functions for the ordered phase: (A) Translational correlation function $g_G$ showing decay faster than $r^{-1/3}$, the theoretical boundary below which the solid phase is no longer stable. (B) Orientational correlation function $g_6$ showing long range correlations. Taken together, the two plots show that the ordered phase is hexatic.
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MARTINI model.

Another feature of a fully hydrated phosphatidylcholine bilayer observed in cooling protocols of multilamellar vesicles and planar bilayers is the appearance of the so-called ripple phase, where the surface of the bilayer appears corrugated [39]. This kind of structure is not observed in the MARTINI model. However, it is has not been conclusively shown whether the ripple structure is a real thermodynamic phase or a metastable structure seen during heating or cooling. It is interesting to note, however, that both tilt as well as the rippled structure disappear upon addition of a small percentage of impurities such as cholesterol [101]. Therefore, despite the absence of a rippled structure, the MARTINI model remains a good candidate to study properties of the the bilayer that rely on that order of the transition.

To summarize, we observe the existence of a hexatic phase in the phase behavior of the MARTINI model for DPPC. Similar to two-dimensional systems of hard disks, the transition between the hexatic and liquid phases is first-order as confirmed by phase coexistence and capillary scaling of the interface between the two phases. These observations lay the ground to better understand the properties of a lipid bilayer and the effect of these properties on lipid bilayer inclusions. Recently, we have shown that a pre-melting layer effect arises in lipid bilayers as a result of the existence of a first-order phase transition in these systems [102]. Understanding the phase behavior of a pure-component bilayer in a model system will guide further understanding of the behavior of multi-component bilayers such as those found in biological membranes.

3.6 Simulation details

The MARTINI model uses the Lennard-Jones potential for non-bonded interactions. The cut-off for these interactions is 1.2 nm. The GROMACS shifting function [103] is used in the range 0.99–1.2 nm. Bond and angle energies are modeled as harmonic potentials with associated force constants 1250 kJmol$^{-1}$nm$^{-2}$ and 25 kJmol$^{-1}$rad$^{-2}$ respectively. We use a time step of 30 fs, within the recommended range of 20–40 fs for this model. Simulations are performed using the GROMACS molecular dynamics package [67] in the $NP_{\perp}\lambda T$ ensemble [104, 23, 72], where $N$ is the total number of particles and $P_{\perp}$ is the component of the stress tensor normal to the bilayer. The Berendsen thermostat is used with a coupling constant of 1.5 ps, and the Berendsen barostat is used with a coupling constant of 3 ps [68]. The compressibility is set to $3 \times 10^{-5}$ bar$^{-1}$. A surface tension of zero is maintained by choosing the components of the stress tensor $P_{zz} = P_{yy} = P_{xx} = 1$ bar. The electrostatic interactions are shifted to zero in the range 0–1.2 nm. A dielectric constant of 15 is used for screening of electrostatic interactions. To calculate the 5–7 pairs, the Triangle mesh generator is used [105].
Chapter 4

The Orderphobic Effect

The clustering of proteins in cell membranes is a controlling factor in biological processes such as endo- and exo-cytosis, cell signaling, and immunological synapses [106, 5, 4]. We find a generic and powerful force that regulates the dynamic arrangement of transmembrane proteins in lipid bilayers. This force is related to the phase transition between the ordered and disordered phases of lipid bilayers and is a pre-transition effect, occurring proximal to a first-order phase transition. Specifically, we show that when a protein with hydrophobic thickness equal to that of the disordered phase is embedded in an ordered bilayer, it induces a disordered region in its vicinity. Such a protein stabilizes an order–disorder interface with a finite line tension. When two such proteins stabilizing disorder in their vicinity approach each other, they self-assemble to reduce the effective line tension, leading to a single order–disorder interface. We refer to this phenomenon as the orderphobic effect. We identify different length scales involved in the orderphobic effect and also show the range of validity of this effect. Furthermore, we demonstrate that the orderphobic force gives rise to the assembly of two proteins and has a range of several nanometers. The orderphobic effect may explain the dynamic arrangement and clustering of membrane inclusions such as proteins and cholesterol.

4.1 Introduction

The fluid mosaic model [3] and the lipid raft hypothesis [4] have guided intuition on how proteins diffuse in a lipid membrane—ordered clusters floating in an otherwise disordered fluid membrane [5, 6]. Recent advances, however, show that the state of membranes containing transmembrane proteins are ordered, even gel-like [107, 108, 109, 110, 111, 89]. It is
difficult to understand how proteins diffuse and assemble within this relatively rigid material. Here, we argue that this question is answered by the fact that transmembrane proteins can pre-melt the ordered phase. Specifically, with molecular simulation, we show that within an otherwise ordered membrane phase, mesoscopic disordered domains surround certain proteins that favor disordered states. The boundary of the domains exhibit a stable, fluctuating order–disorder interface. The dynamic equilibrium established at this boundary allows the protein and its surrounding domain to diffuse.

Moreover, because the interface is associated with a finite line tension, neighboring proteins can experience a membrane-induced force of adhesion, an attractive force that is distinctly stronger and longer ranged than those that can arise from simple, elastic deformations of the membrane [57, 58, 59, 60, 61]. This force between transmembrane proteins is analogous to the hydrophobic force between hydrated particles. In the hydrophobic case, extended oily surfaces nucleate vapor–liquid-like interfaces. This is a nano-scale manifestation of the liquid–vapor phase transition, and appears at ambient conditions, far below the boiling temperature of water [112, 113, 114]. Similarly, in the case of transmembrane proteins, the evidence provided in this chapter shows that a protein favoring the disordered phase nucleates order–disorder-like interfaces in a manifestation of the order–disorder phase transition. Like the lipid raft hypothesis, therefore, clusters do indeed form, but the mechanism for their assembly and mobility emerge as consequences of an order-disorder transition in an otherwise ordered phase.

4.2 Ordered and disordered phases in a model lipid bilayer

To demonstrate the orderphobic force, we choose a model bilayer system consisting of DPPC lipids which exhibits an order–disorder phase transition. The model is a coarse-grained representation of molecules in which every four carbon or equivalent particles are approximated by one ‘bead’ [23]. The physical state of this coarse-grained system depends on two thermodynamic parameters, surface tension (λ) and temperature (T). The different phases of the bilayer can be described by the arrangement of the hydrophobic tails of the lipids. The tails are randomly arranged in the disordered phase and regularly packed in the ordered phase (Figure 4.1). As a result, the hydrophobic thickness of the bilayer is different in the ordered and disordered phase. For this model, the hydrophobic thickness in the ordered phase is $\ell_b^{\text{ord}} = 3.1$ nm, larger than that of the disordered phase $\ell_b^{\text{dis}} = 2.6$ nm at a temperature of 294 K.

The different phases of the bilayer can also be distinguished by the arrangement of the last tail beads of each lipid. In this representation (Figure 4.2), the hexagonal-like packing in the ordered phase and a liquid-like arrangement of the disordered phase is apparent. Inspired by the order parameters in two-dimensional melting [96], we define a translationally and rotationally invariant local order parameter $\phi_6^{(i)}$ to distinguish between a hexagonal packing...
Figure 4.1: Phase diagram: (A) Snapshots from a molecular dynamics simulation of model DPPC bilayers showing a lipid bilayer in the ordered phase (top) and disordered phase (bottom). The hydrophilic head groups of the lipid are shown in gray while the hydrophobic tail groups of the lipid are shown in pink. It can be seen that the tails of the ordered phase are regularly packed while the tails of the disordered phase are randomly arranged. The solvent particles are not shown for clarity.
Figure 4.2: Order parameter $\langle \phi_6^{(i)} \rangle$ showing a finite jump as a function of temperature while crossing the melting temperature during heating. To calculate $\phi_6^{(i)}$ we consider the six nearest neighbors of each particle $i$ as shown (inset) and calculate $\theta_{ij}$, the angle between the bond-vector connecting the particle $i$ and each of its neighbors $j$ with respect to an arbitrary reference axis (the $y-$axis in this case).
and a disordered structure. This order parameter is defined by

$$\phi_6^{(i)} = \frac{1}{36} \left( \sum_{j=1}^{n_n=6} e^{i\theta_{ij}} \right) \left( \sum_{j=1}^{n_n=6} e^{-i\theta_{ij}} \right), \quad (4.1)$$

where $\theta_{ij}$ denotes the angle between the bond-vector connecting the particles $i$ and $j$ with respect to an arbitrary reference axis, and the summation belongs to the six nearest neighbors of particle $i$ (see Figure 4.2 inset). In the extreme case of the particles being perfectly hexagonally packed, $\phi_6^{(i)} = 1$ while for a disordered arrangement $\phi_6^{(i)}$ is close to 0. For our chosen DPPC system, the ensemble averages for the ordered and disordered phases are $\langle \phi_6^{(i)} \rangle_{\text{ord}} \approx 0.45 \pm 0.02$ and $\langle \phi_6^{(i)} \rangle_{\text{dis}} \approx 0.16 \pm 0.02$ at a temperature of 294 K. The variation of $\langle \phi_6^{(i)} \rangle$ across the phase transition from ordered to disordered phase shows a finite jump as shown in Figure 4.2 consistent with a first-order phase transition [115].

We note that any order parameter, such as density or thickness, could be used to demonstrate the first-order phase transition. However, $\phi_6^{(i)}$ is a visually and technically convenient representation to understand the effects of protein-induced changes in the bilayer.

### 4.3 Ideal orderphobes embedded in the ordered phase

This order–disorder phase transition gives rise to a force of self-assembly due to a solute-induced disturbance of the bilayer. The orderphobic force can be explained by the following thought experiment. Consider a bilayer in the ordered phase at zero surface tension close to the order–disorder transition temperature. Consider an ideal cylindrical transmembrane protein with a hydrophobic core of length $\ell_p$ flanked by hydrophilic caps as shown in Figure 4.3. Choosing the hydrophobic length $\ell_p$ equal to the hydrophobic thickness of the disordered phase $\ell_b^{\text{dis}}$ creates a hydrophobic mismatch between the protein and the ordered phase. This mismatch creates a frustration in the arrangement of lipids in the vicinity of the protein, potentially leading to the creation of the disordered phase.

To identify the structural characteristics of the lipids in the vicinity of the protein, we calculate the ensemble-averaged radial distribution of the structural order parameter $\langle \phi_6(r) \rangle$ from the edge of a cylindrical protein. Figure 4.5 shows the order parameter relative to the disordered phase,

$$\langle \phi_6(r) \rangle_{\text{rel}} = \frac{\langle \phi_6(r) \rangle - \langle \phi_6^{(i)} \rangle_{\text{dis}}}{\langle \phi_6^{(i)} \rangle_{\text{ord}} - \langle \phi_6^{(i)} \rangle_{\text{dis}}}, \quad (4.2)$$

for three proteins of different radii. It can be seen that for all three proteins, $\langle \phi_6 \rangle_{\text{rel}} \approx 0$ in the vicinity of the protein, and $\langle \phi_6 \rangle_{\text{rel}} \approx 1$ in the bulk. The shape of the ensemble-averaged density resembles a sigmoidal function, similar to solutions of order parameters.
Figure 4.3: Schematic of an ideal orderphobe: Hydrophobic regions of the bilayer as well as the protein are shown in pink, while the hydrophobic regions are in gray. The ordered phase has a larger hydrophobic thickness $\ell_{\text{ord}}^b$ compared to the hydrophobic thickness of the disordered phase $\ell_{\text{dis}}^b$. We define an ideal ‘orderphobe’ as a protein with a hydrophobic thickness $\ell_p$ equal to that of the disordered phase.

corresponding to two-phase co-existence [93]. The value of $\langle \phi_b \rangle_{\text{rel}} \approx 0$ at the edge of the protein shows that it induces a disordered region in its vicinity. Furthermore, a larger protein induces a larger disordered region in its vicinity. The physics of this process is similar to the physics of liquid–vapor interface formation in the hydrophobic effect [113]. A large, ideal hydrophobe—a solute that simply excludes volume—breaks the hydrogen bond network and stabilizes a vapor region in its vicinity [112]. Modern theories of the hydrophobic effect seek to explain the process of vapor formation through a thermodynamic argument. This vapor formation occurs, even though at room temperature we are far away from the boiling point of water, because the free energy difference between the liquid and vapor phases of water is very small, i.e. room temperature is close to vapor–liquid coexistence. Hence, a solute that excludes volume and therefore prefers the vapor phase in its vicinity, stabilizes a vapor region and a vapor–liquid interface in its surroundings. Similarly, at physiological temperatures, the free energy difference between the ordered and disordered phases could be small enough that orderphobic proteins are able to nucleate disorder around themselves as seen above.

4.4 Soft interface around an orderphobe

Since the proteins stabilize a disordered region, there should exist a soft interface that separates the ordered and disordered phases. To identify and characterize the properties of the
Figure 4.4: Model proteins or ‘orderphobes’ of three different sizes are introduced into an ordered bilayer. The proteins contain a hydrophobic core (magenta) flanked by hydrophilic caps at the top and bottom. An ideal ‘orderphobe’ is a protein with a hydrophobic thickness (i.e. height of the magenta region) equal to that of the disordered phase. These proteins perturb the bilayer inducing a disordered region in their vicinity.
Figure 4.5: The radial variation of the structural order parameter $\langle \phi_6(r) \rangle^{rel}$ shows the disorder in the vicinity of the protein for various sizes. $r$ is the distance from the edge of the protein. At small values of $r$ we see that $\langle \phi_6(r) \rangle^{rel} = 0$ showing that the lipids are disordered very close to the protein. Further away from the protein $\langle \phi_6(r) \rangle^{rel} = 1$, indicating the bulk ordered region. Additionally, we see that larger proteins induce a larger disordered region in the bilayer.
Figure 4.6: Soft order–disorder interface: Top, profile view of the arrangement of lipids in the vicinity of the protein of radius 1.9 nm. Bottom, top view of the membrane showing the arrangement of lipid tail-ends of the top monolayer. It can be seen that protein induces disorder in the vicinity of the protein. The black contour line indicates the position of the order–disorder interface.
defined as the contour of constant density, with a value

$$
\bar{\phi}_6(r, t) = \frac{\langle \phi_6^{(i)} \rangle_{\text{ord}} + \langle \phi_6^{(i)} \rangle_{\text{dis}}}{2}.
$$

(4.3)

The contour is obtained by finding a set of points that traces the division of the system into ordered and disordered regions. The instantaneous soft interface thus constructed is continuous (Figure 4.6) and fluctuates in time.

We compute the Fourier spectrum of radial fluctuations of the interface. As can be seen in Figure 4.7, in the low wave-vector regime, \( k \lesssim 0.1 \) nm, the amplitude \( |\delta R_k|^2 \) of the radial fluctuations for a Fourier mode \( m \) are proportional to \( 1/(m^2 - 1) \), where \( m = \frac{k R_0}{2\pi} \) is the Fourier mode and \( R_0 \) is the mean position of the instantaneous interface. This scaling is a characteristic of capillary fluctuations in thermodynamic interfaces. We also extract the effective line tension from the spectrum of radial fluctuations of the instantaneous interface. The effective line tension of the protein-induced interface as predicted by the long-wavelength capillary fluctuations yields \( \gamma_{\text{od}} = 11 \) pN, and is consistent with that of the free interface at order–disorder coexistence [115]. Moreover, the radial fluctuations at low wave-vector are also consistent with the thermal fluctuations of a free interface at order–disorder coexistence (Figure 4.6). The fluctuation spectrum along with the radial distribution of the order parameter (Figure 4.5) concretely prove that a protein with a smaller hydrophobic thickness compared to the ordered phase, induces disorder in its vicinity.

### 4.5 Demonstration of the orderphobic effect

If there exist two proteins, each stabilizing a disordered region, is there a driving-force for their assembly? To demonstrate the existence of a force for assembly, we begin with two proteins of diameter 1.5 nm separated by a distance of 14 nm. As expected, each of these proteins induces a disordered region in its vicinity and thus an associated order–disorder interface. The energy corresponding to this separated state is \( \approx 2\gamma_{\text{od}} L_0 k_B T \). Initially, the two interfaces fluctuate around their respective proteins (Figure 4.8A). When the proteins are sufficiently proximal to each other, the fluctuations of the interfaces cause the two soft interfaces to merge (Figure 4.8C) and separate (Figure 4.8B several times during the course of a few nanoseconds before they permanently merge as in (Figure 4.8D). The two disordered regions coalesce, leading to a single soft interface around the two proteins. The energy corresponding to the resulting single soft interface is large and hence the extended interface drives the proteins towards each other to decrease its perimeter as well as the associated energy. Finally, the system reaches a configuration where the two proteins are united (Figure 4.8E) with an approximate interface length of \( L \) nm. The energy corresponding to this configuration is \( \gamma_{\text{od}} L k_B T \). Therefore, \( \Delta G \), the free energy difference between the assembled and separated states of the proteins is
Figure 4.7: Fourier spectrum of the fluctuations in the soft order–disorder interface: The fluctuations in the radius of the order–disorder interface induced by the orderphobic protein are consistent with the fluctuations of a free order–disorder interface at coexistence. This further proves that the perturbation around the protein resembles the disordered phase.
Figure 4.8: Demonstration of the orderphobic force: Two proteins separated by a center-to-center distance of 14 nm are simulated at 309 K. Snapshots at various times during the process of assembly are shown. Fluctuations of the interface bring the two proteins together, merging their respective disordered shells. The resulting long interface shrinks to reduce the energy due to the line tension between the two phases, lassoing the two proteins together into one assembled structure. The free energy gain in the process \( 40 \, k_B T \) for a protein of this size.
\[ \Delta G = -\gamma_{od} (L - 2L_0) k_B T \approx -40 k_B T, \] (4.4)

leading to a driving force for assembly.

Analogous driving forces for assembly and organization, modulated by the physics of liquid–vapor phase transitions, form the basis of the hydrophobic effect \[113, 112\]. Indeed, such forces are crucial in the process of protein folding \[64, 116, 117\] and macromolecular self-assembly \[118, 119\]. It is possible that such forces are responsible for protein clustering on the surface of a cell.

### 4.6 Validity of the orderphobic effect

The strength of the orderphobic force depends on the ability of a protein to induce a disordered region. This property is controlled by a) the proximity of the system to phase-coexistence, b) the radius of the protein and c) the extent of the hydrophobic mismatch. The extent of the disordered region increases with proximity to phase coexistence, as shown in Figure 4.9A, i.e. by increasing the temperature of the system, the disordered region is further stabilized. Proximity to phase co-existence can also be achieved by increasing the surface tension of the membrane, driving the system closer to the disordered phase as shown in Figure 4.1. Similarly, increasing the radius of the protein also increases the extent of the disordered region (Figure 4.5).

Similar to the hydrophobic effect, we expect an elastic regime for low temperatures and small solutes, i.e. a regime in which the membrane deforms elastically around the orderphobic solute. For higher temperatures and larger solutes, a disordered region is induced by a solute, and hence there is an associated order–disorder interface with a line tension. The existence of these two regimes is visualized in Figure 4.10. The line tension regime leads to assembly of solutes via the orderphobic effect, while the elastic regime does not. At each temperature, a critical length is required to observe the line tension regime, and hence the orderphobic effect. Very close to the transition temperature \( T_m \), the critical solute size required to observe the orderphobic effect is much smaller compared to lower temperatures, where much larger solutes are required. A similar schematic can be drawn for surface tension instead of temperature.

In addition, we show that for these model proteins, hydrophobic mismatch is a key parameter that determines the degree of orderphobicity of a solute. Figure 4.9B shows that the strength of the effect is maximal for a hydrophobic thickness equal to that of the disordered phase, and decreases as we approach the hydrophobic thickness of the ordered phase. In the case of zero hydrophobic mismatch (protein hydrophobic thickness equal to the ordered phase), and positive hydrophobic mismatch (protein hydrophobic thickness larger than the ordered phase), the lipids in the vicinity of the protein remain ordered. The value of the order parameter in the vicinity of these proteins corresponds to that of the ordered phase. Since these proteins do not induce a disordered region, the orderphobic force vanishes. In reality, it could be possible for proteins to disrupt the ordered phase and favor the disordered
Figure 4.9: Strength of the orderphobic force: (A) Radial variation of the order parameter showing the extent of the disorder region as a function of temperature for a protein of radius 2.7 nm. The average disordered region increases as the melting temperature is approached. (B) Comparison of the radial variation of the order parameter for different hydrophobic mismatches. The proteins with positive or no mismatch ($\ell_m = -0.2$ nm) do not create any disordered region.
Figure 4.10: Schematic of the elastic versus line tension (i.e., orderphobic effect) regimes: At low temperatures, the critical solute size $L$ required to observe the orderphobic effect is large. However, as we approach the transition temperature $T_m$, the critical solute size required to observe the orderphobic effect becomes smaller. In the line tension regime, solutes self-assemble via the orderphobic effect, while in the elastic region there is no such effect.
phase in a variety of ways in addition to the hydrophobic thickness. For example, the side chains of an $\alpha$-helix could disrupt the regular tail packing, and hence be orderphobic.

4.7 Conclusion

To summarize, we have found the existence of a force of self-assembly mediated by phase-transitions, and demonstrated its capability to assemble proteins. We have shown that proteins with negative hydrophobic mismatch embedded in the ordered phase induce a disordered region in their vicinity. These proteins stabilize a soft interface that separates the ordered and disordered regions. The fluctuations of this interface are consistent with that of a free interface at order-disorder coexistence. In addition to providing a force for assembly of proteins, the orderphobic force is also useful in understanding the physical chemistry of the liquid-ordered phase [120, 121, 122, 88] that results from adding cholesterol to single-component lipid bilayers. In this case, cholesterol with a small hydrophilic head and short hydrophobic tail may induce disorder in the ordered phase. This disorder in an otherwise ordered phase may result in a structure that resembles the liquid-ordered phase [122]. Finally, we note that such a phase-transition-mediated force can also occur in reverse when the bilayer is in the disordered phase. In this case, a protein with a hydrophobic thickness equal to that of the ordered phase, when embedded in the disordered phase, would induce order in its vicinity. There would then exist a similar interface separating the order-disorder regions, which may provide a force for assembly of ‘orderphilic’ proteins in membranes. Such phase-transition-mediated forces can be instrumental in understanding the clustering of proteins in biological membranes, as well as processes such as synaptic membrane fusion and cell signaling.

4.8 Details of the simulation

4.8.1 Model

- Lipids:
  
  We use molecular simulation methods for studying the orderphobic effect. To model the interaction between lipids and their interaction with the proteins, we use the MARTINI coarse-grained force field [23]. The non-bonded interactions in the MARTINI force field are modeled by Lennard-Jones interactions. This model is appropriate for studies of a pre-melting layer as it exhibits a first-order order-disorder transition [115] for dipalmitoyl phosphatidylcholine (DPPC) bilayers consistent with experiments.

- Proteins:

  The proteins in our system are idealized proteins, which contain a hydrophobic core with hydrophilic caps. The hydrophobic core is constructed using the same coarse-grained beads as the lipid tails (particle C1 in the MARTINI topology [23]). Similarly,
CHAPTER 4. THE ORDERPHOBIC EFFECT

the hydrophilic caps are constructed using the first bead of the DPPC head group (Q0, in the MARTINI topology). The protein beads also have harmonic bonded interactions where the bond length is 0.45 nm and the bond angle is set to 180°. The associated force constants for the bond lengths and angles are 1250 kJmol$^{-1}$nm$^{-2}$ and 25 kJmol$^{-1}$rad$^{-2}$. Based on the hydrophobic mismatch with the bilayers, the proteins are classified into three categories: (i) positive mismatch ($\ell_p > \ell_{ord}^b$), (ii) negative mismatch ($\ell_p \leq \ell_{dis}^b$), and (iii) no mismatch ($\ell_p \approx \ell_{ord}^b$). To create different mismatches, we alter the number of beads in the protein core. We note that these idealized proteins do not contain charges.

4.8.2 Molecular dynamics simulations

We perform simulations using the GROMACS molecular dynamics package \[67\]. We maintain a fixed number of particle $N$ and a fixed temperature $T$. The time step used is 30 fs. We use periodic boundary conditions in the $x$, $y$ and $z$ directions. The bilayer normal is chosen to be $z$-direction. As in the simulations of the original MARTINI model \[23\], the Berendsen thermostat is used to maintain constant temperature with a coupling constant of 1.5 ps, and the Berendsen barostat is used to maintain constant pressure with a coupling constant of 3 ps \[68\]. The compressibility is set to $3 \times 10^{-5}$ bar$^{-1}$. Zero surface tension is maintained semi-isotropically, by choosing the components of the stress tensor $P_{xx} = P_{yy} = P_{zz} = 1$ bar \[104\]. The cut-off for LJ interactions is 1.2 nm. The standard GROMACS shifting function \[103\] is used in the range 0.99–1.2 nm. The electrostatic interactions are shifted to zero in the range 0–1.2 nm. A dielectric constant of 15 is used for electrostatic screening.

4.8.3 System setup

All points in the phase diagram are calculated using a DPPC bilayer system with 128 lipids and 2000 water beads. A bilayer of consisting of 3200 lipids and 50000 water beads is equilibrated at 279 K for 12 microseconds. Proteins are embedded in the equilibrated bilayer at 279 K. The resulting system is then heated to the required temperature and equilibrated for another 1.2 microseconds. All the subsequent averages are performed using 10 independent trajectories each 600 ns long. The assembly of proteins is also performed using the same DPPC bilayer system with 3200 lipids and 50000 water beads. In this case, two proteins are inserted in this bilayer with centers at a distance of 14 nm and the simulation is carried out at 309 K.

4.8.4 Soft instantaneous interfaces

We provide a procedure that identifies a soft and fluctuating instantaneous interface that separates the disorder from the ordered regions. We use techniques that are well developed to identify liquid–vapor interfaces of water \[92\].
To this end, consider the structural order parameter $\phi^{(i)}_6$ as defined for every particle as in (4.1). Since $\phi^{(i)}_6$ is discrete in space, it is difficult to observe a continuous interface if we use the parameter $\phi^{(i)}_6$ alone. This can be alleviated by coarse-graining the order parameter using a suitable coarse-graining function with an appropriate coarse-graining length. To this end, the coarse-grained $\phi^{(i)}_6$ density is defined as

$$\bar{\phi}_6(r, t) = \sum_i g(|r - r_i|, \xi) \phi^{(i)}_6,$$

(4.5)

where we choose $g$ to be a Gaussian coarse-graining function [92] given by

$$g(r, \xi) = \frac{1}{\sqrt{2 \pi \xi^2}} \exp\left(-\frac{r^2}{2\xi^2}\right),$$

(4.6)

and $\xi$ is the correlation length. The correlation length $\xi$ is obtained from the decay of the auto-correlation function $\phi^{(i)}_6 \phi^{(i+j)}_6$ and is found to be 1.5 nm. Finally, the soft instantaneous interface is a one-dimensional interface defined by a set of points $s$ such that

$$\bar{\phi}_6(s, t) = c,$$

(4.7)

where $c$ is a value chosen to be exactly equal to the mean of the order parameter in the ordered and disordered phases. In this case, $c = 0.30$.

In computing the soft interface, we project the last bead of each of the lipid tails in a monolayer on to the two-dimensional flat plane. We then overlay a spatial square lattice on to the two-dimensional plane and calculate the associated $\bar{\phi}_6$ at every lattice node using (4.5). In calculating $\bar{\phi}_6$, the coarse-graining function $g(r, \xi)$ is truncated and shifted to be zero and continuous at a distance of $3\xi$. The interface is then identified as the set of points where $\bar{\phi}_6 = c$ between any two adjacent lattice sites.
Chapter 5

The Molecular Mechanism of Oil Droplet Formation

Hydrophobic globules surrounded by lipid monolayers are ubiquitous structures found in most cells, prokaryotic and eukaryotic. These structures, called lipid droplets, are hypothesized to accumulate as inclusions of oil-like molecules between the two leaflets of a membrane which grow larger and eventually bud off. The budding process is sub-microscopic and difficult to observe in experiments. Studying it in molecular simulations has thus far presented computational challenges. Using dissipative particle dynamics, we attempt to understand the molecular details of the droplet budding process. We develop a lipid reservoir that can supply lipids to the bulging monolayer as the droplet buds out of the membrane. Our simulations support existing schematic models for the growth and budding process and predict a morphological transition between a partially and completely enveloped intermediate structure. We also propose a molecular mechanism for monolayer scission. Droplets generated using this technique can be useful in further studying the structure and dynamics of the droplet and its unique monolayer-integrated proteome.

5.1 Introduction

The cell contains several kinds of hydrophobic molecules such as triacylglycerols, sterol esters, waxes and biofuels. In the aqueous cytoplasm, these hydrophobic molecules are sequestered into structures called lipid droplets where they are bounded by a lipid monolayer [123]. Lipid droplets are formed within intracellular bilayers such as those found in the endoplasmic reticulum. The initial stages of droplet formation are still not understood, but most models hypothesize that hydrophobic material, synthesized by enzymes in a bilayer, accumulates as an ‘oil lens’ between the two leaflets of the bilayer [124, 125]. As more material accumulates,

“A molecular mechanism for oil droplet formation,” Shachi Katira and Berend Smit, in preparation
the nascent droplet grows larger and may eventually bud off, taking the outer leaflet of the membrane with it by monolayer scission (Figure 5.1). The droplet can vary in size from 20nm to 100µm depending on the cell in which it is formed [123], and can also coalesce with other lipid droplets by monolayer fusion.

Lipid droplets were long thought to be passive organelles which act as storage structures to compartmentalize neutral lipids, but they have recently been identified as key sites for energy metabolism which have their own specific proteome [126]. They have also been linked to lipid storage diseases such as diabetes [127] and atherosclerosis [128], and implicated in the assembly of viruses [129]. However, in this exciting time for lipid droplet studies, very little is known about the sub-microscopic, molecular details of an oil inclusion within a lipid bilayer. Khandelia et al. studied triglyceride molecules between the leaflets of a model lipid bilayer using molecular dynamics simulations [130]. Their work showed that triolein molecules aggregate within a lipid bilayer and form blister-like protrusions which could be the beginnings of lipid droplet formation. Zanghellini et al. used an elastic model of membranes to show that a demixing of different kinds of phospholipids helped minimize the energy of the monolayer surrounding the droplet [131]. Several computational studies have provided insight into the molecular mechanisms of these processes and the specific intermediate structures that can be formed for a bilayer [132, 133, 134, 135]. However, the lipid droplet is a site for monolayer scission and fusion, and these processes have not been studied in molecular detail.

In this work, we attempt to characterize the process of monolayer scission during the formation of an oil droplet from a lipid bilayer and identify the transition states and energy barriers in the process. A membrane simulation with molecular resolution typically has a fixed number of lipids. However, studying lipid droplet growth and formation in a molecular simulation is challenging because the number of lipids surrounding the growing inclusion is constantly increasing asymmetrically in one leaflet of the bilayer, and flip-flops between leaflets are rare [137]. Hence, we develop a lipid reservoir to sustain a fixed chemical potential that is required to automatically set the number of lipids to the equilibrium distribution at each stage. In the case of a budding vesicle or bilayer structure, where the surface area of both leaflets as opposed to one is increasing, a convenient alternative to the reservoir is a constant surface tension simulation as implemented by Baoukina et al. for a membrane tether, where the simulation box shrinks as a tether is pulled out [138]. However, this method cannot be used for an asymmetric structure such as the lipid droplet.

There have been no single gene products shown to be responsible for lipid droplet budding from a bilayer [136] which suggests that it could be a purely physical process which does not necessarily involve, but could be aided by, the presence of proteins. Therefore we begin by adding oil into a lipid bilayer, analogous to enzymatic neutral-lipid synthesis, in a system devoid of any proteins, to be able to observe the structures that can form in a minimal system.
Figure 5.1: Schematic of a model for growth and formation of a lipid droplet adapted from [136]. Oily material (green) is secreted between the leaflets of the membrane (black) by oil-synthesizing enzymes. As the oily phase grows it bulges outward. Lipids flow in from the surrounding regions to increase the surface area of the top leaflet to accommodate the growing droplet. The droplet eventually buds off from the parent membrane, taking the top leaflet of the membrane with it.
5.2 Results and discussion

5.2.1 Simulations of oil in a lipid bilayer

Figure 5.2: Cluster of oil inclusions within a lipid bilayer: lipid head groups are shown in black, tail groups are in gray and oil beads are in red. Water beads have been removed for clarity.
We use a mesoscopic model of lipid bilayers [139, 74] in which three to four heavy atoms are grouped to form one ‘bead’. To this model we added an ‘oil’ bead to represent the hydrophobic contents of a lipid droplet. To reach biologically-relevant length and time scales in our simulation, we employ hybrid dissipative particle dynamics–Monte Carlo (DPD–MC). Dissipative particle dynamics has been shown to preserve correct hydrodynamic behavior [140, 141]. To form oil inclusions within a lipid bilayer, we started with a lipid bilayer system with 16,000 coarse-grained dimyristoylphosphatidylcholine molecules and 720,000 water beads. We simulated this system in the $N_{\text{lipid}}\mu_{\text{oil}}P_{\perp}\gamma T$ ensemble, with a reduced temperature ($T$) corresponding to the lipid bilayer in the fluid ($L_{\alpha}$) phase, zero surface tension ($\gamma$), and oil chemical potential ($\mu_{\text{oil}}$) maintained at a sufficiently high value to allow for an influx of oil beads via grand canonical Monte Carlo to mimic the synthesis of oil. Monte Carlo moves to insert and delete oil particles are attempted throughout the system, but oil preferentially accumulates among the tail beads of the bilayer. We discontinued the grand canonical Monte Carlo moves when the system reached 64,000 oil beads and continued sampling in the $NP_{\perp}\gamma T$ ensemble. A cluster of several small ‘droplets’ is formed as shown in Figure 5.2. The average diameter of the droplets formed is about 10nm.

The curvature of the inclusion is determined by the relative interactions between water, head groups, tail groups and oil. If the composition of the oil was identical to the tail groups, the oil beads would be homogeneously distributed among the tails. As the oil becomes more dissimilar, the surface tension between the oil and the tails causes the oil beads to aggregate into inclusions with a higher curvature. For example, a cholesteryl ester inclusion might have a higher surface tension within a phospholipid bilayer compared to a triacylglycerol inclusion because of the rigid, planar structure of a cholesterol molecule. We parameterized the oil beads to be more hydrophobic compared to the lipid tail groups to be able to observe very high membrane curvatures, and increase the chance of observing a budding event. The size and number of droplets formed in the simulation depends on the rate at which oil is added to the system. If oil is added at a low rate, the individual droplets within the membrane diffuse and coalesce to form larger droplets. Coalescence of small inclusions to form larger ones is also observed in vitro in a solid-supported lipid bilayer system with the enzyme that synthesizes triacylglycerols [142]. In that study, the droplet sizes exceeded 300nm, but it is unclear whether any of them were released from the membrane.

The droplets in our simulation do not discriminate between bulging ‘outwards’ versus ‘inwards’ as they do in the endoplasmic reticulum where they are seen to grow outward into the cytoplasm. This could occur because of the intrinsically high curvature of the endoplasmic reticulum membranes. Membrane bending energy could be minimized by growing outwards on the convex side, rather than inwards on the concave side. The aggregation of proteins on the cytoplasmic side of the bilayer [143] could also contribute to the asymmetric bulging of a lipid droplet.

In several million DPD–MC cycles performed on the cluster of droplets, we observed one
spontaneous budding event where the droplet is released into the water phase. Compared to the timescale of molecular motion, this is a rare event. To better sample the scission event, we perform umbrella sampling on an isolated droplet.

5.2.2 The budding process

Umbrella sampling allows us to (i) calculate the potential of mean force of the process along a chosen coordinate, (ii) reach transition states which we can subsequently use to sample a large number of trajectories which begin at that point and (iii) obtain structures of free droplets with specific surface tensions, by starting with parent bilayers with the desired tension. These structures of free lipid droplets can be used to further study monolayer fusion during the process of droplet fusion \cite{144} and monolayer transmembrane structures found in the lipid droplet proteome \cite{145}. We start with a bilayer patch containing 8000 lipids, 8000 oil beads and about 333000 water beads, to provide a sufficiently large water phase to accommodate the budding lipid droplet. For umbrella sampling, we constrain the droplet center of mass at different heights from the parent lipid bilayer. As the droplet moves out of the membrane, the number of lipids in the ‘outer leaflet’ must increase to accommodate it. To allow for lipids to be added to the system, we introduce a lipid reservoir in the simulation. This approach was inspired by Heffelfinger \textit{et al.} who developed a dual control volume grand canonical molecular dynamics method that allows for spatial control of chemical potential within a system of Lennard-Jones particles \cite{87}. The periphery of the simulation box, a rectangular frame, is treated as a lipid reservoir and maintained at a fixed lipid chemical potential. The reservoir responds to changes in the chemical potential of the system by appropriately adding or removing lipids in the system.

At each stage, as the droplet is constrained at a point above the membrane, the bilayer stretches in response. The reservoir responds to alleviate this increase in membrane tension by adding more lipids, which can diffuse out of the reservoir and restore the native surface tension. At each point above the membrane, the system is equilibrated until the number of lipids and the height of the droplet is stabilized. This process is continued until the droplet is no longer attached to the membrane. Five different stages in the process are shown in Figure 5.3 beginning with the oil ‘lens’ stage in Figure 5.3(a) and ending with the mature droplet stage in Figure 5.3(e). Figure 5.3(c) and Figure 5.3(d) give an idea about the intermediate stages involved. In Figure 5.3(b) we can see the beginnings of negative Gaussian curvature, which becomes more pronounced in Figure 5.3(d) before the neck pinches off.

5.2.3 Budding may require a morphological transition

A theory for the budding of bilayers was developed in \cite{146} for a vesicle containing two aqueous phases separated by an interface as described in \cite{147}. This theory can be extended to monolayers by replacing the interface with a monolayer, and adding the appropriate surface
Figure 5.3: Five different stages in droplet growth. In (a) the droplet is in the ‘oil lens’ stage where it forms an ellipse with an eccentricity that is dependent on the surface tension between the oil and tail groups. In (b), the droplet is asymmetric, but different from (c) where we can see the beginnings of negative Gaussian curvature. The negative Gaussian curvature is emphasized in the formation of a neck in (d), which seals itself to form a free lipid droplet as in (e). The number of lipids in the simulation box increases from (a) to (e) as required to maintain the chemical potential of the system.
Figure 5.4: The surface of a droplet in the (a) partially-wrapped, and (b) completely enveloped states that are possibly separated by a morphological transition.
energy term for the oil phase. The theory predicts a morphological transition during budding from a “partially wrapped” to a “completely wrapped” state, under certain surface tensions. The states in Figure 5.3(c) and Figure 5.3(d) correspond to the shapes predicted in [146] suggesting that a morphological transition is required for monolayer budding as well. Their theory is applicable to lipid droplets where the hydrophobic contents are in the liquid phase, such as triacylglycerols, and change their shape during the budding process. However, pure sterol esters can exist in the solid state [136] in which case the budding process is similar to the case of rigid, colloidal particles as described for bilayers [148] [149].

The result of the morphological transition is an oil droplet that is completely enveloped by the lipid monolayer except for a neck region that still connects it to the parent bilayer. The shape of the monolayer surface is highlighted in Figure 5.4. The time scales involved in the change from partial to complete wrapping in our droplet cluster simulation (Figure 5.2), as well as the range of reaction coordinate over which this change occurred during umbrella sampling, are very small. This further suggests that there is a morphological transition between partial and complete wrapping in the case of monolayers. The individual lipids at the droplet periphery require a concerted reorganization to transition from the partially wrapped to the completely wrapped state. The transition can be aided by lipids with negative spontaneous curvature such as phosphatidyl-ethanolamines, the second most abundant lipid occurring in lipid droplets [126], and those with positive spontaneous curvature, such as lysophosphatidylcholines, which are also found on lipid droplet surfaces [125]. The assembling of proteins on the lipid droplet surface, although not found to be directly responsible for droplet formation, may induce curvature in the membrane and contribute to the transition [143].

To further investigate the transition states, we started several NVT trajectories beginning at the partially, as well as the completely enveloped states. The trajectories started in the partially wrapped state did not show substantial shape changes in 12.5 µs of simulation time. However, the trajectories that were begun in the completely wrapped state almost all showed budding within 2.5–10 µs. The latter trajectories were subsequently used to analyze the neck region of the budding droplet. The neck itself is not always axisymmetric, and sometimes scission can occur as the droplet fluctuates more towards one side of the neck than the other. A closer look at the neck is shown in Figure 5.5. At any point, there are several lipids that are part of both the droplet as well as the parent bilayer. Figure 5.5 highlights two such lipids—one has its head group in the parent bilayer and its tail group in the droplet, while the second has its head group on the droplet surface with its tail embedded in the bilayer. Lipids diffuse back and forth between the droplet and the parent bilayer through the neck. Budding occurs when there is a collective movement in the neck lipids which are part of both structures. As opposed to the ‘splayed’ lipids which enable the formation of a fusion pore [134] scission occurs when the neck lipids belonging to both structures commit to either the parent bilayer, or the droplet. Once the droplet reaches the neck stage, thermal fluctuations can bring about this final release. Electron micrographs of plastoglobules [150]—variants
of lipid droplets in the chloroplast—contain several droplets connected to each other and
the parent thylakoid membrane by extended neck-like structures. These can possibly be
stabilized for longer than a few microseconds by non-bilayer lipids that are typically found
in thylakoid membranes.

5.3 Conclusions

Our simulations provide a molecular view of the submicroscopic formation and budding of oil
droplets in a lipid bilayer, and support the schematics of the process that have been proposed
so far. Using a lipid reservoir that supplies lipids to a growing droplet, we characterize the
different stages involved in lipid droplet formation, and access the transition states involved
in the process, surmounting the computational challenges that this problem has presented
thus far. We hypothesize that there exists a morphological transition between a partially
wrapping and a completely wrapping monolayer structure, which is similar to that proposed
by existing theories for bilayers, and constitute the primary intermediates that occur during
the process. Furthermore, we propose a molecular mechanism for monolayer scission. The
droplets produced by this technique can be tuned to have a desired surface tension and used
for future studies involving droplet fusion and transmembrane organization of the monolayer-
integrated proteome on the droplet surface, which can be substantially different from that
of bilayer-spanning transmembrane segments.

5.4 Details of the simulation

5.4.1 Hybrid dissipative particle dynamics–Monte Carlo

We use the standard dissipative particle dynamics algorithm (DPD) on the coarse-grained
model with periodic boundary conditions. To sample the $NP\gamma T$ ensemble, DPD steps are
interspersed with Monte Carlo attempts to maintain constant surface tension or constant
chemical potential. Constant surface tension is maintained by making Monte Carlo attempts
to change the area of the bilayer while holding the total volume constant. The acceptance
criteria for these attempts is described in [72]. In the constant surface tension simulations,
$\gamma$ is maintained at zero.

5.4.2 Grand canonical Monte Carlo for lipids

Constant chemical potential of a species (oil or lipid) is maintained by performing attempts
to change the number of lipids of that species using grand canonical Monte Carlo. The
density of a lipid bilayer makes successful sampling of the grand canonical ensemble difficult.
To improve the sampling, we use (i) a GPU-accelerated, parallel version of configurational-
bias Monte Carlo [151] without which the reservoir implementation would be untenable, (ii)
density-biased Monte Carlo to take advantage of the anisotropy of the system and make
Figure 5.5: A closer look at the neck of the droplet that precedes budding (the oil beads are not shown for clarity): The lipid head and tail beads are colored cyan and red respectively. Two representative neck lipids are highlighted, both extending between the droplet and the parent bilayer. The neck region consists of several such lipids that are part of both the droplet, as well as the membrane. Budding occurs when these lipids cooperatively commit to either the parent bilayer or the droplet monolayer.
insertion and deletion attempts exclusively in the lipid phase \textsuperscript{85}, and (iii) orientation bias Monte Carlo to orient the attempted configurations into the bilayer. The $\mu_{\text{lipid}}$ value used in the grand canonical Monte Carlo simulations is calculated using Widom insertion \textsuperscript{152} for a single-component, hydrated and tensionless bilayer.
Chapter 6

Conclusion

This work presents results from studies of different kinds of inclusions within model lipid bilayers in an attempt to identify key physical principles operating in the organization and functioning of biological membranes. Firstly, we present the orderphobic effect, a powerful force of lateral assembly within a lipid bilayer borne from the first-order phase transition between the two phases of a lipid bilayer. The nature of this force is generic, and could be applied to any system where two phases are separated by a first-order transition. Although we have presented only one example—the addition of orderphobic inclusions in an ordered bilayer—the reverse effect is also theoretically possible, i.e. the addition of orderphilic (or disorder-phobic) inclusions in a disordered bilayer should give rise to similar force of assembly.

A real biological membrane is, of course, a very rich and complicated multi-component structure. We attempt to understand the physical principles involved in a pure, single-component bilayer and hope to extend these principles to more components in the future. For example, cholesterol, a predominant component of biological membranes, may itself behave as an orderphobe or orderphile. Adding cholesterol to an ordered bilayer is known to form a ‘liquid-ordered’ phase, a material with structure similar to that of the ordered phase but with a diffusion coefficient much higher than the ordered phase. It is possible that the orderphobic effect is responsible in giving rise to these features.

On the other hand, a myriad different forces could be at play in giving rise to the picture of the cell membrane observed using super-resolution microscopy. For example, if several orderphobic inclusions assemble, they would have a large energy due to line tension in their vicinity. At some point, this line tension energy could be high enough to overcome the bending energy of the membrane. This could lead to a buckling, and possible budding, of the membrane to reduce the energy due to the line tension. Considerations of bending, in addition to line tension, could also provide a bound on the cluster size possible in a membrane.

The second part of this work provides computational methods to study asymmetric structures in a lipid bilayer—any structure or process that involves more lipids on one leaflet than the other. Using these methods, we attempt to study the growth and formation of lipid droplets, an organelle about which little is known at the sub-microscopic level. We identify
partially and completely wrapped states that could be a barrier to the budding process that might possibly be aided by curvature generating proteins that are yet to be discovered in the context of lipid droplets. The lipid droplets generated by this method could be used as starting points in the future to embed inclusions specific to lipid droplets and study their physical properties. Lipid droplets have their own dedicated proteome which is different from a regular membrane proteome in that it consists of monolayer proteins. It is entirely possible that effects similar to the orderphobic effect govern protein behavior in the monolayer that coats a lipid droplet.
Appendix A

Testing the Orderphobic Effect with a Soft–Repulsive Potential

A.1 Phase behavior of a soft–repulsive model

Figure A.1: A minimalist, coarse-grained model of dipalmitoyl phosphatidylethanolamine (DPPE) composed of three hydrophilic beads (cyan) corresponding to the head group, and five hydrophobic beads (red) per tail. Details of the bonded and non-bonded interactions are given in Chapter 3.
Here, we report the phase behavior of the minimalist model with a soft, repulsive potential that was introduced in Chapter 2 and subsequently used in Chapter 5 to study lipid droplets. In Chapter 2, we introduced a model for a single-tailed lipid. Here, we examine the phase behavior of a double-tailed lipid parameterized with a head group repulsion $a_{hh} = 10$. This makes head groups prefer each other to water and eliminates the ripples observed during heating and cooling. This lipid is equivalent to dipalmitoyl phosphatidylethanolamine (DPPE) as shown in Figure A.1.

Figure A.2: The ordered (above) and disordered (below) phases observed in the minimalist model with a soft, repulsive potential: The low-temperature ordered phase of the bilayer has erect, ordered chains while the high-temperature disordered phase of the bilayer has scrambled, disordered chains. The ordered phase has a higher hydrophobic thickness and correspondingly, a low area per lipid. The disordered phase has a relatively low hydrophobic thickness and correspondingly, a higher area per lipid. Solvent particles have not been shown for clarity.
This lipid exhibits two phases, a solid-like ordered phase with a large hydrophobic thickness, and a liquid-like disordered phases with a smaller hydrophobic thickness. A snapshot of these two phases is shown in Figure A.2. The aliphatic chains in the ordered phase are erect and ordered while those in the disordered phase are randomly arranged. The ordered phase thus has a lower area per lipid, while the disordered phase has a higher area per lipid, and the areal density (or correspondingly, the hydrophobic thickness) can be used as an order parameter to differentiate between the two phases as in Chapter 3.

In Figure A.3 we plot the variation of the area per lipid as a function of reduced temperature for two different system sizes (2000 lipids and 8000 lipids). For both the larger and the smaller system we see that the variation in the order parameter with temperature is similarly continuous. The transformation from the ordered phase to the disordered phase is continuous and gradual and we observe patterns that resemble spinodal decomposition. These signatures are indicative of a continuous transition. There is no sharp change in the value of the order parameter for the two phases, and the variation does not get sharper with an increase in system size as is expected for a first-order transition. Additionally, Rodgers et al. noted a lack of hysteresis for the order–disorder transition, consistent with a continuous transition [47].

If the ordered phase in this model is hexatic, the lack of a first-order transition is consistent with the results of Kapfer and Krauth who showed that for repulsive potentials $r^{-n}$ with $n < 6$, the hexatic to liquid transition is continuous. The repulsive potential for this minimalist model is a harmonic potential with $n = -2$. This can inform the choice of potential for coarse-grained models for lipid bilayers that seek to reproduce the order of the transition. Since there is no order–disorder transition in this model, the orderphobic effect observed in Chapter 4 should be absent in this minimalist model. To test this hypothesis, we embed idealized orderphobic proteins in the disordered phase exhibited by this model.

### A.2 Orderphobes in a minimalist, soft–repulsive model

In Figure A.4 we embed an orderphobic protein, one with a hydrophobic thickness almost equal to that of the disordered phase, into an ordered bilayer. The protein is modeled as a cylinder that contains a hydrophobic core made of hydrophobic, lipid tail particles flanked at the top and bottom by a hydrophilic head made of hydrophilic, lipid head beads. In addition to a profile view, a top view of the membrane shows the tail end beads of the lipids in one leaflet of the bilayer. We can see that the orderphobic protein causes a perturbation in its vicinity; there appears to be a decrease in the local areal density. The profile view suggests a reduction in membrane thickness around the orderphobic protein.

To quantify this perturbation, we calculate the lipid density as a function of distance from the edge of the protein cylinder in Figure A.5 for orderphobes of three different radii, 0.775 nm (approximately the radius of an $\alpha$-helix), 1.227 nm and 1.68 nm. The bulk lipid densities of
Figure A.3: Variation in the area per lipid as a function of reduced temperature: For two different system sizes, the changes in the area per lipid are similarly continuous. This indicates that the transition is not first-order.
Figure A.4: Orderphobic proteins, i.e. proteins with a thickness approximately equal to the thickness of the disordered phase, are embedded in an ordered bilayer. (Top) The profile view of an orderphobic protein embedded in the ordered phase. (Bottom) a top view of the tail end particles in one leaflet of the bilayer.
the ordered phase and disordered phase are marked as horizontal lines. We observe that at a distance from the protein, the membrane attains the ordered phase density, however, closer to the protein there is no disordered region induced. In fact, proximal to the protein there appears to be an increase in density. As the density decays to the bulk value, we observe a depletion as suggested by the top view in Figure A.4. However, the depletion appears to be caused by an elastic perturbation of the membrane that does not involve a first-order phase transition. Moreover, all three model protein sizes have identical effects on the bilayer, contrary to the observations for the orderphobic effect. This result is consistent with the orderphobic effect, inherently a pre-transition effect, occurring solely in systems that exhibit a first-order transition.
Appendix A. Testing the Orderphobic Effect with a Soft–Repulsive Potential

Figure A.5: Radial distribution function of lipid density around an orderphobic protein: For three different protein radii, the areal density of the lipid bilayer is shown as a function of the distance from the edge of the protein. All three protein sizes have identical effects on the bilayer. There is an enrichment of lipid density surrounding the protein. This is followed by an elastic depletion in density before the density finally reaches the bulk ordered value. There is no orderphobic effect observed here (i.e. there is no disorder induced in the vicinity of the protein).
Appendix B

Quantifying Fluctuations

The pre-transition layer occurs because of proximity to phase coexistence for a first order phase transition, i.e. the free energy difference between the two phases is small. A sampling of the ordered phase in the MARTINI model should therefore reveal glimpses of the disordered phase, however rare. The probability of these events is small far away from the transition temperature, but increase as the transition temperature is approached. We measure the probabilities of occurrence of the disordered regions using two order parameters—the lipid areal density, and a variant of the Halperin Nelson order parameter $\phi_6$ introduced in Chapter 3.

B.1 Density fluctuations

We calculate distributions $P_v(N)$ of observing $N$ particles within a probe area of given radius $R$. For these calculations, we use the tail end particles of each lipid. A low value of $N$ corresponds to the disordered phase, which has a lower areal density compared to ordered phase which has a high average value of $N$. This directly corresponds to other order parameters, such as density or $\phi_6$. Regions with low areal density naturally have a smaller thickness and a smaller $\phi_6$ because they are disordered. Figure B.1 shows the distributions $P_v(N)$ for probe areas of four different sizes. This calculation is performed for two temperatures, one far away from the transition temperature (279 K, Figure B.1 top) and one closer to the transition temperature (309 K, Figure B.1 bottom) in a system of 512 lipids. We can see that at the lower temperature, the fluctuations about the mean $N$ are Gaussian. However, at a higher temperature, low $N$ fluctuations are more frequent and deviations from the Gaussian begin to be seen for all probe area sizes evaluated.

B.2 $\phi_6$ fluctuations

Similar distributions for $\phi_6$ are shown in Figure B.2. At low temperatures (279 K, Figure B.2 top), the probability of observing a cumulative $\phi_6$ value in a probe volume of given radius
Figure B.1: Density fluctuations in a probe area in the ordered phase: Logarithm of the probability distribution of $N$, the number of particles at any instant in time in a probe area of radius $R$ at 279 K (top) and 309 K (bottom). Far away from the transition temperature (at 279 K), the distributions appear Gaussian. As we approach the transition temperature, glimpses of the disordered phase appear as deviations from the Gaussian. Reference Gaussian distributions are shown in dashed lines.
is Gaussian for probe volumes of several different sizes. However, at higher temperatures (309 K, Figure B.2 bottom) deviations from the Gaussian due to glimpses of the disordered phase begin to be seen.

More accurate measures of these probability distributions can be obtained using constrained sampling [153]. The distributions thus observed would give us an estimate of the free energy difference between the two phases. Similar calculations have been performed for the hydrophobic effect [154].
Figure B.2: $\phi_6$ fluctuations in a probe area in the ordered phase: Logarithm of the probability distribution of the cumulative $\phi_6$ in a probe volume of radius $R$. Far away from the transition temperature, at 279 K, the distributions are Gaussian (top). However, as we approach coexistence, the free energy difference between the two phases decreases and we see glimpses of the disordered phase appearing as low $\phi_6$ deviations from the Gaussian (309 K; bottom).
Appendix C

Diffusion of 5–7 Pairs

Solid and hexatic phases are difficult to simulate on a computer because of their long equilibration times. To differentiate the hexatic phase from a relaxing solid, we characterize the diffusion of dislocation defects, or 5–7 pairs in the ordered phase of the MARTINI model. Both the solid and the hexatic phases exhibit dislocations, however, dislocation pairs are bound in a solid while they are freely diffusing in the hexatic phase.

In Figure C.1 we display 5–7 pairs from three snapshots from a molecular dynamics simulations, each 30 ps apart. The 5–7 pairs diffuse rapidly and appear to sample the simulation box ergodically. Further, we do not observe the number of free 5–7 decreasing with time for equilibration times of several $\mu$s. This further confirms that the ordered phase is a hexatic.
Figure C.1: Snapshots from a molecular dynamics simulation of the ordered phase of the MARTINI model exhibiting 5–7 pairs (a site with 5 neighbors is marked in fuchsia, while the site with 7 neighbors is marked yellow). The snapshots are 30 ps apart and show the 5–7 pairs appearing to diffuse ergodically through the box.
Bibliography


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