Computer simulation of liquid/liquid interfaces. II. Surface tension-area dependence of a bilayer and monolayer

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A constant normal pressure-surface tension algorithm for molecular dynamics simulation, developed in the preceding paper, was used to laterally expand and compress the surface area of a dipalmitoylphosphatidylcholine (DPPC) lipid bilayer. Then, from simulations carried out at constant normal pressure and surface area, values of the surface tension and other thermodynamic variables such as the internal energy and system volume were determined at four different values of the surface area per lipid, 60.0, 65.1, 68.1, and 72.1 Å². The surface tension shows dramatic variations with area, going from 6 to 60 dyn/cm at areas per molecule of 65.1 and 68.1 Å², respectively. An approximate thermodynamic analysis indicates that an area of 68.1 Å²/lipid is the closest of the four to the free energy minimum for this system, in agreement with experimental measurements. The effect of surface area changes on the calculated deuterium order parameters, which can be compared with those obtained from nuclear magnetic resonance experiments, is found to be quite large. Additionally, simulations of lipid monolayers were performed at the same surface areas and, though the dependence of the surface tension with area shows qualitative agreement with experiment, the simulation results are more sensitive to area changes than is observed experimentally. The variation in surface tension with area is much greater for the bilayer than the monolayer, suggesting that monolayers are a good model of bilayers only in a narrow range of surface areas.

I. INTRODUCTION

As bilayers, phospholipids form the fundamental structure of cell membranes. Less appreciated is the fact that lipid molecules act as surfactants in living organisms, for example, forming monolayers in the lungs as an integral part of normal respiratory function. The structure and dynamics of lipid bilayers and monolayers are strongly affected by the surface area per molecule. At a constant temperature, phase transitions in the monolayer from a two-dimensional gaseous phase through two distinct liquid phases to a solid phase can be observed experimentally by reducing the surface area per molecule from hundreds of square angstroms to tens of square angstroms; this is typically accomplished using a Langmuir trough. While monolayers tend to spread to fill the available surface area, the surface area per lipid in bilayers in excess water varies in a much narrower range, primarily depending on composition. Nevertheless, bilayers undergo phase transitions (driven by temperature, pressure, or hydration level) from liquid crystal (Lα) to gel (Lβ) states with about a 35% decrease in surface area; even in the Lα phase, the surface area per lipid can be significantly modulated by varying the hydration and, presumably, by pressure.

In this paper, we apply the constant surface tension algorithms developed in the preceding paper (Paper I) with three purposes in mind. First, we demonstrate that because the lipid bilayer is an interfacial system (and thus the pressure tensor is anisotropic) particular care must be taken when applying the technique of constant pressure molecular dynamics. Second, the effect of surface area changes on the surface tension (and other thermodynamic variables) is calculated for bilayers and monolayers. These results are used to test the validity of the assumption that a bilayer can be modeled as two independent monolayers; such equivalence is a matter of considerable practical importance because monolayers can be studied by a variety of experimental techniques not applicable to bilayers. Third, we establish the merit of recent revisions to the CHARMM potential energy parameter set by a comparison of calculated and experimental deuterium order parameters. The remainder of this section discusses these issues in more detail.

The numerical solution to the equations describing the motion of all the atoms in a lipid/water system is so computationally demanding that only a small sample (typically fewer than 100 lipids) can be simulated for a reasonable amount of time (hundreds of picoseconds). To eliminate edge effects in this small system, periodic boundary conditions are employed such that the system interacts with replicas of itself. As with early simulations of interfaces of pure liquids (see references in Paper I), most simulations of lipid bilayers and monolayers have employed a constant volume for the simulation cell. (Monolayer simulations typically include a large region of empty space above the lipid tail.) There is a fundamental difference, however, between simple interfaces (e.g., oil/water) and those formed by lipids and other surfactants. In the former, molecules at the interface can freely exchange with those in the bulk and the interfacial tension is independent of area. Surfactants, due to their amphiphilic nature, are essentially constrained to remain on the interface, and consequently, the interfacial tension is a strong function of surface density. This property leads to a very difficult problem in simulating bilayers (or ultimately cell membranes) at constant volume: the correct
surface area per molecule must be specified in advance because the surface density of lipids is fixed by the lateral area of the simulation cell. While molecular surface areas have been experimentally determined for some lipids at selected temperatures and hydration levels, the simplifying assumptions which are required to interpret the measurements may not be valid under all conditions. Even if the area per molecule of a lipid were known precisely, the area required for insertion of a second molecular species such as a protein must be determined independently. In Ref. 10, where a bilayer containing a gramicidin channel was simulated using molecular dynamics, the interfacial area was chosen by adding the mean surface area per lipid times the number of lipids and the mean surface area of a gramicidin molecule. Though this simple additivity of surface areas may sometimes be appropriate, it will certainly not be universally valid. For example, Langmuir film balance experiments with phospholipid monolayers show that the addition of cholesterol has a large condensing effect on monolayer surface area. Deviation from ideal additivity of mean molecular area is especially large in the Lσ phase relevant for bilayers under physiological conditions.

When accurate values of bilayer spacing or area per molecule are not available from experiment, it is necessary to carry out the simulation at constant pressure. This technique allows the volume, surface area, or even the shape of the simulation cell to adjust from its initial dimensions, and several such simulations of lipid bilayers have already been reported. As was stressed in Paper I, however, constant pressure simulation of an interface requires considerations additional to those generally well understood for isotropic systems. In particular, only the (the component of the pressure tensor normal to the interface) gives a measure of the bulk pressure of the system, while the lateral components of the pressure tensor impose depend on the interfacial tension. Thus, in contrast to a homogenous fluid where only one pair of thermodynamic variables (pressure and volume) determine the size of the simulation cell, two sets of variables determine the size and shape of the interfacial system: ( and the additional conjugate pair, the surface tension and surface area A. (For a planar interface ) Hence, simulating a two phase liquid system with either an applied isotropic pressure or isotropic pressure tensor is inadvisable because when the surface tension is nonzero. As demonstrated in Paper I by a simulation of octane/water, the surface area monotonically decreased when the condition was imposed; i.e., the system was unstable because and cannot, in general, be specified independently (rather and are the appropriate variables). Consequently, it is important to investigate the practical consequences of the different simulation techniques given the qualitative differences between the surfactant/water and oil/water interfaces.

Of the ensembles described in Paper I, the two most natural ones for simulating a lipid bilayer at constant particle number N with flexible cell dimensions are constant normal pressure and constant surface area (NPA), and constant normal pressure and constant surface tension (NPγ)—we drop the subscript on for the remainder of the paper. The latter should be especially useful for simulating the insertion of molecules into the bilayer while allowing the system to evolve dynamically, provided a value of the surface tension is available. Unfortunately, in contrast to monolayers where the determination of γ is relatively straightforward, accurate estimates of the surface tension are very difficult to obtain for bilayers. The surface tension of black lipid films has been measured experimentally by forming a single bilayer onto a Teflon support with a small hole. A hydrostatic pressure is applied on one side of the membrane causing the bilayer to bulge. At the maximum extension of the bilayer, the radius of the bubble is assumed to be equal to the radius of the aperture in the Teflon support. From the pressure difference across the membrane and the radius of the membrane bubble, the surface tensions have been estimated (from the Laplace–Young equation) to be only a few dyn/cm for lipid bilayers. This method, however, does not give the area per molecule of the bilayer and it is not clear that the surface tension of supported bilayers corresponds to that of unilamellar or multilamellar systems. Under very specific conditions, bilayers have been formed as surface films with surface tensions of approximately 20 dyn/cm. The surface area per molecule was not reported, however, and the method is restricted to fully hydrated bilayers at a single critical temperature (depending on composition) at which the surface bilayer will form. Another possibility is to use the surface tensions of monolayers as a guide for assigning bilayer surface tensions—this is one of the many reasons why it is important to better understand the correspondence between bilayers and monolayers.

Given the difficulties of determining bilayer surface tensions experimentally, an alternative is to choose the simulation cell dimensions based on free energy considerations. Experiments on unilamellar vesicles have indicated that the bilayer is in a stress free state, i.e., the free energy minimum. If the potential energy functions used in the simulation are of sufficient quality, then the free energy minimum calculated from simulation studies should be appropriate for the system. The work required to expand or compress the area of a lipid bilayer has been measured experimentally and can thus serve as a check of the accuracy of the calculations. Clearly, however, the accuracy of such estimates depends on the accuracy of the potential energy parameter set used for the simulation. It is therefore essential to establish the validity of the parameters in as many ways as possible.

As a first step towards the study of complex membranes using the algorithms described in Paper I, we have simulated dipalmitoylphosphatidylcholine (DPPC) bilayers and monolayers. In the next section, details of the computer simulation are presented along with a description of the initial conditions used for this study. Section III describes the simulation of bilayers and monolayers at four different surface areas and lists the details of the expansion or contraction needed to reach a given area. The results of expansion/contraction cycles, completed to check for the possibility of hysteresis, are also provided. In Sec. IV, analysis of the simulations is presented including: (1) estimated free energy changes with area, (2) deuterium order parameter profiles from various
bilateral simulations, (3) the effects of parameter refinement on the simulation results, and (4) distribution of lipid atoms and the orientational structure of water.

II. SIMULATION METHODOLOGY

Molecular dynamics computer simulations were carried out using the CHARMM simulation package. The implementation of algorithms described in Paper I for performing simulations of interfacial systems under constant normal pressure and/or surface tension into the standard leapfrog Verlet integrator in CHARMM is described in Ref. 31. All simulations were carried out with a constant normal pressure of 1 atm. An all-atom model, for both the lipids and the water, was employed using the potential energy parameter set PARM22. Bilayer and monolayer simulations at a single surface area were also carried out with a newer release of these parameters (PARM22b1b) to examine the effect of further parameter refinements to the aliphatic chains. Relevant parameter values from the two sets are included as supplementary material. The electrostatic potential was shifted to zero at a distance of 12 Å and the van der Waals energy was smoothly truncated at 12 Å by use of a switching function over a 2 Å interval. A modified TIP3P water model was used in all simulations, with both bonds and angles held fixed with the SHAKE algorithm. The integration time step was 1 fs. Each picosecond of bilayer simulation took approximately 8 h of CPU time on a Hewlett Packard 9000/735 workstation or 2.5 h on a cluster of four workstations over a 2 Å interval. A modified TIP3P water model was used in all simulations, with both bonds and angles held fixed with the SHAKE algorithm. The integration time step was 1 fs. Each picosecond of bilayer simulation took approximately 8 h of CPU time on a Hewlett Packard 9000/735 workstation or 2.5 h on a cluster of four workstations (monolayers took 20% less time). The individual simulations varied in length from 50 to 175 ps, for a total (bilayer and monolayer) of 1.3 ns (equivalent to more than one year of single processor CPU time).

Both the bilayer and monolayer simulations consisted of 72 DPPC and 2511 water molecules; the geometry of the simulation cells is sketched in Fig. 1. A previous simulation of a DPPC bilayer in excess water served as the starting point for the first NPAH bilayer simulation. The original simulation was carried out under conditions of constant particle number, volume, and internal energy (NVE) for 190 ps. The surface area per molecule in square angstroms, A, of 68.1 and the interlamellar spacing were chosen to match that determined experimentally using x-ray diffraction and gravimetric techniques, and the number of water molecules was then chosen to obtain the experimentally determined mass percent H2O. At this water content, the thickness of the water layer is ~20 Å so that little interaction between headgroups on opposing surfaces is expected.

The bilayer systems were transformed into monolayers by separating the two halves of the bilayer and increasing the length of the simulation cell in the z direction to create a large enough void space above the tails of the monolayers so that there is no interaction between tail atoms (Fig. 1). This was accomplished by translating the molecules in the lower half of the simulation cell in the positive z direction for a distance equal to half the bilayer interlamellar spacing. Similarly, the molecules in the upper half were translated in the negative z direction. In this way a monolayer is formed without perturbing the structure of the water. Although the simulation cell is periodic in three dimensions, it is effectively only periodic in the lateral direction because the interatomic forces are truncated at a distance much less than the distance between the monolayers. A water molecule that evaporates through the monolayer, however, will pass through to the opposite side of the simulation cell (this occurred once during the course of the monolayer simulations). This procedure was carried out at the beginning of each of the NPAH bilayer simulations described in Sec. III. Each monolayer was brought to the target temperature of 323 K by 2 ps of velocity rescaling.

III. RESULTS

A. Simulation of hydrated DPPC bilayers

1. NPAH, A~68.1

The coordinates from the end of the previously described NVE simulation were taken as the initial conditions for an NPAH (fixed normal pressure and surface area) simulation with P_n = 1.0 atm and A = 68.1. Figure 2 shows the time evolution of the length of h_z, the simulation cell length in the z direction (which is directly proportional to V since A is constant). The system volume decreased by 1.9%. Much of this change is due to shortcomings in the TIP3P water model used in the simulation. With the treatment of nonbonded interactions used in this study, the density of bulk TIP3P water at 1
The atm pressure is approximately 4% too high. A density increase of 4% by the 2511 water molecules accounts for 95% of the observed volume decrease.

At each time step, the instantaneous value of the surface tension was calculated by the method described in Paper I. In the simulation cell, there are two bilayer/water interfaces and thus the calculated surface tension is given on a per interface basis. This convention is consistent with calculating the interfacial contribution to the enthalpy as $n \gamma A$, where $n$ is the number of lipids. If one wishes to consider the bilayer as a single interface, then the value of the surface tensions reported here should be doubled. Figure 3(c) shows the calculated surface tension values averaged over 0.5 ps intervals. After omitting the first 10 ps of simulation to allow for equilibration, the mean value of the surface tension is 60.3 ± 3.1 dyn/cm, where the standard error has been calculated as described in Sec. IV A of Paper I. This is roughly 5 dyn/cm less than the surface tension calculated for an alkane/water interface with the identical set of potential energy parameters.

2. (a) NP$\gamma$H, $\gamma=0$; NPAH, $\bar{A}=65.1$

It was argued in Paper I that the use of an isotropic constant pressure tensor algorithm was not appropriate for an alkane/water interfacial system. The bilayer system, however, is more complex because the interfacial tension changes with area. As the surface area is decreased from its optimal value, headgroup repulsion reduces the surface tension. To test this idea, the lipid bilayer was simulated in the NP$\gamma$H ensemble with $\gamma=0$. As described in Paper I, this is equivalent to the Nose–Klein constant pressure tensor algorithm with $P_{xx}=P_{yy}=P_{zz}=1.0$ atm.

In contrast to the NPAH simulation described in Sec. III A 1, the NP$\gamma$H simulation allows for $h_x$, $h_y$, and $h_z$ to change. The values of $h_x$ and $h_z$ (we chose to constrain $h_x$ and $h_z$ to be equal) are plotted in Fig. 4. At first the area of the interface decreases, but in contrast to the water/octane simulation reported in Paper I, a plateau is reached at $h_x=48$ Å ($A=65.1$).

At the end of 50 ps of simulation, the area of the simulation cell was fixed at $A=65.1$ and an additional 75 ps of simulation was carried out in the NPAH ensemble. Figure 3(b)—where the surface tension as a function of time for the constant area portion of the simulation is plotted—shows that the system relaxes from the zero surface tension state to one where the mean value is 6.3 ± 4.1 dyn/cm.

3. NP$\gamma$H compression to $\bar{A}=60.0$; NPAH at $\bar{A}=60.0$

Starting from the bilayer system just described, the NP$\gamma$H algorithm was used to impose lower values of the surface tension in a stepwise manner to decrease $A$ from 65.1 to 60.0. This was accomplished over a period of 16 ps by applying surface tensions of $-55$, $-110$, $-165$, and $-220$ dyn/cm. These values of the applied tension were chosen so that the area of the bilayer decreased almost monotonically. The magnitude of the applied tension could be decreased from the values chosen here. This will, however, require a greater simulation length to achieve the same area reduction.

FIG. 3. Surface tension of the DPPC bilayer as a function of time for each of the four NPAH simulations at $\bar{A}=(a) 60.0$, (b) 65.1, (c) 68.1, and (d) 72.1. As discussed in the text, the negative surface tension obtained for $\bar{A}=60.0$ indicates a metastable equilibrium.

FIG. 4. Simulation cell lengths $h_x$ and $h_z$ vs time for the NP$\gamma$H simulation of the DPPC bilayer with $\gamma=0$. 

FIG. 4. Simulation cell lengths $h_x$ and $h_z$ vs time for the NP$\gamma$H simulation of the DPPC bilayer with $\gamma=0$. 

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At the end of this period, \( h_x \) and \( h_y \) were fixed at values corresponding to \( A \approx 60.0 \). The calculated surface tension for the first 25 ps of fixed area simulation is plotted in the lower portion of Fig. 5, showing the dramatic relaxation of the surface tension that occurs after this large and relatively rapid change in surface area. Interestingly, there is almost no relaxation of the potential energy during this period. After this relaxation period, the constant surface area simulation was continued for an additional 75 ps. The calculated surface tension as a function of time is shown in Fig. 3.

At this high surface density of lipid, the interfacial tension is negative indicating that the tangential components of the pressure tensor are positive and much larger in magnitude than the normal pressure. Clearly, this state should be regarded as metastable since on a much longer time scale the system would likely undergo a phase transition to the gel state.

4. NP\( g \)H expansion to \( A \approx 72.1 \); NPAH at \( A \approx 72.1 \)

The bilayer system at \( A \approx 68.1 \) and normal pressure of 1 atm was expanded to \( A \approx 72.1 \) in a manner similar to that just described. In this case, however, the applied surface tension was increased stepwise to expand the surface area toward the target value. The initial value of the applied surface tension was 55 dyn/cm and increased to 110, 165, and 220 dyn/cm. The surface area was then fixed at a value of \( A \approx 72.1 \) and the simulation continued in the NPAH ensemble. Again a large relaxation was observed, as shown in Fig. 5. After this relaxation the surface tension was recorded for another 75 ps [Fig. 3(d)] resulting in a mean value of 44.9 \pm 2.8 dyn/cm. Interestingly at this large value of the area per molecule, the surface tension has decreased from that calculated at \( A \approx 68.1 \).

B. Simulation of DPPC monolayers

The surface tension of the monolayers at \( A \approx 60.0 \) and 72.1 showed a relaxation similar to that observed in the bilayers at corresponding areas (Fig. 5). Presumably, much of this relaxation could have been avoided by forming the monolayers at the end of the bilayer simulations. The monolayers at \( A \approx 65.1 \) and 68.1 required only a short 10 ps equilibration period. Plots of surface tension versus time for the four different areas are presented in Fig. 6. The calculated surface tensions at \( A \approx 60.0, 65.1, 68.1, \) and 72.1 are 16.4 \pm 3.9, 49.8 \pm 3.9, 74.0 \pm 3.8, and 74.4 \pm 3.1 dyn/cm, respectively.

The Langmuir film balance can experimentally measure the surface pressure \( II \) of the monolayer/water interface as a function of surface area per molecule. The surface pressure is related to the monolayer surface tension \( \gamma \) by

\[
II = g_0 - \gamma, \tag{3.1}
\]

where \( g_0 \) is the water/air surface tension. This technique has been used by Somerharju et al. to obtain the surface pressure-area isotherm for DPPC monolayers at 48 °C. Using a value of 67.9 dyn/cm for \( g_0 \) in Eq. (3.1) allows the calculation of \( \gamma \) from the experimental data. The surface tension as a function of area, determined experimentally and by

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**Figures:**

- **Fig. 5.** Surface tension of the DPPC bilayer as a function of time during the relaxation period following area compression to \( A \approx 60.0 \) (lower curve) and expansion to \( A \approx 72.1 \) (upper curve).
- **Fig. 6.** Surface tension of the DPPC monolayer as a function of time for each of the four NPAH simulations at \( A \approx 60.0, 65.1, 68.1, \) and \( 72.1 \).
computer simulation, is plotted in Fig. 7. Although the simulation results give the correct trend of increasing surface tension with increasing area, the magnitude is underestimated at small areas and overestimated at large areas.

An important observation from these simulations is the difference between monolayer and bilayer surface tensions. If the bilayer can be modeled as two independent monolayers, then the following relation should hold:

$$\gamma_b = \gamma_m - \gamma'$$  \hspace{1cm} (3.2)

where $\gamma_b$ and $\gamma_m$ are the surface tensions at a given area of the bilayer and monolayer, respectively, and $\gamma'$ is the surface tension of an alkane/air interface (≈20 dyn/cm). At $A=68.1$, the difference between the monolayer and bilayer surface tensions takes on a minimum value (14 dyn/cm), which is near $\gamma'$. This relation does not hold at the other areas studied (Fig. 7), presumably due to an additional elastic component of the surface tension that is present only in the bilayer.

C. Examination of hysteresis in compression/expansion cycles

Even with the most powerful computers currently available, the simulation of complex biopolymers in full atomic detail requires too many computer resources to allow simulations of much more than a nanosecond. When undertaking a comparison of several large systems, as in the present work, even a few hundred picoseconds per system is impractical. Thus it is important to check the methodology employed to be sure that the systems are reasonably well equilibrated and that the results are reproducible.

To check the reproducibility of the bilayer surface tension calculations, the bilayer at $A=65.1$ was expanded to $A=68.1$. The trajectory from the end of the 75 ps $NP\tilde{H}$ simulation at $A=65.1$ [Fig. 3(b)] was used to start a $NP\gamma\tilde{H}$ simulation. Over the course of 25 ps, the surface area was expanded to its original value of $A=68.1$ by applying a surface tension of 55 dyn/cm for 24 ps followed by a single picosecond of simulation at an applied surface tension of 110 dyn/cm. This simulation was followed by 50 ps of $NP\tilde{H}$ simulation at $A=68.1$. The surface tension values recorded during the constant area simulation are shown in Fig. 8(a), along with the final 50 ps of the original $A=68.1$ simulation. Although the surface tension versus time plots for the two systems are very similar, the mean values of the surface tensions are somewhat different. The second simulation gives an average value of 52.1±3.5 dyn/cm while the original 75 ps simulation gave an average of 60.3±3.1 dyn/cm.

In testing the reproducibility of the monolayer simulations, the consistency of the surface tension measurements was checked as well as the method by which the monolayers at different areas were produced (by splitting the bilayers that had been expanded or contracted). This was done by expanding the monolayer simulated at $A=65.1$ over 17 ps, using applied surface tensions of 76.5, 101.5, 126.5, and 151.5 dyn/cm. Because of the relatively rapid area increase, we simulated the monolayer for 75 ps at the new area $A=68.1$ and saved the final 50 ps for comparison with the original monolayer simulation at $A=68.1$. The surface tension versus time is plotted in Fig. 8(b) along with 50 ps of the original monolayer simulation at this area. The mean value of the surface tension after expansion was 77.0±4.3 dyn/cm, in excellent agreement with the earlier calculation of 74.0±3.8 dyn/cm.

IV. ANALYSIS

A. Free energy estimation

Aside from allowing one to explore a range of possible surface areas, this method may also be able to guide us to the configuration that the system prefers to adopt, i.e., the free energy minimum. To accomplish this task, we must deter-
mine not only the surface tension as a function of surface area but also the free energy as a function of surface area. It is relatively easy to determine all the mechanical variables of the system \( (P, V, T, \gamma, A, \text{and internal energy} E) \) from a computer simulation by taking time averages over a sufficient length of trajectory. More difficult, however, is the determination of entropy differences. In a future work, we will examine this subject in detail and attempt to compare various methods for the estimation of free energy differences in bilayer systems. The development of such methods to determine the optimal dimensions of a model membrane is critical if one hopes to be able to carry out molecular dynamics (MD) simulations of complex lipid systems containing proteins and other embedded molecules. For now, we provide a simple argument for determining which of the four surface areas studied yields the lowest value of the free energy for the \( \text{PARM22b2} \) parameter set.

Under conditions of constant temperature and pressure, the change in Gibbs free energy is given by

\[
\Delta G = \Delta H - T \Delta S,
\]

where \( \Delta S \) is the change in entropy and \( \Delta H \) is the change in enthalpy given by

\[
\Delta H = H_2 - H_1
\]

with

\[
H = E + PV - \gamma A.
\]

The enthalpies of the bilayers and monolayers at each area were determined from the same portion of the trajectory used to compute the mean values of the surface tension for each of the \( \text{NPAH} \) simulations and are listed in Table I. The simulations described in Table I were carried out with an isolated system, i.e., there was no interaction with a heat bath and thus no heat flow to maintain constant temperature. The average temperatures of the various systems (see last column of Table I) show that the expansions and contractions led to only a small difference in the system temperatures. Taking the initial system at \( \hat{A} = 68.1 \) as the reference state, the temperature differences between the systems are small enough that bringing each to a temperature of exactly 325.2 K is a small perturbation resulting in only a change in the internal energy; i.e., we assume that \( \gamma \) and \( V \) would be unchanged by the decrease in temperature. In this case, the change in the internal energy equals the heatflow, \( \Delta E = C_V \Delta T = \gamma q \). Because the term \( -T \Delta S = -q \), the free energy difference at the reference temperature would simply be equal to the original enthalpy difference. Though this may by a crude approximation, it allows us to comment on which area per molecule is the optimal value for the DPPC bilayer. Examination of Table I shows that of the four simulations, the one at \( \hat{A} = 68.1 \) has the lowest value of the enthalpy. This is also the experimentally determined area per enthalpy.

### B. Deuterium order parameters

The measurement of deuterium order parameters by nuclear magnetic resonance (NMR) spectroscopy is a useful probe of lipid orientation. From a MD simulation, the order parameters can be calculated for any given type of carbon atom from

\[
S_{CD} = (\frac{1}{2} \cos^2 \theta - \frac{1}{2}),
\]

where \( \theta \) is the angle between the CD bond and the bilayer normal. One difficulty in obtaining the order parameters from simulation is the extremely long simulation time needed to obtain converged values. For the carbon atoms at the ends of the hydrocarbon chains, the relaxation time associated with the order parameters has been estimated to be approximately 50 ps and for those carbon atoms nearer the headgroup region, it is close to 200 ps. Over the final 50 ps of each \( \text{NPAH} \) simulation, the deuterium order parameters were calculated using Eq. (3.6). The results for chain carbons 9 through 15 are plotted in Fig. 9(a). These carbon atoms have been chosen because they are the only ones that might reasonably converge over the course of these relatively short simulations. From Fig. 9(a), the trend of increasing magnitude of the order parameters with decreasing surface area is apparent. Though all the curves show the same qualitative feature of increasing order as the headgroups are approached, there are clearly quantitative differences in the order param-

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**Table I. Bilayer enthalpy as a function of surface area.**

<table>
<thead>
<tr>
<th>( \hat{A} ) (Å)</th>
<th>( (E + PV - \gamma A)/\text{kcal mol}^{-1} )</th>
<th>( T/\text{K} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>60.0</td>
<td>-10912±24</td>
<td>325.3</td>
</tr>
<tr>
<td>65.1</td>
<td>-11245±24</td>
<td>325.2</td>
</tr>
<tr>
<td>68.1</td>
<td>-11571±28</td>
<td>325.2</td>
</tr>
<tr>
<td>72.1</td>
<td>-11394±21</td>
<td>326.1</td>
</tr>
</tbody>
</table>

**FIG. 9.** The deuterium order parameters for the DPPC bilayer, (a) simulation results at various values of the surface area/lipid using the \( \text{PARM22b2} \) parameter set, (b) experimental results and simulation results at \( \hat{A} = 68.1 \) with \( \text{PARM22b2} \) [same as in (a)] and with \( \text{PARM22b3} \). The error bars represent one standard error and were calculated by treating the time average of each individual lipid as one sample and dividing the variance by \( \sqrt{2} \).
eters corresponding to the various areas. Agreement with experiment is best for the lowest value of the surface area ($\bar{A} = 60.0$), all other simulations give order parameter profiles that are systematically lower in magnitude than that observed experimentally.

C. Effect of parameter refinement

During the period in which these simulations were carried out, further refinement of PARM22b2 was independently carried out, resulting in parameter set PARM22b4b. The revisions primarily involved addition of a 1-fold torsional term for aliphatic carbons which stabilized the trans state. To test the effects of these parameter changes, the $NPH$ bilayer simulation at $\bar{A} = 68.1$ was continued for an additional 175 ps with the new potential energy function. To avoid possibly large temperature drifts, simulations were carried out at constant temperature ($NPAT$ ensemble). The equilibration of the potential energy required roughly 75 ps of simulation time. Using the final 100 ps of simulation, the mean value of the surface tension is $62.3 \pm 2.8$ dyn/cm, which is very close to the result ($60.3 \pm 3.1$ dyn/cm) obtained with PARM22b2.

At the end of the first 100 ps of bilayer simulation, a monolayer was formed as described in Sec. III. The monolayer was then simulated at a constant area ($\bar{A} = 68.1$) for 175 ps. From the final 100 ps of simulation, the mean value of the surface tension was found to be $72.1 \pm 3.0$ dyn/cm. This is very near the value obtained with PARM22b2 ($74.0 \pm 3.8$ dyn/cm) and again is larger than that observed experimentally.

After allowing for a 75 ps equilibration period, the deuterium order parameters were calculated for the lipid bilayer simulation that employed the updated energy parameters. The values of the order parameters for chain carbons 9 through 15 are plotted in Fig. 9 along with the experimentally determined values (where available). The agreement of the order parameters with experiment is very good and greatly improved over those determined at the same surface area with PARM22b2, where the magnitude of the order parameters is systematically low. Only when the area per molecule was reduced to $60.0$ Å$^2$ did the magnitude of the order parameters fall in line with the experimental observation for PARM22b2. As explained in Sec. IV A, free energy considerations suggest that $\bar{A} = 60.0$ is not an appropriate area for DPPC bilayers under these conditions, but rather that the true area per molecule is closer to $68.1$ Å$^2$. On the basis of the large improvement in the order parameter profile at this area, the refinements of the parameter set that led to PARM22b4b seem appropriate. A detailed examination of the dependence of the free energy on surface area, utilizing the potential energy parameter set PARM22b4b will be undertaken in the near future.

D. Interfacial water structure

An area of membrane biophysics where computer simulation may be especially valuable is in determining the origin of the hydration force acting between bilayers. From the trajectories of the longest simulation in this study ($\bar{A} = 68.1$ with PARM22b4b), we have analyzed the structure of key elements of the bilayer/water interface as well as the orientation of the interfacial water. Figure 10(a) shows the density of water and hydrocarbon as a function of the distance from the center of the water layer. We have defined hydrocarbon to include all nonpolar carbon atoms and their hydrogen atoms (C2–C16 of each palmitic acid chain). Figure 10(a) shows that the overlap of water and hydrocarbon is greatly reduced in the lipid bilayer over that observed for a simple oil/water interface (Fig. 7 of Paper I). Figure 10(b) shows the positions and distributions of the phosphorous atoms belonging to the phosphatidylycholine headgroup, and the carbonyl oxygen atoms of the palmitic acid chains. Figure 10(c) demonstrates the strong perturbing effect of the polar groups in the lipid molecule on the water polarization. The orientation of the water molecules extends to near the center of the water layer with a decay length of approximately 2.5 Å. Comparison with Fig. 8 from Paper I reveals that the polarization of water at the bilayer interface is opposite in sign and nearly an order of magnitude greater than at a hydrophobic interface.

In Fig. 11, the probability density for the $z$ component of the water dipole is plotted for slabs of 1 Å thickness at vari-
net polarization of the water molecules changes sign groups. Further to the interior of the headgroup region, the simulating the size of the simulation cell to the pressure. Wilson and Pohorille have reported a very different orientation of the water dipole at the glycerol 1-monooleate (GMO) bilayer/water interface. Their orientational probability plots are broader than ours with the most likely water orientation being parallel to the interface. They do, however, observe the same sign in the net polarization due to some asymmetry in the distribution which favors the hydrogen atoms pointing toward the bilayer. Additionally, the magnitude of the net polarization is approximately five times greater at the DPPC bilayer interface than at the GMO bilayer. This is caused by the smaller dipolar charge density in the GMO headgroup than is found in the phosphatidylcholine headgroup.

V. DISCUSSION

In the past decade numerous MD simulations of phospholipid bilayers have been carried out, most under conditions of constant volume and thus constant surface area. Experimental estimates of the interlamellar spacing and area/molecule have guided these simulations of pure bilayer systems. As the field moves toward more complex problems involving the insertion of molecules (e.g., proteins and steroids) into the membrane, there will be fewer experiments to point the way towards realistic simulations. We have demonstrated a new method for varying the surface area of an interfacial system dynamically, thereby allowing the changes to be observed by computer simulation. Additionally, for these complex systems there can be savings in computer time when compared to building a new simulation cell at the area of interest, due to the long equilibration periods needed.

The anisotropic nature of the pressure tensor for the lipid/water system is clear from the simulations. This shows that simulation techniques which utilize a fully flexible simulation cell should take into account the surface tension of the system. Two other groups have, however, simulated fluid phase DPPC bilayers in excess water with constant pressure tensor methods \((P_{xx} = P_{yy} = P_{zz} = 1 \text{ atm})\). Shinoda et al. obtained a surface area per lipid between 60 and 65 Å² and Berendsen and co-workers obtained \(A = 64.24\). An isotropic pressure tensor simulation imposes a value of zero surface tension on the system, and this constraint must be recognized. As we demonstrated with the test simulation at \(\gamma = 0\) (Sec. III A 2), the result of this constraint is a contraction of the bilayer to a surface area per lipid that is most likely too small. More recently, an anisotropic constant pressure tensor simulation of a lipid bilayer has been reported which implicitly incorporated the surface tension into the simulation through an applied negative lateral pressure. It was pointed out in Paper I, however, that the statistical ensemble corresponding to this technique has not been identified. In summary, it is most natural to set the surface tension if the system area is allowed to vary.

It should be mentioned that two alternative simulation techniques have been applied to lipid bilayers that have elements of a constant pressure simulation (they allow the system density to relax somewhat) without dynamically coupling the size of the simulation cell to the pressure. Wilson
and Pohorille used a periodic simulation cell with a large vapor region above the water hydrating the bilayer. This method imposes a normal pressure of 0 atm (which is essentially equivalent to 1 atm in a simulation), however, and does not allow any other values of the normal pressure to be set. Schulten and co-workers imposed stochastic, rather than periodic, boundary conditions. This requires that the location of the stochastic boundary be specified in advance (which influences the possible values of the area per molecule) and the existence of additional interfaces at the location of the boundaries complicates the analysis of the pressure tensor.

A difficulty with the evaluation of pressure and surface tension is that it depends on the accurate determination of the long range forces between atoms. In this work, a spherical truncation method was used with a large cutoff (12 Å) to minimize errors in the calculation of the electrostatic force. Another factor that makes this a difficult problem is the large fluctuations inherent in the instantaneous pressure (and thus the instantaneous surface tension). Typical fluctuations of the pressure for a complex biopolymer are on the order of hundreds of atmospheres. Thus, long simulation times are required to determine the pressure and surface tension with reasonable precision. These types of problems will undoubtedly become less severe as the power of computers continues to increase. The simulations described here covered a fairly wide range of surface areas, however future simulations of more complex systems may require even a broader study. The inability to obtain quantitative agreement with experiment for the DPPC monolayers suggests additional work may be needed in either the potential energy parameterization or in the evaluation of long range forces. From the present study, nevertheless, we have deduced that the surface tension of the DPPC bilayer is a much stronger function of surface area than is seen in the monolayer, and that the surface tension contribution to the bilayer free energy is very significant.

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