Instabilities due to Charge-Density-Curvature Coupling in Charged Membranes

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Instabilities are caused by the reduction in the electrostatic energy when the membrane is curved with the higher charge density on the bilayer which is stretched by the curvature. In a bilayer where the charges can flip from one lipid layer to the other, there is a thermodynamic instability to a spontaneously curved state with different charge densities on the two sides. If the charges are not permitted to flip, there is a dynamical instability due to the correlated modulation of the charge density and curvature fields. Numerical estimates show that these effects are present in parameter regimes relevant to biological systems.

It is known that biological membranes undergo shape changes due to forces exerted during ion transport in proteins, due to the asymmetry of inclusions in the membrane and their phase separation on the surface, and other nonequilibrium processes [1]. Most of the studies on biological membranes have focused on the structural mechanics of membranes at thermal equilibrium [2,3]. However, it has recently been realized that the forces generated on membranes by inclusions could play a crucial role in the structure and dynamics of membranes. These could be in the form of proteins with head-tail asymmetry, which induce a spontaneous curvature in the membrane [4,5], or in the form of phase segregation of inclusions on the surface [6]. Biological membranes also have charged inclusions on the surface, and it is well known that shape changes are accompanied by variations in the transmembrane potential on the surface and surface charge distributions [7].

Surface charges on vesicles and membranes are responsive to external potentials, and a redistribution of charges occurs under externally applied fields [8]. This surface redistribution has a significant effect on the mobility of the vesicles, and could even cause it to change sign. In addition, a spatial variation of charges on a surface could result in a net force in the presence of an electric field [9]. An important experimental result to emerge recently is the spontaneous formation of vesicles in mixed ionic surfactant systems [10]. This is unusual because the bending energy for these systems is large compared to thermal energy at ordinary temperatures, and unilamellar vesicles are usually formed under nonequilibrium conditions. One suggested mechanism is the "spontaneous curvature" of a mixed surfactant system [11], where a difference in composition of the two lipid layers could result in a nonzero equilibrium curvature of the bilayer.

It has been shown [12] that a difference in the charge densities in the two lipid layers forming a bilayer could stabilize a vesicle, because there is a reduction in electrostatic energy when the higher charge density is on the outside of the vesicle. This could compensate for the increase in the curvature energy. In the present analysis, we consider the coupling between the charge density and curvature of a membrane in the absence of an applied electric field. This is motivated by the observations [7] which indicate that variations in charge distribution coincide with shape changes, and the experimental results [10] which have demonstrated the spontaneous formation of vesicles in mixed surfactant systems. The objective is to examine whether the coupling between the surface charge distribution and the curvature in a bilayer could induce thermodynamic or dynamical instabilities on the flat state of the membrane. When the surface charge density on one side of the bilayer is different from that on the other side, it is shown that there is a reduction in the electrostatic energy if there is a higher charge density on the lipid layer that is stretched by the curvature. Two of the consequences of this are analyzed here.

(1) First, we consider the case where the charges are permitted to flip from one surface of the bilayer to another. In the absence of the curvature–charge asymmetry coupling, it is expected that the charge densities on the two sides are equal in order to maximize entropy, and the membrane is flat to minimize curvature energy. However, in the presence of the curvature–charge density coupling, there is a reduction in the electrostatic energy when the membrane is curved and the charge density is asymmetric. This reduction could compensate for the increase in the bending energy and the reduction in the entropy, leading to a state where the membrane develops a spontaneous curvature with different charge densities on the two sides.

(2) Second, we consider the case where the charges are not permitted to flip from one side to another, but are permitted to diffuse along the surface. In this case, it is shown that there is the possibility of a dynamical instability due to a correlated variation in the charge density and the curvature. The mechanism is explained a little later.

The reduction in the electrostatic energy due to the curvature of the surface with charges on one side is discussed first, and then the above results are derived.

The starting point of our analysis is the Poisson-Boltzmann equation, for the potential ψ, linearized using the Debye-Huckel approximation in the limit $(ze\psi/T) \ll 1$ [13],

$$\nabla^2 \psi = \kappa^2 \psi, \tag{1}$$

where κ, the inverse of the Debye screening length, is $(2N_\infty z^2 e^2 / \epsilon T)^{1/2}$, e is the charge of an electron, z is the number of charges per molecule (assumed to be equal for positively and negatively charged molecules), N_∞ is the concentration of counterions at a large distance from the surface, and T is the product of the Boltzmann constant and the temperature.

The relationship between the surface potential and the charge density for a curved surface is derived in the limit where the curvature C is small compared to the inverse of the Debye screening length κ^{-1}. If the direction is normal to the surface w, then the Laplacian operator, correct to $O(C/\kappa)$, is

$$\nabla^2 \equiv \frac{\partial}{\partial w^2} + C \frac{\partial}{w}. \tag{2}$$

The equation for the potential (1) reduces to

$$\frac{\partial^2 \psi}{\partial w^2} + C \frac{\partial \psi}{\partial w} = \kappa^2 \psi. \tag{3}$$

Here the sign convention used is that C is considered positive if the charges are on the lipid layer which is stretched by the curvature. The above equation can be solved by expanding $\psi = \Psi(w) + (C/\kappa)\Psi'$, and using an asymptotic expansion in the small parameter (C/κ). Here $\Psi(w)$ is the potential at a flat interface given by

$$\Psi(w) = \Psi_0 \exp(-\kappa w), \tag{4}$$

where Ψ_0 is the potential at the surface. The first correction is obtained using an asymptotic expansion in the parameter (C/κ)

$$\partial_w^2 \Psi' + \partial_w \Psi = \kappa^2 \Psi', \tag{5}$$

which is solved to obtain

$$\Psi'(w) = \frac{\Psi_0 \kappa w}{2} \exp(-\kappa w). \tag{6}$$

Therefore, the potential distribution in the Debye layer at a curved surface is given by

$$\psi(w) = \Psi_0 \exp(-\kappa w)\left(1 + \frac{wC}{2}\right), \tag{7}$$

and the charge density in the Debye layer is

$$q(w) = -\frac{\epsilon \kappa^2}{ze} \Psi_0 \exp(-\kappa w)\left(1 - \frac{C}{\kappa} + \frac{Cw}{2}\right). \tag{8}$$

The relationship between the surface potential Ψ_0 and the surface density per unit area of the charged species σ is determined from the charge balance condition

$$\sigma z e = -\int_0^\infty dw \, q(w)(1 + Cw), \tag{9}$$

where $q(w)$ is the total charge $q(w) = n_+(w) - n_-(w)$, z is the number of charges per charged molecule on the

surface, e is the charge on an electron, σ is the number density (per unit area) of the charged species on the surface, and the term $(1 + Cw)$ accounts for the curvature of the differential volume at a distance w from the surface caused by the curvature of the surface. The above integral is easily carried out to provide

$$\sigma = \frac{\Psi_0 \epsilon \kappa}{z^2 e^2}\left(1 + \frac{C}{2\kappa}\right). \tag{10}$$

The free energy due to the charge distribution at a curved surface is calculated next. There are two contributions to the free energy, one required to assemble the charged species at the surface, and the second for establishing the counterion density. In the latter case, it is easy to see that the net change in the free energy is zero, because the reduction in the electrostatic energy is exactly compensated by the reduction in the entropy due to the confinement of the counterions in the diffuse layer [13]. Consequently, it is necessary to consider only the free energy required for assembling the charges at the surface. This free energy per unit area of the surface is determined by considering the energy required to assemble the charged species from zero density to the charge density σ in a potential field which is related to the surface charge density by (10)

$$F_{\mathrm{el}} = \int dS \int_0^\sigma d\sigma' \, \Psi', \tag{11}$$

where dS is the differential surface area. The total free energy due to the surface charges and the counterions is determined by integrating the above free energy over the surface

$$F_{\mathrm{el}} = \int dS \frac{\sigma^2 z^2 e^2}{2\epsilon \kappa}\left(1 - \frac{C}{2\kappa}\right). \tag{12}$$

Note that in the above expression, the charges are assumed to be on one side of the bilayer, and the curvature is assumed to be positive if the charges are on the surface stretched by the curvature. For bilayer charge densities σ_a and σ_b on the two lipid layers, the electrostatic energy due to curvature is

$$F_{\mathrm{el}} = \int dS \frac{(\sigma_a^2 + \sigma_b^2)z^2 e^2}{2\epsilon \kappa} - \frac{(\sigma_a^2 - \sigma_b^2)z^2 e^2}{2\epsilon \kappa}\left(\frac{C}{2\kappa}\right), \tag{13}$$

where C is considered *positive* if the lipid layer with density σ_a is stretched and the lipid layer with density σ_b is compressed due to the curvature. Note that the term proportional to C in (12) is proportional to the curvature, and favors placing a higher charge density on the stretched surface. The next term of $O(C/\kappa)^2$ is proportional to $(\sigma_a^2 + \sigma_b^2)$ [12], and this term augments the curvature energy of the bilayer. Note that in the following analysis we assume that the charges are confined to the surface, so that the total number of charges is conserved, as is applicable to biological membranes, and the electrostatic energy is the only contribution to the free energy. In case the charges

are absorbed from the bulk solution, the number of charges on the surface is not fixed, and it is necessary to consider the energy of adsorption as well.

The effect of the coupling between the charge density and the curvature derived above is first applied to a bilayer in which the charge densities are different on the two sides, and the charges are permitted to flip from one side to the other. In the absence of the charge density–curvature coupling, it is expected that the equilibrium state has equal charges on the two sides, since this maximizes the entropy S of the charge distribution,

$$S = \int dS \left[\sigma_a \log(\sigma_a A) + \sigma_b \log(\sigma_b A) \right], \quad (14)$$

where σ_a and σ_b are the charge densities on the two sides of the membrane, and A is the microscopic area occupied by a charge. For small deviations from the state where the charge densities on the two sides are equal, we set $\sigma_a = \sigma_m(1 + \delta)$ and $\sigma_b = \sigma_m(1 - \delta)$, and the entropy decrease for having different charge densities on the two sides is

$$\Delta S = - \int dS \, \sigma_m \left(\delta^2 + \frac{\delta^4}{6} \right). \quad (15)$$

The change in the free energy contains contributions due to the change in entropy, the change in the bending energy, and the coupling between the charge density and the curvature,

$$F = \int dS \left[T\sigma_m \left(\delta^2 + \frac{\delta^4}{6} \right) + \frac{KC^2}{2} + \frac{(\sigma_a^2 + \sigma_b^2)z^2 e^2}{2\epsilon\kappa} - \frac{(\sigma_a^2 - \sigma_b^2)z^2 e^2}{2\epsilon\kappa}\left(\frac{C}{2\kappa} \right) \right]$$

$$= \int dS \left[T\sigma_m \left(\delta^2 + \frac{\delta^4}{6} \right) + \frac{KC^2}{2} + \frac{(\sigma_a^2 + \sigma_b^2)z^2 e^2}{2\epsilon\kappa} - H\delta C \right]. \quad (16)$$

The above free energy implies a continuous transition from a stable state with zero curvature for $T\sigma_m > (H^2/2K)$ to a stable state with a nonzero curvature for $T\sigma_m < (H^2/2K)$, where $H = (\sigma_m^2 z^2 e^2/\epsilon\kappa^2)$. The stable state with nonzero curvature has a curvature and charge density difference given by

$$\delta = \pm \left[3\left(\frac{H^2}{2KT\sigma_m} - 1 \right) \right]^{1/2},$$

$$C = \pm \frac{H^2}{K}\left[3\left(\frac{H^2}{2KT\sigma_m} - 1 \right) \right]^{1/2}. \quad (17)$$

It is of interest to determine the parameter regime where the transition from a zero curvature state to a nonzero curvature state occurs. The condition for the transition can be written as

$$\frac{H^2}{2K} > T\sigma_m,$$

$$\frac{\sigma_m^3 z^4 e^4}{2\epsilon^2\kappa^4 KT} > 1. \quad (18)$$

The above relation indicates that the transition is induced if the temperature is decreased, the Debye length is increased, or the total charge density on the surface is increased. The dependence on the total charge density and the Debye length are sensitive, since the term on the left is proportional to the third and fourth powers, respectively. It is of interest to determine numerically the values where the transition may be expected. Using estimates $e = 1.6022 \times 10^{-19}C$, $\kappa = 10^8$ m^{-1}, $\epsilon = 1.6 \times 10^{-10}$ F m^{-1} for water, and $K = 10^{-19}$ J for typical bilayers, the transition is expected at $\sigma_m \sim 10^{16}$ m^{-2}, to within a constant of order unity. This works out to about a charge for every 100 nm^2, which is the order of magnitude certainly encountered in biological systems, but it should be noted that there is a sensitive dependence of the density on the Debye length. It is also of interest to examine the order of magnitude of the curvature expected in such systems. From Eq. (17), the curvature scale is $C \sim (\sigma_m^2 z^2 e^2/\epsilon\kappa^2 K)$, which is $O(10^7$ m$^{-1})$ for the parameter values quoted above. Therefore, the charge density–curvature coupling could lead to the stabilization of structures of the micron scale.

The second application of the coupling between the charge density and curvature is the dynamical instability of a membrane where the charges are not permitted to flip from one side to the other, but are permitted to diffuse along the two surfaces. There are two number density fields, $\sigma_a'(\mathbf{y}, t)$ and $\sigma_b'(\mathbf{y}, t)'$, which describe the deviation of the local charge density from the mean value σ_m due to diffusion along the surface, where $\mathbf{y}$ is a two-dimensional displacement field along the membrane surface. The membrane surface is described by a height field $h(\mathbf{y}, t)$ which is the normal displacement of the surface from its equilibrium position. The local curvature is given by $C = \nabla_s^2 h$, when the displacement is small compared to the wavelength of the perturbations, where ∇_s is the gradient operator along the surface. The free energy functional is written as

$$F[h, \sigma_a', \sigma_b'] = \frac{1}{2} \int dS$$

$$\times \left[K(\nabla_s^2 h)^2 + \Gamma(\nabla_s h)^2 + A(\sigma_a'^2 + \sigma_b'^2) + \frac{2\sigma_m z^2 e^2}{\epsilon\kappa^2}(\sigma_a' - \sigma_b')\nabla_s^2 h \right], \quad (19)$$

where K is the bending modulus, Γ is the surface tension; the term proportional to A provides an energy penalty for changes in the surface charge density about an equilibrium value.

The dynamical stability of the system is determined by writing dynamical equations for the charge density and height variations. For the present purpose, it is sufficient to write an equation for the difference in the charge densities $\sigma' = \sigma'_a - \sigma'_b$, which is obtained by writing equations for σ'_a and σ'_b and subtracting the two,

$$\frac{\partial \sigma'}{\partial t} = D\nabla_s^2\left(\frac{\delta F}{\delta \sigma'}\right)$$
$$= D\nabla_s^2(A\sigma' + 2G\nabla_s^2 h) + \xi, \qquad (20)$$

where $G = (\sigma_m z^2 e^2/\epsilon\kappa^2)$, the Gaussian white noise ξ has zero mean, the correlation is given by

$$\langle \xi(\mathbf{y},t)\xi(\mathbf{y}',t')\rangle = 2TD\nabla_s^2\delta(t - t')\delta(\mathbf{y} - \mathbf{y}'), \quad (21)$$

and D is an Onsager coefficient. The equation for the height fluctuations is determined from the stress balance condition, which relates the stress exerted by the membrane to the fluid velocity field. The tangential velocity at the surface is set equal to zero when the amplitude of the height fluctuations is large compared to the thickness of the membrane. For the normal velocity, the stress balance condition has the following form for a Stokes flow in the absence of fluid inertia [5]:

$$v_n = -\int d\mathbf{k} \exp(i\mathbf{k} \cdot \mathbf{y})\frac{1}{4\eta k}\frac{\delta F}{\delta h_{\mathbf{k}}}, \qquad (22)$$

where $h_{\mathbf{k}}$ is the Fourier transform of the height fluctuations. There is a contribution to the electrostatic stress due to the curvature of the membrane, but this is bilinear to the curvature and the difference in the charge densities on the two sides, and does not enter into a linear analysis.

If there is no permeation of the fluid through the membrane, the normal velocity is equal to the rate of change of height, and the equation for the height fluctuation field, in Fourier space, reduces to

$$\partial_t h_{\mathbf{k}} = -\frac{1}{4\eta k}(\Gamma k^2 h_{\mathbf{k}} + Kk^4 h_{\mathbf{k}} - Gk^2\sigma'_{\mathbf{k}}) + \zeta_{\mathbf{k}},$$
$$(23)$$

where the noise $\zeta_{\mathbf{k}}$ is Gaussian white noise. The Fourier transform of the equation for the charge density (20) is

$$\partial_t \sigma'_{\mathbf{k}} = -Dk^2(A\sigma'_{\mathbf{k}} - 2Gk^2 h_{\mathbf{k}}) + \xi_{\mathbf{k}}. \qquad (24)$$

The above equations predict that a flat membrane, with no tension, $\Gamma = 0$, is linearly unstable for sufficiently large values of $G^2 > (KA/2)$ even in the long wave limit $k \to 0$, and the growth rate of the perturbations is $O(k^3)$ in this limit. When the tension is nonzero, the long wave perturbations are always stable, but modes with wavelength $k^2 > (\Gamma A/2G^2)$ become unstable. The physical mechanism for this instability is as shown in Fig. 1. A fluctuation which tends to curve the membrane as shown in Fig. 1(a) will result in an accumulation of the charges on the crests of the wave in the upper lipid layer, and in the troughs of the wave in the lower lipid layer. This accu-

(a)

(b)
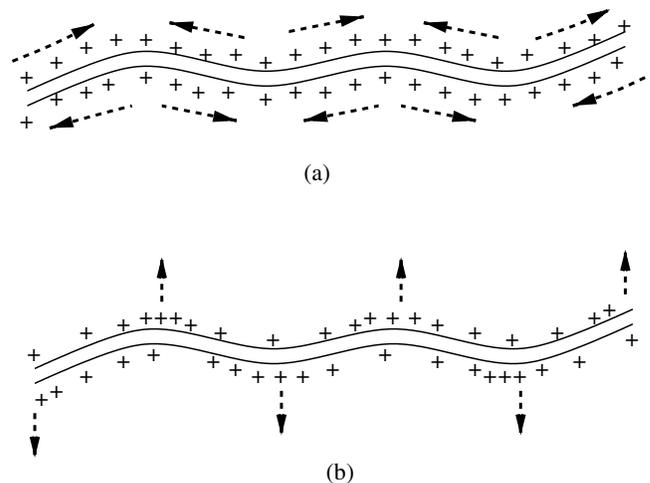
FIG. 1.   Physical mechanism for dynamical instability in a charged bilayer when the charges cannot be transferred from one lipid surface to the other.

mulation will further result in a force which tends to increase the amplitude of the perturbation due to the coupling between the charge density and curvature. This destabilizing effect is opposed by the surface diffusion of the charges, which favors a uniform concentration, and the surface tension or bending elasticity which tends to reduce the curvature. When the destabilizing effect of the charge density–curvature coupling exceeds the stabilizing effect of surface diffusion and surface tension, the membrane becomes unstable.

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