Biophysical Chemistry 150 (2010) 112–118

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

A model for the electrostatic contribution to the pH-dependent nonideal mixing of a binary charged–zwitterionic lipid bilayer

Demmelash H. Mengistu [a], Klemen Bohinc [b], Sylvio May [a,*]

[a] Department of Physics, North Dakota State University, Fargo, ND 58105-5566, USA
[b] Faculty of Health Sciences, Poljanska 26a, University of Ljubljana, 1000 Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 16 December 2009
Received in revised form 29 January 2010
Accepted 30 January 2010
Available online 6 February 2010

Keywords:
Lipid bilayer
Gouy–Chapman
Poisson–Boltzmann
Phase behavior
Nonideal mixing
Binodal

ABSTRACT

Nonideal mixing provides the physical basis of domain formation and macroscopic phase separation in lipid bilayers. We present a model for the electrostatic contribution to the nonideality of a two-component acidic–zwitterionic lipid membrane. Our model is based on the mean-field Poisson–Boltzmann approach; it includes a protonation/deprotonation equilibrium applicable to acidic lipids such as phosphatidic acid or phosphatidylglycerol. It also includes an electrostatic model for zwitterionic lipids such as phosphatidylcholine or phosphatidylethanolamine that accounts for the spatial separation of the two headgroup charges and the orientational freedom of the headgroup. Modeling the nonelectrostatic contribution to the free energy using the Bragg–Williams approximation of a binary lattice gas enables us to compute binodal lines that reflect the influence of membrane electrostatics on the nonideal mixing properties of the bilayer. If we neglect the zwitterionic lipid's electrostatic contribution, then increasing pH is predicted to always oppose demixing, stabilizing the membrane due to the repulsion between the charged lipids. If the electrostatic properties of the zwitterionic lipids are accounted for, this opposing tendency weakens and may even reverse. In this case, increasing the fraction of charged lipids through increasing pH would, somewhat unexpectedly, promote domain formation. We discuss the corresponding physical mechanism.

1. Introduction

Cellular membranes contain a significant fraction of charged lipids; electrostatics is thus expected to ubiquitously contribute to the energetics and stability of biomembranes. Of the numerous biological functions that take place at the membrane many are influenced (or even dominated) by electrostatic interactions [1]. It is well recognized that the complex interplay of interactions within a lipid bilayer leads, in general, to nonideal mixing properties [2] which enables a membrane to laterally organize its structure, form domains, and locally adjust lipid composition. Indeed, the emergence of the lipid raft concept [3] underscores the functional significance of nonideal mixing in biological membranes. Experimental evidence for how nonideal lipid mixing depends on membrane electrostatics has been collected using various binding assays [4–7] and other methods [8–10]. In binary fluid membranes, the degree of nonideality is usually insufficient to form macroscopic domains; ternary membranes such as lipid mixtures with cholesterol [11] or associated proteins [12] may, however, undergo macroscopic lateral phase separation.

One of the experimental approaches to characterize nonideal mixing in lipid membranes has been developed by Alfred Blume et al. [13,14]. It is based on differential scanning calorimetry and simulations of the corresponding heat capacity curves using regular solution theory [14]. The simulations yield sets of nonideality parameters χ and τ for a binary lipid mixture in both the gel and liquid crystalline phases. Specifically, in each phase (gel and liquid crystalline) the model is based on the expression for the free energy per lipid $f/k_BT = \phi ln\phi + (1-\phi)ln(1-\phi) + \phi(1-\phi)[\chi + \tau(2\phi-1)]$, measured in units of the thermal energy k_BT (where k_B is Boltzmann's constant and T the absolute temperature). Here, ϕ is the composition of the membrane (mole fraction of one lipid type), χ represents a nonideality parameter familiar from the Bragg–Williams approximation [15], and τ is a second nonideality parameter which introduces an asymmetry in the phase diagram. Note that the role of τ in introducing asymmetry can be seen by calculating the critical point $\chi = \chi_c$ and corresponding critical composition $\phi = \phi_c$ predicted by f. Vanishing second and third derivatives of the free energy, $d^2f(\phi)/d\phi^2 = d^3f(\phi)/d\phi^3 = 0$, yield for small τ the values $\chi_c = 2 - (9/8)\tau^2$ and $\phi_c = 1/2 + 3\tau$. Nonideality parameters χ and τ for the gel and liquid crystalline phase state have been extracted for binary mixtures of phosphatidylcholine (PC) with phosphatidic acid (PA) [16] or phosphatidylglycerol (PG) [17], lipid mixtures of PG with phosphatidylethanolamine (PE) [18], lipid mixtures that only differed in chain length [19] or were exposed to the influence of divalent cations [20,21]. In some of the studies was the pH used to tune the charge of the involved acidic lipids, which allows to monitor the change in nonideality as a function of electrostatic membrane properties. Protonation of acidic lipids (PA [16] and PG [17]) for sufficiently small pH was, generally,

* Corresponding author. Tel.: +1 701 231 7048; fax: +1 701 231 7088.
E-mail address: Sylvio.May@ndsu.edu (S. May).
URL: http://physics.ndsu.edu/people/faculty/may/ (S. May).

0301-4622/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2010.01.012

observed to increase the nonideality parameter χ in the fluid phase. This is expected because the electrostatic contribution to the free energy disfavors the formation of clusters that contain charged lipids. The details of the relation χ(pH) depend in a complex way on additional factors such as chain length differences and ability to form hydration bonds between the involved lipids. To what extent the electrostatic properties of the zwitterionic lipids can be expected to influence the nonideality is the subject of the present study.

Existing modeling approaches of membrane electrostatics range from continuum Poisson–Boltzmann theory [22] to all-atom Molecular Dynamics simulations [23]. The former, continuum Poisson–Boltzmann theory, involves a number of drastic simplifications such as point-like ions, smeared charge distributions, and the neglect of both solvent structure and ion–ion correlations. On the other hand, the simplicity of the Poisson–Boltzmann model often allows for a transparent interpretation of its predictions, and it can easily be combined with other approaches such as lattice gas models or chemical dissociation equilibria. In fact, dissociation equilibria have received considerable attention [24–29], because electrostatic properties can be regulated through the adsorption of ions or changes in pH. Lipid bilayers that contain a mixture of acidic and zwitterionic lipids can, in principle, be described by similar approaches. However, the additional presence of zwitterionic lipids can be expected to influence the electrostatic membrane properties, including the protonation/deprotonation equilibrium of the acidic lipid. The large dipole moment of a zwitterionic headgroup such as PC makes it likely that this influence is significant.

In the present work we analyze the electrostatic contribution to the nonideality of a mixture between zwitterionic lipids (such as PC or PE) and acidic lipids (such as PA or PG). We include into our model (i) the electrostatic properties of the zwitterionic lipids, (ii) the protonation/deprotonation equilibrium of the acidic lipids, and (iii) a nonelectrostatic nonideality based on the mean-field Bragg Williams approximation of a binary lattice gas [15]. We do not aim to extract nonideality parameters from experimental data (work along this line is available [6,30]). Instead, our objective is to characterize the physical mechanism how zwitterionic lipids impact the protonation/deprotonation equilibrium of the acidic lipids and what the implications for the stability of the membrane are.

We shall employ, within the Poisson–Boltzmann framework, a structural model for a zwitterionic lipid headgroup that has been suggested previously by Mbamala et al [31]. This model reproduces typical experimentally observed differences between mixed cationic and anionic lipid bilayers [31], including differences in the adsorption of charged macroions such as proteins or DNA [32]. The model also leads in some limits (the Debye–Hückel limit [33] and the dipole limit [34]) to analytical results for the electrostatic free energy. It has finally been used to characterize the adsorption of DNA onto zwitterionic bilayers mediated by divalent cations [35]. In the present work, we introduce a protonation/deprotonation equilibrium for the acidic lipids that leads to the nominal probability

$$\eta_0 = \frac{1}{1 + 10^{\mathrm{pKa}-\mathrm{pH}}} \tag{1}$$

for a headgroup of given pKa and ambient pH to carry a single negative charge. The presence of zwitterionic lipids will affect the electrostatic potential and this will modify the protonation/deprotonation equilibrium of the acidic lipids. Finally, our present work will include a nonelectrostatic contribution to the nonideal mixing which, as mentioned above, will be modeled using the Bragg–William free energy f_{BW}; see below in Eq. (5). The total free energy $f = f_{BW} + f_{el}$ will contain the additional electrostatic contribution f_{el}. Calculating how the critical point $\chi = \chi_c$ shifts as function of η_0 reveals how the dipolar character of the zwitterionic lipids affects the mixing properties of the membrane.

2. Model

We consider a planar, binary lipid bilayer that contains an acidic (such as PA or PG) and a zwitterionic lipid type (such as PC or PE), present with mole fractions ϕ and $1-\phi$, respectively. For sufficiently small pH, the phosphate group of the acidic lipid becomes protonated. In the present study we explicitly account for the corresponding protonation/deprotonation equilibrium. Let η denote the fraction of the acidic lipids that carry a deprotonated phosphate group (and thus a single negative charge). Below we refer to η as the degree of deprotonation. The remaining fraction of acidic lipids, $1-\eta$, is protonated (and thus electrically neutral). For simplicity we assume that the phosphate group of the zwitterionic lipid headgroup remains negatively charged. We can thus characterize the state of the bilayer by the three mole fractions $\phi_1 = 1-\phi$, $\phi_2 = \eta\phi$ and $\phi_3 = (1-\eta)\phi$ of the zwitterionic, deprotonated, and protonated lipids, respectively, as is illustrated in Fig. 1. We describe the energetics of the bilayer on the mean-field level; all properties (the electrostatic potential, concentrations of salt ions, etc.) depend only on the distance x away from the bilayer, but not on the in-plane location.

The headgroups of zwitterionic lipids such as PC or PE have a large intrinsic dipole moment. Using a distance of $\approx$0.5 nm between the charges carried by the phosphate and amine groups, we obtain a dipole moment of about 24 Debye, an order of magnitude more than that of water (1.85 Debye). This is likely to influence the electrostatic properties of the bilayer. In the present work we employ an electrostatic model of zwitterionic lipid headgroups that was suggested and analyzed previously [31]. Briefly, the two headgroup charges are both modeled explicitly, with the negative charge (i.e. the phosphate group for phospholipids) residing immobilized at the hydrocarbon core–headgroup interface (at $x=0$ as illustrated in Fig. 1). The positive headgroup charge, which is located at fixed distance l away from the corresponding negative headgroup charge, is allowed to adopt headgroup tilt angles θ with $0 \le \theta \le \pi/2$. Below we use the probability $P(x)$ with $x = l\cos\theta$ to describe the tilt angle distribution

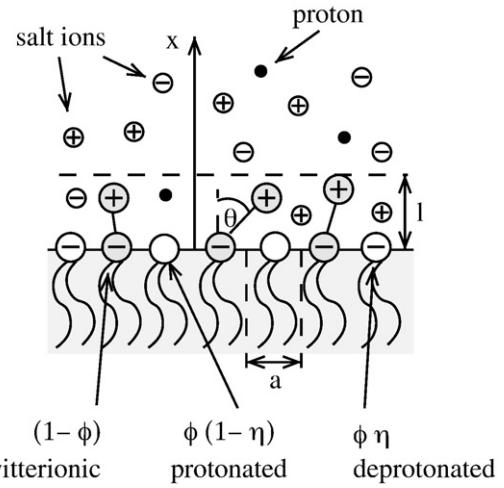

Fig. 1. Illustration of a mixed acidic–zwitterionic lipid layer. The mole fraction of the acidic lipid is ϕ, and that of the zwitterionic lipid is $1-\phi$. For the acidic lipid we account for a protonation/deprotonation equilibrium, where deprotonated and protonated lipids are present with mole fractions $\phi\eta$ and $\phi(1-\eta)$, respectively. Acidic lipids (protonated and deprotonated) as well as the negative charges of the zwitterionic lipids are confined to the plane $x=0$ whereas the positively charged ends of the zwitterionic lipids are able to reside at any position $x = l\cos\theta$ within the headgroup region ($0 \le x \le l$), corresponding to a headgroup tilt angle θ. The cross-sectional area a is assumed to be constant and the same for all lipids. Charges belonging to zwitterionic lipid headgroups are shaded. The horizontal broken line marks the outer boundary of the headgroup region at distance l away from the polar–apolar interface, $x=0$. The aqueous solution contains protons and salt ions.

of the headgroups. We use the normalization $(1/l)\int_0^l dx P(x)=1$. Note that $P(x)=0$ for $x>l$ and for $x<0$; we thus refer to the region $0\le x\le l$ as the *headgroup region*.

Assuming each lipid (irrespective of chemical structure and protonation state) occupies the same and fixed cross-sectional area a implies a surface charge density $\sigma=-(1-\phi+\eta\phi)e/a$ at position $x=0$ of the lipid layer, where e denotes the elementary charge. Note that the contribution $-(1-\phi)e/a$ results from the negative charges of the zwitterionic lipids whereas the remaining part, $-\eta\phi e/a$, is due to the deprotonated fraction of the acidic lipids. The aqueous solution contains salt with local concentrations n_+ and n_- of positively and negatively charged monovalent ions. In addition, protons are present with local concentration n_p. The bulk values of n_+, n_-, and n_p are n_0, $n_0+n_p^0$, and n_p^0, respectively. Note that this choice ensures electroneutrality in the bulk. (That is, for each proton a negatively charged salt ion is added in the bulk.) Within the headgroup region, the positive charges of the zwitterionic headgroups contribute $e(1-\phi)P(x)/(al)$ to the local volume charge density $\rho(x)$. The total value for the local volume charge density $\rho(x)$ can thus be expressed by

$$\frac{\rho(x)}{e}=\begin{cases} n_+ + n_p - n_- + \dfrac{(1-\phi)}{al}P(x) & 0<x<l \\ n_+ + n_p - n_- & l\le x<\infty. \end{cases} \tag{2}$$

Known ρ allows us to calculate the electrostatic potential Φ through Poisson's equation $\Phi''(x)=-\rho(x)/(\epsilon_W\epsilon_0)$ where (here and in the following) a prime denotes the derivative with respect to the argument. In addition, $\epsilon_W\approx 80$ is the dielectric constant of water, and ϵ_0 is the permittivity in vacuum. The discussion of physical models often benefits from expressing quantities in dimensionless form or in terms of lengths. For the Poisson–Boltzmann model it is convenient to use the dimensionless potential $\Psi=e\Phi/k_BT$ and to express ϵ_W in terms of the Bjerrum length $l_B=e^2/(4\pi k_BT\epsilon_W\epsilon_0)\approx 0.7$ nm. Hence, the Poisson equation reads $\Psi''(x)=-4\pi l_B\rho(x)/e$.

We decompose the free energy per lipid

$$f=f_{el}+f_{BW} \tag{3}$$

into an electrostatic (f_{el}) and nonelectrostatic (f_{BW}) contribution. On the mean-field level, we write for the electrostatic contribution, measured per lipid and in units of the thermal energy k_BT,

$$\begin{aligned} \frac{f_{el}}{k_BT} &= \frac{a}{8\pi l_B}\int_0^\infty dx\left(\Psi'(x)\right)^2 \\ &+ a\int_0^\infty dx\left[n_+ ln\frac{n_+}{n_0} - n_+ + n_0\right] \\ &+ a\int_0^\infty dx\left[n_- ln\frac{n_-}{n_0+n_p^0} - n_- + n_0 + n_p^0\right] \\ &+ a\int_0^\infty dx\left[n_p ln\frac{n_p}{n_p^0} - n_p + n_p^0\right] \\ &+ (1-\phi)\frac{1}{l}\int_0^l dx P(x) lnP(x) \\ &+ \phi\left[\eta ln\frac{\eta}{\eta_0} + (1-\eta) ln\frac{1-\eta}{1-\eta_0}\right]. \end{aligned} \tag{4}$$

The first line in Eq. (4) describes the electrostatic field energy. The integration runs from the hydrocarbon core–headgroup interface, at $x=0$, to infinity. Ignoring the electrostatic contribution within the hydrocarbon core of the lipid bilayer is appropriate because of the small value of the dielectric constant inside the membrane (as compared to water). The second, third, and fourth lines in Eq. (4) account for the ideal mixing free energy contributions of the positive salt ions, negative salt ions, and protons, respectively. Line five denotes the orientational entropy contribution of the zwitterionic headgroups where, as pointed out above, $P(x)$ is the probability of the zwitterionic lipid's positive charge to be found at position x. The last term in Eq. (4) accounts for the demixing free energy contribution of the protonation/deprotonation equilibrium. Here, η_0 is the nominal (i.e., in the absence of an external electrostatic field) probability to find the acidic lipid negatively charged. Eq. (1) relates η_0 to the pH and pKa value of the acidic lipid.

For the nonelectrostatic contribution to f we employ the Bragg–Williams model which is based on a random mixing approximation of a binary lattice gas [15]. Note that the Bragg–Williams model neglects correlations, as does the Poisson–Boltzmann model. The two models that we combine in the present study thus describe the membrane using the same mean-field level of approximation. The Bragg–Williams free energy per lipid can be written as

$$f_{BW}=k_BT[\phi ln\phi + (1-\phi)ln(1-\phi) + \chi\phi(1-\phi)]. \tag{5}$$

with nonideality parameter χ. Increasing χ acts toward demixing and cluster formation of like molecules. Eq. (5) is similar to the model used by Johann et al. [13]; yet without the asymmetry parameter τ. In our model, the nonideality parameter χ represents only the nonelectrostatic contribution to the nonideal behavior of the lipid layer (electrostatics is explicitly accounted for by f_{el}). Also, we assume that f_{el} does not affect the nonelectrostatic contribution to the nonideality. If it did, we would not only have to allow for a dependence of χ on the electrostatic properties of the membrane, but two more nonideality parameters would appear. That is, the total number of nonelectostatic nonideality parameters would be three, related to the nonelectostatic interactions between protonated and deprotonated acidic lipids, between protonated acidic and zwitterionic lipids, and between deprotonated acidic and zwitterionic lipids. Even an additional three-body interaction term could appear. Although we cannot rule out a coupling between electrostatic and nonelectrostatic interactions, we ignore its possible existence in the present work.

The free energy $f=f(n_+,n_-,n_p,P,\eta)$ given in Eqs. (4) and (5) fully specifies the behavior of the lipid layer, including the fractions of protonated/deprotonated lipids and the degree of nonideality. In thermal equilibrium f adopts a minimum with respect to the functions $n_\pm(x)$, $n_p(x)$, and $P(x)$ as well as with respect to the degree of deprotonation η. Variation of f leads to

$$\begin{aligned} \frac{\delta f}{k_BT} = a\int_0^\infty dx\Bigg\{ &\delta n_+\left(ln\frac{n_+}{n_0}+\Psi\right) + \delta n_p\left(ln\frac{n_p}{n_p^0}+\Psi\right) + \delta n_-\left(ln\frac{n_-}{n_0+n_p^0}-\Psi\right)\Bigg\} \\ &+ \delta\eta\phi\left[ln\left(\frac{\eta(1-\eta_0)}{\eta_0(1-\eta)}\right)-\Psi(0)\right] + (1-\phi)\frac{1}{l}\int_0^l dx\,\delta P(lnP+\Psi+lnq) \end{aligned} \tag{6}$$

where $\Psi(0)$ is the (dimensionless) potential at $x=0$. The constant q ensures proper normalization of $P(x)$. Vanishing of δf gives rise to the Boltzmann distributions $n_+=n_0exp(-\Psi)$, $n_p=n_p^0exp(-\Psi)$, $n_-=(n_0+n_p^0)exp(\Psi)$, and $P=exp(-\Psi)/q$, with $q=(1/l)\int_0^l dxexp(-\Psi)$ appearing as the corresponding partition sum. Moreover, for the degree of deprotonation we obtain

$$\eta=\frac{1}{1+\frac{1-\eta_0}{\eta_0}e^{-\Psi(0)}}. \tag{7}$$

Inserting the Boltzmann distributions into Poisson's equation yields the Poisson–Boltzmann equation

$$l_D^2\Psi''(x)=\begin{cases} sinh\Psi(x)-2(1-\phi)p_0\dfrac{l_D}{l}\dfrac{e^{-\Psi}}{q} & 0<x<l \\ sinh\Psi(x) & l\le x<\infty, \end{cases} \tag{8}$$

where the Debye screening length l_D is defined through $1/l_D^2=8\pi l_B(n_0+n_p^0)$, and where we have introduced the dimensionless parameter $p_0=2\pi l_B l_D/a$. The Poisson–Boltzmann equation splits into two

parts, one within the headgroup region $0<x<l$ (upper line in Eq. (8)) and one in the aqueous region $l\le x<\infty$ outside the headgroups (lower line in Eq. (8)). At $x=l$ the potential must be continuous and smooth. The presence of the partition sum q renders the Poisson–Boltzmann equation to be an integral–differential equation. The two boundary conditions for Eq. (8) are $\Psi'(x=0)=-4\pi l_B\sigma/e$ and $\Psi'(x\to\infty)=0$. The condition at $x=0$ reflects the surface charge density $\sigma=-(1-\phi+\eta\phi)e/a$ of the membrane. Inserting η from Eq. (7) leads to

$$l_D\Psi'(0)=2p_0\left(1-\phi+\frac{\phi}{1+\frac{1-\eta_0}{\eta_0}e^{-\Psi(0)}}\right). \tag{9}$$

Solving the Poisson–Boltzmann equation numerically yields $\Psi(x)$ and from that the ion concentrations $n_+(x)$, $n_p(x)$, $n_-(x)$, the degree of deprotonation η, and the probability distribution $P(x)$. With that we are able to calculate the free energy f in Eq. (3) numerically.

The nonideality parameter χ represents only nonelectrostatic lipid–lipid interactions. We employ χ to calculate the binodal line beyond which, hypothetically, the membrane becomes unstable with respect to lateral phase separation. Shifts of the binodal line upon changing the solution pH reflect the influence of electrostatic interactions on the membrane's degree of nonideality. Calculating these changes for different l (the separation of the charges within the zwitterionic headgroup) reveals the role of the zwitterionic lipids. This is the approach pursued in the present work. Alternatively, one could set $\chi\equiv 0$ and lump the electrostatic interactions into a corresponding nonideality parameter χ_{el} (and possibly also an asymmetry parameter τ_{el}). However, we find this approach less appropriate because $f_{el}(\phi)$, reflecting long-ranged interactions, does generally not follow a simple $\chi_{el}\phi(1-\phi)[\tau_{el}(2\phi-1)]$ dependence.

3. Results and discussion

In all calculations we use a cross-sectional area per lipid $a=0.65$ nm^2, Bjerrum length $l_B=0.7$ nm, and Debye length $l_D=1$ nm, corresponding to a 0.1 M salt solution. We thus have $p_0=2\pi l_B l_D/a=6.9$.

Our model accounts for the presence of the zwitterionic lipids by the length l between the two headgroup charges. We first consider the limit $l=0$ where the zwitterionic lipids are modeled as neutral entities that do not influence the electrostatic properties of the membrane. In this case, the Poisson–Boltzmann equation, Eq. (8), reduces to $l_D^2\Psi''(x)=\sinh\Psi(x)$ in the entire region $0\le x<\infty$, and the boundary condition in Eq. (9) becomes

$$l_D\Psi'(0)=\frac{2p_0\phi}{1+\frac{1-\eta_0}{\eta_0}e^{-\Psi(0)}}. \tag{10}$$

Integrating the Poisson–Boltzmann equation once yields $\Psi'(x)=-(2/l_D)\sinh(\Psi(x)/2)$. At the particular position $x=0$ we can combine that equation with the boundary condition in Eq. (10), resulting in the transcendental equation

$$\Psi(0)=-2\operatorname{arcsinh}\left[\frac{p_0\phi}{1+\frac{1-\eta_0}{\eta_0}e^{-\Psi(0)}}\right] \tag{11}$$

for the surface potential $\Psi(0)$. Eq. (11) can be solved numerically; results are shown in Fig. 2. For $\eta_0=1$ we obtain the familiar expression $\Psi(0)=-2\operatorname{arcsinh}(2\pi l_B l_D\phi/a)$ [36] for a planar surface of lateral area a/ϕ per charge and Debye screening length l_D. The inset in Fig. 2 displays the corresponding relation $\eta=\eta(\eta_0)$ according to Eq. (7). The negative surface potential $\Psi(0)$ favors protonation, leading to $\eta\le\eta_0$. With known $\Psi(0)$ we can calculate the free energy f_{el} in Eq. (4). In fact, the calculation of f_{el} in the limit $l=0$ becomes straightforward because it can be expressed directly in terms of $\Psi(0)$, η_0, and ϕ, namely

$$\frac{f_{el}}{k_BT}=\frac{2}{p_0}\left[\frac{\Psi(0)}{2}\sinh\frac{\Psi(0)}{2}-\cosh\frac{\Psi(0)}{2}+1\right]+\phi\left[\eta\ln\frac{\eta}{\eta_0}+(1-\eta)\ln\frac{1-\eta}{1-\eta_0}\right] \tag{12}$$

where η is given in Eq. (7). Let us briefly discuss the two limiting cases, $\eta_0=0$ and $\eta_0=1$, corresponding to pH $\ll$ pKa and pH $\gg$ pKa, respectively. For $\eta_0=0$ none of the membrane lipids carries charge anymore because all acidic lipids are protonated and the zwitterionic lipids are treated as neutral entities in the limit $l=0$. The free energy $f=f_{BW}$ then reduces to the familiar Bragg–Williams expression in Eq. (5). In the other limit, for $\eta_0=1$, all acidic lipids are deprotonated, and the free energy $f=f_{BW}+f_{el}$ contains the additional electrostatic contribution

$$\frac{f_{el}}{k_BT}=2\phi\left[\frac{1-\sqrt{1+(\phi p_0)^2}}{\phi p_0}+\ln\left(\sqrt{1+(\phi p_0)^2}+\phi p_0\right)\right]. \tag{13}$$

As discussed previously [37], for $p_0\gg 1$ the critical point shifts from $\chi_c=2$ for $\eta_0=0$ to $\chi_c=2+\sqrt{3}\approx 3.7$ for $\eta_0=1$. The increase in χ_c upon adding f_{el} reflects the repulsive nature of the electrostatic lipid–lipid interactions. Hence, changes in pH (and thus η_0) will affect the nonideality of the membrane. This is demonstrated in Fig. 3 which shows two examples for $f(\phi)$, one calculated for $\eta_0=0.30$ and the other for $\eta_0=0.99$ (the two solid lines), both for fixed $\chi=2.8$ and $l=0$. The curve for $\eta_0=0.30$ allows for a common-tangent construction, indicating separation into two coexisting phases of composition ϕ_1 and ϕ_2. In contrast, the curve for $\eta_0=0.99$ is convex everywhere and thus corresponds to a laterally stable membrane. Calculating the coexisting compositions for different χ leads to a binodal line that can

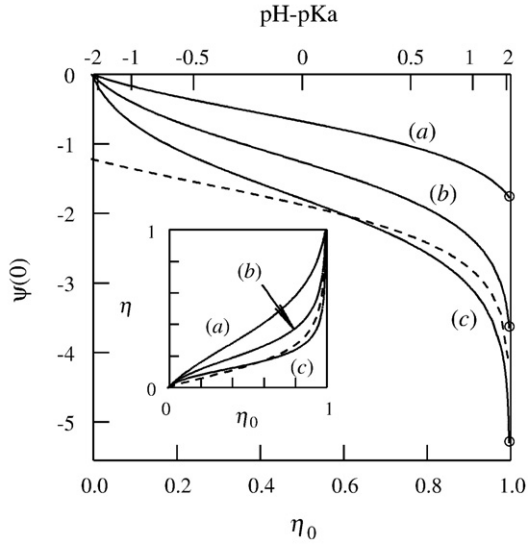

Fig. 2. The surface potential $\Psi(0)$ for $l=0$ and $l_D=1$ nm as function of η_0 for $\phi p_0=1$ (a), $\phi p_0=3$ (b), and $\phi p_0=6.9$ (c). The circles mark the finite values for $\Psi(0)$ at $\eta_0=1$ (which according to Eq. (1) corresponds to the hypothetical limit pH–pKa $\to\infty$) where the deprotonation is complete. In the other limit, for $\eta_0=0$ (corresponding to pH–pKa $\to-\infty$), all acidic lipids are protonated, and the potential $\Psi(0)$ must vanish. Note that $\Psi(0)$ is calculated by numerically solving Eq. (11). The inset shows the corresponding relation $\eta(\eta_0)$, again for $\phi p_0=1$ (a), $\phi p_0=3$ (b), and $\phi p_0=6.9$ (c). The broken line (in both the main figure and the inset) is derived for $l=0.5$ nm, $l_D=1$ nm, and $\phi=0.44$, leading $\phi p_0=3$.

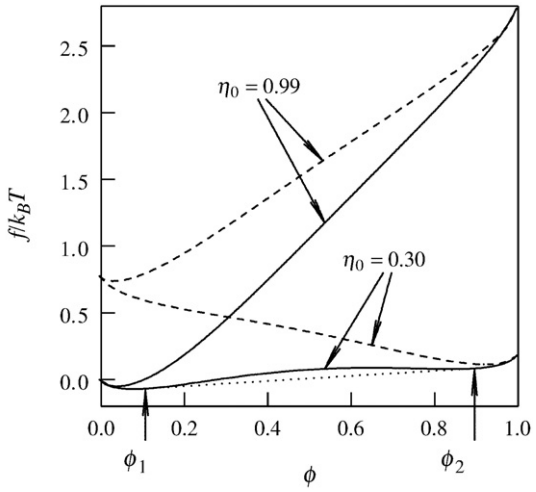

Fig. 3. The total free energy per lipid f (in units of k_BT) as function of ϕ for $\chi = 2.8$. Broken and solid lines correspond to $l = 0.5$ nm and $l = 0$, respectively. Values of η_0 are indicated. For one curve ($l = 0$ and $\eta_0 = 0.30$) the common tangent (dotted line) is shown, and the two corresponding coexisting compositions ϕ_1 and ϕ_2 are marked.

be plotted in a phase diagram $1/\chi$ vs. ϕ. Fig. 4 displays such a phase diagram, derived for $l = 0$. Different binodals correspond to different values of η_0. The inset shows how the critical point χ_c (i.e., the point where $\chi'(\phi) = 0$) shifts as function of η_0. The results suggest that increasing pH (and thus increasing η_0) generally increases the stability of the membrane. In agreement with this, χ_c increases monotonously with η_0 in the inset of Fig. 4.

Let us now consider the case $l = 0.5$ nm which accounts for the dipolar character of the zwitterionic lipid headgroups. In this case, we have solved the modified Poisson–Boltzmann equation, Eq. (8), subject to the boundary condition given by Eq. (9). Fig. 2 displays an example for how η_0 affects the potential $\Psi(0)$ and the degree of deprotonation η (see the broken line in the main diagram and in the inset), both derived for $\phi = 0.44$, corresponding to $\phi p_0 = 3$. Clearly, the electrostatic contribution of the zwitterionic lipids shifts the potential $\Psi(0)$ to more negative values and thus leads to a lower degree of deprotonation η (compare curve (b) with the broken line in Fig. 2 and in the inset of Fig. 2). These results are a consequence of the close proximity of the phosphate groups belonging to the zwitterionic and acidic lipids, both being located at $x = 0$.

For known $\Psi(x)$ we can calculate the electrostatic free energy in Eq. (4) conveniently using the expression

$$\begin{aligned}\frac{f_{el}}{k_BT} &= -\frac{1}{2}\Psi(0)(1-\phi+\eta\phi)\\ &+ \frac{1}{4p_0 l_D}\int_0^\infty dx[\Psi sinh\Psi - 2cosh\Psi + 2]\\ &-(1-\phi)\left\{\frac{1}{2}\frac{\int_0^l dx\Psi e^{-\Psi}}{\int_0^l dx e^{-\Psi}} + ln\left[\frac{1}{l}\int_0^l dx e^{-\Psi}\right]\right\}\\ &+\phi\left[\eta ln\frac{\eta}{\eta_0} + (1-\eta)ln\frac{1-\eta}{1-\eta_0}\right]\end{aligned} \tag{14}$$

where η is given in Eq. (7). Note that we obtain Eq. (14) from Eq. (4) after some algebra, using the Poisson–Boltzmann equation (Eq. (8)) and the equilibrium expressions for $n_\pm$, n_p, P and η. In Fig. 3 we show two calculations of f with $\chi = 2.8$. The two broken lines in Fig. 3 both correspond to $l = 0.5$ nm; one is derived for $\eta_0 = 0.30$ and the other for $\eta_0 = 0.99$. Here, in contrast to the case $l = 0$, both values for η_0 lead to an unstable membrane for $\chi = 2.8$. Hence, because for $\eta_0 = 0.99$ and $l = 0$ we obtain a stable membrane but an unstable membrane for $\eta_0 = 0.99$ and $l = 0.5$ nm, we conclude that, at least in this particular case, the electrostatic contribution of the zwitterionic lipids renders the membrane more unstable. With regard to Fig. 3 we also note that the free energy for $\phi = 0$ corresponds to a membrane consisting of only zwitterionic lipids. For $l = 0$ the membrane appears as a neutral surface and f thus vanishes. Yet, for $l = 0.5$ nm the zwitterionic lipids contribute significantly to the electrostatic free energy. In fact, this free energy is larger than that of a pure ($\phi = 1$) acidic membrane as long as $\eta_0 \lesssim 0.7$. We have calculated the phase diagram for $l = 0.5$ nm; it is displayed in Fig. 5. Different binodal lines correspond to different η_0 as indicated. The inset shows the critical point χ_c as function of η_0. Comparison of the phase diagrams in Figs. 4 and 5 reveals considerably smaller shifts of the binodal lines as η_0 changes. This is the central finding of the present work. Hence, *the electrostatic properties of the zwitterionic lipids generally weaken the tendency of acidic lipids to increase the lateral membrane stability as η_0 increases*. For $\eta_0 \lesssim 0.8$ this stabilizing tendency may even be reversed. This is because the binodal lines do not change monotonously; for growing η_0 they first move up in the $1/\chi$ vs. ϕ diagram, reaching maximal values at about $\eta_0 \approx 0.8$, and then decrease. Differently expressed,

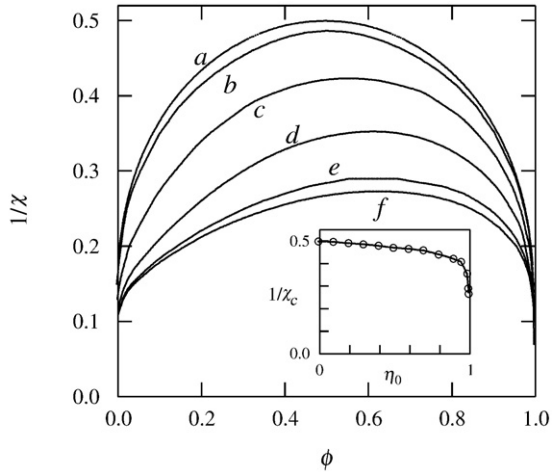

Fig. 4. Binodal lines for $\eta_0 = 0.0$ (a), $\eta_0 = 0.3$ (b), $\eta_0 = 0.9$ (c), $\eta_0 = 0.99$ (d), $\eta_0 = 0.999$ (e), and $\eta_0 = 1.0$ (f). All binodals are calculated for $l = 0$ thus ignoring the electrostatic properties of the zwitterionic lipids. The inset shows the dependence of the inverse critical point $1/\chi_c$ on η_0. Here, calculated points are marked by open circles connected by straight lines.

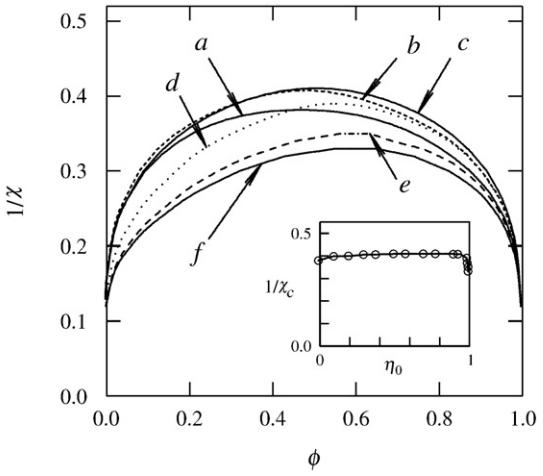

Fig. 5. Binodal lines for $\eta_0 = 0.0$ (a), $\eta_0 = 0.3$ (b), $\eta_0 = 0.9$ (c), $\eta_0 = 0.99$ (d), $\eta_0 = 0.999$ (e), and $\eta_0 = 1.0$ (f). All binodals are calculated for $l = 0.5$ nm thus accounting for the electrostatic properties of the zwitterionic lipids. The inset shows the dependence of the inverse critical point $1/\chi_c$ on η_0. Here, calculated points are marked by open circles connected by straight lines.

deprotonation of the acidic lipid would always be predicted to increase membrane stability if the electrostatics of the zwitterionic lipids is neglected ($l=0$). Without neglecting it ($l=0.5$ nm) deprotonation leads to considerably smaller membrane stabilization or may even act toward destabilization. In summary, our model predicts the electrostatic properties of zwitterionic lipids to have a significant influence on the nonideality of the membrane. Zwitterionic lipids moderate (or even reverse) the stabilizing influence that the charges of acidic lipids exert.

We provide an interpretation of our findings concerning the influence of zwitterionic lipids on the stability of a mixed acidic–zwitterionic membrane. To this end, consider the two limiting cases $l=0$ and $l\gg l_D$, each for $\eta_0=0$ and $\eta_0=1$. For $l=0$ and $\eta_0=0$ all acidic lipids are protonated (and hence uncharged) and remain so for any composition ϕ. At the same time, the headgroups of the zwitterionic lipids do not contribute to the electrostatic energy. Hence, the free energy reduces to its nonelectrostatic contribution f_{BW}, implying the binodal line of curve (a) in Fig. 4. For $l=0$ and $\eta_0=1$ all acidic lipids carry a single negative charge, and the corresponding electrostatic repulsion opposes any nonelectrostatic tendency to demix. Thus, electrostatics acts toward stabilizing the membrane, shifting the binodal in Fig. 4 from curve (a) to curve (f). Now consider the case $l\gg l_D$. Here, the positively charged moiety of the zwitterionic headgroup is able to reside at any position x within the diffuse double layer normal to the membrane. Hence, it effectively acts as a counterion for the negative charges at $x=0$ (and is indistinguishable from the other positively charged mobile salt and coions in the system). For $\eta_0=0$ the electrostatic contribution to the free energy is then given by f_{el} according to Eq. (13) with the replacement $\phi\rightarrow(1-\phi)$. The corresponding binodal line is that of curve (f) in Fig. 4. Finally, for $\eta_0=1$ all phosphate groups are charged irrespective of whether they belong to an acidic or zwitterionic lipid. The electrostatic contribution to the free energy is a constant for all compositions, namely f_{el} according to Eq. (13) with $\phi=1$. Thus, because only the nonelectrostatic contribution to f depends on composition, the binodal corresponds to curve (a) in Fig. 4. In summary, we see that for $l=0$ and $l\gg l_D$ we obtain the same pair of binodal lines but with an exchange of $\eta_0=0$ and $\eta_0=1$. That is, for $l=0$ growing pH opposes a tendency of the membrane to demix whereas for $l\gg l_D$ it enhances that tendency. If l increases from $l=0$ to $l\gg l_D$ the binodal lines for $\eta_0=0$ and $\eta_0=1$ switch their places in the phase diagram. Intermediate l (with l being on the same order of magnitude as l_D) are then expected to give rise to a narrower, more condensed set of binodal lines. This is indeed what we observe in Fig. 5 for the particular case $l=l_D/2=0.5$ nm.

Our predictions are derived for fixed temperature and for a planar bilayer. With regard to changing the temperature we point out that, indeed, our model would allow to compute the change of the electrostatic free energy. More important, however, is the temperature dependence of the membrane-associated solvent, which is not included in our model. Hence, the prediction of calorimetric quantities is beyond the scope of the present model. In contrast to temperature, membrane curvature can be included into our model by considering curved (i.e. cylindrical and spherical) geometry. Given a motivating experimental observation, this would be an interesting extension of the present model.

4. Conclusions

We have analyzed a model (based on the Poisson–Boltzmann approach) for the influence of pH changes on the degree of nonideality in a binary acidic–zwitterionic lipid bilayer. Our model predicts that, if the electrostatic properties of the zwitterionic lipids are ignored, then increasing the charge of acidic lipids through an elevation in pH strongly opposes any tendency of the lipids to demix [25]. Accounting for the electrostatic properties of the zwitterionic lipids moderates this tendency (and may even reverse it to a small extent). Hence, increasing pH may not, or only to a minor degree, contribute to an electrostatic membrane stabilization. A stabilizing effect (as observed for PA [16] and some mixtures involving PG [17]) may still occur, indirectly, through changes in the cross-sectional area per lipid and corresponding modifications of nonelectrostatic interactions.

We finally reiterate that our present mean-field approach does not intend quantitative comparison with experimental results. This is even less so because we employ a number of structural assumptions that are known to be inaccurate. Among these are the neglect of the solvent structure and of the finite sizes of all involved ions, the assumption of a smooth and planar hydrocarbon core–headgroup interface, and the usage of a constant and uniform dielectric constant for water even within the headgroup region. On a qualitative level, however, our model has been used previously to reproduce experimentally and computationally observed differences of the average area per lipid and headgroup tilt angles for mixed zwitterionic-anionic and mixed zwitterionic–cationic membranes [31]. This makes us confident that our present model is capable to also reveal the mechanism of how zwitterionic lipids affect the stability of acidic membranes that exhibit a protonation/deprotonation equilibrium.

Acknowledgment

This work was supported by NSF through grant DMR-0605883. The authors would like to thank Dr. Alexander Wagner for illuminating discussions.

References

[1] M. Langner, K. Kubica, The electrostatics of lipid surfaces, Chem. Phys. Lipids 101 (1) (1999) 3–35.
[2] P.F.F. Almeida, Thermodynamics of lipid interactions in complex bilayers, Biochim. Biophys. Acta Biomembr. 1788 (1) (2009) 72–85.
[3] K. Simons, E. Ikonen, Functional rafts in cell membranes, Nature 387 (1997) 569–572.
[4] J.Y. Huang, J.E. Swanson, A.R.G. Dibble, A.K. Hinderliter, G.W. Feigenson, Nonideal mixing of phosphatidylserine and phosphatidylcholine in the fluid lamellar phase, Biophys. J. 64 (2) (1993) 413–425.
[5] T. Heimburg, B. Angerstein, D. Marsh, Binding of peripheral proteins to mixed lipid membranes: effect of lipid demixing upon binding, Biophys. J. 76 (5) (1999) 2575–2586.
[6] A. Hinderliter, P.F.F. Almeida, C.E. Creutz, R.L. Biltonen, Domain formation in a fluid mixed lipid bilayer modulated through binding of the C2 protein motif, Biochem. 40 (13) (2001) 4181–4191.
[7] J. Zhang, B. Jing, V. Janout, S.L. Regen, Detecting cross talk between two halves of a phospholipid bilayer, Langmuir 23 (17) (2007) 8709–8712.
[8] T. Ahn, C.H. Yun, Phase separation in phosphatidylcholine/anionic phospholipid membranes in the liquid-crystalline state revealed with fluorescent probes, J. Biochem. 124 (3) (1998) 622–627.
[9] A. Hermelink, G. Brezesinski, Do unsaturated phosphoinositides mix with ordered phosphadidylcholine model membranes? J. Lipid Res. 49 (9) (2008) 1918–1925.
[10] E.R. Lamberson, L.R. Cambrea, J.C. Rochet, J.S. Hovis, Path dependence of three-phase or two-phase end points in fluid binary lipid mixtures, J. Phys. Chem. B 113 (11) (2009) 3431–3436.
[11] S.L. Veatch, S.L. Keller, Seeing spots: complex phase behavior in simple membranes, Biochim. Biophys. Acta 1746 (3) (2005) 172–185.
[12] J.A. Poveda, J.A. Encinar, A.M. Fernandez, C.R. Mateo, J.A. Ferragut, J.M. Gonzalez-Ros, Segregation of phosphatidic acid-rich domains in reconstituted acetylcholine receptor membranes, Biochem. 41 (40) (2002) 12253–12262.
[13] C. Johann, P. Garidel, L. Mennicke, A. Blume, New approaches to the simulation of heat-capacity curves and phase diagrams of pseudobinary phospholipid mixtures, Biophys. J. 71 (6) (1996) 3215–3228.
[14] P. Garidel, C. Johann, A. Blume, The calculation of heat capacity curves and phase diagrams based on regular solution theory, J. Therm. Anal. Calorim. 82 (2) (2005) 447–455.
[15] H.T. Davis, Statistical Mechanics of Phases, Interfaces and Thin Films, VCH publishers, New-York, 1996.
[16] P. Garidel, C. Johann, A. Blume, Nonideal mixing and phase separation in phosphatidylcholine phosphatidic acid mixtures as a function of acyl chain length and ph, Biophys. J. 72 (5) (1997) 2196–2210.
[17] P. Garidel, C. Johann, L. Mennicke, A. Blume, The mixing behavior of pseudobinary phosphatidylcholine phosphatidylglycerol mixtures as a function of pH and chain length, Eur. Biophys. J. 26 (6) (1997) 447–459.

[18] P. Garidel, A. Blume, Miscibility of phosphatidylethanolamine-phosphatidylglycerol mixtures as a function of pH and acyl chain length, Eur. Biophys. J. 28 (8) (2000) 629–638.
[19] P. Garidel, A. Blume, Miscibility of phospholipids with identical headgroups and acyl chain lengths differing by two methylene units: Effects of headgroup structure and headgroup charge, Biochim. Biophys. Acta, Biomembr. 1371 (1) (1998) 83–95.
[20] P. Garidel, A. Blume, Interaction of alkaline earth cations with the negatively charged phospholipid 1, 2-dimyristoyl-sn-glycero-3-phosphoglycerol: A differential scanning and isothermal titration calorimetric study, Langmuir 15 (17) (1999) 5526–5534.
[21] P. Garidel, A. Blume, Calcium induced nonideal mixing in liquid-crystalline phosphatidylcholine-phosphatidic acid bilayer membranes, Langmuir 16 (4) (2000) 1662–1667.
[22] D. Andelman, in: R. Lipowsky, E. Sackmann (Eds.), Electrostatic properties of membranes: the poisson–boltzmann theory, 2nd Edition, Structure and Dynamics of Membranes, vol. 1, Elsevier, Amsterdam, 1995, pp. 603–642, Section 12.
[23] M.L. Berkowitz, D.L. Bostick, S. Pandit, Aqueous solutions next to phospholipid membrane surfaces: Insights from simulations, Chem. Rev. 106 (4) (2006) 1527–1539.
[24] B.W. Ninham, W. Parsegian, Electrostatic potential between sufraces bearing ionizable groups in ionic equilibrium with physiologic saline solution, J. Theoretical Biol. 31 (3) (1971) 405–428.
[25] H. Trauble, M. Teubner, P. Woolley, H. Eibl, Electrostatic interactions at charged lipid-membranes.1. Effects of ph and univalent cations on membrane structure, Biophys. Chem. 4 (4) (1976) 319–342.
[26] D.C. Prieve, E. Ruckenstein, Surface-potential of a double-layer interaction force between surfaces characterized by multiple ionizable groups, J. Theoretical Biol. 56 (1) (1976) 205–228.
[27] S.H. Behrens, M. Borkovec, Electric double layer interaction of ionizable surfaces: charge regulation for arbitrary potentials, J. Chem. Phys. 111 (1) (1999) 382–385.
[28] N. Dan, Interactions between charge-regulating surface layers, Langmuir 18 (9) (2002) 3524–3527.
[29] P.M. Biesheuvel, Electrostatic free energy of interacting ionizable double layers, J. Colloid Interface Sci. 275 (2) (2004) 514–522.
[30] A. Hinderliter, R.L. Biltonen, P.F.F. Almeida, Lipid modulation of protein-induced membrane domains as a mechanism for controlling signal transduction, Biochem. 43 (22) (2004) 7102–7110.
[31] E.C. Mbamala, A. Fahr, S. May, Electrostatic model for mixed cationic–zwitterionic lipid bilayers, Langmuir 22 (11) (2006) 5129–5136.
[32] A. Haugen, S. May, The influence of zwitterionic lipids on the electrostatic adsorption of macroions onto mixed lipid membranes, J. Chem. Phys. 127 (21) (2007) 215104.
[33] D.H. Mengistu, S. May, Debye–Huckel theory of mixed charged–zwitterionic lipid layers, Eur Phys. J. E 26 (3) (2008) 251–260.
[34] D.H. Mengistu, S. May, Nonlinear Poisson–Boltzmann model of charged lipid membranes: accounting for the presence of zwitterionic lipids, J. Chem. Phys. 129 (12) (2008) 121105.
[35] D.H. Mengistu, K. Bohinc, S. May, Binding of DNA to zwitterionic lipid layers mediated by divalent cations, J. Phys. Chem. B 113 (36) (2009) 12277–12282.
[36] D.F. Evans, H. Wennerström, The Colloidal Domain, Where Physics, Chemistry, and Biology Meet, 2nd EditionVCH publishers, 1994.
[37] S. May, Stability of macroion-decorated lipid membranes, J. Physics-condensed Matter 17 (32) (2005) R833–R850.