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On Simulating Lipid Bilayers with an Applied Surface Tension: Periodic Boundary Conditions and Undulations

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Recently, computer simulations of phospholipid bilayers have been carried out with applied surface tensions of nonzero (Chiu et al., 1995) and zero (Marrink et al., 1993; Tu et al., 1996). Feller et al. (1995) have simulated bilayers at constant surface area and have reported nonzero surface tensions. We argue in this note that because long wavelength undulations of the bilayer are absent in the simulation cell (as constrained by periodic boundary conditions), the surface tension evaluated from a simulation is expected to be significantly greater than the macroscopic surface tension. A consequence of this "small system effect" is that an applied nonzero surface tension is required when modeling even flaccid bilayers to obtain the correct experimental surface area per lipid. This approach is consistent with the thermodynamics of the system and with a macroscopic surface tension of approximately zero. Additionally, factors such as hydration level and osmotic or applied pressure could require further adjustment of the applied surface tension when simulating multilayers or other bilayer systems under thermodynamic stress. Values in the range of 10–50 dyn/cm for the surface tension of an undulation-free microscopic patch are then estimated from several theories. Molecular dynamics simulations of a lipid bilayer at progressively smaller system size show the expected increase in surface tension.

BACKGROUND

A critical step in the progress of computer studies of lipid bilayers has been the introduction of flexible simulation cells to replace cells of fixed dimensions. Ideally, this technique enables the dimensions of the simulation cell to adjust dynamically to the appropriate surface area per lipid and lamellar spacing. This is important even for one-component bilayers, because accurate values of surface areas are difficult to determine experimentally (Rand and Parsegian, 1989; Nagle et al., 1996) and, furthermore, are only available for a relatively small number of lipids. The addition of proteins, peptides, or other substituents further complicates the determination of molecular dimensions.

As is well known, the conjugate thermodynamic variable for the surface area is the surface tension, γ. Therefore, the value for an applied γ must be specified when simulating an interfacial system when the area is allowed to fluctuate; conversely, γ may be calculated when the surface area is fixed (Zhang et al., 1995). The value of γ for bilayers, however, is controversial. Analogy to a shielded oil/water interface suggests a value around 20 dyn/cm (Parsegian, 1966), although it is easy to argue that interfacial tensions should be closer to fatty acid/water values, 5–10 dyn/cm. Estimates using monolayer data must proceed with numerous assumptions (Nagle, 1980; Evans and Waugh, 1977): the monolayer tension is divided into independent headgroup/water and chain/air parts, with the latter approximated by alkane/air values and the former associated with the bilayer at the same surface area. McDonald and Simon (1987) derived an interfacial tension near zero for dimyristoyl phosphatidylcholine bilayers using this method; in contrast, we obtain $\gamma = 15$ dyn/cm (per interface) for dipalmitoyl phosphatidylcholine (DPPC) bilayers at 48°C based on $\gamma = 40$ dyn/cm at 68 Å^2/lipid for the monolayer (Somerharju et al., 1985) and $\gamma = 25$ dyn/cm for hexadecane (Small, 1986). Experimental measurements on black lipid membranes typically yield surface tensions in the 1–5 dyn/cm range (Tien, 1989), though the values may be perturbed by the Plateau-Gibbs border and cosolvents.

An apparently quite different point of view has been taken by a number of other workers. Brochard et al., (1976) distinguished three classes of bilayer systems: 1) open (where lipids are free to exchange with the environment, such as in a black lipid membrane); 2) closed and under stress (e.g., a sealed vesicle with an internal pressure greater than that of the surrounding solvent); and 3) closed and unstressed (a flaccid vesicle). These authors then argued that the interfacial tension of a closed, unstressed system must equal zero and interpreted the shape fluctuations of red blood cells under this ansatz. These notions resemble those of Evans and Waugh (1977), who derived thermodynamic relationships for changes in projected area and applied tension. In their formulation, expanded in Evans and Skalak (1980), an effective surface tension or lipid/water interfacial free energy (denoted γ and estimated to be 35 dyn/cm) is balanced by a surface pressure π resulting in a stress-free state for flaccid vesicles. Tanford (1979) argued that small vesicles should have zero surface tension (partly based on a cancellation of tensions in the inner and outer surfaces), though White (1980) shortly afterward questioned some of his assumptions. Tu et al. (1996) and Jahnig (1996) have reviewed arguments as to why bilayer surface tensions should be zero when carrying out computer simulations.

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ENFORCING PERIODIC BOUNDARY CONDITIONS

Begin by considering a flaccid bilayer vesicle (Fig. 1). Flaccid vesicles exhibit large undulations that can be modeled as purely entropic (Safran, 1994; Evans, 1991). In such a model the area per lipid, A_0, is fixed (i.e., the membrane does not stretch), whereas the projected area of a large patch appears to fluctuate with no energy cost. (Analogous behavior occurs in random coil polymers, where the distribution of chain lengths is independent of energy.) The size of this patch ($\sim$1 μm) is many orders of magnitude larger than is presently feasible in molecular dynamics (MD) simulations, where, because of computer limitations, only 50–100 lipids (patches of $\sim$50 Å/side) are typically studied (Fig. 1).

Fig. 2 illustrates the correspondence of a hypothetical (microscopic and undulation-free) simulation cell (I) and a macroscopic patch (V) of a flaccid vesicle introduced in Fig. 1; for notational convenience, the systems in Figs. 1 and 2 will be referred to by Roman numeral alone. Because the bilayer depicted in I is flat, the area of the cell, A_I, equals NA_0, where N is the number of lipids per half. The surface tension of I equals γ_0, though we do not, a priori, exclude the possibility that γ_0 could be very small or even zero. The numerical value of γ_0 could be evaluated by simulation from the difference in the normal and tangential components of the pressure tensor (Hill, 1960; Feller et al., 1995); alternatively, γ_0 is the surface tension that must be applied to keep A_I from expanding or contracting if the cell dimensions are allowed to fluctuate.

Next we assume that I is fully hydrated and, hence, can be expanded to II by adding bulk water with no change of surface tension. II is then replicated M times to III, maintaining the microscopically flat surface and constant surface area per lipid. Because III is undulation free, $A_{III} = M A_I$ and $\gamma_{III} = \gamma_0$. III clearly does not correspond to a patch in the flaccid bilayer (V): some tension, denoted γ_1, must be removed to allow undulations. For very large M we may assume that $\gamma_1 \approx \gamma_0$, so that $\gamma_V \approx 0$. In this sense, V could also represent a patch of a black lipid membrane.

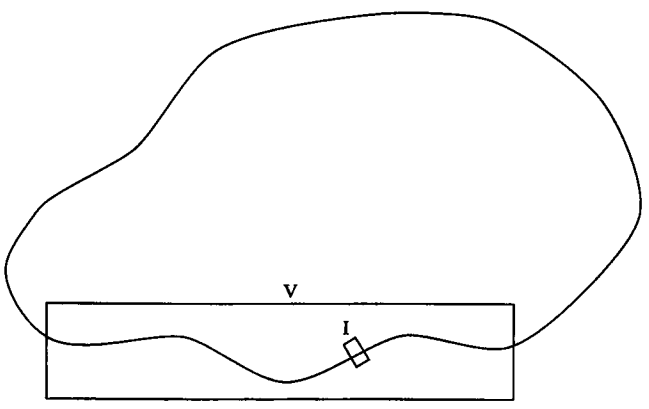

FIGURE 1 A schematic of a flaccid bilayer vesicle, a micrometer-sized patch (V), and a 50-Å simulation cell (I).

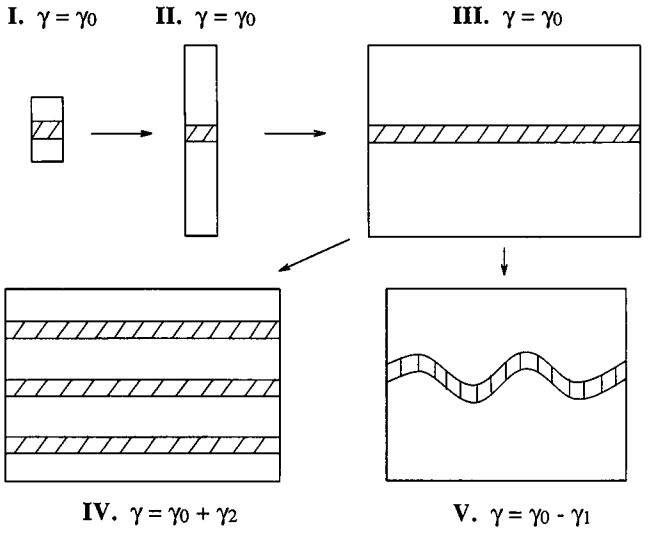

FIGURE 2 The correspondence between a microscopic simulation cell, I, containing a lipid bilayer and excess water, and a macroscopic (micrometer-sized) flaccid bilayer, V, or multilayer system, IV. The hatched and clear regions depict bilayer and water, respectively, and it is assumed that the area per lipid is the same for each system. As described in the text, II contains the same number of lipids as I, but a greatly increased volume of water. System III is hypothetical, in that it contains a large number of lipids and is microscopically flat. The surface tension (as might be evaluated from the difference in the normal and tangential pressures) is noted for each system.

It is essential to note that the effects of subtracting γ_1 (or γ_0) from I and III are very different: the area of the macroscopic cell III decreases to accommodate the undulations, without change in A_0 (i.e., it goes to V). In contrast, because I is too small to support undulations, the surface area per lipid would shrink slightly and the value of A_0 is not preserved; we have shown this explicitly for a DPPC bilayer, where the surface area shrank from 68.1 to 65.5 Å^2/lipid when the system was simulated with zero surface tension (cf. Fig. 4 in Feller et al. (1995)).

ESTIMATE OF γ_0

The preceding arguments have been qualitative. From a practical point of view, if γ_0 is very small (less than a dyn/cm) the predicted contraction of surface area in a simulation carried out with zero applied surface tension would be negligible compared with statistical fluctuations. We now show that γ_0 is likely in the range of 10–50 dyn/cm using a combination of theory and experiment.

Our route to γ_0 of Fig. 1 is to estimate the surface tension required to reduce the transverse displacement, $<u^2>^{1/2}$ from that of a macroscopic patch of unstressed membrane (V) to that typical of an undulation-free patch (III). Following Safran (1994), the mean square amplitude of an undulation mode, u_q, with wavevector, q, where q takes on values of $(\pi i/L)$ (i = integer), is

$$\langle u_q^2 \rangle = \frac{k_B T}{K_c q^4 + \gamma q^2} \quad (1)$$

where L is the longest undulation wavelength, k_B is Boltzmann's constant, T is temperature, and K_c is the bending modulus. For an unstressed bilayer, γ can be set to zero in Eq. 1 and the summation over modes carried out to yield the average transverse displacement

$$\langle u^2 \rangle \approx \frac{k_B T}{4\pi^3 K_c} L^2 \tag{2}$$

Experimental values of $<u^2>^{1/2}$ for flacid stearoyloleoyl phosphatidylcholine vesicles are ~100 Å (Radler et al., 1995). Assuming $K_c = 35\ k_B T$ (the value for stearoyloleoyl phosphatidylcholine of Duwe and Sackmann (1990)) and $L = 0.8\ \mu$m, we obtain $<u^2>^{1/2} \approx 120$ Å. In contrast, experimental values of $<u^2>^{1/2}$ for undulation-free bilayers are 3–5 Å (Weiner and White, 1992); this value is in accord with most simulations (e.g., Feller et al., 1995). To transform the flaccid bilayer into a flat bilayer, a surface tension must be applied. For a bilayer having undulations of a few Å in magnitude, the contribution from curvature energy in Eq. 1 can be assumed to be zero and

$$\langle u_q^2 \rangle = \frac{k_B T}{\gamma q^2} \tag{3}$$

Summing Eq. 3 over modes from $q_{min} = \pi/L$ to $q_{max} = \pi/a$ yields

$$\langle u^2 \rangle = \frac{k_B T}{\gamma 2\pi} \ln\left(\frac{L}{a}\right) \tag{4}$$

where a is the lower bound for the wavelength of an undulation, assumed to be $\sqrt{A_0}$. (Equation 4 is the expression for capillary wave height of a planar interface with surface tension γ.) Upon rearrangement

$$\gamma = \frac{k_B T}{\langle u^2 \rangle 2\pi} \ln\left(\frac{L}{a}\right) \tag{5}$$

Estimates of $<u^2>^{1/2}$ for a simulation-sized patch depend on the definition of the surface; e.g., $<u^2>^{1/2} \approx 3$ Å for the phosphate distribution, but is closer to 4 Å for the cholines and 5 Å for the water. Using this range in Eq. 5 and $L/a = 1000$, $\gamma = 20$–50 dyn/cm.

It is important to point out at this juncture that the value of γ_0 will be difficult to obtain directly from experiment, at least for a large unilamellar vesicle. This is because it may not be possible to suppress undulations on a microscopic (e.g., 50 Å) length scale without also stretching (and ultimately rupturing) the membrane. For example, in pipette aspiration studies of flaccid bilayers, Kwok and Evans (1981) found that at low tensions (<0.5 dyn/cm) the projected area increases at constant A_0 as the longer wavelength undulations are removed, but at tensions of 5–10 dyn/cm, the area/lipid begins to increase and the vesicle bursts. The preceding calculations indicate that undulations are still present at tensions in the 5–10 dyn/cm range, implying that a large though microscopically flat bilayer (as depicted in Fig. 1(III)) is an idealization.

MD SIMULATIONS

The arguments of the previous section predict that for systems with the identical value of the area per molecule, the calculated surface tension will increase as the number of lipids in the patch decreases. To investigate this size dependence, we performed MD simulations of three different DPPC bilayers containing a total of 18, 32, and 72 lipids, respectively, with 32 waters/lipid (i.e., excess water). Simulations were carried out at a constant area of 65.5 Å^2/lipid and a constant normal pressure and temperature (Feller et al, 1995) using the parameter set PARMM22b4b (Schlenkrich et al., 1996), with Ewald summation of the coulombic interactions. The two smaller systems were simulated for 1 ns each and the larger one for 800 ps. A detailed account of the methodology (including generation of initial conditions) and results will be reported elsewhere (Feller et al., manuscript in preparation). Here we restrict our attention to the calculated surface tensions. Fig. 3,*a* and *b* plots the 0.5-ps block averages of the surface tension calculated from the two smaller systems. Fig. 3 *c* shows that the cumulative averages of the surface tensions (per interface) are 33.5, 39.2, and 57.2 dyn/cm for 72, 32, and 18 lipids, respectively; a statistical analysis based on subaverages over independent blocks yields a standard error of ~3 dyn/cm. This demonstrates the system size dependence of surface tension.

We note that the value of γ for the 72 lipid simulation differs from our previously reported value of 6.3 dyn/cm for 65.5 Å^2/lipid (Feller et al, 1995). The difference resulted from changes in the potential energy parameter set and the replacement of spherical truncation of electrostatic interactions by Ewald summation, as well as a longer period of equilibration.

CONNECTION TO THERMODYNAMICS

The distinction between macroscopic and microscopic surface tensions is natural when one recalls that there are two very different definitions used when discussing membrane area. The macroscopic area of the unstressed membrane is actually the area of the surface projected onto the plane normal to the surface. As discussed previously, this area can change with little or no energy cost leading to huge shape fluctuations. But the area per molecule, a microscopic property, is fixed in a stress-free membrane and there is a significant energy cost associated with changing A_0.

With these two very different length scales in mind, we comment on the thermodynamics of the system. As pointed out by White (1980) the system consists of the phospholipid bilayer and the waters associated with it, leading to the following expression for the total (water + lipid) Helmholtz free energy at constant temperature

$$dF = -P\,dV + \gamma dA + \mu_l dn_l + \mu_w dn_w \tag{6}$$

where the subscripts l and w indicate lipid and water, respectively. To a good approximation, the macroscopic

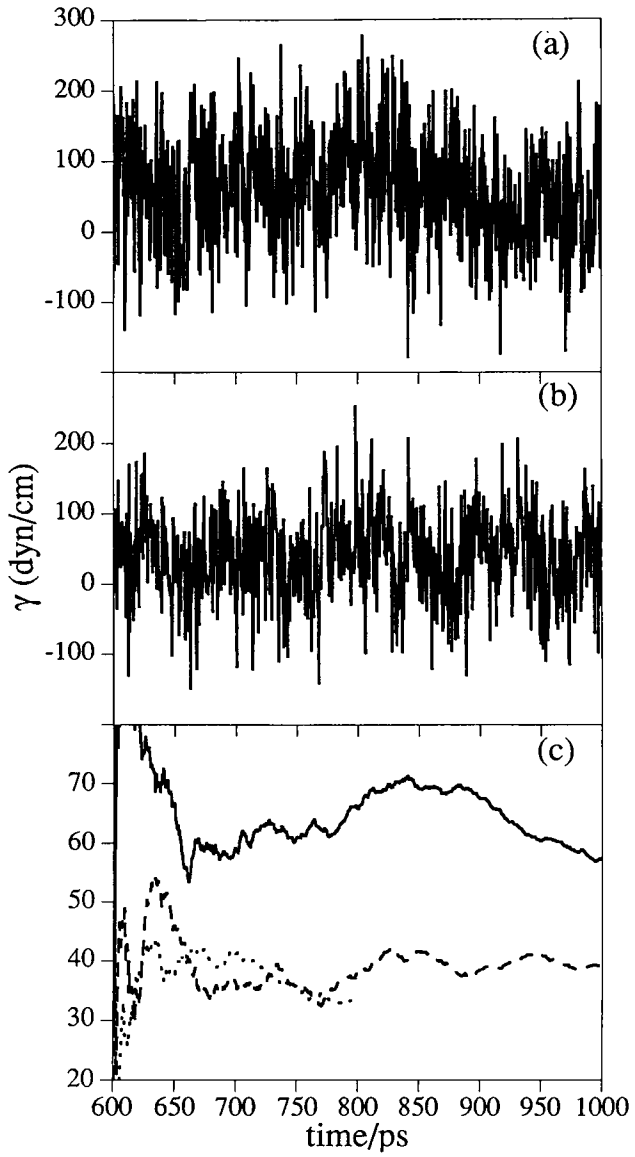

FIGURE 3 Calculated surface tensions (per interface) as a function of time from molecular dynamics simulations of DPPC bilayers. The upper panels show the 0.5-ps block averages for patches containing (*a*) 18 and (*b*) 32 lipids. Fig. 3 *c* shows the cumulative average of the surface tension for patches of 18 (*solid*), 32 (*dashed*), and 72 (*dotted*) lipids.

area of an unstressed bilayer can adjust independently of the remaining thermodynamic variables:

$$\frac{dF}{dA} = \left(\frac{\partial F}{\partial A}\right)_{T,V,n_l,n_w} = \gamma \tag{7}$$

Because at equilibrium the free energy is a minimum, we have $\gamma = 0$ for an unstressed bilayer, where A is the macroscopic area. These relations have been used by Tu et al. (1996) and Jahnig (1996) to argue that bilayer simulations should be carried at zero surface tension. There are two points to be made in this regard. First, the assumptions underlying Eq. 7 are not valid when the bilayer is under stress, as is demonstrated, for example, by the dependence of the surface area of multilayers with applied osmotic pressure (Lis et al., 1982); consequently, for a bilayer under thermodynamic stress, a nonzero macroscopic surface tension is possible. Second, as recognized by White (1980), Eq. 7 does not follow from Eq. 6 for the *microscopic* case (where γ_0 and A_0 must replace γ and A in Eq. 6). Most importantly, the number of hydrating water molecules per lipid is a function of A_0, i.e., the area per molecule cannot vary independently to minimize the total free energy of the system. The equilibrium condition is instead met by an adjustment of several variables leading to a possibly nonzero value of the microscopic surface tension. The free energy is minimized (ignoring volume changes) by a balance between the hydrophobic-like term $\gamma_0 dA_0$ (which favors smaller A_0) and the hydrophilic-like term $\mu_w dn_w$ (which favors larger A_0 and greater hydration of the headgroups). These arguments hold for both stressed and unstressed bilayers.

MULTILAYERS

To introduce the final topic, we note that bilayers exist in numerous states, including very stable multilayer dispersions, which are the source of most structural experimental data. A hypothetical multilayer with all undulations suppressed is depicted as IV in Fig. 1. Here the surface tension includes an additional contribution, γ_2, that depends on the multilayer spacing. The value of γ_2 is related to any changes in the volume, number of hydrating waters, or chemical potentials of the system. This implies that if a simulation cell is meant to model a multilayer rather than a flaccid vesicle, an adjustment of surface tension may be required. The adjustment is expected to be small if the multilayer dispersion is at excess water.

We first must reconsider the surface tension, γ_1, required to remove undulations. As discussed previously, isolated membranes take on fluctuations of order 100 Å. The fluid spacing between fully hydrated bilayers is only tens of angstroms, indicating that the multilamellar geometry acts to suppress undulations. Following Helfrich (1978), the transverse displacement in multilamellar geometry can be written

$$\langle u^2 \rangle = c\bar{z}^2 \tag{8}$$

where c is between 0 and 1, and z is the thickness of the water spacing. Because the magnitude of the fluctuations in the unstressed isolated membrane is proportional to the lateral correlation length squared (Eq. 2), confinement (i.e., limiting the magnitude of $<u^2>$) would be expected to decrease the lateral correlation length. From Evans' (1991) theory of membrane undulation with parallel confinement,

$$\langle u^2 \rangle = \frac{k_B T}{4\pi\gamma} \ln\left(1 + \frac{\gamma L^2}{4\pi K_c}\right) \tag{9}$$

In the limit of negligible surface tension ($\gamma L^2/4\pi K_c \ll 1$),

$$\langle u^2 \rangle = c\bar{z}^2 = \frac{k_B T}{16\pi^2 K_c} L^2 \tag{10}$$

From Eq. 10 with $z = 30$ Å and $c = 0.1$ (the values assumed by Evans), $L = 430$ Å or $\sim .04$ μm. (We have used $K_c = 13k_BT$, (Evans and Rawicz, 1990) the value for dimyristoyl phosphatidylcholine that is most closely related to the DPPC systems we have simulated.) This is likely a lower bound for the correlation length because we have assumed zero tension upon bringing the bilayers together, i.e. $\gamma_2 = 0$. Nevertheless, we can use this estimate of L in Eq. 9 to calculate the tension required to bring the multilamellar system to a state where its fluctuations are comparable to those found in the MD simulation:

$$10 \times 10^{-16}\,\mathrm{cm}^2 = \frac{4.5 \times 10^{-14}\,\mathrm{ergs}}{4\pi\gamma} \ln\left(1 + \frac{\gamma(430 \times 10^{-8}\,\mathrm{cm})^2}{4\pi \times 5.6 \times 10^{-13}\,\mathrm{ergs}}\right) \quad (11)$$

or $\gamma = 13$ dyn/cm. If one assumes that the lateral correlation length is greater than the fraction of a micrometer assumed here, then a larger value of the surface tension is obtained. For example, using $c = 1/6$ (the value proposed by Helfrich) and assuming an initial surface tension of 1.0 dyn/cm results in $L = 2140$ Å and $\gamma = 27$ dyn/cm.

The surface tension of flat multilayers (in excess water) is thus approximately that of single bilayers because most of the change in surface tension comes as the membrane is approaching the flat state, i.e., the large amplitude undulations present only in the isolated bilayer are damped out quickly by the first dyn/cm change in the surface tension.

SUMMARY

As sketched in Fig. 1, a current molecular dynamics computer simulation of a lipid bilayer fails to capture significant features of the macroscopic system, including long wavelength undulations. Such fluctuations are intrinsically connected to the value of the macroscopic (or thermodynamic) surface tension (cf. Eqs. 1 and 9; for a related treatment, see Brochard et al., 1975, 1976). Consequently, the surface tension that might be evaluated in an MD simulation should not be expected to equal the surface tension obtained from macroscopic measurements. Put another way, the largest of the three simulations presented here contained over 16,000 atoms and required substantial computer time to complete, but modeled a system of only 36 lipids per side. From this perspective it is not surprising that the system is not at the thermodynamic limit. An important practical consequence of this effect is that simulations with fluctuating area should be carried out with a nonzero applied surface tension (γ_0 of Fig. 2) even when the macroscopic tension is zero, or close to zero.

Computer simulations at fixed surface area, which can explicitly determine pressure anisotropy at the molecular level, should ultimately lend insight into the value of γ_0, including its dependence on lipid composition and other membrane components. As we have noted and will describe further in separate publications (Feller et al., 1996; Feller et al., manuscript in preparation), surface tensions obtained from simulations can be distorted by inadequate initial conditions and convergence, and are sensitive to potential energy functions, force truncation methods, and system size; it is not difficult, in fact, to tune terms in the potential energy function so as to yield surface tensions close to zero. This is why parameters should be tested extensively on simpler systems, for example, monolayers.

The estimates of γ_0 that we have presented here should be regarded as qualitative, and primarily underscore the assertion that the surface tension of a microscopically flat, simulation-sized patch is significantly greater than zero. As the simulation cell length increases, the surface tension that would be evaluated (or should be applied) decreases; in the limit of micrometer-sized simulation cells, γ would approach zero or its appropriate thermodynamic value. The theories presented here also imply that the estimation of bilayer surface tension from monolayer data should take the degree of flatness into account. These conclusions are independent of the precise values of parameters such as bending constants.

In conclusion, from the simulator's perspective, the question "What is the surface tension of a bilayer?" is better phrased as "What is the value of the applied surface tension necessary to simulate a particular experimental system with a given number of lipids?". As we have shown, the answer to the second question varies, but it should not be assumed a priori to equal zero.

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