Lipid Demixing and Protein-Protein Interactions in the Adsorption of Charged Proteins on Mixed Membranes

Sylvio May,* Daniel Harries,† and Avinoam Ben-Shaul†

*Institut für Biochemie und Biophysik, Friedrich-Schiller-Universität Jena, Philosophenweg 12, 07743 Jena, Germany, and †Department of Physical Chemistry and the Fritz Haber Research Center, The Hebrew University, Jerusalem 91904, Israel

ABSTRACT The adsorption free energy of charged proteins on mixed membranes, containing varying amounts of (oppositely) charged lipids, is calculated based on a mean-field free energy expression that accounts explicitly for the ability of the lipids to demix locally, and for lateral interactions between the adsorbed proteins. Minimization of this free energy functional yields the familiar nonlinear Poisson-Boltzmann equation and the boundary condition at the membrane surface that allows for lipid charge rearrangement. These two self-consistent equations are solved simultaneously. The proteins are modeled as uniformly charged spheres and the (bare) membrane as an ideal two-dimensional binary mixture of charged and neutral lipids. Substantial variations in the lipid charge density profiles are found when highly charged proteins adsorb on weakly charged membranes; the lipids, at a certain demixing entropy penalty, adjust their concentration in the vicinity of the adsorbed protein to achieve optimal charge matching. Lateral repulsive interactions between the adsorbed proteins affect the lipid modulation profile and, at high densities, result in substantial lowering of the binding energy. Adsorption isotherms demonstrating the importance of lipid mobility and protein-protein interactions are calculated using an adsorption equation with a coverage-dependent binding constant. Typically, at bulk-surface equilibrium (i.e., when the membrane surface is "saturated" by adsorbed proteins), the membrane charges are "overcompensated" by the protein charges, because only about half of the protein charges (those on the hemispheres facing the membrane) are involved in charge neutralization. Finally, it is argued that the formation of lipid-protein domains may be enhanced by electrostatic adsorption of proteins, but its origin (e.g., elastic deformations associated with lipid demixing) is not purely electrostatic.

INTRODUCTION

Unlike solid surfaces, multicomponent ("mixed") lipid bilayers can respond to interactions with peripheral macromolecules (e.g., proteins or DNA) through two, often coupled, mechanisms. First, above the lipid chain melting transition, the lipid membrane is a two-dimensional (2D) fluid mixture. Consequently, the lipid species that interact more favorably with the adsorbing macromolecule can migrate toward the interaction zone, exchanging with the less favorably interacting lipids, which migrate away from this zone. The extent of this lipid "demixing" process, which involves a local deviation from the average lipid composition, is determined by the balance between the gain in adsorption energy and the loss of 2D lipid mixing entropy, as dictated by the minimum of the total interaction free energy. The second mechanism by which a lipid bilayer can lower the interaction free energy is associated with the elasticity of the membrane. Namely, because the membrane is elastic with respect to stretching and/or bending deformations, it may lower the interaction free energy with an adsorbing molecule by changing its local area and (usually more easily) its curvature. A dramatic example of such changes is provided by the formation of hexagonal cationic lipid-DNA complexes upon adding DNA to an aqueous solution of cationic vesicles (Koltover et al., 1998). The elastic and compositional degrees of freedom of a mixed lipid bilayer are also apparent when macromolecules, e.g., hydrophobic integral proteins, are incorporated into the lipid membrane. The presence of proteins within the membrane can result in lipid sorting (Sperotto and Mouritsen, 1993; Gil et al., 1998), lipid-mediated (attractive or repulsive) elastic interactions between the proteins (Harroun et al., 1999; Nielsen et al., 1998; Aranda-Espinoza et al., 1996; Fournier, 1998; Ryba and Marsh, 1992; May and Ben-Shaul, 1999; Bruinsma and Pincus, 1996), and morphological transitions between different (e.g., lamellar and inverse-hexagonal) lipid phases (Killian et al., 1996; May and Ben-Shaul, 1999).

Our interest in this paper is focused on the role of lipid lateral mobility in the adsorption of electrically charged macromolecules on the surface of a binary, oppositely charged, lipid membrane. That is, one lipid component carries a headgroup charge of opposite sign to that of the adsorbing macromolecule, whereas the other is electrically neutral. More specifically, we consider a model system for the adsorption of, say, positively charged (basic) globular proteins on a membrane containing varying proportions of acidic lipids. The protein is modeled as a rigid sphere of low dielectric constant, with positive charges uniformly smeared over its surface. This is a special case of the interaction between two oppositely charged, unequal spheres, which has recently been investigated within Poisson-Boltzmann (PB) theory for various boundary conditions on the spheres,

Received for publication 18 April 2000 and in final form 10 July 2000.

Address reprint requests to Dr. Avinoam Ben-Shaul, Dept. of Physical Chemistry and the Fritz Haber Research Center, The Hebrew University, Jerusalem 91904, Israel. Tel.: 972-2-6585271; Fax: 972-2-6513742; E-mail: abs@fh.huji.ac.il.

0006-3495/00/10/1747/14 $2.00

accounting for constant charge density, constant potential, and ionizable surface charges (Ninham and Parsegian, 1971; Carnie et al., 1994; Warszyńsky and Adamczyk, 1997; Palkar and Lenhoff, 1994; McCormack et al., 1995; Jönsson and Stahlberg, 1999).

A special feature of our model is that as the protein approaches the membrane surface the charged lipids are allowed to migrate toward (or away from) the interaction zone. This exchange, or "demixing," of charged and neutral lipids results in a locally varying lipid composition profile. The lipid charge modulation (or "polarization") profile varies with the distance of the protein from the membrane surface. In general, the deviation of the local charge distribution from the average (say, uniform) distribution increases as the protein approaches the surface, becoming most pronounced at the equilibrium distance.

Another important factor affecting the charge modulation profile and adsorption free energy is the lateral density of the adsorbed proteins, reflecting the combined effects of protein-membrane and protein-protein interactions. These interactions play a major role in determining the equilibrium density ("surface coverage") of proteins on the membrane, i.e., the adsorption isotherm, as dictated by the equality of the protein chemical potentials on the membrane surface and in the bulk solution.

Our theoretical approach for the analysis of the adsorption process is based on a mean-field free energy functional that takes into account all the relevant electrostatic contributions to the free energy of the lipid-protein "double layer" and the 2D lipid mixing entropy in the membrane plane. The adsorption free energy and the lipid distribution profiles are determined by a minimization of this functional with respect to both the spatial distribution of the mobile counterions and the 2D distribution of the lipids in the membrane plane. 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. This boundary condition, reflecting the competition between the mobility of lipid charges and the demixing entropy penalty, expresses the requirement for constant *electrochemical* potential of the membrane lipids. The resulting equation for the electrostatic potential at the membrane surface must be solved self-consistently with the PB equation. A similar type of boundary condition appears in the "charge regulation" model for the electrostatic interaction between colloidal particles involving ionizable surface groups (Ninham and Parsegian, 1971; Carnie and Chan, 1993; Carnie et al., 1994). In these systems, the equilibrium surface charge is adjustable, and determined self-consistently by the interplay between the chemical dissociation reaction and the electrostatic interaction between the charged surfaces.

Our constant electrochemical potential boundary condition is as an intermediate case between the two familiar boundary conditions corresponding to surfaces of constant charge density and constant surface potential. As we shall see in the next section, in the (hypothetical) limit corresponding to infinite lipid demixing entropy, this special boundary condition reduces to the case of constant ("frozen") charge density. In the opposite (again, hypothetical) limit of zero demixing entropy penalty, the surface charges are fully mobile, as if the membrane were a conductor, implying constant surface potential.

The validity of the PB theory for treating the interaction between charged surfaces and colloidal particles in aqueous salt solutions has been examined by various authors based on comparisons to either non-mean-field (integral equation) or computer simulation studies (Linse and Jönsson, 1982; Wennerström et al., 1982; Das et al., 1995; Deserno, 2000); for reviews see Andelman (1995) and Vlachy (1999). The conclusion from these studies is that PB theory is adequate for aqueous solutions containing monovalent electrolyte for salt concentrations not exceeding $\approx$0.1 M. The aqueous solutions considered in the present work fulfill this condition.

Once the adsorption free energy has been evaluated as a function of protein density, and using an appropriate model for the configurational entropy of the adsorbed protein layer, one can calculate the chemical potential of the protein in the adsorbed state, and hence the adsorption isotherm. We shall adopt here a simple model for the configurational entropy of the adsorbed protein layer, resulting in a Langmuir-like adsorption equation, but with coverage-dependent adsorption energy. Our main goal in presenting these isotherms is to demonstrate the important effects of lipid lateral mobility (or "surface relaxation") and protein-protein interactions on the adsorption behavior of charged proteins on mixed fluid layers. Qualitatively, our conclusions should be relevant to a variety of adsorption processes involving charged macromolecules; e.g., oligonucleotides, colloidal particles, or polyelectrolytes.

The adsorption of charged proteins on oppositely charged membranes has been studied by many groups, both experimentally and theoretically. The electrostatic binding of various peptides on lipid membranes was calculated and compared to experiment by Ben-Tal et al. (1996, 1997; Murray et al., 1999), based on solutions of the nonlinear PB equation for atomic models of the lipid bilayer and the peptides. Assuming a "frozen" lipid distribution in the mixed membrane, these authors calculated peptide binding constants as a function of salt concentration, finding good agreement with experiment. Using linear PB theory, Roth et al. (1998) have modeled protein-surface binding as the adsorption of a charged sphere on a uniformly charged planar surface. Analyzing the enthalpic and entropic contributions to the adsorption free energy as a function of the protein-surface charge density ratio, they conclude that the entropic component associated with the release of mobile counterions provides the major contribution to the binding free energy. This conclusion is in line with the common notion that counterion release is the main driving force for

electrostatic attraction between oppositely charged macromolecules (see, e.g., Record et al. (1978); Wagner et al. (2000)).

At least two theoretical models have recognized and emphasized the important role of lipid mobility and demixing in determining the protein binding free energy and adsorption isotherms. One of these models, by Denisov et al. (1998), has further suggested that protein-induced lipid demixing is the mechanism underlying the formation of lipid-protein domains in membranes. The domains are membrane regions (phases) characterized by a large lateral density of adsorbed proteins and "adsorbing" lipids, coexisting with other regions ("nondomains") of lower protein density. Based on Gouy-Chapman theory, these authors have calculated the adsorption free energy of pentalysine on the surface of a mixed membrane, composed of acidic and neutral lipids, and found it to increase with the mole fraction of acidic lipid in the membrane. Their calculations show that the gain in electrostatic free energy associated with the adsorption of proteins on the phase-separated membrane overrides the concomitant loss in lipid mixing entropy, suggesting that domain formation is thermodynamically favorable. It should be noted, however, that this calculation does not account for two important (and coupled) effects. First, assuming uniformly smeared surface charge distributions (in both the domain and nondomain regions), the model cannot account for *local* lipid demixing, i.e., for the accumulation of acidic lipids in the immediate vicinity of an adsorbed basic peptide. Second, the model assumes that the basic peptides neutralize a certain fraction of the acidic lipid charges, thus reducing the net surface charge density. The structural characteristics of the adsorbed peptides and, consequently, the lateral electrostatic repulsion between them, are not included in the model. This direct interaction between peptides has been studied by Murray et al. (1999) by calculating the adsorption energy of a peptide onto a vacant membrane adsorption "site" surrounded by pre-adsorbed peptides. These authors find that the adsorption energy indeed decreases, though not to the extent predicted by models assuming uniformly smeared (lipid and protein) surface charges. However, this latter calculation does not allow for local demixing of the lipids. Qualitatively, recalling that the membrane is a 2D fluid mixture, one expects that the already adsorbed peptides will deplete the charged lipids from the vacant regions, thereby reducing the adsorption energy of an additional peptide and hence enhancing the effects of adsorbate-adsorbate repulsion.

Clearly, if lipid demixing can take place locally, i.e., in the vicinity of singly adsorbed peptides, there is no thermodynamic incentive for adsorbate aggregation. This conclusion is consistent with the general result that, at least according to PB theory, the interaction between like-charged colloidal particles is always repulsive, whether in the bulk or in the vicinity of a confining wall (Neu, 1999; Sader and Chan, 1999a, 1999b). This, in turn, suggests that protein domain formation is most likely driven by a nonelectrostatic mechanism, e.g., a lipid-mediated protein attraction resulting from elastic membrane deformations (and hence line tension) around the protein-membrane interaction zone (Sperotto and Mouritsen, 1993; Gil et al., 1998).

Another theoretical model allowing for lipid redistribution upon protein adsorption on mixed lipid membranes has been presented by Heimburg et al. (1999; Heimburg and Marsh, 1995). Here, too, the electrostatic adsorption energy is calculated using Gouy-Chapman theory, assuming that every adsorbed peptide neutralizes a certain number of charged lipids. The charged and neutral lipids are allowed to exchange, as in chemical equilibrium, between the "protein covered" and vacant regions. The equilibrium compositions in these regions are determined by the interplay between adsorption energy and mixing entropy. Then, using either van der Waals or scaled particle theory to account for *nonelectrostatic* lateral interactions between the adsorbed proteins, the authors derive adsorption isotherms for membranes of varying (average) lipid compositions. With appropriate choice of interaction parameters the model shows good agreement with experimental adsorption isotherms of cytochrome c on mixed dioleoyl phosphatidylglycerol/dioleoyl phosphatidylcholine membranes.

In both models outlined above the lipid composition in the protein adsorption domains (whether local or global) is different from that of the protein-deficient regions. Both models do not allow for local variations in lipid composition, on a molecular scale, within and around the protein-membrane interaction zone, nor for the dependence of the composition profile on protein lateral density, and hence on protein-protein repulsion. These rather subtle yet important effects are reflected, for example, by the different adsorption isotherms corresponding to fluid versus "frozen" lipid membranes and interacting versus noninteracting protein layers. As we shall see in the next sections they can be treated based on one general free energy functional.

THEORY

We model the proteins as positively charged spherical particles of radius R_P, and the membrane surface as an incompressible 2D fluid mixture composed of acidic and neutral lipids, both of the same headgroup area, a. The headgroup of the acidic lipid carries a single negative charge. The membrane and proteins are embedded in an aqueous solution containing a symmetric 1:1 electrolyte of concentration n_0, corresponding to the Debye length $l_D = (8\pi n_0 l_B)^{-1/2}$, where $l_B = 7.14$ Å is the Bjerrum length in water. The average charge density of the lipid membrane is $\bar{\sigma} = -\bar{\phi}e/a$ where e is the elementary charge and $\bar{\phi}$ is the (overall) mole fraction of charged lipids in the membrane. The positive charge is assumed to be uniformly distributed on the surface of the protein, with σ_P denoting the (fixed) surface charge density. One of the most relevant variables in our model is

$\chi = -\sigma_P/\bar{\sigma}$, the ratio between the charge densities on the protein and membrane surfaces; $\chi > 0$ to ensure opposite signs of the two macroion charges. For the purpose of presentation we find it convenient to introduce the quantity $\phi_P = \chi\bar{\phi}$, expressing the "equivalent composition" of the protein surface. That is, if the protein surface is regarded as composed of (positively) charged and neutral groups, each of area a (identical to the lipid headgroup area), then ϕ_P is the fraction of charged protein groups.

The equilibrium partitioning of proteins between the bulk solution and the adsorption layer is dictated by the equality of chemical potentials in these two phases. The chemical potential of the adsorbed proteins depends on their adsorption free energy and the 2D translational entropy, both depending on the lateral density of the adsorbed layer. We shall first consider the adsorption free energy and then describe our model for the protein chemical potential and adsorption isotherms.

Adsorption free energy

When the surface density of adsorbate is low, interprotein interactions are weak and the adsorption energy is nearly equal to that of an isolated protein. This is the limit in which lipid demixing or, more precisely, local composition modulations, are expected to be most important, especially at low surface charge densities (large χ). Protein-protein interactions become increasingly important upon increasing the lateral density of adsorbate. On the average, a given adsorbed protein is surrounded by a radially symmetric distribution of its neighbors. Based on this notion we shall adopt a mean-field scheme whereby every adsorbed protein defines a cylindrical cell whose main axis (which passes through the protein's center) is normal to the membrane plane. Its projection on the membrane surface is a circular, Wigner-Seitz cell of radius R ($R > R_P$) and area $A = \pi R^2$, as depicted in Fig. 1. Cell models of this kind have been used to describe a variety of electrostatic interaction phenomena in both two- and three-dimensional systems; e.g., the adsorption of divalent surfactants on solid surfaces (Ström et al., 1999), the concentration polarization of colloidal particles at membrane surfaces (Jönsson and Jönsson, 1996), the ionic atmosphere around sphericle micelles and other colloidal particles (Linse and Jönsson, 1982; Wennerström et al., 1982), and the classical theory of Lifson and Katchalsky (1954) for calculating the electrostatic free energy of hexagonally packed (rigid) polyelectrolytes.

Based on the cell model scheme, one can calculate the adsorption energy as a single particle property, with interprotein interactions treated in a mean-field approximation. At the cell limits we have the boundary condition $(\partial\Phi/\partial r)_R = 0$, where Φ is the electrostatic potential and r is the radial coordinate, measuring the distance from the center of the protein in the x, y plane, parallel to the membrane surface, as described in Fig. 2. The minimal distance of the protein surface from the membrane plane, measured along the membrane normal axis (z) will be denoted by h. Any point within the cylinder defined by the circular Wigner-Seitz cell is specified by the three coordinates $\{r, z, \alpha\}$, with α denoting the azimuthal angle (see Fig. 2). By symmetry, $\Phi = \Phi(r, z)$ is independent of α. Similarly, the lipid composition profile around a given protein is a function of r (and h), but is independent of α.

The mean distance between adsorbed proteins, $2R$, is dictated by their 2D density, $\rho \propto 1/A \propto 1/R^2$. Thus, the effects of protein lateral interactions on the adsorption free energy enter our model through the dependence on R of the electrostatic free energy per unit cell (or, per protein), F. Of course, this treatment is approximate because it neglects the positional (both angular and radial) fluctuations of the protein 2D distribution. Note, however, that at very high surface densities the proteins tend to organize into a quasi-

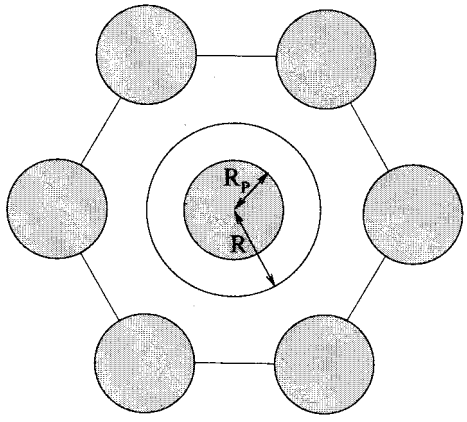

FIGURE 1 Schematic view of the Wigner-Seitz cell.

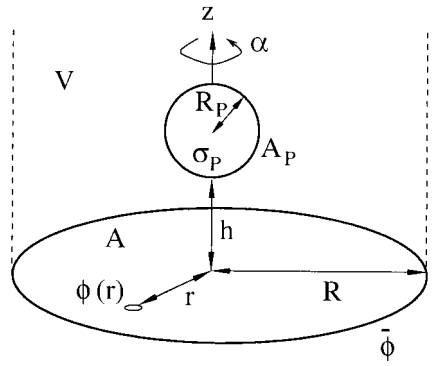

FIGURE 2 Schematic illustration of a spherical protein adsorbed on a mixed planar lipid membrane. The protein radius is R_P and its (uniform) surface charge density is σ_P. The minimal distance between the protein and membrane surfaces is h. A circular membrane region of radius R (and corresponding area $A = \pi R^2$) defines the basis of the cylindrical "cell" corresponding to one adsorbed protein. $\phi = \phi(r)$ is the locally varying mole fraction of charged lipid in the interaction zone.

crystalline hexagonal lattice, as illustrated in Fig. 1. In this limit, where the lateral interactions are most pronounced, the main approximation corresponds to assuming that the nearest neighbor shell is perfectly circular rather than hexagonal. At low surface densities the lateral interactions, and hence their effects on the adsorption energy, are rather weak. In particular, when $R \to \infty$ our model describes the adsorption of an isolated protein.

The adsorption free energy, per protein, is $\Delta F = F(h = h_{eq}, \rho) - F(h = \infty, \rho = 0)$, where $F(h, \rho)$ is the electrostatic (charging) energy of one protein and a membrane of surface area A (as defined by the cylindrical cell volume prescribed in Fig. 2) when the protein is at distance h from the membrane surface and surrounded by identical neighbors at distance $2R \propto \rho^{-1/2}$; h_{eq} is the equilibrium distance of the protein, corresponding to the minimum value of F. The electrostatic potential, Φ, the local lipid composition profile within the interaction zone, $\phi(r)$, and the electrostatic free energy, F, are all functions of h and depend, parametrically, on the average lipid composition ($\bar{\phi}$), the size (R_P) and surface charge density (σ_P) of the protein, the salt concentration (n_0), and the linear dimension of the Wigner-Seitz cell, R. (Of course, $2R$ can be interpreted as the equilibrium distance between the adsorbed proteins only when $h = h_{eq}$.) We shall calculate F, Φ, and $\phi(r)$ based on the nonlinear PB theory, thus neglecting the spatial correlations and finite sizes of the mobile salt ions. However, we shall explicitly account for protein-induced modulations in the lipid charge distribution and protein-protein interactions. We shall assume that in the "unperturbed" membrane (i.e., when $h \to \infty$ or at $r \approx R$ when $R \to \infty$) the acidic and neutral lipids are mixed ideally.

Using $\Psi = e\Phi/k_BT$ to denote the reduced electrostatic potential, where k_B is Boltzmann's constant and T the temperature, our starting point is the free energy functional for the electrolyte solution and the charged surfaces,

$$\frac{F}{k_BT} = \frac{1}{2}\epsilon\left(\frac{k_BT}{e^2}\right)\int_V (\nabla\Psi)^2 dv + \int_V \left[n_+ \ln\frac{n_+}{n_0} + n_- \ln\frac{n_-}{n_0} - (n_+ + n_- - 2n_0)\right] dv + \frac{1}{a}\int_A \left[\phi \ln\frac{\phi}{\bar{\phi}} + (1-\phi)\ln\frac{1-\phi}{1-\bar{\phi}}\right] ds + \lambda\frac{1}{a}\int_A (\phi - \bar{\phi}) ds \quad (1)$$

with $\epsilon = \epsilon_0\epsilon_r$, ϵ_0 denoting the permittivity of vacuum and $\epsilon_r = 78$ the dielectric constant of the solution.

The first term in the last equation is the electrostatic energy of the system, with the integration extending over the entire aqueous volume of the cylindrical region corresponding to our unit (Wigner-Seitz) cell, (including the volume "above" the protein). The second integral accounts for the translational ("mixing") entropy of the mobile ions (of local concentrations n_+ and n_-), relative to their entropy far away from the charged macromolecules where $n_+ = n_- = n_0$; within the interaction region $n_\pm = n_\pm(r, z)$. The third integral, where $\phi = \phi(r) = -e\sigma(r)/a$ is the local mole fraction of acidic lipid in the membrane, represents the 2D demixing entropy of the lipid distribution; the integration extending over the membrane surface from $r = 0$ to $r = R$ ($ds = 2\pi r dr$). The last term in F has been added to the thermodynamic potential to account for the lipid charge conservation, namely, for the condition $\int_A \phi ds = \bar{\phi}A$. 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.

Note that the free energy functional in Eq. 1, which we shall treat as the total free energy of the system, does not include any contribution from the inner (hydrophobic) regions of the membrane and the protein. Namely, we disregard the dielectric properties of these regions, treating them as decoupled from those of the electrolyte solution (and charged surfaces). Formally, this decoupling is equivalent to setting $\epsilon = \epsilon_{int} = 0$ within the hydrophobic regions. Qualitatively, one expects that because any molecular polarization within the hydrophobic regions provides the system with an additional degree of freedom, the interaction free energy between the particles will be lower for all $\epsilon_{int} > 1$. Detailed numerical studies, based on solving the PB equation in the electrolyte solution and the Laplace equation within the (charge-free) hydrophobic regions, corroborate this notion. Yet, the magnitude of these effects for $\epsilon_{int} \approx 2$ (as appropriate for hydrophobic media) are negligibly small for all relevant interparticle separations (Carnie et al., 1994; Carnie and Chan, 1993).

The minimization of F with respect to the mobile ion distributions in the aqueous region, $n_\pm(r, z)$, and the mobile lipid charges in the membrane plane, $\phi(r)$, results in the familiar PB equation

$$\Delta\Psi = l_D^{-2} \sinh\Psi, \quad (2)$$

and the special boundary condition at the membrane surface

$$\phi = \frac{\exp(\Psi - \lambda)}{\frac{1-\bar{\phi}}{\bar{\phi}} + \exp(\Psi - \lambda)} = \frac{l_D}{2p_0}\left(\frac{\partial\Psi}{\partial z}\right)_{z=0}. \quad (3)$$

which should be solved self-consistently with the PB equation. The second equality (where $p_0 = 2\pi l_B l_D/a$), relates the local lipid charge density and the normal derivative of the

electrostatic potential at the membrane surface through Gauss' theorem.

Two additional boundary conditions on Ψ are

$$\left(\frac{\partial\Psi}{\partial n}\right)_{\mathrm{p}} = 2\frac{\chi\bar{\phi}p_0}{l_{\mathrm{D}}}, \quad \left(\frac{\partial\Psi}{\partial r}\right)_{\mathrm{r=R}} = 0. \tag{4}$$

The first of these conditions fixes the normal derivative of the reduced potential at the surface of the protein, as implied by its uniform charge density $\sigma_{\mathrm{P}} = e\chi\bar{\phi}/a = e\phi_{\mathrm{P}}/a$. The second condition follows from our construction of the Wigner-Seitz cell.

Returning to Eq. 3, we note that for large R ($R \gg l_{\mathrm{D}}$), i.e., low density of adsorbed proteins, the local lipid composition far away from the adsorption site should equal the unperturbed composition of the membrane, that is, $\phi \rightarrow \bar{\phi}$. Similarly, the membrane potential Ψ should equal the electrostatic potential corresponding to an unperturbed membrane, Ψ_0; ($\Psi_0 = -2\ \mathrm{arcsinh}(\bar{\phi}p_0)$). From Eq. 3 we see that this implies $\lambda = \Psi_0$. The limit just described corresponds to the adsorption of a single protein on a lipid membrane that is in contact with a lipid reservoir of composition $\bar{\phi}$ and electrostatic potential Ψ_0. It can be shown that the adsorption free energy in this system is, indeed, given by Eq. 1 with $\lambda = \Psi_0$. The last term in Eq. 1 then becomes $\Psi_0 \int_{\mathrm{A}}(\phi - \bar{\phi})ds/a$, expressing the change in the electrostatic energy of the reservoir, associated with the transfer of charged lipids into (or out of) the interaction zone.

The protein-induced lipid charge modulation is driven by the tendency of the membrane charges to provide optimal charge matching conditions between the membrane and protein surfaces. This tendency is opposed by the demixing entropy penalty. The actual, optimal, lipid composition profile reflects the compromise between these two conflicting tendencies. If no free energy price was involved in lipid demixing, the lipid charges could freely move on the membrane surface, lowering even further the electrostatic binding energy. This case, resembling a conducting surface, corresponds to a constant surface potential $\Psi(z = 0) = \Psi_0$. The free energy functional corresponding to this case is obtained by omitting the lipid demixing term in Eq. 1 and replacing the boundary condition in Eq. 3 by $\Psi(z = 0) = \Psi_0$. In the opposite limit the lipids are forced to maintain a constant ("frozen") composition throughout the membrane, implying $\phi \equiv \bar{\phi}$ in Eq. 1 and replacing Eq. 3 by $(l_{\mathrm{D}}/2p_0)(\partial\Psi/\partial z)_{z=0} = \bar{\phi}$. This is the limit of a solid mixed membrane, appropriate for membranes below the chain melting temperature.

It will be interesting to compare the binding characteristics in the two limits above to the ones derived from our model. The adsorption free energies corresponding to constant-uniform lipid composition and constant membrane potential will be denoted as $\Delta F_{\bar{\phi}}$ and ΔF_{Ψ}, respectively. We obviously expect that $\Delta F_{\Psi} \leq \Delta F \leq \Delta F_{\bar{\phi}}$ for all values of h and R.

Adsorption isotherms

To examine the effects of lipid mobility and protein-protein interactions on the thermodynamics of protein binding to mixed lipid membranes, we shall present, in the next section, several representative adsorption isotherms. Our main goal here is to compare adsorption isotherms calculated with, and without, these effects taken into account. Because there is no exact statistical-thermodynamic model for a layer of electrostatically interacting particles (nor for such particles in solution) we shall adopt here an approximate scheme, involving no adjustable parameters.

The finite size of the proteins and the strong electrostatic repulsions between them, in the adsorbed state, are explicitly taken into account in our calculation of ΔF. We shall not include in our model long-range nonelectrostatic (van der Waals) attractions between the proteins, as these may vary from one system to another and are generally weak compared to the electrostatic forces. Thus the "energetic" contribution to the (Helmholtz) free energy of the protein surface layer, $\mathcal{F}_{\mathrm{s}} = \mathcal{E}_{\mathrm{s}} - \mathrm{T}\mathcal{S}_{\mathrm{s}}$, is given by $\mathcal{E}_{\mathrm{s}} = N_{\mathrm{P}}\Delta F$, with N_{P} denoting the number of adsorbed proteins and $\Delta F = \Delta F(h_{\mathrm{eq}}, R)$ the electrostatic adsorption energy per protein. The configurational entropy of the adsorbed layer will be modeled using a 2D lattice gas model, whereby the membrane surface is regarded as a (say, hexagonal) array of N adsorption sites, each of which can accommodate, at most, one protein (thus accounting for excluded volume interactions). Using $\theta = N_{\mathrm{P}}/N$ to denote the fraction of occupied sites, the configurational entropy is given by the familiar expression, $\mathcal{S}_{\mathrm{s}} = -Nk_{\mathrm{B}}[\theta \ln \theta + (1 - \theta)\ln(1 - \theta)]$. Thus,

$$\mathcal{F}_{\mathrm{s}} = N\{\theta\Delta F(\theta; h_{\mathrm{eq}}) + k_{\mathrm{B}}T[\theta \ln \theta + (1 - \theta)\ln(1 - \theta)]\} \tag{5}$$

The explicit dependence of ΔF on θ has been indicated to emphasize that unlike in simple Langmuir adsorption, the adsorption energy here depends on surface coverage.

We still need to define the size of the adsorption cell and hence the value of θ corresponding to a given surface density of proteins. Quite generally, we can set $\theta = \alpha(R_{\mathrm{P}}/R)^2$, where R_{P} is the radius of the protein sphere and R the radius of the Wigner-Seitz cell defining the area (πR^2) per protein on the membrane surface. The parameter α ($\alpha > 1$), expresses the extent to which the "actual" cell size exceeds the projected area (πR_{P}^2) of the bare protein. For a given experimental system it may be determined based on the saturation coverage of this system. We shall simply use $\alpha = 1$.

Using Eq. 5, the chemical potential of the adsorbed proteins, $\mu_{\mathrm{s}} = ((\partial\mathcal{F}_{\mathrm{s}}/\partial N_{\mathrm{P}}) = \partial(\mathcal{F}_{\mathrm{s}}/N)/\partial\theta)$, is given by

$$\mu_{\mathrm{s}} = \Delta F + \theta\left(\frac{\partial\Delta F}{\partial\theta}\right) + k_{\mathrm{B}}T\ln\left(\frac{\theta}{1-\theta}\right) \tag{6}$$

Recalling that the adsorption energy, ΔF, is measured with respect to the charging energy of the separated macroions, the energetic term in the chemical potential of the

free proteins in solution is zero. The configurational entropy contribution to this chemical potential can be derived based on a 3D lattice model description, analogous to the one used for the adsorbed layer, yielding $\mu_f = k_B T \ln[c/(1 - c)]$ for the chemical potential of the proteins in the bulk solution, with c denoting their volume fraction in this phase. Because we ignore interprotein interactions in solution we shall only consider the dilute solution limit, implying $\mu_f = k_B T \ln c$.

Comparing the chemical potentials of the protein in the adsorbed and free states, we obtain a Langmuir-like adsorption equation

$$\theta = \frac{\kappa(\theta)c}{1 + \kappa(\theta)c}, \tag{7}$$

with the caveat that the binding constant depends on surface coverage,

$$\kappa = \exp\left\{-\left[\frac{\Delta F + \theta(\partial \Delta F/\partial \theta)}{k_B T}\right]\right\} \tag{8}$$

It should be mentioned that coverage-dependent adsorption constants have also been derived by Heimburg et al. (1999). Their expression for $\kappa(\theta)$ takes into account excluded volume and other, nonelectrostatic, interactions between the adsorbed proteins, but not the direct electrostatic interactions.

RESULTS AND DISCUSSION

The interaction between two planar and parallel surfaces, uniformly and oppositely charged with *exactly* the same charge density, is attractive. The origin of this attraction is the entropic gain associated with the release of counterions originally present in the vicinity of the charged surfaces. Eventually, when the two surfaces are very close to one another, all counterions can be released and electroneutrality is achieved by the fixed surface charges. This is no longer the case when the charge densities of the two surfaces are not equal. In such cases, a certain fraction of the counterions must remain within the gap between the surfaces. Consequently, the interaction between the surfaces, which can be attractive at large surface separations, becomes repulsive at close approach, owing to the increasing osmotic pressure of the remaining counterions. This short-range repulsion is stronger the larger the "charge mismatch" between the surfaces (Parsegian and Gingell, 1972; Lau and Pincus, 1999).

Qualitatively similar effects prevail, though to a lesser extent, when one or both surfaces are curved. For example, according to PB theory, when a charged sphere approaches an oppositely charged planar surface (of different but fixed charge density) the interaction turns repulsive only at very small distances. When lipid demixing (surface charge redistribution) is allowed, the interaction (according to PB theory) may be attractive at all distances. This scenario may prevail in the adsorption of charged proteins on mixed lipid membranes containing oppositely charged lipid molecules. In the terminology of the previous section, it is possible that $\Delta F_{\bar{\phi}}$ and ΔF (and even more so, ΔF_{Ψ}) will differ not only in magnitude, but also in sign. The differences are expected to depend sensitively on the charge density ratio $\chi = -\sigma_P/\bar{\sigma}$, becoming pronounced for large χ and small $\bar{\sigma}$. Note, however, that our PB calculations, which do not take into account the finite size of the ions and water molecules, are not applicable for distances shorter than $h_{min} \approx 3$ Å, corresponding to the range of strong hydration repulsion. In the following discussion we present calculations of $\Delta F(h)$ and $\phi(r)$ as a function of h/l_D, extending to separations as small as $h/l_D = 0.1$. Clearly, our calculations are only relevant for $h > h_{min}$.

We shall begin the discussion with a comparison of the adsorption characteristics of an isolated protein ($R \gg R_P + l_D$) on "frozen" and fluid ("annealing") membrane. We shall then consider the effects of protein crowding on the binding behavior and their reflection in adsorption isotherms. We shall conclude with a simple analytical model for the adsorption of an isolated protein.

In all the calculations presented below we shall use the same set of values for the cross-sectional area per lipid, $a = 65$ Å^2; the Debye length, $l_D = 10$ Å; and the radius of the protein sphere, $R_P = l_D = 10$ Å.

Numerical solutions of the PB equation are derived using a fourth-order collocation method combined with a Newton-Raphson iteration scheme (Houstis et al., 1985). Calculations were carried out on an appropriately chosen rectangular 50×50 grid, yielding a 4–5-digit accuracy in the free energy, F. The method has been used recently for a number of related problems, including calculation of the forces between colloidal spheres (Carnie et al., 1994) and cylinders (Harries, 1998), and the formation free energies of DNA-cationic lipid assemblies (Harries et al., 1998).

Adsorption of a single protein

Equal surface charge densities

Our first set of calculations is presented for a lipid membrane where half of the lipids are acidic and the rest are neutral, i.e., $\bar{\phi} = 0.5$, corresponding to one negative charge per 130 Å^2 of membrane surface. The protein charge density matches exactly the membrane charge density, i.e., $\chi = 1.0$, corresponding to a protein carrying about 10 positive charges, uniformly smeared on its surface.

Fig. 3 shows the adsorption free energies ΔF_{Ψ}, ΔF, and $\Delta F_{\bar{\phi}}$ as a function of the membrane-protein distance, h.

Although, as expected, $\Delta F_{\Psi} < \Delta F < \Delta F_{\bar{\phi}}$, the adsorption free energies corresponding to the three cases considered are hardly discernible. This appears reasonable in view of the fact that the average charge density of the membrane matches the one on the protein surface. Nevertheless, as

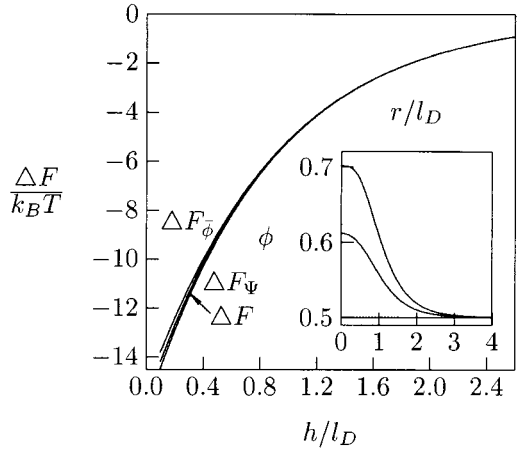

FIGURE 3 Adsorption free energies, ΔF_Ψ, ΔF, and $\Delta F_{\bar{\phi}}$, as a function of the protein-membrane distance, h, for $l_D = 10$ Å, $R_P = l_D$, $a = 65$ Å^2, $\bar{\phi} = 0.5$, and $\chi = 1.0$. The *inset* shows the local composition profile, $\phi(r)$, at $h/l_D = 0.3$, for membranes with constant surface potential (*top curve*), a mixed fluid membrane (*middle curve*), and a frozen lipid distribution (*bottom, horizontal curve*).

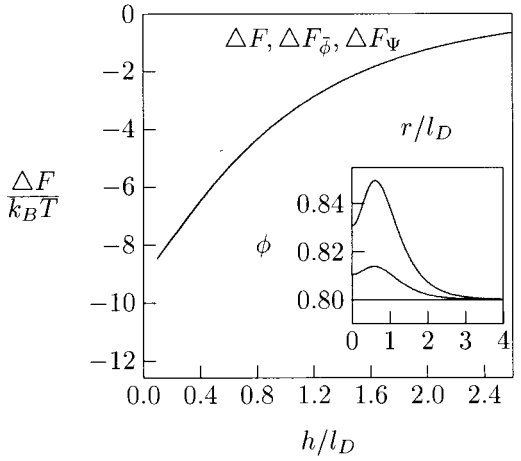

FIGURE 4 Adsorption free energies as a function of the protein-membrane distance, h, for $l_D = 10$ Å, $R_P = l_D$, $a = 65$ Å^2, $\bar{\phi} = 0.8$, and $\chi = 0.375$. All adsorption free energies (ΔF_Ψ, ΔF, and $\Delta F_{\bar{\phi}}$) are essentially equal. The *inset* shows the local composition profile, $\phi(r)$, at $h/l_D = 0.3$, for membranes with constant surface potential (*top curve*), a mixed fluid membrane (*middle curve*), and a frozen lipid distribution (*bottom, horizontal curve*).

indicated by the charge modulation profiles shown in the inset of Fig. 3, the extent of charged lipid recruitment to the immediate vicinity of the protein is non-negligible. (Recall that the calculations shown in Fig. 3 are only relevant for $h > h_{min}$.)

A qualitative argument explaining why the substantial variations in local lipid composition are not reflected to the same extent in the binding free energy curves can be given as follows. As the protein approaches the membrane surface, the charged lipids in its immediate vicinity are essentially neutralized, thus lowering the electrostatic potential in the contact zone. When the lipids are mobile, they tend to diffuse from the surroundings toward the interaction zone, attempting to restore a uniform electrostatic potential throughout the membrane. The gain in electrostatic energy by the stronger adsorption is largely offset by the entropy loss associated with the concomitant transport of counterions into the confines of the interaction region. Later in this section we present a simple model (based on linear PB theory and the constant potential boundary condition on the membrane) that accounts for this mechanism.

Highly charged membrane, weakly charged protein

Our next representative case corresponds to a membrane where most lipids are acidic, $\bar{\phi} = 0.8$, adsorbing a relatively weakly charged protein with $\phi_P = 0.3$ ($\chi = 0.375$). The adsorption free energies and charge modulation profiles for the three types of adsorbing membranes are shown in Fig. 4.

As expected, because the protein is weakly charged, the magnitude of the adsorption free energy is considerably smaller than in the previous case. However, the distinction between the three different types of membranes is completely lost; all three ΔF values are essentially identical. Nevertheless, noticeable, though small, differences appear again in the charge modulation profiles. Our calculation thus suggests that the adsorption energy of weakly charged proteins on highly charged membranes is not affected appreciably by the degree of lipid demixing. For small values of h/l_D charged lipids are depleted from the center of the interaction zone but concentrate at its rim, resulting in a nonmonotonic composition profile.

Highly charged proteins on weakly charged membranes

The case of greatest biological relevance is that of highly charged basic proteins interacting with weakly charged acidic membranes. This is also the type of system where the effects of lipid charge modulation are most pronounced.

The adsorption free energies, ΔF_Ψ, ΔF, and $\Delta F_{\bar{\phi}}$, for a system characterized by $\bar{\phi} = 0.2$ and $\chi = 3.5$ ($\phi_P = 0.7$), are presented in Fig. 5. The inset shows the lipid composition profiles corresponding to the three types of adsorbing membranes for $\phi(r)$ at $h/l_D = 0.3$.

In this case the effects of lipid mobility are apparent in both the adsorption free energy and the composition profile. The magnitude of the binding free energy on a membrane of uniform, frozen, lipid composition ($\phi \equiv \bar{\phi}$) is considerably smaller than that on a fluid membrane. We also note that $\Delta F_{\bar{\phi}}$ shows a minimum at some very small (albeit unrealistic) value of h/l_D, reflecting the osmotic pressure due to counterion confinement in the contact region. This minimum disappears when lipid demixing is allowed to take

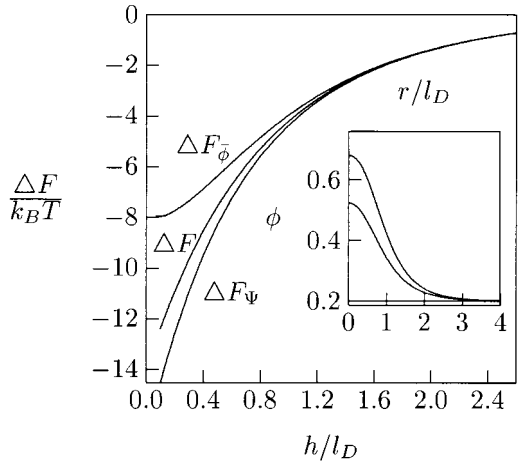

FIGURE 5 Adsorption free energies, ΔF_Ψ, ΔF, and $\Delta F_{\bar{\phi}}$, as a function of the protein-membrane distance, h, for $l_D = 10$ Å, $R_P = l_D$, $a = 65$ Å^2, $\bar{\phi} = 0.2$, and $\chi = 3.5$ ($\phi_P = 0.7$). The *inset* shows the local composition profile, $\phi(r)$, at $h/l_D = 0.3$, for membranes with constant surface potential (*top curve*), a mixed fluid membrane (*middle curve*), and a frozen lipid distribution (*bottom, horizontal curve*).

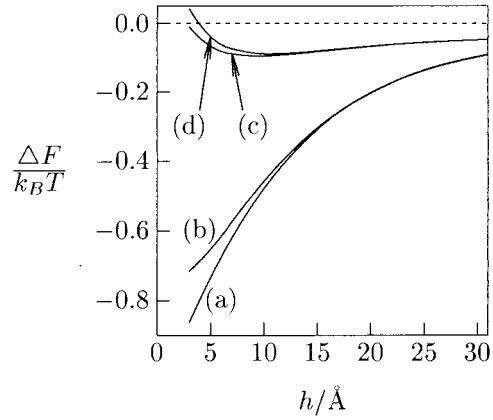

FIGURE 6 The protein adsorption energy ΔF as a function of the protein-membrane distance h for $\phi_P = 0.2$. Curves (*a*) and (*b*) correspond to $\bar{\phi} = 0.05$. Curves (*c*) and (*d*) correspond to $\bar{\phi} = 0.01$. For curves (*a*) and (*c*) the lipids are mobile, whereas for curves (*b*) and (*d*) the local membrane composition $\phi = \bar{\phi}$ is fixed.

place, as charged lipids move toward the interaction zone to achieve charge matching, concomitantly releasing the confined counterions into the bulk solution. The tendency for charge matching is clearly visible in the inset of Fig. 5. The demixing entropy penalty associated with this process is reflected in the difference (of order 1 k_BT) between ΔF and ΔF_Ψ. In this case, in contrast to the two previous cases considered, the diffusion of charged lipids into the interaction zone is accompanied by counterion release and concomitant gain in binding free energy.

Increasing the charge mismatch between the protein and the membrane surface lowers the adsorption energy and may result in the appearance of a minimum in the energy-distance curve at relatively large separations. This behavior is illustrated in Fig. 6 for a protein with a relatively small surface charge ($\phi_P = 0.2$, corresponding to four elementary charges on the surface of our protein sphere) and membranes containing a small fraction of acidic lipid. The figure shows ΔF and $\Delta F_{\bar{\phi}}$ for membranes of composition $\bar{\phi} = 0.05$ and $\bar{\phi} = 0.01$. For $\bar{\phi} = 0.05$ we find the same qualitative behavior as that found for larger surface charge densities (Fig. 5). The magnitude of the binding energy is smaller because the charge densities are smaller. For the membrane containing only one percent of charged lipids the interaction is weak and attractive at large distances, turning repulsive at $h \sim l_D$. In this case, because of the very low "background" concentration of acidic lipids, importing these lipids into the interaction zone implies a severe demixing entropy penalty, which the system is reluctant to pay. Consequently, the binding energy remains small in both the fluid and frozen membranes.

Protein lateral interactions and adsorption isotherms

Two important effects come into play when charged proteins begin to crowd on the surface of a (relatively weakly) charged mixed membrane. First, they compete in recruiting charged lipids into their immediate vicinity. (In the opposite case, i.e., when the membrane charge density is larger than that of the protein, the adsorbed proteins compete in recruiting neutral lipids.) Second, lateral interprotein repulsion becomes significant, resulting in smaller adsorption free energies. The magnitude of these effects depends sensitively on the protein-membrane charge ratio and, of course, the degree of surface coverage $\theta = (R_P/R)^2$.

In Fig. 7 we show how the lipid composition profile in the vicinity of an adsorbed protein, $\phi(r)$, depends on the aver-

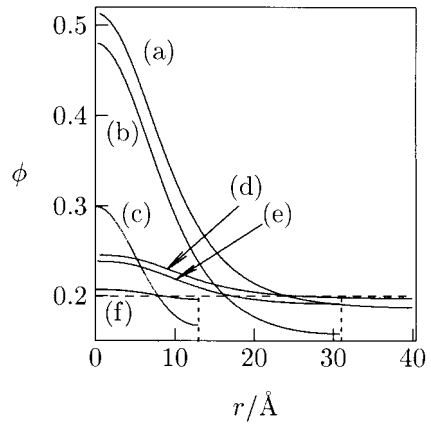

FIGURE 7 The local membrane composition, $\phi(r)$, for $\phi_P = 0.7$, $\bar{\phi} = 0.2$, and $R = 60$ Å (*a*); $R = 31$ Å (*b*); and $R = 13$ Å (*c*). Curves (*d*), (*e*), and (*f*) correspond to $\phi_P = \bar{\phi} = 0.2$ for the same values of R as above. In all cases $h = h_{eq} = 3$ Å.

age distance between the adsorbed proteins. (Recall that $2R$ is the distance between adjacent protein centers; the smallest distance between their charged surfaces is $2(R - R_P)$.) Calculated composition profiles are shown for basic proteins of two different surface charge densities, $\phi_P = 0.7$ and $\phi_P = 0.2$, interacting with a mixed membrane containing $\bar{\phi} = 0.2$ acidic lipids. When $\phi_P = \bar{\phi} = 0.2$ ("charge matching") the extent of charge modulation, $\phi(r) - \bar{\phi}$, is small, and mainly apparent at large interprotein separations.

Pronounced lipid composition modulations are expected, and observed, for large R, especially when the surface charge density of the protein is significantly larger than that of the membrane, as seen for $\phi_P = 0.7$, $\bar{\phi} = 0.2$, and for $R = 60$ Å in Fig. 7. In this case charged lipids accumulate in the immediate vicinity of the protein, thereby reducing the charged lipid concentration at larger distances, $r \sim R$. The accumulation of charged lipids near the protein is somewhat smaller when $R = 31$ Å, yet their depletion from the "central region," $r \sim R$, becomes more pronounced. The charge modulations are rather weak when the proteins are densely packed ($R = 13$ Å in Fig. 7). In this limit the driving force for lipid polarization is diminished because the charged lipids in between neighboring proteins favorably interact with both of them.

Finally, in Fig. 8 we compile a series of calculations demonstrating the effects of lipid mobility and protein-protein interactions on the adsorption free energy, and how they are reflected in the adsorption isotherms, as calculated using Eqs. 7 and 8. The two lower diagrams show the binding free energy, as a function of the distance between adsorbed protein, $2R$, for highly ($\phi_P = 0.7$, *left*) and moderately ($\phi_P = 0.2$, *right*) charged proteins on mixed membranes with varying proportions of charged lipids in the range $\bar{\phi} = 0.05$–0.7. Four curves are shown for every ϕ_P, $\bar{\phi}$ combination. One of these curves corresponds to the "real" case, where the lipids are allowed to demix (paying the necessary price of demixing entropy) and the adsorbed proteins interact with each other. The other three curves, shown only for comparison, were calculated with either one, or both, of these effects artificially turned off. The adsorption isotherms corresponding to the various cases are shown in the two top diagrams.

A general conclusion from these calculations concerns the rather dramatic role of interprotein interactions. Whether lipid demixing is allowed or arrested, for all sets of ϕ_P, $\bar{\phi}$, we find that the magnitude of the adsorption free energy is steeply decreasing once the separation between adjacent protein surfaces, $2(R - R_P)$, falls below $\sim 2l_D$; that is when the counterion clouds surrounding the proteins begin to overlap. For our choice of molecular parameters this happens at $R \sim 20$ Å. At larger distances interprotein interactions are negligible. This conclusion is in line with the calculation of Murray et al. (1999) for pentalysine adsorption on mixed (frozen) membranes.

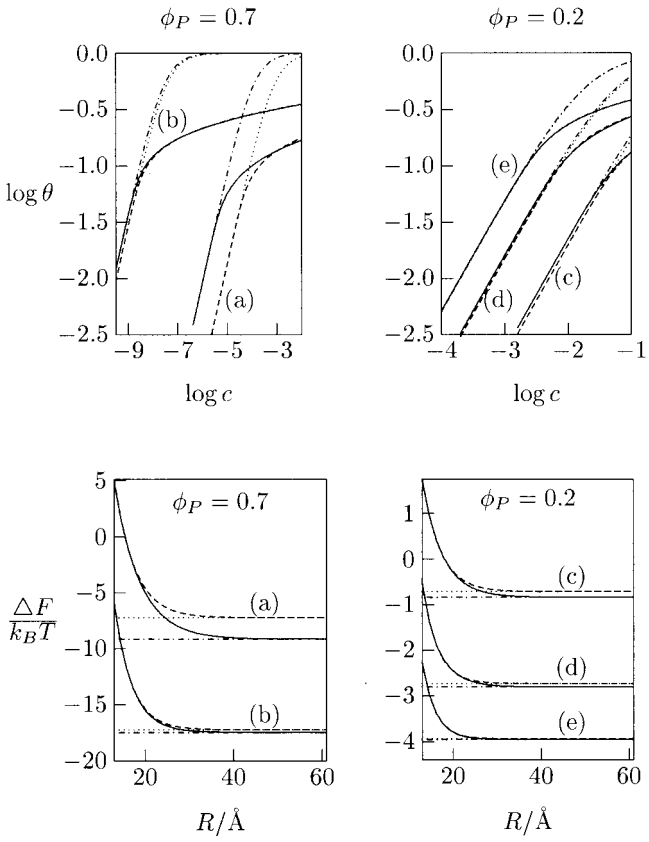

FIGURE 8 Adsorption isotherms $\theta(c)$ (*top panels*) and adsorption free energies $\Delta F(R)$ (*bottom panels*) for several combinations of protein and membrane charge densities. The two figures on the left correspond to the adsorption of highly charged proteins ($\phi_P = 0.7$) on membranes with a smaller charge density, $\bar{\phi} = 0.2$ (curves (*a*)), and an equal charge density, $\bar{\phi} = 0.7$ (curves (*b*)). The figures on the right are for $\phi_P = 0.2$; the curves marked (*c*), (*d*), and (*e*) correspond to $\bar{\phi} = 0.05$, 0.2, and 0.5, respectively. In addition to the solid curves, which represent the results obtained from the full calculation, including the effects of lipid mobility (mixing) and protein-protein interactions, we also show, for comparison, three other curves, corresponding to free energies and adsorption isotherms calculated for: immobile lipids but with interprotein electrostatic interactions (*dashed curves*); mobile lipids but without protein-protein interactions (*dashed-dotted curves*); immobile lipids and no protein-protein interactions (*dotted curves*). All calculations are for $h_{eq} = 3$ Å.

As expected, with these interactions taken into account, the adsorption isotherms begin to saturate at much smaller values of the protein concentration in the bulk solution (c), reaching a much smaller saturation value, θ_{max}, considerably smaller than 1. These findings suggest that the simple Langmuir adsorption scheme may provide a reasonable approximate description of the adsorption equilibrium, provided the linear dimension of an adsorption site is taken as $\sim R_P + l_D$.

Whereas the effects of interprotein interactions become increasingly pronounced at higher surface coverage, the role of lipid mobility is mainly apparent when these interactions subside. As shown in Fig. 8, local demixing of the lipids in

the vicinity of the adsorbed proteins can result in significant enhancement of the adsorption free energy, especially when the protein charge density is considerably higher than the average charge density of the membrane. The difference in free energy can be a substantial fraction of the total free energy. The adsorption isotherms corresponding to mobile versus frozen lipid distributions show even greater differences because their dependence on ΔF is exponential.

Surface overcharging

The charges on the *apposed* faces of the membrane and the protein provide partial, possibly complete, charge neutralization, depending on the degree of surface coverage θ. In contrast, the "outer" surfaces of the proteins, those facing the aqueous solvent, hardly interact with the charged lipid surface and are not "compensated" by other "fixed" (macroion) charges. The apparent surface charge density corresponding to an adsorbing membrane, partially covered by proteins, is given by

$$\sigma_{\rm net} = \bar{\sigma} + \sigma_{\rm P} A_{\rm P}/A = \bar{\sigma}(1 - 4\chi\theta) \qquad (9)$$

where, as before, $\chi = -\sigma_{\rm P}/\bar{\sigma}$ is the protein-membrane charge density ratio. The second equality corresponds to the special case of spherical proteins, where $A_{\rm P}/A = 4\pi R_{\rm P}^2/\pi R^2 = 4\theta$.

From the adsorption isotherms shown in Fig. 8 it follows that for all cases and conditions considered, *at saturation* $\sigma_{\rm net} > 0$, i.e., the total protein charge *overcompensates* the negative charge of the lipid membrane. As a specific example, consider the case $\phi_{\rm P} = 0.7$, $\bar{\phi} = 0.2$, ($\chi = 3.5$) (with mobile lipids). The saturation coverage corresponding to this case is $\theta_{\rm sat} \approx 0.16$, implying $\sigma_{\rm net} \approx -1.2\bar{\sigma}$. That is, the effective charge of the membrane, after adsorption, is approximately reversed. As qualitatively argued above, this is a consequence of the fact that only about half of the protein charges (those on the hemisphere facing the membrane) are compensated. In general, we expect $\sigma_{\rm net}/\bar{\sigma}$ to be a function of the geometry of the adsorbing particles and the charge distribution on their surface.

It should be mentioned that the phenomenon of charge overcompensation and reversal is commonplace in the adsorption of colloidal particles and polyelectrolytes on oppositely charged surfaces (Châtellier and Joanny, 1996; Joanny, 1999; Borukhov et al., 1998; Park et al., 1999). In fact, surface charge overcompensation (sign reversal of the apparent surface charge) has also been observed experimentally (Kékicheff et al., 1993) and predicted theoretically for ordinary, especially multivalent, electrolyte solutions (for a comprehensive discussion of these effects see Greberg and Kjellander (1998) and references therein). In these systems surface charge reversal is the consequence of ion-ion correlations in the bulk solution and the vicinity of the charged surfaces. Of course these correlations are not accounted for by the mean-field PB theory. Nevertheless, the fact that we predict surface charge over compensation in our protein-membrane system is hardly surprising, even though our treatment of the electrolyte solution is based on PB theory. This is because in our problem the analogs of the multivalent counterions in electrolyte solutions are not the small monovalent salt ions (that we treat in a mean-field fashion) but, rather, the charged colloidal (protein) particles. Spatial correlations between these macro-counterions, as well as excluded volume constraints between the protein counterions and the membrane surface, are explicitly accounted for by the use of the cell model.

A simple model for protein-induced membrane charge polarization

Earlier in this section we have shown that the ability of a mixed fluid membrane to adjust its local charge to that of an approaching protein results in significant enhancement of the adsorption free energy. Moreover, this charge polarization tendency was found to be stronger than the entropic resistance to lipid demixing. This is reflected by the fact that ΔF is not very different from ΔF_Ψ, as compared to $\Delta F_{\bar{\phi}}$ (see, e.g., Fig. 5). Replacing ΔF by ΔF_Ψ, i.e., omitting the (positive) demixing entropy contribution to the adsorption free energy, we can treat the membrane as a surface of constant electrostatic potential, $\Psi = \Psi_0$. Based on this approximation a simple, closed-form model for the adsorption of a single protein on the membrane surface can be presented, as follows.

Suppose first that a charged and flat, say disklike, protein is approaching the membrane. The charge density on the surface of the protein, $\sigma_{\rm P} = -\chi\bar{\sigma}$, is generally different from the average charge density on the membrane surface, $\bar{\sigma} = -\bar{\phi}e/a$. Thus, the lipid composition in the contact region, $\phi = -a\sigma/e$, is no longer equal to the composition in the rest of the membrane (which can be treated as infinitely large). If the area of the protein, $A_{\rm eff}$, is large compared to the cross-sectional area per lipid headgroup, we can neglect edge effects and assume that ϕ is uniform within the contact area; ϕ depends, of course, on the disk-surface distance h, ($\phi(h \to \infty) = \bar{\phi}$).

Within the framework of *linearized* PB theory (valid for $\Psi \ll 1$) an expression for the interaction free energy between two arbitrarily charged planar surfaces was derived by Parsegian and Gingell (1972). Based on this expression the electrostatic free energy of our disk-membrane system is given by

$$\frac{F}{k_{\rm B}T} = \frac{A_{\rm eff}p_0}{a}\left[\frac{(\phi^2 + \chi^2\bar{\phi}^2)\cosh(h/l_{\rm D}) - 2\phi\chi\bar{\phi}}{\sinh(h/l_{\rm D})} - 2\bar{\phi}(\phi - \bar{\phi})\right] \qquad (10)$$

where $p_0 = 2\pi l_{\rm B} l_{\rm D}/a$. To obtain the result in Eq. 10, we have used the expression for the reduced potential of an

unperturbed membrane $\Psi_0 = -2\bar{\phi}p_0$, as is known from linear PB theory. The second term in this expression is the change in the electrostatic free energy in the reservoir associated with the transfer of lipids into the interaction zone. The membrane composition in the interaction zone is unknown. We find it by minimizing F with respect to ϕ, resulting in

$$\phi = \bar{\phi}\frac{\chi + \sinh(h/l_D)}{\cosh(h/l_D)} \tag{11}$$

The dependence of ϕ on the protein-surface separation, as predicted by Eq. 11, is shown in Fig. 9 for three different values of χ. For $h \gg l_D$ we indeed find $\phi = \bar{\phi}$. Interestingly, for all χ a maximum in $\phi/\bar{\phi}$ appears at some intermediate separation. In the limit $h \to 0$, the charge density on the membrane surface becomes equal to that on the protein surface. That is, at small separations the adsorption free energy is minimal (maximal in magnitude) when the protein-membrane "complex" is *isoelectric*, at which point all the counterions (which otherwise would be confined between the surfaces) are released into the bulk solution.

The electrostatic interaction model outlined above can be extended to the case of a nonplanar, e.g., spherical protein, using a Derjaguin-like approximation (Evans and Wennerström, 1999). In this approximation, the interaction between two curved (or curved and planar) surfaces is expressed as a sum of interactions between planar and parallel area elements belonging to the two surfaces, appropriately weighted according to the curvatures of the interacting surfaces. The interaction energy between area elements is derived from the corresponding expression for infinite surfaces. The Derjaguin approximation is appropriate for moderately curved surfaces. For our problem, of a charged spherical protein interacting with a planar membrane, this requires $R_P > h$ and $R_P > l_D$.

In brief, to apply the Derjaguin approximation to the sphere-membrane problem we divide the sphere surface into circular-stripe elements, cylindrically surrounding the symmetry axis. With each such element we associate a circular shell of equal area on the membrane surface. The distance between the area element on the membrane (at distance r from the axis) and the corresponding area element on the sphere is $l(r) = \sqrt{(r - r_\xi)^2 + h(r_\xi)^2}$, with $h(r_\xi) = d + R_P - \sqrt{R_P^2 - r_\xi^2}$ and $r_\xi = R_P \sin\xi$, where $\xi = 2\arcsin(r/2R_P)$ is the angle between the membrane normal and the radius vector connecting the protein center with the area element on the protein surface. (The interaction range on the membrane, as measured by r, exceeds the projected radius of the protein, R_P. We let the angle ξ vary over the protein hemisphere facing the membrane, i.e., ξ varies between 0 and $\pi/2$, implying that on the membrane the interaction zone is bounded by $r_{max} = \sqrt{2}R_P$.) Then, using Eq. 11, we calculate $\phi(l(r)) = \phi(r)$, the lipid composition at distance $l(r)$ from the sphere or, equivalently, at distance r from the symmetry axis, in the membrane plane.

The results of these calculations are shown in Fig. 10 for three values of the protein-membrane charge ratio, namely $\chi = 0.5$, $\chi = 1$, and $\chi = 2$. To ensure the validity of the Derjaguin approximation we have used here a large protein radius $R_P = 30$ Å. The other parameters in this series of calculations are $l_D = 10$ Å, $\bar{\phi} = 0.1$, and $h/l_D = 0.2$. Also shown in the figure are the numerical solutions for $\phi(r)$ according to the nonlinear PB theory. The simple model in Eq. 11 is in good qualitative agreement with PB theory. It correctly predicts the accumulation of charged lipids near the adsorbed protein and the tendency toward charge matching at the binding site of the sphere.

CONCLUDING REMARKS

Based on a general free energy expression we have analyzed the role of lipid mobility (hence demixing) and lateral

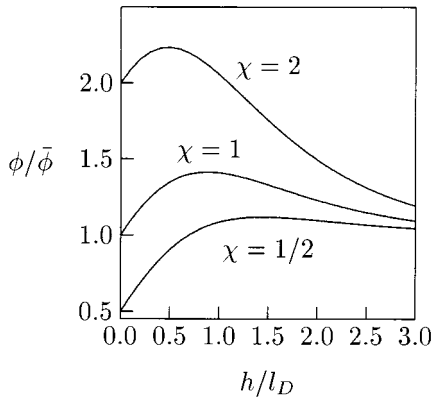

FIGURE 9 The membrane charge density in the interaction region of a "flat protein" as a function of the distance between the membrane surface and the protein. The figure shows $\phi/\bar{\phi} = (\chi + \sinh h/l_D)/\cosh h/l_D$, as given by Eq. 11, for three representative values of the charge density ratio χ.

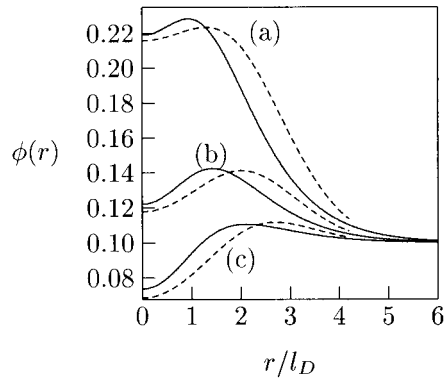

FIGURE 10 The lipid distribution $\phi(r)$ according to nonlinear PB theory (*solid curves*) and the Derjaguin-like approximation (*dashed curves*), for $l_D = 10$ Å, $\bar{\phi} = 0.1$, $R_P = 30$ Å, and $h/l_D = 0.2$. Curves (*a*), (*b*), and (*c*) correspond to $\chi = 2$, 1, and 0.5, respectively.

adsorbate interactions, on the adsorption free energy of globular charged proteins onto mixed lipid membranes. We found that the binding energy is significantly enhanced by the ability of the charged lipids to adjust their local concentration in the vicinity of the adsorbed protein. The effects of this lipid-mobility degree of freedom are particularly pronounced when the protein is highly charged and the membrane is weakly charged. In this case, the extent of local membrane charge modulation is substantial, especially at low protein densities. Interprotein repulsions within the adsorbed layer become important, as expected, when the counterion atmospheres of neighboring proteins begin to overlap. Both the lipid demixing degree of freedom and the lateral interactions between the proteins are reflected in the calculated adsorption isotherms. Assuming that the lipid charge in the vicinity of the adsorbed protein matches the ("membrane facing") protein charge, and that the minimal distance between protein is governed by their counterion screening clouds, provides an approximate scheme for calculating (Langmuir-like) adsorption isotherms.

At saturation the charges on the membrane and the protein regions facing the membrane are nearly equal. The protein charges facing the aqueous solution remain uncompensated, implying "charge reversal" of the adsorbing surface.

The free energy functional presented in this paper can be extended to include other relevant effects and degrees of freedom, such as nonideal lipid mixing (Gelbart and Bruinsma, 1997) or elastic membrane perturbations (see, e.g., Dan (1996); Dan et al. (1993); Fournier (1999)). Local elastic deformations, induced and enhanced by the adsorption of proteins on the membrane surface, could possibly explain the formation of high-density protein "patches" (domains) in lipid membranes.

The financial support of the Israel Science Foundation (Excellence Center, Grant No. 8003/97) and the U.S.-Israel Binational Science Foundation (Grant No. 94/130) is gratefully acknowledged. S.M. thanks the DFG for support through SFB 197. D.H. thanks the Clore Foundation for a doctoral fellowship. The Fritz Haber research center is supported by the Minerva Foundation, Munich, Germany.

REFERENCES

Andelman, D. 1995. Electrostatic properties of membranes: the Poisson-Boltzmann theory. *In* Structure and Dynamics of Membranes, 2nd Ed., Vol. 1., Section 12. R. Lipowsky and E. Sackmann, editors. Elsevier, Amsterdam. 603–642.

Aranda-Espinoza, H., A. Berman, N. Dan, P. Pincus, and S. A. Safran. 1996. Interaction between inclusions embedded in membranes. *Biophys. J.* 71:648–656.

Ben-Tal, N., B. Honig, C. Miller, and S. McLaughlin. 1997. Electrostatic binding of proteins to membranes. Theoretical predictions and experimental results with charybdotoxin and phospholipid vesicles. *Biophys. J.* 73:1717–1727.

Ben-Tal, N., B. Honig, R. M. Peitzsch, G. Denisov, and S. McLaughlin. 1996. Binding of small basic peptides to membranes containing acidic lipids: theoretical models and experimental results. *Biophys. J.* 71: 561–575.

Borukhov, I., D. Andelman, and H. Orland. 1998. Scaling laws of polyelectrolyte adsorption. *Macromolecules.* 31:1665–1671.

Bruinsma, R., and P. Pincus. 1996. Protein aggregation in membranes. *Curr. Opin. Solid St. M.* 41:401–406.

Carnie, S. L., and D. Y. C. Chan. 1993. Interaction free energy between identical colloidal particles: the linearized Poisson-Boltzmann theory. *J. Colloid Interface Sci.* 155:297–312.

Carnie, S. L., D. Y. C. Chan, and J. Stankovich. 1994. Computation of forces between spherical colloidal particles: nonlinear Poisson-Boltzmann theory. *J. Colloid Interface Sci.* 165:116–128.

Châtellier, X., and J.-F. Joanny. 1996. Adsorption of polyelectrolyte solutions on surfaces: a Debye-Hückel theory. *J. Physique II (France).* 6:1669–1686.

Dan, N. 1996. Formation of ordered domains in membrane-bound DNA. *Biophys. J.* 71:1267–1272.

Dan, N., P. Pincus, and S. A. Safran. 1993. Membrane-induced interactions between inclusions. *Langmuir.* 9:2768–2771.

Das, T., D. Bratko, L. B. Bhuiyan, and C. W. Outhwaite. 1995. Polyelectrolyte solutions containing mixed valency ions in the cell model: a simulation and modified Poisson-Boltzmann study. *J. Chem. Phys.* 107: 9197–9207.

Denisov, G., S. Wanaski, P. Luan, M. Glaser, and S. McLaughlin. 1998. Binding of basic peptides to membranes produces lateral domains enriched in the acidic lipids phosphatidylserine and phosphatidylinositol 4,5-biphosphate: an electrostatic model and experimental results. *Biophys. J.* 74:731–744.

Deserno, M., C. Holm, and S. May. 2000. The fraction of condensed counterions around a charged rod: comparison of Poisson-Boltzmann theory and computer simulations. *Macromolecules.* 33:199–205.

Evans, D. F., and H. Wennerström. 1999. *The Colloidal Domain, where Physics, Chemistry, and Biology Meet,* 2nd Ed. VCH Publishers, New York.

Fournier, J. B. 1998. Coupling between membrane tilt-difference and dilation: a new "ripple" instability and multiple crystalline inclusions phases. *Europhys. Lett.* 43:725–730.

Fournier, J. B. 1999. Microscopic membrane elasticity and interactions among membrane inclusions: interplay between the shape, dilation, tilt and tilt difference modes. *Eur. Phys. J.* 11:261–272.

Gelbart, W. M., and R. Bruinsma. 1997. Compositional-mechanical instability of interacting mixed lipid membranes. *Phys. Rev. E.* 55:831–835.

Gil, T., J. H. Ipsen, O. G. Mouritsen, M. C. Sabra, M. M. Sperotto, and M. J. Zuckermann. 1998. Theoretical analysis of protein organization in lipid membranes. *Biophys. Biochim. Acta.* 1376:245–266.

Greberg, H., and R. Kjellander. 1998. Charge inversion in electric double layers and effects of different sizes for counterion and coions. *J. Chem. Phys.* 108:2940–2953.

Harries, D. 1998. Solving the Poisson-Boltzmann equation for two parallel cylinders. *Langmuir.* 14:3149–3152.

Harries, D., S. May, W. M. Gelbart, and A. Ben-Shaul. 1998. Structure, stability, and thermodynamics of lamellar DNA-lipid complexes. *Biophys. J.* 75:159–173.

Harroun, T. A., W. T. Heller, T. M. Weiss, L. Yang, and H. W. Huang. 1999. Theoretical analysis of hydrophobic matching and membrane-mediated interactions in lipid bilayers containing gramicidin. *Biophys. J.* 76:3176–3185.

Heimburg, T., and D. Marsh. 1995. Protein surface distribution and protein-protein interactions in the binding of peripheral proteins to charged lipid membranes. *Biophys. J.* 68:536–546.

Heimburg, T., B. Angerstein, and D. Marsh. 1999. Binding of peripheral proteins to mixed lipid membranes: effect of lipid demixing upon binding. *Biophys. J.* 76:2575–2586.

Houstis, E. N., W. F. Mitchell, and J. R. Rice. 1985. Collocation software for second order elliptic partial differential equations. *ACM Trans. Math. Software.* 11:379–418.

Joanny, J.-F. 1999. Polyelectrolyte adsorption and charge inversion. *Eur. Phys. J. B.* 9:117–122.

Jönsson, A-S., and B. Jönsson. 1996. Ultrafiltration of colloidal dispersions—a theoretical model of the concentration polarization phenomena. *J. Colloid Interface Sci.* 180:504–518.

Jönsson, B., and L. Stahlberg. 1999. The electrostatic interaction between a charged sphere and an oppositely charged planar surface and its application to protein adsorption. *Colloids and Surfaces B.* 14:67–75.

Kékicheff, P., S. Marčelja, T. J. Senden, and V. E. Shubin. 1993. Charge reversal seen in electrical double layer interaction of surfaces immersed in 2:1 calcium electrolyte. *J. Chem. Phys.* 99:6098–6113.

Killian, J. A., I. Salemink, M. R. R. de Planque, G. Lindblom, R. E. Koeppe, and D. V. Greathouse. 1996. Indication of nonbilayer structures in diacylphosphatidylcholine model membranes by transmembrane α-helical peptides: importance of hydrophobic mismatch and proposed role of tryptophans. *Biochemistry.* 35:1037–1045.

Koltover, I., T. Salditt, J. O. Rädler, and C. R. Safinya. 1998. An inverted hexagonal phase of cationic liposome-DNA complexes related to DNA release and delivery. *Science.* 281:78–81.

Lau, A. W. C., and P. Pincus. 1999. Binding of oppositely charged membranes and membrane reorganization. *Eur. Phys. J. B.* 10:175–180.

Lifson, S., and A. Katchalsky. 1954. The electrostatic free energy of polyelectrolyte solutions. II. Fully stretched macromolecules. *J. Polym. Sci.* 13:43–55.

Linse, P., and B. Jönsson. 1982. Electrostatic interactions in micellar solutions. A comparison between Monte Carlo simulations and solutions of the Poisson-Boltzmann equation. *J. Phys. Chem.* 86:413–421.

May, S., and A. Ben-Shaul. 1999. Molecular theory of lipid-protein interaction and the L_α–H_{II} transition. *Biophys. J.* 76:751–767.

McCormack, D., S. L. Carnie, and D. Y. C. Chan. 1995. Calculations of electric double-layer force and interaction free energy between dissimilar surfaces. *J. Colloid Interface Sci.* 169:177–196.

Murray, D., A. Arbuzova, G. Hangyás-Mihályné, A. Gambhir, N. Ben-Tal, B. Honig, and S. McLaughlin. 1999. Electrostatic properties of membranes containing acidic lipids and adsorbed basic peptides: theory and experiment. *Biophys. J.* 77:3176–3188.

Neu, J. C. 1999. Wall-mediated forces between like-charged bodies in an electrolyte. *Phys. Rev. Lett.* 82:1072–1074.

Nielsen, C., M. Goulian, and O. S. Andersen. 1998. Energetics of inclusion-induced bilayer deformations. *Biophys. J.* 74:1966–1983.

Ninham, B. W., and V. A. Parsegian. 1971. Electrostatic potential between surfaces bearing ionizable groups in ionic equilibrium with physiologic saline solutions. *J. Theor. Biol.* 31:405–428.

Palkar, S. A., and A. M. Lenhoff. 1994. Energetic and entropic contributions to the interaction of unequal spherical double layers. *J. Colloid Interface Sci.* 165:177–194.

Park, S. Y., R. F. Bruinsma, and W. M. Gelbart. 1999. Spontaneous overcharging of macro-ion complexes. *Europhys. Lett.* 46:454–460.

Parsegian, V. A., and D. Gingell. 1972. On the electrostatic interaction across a salt solution between two bodies bearing unequal charges. *J. Biophys.* 12:1192–1204.

Record, M. T., Jr., C. F. Anderson, and T. M. Lohman. 1978. Thermodynamic analysis of ion effects on the binding and conformational equilibria of proteins and nucleic acids: the roles of ion association on release, screening, and ion effects on water activity. *Q. Rev. Biophys.* 11:103–178.

Roth, C. M., J. E. Sader, and A. M. Lenhoff. 1998. Electrostatic contribution to the energy and entropy of protein adsorption. *J. Colloid Interface Sci.* 203:218–121.

Ryba, N. J. P., and D. Marsh. 1992. Protein rotational diffusion and lipid/protein interactions in recombinants of bovine rhodopsin with saturated diacylphosphatidylcholines of different chain lengths studied by conventional and saturation-transfer electron spin resonance. *Biochemistry.* 31:7511–7518.

Sader, J. E., and D. Y. C. Chan. 1999a. Electrical double-layer interaction between charged particles near surfaces and in confined geometries. *J. Colloid Interface Sci.* 218:423–432.

Sader, J. E., and D. Y. C. Chan. 1999b. Long-range electrostatic attractions between identically charged particles in confined geometries: an unresolved problem. *J. Colloid Interface Sci.* 213:268–269.

Sperotto, M. M., and O. G. Mouritsen. 1993. Lipid enrichment and selectivity of integral membrane proteins in two-component lipid bilayers. *Eur. Biophys. J.* 22:323–328.

Ström, C., B. Jönsson, O. Söderman, and P. Hansson. 1999. Adsorption of a divalent cationic surfactant onto a silica surface. *Colloids and Surfaces A.* 159:109–120.

Vlachy, V. 1999. Ionic effects beyond Poisson-Boltzmann theory. *Annu. Rev. Phys. Chem.* 50:145–165.

Wagner, K., D. Harries, S. May, V. Kahl, J. O. Rädler, and A. Ben-Shaul. 2000. Counterion release upon cationic lipid-DNA complexation. *Langmuir.* 16:303–306.

Warszyńsky, P., and Z. Adamczyk. 1997. Calculations of double-layer electrostatic interactions for sphere/plane geometry. *J. Colloid Interface Sci.* 187:283–295.

Wennerström, H., B. Jönsson, and P. Linse. 1982. The cell model for polyelectrolyte systems. Exact statistical mechanical relations, Monte Carlo simulations, and the Poisson-Boltzmann approximation. *J. Chem. Phys.* 76:4665–4670.