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Modeling Biomembranes

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Modeling Biomembranes

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Abstract

Understanding the properties and behavior of biomembranes is fundamental to many biological processes and technologies. Microdomains in biomembranes or ‘lipid rafts’ are now known to be an integral part of cell signaling, vesicle formation, fusion processes, protein trafficking, and viral and toxin infection processes. Understanding how microdomains form, how they depend on membrane constituents, and how they act not only has biological implications, but also will impact Sandia’s effort in development of membranes that structurally adapt to their environment in a controlled manner. To provide such understanding, we created physically-based models of biomembranes. Molecular dynamics (MD) simulations and classical density functional theory (DFT) calculations using these models were applied to phenomena such as microdomain formation, membrane fusion, pattern formation, and protein insertion. Because lipid dynamics and self-organization in membranes occur on length and time scales beyond atomistic MD, we used coarse-grained models of double tail lipid molecules that spontaneously self-assemble into bilayers. DFT provided equilibrium information on membrane structure. Experimental work was performed to further help elucidate the fundamental membrane organization principles.

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Chapter 1

Introduction

Understanding the properties and behavior of biomembranes, including their bound proteins, is fundamental to many biological processes. For example, microdomains in biomembranes or ‘lipid rafts’ are now known to be an integral part of cell signaling, vesicle formation, fusion processes, protein trafficking, and viral and toxin infection processes. Understanding how microdomains form, how they depend on membrane constituents, and how they act not only has biological implications, but also will impact Sandia’s interest in developing membranes that structurally adapt to their environment in a controlled manner. We also seek to understand the effect of membrane proteins on the bilayer structure and dynamics, and vice versa. This includes protein organization within a bilayer and insertion into the bilayer.

One of the defining characteristics of biomembranes is their fluidity. The lipid molecules diffuse within the 2D bilayer. Without fluidity none of the above membrane processes could occur. In the development of supported bilayers, maintaining fluidity of the bilayer is viewed as an essential ingredient. The fluid dynamics of bilayers occurs on time scales beyond the current capability of atomistic simulations. Thus, different models and methods must be developed in order to treat membrane phenomena.

We propose to create physically-based, coarse-grained models of biomembranes. Using these models, molecular dynamics (MD) simulations and density functional theory (DFT) will be applied to phenomena such as microdomain formation, membrane fusion, pattern formation, and membrane-protein interactions. A key point is that the organization and dynamics of membrane constituents can be simulated by MD and DFT using coarse-grained models. In addition, we will develop the dissipative particle dynamics (DPD) method to further increase the range of treatable dynamics. While the main focus of this project is on theoretical understanding and methods, complementary experimental work will be performed. We have performed significant preliminary work to the proposed project. In the following paragraphs we summarize our efforts for modeling lipid bilayers with MD and DFT approaches.

Figure 1.1. Bead-spring model.

To simulate bilayer membranes, a bead-spring model of a double tail lipid molecule has been tested. In this model a bead corresponds to several atoms in a lipid. The head consists of three (blue) beads and each tail consists of four (red) beads. Self-assembly is induced by a simple Lennard-Jones (LJ) potential for the bead-bead interactions. For two beads of the same type the full LJ potential is used. For two beads of a different type, the LJ potential is cutoff at the minimum and shifted yielding a purely repulsive interaction. The solvent is treated as a single bead (cyan) that has the full attractive LJ interaction with the lipid headgroup and a repulsive interaction with the tail beads.

Using the above model, MD simulations starting from a random configuration of lipids and water self assemble into a *fluid* bilayer membrane. Membrane fluidity was demonstrated by calculations of the lateral diffusion constant and the fact that individual lipid molecule diffuse completely across the system. We have also started investigations of fusion processes. Initially, two distinct bilayers were created between two solid surfaces a la supported bilayers (Fig. 1.2(a)). This provides a route to creating fusion intermediates such as shown in Fig. 1.2(b). Finally, we have also simulated a small vesicle (Fig. 1.2(c)-(d)), demonstrating that vesicle simulations are feasible, including simulations of two vesicles fusing.

These simulations are about 1000 times faster than atomistic simulations. This speed-up is due to the coarse-grained model requiring calculation of fewer interactions and having fewer particles per volume. The simulation size ranges from about 10,000 beads for a single bilayer to 200,000 for vesicle simulations. The vesicles simulations used 64 CPlant processors while the single bilayer simulations need only a few processors. These simulations take on the order of 1 day of CPU time.

Figure 1.2. Biomembrane images: a) two bilayers formed between two solid surfaces; b) two bilayers with connector formed between two surfaces; c) slice through a spherical vesicle early in simulation, d) a later image showing growth of bud and e) AFM image of microdomains. The solvent is transparent in a) and b).

Chapter 2

Coarse-grained simulations of lipid bilayers

2.1 Introduction

Lipid bilayers are one of the fundamental components of cell membranes and are the prototypical self-assembly system.[1, 2] Lipid molecules are amphiphiles with a hydrophilic head group and (typically) two hydrophobic tails. They self assemble into planar bilayers as well as other structures.[2] There are many biological phenomena for which lipid bilayers are an essential component, for example membrane fusion.[3] Our understanding of the importance of the cell membrane to biological functions is growing.[4] The cell membrane used to be viewed as a nonparticipating matrix for membrane proteins which did all the biological function. More and more examples show that the cell membrane acts as a major participant in biological function.[5, 4]

The role of lipid membranes should not be a surprise to physical chemists. Work over the last two decades has shown that the fundamental characteristics of membranes can affect not only the structure of the membrane, but also exert forces on inclusions such as membrane proteins. [6, 7, 5, 8, 9, 10] The variety of lipid types allows a wide variety of interactions with other membrane components as well as the variety of self-assembled structures. From an engineering or evolutionary perspective, much can or has been accomplished with this variety.

Lipid bilayers in cell membranes are a two-dimensional (2D) fluid membrane. The lipid molecules diffuse laterally within the bilayer. This is a essential feature for transport in cell membranes. While cell membranes are fluid, they maintain a structural integrity with curvature deformations playing a significant role in bilayer shape. A basic understanding of the mechanics of lipid bilayers has been developed through theoretical analysis of the membrane elasticity. [11, 12]

Molecular simulations offer a means to understand how the dynamics and structure of lipid bilayer depend on the molecular aspects of the lipid molecules. There have been many atomistic simulations of lipid bilayers.[13, 14] These simulations have given much insight into the nature of lipid structure within the bilayer. A key limitation of atomistic simulations is the slow (lateral) diffusion of lipid molecules within in the bilayer. A typical diffusion constant for a phosphatidylcholine lipid is about $5 \mu\text{m}^2/\text{s}$ which yields about 50 ns for time to diffusion just to the nearest neighbor position. Since the upper range of atomistic simulations is presently in this range, the dynamics of lipids in the bilayer is not treatable with atomistic molecular dynamics (MD). Thus, most phenomena of lipid bilayers which have functional significance are beyond the times accessible by atomistic MD simulations. This is a similar situation to problems of polymer physics including entanglement dynamics and protein folding. One of the successful methods of treating long time dynamics in polymers has been the use of coarse-grained models in simulations.[15, 16] Minimal models for proteins have developed significantly in the last several years.[16, 17] Coarse-grained models of lipid molecules have been recently developed. [18, 19, 20, 3, 21] Simulations using such models have shown that the bilayer can self assemble from random starting states and that the lipid molecule can diffuse well beyond nearest neighbor distances.[18]

Here, we present a coarse-grained model of a lipid molecule that was used to study membrane fusion,[3] and will be used for other membrane problems. The focus here is on the fundamentals of the model. The interactions within the model are described, including models for saturated and unsaturated lipids in section 5.2. The main calculations are the area per lipid as a function of chain length and temperature which are presented in section 5.4. Within these simulations the liquid to gel transition is determined, and the liquid and gel state structures are characterized. The elastic moduli are also calculated to provide a connection to membrane elasticity theories. Discussion and conclusions are presented in section 2.4.

2.2 Simulation Method and Model

The lipid is treated a bead-spring, double tail molecule as shown in Fig. 9.1.1. The model is based on coarse-grained polymer and related models.[15, 22] This model will work best for neutral lipids such as phosphatidylcholine lipids. Each tail is composed of N_T hydrophobic beads of type T and the head is composed of three hydrophilic beads of type H. The solvent (S type) is represented by a single bead; the S and H types are equivalent in this work. Beads interact through the Lennard-Jones (LJ) potential

$$u_{\text{LJ}}(r) = 4\epsilon[(\sigma/r)^{12} - (\sigma/r)^6] \quad (2.1)$$

To keep the model simple, all monomeras interact with the same interaction strength ϵ . The TT, HH, HS and SS pair interactions have the LJ potential with the standard cutoff

Figure 2.1. Schematic of lipid model with $N_T = 4$.

$r_c = 2.5\sigma$. The hydrophobic HT and TS interactions are taken to be purely repulsive by using a cutoff $r_c = 2^{1/6}\sigma$. The bond potential is the same as used in many bead-spring polymer simulations.[23] It is the sum of the attractive finitely extensible, nonlinear elastic (FENE) potential and the purely repulsive LJ potential u_{LJ} with cutoff equal to $2^{1/6}\sigma$,

$$U_{\text{bond}}(r) = -\frac{1}{2}k_0R_0^2\ln(1 - r^2/R_0^2) + u_{LJ}(r), \quad (2.2)$$

where $k_0 = 30\epsilon/\sigma^2$ and $R_0 = 1.5\sigma$. This bond potential has the advantage of being integrable with the same time step as the LJ potential. This is in contrast to a similar model which has a very stiff bond potential that requires a time step 10 times smaller.[18] However, while computationally there is an advantage with our model, we expect the two models to yield similar results, since the differences are slight (r^{-9} vs. r^{-12} hydrophobic repulsion and $\cos\theta$ vs. θ^2 angle potentials). Angle terms are included via the harmonic potential

$$U_{\text{bend}}(\theta) = k_\theta(\theta - \theta_0)^2, \quad (2.3)$$

where θ is the angle between neighboring bonds. To give a persistence length of about 2σ for the tails $k_\theta = 2\epsilon/\text{rad}^2$ and $\theta_0 = 180^\circ$. Other angle terms are included for the head group geometry. Following the labeling in Fig. 9.1.1, $\theta_0 = 120^\circ$ for triplet 1-2-3 and $\theta_0 = 105^\circ$ the triplets 2-3-4 and 1-2-8. A double bond is modeled by including a kink in the tail chain. A harmonic angle term with spring constant $k_\theta = 10\epsilon/\text{rad}^2$ and equilibrium angle of 120° is included in the potential energy to yield the kink. This model system spontaneously self-assembles into a lamellar bilayer when started from a random configuration of lipids and solvent at appropriate lipid concentrations (see also ref. [18]).

For simulations of the area per lipid A as a function of temperature T , the starting state is formed by placing the lipids on a triangular lattice with the chosen value of A . The total number of lipids in the simulation is N_{lip} . The number of solvent particles N_s was chosen so that the single bilayer in the simulations was well separated (at least $> 10\sigma$)

from its periodic image. Molecular dynamics (MD) simulations using a Verlet integrator with a time step of 0.005τ were performed, where τ is the LJ time unit. Simulations were primarily performed in the NPT ensemble using the Nosé-Hoover equations of motion.[24] The pressure is $0.5\epsilon/\sigma^3$. This value is chosen to approximate atmospheric pressure. In our NPT simulations each of the cell dimensions is independently controlled so that the area per lipid is determined by the interactions and is not dependent on the initial value. In addition, the surface tension γ is automatically zero in this case consistent with direct calculations.[25]

The surface tension is directly calculated in NVT simulations using A determined in NPT simulations. The calculation of the surface tension is through the usual integration of the local stress tensor components.[18] The local stress can be calculated by more than one method.[18, 26, 27] They all involve binning the stress components in slabs parallel to the bilayer. We use the Hardy method[26] as it is generalizable beyond planar geometries.

2.3 Results

A key aspect of coarse-grained simulations of a lipid bilayer is that in the liquid phase diffusion well beyond near neighbor distances should be readily obtainable. Figure 2.2 shows the mean square displacement for a system with $N_T = 6$ at two different temperatures. In the liquid state at $T = 1.2\epsilon$ the average displacement is about 9σ within a time period of 5000τ . The area per lipid at this temperature is $A = 3.63\sigma^2$. The nearest neighbor distance is approximately $\sqrt{A} = 1.9\sigma$. Thus, in this short time, the average lipid has traveled several nearest neighbor distances. At $T = 1.0\epsilon$ where the bilayer is in a gel state, the diffusion rate has dropped significantly as expected. The diffusion constant is $D = 4 \cdot 10^{-3}\sigma^2/\tau$ at $T = 1.2\epsilon$ in the liquid state and $9 \cdot 10^{-4}\sigma^2/\tau$ in the gel state at $T = 1.0\epsilon$. These diffusion constants were calculated using the 2D mean squared displacement: $r^2 = 4Dt$. At least for this system, even in the gel state the dynamics beyond the initial starting structure is obtainable with the bead-spring model.

An approximate map of τ to seconds can be obtained from the diffusion constant. If we take $5 \mu\text{m}^2/\text{s}$ as a typical value of the diffusion constant for dilauroylphosphatidylcholine,[28] and use $\sigma \simeq 0.5 \text{ nm}$, then $\tau = 0.20 \text{ ns}$. Thus, 5000τ corresponds to about $1.0 \mu \text{ s}$. Using this map, our simulations are in the μs to ms time range.

The area per lipid A is a key quantity defining lipid bilayers. The simulation cell area divided by the number of lipids in one of the leaflets of the bilayer is taken to be A . Fig. 2.3 shows the calculated values of A as a function of temperature for different N_T . For a given N_T , A increases linearly as a function of temperature in the liquid phase. There is a discontinuity at the liquid to gel transition temperature T_m . In the gel phase at $T < T_m$, A is basically constant at $2.35 \sigma^2$.

In the liquid phase A decreases as N_T increases for most temperatures. The longer tails

Figure 2.2. Plot of mean square displacement (r^2) as a function of time for a $N_T = 6$ simulation of a liquid state (larger slope) at $T = 1.2$ and a gel state at $T = 1.0$. The dotted lines are least square fits that yield the diffusion constants. $N_{lip} = 448$.

Figure 2.3. Plot of area per lipid (A) as a function of temperature (T) and of the number of beads in tail (N_T). The solid lines are least square fits to the liquid state portion of the data. The dashed line is a guide to the eye for the gel phase data. $N_{lip} = 448$ and $N_s = 8728$.

have a larger tail-tail van der Waals attractive energy which promotes denser structures. At $T \simeq 1.17\epsilon$, A is approximately equal for all N_T , while for $T \geq 1.17\epsilon$, A increases with increasing N_T . At these temperatures there are strong undulations in the membrane structure particularly for the shorter lipids. (See the calculation of the bending modulus discussed below.) In these cases, the contour surface area may be noticeably larger than the projected area and larger for short tails compared to long tails. However, such a ‘correction’ would imply that A is nonlinear in this regime.

Since the liquid-gel transition exhibits a discontinuity, simulations were performed with sequentially decreasing and increasing temperature. That is, the starting point in the decreasing sequence is the final configuration of the simulation at the temperature just above it. Figure 2.4 shows the data for $N_T = 6$. There is a large hysteresis which makes the uncertainty for the liquid-gel transition temperature quite large. In order to reduce this uncertainty, simulations were performed on a system composed of a half which is ordered and a half which is liquid. In such a system, the nucleation barrier to create either the liquid or gel phase is greatly reduced which should reduce the hysteresis.[29] This system was constructed by constraining half of the lipids in their initial triangular lattice, while the other half was equilibrated at $T = 1.2\epsilon$. This results in a system with the lipids in which half are in the liquid state and the other half are in an ordered structure. Simulations are then run at the desired T and the phase at that T determined. Figure 2.4 shows the decreasing temperature runs are sufficiently accurate in determining the area per lipid. In fact, only the increasing T data is distinct. The other three data sets are almost identical.

An important issue in simulations involving different phases is the system size dependence. We find that the area per lipid in the liquid phase is not noticeably different for simulations containing up to 6696 lipids. However, we note that particularly for $N_T = 8$ that structures with some characteristics of a ripple phase occur.[30, 31] To observe a ripple phase, the simulation cell must be at least the length the wavelength of the ripple structure. Performing simulations with a large number of wavelengths per cell is difficult. In addition, issues concerning commensurability with the cell can affect the simulation results. Given that the interpretation of such simulations raises additional questions, these results are only noted here.

In Fig. 2.5 the melting temperature is plotted as a function of the number of carbon atoms N in the lipid tail. The experimental data is for phosphatidylcholine lipids. To convert the simulation temperature data to units of K, we set $\epsilon = 320$ K. The mapping between N and N_T is $N = 2.4N_T$. The result is very good with the exception of the $N_T = 10$ point. This map is not unique, for the simulation data can be moved up or down along the experimental line of data so long as the map preserves the slope. For example, $N = 2.0N_T$ and $\epsilon = 300$ K also give a good fit with the simulation data lower on the fit curve. The figure shows that the value of the T_m decreases with N_T similarly to the experimental data, and gives a rough mapping of 1 bead to 2 or 2+ CH_2 groups per coarsened grained monomer. This is similar to that found in polymer modeling.[15]

Figure 2.6 shows images of the liquid and gel phase. The higher degree of order and the presence of a preferred tilt angle in the gel phase is evident from the figure. In the gel

Figure 2.4. Plot of area per lipid (A/σ^2) and bilayer thickness (h/σ) as a function of temperature for $N_T = 6$. The open circles represent simulations decreasing in T starting from $T = 1.2\epsilon$. The open squares represent runs starting from the $T = 0.99\epsilon$ run and increasing in temperature. For these two sets $N_{lip} = 448$. The solid circles represent simulations with starting configurations composed of half solid and half liquid (see text for more details). For this set $N_{lip} = 3024$ and $N_s = 50000$. The solid squares represent data of Fig. 2.3 which are often hidden by the solid circles.

Figure 2.5. Plot of the melting temperature (T_m) as a function of the number of C atoms in a lipid tail. Solid points are for experimental data,[32] and the open points are the scaled simulation data. The solid line is a least square fit to the experimental data.

images the gap between the two leaflets of the bilayer is artifact of the bond representation for the image; a ball and stick representation shows that volume filled, but also occludes the gel phase ordering. In the liquid phase, there is some overlap in the density profiles of the two leaflets. The overlap in the density profile of the top on the bottom leaflet goes to zero in 2σ . Thus, there is some overlap of the terminal beads in the tails, but that is about it. Statistically, the structure of the phases as well as the liquid-gel transition were examined by calculating such quantities as the tilt angle and radial distribution functions. The tails straighten as the temperature decreases; this is equivalent to the number of gauche defects decreasing as the temperature decreases. In the gel state, most of the tails are in the completely straight, all trans state. The tilt angle is calculated as the average of the angles between end-to-end vector of each tail with z -axis, the bilayer normal. In the liquid state there is a very broad distribution of tilt angles. As the temperature decreases the distribution narrows considerably. In the gel state the tilt angle has a narrow distribution about 25.5° (width at half maximum is 7°). Experimentally, the tilt angle for dimyristoyl-phosphatidylcholine varies from 26° to 30° . [33] The value is surprisingly close. The main physical origin is basically the same; the tilt angle represents the best packing angle for the bead-spring lipid model. Given the similar values of simulation and experiment, the effect of the details of the head group interaction appears to be small for the tilt angle. The distance between the peaks in the density profile for the head group beads is taken to be the bilayer height or thickness h . The thickness increases as T decreases (see Fig. 2.4). This is a consequence of A decreasing, the tails straightening and the tilt angle distribution narrowing as T decreases. Basically, as T decreases, the single chain structure becomes

Figure 2.6. Images of lipid bilayers using the bond representation for $N_T = 6$ at (top) $T = 1.2\epsilon$ in the liquid state and at (bottom) $T = 1.0\epsilon$ in the gel state. The H beads are dark and the tail beads are light gray. $N_{lip} = 960$ and $N_s = 18700$.

more ordered.

The order among the chains was examined through the bead-bead in-plane radial distribution function $g_{xy}(r)$. In the liquid state, $g_{xy}(r)$ has a correlation hole and no peak. In the gel state a series of peaks occur at small r corresponding to the ordering of the lipid chains in the bilayers. Images parallel to the bilayer normal of the gel state show predominantly a triangular lattice order of the molecules. The order extends beyond the nearest neighbor, but is not globally present, i.e. not a crystal state.

The structural order within the bilayer can be disrupted by double bonds (unsaturated bonds) in the lipid tails. Including unsaturated lipids in the membrane is a mechanism for the cell to control the fluidity of the membrane; unsaturated lipids have lower T_m . Simulations were performed for $N_T = 6$ and $N_{lip} = 448$ with a kink angle centered at the third bead from the top in the tail chain. Simulations were performed with one unsaturated tail chain and with both tails singly unsaturated. Figure 2.7 shows A and bilayer thickness as a function of temperature for these two cases along with the case without double bonds. As the number of double bonds per lipid increases, A increases as the kink requires a larger area. Similarly, the bilayer thickness decreases as the (almost) all trans state has a shorter length for unsaturated molecules due to the kink. The liquid to gel transition temperature (T_m) decreases with increasing number of double bonds, as it should.

Theories of liquid membranes typically start by characterizing the membrane structure and dynamics in terms of the elastic moduli.[9] In the Monge representation in which the height function $h(x,y)$ is zero at the bilayer center,[9] the Hamiltonian is

$$H(h) = \int d\mathbf{x} \left[\frac{\gamma}{2} (\nabla_x h)^2 + \frac{\kappa}{2} (\nabla_x^2 h)^2 + \dots \right]. \quad (2.4)$$

Figure 2.7. The effect of double bonds on the area per lipid and bilayer thickness for $N_T = 6$ with no double bonds (▲), with one tail with a double bond (■) and with one double bond on each tail (●).

Here γ is the surface tension and κ is the bending modulus. Calculating γ and κ is consequently fundamental to such theories. The interfacial structure is described in terms of the height-height correlation in Fourier space,

$$S(\mathbf{q}) \equiv \langle |h(\mathbf{q})|^2 \rangle = \int_A d\mathbf{x} e^{-i\mathbf{q} \cdot \mathbf{x}} h(\mathbf{x}). \quad (2.5)$$

Neglecting the higher order terms in Eq. 2.4, one finds

$$S(\mathbf{q}) = \frac{2k_B T}{\gamma q^2 + \kappa q^4 + \mathcal{O}(q^6)}. \quad (2.6)$$

The height function $h(x, y)$ is calculated separately for the top and bottom interfaces. The positions of the interfaces are calculated based on solvent positions and head group positions. The particle positions are binned in a $N_x \times N_y$ grid in the xy -plane. For the solvent in the top half, we define $h(x, y)$ to be the minimum of the solvent positions in the (x, y) bin. On the bottom half, the maximum is used. For the head group, we average the position of all head group beads in the (x, y) bin. The four sets of $h(q)$ are typically very similar, although there are cases of significant differences.

To calculate $h(x, y)$ we performed NVT simulations with the area per lipid and density determined from our constant pressure simulations. These NPT simulations yield a tensionless bilayer.[25] By varying the simulation cell area in NVT simulations nonzero

Figure 2.8. For $N_T = 6$ the area compressibility is obtained from the slope of the plot of surface tension γ as a function of the differential area per lipid $(A - A(0))/A(0)$, where $A(0)$ is the area per lipid at $\gamma = 0$. The symbols represent $T = 1.05\epsilon$ (■), $T = 1.11\epsilon$ (□) and $T = 1.20\epsilon$ (▲). $N_{lip} = 960$ and $N_s = 18700$.

γ are obtained, and the area compressibility k_A can be calculated. Near $\gamma = 0$ the slope of γ vs. $(A - A_0)/A_0$ is k_A , where A_0 is the area per lipid at $\gamma = 0$. In Fig. 2.8 we plot γ vs. $(A - A_0)/A_0$ for $N_T = 6$ at different temperatures. Table 2.1 gives the values of k_A calculated from this data as well as values of the bending modulus using the formula

$$\kappa = k_A h^2 / b \quad (2.7)$$

where b is a geometrical factor. Here we use $b = 48$ which has been used in other simulations,[19, 34, 21, 35] Our value of κ are consistent with the coarse-grained simulations.[19] In the evaluation of experimental data[36] $b = 24$ has been used. However, the membrane thickness used was $h - h_0$, where $h_0 = 1$ nm is the nondeformable length and determined from plots of $(\kappa/k_A)^{1/2}$ vs. h . The effect of using $h - h_0$ instead of h is to reduce the value of κ by 1/2. Thus, using eq. 2.7 with $b = 48$ yields the same values as using $b = 24$ and substituting $h - h_0$ for h . Consequently, the simulation and experimental values are in agreement.[36] Finally, we note that as the temperature approaches the liquid-gel transition temperature κ increases which is expected as the bilayer becomes stiffer.[37, 38]

The calculation of κ from the height-height correlation function as outlined above produces similar values. Figure 2.9 shows a plot of $1/(q^2|h(q)|^2)$ vs. q^2 for $N_T = 6$ at $T = 1.2\epsilon$. The value of κ is $k_B T$ times the slope which yields $\kappa = 8k_B T$. Compared to κ obtained from Eq. 2.7 given in Table 2.1, this is slightly smaller. For this T we find that $|h(q)|^2$ scales

Table 2.1. Values of area compressibility for $N_T = 6$. κ_1 is calculated from Eq. 2.7. κ_2 is calculated from Eq. 2.6.

$T(\epsilon)$	$k_A(\epsilon/\sigma^2)$	$\kappa_1(k_B T)$	$\kappa_2(k_B T)$
1.05	9.4	21	26
1.11	7.8	14	14
1.20	6.6	10	8

as $q^{-4.1}$ at low q which is quite close to the scaling of Eq. 2.6. At $T = 1.11\epsilon$ the value of κ ranges between 10 and 16 ϵ depending on the number of points used in the fit; the average of the two values is given in Table 2.1. Even though the system has a relatively large number of lipids compared to previous simulations, the small q regime has only a few points in the q^{-4} scaling regime at low q which yields a relatively large uncertainty. To obtain more points at low q would require a system size beyond present resources. For $T = 1.05\epsilon$ ($T_m = 1.025\epsilon$) κ_1 increases further to 26 ϵ , somewhat larger than calculated from Eq. 2.7, however the uncertainty is comparable to the difference. Overall, the calculation of κ using Eqs. 2.6 and 2.7 with $b = 48$ yield similar values. Using $b = 24$ yields values of κ_1 smaller than κ_2 .

The variation of κ with tail length has been calculated at $T = 1.2\epsilon$ using Eq. 2.6. For $N_T = 4, 6$ and 8 , we obtain $\kappa = 4, 8$ and $14 k_B T$, respectively. The data shows that the bilayer becomes stiffer as the tail becomes longer. As expected, this is consistent with area per lipid decreasing with N_T yielding a tighter, more stiff bilayer.

2.4 Discussion and Conclusions

This work lays a foundation for minimal coarse-grained lipid models and simulations. Such models are necessary for the treatment of many interesting biophysical problems, including membrane fusion.[3, 21] In protein simulations minimal models are commonly used and have reached new levels of sophistication.[16] For lipids, minimal models are just at the beginning, but key aspects of membrane can already be simulated. With coarse-grained lipid models the fluid character of the lipid dynamics can be explicitly simulated in contrast to atomistic simulations. This is a very important distinction for coarse-grained simulations, because the fluidity of the membrane is crucial its many biological functions. This feature along with the ability to perform simulations in the μs to ms time range are attainable which opens up many interesting problems.

The perspective for the minimal lipid model presented here is similar to the minimal models used for proteins (in protein folding simulations). As the development of minimal lipid models is just beginning, the model is basically at the level of the HP (hydrophobic-polar) protein models. [39] In this work we determined what can be accomplished with

Figure 2.9. For $N_T = 6$ at $T = 1.2\epsilon$ the bending modulus κ is obtained from the slope of the plot of $1/(q^2|h(q)|^2)$ vs. q^2 at small q . The circles represent the calculation for top surface and the squares for the bottom surface. The solid points are for the solvent defined surface and the open points are for the head group defined surface. The lines are least square fits to each data set. The average slope of the lines is 8. $N_{lip} = 3024$ and $N_s = 50000$.

such a simple model. It is expected that the model will evolve. The simplicity of the model is partly driven by the need to efficiently integrate to the long time scales necessary for problems like membrane fusion. Another goal which is intrinsic to minimal models is to develop a model with only the essential characteristics of lipid molecules for the problems of interest. One of the main results and advantages of minimal model simulations is the determination of the essential physical features to be included in the model. By starting with a very simple model and adding (and subtracting) new features one at a time, one can determine the necessary features or, in other words, the effect of individual features. Here, the minimal lipid models contains two main features: the force-field and the molecular geometry. The force-field consists of just the hydrophobic and hydrophilic interactions which are essential for self-assembly. The double tail geometry helps stabilize the lamellar phase. We show in this paper that from this minimal model, a whole set of results follows. The liquid and gel states of lipid bilayers are present. The area per lipid dependence as a function of temperature is reasonable. The shape fluctuations and the bending moduli correspond to experimental ranges and variation with lipid tail length and temperature.

There is a variety of lipid types in cell membranes. Modeling this variety will require additions and/or variations in the lipid model presented here. Clearly, different models of the head group will be needed to model the different lipid types. As one example, we have modeled the double bonds in lipid tails by imposing a kink angle. The effect of the kink is to reduce ordering within the bilayer which results in an increased area per lipid. Thus, the kink has successfully modeled the main effects of a single double bond in the tail.

In the vein of minimal models, we can compare the simulation results with experiment. Several features confirm that the basic model has a good foundation. The area per lipid data has the correct temperature dependence when compared to experimental data. The T dependence of A is decreasing and linear in the liquid phase. A discontinuity occurs at the liquid to gel transition, which is consistent with the second order nature of the phase transition; however, for simulations to confirm a second order phase transition require system size studies beyond what is considered here. Finally, the melting temperature decreases with N_T . For fully hydrated phosphatidylcholine lipids, A as a function of T and tail length has been calculated from NMR data.[40] There is one difference between this experimental data and the simulation data. The lines for different tail lengths (Fig. 2.3) intersect in the simulation data, but are parallel in experimental data. The calculations of A from the experimental data involve various assumptions. In fact, there are two sets of distinct values of A for two different models used in the calculations. Both yield parallel curves for the different tail lengths. At this stage it is uncertain whether the intersection is something in the model that needs improvement, or whether the experimental data needs improvement.

The basic features of the gel phase are consistent with experimental measurements. The lipid molecules tilt relative to the bilayer normal. The tails are well ordered equivalent to an all trans state. The bilayer thickness is larger for the gel than the liquid state. Locally in the bilayer, the lipids have a triangular lattice ordering. The diffusion in the plane is significantly reduced relative to the liquid state.

Since a key input to the theoretical models of biomembranes is the bending modulus

κ , we calculated κ for several different systems. In the liquid phase as the temperature decreases toward the melting temperature, we find that κ increases. One expects the membrane to be stiffer at lower temperatures, and this is observed in experiments.[37, 38] The experiments also observe a decrease in κ in a narrow range of temperatures at the liquid to gel transition. Presently, it is difficult to calculate κ accurately. Near a phase transition the situation is even more difficult due to the strong system size effects. We thus have not made any attempt to calculate κ near T_m . In addition, our system sizes show small fluctuations in the gel phase suggesting that experimental observations involve a large scale dynamics (of domains, possibly?) not present in our systems. Finally, we note that as T increases so does A ; therefore, we also find for constant N_T that κ decreases with increasing A which is consistent with theoretical predictions.[41].

Recently, there have been new simulations of the dependence of κ upon chain length for surfactants.[42] They find that κ increases with N_T as we do. They give an extensive examination of the functional dependence which is not possible for our data, although our data does appear quite linear, consistent with surfactant simulations. Overall, we find the mechanical behavior of the coarse-grained lipid bilayers has correct dependences. These lipid models can now be used to study more complex phenomena and the prediction of membrane theories (e.g. membrane inclusions). [43]

With this work, the foundation has been laid for a set of coarse-grained lipid models. The appropriate lipid per area can now be chosen based on the data provided for simulations of more complex phenomena such as membrane fusion. The development of minimal models for different lipid types now has a stronger basis for comparison.

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Chapter 3

Insights into the molecular mechanism of membrane fusion from simulation: evidence for the association of splayed tails

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3.1 Introduction

The molecular details of the fusion between two lipid bilayers is one of the most vexing problems in membrane biophysics today [1, 2]. Fusion models based on continuum mechanics predict initial fusion of the outer leaflets to form a stalk-like structure, and the subsequent merger of the two sides [2, 3, 4]. Here we present coarse-grained simulations of fusion between two liposomes pushed into contact, from which a detailed picture of lipid movements emerges. The results have interesting implications for membrane fusion, and suggest new possibilities for designing molecules that control fusion.

The process of membrane fusion is central to biology, and plays a role in events such as vesicular trafficking, fertilization, viral entry and mitosis [5, 6]. While fusion between two biological membranes is normally a process mediated by proteins, many pure lipid bilayer systems will spontaneously fuse implying important similarities in the underlying physical chemistry [1, 2, 7]. In addition, mechanisms of fusion between pure lipid systems have a number of technological applications such as liposome based drug delivery [8, 9], and the formation of supported lipid bilayer biosensors and other devices [10]. As a result, fusion between pure lipid membranes has been the subject of a wide range of experimental, computational and theoretical studies. These efforts have produced a number of models,

typically involving the local deformation of a membrane, the formation of a hemifused inverted hexagonal phase like structure as an intermediate, and the subsequent complete merger of the two sides [2, 3, 4, 11]. In the hemifused intermediate the outer leaflets are fused while the inner leaflets remain separate, to form a connection between membranes referred to as a stalk. However, the molecular aspects of membrane fusion are not treated by the continuum models and have yet to be determined experimentally. Here we have performed molecular dynamics (MD) simulations that suggest new processes in membrane fusion and reveal novel molecular details of lipid movement.

Experimental measurements of fusion suggest a process that occurs beyond the time-scale that is available to traditional molecular dynamics methods [1, 12]. Two aspects of our simulations of liposome fusion are critical for overcoming this time scale difficulty. First, we coarse grain the system using bead-spring models for the lipid molecule. This approach has been extensively used in polymer simulations, where these models have been shown to capture central physical and chemical features [13]. More recently the approach has been applied to lipids, where it has been shown to reproduce the spontaneous process of lipid self-assembly into a bilayer [14]. Simulations of trimer amphiphiles without solvent have produced vesicle fusion [15]. However, even when using coarse grained lipid models, the time for two liposomes in near contact to spontaneously fuse is prohibitive and to overcome that barrier we push the liposomes together. This will bias the simulations with respect to spontaneous fusion, but methods exist to compensate for this biasing [16, 17]. Moreover, the function of some fusion proteins and other fusion molecules may be to act in lowering the fusion barrier in a similar manner. There are both qualitative and quantitative aspects to a full understanding of the process. Here we focus on qualitative mechanistic insights into the geometry of membranes and the conformational changes of the individual lipids during fusion, and leave a quantitative analysis for future publication.

3.2 Method

The lipid model used for the simulations is composed of 11 beads, four for each aliphatic tail (T beads) and three for the headgroup (H beads). The solvent (S beads) is represented by a single bead. Beads interact through the Lennard-Jones (LJ) potential

$$u_{\text{LJ}}(r) = 4\epsilon[(\sigma/r)^{12} - (\sigma/r)^6] \quad (3.1)$$

The size of a single bead is given by the LJ diameter σ which is approximately 0.5 nm [18]. The TT, HH, HS and SS interactions have the LJ potential with a cutoff $r_c = 2.5\sigma$. The HT and TS interactions are purely repulsive with cutoff $r_c = 2^{1/6}\sigma$. The bond potential [19] is the sum of the attractive finitely extensible, nonlinear elastic (FENE) potential and the purely repulsive LJ potential u_{LJ} with cutoff equal to $2^{1/6}\sigma$,

$$U_{\text{bond}}(r) = -1/2k_0R_0^2\ln(1 - r^2/R_0^2) + u_{\text{LJ}}(r), \quad (3.2)$$

where $k_0 = 30\epsilon/\sigma^2$ and $R_0 = 1.5\sigma$. Angle terms are included via the harmonic potential

$$U_{\text{bend}}(\theta) = k_\theta(\theta - \theta_0)^2, \quad (3.3)$$

where θ is the angle between neighboring bonds and $k_\theta = 2\epsilon/\text{rad}^2$ and $\theta_0 = 180^\circ$ to give a persistence length of about 2σ for the tails. Other angle terms are included for the head group geometry. This model system spontaneously self-assembles into a lamellar bilayer when started from a random configuration of lipids and solvent at appropriate lipid concentrations (see also ref. [14]). Massively parallel MD simulations using a Verlet integrator with a time step of 0.005τ were performed on a CPlant cluster [20]. Simulations were performed at constant temperature $T = 1\epsilon$ using the Langevin thermostat [21].

To form the starting state, we create a spherical liposome by placing lipids on a sphere with their tails in contact. For our calculations, we created liposomes with outer diameters D of 30σ and 50σ . The number of lipids is determined by the area per lipid A which is chosen to be $4.0 \sigma^2$, slightly larger than the values obtained in simulations of lamellar bilayers. Solvent particles are placed randomly in the unoccupied volumes inside and outside the liposome. The effective osmotic pressure is controlled through our choice of the amount of solvent placed in these two regions. A nonzero osmotic pressure was found to speed up the fusion process. However, zero osmotic pressure simulations yielded the same qualitative dynamics. For a fusion simulation, this single liposome is first equilibrated for about $300 \mu s$ to ensure stability. The liposome is copied and two equilibrated liposomes are placed with their outer diameters about 10σ apart along the axis of approach (x -axis). For liposomes with $D = 30\sigma$, the total system has $N = 330,000$ beads. Two liposomes are brought together by applying a small biasing force of $0.0025 - 0.001\epsilon/\sigma$ in the x -direction to each bead in the lipid molecules. Once a few lipids have been exchanged between the two liposomes, the force is released and the process allowed to proceed. Once contact occurs the work by the biasing forces goes into deforming the liposome and promoting fusion as well as translation. The total work by the biasing force from contact to initial fusion is upper bound of the fusion barrier. For the smallest biasing force the total work is $41 kT$ which is consistent with calculations [22].

In a typical fusion simulation, the process begins after the liposomes contact and a flattened contact forms between the liposomes (Fig. 3.1A). This causes membrane bending at the contact edge bringing two strained points on the membranes into close proximity. Fusion between the outer membrane leaflets initiates at the edge of the contact surface (Fig. 3.1B). There are several factors that promote fusion at the contact edge, including strain that is relieved by fusing with the neighboring liposome and an increase in area per lipid giving more mobility to molecules in this region. Recent calculations show that edges in the internal leaflets of membrane greatly reduce the free energy cost of the stable intermediate [23]. Similar calculations should show that the cost of forming the external edge is within reason. While this finding of edge originated fusion is intuitive, it offers a contrasting view to that described in existing models. For example, Kuzmin and coworkers have suggested that formation of a nipple shaped protrusion in opposing bilayers, serves as a point of initiation for fusion, and leads to the formation of a stable intermediate [22]. The

Figure 3.1. Cross sections (10σ thick) of fusing liposomes showing sequence of events to complete fusion. (A) flat interface at $t = 55\mu s$ (B) initial stalk at $t = 94\mu s$ (C) growth of stalk to other side with solvent cavity at $t = 140\mu s$ (D) dissolution of one connector and solvent cavity $t = 149\mu s$ (E) intermediate fusion state at $t = 204\mu s$ (F) complete fusion at $t = 231\mu s$ (18). Coloring scheme: right (left) liposome has H blue (cyan) and T red (yellow). Solvent is not displayed. The system is under osmotic pressure with internal solvent density 20% larger than external density.

Figure 3.2. Lipid conformations and associations in the early stages of fusion. System and coloring scheme is same as in Fig. 1. The left side is at $t = 58\mu s$ and right side at $t = 66\mu s$. A) and C) show splaying of lipids that bridges the two outer leaflets. There is a tendency for the aliphatic tails of the splayed lipids to oppose each other. B) and D) show association of splayed lipids and the beginning of a hydrophobic core that spans the space between the two liposomes. The line in D) is a guide to the eye to indicate the ordering of the upper bilayer to become part of the stalk. For evolution beyond $t = 66\mu s$ the external force was turned off.

present work suggests a second although not mutually exclusive mechanism in which first a membrane-membrane contact forms (here through an initially applied force). This causes membrane bending at the contact edge bringing two strained points on the membranes into close proximity where fusion initiates. Hence the point of fusion can be significantly distal to the initial point of closest approach.

The simulations also provide direct insight into the dynamics of individual lipids during the early stages of fusion. To begin with there is a tilting of the lipids at the presumptive point of fusion, similar to that proposed for the modified stalk model [4, 24] which appears to be facilitated by the local increase in the area per lipid. The first exchange between the membranes occurs when an aliphatic tail rotates out of the parent membrane and inserts into the opposing membrane, resulting in a tilted lipid with the aliphatic tails in a splayed (trans) conformation that is shared between the two fusing bilayers (Fig. 3.2). This immediately suggests a mechanism for accommodating curvature strain on a molecular level. The possibility of splayed lipid conformations has been considered in some models for membrane fusion [25, 26] however the picture that emerges here is distinctly different. In our simulations the splayed lipids tend to orient such that their aliphatic tails contact each other, thus creating the beginnings of a new hydrophobic core. As additional (tilted) lipids associate with the splayed bridging lipids, the aliphatic tails of these molecules extend into a more cis-like conformation, establishing a hydrophobic core and eventually forming a

Figure 3.3. Cross sectional images of fusion in plane of fusion (yz): A) Initial stalk at time $t = 69 \mu s$, growing stalk at B) $86 \mu s$, C) $108 \mu s$, and D) almost complete fusion of outer leaflets at $t = 140 \mu s$. Only lipids in outer leaflets are shown and slice is about 10σ thick.

classical stalk.

A somewhat surprising finding is that following the initial formation of the stalk, this structure expands highly asymmetrically along the strained membrane at the contact edge (Fig. 3.3). In current fusion models the stalk forms in a central location and growth occurs radially outward from the center with cylindrical symmetry. A consequence of fusion along the contact edge is the formation of a partially confined solvent cavity between the two liposomes (see Fig. 3.1C). Some of the confined solvent joins the external solvent, and some empties into the left liposome as the inner bilayer dissolves and an extended hemifused structure forms (Fig. 3.1D). We also note that in the simulation of the larger system, multiple, independent stalks initially formed as one might expect for a system large enough to allow well separated initiation sites. The dynamics becomes more complex in this case as stalks merge and dissolve. We present here the simpler, single stalk case whose dynamics forms the basis of the more complex possibilities contained in larger systems.

3.3 Conclusion

While many of the general features of our simulations agree well with models based on a continuum mechanics framework, the present work offers significant insights into the molecular details of the early stages of fusion. In particular the simulations suggest a

model for specific molecular rearrangements that result in the formation of a stalk (Fig. 3.4). This model builds on the notion of tilted lipids in a strained pre-stalk structure [4, 24], which here facilitates the splaying of lipids to connect the two fusing membranes. A critical event is then the association of the aliphatic tails of splayed lipids, which nucleates a new hydrophobic core that spans the gap between the original membranes. This core grows as the chains extend and resolves completely the fusion between the outer leaflets. In addition, the importance of mechanisms that accommodate changes in membrane curvature is highlighted. Continuum models of the stalk do not provide a clear molecular view of how the stresses are resolved during the fusion process [23].

A number of interesting considerations arise from these results, including new ways in which proteins might mediate the fusion process. For example, proteins might act directly to facilitate splaying of lipid tails or stabilize bridging lipid molecules. Proteins might also act more indirectly as adhesion molecules that promote the formation of a flat contact that produces a strained contact edge. In such a picture, proteins would serve a relatively passive function by keeping liposomes apposed, while fusion occurs away from the actual protein mediated contact. The results also suggest that conformational properties of lipids that have not previously been closely examined play an important role in fusion. In that context, it is interesting to consider the possibility of rationally designing lipids with an improved ability to adopt splayed conformations, or lipids that can controllably be switched between a cis and trans conformation of the tails, or other novel molecules that might be used to facilitate or control membrane fusion.

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Figure 3.4. Schematic of the early stages of fusion and association of splayed lipids suggested by the simulations. A) Two liposomes are brought together and B) a flat contact forms where, at the edge, the area per lipid in the outer leaflet increases as the membrane is strained. C) Lipids tilt at strained contact and D) subsequently the aliphatic tails of some molecules begin to splay. Although not shown here, lipid exchange occurs early in the process. E) Splayed lipids then associate by their tails to form a new hydrophobic core, F) which expands as the tails extend to form a classical stalk-like structure.

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- [17] In full generality, various force biasing methods can be used to calculate the free energy changes along a reaction coordinate. Here we employ a computational biasing of our reduced complexity system that brings the liposomes in contact and then remove the biasing force. While there is no calculation of the free energy throughout the transition, there is a significant improvement of sampling for the transition event.
- [18] The conversion of the LJ units for the coarse-grained model to metric values is not unique as the real structure and dynamics will map in more than one way to the coarse-grained model. For hydrocarbon tails, one map involves equating the persistence length of the flexible coarse-grained model to that of a true lipid. For polyethylene this yields $\sigma = 0.5$ nm. The energy scale is set by the temperature T and the relation $\epsilon = kT$. By equating the simulation value of the diffusion constant for a lipid molecule in a lamellar bilayer to a measured value of 10^{-8} cm²/s, the LJ time unit τ is mapped to 11 ns. We use this value of τ in the rest of the discussion.
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Chapter 4

Complementary matching in domain formation within lipid bilayers

The importance of lipid bilayers in biological processes is being more and more recognized as fundamental to biological function.[1] Structures within the lipid bilayers have been observed for a while now and have been shown to influence the function of membrane proteins, for example.[2, 3, 4] The most simple systems that possess domains within bilayers are binary lipid systems in which one lipid type has a bulk liquid phase and the other lipid type has a bulk gel phase at the given temperature.[5, 6, 7] liquid and gel domains can occur in such binary systems. The molecular scale structure of these coexisting systems is not fully understood. Recently, transbilayer complementarity has been demonstrated by nearest-neighbor recognition (NNR) experiments.[8] This method uses thiolate-disulfide interchange reactions to effectively take snapshots of the nearest neighbor organization in membranes.[9] For a binary lipid system with the two lipid types differing only in the tail length, the NNR method demonstrated a complementary organization across the bilayer.⁸ Where a long lipid is in one monolayer, a short lipid exists underneath in the bottom monolayer (Figure 1A).

In this paper, the dynamics and structure of such mixed bilayers is studied by molecular dynamics simulations that use a coarse grained model of the lipid molecules.[10, 11] The lipid is treated as a bead-spring molecule. The model is based on coarse-grained polymer models and related to minimal models used to treat protein folding.[12, 13] The coarse-graining enables the treatment of long time dynamics such as that in membrane fusion.[14] The present model works best for neutral lipids such as saturated phosphatidylcholine lipids. Each tail is composed of N_T hydrophobic beads of type T and the head is composed of three hydrophilic beads of type H. The solvent is represented by a single bead, which is equivalent to the head type. The details of the model have been described elsewhere, including the calculation of the liquid-gel phase transition for lipid with different tail lengths.[15] A single tail bead corresponds to about 3 C atoms in a lipid tail.

Simulations of two binary lipid mixtures in a single bilayer have been performed. The

Figure 4.1. Schematics of possible gel domain structures. (A) shows the complementary structure of a domain in which a long tail lipid in the top monolayer has a short tail lipid beneath it and vice versa. (B) shows the fully phase separated structure with only long tail lipids in the gel domain with tilt. Both cases are surrounded by the small lipid liquid phase.

two types differ only in their tail length and will be referred to as the short (S) and long (L) lipid types. Each system is composed of 12096 total lipids and about 300000 solvent particles. System I has $N_T = 4$ and 8 beads per tail in a 2:1 mixture. System II has $N_T = 4$ and 6 in a 1:1 mixture. Since each tail bead corresponds to about 3 C atoms, the $N_T = 8$ is long with respect to typical bilayers, but the large length difference makes the effects more pronounced. The second system is one of the experimentally studied systems (DLPC:DSPC),[5] and demonstrates that complementarity occurs in these simulated systems as well. Each system is initially constructed by random placement of the two lipid types in a bilayer and equilibration at a temperature above the melting temperatures of both lipid types. This equilibration removes any memory of the initial state. To study the domain formation dynamics, the system was cooled to a temperature between the melting temperatures of the two lipid types. Specifically, we simulated at the temperature $T = 1.05T_m$, where T_m is the melting temperature for the $N_T = 4$ lipids.[15] The simulations were run in the constant pressure and temperature ensemble for about 30 million time steps corresponding to 0.7ms. The lipid dynamics has been visualized in movies, which are available in the supplemental information. The movie shows multiple, distinct gel domains form and grow as the simulation progresses. The lipids within a domain move as a collective body performing Brownian dynamics in the background fluid composed primarily of the short lipid. Figure 2A shows the state of the final configuration for system I. Only the tails of the lipids in the top monolayer are shown for visual clarity. The gel domains are visible in Figure 2A by the clustering of the green tails of the L lipids. Also, Figure 2A shows there are S lipids within the cluster of L lipids. The movie shows that the S lipids are dynamically part of the gel domain, i.e. S lipids move as a unit with the domain. In between these domains are regions primarily composed of S type lipids in a liquid phase. For these S lipids in the liquid phase the diffusion is always faster than the L lipids. As the domains form and grow, the diffusion rate for both types decreases.[7] Since the diffusion rate is a sum of lipids in the liquid and gel phases, the rate decreases as the gel domains grow increasing the smaller contribution of the gel phase. In addition, the path of the S lipids in the liquid phase is constrained by the gel domains reducing their diffusion rate.

Figure 4.2. Images of domain formation for system I. (A) shows only the tail beads of the top monolayer. (B) shows a transmembrane slice marked by the two black lines in (A). The subsequent images show magnified views of this slice. (C) is part of a domain and exhibits the complementary matching of the lipid types between monolayers. (D) shows the liquid region is primarily composed of the short lipid. Colors: for $N_T = 8$ lipids, head is cyan and tail is green; for $N_T = 4$ lipids, head is blue and tail is red. The image uses smaller spheres (0.7 diameter) than actual size for clarity.

The domains are composed of both lipid types in a complementary match between the two monolayers.[8] Figure 2B shows a slice through the system, which is marked by the two black lines in Figure 2A. The slice shows the variation in the bilayer thickness as a function of position. Where the long lipids have clustered to form the gel domains, the bilayer is thick. The remaining region primarily composed of the short lipids in the liquid state is thin. Figure 2C shows a magnification of a gel domain. This thin slice shows that the lipids in the top and bottom monolayers form a complementary match of the lipid types: where a L lipid is on the top monolayer, an S lipid occurs on the bottom monolayer and vice versa as seen in the recent NNR experiments.[8] Figure 3 shows quantitatively the 2D radial distribution functions for a chain of type α in one layer and a type β in the other layer. In both systems, there is a peak at $r = 0$ in the S:L distribution demonstrating that it is more likely that the opposite chain type is below the given chain. The oscillations for system I in the S:L curve imply that in a layer there is a switching between S and L types which confirms the picture given in Fig. 2A. System II, with the shorter L lipid and thus weaker tail-tail interactions, does not exhibit such longer ranged correlations. In single lipid simulations at the temperature T , the $N_T=4$ lipid bilayer has a thickness $t = 8.0\sigma$, where σ is the Lennard-Jones particle diameter.[13] For the $N_T=8$ lipid bilayer $t = 14.4\sigma$. Thus, a mixed L-S bilayer (system I) should have $t = 11.2\sigma$. However, the thickness of the gel domains in the binary system is larger, about 13σ , which is between the values given above. The reason that $t > 11.2\sigma$ is that the lipids do not tilt in the gel domains of system I. The combined lengths of the long and short lipids in the gel domain without tilt yield a thickness closer to that of a gel domain containing only long lipids that tilt. This example provides a caution that one should take in interpreting experimental thickness data (esp. AFM) without sufficient other geometrical detail of the molecular orientation.

One of the issues in a system with two coexisting phases is how the boundary structure ameliorates the cost of the boundary mismatch (cf. Figure 1). With the gel and liquid phases having different thicknesses, there would be a hydrophobic mismatch at the boundary, unless the boundary structure adapts. Figure 2B shows that in the present case the boundary structure adapts to yield a smooth head group position that limits the water contact with lipid tails. At the boundaries, the surface of the bilayer is typically more curved allowing the transition in bilayer thickness to occur smoothly. On the molecular scale, examination of individual lipid conformations shows that the L lipids near the boundary adopt the structure of the liquid phase thereby reducing the bilayer thickness.

Figure 4.3. Radial distribution functions between chain types in different leaflets of the bilayer. Top figure is for $N_T = 4:8$ mixture (System I) and bottom is for $N_T = 4:6$ mixture (System II). Both cases have at $r = 0$ a peak in the S:L data showing that it is more likely to occur.

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Chapter 5

A density functional theory approach for coarse-grained lipid bilayers

5.1 Introduction

The lipids making up cell membranes play an active role in a variety of biological processes. Many of these processes, such as the formation of lipid domains or rafts, the interactions of transmembrane proteins, and membrane fusion, occur on length and time scales inaccessible to atomistic simulations. As a result, there has been increasing interest in developing more coarse-grained models for lipid membranes. Many coarse-grained models have been developed for either molecular dynamics (MD) or Monte Carlo simulations, ranging from united-atom type models [1, 2, 3], to bead-spring [4, 5] or liquid-crystalline rigid rod models [6].

Theoretical approaches that can directly calculate the equilibrium structure of model membranes can be attractive for their low computational cost and clear parsing of the critical physics. A historically important approach treats membranes as a continuum elastic sheets [7]. More recently molecular level theories have been proposed. May and Ben-Shaul [8, 9] studied lipid-protein interactions with a theory that described the lipids with a phenomenological free energy that included head-group repulsions, a water/membrane interfacial energy, and a contribution from the configurational entropy of the tails.

Several groups have used self-consistent field (SCF) theory to study lipid phase behavior and membranes. SCF theory is a mean-field theory that in bilayer applications typically includes van der Waals-type interactions between various species, and the configurational entropy of the lipid tails. Most often lipids are modeled as infinitely thin “threads” and the fluid is assumed to be incompressible. Leermakers and coworkers [10, 11, 12] have developed a lattice SCF theory for lipids that includes chain stiffness and anisotropic interactions between tail segments. They are able to predict a fluid to gel phase transition for their bilayers. Similar lattice SCF theories are used by other groups [13, 14, 15]. Schick

and coworkers have studied lipid phase diagrams using off-lattice SCF theories [16, 17].

Another class of theories is based on liquid state theories. In one extreme, Somoza and coworkers [18] neglected internal degrees of freedom of the lipids, and developed a density functional theory (DFT) for anisotropic, rigid amphiphilic molecules. They were able to calculate the structure of bilayers and micelles [18]. Lagüe and coworkers [19, 20, 21] developed an integral equation theory based on the laterally-averaged response of the lipid hydrocarbon tails (obtained from atomistic MD simulations). They used the theory to calculate the potential of mean force between transmembrane proteins, modeled as rigid cylinders. However, the theory neglects the lipid headgroups, the entropy of the tails, and any fluctuations normal to the bilayer.

In this paper we consider the molecular density functional theory (DFT) of Chandler, McCoy, and Singer (CMS) [22, 23, 24], as extended to polymeric molecules by McCoy, Curro and coworkers [25, 26, 27]. We use the implementation first suggested by Donley et al. and detailed further by Frischknecht et al. [28, 29, 30]. The lipids are modeled as coarse-grained spherical interaction sites. Like SCF theory, the DFT is a mean-field theory and includes the energetics of hydrophobic/hydrophilic interactions and the entropy of the lipid tails. Furthermore both SCF and our DFT approach treat the tail configurations as random walks. However, the DFT approach includes compressibility and packing effects via explicit liquid state structure as determined from an integral equation theory of polymeric molecules (PRISM theory). Thus the DFT includes two length scales in predicting bilayer structure, the length scale of the lipid chains and the length scale of the beads on the chain.

We describe our model system in Sec. 5.2 and computational methods in Sec. 6.2. We present our results for bilayer structure and phase behavior in Sec. 5.4. In this paper we restrict our results to morphologies that vary in only one dimension, since we are primarily interested in lipid bilayers. Our implementation of the theory can however treat systems in two or three dimensions; an example is given in Sec. 5.4.5 where we show some preliminary results for the effect of an inclusion in the bilayer. Details of the calculations can be found in the appendices. Finally, comparison of the DFT results found here with molecular dynamics simulations can be found in the following paper [31].

5.2 The model system

Our approach is to coarse-grain the lipid molecules while retaining both the flexible nature of the tails as well as the different size and energetic characteristics of hydrocarbon tails and polar head groups. The lipid molecules consist of freely-jointed, tangent spherical sites or “beads”. Typical lipids found in biological systems have tails composed of 14-20 carbon atoms. There are a wide variety of head groups possible both in nature and in engineered lipid systems. In this work we focus on a specific coarse-grained lipid model in which the tails are 8 beads long (roughly two CH_2 groups per bead), and the head groups are lumped into two larger beads with energetic properties different from the tail beads. This 8-2-8

model is then a linear copolymer chain, as shown in Fig. 5.1. The different sites in the lipid interact with standard Lennard-Jones (LJ) potentials,

$$u_{\alpha\beta}(r) = u_{\alpha\beta}^{LJ}(r) - u_{\alpha\beta}^{LJ}(r_c) \quad (5.1)$$

$$u_{\alpha\beta}^{LJ}(r) = \frac{4\varepsilon_{\alpha\beta}}{kT} \left[\left(\frac{\sigma_{\alpha\beta}}{r} \right)^{12} - \left(\frac{\sigma_{\alpha\beta}}{r} \right)^6 \right], \quad (5.2)$$

where r_c is the cutoff distance where the potential goes to zero, k is Boltzmann's constant and T is the temperature.

We are interested in bilayer-forming lipids and thus chose the ratio of head to tail bead diameters to be $\sigma_h/\sigma_t = 1.44$, giving an overall head group volume fraction of 0.27. On the basis of simple packing arguments and previous theoretical work on similar lipid models [17], we expect this ratio to result in lamellar/bilayer-forming lipids. In addition to the lipid molecules, the model has a single site solvent with diameter $\sigma_s = \sigma_t \equiv \sigma = 1$. We set the cross-terms in the bead diameters from the usual Berthelot scaling rules, so that $\sigma_{\alpha\beta} = 0.5(\sigma_\alpha + \sigma_\beta)$. The self-assembly of the lipids into bilayers is driven by the various interactions in the system. We have chosen the tail-solvent and tail-head interactions to be purely repulsive with $r_c = 2^{1/6}\sigma_{ts}$ and $r_c = 2^{1/6}\sigma_{th}$ respectively. Solvent-solvent, solvent-head, head-head, and tail-tail interactions are all uniformly attractive with a cutoff of $r_c = 3.5\sigma$. Finally, we set all of $\varepsilon_{\alpha\beta} \equiv \varepsilon = 1$. This combination of parameters allows for a self-assembling bilayer to form. We note that one could choose model parameters in a more physical way to represent specific lipids, as has been done by others [11]. Here we take a general approach, inspired by previous coarse-grained MD simulations [4, 5] and SCF theories [16, 17], and explore the predictions of the DFT for this initial simple model. We will report all lengths in units of σ and energies in units of ε/kT .

5.3 Methods

The details of the CMS-DFT applied here to coarse-grained lipid models, along with the numerical methods used to solve the theory, have been enumerated elsewhere [29, 32]. Briefly, the basic quantity in the theory is the inhomogeneous site density profile $\rho(\mathbf{r}) = \sum_\alpha \rho_\alpha(\mathbf{r})$, where $\rho_\alpha(\mathbf{r})$ is the density of site type α at $\mathbf{r}$. The basic idea in CMS-DFT is to replace the inhomogeneous, interacting system of interest with a reference system of ideal, noninteracting chains and solvent, in a medium induced potential $U_\alpha(\mathbf{r})$ which captures the effects of the site interactions. The theory is formulated in the grand canonical ensemble (GCE), with the grand potential free energy Ω of the inhomogeneous system of interest measured relative to the free energy Ω_b of the bulk, homogeneous lipid-solvent mixture which serves as the reservoir for the inhomogeneous system. Then the grand potential free energy difference $\Delta\Omega = \Omega - \Omega_b$ is given by a Taylor series expansion about the noninteracting reference system, truncated at second order. A functional minimization of $\Delta\Omega$ leads

to the DFT equations to be solved. For completeness, we review this system of equations in Appendix A. Here we give details of the calculations presented in this paper.

5.3.1 Density Functional Calculations

A state point in the GCE is specified by μ_s , μ_L , V , and T where μ_s is the solvent chemical potential, μ_L is the lipid chemical potential, V is the system volume, and T is the temperature. The μ variables are defined indirectly in the CMS-DFT by specifying the site number densities of solvent and lipid in a mixed bulk reservoir fluid. We report here the total site number density in the reservoir ρ_b , and the number fraction of solvent, x_s , in that reservoir.

There is no way in this grand canonical ensemble to guess *a priori* how the state variables $\{T, \rho_b, x_s\}$ should be chosen in order to obtain a bilayer structure, or furthermore to guarantee the constraint of zero surface tension that is expected for biologically relevant structures. For this work, we chose to keep the value of ρ_b fixed at $\rho_b \sigma^3 = 0.59$. We restricted our solutions to have planar symmetry so that the densities were nonuniform only perpendicular to the bilayer, and analytical integration of the two dimensions parallel to the bilayer was possible. While a homogeneous solution of mixed lipids and solvent at constant bulk density is always a stationary state of this DFT approach, it is clearly not the solution of interest. In order to obtain bilayer-like solutions, suitable initial guesses were required. Initial guesses consisting of step function density profiles were used (with some trial and error) to generate a nonuniform bilayer-like solution. Arc-length continuation algorithms were used to step from one converged solution to another (see Appendix B for more details) [33]. We performed exhaustive arc-length continuation calculations as a function of T and x_s to find the possible thermodynamic states of the system. Finally, bilayer states of interest (e.g. zero tension bilayers) were identified.

Most of the calculations presented here had a total computational domain size of 40σ with reflecting boundary conditions on either side of the domain. Reflecting boundaries assume $\rho_{B+i} = \rho_{B-i}$ where B is the node at the boundary, i is an integer, and ρ is any variable in the calculation. For single bilayer solutions, we often locate the center of the bilayer at one of the reflecting boundaries. This ensures symmetric bilayer solutions, and removes any numerical instabilities due to drift of bilayer-solvent interfaces in the computational domain. Note that in this case the reflecting boundaries result in a periodic stack of bilayers with neighbors separated by 80σ . This separation is always large enough to ensure that neighboring bilayers are independent.

5.3.2 Properties of bilayers

Lipid bilayers are inhomogeneous fluids with soft boundaries. An extensive literature exists that describes the physics of fluids at hard boundaries [34]. Much of the analysis from those physical systems can be transferred directly to bilayers with the caveat that the Gibbs

dividing surface may be trickier to define. In general, the properties of interfacial fluids are defined by the excess surface free energy (see Eq. (5.15) for the particulars of our theory). The excess surface free energy Ω^{ex} is the free energy difference between the bilayer-solvent system of interest and a pure solvent. The pure solvent density needed to calculate Ω^{ex} is not known *a priori*, but is observed as the solvent density far from the bilayer in any inhomogeneous solution. This excess free energy is also precisely the surface tension, γ ,

$$\gamma = \Omega^{ex} = \frac{(\Delta\Omega[\rho(\mathbf{r})] - \Delta\Omega^s)}{A}, \quad (5.3)$$

where $\Delta\Omega^s$ is the difference in free energy between the pure solvent and the bulk homogeneous reservoir, and A is the total area.

Equation (5.3) is an energetic route to the surface tension. It is also possible to use a mechanical route to compute the surface tension [35],

$$\gamma = \int_{-\infty}^{\infty} dx s(x) = \int_{-\infty}^{\infty} dx [P_N(x) - P_T(x)], \quad (5.4)$$

where the stress profile across the bilayer is $s(x) = P_N(x) - P_T(x)$, x is the direction normal to the bilayer, $P_N(x)$ is the normal component of the pressure tensor (a constant for all x as dictated by mechanical equilibrium), and $P_T(x)$ is the tangential component of the pressure tensor. Thermodynamically, the grand free energy density $\Omega(x)/V$ can be identified with the lateral pressure $P_T(x)$ [36, 37], and thus a stress profile is rather straightforward to compute with DFT (see Appendix A for a precise derivation).

The basic structural quantities that define a given bilayer are the bilayer thickness t and the density of lipids in the bilayers, usually reported as the area per lipid A_L . Reported thicknesses are given as the distance between head group density peaks, while A_L is calculated from

$$A_L = (\Gamma_L/A)^{-1} \equiv \left(N_{\alpha}^{-1} \int \rho_{\alpha}(x) dx \right)^{-1} \quad (5.5)$$

where $\rho_{\alpha}(x)$ is the density profile of any single site type α on the lipid and Γ_L is the total number of lipid molecules in the system.

5.4 Results

While the coarse-grained model presented in section II seems quite simple, the complexity of the solution space is significant. Appendix B details this complexity, along with computational strategies for locating solutions of interest. We begin this section with a description of the most biologically relevant bilayer structures that we find in the model. We then go on to explore the phase diagram of our model in more detail.

5.4.1 Bilayer structure

In biological membranes, the surface tension is expected to be zero. The $\gamma = 0$ bilayer for our model at the state point $kT/\epsilon = 1.3$ and $\rho_b\sigma^3 = 0.59$ is shown in Fig. 5.2A. The density profile across the bilayer has tail beads in the inner part of the bilayer, head groups clustered at the bilayer-solvent interface, and solvent excluded from the bilayer. The density profiles do not exhibit significant packing structure indicating that the bilayer is in a fluid state. This zero tension bilayer is not the only solution of the DFT found for this model at this state point. Rather, a whole series of solutions are obtained with different integral numbers of bilayers in the computational domain (see Appendix B). Eventually the space is completely filled with a lamellar state as is shown in Fig. 5.2B.

We can compare the structure of our model bilayers to experiment by a rough mapping of the coarse-grained model. The ratio of head group volume to tail volume in our lipid is similar to that in dipalmitoylphosphatidylcholine (DPPC); if we assume that the volume of one of our tail beads is equivalent to two CH_2 groups in DPPC, we can identify $\sigma = 4.75 \text{ \AA}$. Here we have used a volume of $28.1 \text{ \AA}^3$ for the CH_2 groups in DPPC [38]. We can then compare the thicknesses of our bilayers, measured as the distance between the head group density peaks, and the areas per lipid with values in the literature. For fully hydrated DPPC at 50°C , the head-to-head distance $t = 38.3 \text{ \AA}$ and the area per lipid $A_L = 64 \text{ \AA}^2$ [39]. These conditions should apply to our fluid bilayers at zero tension. Using our mapping for σ , we find that the thickness for bilayers in this model varies from $38 \text{ \AA}$ to $35.2 \text{ \AA}$ and the area per lipid from $77.4 \text{ \AA}^2$ to $110.3 \text{ \AA}^2$ for zero tension bilayers where the temperature ranges from $kT/\epsilon = 0.92$ to 1.3 . The best fit to the DPPC data is thus at low temperatures in the model, close to the transition to ordered bilayers (see below). While arguments could be made for making adjustments to the model (adding stiffness or changing coarsening strategy), and while we have limited this analysis in phase space by constraining $\rho_b\sigma^3 = 0.59$, we nevertheless see that this model does predict bilayers with properties reasonably close to experimental lipid bilayers.

In addition to stacked bilayers, we find that the model exhibits a morphology where two bilayers are fused. Figure 5.3 shows these fused bilayers at the zero tension point for the assembly. In this case some of the tails leak out beyond the head groups, fusing the two bilayers together. Note that the chemical potential of the $\gamma = 0$ point for the fused assembly is different than the chemical potential for the $\gamma = 0$ point of the independent bilayers described above.

While zero tension bilayers are clearly important, a variety of experiments on bilayers or self-assembled monolayer analogs are performed at nonzero tension. The DFT approach can be used to study expected structural variations in the lipid bilayers away from the zero tension point. Figure 5.4 shows two extremes in lipid structure when the bilayer is under either tension (Fig. 5.4A) or compression (Fig. 5.4B). These two extremes in structure were found at turning points located using arc-length continuation algorithms (see Appendix B).

At low temperatures we obtain bilayers with strongly ordered tails from the DFT. As

with the fluid systems, the thickness of the bilayers varies with state point. In this case, we observe an increasing number of ordered peaks as the bilayers become wider. Figure 5.5 shows two examples of ordered profiles. While this order-disorder transition may be akin to the main gel transition observed in real lipid systems, we note that in contrast to experiments, the character of the transition observed here is continuous (discussed further below). Furthermore while experimental gel phases are characterized by nearly fully extended lipid chains, we never observed fully separated leaflets with 16 peaks in the DFT calculations.

The characteristics of our ordered states likely arise from the flexibility in the chains. Previous theoretical SCF descriptions of the gel phase required the inclusion of both some stiffness in the lipid tails and an anisotropic packing interaction [10, 40, 41]. Our DFT does include packing effects and since the theory is nonlocal (in contrast with SCF theories), one may expect that some anisotropy can arise in highly ordered profiles. However, the large entropy associated with flexible chains hinders the formation of gel phases; previous CMS-DFT studies of polyethylene required some chain stiffness in order to obtain a freezing transition [42]. Thus the ordered phases predicted by our theory are likely driven by packing effects.

5.4.2 Bilayer Hydration

As is clear from Fig. 5.2, these model systems can exhibit variable amounts of bilayer hydration. The structure of bilayers is generally studied experimentally by considering multilamellar assemblies, and using X-ray scattering to measure precise peak positions. Thus it is quite important to understand bilayer hydration, and any possible transitions from independent bilayers to interacting lamellar assemblies.

In order to investigate the nature of different hydration states, we considered a computational domain with periodic boundaries that contained only one bilayer in an infinite stack. The size of the computational domain was varied in order to determine whether there is a preferred hydration state with minimal free energy. A first order phase transition requires that there will be a free energy barrier between competing hydration states that have the same free energy at the same state point. Thus the repeat period of the stack, λ is the order parameter of interest.

Figure 5.6 shows Ω^{ex} as a function of λ for several $\{T, x_s, \rho_b \sigma^3 = 0.59\}$ state points where the lamellar morphology is at $\gamma = 0$. There are clearly two competing thermodynamic phases with an energy barrier in between them in all of the curves [43]. At large λ , Ω^{ex} becomes a constant indicating that the bilayer is fully hydrated and independent of neighboring bilayers. In an infinite system, the observed spacing between bilayers will depend only on the amount of solvent in the system. The local minima at smaller λ correspond to the lamellar morphologies. Figure 5.6 shows that lamellar morphologies are stable at high temperatures, and independent bilayers are stable at low temperatures. Given the free energy barrier between these two states, the point where the free energies are the same ($kT/\epsilon \approx 1.154$) locates a first order phase transition. This transition occurs at the $\gamma = 0$

point for both the lamellar and bilayer phases where a pure solvent is also stable. Thus this transition occurs at a triple point.

The distinct nature of these two phases is apparent when bilayer properties such as the area per lipid and bilayer thickness are plotted as a function of the order parameter λ . The properties of the lamellar phase are quite sensitive to the order parameter while the properties of independent bilayers are not. Generally, A_L increases and the bilayer thickness t decreases with decreasing λ in the lamellar phase. Finally, we note that on increasing λ the bilayer structure is fixed (with A_L and t becoming constant) before the total free energy is minimized in the independent bilayer phase. We conclude that the free energy maximum that differentiates the lamellar and bilayer phases is primarily due to solvent packing effects in this model.

Although this phase transition from a lamellar to an independent bilayer phase as the temperature is lowered is a sort of unbinding transition, it is not the usual unbinding transition discussed in the literature. Elastic membrane theoretical studies have suggested that on increasing temperature in the L_α phase, steric undulations should dominate and unbinding from a lamellar stack to isolated bilayers will occur in a continuous fashion [44]. The transition found here is by contrast first order and occurs with decreasing temperature. Since our calculations are based on a mean field theory, unbinding transitions due to steric undulations are not possible, and the membranes are in some sense infinitely rigid. However, an “anomalous” swelling regime has been documented in experiments where on decreasing the temperature toward the main (e.g. fluid to gel) transition, the lamellar spacing increases nonlinearly [45, 46]. This trend is in the same direction as the predictions of the current theory where we have found that solvent packing effects are important. It remains to be seen whether this is an artifact of our coarse-grained model or has broader implications for the experimental systems.

5.4.3 Phase Diagrams

The previous sections present bilayer structures and discuss hydration of bilayers in the context of biologically relevant structures at zero tension. In this section we take a broader perspective to discuss the phase behavior of the particular coarse grained model presented here. Clearly at high enough temperatures, macroscopic mixing must occur, and so there are limits to the existence of the microstructures presented previously.

In order to construct phase diagrams, it is necessary to identify all possible thermodynamic phases, and then locate coexistence curves as a function of appropriate state parameters. We have identified 4 distinct fluid phases and a series of ordered phases. On the fluid side we have the independent bilayer and lamellar microphases presented above, and two macrophases consisting of a solvent rich homogeneous solution and a lipid rich homogeneous solution (SRM and LRM respectively).

The phase diagram in the $\{T, x_s\}$ plane for the case where $\rho_b \sigma^3 = 0.59$ is shown in

Fig. 5.7. At very high temperatures (not shown), all species are mixed in a uniform homogeneous phase. There is a high temperature critical point (also not shown in plot) at $\{kT/\epsilon \approx 11, x_s \approx 0.75\}$, above which the system is mixed, and below which the system phase separates into a SRM and a LRM. An example of an interface between the LRM and SRM as calculated in the DFT is shown in Fig. 5.8. In this particular density profile, the total spatial extent of the LRM is quite small, and so interfacial oscillations are seen throughout the lipid rich domain.

As the temperature is lowered further, the lipids are able to self-assemble into the microphase-separated states. As discussed above, there is first a lamellar phase, followed by an independent fluid bilayer phase at lower temperatures. The surface tension is zero along the coexistence lines between the region of the SRM and either type of microphase, and a triple point occurs at $\{kT/\epsilon = 1.154, x_s = 0.404\}$. Another triple point is found at $\{kT/\epsilon = 2.15, x_s = 0.527\}$ at the coexistence point of the SRM, the LRM, and the lamellar microphase. The SRM-bilayer line enters the ordered regime continuously at $\{kT/\epsilon \approx 0.91, x_s = 0.33\}$. In addition this plot shows several critical points at low temperature that mark the termination of various otherwise first order layering transitions found for ordered bilayers.

The nature of the transition between the fluid and ordered bilayers is further demonstrated in Fig. 5.9. In essence, first order phase transitions between bilayers with different numbers of peaks in the tail region were found at low temperature. The two-phase envelopes for several of these ordering transitions are shown in the figure. The area per lipid A_L is an order parameter much like density for liquid-vapor transitions; the plot shows pairs of data where each pair is found at the same state point and excess surface free energy, but has different morphology. As the temperature is increased, these ordering transitions terminate in critical points as the phase envelopes close. We note that phase transitions between ordered phases with 6 and 7 peaks as well as 10 and 11 peaks were found, but the complete phase envelopes were not computed.

Figure 5.10 shows arc length continuation calculations using both the chemical potential variable x_s and the temperature as the continuation variables. The lack of discontinuities in the temperature continuation data further confirms that the transition from fluid to ordered bilayer phases in this theory is continuous (second order). The chemical potential continuation results highlight the first order transitions among different ordered phases, demonstrating that there are multiple solutions at many state points, and that various phases exhibit hysteresis beyond the thermodynamic transitions. As was discussed previously, stiffness in real lipid systems may preclude the complexity observed here for the ordered phases.

Finally, in order to facilitate comparison with experiment and other theoretical approaches in closed ensembles, we have generated a phase diagram in $\phi_s - T$ space where ϕ_s is the number fraction of solvent particles in the solution computed via

$$\phi_s = \frac{\Gamma_s}{\Gamma_L + \Gamma_s}. \quad (5.6)$$

For incompressible models such as those used in self-consistent field theories [17], this number fraction could be easily converted to a volume fraction.

Figure 5.11 shows this representation of the phase space. This plot was generated by taking several points along the coexistence curves in Fig. 5.7. At each point, the solvent number fractions of the two coexisting phases were computed. The solvent fractions for the independent bilayer phase were computed based on the λ of minimal hydration. This λ was established by computing Ω^{ex} as a function of λ as was done in Sec. 5.4.2. The properties of the lamellae were also found from the small λ minima in these Ω^{ex} vs. λ curves.

We see that at higher temperatures, there is a region of pure lamellae at low solvent fractions, and region of two phase coexistence between lamellae and an excluded SRM phase at higher ϕ_s . This behavior is seen experimentally for aqueous lipid solutions, in which there is a limit to how much a lamellar phase can be swollen, and beyond which one has a maximally hydrated lamellar phase coexisting with excess water [39]. At lower temperatures and low ϕ_s , lamellae are again found. Then with increasing ϕ_s , we find lamellae-bilayer mixtures, pure independent bilayers, independent bilayers swollen with excess solvent, and eventually pure solvent at $\phi_s \approx 1$.

Since we have limited our calculations to planar assemblies, our phase diagrams do not show 3-dimensional morphologies (e.g. the hexagonal phase) discussed by others. Nevertheless, we can make some comparisons with previous work. Both [16] and [17] used SCF theory to compute phase diagrams for lipid-like models. In the former case a single tailed model was used while in the latter case a double tailed lipid was studied. Both studies found a lamellar phase in a relatively large region of the phase diagram for low ϕ_s . In the work of Li and Schick, the shape of the boundary between lamellar and solvent-lamellar two phase regions was similar to the shape of the coexistence curve in Fig. 5.11.

5.4.4 Thermodynamic stability of fused bilayers

In Fig. 5.3 we showed a profile for two fused bilayers. Given the above energetic analysis, it is natural to inquire about the thermodynamic stability of two fused bilayers relative to two stacked bilayers. Figure 5.12 shows the free energy difference between these two cases at three temperatures along the $\gamma = 0$ coexistence curve (see Fig. 5.7). In all cases the free energy of the fused state is higher than that of the stacked bilayers, although the fused system becomes relatively more stable at higher temperatures. This model then indicates that fused states are metastable and they would exist as fluctuation driven phenomena. For this model thermal fluctuations could be expected to be sufficient to observe fusion at higher temperatures. However, higher temperatures would also correspond to bilayers exhibiting increased undulations (not captured in the present theory) so further investigation of the interplay between fused states and undulation is required.

Much of the literature on fusion is currently concerned with determination of the structures that form when biological membranes fuse. The density profiles presented here do not

give insight into the three-dimensional structure, but do demonstrate that fused structures are a natural observation in DFT calculations of the coarse grained models considered here.

5.4.5 Inclusions in bilayers

Finally we note that one of the strengths of this DFT approach in comparison with other theoretical approaches applied to study lipid bilayers is that it can capture both nanostructure and mesostructure associated with inclusions in the lipid bilayer. As a demonstration, we have performed a 2-dimensional calculation where a rod-like inclusion was inserted in the model bilayer. Figure 5.13 shows both the tail density distribution and the head group density distribution around this inclusion. Clearly, the bilayer bends to accommodate this large object. In addition, complex structure is found within the tail region of the bilayer near the inclusion. Although beyond the scope of this paper, various effects of the inclusion on the bilayer could be calculated from the DFT, such as the elastic length scale associated with the decay of perturbations away from the inclusion [47], or the details of the tail configurations and packing near the inclusion. While these results are preliminary, they demonstrate that this approach may be viable for investigation of a number of complicated phenomena, from hydrophobic matching to the interactions of membrane-bound proteins.

5.5 Summary

In this paper we present in detail the behavior of a density functional theory approach to lipid bilayers based on a particular (8-2-8) coarse grained model. We demonstrated that this theoretical approach may be suitable for studying bilayers as reasonable density profiles (tail groups to the center of the bilayer, head groups at the tail-solvent interface, and solvent excluded from the bilayer) are found. Furthermore, calculated properties for fluid bilayers (thickness and area per lipid) agree reasonably well with experimental systems at zero tension. At low temperatures, the model predicts ordered bilayers in contrast to earlier work using self-consistent field theories on flexible chain lipids. This suggests that the observed ordering results from packing effects not present in the SCF approach.

In addition to studying zero tension bilayers, we considered bilayer hydration, and more generally the phase diagram of bilayer morphologies that vary only perpendicular to the bilayer. We found that there is a first order phase transition from a lamellar phase with low hydration to an independent bilayer phase with potentially large hydration on lowering temperature. This cannot be the unbinding transition previously studied in theoretical investigations, but it may be related to anomalous swelling observed in experiments. We find that this effect is related to solvent packing.

While this particular model may not ultimately be the ideal choice for studying a particular lipid-solvent system, the computational techniques and fundamental groundwork

(including observation of fluid and ordered phases, and elucidation of the model phase diagram) provide a fundamental building block for further investigations of different lipid models, proteins interacting with bilayers, more complete physics (e.g. charged bilayers or lipid mixtures), and improved density functionals.

5.6 System of Equations

5.6.1 Density Functional Theory

As explained briefly in Sec. 6.2, the basic idea in CMS-DFT is to replace the inhomogeneous, interacting system of interest with a reference system of ideal, noninteracting chains and solvent, in a medium induced potential $U_\alpha(\mathbf{r})$ which captures the effects of the site-site interactions. This unknown field is the field required for the ideal system to ultimately have the same density distribution, $\rho(\mathbf{r}) = \sum_\alpha \rho_\alpha(\mathbf{r})$, as the system of interest in the *known* external field $V_\alpha(\mathbf{r})$.

The theory is formulated in the grand canonical ensemble, with the grand potential free energy Ω of the inhomogeneous system of interest measured relative to the free energy Ω_b of the bulk, homogeneous system which serves as the reservoir for the inhomogeneous system. Then the grand potential free energy difference $\Delta\Omega = \Omega - \Omega_b$ is given by a Taylor series expansion about the noninteracting reference system, truncated at second order. A functional minimization of $\Delta\Omega$ with respect to $\rho_\alpha(\mathbf{r})$ and $\mu_\alpha - U_\alpha(\mathbf{r})$ leads to

$$U_\alpha(\mathbf{r}) = V_\alpha(\mathbf{r}) - \sum_\beta \int d\mathbf{r}' c_{\alpha\beta}(\mathbf{r} - \mathbf{r}') (\rho_\beta(\mathbf{r}') - \rho_{b\beta}), \quad (5.7)$$

where μ_α is the chemical potential of site type α , $\rho_{b\beta}$ is the bulk density for site type β , and $c_{\alpha\beta}$ is the direct correlation function (DCF) between the α and β site types in the bulk fluid. The direct correlation function can be calculated from liquid state theory or obtained from simulations, and essentially describes the packing and interactions between different sites in the bulk homogeneous fluid. The calculation of $c_{\alpha\beta}(\mathbf{r})$ used in this work is described below in section 5.6.2.

Equation (5.7) has two unknown functions, the density distribution of interest, $\rho_\alpha(\mathbf{r})$, and the mean field, $U_\alpha(\mathbf{r})$. Clearly the relationship between the two must be defined. For the single site solvent, we simply have the usual Boltzmann distribution

$$\rho_s(\mathbf{r}) = \rho_{b,s} \exp[-\beta U_s(\mathbf{r})], \quad (5.8)$$

where $\beta = 1/kT$. For the ideal gas of lipids, this relationship is

$$\rho_\alpha(\mathbf{r}) = \frac{V}{N_L} \rho_{b,L} \left\langle \delta(\mathbf{r} - \mathbf{r}_\alpha) \exp \left[-\beta \sum_\gamma U_\gamma(\mathbf{r}_\gamma) \right] \right\rangle_S \quad (5.9)$$

where N_L is the total number of sites in the lipid, $\rho_{b,L}$ is the lipid site density in the bulk reservoir, and the brackets denote a configurational integral over all possible configurations, $\{\mathbf{r}\} = \{\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_{N_L}\}$, based on the configuration probability $S\{\mathbf{r}\}$. For a single random walk chain, the configurational probability can be written as

$$S(\mathbf{r}_1, \dots, \mathbf{r}_{N_L}) = \prod_{s=2}^{N_L} [\omega_{\alpha\beta}(\mathbf{r}_s - \mathbf{r}_{s-1})], \quad (5.10)$$

where $\omega_{\alpha\beta}(\mathbf{r} - \mathbf{r}')$ is the probability of a bond of a certain length between sites of type α at $\mathbf{r}$ and type β at $\mathbf{r}'$. In this work we will consider freely jointed chains with bond lengths $\sigma_{\alpha\beta}$, for which we have

$$\omega_{\alpha\beta}(\mathbf{r}) = \frac{1}{4\pi\sigma_{\alpha\beta}^2} \delta(|\mathbf{r}| - \sigma_{\alpha\beta}). \quad (5.11)$$

Given the definition for $S\{\mathbf{r}\}$ in Eq.(5.10), the density distribution for sites of type α on a linear chain lipid becomes

$$\rho_\alpha(\mathbf{r}) = \frac{\rho_{b,\alpha}}{N_\alpha} \sum_{s \in \alpha} \frac{G_s(\mathbf{r}) G_s^{inv}(\mathbf{r})}{\exp[-\beta U_\alpha(\mathbf{r})]}, \quad (5.12)$$

where the sum over s is a sum over all the sites of type α in the molecule, N_α is the total number of sites of type α , and G and G^{inv} are propagator functions that describe the configurational probability based on chain connectivity. The propagator functions in Eq. (5.12) obey the recursion relations

$$G_s(\mathbf{r}) = \exp[-\beta U_{\alpha(s)}(\mathbf{r})] \int d\mathbf{r}' \omega_{\alpha\beta}(\mathbf{r} - \mathbf{r}') G_{s-1}(\mathbf{r}'), \quad (5.13)$$

$$G_s^{inv}(\mathbf{r}) = \exp[-\beta U_{\alpha(s)}(\mathbf{r})] \int d\mathbf{r}' \omega_{\alpha\beta}(\mathbf{r} - \mathbf{r}') G_{s+1}^i(\mathbf{r}'), \quad (5.14)$$

for the $2 \dots N_L$ sites in the case of Eq. (5.13) and the $N_L - 1 \dots 1$ sites in the case of Eq. (5.14). They satisfy the “initial” conditions $G_1(\mathbf{r}) = \exp[-\beta U_{\alpha(1)}(\mathbf{r})]$ and $G_{N_L}^{inv}(\mathbf{r}) = \exp[-\beta U_{\alpha(N_L)}(\mathbf{r})]$. Here $\alpha(s)$ indicates the site type of bead s . Note that Eq. (5.12) reduces to Eq. (5.8) in the case of an atomic liquid. In our calculations, Eqs. (5.7), (5.12), (5.13), and (5.14) for

the lipid and Eqs. (5.7) and (5.8) for the solvent are solved simultaneously in real space on a cartesian grid. Our numerical methods have been enumerated extensively elsewhere [29, 32]; here we simply state that we have used a Newton's method approach with update dampening as required for good convergence.

The difference between the free energy of the inhomogeneous system of interest and a bulk homogeneous system is

$$\begin{aligned} \Delta\Omega = & - \int d\mathbf{r} (\rho_s(\mathbf{r}) - \rho_{b,s}) - \frac{1}{N_L} \sum_{\alpha \in \{L\}} \int d\mathbf{r} (\rho_\alpha(\mathbf{r}) - \rho_{b,\alpha}) + \\ & \frac{1}{2} \sum_{\alpha\beta} \int \int d\mathbf{r} d\mathbf{r}' c_{\alpha\beta}(\mathbf{r} - \mathbf{r}') [\rho_\alpha(\mathbf{r}) \rho_\beta(\mathbf{r}') - \rho_{b,\alpha} \rho_{b,\beta}], \end{aligned} \quad (5.15)$$

where the second term is summed over all lipid sites and the sum in the last term is over all species (solvent and lipid). Note that $c_{\alpha\beta}(\mathbf{r} - \mathbf{r}')$ contains the nonideal interactions of the model of interest.

The lateral pressure profile across fluid bilayers is thought to play a significant role in membrane and membrane protein function [13, 48] and is also related to the curvature elasticity of the membrane, and is hence a quantity of interest (which we explore in the following paper). Based on Eqs. (5.3), (5.4) and (5.15), we can identify the stress profile with the excess surface free energy density, i.e. $s(x) = \Omega^{ex}(x)$ so that

$$\begin{aligned} s(x) = & - (\rho_s(x) - \rho_{b,s}) - \frac{1}{N_L} \sum_{\alpha \in \{L\}} (\rho_\alpha(x) - \rho_{b,\alpha}) + \\ & \frac{1}{2} \sum_{\alpha\beta} \int dx' c_{\alpha\beta}(|x - x'|) [\rho_\alpha(x) \rho_\beta(x') - \rho_{b,\alpha} \rho_{b,\beta}] - S, \end{aligned} \quad (5.16)$$

where S is a constant arising from the pure solvent contribution, and the first sum is taken over the beads in the lipid. In a pure solvent $P_T \rightarrow 0$ and so S is the isotropic pressure in a pure solvent where

$$\begin{aligned} -S = & (\rho_{ps,s} - \rho_{b,s}) - \frac{\rho_{s,ps}^2}{2} \int dx' c_{ss}(|x - x'|) - \\ & \frac{1}{2} \sum_{\alpha\beta} \int dx' c_{\alpha\beta}(|x - x'|) \rho_{b,\alpha} \rho_{b,\beta} \end{aligned} \quad (5.17)$$

and $\rho_{ps,s}$ is the density of the solvent in a solution that has a pure uniform solvent.

5.6.2 Liquid state input

The direct correlation function $c_{\alpha\beta}(r)$ in Eq. (5.7) is calculated using the polymer reference interaction site model (PRISM) liquid state theory with the Percus-Yevick (PY) closure [49, 50]. In this work we employ a self-consistent version of PRISM theory to calculate the DCFs. In self-consistent PRISM theory, the intramolecular structure factor $\omega(k)$ is determined from a single-chain Monte Carlo (MC) simulation of the system in a solvation potential which is determined essentially by the intermolecular correlations. The PRISM equation for the intermolecular correlations is solved using the determined $\omega(k)$, and then the cycle is repeated to convergence. Thus, the intramolecular and intermolecular correlations are calculated self-consistently. This PRISM/MC approach has been shown to be in good agreement with bulk molecular dynamics simulations for a variety of polymer melts [51, 52]. Good agreement with simulation was also found for PRISM/MC calculations of athermal polymer solutions with a single-site solvent such as we use here [53]. We note that in our work the single-chain structure is not completely consistent as it is taken to be of freely-jointed form in the DFT but not in the PRISM/MC calculations. A recent study of the accuracy of this combined “random walk CMS-DFT” (RWCMS-DFT) and PRISM/MC theory found good qualitative agreement with simulation for density profiles of homopolymer melts near surfaces [54].

In this work we are interested in a wide range of attractive systems, but PRISM theory is known to be more accurate for harshly repulsive systems. Therefore, we take a perturbation approach in our treatment of the intermolecular potentials and hence of the direct correlation functions. Specifically, we apply the random phase approximation (RPA) [55]. The RPA splits $c(r)$ into two parts, a short-range, repulsive core contribution and a long-range attractive contribution,

$$c_{\alpha\beta}(r) = c_{\alpha\beta}^{core}(r) - \beta u_{\alpha\beta}^{att}(r). \quad (5.18)$$

The core term, c^{core} , is calculated from PRISM theory using a repulsive lipid molecule with the same architecture as the molecule of interest and with purely repulsive interactions set to $u^{rep}(r)$. The long range part is taken to be directly proportional to the attractive part of the pair interaction potential $u^{att}(r)$, as is indicated above. Here we define $u^{rep}(r)$ and $u^{att}(r)$ to be

$$\begin{aligned} u_{\alpha\beta}^{rep}(r) &= \begin{cases} u_{\alpha\beta}^{LJ}(r) + \varepsilon, & \text{if } r \leq r_{min} \\ 0, & \text{otherwise,} \end{cases} \\ u_{\alpha\beta}^{att}(r) &= \begin{cases} 0, & \text{if } r \leq r_{min} \\ u_{\alpha\beta}(r), & \text{otherwise,} \end{cases} \end{aligned} \quad (5.19)$$

where the minimum of $u_{\alpha\beta}^{LJ}$ is located at $r = r_{min}$. Note that a true Weeks, Chandler, Anderson approximation would instead have, for $r \leq r_{min}$, $u_{\alpha\beta}^{att}(r) = -u_{\alpha\beta}(r_{min})$ and $u_{\alpha\beta}^{rep}(r) =$

$u_{\alpha\beta}^{LJ}(r) + u_{\alpha\beta}(r_{min})$ [55, 56]. We do not include the temperature dependence in the calculation of $c^{core}(r)$ in order to prevent the need for new PRISM calculations at every temperature and also to ensure that we remain in the harshly repulsive regime appropriate for PRISM calculations. Given this constraint of fixed $c^{core}(r)$, it would be difficult to apply the WCA approximation because then for $r \leq r_{min}$ some terms (specifically, solvent-solvent, head-head, tail-tail, and head solvent) would be shifted significantly, by ϵ/kT , while the others would not be shifted at all due to their purely repulsive character ($r_c = r_{min}$). Shifting the relative interaction strengths for $r \leq r_{min}$ significantly affects self-assembly for these systems. However, using the approximation of Eq. (5.19) is also somewhat problematic since it results in a small discontinuity at r_{min} in the $c(r)$ used in the DFT calculations. While this combination of approximations seems to work adequately for both attractive homopolymers near attractive walls and for the fluid bilayers we consider here, it still remains to completely quantify the impact of these approximations [57].

5.7 Complexity of the state space

To locate possible solutions for the model lipid system, we applied an arc-length continuation algorithm [33]. Once a solution to the DFT equations has been found, this algorithm is able to follow that solution, as a function of any continuous system parameter, along stable, metastable, or unstable solution branches and around turning (spinodal) points. This algorithm was instrumental in understanding the thermodynamic behavior of our system, since a given initial guess does not necessarily lead to the lowest free energy solution.

In order to perform these state tracking calculations, each new DFT solution requires a different $c^{core}(r)$. Since these enumerations require hundreds to thousands of solutions, it is not practical to embed the PRISM calculation within the DFT calculation. Therefore as an approximation, we typically interpolate among four $c^{core}(r)$ for a given $\rho_b\sigma^3$. For all of the calculations presented here, the four $c^{core}(r)$ functions were calculated at $x_s = 0.001$, $x_s = 0.333$, $x_s = 0.667$, and $x_s = 0.999$. As a result, small errors can be expected at the extremes in x_s ($x_s < 0.01$ and $x_s > 0.99$). However, we find that most of the interesting physics occurs in the region $0.2 < x_s < 0.8$ where the interpolation should be adequate. Figure 5.14 shows the core repulsive part of the direct correlation functions at the four chosen x_s values for all of the interactions at $\rho_b\sigma^3 = 0.59$ and $kT/\epsilon = 1.3$. We see that the changes in $c^{core}(r)$ with concentration are relatively small for this system.

Figure 5.15 shows arc-length continuation data at the state point $kT/\epsilon = 1.3$ and $\rho_b\sigma^3 = 0.59$ for the excess free energy Ω^{ex} as a function of x_s . The complex solid curve has three branches that correspond to solutions with different numbers of bilayers (1, 2, and 3) in the computational domain. Turning points correspond to the limits of stability of the various morphologies. The branch where two bilayers are found has two parts. One corresponds to stacked bilayers, while the other corresponds to fused bilayers (see Fig. 5.3).

Other curves are also included in Fig. 5.15. The excess free energy of a solution con-

sisting only of solvent is zero by definition (see Eq. 5.3). The lamellar data shown could not be generated by arc length continuation since the free energy for this phase must be minimized with respect to the lamellar period, i.e. the computational domain size, which in our Cartesian coordinate system is not a continuous variable. These data were thus generated by individual solves of the DFT equations. We note that zero tension assemblies occur at $\gamma = \Omega^{ex} = 0$ which corresponds to a multinodal point where several branches cross in this model.

Finally, our code also includes algorithms that track binodal points in parameter space [33]. The strategy here is to solve for two solutions of differing structure simultaneously while imposing the constraint that the two solutions have identical free energies. This allows us to track binodal points, such as the multinodal point between bilayer, lamellar, and solvent solutions shown in Fig. 5.15, as a function of T . These binodal calculations are straightforward provided that optimization of the computational domain size is not required. So, we can easily compute the coexistence curves between solvent rich and lipid rich macrophases. We can also compute coexistence between bilayers with a large excess pure solvent region and a pure solvent solution because the bilayers are not constrained by the computational domain and will find their optimal structure. This allows us to find all $\gamma = 0$ bilayers as a function of temperature in our model in an automated way.

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Figure 5.1. A sketch of the model lipid-solvent system. The tail beads (white) and the solvent (black) are the same size. The head group beads (grey) are larger by a factor of 1.44.

Figure 5.2. Density profiles for zero tension bilayers, showing the head groups (bold solid curve), tail groups (solid curve), and solvent (dotted curve). The two cases shown are a single isolated bilayer (A), and a lamellar stack (B). The lamellar profile has a repeat period of $\lambda = 9.05\sigma$. The distance between head group peaks on either side of the bilayers is found to be 7.4σ for the isolated bilayer and 7.5σ for the lamellar bilayers. The state point for both profiles is $\{x_s = 0.415, \rho_b\sigma^3 = 0.59, kT/\epsilon = 1.3\}$.

Figure 5.3. Density profile for two fused bilayers at zero tension at the state point $\{kT/\varepsilon = 1.3, \rho_b\sigma^3 = 0.59, x_s = 0.465\}$ (curves as in Fig. 5.2).

Figure 5.4. Density profiles for lipid bilayers at the two extremes of stability for $kT/\varepsilon = 1.3$ and $\rho_b\sigma^3 = 0.59$. The solvent fractions and surface tensions at the two extremes are $x_s = 0.303$, $\gamma = 0.036kT/\sigma^2$ (A) and $x_s = 0.627$, $\gamma = -0.300kT/\sigma^2$ (B) (curves as in Fig. 5.2; note that these two states are the two extremes of the 1 bilayer curve shown in Fig. 5.15).

Figure 5.5. Density profiles for two ordered bilayers. The narrow bilayer in (A) was found at $\{kT/\varepsilon = 0.877, x_s = 0.3, \rho_b\sigma^3 = 0.59\}$. The thicker bilayer in (B) was found at $\{kT/\varepsilon = 0.943, x_s = 0.668, \rho_b\sigma^3 = 0.59\}$ (curves as in Fig. 5.2).

Figure 5.6. Excess surface free energy as a function of the period of a uniform stack of bilayers for different temperatures, all with the lamellar phase at zero tension.

Figure 5.7. Phase diagram in the $T - x_s$ phase space.

Figure 5.8. Density profiles demonstrating properties of the interfaces between coexisting lipid rich and solvent rich macrophases at $kT/\varepsilon = 2.2$ and $x_s = 0.5312$.

Figure 5.9. Four different phase envelopes between different ordered phases exhibiting different numbers of peaks in the tail region of the bilayer density distribution.

Figure 5.10. Arc-length continuation calculations highlighting the nature of the ordered phases exhibited by this model and theory. First order transitions are highlighted by the $kT/\epsilon = 0.9427$ isotherm in (A) where multiple solutions are shown at many state points and hysteresis (dotted lines show metastable branches) is found around thermodynamic transitions. The continuous nature of the order-disorder transition is shown in (B) where arc-length continuations in temperature at several x_s show no discontinuities.

Figure 5.11. Phase diagram in the $\phi_s - T$ space. Lamellar and two phase lamellar-SRM regions are marked. The pure SRM phase is found on the line $\phi_s \approx 1$. Regions I, II, and II were not fully resolved, and only solid lines are based on data obtained from calculations. Nevertheless it is clear that at these low temperatures, from left to right there will be a pure lamellar phase, a mixture of lamellar and independent bilayers (region I), pure bilayers with minimal hydration (region II), swollen bilayers that take up excess solvent (region III), and finally the SRM.

Figure 5.12. Free energy difference between stacked bilayers and fused bilayers as a function of temperature.

Figure 5.13. Density distributions of (A) tail segments and (B) head segments in the vicinity of a rod-like inclusion. Note that the white regions are where the respective densities of the two species are less than $\rho\sigma^3 = 10^{-8}$.

Figure 5.14. Direct correlation functions for all $\alpha\beta$ pairs as a function of bulk solvent number fraction x_s at a total bulk density of $\rho_b\sigma^3 = 0.59$. Figure A shows the direct interactions, c_{tt}, c_{hh}, c_{ss} while figure B shows the cross terms c_{th}, c_{ts}, c_{hs} . Note that the subscripts indicate tail beads, head group beads and solvent. The various curves are for $x_s = 0.001$ (solid line), $x_s = 0.333$ (dashed line), $x_s = 0.666$ (dotted line), and $x_s = 0.999$ (dashed-dotted line) for all cases.

Figure 5.15. Excess free energy per unit volume as a function of x_s for the state point $kT/\epsilon = 1.3$ and $\rho_b \sigma^3 = 0.59$. The numbers in the figure indicate the number of bilayers in the 40σ computational domain for various branches. The various curves are as follows: bilayer solutions are shown in solid lines, space filling lamellar solutions are shown with dotted lines and are marked for clarity, a lipid rich uniform macrophase solution is shown in dashed lines, and the pure solvent solution is the dashed-dot-dot line at $\Omega^{\text{ex}} = 0$.

Chapter 6

A comparison of density functional theory and simulation of fluid bilayers

6.1 Introduction

Lipid bilayer membranes play important functional roles in biological systems. A detailed understanding of their structure and mechanical properties can help to elucidate the behavior of lipid domains and membrane proteins. Since the biologically relevant phase of lipid bilayers is the disordered, fluid phase, it is difficult to extract detailed information on bilayer properties from experiment. Computer simulations have played a complementary role in obtaining and understanding this information. However, atomistic simulations are limited to space and time scales that are too short to capture many interesting processes and features of lipid bilayers, such as the formation of lipid domains or rafts, the interactions of transmembrane proteins, and membrane fusion. As a result, there has been increasing interest in developing more coarse-grained models to describe lipid membranes [1].

These models have taken a variety of forms. The shapes and internal degrees of freedom of the model lipids vary, from rigid spherocylinders or rigid chains of beads with no internal degrees of freedom, to more flexible chains of spherical beads. Both lattice and off-lattice simulations have been performed on coarse-grained lipid models. Techniques include molecular dynamics (MD), Monte Carlo, and dissipative particle dynamics (DPD). Recently, efforts have been made to build united-atom-type models based on atomistic force fields, in order to obtain coarse-grained models that can give relatively quantitative results [2, 3].

A more generic coarse-grained model that maintains the internal degrees of freedom of the lipids is that introduced (for lipid bilayers) by Goetz and Lipowsky [4]. Their model consists of chains of spherical interaction sites, which interact through Lennard-Jones interactions, a bonding potential for bonded neighbors, and angle potentials. This model has been used in both MD and DPD simulations, and has been shown to self-assemble from

random initial conditions into bilayers. The model has also been used to obtain bilayer properties such as the elastic constants, to study the liquid to gel phase transition, and to simulate vesicle fusion [4, 5, 6, 7, 8].

These kinds of spherical interaction site models can also be treated using classical density functional theory (DFT). In the previous paper ([9], hereafter referred to as paper I), we presented a theoretical framework for the equilibrium properties of lipid bilayers that treats the lipid chains in a coarse-grained molecular fashion using a DFT approach previously developed for inhomogeneous and self-assembling polymers [10, 11, 12, 13, 14, 15]. In paper I we presented bilayer structures, an analysis of bilayer hydration, and phase diagrams based on 1-dimensional morphologies for the model system.

Here we explore the accuracy of the DFT approach by comparison to MD simulations of flexible chain lipids. To our knowledge, all previous MD simulations of coarse-grained lipids in the literature [4, 5, 7, 8, 3] have treated the lipid tails as semiflexible, by including an angular bending potential between monomers. Since our DFT treats the lipid tails as freely-jointed, random walks, we need to compare to MD simulations on flexible-tail lipids. Thus in this paper we perform MD simulations on the model lipid system introduced in paper I. Section 6.2 provides a summary of the DFT and details of the MD simulations, Sec. 6.3 presents calculations of lipids and solvated lipids at surfaces along with a discussion of the solvent model, Sec. 6.4 compares bilayer structures predicted from the theory to MD calculations, and finally Sec. 6.5 compares physical properties of the membrane to predictions from the DFT. Generally we show that the agreement between the methods is adequate for fluid bilayers, with room for improvement in the quality of the solvent model.

6.2 Methods

6.2.1 Model system

Our coarse grained lipid model (see paper I for details) consists of a freely-jointed chain of tangent sites, with a head group and two tails. The tails consist of 8 interaction sites or “beads” while the head group is composed of 2 larger beads. This 8-2-8 model roughly groups two CH_2 groups per bead, and the ratio of head to tail bead diameters ($\sigma_h/\sigma_t = 1.44$) was chosen on the basis of simple packing arguments that predict lamellar or bilayer assemblies. The model also includes a single site solvent.

The interactions between different site types in the model are based on Lennard-Jones (LJ) interactions, with the various energetic constants set to bias the system towards self-assembly. Specifically, the interaction potentials are

$$u_{\alpha\beta}(r) = u_{\alpha\beta}^{LJ}(r) - u_{\alpha\beta}^{LJ}(r_c), \quad (6.1)$$

$$u_{\alpha\beta}^{LJ}(r) = \frac{4\epsilon_{\alpha\beta}}{kT} \left[\left(\frac{\sigma_{\alpha\beta}}{r} \right)^{12} - \left(\frac{\sigma_{\alpha\beta}}{r} \right)^6 \right], \quad (6.2)$$

where r_c is the cutoff distance where the potential goes to zero, k is Boltzmann's constant and T is the temperature. In addition to the lipid molecules, the model has a single site solvent with diameter $\sigma_s = \sigma_t \equiv \sigma = 1$. The cross-terms in the bead diameters are found from the usual Berthelot scaling rules, so that $\sigma_{\alpha\beta} = 0.5(\sigma_\alpha + \sigma_\beta)$. The tail-solvent and tail-head interactions are purely repulsive with $r_c = 2^{1/6}\sigma_{ts}$ and $r_c = 2^{1/6}\sigma_{th}$ respectively. Solvent-solvent, solvent-head, head-head, and tail-tail interactions are all uniformly attractive with a cutoff of $r_c = 3.5\sigma$. Finally, we set all of $\epsilon_{\alpha\beta} \equiv \epsilon = 1$. This combination of parameters allows for a self-assembling bilayer to form. We will report all lengths in units of σ and energies in units of ϵ/kT .

6.2.2 DFT calculations

The DFT is a mean-field theory for inhomogeneous chain liquids, based on the DFT of Chandler, McCoy, and Singer (CMS) [10, 11, 12]. The properties of the bulk system are calculated using the polymer reference interaction site model (PRISM) theory [16, 17]. The direct correlation functions $c_{\alpha\beta}(r)$ for the effective interactions between sites in the bulk fluid are obtained from this theory and used as an input to the CMS-DFT.

The DFT calculations are done in the grand canonical ensemble where the state variables are the volume V , the temperature T , and the chemical potentials of the solvent and lipid species, μ_s and μ_L respectively. In our formulation, μ_s and μ_L are manipulated via a total site density ρ_b and the solvent number fraction x_s in a bulk reservoir fluid in equilibrium with the fluid in the computational domain. Given a thermodynamic state, the calculations produce density profiles, $\rho_t(x)$, $\rho_h(x)$, and $\rho_s(x)$, for tail beads, head beads, and solvent respectively. Note that x is in the direction of the bilayer normal.

The total density of lipid chains is calculated as the area per lipid chain, A_L , via

$$A_L = \left(N_\alpha^{-1} \int \rho_\alpha(x) dx \right)^{-1} \quad (6.3)$$

where N_α is the number of beads of type α on the lipid chain. The surface tension of a given bilayer is

$$\gamma = (\Delta\Omega[\rho_\alpha(\mathbf{r})] - \Delta\Omega^s)/A, \quad (6.4)$$

where the Δ indicates that free energies are measured relative to a homogeneous mixed lipid-solvent system, Ω is the free energy for the inhomogeneous system containing a bilayer, Ω^s is the free energy of a pure solvent at the density observed far from the bilayer in the inhomogeneous system, and A is the total area.

6.2.3 Molecular Dynamics Simulations

In the MD simulations bonding is enforced with a harmonic bond potential of the form

$$V_{bond,\alpha\beta} = k_b(r - \sigma_{\alpha\beta})^2, \quad (6.5)$$

where r is the distance between two bonded sites. We set $k_b = 10^3 \epsilon \sigma^{-2}$, of the same order of magnitude as used by Goetz and Lipowsky [4]. Because we did not constrain the bond lengths to be rigidly fixed, this model differs slightly from that used in the DFT calculations; however, with the relatively large value of k_b the average bond length is close to the long time average of σ [4].

MD simulations were carried out using the parallel LAMMPS MD code [18], using 8 processors on the Sandia Cplant cluster. A timestep of 0.005τ (in reduced LJ units) was used. The temperature T was controlled with a Nose-Hoover thermostat using a coupling frequency of $10\tau^{-1}$. Constant-pressure simulations employed an anisotropic Nose-Hoover barostat which allowed all three box dimensions to fluctuate independently.

We performed two different kinds of simulations. The first set consisted of a liquid of either melt or solvated lipids near surfaces, which were run in the NVT ensemble. Initial states for these simulations were prepared by randomly placing lipid molecules and solvent sites in the simulation box, and overlaps were removed using a soft nonbond potential. The systems were equilibrated for typically 3×10^5 time steps, and statistics to obtain smooth density profiles were collected for 10^6 to 1.5×10^6 time steps. The melt lipid/wall systems consisted of 1000 lipid molecules confined between two surfaces as described below, with periodic boundaries in the other two directions. The lipid/solvent/wall systems consisted of 600 lipid molecules and 17621 solvents for the repulsive systems, and 400 lipid molecules with 11747 solvents for the attractive systems.

We also simulated solvated lipid bilayers, using periodic boundary conditions. These systems were prepared in two ways. First we verified that our lipid model would self-assemble into a bilayer by starting from random configurations of lipids and solvent. These systems were run in the NPT ensemble until a bilayer formed. These bilayers were randomly oriented in the simulation box. To extract density profiles, we created initial configurations consisting of a bilayer oriented parallel to two of the simulation box directions, with the lipids initially packed on a hexagonal lattice. The bilayer was then equilibrated in the NPT ensemble for up to 2.5×10^6 time steps until the system reached its equilibrium area per lipid. This approach led to a well-equilibrated bilayer that remained parallel to two of the simulation box directions. We also note that all three box dimensions were independently controlled. The area per lipid was thus controlled by the interactions and not by the initial box size. Furthermore, we kept all three components of the pressure tensor equal ($P_{xx} = P_{yy} = P_{zz}$) so that the surface tension $\gamma = 0$ [19]. Results reported for areas per lipid are thus for zero tension bilayers. In order to calculate density profiles, we ran the equilibrated systems in the NVT ensemble for 5×10^5 timesteps for equilibration, followed

by another 8×10^5 timesteps to collect statistics. We simulated several different sized systems, consisting of either $n_L = 220$ or $n_L = 450$ lipid molecules and from 50% to 60% site density of solvent.

6.3 Verifying the approach

There are several possible sources for error in the DFT approach of paper I. While it is difficult to tease these errors apart to assess them separately, this section explores several example systems to rigorously test the predictions of the DFT in order to determine the net effect of the approximations in studies of lipid bilayers. Briefly, we expect discrepancies could come from the following sources. First, the CMS-DFT is based on a second order expansion of the free energy, and so precludes liquid-vapor or wetting transitions. Second, the “HNC” form of the mean field used in the CMS-DFT is known to overpredict structure in fluids near surfaces. Third, the DFT incorporates liquid state structure from PRISM theory, and so may have errors that propagate from the liquid state calculations. We note that errors due to the HNC field and due to approximations in PRISM theory are known to partially compensate each other [14]. Fourth, attractions are introduced in the DFT calculations via a perturbation approach with an unshifted core term (see Appendix A of paper I). Fifth, the chain statistics are not the same in the simulation and the DFT. This is because the simulations enforce excluded volume, so that the chain statistics are not Gaussian on short length scales. By contrast, in the DFT the chains follow random walks and thus allow chain overlaps (although the repulsive part of the LJ interactions will counter this effect). And finally, while DFT holds bond lengths constant, the MD bonds can fluctuate slightly (see Eq. (6.5)).

6.3.1 Lipids at surfaces

The CMS-DFT has not previously been used to treat an asymmetric triblock copolymer such as our model 8-2-8 lipid. As a rigorous test on fluid structure, we performed both density functional and molecular dynamics calculations of the lipid molecules at surfaces. In these calculations, the size asymmetry of the head and tail beads is preserved, but the interactions are all taken to be identical ($\epsilon_{\alpha\beta} = 1$, with equal cutoffs r_c for all species). Thus no self-assembly is expected. While previous work found that the CMS-DFT with Lennard-Jones interactions was accurate in describing the self-assembly of diblock copolymers, both in the bulk and in thin films [20, 21], only symmetric diblocks were studied in those cases. Symmetric diblocks are a special case because while the individual $\rho_\alpha(\mathbf{r})$ show microphase separation, they are decoupled by symmetry from the total density $\sum_\alpha \rho_\alpha(\mathbf{r})$ which controls packing effects [22, 21]. Thus we anticipate that there may be more interplay between total density and concentration effects in our asymmetric lipid model.

We considered three kinds of surface-lipid systems all at $kT/\epsilon = 1.0$. The first case had

purely repulsive interactions both between the chains and between the walls and the chains (case RR). Another calculation had attractive interactions between the chains as well as between the surfaces and the chains (case AA). A final calculation had attractive chain-chain interactions, but repulsive chain-wall interactions (case AR). Attractive chain interactions were generated by setting longer LJ cutoffs for the AA and AR cases. Specifically the AR and AA cases had $r_c = 3.5\sigma$ while the RR case had $r_c = 2^{1/6}\sigma_{\alpha\beta}$. Surface-fluid interactions were a function of the distance x between a given point in the fluid and the surface, and were of an integrated LJ form,

$$V_\alpha(x) = \frac{2\pi\epsilon_{w\alpha}}{3} \left[\frac{2}{15} \left(\frac{\sigma_{w\alpha}^{12}}{x^9} \right) - C \left(\frac{\sigma_{w\alpha}^6}{x^3} \right) \right], \quad (6.6)$$

where $C = 0$ for the RR and AR cases while $C = 1$ for the AA case. Note that the wall-site interaction parameters were $\epsilon_{w\alpha} = 1.0$ and $\sigma_{w\alpha} = 1.44$. The bulk density ρ_b in the fluid needed for the DFT calculations was chosen to match the density far from the surfaces in the MD simulations. The bin size for the MD density profiles and the mesh size for DFT calculations were both set to $\Delta x = 0.1$.

A comparison of DFT and molecular dynamics calculations is shown in Fig. 6.1. The DFT reproduces structure reasonably well in both the RR and AA cases. This indicates that the theory is able to handle the packing of the different size sites in the lipid molecule correctly. However, in the AR case, the DFT significantly overpredicts structure at the interface. A similar disagreement has been observed for attractive homopolymers near repulsive surfaces [23]. In that paper, it was shown that an *ad hoc* correction of the repulsive (PRISM) part of the direct correlation functions by a constant factor improved the result. A similar correction is applied in Fig. 6.1D where the agreement is now quite good. However, this correction is completely empirical, and so we don't pursue it further here except to note that the theory may not work well for lipids near strongly solvophobic surfaces.

Figure 6.2 shows results for a mixture of lipids and solvent next to a surface. Again, all the site-site interactions were identical so that we are again probing the ability of the theory to predict packing. Figure 6.2 shows the completely repulsive RR case and the completely attractive AA case. In both cases, although the shapes of the density profiles are in agreement with the MD simulations, the DFT predicts a much higher head group peak near the surface, whereas the MD simulations predict that more of the solvent goes to the surface. The DFT result is somewhat surprising, as one might expect that the lipids could gain more configurational entropy by not being too close to the surface, but putting the solvent there instead. The discrepancy is presumably related to the balance of approximations in the HNC field and the PRISM input, as mentioned above. The agreement is not as good as for the melt of lipids shown in Fig. 6.1, which must be due to inclusion of the solvent. We examine the quality of our solvent model in the next section. However, we note that in lipid bilayers, the solvent is excluded from the bilayer, and so we expect the quality of the solvent model to be less important for lipid bilayers than it is in the mixtures discussed here.

6.3.2 The solvent model

Given the approximations of the DFT detailed above, it is clear that the single site solvent in this theory will not be the same as the LJ fluid used in simulations. This is illustrated in Fig. 6.3 where we present pressure-density curves for pure solvents at three different temperatures. We calculated the DFT solvent pressure using the contact density ($P/kT = \rho_s(x=0)$ [24]) for this solvent at a hard wall. We find that the pressure is significantly higher in our solvent than it is in a true Lennard-Jones fluid. For liquid-like densities, P is even higher than in a hard-sphere system described by the Carnahan-Starling equation of state. This result was noted previously by Hooper et al. for hard-sphere sites, and is due at least in part to errors in the HNC form of the mean field used in the DFT [14, 25]. Taking both the absence of the liquid-vapor transition in this DFT, and the high pressures in Fig. 6.3, one concludes that this model solvent will behave more like a purely repulsive (e.g. hard sphere or WCA) fluid than like a Lennard-Jones fluid.

While these shortcomings of the CMS solvent are no doubt a significant factor in the poorer performance of the CMS-DFT in Fig. 6.2, there are some advantages to using this particular solvent model. First, it is computationally simple to implement. While there are more accurate density functionals available for inhomogeneous Lennard-Jones fluids, it is not *a priori* clear how to couple these to the CMS polymer-DFT used here to treat the lipids. Since we are interested in solutions that exhibit microphase separation, and since we are principally interested in the structure of the lipid-rich bilayer phase, a highly accurate solvent treatment may not be required.

6.4 Structure of Lipid bilayers

We now turn to lipid bilayers. It would be reasonable to expect that lipid bilayers based on flexible lipids would exhibit significant interpenetration of the two bilayer leaflets. However, this does not appear to be the case. Figure 6.4 shows a snapshot of a lipid bilayer at temperature $kT/\epsilon = 1$ and a pressure of $P = 0.5\epsilon/\sigma^3$ (chosen to approximate atmospheric pressure). The two different leaflets are colored differently, and we see that they only interpenetrate by one or two beads.

For bilayers, attractive interactions drive the self-assembly, creating fluid interfaces rather than rigid ones. The fluid nature of the interface presents a challenge for comparing DFT- and MD-generated results explicitly. Specifically, the MD simulations exhibit both small scale density fluctuations and long range interface fluctuations of the bilayer. As a result a simple binning of the computational domain to calculate density profiles necessarily results in structures that are more broad than those found in the mean field DFT.

In order to compare density profiles, we have attempted to correct for the MD interface fluctuations by averaging density profiles locally based on the center of mass of the bilayer at a given position (y, z) in space. (Note that x is taken to be the direction perpendicular to

the bilayer-water interface). The correction was made as follows. For each tail bead, we find all the headgroups in a cylinder of radius R_{cyl} parallel to the x axis and centered on the tail bead. We average the x -positions of the head groups on each side of the bilayer in this cylinder to produce an average x position x_{top} for the top leaflet and x_{bot} for the bottom leaflet of the bilayer, and use these to find the midpoint of the bilayer as $x_{mid} = 0.5(x_{top} + x_{bot})$. The x position of the tail bead is then corrected by the difference between x_{mid} and the actual midpoint of the simulation box. An identical correction is done for all the solvent molecules. The head group beads are corrected by the same factor as the tail beads to which they are bonded. This correction procedure is analogous to fitting a surface over the fluctuating bilayer and then correcting that surface to be flat, and is similar to a method used previously [6].

We tried several values of the radius of the averaging cylinder. We need R_{cyl} to be large enough to contain enough head groups for good statistics, while still being significantly smaller than the average spatial extent of the interface fluctuations. We found $2 \leq R_{cyl} \leq 4$ to give indistinguishable results. A comparison of uncorrected and locally averaged MD density profiles is shown in Fig. 6.5 for two temperatures, $kT/\epsilon = 1$ and $kT/\epsilon = 1.3$. The local averaging sharpens the density profile of the head groups and the interface between the lipids and the solvent considerably, and narrows the density profile of the tail beads slightly as well. In addition, there is an apparent depletion of tail beads in the center of the bilayer that is not obvious if bilayer midpoint corrections are not applied.

Since DFT and MD calculations are done in different ensembles, it is necessary to establish a strategy for mapping between ensembles. If the theory were exact we would expect all possible mappings to yield identical results; however this is not the case. While temperature can be set identically for both calculations, the remaining two state variables (ρ_b and x_s in the DFT calculations) are set based on observables from MD simulations. Many observables could be used, but we consider (1) the solvent density far from the bilayer, (2) the constraint of zero surface tension, and (3) the observed A_L in the MD bilayer simulations in Fig. 6.5.

Figure 6.6 provides the data used to identify the DFT state points that correspond most closely with the MD profiles in Fig. 6.5. Beginning with the solvent density, in the MD simulations we found that far from the bilayer $\rho_s \sigma^3 = 0.74$ at $kT/\epsilon = 1.0$, and $\rho_s \sigma^3 = 0.61$ at $kT/\epsilon = 1.3$. Figures 6.6A and B show DFT predictions for the variation of the solvent density far from the bilayer as a function of ρ_b , x_s , and T . Since the solvent density is relatively insensitive to x_s at least over the range $0.2 < x_s < 0.8$, we use the data in Fig. 6.6A to set $\rho_b \sigma^3 = 0.68$ for $kT/\epsilon = 1.0$, and $\rho_b \sigma^3 = 0.59$ for $kT/\epsilon = 1.3$.

Figure 6.6C shows DFT calculations of the area per lipid as a function of x_s while holding $\{\rho_b \sigma^3 = 0.68, T = 1.0\}$ or $\{\rho_b \sigma^3 = 0.59, T = 1.3\}$ constant. The MD simulations had $A_L = 5.1 \sigma^2$ at $kT/\epsilon = 1$ and $A_L = 6.2 \sigma^2$ at $kT/\epsilon = 1.3$. To match these values, Fig. 6.6C can be used to identify $x_s = 0.392$ and $x_s = 0.364$ for $kT/\epsilon = 1.0$ and 1.3 respectively.

If we choose instead to constrain the solvent densities to the MD values and the surface tension to zero, the two state points are found to be $\{x_s = 0.442, \rho_b \sigma^3 = 0.68, kT/\epsilon = 1\}$

and $\{x_s = 0.415, \rho_b \sigma^3 = 0.59, kT/\epsilon = 1.3\}$. Paper I discusses the identification of zero tension bilayers; we just note here that there is only one zero tension point in a fixed $\{\rho_b, T\}$ slice of the phase space.

Finally, Fig. 6.6D shows the area per lipid as a function of ρ_b for zero tension bilayers at $kT/\epsilon = 1.3$. This figure shows that the DFT cannot predict areas per lipid as large as those observed in zero tension MD bilayers. This may be a consequence of the high solvent pressures in this theory. Therefore, it is not possible to use $\gamma = 0$ and A_L as simultaneous constraints on the mapping of ensembles. However we note that in designing DFT approaches for bilayers, achieving this mapping would be optimal.

Figure 6.7 shows DFT predictions for the bilayers using the $\{\rho_s, A_L\}$ and $\{\rho_s, \gamma = 0\}$ constraints to determine state points as detailed above. These structures should be compared to the MD results in Fig. 6.5. Overall the agreement is quite good. The basic structure of the bilayer (tails to the center, head groups at the interface, solvent excluded) is found by both methods. The magnitudes of the density profiles are similar as well, and both calculations predict fluid bilayers. However, there are some notable differences. The DFT predicts bilayers that are slightly narrower with a higher tail peak density and some additional structure in the center of the bilayer. These DFT profiles do not have the depletion of tails observed in the center of mass corrected MD profiles, although such features are predicted at other state points (see Figs. 2 and 4B of paper I). We expect that an improved solvent model would have lower pressure at a given bulk density, and would likely result in bilayers with larger A_L .

For more detail on the structure, we compare the densities of the ends of the lipid tails in the two approaches. The MD snapshot shown in Fig. 6.4 implies that there is only a small amount of interpenetration of the two leaflets in the bilayer. We infer a similar result for the DFT bilayers based on the density profiles for the last site in the lipid tails, shown in Fig. 6.8A. We see that the end densities are qualitatively similar in the DFT and MD profiles. In particular, there is very little overlap in the distributions of the ends of the tails and the head groups. This implies that the leaflets cannot be fully interdigitated.

Further evidence for this picture is shown in Fig. 6.8B where we plot the site density distribution for each of the sites on the chain from the DFT calculations. Note that the chain is symmetric, and these site densities include contributions from each pair of identical beads (in the two tails). This figure shows that while the density distributions for beads 1–3 are overlapping with a single peak in the center of the bilayer, beads 4–8 each have a distinct peak getting nearer to the head groups. Thus, the bilayer is most mixed in the center, with overlap of the leaflets most likely between the three end beads of the lipids on opposing monolayers.

In Fig. 6.9 we show both the area per lipid and the thickness of the bilayer as a function of temperature. DFT data along the zero tension line as well as along the single bilayer turning points, which define the limits of stability of the isolated bilayer, are included in the plots. (See Appendix B of paper I for a description of turning points.) As the bilayer thickness increases, the area per lipid decreases. For the physically realistic system along

the $\gamma = 0$ line, the area per lipid decreases roughly linearly with temperature in both the DFT and MD cases, in agreement with experiment [26] and previous simulations [8]. Given the approximations of the model, the agreement of the $\gamma = 0$ DFT bilayer properties with MD are quite good.

On decreasing temperature below about $kT/\epsilon = 0.95$, the DFT predicts ordered bilayers (see paper I). The transition from the fluid bilayer phase to the ordered bilayer phase was continuous, and there were first order phase transitions between ordered bilayers with different numbers of density peaks in the tail region. We looked for these ordered bilayers in our MD simulations, but did not find them. Instead, we found that as we lowered temperature the solvent eventually froze into a crystal while the lipid bilayer remained disordered and fluid.

In order to assess the impact of the approximate solvent model, we ran MD simulations with purely repulsive interactions for the solvent, at an external pressure of $P = 4\epsilon/\sigma^3$. At $kT/\epsilon = 0.6$, there was a small amount of ordering in the tails, with 9 peaks of amplitude $\approx 0.2\rho\sigma^3$ in the tail densities. However, this case still had a fairly liquid-like area per lipid of $A_L = 3.75\sigma^2$.

Finally, we also performed a simulation on one of the stiff 8-bead tail lipids from Ref. 8. We started the system in the gel phase at $kT/\epsilon = 1.05$ and then turned off the angle potential between beads. The bilayer melted. We then began again in the gel phase, lowered the temperature in the gel phase to $kT/\epsilon = 0.75$ and then turned off the angle potentials; we again found that the bilayer melted into a fluid phase. This is clearly seen in the area per lipid; for the stiff lipids in the gel phase at $kT/\epsilon = 0.75$ the area per lipid is $A_L = 2.30\sigma^2$ whereas for the flexible lipids at the same conditions we find $A_L = 3.87\sigma^2$.

Thus, it seems that the flexible lipid tails preclude a gel transition in the MD simulations. In comparing ordered DFT predictions with ordered bilayers based on stiff lipids, we find that ordering in DFT occurs in the same range of temperatures and ordered bilayers have similar A_L as in previously published MD simulations of the gel phase [8]. However, the stiff lipid MD simulations had bilayers with well separated leaflets where the number of observed density peaks in the tail region was the same as the number of tail beads from the end to the head group. We never observed fully separated leaflets with 16 peaks in the DFT calculations. Thus while ordering is an important feature of lipid bilayers, flexible chain models are likely not optimal for further investigations of ordered states.

6.5 Mechanical Properties of Lipid Bilayers

In this section we compare two fundamental mechanical properties of the fluid bilayers as obtained from the DFT and the MD simulations, namely the lateral stress profiles and the area compressibility modulus. Other important elastic constants of bilayer membranes include the bending modulus κ and the splay modulus $\bar{\kappa}$. These moduli are related to the

lateral pressure profiles, but to calculate them from our DFT would require solutions in curved geometries [27, 28], which is beyond the scope of this paper.

6.5.1 Lateral stress profiles

As described in paper I, the surface tension across the entire bilayer is given by

$$\gamma = \int_{-\infty}^{\infty} dx s(x) = \int_{-\infty}^{\infty} dx [P_N(x) - P_T(x)], \quad (6.7)$$

where the stress profile is $s(x) = P_N(x) - P_T(x)$, x is the direction normal to the bilayer, $P_N(x)$ is the normal component of the pressure tensor (a constant for all x as it must be for mechanical equilibrium), and $P_T(x)$ is the tangential component of the pressure tensor. The stress profile itself (or the lateral pressure profile, $\pi(x) = -s(x)$) is a quantity of considerable interest because it is related to the curvature elasticity of the membrane [27, 28]. Furthermore, changes in lateral stress in e.g. mixed lipid bilayers affect membrane protein conformational changes and activity [29, 30].

However, the lateral pressure $P_T(x)$ and hence the stress tensor $s(x)$ are not uniquely defined, as shown by Schofield and Henderson [31]. We can see this by noting the virial expression for the pressure tensor $\mathbf{P}$:

$$\mathbf{P}V = NkT\mathbf{I} + \frac{1}{2} \sum_{i=1}^N \sum_{j=1}^N \langle \mathbf{f}_{ij} \mathbf{r}_{ij} \rangle \quad (6.8)$$

where N is the number of particles, $\mathbf{I}$ is the unit tensor, and $\mathbf{f}_{ij}$ is the force between particles i and j separated by the vector $\mathbf{r}_{ij}$. The first term above is the kinetic contribution and the second is the interaction contribution. The nonuniqueness of $\mathbf{P}$ arises because the path between particles is not specified. The surface tension γ is invariant with respect to the choice of path, but $\mathbf{P}$ is not [31]. Choosing a straight line between particles i and j leads to the Irving-Kirkwood (IK) expression for the pressure [32], which we will use here in computing $P_T(x)$ from our MD results. However, other choices of path are possible.

We calculate the components of the IK pressure tensor directly in the MD simulations, using the expressions derived by Goetz and Lipowsky [4] to include the many-body terms in the interaction potentials. The Hardy method [33] is used to bin the stress into slices perpendicular to the x axis and to determine the contours between pairs of sites. Because $s(x)$ fluctuates considerably, we calculated it using the small system of 220 lipids, at $kT/\epsilon = 1.0$ and $kT/\epsilon = 1.3$, averaging over 10^7 and 3×10^7 timesteps, respectively. The resulting MD stress profiles are shown in Fig. 6.10. We find by integration of $s(x)$ that the surface tension is essentially zero, $\gamma = -0.037\epsilon/\sigma^2$, for $kT/\epsilon = 1$. For the higher temperature of $kT/\epsilon = 1.3$ we find $\gamma = -0.110\epsilon/\sigma^2$, so that in this case the bilayer is under a small

compression. We note that the stress profiles look very similar to that for a bilayer of more stiff coarse-grained lipids obtained by Goetz and Lipowsky [4]. They considered a coarse-grained lipid with four tail beads per tail and angle potentials between all sites so that the tails were semiflexible (they preferred to be straight). For both lipid models, one finds a total stress profile with several different peaks. The physics underlying the spatial variations in $s(x)$ is fairly clear. As it must be, the total stress is zero in the solvent region, and reaches a peak at the solvent/head group interface corresponding to the SH interfacial tension. The adjacent negative (compressive) region is associated with the attractive HH potential, and is followed by a second peak at the HT interface corresponding to the HT interfacial tension. The stress profile in the center of the bilayer tail region is negative, corresponding to a compression of the tails.

We note that we only expect $s(x)$ to be nonunique on a length scale of the order of the range of the interparticle interactions. In this work the largest cutoff was 3.5σ , clearly much shorter than the total thickness of the lipid bilayer. Variations in $s(x)$ on length scales longer than this should thus be unique. In atomistic simulations of lipid bilayers, the typical cutoffs for LJ interactions (10-12 Å) are a smaller proportion of typical bilayer thicknesses (e.g. 60 Å or so) than in this work; thus lateral pressure profiles calculated in atomistic simulations may be even more physically meaningful than those calculated here. Some (IK) lateral pressure profiles from atomistic simulations have been published recently in the literature, and show some of the same qualitative features as the coarse-grained models [34, 35].

The stress profile can also be calculated using the DFT from the excess surface free energy, as discussed in paper I. This calculation identifies the surface excess grand free energy density directly with the stress profile. Due to the nonuniqueness of $s(x)$, it is not *a priori* clear that this stress profile will be the same as the IK stress profile derived from the virial route. However, previous work on mean-field theories found that the same physics applied to the spatial behavior of $P_T(x)$ calculated from the energetic and virial routes [36]. We find that this is also the case here.

Figure 6.11 shows stress profiles calculated from Eq. (A10) in paper I and shifted by a constant so that $s(x) = 0$ in the bulk solvent region. The overall shape of the stress profiles is quite similar to the MD case, with the same number of major peaks. There is more structure in the profile at $kT/\epsilon = 1.0$, corresponding to the additional order in the bilayer itself as seen in Fig. 6.7A,B. In both methods the magnitudes of the peaks decrease with increasing temperature. For example, for the head groups the height of the peak in $s(x)$ decreases by a factor of 3.3 for the MD and 3.0 for the DFT calculations on going from $kT/\epsilon = 1.0$ to $kT/\epsilon = 1.3$. However, the magnitudes of the stress are much larger in the DFT than in the MD. In part this is related to the higher densities of the lipids in the DFT density profiles as compared to the corresponding MD calculations (see Fig. 6.7). We also note that no bilayer midpoint corrections were applied in the MD calculation of the stress profiles, so these must be broadened by transverse fluctuations of the bilayer. From Fig. 6.7 we see that the broadening effect in the density profiles reduces, for example, the head group density peaks by factors of about 1.5 and 2 for $kT/\epsilon = 1.0$ and $kT/\epsilon = 1.3$,

respectively. Thus some of the difference in the results is due to interface fluctuations.

Several previous authors have also calculated lateral stress profiles from mean-field theories of lipid bilayers [37, 38, 29]. All of these theories have assumed that the lipid tails form an incompressible liquid, and furthermore they calculate only the contribution of the tails to the lateral pressure profile. The general result is that there is a peak in the lateral pressure $\pi(x)$ in the middle of each leaflet of the bilayer, with the lateral pressure falling in the middle of the bilayer and near the tail/head interface. Recalling that $\pi(x) = -s(x)$, this is also what we see in the tail region of the bilayer. In previous work the lateral pressure is positive (and thus the stress profile is negative) throughout the tail region, as it must be since it is calculated as the constraint which must be applied to impose incompressibility and thus a constant density through the tail region. In contrast, we find that the peak in $s(x)$ in the tail region in the DFT goes above zero for $kT/\epsilon = 1.3$, indicating that the ends of the tails are under tension in our model for this state point.

6.5.2 Area compressibility

A final quantity that is straightforward to compare is the area compressibility modulus K_A of the membrane. This modulus can be obtained from the dependence of the surface tension on the area per lipid near the zero tension point,

$$\gamma \approx K_A (A_L - A_{L0}) / A_{L0} \quad (6.9)$$

for small deviations of A_L from its value A_{L0} at $\gamma = 0$.

We performed several MD simulations at $kT/\epsilon = 1$ and at constant area and constant normal pressure $P_N \sigma^3 / \epsilon = 0.5$ to obtain K_A from the variation of γ with A_L . The results are shown in Fig. 6.12A. From the linear fit we obtain $K_A = 15.2\epsilon/\sigma^2$. This value is comparable with previous results from coarse-grained MD simulations. [4] obtained $K_A = 14.3\epsilon/\sigma^2$ at $kT/\epsilon = 1.35$ for their stiff, 4-bead tail lipid. Using a similar model, [8] found a value of $K_A = 9.4\epsilon/\sigma^2$ for lipids with 6-bead tails at $kT/\epsilon = 1.05$.

For the DFT calculations, we note that the solvent density far from the bilayer is nearly constant as we change the area per lipid by changing x_s , as shown in Fig. 6.6B. Thus, $\gamma(A_L)$ can be obtained simply from the arc-length continuations done at constant temperature in paper I. The results for two different temperatures in the linear region around $\gamma = 0$ are also shown in Fig. 6.12A. For larger magnitudes of γ the curves become nonlinear and saturate at large values of A_L as shown in Fig. 6.12B, a result found previously [4, 6]. We find that $K_A = 9.72\epsilon/\sigma^2$ for $kT/\epsilon = 1.0$ and $K_A = 5.31\epsilon/\sigma^2$ for $kT/\epsilon = 1.3$. As expected, the compressibility is lower (higher modulus) at lower temperatures. The DFT is more compressible than the MD calculation, but is of the same order of magnitude. Because the DFT does not strictly enforce excluded volume in the chain configurations, we would expect it to yield a somewhat more compressible bilayer.

6.6 Summary

We have compared results of CMS-DFT and MD calculations for a freely-jointed coarse-grained model of lipids. The MD simulations showed that these flexible model lipids will self-assemble into a bilayer, and that the two leaflets of the bilayer are only slightly interdigitated. Comparisons of DFT calculations and MD simulations of lipids near surfaces demonstrated that the theory predicts packing in these asymmetric molecules rather well. Our single site solvent model is somewhat different than the LJ fluid used in the simulations, but is nevertheless simple to use.

We are able to obtain fluid bilayer solutions of the DFT which are quite similar to the corresponding MD bilayer profiles, once those profiles have been corrected for transverse fluctuations of the bilayer. We did not find an ordered or gel phase in the MD simulations, although ordered phases are predicted by the DFT; thus the theory does not compare well with simulation at low temperatures. However, the physical properties of the fluid bilayers are also similar in the DFT and MD calculations, and they do exhibit the same trends with temperature. Calculation of the lateral stress profile is particularly straightforward with the DFT and gives similar shapes as the Irving-Kirkwood stress profiles calculated with MD. We also showed that the area compressibility modulus compares favorably between the DFT and MD calculations, although the DFT system is somewhat more compressible. The DFT thus provides an attractive way to calculate mechanical properties of more complex systems such as mixed lipid bilayers or assemblies of proteins and lipids.

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Figure 6.1. A comparison of density functional (curves) and molecular dynamics (symbols) calculations on a liquid of model lipids near surfaces. We show density profiles for the head groups (plus signs) and tail beads (circles) near a surface located at $x = 0$. The various cases are: (A) Repulsive chains at a repulsive wall with a bulk density of $\rho_b\sigma^3 = 0.711$, (B) attractive chains at an attractive wall with a bulk density of $\rho_b\sigma^3 = 0.695$, (C) attractive chains at a repulsive wall with $\rho_b\sigma^3 = 0.746$, and (D) a modified prediction to the attractive chain repulsive wall case where the repulsive part of the direct correlation functions were reduced in magnitude by a factor of 2.5.

Figure 6.2. A comparison of density functional (solid curves) and molecular dynamics (symbols) calculations on solvated lipids near surfaces. The two cases are: RR with a bulk density of $\rho_b\sigma^3 = 0.709$ and a solvent fraction of $x_s = 0.630$, showing A) headgroups (solid circles) and tails (plus symbols), and B) solvent density profiles (triangles); and AA with a bulk density of $\rho_b\sigma^3 = 0.683$ and solvent fraction $x_s = 0.635$, showing C) headgroups (solid circles) and tails (plus symbols), and D) solvent density profiles (triangles).

Figure 6.3. Pressure as a function of solvent density for three temperatures: $kT/\epsilon = 0.85$ (dashed curves), $kT/\epsilon = 1.0$ (dotted curves), and $kT/\epsilon = 2.0$ (solid curves). The upper curves are calculated with the current DFT theory, the lower set of curves is from the equation of state for Lennard-Jones fluids determined by simulation [39], and the dash-dotted curve is the Carnahan-Starling (CS) equation of state for hard-sphere fluids [40].

Figure 6.4. Snapshot of an MD simulation of a solvated lipid bilayer at $kT/\epsilon = 1.0$ and $P = 0.5\epsilon/\sigma^3$, with $n_L = 220$. The large blue (dark) spheres are the headgroups, and the smaller cyan (gray) spheres on the outside of the bilayer are the solvent. The lipid tails have been colored according to which leaflet they belong to; upper (red or dark gray) and lower (yellow or light gray) leaflets of the bilayer only interpenetrate over a small distance in the center of the bilayer.

Figure 6.5. Density profiles from two MD simulations. Both were run at $P\sigma^3/\epsilon = 0.5$, but the first (A) and (B) was run at $kT/\epsilon=1.0$ while the second (C) and (D) was run at $kT/\epsilon = 1.3$. Both (A) and (C) show density profiles calculated by straightforward binning. (B) and (D) show profiles in which bilayer midpoint corrections have been applied. In all profiles, the tail beads are shown in solid lines, the head groups are shown in bold lines, and the solvent is shown in a dotted line.

Figure 6.6. Data used to pin the DFT ensemble in order to compare with MD simulations on lipid bilayers. (A) shows the relationship between observed solvent density far from the bilayer and total density in the bulk fluid for $kT/\epsilon = 0.9$ (dotted lines), $kT/\epsilon = 1.3$ (dashed lines), and $kT/\epsilon = 2.5$ (solid lines) at both $x_s = 0.1$ (no symbols) and $x_s = 0.5$ (with symbols). (B) shows the variation of the solvent density with x_s for the particular case of $\rho_b \sigma^3 = 0.59$ and $kT/\epsilon = 1.3$, (C) shows the relationship between the area per lipid molecule in the bilayer and the chemical potential variable x_s at the two state points $\{kT/\epsilon = 1.3, \rho_b \sigma^3 = 0.59\}$ (open circles) and $\{kT/\epsilon = 1.0, \rho_b \sigma^3 = 0.68\}$ (closed circles), and (D) shows the area per lipid molecule measured at the point of zero surface tension varying with total bulk density $\rho_b \sigma^3$, when the temperature is held constant at $kT/\epsilon = 1.3$.

Figure 6.7. DFT predictions for lipid bilayer profiles for comparison with MD predictions in Figs. 5B and 5D. (A) and (B) show results for $kT/\epsilon = 1.0$ while (C) and (D) show results for $kT/\epsilon = 1.3$. The DFT state points for (A) and (C) were set based on matching solvent density far from the bilayer and area per lipid in the observed bilayer. The state points for (B) and (D) were chosen by matching the solvent density and requiring the bilayer to be at zero surface tension.

Figure 6.8. Site density distributions in zero tension bilayers at $kT/\epsilon = 1.0$. (A) compares the end site density distribution from MD (thick solid curve) and DFT (thin solid curve), as well as the head group densities from MD (dotted curves) and DFT (dashed curves). (B) shows DFT predictions for all the different sites on the lipid, as labeled. In both cases, the center of the bilayer is found at $x/\sigma = 0$.

Figure 6.9. Bilayer properties for the coarse-grained lipid models: (A) the area per lipid, and (B) the bilayer thickness. Dashed lines show DFT bilayer properties on the turning points (the limits of stability of the isolated bilayer), while the solid line shows the DFT data along the $\gamma = 0$ line. The MD simulations, shown in the filled circles, were performed for systems with $n_L = 450$ and at a pressure of $P\sigma^3/\epsilon = 0.5$, for which γ is close to zero.

Figure 6.10. Stress profiles calculated from an MD simulation of 220 lipids at $P\sigma^3/\epsilon = 0.5$ and (A) $kT/\epsilon = 1$ and (B) $kT/\epsilon = 1.3$. The profile in (B) was averaged about the middle of the bilayer.

Figure 6.11. Stress profiles calculated from DFT calculations at (A) $\{kT/\varepsilon = 1, \rho_b \sigma^3 = 0.68, \gamma = 0\}$ and (B) $\{kT/\varepsilon = 1.3, \rho_b \sigma^3 = 0.59, \gamma = 0\}$, corresponding to the same state points as Fig. 7.

Figure 6.12. Surface tension as a function of area per lipid, relative to the zero tension area per lipid. (A) MD results for $kT/\epsilon = 1.0$ (square symbols with best linear fit) and DFT results for $kT/\epsilon = 1.0$ (dotted line) and $kT/\epsilon = 1.3$ (dashed line), corresponding to the same state points as Fig. 7. (B) shows the full nonlinear behavior of the DFT results over a wider range of $(A_L - A_{L0})/A_{L0}$.

Chapter 7

Comparisons of different lipid models with DFT

7.1 Motivation

In this section we examine the effects of varying some of the parameters of the coarse-grained lipid model used in the DFT calculations. These results should be compared with those in the section, “A density functional theory approach for coarse-grained lipid bilayers”. We had several motivations to look at different lipid models:

1. Can we find models that give consistent values for lipid properties and that are reasonable models for real lipid bilayers?
2. Can we find a lamellar phase with a reasonable amount of solvent between the bilayers by going to smaller solvent size?
3. What happens to our “unbinding” transition as we change the model?
4. Do we still get ordering with overlapped spheres or was that an artifact of tangent spheres?

7.2 Models

We attempt to answer these questions by looking at two modifications to the model. Our original tangent site model with headgroups of size $\sigma_h = 1.44\sigma$ and tail and solvent groups of sizes $\sigma_t = \sigma_s = \sigma$ will be denoted by Model I.

7.2.1 Model II: Tangent spheres, small solvent

Here we consider lipid chains still consisting of tangent spheres, with $\sigma_h = 1.44\sigma$ and $\sigma_t = \sigma$. However, we consider a more realistic size for the solvent spheres. For DPPC, the total volume of the head group is $320.9 \text{ \AA}^3$ while the volume of a water molecule is about $30 \text{ \AA}^3$, so the ratio of water volume to head group volume for this case is about 0.093 [1]. If we enforce the same ratio in our model lipid, we obtain $\sigma_s = 0.82\sigma$. For simplicity, we explore this model keeping the total site density fixed at $\rho_b\sigma^3 = 0.59$ as before. This means that at the same value of x_s , the actual volume of solvent will be smaller than for the larger solvent we used previously.

7.2.2 Model III: Overlapped spheres, large solvent

Here we consider lipid chains in which the monomers are allowed to overlap. This should give a more realistic representation of the lipid molecule, since generally bond lengths are considerably shorter than van der Waals radii in real molecules. To define the bond length, we consider the bond length between CH_2 groups which is approximately $1.54 \text{ \AA}$. Then the effective bond length between groups of 2 CH_2 groups each is about $2.58 \text{ \AA}$. Assuming that each tail bead represents two CH_2 groups, we can identify $\sigma = 4.75 \text{ \AA}$ by equating volumes. Then we find that a bond length of $2.58 \text{ \AA}$ corresponds to $l_{tt} = 0.54\sigma$. We arbitrarily set the head-head and head-tail bond lengths to have the same ratio of $l_{tt}/\sigma = 0.54$, so that $l_{hh} = 0.78\sigma$ and $l_{th} = 0.66\sigma$.

The next question is what bulk density to use in this model. Since the sites in the lipid molecules now overlap, the lipids occupy a smaller total volume than before. The volume can be calculated following work by Abrams and Kremer [2]. We find for the bead sizes chosen here, the lipid now has a volume of about $8.26\sigma^3$, compared to the tangent site lipids which have a volume of about $11.5\sigma^3$. We attempted to set the bulk site density for this model by maintaining the same lipid packing fraction as at the bilayer-lamellar-solvent triple point in the tangent site model. This gives a total site density of $\rho_b\sigma^3 = 0.728$. However, we had some numerical difficulties at this density and so mostly looked at a system with a higher bulk site density of $\rho_b\sigma^3 = 0.825$.

7.3 Results

Here we compare our results to those obtained for Model I. First we note that in all cases, the direct correlation functions $c(r)$ are much more concentration dependent than before. This isn't surprising since in the original model the solvent was quite similar to the lipid tails, so the environment in model I in the homogeneous phase was relatively concentration independent. Fig. 1 shows the $c(r)$'s for Model II, and Fig. 2 shows those for model III.

We checked that the interpolation of the $c(r)$'s made sense by running a few independent PRISM calculations, and found quite good agreement for the case of overlapped spheres and large solvent at $\rho_l \sigma^3 = 0.825$, as shown in Fig. 2C,D.

Figure 7.1. Model II: Direct correlation functions for all $\alpha\beta$ pairs as a function of bulk solvent number fraction x_s at a bulk density of $\rho\sigma^3 = .59$. Figure A shows the direct interactions c_{tt} , c_{hh} , c_{ss} , while figure B shows the cross terms c_{th} , c_{ts} , c_{hs} . Note that the subscripts indicate tail beads, head group beads and solvent. The various curves are $x_s = .001$ (solid line), $x_s = .333$ (dashed line), $x_s = .667$ (dotted line) and $x_s = .999$ (dashed-dotted line) for all cases.

7.3.1 Tangent spheres, small solvent

Here the lipid molecules still consist of tangent spheres, but now $\sigma_s = 0.82\sigma$. In general, we found that this model behaved quite similarly to Model I. The main results can be summarized as follows:

1. We found that both bilayers and lamellar phases look qualitatively similar to those found from Model I. In particular, the lamellar phase is still not very hydrated, at least at the binodal point at $T = 1.73$. See Fig. 3. The density profiles at the two extremes of the single bilayer stability also looked similar to those in Model I.
2. The continuation curve at $T = 1.73$ had less structure than that for model I at $T = 1.3$ (we note that it's not necessarily clear how the temperatures change with the model). In particular, we did not find the two fused bilayer state that we found with model I, although perhaps we looked at the wrong state point. The lamellar phase no longer crosses through the multinodal point as before, but instead occurs slightly to the left in a plot of free energy vs x_s at $T = 1.73$.
3. The shape of the bilayer binodal line is rather different than before, and in particular it intersects the high x_s curve describing the bilayer limit of stability near $T = 2.5$. The binodal appears to become independent of x_s at lower temperatures, around $T = 1.4$. Thus the range of existence for zero tension bilayers is smaller than before.
4. Elements of the phase diagram are shown in Fig. 4. We still have a critical point at high temperature. The transition from macrophases to microphases occurs near $T = 2.1$, similar to before, but the slopes of the bilayer/solvent and 1 bilayer-3 bilayer coexistence curves are much more shallow than before, and don't extend to as low a value of x_s . Also the bilayers seem to stop at higher temperatures. The biggest change from Model I is that the isolated bilayer phase no longer appears to be lowest in free energy anywhere; instead we have the lamellar phase. We performed a few calculations of the excess free energy curves for the lamellar and independent bilayer states, at temperatures of $T = 2.0, 1.73, 1.46$, and in all cases the lamellar phase had the lower free energy. So, the "unbinding" transition we had before seems to not exist in this model.
5. Fig. 5 shows some bilayer properties. We see that the area per lipid has similar magnitudes as in Model I but a different temperature dependence.
6. Finally, at sufficiently low temperatures the lipid chains still order, so this phenomenon was not dependent on having solvent sites of the same size as the tail sites. We did not explore the ordering in detail.

7.3.2 Model III: Overlapped spheres, large solvent

For the overlapped spheres model, we first look at results for $\rho_b \sigma^3 = 0.825$:

1. The continuation curve at $T = 2$ consists solely of different numbers of bilayer stackings, with no extra loops of any kind, and thus none consisting of fused bilayers.
2. The bilayer profiles are quite narrow and have pronounced depletions in the centers. The lamellar phase again looks very similar to the bilayer profiles at the multinodal

point. The densities in the tail regions are high because the bilayers are so narrow. See Fig. 6.

3. We again found similar behavior for the zero-tension bilayers. The coexistence curves that we calculated are shown in Fig. 7. We did not examine the relative stability of the lamellar and bilayer phases.
4. We hoped that this model might work better for comparing bilayer properties to those of real lipids, since in this model the volumes and bond lengths in the lipid molecule are consistent with reasonable values. For zero tension bilayers we find areas per lipid in the range of $A_L/\sigma^2 = 3 - 4\sigma^2$, which corresponds to areas of 67.7 to 90.2 $\text{\AA}^2$. The thickness of the bilayers is in the range of 5σ , which corresponds to a thickness of 23.8 $\text{\AA}$. The areas per lipid are well within the experimental range for phospholipids such as DPPC, but the thickness is too small. This is presumably due to the excess flexibility of our lipid tails. We conclude that simultaneously obtaining a physically realistic area per lipid and bilayer thickness requires introduction of some chain stiffness into the model.

Finally, at the lower density of $\rho_b\sigma^3 = 0.728$, things become quite different. We initially had difficulties finding more than one bilayer in the computational domain. We finally did construct a two-bilayer state and found, at $T = 2.3$, that the two bilayer branch is completely disconnected from the single bilayer and solvent branches. This made it more difficult to sort out the behavior of this model as we had done previously. We thus did not pursue this further. At this density, the zero-tension single bilayer appeared to be stable over only a narrow range of temperatures, from about $T = 2.2$ to $T = 2.6$.

7.4 Conclusions

To answer the questions originally posed in the motivation section: first, for our coarse-grained lipid bilayers to have geometrical properties corresponding to real bilayers will require introducing some chain stiffness into the model. The random-walk chains are too coiled and lead to overly thin bilayers. In both new models, our lamellar phases were still not very hydrated in these models, so the solvent size does not appear to have been the problem. We found that the lamellar to independent bilayer unbinding transition is indeed model-dependent, as it is absent in Model II where the solvent size is reduced. This is perhaps not suprising, since this transition appears to be due to packing effects, which change when one changes the size of the solvent. Finally, although we did not explore this fully, we did not find clear signs of ordering in the overlapped lipids. This was difficult to determine however since the overlapped lipids make such thin bilayers even in the fluid phase.

The calculations presented here were done as a summer project by Julieanne Heffernan, a student intern at Sandia during the summer of 2004.

Figure 7.2. Model III: Direct correlation functions for all $\alpha\beta$ pairs as a function of bulk solvent number fraction x_s at a bulk density of $\rho_b \sigma^3 = .825$. A) shows the direct interactions c_{tt} , c_{hh} , c_{ss} , while B) shows the cross terms c_{th} , c_{ts} , c_{hs} . The various curves are $x_s = .001$ (solid line), $x_s = .333$ (dashed line), $x_s = .667$ (dotted line) and $x_s = .999$ (dashed-dotted line) for all cases. C) and D) show a comparison between $c(r)$ data generated by PRISM and interpolated linearly: $x_s = .333$ (dashed line), $x_s = .667$ (dotted line), $x_s = .5$ by PRISM (solid line), and $x_s = .5$ interpolated between the other two (dashed-dotted line).

Figure 7.3. Model II: Density profiles at the multimodal point $x_s = .5439$, $T = 1.73$. The two cases shown are the single isolated bilayer (A) and the infinite stack of lamellae (B).

Figure 7.4. Model II: Coexistence lines shown in the $T - x_s$ space at two different resolutions. The various curves show: 1 bilayer-3 bilayer coexistence (solid-line), solvent macrophase-bilayer coexistence (dash-dotted line) and the solvent macrophase-lipid macrophase coexistence (dashed-line).

Figure 7.5. Model II: Area per lipid A_L and thickness t along the binodal (solid line) and along the spinodals (dashed lines) for a single bilayer as a function of temperature.

Figure 7.6. Model III: Density profiles at the multinodal point $T = 2.0, x_s = .3037$. The two cases shown are the single isolated bilayer (A) and the infinite stack of lamellae (B).

Figure 7.7. Model III: Coexistence curves showing: 1-bilayer-3bilayer coexistence (solid-line), solvent macrophase-bilayer coexistence (dotted line) and the solvent macrophase-lipid macrophase coexistence (dashed-dot-line).

References

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Chapter 8

Control of Membrane Structure and Organization through Chemical Recognition

8.1 Introduction

The recognition of molecules and ions at the cell membrane surface is the basis of cellular communication. The binding of these analytes from solution typically induces a reorganization of membrane components to form supramolecular structures that direct a specific membrane function or signaling process. A prominent example of molecular recognition induced membrane reorganization is the activation of T cells to antigen presenting cells. It is known that on the T cell membrane a $\alpha\beta$ heterodimer, along with other subunits, organize into a supramolecular structure to recognize and elicit specific immune responses to the antigen presented.^{1,2} Furthermore, the signaling from these organized subunits is facilitated by the aggregation of peripheral receptors. Aggregation of these receptors into the so-called “immunological synapse” has been observed at the microscopic scale via fluorescent labeling of the receptors.^{3,4} The aggregation of receptors, either in specific organized assemblies of a few components or in large, but less well defined structures, is a common theme for cell-cell and cell-environment communication.

Another type of organized assembly of molecules in cell membranes that has been linked to specific cellular functions is the lipid raft.⁵ These sub-micron structures are composed of various glycosphingolipids, cholesterol, long, saturated phosphatidylcholine lipids,⁶ and lipid-modified proteins assembled in ordered, liquid phase structures. These lipid components typically have elevated crystal (or gel)-to-liquid crystalline phase transition temperatures (T_m) relative to the rest of the membrane lipids, and their straight chain structure allows them to pack in ordered assemblies that are resistant to dissolution by detergents. Lipid rafts are believed to be associated with signal transduction⁷ (e.g., T cells, Ras signaling, etc.), as well as toxin and pathogen entry⁸ due to the selective partitioning of

gangliosides and specific proteins into these ordered liquid phase domains. The formation of these specialized structures and the trafficking of lipids and proteins into and out of them have spurred a flurry of recent interest in the biological community.

The chemical and physical properties of membrane components dictate their relative positioning in the membrane and their inter-molecular associations. Clearly, these associations and positions are momentary as signaling and molecular trafficking are dynamic processes. The binding of specific analytes from solution or, alternatively, the chemical modification of membrane components, is the trigger that determines the spatial and temporal positioning of these components and the structures they form. Understanding the forces that drive these reversible reorganizations in lipid membranes would impact not only the biological sciences but may also provide a means to control nanostructure in a dynamic medium.

Most of these membrane associations are complex, involving numerous long- and short-range interactions. A study of these interactions will require a simplification of the system, both with the membrane component and the guest substrates, to isolate specific interactions. This review describes the study of host-guest systems in lipid membranes using simple lipid structures to bind metal ions, small molecules, polyelectrolytes, and proteins, and the reorganization of the membrane components as they respond to the binding event. Using a variety of techniques it is possible to show that molecular reorganization in lipid membranes occurs at the micron- to nano-scale. From these studies we can begin to gain insight into the subtle interactions that play various roles in receptor aggregation or even lipid raft formation, and the temporal and spatial control that is possible with such interactions. Furthermore, an understanding of such self-organized systems may aid in the development of unique biocompatible and reversible sensor materials, selective affinity surfaces for separations, and templates for nanoarchitecture.

8.2 Phase Separation in Phospholipid Membranes Induced by Cation Binding

As mentioned above, the formation of phase separated domains in cell membranes is known to be caused by differences in the structures of lipid components. Model studies performed on Langmuir monolayers⁹ and lipid bilayers¹⁰ have confirmed this in phospholipid films containing two or more lipid structures. Phase separation can also be induced in membranes with initially miscible components through the binding of cations. This is due to the changes in the phase transition temperature upon cation binding, charge neutralization of anionic headgroups, and bridging interactions of those anionic lipids with metal ions.

In general, cation binding with phospholipid membranes increases the phase transition temperature.¹¹ This effect is attributed to changes in the electrostatic interactions at the headgroup position, conformation and packing of the lipids, and the headgroup hydration. Even protonation of the lipid's headgroup can lead to considerable increases in phase tran-

sition temperature.¹² The transition temperature of dimyristoylphosphatidylethanolamine (DMPE), for example, exhibits an increase of 30 °C upon changing the pH from 13 to 0. DMPE undergoes two pH dependent transitions corresponding to the two ionizable moieties of the headgroup. Protonation of the amino group increases the T_m from 24 °C to 49.5 °C, while protonation of the phosphate diester, at lower pH, causes a subsequent increase to 54 °C. Phosphatidylcholine lipids respond similarly to changes in pH, but experience only a single change in transition temperature at low pH (< 3) upon protonation of the phosphodiester. In this case increases of ~ 10 °C are observed (e.g., dimyristoylphosphatidylcholine [DMPC] $\Delta T_m = 13$ °C, dipalmitoylphosphatidylcholine [DPPC] $\Delta T_m = 8$ °C).

Both divalent and monovalent cations induce changes of the phase transition temperature of phospholipid bilayers, although to dramatically different extents. Monovalent ions, such as Na^+ , K^+ , Rb^+ , and NH_4^+ , induce small increases in T_m (several degrees) of phospholipid membranes at concentration levels ≥ 1 M.¹³ The exception to this is Li^+ , which can induce large changes in transition temperatures ($\Delta T_m = 40 - 50$ °C) for phosphatidylserine (PS) bilayers with LiCl concentrations of < 1 M. X-ray diffraction studies find that Li^+ causes a change in packing of the lipids and even induces phase separated domain formation.¹⁴ In contrast, Na^+ and K^+ ions do not induce any change in membrane structure indicating relatively weak associations with the lipid headgroups.

The biologically relevant divalent metal ions Ca^{2+} and Mg^{2+} have much larger effects on the membrane's phase transition compared to monovalent ions, although their binding constants (K_a) differ by only about an order of magnitude. An example of a large phase transition change occurs in bilayers of dimyristoylphosphatidylserine (DMPS), which exhibit a T_m of ~ 40 °C in the presence of ammonium ion, but registers an increase to 155 °C with the addition of 0.5 M CaCl_2 .¹⁵ With Mg^{2+} the effect is not as pronounced but is still significantly higher than the monovalent ions. X-ray diffraction, infrared spectroscopy, and ^{31}P NMR spectrometry have determined that Ca^{2+} interacts mainly with the phosphodiester moiety displacing waters of hydration and altering the headgroup conformation. The interaction of Ca^{2+} with initially homogeneous lipid bilayers is also believed to cause phase separation within the membrane leading to fusion of phospholipid vesicles.¹⁶

Lateral phase separation in mixed membrane systems induced through Ca^{2+} or Mg^{2+} binding has been an important topic of investigation over the past three decades. The introduction of temporal and spatial changes in membrane structure through reversible chemical ligation is an intriguing possibility as a method to direct and enable specific membrane function. Thus, a number of studies have been used to evaluate lipid chemistry, membrane composition, and the effects of metal ion affinity upon phase separation. Some of the first studies were performed by calorimetry. In these studies¹⁷ bilayers composed of 66% PS lipids with 34% diphosphatidylcholine (DPPC) were found to be initially miscible, exhibiting a broad transition centered around 16 °C. Upon the addition of CaCl_2 (10 mM) the two lipids phase separate into pure DPPC domains and areas rich in Ca^{2+} bound PS lipids. A similar effect was seen with membranes composed of phosphatidic acid (PA) and PC lipids.¹⁸ Spectroscopic methods provide an alternative means to observe lateral phase separation phenomena, allowing measurements to be conducted on bilayers containing smaller

mole fractions of specific lipids. Electron spin resonance (ESR) studies of bilayers containing spin-labeled PC lipids with unlabeled PS lipid have shown that Ca^{2+} can cause aggregation of the PS lipids into solid phase domains that excluded the fluid phase PC lipids.¹⁹ Fluorescence spectroscopy can detect phase separation of phosphoinositide (PI) lipid in PC lipid bilayers using the excimer and monomer fluorescence of pyrene-labeled PI lipids.²⁰ Calcium induced clustering of PA lipids in an egg-PC bilayer can even be observed at the micron level via fluorescence microscopy.²¹

From the examples mentioned above, and in many others not cited here, it has been shown that divalent metal ions actuate the phase separation of negatively charged lipids from charge neutral lipids (Figure 1). The observed phase separation is partially due to the change in miscibility of the negatively charged lipids as their T_m increases upon metal ion binding, as described previously. However, another important factor is the neutralization of anionic charge, which eliminates electrostatic repulsion allowing lipids of like structure to associate. Additionally, the divalent metal ion can act as an ionic bridge, attracting and interacting with more than one lipid in a local environment. The interaction of lipid membranes to divalent metal ions is not limited to Ca^{2+} and Mg^{2+} . Indeed, as will be described in the next section, it is possible to tailor the membrane surface to bind an array of multivalent metal ions with varied membrane reorganizational responses.

8.3 Metal Ion Recognition with Synthetic Lipids

Although metal ion interaction with biological lipids can yield remarkable phase changes, these naturally occurring lipids possess a limited range of functionality. Tailoring of the membrane with alternative functionality expands the diversity of membrane interactions with aqueous phase analytes leading to unique responses and structures formed within the membrane. Furthermore, labeling of the lipids with fluorophores and chromophores facilitates the spectroscopic and microscopic monitoring of the membrane as the lipids change aggregational state in response to metal ion recognition.

Earlier work by Kunitake²² and Singh²³ found that amine functionalized amphiphiles aggregated via complexation with divalent ions. Their synthetic amphiphiles contained azobenzene chromophores in the midsection of the hydrophobic tail that report on the lipid aggregation and packing structure. The diamino-functionalized amphiphile **1**, shown in Figure 2, is initially immiscible in the dihexadecyldimethyl ammonium bromide (**3**) bilayer yielding an absorption maximum (λ_{max}) of 312 nm, indicative of H-like (vertically oriented, close packing) aggregation of the azobenzene chromophores. Upon protonation of the amino headgroup with acid the λ_{max} shifts to 355 nm revealing an electrostatically induced dispersion of **1**. Subsequent addition of 0.4 mM CuSO_4 to the mixture produces a reaggregation. For this system, the dianionic sulfate group, not the metal ion, was attributed as the cause of amphiphile aggregation resulting from electrostatic attraction. On the other hand, an azacrown derivative (**2**) of the above mentioned azobenzene amphiphile aggregates in response to the binding of Cu^{2+} ion. Using the same bilayer matrix lipid **3**,

and the same optical detection scheme, the azacrown amphiphile changes from an initially dispersed state to one that is aggregated as the metal ion complexes with more than one amphiphile (bottom of Figure 2).

Converse to the lipid cluster formation observed above, metal ion binding can also produce lipid dispersion in membranes. A number of lipids with metal ion recognition sites at the headgroup position have been synthesized in our laboratory. Each of these lipids were designed to mimic biological lipids with two hydrophobic tails off the C1 and C2 carbon positions of a glycerol backbone and a hydrophilic spacer and headgroup off the C3 carbon. The lipids were tagged with a pyrene fluorophore on one of the hydrophobic tails to allow an evaluation of the aggregational state of these receptor-lipids upon the recognition and chelation of their target metal ions. By monitoring the changes in the pyrene excimer ($\lambda_{max} = 470$ nm) to monomer ($\lambda_{max} = 375$ nm) emission a relative assessment of the lipid aggregation, or phase separation, could be attained. Some excellent examples using pyrene-labeled lipids to evaluate phase transition temperatures, phase separation, and diffusion rates can be found elsewhere.^{24,25,26}

Figure 3 shows the basic lipid structure and the headgroups of three synthetic lipids – PSIDA, PSDSDA, and PS18C6. Lipid membranes prepared with 5% of a pyrene-labeled lipid and 95% of distearylphosphatidylcholine (DSPC) exhibited a selective fluorescence response to particular divalent metal ions. Bilayers prepared with PSIDA²⁷ responded selectively to the presence of Cu^{2+} , PSDSDA²⁸ was selective for Hg^{2+} , and PS18C6²⁹ selective for Pb^{2+} . The sensitivities were in the sub-ppb range with linear responses covering approximately three orders of magnitude in metal ion concentration. Unlike the previously mentioned phase separation that occurred upon metal ion chelation with negatively charge phospholipids, these pyrene-labeled lipids initially form aggregated structures that dispersed into the DSPC matrix upon metal ion binding. The pyrene-labeled lipids are fluid phase at room temperature whereas DSPC is in the solid phase ($T_m = 55$ °C), thus the two lipids spontaneously phase separate into domains. Further, the pyrene-labeled lipids are charge neutral at $\sim$ pH 7. Unlike the anionic phospholipids mentioned above, where the binding of metal ions extinguishes the negative charge repulsion between lipids allowing or promoting lipid aggregation, instead the headgroups of the pyrene lipids become positively charged upon metal ion binding. The metal bound lipids thus become electrostatically repulsive resulting in their dispersion into the DSPC matrix. A schematic illustration of the metal ion induced membrane reorganization is shown in Figure 4.

The nanoscale structural changes in the membrane that were produced with the addition, and subsequent removal, of metal ions in solution were monitored using *in situ* atomic force microscopy (AFM) on supported lipid bilayers. AFM images were obtained of PSIDA/DSPC³⁰ and PS18C6/DSPC²⁹ bilayers formed on mica via vesicle fusion. The pyrene-labeled lipids were designed with a triethylene glycol spacer so that the headgroup may extend 8 – 10 Å further into solution from the phosphocholine headgroups of DSPC. This height difference provided a means to distinguish the areas rich in the pyrene lipids from those rich in DSPC. Figure 5 shows AFM images of the 20% PS18C6/DSPC bilayer before and after addition of $\text{Pb}(\text{NO}_3)_2$ at 100 μM . Addition of the metal ion caused a dra-

matic loss in density and structure of the PS18C6 aggregates, as the metal bound lipids dispersed into the DSPC matrix. Removal of the Pb^{2+} ions from the membrane, with the addition of ethylenediamine tetraacetic acid (EDTA), allowed reaggregation to occur and many of the nanostructural features in the initial membrane structure were recovered. Such temporal and spatial control over self-assembled materials may be useful in the development of actuated platforms for nanoscale engineering and materials architecture.

These pyrene-labeled membranes respond to different metal ions with sensitivities determined by the affinity of the receptor at the headgroup position.³¹ Thus, the selectivity of a membrane can be readily tuned by synthetically substituting the headgroup with functionality selective for targets of interest. The bilayers are responsive to di- and trivalent metal ions, however, no response has been observed for monovalent ions (e.g., Na^+ , K^+ , Rb^+ , Cs^+ , Ag^+) at concentrations up to 1 M. Apparently, either the headgroup on the membrane surface has little or no affinity for monovalent ions or the electrostatic charge produced by the binding of the monovalent ion produces insufficient electrostatic repulsion to overcome the energetic barrier that separates the fluid from the solid phase domains. Further details into the mechanism of the metal ion induced lipid dispersion will require efforts in modeling and simulation to estimate the energetics of interactions within this dynamic and multi-molecular system.

Association with Small Molecules and Polyelectrolytes

Membrane functionality can be further tailored to selectively bind with small molecules. Specific interactions that occur between the membrane and the small molecule guests can, like the metal ions, invoke a change in lipid packing and orientation relative to the interface. Such structural changes in the membrane can subsequently result in aggregation or fusion of lipid bilayer vesicles.³² An investigation of the relationship between molecular recognition on the membrane surface and the organization of lipid components in the membrane, however, requires analyses at the molecular to nanoscale in contrast to these macroscale phenomena. Membrane imbibed optical probes provide a means to monitor the structural changes of the membrane upon host-guest interaction, while scanning probe techniques could yield information on the nanoscale structural changes. Binding isotherms can also render insights into the reorganization of membrane components through the associations between the guest molecule and the membrane. Liposome and supported bilayer studies are, however, difficult experiments to conduct. So, for ease of study the bulk of previous investigations have been conducted upon functionalized Langmuir monolayers, which provide good models for host-guest complexation studies with lipid membranes.

Amphiphiles with a variety of functionalities at the headgroup position and tail structures, some containing optical reporters, are reported extensively within the literature. Langmuir monolayer studies offer an array of spectroscopic, microscopic, and other physical measurements on the film either on the air-water interface or as deposited onto solid substrates as Langmuir-Blodgett films. The surface pressure – area (π -A) isotherm is the most

common method used to characterize membrane structural change upon substrate binding. In most cases, molecular recognition results in an expansion of the isotherm indicating a loss in packing order from the increase in steric bulk at the headgroup as the guest molecule intimately associates. UV-vis absorption and X-ray diffraction³³ are a couple of examples of spectroscopic techniques that can be applied to the monolayer as it exists at the air-water interface. In the case of UV-vis absorption, the amphiphile, or host molecule, must contain a chromophore that responds to aggregational change. X-ray diffraction, on the other hand, does not require any special labeling of either species. However, the technique is dependent upon specialized high-energy sources. Other techniques, such as Fourier Transform infrared (FT-IR) spectroscopy, X-ray photoelectron spectroscopy (XPS), and scanning probe analysis, are conducted upon monolayers transferred onto solid substrates. Although the data collected from these latter techniques have corresponded well with the general characterization of the monolayer, they do carry with them the implicit assumption that the stoichiometry and mode of interaction at the air-water interface has transferred faithfully.

Through careful design to create uniquely functionalized monolayer films, hydrogen bonding, electrostatic, and van der Waals interactions with aqueous phase guest molecules, can be probed individually or collectively. Lipids functionalized with nucleobase and nucleoside headgroups have exhibited selective affinity for their complementary base pair either as substrates dissolved in the subphase³⁴ or through inter-lipid association within the membrane.³⁵ An expansion of the monolayer indicated the 1:1 binding interaction between the headgroup and a complementary but sterically bulky nucleobase (Figure 6). Interactions between the host and guest have also been probed with FT-IR spectroscopy confirming the tight pairing of melamine-barbituric acid hydrogen bond networks,³⁶ N-heterocycles with Kemp's acid derivatives,³⁷ and peptide-peptide interactions,³⁸ to mention just a few examples.

An excellent illustration of how molecular recognition can influence lipid aggregation is in the study of monovalent versus multivalent interactions.^{39,40} A guanidine-functionalized amphiphile, containing an azobenzene chromophore in the hydrophobic tail, can bind with guest substrates through electrostatic as well as hydrogen bonding interactions. In the presence of adenosine monophosphate (AMP) the guanidine-functionalized monolayer exhibited an expansion, as determined by surface pressure measurements and UV-vis spectroscopy of the azobenzene chromophores that found the aggregation of the amphiphiles to be disordered. Adenosine triphosphate (ATP), on the other hand, induced a condensation in the film and produced H-like aggregation of the amphiphiles. XPS analyses of the films found that the monolayer binds with a 1:1 stoichiometry of phosphate groups to the guanidines. Collectively, the data revealed that the 1:1 interaction with AMP created an increase in the amphiphile's molecular area due to the steric bulk of the bound guest molecule, whereas the 1:3 interaction of ATP to amphiphile produced an aggregation of the lipids as a result of multiple interactions between the guest molecule and the film (Figure 7).

Multiple-point binding interaction has also been observed with polyelectrolytes binding to lipid bilayers as liposomes and supported membranes. In one system, polymethacrylic acid was found to associate with the zwitterionic headgroup of DMPC via localized electro-

static interactions.⁴¹ Infrared spectroscopic analysis of the supported bilayer-polyelectrolyte complex found evidence of domain formation within the membrane. In another example, lipid “demixing” was observed in two-component lipid membranes containing an anionic lipid (i.e., PS, phosphatidylglycerol (PG), phosphatidylinositol 4,5-bisphosphate (PIP₂)) and PC lipid upon interaction with polycations.^{42,43} Initially, the lipids are mixed homogeneously in the membrane, but upon association with the oligomers of lysine the anionic lipids aggregated into domains. The oligopeptide complexation and subsequent domain formation was followed optically by fluorescence microscopy while the binding stoichiometry and thermodynamics were determined through binding isotherms of the peptide-membrane complex. Thermodynamic analyses of the data found that the free energy of electrostatic binding of the polypeptide overrides the loss in free energy produced by the “demixing” or phase separation of the membrane. Similar data were also found with the binding of cationically charged proteins, such as MARCKS and cytochrome c.^{44,45} Lipid demixing upon protein adsorption with these systems has been given in depth theoretical analysis with consideration of the adsorption free energy and the lateral interaction of bound proteins and lipids.⁴⁶

Recognition of Proteins

Protein-membrane recognition in synthetic systems is an effective model to study the complex interactions between two self-organized structures, each composed of an array of strong and subtle forces. This complexation, albeit simplified from the real biological system, is nonetheless complicated by multivalent, multifunctional interactions and dynamic structural changes in the protein that can introduce subsequent non-specific interactions. Focusing on the specific interactions and, to simplify matters, disregarding the ever present possibility of protein denaturation, membrane components can be induced to undergo various reorganizational processes upon protein recognition. Functionalized lipids that act as receptors for proteins can change from randomly distributed to aggregated, or clustered, structures as a result of multivalent interactions. Conversely, proteins can also bring about the dispersion of initially aggregated lipid species into the membrane as a consequence of the physical separation of the receptor sites on the protein. These membrane organizational changes can be most readily monitored spectroscopically,⁴⁷ although all the techniques previously mentioned have been applied.

As in the studies with small molecules, the Langmuir monolayer provides a model system that offers control over the lipid film structure while allowing spectroscopic and microscopic measurements to be made. Experiments performed at either constant surface pressure or constant area have indicated that the adsorption and denaturation of proteins to the monolayer surface is dependent upon the film’s pressure.⁴⁸ Low film pressure leads to rapid and extensive denaturation, whereas the protein structure is maintained with higher pressures. This important finding helped to establish that the membrane structure dictates to some degree the extent of non-specific interactions, which can dominate protein-membrane association. A correlated aspect of this finding was the observation that cytochromes *b*₅

and *c* preferentially adsorb to fluid phase portions of phospholipid films existing at the gel-fluid state.⁴⁹ The proteins are attracted not only to the chemical nature (i.e., electrostatic interaction between cytochrome and a dimyristoylphosphatidic acid (DMPA) membrane) of the surface but also the physical structure, which can promote hydrophobic interactions between the protein and the membrane.

Protein adsorption to monolayers functionalized with optically-labeled “receptor” lipids can be used to monitor reorganization of lipid components as the film responds to interactions with specific functionality on the protein surface. Some examples, featuring lipids with iminodiacetic acid- Cu^{2+} and mannose headgroups, were monitored by fluorescence microscopy to find that His-tagged and lectin proteins induced receptor aggregation through multivalent interactions.^{50,51} An important corollary for protein-targeted, functionalized films is their use as substrates for two-dimensional crystal growth.⁵² With controlled surface pressure, strong affinity for proteins, and dynamic restructuring of the self-organized lipid film Langmuir monolayers provide an excellent medium for crystal growth of membrane-associated proteins.

Bilayer membranes are, of course, more suitable models for understanding cell membrane-protein interactions. However, their structure and environmental conditions requires a different set of characterization techniques, which are typically challenging to perform and the data can be difficult to evaluate. Protein binding to the membrane surface can be detected by a number of methods that measure mass or viscosity changes in the membrane, but monitoring the change in membrane organization has been mostly limited to spectroscopy. Cytochrome *c*, for example, has been observed by ESR spectroscopy to induce lateral phase separation of anionically charged phospholipids.⁵³ Solid state NMR has been used to detect mastoparan (wasp venom) induced phase separation of dimyristoylphosphatidylglycerol (DMPG) lipid bilayers.⁵⁴ And, fluorescently-labeled lipids have served as excellent probes to monitor domain formation, such as the binding of a virus matrix protein on a mixed DPPC/DPPG bilayer.⁵⁵

Membrane reorganization as a response to the binding of specific proteins can be a potent signal transducer of a molecular recognition event on the cell membrane surface. The biocompatibility of the lipid membrane, the dynamic nature of the membrane components, and the inherent physical separation of the recognition site (at the aqueous phase) from the optical signal transducer (in the hydrophobic interior of the bilayer) make the functionalized lipid membrane an ideal sensor material for proteins and cells. Sensor materials based on fluorescence resonance energy transfer (FRET)⁵⁶ and fluorescence quenching⁵⁷ have been developed for the detection of cholera toxin. In these membranes a fluorescent labeled ganglioside, GM_1 , aggregates in response to multivalent binding with the B subunit pentamer producing either an increase in the FRET signal or loss of fluorescence due to quenching, depending on the system used (Figure 8). Another unique sensor material, based on the color changing property of polydiacetylene, also has its detection scheme based on the reorganization of membrane components. This sensor material has a number of versions with different biological and chemical targets. One of the original material designs, which used a sialic acid functionalized lipid in a bilayer containing the polydi-

acetylene backbone, illustrates the material properties well.⁵⁸ Recognition of influenza virus to the membrane causes membrane reorganization, most likely due to the multivalent interactions between the virus and membrane, resulting in the reorientation of the ene-yne backbone of the polydiacetylene and subsequent blue to red color change in the material.

In contrast to the common observation of protein-induced clustering, or phase separation, of lipids in the bilayer we have recently found that protein binding can also induce a dispersion of receptor-lipids.⁵⁹ Liposomes of DSPC and a pyrene-labeled, mannosyl functionalized lipid (PSMU) were analyzed with fluorescence spectroscopy to find that the glycolipids spontaneously aggregated within the bilayer, due to differences in their T_m . Upon addition of Concanavalin A (Con A) to the solution the glycolipids dispersed producing a large inversion in the excimer to monomer emission ratio. The reason for the lipid dispersion is believed to be steric factors. That is, the distance between the mannosyl binding sites of the Con A tetramer and the sheer steric bulk of the protein structure physically separate the glycolipids subsequently reducing the collision frequency of pyrene (Figure 9). *In situ* AFM imaging discovered that the glycolipids aggregated in 10 – 20 nm wide branched structures throughout the membrane and that the protein adsorbed preferentially to these glycolipid-rich areas. These results are some of the first to link the molecular scale and nanoscale reorganizational events occurring in lipid membranes with protein recognition.

8.4 Summary

Lipid membranes are dynamic systems composed of components that are in constant motion within the membrane but partition to specific areas as needed. Chemical recognition provides the means by which these components are directed to those areas while also accommodating for the timing of when those components are needed or when they should dissipate. In this review it was shown that the recognition of metal ions, small molecules, polyelectrolytes, and proteins dramatically alter the structure of the membrane and the relative organization of the components. Electrostatic interactions play an important role in the phase transition temperature of the membrane, as well as the phase separation within single component and multi-component bilayer assemblies. Short range, specific interactions, such as hydrogen bonding, van der Waals, and metal ion coordination, also contribute significantly to the recognition of specific molecular guests and the organization of specific lipids in the membrane. Whether these host-guest interactions play a role in cellular membrane phenomena, such as lipid raft formation, is yet to be determined. The current focus of that research still remains on the complex issues of phase separation based upon lipid structure. But, from the same principals presented in this review ever more complex schemes for signal transduction in synthetic systems are being developed that integrate transmembrane structures⁶⁰ or enzymatic reactions⁶¹ showing the versatility of the chemical recognition concept. The insights gained through these model membrane systems will provide a fundamental understanding of cell membrane structure and also aid in the future development of novel lipid membrane based materials.

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Figure 8.1. Phase separation of a mixed membrane, composed of anionic and charge neutral lipids, induced by the binding of a divalent metal ion. The binding of the metal ion not only neutralizes the anionic charge at the lipid's headgroup but also increases the T_m of the aggregated lipids.

Figure 8.2. Molecular structures of azobenzene containing amphiphiles from references 22 and 23. The bottom illustration shows how metal ion binding results in amphiphile aggregation.

Figure 8.3. Molecular structures of pyrene-labeled lipids functionalized with metal ion receptors from references 27, 28, and 29.

Figure 8.4. Divalent metal ion recognition with the receptor lipid creates electrostatic repulsion between metal bound lipids inducing a dispersion of these lipids into the solid phase domains of DSPC. The decrease in local pyrene concentration from lipid dispersion produces a significant decrease in the excimer to monomer fluorescence ratio.

Figure 8.5. *In situ* AFM topographic images of a 20% PS18C6/DSPC bilayer in an aqueous 0.1 M NaCl solution supported on mica (A) before and (B) after addition of 0.1 mM $\text{Pb}(\text{NO}_3)_2$. The lighter, taller areas, representing aggregated PS18C6 lipids, are rapidly dispersed by the recognition of Pb^{2+} ion (reference 29). There also appear in the images tall, sharp peaks that do not correspond to any structures formed by aggregated lipids. The origin of these nanoscale particulates is unknown, although they were observed sporadically throughout the membrane and were sometimes in fixed positions relative to the AFM imaging area, such as the peak located slightly left of center (for a full set of images see ref. 29).

Figure 8.6. Molecular recognition of adenine at the air-water interface with a uridine functionalized lipid causes an expansion of the monolayer due to increases in steric interactions at the head-group position.

Figure 8.7. Monovalent versus multivalent interactions of a guanidine functionalized monolayer with nucleotides. Monovalent interaction with AMP causes expansion and disorder in the monolayer, whereas multivalent interactions with ATP induce lipid aggregation and highly ordered packing.

Figure 8.8. Ganglioside GM₁ (depicted as lipids with diamond-shaped headgroups), labeled with fluorescent donor and acceptor probes, mixed homogeneously in a fluid PC bilayer producing little fluorescence resonance energy transfer (FRET) upon illumination. Binding of the cholera toxin pentamer on the membrane surface resulted in the clustering of the gangliosides and a subsequent increase in FRET as a result of the change in proximity between the donor and acceptor fluorophores.

Figure 8.9. Initially the glycolipid PSMU phase separates from the solid phase matrix of DSPC, as shown on the left side of the figure. Upon recognition of Concanavalin A (Con A) the glycolipids become separated due to specific interactions with widely separated mannosyl binding sites on the tetrameric protein structure (right side of figure).

Chapter 9

Code development

9.1 Point-Dipole Model and Implementation in LAMMPS

One of the goals of this project was to develop and implement a point-dipole model within Sandia’s molecular dynamics code LAMMPS [1, 2] that would be suitable for coarse-grain simulations of lipid/solvent mixtures. The motivation for this task is to include interactions between a dipolar solvent (e.g. water) and lipid molecules containing charged sites (e.g. head groups) in a coarse-grain simulation so that charge and dipole effects on membrane self-assembly, structure, and dynamics can be modeled, similar to the simulation results presented earlier in the report for uncharged systems. In the following sub-sections we describe the point-dipole model itself, discuss how LAMMPS was generalized to enable such models to be added, and present preliminary results for how the model performs.

9.1.1 Point-Dipole Model

A schematic of a system containing charged and dipolar particles is shown in Figure 9.1.1. In the most general sense an individual particle (atom or coarse-grain entity) can be neutral or charged, non-polar or dipolar, or be assigned both a charge and dipole strength. Each particle also interacts with its neighbors via the usual Lennard-Jones (VanderWaals) potential and can be bonded to other particles to form molecules. Computationally, the VanderWaals, charge, and dipolar interactions between particles are treated as pairwise interactions. When a pair of particles is part of the same molecule, these pairwise terms may be weighted to adjust for the additional bond, angle, and dihedral terms in the molecular force field that include the two particles.

Prior to this LDRD, LAMMPS already included force-fields for charged particles interacting via Coulombic potentials which describe the energy E of a system of particles

Figure 9.1. Individual particles in the simulation domain can be uncharged/charged, non-polar/dipolar (small arrows), or any combination thereof. Each particle is also assigned VanderWaals parameters (ϵ, σ) and can be part of a molecular structure (bonded to other particles).

as

$$E = \sum_i \sum_{j>i} \frac{q_i q_j}{\epsilon r_{ij}}$$

where q_i is the charge on particle i , r_{ij} is the distance between particles i and j , and ϵ is the dielectric constant. For a collection of particles in a periodic box, Coulombic interactions can be computed within a cutoff distance (with various smoothing options) or without a cutoff (full long-range interactions). The latter option is performed in two parts: a short-range pairwise portion using the above equation multiplied by an $\text{erfc}(\beta r_{ij})$ term, and a long-range portion performed via Ewald summation. β is a user-specified parameter that partitions the work between the two portions. For efficiency LAMMPS has a particle-particle-mesh (PPPM) solver that computes the Ewald summation by interpolating particle charge to a 3d grid that overlays the simulation domain, solving Poisson's equation on the grid via FFTs, and interpolating the grid solution back to the particle positions to compute energy and force (electric field) on each particle.

As part of this LDRD, two additional Coulombic options were added to LAMMPS. The first was a distance-dependent dielectric which adds an additional $1/r$ term to the Coulombic energy formula above. It can be used as a simple implicit solvent model that includes solvent screening effects to reduce the interaction between distant charged particles without the computational expense of adding large numbers of explicit water (or other coarse-grain solvent) molecules to the simulation.

The second Coulombic option is an implicit solvent model with a Debye potential of the form

$$E_{ij} = \frac{q_i q_j}{\epsilon r_{ij}} \exp(-\kappa r_{ij})$$

where κ is the Debye length and the exponential term provides a solvent screening effect.

For dipolar systems, the formulation outlined in [3] was followed, to derive a dipole force field for implementation in LAMMPS. A brief listing of the resulting equations is given here.

The potential at a point i due to a charge/dipole particle at point j a distance r away is given by

$$V_i = (q_j - \vec{\mathbf{p}}_j \bullet \nabla_i) \left(\frac{1}{r} \right)$$

from which the energy of two particles each with their own charge and dipole terms can be computed as

$$E_{ij} = (q_i + \vec{\mathbf{p}}_i \bullet \nabla_i)(q_j - \vec{\mathbf{p}}_j \bullet \nabla_i) \left(\frac{1}{r} \right)$$

since $E = qV$ for a point charge in a potential V , and $E = -\vec{\mathbf{p}} \bullet \vec{\mathbf{Z}} = \vec{\mathbf{p}} \bullet \nabla V$ for a point-dipole in a potential V , where $\vec{\mathbf{Z}}$ is the electric field induced by the potential.

The various terms of this generalized formula lead to the familiar expressions for charge-charge (qq), charge-dipole (qd), and dipole-dipole interactions (dd), namely

$$E_{qq} = \frac{q_i q_j}{\mathbf{r}}$$

$$E_{qd} = \frac{q}{r^3} (\vec{\mathbf{p}} \bullet \vec{\mathbf{r}})$$

$$E_{dd} = \frac{1}{r^3} (\vec{\mathbf{p}}_i \bullet \vec{\mathbf{p}}_j) - \frac{3}{r^5} (\vec{\mathbf{p}}_i \bullet \vec{\mathbf{r}})(\vec{\mathbf{p}}_j \bullet \vec{\mathbf{r}})$$

where $\vec{r} = \vec{r}_i - \vec{r}_j$ is the vector of length r_{ij} from point j to point i . The force $\vec{F}$ and torque $\vec{T}$ acting between the charges and dipoles of two particles can be derived from $\vec{F} = -\nabla E$ and $\vec{T} = \vec{p} \times \vec{Z}$ to yield

$$\vec{F}_{qq} = \frac{q_i q_j}{r^3} \vec{r}$$

$$\vec{F}_{qd} = -\frac{q_i}{r^3} \vec{p}_j + \frac{3q_i}{r^5} (\vec{p}_j \bullet \vec{r}) \vec{r}$$

$$\vec{F}_{dd} = \frac{3}{r^5} (\vec{p}_i \bullet \vec{p}_j) \vec{r} - \frac{15}{r^7} (\vec{p}_i \bullet \vec{r})(\vec{p}_j \bullet \vec{r}) \vec{r} + \frac{3}{r^5} [(\vec{p}_j \bullet \vec{r}) \vec{p}_i + (\vec{p}_i \bullet \vec{r}) \vec{p}_j]$$

$$\vec{T}_{dq} = \frac{q_j}{r^3} (\vec{p}_i \times \vec{r})$$

$$\vec{T}_{dd} = -\frac{1}{r^3} (\vec{p}_i \times \vec{p}_j) + \frac{3}{r^5} (\vec{p}_j \bullet \vec{r})(\vec{p}_i \times \vec{r})$$

which are the force and torque on a charge/dipole at point i due to a charge/dipole at point j . Note that $\vec{F}_{ji} = -\vec{F}_{ij}$ in all 3 cases, but the torque for the dipole/dipole interaction is not symmetric, namely $\vec{T}_{ji} \neq -\vec{T}_{ij}$.

These equations were implemented in LAMMPS with a cutoff as a short-range dipolar force field. Because the energy due to dipole/dipole interactions falls off relatively slowly as $1/r^3$, a long-range option was also implemented. Similar to the PPPM discussion above, this is done in two parts.

The interactions for the short-range pairwise portion are derived from

$$E_{all} = \sum_{ij} (q_i + \vec{p}_i \bullet \nabla_i)(q_j - \vec{p}_j \bullet \nabla_i) \frac{\text{erfc}(\beta r)}{r}$$

$$E_{wt} = -\sum_M (1 - w_{ij})(q_i + \vec{p}_i \bullet \nabla_i)(q_j - \vec{p}_j \bullet \nabla_i) \frac{1}{r}$$

where E_{all} is a sum over all pairwise interactions within the cutoff distance and E_{wt} is a sum over only the M interaction pairs with a weighting factor $0 \leq w_{ij} < 1$ applied when atoms i and j are in the same molecule and are 1, 2, or 3 bonds apart in a topological sense. These weighting factors are user-specified in LAMMPS. As mentioned above they are used in conjunction with bond, angle, dihedral potentials to create consistent molecular force fields.

Following [3], these equations can be rewritten in the following form

$$E_{all} = \sum_{ij} w_{ij} [B_0 G_0 + B_1 G_1 + B_2 G_2]$$

$$E_{wt} = \sum_M (1 - w_{ij}) [D_0 G_0 + D_1 G_1 + D_2 G_2]$$

$$G_0 = q_i q_j$$

$$G_1 = -q_j (\vec{\mathbf{p}}_i \bullet \vec{\mathbf{r}}) + q_i (\vec{\mathbf{p}}_j \bullet \vec{\mathbf{r}}) + \vec{\mathbf{p}}_i \bullet \vec{\mathbf{p}}_j$$

$$G_2 = -(\vec{\mathbf{p}}_i \bullet \vec{\mathbf{r}})(\vec{\mathbf{p}}_j \bullet \vec{\mathbf{r}})$$

$$B_0 = \frac{\text{erfc}(\beta r)}{r} \quad B_n = \frac{1}{r^2} \left[(2n-1)B_{n-1} + \frac{(2\beta^2)^n}{\beta\sqrt{\pi}} \exp(-\beta^2 r^2) \right]$$

$$D_0 = \frac{-\text{erf}(\beta r)}{r} \quad D_n = \frac{1}{r^2} \left[(2n-1)D_{n-1} + \frac{(2\beta^2)^n}{\beta\sqrt{\pi}} \exp(-\beta^2 r^2) \right]$$

which is convenient computationally since the B_n and D_n terms are defined recursively.

Force and torques can be similarly derived to yield the force and torque on atom i as

$$\vec{\mathbf{F}}_{all_i} = \sum_j w_{ij} [G_0 B_1 \vec{\mathbf{r}} + G_1 B_2 \vec{\mathbf{r}} - B_1 \nabla_i G_1 + G_2 B_3 \vec{\mathbf{r}} - B_2 \nabla_i G_2]$$

$$\vec{\mathbf{F}}_{wt_i} = \sum_{M_i} (1 - w_{ij}) [G_0 D_1 \vec{\mathbf{r}} + G_1 D_2 \vec{\mathbf{r}} - D_1 \nabla_i G_1 + G_2 D_3 \vec{\mathbf{r}} - D_2 \nabla_i G_2]$$

$$\vec{\mathbf{Z}}_{all_i} = \sum_j w_{ij} [q_j B_1 \vec{\mathbf{r}} - B_1 \vec{\mathbf{p}}_j + B_2 (\vec{\mathbf{p}}_j \bullet \vec{\mathbf{r}}) \vec{\mathbf{r}}]$$

$$\vec{\mathbf{Z}}_{wt_i} = \sum_{M_i} (1 - w_{ij}) [q_j D_1 \vec{\mathbf{r}} - D_1 \vec{\mathbf{p}}_j + D_2 (\vec{\mathbf{p}}_j \bullet \vec{\mathbf{r}}) \vec{\mathbf{r}}]$$

$$\vec{\mathbf{T}}_i = \vec{\mathbf{p}}_i \times (\vec{\mathbf{Z}}_{all_i} + \vec{\mathbf{Z}}_{wt_i})$$

$$\nabla_i G_1 = q_i \vec{p}_j - q_j \vec{p}_i$$

$$\nabla_i G_2 = -(\vec{p}_j \bullet \vec{r}) \vec{p}_i - (\vec{p}_i \bullet \vec{r}) \vec{p}_j$$

where the 2nd of the $\vec{F}$ and $\vec{Z}$ equations is now a sum over the bonded neighbors of atom i .

The long-range portion of the charge/dipole potential is rigorously derived in the Touk-maji paper [3] for a particle-mesh Ewald implementation (slightly different than the PPPM algorithm in LAMMPS). It requires many more FFTs than a charge/charge solution (15 vs 4) and thus is expensive, particularly in parallel, due to the poor scalability of 3d FFTs on many platforms.

For this LDRD, we instead implemented an alternative formulation that is more approximate but was easier to implement and required the same number of FFTs as the standard charge-charge solution. The basic idea for the approximation is described here. Issues with its accuracy and performance are discussed below.

In the long-range PPPM solver, point-dipoles are treated as 2 separated point charges. The user specifies the separation value a and the code sets q for the two positive/negative charges to give the correct magnitude of the dipole $p = aq$. This conversion from a point dipole to a pair of separated charges occurs twice during the PPPM solve. When charge is interpolated from the particle positions to the grid, each dipole is converted to two point charges (taking into account the current orientation of the dipole), and their charge is assigned to neighboring mesh points. The PPPM Poisson solver then proceeds in the customary fashion except that the number and magnitude of the charges (quantities used in the PPPM equations) are adjusted to account for the extra dipole charges. Following the FFT-based Poisson solve, the electric field is interpolated from the grid back to the pair of charges to compute a net force and torque on the dipole.

9.1.2 LAMMPS Implementation

Implementation of the charge/dipolar force field in LAMMPS (or any molecular dynamics code) requires more effort than coding the equations described in the previous section. Performing dynamics with a collection of point dipoles uses different formulas for time integration and thermodynamic computations. New boundary conditions (e.g. application of an external field) are useful. For a parallel code like LAMMPS that uses a spatial decomposition of the simulation domain to assign particles to processors, there are additional issues to be addressed. For example, there are new particle attributes (orientation, torque, etc.) which have to be stored and communicated between processors at different stages of the simulation as particles move through the domain.

At the beginning of this LDRD, we recognized various drawbacks to implementing dipoles and their force fields in our current Fortran version of LAMMPS which was de-

signed originally to do a single kind of simulation with various options (particles interacting via Lennard-Jones potentials with optional Coulombic and molecular potentials). While adding dipoles was possible it would make the code considerably more complex and create a variety of new tests and dependencies that would potentially slow down or introduce bugs in existing capabilities.

We instead took the opportunity afforded by this LDRD (and other related funding) to create a new version of LAMMPS that was designed from the outset with more flexibility and modularity in mind. The new version was written in C++, which allows different categories of capabilities to be written so that individual options within a category have no interaction with each other, either in terms of their coding or their execution. The former means less bugs; the latter means fast code no matter how many options are eventually implemented.

As a concrete example pertinent to this LDRD, there is an “atom style” defined in the new LAMMPS version that the user specifies and which determines what attributes are stored with a particle. All styles store common attributes such as particle type, coordinates, and velocities. The vanilla “atomic” style stores nothing else. The “molecular” style stores additional bond, angle, and dihedral topology information with each particle. The “dipole” style stores charge and dipole orientation information with each particle, as well as arrays for rotational velocity and torque accumulation. The C++ class for each “atom style” does more than allocate and store atom attributes; it also contains functions specific to that style to read particle attributes from LAMMPS input data files, to communicate atom attributes when forces are computed, and to bundle atom attributes into buffers when atoms migrate to other processors, etc. This organization of LAMMPS into categories of related functionality was done for several kinds of molecular simulation features: integrator styles, boundary condition styles, thermodynamic output styles, force field styles, etc.

At a low-level, the data structures and algorithms were retained that made the original LAMMPS version fast and scalable. These include data structures for numeric particle attributes, neighbor lists, and molecular connectivities, as well as algorithms for neighbor-list construction, force evaluation, and parallel data exchanges.

The result is new version of LAMMPS that retains the features and speed of the older version, while adding considerable new functionality. Metals, granular materials, and now dipoles can be modeled, a variety of new pairwise potentials are available, and various new boundary conditions and simulation styles (parallel tempering, targeted molecular dynamics) have been added. The new code has also been made open-source and is freely distributed under the GNU Public License (GPL) from this WWW site: www.cs.sandia.gov/sjplimp/lammps.html. The site also contains extensive documentation, example inputs and simulation output, and serves as an archive for papers and simulation results produced using the code. The various versions of LAMMPS have been downloaded 3200 times since the open-source distribution began a year ago in September 2004.

The specific features added to this new version of LAMMPS pertinent to the point-dipole model are as follows:

- Dipolar atom style that stores and communicates dipolar attributes with other processors and files.
- Short-range dipolar force fields with cutoff or long-range corrections, as outlined in the previous section.
- Long-range dipolar PPPM solver using the separated-charge approximation described in the previous section.
- Time integrator for constant NVE simulation with dipoles that includes rotational degrees of freedom for dipole particles with an appropriate moment of inertia.
- Boundary conditions for applying an external field that interacts with particle dipoles.
- New commands for assigning dipole magnitudes to atom types.
- Thermodynamic computations and output that include dipolar rotational energies.
- Options to include dipolar orientation and torque in output snapshot files.

9.1.3 Current Status

All of the dipole options described in the previous sections are implemented in the current version of LAMMPS, including the pairwise charge/dipole and dipole/dipole potentials with both cutoff and long-range options. Testing of the pairwise force-field implementation was done by comparing LAMMPS snapshot output that included per-particle force and torque quantities to independent implementations of the formulas listed above in Python scripts (separate from LAMMPS), as well as by energy conservation tests of LAMMPS dynamics.

The long-range PPPM dipole implementation has been more problematic, both to test for correctness and to judge its accuracy. We have made comparisons with simulations using very-long cutoff pairwise simulations as well as two-particle dipole models (using SHAKE constraints to keep the dipole's bond-length constant) as part of this testing, but the results are still inconclusive.

One key unanticipated issue is that the separated-charge approximation, while simple, appears to contribute to poor energy conservation, particularly at the cutoff distance where the pairwise point dipole (short-range) is converted to two separated charges (long-range). When the separation parameter is decreased, this transition error at the cutoff is decreased, but then large, closely-spaced charge pairs are being mapped to the PPPM grid. This decreases the accuracy of PPPM itself, through the β factor which depends on the sum of squared charge values, and through the interpolation of values to and from the mesh. This can be partly compensated for by using finer grids, but that can incur a significant computational cost which negates the benefit of the approximation as originally designed! The

current status is that we are still trying to work out the trade-offs between these various parameter choices to see if the method can be made sufficiently accurate. If not, a new Sandia post-doc (Ahmed Ismail) has been working through the details of a true point-dipole long-range PPPM solution, which may be the alternative we end up choosing over the current separated-charge approximation.

Unfortunately, without a fully operational long-range component of the force field we have not yet been able to perform long-timescale energy-conserving simulations of a coarse-grained dipolar membrane system, as originally planned in our LDRD goals.

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