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Compositional-mechanical instability of interacting mixed lipid membranes

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We consider lateral phase separations in mixed vesicles that are comprised of neutral and charged lipids and which interact with each other at close distances. Their demixing transition is driven by the electrostatic interaction between the bilayers, and is due to enhanced screening of the in-plane repulsions by counterions shared between the two surfaces. Buckling of the membranes further facilitates the phase separation and lowers the interbilayer energy. We propose this instability as a precursor for vesicle fusion. [S1063-651X(97)01401-3]

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Biological cell membranes are essentially molecular bilayers comprised of several kinds of phospho- and glycolipids, with incorporation of many different proteins and steroids [1]. The functioning of such a membrane often depends crucially on its ability to reorganize the spatial distribution of its constituents in response to changes in temperature, pH, and salt concentration, etc., and to interactions with other membranes. (In this respect, multicomponent biomembranes are fundamentally different from the simpler one-component surfactant bilayers whose properties have been studied in considerable detail under highly controlled conditions [2]). Motivated by the need to understand these biologically related phenomena, lateral phase separations within the individual monolayers of unilamellar vesicles consisting of mixtures of neutral and charged lipids have been extensively investigated [3,4]. Divalent cations such as Ca^{2+} have been shown to be especially effective, both in driving the lateral demixing of components [5] and in promoting eventual *fusion* between vesicles [6]. In all of this work, however, the lipid phase separation is assumed to occur in isolated bilayers in the absence of interactions with other membranes. Theoretical attention has been focused on the extent to which the demixing of lipids in simple bilayers is affected by their being charged [7]: a key result of these studies is that electrostatic interactions between charged lipids have a strongly *stabilizing* effect, i.e., keeping them mixed and inhibiting segregation.

Charge inhomogeneity in fluid amphiphilic membranes has been addressed recently by Fogden and Ninham [8] and Guttman and Andelman [9], who were primarily concerned with estimating the role of electrostatic contributions in determining the bending elasticity of amphiphilic mono- and bilayers [10]. They considered both small amplitude, long wavelength, *height* perturbations of a single surface *and* spatial modulation of its charge density, in the linearized Poisson-Boltzmann regime. It was shown, for example, that in this approximation the lowest free-energy state for a planar surface corresponds to uniform charge density. If, on the other hand, the height profile is allowed to include undulations, then ''in-phase'' spatial modulation of the charge density is favored: regions with higher positive curvature are found to have greater concentrations of charged species, since the associated counterions there enjoy larger local volumes. Related work involving the coupling of local bending and concentration fluctuations has been reported in studies of mixed mono- and bilayers in which two charge-*neutral* amphiphilic components prefer different local curvatures [11]: important examples include the complications of thermodynamic phase separation [12,13] and the special case of diblock copolymer mixtures [14]. As far as we are aware, however, most (see below) studies of the above kinds have been restricted exclusively to a single surface.

Much work has, of course, been done on the explicit nature of the forces between a *pair* of charges surfaces in aqueous solution [15]. But in this classic problem the density of charge on each surface is generally assumed to remain uniform, i.e., there is no possibility of charge reorganization that might result in lateral spatial inhomogeneity, and the surface *curvature* is also taken to be constant (corresponding to, say, a planar, or spherical, geometry). The total interaction free energy, as a function of distance, is then evaluated within various continuum (e.g., Poisson-Boltzmann) [15], integral equation [16], and computer simulation (Monte Carlo) [17] approaches. Attention has also been focused on the possibilities for coupling between electrostatic repulsions and the thermally excited curvature undulations of interacting bilayers [18], with specific emphasis on the effective bending elasticity [19,20] and thermodynamic stability of multimembrane states and lamellar phases [21]. In all of these cases, however, the distribution of surface charge density is implicitly assumed to remain constant; see, however, the papers cited in recent reviews [22] for discussions that are relevant to the interaction between a pair of heterogeneous and charged membranes.

For a number of important biological problems such as cell-cell or vesicle fusion, one needs to address directly the spatial reorganization of surface charge *and* the deformation of nearby, strongly *interacting*, membranes. In the present work we explore the possibility that interactions *between* bilayers of neutral and charged lipid mixtures can affect significantly the onset and nature of the lateral demixing between their components. We show in particular that these interactions serve to *promote* this phase separation, e.g., that the intrabilayer (lateral) demixing occurs at higher temperatures and lower concentrations than for isolated vesicles. Equivalently, we conclude that interbilayer repulsive interactions are lowered by the onset of phase separation in the individual membranes. This demixing is shown to be further enhanced by *buckling* deformations that arise in response to the spatial inhomogeneity in surface charge density, a phenomenon that we argue is intimately related to the more general coupling between strain and composition in binary elastic systems [23–25].

In treating first the case of flat membranes, we start with the classic mean-field theory of the electrostatic free energy of interaction of two apposing charged surfaces in aqueous solution. Since we will show that the important coupling between interbilayer interactions and intramembrane phase separation occurs in the small distance limit, where the effects of added salt become irrelevant [15b], it is convenient to ignore electrolytes in the solution and to treat the case of pure counterion screening. Our results will be valid, then, in the limit where the separation between surfaces is small compared to *both* the Debye length *and* the Chapman length [defined below in the discussion of Eq. (2)]. Note also that we do not include penetration of the bilayers by the electric field, nor do we take into account here the finite thickness (or any other structural properties) of the membranes, in formulating their electrostatic interactions.

Let σ denote the number density of (positive, say) charge per unit area on each surface, and $n(\mathbf{r})$ the number density of (negative) counterions at point $\mathbf{r}$ in the intervening aqueous medium. The dimensionless electrostatic potential at $\mathbf{r}$ is denoted by $e\psi/kT=\phi(\mathbf{r})$. Then the total (Helmholtz) free energy of the system (volume V), in units of kT, can be written in mean-field approximation as

$$F_{\mathrm{el}}=\int_V d^3r\{n(\mathbf{r})\ln[v_0 n(\mathbf{r})]-\tfrac{1}{2}n(\mathbf{r})\phi(\mathbf{r})\} + \int_S d^2r\,\tfrac{1}{2}\sigma(\mathbf{r})\phi(\mathbf{r}). \tag{1}$$

Here v_0 is a volume typical of each counterion, and the second integral extends over both surfaces (S), assumed to lie at a distance $2D$ from one another. Within Poisson-Boltzmann theory, the electrostatic free energy per unit area, $\gamma_{\mathrm{el}}=F_{\mathrm{el}}/A$, can straightforwardly be, shown to have the form

$$\gamma_{\mathrm{el}}=\begin{cases} 2\sigma[\ln(2\pi l_B\sigma^2 v_0)-1]+\dfrac{\pi}{4l_B D} \quad D\gg 1/l_B\sigma & \text{(2a)}\\[2ex] 2\sigma\ln\left(\dfrac{\sigma_{V_0}}{D}\right)+4\pi\sigma^2 l_B D \quad D\ll 1/l_B\sigma. & \text{(2b)}\end{cases}$$

Here $l_B=e^2/\varepsilon kT$ is the usual Bjerrum length [15] and ε is the dielectric constant of water.

Note that the characteristic concentration-dependent length scale is the Chapman length $\xi=l/l_B\sigma$, separating as it does the above two distance regimes for D, which we shall refer to henceforth as the strong ($\xi\ll D$) and weak ($\xi\gg D$) coupling limits, respectively. The electrostatic repulsive force per unit area between the membranes, $\Pi=-\delta\gamma_{\mathrm{el}}/\delta D$, diverges as $2\sigma/D$ in the $\xi\gg D$ limit, and this repulsion has an obvious inhibitive effect on membrane fusion. Note also that in Eq. (2b)—as distinct from in Eq. (2a)—each term involves a *non*separable combination of D and σ, a fact which will prove crucial in our discussion below of the coupling between intermembrane interaction and intrabilayer phase separation.

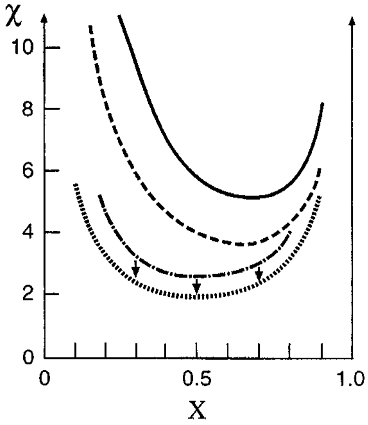

FIG. 1. Spinodal curves, plotted in the form of inverse dimensionless temperature χ vs mole fraction X of charge lipid, for different physical situations considered in the text: familiar uncharged case, · · · ·; one lipid charged, but interaction distance large ($D\gg\xi$), ——; same, but D small ($\ll\xi$); — — —; and buckling included, small D, —·—·—·— (see text).

The free energy formulated above only includes the electrostatic contributions relevant to a pair of *uniformly* charged membranes apposing each other in aqueous solution, whereas the membranes we have in mind are planar bilayers composed of a *mixture* of neutral and charged phospholipids. Let X denote the molefraction of charged lipid, so that the surface density σ of charged molecules can be written in the form $\sigma=X/a$, where a is the area per molecule. The complete surface free-energy density γ_{total} is then the sum of the electrostatic contribution $\gamma_{\mathrm{el}}(X,D)$ plus a term describing the lipid mixture in the *absence* of charge. For this second term we use the Flory mean-field theory of binary mixtures [26] (assuming the same area per molecule for the two lipid species):

$$\gamma_{\mathrm{total}}=\gamma_{\mathrm{el}}(X;D)+\frac{1}{a}[X\ln X+(1-X)\ln(1-X) + \chi X(1-X)]. \tag{3}$$

Here χ is the usual parameter expressing the preference ($\chi>0$), in units of kT, for like-like intermolecular interactions in the binary mixture. For example, in the *absence* of electrostatic effects (i.e., $\gamma_{\mathrm{el}}\equiv 0$), setting equal to zero the second derivative of γ with respect to X gives the familiar mean-field equation [26] for the demixing spinodal curve: $\chi_{\mathrm{sp}}(X)=(1/2)/X(1-X)$. Thus, *neglecting* electrostatic effects, the lipid mixture becomes unstable (with respect to phase separation) at the χ-dependent molefractions shown by the dotted curve in Fig. 1. The critical consolute point is at $X_c=1/2$ with $\chi_c=2$.

Now consider the effects of electrostatics on the neutral and charged lipid phase separation. First, treating the *large D* limit ($D\gg\xi$), we substitute Eq. (2a) for γ_{el} in Eq. (3) and replace σ by X/a. Setting equal to zero the second derivative (with respect to X) of the resulting expression for γ_{total}, we have

$$\chi_{\text{sp}}(X)=\frac{(5/2)-2X}{X(1-X)}, \quad D \gg \xi \tag{4}$$

for the spinodal line (see the solid curve in Fig. 1). Note that the effect of charge is to suppress the demixing relative to the *non*electrostatic situation: χ_c is considerably increased from 2 to 5.2, and the limiting molefractions at which the mixed phase becomes unstable increase (i.e., the solid curve spinodal lies above, and *inside*, the dotted one). This large D behavior is consistent with the lowering of the transition temperature ($T\alpha 1/\chi$) discussed by Trauble *et al.* [17] in their study of lateral phase separation in single ($D\rightarrow\infty$) bilayers of mixed neutral and charged lipids.

In the *small* D limit, however, replacing γ_{el} in Eq. (3) by Eq. (2b), the spinodal becomes

$$\chi_{\text{sp}}(X)=\frac{(3/2)-X}{X(1-X)}+\frac{4\pi l_B D}{a}, \quad D \ll \xi. \tag{5}$$

This spinodal is shown by the *dashed* curve in Fig. 1 and, most significantly, is found to lie *below* the solid one (χ_c decreasing, for example, from 5.2 to 3.7—here we have neglected the second term in Eq. (5), consistent with the small D limit). That is, as the charged membranes approach to distances D comparable to ξ, de-mixing is enhanced. At a temperature corresponding to $\chi=4$, say, the *the lipids mix in all proportions if and only if the bilayers are sufficiently far apart*: only at spacings smaller than ξ will phase separation be induced by the intermembrane interactions. Note that the effect of one membrane component being charged is not simply to introduce D dependence into the Flory χ parameter when the bilayers approach to small distances. Rather it is the effect of counterion entropy which is dominant, as seen by the fact that the relevant spinodal [cf. Eq. (5), even upon the neglect of the second term on the right-hand side] has a different form (X dependence) than that in the case of both lipids being neutral.

In the demixed phase, inside each bilayer, we must encounter interfacial boundary lines separating regions that are rich in charged lipids (with molefraction X) from regions that are poor in charged lipids (molefraction $X'<X$). Equilibrium demands that the chemical potential of the counterions μ and that of the charged lipids μ_L are each constant across these boundaries. Recalling that $\phi(z)$ is the dimensionless electrostatic potential at z (with $z=0$ and $z=D$ denoting the positions of the midplane and right-hand surface, respectively), we have

$$\mu/k_BT=-\phi(0)+\ln[v_0 n(0)], \tag{6a}$$

and

$$\mu_L/k_BT=+\phi(D)+\ln X+\chi(1-X)^2. \tag{6b}$$

Requiring that μ and μ_L each be constant, however, is a condition that *has no solution for flat membranes*. Rather, we are forced to introduce a discontinuity in the layer spacings D and D' associated with the regions on opposite sides of the phase boundaries. Using the limiting form of $\phi(D)$ appropriate to small separations ($D,D' \ll \xi$), we find that μ_L is constant across the interface *if* the spacings satisfy

$$\frac{D}{D'}=\frac{X^2}{X'^2}\exp[\chi(X^2-X'^2)-2(X-X')]. \tag{7}$$

Evaluating the above in the region of the critical point for the small D limit ($X_c=0.63$ and $\chi_c=3.7$–see dashed curve minimum in Fig. 1), i.e., substituting $X=X_c+\varepsilon$, $X'=X_c-\varepsilon$, $\chi=\chi_c+\varepsilon'$, we find $D>D'$. It follows that the spacing is reduced in regions that are poor in charged lipids, while it is increased in those that are rich in charged lipids. *Phase separation inside charged interacting membranes thus unavoidably produces mechanical instability*. In the large D limit, on the other hand, we find that flat membranes decompose *without* mechanical instability [consistent with the separable, uncoupled from of Eq. (2a) with respect to D and X].

The membrane deformation across a phase boundary suggests that lipid demixing for $D<\xi$ is a combined compositional-mechanical instability. To explore this possibility further, we impose a simultaneous modulation in both composition X and spacing D along both membranes. More explicitly, we write

$$X\rightarrow\tilde{X}=X+X_q\cos qy \tag{8a}$$

and

$$D\rightarrow\tilde{D}=D+D_q\cos qy, \tag{8b}$$

and determine when this modulation lowers the overall free energy of the interacting system. In doing so, we need to add–to the integral of Eq. (3), with $X,D\rightarrow\tilde{X},\tilde{D}(y)$, and using Eq. (2b) for $\gamma_{\text{el}}(D\ll\xi)$–the energy cost associated with gradients in D and X:

$$F_{\text{grad}}=\int d^2r\left\{\kappa\left(\frac{d^2\tilde{D}}{dy^2}\right)^2+\Gamma\left(\frac{d\tilde{X}}{dy}\right)^2\right\} \tag{9}$$

with the integration extending over the area of each membrane. Here κ is the bending energy of the membranes [2] (Chapters 1 and 5) and Γ is the usual coefficient giving rise to surface tension between the two components of the mixture [23]. We expand in powers of X_q and D_q and keep all terms except for those which are third order or higher. Then, integrating over the surfaces, we obtain for the overall free energy per unit area the result

$$\gamma=\gamma_{\text{total}}(X,D)+\frac{1}{4a}\left\{-2\chi+\frac{8\pi l_B D}{a}+\frac{3}{X}+\frac{1}{1-X}+2\Gamma a q^2\right\}X_q^2+\left[\frac{1}{2a}\frac{X}{D^2}+\frac{\kappa}{2}q^4\right]D_q^2-\frac{1}{a}\frac{1}{D}X_qD_q. \tag{10}$$

Note that the *average* interlayer spacing D is fixed, while the *fluctuation amplitude* (qth Fourier component) D_q is determined from a variational calculation.

More explicitly, minimizing the above expression with respect to D_q gives a simple proportionality (in the long wavelength $q \rightarrow 0$ limit) between the amplitudes of the buckling and composition fluctuations:

$$\frac{D_q}{D} = \frac{X_q}{[X + aD^2\kappa q^4]} \approx \frac{X_q}{X}. \tag{11}$$

Using this result to substitute for D_q in Eq. (10) leads to a free energy per unit area $\gamma(X,D;X_q)$, the vanishing of whose second derivative (with respect to X_q) corresponds, in the $q \rightarrow 0$ limit, to the interaction and *buckling-renormalized* spinodal given by

$$\chi_{\mathrm{sp}}(X) = \frac{1/2}{X(1-X)} + \frac{4\pi l_B D}{a}. \tag{12}$$

It follows immediately that the spinodal is lowered still further (see the dot-dashed curve shown in Fig. 1) beyond the *un*buckled small D result (dashed). Note that the first term in Eq. (12) is identical to the spinodal obtained in the familiar case of purely charge-neutral mixtures. That is, the net small D effect of counterion sharing (intermembrane interaction), and coupling between composition and buckling, is to offset, essentially completely, the electrostatic terms that discourage phase separation. Note further that the proportionality between the amplitudes D_q and X_q given by Eq. (11) is consistent with our having found earlier that the constancy of the chemical potentials in Eq. (6) implies that D increases with X according to Eq. (7): an expansion of Eq. (7) about the critical point yields the proportionality in Eq. (11). Indeed, Eq. (11) followed from a free-energy minimization that is equivalent to guaranteeing the equality of μ's.

In the present description then, our scenario for vesicle fusion induced by intramembrane decomposition would be as follows. For large D spacings the membrane bilayers of two respective vesicles, which both contain the same concentration of charged lipids, are stabilized against demixing by electrostatic repulsion (solid curve in Fig. 1). As long as D greatly exceeds ξ, the electrostatic interaction *between* them does not affect their phase behavior. When the D spacing drops below ξ, however, a *a composition-mechanical instability is triggered*. The separation D in regions that are depleted in charged lipid decreases significantly and fusion becomes possible due to the local reduction in electrostatic repulsion.

As alluded to in our introductory remarks, the above scenario bears a striking similarity to the behavior of metal alloys under external stress, where it has been shown [23,24] that decomposition of a solid solution into its constituent components can be triggered by applied loads. The theory presented in the present communication is closely analogous if one replaces external stress by the intermembrane electrostatic repulsion and internal strain by the D spacings. Indeed, in the original work of Cahn [23], the coupling of composition to ‘‘coherency (lattice mismatch)’’ strain significantly shifts the *zero*-load spinodal, although the effect happens to involve a *lowering* of the demixing temperature, rather than a *raising*, as in our present work. The sign of the coupling effect in the Cahn problem is associated with the fact that the average elastic strain energy density is necessarily *positive*, whereas in our case the phase separation is made *easier* because of the enhanced electrostatic screening arising from shared counterions (from both surfaces) at short interaction distances. Related, more recent treatments of this basic physical phenomenon are found in the theory and simulations of Desai aand coworkers [25] on spinodal decomposition in order-disorder systems with long-range forces, and in the microscopic model by Laberge, Fratzl, and Lebowitz [27] who treat explicitly the directional coarsening–‘‘rafting’’–observed in lattice-mismatched binary alloys subjected to uniaxial external loads.

Finally, we note that for simplicity in our work we have invoked the usual mean-field continuum (Poisson-Boltzmann) theory in describing the consequences of electrostatics on compositional-mechanical instabilities in interacting mixed membranes. While correlation corrections and atomic scale ion-solvent-head group effects are surely operative in the small D limit of interest to us, we do not believe they are relevant to a *qualitative* understanding of the phenomena discussed in this paper. Note also that, because we are specifically treating phospholipid bilayers, we have implicitly assumed that values of the bending energies (κ) of the membranes are sufficiently large that undulation forces between them are negligible. Accordingly, when the intermembrane electrostatic repulsions are reduced by the counter-ion-sharing, lateral-phase-separation and buckling mechanism, attractive forces between the surfaces can become more effective in promoting eventual fusion. Similarly, because we consider only the *initial* instability (and do not investigate, for example, the wavelengths that characterize the ensuing spinodal decomposition), the actual values of the bending energy–and its spatial variation with charge density–are of no consequence.

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