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2005 J. Phys.: Condens. Matter 17 R833

(http://iopscience.iop.org/0953-8984/17/32/R01)

INSTITUTE OF PHYSICS PUBLISHING JOURNAL OF PHYSICS: CONDENSED MATTER

J. Phys.: Condens. Matter **17** (2005) R833–R850 doi:10.1088/0953-8984/17/32/R01

TOPICAL REVIEW

Stability of macroion-decorated lipid membranes

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Received 6 June 2005
Published 29 July 2005
Online at stacks.iop.org/JPhysCM/17/R833

Abstract

Adsorption of macroions such as colloidal particles, proteins, or other rigid biopolymers onto oppositely charged, mixed lipid membranes is a ubiquitous phenomenon encountered in biotechnology, drug delivery, and cellular biology. The softness and self-assembled nature of the membrane enable the macroion–membrane complex to laterally reorganize via forming macroion clusters, lipid domains, or separate phases, and to exhibit curvature modulations or even morphological transitions. Almost always, the lateral organization of the membrane and associated macroion layer mutually depend on each other so that neither of the two extreme views—macroion-induced membrane domain formation or membrane-mediated macroion clustering—strictly accounts for the underlying energetics. We review and discuss some recent efforts to describe the lateral organization and stability of macroion-decorated lipid membranes using different levels of mean-field electrostatics, thereby focusing on binary membranes and the destabilizing role of compositional gradients.

Contents

1. Introduction

All living cells are surrounded by a lipid membrane that serves as a permeability barrier between the cytoplasm and the extracellular space. The membrane consists of a multitude

of different lipid species and membrane-associated proteins. Most lipids share the same structural pattern: two hydrocarbon chains are linked to a polar, sometimes charged, headgroup. This architecture renders lipids amphiphilic. That is, in aqueous solution they tend to spontaneously [1] assemble into an aggregation geometry that allows the headgroups to shield the hydrophobic tails from the unfavourable contact with water. Formation of the planar bilayer geometry reflects the preferred packing properties of the most abundant double-chained lipids such as the zwitterionic phosphatidylcholine (PC) or the monovalently charged phosphatidylserine (PS). Yet, biomembranes also contain a fraction of non-bilayer-forming lipids such as phosphatidylethanolamine (PE), the 3–4-valent lipid phosphatidylinositol 4,5-bisphosphate (PIP_2), or diacylglycerol (DAG). These lipids tend to perturb the lamellar bilayer structure and are often involved in fusion, signalling, and trafficking processes [2, 3].

The lateral organization of lipid membranes is currently an area of active research. The renewed interest originates in the discovery of so-called *lipid rafts* [4] in biological membranes that are associated with a multitude of specific biological functions. Lipid rafts are believed to be dynamically changing small domains or inhomogeneities, enriched in certain lipids and cholesterol, that spatially organize the membrane and provide a platform for the functioning of raft-associated proteins. Lateral domains are also found in model membranes, particularly for certain biomembrane-mimicking lipid compositions. In fact, our current understanding about the energetics of lateral domains in fluid-like membranes derives almost exclusively from studies on model systems that contain few lipid species at well defined compositions [5].

Most research on domain formation in model membranes has been and still is being performed in the absence of membrane-associated proteins. This is justified in view of the complexity of even the bare membrane. However, biomembranes contain proteins, and these do affect the formation of membrane domains. The way proteins and lipids mutually influence their lateral organization is thus likely to increasingly attract research efforts [6]. To illustrate the role of proteins for domain formation in biomembranes, we mention the unresolved problem of how domains are coupled across a biomembrane [7, 8]: the composition in the outer leaflet of the plasma membrane seems to favour domain formation much more strongly than in the inner leaflet. Yet, membrane rafts exist on the inner and outer faces of the plasma membrane.

Domains are an issue not only in biomembranes but also in lipid-based drug delivery systems. Cationic lipid–DNA complexes—also called *lipoplexes*—are among the most promising non-viral gene delivery vectors. The structural properties of lipoplexes are among the key determinants of transfection efficiency [9]. Here too, the cationic membrane is usually composed of several lipid species, typically a cationic one and a helper lipid. As for the mixed membrane–protein system, the cationic membrane is able to adjust its composition upon interaction with DNA, opening the possibility of lipid domain formation or phase separation. Lipoplexes are composite materials that extend into three dimensions with adjacent lipid layers sufficiently close to interact with each other. Hence, in addition to DNA–membrane interactions, membrane–membrane interactions contribute to the energetics of lipoplexes.

A characteristic feature of lipid membranes is the possibility of curvature modulations. Macroions are able to induce bending deformations in charged membranes [10], giving rise to various phenomena such as membrane-mediated elastic interactions between macroions or wrapping of the membrane around the macroion. Cellular membranes bear potential applications such as the internalization of drug delivery systems [11] or membrane traffic [10].

This review reports on recent efforts to understand the physical principles underlying macroion-induced domain formation in lipid membranes. We shall focus on modelling studies that involve electrostatically charged membranes onto which oppositely charged macroions such as colloids, proteins, or DNA are adsorbed. Frequently, these systems are described on the basis of Poisson–Boltzmann (PB) theory; we shall therefore, in section 2, start with

a short account of this mean-field method. Charged lipid membranes often contain at least one uncharged component such as a zwitterionic lipid species or a sterol. These membranes thus represent mixed systems with specific lateral mixing properties that are affected by both electrostatic and non-electrostatic interactions. In section 3 we discuss the stability of bare membranes where macroions are absent. The next part, section 4, is devoted to the adsorption of a macroion onto a binary flat membrane. The macroion is able to laterally polarize the membrane, inducing lipid migration and thus formation of macroion-coupled microdomains. Effective attraction between lipids of the same species can render the domain formation a macroscopic phase separation. Underlying physical mechanisms are outlined in section 5, followed by a brief account of cationic lipid–DNA complexes; see section 6. Finally, section 7 discusses the ability of macroions to induce membrane curvature modulations.

2. Poisson–Boltzmann theory

Most macroions encountered in cellular systems share a number of common properties: (i) the charged residues are typically distributed on the macroion surface A; (ii) the interior space of the macroion is uncharged and has low dielectric constant $\epsilon_{\mathrm{L}} \approx 2$–$4$; (iii) the macroion is embedded in aqueous solution of large dielectric constant $\epsilon_{\mathrm{W}} \approx 80$; (iv) under physiological conditions the aqueous solution contains salt ions which screen electrostatic interactions. The corresponding screening length, referred to as the Debye length, is $l_{\mathrm{D}} \approx 10$ Å. Indeed, this structural pattern is the same for three basic biomolecular structures: membranes, proteins, and DNA. The difference is the dimensionality with membranes extending into two spatial dimensions, DNA forming a linear polyelectrolyte, and proteins typically adopting a globular structure. Another difference is the degree of mobility of the surface charges. The phosphate groups of DNA are firmly attached to the DNA backbone whereas the charged residues of proteins can be somewhat adjustable depending on the conformational flexibility of the protein. Fluid-like membranes, on the other hand, are able to laterally reorganize and thus to adjust their local charge density through compositional changes.

A typical situation in which a basic protein is adsorbed onto an acidic membrane is displayed in figure 1. The starting point of PB theory is a mean-field expression for the electrostatic free energy, $F_{\mathrm{EL}}(\Phi, n_+, n_-)$, in terms of the initially unknown electrostatic potential $\Phi = \Phi(\mathbf{r})$, and local concentrations, $n_+ = n_+(\mathbf{r})$ and $n_- = n_-(\mathbf{r})$, of positively and negatively charged salt ions, respectively, at positions $\mathbf{r}$ within the aqueous environment V. For symmetric 1:1 salt, this expression can be written as

$$F_{\mathrm{EL}} = \frac{\epsilon_{\mathrm{W}}\epsilon_0}{2}\int_V \mathrm{d}v\,(\nabla\Phi)^2 + kT\int_V \mathrm{d}v\left[n_+\ln\frac{n_+}{n_0} + n_-\ln\frac{n_-}{n_0} - (n_+ + n_- - 2n_0)\right]. \tag{1}$$

The first term describes the energy stored in the electrostatic field $-\nabla\Phi$ (ϵ_0 is the permittivity of vacuum), and the second term contains the (mean-field) translational entropy of the mobile salt ions where $n_0 = n_\pm(\mathbf{r} \to \infty)$ is the salt concentration in the bulk and kT is the thermal energy. Both integrations run over the aqueous environment and need not include the interior regions of the macroions, which is a consequence of $\epsilon_{\mathrm{L}} \ll \epsilon_{\mathrm{W}}$. First variation of the free energy, thereby taking into account the Poisson equation, $\epsilon_{\mathrm{W}}\epsilon_0\Delta\Phi = -e(n_+ - n_-)$, where e denotes the elementary charge and Δ the Laplacian, leads to

$$\delta F_{\mathrm{EL}} = \int_A \mathrm{d}a\,\Phi\delta\sigma + kT\int_V \mathrm{d}v\left\{\delta n_+\left[\ln\frac{n_+}{n_0} + \frac{e\Phi}{kT}\right] + \delta n_-\left[\ln\frac{n_-}{n_0} - \frac{e\Phi}{kT}\right]\right\}. \tag{2}$$

The first term is the variation of the electrostatic energy at the macroion surfaces. It vanishes for fixed surface charge density, σ, or contributes to the variation of additional energy

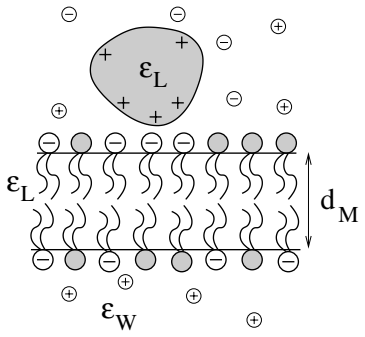

Figure 1. Schematic representation of a basic protein adsorbed onto an acidic lipid bilayer. Both involved macroions, the membrane and the protein, have low interior dielectric constant ϵ_{L}, as opposed to the large dielectric constant ϵ_{W} of the surrounding aqueous solution. To a good approximation, all macroion charges are surface charges. However, while the protein charges are fixed, the charged lipids of a binary membrane (which is of thickness d_{M}) are mobile and can migrate towards the protein adsorption site. Some mobile (salt) ions are depicted.

contributions associated with mobile surface charges; see equations (7) and (12) below. Vanishing of the remaining two contributions in δF_{EL} gives rise to the familiar Boltzmann distributions, $n_{\pm} = n_0 \exp(\mp\Psi)$, which are written in terms of the reduced electrostatic potential $\Psi = e\Phi/kT$. Upon insertion of the Boltzmann distributions into the Poisson equation we obtain the PB equation

$$l_{\mathrm{D}}^2 \Delta\Psi = \sinh\Psi. \tag{3}$$

From equation (2) we also conclude that for fixed surface charge density σ the free energy F_{EL} can be calculated through the charging process, $F_{\mathrm{EL}} = \int_A \mathrm{d}a \int_0^\sigma \mathrm{d}\bar{\sigma}\, \Phi(\bar{\sigma})$, carried out at thermal equilibrium. A large body of work on macroion interaction is based on the use of PB theory, and excellent reviews are available [12–15]. Generally, PB theory predicts at least qualitatively correctly co-and counterion distributions in monovalent salt solution, even for some of the largest surface charge densities encountered in cellular systems (such as DNA [16]). Yet, PB theory is doomed to fail if ions of high valence are involved because correlation effects (which are neglected in mean-field approaches) are no longer negligible [17]. In fact, a number of recent theoretical and computational studies are devoted to understanding electrostatic correlation effects in systems of *uniform* dielectric constant [18, 19]. Accounting for spatially changing dielectric constant, while being simple within PB theory, is still a challenging problem beyond the mean-field level.

3. Stability of bare membranes

For an isolated and planar membrane, the PB equation can be solved and F_{EL} can be calculated. More specifically, if a single lipid layer consists of N lipids, of which ϕN are monovalently charged and $(1-\phi)N$ are uncharged, the composition-dependent free energy is given as [20, 21]

$$F_{\mathrm{EL}} = 2\phi kTN\left[\frac{1-q}{p} + \ln(p+q)\right] \tag{4}$$

where $q^2 = p^2 + 1$ and $p = \phi p_0$. The constant $p_0 = 2\pi l_{\mathrm{B}} l_{\mathrm{D}}/a$ reflects the Bjerrum length $l_{\mathrm{B}} = e^2/4\pi kT\epsilon_{\mathrm{W}}\epsilon_0 \approx 7$ Å, the Debye length $l_{\mathrm{D}} = (8\pi n_0 l_{\mathrm{B}})^{-1/2} \approx 10$ Å (at physiological 0.1 M salt solution), and the cross-sectional area per lipid, $a \approx 65$ Å^2 (assumed to be the

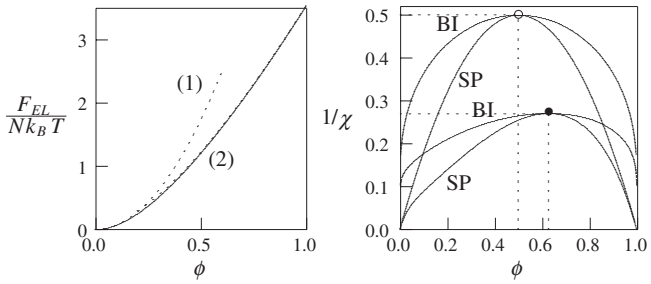

Figure 2. Left: plot of the PB free energy, $F_{\rm EL}(\phi)$, for a bare membrane according to equation (4) (solid line). It is $a = 65$ Å^2 and $l_{\rm D} = 10$ Å, implying $p_0 = 6.9$. The two broken lines, marked (1) and (2), denote the linear Debye–Hückel regime (where $F_{\rm EL}/NkT = p_0\phi^2$) and the high charge limit (where $F_{\rm EL}/NkT = 2\phi[\ln(2p_0\phi) - 1] + 2/p_0$), respectively. Right: the spinodal ('SP') and binodal ('BI') for a charged (bottom) and for an uncharged (top) lipid layer. The uncharged lipid layer corresponds to the limit of vanishingly small Debye length; $l_{\rm D} \to 0$. Upon charging the lipid layer the critical point upshifts from $\chi_{\rm C} = 2$, $\phi_{\rm C} = 0.5$ (○) to $\chi_{\rm C} = 3.7$, $\phi_{\rm C} = 0.63$ (•).

same for both lipid species). The consequence of $p_0 \gg 1$ is that only for small compositions, $\phi \lesssim 0.05$, the lipid layer resides in the Debye–Hückel regime for which the PB equation linearizes; see the left diagram of figure 2.

What implications have electrostatic interactions for the compositional stability of an undressed (bare) membrane? Since $F_{\rm EL}(\phi)$ is a convex function (see figure 2), electrostatic interactions are expected to stabilize mixed membranes. An illustrative case is to add to $F_{\rm EL}$ a non-electrostatic, membrane-destabilizing contribution. Effective short-range attraction between lipids of the same species, treated on the mean-field level of the lattice gas model, gives rise to the familiar Bragg–Williams free energy [22]

$$F_{\rm BW} = kTN\,[\phi\ln\phi + (1-\phi)\ln(1-\phi) + \chi\phi(1-\phi)] \tag{5}$$

where $\chi > 0$ is the non-ideality parameter that characterizes the attraction strength. (Note that using cooperative models [23] or more than one order parameter [24] may be more appropriate to describe the energetics of mixed lipid membranes, particularly in presence of cholesterol.) Sufficiently large $\chi > \chi_{\rm BI}$ gives rise to *global* instability of the membrane with respect to lateral phase separation; $\chi_{\rm BI} = \chi_{\rm BI}(\phi)$ is the *binodal* line. *Local* instability requires even larger $\chi > \chi_{\rm SP} \geqslant \chi_{\rm BI}$; the line $\chi_{\rm SP} = \chi_{\rm SP}(\phi)$ is the *spinodal*. The joint (local) minimum of $\chi_{\rm BI}$ and $\chi_{\rm SP}$ is the critical point $\chi_{\rm C}$. Critical points, spinodals, and binodals for $F_{\rm BW}$ and $F_{\rm BW} + F_{\rm EL}$ are shown in the right diagram of figure 2. It can be seen that, indeed, electrostatic interactions stabilize the lipid layer: upon switching on electrostatic interactions, the spinodal line $\chi_{\rm SP} = 1/[2\phi(1-\phi)]$ acquires the additional *positive* contribution p_0/q, which for $p_0 \gg 1$ becomes $1/\phi$. In this high charge limit the spinodal adopts its minimum at $\chi_{\rm C} = 2+\sqrt{3} = 3.7$ and corresponding $\phi_{\rm C} = (3-\sqrt{3})/2 = 0.63$. We thus infer the upshift [25, 26] in the critical point $\chi_{\rm C} = 2 \to 3.7$ (and corresponding critical composition $\phi_{\rm C} = 0.5 \to 0.63$); see the right diagram of figure 2.

The model based on equations (4) and (5) takes only one single, isolated lipid layer into account. Yet, a lipid bilayer consists of two apposed monolayers whose demixing properties possibly influence each other through either structural or electrostatic trans-monolayer coupling. The structural coupling was recently analysed on the basis of a phenomenological model by Hansen *et al* [27]. Indeed, experiments have demonstrated that the phase-separated domains of a mixed lipid vesicle are spatially in register across the membrane [28]. The microscopic origin of this coupling is currently unknown; yet, it was speculated that the

penetration of the lipid chains from one into the apposed monolayer could give rise to the registry [29]. The second mechanism, electrostatic coupling, can be analysed on the basis of PB theory [30, 31]. The degree of the coupling is conveniently expressed by the parameter [32] $H = \epsilon_L l_D/\epsilon_W d_M$ where d_M is the membrane thickness; see figure 1. The spinodal of a binary, charged, symmetric bilayer is then [31]

$$\chi_{SP} = \frac{1}{2\phi(1-\phi)} + \frac{p_0}{\sqrt{1 + p_0^2\phi^2} + 2H}. \tag{6}$$

For large coupling, where $H \to \infty$ while p_0 remains finite, electrostatics does not affect the membrane stability. More relevant for lipid membranes, however, is $H \ll 1$, and the two membrane leaflets are electrostatically decoupled. Interestingly, even in the low salt limit (where $l_D \to \infty$), the electrostatic trans-monolayer coupling can safely be neglected.

Experimental results support the notion of electrostatic interactions stabilizing binary membranes [33–35]. Particularly notable is a recent calorimetric study [36] on the miscibility of PC with phosphatidylglycerol (PG), which acquires a single negative charge through deprotonation upon increasing the pH from 2 to 7. The non-ideality parameters at each of the two pH values were estimated from fitting the calorimetric data to a thermodynamic model, yielding $\chi_{eff} = 1.3$ for a pH of 2 and $\chi_{eff} = 0$ for a pH of 7. (Unlike χ, the experimentally determined non-ideality parameter χ_{eff} contains both electrostatic and non-electrostatic contributions.) Thus, after charging the lipid mixture, the demixing tendency was entirely suppressed, reflecting the compensation of attractive non-electrostatic interactions with electrostatic repulsion. Note that the charge-induced change in the non-ideality parameter, $\Delta\chi_{eff} = 1.3$, compares quite well with the prediction $\Delta\chi = 1.7$ from PB theory.

While electrostatic interactions tend to stabilize binary membranes with respect to lateral phase separation, they can also destabilize the bare membrane via a different route, namely through the formation of pores. There are at least two mechanisms of how this can happen. The first is that at the rim of a membrane pore the counterion cloud gains translational entropy as compared to the planar membrane, thus lowering the electrostatic free energy with growing pore size. This mechanism becomes effective for highly charged membranes, at large Debye length, and above a critical pore size, as was recently discussed by Betterton and Brenner [37]. The second mechanism is that headgroup charges tune the preferred packing geometry of the lipids toward micellar structures, thus lowering the line tension of a pore and ultimately rendering it negative [38]. The experimental relevance of these considerations is currently not clear as studies exist that argue in favour or disfavour of charged lipid-induced facilitation of membrane rupture [39, 40].

4. Adsorption of macroions onto mixed membranes

The electrostatic adsorption of macroions onto flat surfaces is a phenomenon of fundamental importance in colloid science, biotechnology, and cellular biology [41]. It is thus no surprise that considerable effort has been put into calculating the electrostatic contribution to the interaction between colloidal particles and a flat surface on the basis of non-linear PB theory [42–45]. Note that most studies considered only one single spherical macroion adsorbed onto a flat surface; cylindrical symmetry then renders the PB equation two dimensional. Recent computations of ensembles of colloidal particles have provided new insights about the presence and importance of multi-body interaction terms [46–48]. Still, non-linear PB theory is far from becoming routinely implemented into computer simulations. Instead, the two-body Yukawa interaction potential $U(r)/kT = l_B \exp(-r/l_D)/r$, which is valid within the Debye–Hückel

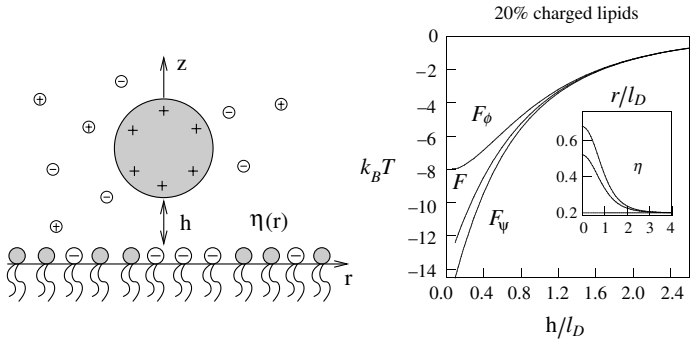

Figure 3. Left: a uniformly charged sphere adsorbs onto a flat two-component lipid layer. The individual membrane lipids are mobile, implying the charged lipid species can optimize its radial composition $\eta(r)$ for any given macroion-to-membrane distance h. Right: predictions from PB theory for the adsorption of a single sphere (of radius $R = 10$ Å with uniform charge density corresponding to seven positive charges) onto a mixed membrane that contains 20% negatively charged lipids (reproduced from May *et al* [52], with permission). The three adsorption free energies (in units of $k_B T$) correspond to fixed surface charge density (F_ϕ), mobile lipids (F), and constant membrane surface potential (F_Ψ). The inset shows the local membrane composition, η, for the three cases at $h/l_D = 0.3$. The Debye length is $l_D = 10$ Å.

limit (that is, within linearized PB theory), is commonly used to represent screened electrostatic interactions [49–51].

There are two special issues concerning the electrostatic adsorption of macroions onto lipid membranes: lipid membranes are deformable and fluid-like. That is, the membrane can respond to the adsorption by adopting curvature deformations or—in case of mixed membranes—by lateral reorganization. As the former will be discussed in section 7, we shall first focus on the limit of high bending stiffness, for which the membrane remains flat. Lateral fluidity of a mixed membrane implies the possibility of macroion-induced lipid sequestering. That is, lipids that preferentially interact with the macroion (electrostatically and possibly also non-electrostatically) can migrate towards the adsorption site, thus creating compositional, macroion-coupled inhomogeneities within the membrane plane. This mechanism is schematically illustrated in the left diagram of figure 3 for a two-component membrane, consisting of a charged and a zwitterionic (uncharged) lipid species. The local composition of the membrane η may differ from the average composition ϕ which entails an in-plane demixing penalty of the free energy

$$F_{\rm D} = \frac{kT}{a}\int_A {\rm d}a\left[\eta\ln\frac{\eta}{\phi} + (1-\eta)\ln\frac{1-\eta}{1-\phi} + \lambda(\eta-\phi)\right] \tag{7}$$

where the integration runs over the membrane plane and where the Lagrange multiplier λ ensures conservation of the number of charged lipids. Adding $F_{\rm D}$ to $F_{\rm EL}$ gives rise to the local equilibrium composition

$$\eta = \frac{1}{1+\frac{1-\phi}{\phi}\exp(\lambda-\Psi)} = \frac{l_{\rm D}}{2p_0}\left(\frac{\partial\Psi}{\partial z}\right)_{z=0} \tag{8}$$

which enters as the boundary condition at the membrane ($z = 0$) and must be solved self-consistently together with the non-linear PB equation [53, 52]. Calculations of the adsorption free energy reveal a notable influence of the lipid demixing on the adsorption isotherm [52], particularly for highly charged macroions and small ϕ. The right diagram of figure 3 shows the adsorption free energy, $F = F_{\rm EL} + F_{\rm D}$ (normalized so that $F(h \to \infty) = 0$), of the

macroion, and the inset indicates the corresponding degree of demixing. Note that two other, thermodynamically limiting cases are also displayed in figure 3. One is the hypothetical limit of constant surface potential (F_Ψ), where demixing does not invoke the penalty in equation (7), and the other is the limit of fixed surface charge density (F_ϕ) for which $\eta \equiv \phi$. The differences in the corresponding free energies of the adsorbed macroions are substantial. Thus, electrostatically driven lipid migration can lead to a large gain in free energy, even though it is opposed by the demixing free energy.

The relevance of these considerations is clearly reflected in recent work on the basic effector domain of myristoylated alanine-rich C kinase substrate (MARCKS) which is suggested to sequester the multivalent lipid PIP_2 on the inner leaflet of the plasma membrane [54, 55]. The sequestration of multivalently charged membrane lipids was also supported by two recent PB-level calculations. One work presents a simple formalism to estimate the ability of a macroion to induce sequestration of charged lipids of different valence [56]. Based on a two-state model, an equation is derived that relates the mole fractions of the lipids inside and outside the protein adsorption zone to their valence, providing a handy tool to estimate the degree of lipid demixing. The other work employs a finite difference method to solve the non-linear PB equation in three dimensions for atomistic-level representation of the involved macroions, including the membrane [57]. The detailed nature of this method, which does capture the discreteness of the involved charges rather than relying on the smeared charge representation, has previously led to a number of quantitative predictions concerning the energetics of protein adsorption onto membranes of predefined and homogeneous membrane composition [58, 59]. The major challenge concerning the in-plane lipid demixing is to faithfully sample the lateral lipid distribution in thermal equilibrium.

It should be noted that the degree of lipid demixing induced by membrane-bound macroions can be influenced by kinetic or energetic contributions that are not accounted for by PB theory. In fact, there are experimental indications that small peptides such as MARCKS or Lys13 leave the local composition in monovalently charged lipid membranes essentially unaffected (private communication with Stuart McLaughlin). A possible explanation contrasts the long time to form a cluster of charged lipids (onto which the peptide could tightly adsorb) to the fast diffusion of the peptide, trapping the peptide kinetically to interact with a uniformly charged membrane.

We note that with increasing concentration of macroions in solution the macroion density on the membrane will grow and eventually approach saturation. In this case, the demixing properties of the membrane substrate tend to be less important as the membrane is uniformly covered with macroions. Yet, other issues such as direct interactions between macroions (van der Waals, electrostatic, or dehydration forces) as well as the distributional entropy of the macroion array become crucial factors for the macroion adsorption behaviour [60]. This notion is also supported by recent work on membrane adsorption of cytochrome c where, besides a simple electrostatic membrane-protein neutralization model, scaled particle theory and the van der Waals gas model were used to reproduce experimental binding isotherms [61]. The model was also used to predict the degree of lipid demixing upon adsorption of the protein [62].

5. Stability of macroion-decorated planar membranes

Electrostatic adsorption of macroions on a two-component membrane with ideal mixing properties can lead to compositional inhomogeneities at the macroion adsorption sites, but does not lead to macroscopic phase instability of the membrane, at least not if the membrane remains perfectly flat and if there is no direct macroion–macroion attraction. Yet, lateral phase separation becomes possible if attractive interactions between lipids of the same species are

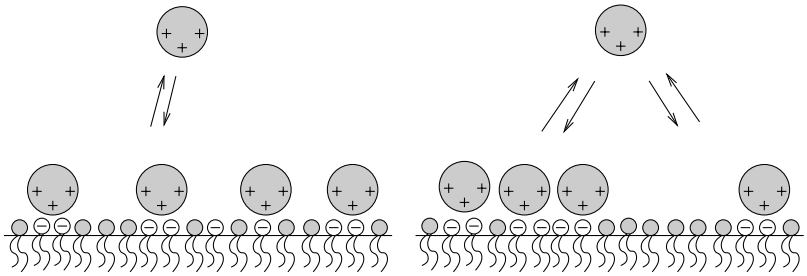

Figure 4. Left: Membrane-adsorbed macroions induce local lipid demixing but remain uniformly distributed on a macroscopic scale. Right: sufficiently strong effective attraction between lipids of the same species leads—in addition to local demixing—also to macroscopic phase separation of the macroion-decorated membrane. The corresponding driving force is the line tension associated with macroion-induced compositional gradients within the membrane.

present. The presence of usually small but notable lipid–lipid attraction is well documented for many lipid mixtures [34]. In biomembranes, the possibility of protein-induced membrane domain formation or, reversing the emphasis, of membrane-mediated protein clustering is a functionally relevant issue which currently attracts significant research efforts. Indeed, raft formation in biomembranes is likely to reflect the energetics of the composed lipid–protein mixture rather than lipid properties alone [6]. From a physical point of view it is thus interesting to ask under which conditions macroions are able to enhance domain formation or to even induce macroscopic phase separation. The latter process is schematically illustrated in figure 4 where like-charged (and thus repelling) macroions are driven toward phase separation through the underlying non-ideality of the lipid mixture, characterized by $\chi > 0$; see equation (5).

The presence of adsorbed macroions such as proteins or colloidal particles on a flat binary lipid membrane provides the system with an additional degree of freedom. That is, apart from the lipid composition, ϕ, the membrane is also able to adjust the number of adsorbed macroions, conveniently expressed as the area fraction, θ, of the membrane covered by macroions (for $\theta = 0$ there are no macroions adsorbed whereas for $\theta = 1$ the membrane coverage is maximal). To investigate the influence of electrostatic interactions on the stability of the macroion-decorated membrane, it is appropriate to decompose the overall free energy

$$F(\phi, \theta) = F_{\mathrm{BW}}(\phi) + F_P(\theta) + F_{\mathrm{EL}}(\phi, \theta) \tag{9}$$

into additive contributions of which only the electrostatic free energy F_{EL} depends on both ϕ and θ. In the simplest case, the contribution F_{P} describes the ideal mixing free energy of the membrane-adsorbed macroions; $F_{\mathrm{P}} = kTM[\theta \ln\theta + (1-\theta)\ln(1-\theta)]$ where M denotes the number of adsorbed macroions. Local stability requires the matrix

$$\begin{pmatrix} \partial^2 F/\partial\phi^2 & \partial^2 F/\partial\phi\partial\theta \\ \partial^2 F/\partial\phi\partial\theta & \partial^2 F/\partial\theta^2 \end{pmatrix} \tag{10}$$

to be positive definite. Vanishing of its determinant marks the spinodal line. Because the coupling of the two membrane degrees of freedom, ϕ and θ, enters only through F_{EL}, any prediction about membrane stability depends crucially on the choice of a reasonable electrostatic model for the macroion–membrane complex. To illustrate this we discuss three recently suggested electrostatic models and their consequences for the spinodal and corresponding critical point.

In the first model [63] (for related electrostatic models see [62, 64]) the macroions merely contribute to the electrostatic shielding of the membrane charges; they are treated as structureless ions of valence z_{P}, and they do not affect the homogeneous lateral distribution

of the lipids at or near the macroion adsorption sites. This behaviour can be expressed by writing the electrostatic free energy as $F(\phi, \theta) = F(\phi^{\rm eff}, 0)$ where $\phi^{\rm eff} = \phi - z_{\rm P}\theta a/a_{\rm P}$ is an effective membrane composition, determined by the difference of the bare membrane charges and the (opposite) charge contributed by the absorbed macroions ($a/a_{\rm P}$ is the ratio of the lipid's and macroion's cross-sectional areas). Within this model the spinodal is given as

$$\chi_{\rm SP} = \frac{1}{2\phi(1-\phi)} + \frac{p_0}{q + 2p_0\theta(1-\theta)z_{\rm P}^2 a/a_{\rm P}} \tag{11}$$

with $q^2 = 1 + p_0^2(\phi - z_{\rm P}\theta a/a_{\rm P})$. Not surprisingly, equation (11) places the critical point always between that of a fully charged ($\chi_{\rm C} = 3.7$) and completely neutralized ($\chi_{\rm C} = 2$) membrane. The conclusion within this model is that macroions—particularly highly charged ones—are able to induce phase separation, but only if the membrane substrate already has a large intrinsic tendency to demix. In fact, this tendency needs to be so large that the bare membrane only remains stable due to electrostatic stabilization.

The second model—a two-state model—predicts a qualitatively different scenario [26]. The model assumes that the charged lipids can reside either in the unperturbed, bare membrane or within the macroion adsorption region where they participate in the electrostatic macroion–membrane interaction. The compositions, $\phi_{\rm P}$ and $\phi_{\rm L}$, within the adsorption region and bare membrane, respectively, are in general different but related through $\theta\phi_{\rm P} + (1-\theta)\phi_{\rm L} = \phi$. In thermal equilibrium, the compositions are allowed to adjust so as to minimize the overall free energy. The model becomes tractable by assuming that the macroion–membrane interaction can be modelled through two planar charged surfaces of given distance, embedded in electrolyte solution. Of crucial importance is the appearance of a *line tension*, Λ, along the circumference, $\sim\sqrt{a_{\rm P}}$, of the macroion adsorption region. The line tension, $\Lambda = kT\chi(\Delta\phi)^2/\sqrt{a_{\rm P}}$, arises from non-electrostatic lipid–lipid interactions (expressed by $\chi > 0$; see equation (5)) in the presence of a compositional gradient $\Delta\phi = \phi_{\rm P} - \phi_{\rm L}$ in the membrane plane. It is the line tension (but not electrostatic interactions) that provide the driving force for lateral phase separation. We note that *imposing* compositional homogeneity, $\phi = \phi_{\rm P} = \phi_{\rm L}$, eliminates the line tension. In this hypothetical case, macroscopic phase separation can still take place, in fact even for $\chi = 0$, and would result from the tendency of the membrane to adjust its composition so as to optimize the electrostatic energy between membrane and macroion [64, 65]. Yet, this behaviour reflects the imposition of local compositional homogeneity while allowing for macroscopic phase separation. If the local compositions are allowed to adjust, lateral phase separation requires $\chi > 0$. In fact, it was shown [26] that in the strong electrostatic interaction regime the critical point is $\chi_{\rm C} = 2\sqrt{a/a_{\rm P}}/\phi_{\rm P}^2$, implying that for sufficiently large macroions ($a_{\rm P} \gg a$) the critical point can fall below the limit of the fully screened bare membrane (where $\chi_{\rm C} = 2$). In summary, electrostatic adsorption of macroions induces lipid migration, thus creating compositional gradients in the membrane plane. These gradients induce a line tension if the membrane exhibits non-electrostatic attraction between lipids of the same species (that is $\chi > 0$). The line tension represents an effective attractive force between the macroions which can drive macroscopic phase separation. Hence, electrostatics mediates but does not drive the phase separation. Indeed, the same mechanism was recently analysed by Netz [66] for flocculation of non-interacting colloids immersed in a host that exhibits attractive interactions.

The third model is based on and extends the microscopic-level PB treatment of a spherical macroion adsorbing onto a flat binary membrane, discussed above (see equations (7) and (8) and figure 3). As there, the membrane is allowed to adjust its local composition η. Yet, apart from the ideal mixing free energy, $F_{\rm D}$, given in equation (7), a non-ideality contribution

$$\frac{F_N}{kT} = -\frac{\chi}{a}\int_A {\rm d}a\,(\eta - \phi)^2 + \frac{\chi}{6}\int_A {\rm d}a\,(\nabla\eta)^2 \tag{12}$$

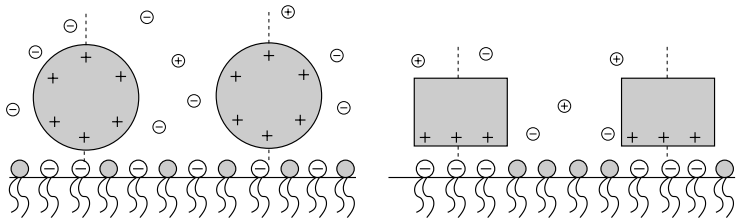

Figure 5. Two examples for macroions with different propensity to induce phase separation in a planar membrane that exhibits non-ideal lipid demixing with $\chi > 0$. Left: uniformly charged spherical macroions. Right: cylinder-like macroions that carry charges only on their bottom face. The critical point χ_C is larger for the spherical macroions because of small line tension (resulting from small compositional gradients) and direct electrostatic repulsion between the macroions.

is added that also accounts for in-plane compositional gradients, $\nabla\eta$, of the membrane. As a result of the non-ideality, the boundary condition

$$a\frac{\chi}{3}\nabla^2\eta = \ln\frac{\eta(1-\phi)}{\phi(1-\eta)} - 2\chi(\eta-\phi) + \lambda - \Psi \tag{13}$$

at the membrane for solving the PB equation becomes a differential equation for the local composition η. Analysis of the spinodal lines and the critical point was performed recently for macroions of various sizes and shapes [67]. As for the above-mentioned two-state model, it is the line tension contribution that drives lateral phase separation. Indeed, omitting the second contribution in equation (12) eliminates any phase separation occurring for $\chi < 3.7$ where the bare membrane becomes unstable. Generally, the underlying compositional gradients within the membrane are weaker compared to the step-profile of the two-state model. In addition, phase separation is counteracted by direct electrostatic repulsion between the macroions which is not accounted for in the two-state model. As a result, the macroion-dressed membrane generally gains stability in the microscopic-level PB treatment as compared to the two-state model. In fact, there is a delicate interplay between the macroion's shape and the charge distribution on it. For example, uniformly charged spherical macroions are not potent candidates because they induce relatively small compositional gradients while direct electrostatic repulsion is strong. On the other hand, for a cylinder-like disc with only its bottom face charged there is a considerable reduction in χ_C, in good agreement with the two-state model. Figure 5 schematically illustrates these two cases.

As all three models suggest, the stability of the membrane depends sensitively on the accuracy used to model electrostatic interactions. The corresponding contribution to the free energy, $F_{EL}(\phi, \theta)$, has been calculated so far only for a few simple cases within PB theory. New perspectives will be opened by accounting for dipolar (or higher order electrostatic) moments of the adsorbed macroions and for non-electrostatic interactions not only between the lipids but also between the macroions. Perhaps even more relevant for understanding biological membrane rafts, more accurate lipid–lipid interaction models will be needed to describe the cooperative features encountered particularly in cholesterol-containing membranes [23].

Recently, the ability of macroions to induce domains in binary membranes has also been investigated experimentally. An interesting system is the C2A domain of synaptotagmin I which is involved in triggering fast fusion of synaptic vesicles with the plasma membrane and subsequent release of neurotransmitter into the synaptic cleft [68]. The interaction of this domain with charged lipids—in particular with PS—is mediated by Ca^{2+}. Binding studies of C2A on mixed PS/PC membranes were analysed by Monte Carlo simulations and revealed a pronounced tendency of C2A to induce lipid segregation [69]. Even more, it was shown that

small variations in lipid structure, such as varying the length of a hydrocarbon chain for one lipid species, notably influenced the domain sizes, indicating that the observed domain formation was mediated by non-specific interactions between the lipids [70]. The corresponding Monte Carlo simulations were performed on a lattice with all interactions (electrostatic and non-electrostatic) being lumped into a set of two-body lipid–lipid and lipid–protein interaction parameters.

In another combined experimental/theoretical study, Denisov *et al* [64] have analysed—using fluorescence microscopy—the ability of pentalysine to induce membrane domains enriched in PS and PIP_2. However, as Murray *et al* pointed out recently (see the appendix of [71]), there are a number of complementary experimental methods that argue against phase separation induced by small peptides such as pentalysine in PS/PC membranes. The underlying theoretical model of Denisov *et al* [64] is based on the peptide's ability to change the electrostatic free energy. In fact, a simple model similar to the above-mentioned electrostatic shielding of the membrane charges was used (with effective membrane composition $\phi^{\mathrm{eff}} = \phi - z_{\mathrm{P}}\theta a/a_{\mathrm{P}}$), yet without allowing for local compositional changes [64]. As mentioned, the suppression of local compositional changes greatly promotes phase separation: it can take place even in absence of attractive forces between lipids of the same species (that is, for $\chi = 0$).

6. Cationic lipid–DNA complexes

Charged lipid membranes can also be decorated by DNA. However, because DNA is highly negatively charged (one elementary charge per $l = 1.7$ Å DNA length), cationic instead of anionic lipids are commonly being used. The background for the large number of investigations concerning cationic lipid–DNA complex formation is their potential use as non-toxic gene delivery vehicles [72, 9]. The crucial issue concerning non-viral gene delivery is to improve the transfection efficiency, which depends (though in a non-trivial way) on the structural and physical properties of the cationic lipid–DNA complex. Upon interaction of cationic vesicles with DNA, a rigorous structural reorganization takes place that can lead to the formation of different aggregation geometries. Cationic membranes often contain—besides the actual cationic species—an uncharged zwitterionic species, also called a helper lipid, which reduces the toxicity of the complexes and improves the transfection rate. An essential determinant of lipoplex structure is the type of helper lipid, promoting either formation of the planar L_α-phase or of the inverse hexagonal H_{II}-phase [73]. That is, bilayer-forming helper lipids such as PC tend to favour the so-called L_α^C [74] complex, which is an alternating stack of cationic bilayers and linear DNA arrays. In contrast, PE, which promotes the inverse hexagonal H_{II}-phase, favours the so-called H_{II}^C structure, in which the DNA is intercalated within the aqueous tubes of an inverse hexagonal lipid matrix [75]. Also other structures have been identified, such as DNA-attached lipid micelles [76] or the spaghetti-like complex [77, 78] in which single DNA molecules are wrapped individually by a cationic bilayer.

At the physical basis of lipoplex formation stands the electrostatic interaction between the rigid macroion DNA and a mixed cationic membrane, leading to macroion-decorated membrane structures similar to those discussed in the preceding sections. It is thus no surprise that among other methods (including molecular dynamics [79], Monte Carlo simulations [80], and a simple electrostatic model [81]), PB theory has frequently been employed to model lipoplex structure and stability We shall briefly illustrate the use of PB theory for describing the lamellar L_α^C complex structure (for a more extensive review see May and Ben-Shaul [82]). A cross-section through the L_α^C complex is displayed in figure 6. Membrane and DNA are modelled as planar sheet and rigid rod, respectively. The rectangular shaded region in figure 6 marks a single unit cell in which the PB equation is to be solved. In fact, solving it in one-quarter

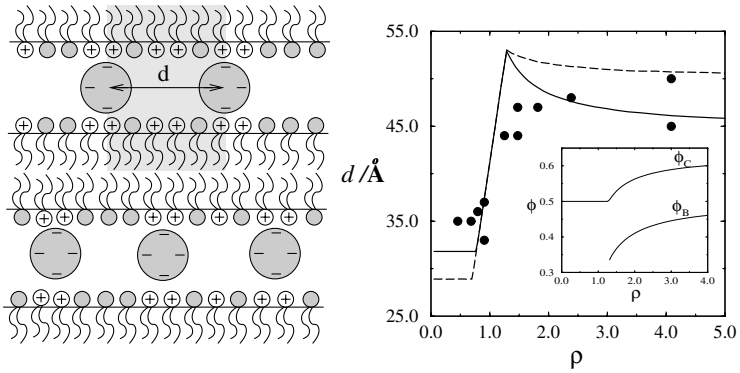

Figure 6. Left: schematic cross-section through the lamellar L_α^C complex, consisting of a cationic membrane stack into which linear arrays of DNA are intercalated. The average composition of the membrane is ϕ and the DNA-to-DNA distance is denoted by d. The rectangular shaded region marks the cross section of a unit cell. Right: the DNA-to-DNA distance, d, as a function of the cationic lipid-to-DNA charge ratio ρ for a membrane composition $\phi = 0.5$ and for a Debye length of $l_D = 50$ Å (solid line) and $l_D = 10$ Å (dashed line). The dots mark experimental data from Rädler *et al* [74]. The inset shows the variation in lipid composition in the complex (ϕ_C) and free bilayer (ϕ_B) as a function of the charged lipid-to-DNA ratio, for $l_D = 50$ Å. Reproduced with permission from Harries *et al* [53].

of the aqueous part of the unit cell is sufficient. Also, invariance of the complex along the DNA strands renders the PB equation two dimensional. Two degrees of freedom determine the physical state of the lipoplex, the composition ϕ and the cationic lipid-to-DNA charge ratio ρ. (Note that for the L_α^C complex ρ is a more convenient measure than the macroion coverage, θ, but both quantities are related.) As discussed in section 4, the mixed membrane can adjust its *local* composition, η, so as to optimize the electrostatic interaction with the DNA strands. Thus, assuming that there is no non-ideal lipid demixing, equation (8) describes the local compositional adjustment of the membrane (and serves as the membrane boundary condition for the PB equation). Analysis of the solutions of the PB equation and of the corresponding free energy reveal that the optimal free energy almost exactly corresponds to the *isoelectric* state $\rho = 1$ where the number of cationic lipids is identical to the number of DNA phosphate groups. At the isoelectric state, where $d = d^\star = a\phi/l$, nearly all co- and counterions are released into solution, which is not only a theoretical prediction but has been verified experimentally [83]. Notably, the experimentally observed DNA-to-DNA distance d can but does not necessarily have to correspond to $d^\star$. In fact, initially isoelectric complexes easily incorporate additional cationic membrane, thereby *overcharging* the complex and increasing d beyond $d^\star$ [84]. In this respect, the L_α^C complex does not behave differently from the macroion-adsorbed membrane where in the absence of non-ideal lipid mixing no phase separation occurs; see section 5. However, beyond a certain amount of added cationic membrane, the system splits into two phases, the L_α^C complex and bare cationic membrane, leaving d essentially unchanged upon further increase of ρ. The reason for this phase separation originates in the stacked structure of the L_α^C complex where neighbouring membranes experience electrostatic repulsion. For sufficiently large d, this repulsion becomes intolerably high, thus inducing the formation of isolated excess membrane. Note that also upon adding DNA to initially isoelectric complexes d decreases until (nearly) steric contact between neighbouring strands stops further overcharging. In summary, the composite structure of the L_α^C complex, with the membrane-to-membrane distance roughly being fixed by the diameter of the DNA, brings about a phase separation that has no equivalent for a single macroion-decorated membrane.

The phase behaviour of the lamellar L_α^C complexes has been studied experimentally [85] and is qualitatively reproduced by PB theory [53, 84, 86]. As an illustration, the right diagram of figure 6 shows $d(\rho)$ according to experiment [74] and PB theory [53].

Beyond the electrostatics of the lamellar L_α^C complex, PB theory has also been employed to investigate the adsorption of DNA onto a single cationic membrane with mobile lipids [87], particularly also including pH-dependent chemical charge regulation [88], or analysing the role of image-charges on the degree of counterion release [89]. In another set of studies, PB theory was used to model the stability of non-lamellar cationic lipid–DNA complex structures, namely of the H_{II}^C and of the spaghetti-like complexes [90, 91], arguing that the latter one is likely to represent a metastable structure. The presence of a non-bilayer-forming helper lipid such as PE has profound consequences on the phase behaviour of cationic lipid–DNA complexes. For small membrane composition ϕ, where the helper lipid PE dominates, formation of the H_{II}^C structure is favoured, whereas for large ϕ the (usually bilayer-forming) cationic lipid furthers the lamellar L_α^C structure. Detailed calculations on the basis of non-linear PB theory [92] predict complex phase behaviour, sometimes with coexisting L_α^C and H_{II}^C complexes, three-phase regions, or re-entrant transitions. Some of these qualitative predictions have been verified experimentally [93, 94]. Another issue that has attracted theoretical interest is DNA-induced curvature deformations of the cationic membrane. They fall into the general framework of macroion-induced membrane bending that is discussed in the following section.

7. Macroion-induced curvature deformations of membranes

Lipid membranes have a low bending stiffness of $\kappa \sim 10kT$, which enables them to adjust their curvature in response to the interaction with rigid particles such as colloids or biopolymers. Curvature modulations give rise to short- and long-range interactions between membrane-associated or membrane-inserted particles (the latter usually being referred to as *membrane inclusions*), that have attracted considerable theoretical interest, starting over a decade ago [95]. From a physical point of view it is interesting to note that the interactions between membrane inclusions are non-additive and depend sensitively on the inclusion size, shape, and coupling strength. This is where most recent theoretical approaches either focus on those many-body effects on the basis of given membrane-inclusion interaction strength [96, 97], or calculate (mostly for a single inclusion or an inclusion pair) how the interaction strength depends on the various microscopic forces between membrane and inclusion [98–100]. As the present review deals with charged membranes and macroions, we shall only be concerned with the latter case, focusing on the coupling of electrostatics and bending elasticity.

A system that has been investigated to some extent is a cationic lipid–DNA complex, namely the lamellar L_α^C structure. Based on experiments, it was suggested that the DNA galleries within the lamellar complex are orientationally aligned [101, 102], implying that there must be some kind of coupling across the cationic membrane. A possible mechanism is the interlocking of the galleries through DNA-induced curvature modulations of the cationic membrane stack, which was investigated analytically on the basis of linear PB theory [103, 104] and numerically using non-linear PB theory [105]. Beyond this, curvature modulations were also suggested to contribute to—or even to be a major determinant of—the L_α^C complex stability [106]. The modelling of the interplay between membrane bending elasticity and electrostatic interactions suggests that, quite generally, highly charged macroions such as DNA are able to substantially affect membrane curvature. A notable consequence of the ensuing curvature-mediated interactions between membrane-adsorbed rods (and similarly for spherical particles) is lateral phase separations which have been analysed theoretically on various levels [107–109].

The profound ability of DNA to impose membrane curvature is clearly reflected in the formation of the spaghetti-like complex where the DNA is entirely wrapped by a single lipid bilayer. A simple *capacitor model* [90] illustrates the two competing forces: electrostatic attraction between the DNA and the cationic membrane, and bending elasticity of the bilayer. The inner one of the two concentric cylinders of the capacitor models the DNA (with charge density $\sigma = -e/2\pi Rl$ where $R \approx 10$ Å is the DNA radius, and $l = 1.7$ Å is the charge-to-charge distance along the DNA rod), and the outer cylinder (being of radius R^I) represents a cationic lipid layer of charge density $\sigma^I = \sigma R/R^I$, which ensures charge neutrality. The overall free energy is $F = F_{\mathrm{EL}} + F_{\mathrm{C}}$, where $F_{\mathrm{EL}} = L(l_{\mathrm{B}}/l^2)kT \ln(R^I/R)$ is the electrostatic energy of the capacitor (L is the length of the capacitor) and $F_{\mathrm{C}} = A\kappa(c - c_0)^2/2$ is the membrane bending energy, with $A = 2\pi R^I L$ being the area of the outer cylinder, κ the bending stiffness, and $c = 1/R^I$ the curvature of the cationic lipid layer. Assuming a vanishing spontaneous curvature, $c_0 = 0$, minimization of $F(R^I)$ leads to the equilibrium area

$$R^I = \pi \frac{\kappa l^2}{kT l_{\mathrm{B}}} \tag{14}$$

which for $\kappa \approx 10kT$ predicts $R^I = 13$ Å just slightly larger than the DNA radius.

Wrapping of macroions by an oppositely charged membrane has been investigated in much greater detail than using the simple capacitor model. Potential applications of passive wrapping range from viral budding [110] to the cellular internalization of drug delivery systems such as DNA carriers [111] or peptide shuttles [11]. Harries *et al* [112] employed PB theory to describe the wrapping transition of a charged, spherical model protein, initially adsorbed onto a flat, mixed membrane. In this state, lipid mobility was accounted for on the level of ideal mixing, leading to the membrane boundary condition given in equation (8). The fully macroion-wrapped state is electrostatically favourable but entails a penalty in bending energy. The theoretical analysis of the two limiting states—membrane adsorbed and fully membrane coated—showed that the protein's crossover charge (above which wrapping becomes energetic favourable) is generally high but realizable in biomolecules such as charged polypeptides. Theoretical investigations of macroion (or colloidal) wrapping beyond the two limiting states generally requires us to determine the membrane shape profile. Assuming an adhesion energy proportional to the contact area between membrane and particle allows us to use a set of previously well studied shape equations [113] that are based on the Helfrich elastic energy [114]. As Deserno and Bickel showed [115], partial wrapping requires lateral membrane tension and leads to a discontinuous transition to the fully wrapped state. Due to hysteresis, colloid binding and unbinding transitions are not equivalent [116]. Finally, accounting explicitly for long-range electrostatic interactions on the basis of Debye–Hückel theory affects the membrane shape, shifting it from catenoid-like for large salt content to volcano-like for small salt content [117].

8. Concluding remarks

Macroions 'routinely' encounter charged membranes, giving rise to various interesting phenomena regarding the conformation and stability of the ensuing macroion–membrane complex. Being soft and self-assembled materials, lipid membranes are able to simultaneously adjust their local charge density through compositional changes as well as their curvature through elastic deformations. Both responses have been addressed in theoretical studies, yet in most cases separately. This is likely to change if future experiments continue to highlight the importance of coupled compositional and shape changes of charged membranes, particularly biomembranes, including the dependence of this coupling on associated macroions [118].

Membrane budding, fusion and fission, peptide-induced pores, biomembrane rafts, and drug delivery systems are among the most intensively studied candidates to impact the direction of future theoretical research. Related to this is the challenge to simultaneously model electrostatic interactions and membrane elasticity. It is not yet well known to what extent the charged residues of membrane-active biopolymers such as peripheral proteins or amphipathic peptides [119] are involved in inducing elastic membrane deformations and whether these deformations are crucial for biological function. In this respect, an interesting candidate to employ electrostatic interaction to bend a membrane is the BAR domain, contained in amphiphysin, arfaptin2, and other proteins. The crescent shape of the domain and the presence of positive charges on its concave face visually suggest the possibility of bending charged membranes [120]. Indeed, at sufficiently high concentrations, the domain induces the formation of lipid tubules. A rough estimate of the energetic feasibility of the electrostatic bending mechanism was recently provided by Zimmerberg and McLaughlin [121].

The present review has largely focused on PB theory, which is a reasonable approach for monovalent ions, where ion–ion correlations can largely be ignored. A strength of PB theory is that the different dielectric constant in macroions as compared to that in the aqueous solution can easily be accounted for. In addition, solving the PB equation becomes computationally simple for systems with high symmetry. (In fact, it is often possible to introduce reasonable approximations that allow us to analytically express the electrostatic free energy.) Extending the applicability of PB theory to new physical scenarios and coupling it to other, non-electrostatic energy contributions (for example polyelectrolyte adsorption [122] or rod-like mobile ions [123]) is likely to remain an active area of research.

Acknowledgments

The author thanks all collaborators who have contributed to this work during recent years, particularly Avinoam Ben-Shaul, Daniel Harries, and Emmanuel Mbamala. Illuminating discussions with Anne Hinderliter, Stuart McLaughlin, and Aleš Iglič are also acknowledged. This work was supported by ND EPSCoR through grant no. EPS-0132289.

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