Phase Behavior of Lipid Bilayers under Tension

Mark J. Uline,[†*] M. Schick,[‡] and Igal Szleifer[§]
[†]Department of Chemical Engineering, University of South Carolina, Columbia, South Carolina; [‡]Department of Physics, University of Washington, Seattle, Washington; and [§]Department of Biomedical Engineering, Northwestern University, Evanston, Illinois

ABSTRACT Given the proposed importance of membrane tension in regulating cellular functions, we explore the effects of a finite surface tension on phase equilibrium using a molecular theory that captures the quantitative structure of the phase diagram of the tensionless DPPC/DOPC/Cholesterol lipid bilayer. We find that an increase in the surface tension decreases the temperature of the transition from liquid to gel in a pure DPPC system by ~1.0 K/(mN/m), and decreases the liquid-disordered to liquid-ordered transition at constant chemical potentials by approximately the same amount. Our results quantitatively isolate the role of tension in comparison to other thermodynamic factors, such as pressure, in determining the phase behavior of lipid bilayers.

INTRODUCTION

The tension of the plasma membrane of eukaryotic cells plays a role in regulating cell functions that involve membrane deformation or a change in cell shape such as endocytosis, exocytosis, cell motility, and cell spreading (1). Molecular contacts between the plasma membrane and the underlying actin cytoskeleton are known to make contributions to this tension (1,2). Experimental studies on renal epithelial cells have shown that the tension in blebs, regions where the membrane detaches from the actin cytoskeleton, can be an order of magnitude or more smaller than in membranes that are supported by the cytoskeleton (3).

Despite the possible importance of tension on various functions, little is known quantitatively about its effect on such basic phenomena as phase transitions, in contrast to the effect of pressure whose effect on the main chain transition of lipids, for example, is well known (4). Demixing phase transitions in model membranes have been studied extensively due to their possible connection to the origin of lipid rafts (5). Here too, the effect of tension is of possible interest due to the observation of phase separation in giant plasma membrane vesicles that were isolated directly from living cells and retain most of their compositional complexity. These systems exhibited transition temperatures in the range of 15–25°C (6,7). The observation of phase separation, even at these relatively low temperatures, contrasts with the lack of such observations in living cells at such temperatures. This difference in behavior could have several possible causes (7): a reduction in the vesicles of the asymmetry between membrane leaflets, the severance of the connection to active processes, like lipid recycling, and the loss of the cytoskeleton. Without knowledge of the effect of tension on the temperatures of demixing transitions, it is not possible to determine the relevance of the cytoskeleton, or its absence, on the observed transitions.

The purposes of this article are: 1), to display clearly the thermodynamic argument relating changes of tension to changes of transition temperatures; 2), to show that in the particular case of the transition between liquid-ordered and liquid-disordered phases, an increase in tension leads to a decrease in the transition temperature; and 3), to calculate the magnitude of the reduction of this transition temperature with tension in model membranes.

THERMODYNAMICS OF LIPID PHASE TRANSITIONS

The fact that applying tension to a lipid bilayer must reduce the temperature at which phase separation occurs is easily deduced from thermodynamics as follows. The internal energy, U, of a membrane is an extensive function of its entropy S, volume V, area A, and the number of molecules N_i of component i,

$$U = TS - pV + \gamma A + \sum_i \mu_i N_i,$$

with T, p, γ, and μ_i the temperature, pressure, surface tension, and the chemical potential of the i^{th} component, respectively. The variation of the energy with the extensive quantities, the combined first and second laws, is

$$dU = TdS - pdV + \gamma dA + \sum_i \mu_i dN_i.$$

Comparison of the two statements leads immediately to the Gibbs-Duhem equation that relates the variation of the intensive quantities,

$$\sum_i x_i d\mu_i = -sdT + vdp - ad\gamma, \qquad (1)$$

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*Correspondence: uline@cec.sc.edu

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with s, v, and a the entropy, volume, and area per molecule, and x_i the mole fraction of the i^{th} component.

For a pure component bilayer, the left-hand side of Eq. 1 only has one term. The Gibbs-Duhem for the pure component bilayer is then $d\mu = -sdT + vdp - ad\gamma$, and this equation can be applied to each of the two coexisting phases. Noting that the change in chemical potential at coexistence with changes in temperature, pressure, and surface tension must be the same in each phase, we obtain the following expression for the differential of the transition temperature along coexistence,

$$dT = \frac{\Delta v}{\Delta s}dp - \frac{\Delta a}{\Delta s}d\gamma, \tag{2}$$

where Δv, Δa, and Δs are the differences in the volumes, areas and entropies per molecule in the two phases. The magnitude and sign of the change in the transition temperature with pressure is well known for many lipids. The $(\partial T/\partial p)_\gamma$ along gel-fluid phase coexistence for pure component DPPC is ~0.02 K/bar (4). For DPPC bilayers, the second term in the above equation can be estimated at zero tension from measurements of the change in area per molecule, $a_k - a_g \approx 0.15$ nm^2 (8) and of the enthalpy of the transition, $T(s_k - s_g) = 8.5$ kcal/mol at $T = 314.7$ K (9). From these values, one finds from Eq. 2 that the liquid crystal to gel transition temperature, at constant pressure, decreases with increasing tension by an amount $(\partial T/\partial\gamma)_p \approx -0.8$ K/(mN/m). That the temperature of the transition from liquid crystalline to gel phase should decrease with increased tension is clear from simple physics. The increased tension increases the area per lipid so that the tails are further apart and can, therefore, only be ordered if the temperature is lowered. Conversely, a negative tension (i.e., a positive external surface pressure) pushes the tails closer together, making it easier for them to order and increases the transition temperature. This effect has been observed in simulation (10).

A bilayer consisting of two components can be characterized by four independent intensive variables, T, p, γ, and η_1, where $\eta_1 \equiv \mu_1 - \mu_2$. Coexistence of two phases can be represented by a three-dimensional surface, $T(p, \gamma, \eta_1)$, in this space. If we consider the intersection of this surface with the constraint that η_1 be fixed, then the transition temperature depends only on two variables, $T(p, \gamma)$. Application of the Gibbs-Duhem equation to the coexisting phases will yield

$$dT|_{\eta_1} = \frac{\Delta v}{\Delta s}dp|_{\eta_1} - \frac{\Delta a}{\Delta s}d\gamma|_{\eta_1}, \tag{3}$$

where η_1 is fixed. This equation applies to an experimental system that is in contact with reservoirs of the two components, for then each chemical potential, μ_1 and μ_2 is held fixed as is their difference η_1.

We now consider a three-component bilayer that exhibits coexistence of two phases. A bilayer consisting of three components can be characterized by five independent intensive variables, T, p, γ and $\eta_{j=1,2}$ with $\eta_j \equiv \mu_j - \mu_3$. Coexistence of two phases can be represented by a four-dimensional surface, $T(p, \gamma, \eta_1, \eta_2)$, in this space. If we consider the intersection of this surface with the constraints that the differences of two chemical potentials relative to the third, η_1 and η_2 be fixed, then we obtain, for the change in temperature along phase coexistence, the result

$$dT|_{\eta_{j=1,2}} = \frac{\Delta v}{\Delta s}dp|_{\eta_{j=1,2}} - \frac{\Delta a}{\Delta s}d\gamma|_{\eta_{j=1,2}}. \tag{4}$$

This expression holds a fortiori when the chemical potentials of all three components are fixed at coexistence. Again, this can be brought about by keeping the vesicle, on which the transition is observed, in contact with a particle reservoir, as is the case in which the vesicle remains attached to a micropipette (T. Portet, S. E. Gordon, and S. L. Keller, unpublished). For the liquid-ordered to liquid-disordered transition, the sign of the second term is known because the liquid-disordered phase has a larger entropy per molecule and larger area per molecule than the liquid-ordered phase. Thus the transition temperature, at constant pressure and constant chemical potential of two of the components, decreases with an increase in surface tension. However, the magnitude of this effect is unknown. The remainder of this article is devoted to a calculation of this shift in phase equilibria of lipid bilayers due to tension.

We note that a recent experiment increased the lateral tension of a vesicle containing a ternary mixture by subjecting the system to osmotic pressures (12). An increase in transition temperature was observed and ascribed to the increased tension. This experiment has a glucose-water solution both inside and outside of the lipid vesicles, and an osmotic pressure is achieved by diluting the exterior phase of the vesicles by adding water and therefore lowering the concentration of glucose exterior to the vesicles. Because water can pass through the lipid bilayer and glucose cannot, we have an osmotic equilibrium.

Our thermodynamic analysis above is valid for situations where the pressure is uniform throughout the system. For the case of an osmotic pressure system, the inner and outer leaflets of the bilayer are subjected to different pressures (upward of 5 bar in the experiments of Hamada et al. (12), if one assumes an ideal solution between glucose and water). We are therefore unable to make a direct comparison between our work and the experiments in Hamada et al. (12). Work on theoretically quantifying the effects of osmotic pressure on the transition temperature is currently underway.

THE MODEL SYSTEM

We consider a fluid, planar, symmetric lipid bilayer of which x_c is the mole fraction of cholesterol molecules, x_s is that of

saturated lipid, DPPC (dipalmitoyl-phosphatidylcholine), and x_u is that of unsaturated lipid, DOPC (dioleoylphosphatidylcholine). We consider the bilayer system to be incompressible, which means that the volume of the system only changes due to a change in the number of molecules in the system (i.e., $dv = \sum v_i dx_i$, where v_i is the volume per molecule of the i^{th} species).

This assumption of incompressibility is in accord with direct experimental measurements on the gel-fluid transition of DMPC (dimyristoyl phosphatidylcholine) that show only a 3% volume change at pressures <500 bar (4). The system is taken to be homogenous in the (x,y)-plane with an area per molecule, a, in this plane. All inhomogeneities within a phase are only a function of the z coordinate that is directed along the bilayer normal. The Helmholtz free-energy per molecule, f, of the system divided by $k_B T = \beta^{-1}$ is

$$\begin{aligned}\beta f(T, x_i, a) \;=\; & \sum_i \frac{x_i}{2} n_i^{tails} \sum_{\alpha_i} P_i(\alpha_i)(\ln P_i(\alpha_i) + \beta\epsilon(\alpha_i)) \\ & - \sum_i \sum_j \beta J_{i,j} \frac{x_i}{2} \frac{x_j}{2} \frac{n_i^{tails} n_j^{tails}}{2 a v_s} \\ & \times \int \langle \xi_i(z) \rangle \langle \xi_j(z) \rangle dz + \beta \gamma_0 a \\ & + \sum_{i\,=\,s,u,c} \frac{x_i}{2} \ln\left(x_i \lambda_i^2 / a\right). \end{aligned} \tag{5}$$

The first term in the free energy accounts for the conformational entropy and internal energy of the many different configurations of the single hydrophobic chains. The number of chains of component i is denoted n_i^{tails} with $n_s^{tails} = n_u^{tails} = 2$, $n_c^{tails} = 1$. The chains are generated using Flory's rotational isomeric states model (13) in which each CH_2 group is in one of three configurations: the lowest energy *trans*, *gauche-plus*, or *gauche-minus* (the last two of which are of an energy 500 cal/mol or $\approx 0.8\ k_B T$, greater than that of the *trans* configuration). Further $P(\alpha_j)$ is the probability of finding the chain of component j in a particular conformation specified by the index α_j, one with a total internal energy $\varepsilon(\alpha_j)$. The second term in the free energy describes the orientational interaction between the chains. The volume v_s is that of a CH_2 group and is taken to be 0.0273 nm^3. The ensemble average $\langle \xi_i(z) \rangle$ is that of the local density of bonds weighted by their relative orientation to the bilayer normal. The $J_{i,j}$ are the strengths of the orientational interactions. Our molecular mean-field theory treats separately the contributions to the free energy arising from the lipid hydrocarbon chains and from the polar headgroups. The third term is the free energy that arises from the interaction of the chains with the polar headgroups and external water, where $\gamma_0 a$ is the interfacial free energy per molecule with γ_0 taken to be 12 $k_B T/nm^2$. The last term in the free energy is the entropy of mixing of the three components, with λ_i the thermal wavelength of species i. We minimize the free energy under the incompressibility constraint, which assures that the local volume fraction of all species sum to unity at every location in the hydrophobic region. An extended discussion of the details of the molecular theory that is used in this work can be found in Uline et al. (14).

RESULTS AND DISCUSSION

Given the Helmholtz free energy per molecule, $f(T, x_i, a)$, the phase behavior at a specified value of the surface tension, γ_{ex}, is most easily determined from the variation with the area per particle, a, of the ancillary function $\overline{w}(T, x_i, a, \gamma_{ex}) \equiv f(T, x_i, a) - \gamma_{ex} a$. This ancillary function is not, in general, the Legendre transform of the Helmholtz free energy because γ_{ex} is not, in general, equal to the thermodynamic tension $\gamma = (\partial f/\partial a)_{T,x_i}$. However, one sees from its definition that, at the extrema of $\overline{w}(T, x_i, a, \gamma_{ex})$, with respect to a, one obtains $(\partial f/\partial a)_{T,x_i} - \gamma_{ex} = 0$—so that at these points the externally specified tension, γ_{ex}, *is* equal to the thermodynamic tension, and the ancillary function $\overline{w}(T, x_i, a, \gamma_{ex})$ is equal to the thermodynamic potential $w(T, x_i, \gamma) = f(T, x_i, a) - \gamma a$, the Legendre transform of $f(T, x_i, a)$. For this incompressible system, the variation of the thermodynamic potential w is

$$dw \;=\; -s dT + \sum \mu_i' dx_i - a d\gamma,$$

where $\mu' \equiv \mu_i - p v_i$. Two-phase coexistence is indicated by the existence of two minima in the ancillary function that have the same horizontal tangent.

We first consider the pure DPPC bilayer. The main-chain transition occurs at that temperature at which a gel phase, characterized by a small area-per-molecule, and large saturated chain order, coexists with the liquid crystalline phase, characterized by a larger area-per-molecule and less order in the chains. For pure DPPC the main chain transition temperature occurs at 315 K. We use this state point to determine J_{ll} (14). The inset of Fig. 1 *A* shows $\beta\overline{w}(T, a, 0)$, the ancillary function divided by $k_B T$, calculated for pure DPPC at 315 K and zero imposed tension as a function of the area per lipid. This function exhibits two minima at equal free energies that corresponds to the coexistence of the gel and liquid crystalline phases. From the difference in the values of the area and entropy per molecule in the coexisting phases, we find from Eq. 2 that the slope of the transition temperature with tension is equal to $(dT/d\gamma)_{[k-g]} = -0.985$ K/(mN/m). This is quite close to the value obtained from experiment, −0.8 K/(mN/m) noted above, and gives us confidence that our molecular theory captures the effect of tension on the transition temperature.

Fig. 1 *A* shows $\beta\overline{w}(T, x_i, a, \gamma_{ex})$ at 300 K for three different values of the imposed tension. The black curve corresponds to zero tension, and clearly shows that the gel phase is the equilibrium one. As the tension increases at constant temperature, the minimum representing the liquid crystalline phase decreases in relation to the one of the gel

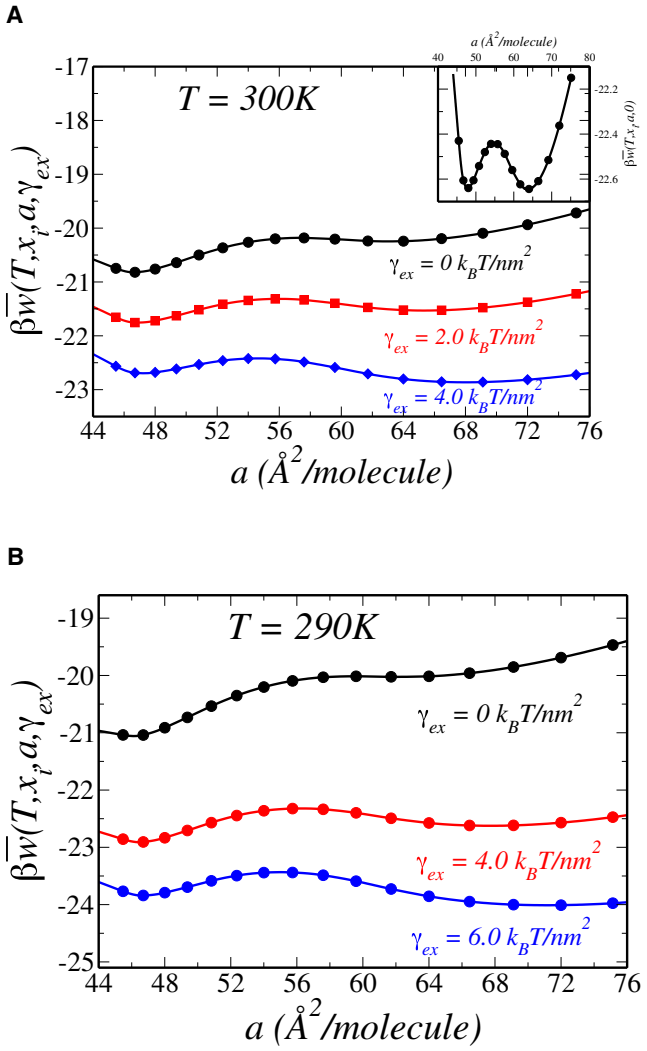

FIGURE 1 Ancillary function, $\beta\overline{w}(T,x_i,a,\gamma_{ex})$, for a pure DPPC bilayer as a function of the area per molecule, a, at several values of the imposed tension γ_{ex}. As the surface tension increases, the minimum in the function shifts from a small value of a, which corresponds to the gel phase, to a larger a, which corresponds to the liquid crystalline phase. (*A*) The results at 300 K. (*Inset*) Temperature of 315 K at zero tension. (*B*) The results at 290 K.

phase, and the minima are comparable at a tension of 2 k_BT/nm^2. The liquid crystalline phase is certainly the equilibrium phase at a tension of 4 k_BT/nm^2. The value of the tension at the $T = 300$ K transition is determined from Eq. 3 to be 3.6 k_BT/nm^2 = 14.6 mN/m. This is ~4% lower than the value $\gamma = 15.2$ mN/m that we would obtain were we to assume that the derivative of the transition temperature with tension was unchanged from its value at 315 K. Fig. 1 *B* shows the function $\beta\tilde{w}(a,\gamma_{ex})$ at 290 K for three different tensions. At this temperature, we obtain by direct calculation that the tension at the transition is 5.5 k_BT/nm^2 = 22.3 mN/m. A naive extrapolation from the result at 315 K would produce a tension of $\gamma = 25.4$ mN/m.

The area compressibility modulus, K_A, is directly related to the second derivative of the thermodynamic potential,

$$K_A = a\left(\frac{\partial^2 w(T,x_i,a,\gamma)}{\partial a^2}\right)_{T,x_i,\gamma}. \quad (6)$$

The direct area compressibility modulus has been experimentally measured for LC-phase diacyl PC bilayers with chain lengths from 13 to 22 carbons and a range of unsaturation from 0 to 6 double bonds to be ~0.24 J/m^2 for all of the bilayers studied (15). We calculate the area compressibility modulus for the LC-phase of the pure component DPPC bilayer under zero imposed tension at 315 K to be 0.26 J/m^2, which is in excellent agreement with the experimental observations. When the imposed tension is high enough in the lower temperature systems to stabilize the LC-phase, we see a slightly lower K_A and a slightly larger area per molecule as compared to those quantities seen in the tensionless LC-phase at higher temperatures. We can also report the area compressibility modulus for the pure DPPC bilayer in the tensionless gel-phase to be $K_A = 0.71$ J/m^2 for $T = 315$ K, and $K_A = 1.65$ J/m^2 for $T = 300$ K.

We now consider the liquid-ordered to liquid-disordered phase equilibrium in the three-component DPPC/DOPC/cholesterol system. We calculated the discontinuities in area and entropy per molecule at fixed chemical potentials to obtain the slope of the liquid-disordered to liquid-ordered transition temperature from Eq. 4 for several l_o-l_d coexistence points at both $T = 300$ K and $T = 290$ K. We found that this derivative is approximately $(\partial T/\partial\gamma)_{[ld-lo];\mu_i'} = -1.0$ K/(mN/m). This value is almost the same as that for the slope of the gel-liquid crystal transition in the pure DPPC bilayer, despite the obvious differences between the two systems and their transitions.

We show the effect of imposing tension on the bilayer when the tensionless phase is a stable l_o phase by studying the tertiary system at composition $x_s = 0.25$, $x_u = 0.33$, and $x_c = 0.42$. Fig. 2 *A* shows the function $\beta\overline{w}(a,\gamma_{ex})$ at $T = 300$ K as a function of the area per particle for several tensions and Fig. 2 *B* for $T = 290$ K. As the tension is increased, the l_o phase becomes less stable and a first-order transition takes place to the l_d phase. For the system at $T = 300$ K we find that the tension at the transition is $\gamma = 1.5$ k_BT/nm^2 = 6.1 mN/m, whereas at $T = 290$ K the tension at the transition is 1.7 k_BT/nm^2 = 7.2 mN/m.

For completeness, we have calculated the limit of thermodynamic stability with tension of the liquid-ordered phase. With the two independent chemical potentials taken to be $\eta'_u \equiv \mu'_u - \mu'_c$ and $\eta'_s \equiv \mu'_s - \mu'_c$, stability requires the positivity of (16),

$$\Phi(T,\gamma,x_s,x_u) = \left[\left(\frac{\partial\eta'_s}{\partial x_s}\right)_{T,\gamma,x_u}\left(\frac{\partial\eta'_u}{\partial x_u}\right)_{T,\gamma,x_s} - \left(\frac{\partial\eta'_s}{\partial x_u}\right)^2_{T,\gamma,x_s}\right], \quad (7)$$

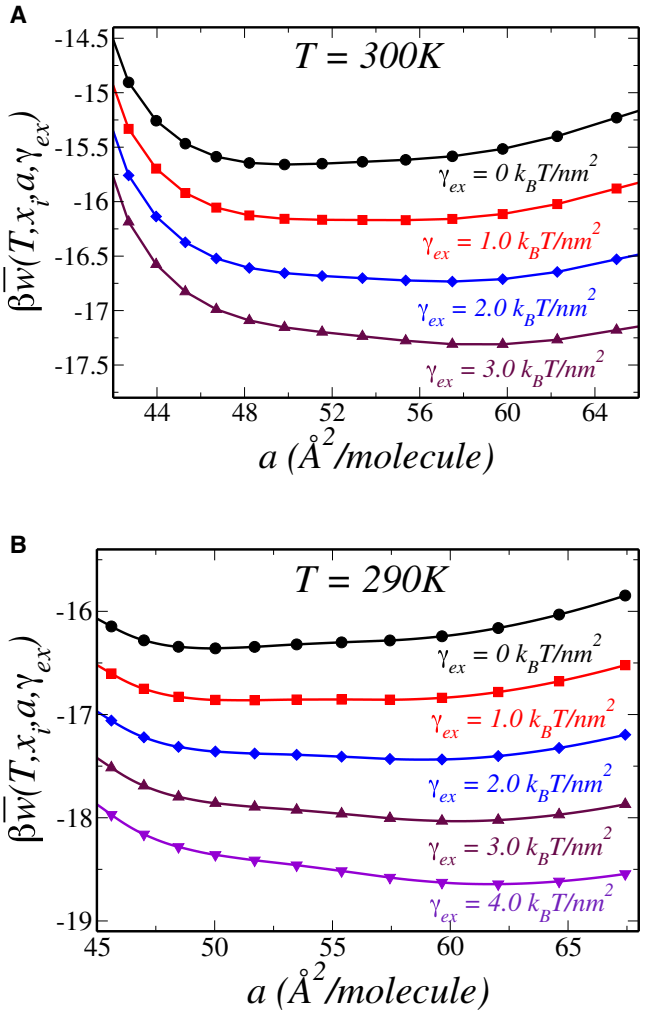

FIGURE 2 Ancillary function, $\beta\overline{w}(T, x_i, a, \gamma_{ex})$, as a function of area per molecule, a, for the three-component bilayer with $x_s = 0.25$, $x_u = 0.33$, and $x_c = 0.42$ at several values of the imposed tension, γ_{ex}. The temperature is (*A*) 300 K and (*B*) 290 K.

where x_c is varied in each of the derivatives to assure that $\sum_i dx_i = 0$. Fig. 3 is a plot of the spinodal, the locus in the x_s, x_u plane, of $\Phi(T, \gamma, x_s, x_u) = 0$ for $T = 290$ K with $\gamma = 0$ k_BT/nm^2 for the solid/black curves, $\gamma = 2.0$ k_BT/nm^2 for the dashed/red curves, and $\gamma = 4.0$ k_BT/nm^2 for the dotted-dashed/blue curves. The system is unstable between the two curves, whereas the liquid-ordered phase is stable for small x_u and the liquid-disordered phase is stable for large x_u. As the tension increases, the location of the spinodal shifts to lower values of the mole fraction of the unsaturated lipid. This implies that as the tension increases, more cholesterol is needed to drive phase separation. We also see in Fig. 3 that, as the tension increases, the area of the unstable region gets smaller. By examining the behavior of the spinodal with increasing tension, we find that the system is stable at $T = 290$ K for all compositions when the tension exceeds 24.0 mN/m.

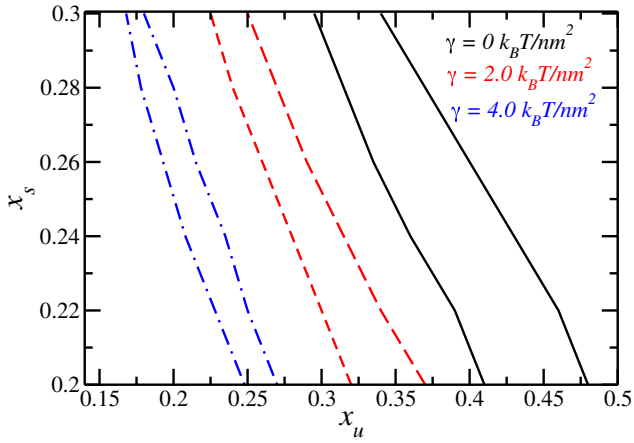

FIGURE 3 Plot of the spinodal, $\Phi(T, \gamma, x_s, x_u) = 0$, as a function of the DPPC fraction, x_s, and the DOPC fraction, x_u. (*Solid black curves*) Temperature is 290 K and the tension is $\gamma = 0$ k_BT/nm^2. (*Dashed red curves*) Tension is $\gamma = 2.0$ k_BT/nm^2. (*Dot-dashed blue curves*) Tension is $\gamma = 4.0$ k_BT/nm^2.

CONCLUSIONS

In summary, we have shown that increasing the tension on the bilayer decreases the transition temperature of the gel-liquid crystalline transition in the pure DPPC bilayer and the l_o-l_d transition in the DPPC/DOPC/cholesterol bilayer. The numerical value of the change in the transition temperature with tension is on the order of 1.0 K/(mN/m) for both the pure DPPC bilayer and the three-component mixture at constant chemical potentials. For the one-component system, this prediction is in excellent agreement with experimental data. We found that the value of the surface tension in the three-component bilayer system at which the liquid-disordered phase is stabilized at all compositions is temperature-dependent with a value of 16.6 mN/m at 300 K and 24.0 mN/m at 290 K. Our results have implications for the evolving understanding of the lipid bilayer in the cell membrane.

To our knowledge, this is the first calculation of the change in the l_o-l_d transition temperature with tension for a lipid bilayer. It is clear from our analysis that if there is phase separation in a cell membrane with its cytoskeleton, it would occur at a lower temperature than in a bleb without one. The experimentally determined value of the amount of tension imposed by the actin cytoskeleton is ~0.02 mN/m (3), which, when combined with our result for the rate at which the transition temperature decreases with tension, implies that this tension is insufficient to change the transition temperature dramatically. Thus, we conclude that the tension of the cytoskeleton is not the source of the difference seen experimentally between the macroscopic phase separation shown in giant plasma membrane vesicles obtained from living cells and the lack of macroscopic phase separation in living cells.

Lastly, it has been shown both experimentally and theoretically that curvature can control the lateral sorting of

membrane components (17,18). We have demonstrated here that the separation into different phases, which are characterized by different partition coefficients (6,14,19,20), can also be controlled by lateral tension. To couple these two effects is an ongoing effort.

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