Enveloping of Charged Proteins by Lipid Bilayers

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The ability of a mixed lipid bilayer composed of neutral and charged lipids to encapsulate an oppositely charged protein is studied with use of a simple theoretical model. The free energy of the bilayer-enveloped protein complex is expressed as a sum of electrostatic and curvature elasticity contributions, and compared to that of a protein adsorbed on a mixed planar bilayer. The electrostatic adsorption energy on the planar bilayer is calculated by using an extended Poisson−Boltzmann approach, which allows for local lipid charge modulation in the adsorption zone. We find that the electrostatic interactions favor the wrapped state, while the bending energy prefers the planar bilayer. To enable the transition from the adsorbed to enveloped protein geometry, there is a minimal necessary protein charge. This "crossover" charge depends on the bending rigidity of the lipid membrane and the (composition dependent) spontaneous curvature of its constituent monolayers. The values for the crossover charge predicted by the theory are in line with the charge necessary for peptide shuttles to penetrate cell membranes.

1. Introduction

Interactions between biopolymers and lipid membranes play a central role in a large variety of biological and drug delivery processes. Among those is protein endocytosis, whereby, following adsorption onto the outer leaflet of the plasma membrane, the protein becomes encapsulated by the lipid membrane and eventually released into the cell interior, coated by a lipid bilayer envelope. Effective membrane crossing of this kind is key to the proper delivery of drugs, especially in cases where the protein−membrane interaction is *nonspecific* (i.e., not mediated by a specific ligand−receptor interaction) or energetically activated by a membrane protein. While not much is known about the mechanism of such nonspecific endocytosis, it is clear that its effectiveness must depend on the strength and type of protein−membrane interaction, as well as on the curvature elasticity of the membrane, which dictates the ease of protein enveloping. Both the protein−membrane interaction and the membrane elastic properties depend, in turn, on the chemical composition of the lipid bilayer which, in general, comprises many lipid species. Because the lipid membrane is a two-dimensional (2D) *fluid mixture*, those lipid species which interact more favorably with the adsorbing protein tend to diffuse into the interaction zone, thus enhancing protein−membrane binding, concomitantly generating local variations in lipid composition.

A number of recent studies have demonstrated the use of peptide shuttles for the delivery of drugs across the plasma membrane, into the cytoplasm and the cell nucleus.[1−5] In some cases, the delivery appears to proceed through endocytosis, without the use of ATP by the cell.[6] In common to all the peptide shuttles studied is that they must possess several charged amino acid residues, indicating that electrostatic interactions play a crucial role in this process. Motivated by such experiments and by the general interest in drug delivery processes, our goal in this paper is to analyze, theoretically, the interplay between electrostatic protein−membrane interactions and membrane curvature elasticity, and its consequences with respect to protein membrane crossing. In addition to being a necessary step for endocytosis, protein (or other drug) encapsulation by a lipid bilayer may provide an efficient means for its storage and transport. Because both the electrostatic and elastic interactions depend on membrane composition, by choosing the appropriate bilayer composition, one should be able to control the structure and stability of the desired protein−membrane complex. Conversely, through knowledge of the composition of naturally occurring biological membranes, it may be possible to predict the necessary protein charge that will induce its wrapping by the cell membrane.

Examining the interaction between (model) proteins carrying different amounts of electrical charge and lipid membranes of varying compositions (and hence electrical and elastic characteristics), our specific objective in this study is to determine the threshold conditions favoring protein encapsulation over adsorption. To this end we shall compare the stabilization free energies of the two relevant geometries: (i) a protein adsorbed on a planar lipid membrane and (ii) a protein enveloped by a lipid bilayer vesicle, as illustrated in Figure 1.

In our model, a uniformly charged sphere is used to represent a globular protein. The membrane is treated as a 2D, *binary, fluid mixture* of neutral and electrically charged lipid molecules. For the sake of concreteness, we shall assume that the protein is positively charged, while the charged lipids (e.g., phosphatidyl serine) are anionic, as is often the case for biological membranes. Membrane fluidity is explicitly accounted for by allowing the lipids to demix (bilayer annealing) upon protein adsorption.

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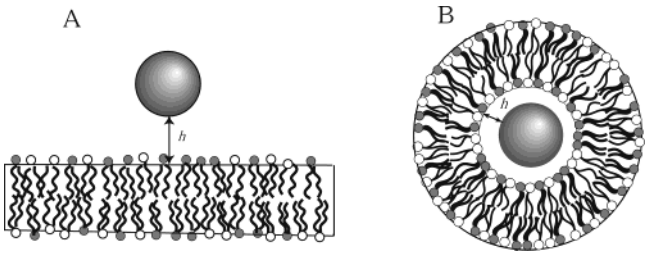

Figure 1. Schematic illustrations of (A) spherical protein adsorbing on a mixed planar lipid membrane. The local lipid composition in the interaction zone, $\eta = \eta(r)$, can adjust so as to minimize the interaction free energy with the approaching protein. (B) Protein wrapped by a lipid bilayer.

Furthermore, the lipid composition of the curved bilayer surrounding the complexed protein is allowed to differ from the original (bare) membrane composition, reflecting the ability of the lipid bilayer to adjust both its composition and curvature (i.e., the radius of the spherical bilayer sheath) to minimize the free energy of the protein−membrane complex. Noting that the spherical shape of the lipid envelope implies highly asymmetric environments for the lipids in the two monolayers (namely, opposite curvatures), our model allows for different lipid compositions of the inner and outer leaflets. Qualitatively, the inner monolayer composition is expected to be largely governed by the requirement for charge matching between the protein and the surrounding lipids.[7] On the other hand, the composition of the outer monolayer will mainly be determined by the optimal curvature free energy. These expectations are largely based on previous studies involving the formation of composite DNA−lipid phases.[8−10] Some of the methods used in those analyzes are implemented in the present study.

Representing a globular protein by a uniformly charged sphere is, obviously, a rather drastic approximation. Similarly approximate is the representation of a multicomponent biological membrane by a two-component lipid bilayer. Limiting our calculations to the initial (adsorption) and final (enveloped) states of the protein, thereby disregarding the intermediate (presumably high energy[11]) states encountered during the passage from the initial to the final state, is yet another simplification of our model. Nevertheless, the simple theoretical scheme described in the following sections should provide the basic qualitative principles dictating the (necessary) conditions for the electrostatically mediated passage of charged proteins through fluid lipid membranes.

The next section reviews the approach that we use to calculate the electrostatic and elastic free energies associated with protein adsorption and its subsequent encapsulation by a mixed lipid bilayer. In this section we also describe our electrostatic−elastic model for the bilayer-coated protein. The predictions of the theory are presented in the subsequent section, closing with a short summary of the main conclusions.

2. Theoretical Model

In this section we present the model used to determine the free energy of an adsorbed protein on a planar bilayer and of a membrane enveloped protein. We consider the competition between the electrostatic and elastic contributions to the free energy, to determine the range of membrane lipid compositions resulting in the encapsulation of a protein of given size and charge. We reiterate that in this study we consider only two possible equilibrium structures: namely, either the adsorbed protein on the planar bilayer, or the fully encapsulated protein, as illustrated in Figure 1. Depending on the relative free energies of these two states the protein either remains adsorbed or is transferred across the membrane surrounded by a lipid bilayer. We do not consider the intermediate states, which may involve rather complicated and largely undetermined geometries.

Our model protein−lipid system, Figure 1, involves a single, positively charged, spherical protein adsorbing onto a planar mixed bilayer (Figure 1A), which, under certain conditions that we wish to determine, becomes enveloped by the membrane, as illustrated in Figure 1B. We use R_p and $q_p = n_p e$ to denote the radius of the protein and its total charge, respectively, e being the elementary charge and n_p the number of positive protein charges. The charge will be treated as uniformly smeared over the surface of the protein. The bilayer is composed of a (random) 2D mixture of anionic and neutral lipids. The mole fraction of charged lipid in the planar membrane is ϕ. The bilayer thickness is denoted by δ and the area per molecule at the hydrocarbon−water interface, which we assume to be the same for both lipids, is a. We assume that a and δ do not change upon forming the enveloped structure. The distance between the protein surface and the bilayer is denoted by h. Note that the optimal spacing h and lipid composition ϕ need not be the same in the initial (A) and final (B) configurations considered in Figure 1.

For both geometries, the total free energy can be expressed as a sum of two major contributions, $F = F^{es} + F^{el}$. The first, $F^{es} = F^{es}(h,\phi)$, is the electrostatic interaction free energy between the protein and the lipid membrane, the latter being planar in the initial state and spherical in the final state. (We ignore nonelectrostatic membrane−protein interactions.) The second term, $F^{el} = F^{el}(h,\phi)$, is the curvature elastic energy of the mixed lipid membrane.

The curvature elastic free energy of a lipid bilayer is, to a very good approximation, a sum of the curvature energies associated with its two leaflets. For a planar and symmetric bilayer, as we assume to be the case in the initial state (Figure 1A), the two monolayers contribute equally to F^{el}. When the bilayer forms a spherical shell, as in Figure 1B, the two leaflets involve opposite curvatures and generally also different lipid compositions. Consequently, their curvature energies may be markedly different. All these effects are adequately accounted for by the familiar Helfrich free energy[12]

$$\frac{F^{el}}{A} = \frac{1}{2}k(c - c_0)^2 \qquad (1)$$

In this equation, which is often used to express the curvature elastic energy of a thin film such as a lipid monolayer (or bilayer) of area A, k is the "splay modulus", or the *bending rigidity* of the film, $c = 1/R$ is the film curvature (R being the radius of curvature; for the planar membrane $c = 0$), and c_0 is the *spontaneous curvature*, i.e., the curvature at which the free energy is minimal. It may be noted that we have used here a simplified version of the elastic free energy, ignoring the contribution of the (generally unknown) saddle-splay (Gaussian) curvature.[12] Also, in general, two principal curvatures are needed to specify the local geometry. However, for the planar and spherical geometries of interest here, these two principal curvatures are identical ($c = 0$ for the planar bilayer and $c = 1/R$ for the spherical vesicle).

In general, both material constants, k and c_0, are functions of the (local) composition of the film. For the sake of simplicity we assume that only the spontaneous curvature depends on the composition of the monolayer. This is a good approximation if the two lipid species have similar hydrophobic chain lengths.[13]

For the composition variation of the spontaneous curvature we assume a linear dependence of the form

$$c_o(\phi) = c_o^n + \phi(c_o^c - c_o^n) \quad (2)$$

where c_o^n and c_o^c are the spontaneous curvatures of single-component monolayers composed of neutral and charged lipids, respectively.[14,15]

Our calculation of the electrostatic free energies of the two geometries depicted in Figure 1 is based on Poisson−Boltzmann (PB) theory. More precisely, the electrostatic free energy of a charged spherical protein interacting with a mixed, oppositely charged, lipid bilayer is calculated by using an extended PB free energy functional, which allows for local lipid charge modulations in response to interactions with nearby charged particles.[9,16]

By symmetry, no lipid charge modulation should appear in the spherically symmetric protein−bilayer complex of Figure 1B. Furthermore, on the basis of previous detailed PB calculations for similar geometries we know that the free energy of the complex obtains a sharp minimum at the *isoelectric point*, where the total positive (in this case protein) surface charge is exactly equal to the total negative (lipid bilayer) surface charge.[8] At this point the complex is, in fact, a concentric spherical capacitor, whose charging energy is known from classical electrostatics. We shall use this classical expression for F^{es} of the spherical complex. The following discussion elaborates on the calculation of the free energy of the two membrane−protein configurations.

2.1. A Protein Interacting with a Planar Membrane. The elastic free energy of a symmetric planar *bilayer* ($c = 0$) of lipid composition ϕ and area A is $F_{pl}^{el}(\phi) = Akc_0(\phi)^2$, see eq 1. Protein adsorption may induce a local variation in ϕ around the adsorption site, so that F_{pl}^{el} is, in fact, an integral over different local contributions. Local composition variations of this kind play a significant role in determining the electrostatic adsorption energy (see below), but their effect on the elastic energy, F_{pl}^{el}, is negligible. We shall thus calculate $F_{pl}^{el}(\phi)$ using the average membrane composition, ϕ.

Consider now the electrostatic free energy of a positively charged protein adsorbing onto a negatively charged membrane, both immersed in a 1:1 salt solution of concentration n_0, corresponding to the Debye length $l_D = (8\pi n_0 l_B)^{-1/2}$, $l_B = e^2/4\pi\epsilon_r\epsilon_0 k_B T = 7.14$ Å denoting the Bjerrum length in water, ϵ_r the dielectric constant of the solvent, and ϵ_0 the permittivity of the vacuum.

To calculate $F_{pl}^{es}(h,\phi)$ we follow the procedure described in a previous study, treating the adsorption of a charged protein onto an oppositely charged membrane using a "cell-model".[16] The cell model dictates simple boundary conditions and enables analyzing the adsorption characteristics as a function of the 2D density of the protein adlayer. More specifically, in this model a cylindrical (Wigner−Seitz) cell of radius R, perpendicular to the membrane plane, is associated with each protein and the circular membrane area "underneath". Protein concentration ($\propto 1/R^2$) effects can be derived from the R dependence of $F_{pl}^{es}(h,\phi)$. Here, however, we are not interested in interprotein interactions, but rather with a single adsorbing protein. Thus, henceforth we shall only consider the $R \rightarrow \infty$ limit.

We model the membrane as a flat, low dielectric object with local surface charge density $\sigma(r) = -e\eta(r)/a$, where η is the local mole fraction of charged lipids in the membrane, r denoting the distance from the center of the adsorption cell (which coincides with the projection of the center of the protein). The average charge density of the lipid membrane is $\bar{\sigma} = -\phi e/a$. The protein is treated as a rigid sphere of low dielectric constant with a uniform (positive) surface charge density, $\sigma_p = en_p/4\pi R_p^2$.

Our extended PB free energy functional allows for the possibility of spatial local inhomogeneities in the membrane surface charge density, in response to interactions with the cationic protein. This is accounted for by adding a mixing free energy contribution to the electrostatic membrane free energy, reflecting the compositional degree of freedom associated with mobile lipids in mixed fluid bilayers.[9,10,16] We include this effect in a self-consistent manner when deriving the electrostatic potential φ by first writing the electrostatic free energy functional of a unit cell

$$\frac{F_{pl}^{es}}{k_B T} = \frac{\epsilon k_B T}{2e^2}\int_V (\nabla\psi)^2 \, d\upsilon + \int_V \left[n_+ \ln\frac{n_+}{n_0} + n_- \ln\frac{n_-}{n_0} - (n_+ + n_- - 2n_0)\right] d\upsilon + \frac{1}{a}\int_A \left[\eta \ln\frac{\eta}{\phi} + (1-\eta)\ln\frac{1-\eta}{1-\phi}\right] ds + \lambda\frac{1}{a}\int_A (\eta - \phi)\, ds \quad (3)$$

The first term in eq 3 represents the electrostatic energy of the system, with the integration extending over the entire aqueous volume; $\psi = e\varphi/k_B T$ is the scaled (dimensionless) electrostatic potential, and $\epsilon = \epsilon_0\epsilon_r$.[17] The second integral accounts for the translational ("mixing") entropy of the mobile ions (of local concentrations n_+ and n_-), relative to their entropy in the bulk solution, and away from any macromolecules, where $n_+ = n_- = n_0$. The third integral represents the 2D (nonideal) demixing entropy of the lipid distribution, the integration extending over the membrane surface from $r = 0$ to the radius of the adsorption cell, $r = R$ ($ds = 2\pi r\, dr$). The last term in F_{pl}^{es} has been added to the thermodynamic potential to account for the lipid charge conservation, namely, for the condition $\int_A \eta\, ds = \phi A$. The Lagrange parameter, λ, expressing the chemical potential of the charged lipid is determined (following minimization of the system free energy) by the charge conservation condition.

The adsorption free energy, $\Delta F = F(h = h_{eq}, R) - F(h = \infty, R = \infty)$, and the local lipid composition, $\eta(r)$, are determined by a minimization of the functional F_{pl}^{es} with respect to both the spatial distribution of the mobile counterions and the 2D distribution of the lipids in the membrane plane for a specific ϕ. The minimization results in the familiar nonlinear PB equation for the electrostatic potential in the system, supplemented by a special boundary condition on the electrostatic potential at the membrane surface.[9,16] The set of differential equations is then solved numerically, as has been described elsewhere.[9,16] The calculations reveal that under most conditions the optimal value of h is nearly zero. Since this conclusion is based on treating the solvent as a continuum, thus disregarding the molecular nature of water, in all results presented in the next section we have imposed $h = h_{min} = 3$ Å as the minimal possible value of h. This reflects the minimal approach distance of the protein to the membrane resulting from a repulsive hydration interaction at close distances.

2.2. Membrane-Enveloped Protein. The concentric geometry of the spherical complex (Figure 1B), in which the oppositely charged protein and membrane surfaces are facing each other in all directions, enables a substantially more favorable electrostatic interaction, as compared to that of a protein interacting with a planar membrane (Figure 1A). This enhanced electrostatic energy is the major driving force for protein encapsulation by the membrane. Recall, also, that this

interaction is particularly favorable at the isoelectric point, where the total membrane charge is equal in magnitude and opposite in sign to the total protein charge, at which point the complex can be treated as a concentric capacitor. It should be noted that since the protein charge is fixed, the membrane charge density (hence lipid composition) ensuring isoelectricity is, in general, different from that of the noninteracting membrane. The ability of the lipid membrane to adjust its (local) surface charge so as to ensure isoelectricity is a direct consequence of the fact that the lipid membrane is a *flexible 2D fluid mixture*, allowing for changes in lipid composition and membrane curvature. Thus, in our calculation below of the electrostatic free energy of the membrane-wrapped protein, F^{es}_{sph}, we shall assume that the composition, ϕ_I, of the inner lipid leaflet (the one facing the protein) is always adjusted so as to ensure isoelectricity. Furthermore, the membrane radius is adjusted so as to ensure a minimal free energy configuration. For simplicity, the outer monolayer is assumed to have the same composition as in the original, unperturbed, bilayer, $\phi_O = \phi$. We shall ignore the small change in the electrostatic energy of the outer monolayer resulting from the passage from the planar to the spherical geometry.

The number of anionic lipid molecules in the inner lipid monolayer is $4\pi(R_p + h)^2\phi_I/a$, implying that the isoelectric lipid composition depends on the distance, h, between the lipid and protein surfaces. Explicitly,

$$\phi_I = \frac{an_p}{4\pi(R_p + h)^2} \tag{4}$$

The equilibrium value of h is determined by the minimization of the total free energy of the complex, $F_{sph} = F^{es}_{sph} + F^{el}_{sph}$, with respect to h.

The charging energy of a spherical capacitor, composed of two concentric surfaces of radii R_p and $R_p + h$, each carrying a charge of magnitude q_p, is given by

$$W(h) = \frac{q_p^2}{8\pi\epsilon}\left(\frac{1}{R_p} - \frac{1}{R_p + h}\right) \tag{5}$$

In this expression the energy is zero when the two surfaces are in contact. To use the same reference state as that of a protein interacting with a planar bilayer, we shall determine F^{es}_{sph} by subtracting from eq 5 the capacitor's energy at infinite separation, i.e., $F^{es}_{sph} = W(h) - W(\infty)$, or

$$\beta F^{es}_{sph}(h) = \beta[W(h) - W(\infty)] = -\frac{l_B}{2}\frac{n_p^2}{R_p + h} \tag{6}$$

with $\beta = 1/k_BT$.

The electrostatic free energy is minimal at contact between the protein and the membrane. As in the case of a protein interacting with a planar membrane, to implicitly account for the effect of hydration forces, we do not allow h to be smaller than $h = h_{min} = 3$ Å. That is, if the minimum of $F_{sph} = F^{es}_{sph} + F^{el}_{sph}$ happens to fall below h_{min}, we set $h = h_{min}$. Our qualitative conclusions do not depend on the precise choice of h_{min}.

The curvature elastic free energy of the spherical membrane shell around the protein is obtained by multiplying the area of each monolayer by its corresponding free energy density, as given by eqs 1 and 2. Measuring the curvature elastic energy

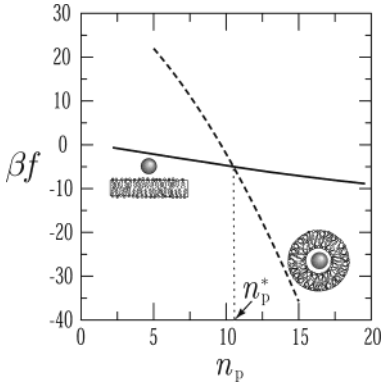

Figure 2. The free energy of the protein adsorbed on the planar bilayer (full line) and that of the wrapped protein (dashed line) as a function of the number of charges in the protein. The calculations correspond to $R_p = 15$ Å, $\phi = 0.15$, $\beta k = 10$, and $c_0^n = 1/30$ Å^{-1}.

with respect to that of the planar bilayer we find,

$$\Delta F^{el} = F^{el}_{sph} - F^{el}_{pl} = 2\pi k[1 + 2c_0(\phi_O)(R_p + h + \delta)]^2 - 2\pi k\left(1 + \frac{h}{R_p} + \frac{\delta}{R_p}\right)^2 c_0^2(\phi) + 2\pi k[1 - 2c_0(\phi_I)(R_p + h)]^2 - 2\pi k\left(1 + \frac{h}{R_p}\right)^2 c_0^2(\phi) \tag{7}$$

The first two terms in this equation describe the elastic free energy cost associated with changing the curvature and composition of the outer lipid monolayer; the last two terms correspond to the inner monolayer. Note that we include the important elastic energy cost associated with bending the outer monolayer, and recall that we assume $\phi_O = \phi$. The equilibrium spacing, $h = h_{eq}$, is determined by minimizing the sum of the electrostatic and elastic free energies, eqs 6 and 7, respectively, with respect to h.

3. Results

Once the equilibrium free energies of the adsorbed and enveloped protein states have been evaluated, we can determine the conditions preferring one state over the other. Protein encapsulation by the lipid membrane may take place when the wrapped state is of lower free energy than the adsorbed state.

All the numerical results presented below were derived for $c_0^c = 0$, i.e., the charged lipid prefers the planar film. This is a reasonable, approximate, value for a typical charged lipid such as DOTAP[18] or DOPS.[19] In addition, all results were derived for $l_D = 10$ Å, corresponding to a bulk salt concentration of $\approx$0.1 M.

By varying the bending rigidity k, the composition of charged lipids ϕ, the size of the protein R_p, and the spontaneous curvature of the neutral lipid c_0^n we are able to compare the relative stabilities of the adsorbed and enveloped protein geometries, over a wide range of experimentally relevant conditions.

Figure 2 shows the free energy of the adsorbed and wrapped proteins as a function of the protein charge. In this, and all other, calculations the area per lipid headgroup is $a = 65$ Å^2 and the bilayer thickness is $\delta = 30$ Å. As expected, the wrapped state is favored by high protein charge, consistent with the more favorable electrostatic energy of this geometry. Quite generally then, a transition from the planar to the wrapped geometry will take place when the protein charge exceeds a certain "crossover" value, n_p^*, which depends on protein size as well as on the lipid

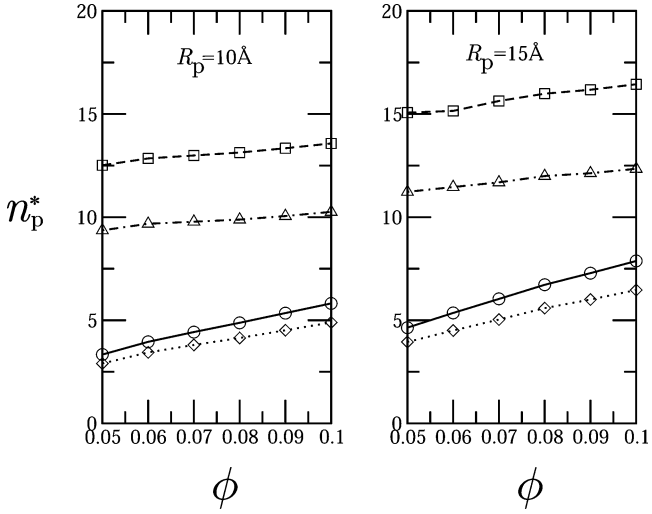

Figure 3. The crossover protein charge as a function of the mole fraction of charged lipids in the bilayer. Two different protein radii are shown. The curves correspond to the following: squares, $\beta k = 10$, $c_0^n = 1/50$ Å^{-1}; triangles, $\beta k = 5$, $c_0^n = 1/50$ Å^{-1}; circles, $\beta k = 10$, $c_0^n = 1/30$ Å^{-1}; and diamonds, $\beta k = 5$, $c_0^n = 1/30$ Å^{-1}.

membrane characteristics, such as the spontaneous curvature, bending rigidity, and composition.

Figure 3 shows how n_p^* varies with the lipid composition of the planar bilayer. We consider two different protein radii, $R_p = 10$ and 15 Å, and four representative combinations of spontaneous curvatures and bending rigidities. All combinations are in the range found experimentally for typical lipids or synthetic surfactants. In all cases the crossover charge, n_p^*, increases with the concentration of charged lipid. Two effects are responsible for this behavior. First, as can be shown by a detailed calculation of the electrostatic interaction energies, as ϕ increases, the difference between the electrostatic stabilization energies in the two geometries decreases, thus diminishing the tendency for protein wrapping by the membrane. Second, the elastic bending energy of the outer monolayer increases with ϕ owing to the vanishing spontaneous curvature of the charged lipid.

Figure 3 reveals that n_p^* depends sensitively on the spontaneous curvature and bending rigidity of the lipid membrane. More explicitly, we note that (for a given c_0^n and ϕ) the crossover charge decreases with the bending rigidity, consistent with the notion that a lower k implies a lower bending energy of the membrane's outer monolayer, whose area is much larger than that of the inner monolayer. (The ratio of areas is $[(R_p + h + \delta)/(R_p + h)]^2$, which, for typical protein radii R_p and membrane thicknesses δ is of order 10.) Similarly, in our case a lipid monolayer of high spontaneous curvature, $c_0^n = 1/30$ Å^{-1}, implies a smaller bending free energy cost for a highly curved membrane such as the one enveloping the protein.

The effect of the bending rigidity on the crossover charge is shown in Figure 4. We note that the rate of change of n_p^* as a function of k depends strongly on the spontaneous curvature of the neutral lipid, c_0^n. For the smaller value of c_0^n, which is closer to the curvature of the protein's surface, the rate of change is small because the elastic energy penalty is small throughout the range considered. Larger elastic energy penalties are involved when the actual membrane curvature deviates significantly from the spontaneous curvature, implying a strong increase in the crossover charge with the membrane's bending rigidity. (Recall that the elastic energy penalty varies quadratically with the difference in curvatures.)

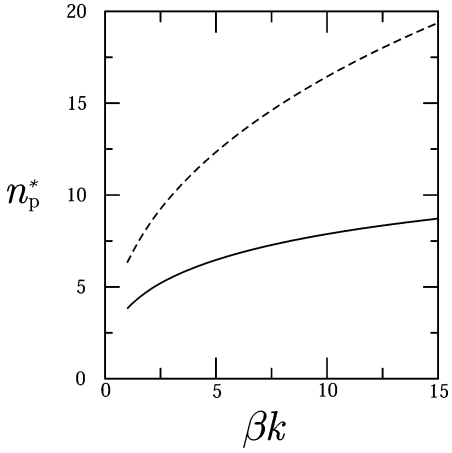

Figure 4. The crossover protein charge as a function of the bending elastic constant of the lipid bilayer for two different spontaneous curvatures: solid line, $c_0^n = 1/30$ Å^{-1} and dashed line $c_0^n = 1/50$ Å^{-1}. For both cases $\phi = 0.1$ and $R_p = 15$ Å.

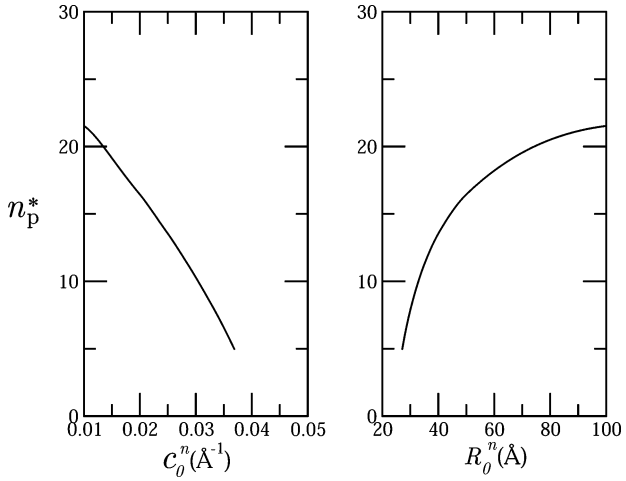

Figure 5. The crossover protein charge as a function of the spontaneous curvature of the pure neutral lipid (left) and as a function of the spontaneous radius (right). The calculations correspond to $\phi = 0.1$, $\beta k = 10$, and $R_p = 15$ Å.

Finally, in Figure 5 we show the effect of changing the spontaneous curvature of the neutral lipid on n_p^* for fixed composition and rigidity constant. The crossover charge increases as the spontaneous curvature (radius) decreases (increases). As argued above, this behavior reflects the increasing elastic energy cost associated with bending the outer leaflet of the membrane.

4. Summary and Conclusions

The ability of mixed lipid bilayers to envelop colloidal particles such as proteins provides a possible mechanism for drug delivery through cell membranes. In addition, it poses a potential method for storage of charged polypeptides. Here, we have considered the free energy difference between a protein adsorbed on a mixed planar lipid bilayer as compared to that of a spherically enveloped protein, focusing on the interplay between the electrostatic and elastic contributions to the free energy of the protein−membrane system.

The large number of degrees of freedom may confound the most important energy terms determining the balance of forces in the problem. However, insights may be gained from the following highly simplified model system, whose only degrees of freedom are elastic and electrostatic. We treat the membrane

as a uniform, one component bilayer with a single (average) radius of curvature. We notice in Figure 2, that the *change* in free energy of the membrane adsorbed protein with the number of protein charges is considerably smaller than the corresponding change for the membrane-wrapped protein. Therefore, the value of n_p^* will not change much if we assume the protein−membrane adsoption energy to be zero. Under this assumption, n_p^* is determined solely by the balance of the elastic energy penalty for wrapping the protein, and the electrostatic gain from wrapping the charged protein with an oppositely charged membrane. Defining the mean radius of the membrane bilayer as r, the radius of the inner membrane leaflet apposing the protein as $r - \Delta$, and the spontaneous radius of curvature for the membrane r_0, the equality of elastic and electrostatic energy (given in eq 6 and 7) at n_p^* is reduced to

$$4\pi\beta\kappa r^2\left(\frac{1}{r}-\frac{1}{r_0}\right)^2 = \frac{l_B}{2}\frac{n_p^{*2}}{r-\Delta} \tag{8}$$

We thus derive the following simple expression for n_p^*,

$$n_p^* = \sqrt{\frac{8\pi\beta\kappa(r-\Delta)}{l_B}}\left(1-\frac{r}{r_0}\right) \tag{9}$$

For concreteness, we use similar values to those used in the more detailed model, considering a protein of $R_P = 10$ Å: $r = R_P + 18$ Å, $\Delta = 15$ Å, $\beta\kappa = 5$. We find $n_p^* \approx 15[1 - (r/r_0)]$. The value for the minimal charge needed to achieve membrane wrapping sensitively depends on the membrane spontaneous curvature. By changing membrane spontaneous curvature, the penalty for wrapping can be minimized, lowering the charge needed to (electrostaticaly) compensate for bending. The results of the detailed model, which includes in addition the compositional and local demixing degrees of freedom (Figures 3 and 4), are in accord with this conclusion. The range of n_p^* found in the detailed model is 3−15, reflecting the effect of curvature energy contribution.

More generally, using the detailed model, we have shown that the critical protein charge resulting in membrane wrapping depends on the fraction of charged lipids in the planar bilayer, as well as on the bending rigidity and spontaneous curvature of the lipid monolayers. The values calculated for the minimal protein charge needed for protein encapsulation, for reasonable values of the elastic constants of the bilayer, are within the range of experimentally accessible charges.[3,5] These may be found, for instance, in short polypeptides used as anchors in drug delivery systems. This may explain the particular necessary charge for peptides of the nuclear localization signal to penetrate cell membranes without the use of external energy sources.[20,3,5]

Within our model, we showed how the interplay between electrostatic and composition dependent elastic contributions can be manipulated to stabilize a given aggregate geometry. However, there are several contributions to the free energy that were not considered here. We have assumed that the outer monolayer of the wrapping bilayer is always of the same composition as the planar bilayer. Also, the bending constant of the monolayers was assumed to be independent of composition. The electrostatic free energy in the enveloped geometry was approximated by a capacitor model assuming that isoelectricity is achieved by balancing the protein charges with the lipids in the bilayer, without explicitly taking into account the layer of solvent between the protein and the film, which may contain ions from solutions. We believe that while these contributions are missing, the main effects responsible for the predicted behavior are properly accounted for within our approach.

The calculations presented here attempt to answer the following question: Under what conditions it is possible for a mixed bilayer to wrap a protein? Yet, we have not considered the mechanisms, or pathways, by which a planar bilayer becomes spherical in the wrapping process. Clearly, further study of the possible mechanisms, while desirable, is not trivial.

To summarize, we have found, using a simple theoretical approach, that enveloping of charged proteins is feasible for mixed (charged-neutral) lipid bilayers, provided the spontaneous curvature of the neutral lipid is not too far from the radius of the protein. The driving force for the formation of the wrapped aggregates is the gain in electrostatic free energy, at the cost of the elastic free energy of the film. Our results can serve as guidelines in the design of drug carrier systems.

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