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Colloids and Surfaces
A: Physicochemical and Engineering Aspects 208 (2002) 41–50

COLLOIDS AND SURFACES A

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Adsorption of charged macromolecules on mixed fluid membranes

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Received 11 June 2001; accepted 19 November 2001

Abstract

The adsorption of charge rigid macromolecules, such as proteins from solution, on mixed (charged and neutral) lipid membranes is affected by several important factors. First, the mobile lipids in the membrane may rearrange, and demix locally to match the charge density of the apposed macromolecule, thus lowering the adsorption free energy. On the other hand, the (electrostatic) interaction between adsorbed macromolecules tends to lower the saturation coverage of the membrane. Additional factors, such as non-ideal lipid demixing or an elastic membrane response, enhanced by the presence of the charged macromolecules, may be at the base of the experimentally observed formation of high density protein domains and lateral macro-phase separation in lipid membranes.

Keywords: Macromolecules; Electrostatic interactions; Lipid membranes

1. Introduction

Charge is carried by many biological molecules such as proteins, polynucleotides (e.g. DNA) and lipid membranes. The electrostatic interaction between these macromolecules is at the base of many biological processes, such as protein–DNA binding, the adsorption of peripheral proteins onto cell membranes and the condensation of DNA in cell nuclei or viral capsids. Particular experimental and theoretical effort has been invested in the study of the adsorption of proteins onto charged lipid membranes since many biological processes, e.g. membrane activated enzymatic and signal transduction activity, occur at the membrane surface. This adsorption is also a primary step in other processes such as the formation of ion channels in cell membranes by self-assembled amphipathic peptides.

A large number of experimental studies based mainly on fluorescence labeling and NMR techniques reveal that the adsorption process may occur in several stages [1–14]. At first, the basic proteins bind to the mixed, acidic and neutral lipid membrane. The fluid nature of the lipid bilayer allows the lipid constituent which interact

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0927-7757/02/$ - see front matter
PII: S0927-7757(02)00100-0

more favorably with the adsorbing protein (i.e. the acidic component) to migrate laterally towards the protein's vicinity, thus modulating locally the lipid composition. Conversely, the less favorably interacting (neutral) lipids, migrate away from this area. As will be shown, the (often substantial) gain in free energy associated with the charge rearrangement process is opposed by the inevitable penalty of demixing entropy. The balance between these two forces determines the final local lipid composition. This effect can be expected to be most pronounced at small equilibrium protein–membrane distances. The adsorption isotherms, and membrane coverage at equilibrium is also expected to be strongly dependent on the interaction between proteins: in general, repulsive interactions will tend to lower the coverage of bilayers by proteins.

Experiments also show that in some cases, adsorbed proteins and the underlying anionic lipids may further colocalize into domains. It was further shown that the radius of curvature of these domains was higher than that of the surrounding lipid. These domains were observed to bud and pinch off in the form of vesicles [13]. This process of domain-formation pertains to the second mechanism by which a lipid bilayer can lower the interaction free energy. Since the membrane can deform by stretching and bending, it may lower the interaction free energy with an adsorbing molecule through such an elastic response.

The perturbation to the underlying lipid bilayer and lipid migration may be enhanced by non-ideal lipid demixing which may subsequently lead to a 'line-tension' in the lipid bilayer. More explicitly, the non-ideal contribution to the membrane free energy may favor the segregation of adsorbed proteins, in order to satisfy the natural tendency of the two underlying lipid species to demix. Thus, the membrane can lower the free energy associated with 'rims' of the lipid domains, minimizing them by coalescing smaller domains into larger ones, hence forming macroscopic lateral domains. A wide variety of models have been suggested to account for some or all of these effects.

We concentrate on one particular model which we have recently presented [15] for studying the interaction between rigid macromolecules and mixed (charged/uncharged) membranes, and then discuss some emerging results. We then briefly comment on other possible models that have been proposed. Models of the adsorption of other, more flexible macromolecules, such as polyelectrolytes have been presented elsewhere (see e.g. [16–19] and references therein).

2. Model

2.1. Cell free energy

Consider a globular, positively charged, protein adsorbing to a negatively charged membrane, both immersed in a 1:1 salt solution. Within Poisson–Boltzmann theory we treat the system of electrolyte with 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 effective interactions between adsorbed proteins will be treated using a 'cell-model' [15], whereby every adsorbed protein defines a cylindrical (Wigner–Seitz) cell of radius R as depicted in Fig. 1. The membrane is modeled as a flat, low dielectric, surface with local surface charge density $\sigma(r) = -e\phi(r)/a$, where a is the area per (both types of) lipid molecule, ϕ is the (local) mole fraction of charged lipids in the membrane, and e the elementary charge, (Fig. 2). The average charge density of the lipid membrane is $\bar{\sigma} = -\bar{\phi}e/a$ where $\bar{\phi}$ is the (overall) mole fraction of charged lipids in the membrane. The protein is modeled as a rigid sphere of low dielectric con-

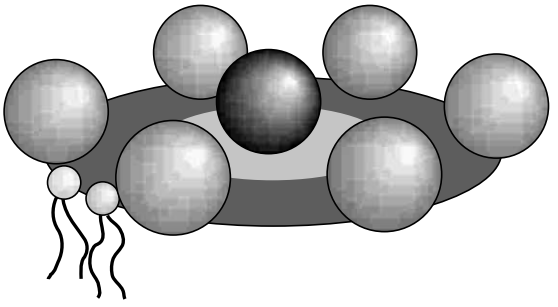

Fig. 1. Schematic view of the Wigner–Seitz cell.

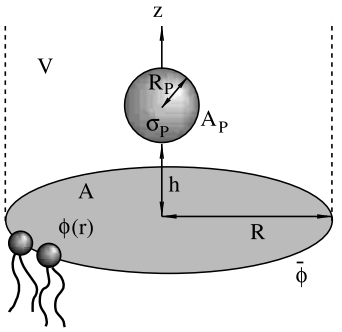

Fig. 2. Schematic illustration of a spherical protein adsorbed on a mixed planar lipid membrane. The protein radius is R_P and its (uniform) surface charge density is σ_P. The minimal distance between the protein and membrane surfaces is h. A circular membrane region of radius R (and corresponding area $A=\pi R^2$) 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.

stant with a uniform (positive) surface charge density $\sigma_P = e\phi_P/a$. The distance between the protein and the membrane will be denoted by h.

Within our model we allow for the possibility of spatial local inhomogeneities in the membrane surface charge density, in response to interactions with the cationic protein. A (possibly non-ideal) mixing free energy contribution must be added to the membrane free energy. Lipid demixing turns out to be significant in many relevant cases, and reflects the compositional degree of freedom associated with mobile lipids in mixed fluid bilayers. We include this effect in a self-consistent manner, by first writing the free energy functional of a unit cell

$$\begin{aligned}\frac{F}{k_BT} &= \frac{1}{2}\varepsilon\left(\frac{k_BT}{e^2}\right)\int_V (\nabla\Psi)^2 \mathrm{d}v \\ &+ \int_V \left[n_+ \ln\frac{n_+}{n_0} + n_- \ln\frac{n_-}{n_0} \right. \\ &\left. - (n_+ + n_- - 2n_0) \right] \mathrm{d}v \\ &+ \frac{1}{a}\int_A \left[\phi \ln\frac{\phi}{\bar{\phi}} + (1-\phi)\ln\frac{1-\phi}{1-\bar{\phi}} \right] \mathrm{d}s \\ &+ \frac{1}{a}\int_A \chi[\phi(1-\phi) - \bar{\phi}(1-\bar{\phi})] \mathrm{d}s \\ &+ \lambda\frac{1}{a}\int_A (\phi - \bar{\phi}) \mathrm{d}s \end{aligned} \quad (1)$$

The first term in Eq. (1) represents the electrostatic energy of the system, with the integration extending over the entire aqueous volume. 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 and fourth integrals, represent the 2D (non-ideal) demixing entropy of the lipid distribution; the integration extending over the membrane surface from $r=0$ to $r=R$ ($\mathrm{d}s = 2\pi r\,\mathrm{d}r$). The (phenomenological, mean-field) interaction parameter χ accounts for the non-ideal mixing contribution to the free energy of the lipid molecules. Non-ideal lipid mixing is commonplace in biological membranes [20–22], resulting, for instance, from the different molecular structure of the lipid tails. The last term in F has been added to the thermodynamic potential to account for the lipid charge conservation, namely, for the condition $\int_A \phi\,\mathrm{d}s = \bar{\phi}A$. 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. The adsorption free energy, $\Delta F = F(h=h_{eq},R) - F(h=\infty,R=\infty)$, and the local lipid composition $\phi(r)$ are determined by the minimization of the functional F 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 non-linear PB equation for the electrostatic potential in the system, supplemented by a special boundary condition on the electrostatic potential at the membrane surface [23,15]. This boundary condition may be interpreted as the requirement for a 'constant electro-chemical' potential reflecting the balance between the chemical and electrostatic potentials of the mobile lipids. The set of differential equations is then solved numerically.

2.2. Evaluating adsorption isotherms

Once derived, ΔF may be further used to determine adsorption isotherms, which clearly reflect the effects of lipid mobility and protein lateral interactions on the adsorption free energy.

We apply a Langmuir-like approach to the adsorption of charged proteins from a solution of a certain protein concentration c. The (Helmholtz) free energy of a layer of N_P adsorbed proteins is $\mathscr{F} = \mathscr{E} - \mathrm{TS}$ where the energetic contribution is $\mathscr{E} = \mathrm{N_p}\Delta\mathrm{F}$ with N_P denoting the number of adsorbed proteins. Using a two dimensional lattice gas model for evaluating the configurational entropy of the adsorbed protein layer, the entropic contribution to $\mathscr{F}$ is $\mathrm{S} = -\mathrm{N_p}[\theta\ln\theta + (\infty - \theta)\ln(\infty - \theta)]$, with N denoting the number of adsorption sites, and $\theta = N_P/N$. In the following we choose to define the coverage as $\theta = (R_\mathrm{P}/R)^2$, where R_P is the radius of the protein and R is the radius of the unit cell. The chemical potential of the adsorbed proteins, μ_l, can now be determined:

$$\mu_\mathrm{l} = \left(\frac{\partial \mathscr{F}}{\partial N_\mathrm{P}}\right) = \Delta F + \theta\left(\frac{\partial \Delta F}{\partial \theta}\right) + k_\mathrm{B}T\ln\left(\frac{\theta}{1-\theta}\right) \tag{2}$$

The chemical potential of proteins in solution may be evaluated in a similar fashion. Assuming dilute solution behavior, we set the electrostatic energy of the free proteins in solution (measured with respect to the isolated protein) as $\mathscr{E}_\mathrm{s} = 0$. To determine the configurational entropy of proteins we employ a three dimensional lattice model, resulting in $\mu_\mathrm{s} = k_\mathrm{B}T\ln[c/(1-c)]$ for the chemical potential of the proteins in the bulk solution.

Equating the chemical potential of the adsorbed protein layer with that of the proteins in the bulk solution we obtain the adsorption equations of the form:

$$\theta = \frac{\kappa(\theta)c}{1 + \kappa(\theta)c} \tag{3}$$

In contrast to the Langmuir adsorption isotherms, here the binding constant, κ is dependent on surface coverage through ΔF:

$$\kappa = \exp\left\{-\left[\frac{\Delta F + \theta(\partial \Delta F/\partial\theta)}{k_\mathrm{B}T}\right]\right\} \tag{4}$$

It should be mentioned that coverage dependent adsorption constants have previously been presented to describe the effect of lateral interaction between adsorbates. Such are the Davies [24] and Frumkin [25] isotherms, which are suitable for systems where adsorbates interact weakly. Here, however, the adsorbed proteins interact weakly at distances larger than $\approx l_\mathrm{D}$, but interact strongly when $R - R_\mathrm{P} \lesssim l_\mathrm{D}$. We therefore use the complete form of $\Delta F(\theta)$ in our equations. We note, that Heimburg et al. [26] have also considered coverage dependent adsorption constants. Their expression for $\kappa(\theta)$ takes into account excluded volume and other, non-electrostatic, interactions between adsorbed proteins, but not the direct electrostatic interactions.

In the following we will demonstrate the importance of the different degrees of freedom, as they apply to one specific example. More explicitly, we shall examine the case in which a protein with $\phi_\mathrm{P} = 0.7$ is adsorbed on a membrane with $\bar{\phi} = 0.2$. Unless otherwise stated, all results pertain to ideally mixed membranes, i.e. $\chi = 0$, corresponding to the more simple 'ideal mixing' case. In this type of system (where a highly charged protein interacts with a weakly charged membrane) effects of lipid charge modulation, and subsequent contribution to ΔF are found to be most pronounced. The case of highly charged basic proteins interacting with weakly charged acidic membranes is also the one of greatest biological relevance.

The case of protein adsorption on non-ideally mixed ($\chi \neq 0$) membranes will be briefly discussed in Section 3.4.

3. Results and discussion

3.1. Single protein adsorption

To demonstrate the role of lipid demixing in protein adsorption we consider three types of adsorbing surfaces. In one case the surface charge density is uniform and constant throughout the membrane, i.e. $\phi(r) = \bar{\phi} = \text{const}$. This corresponds, for example, to a lipid membrane in the 'gel' state, where the lipids are 'frozen'. The other limiting case, corresponding to 'constant electrical potential' resembles the adsorption on metal surfaces where charges move freely. We model this limit by 'turning off' all

lipid mixing terms in Eq. (1). The third and most relevant case, corresponding to a mixed fluid membrane, is that of constant electro-chemical potential, as discussed in the previous section. The adsorption free energies for these three different boundary conditions, for a system characterized by $\bar{\phi} = 0.2$ and $\phi_P = 0.7$, are presented in Fig. 3. The inset shows the lipid composition profiles corresponding to the three types of adsorbing membranes for $h/l_D = 0.3$.

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. The free energy gain increases when lipid mobility is permitted to take place, as charged lipids move towards the interaction zone so as to achieve charge matching, concomitantly releasing the confined counterions into the bulk solution [27,28]. The tendency for charge matching is clearly seen in the inset of Fig. 3. The demixing entropy penalty associated with this process is reflected in the difference (of order 1 $k_B T$) between ΔF for fixed electrical potential as opposed to fixed 'electro-chemical' potential.

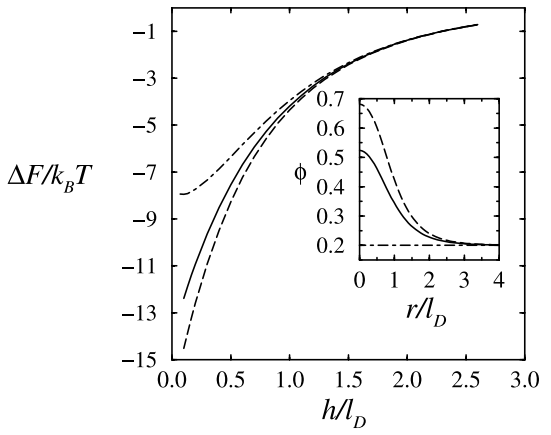

Fig. 3. Adsorption free energies, as a function of the protein–membrane distance, h, for $l_D = 10$ Å, $R_P = l_D$, $a = 65$ Å^2, $\phi = 0.2$, $\phi_P = 0.7$, and $\chi = 0$. The three curves correspond to the cases of constant membrane density boundary condition (dot-dashed line), constant potential density (dashed line), and constant 'electro-chemical' potential (solid line). The inset shows $\phi(r)$, the local composition profile, at $h/l_D = 0.3$ for membranes with constant surface potential (dashed), a mixed fluid membrane (solid), and for an arrested lipid distribution (lower, dot-dashed, curve).

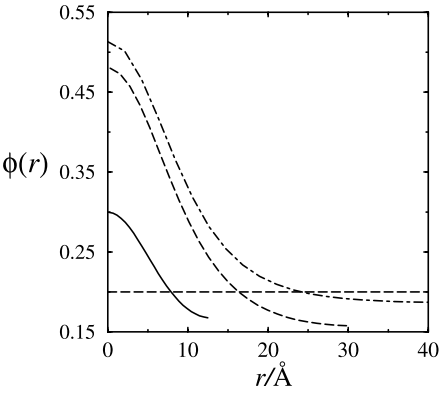

Fig. 4. The local membrane composition, $\phi(r)$, for $\phi_P = 0.7$, $\phi = 0.2$ and $\chi = 0$, for the cases where $R = 60$ Å (dot-dashed line) $R = 31$ Å (dashed line), and $R = 13$ Å (solid line).

3.2. Effect of cell size on charge density

Fig. 4 shows the effect of adsorbate coverage and lateral protein–protein interaction, reflected in the cell size R (hence θ), on the modulation of membrane charge density $\phi(r)$. When the charged proteins begin to crowd on the surface of a relatively weakly charged mixed membrane, they attempt to attract charged lipids to their proximity to achieve optimal charge matching. However, this becomes more difficult once the cell size, and therefore number of charged lipids, available to each protein becomes small. It is then that a smaller charge density modulation is observed.

3.3. Adsorption isotherms

In Fig. 5 we demonstrate the effects of lipid mobility and protein–protein interactions on the adsorption free energy, and the way they are reflected in the adsorption isotherms, as calculated using Eqs. (3) and (4).

The inset shows the binding free energy as a function of the distance between adsorbed proteins, $2R$, for highly charged proteins, $\phi_P = 0.7$, on mixed membranes with a rather low charge density, $\bar{\phi} = 0.2$. Four curves are shown in the inset. One corresponds to the case where the lipids are allowed to demix for an 'ideal' demixing penalty and the adsorbed proteins interact electrostatically with each other. The other three curves

were calculated with either one, or both, of these effects specifically turned off. The adsorption isotherms corresponding to the various cases are also shown.

In general, whether lipid demixing is allowed or arrested, we find that the magnitude of the adsorption free energy is strongly increasing 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 interact. For our choice of l_D this happens at $R \sim 20$ Å. At larger distances inter-protein interactions are negligible. This conclusion is in line with the calculation of Murray et al. [29] for pentalysine adsorption on mixed (frozen) membranes.

As expected, when lateral interactions between adsorbates are taken into account, the adsorption isotherms reach saturation at much smaller protein concentration in the bulk solution, c. The surface saturation coverages are also much smaller. We therefore 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 to be $\sim R_P + l_D$.

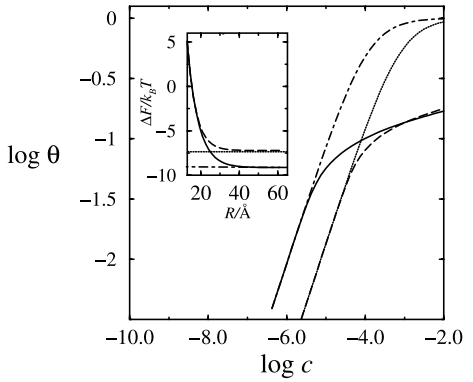

Fig. 5. Adsorption isotherms $\theta(c)$ and adsorption free energies $\Delta F(R)$ (inset) corresponding to the adsorption of highly charged proteins ($\phi_P = 0.7$) on membranes with a smaller charge density, $\bar{\phi} = 0.2$. In addition to the solid curves, which represent the results obtained from the full calculation, including the effects of lipid mobility (mixing) and protein–protein interactions, we also show, for comparison, three other curves. These correspond to free energies and adsorption isotherms calculated for: immobile lipids but with inter-protein electrostatic interactions (dashed curves); mobile lipids but without protein–protein interactions (dash-dotted curves); immobile lipids and no protein–protein interactions (dotted curves). All calculations are for $h_{eq} = 3$ Å and $\chi = 0$.

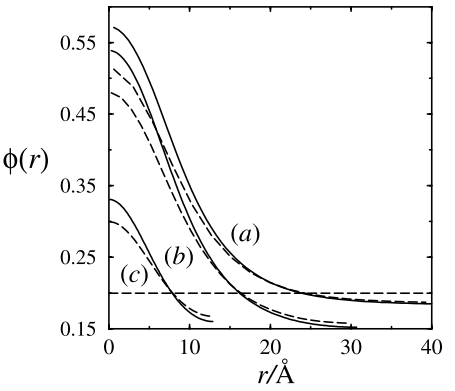

Fig. 6. The local membrane composition, $\phi(r)$, for $\phi_P = 0.7$, $\bar{\phi} = 0.2$ for $\chi = 0$ (dashed) and $\chi = 1\ k_BT$ (solid), for the cases where (a) $R = 60$ Å, (b) $R = 31$ Å, and (c) $R = 13$ Å.

While the effects of inter-protein interactions become increasingly pronounced at shorter average protein–protein distances, the role of lipid mobility is mainly apparent when these are large. As shown in (the inset of) Fig. 5, local demixing of the lipids in the vicinity of the adsorbed proteins can result in significant enhancement of the adsorption free energy. The difference in free energy can be a substantial fraction of the total free energy. The adsorption isotherms corresponding to mobile vs. frozen lipid distributions show even greater differences because their dependence on ΔF is exponential.

3.4. Non-ideal mixing

Let us now examine the effect of a non-ideal contribution to the free energy (i.e. a non-zero χ value) on the migration of lipids in the vicinity of the charged protein. Fig. 6 shows the charge density modulation for $\chi = 0$ and $\chi = 1$, for a protein of $\phi_P = 0.7$ and membrane with $\bar{\phi} = 0.2$, for several cell sizes, R. The effect of the non-ideal mixing is apparent: the higher the value of χ, the stronger the compositional modulations of the lipids. In fact, such a non-ideal mixing term may result in charge density modulations yet stronger than in the case of a similar surface without a

non-ideal mixing contribution but with constant potential boundary condition.

It is well known [30] that a sufficiently large non-ideal mixing parameter will eventually render any system unstable with respect to macroscopic phase separation. The smallest value of χ at which this occurs is called the critical point χ_c. Similarly for a membrane with adsorbed proteins on it, we expect the formation of lateral domains within the membrane for $\chi > \chi_c$. That is, instead of varying the composition only locally, the lipids may rearrange macroscopically to take advantage of the favorable non-ideal mixing contribution. This results in macroscopic membrane domains that do not only differ in their lipid composition but also in the density of adsorbed proteins.

An interesting question is whether a protein-free membrane can be stable while the same membrane with adsorbed proteins on it would phase separate. In other words, can the adsorption of proteins onto membranes induce phase separation?

A simple qualitative illustration of this idea can be provided by the following model. Start with an uncharged, two-component, protein-free lipid bilayer. Within a 'Bragg–Williams', mean-field approximation the free energy per lipid molecule in the membrane is $f = f_{nm}$ with

$$f_{nm} = k_B T[\phi \ln\phi + (1-\phi)\ln(1-\phi)] + \chi\phi(1-\phi), \quad (5)$$

where the first two terms correspond to the ideal 'mixing' of the lipids within the membrane, and the last term represents the non-ideal mixing contribution. As is well known, Eq. (5) gives rise to a critical point $\chi_c = 2$ at $\phi = \phi_c = 0.5$.

Consider now a two-component, protein-free lipid bilayer that has one of its components carrying an electrical charge. We account for the corresponding electrostatic free energy by adding to the molecular free energy $f = f_{nm} + f_{el}$ the term [31]

$$f_{el} = 2k_B T\phi\left[\frac{(1-q)}{p} + \ln(p+q)\right] \quad (6)$$

where $p = p_0\phi$, with $p_0 = 2\pi l_B l_D/a$ and $q = \sqrt{p^2+1}$. Eq. (6) is the charging free energy of a homogeneously charged membrane of composition ϕ within Poisson–Boltzmann theory. Again, above a certain value $\chi > \chi_c$ there appears an instability in the membrane with respect to demixing. The value of the critical point depends on p_0 For $p_0 \ll 1$ it is $\chi_c = 2 + p_0$, increasing to $\chi_c = 2 + \sqrt{3}$ for $p_0 \gg 1$. In Fig. 7 (curve a), the behavior of χ_c is plotted as a function of p_0. The new critical value, χ_c, is always higher than that for an uncharged membrane. The reason for this is the additional repulsion between the head group charges, which renders the mixing process more favorable.

The presence of adsorbed proteins can regain the instability. By neutralizing some (or most) of the lipids in the membrane the additional electrostatic free energy contribution is diminished. The combined protein–membrane system is then expected to display a lower critical demixing parameter χ_c, intermediate between that of a neutral and protein-free, charged membrane. Turning to our illustrative model we adsorb onto the charged membrane proteins of charge z_P and cross-sectional area a_P with coverage θ. This will modify the molecular free energy per lipid $f = f_{nm} + f_{el} + f_{im}$. First, the charging energy f_{el} will be lowered due to the neutralizing effect of the adsorbed proteins. Second, f contains the additional contribution $f_{im} = k_B T(a/a_P)[\theta\ln\theta + (1-\theta)\ln(1-\theta)]$, accounting for the mixing entropy of the protein layer. While in Fig. 6 f_{el} was calculated based on a microscopic model, it is sufficient for

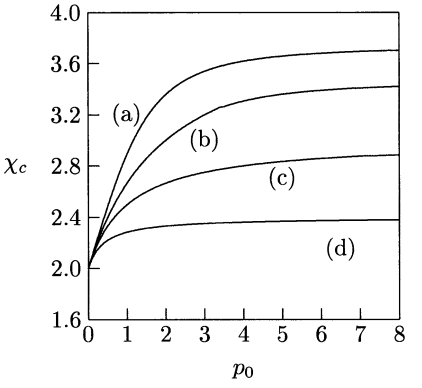

Fig. 7. The critical point χ_c of a mixed membrane with an adsorbed protein layer as a function of $p_0 = 2\pi l_B l_D/a$ for no adsorbed proteins (a), $a_P = a$ and $z_P = 1$ (b), $a_P = 2a$ and $z_P = 2$ (c), and $a_P = 5a$ and $z_P = 5$ (d).

our present purpose to consider a highly simplified description of f_{el}. To this end, we shall assume that each adsorbed protein lowers the charge on the membrane by an amount of z_P, without inducing charge density modulations within the membrane. That is, the membrane is treated to remain homogeneously charged, but with an effective composition $\phi^{eff} = \phi - z_P\theta a/a_P$. Consequently, the charging energy per lipid is now given by Eq. (6) where ϕ is replaced by ϕ^{eff}. Clearly then, for $a_P\phi = az_P\theta$ all charged lipids would be neutralized by corresponding protein charges, rendering the membrane uncharged (implying $f_{el} = 0$ and thus $\chi_c = 2$). This however is not what the model predicts. Rather, the demixing contribution of the adsorbed protein layer (f_{im}) provides an additional contribution that opposes macroscopic phase separation which gives rise to an increase of the critical point with respect to that of a neutral membrane. In fact, the critical point for a membrane with an adsorbed protein layer is given by the minimum of the expression

$$\chi_c = \frac{1}{2\bar{\phi}(1-\bar{\phi})} + \frac{p_0}{q + 2p_0\bar{\theta}(1-\bar{\theta})z_P^2 a/a_P} \quad (7)$$

with respect to $\bar{\phi}$ and $\bar{\theta}$ where q is given by the expression

$$q = \sqrt{1 + p_0^2(\bar{\phi} - z_P\bar{\theta}a/a_P)^2} \quad (8)$$

In the derivation of Eq. (7) all relevant degrees of freedom must be included. That is, the membrane is able to adjust not only its composition but also the amount of adsorbed proteins in each of its sub-phases. The only two conserved quantities are the average composition, $\bar{\phi}$, of the membrane and the bulk concentration of the proteins in solution which determines the average coverage, $\bar{\theta}$, of the protein layer. Fig. 7 displays χ_c according to Eq. (7) as a function of $p_0 = 2\pi l_B l_D/a$ for several choices of z_P, a_P, and a.

We see that, indeed, χ_c is intermediate between that of a protein-free and fully neutralized membrane. Fig. 7 also suggests that large and highly charged proteins are more efficient in reducing the critical point. At this point we emphasize again the approximative nature of the model. It neither takes into account any structural details of the adsorbed proteins nor does it allow for modulations of the membrane compositions within each phase. Moreover, it neglects the electrostatic repulsion between adsorbed proteins. Thus, it cannot replace detailed numerical calculations. However, it points at a mechanism by which lipid membranes may mediate the accumulation of proteins into domains.

3.5. Other theoretical models

Several other, previous, theoretical studies have been proposed to model the interaction of charged proteins with mixed membranes. Models in which the lipid composition is assumed to be uniform were proposed by Ben-Tal et al. [32,33,29], based on solutions of the non-linear PB equation. These authors calculated peptide binding constants as a function of salt concentration, finding good agreement with experiment.

Employing linear PB theory, Roth et al. [34] demonstrated the importance of the entropy of released mobile counterions upon adsorption, as the underlying force for electrostatic attraction between the proteins and membranes.

The important role of lipid mobility was demonstrated in the works of Denisov at al. [14] and Heimburg et al. [26,35]. Both models consider macroscopic, non-local demixing of lipids in the vicinity of adsorbed domains. The work of Denisov et al. also demonstrates that this compositional degree of freedom may induce a lateral phase separation in the lipid membrane. However, if lipid demixing can take place *locally*, i.e., in the vicinity of singly adsorbed peptides there is no electrostatically based advantage for adsorbate aggregation. Rather, adsorbed proteins could gain a further translational-entropy contribution from spreading uniformly on the membrane surface. This conclusion is consistent with the general result that, at least within (mean-field) PB theory, the interaction between like-charged colloidal particles is always repulsive, whether in the bulk or in the vicinity of a confining wall [36–38]. This, in turn, suggests that protein domain formation is most likely driven by a non-electrostatic mechanism, e.g. non-ideal mixing of lipid tails or a lipid-mediated protein attraction resulting from elastic membrane deformations (and hence line

tension) around the protein–membrane interaction zone [39,40].

The importance of the direct interaction between peptides has been studied by Murray et al. [29], by calculating the adsorption energy of a peptide onto a vacant membrane adsorption 'cavity', 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. Here, no local demixing is accounted for within the model.

4. Conclusions

We have demonstrated the important role of lipid mobility and lateral 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. Inter-protein 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 apposed 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. The addition of a non-ideal lipid mixing contribution to the free energy leads to a stronger membrane charge density modulation. This may be at the base of the experimentally observed formation of high density protein domains in lipid membranes.

Acknowledgements

The financial support of the Israel Science Foundation (Excellence Center grant No. 8013/00) and the US–Israel Binational Science Foundation (grant No. 94/130) is gratefully acknowledged. SM thanks the DFG for the support through SFB 197. DH thanks the Clore Foundation for a doctoral Fellowship. The Fritz Haber research center is supported by the Minerva foundation, Munich, Germany. A considerable part of this work is based on a recent paper by May, Harries and Ben-Shaul, Biophysical J. 79 (2000) 1747.

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