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# Stability of charged, mixed lipid bilayers: effect of electrostatic coupling between the monolayers

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## Abstract

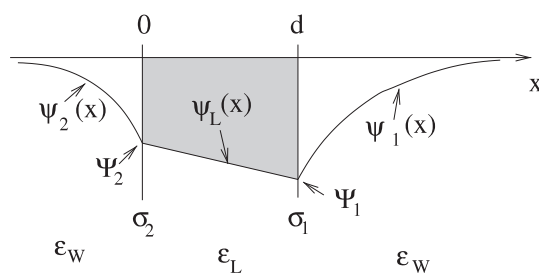
The stability of a planar, two-component, charged lipid bilayer with respect to lateral phase separation is analysed. Particular focus is put on the influence of electrostatic coupling between the two apposed monolayers. We describe the membrane on the mean-field level by a lattice gas model, supplemented by the Poisson–Boltzmann theory for electrostatic interactions. The spinodal line is calculated analytically for a symmetric membrane. We demonstrate that the critical non-ideality parameter decreases with the extent of electrostatic coupling across the bilayer. In the limit of strong coupling it approaches the value of an uncharged membrane.

## 1. Introduction

Stability of multi-component lipid membranes with respect to lateral demixing plays an important role in biological systems where a multitude of cellular processes (like cell adhesion, transport, signalling) are influenced by the physical properties of the membrane. Fluid-like model membranes are known to exhibit non-ideal mixing behaviour [1] which may lead spontaneously to the formation and coexistence of lateral phases of different lipid composition [2]. Electrostatic interactions are among the key factors that determine the mixing properties of lipids in charged membranes. Previous calculations concerning the stability of binary charged lipid monolayers reveal the stabilizing role of the electrostatic repulsion between similarly charged lipids. That is, the spinodal line is shifted up to higher temperatures [3]. Interestingly, additionally adsorbed macroions that interact electrostatically with lipid monolayers are able to decrease the membrane stability, even beyond the level of neutral membranes [4].

In the present work we investigate the stability of a membrane modelled as a two-component lipid bilayer and we focus on the effects imposed by the electrostatic coupling

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**Figure 1.** Schematic representation of the electrostatic potential for the charged bilayer. The head group regions of the two monolayers (indexed ‘1’ and ‘2’) are located at  $x = d$  and  $0$ ; the (dimensionless) electrostatic potential is denoted by  $\psi_1(x)$  for  $x \geq d$ ,  $\psi_L(x)$  for  $0 \leq x \leq d$ , and  $\psi_2(x)$  for  $x \leq 0$ . The surface charge densities are  $\sigma_1$  and  $\sigma_2$  and the corresponding surface potentials  $\Psi_1 = \psi_1(d) = \psi_L(d)$  and  $\Psi_2 = \psi_2(0) = \psi_L(0)$ . The dielectric constants in the aqueous regions ( $x < 0$  and  $x > d$ ) and within the membrane interior ( $0 < x < d$ ; shaded region) are  $\epsilon_W$  and  $\epsilon_L$ , respectively.

between the two charged faces of the membrane. To this end, we separate the free energy of the bilayer into non-electrostatic and electrostatic contributions. Both contributions are treated on the mean-field level, the former using the lattice gas description of a binary fluid mixture and the latter on the basis of non-linear Poisson–Boltzmann theory. We shall show that the electrostatic coupling across the bilayer generally lowers the stability with respect to lateral phase separation.

## 2. Free energy of a two-component, charged lipid bilayer

We consider a two-component lipid bilayer that consists of one negatively charged and one neutral species. The membrane is assumed to be flat and is immersed in aqueous monovalent salt solution characterized by Debye length  $l_D = 1/\kappa$  and dielectric constant  $\epsilon_W$ . Let us label the two individual monolayers by the indices ‘1’ and ‘2’. The corresponding mole fraction of charged lipids in each monolayer (further referred to as ‘composition’) is  $\phi_1$  and  $\phi_2$ . Assuming that all lipids—charged and uncharged ones—have the same cross-sectional area  $a$ , the (negative) charge densities of the two monolayers can be expressed as  $\sigma_1 = -\phi_1 e/a$  and  $\sigma_2 = -\phi_2 e/a$  where  $e$  is the elementary charge. The hydrophilic lipid head groups separate the aqueous phase from the hydrophobic interior of the bilayer. We denote the corresponding thickness of the hydrocarbon core by  $d$  and its dielectric constant by  $\epsilon_L$ .

It is convenient to incorporate the constants  $e/k_B T$  into the electrostatic potential  $\Phi$  to render it unitless through the definition  $\psi = e\Phi/k_B T$  (where  $k_B$  is Boltzmann’s constant and  $T$  is the absolute temperature). Due to the planar symmetry of the bilayer, the potential  $\psi = \psi(x)$  will only be a function of the  $x$ -coordinate pointing normal to the membrane plane. Figure 1 shows a schematic cross-section through the bilayer and illustrates the principal behaviour of the electrostatic potential.

In our expression for the overall free energy,  $F = Nf(\phi_1, \phi_2)/2$ , of the membrane (note that  $N/2 = N_1 = N_2$  is the constant number of lipids in either one of the two monolayers) we separate the electrostatic free energy contribution ( $f_{el}$ ) from all non-electrostatic interactions ( $f_{mix}$ )

$$f(\phi_1, \phi_2) = f_{el}(\phi_1, \phi_2) + f_{mix}(\phi_1) + f_{mix}(\phi_2). \quad (1)$$

In the following we express all energies in units of  $k_B T$ . The non-electrostatic contribution to the free energy of the bilayer is modelled within the well-known mean-field treatment of

regular solution theory [5]

$$f_{\text{mix}}(\phi) = \phi \ln \phi + (1 - \phi) \ln(1 - \phi) + \chi \phi(1 - \phi) \quad (2)$$

which describes each monolayer as an incompressible binary fluid exhibiting only nearest neighbour interactions. The first two terms of (2) represent the ideal mixing free energy, whereas the last term takes the non-ideality of the mixture into account;  $\chi$  is the non-ideality parameter that measures the chemical mismatch between the two types of lipids. For  $\chi > 0$  attractive short-range interactions between lipids of the same species dominate. Above a critical value  $\chi > \chi_c$  lateral phase separation occurs. In the absence of electrostatic interactions (2) gives rise to  $\chi_c = 2$  and a corresponding critical composition  $\phi^c = 1/2$ .

In order to calculate the electrostatic free energy we employ a charging process of the two apposed monolayers. This requires only knowing how the membrane surface potentials,  $\Psi_1 = \Psi_1(\phi_1, \phi_2)$  and  $\Psi_2 = \Psi_2(\phi_1, \phi_2)$ , depend on the monolayer compositions  $\phi_1$  and  $\phi_2$ .

$$f_{\text{el}}(\phi_1, \phi_2) = - \int_0^{\phi_1} \Psi_1(\tilde{\phi}_1, 0) d\tilde{\phi}_1 - \int_0^{\phi_2} \Psi_2(\phi_1, \tilde{\phi}_2) d\tilde{\phi}_2. \quad (3)$$

The particular form of (3) assumes first charging of the monolayer '1' and then '2', but the final result for  $f_{\text{el}}(\phi_1, \phi_2)$  does, of course, not depend on that order. According to Poisson–Boltzmann theory, the electrostatic potentials  $\psi_1$  and  $\psi_2$  in the aqueous regions and  $\psi_L$  inside the membrane satisfy the equations

$$\psi_1'' = \kappa^2 \sinh \psi_1, \quad \psi_2'' = \kappa^2 \sinh \psi_2, \quad \psi_L'' = 0 \quad (4)$$

where here and in the following the prime denotes the derivative with respect to  $x$ . The corresponding boundary conditions at the head group regions of both monolayers read

$$\begin{aligned} \psi_1'(d) - Hd\kappa\psi_L'(d) &= 2\kappa p_0\phi_1 \\ \psi_2'(0) - Hd\kappa\psi_L'(0) &= -2\kappa p_0\phi_2 \end{aligned} \quad (5)$$

where we have introduced the dimensionless constant  $p_0 = 2\pi l_B l_D / a$  with  $l_B = e^2 / 4\pi k_B T \epsilon_W$  being the Bjerrum length. The degree of electrostatic coupling between the two monolayers is taken into account by the coupling parameter

$$H = \frac{\epsilon_L l_D}{\epsilon_W d}. \quad (6)$$

The electrostatic potential vanishes at very large distances from the isolated membrane,  $\psi_1(x \rightarrow \infty) = 0$  and  $\psi_2(x \rightarrow -\infty) = 0$ . Integration of (4) subject to these boundary conditions yields the surface potentials

$$\Psi_1 = -2 \operatorname{arcsinh}(p_0 \bar{\phi}_1), \quad \Psi_2 = -2 \operatorname{arcsinh}(p_0 \bar{\phi}_2) \quad (7)$$

where the quantities  $\bar{\phi}_1 = \phi_1 + H \Delta \Psi / 2 p_0$  and  $\bar{\phi}_2 = \phi_2 - H \Delta \Psi / 2 p_0$  appear as effective monolayer compositions;  $\Delta \Psi = \Psi_1 - \Psi_2$  is the potential difference across the membrane. Insertion of the surface potentials into (3) results in the charging free energy

$$f_{\text{el}}(\phi_1, \phi_2) = \frac{H}{4p_0} (\Delta \Psi)^2 + f_{\text{ml}}(\bar{\phi}_1) + f_{\text{ml}}(\bar{\phi}_2) \quad (8)$$

which appears as the sum of three terms. The first one is the electrostatic energy of a planar capacitor with potential difference  $\Delta \Psi = \Delta \Psi(\phi_1, \phi_2)$  and lateral extension  $a$ . The remaining two terms correspond to the charging free energies of two isolated monolayers with effective compositions  $\bar{\phi}_1$  and  $\bar{\phi}_2$ , respectively. Note that the well-known charging free energy [6, 7] of a single monolayer of composition  $\phi$  is

$$f_{\text{ml}}(\phi) = 2\phi \left[ \frac{1-q}{p} + \ln(p+q) \right] \quad (9)$$

with  $p = p_0\phi$  and  $q = \sqrt{p^2 + 1}$ . The electrostatic coupling between the two monolayers is contained in the two effective compositions  $\bar{\phi}_1$  and  $\bar{\phi}_2$  and also in the potential difference  $\Delta\Psi$  that is the solution of the transcendental equation

$$\frac{\Delta\Psi}{2} = -\operatorname{arcsinh}\left(p_0\phi_1 + \frac{H}{2}\Delta\Psi\right) + \operatorname{arcsinh}\left(p_0\phi_2 - \frac{H}{2}\Delta\Psi\right). \quad (10)$$

If we artificially exclude any electric field inside the membrane ( $H = 0$ ) the electrostatic free energy of the bilayer,  $f_{\text{el}} = f_{\text{ml}}(\phi_1) + f_{\text{ml}}(\phi_2)$ , is the sum of the charging free energies of two isolated monolayers of compositions  $\phi_1$  and  $\phi_2$ . In the other limit ( $H \rightarrow \infty$ ) we find that  $H\Delta\Psi = p_0(\phi_2 - \phi_1)$  and thus  $f_{\text{el}}(\phi_1, \phi_2) = 2f_{\text{ml}}[(\phi_1 + \phi_2)/2]$ .

### 3. Local stability analysis

The boundary of local stability with respect to lateral phase separation is conveniently described by the spinodal line [5]. Since the lipid bilayer has two degrees of freedom (namely the two compositions  $\phi_1$  and  $\phi_2$ ) the spinodal is determined by the solution for  $\chi$  of the equation

$$\left(\frac{\partial^2 f}{\partial\phi_1^2}\right)\left(\frac{\partial^2 f}{\partial\phi_2^2}\right) - \left(\frac{\partial^2 f}{\partial\phi_1\partial\phi_2}\right)^2 = 0. \quad (11)$$

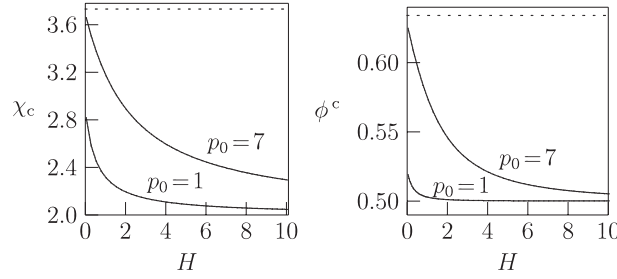
The left-hand side of the last equation can be understood as the determinant of the stability matrix  $\partial^2 f/\partial\phi_i\partial\phi_j$  with  $i, j = 1, 2$ . We note that (11) accounts for the possibility that the compositional changes upon phase separation in the two monolayers are coupled to each other. On the other hand, vanishing of only a minor determinant of the stability matrix (either  $\partial^2 f/\partial\phi_1^2 = 0$  or  $\partial^2 f/\partial\phi_2^2 = 0$ ) does not include this degree of freedom. Hence (11) is the relevant local stability criterion. We recall that the total free energy of the bilayer in (1) is a sum of electrostatic and non-electrostatic terms. Inserting them into (11) yields

$$\left(\frac{1}{\phi_1(1-\phi_1)} + \frac{\partial^2 f_{\text{el}}}{\partial\phi_1^2} - 2\chi\right)\left(\frac{1}{\phi_2(1-\phi_2)} + \frac{\partial^2 f_{\text{el}}}{\partial\phi_2^2} - 2\chi\right) = \left(\frac{\partial^2 f_{\text{el}}}{\partial\phi_1\partial\phi_2}\right)^2 \quad (12)$$

which is a quadratic equation in  $\chi$ . The smallest solution of (12) corresponds to the spinodal. Based on (8) we calculate the first and second derivatives of  $f_{\text{el}}(\phi_1, \phi_2)$ ; the results are  $\partial f_{\text{el}}/\partial\phi_1 = f'_{\text{ml}}(\bar{\phi}_1) = -\Psi_1(\bar{\phi}_1)$  and  $\partial f_{\text{el}}/\partial\phi_2 = f'_{\text{ml}}(\bar{\phi}_2) = -\Psi_2(\bar{\phi}_2)$  and

$$\begin{aligned} \frac{\partial^2 f_{\text{el}}}{\partial\phi_1^2} &= \frac{f''_{\text{ml}}(\bar{\phi}_1) + \frac{H}{2p_0}f''_{\text{ml}}(\bar{\phi}_1)f''_{\text{ml}}(\bar{\phi}_2)}{1 + \frac{H}{2p_0}[f''_{\text{ml}}(\bar{\phi}_1) + f''_{\text{ml}}(\bar{\phi}_2)]} \\ \frac{\partial^2 f_{\text{el}}}{\partial\phi_2^2} &= \frac{f''_{\text{ml}}(\bar{\phi}_2) + \frac{H}{2p_0}f''_{\text{ml}}(\bar{\phi}_1)f''_{\text{ml}}(\bar{\phi}_2)}{1 + \frac{H}{2p_0}[f''_{\text{ml}}(\bar{\phi}_1) + f''_{\text{ml}}(\bar{\phi}_2)]} \\ \frac{\partial^2 f_{\text{el}}}{\partial\phi_1\partial\phi_2} &= \frac{\frac{H}{2p_0}f''_{\text{ml}}(\bar{\phi}_1)f''_{\text{ml}}(\bar{\phi}_2)}{1 + \frac{H}{2p_0}[f''_{\text{ml}}(\bar{\phi}_1) + f''_{\text{ml}}(\bar{\phi}_2)]} \end{aligned} \quad (13)$$

where from (9) it follows that  $f'_{\text{ml}}(\phi) = 2\operatorname{arcsinh}(p_0\phi)$  and  $f''_{\text{ml}}(\phi) = 2p_0/q$ . Insertion of (13) into (12) together with the calculation of the effective compositions,  $\bar{\phi}_1$  and  $\bar{\phi}_2$  (see (10)), allows us to determine the spinodal surface  $\chi(\phi_1, \phi_2)$ . Numerical minimization of  $\chi(\phi_1, \phi_2)$  gives rise to the critical non-ideality parameter  $\chi_c$  and reveals that the corresponding critical compositions,  $\phi_1^c = \phi_2^c = \phi^c$ , are equal for all choices of  $p_0$  and  $H$ . The lower the value of  $\chi_c$  the more unstable is the membrane with respect to lateral phase separation. Figure 2 shows the dependence of  $\chi_c$  and  $\phi^c$  as a function of  $H$  for different values of  $p_0$ . Increasing values of the coupling parameter  $H$  shift down the critical point. The finding  $\phi_1^c = \phi_2^c = \phi^c$



**Figure 2.** The critical non-ideality parameter,  $\chi_c$ , and the corresponding critical compositions  $\phi_1^c = \phi_2^c = \phi^c$ , as a function of  $H$  for different values of  $p_0$ . We obtained  $\chi_c$  from a minimization of the spinodal,  $\chi(\phi_1, \phi_2)$ ; see (12). The dashed lines are located at  $\chi_c = 2 + \sqrt{3}$  (left) and  $\phi^c = (3 - \sqrt{3})/2$  (right).

implies that minimization of the spinodal line  $\chi(\phi_1 = \phi_2 = \phi)$  is sufficient for the calculation of  $\chi_c$ . Indeed, this case corresponds to the particular but most relevant case of a symmetric membrane ( $\phi_1 = \phi_2$ ) for which we obtain the following explicit expression for the spinodal

$$\chi = \frac{1}{2\phi(1-\phi)} + \frac{p_0}{\sqrt{1+p_0^2\phi^2+2H}}. \quad (14)$$

The first part on the right-hand side of (14) is the non-electrostatic contribution to the spinodal line, whereas the second one describes the influence of electrostatic interactions.

The limit of high salt concentrations ( $p_0 \ll 1$ ) corresponds to the Debye–Hückel regime. In this case, the spinodal  $\chi = 1/(2\phi(1-\phi)) + p_0/(1+2H)$  implies a critical composition  $\phi_c = 1/2$  and a corresponding critical non-ideality parameter  $\chi_c = 2 + p_0/(1+2H)$ . For sufficiently small Debye length, electrical charges on both sides of the bilayer are strongly screened by the counterion clouds and the critical point is located close to that of an uncharged membrane ( $\chi_c \rightarrow 2$ ).

In the low salt concentration regime ( $p_0 \gg 1$ ), (14) can be written as  $\chi = 1/(2\phi(1-\phi)) + 1/(\phi + 2H/p_0)$ . The non-ideality parameter  $\chi$  is no longer a function of  $l_D$  and  $\varepsilon_W$  but only depends on the intrinsic bilayer properties through the ratio  $H/p_0 = 2a\varepsilon_L k_B T/e^2 d$ . Compared to water the hydrophobic core of a lipid membrane has a low dielectric constant ( $\varepsilon_L/\varepsilon_W \approx 1/40$ ) so that for not too high values of the Debye length the coupling constant is small ( $H \ll p_0$ ). Series expansion with respect to  $H/p_0$  of the critical point results in  $\chi_c = (2 + \sqrt{3})(1 - 4H/3p_0)$  and critical composition  $\phi^c = (3 - \sqrt{3})/2 - 2(\sqrt{3} - 1)H/3p_0$ . Note that for an electrostatically decoupled bilayer ( $H = 0$ ), the results recover the previously derived [4] critical values  $\chi_c = 2 + \sqrt{3}$  and  $\phi^c = (3 - \sqrt{3})/2$ ; see figure 2. In fact, the latter case is supposed to serve as a sufficiently accurate approximation for biological membranes which are typically characterized by  $p_0 \approx 7$  and  $H \approx 0.01$ .

Generally, the presence of a non-vanishing coupling parameter decreases the stability of a bilayer. In the hypothetical limit  $H \rightarrow \infty$  we always find  $\chi_c = 2$  and  $\phi^c = 1/2$ , characterizing the stability of an uncharged membrane. This finding is a manifestation of the coupling between the lateral phase separation within the two monolayers. That is, local charge accumulation within one monolayer correlates with a corresponding local charge depletion on the other side of the membrane. In the limit of strong electrostatic coupling ( $H \rightarrow \infty$ ) the two effects neutralize each other such that the membrane behaves as if it was uncharged.

To summarize, we demonstrate that electrostatic coupling across a two-component, charged bilayer generally tends to lower the stability with respect to lateral phase separation.

The use of mean-field theory does not permit quantitative prediction of the critical point, but can be expected to capture correctly the qualitative influence of electrostatic coupling through the membrane.

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