# Macroion-Induced Compositional Instability of Binary Fluid Membranes

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Macroion adsorption on a mixed, fluid, lipid membrane containing oppositely charged lipids induces local changes in lipid composition at the interaction zones, and gradients at their boundaries. Including these effects in the free energy of the macroion-dressed membrane we derive its spinodal equation, and show that nonideal lipid mixing can lead to (lipid-mediated) attraction between macroions and lateral phase separation in the composite membrane. The critical nonideality for this transition is substantially smaller than that of the bare lipid membrane, decreasing with macroion size and charge. That is, the lipid membrane is destabilized by macroion adsorption.

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Biomembranes are two-dimensional (2D) *fluid mixtures*, composed of various lipid species and membrane-associated proteins. Since the lipids are mobile, the membrane can respond to interacting macromolecules by locally changing its composition. Consider, for instance, the adsorption of a highly charged cationic protein onto a membrane containing a small fraction of anionic lipids, initially randomly dispersed among nonionic lipids. Upon adsorption, anionic lipids diffuse into the interaction zone (thus displacing neutral lipids) so as to minimize the electrostatic interaction free energy. For two infinite, oppositely charged, planar surfaces this minimum is reached when their charge densities are equal [1,2], allowing maximal release of counterions into the bulk solution. Similar principles govern macroion adsorption, with variations depending on the macroions' (finite) size, shape, and charge. More significantly, the "lipid demixing" induced by macroion adsorption is partially inhibited by the concomitant entropy loss. The actual composition profile of the *annealed, macroion-dressed*, membrane is governed by the balance between these opposing forces [3].

*Local* changes in lipid composition were reported for both protein-membrane [4,5] and DNA- (cationic) membrane [6] systems. Experiments also indicate that, upon adsorption, charged proteins [4] or colloidal particles [7] may aggregate into macroscopic *domains*, presumably enriched with the macroions' favorite lipids. Monte Carlo simulations [8,9] of lipid-protein membranes, featuring nonelectrostatic interactions, indicate that domain formation can indeed take place. More generally, it was shown theoretically that the critical demixing temperature of a binary membrane increases upon (nonelectrostatic) adsorption of colloidal particles which interact preferentially with one lipid component [10].

Theoretical models for macroion-induced phase separation of mixed lipid membranes were previously proposed by two groups [4,7]. Though using very different electrostatic adsorption models (yet both favoring macroion-membrane charge matching), both models predict macroion-induced phase splitting even for *ideal lipid mixture*. However, a major deficiency underlying these treatments is the (tacit) assumption that the dressed membrane is *spatially uniform*, thus neglecting *local changes* in lipid composition and the role of *macroion size*. In our theory, nonideal lipid mixing, local changes in membrane composition and macroion size, and charge are crucially important.

Thermodynamically, domain formation is a 2D phase separation, indicating that the interaction between macroions is *effectively attractive*. Since like-charged macroions repel each other [at least, according to Poisson-Boltzmann (PB) theory], this attraction must be mediated by the "underlying" lipid substrate; involving *simultaneous splitting* of both the macroion and lipid layers. In this Letter we derive the dependence of the membrane-mediated attraction on macroion size and charge, and membrane composition. Also, while arguing that nonideal lipid (chain) mixing is a necessary condition for phase separation in the dressed membrane, we will show that the critical chain nonideality is substantially lower than that of the bare lipid membrane.

Consider a binary membrane of area $A$, composed of $N$ molecules; $N_a = \phi N$ are charged lipids and $N_n = (1 - \phi)N$ are nonionic. We assume that the charged lipids carry a monovalent anionic headgroup, and the area per molecule, $a_l = A/N$, is the same for both species. Thus $\phi$ is both the molar and area fraction of charged lipid, measuring also the surface charge density, $-e\phi/a_l$; $e$ denoting the elementary charge. The membrane is embedded in an aqueous electrolyte solution, characterized by its Debye length, $l_D$ [2]. The solution also serves as a reservoir of macroions with chemical potential $\mu_p = \epsilon + k_B T \ln\lambda$; $\epsilon$ is the transfer free energy of a macroion from membrane to solution, and $\lambda$ the macroions' activity (approaching the volume fraction in dilute solution).

  

Hereafter all energies will be measured $k_BT$'s; $k_B$ is Boltzmann's constant and $T$ the temperature.

The 2D density of the macroion adlayer will be expressed in terms of $\theta = M/M_{\max}$; $M$ and $M_{\max}$ denoting, respectively, the actual and maximal (close-packed in 2D) number of adsorbed macroions. Thus, $a_p = A/M_{\max}$ is the minimal (projected) area per macroion, $a_p/a_l \equiv \sigma$ serving as a convenient measure of macroion size ($\sigma \sim 10$ for typical lipid-protein membranes). For concreteness, as illustrated in Fig. 1, the macroions may be depicted as disklike particles of effective area $a_p$, each carrying a net positive charge $ez_p$, uniformly smeared over its "membrane-apposed" face.

The free energy of the dressed membrane, $F = Nf(\phi, \theta)$, can be expressed as a sum of three terms,

$$F = Nf_{\rm el}(\phi, \theta) + M_{\max}[\theta \ln\theta + (1-\theta)\ln(1-\theta)] + N[\phi \ln\phi + (1-\phi)\ln(1-\phi) + \chi\phi(1-\phi)]. \quad (1)$$

The first term is the electrostatic charging free energy of the system, already minimized with respect to local changes in lipid composition and macroion-membrane distance, $h$. Also included in $f_{\rm el}$ are the lateral electrostatic interactions between adsorbed macroions [11]. Excluded area interactions, and the translational entropy of the macroion adlayer are accounted for by the 2D lattice gas model in the second term. The last term in Eq. (1) includes all *nonelectrostatic* (mainly lipid chain) contributions, representing the free energy of a *bare neutral membrane*. It is modeled here as an incompressible binary mixture, with $\chi$ measuring the extent of nonideal lipid mixing in mean field approximation. This model predicts a critical point at $\chi_c = 2$, $\phi_c = 1/2$ [12], i.e., phase separation occurs when $\chi > 2$.

For $\theta = 0$ Eq. (1) is the free energy of the *charged bare membrane*. Now $\chi > 2$ may not suffice for lipid phase separation, because electrostatic repulsions between the charged lipid headgroups counteract the chains' demixing tendency. Indeed, we shall show below that $\chi_c$ of the charged membrane is nearly twice as large as $\chi_c$(neutral) at low salt concentrations, decreasing to the neutral membrane value at the high salt limit ($l_D \to 0$), where Coulomb forces are fully screened. This result will be obtained as a special ($\theta = 0$) case of the macroion-dressed membrane whose spinodal equation is derived below.

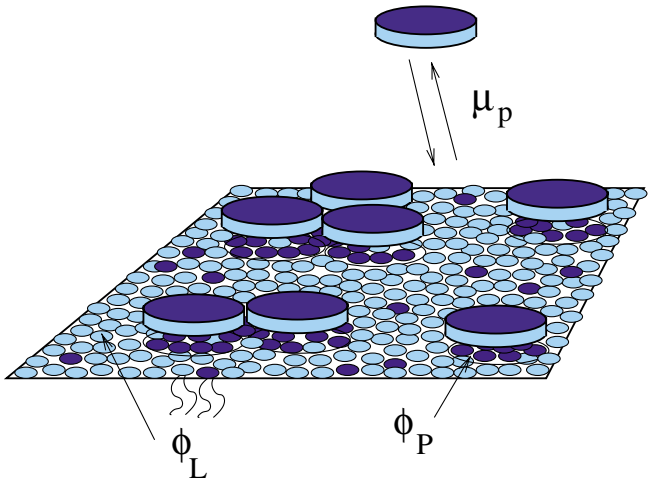


FIG. 1 (color online). Adsorption of macroions (of chemical potential $\mu_P$) from solution onto a binary fluid membrane, inducing lipid "demixing," i.e., different local compositions, $\phi_P$ and $\phi_L$, in interacting and bare membrane regions, respectively.

At equilibrium the chemical potentials of adsorbed and solvated macroions are equal, $\partial F/\partial M = (\partial F/\partial\theta) \times (\sigma/N) = \mu_p$. Using Eq. (1) we find

$$\mu_p = \sigma \frac{\partial f_{\rm el}(\phi, \theta)}{\partial\theta} + \ln\frac{\theta}{1-\theta}. \quad (2)$$

The solution of Eq. (2), $\theta = \theta_{\rm eq}(\phi; \mu_p)$, is the equilibrium coverage of macroions on a membrane of composition $\phi$. If the dressed membrane separates into coexisting phases characterized by $\theta_1, \phi_1$ and $\theta_2, \phi_2$, then Eq. (2) must hold for both phases, relating $\theta_i = \theta_{i,\rm eq}(\phi_i; \mu_p)$, $(i = 1, 2)$ to $\phi_i$. (Of course $\mu_p = \mu_{p,1} = \mu_{p,2}$.) The equilibrium values of $\phi_1, \phi_2$ can be derived from the requirement for the equality of the anionic (or the nonionic) lipid chemical potential in the two phases ($\mu_{a,i} = \partial F_i/\partial N_{a,i}$) and the equality of the 2D pressures $-\partial F_i/\partial N_i$ [12]. Solving these coexistence equations is equivalent to the "common tangent construction" for the thermodynamic potential $\xi(\phi; \mu_p) = f[\phi, \theta_{\rm eq}(\phi; \mu_p)] - \mu_p\, \theta_{\rm eq}(\phi; \mu_p)/\sigma$; i.e., $d\xi/d\phi$ and $\xi - \phi d\xi/d\phi$ should be equal in both phases. Rather than solving the coexistence equations we shall suffice here in deriving the spinodal curve, $\chi(\phi)$, defined by the solution of $\xi''(\phi) = d^2\xi/d\phi^2 = 0$. The spinodal marks the border between the metastable (single phase) and unstable (phase separated) regions and its minimum, $\chi_c(\phi = \phi_c)$, is the critical point.

Using Eqs. (1) and (2) in $\xi''(\phi) = 0$ one can derive the *general spinodal equation*,

$$\chi = \frac{1}{2\phi(1-\phi)} + \frac{1}{2}\frac{d}{d\phi}\left(\frac{\partial f_{\rm el}(\phi, \theta)}{\partial\phi}\right), \quad (3)$$

where it should be noted that the $d/d\phi$ operation must be performed after substituting $\theta = \theta_{\rm eq}(\phi)$ from Eq. (2).

Below we apply Eq. (3) to two models of $f_{\rm el}(\theta, \phi)$. First, mainly for comparison, we briefly discuss an adsorption scheme whereby the macroions do not induce any modulations in membrane charge. (For $\theta = 0$, this model provides an adequate description of the bare membrane.) The second model accounts explicitly for local, macroion-induced, charge modulations. The two schemes agree only in the high salt (weak adsorption) limit.

Macroion adsorption reduces the average membrane charge density from $\phi$ to $\tilde{\phi} = \phi - z_p\theta/\sigma$. Treating this "rescaled" charge as uniformly *delocalized* over the membrane surface, the membrane free energy can be calculated using the PB expression [2],

$$g(\phi) = 2\phi[(1-q)/p + \ln(p+q)] \quad (4)$$

for the charging energy (per molecule) of a uniform planar membrane of composition $\phi$. Here, $q = \sqrt{p^2+1}$, $p = p_0\phi$, $p_0 = 2\pi l_B l_D/a_l$, and $l_B$ is the Bjerrum length. Setting $f_{\rm el}(\phi,\theta) = g(\tilde{\phi})$ in Eq. (3) we find

$$\chi = \frac{1}{2\phi(1-\phi)} + \frac{p_0}{\tilde{q} + 2p_0(1/\sigma)z_p^2\theta(1-\theta)}, \quad (5)$$

with $\tilde{q} = [1 + (p_0\tilde{\phi})^2]^{1/2}$ and $\theta = \theta_{\rm eq}$, as given by the solution of Eq. (2). [In fact, Eq. (5) holds for any given $\theta$.]

The first term in Eq. (5) is the spinodal of a *neutral lipid membrane* [$f_{\rm el} \equiv 0, \theta = 0$ in Eq. (3)], yielding $\chi_c = 2$; $\phi_c = 1/2$. For $\theta = 0$, Eq. (5) becomes the spinodal of the *bare charged membrane*: $\chi = 1/2\phi(1-\phi) + p_0/\sqrt{1+(p_0\phi)^2}$; yielding $\chi_c \to 2 + p_0 \to 2$; $\phi_c \to 1/2$ in the high salt ($p_0 \ll 1$) limit. $\chi_c$ increases rapidly with $p_0$ (e.g., $\chi_c \approx 3$ for $p_0 = 2$) saturating at $\chi_c \to 2 + \sqrt{3} \approx 3.7$ [with $\phi_c \to (3-\sqrt{3})/2 \approx 0.63$] in the low salt limit (see also [13]). Under physiological salt conditions $p_0 \approx 7$.

For the dressed membrane Eq. (5) predicts a rapid decrease of $\chi_c$ with $z_p$, reflecting the enhanced adsorption of highly charged macroions and their efficient screening of lipid charge. Still, $\chi_c$ cannot fall below the neutral membrane value, $\chi_c = 2$. Recall, however, that this conclusion is based on the "charge smearing" approximation, ignoring the special ability of a fluid membrane to adjust its local composition at the macroion adsorption site.

The theory described in the remainder of this Letter features the *localized* nature of macroion adsorption, allows for local modulations in membrane composition, and emphasizes the qualitatively different phase behavior predicted by this approach, as compared to Eq. (5).

The area of the membrane "patch" affected by the adsorption of a single macroion must be of the order of $a_p$, as illustrated in Fig. 1 (see also [3]). The exact lipid composition profile within and around the interaction patch depends generally on the macroion size, shape and charge, as well as on $\theta$ and $\phi$. We shall ignore these details here and assume that the interaction zone is bounded by a narrow stripe, of width comparable to one molecular diameter ($\sim\sqrt{a_l}$). The mobile lipids in the fluid membrane diffuse into and out of the "macroion covered" ("$P$") and bare membrane regions ("$L$"), adjusting the local compositions $\phi_P$ and $\phi_L$ in order to minimize the dressed-membrane free energy, subject to the conservation condition $\theta\phi_P + (1-\theta)\phi_L = \phi$.

The electrostatic free energy of the dressed membrane can be expressed as a ($\theta$-weighted) sum of contributions from the $P$ and $L$ regions, and an interfacial, line energy, term for the separating boundary

$$f_{\rm el}(\phi,\theta) = \theta f_P^\star(\phi_P,\phi) + (1-\theta)f_L^\star(\phi_L,\phi) + \frac{\Lambda}{\sigma}\,\theta(1-\theta). \quad (6)$$

Here $f_P^\star(\phi_P,\phi) = f_P(\phi_P) + \triangle f_{\rm mix}(\phi_P,\phi)$ is the sum of the electrostatic energy of $P$ regions, $f_P(\phi_P) = f_{\rm el}(\phi_P,\theta=1)$, and the lipid "demixing" term,

$$\triangle f_{\rm mix}(\phi_P,\phi) = \phi_P \ln\frac{\phi_P}{\phi} + (1-\phi_P)\ln\frac{1-\phi_P}{1-\phi} + \chi[\phi_P(1-\phi_P) - \phi(1-\phi)]. \quad (7)$$

Similarly, $f_L^\star(\phi_L,\phi) = f_L(\phi_L) + \triangle f_{\rm mix}(\phi_L,\phi)$, with $f_L(\phi_L) = f_{\rm el}(\phi_L,\theta=0) = g(\phi_L)$. Note that upon substituting Eq. (6) into Eq. (1), the third (uniform lipid phase) term in Eq. (1) is replaced by the weighted sum of lipid free energies in the $P$ and $L$ regions.

The last term in Eq. (6) accounts for the *line energy* associated with the gradients in lipid composition across the boundaries between $P$ and $L$ regions. It is the product of the boundary length [$\sim\theta(1-\theta)$] and the line energy density ($\sim\Lambda$). Actually, $\Lambda$ is the line energy corresponding to the perimeter length of one macroion (measured in units of $\sqrt{a_l}$). Its general form for a circular interaction zone (applicable to any composition profile) is $\Lambda = (\chi/3)\int 2\pi r(\partial\phi/\partial r)^2 dr$. For our narrow $P-L$ boundary where $\phi_P$ changing sharply to $\phi_L$,

$$\Lambda = \sigma^{1/2}\chi\,(\triangle\phi)^2, \quad (8)$$

with $\triangle\phi = \phi_P - \phi_L$. The last equation could also be derived using a 2D lattice model with nearest neighbor interlipid interactions, $\omega(i,j)$; [$i,j$ = anionic ($a$) or nonionic ($n$)]. Equation (8) is obtained by calculating the number of $a,a$, $n,n$, and $a,n$ contacts across the boundary, using $\chi = (c/2k_BT)[2\omega(n,a) - \omega(a,a) - \omega(n,n)]$; with $c$ the lattice coordination number [12].

Lateral aggregation of the macroion-lipid *"clusters"* (as defined by the $P$ regions) lowers the total $P$-$L$ boundary length, thus favored by large positive $\Lambda$. If strong enough, this tendency can overcome the translational entropy of the 2D "cluster gas," resulting in phase separation of the dressed membrane. Consistent with Eq. (8) the attraction between clusters increases with their *perimeter length*, the degree of *lipid nonideality*, and the *composition gradient*, $\triangle\phi$, on which we focus next.

For any given $\phi$ and $\theta$, the segregated lipid populations should adjust their compositions, $\phi_L = \phi - \theta\triangle\phi$ and $\phi_P = \phi + (1-\theta)\triangle\phi$, so as to minimize $f_{\rm el}(\phi,\theta)$ in Eq. (6); resulting in equal lipid chemical potentials in the $P$ and $L$ regions: $\partial f_{\rm el}(\phi,\theta)/\partial\triangle\phi = 0$. Combining Eqs. (6)–(8) with the general spinodal equation, Eq. (3), we find (after some algebra) that the spinodal equation for the *localized adsorption* model reads

$$\chi = \frac{1}{2\sigma^{1/2}\,\triangle\phi\,\theta\,(1-\theta)}\,\frac{d\theta}{d\phi}, \quad (9)$$

where, as before, $d\theta/d\phi$ must be obtained from Eq. (2), and $\triangle\phi$ must satisfy $\partial f_{\rm el}(\phi,\theta)/\partial\triangle\phi = 0$.

Equation (9) is valid for any model of $f_L(\phi_L)$ and $f_P(\phi_P)$. For instance, in the "high salt" ($p_0 \ll 1$, small $l_D$) limit one can use the Debye-Hückel expressions,

$f_L(\phi_L) = g(\phi_L)_{p_0 \to 0} = p_0 \phi_L^2$ and $f_P(\phi_P) = p_0 |\phi_P^2 - (z_p/\sigma)^2|$. It can be shown that for weakly charged macroions ($z_p/\sigma < \phi$) lipid demixing is negligible ($\triangle \phi = \phi_P - \phi_L \to 0$), and adsorption is weak ($\theta_{eq} \ll 1$ and $d\theta/d\phi \to 0$). Furthermore, the limit of $(d\theta/d\phi)/\triangle\phi$ in Eq. (9) is such that $\chi \to 1/2\phi(1-\phi) + p_0$, which coincides with the high salt ($p_0 \ll 1$) limit of Eq. (5), the spinodal equation of the delocalized charge model. The same result is obtained by using the Debye-Hückel expression $f_{el}(\phi, \theta) = \theta f_P(\phi) + (1-\theta) f_L(\phi) = p_0(\phi^2 - \theta z_p^2/\sigma^2)$ in the general spinodal equation, Eq. (3).

The *low salt* ($p_0 \gg 1$) limit is much more interesting, and more relevant for biological systems where, typically, $p_0 \approx 7$. These conditions imply *strong adsorption* for highly charged (say $z_p \simeq 10$, $\sigma \simeq 10$) macroions, because the adsorption free energy is many $k_B T$'s per macroion. The adsorption is particularly favorable under charge matching conditions, i.e., when $\phi \approx z_p/\sigma$. In general, the average membrane charge density $\phi$ is different (say smaller) than $z_p/\sigma$. However, because the gain in adsorption energy overwhelms the entropic demixing penalty we may safely assume perfect charge matching, $\phi_P = z_p/\sigma$. Furthermore, because the adsorption is highly favorable, macroions will continue to adsorb until all charged lipids are "bound," i.e., until $\theta = \theta_{eq}(\phi) = \phi\sigma/z_p = \phi/\phi_P$ and $\phi_L = 0$. Thus, $d\theta_{eq}/d\phi = 1/\phi_P$ and $\triangle\phi = \phi_P = z_p/\sigma$. Using these results in Eq. (9) and minimizing $\chi$ with respect to $\phi$ we find the critical parameters,

$$\chi_c = \frac{2}{\phi_P^2 \sqrt{\sigma}}; \qquad \phi_c = \frac{\phi_P}{2}. \tag{10}$$

Recall that the critical nonideality of a bare membrane in the low salt regime can be as high as $\chi_c = 3.7$. Equation (10) reveals that a much smaller $\chi$ may lead to phase separation of the *dressed membrane*; e.g., for $\sigma \approx 10$ and $z_p \approx 10$ we find $\chi_c \approx 2/3$, smaller even than the neutral membrane value $\chi_c = 2$. In other words, macroion adsorption onto a uniform and stable [$\chi < \chi_c$(bare)] lipid membrane can destabilize the system, leading to 2D phase splitting of the dressed membrane. From Eq. (10) we conclude that large ($\sigma \gg 1$) and highly charged ($z_p/\sigma \approx \phi_P \approx 1$) macroions facilitate the phase separation, owing to their low $\chi_c$. Interestingly, in the opposite (formal) limit of "small, monovalent, and tightly bound" macroions ($z_p = 1$, $\sigma = 1$, $\phi_P = 1$) Eq. (10) recovers the neutral membrane values $\chi_c = 2$, $\phi_c = 1/2$.

An instructive interpretation of the localized adsorption scheme can be given as follows. Substituting $\chi_c$ from Eq. (10) into Eq. (8) (with $\triangle\phi = \phi_P$) we find $\Lambda_c = 2$ for $\Lambda$ at the critical point of the dressed membrane. Also, at this point $\theta_c = \phi_c/\phi_P = 1/2$. These results are valid in the strong adsorption limit, where all charged lipids are bound to macroions, forming a 2D "gas" of $P$ clusters (with $\phi_P = z_p/\sigma$), embedded in a neutral membrane ($\phi_L = 0$); see Fig. 1. The free energy of this system is $\tilde{F}/M_{max} = \theta \ln\theta + (1-\theta)\ln(1-\theta) + \Lambda\theta(1-\theta)$ plus linear terms in $\theta$ that do not effect $\Lambda_c, \theta_c$. Now, it is easily verified that (in the strong adsorption limit) substitution of $f_{el}$ from Eq. (6) into Eq. (1) yields a dressed-membrane free energy, $F$, identical to $\tilde{F}$. In other words, the phase behavior of the composite membrane is governed by the ($\chi, z_p, \sigma$-dependent) interaction between *clusters*. In contrast, the phase behavior predicted by the delocalized adsorption scheme is dictated by the interaction between *lipids*, partially screened by the adsorbed macroions.

The theoretical formulation in this Letter assumed PB theory for the electrostatic interactions, and random (local) mixing of the lipid species. These mean field approximations may affect our calculated critical parameters, but cannot detract from our conclusions regarding the crucial roles of lipid mobility and localized macroion adsorption in the membrane phase behavior.

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