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# Electrostatics of DNA-cationic lipid complexes: isoelectric instability

R. Bruinsma[a]

Physics Department, University of California, Los Angeles, CA, 90024, USA

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**Abstract.** We propose a Poisson-Boltzmann electrostatic theory for DNA/cationic lipid complexes modeled as a stack of aligned DNA chains intercalated with lipid bilayers, a structure suggested by the recent X-ray synchrotron studies of Radler *et al.* Poisson-Boltzmann theory is shown to predict that the isoelectric point – where the DNA and cationic lipid charges are in balance – is *unstable* against absorption of extra DNA or lipid material. The instability is caused by the entropy gain obtained following the release of small ions inside the complex and is manifested by singular behavior of the rod-rod spacing near the isoelectric point. We apply the theory to a discussion of the results of Radler *et al.*

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## 1 Introduction

The phase behavior of DNA chains dissolved in aqueous media has been extensively studied, partly because of biological interest and partly because the phase-diagram contains fascinating liquid crystalline phases [1]. Studies [2] of the interactions between DNA chains in aligned bundles report that the dominant interaction between chains at larger inter-chain spacings is electrostatic in nature, a consequence of the high charge per unit length of DNA (one charge per 1.7 Å). Recently, there has been a growing interest in the phase-behavior of aqueous mixtures of DNA with *cationic lipids* in the context of non-viral gene therapy strategies [3]. DNA can form complexes – called "lipoplexes" – with cationic lipids which can be used as vectors for injecting DNA into cells [4]. The cationic lipids neutralize the negative charge of DNA and allow the complex to approach the negatively charged surface of a cell and fuse with the cell, which is somewhat more difficult for naked DNA. Lipoplexes are undergoing clinical trials to test their therapeutic efficacy [5]. More speculatively, it is interesting from the viewpoint of the early evolution of cells to explore spontaneous association between DNA and lipids since this could give insight into the formation of cellular membranes [6].

The stability of lipoplexes raises interesting physical questions: why do they want to form in the first place and what is the nature of the interaction between DNA strands inside a lipoplex? The answer to the first question seems obvious: the negatively charged DNA should favor association with positively charged lipids. However, both positively charged liposomes and negatively charged DNA chains in solution are *already* closely surrounded by clouds of oppositely charged small ions, the "counter-ions". Bringing DNA chains in contact with cationic lipids is not by itself going to greatly reduce the free energy. The second question arises because it would be natural to assume that the internal structure of a lipoplex could be understood once we know the effective interaction between DNA chains inside the lipoplex.

The internal structure of a lipoplex was studied in recent X-ray scattering studies by Radler *et al.* [7]. A solution of DNA chains extracted from $\lambda$-phage virus was found to form lipoplexes when mixed with positively charged liposomes (bilayer vesicles with radii in the range of 500-700 Å). The liposomes themselves were mixtures of neutral lipids (such as DOPC or DOPE) and cationic lipids (such as DOTAP). Under salt-free conditions, this ternary mixture contained micron-sized lipoplexes which X-ray diffraction revealed to consist of well-organized stacks of lipid bilayers intercalated by aqueous layers containing parallel arrays of DNA chains. The structure of the lipoplexes was thus similar to that of a three-dimensional (3-D) smectic liquid crystal phase.

Radler *et al.* suggested that lipoplexes form through a mechanism familiar in biochemistry, but less studied in the physics literature, namely *counter-ion release* [8]. Applied to DNA/cationic lipid interaction, the counter-ion release mechanism works as follows. Start with a single DNA chain in solution. A certain fraction of its positive counter-ions are highly confined to the neighborhood of the chain ("Manning condensation" [9]). Similarly, a certain fraction of the negative counter-ions of cationic liposomes are confined to the liposome surface. If the DNA chain fuses with a cationic liposome, then equal numbers of positive and negative counter-ions can be released to the

[a] e-mail: bruinsma@physics.ucla.edu

bulk solution which allows them to increase their entropy. This entropic contribution of the counter-ions to the free energy is, as we will see below, of a magnitude comparable to the direct electrostatic contribution, so the "hidden physics" of counter-ion release could play an important role for the organization of the lipoplexes.

This is an attractive suggestion but it appears to lead to a difficulty: counter-ion release would seem to favor the "isoelectric point". The isoelectric point refers to that particular DNA/cationic lipid mixing ratio for which there is perfect charge compensation (see Fig. 1a). For a given cationic lipid surface charge, the isoelectric condition imposes a value on the rod spacing which we will denote by $R_{iso}$. The isoelectric spacing is determined by the condition of charge neutrality of an array of DNA chains and the two associated layers of cationic lipids. This is the case if $R_{iso} \equiv \dfrac{|\lambda|/e}{2\sigma_c}$ with $\lambda$ the charge per unit length of the DNA chains and with $\sigma_c$ the cationic lipid surface density (see Fig. 1). $R_{iso}$ is about 40 Å for the experiments of Radler *et al.* If the mixing ratio deviates from this isoelectric point, we should expect a certain amount of either excess DNA or excess lipid material (in the form of liposomes) to be in solution in phase coexistence with the complex. Chemical equilibrium between the solution with excess DNA or liposomes and the lipoplexes would lead us to expect rod spacings which are somewhat less than $R_{iso}$ for the case of excess DNA and rod spacings which are somewhat greater than $R_{iso}$ for the case of excess lipid. This was indeed observed but, surprisingly, when the lipid/DNA mixing ratio was varied (while keeping fixed the neutral/ionic lipid mixing ratio), the $R$ spacing appeared to undergo a discontinuous jump across $R_{iso}$, as if the lipoplex was trying to *avoid* the isoelectric point. Since, away from the isoelectric point, at least some of the bulk counter-ions must be confined to the lipoplex to maintain local charge neutrality (see Figs. 1b and c), it seems unclear how to interpret the apparent instability of the isoelectric point in the light of counter-ion release. The instability indicates that an isoelectric lipoplex has a high chemical "affinity" for both DNA chains and cationic lipid material. Isoelectric lipoplexes could be similarly "reactive" at the macromolecular level for other charged macromolecules, like proteins, and the reactivity could affect their fusion kinetics which may be important for the clinical applications.

Another problem with lipoplexes concerns the nature of the interaction energy between DNA rods inside a lipoplex. Suppose, as suggested by Radler *et al.*, that we consider the array of DNA chains as a two-dimensional (2-D) *lyotropic smectic*, with the DNA chains as the smectic lines and the lipid molecules as the embedding solvent. Adding extra lipid to a lipoplex is then analogous to diluting a 3-D lyotropic smectic by adding solvent. When, in the experiments of Radler *et al.*, the lipid concentration was significantly increased beyond the isoelectric point (by increasing the amount of cationic liposomes), the DNA spacing was found to saturate at a value of around 47 Å. Saturation of the layer spacings in 3-D lyotropic

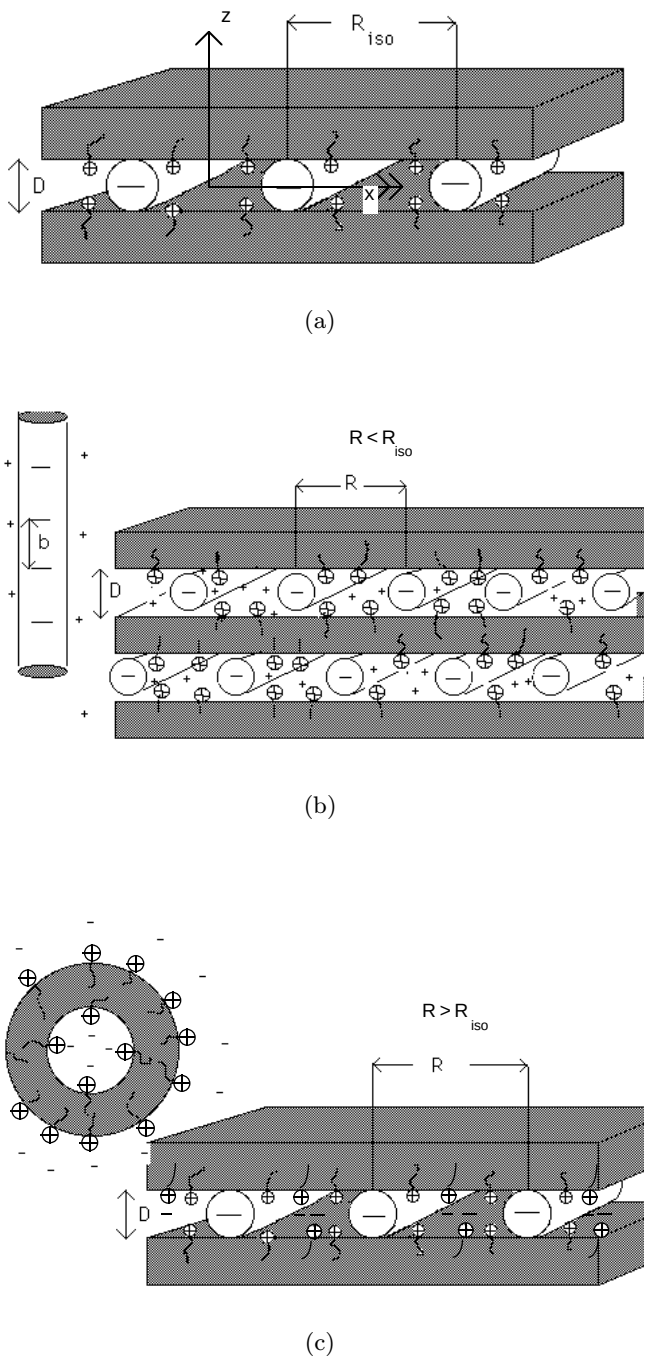


**Fig. 1.** Geometry used in the calculation. A parallel array of rods with spacing $R$ in an aqueous medium is confined between two dielectric slabs separated by a distance $D$. The rods are negatively charged, with mobile positive cations confined within the two boundary plates at $z = D/2$ and $z = -D/2$. Figure 1a shows the isoelectric point, where the cationic lipid charge compensates the rod charge. Figure 1b shows the case of excess DNA, and Figure 1c the case of excess liposome. The structures are not drawn to scale: in Figure 1b, $b$ is in reality less than 0.1 of $D$ while the liposome radius in Figure 1c should be more than 10 times $D$.

smectics upon dilution is generally associated with a situation where longer-range attractive interactions compete with shorter range (usually electrostatic) repulsive interactions, leading to an optimal spacing [10]. Attempts to swell much beyond this optimal spacing would produce phase separation. If the same explanation also holds for the saturation of the DNA chain spacing in the lipoplexes, then there should exist a fairly long-ranged attraction which competes with a shorter range electrostatic interchain repulsion. Van der Waals attraction or membrane deformation by the DNA chains indeed could produce DNA-DNA attractions [11]. The chain-chain interaction can be studied by analysis of the thermal diffuse contribution to the X-ray diffraction peaks [12]. Salditt *et al.* have analyzed the X-ray data of lipoplexes in this manner [13], but (so far) only found evidence for a long-range repulsion between the DNA chains. The saturation of the rod spacing is thus presenting us with a second puzzle.

A third problem is the *order of magnitude* of these rod-rod forces. In an earlier paper [14] we found that, within the mean-field Poisson-Boltzmann theory, the electrostatic force $f(R)$ per unit length between two charged rods inside a salt-free aqueous slab bounded by slabs with a low dielectric constant should depend on their spacing $R$ as $f(R) \approx \frac{k_B T D}{l_B R^2}$ (for $R \gg D$, with $D$ the spacing between the lipid bilayers and $l_B$ the Bjerrum length (of order 7 Å)). Both the order of magnitude of $f(R)$ and the dependence of $f(R)$ on $R$ are reasonably consistent with the X-ray analysis of Salditt *et al.* [13]. If attractive forces indeed play no important role, we would expect that the equilibrium spacing could be found by comparing the repulsive force $f(R)$ to $\tau/R$, with $\tau$ being the absorption energy per unit length of a DNA chain on a pair of layers of cationic lipids. This absorption energy, as will be shown below, is of order $k_B T/b$ (see Eq. (3.13)) with $1/b$ the line density of rod charges. Using the quoted result for $f(R)$, the rod-rod spacing would be expected to be of the order of $bD/l_B$. For DNA, $b$ is of order 1.7 Å and $D$ is of order 20 Å: the resulting rod-rod force spacing (about 5 Å) is much too short. The rod-rod repulsion would be too weak to prevent continued absorption until $R$ is of order $D$ when short-range forces like the hydration force become important. This is clearly in disagreement with experiment. Thus, even though the electrostatic repulsion $f(R)$ between the rods is probably important for long-wavelength thermal fluctuations of the rods around the mean spacing – as inferred from the analysis of thermal diffuse X-ray diffraction – it apparently does not play a significant role in determining the mean rod-rod spacing itself! Thus, we also run into a puzzle if we assume that repulsive forces of the form of $f(R)$ determine the rod spacing.

The formation of the DNA/lipid lipoplexes is clearly a less simple matter then would appear at first sight. In this paper we will study the electrostatic nature of lipoplexes from the viewpoint that *the dominant interaction is electrostatic* and for the simple model geometry of Figure 1. We will focus on the case that the rod-rod spacings are large compared to the rod diameter. This will allow us to avoid treating the detailed architecture of DNA chains and their association with cationic lipids and to focus instead on constructing a simple, analytically tractable, model which will let us examine the generic features of the puzzling electrostatics of the lipoplex formation, in particular the role of counter-ion release when a lipoplex is in chemical equilibrium with excess DNA or excess liposome material. The price is that we are restricted to lower surface concentrations of cationic lipids than those used experimentally. The aim of the study is in fact not so much to provide an analysis of the experimental results but rather to gain theoretical insight into the underlying physics and the "design criteria" of lipoplexes: what physical mechanism sets the rod spacing and what is the nature of the internal electrostatic structure of a lipoplex?

With these caveats in mind, we can summarize the results of the model as follows. An isoelectric complex is indeed found to have a high affinity for both DNA chains and for cationic lipid material. The driving mechanism is *partial* counter-ion release. If, for instance, a charged rod enters an isoelectric complex from solution, then the counter-ions of the rod still lower their electro-chemical potential $\mu = e\phi + k_B T \ln c$ – with $\phi$ the electrostatic potential and $c$ the counter-ion concentration – even though they must stay inside the complex (the counter-ions cannot be released into bulk since this is forbidden by charge neutrality). The reason is that, near the isoelectric point, the concentration $c$ of counter-ions inside a complex is still significantly lower than in the Manning-condensed layer of counter-ions near the rod. As a consequence, the entropic contribution to the electrochemical potential is higher for counter-ions inside a Manning layer. Counter-ions of a rod entering the complex in fact both can reduce the entropic contribution to their chemical potential and lower the electrostatic contribution, since repulsive interactions between counter-ions are weakened inside the complex as the counter-ions can move away from each other upon release. The entropic free energy gain is further enhanced by the fact that the condensed counter-ions on a rod suffer 1-D confinement, while in the complex they are only 2-D confined.

When the number of excess rods inside the complex starts to grow, the counter-ion concentration increases and the corresponding free energy gain decreases until an equilibrium spacing $R^*$ is reached. Within our model, we find that on the DNA rich side of the isoelectric point, this equilibrium spacing is given by:

$$\left(1 - \frac{R^*}{R_{iso}}\right) \cong (\Phi_{DNA})^{\frac{1}{2\xi}} \exp\left\{-\left[\ln\left(\frac{\pi^2\xi^2}{4(1-\xi)^2}\right) + \frac{2}{\xi}\ln(1-\xi)\right]\right\} \tag{1.1}$$

with $\Phi_{DNA}$ the volume fraction of excess DNA in solution (assumed small here) and with $\xi = l_B/b$ the Manning parameter (see Sect. 2). Note the singular dependence of the rod spacing on the volume fraction: for typical values of $\xi$ (around 4.1) the rod spacing increases very steeply close

to the isoelectric point $\Phi_{DNA} = 0$. This result is somewhat reminiscent of the behavior of domain wall spacings near a commensurate-incommensurate (CI) transition [15], but unlike the CI transition, the line-line pair interaction plays no important role in equation (1.1). The key is the dependence of the adsorption energy per unit length $\tau_{complex}(R)$ on the rod spacing $R$ through the overall counter-ion entropy which depends on $R$ (see for instance Eq. (3.12)).

A qualitative understanding of the origin of equation (1.1) can be obtained by considering the free energy of the counter-ions. The average concentration of the counter-ions inside the lipoplex must be $c = \frac{1}{D}\left(\frac{|\lambda|}{eR} - 2\sigma_c\right)$. The reason is that the lipoplex as a whole must be charge neutral so if we add the total charge per unit volume due to the counter-ions, the cationic lipids, and the DNA chains we must get zero. The entropic contribution to the free energy per unit length of a rod in the lipoplex due to counter-ion release then is of the order of $\frac{k_B T}{b} \ln\left(1 - \frac{R}{R_{iso}}\right)$ using the fact that $R_{iso} \equiv \frac{|\lambda|/e}{2\sigma_c}$. On the other hand, let $\lambda^* = \lambda/\xi$ be the well-known "Manning-renormalized" effective charge per unit length of a charged rod. Assume that the rod is in a dilute solution of rods with a volume fraction $\Phi = D^2/L^2$. The electrostatic self-energy per unit length of a rod is then of the order $\lambda^{*2} \ln L$ (recalling that infinite rods have an electrostatic potential which depends logarithmically on distance). To obtain chemical equilibrium between the rods in solution and rods inside the lipoplex, we equate the counter-ion free energy per unit length of rods inside the lipoplex to the electrostatic energy per unit length of rods in solution. Recalling that $\xi = l_B/b$ this condition leads to $\left(1 - \frac{R^*}{R_{iso}}\right) \propto (\Phi_{DNA})^{\frac{1}{2\xi}}$, which gives us the singular part of equation (1.1).

For the case of a lipoplex in contact with a dilute solution of cationic liposomes, we find a stable rod spacing which is essentially independent of the liposome concentration:

$$\left(\frac{R^*}{R_{iso}} - 1\right) \cong \sqrt{\frac{eD}{4l_c}} \tag{1.2}$$

with $l_c$ the Chapman length (defined in Sect. 2). The reason for the discontinuity is again partial counter-ion release. The negative counter-ions of a cationic liposome are already constrained to move along a surface: there is no "dimensionality effect". However, since there are again no counter-ions inside a perfectly isoelectric complex, the counter-ions attached to a liposome still can greatly reduce their electro-chemical potential by entering the complex.

The PB calculation which leads to equation (1.2) provides intuitive insight into the question why the rod-rod spacing does not grow indefinitely when we increase the lipid/DNA mixing ratio beyond the isoelectric point. Saturation of the rod spacing is found to be due to the electrostatic repulsion between the lipid layers. Imagine a sandwich consisting of two flexible parallel surfaces of area $L$ by $L$ that repel each other. Assume that the two surfaces are held together by a certain number $N$ of aligned rods of length $L$, located in between the two surfaces. The rods locally fix the spacing between the two surfaces to be $D$. The spacing $R$ between the rods is adjustable. If the rods are uniformly distributed over the sandwich, then $R = L/N$. If we now reduce $N$, we must increase $R$ to maintain the uniform distribution. However, in the small $N$ limit it is energetically more favorable to collect all the rods in a small part of the sandwich in an array with $R$ finite, and to allow the two surfaces in the remaining part of the sandwich to separate, leading to spacings large compared to $D$. This mechanism applies even if the rods repel each other. We find that it dominates for rod spacings large compared to an effective Debye screening length. In that regime, the cationic lipid material in one layer repels the other layer in much the same way as the cationic lipid material in the bilayer of a liposome repels the other side. The separation of the two surfaces in the toy model just discussed corresponds to the formation of liposome material. This mechanism competes with the counter-ion release effect. The balance between these effects is represented by equation (1.2).

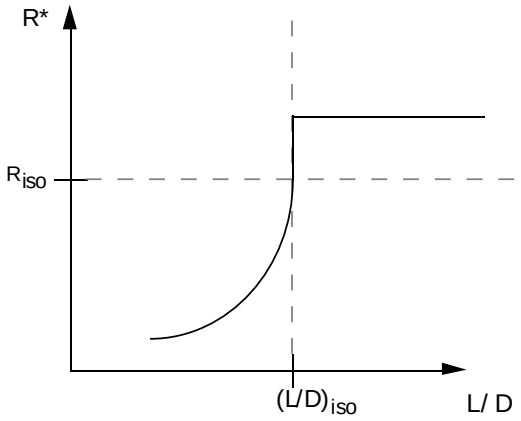


**Fig. 2.** Dependence of the rod spacing $R^*$ on the lipid/DNA ratio $L/D$, for fixed neutral/ionic lipid ratio, as predicted by equations (1.1, 1.2). $(L/D)_{iso}$ is the isoelectric point and $R^*_{iso}$ is the spacing at the isoelectric point.

The combined dependence of the DNA spacing upon the lipid-to-DNA mixing ratio predicted by equations (1.1, 1.2), is shown in Figure 2. We conclude that the DNA spacing indeed appears to avoid the isoelectric point: the instability of the isoelectric point is consistent with PB theory through the mechanism of counter-ion release.

We now will define our model (in Sect. 2) and then proceed to substantiate, in Sections 3 and 4, the above claims. We finish in Section 5 with a summary, a discussion of the limitations of the model, and possible experiments which could be done to test the validity.

## 2 Poisson-Boltzmann theory of model lipoplexes

Based on the experiments of Radler *et al.*, we model the lipoplex as a parallel array of cylindrical rods with a negative charge per unit length equal to $-\lambda$, and a rod-rod spacing $R$ (see Fig. 1a). The rods are oriented along the $y$-axis with the center-lines located at $[x = \pm(m + 1/2)R,\ z = 0]$ ($m = 0, 1, 2,...$ is an integer) and they lie inside an aqueous slab of thickness 2D and a high dielectric constant $\varepsilon_w$. The slab is sandwiched between two semi-infinite volumes whose dielectric constant is assumed to be very small compared to that of water. The aqueous slab contains either positive counter-ions (for the case of excess liposome) or negative counter-ions (for the case of excess DNA) to assure charge neutrality.

Localized on the two boundary planes of the slab at $z = \pm D/2$ are equal amounts of mobile, positive charges with an average surface concentration $\sigma(x)$ which can vary with position $x$. They represent a 2-D solution of cationic lipids in a matrix of neutral lipids. We will exclude any specific association between DNA and cationic lipids (such an association would depend on the molecular architecture of the DNA and the lipids, which – as mentioned – is beyond the scope of the present calculation). By symmetry, the surface number density of cationic lipids must be the same on the two planes while, again for symmetry reasons, $\sigma(x) = \sigma(-x)$.

The lipoplex is assumed to be in chemical equilibrium with a salt-free solution containing either excess DNA rods, or excess lipid material (in the form of cationic liposomes), *but not both*. In other words the gain in free energy upon self-assembly of the lipoplex is sufficiently large that lipoplexes in a DNA/cationic lipid solution will continue to form until either the supply of lipid or the supply of DNA has run out. In the first case (see Fig. 1b), chemical equilibrium requires that the number of DNA chains inside the lipoplex is somewhat greater than that at the isoelectric point. The lipoplex will then contain only positive counter-ions to maintain charge neutrality. In the second case (see Fig. 1c), the amount of cationic lipid material inside the complex exceeds the amount required for isoelectricity, and the lipoplex will contain only negative counter-ions. The lipoplex thus will never contain both positive and negative counter-ions. We can come to the same conclusion that the slabs cannot contain both positive and negative counter-ions by noting that we could remove pairs of positive and negative counter-ions from the lipoplex without violating charge neutrality and transfer them to the (salt-free) solution where they would gain a free energy of order $k_BT \ln V$, with $V$ the system volume. In the limit $V \to \infty$ this would always off-set any finite loss in electrostatic enthalpy suffered by the removal of the counter-ions from the lipoplex.

Let $c(\mathbf{r})$ be the number density of either positive or negative counter-ions inside the slab. Charge neutrality and translational invariance require that:

$$\pm e \int_{-D/2}^{D/2} dz \int_{-R/2}^{R/2} dx\ c(x,z) + 2e \int_{-R/2}^{R/2} dx\ \sigma(x) = |\lambda| \tag{2.1}$$

with the upper sign referring to excess DNA and the lower sign to excess lipid. The electrostatic potential $\phi(x,z)$ and the number density $c(x,z)$ must obey Poisson's equation:

$$\frac{\partial^2\phi(x,z)}{\partial x^2} + \frac{\partial^2\phi(x,z)}{\partial z^2} = \mp\frac{4\pi e}{\varepsilon_w} c(x,z). \tag{2.2}$$

In Poisson-Boltzmann (PB) theory [16], it is assumed that both the (monovalent) lipid and counter-ion concentrations obey the Boltzmann distribution:

$$\sigma(x) = \sigma_0 \exp\left[-\left(\frac{e\phi(x, z = \pm D/2)}{k_BT}\right)\right]$$
$$c(x,z) = c_B \exp\left[\mp\left(\frac{e\phi(x,z)}{k_BT}\right)\right]. \tag{2.3}$$

Here, $\sigma_0$ and $c_B$ are constants independent of position.

The constant $c_B$ is determined as follows. The counter-ions inside the lipoplex must be in chemical equilibrium with those in solution. If we set the electrostatic potential to zero in the bulk solution (away from excess DNA or cationic liposomes) then the constant $c_B$ must correspond to the ion concentration in solution. The constant $\sigma_0$ is determined by the following condition. For the case of excess DNA, the lipoplexes have exhausted all the lipid material. The average cationic lipid surface concentration then must be equal to $\sigma_c$, the (surface) concentration of cationic lipids of the liposomes from which the DNA/lipid solution was prepared. We thus must demand:

$$\int_{-R/2}^{R/2} dx\ \sigma(x) = R\sigma_c. \tag{2.4}$$

To find $\sigma_0$ for the case of excess liposomes, recall that the lipoplexes are produced by the fusion of a certain number of liposomes whose (surface) concentration of cationic lipid was $\sigma_c$. This means that equation (2.4) must be valid as well for a newly formed lipoplex. There are now two possibilities: if the lipid molecules are (even weakly) hydrosoluble then lipid material can be exchanged between the liposomes and the lipoplexes. In that case, $\sigma_0$ is determined by the condition that the cationic lipids in the lipoplexes and in the liposomes have the same electrochemical potential so equation (2.4) would not be valid. If the lipids are insoluble, as we will assume in this paper, then equation (2.4) remains valid also for excess liposomes.

It is helpful to define at this point a number of characteristic length scales. The Bjerrum length is defined as $l_B = e^2/\varepsilon_w k_BT$ (equal to 7.1 Å at room temperature for $\varepsilon_w \approx 80$); it is the separation between two unit charges when their Coulomb energy is equal to $k_BT$. The spacing between the rods at the isoelectric point is related to the mean cationic lipid surface concentration $\sigma_c$ by

$R_{iso} \equiv \dfrac{|\lambda|/e}{2\sigma_c}$, as noted earlier. Note also that $|\lambda|/e = 1/b$. From $\sigma_c$ we can also construct another important length scale namely $l_c = \dfrac{\varepsilon_w k_B T}{e^2 \sigma_c}$, which is the Chapman length. For a 2-D charged surface, the Chapman length is approximately the width of the layer of condensed counter-ions near the surface. We can write the mean cationic lipid surface density in terms of the Chapman and Bjerrum lengths as $\sigma_c = \dfrac{1}{l_B l_c}$ and the isoelectric spacing as $R_{iso} = \dfrac{\xi}{2} l_c$. The Manning parameter $\xi = l_B/b$ is the ratio of the characteristic monovalent rod electrostatic energy $\left(\dfrac{e^2}{\varepsilon_w b}\right)$ over the thermal energy $k_B T$. For $\xi$ larger than one, the DNA chains are surrounded by a sheath of counter-ions screening the bare charge such that the renormalized charge is a fraction $1/\xi$ of the bare charge [17].

If we insert equation (2.3) into equation (2.2) we recover the well-known Poisson-Boltzmann (PB) equation:

$$\frac{\partial^2 \phi(x,z)}{\partial x^2} + \frac{\partial^2 \phi(x,z)}{\partial z^2} = \mp \frac{4\pi e}{\varepsilon_w} c_0 e^{\mp\left(\frac{e\phi(x,z)}{k_B T}\right)}. \quad (2.5)$$

The *mixed* boundary conditions for equation (2.5) at $z = \pm D/2$ involving both the electrostatic potential and the normal electrical field are found by combining Gauss' Law with equation (2.3):

$$\frac{\partial \phi(x, z = \pm D/2)}{\partial z} = \pm \frac{4\pi e \sigma_0 e^{-\left(\frac{e\phi(x, z=\pm D/2)}{k_B T}\right)}}{\varepsilon_w} \cdot \quad (2.6)$$

(We neglect in Eq. (2.6) the electrical displacement outside the aqueous slab; in an earlier paper [14] (I) it was shown that this is a reasonable assumption provided $R \ll \dfrac{\varepsilon_w}{\varepsilon_l} D$ with $\varepsilon_l$ the lipid dielectric constant.) The cylindrical rods are assumed to have a surface charge density $\lambda/\pi D$ so the normal component of the electrical field at the rod surface is $E_n = -\dfrac{4\lambda}{\varepsilon_w D}$, thereby providing a complete set of boundary conditions.

After solving the PB equation with these mixed, nonlinear boundary conditions, we must compute the PB free energy $F(R)$ per rod per unit length:

$$\begin{aligned} F(R) = & \int_{-R/2}^{R/2} dx \int_{-D/2}^{D/2} dz \Big\{ k_B T c(x,z)[\ln(c(x,z)/c_0) - 1] \\ & \pm \frac{1}{2} e c(x,z) \phi(x,z) \Big\} \\ & + 2 \int_{-R/2}^{R/2} dx \Big\{ k_B T \sigma(x) \ln(\sigma(x)/\sigma_L) \\ & + k_B T (\sigma_L - \sigma(x)) \ln(1 - \sigma(x)/\sigma_L) \\ & + \frac{1}{2} e \sigma(x) \phi(x, z = D/2) \Big\} \\ & + \frac{\lambda}{2\pi D} \oint ds \phi(s). \end{aligned} \quad (2.7)$$

The first terms in curly brackets constitute the entropic contribution of the counter-ions to the free energy and their electrostatic energy; $c_0$ is the number concentration of bulk solvent molecules. The next set of terms involve the entropic and electrostatic contributions of the lipids, with $\sigma_L$ the total surface number concentration of lipids so $\sigma(x)$ and $\sigma_L - \sigma(x)$ are the surface number densities of charged and neutral lipids, respectively. We are assuming that the surface concentration of cationic lipids is low enough to allow usage of ideal-mixing theory. The last term is the electrostatic energy of the rods with the integral over $s$ running over the rod circumference.

The calculation outlined so far can be performed for any $R$, so it does not select any particular spacing between the rods. To select the $R$ value, we impose the condition that the free energy of the lipoplex cannot be lowered by absorbing or releasing either DNA chains or liposomes. In other words, we are demanding the equality of chemical potentials for either: (i) DNA inside the lipoplex and excess DNA; or (ii) lipid material inside the lipoplex and in the excess liposomes. This equilibrium condition translates into the following constraints. For case (i) (excess DNA) let the free energy of the lipoplex be $NF(R)$ with $N$ the number of chains in the lipoplex (and with $F(R)$ computed from Eq. (2.7)). Now insert one more chain *while keeping the total lipoplex surface area* $A = 2RNL$ *fixed* (with $L$ the length of the rods). We must impose this condition since the lipoplex has already exhausted all of the lipid material. The free energy cost per unit length for this chain-insertion operation is $\tau_{complex}(R) = \dfrac{\partial}{\partial N}[NF(R)]_{Fixed\ A}$, or:

$$\tau_{complex}(R) = F(R) - R \frac{d}{dR} F(R). \quad (2.8)$$

Our stability condition is satisfied provided $\tau_{complex}(R)$ equals the free energy per unit length $\tau_{solution}$ of a DNA chain in the bulk solution, computed within PB theory. We may consider $\tau_{complex}(R)$ as the chemical potential of DNA.

For case (ii) of excess liposome, let $L' = NR$ be the lateral size of the array so $NF(L'/N)$ is the free energy of the lipoplex. The free energy cost per unit area $\gamma_{complex}(R)$ required to introduce extra lipid material is found by taking the derivative of $NF(L'/N)$ with respect to $L'$ *while keeping the number* $N$ *of chains fixed*. since the lipoplex has exhausted the supply of DNA chains. The result is that:

$$\gamma_{complex}(R) = \frac{dF(R)}{dR} \quad (2.9)$$

which we can consider as the chemical potential of lipid material (at fixed neutral/ionic lipid mixing ratio). Stability of the lipoplex requires that $\gamma_{complex}(R)$ equals the free energy per unit area of the DNA-free liposome, again to be computed within the PB approximation.

### Limit of low cationic lipid concentrations

The program outlined above for the calculation of the rod spacing still presents a formidable analytical challenge since it requires solution of a non-linear [18] partial differential equation with complex, mixed non-linear boundary conditions (see Eq. (2.6)). We will restrict ourselves in this paper to studying the case of low cationic lipid concentrations, since this allows analytic treatment. To examine this regime, first define the $z$-averaged potential $\phi(x)$:

$$\phi(x) = \frac{2}{D}\int_0^{D/2} dz\phi(x,z). \tag{2.10}$$

Integrating the PB equation (Eq. (2.5)) over $z$ and utilizing the mixed boundary condition equation (2.6), we find that $\phi(x)$ obeys:

$$\frac{d^2}{dx^2}\phi(x) + \frac{8\pi e\sigma_0}{\varepsilon_w D}e^{-\left(\frac{e\phi(x,z=D/2)}{k_BT}\right)} = \mp\frac{8\pi e c_B}{\varepsilon_w D}\int_0^{D/2} dz e^{\mp\left(\frac{e\phi(x,z)}{k_BT}\right)}. \tag{2.11}$$

It follows from equation (2.6) that, far from the charged rods, the variation $\delta\phi$ of the electrostatic potential across the normal $z$-direction of the slab is of order $\delta\phi \cong \frac{e\sigma_c D}{\varepsilon_w}$. If $\frac{D}{l_c} \ll 1$, as indeed holds when $\sigma_c$ is small enough recalling that $l_c = 1/\sigma_c l_b$, then $e\delta\phi$ is small compared to $k_BT$, and the normal variation of the potential can be neglected in equation (2.11). Equation (2.11) then reduces to an *ordinary* non-linear differential equation:

$$\frac{d^2}{dx^2}\phi(x) + \frac{8\pi e\sigma_0}{\varepsilon_w D}e^{-\frac{e\phi(x)}{k_BT}} \cong \mp\frac{4\pi e}{\varepsilon_w}c_B e^{\mp\frac{e\phi(x)}{k_BT}}. \tag{2.12}$$

Equation (2.12) actually only holds provided we are at least a distance $D$ away from the surfaces of the charged rods since near the rods there is obviously a rapid normal variation of the potential.

To find the boundary conditions near $x = \pm R/2$ for the simplified differential equation (2.12), recall that $R_{iso} = \frac{\xi}{2}l_c$, so $R_{iso}$ is large compared to $D$ in the region of interest. Let $\Delta$ (of order $D$) be the distance away from the rod center beyond which equation (2.12) starts to hold. Applying Gauss' Law to a rectangular box of width $\Delta$ and height $D$ surrounding a rod, it follows that:

$$\frac{d}{dx}\phi(-R/2+\Delta/2) \approx \frac{2\pi\lambda^*}{\varepsilon_w D} \tag{2.13}$$

with a similar condition near $x = R/2$. Here, $\lambda^*$ is the effective charge per unit length of the rods, including cationic lipids located within a distance $\Delta$ of the rods. We will show below that the width of the strip of cationic lipids near a rod is of order $\sqrt{Dl_c}$. We are assuming that the Chapman length is large compared to $\Delta$ (and thus $D$) so the number of cationic lipids within a distance $\Delta$ of the rods must be modest and we will take $\lambda^* \cong \lambda$. Finally, for $R$ spacings in the interesting regime around $R_{iso} = \frac{\xi}{2}l_c$, $R$ is large compared to $D$ so equation (2.13) simplifies to:

$$\frac{d}{dx}\phi(-R/2) \approx \frac{2\pi\lambda}{\varepsilon_w D}. \tag{2.14}$$

The PB free energy, equation (2.7), simplifies for $\frac{D}{l_c} \ll 1$ to

$$F = \frac{k_BT}{b}\left(1-\frac{R}{R_{iso}}\right)[\ln(c_B/c_0)-1] + \frac{k_BT}{b}\left(\frac{R}{R_{iso}}\right)[\ln(\sigma_0/\sigma_L)-1] - \frac{k_BT}{b} + RDk_BT\left(c_B+\frac{2\sigma_0}{D}\right)e^{-\frac{e\overline{\phi}}{k_BT}} + \lambda\phi(R/2) \quad \text{(excess DNA)} \tag{2.15}$$

$$F = \frac{k_BT}{b}\left(\frac{R}{R_{iso}}-1\right)[\ln(c_B/c_0)-1] + \frac{k_BT}{b}\left(\frac{R}{R_{iso}}\right)[\ln(\sigma_0/\sigma_L)-1] - \frac{k_BT}{b} + RDk_BT\left(\frac{2\sigma_0}{D}e^{-\frac{e\overline{\phi}}{k_BT}} - c_B e^{\frac{e\overline{\phi}}{k_BT}}\right) + \lambda\phi(R/2) \quad \text{(excess liposome)} \tag{2.16}$$

as shown in the appendix. We are now in a position to apply this formalism, which we will do first for the case of excess DNA in the next section.

## 3 Excess DNA

For the case of excess DNA (and hence of positive counterions inside the lipoplexes) we should take the minus sign in equation (2.12) which can then be rearranged as:

$$\frac{d^2}{dx^2}\phi(x) \cong -\frac{4\pi e}{\varepsilon_w}\left(c_B+\frac{2\sigma_0}{D}\right)e^{-\frac{e\phi(x)}{k_BT}}. \tag{3.1}$$

This equation is actually well-known from the study of electrostatic interactions between charged plates in salt-free solution and we will closely follow that analysis [19]. The appropriate solution of equation (3.1) is:

$$\phi(x) = \overline{\phi} + \frac{k_BT}{e}\log(\cos^2(\kappa x)) \tag{3.2}$$

with $\overline{\phi}$ an integration constant. The physical meaning of this constant is that of the voltage difference between the bulk solution and a point inside the lipoplex halfway between two rods (*i.e.* $x = 0$). The parameter $\kappa$ in equation (3.2) has units of $L^{-1}$ and is the effective Debye parameter for the combined screening action of counter-ions

and cationic lipids. Equation (3.2) is a solution of equation (3.1) under the condition that

$$\kappa^2 \equiv 2\pi l_B \left( c_B + \frac{2\sigma_0}{D} \right) e^{-\frac{e\overline{\phi}}{k_B T}}. \tag{3.3}$$

Imposing the boundary condition equation (2.14) on equation (3.2) requires the Debye parameter to obey:

$$\kappa \tan(\kappa R/2) \approx \frac{\pi\xi}{D} \cdot \tag{3.4}$$

There are two interesting limits for treating equation (3.4). For $\xi R \gg D$ the solution of equation (3.4) is:

$$\kappa \approx \frac{\pi}{R} - \frac{2D}{\xi R^2} \cdot \tag{3.5}$$

The screening length $\kappa^{-1}$ is thus of order the spacing between the rods. Using equation (3.5) in equation (3.2), it follows that the voltage drop between the rod surface and the area in between the rods is large compared to $k_BT/e$. The cationic lipids and the counter-ions are largely confined to the regions near the rods. For the present calculation with $R \gg D$, we in fact are in this regime, but it is useful to look also at the case $\xi R \ll D$. In that case, we find a Debye parameter $\kappa \approx \sqrt{\xi/RD}$ from equation (3.5), while the voltage difference is of order $k_BT/e$, and the surface charge density is nearly uniform. The cross-over distance $D/\xi$ between the two regimes can be interpreted as the *width* of the strip of cationic lipids surrounding a rod. In terms of the Chapman length, this width is of order $\sqrt{Dl_c}$, the geometrical mean of the layer spacing and the Chapman length. Recall that for charged plates the Chapman length itself is the width of the condensed layer. We thus could interpret $\sqrt{Dl_c}$ as a "Chapman length" for the reduced dimensionality problem of a charged line in the presence of a plate with mobile charges.

We can use equation (3.5) in equation (3.3) to find the potential drop:

$$\overline{\phi} \cong \frac{k_BT}{e} \ln\left( \frac{2l_BR^2}{\pi} \left( c_B + \frac{2\sigma_0}{D} \right) \right) \cdot \tag{3.6}$$

The potential $\phi(R/2)$ at the surface of the rods is found by integrating the PB equation:

$$\phi(R/2) = - \frac{k_BT}{e} \ln\left( \frac{\pi\lambda^2}{2D^2 k_BT \varepsilon_w (c_B + 2\sigma_0/D)} + e^{-e\overline{\phi}/k_BT} \right). \tag{3.7}$$

Using equations (3.3–3.7) in the expression for the free energy equation (2.15), gives:

$$F(R) = \frac{k_BT}{b} \left( 1 - \frac{R}{R_{iso}} \right) [\ln(c_B/c_0) - 1] + \frac{k_BT}{b} \left( \frac{R}{R_{iso}} \right) [\ln(\sigma_0/\sigma_L) - 1] - \frac{k_BT}{b} + \frac{\pi D k_BT}{2Rl_B} + \frac{k_BT}{b} \ln\left( \frac{\pi\xi^2}{2l_BD^2(c_B + 2\sigma_0/D)} \right) \cdot \tag{3.8}$$

The only unknown left in equation (3.8) is the parameter $\sigma_0$. Using equation (3.2) in equations (2.3, 2.4), and $\kappa \approx \frac{\pi}{R}$ as is appropriate in the $\xi R \gg D$ limit, we find

$$\sigma_0/\sigma_c \approx \frac{\pi D}{2\xi R} e^{e\overline{\phi}/k_BT}. \tag{3.9}$$

Eliminating the voltage difference $\overline{\phi}$ by inserting equation (3.6) provides the following self-consistency condition:

$$\sigma_0 \approx \frac{Dc_B/2}{R_{iso}/R - 1} \cdot \tag{3.10}$$

Note that $\sigma_0$ *diverges* at the isoelectric point $R = R_{iso}$. The in-out voltage difference also diverges at the isoelectric point:

$$e\overline{\phi}/k_BT = \ln\left( \frac{\xi R c_B/\pi\sigma_c}{R_{iso}/R - 1} \right) \cdot \tag{3.11}$$

Using equation (3.10) in equation (3.8) gives the free energy:

$$F(R) = \frac{k_BT}{b} \left[ \ln\left( \frac{\pi\xi}{2D^2c_0b} \right) - 2 \right] + \frac{\pi}{2} \frac{Dk_BT}{l_BR} + \frac{k_BT}{b} \left\{ \left( 1 - \frac{R}{R_{iso}} \right) \ln\left( 1 - \frac{R}{R_{iso}} \right) + \frac{R}{R_{iso}} \ln\left( \frac{R}{R_{iso}} \right) \right\} + const\ R. \tag{3.12}$$

Terms linear in $R$ play no role since they do not contribute to the line energy $\tau_{complex}(R)$ – or rather the DNA chemical potential (see Eq. (2.8)). Note that $F(R)$ does not depend on $c_B$.

From equation (2.8) we find for the line energy for introducing a rod:

$$\tau_{complex}(R) = \frac{k_BT}{b} \left[ \ln\left( \frac{\pi\xi}{2D^2c_0b} \right) - 2 \right] + \frac{\pi D k_BT}{l_BR} + \frac{k_BT}{b} \ln\left( 1 - \frac{R}{R_{iso}} \right) \cdot \tag{3.13}$$

The first term of equation (3.13) is the "self" energy per unit length of an isolated rod inside the complex. Note that it is of order $k_BT$ times the number density $1/b$ of counter-ions, as claimed in the introduction, and that it does not depend on the charge per unit area $\sigma_c$ of the lipid surface. The second term can be identified as the contribution to the line energy from the electrostatic repulsion between two rods at the isoelectric point, as shown in the earlier paper by a different method. It is just the 2-D analog of the Langmuir equation for the electrostatic repulsion between charged plates [20]. Note that it is less than the first term by a factor $D/R\xi$ which is small compared to one, so electrostatic rod-rod repulsion between rods will not play a dominant role in setting the value of $R$. The third term in equation (3.13) is of central importance: it derives from the third term in equation (3.12) for the free energy. To interpret this contribution physically,

note that $\frac{1}{b}\left(1-\frac{R}{R_{iso}}\right)$ is the number of extra counter-ions per unit length of chain which must be introduced into the lipoplex to maintain charge neutrality when we reduce $R$ below $R_{iso}$. The first part of this term in equation (3.12) is then clearly the translational entropy gain obtained upon introducing these extra counter-ions into the complex. The second part of the third term in equation (3.12) can be interpreted as the loss in translational entropy of counter-ions and of mobile cationic lipids if we reduce the spacing between rods. Taken together the third term of equation (3.12) has the form of a *mixing entropy*; note that it is minimized by $R = (1/2)R_{iso}$.

Thermodynamic stability of the complex requires that $\tau_{complex}$ is the line energy of a chain in solution. The bulk solution is modeled as a set of long, parallel chains with a charge per unit length $-\lambda$ and a volume fraction $\Phi_{DNA}$ (so $\Phi_{DNA} = 0$ marks the isoelectric point). The PB free energy of such a chain was computed by Lifson and Katchalsky [21]. In the limit of low volume fractions $\Phi_{DNA}$ they found:

$$\tau_{solution} = \frac{k_BT}{b}\left\{\ln\left(\frac{2(1-\xi)^2}{\pi c_0 b D^2 \xi}\right) - 1\right\} + \frac{k_BT}{b\xi}\left\{\frac{1}{2}\ln\Phi_{DNA} - 2\ln(1-\xi) - \xi\right\}. \quad (3.14)$$

Equating $\tau_{solution}$ to $\tau_{lipoplex}$ gives the following self-consistency condition on the DNA-DNA spacing $R^*$:

$$\left(1-\frac{R^*}{R_{iso}}\right) = (\Phi_{DNA})^{\frac{1}{2\xi}} \times \exp\left\{-\left[\ln\left(\frac{\pi^2\xi^2}{4(1-\xi)^2}\right)+\frac{2}{\xi}\ln(1-\xi)\right] - \frac{\pi D}{\xi R^*}\right\}. \quad (3.15)$$

For $R$ large compared to $D$, the second term in the exponent on the right hand side (due to the rod-rod repulsion) is small compared to the first term and we obtain:

$$\left(1-\frac{R^*}{R_{iso}}\right) \cong (\Phi_{DNA})^{\frac{1}{2\xi}} \times \exp\left\{-\left[\ln\left(\frac{\pi^2\xi^2}{4(1-\xi)^2}\right)+\frac{2}{\xi}\ln(1-\xi)\right]\right\} \quad (3.16)$$

which is equation (1.1) of the introduction.

As we approach the isoelectric point, by reducing the volume fraction of DNA in solution, the separation between the rods approaches the isoelectric separation as a steep power law of the DNA volume fraction. This curious result represents the competition between the entropic free energy gain obtained upon release of counter-ions out of the Manning layer of the chains and into the complex – which favors introduction of more chains from the solution into the complex – and the fact that the line energy of a chain in solution decreases as $\frac{k_BT}{b\xi}\ln\Phi_{DNA}$ when we approach the isoelectric point. Note that the singular power law dependence disappears at the onset of Manning condensation $\xi = 1$, presumably since without Manning condensation there is no significant entropic gain for the counter-ions to enter the complex and be released.

We have assumed that *all* of the excess positive counter-ions stay inside the lipoplex. In actuality, a certain number can leave until the effective negative charge of the lipoplex becomes sufficiently strong to prevent further ionization. Applying Oosawa's theory of charge renormalization to this case [17], it is easy to show that the resulting ionization level $Z^*$ is of order $R_{lipo}/l_B$, with $R_{lipo}$ the radius of the lipoplex. This degree of ionization is however very small compared to the total number of excess counter-ions inside the complex unless we are extremely close to the isoelectric point.

## 4 Excess liposome

We now must solve a different PB equation, which is obtained by taking the plus sign on the RHS of equation (2.12):

$$\frac{d^2}{dx^2}\phi(x) \cong -\frac{4\pi e}{\varepsilon_w}\left(\frac{2\sigma_0}{D}e^{-\frac{e\phi(x)}{k_BT}} - c_B e^{\frac{e\phi(x)}{k_BT}}\right). \quad (4.1)$$

The general solution of equation (4.1) is an incomplete elliptical integral, which leads to mathematical complexities, but the solution simplifies in the "critical" regime of $R$ very close to $R_{iso}$, and in the "screening" regime at small but finite values of $(R/R_{iso} - 1)$.

### 4.1 Critical regime

Near the isoelectric point, we will use perturbation theory with $\phi(x) = \phi_0(x) + \phi_1(x) + \ldots$ to solve equation (4.1). The lowest order term, $\phi_0(x)$, is the solution of the PB equation right at the isoelectric point – where $c_B = 0$ – while $\phi_1(x)$ is the lowest order correction term for finite values of the bulk concentration $c_B$ of counter-ions. The dimensionless expansion parameter of the perturbation theory will actually turn out to be $(R/R_{iso} - 1)$.

#### (i) Zero order

The solution of the PB equation (4.1) for $c_B = 0$ is found by setting $c_B = 0$ in equation (3.1):

$$\phi_0(x) = \overline{\phi} + \frac{k_BT}{e}\log(\cos^2(\kappa x)). \quad (4.2)$$

The Debye parameter is given by:

$$\kappa^2 \equiv \frac{4\pi l_B \sigma_0}{D}e^{-\frac{e\overline{\phi}}{k_BT}}. \quad (4.3)$$

To satisfy the boundary conditions at the surface of the rods, we recover the condition $\kappa \approx \frac{\pi}{R} - \frac{2D}{\xi R^2}$. Using this

in equation (4.3), we find for the voltage difference $\overline{\phi}$:

$$\overline{\phi} \equiv \frac{k_B T}{e} \ln\left(\frac{4R^2 l_B \sigma_0}{\pi D}\right). \tag{4.4}$$

To determine explicitly the parameters $\sigma_0$ and $\overline{\phi}$, we need however the first-order correction.

### (ii) First order

The first order correction is found by linearizing the PB equation (4.1) around $\phi_0(x)$:

$$\frac{d^2}{dx^2}\phi_1(x) \cong \frac{2\kappa^2}{\cos^2(\kappa x)}\phi_1(x) + \left(\frac{4\pi e c_B e^{\frac{e\overline{\phi}}{k_B T}}}{\varepsilon_w}\right)\cos^2(\kappa x) \tag{4.5}$$

where we have used equation (4.2). The boundary conditions for equation (4.5) at the rod positions are $\frac{d}{dx}\phi_1(x = \pm R/2) = 0$, since the zero'th order solution already accounts for the rod charges. We also must demand that $\phi_1(x)$ vanishes for $c_B = 0$ since in that limit $\phi(x)$ must reduce to $\phi_0(x)$. The appropriate solution of equation (4.5) has the general form:

$$\phi_1(x) = \left(\frac{4\pi e c_B e^{\frac{e\overline{\phi}}{k_B T}}}{\varepsilon_w \kappa^2}\right) f(\kappa x) \tag{4.6}$$

where the function $f(y)$ must obey the following, parameter-free, differential equation:

$$\frac{d^2}{dy^2}f(y) - \frac{2}{\cos^2(y)}f(y) = \cos^2(y). \tag{4.7}$$

We must solve equation (4.7) under the boundary conditions $\frac{d}{dy}f(\pm\pi/2) = 0$.

The solution of equation (4.7) can be obtained numerically but the only properties of the solution we really need for the following can be demonstrated analytically. They are:
(a) Near $y = \pi/2$, $f(y)$ vanishes as

$$f(y) \cong \frac{1}{3}(\pi/2 - y)^2 \ln(\pi/2 - y),$$

while a similar condition holds at $y = -\pi/2$.
(b)

$$\int_{-\pi/2}^{\pi/2} \frac{f(y)}{\cos^2(y)} dy = -\frac{\pi}{4}\cdot \tag{4.8}$$

To determine $\sigma_0$, we integrate the surface density of cationic lipids to first order in the perturbation theory:

$$\sigma_0 \int_{-R/2}^{R/2} dx e^{-\frac{e\phi_0(x)}{k_B T}}\left(1 - \frac{e\phi_1(x)}{k_B T}\right) = R\sigma_c. \tag{4.9}$$

Using equations (4.7, 4.8) in equation (4.9):

$$\sigma_0 \cong \frac{\pi D \sigma_c}{2\xi R} e^{e\overline{\phi}/k_B T}\left(1 - \frac{l_B c_B R D}{2\xi} e^{e\overline{\phi}/k_B T}\right). \tag{4.10}$$

Using equation (4.5) to eliminate the voltage difference $\overline{\phi}$ finally gives:

$$\frac{\sigma_0}{\sigma_c} \cong \frac{\pi}{c_B l_B R_{iso}^2}\left(\frac{R}{R_{iso}} - 1\right). \tag{4.11}$$

The parameter $\sigma_0$ now *vanishes* as we approach the isoelectric point, where as on the DNA-rich side it diverged. The potential difference

$$\overline{\phi} = \frac{k_B T}{e} \ln\left(\frac{4\sigma_c}{D c_B}\left(\frac{R}{R_{iso}} - 1\right)\right) \tag{4.12}$$

still diverges, but it goes to *minus* infinity as we approach the isoelectric point, whereas on the DNA-rich side it went to positive infinity. These results indicate that, within PB theory, the isoelectric point represents a mathematical singularity. If we use equations (4.11, 4.12) in the RHS of equation (4.10), we find that the small parameter of the perturbation expansion is $(R/R_{iso} - 1)$.

Inserting the above results into equation (2.16) for the free energy per rod per unit length, and using the fact that $f(y)$ vanishes near the rods, we find for the surface energy $\gamma_{complex}(R) = \frac{dF(R)}{dR}$:

$$\begin{aligned}\gamma_{complex}(R) = {} & 2\sigma_c k_B T\left(\ln\left(\frac{\sigma_c}{\sigma_L}\right) - 1\right) \\ & + 2\sigma_c k_B T \ln\left(\frac{l_B \sigma_c^2}{2c_0}\right) \\ & + 2\sigma_c k_B T \ln\left(\left(\frac{8\pi}{\xi^2}\right)\left(\frac{R}{R_{iso}} - 1\right)\right) - \frac{\pi D k_B T}{2R^2 l_B}\cdot\end{aligned} \tag{4.13}$$

This must be compared to the PB free energy per unit area of a cationic liposome. The free energy per unit area of a flat, infinite charged surface is well-known [22]. For a charged bilayer:

$$\gamma(lip) = 2\sigma_c k_B T\left(\ln\left(\frac{\sigma_c}{\sigma_L}\right) - 1\right) + 2\sigma_c k_B T \ln\left(\frac{l_B \sigma_c^2}{2c_0}\right)\cdot \tag{4.14}$$

The first term is the entropic contribution to the free energy per unit area of an ideal mixture of cationic lipids. The second term is the PB free energy per unit area of a charged surface with counter-ions.

Turning a flat cationic bilayer into a collection of spherical cationic liposomes leads to two kinds of correction terms to equation (4.14). The entropic cost per unit area of confining half of the counter-ions inside a sphere of radius $R_{lip}$ is of order $\frac{k_B T}{l_B R_{lip}}$. The second correction is due to the fact that a charged sphere, like a liposome, cannot hold on to all of its counter-ions: the liposomes must have

an overall effective positive charge $Z^*$. From the PB theory of colloids [23], it is known that the renormalized charge is of order $Z^* \approx R_{lip}/l_B$, independent of the bare charge, over a wide range of volume fractions. This "ionization" process produces another correction term of order $\dfrac{k_BT}{l_BR_{lip}}$ to the surface energy. The surface energy equation (4.14) itself is of order $\dfrac{k_BT}{l_Bl_C}$ so these corrections can be neglected for the relevant case of liposome radii large compared to the Chapman length. The only exception is the limit of extremely low liposome volume fractions. For liposome volume fractions of the order of $\left(\dfrac{\sigma_c a^3}{l_c}\right) e^{-a/l_c}$ or less, the effective charge $Z^*$ of the liposome starts to approach the bare charge $\sigma_c a^2$ of the liposomes. Such levels of dilution are probably beyond experimental control and we will restrict ourselves to the regime where $Z^* \approx R_{lip}/l_B$.

Using now equation (4.14) in equation (4.13) we find a simple result:

$$\gamma_{complex}(R) \cong \gamma(lip) + 2\sigma_c k_B T \ln\left(\left(\frac{8\pi}{\xi^2}\right)\left(\frac{R}{R_{iso}} - 1\right)\right) - \frac{\pi D k_B T}{2R^2 l_B}\,. \tag{4.15}$$

Near the isoelectric point, the surface energy of the complex $\gamma_{complex}(R)$ is, according to equation (4.15), always *less* than the surface energy $\gamma(lip)$ of the liposome due to the second and third negative terms in equation (4.15). The second term on the RHS of equation (4.15) can be traced back to the counter-ion entropy. Note that it goes to minus infinity at the isoelectric point and that this negative divergence is enhanced by the Manning parameter $\xi$. The third term is clearly due to rod-rod repulsion. It is smaller than the second term by an amount of order $(D/(\xi R_{iso}))^2$ which is small compared to one. Direct rod-rod interaction thus plays again only a minor role.

There is apparently no solution for the stability condition $\gamma_{complex}(R) = \gamma(lip)$ in the critical regime. The immediate neighborhood of the isoelectric point is thus unstable: the complex *must* absorb a non-zero amount of lipid material until $R$ is sufficiently large compared to $R_{iso}$ to be out of the critical regime. We conclude that, on the liposome-rich side, the parameter $(R/R_{iso}-1)$ will assume a *non-zero* value as we approach the isoelectric point. To find this value, we must go beyond perturbation theory.

### 4.2 Screening regime

To find a non-perturbative solution, note that the cationic lipids and the extra negative counter-ions should mimic the effects of a finite concentration of added salt at higher concentrations (see Fig. 1c) and thereby produce an effective Debye screening length. For rod spacings large compared to this Debye screening length, the screening action should produce an electrostatic potential in the region between the rods which is (nearly) constant, and which is (nearly) charge neutral. We will call this the "screening regime". We will determine below the validity criteria of the screening regime more carefully.

Let $\overline{\phi}$ be the value of the potential in the screened region between the rods. Its value is found by looking for solutions to equation (4.1) which are constant:

$$\overline{\phi} = \frac{k_BT}{2e} \ln\left(\frac{2\sigma_0}{Dc_B}\right)\,. \tag{4.16}$$

Alternatively, equation (4.16) can be found by demanding that the positive and negative surface charge densities – respectively $2\sigma_0 \exp\left(-\frac{e\phi}{k_BT}\right)$ and $(c_B/D)\exp\left(\frac{e\phi}{k_BT}\right)$ – are equal in magnitude to assure charge neutrality for the lipid/coun-terion "added salt". The Debye screening parameter is found by linearizing equation (4.1) around $\overline{\phi}$ and looking for solutions which approach $\overline{\phi}$ exponentially. The result is that:

$$\kappa^2 \cong 8\pi l_B \sqrt{\frac{2\sigma_0 c_B}{D}}\,. \tag{4.17}$$

By comparing with the normal expression for the Debye parameter in salt solution, we see that the geometrical mean $\sqrt{\dfrac{2\sigma_0 c_B}{D}}$ plays the role of the added salt concentration. As before, we still must determine the constant $\sigma_0$.

To find $\sigma_0$, we note that the explicit ("Gouy-Chapman") solution of the Poisson-Boltzmann equation equation (4.1), near a rod at $x = -R/2$, which approaches $\overline{\phi}$ far from the rod is:

$$\phi(z) = \overline{\phi} + \frac{2k_BT}{e} \log\left(\frac{1 - \tanh\left(\frac{e\Delta\phi}{4k_BT}\right) e^{-\kappa z}}{1 + \tanh\left(\frac{e\Delta\phi}{4k_BT}\right) e^{-\kappa z}}\right) \tag{4.18}$$

with $z = x + R/2$ and $\Delta\phi$ the potential drop between the asymptotic region in between the rods and the rod surface (there is a corresponding solution at $x = R/2$). We obviously must demand that the rod spacing $R$ is large compared to the Debye screening length $\kappa^{-1}$ for this solution to hold. The potential drop $\Delta\phi$ is found by imposing the boundary condition equation (2.14) on equation (4.18):

$$\sinh\left(\frac{e\Delta\phi}{2k_BT}\right) = \frac{\pi\xi}{\kappa D}\,. \tag{4.19}$$

Now recall that the average surface concentration of cations must equal $\sigma_c$. Integrating $2\sigma_0 \exp\left(-\frac{e\phi}{k_BT}\right)$ from $-R/2$ to $R/2$ and using the above solution in equation (2.4) leads to the following result:

$$\kappa^2 \cong \frac{16\pi}{l_C D}(1 - R_{iso}/R). \tag{4.20}$$

For $R \gg R_{iso}$, the screening length approaches a finite value, which is approximately the geometrical mean of the layer spacing and the Chapman length (consistent with the result of the previous section that the width of the strip of cationic lipids surrounding a rod is of order $\sqrt{l_C D}$).

The validity condition for the screening regime, $\kappa R \gg 1$, is then:

$$\left(\frac{R}{R_{iso}} - 1\right) \gg \frac{D}{4\pi\xi^2 l_c}\,. \tag{4.21}$$

The parameter on the right hand side is quite small near the isoelectric point since the Manning parameter is of order 4-5 so the screening regime actually starts very close to the isoelectric point. The parameter $\sigma_0$ follows from equation (4.17):

$$\sigma_0/\sigma_c \cong \frac{2\sigma_c}{c_B D}(1 - R_{iso}/R)^2. \tag{4.22}$$

It saturates to a finite value for $R$ large compared to $R_{iso}$. If we check for what $R$ value equation (4.22) is equal to equation (4.11) (for the critical regime) we find that this takes place near $\left(\frac{R}{R_{iso}} - 1\right) \approx \frac{D}{\xi^2 l_c}$. Comparing this with equation (4.21) we see that we can assume that the cross-over between the regimes indeed takes place at this spacing.

The free energy per rod in the screening regime is found using equations (4.17–4.20, 4.22) in equation (2.16):

$$F(R) \cong \frac{k_B T}{b}\left(\frac{R}{R_{iso}}\right)\left[\ln\left(\frac{2\sigma_c^2}{c_0 D \sigma_L}\right) - 2\right] + 2\frac{k_B T}{b}\left(\frac{R}{R_{iso}} - 1\right)\ln\left(1 - \frac{R_{iso}}{R}\right) + const. \tag{4.23}$$

The second term in equation (4.23) is obviously again entropic. Note that for $R \gg R_{iso}$ it approaches a constant. The corresponding free energy per unit area $\gamma = \frac{dF(R)}{dR}$, or rather the lipid chemical potential at fixed neutral/ionic lipid ratio, is:

$$\gamma_{complex}(R) \cong \gamma(lip) + 2\sigma_c k_B T\left(\ln\left(\frac{4l_c}{eD}\right) + 2\ln\left(1 - \frac{R_{iso}}{R}\right) + 2\frac{R_{iso}}{R}\right) \tag{4.24}$$

is the sum of three terms. The first term is the surface energy of the liposome. The second term is the dominant correction to $\gamma(lip)$ in the limit of large $R$. This term can be derived in a more intuitive manner as follows. Suppose we compute the repulsive electrostatic disjoining pressure $\Pi(d)$ between two charged plates in a salt free solution with a positive surface charge $\sigma_c$, spacing $d$, and with compensating negative counter-ions in between. This geometry corresponds approximately to the charge distribution for our case away from the rods. A well-known result of PB theory [24] is that:

$$\Pi(d) \cong \begin{cases} 2\sigma_c \dfrac{k_B T}{d} & (d \ll l_c) \\ \dfrac{\pi k_B T}{4 l_B d^2} & (d \gg l_c). \end{cases} \tag{4.25}$$

If we use equation (4.25) to calculate the work per unit area $W(D)$ done against the repulsive disjoining pressure in bringing the plates from $d = \infty$ down to $d = D$ we find $W(D) \approx 2\sigma_c k_B T \ln\left(\frac{l_c}{D}\right)$, which agrees with the second term of equation (4.24). This term in the difference between $\gamma_{complex}(R)$ and $\gamma(lip)$ in the large $R$ limit thus corresponds to the work done in compressing the two charged surfaces together. Since the spacing between opposite surfaces of the liposome is large compared to $D$, we always must pay an extra free energy price in compressing the layers of a liposome to construct a complex.

The last two terms in equation (4.24) are entropic. Far from the isoelectric point they cancel, but as we approach $R_{iso}$ they give a negative contribution which can compensate the work done by the disjoining force. Equating $\gamma_{complex}$ to $\gamma(lip)$ gives the following stable spacing:

$$\left(\frac{R^*}{R_{iso}} - 1\right) \cong \sqrt{\frac{eD}{4l_c}} \tag{4.26}$$

which is equation (1.2) of the introduction. Since $l_c$ is, by assumption, large compared to $D$ this stable spacing is close to the isoelectric spacing. Consistency requires that we still must be in the screening regime. Using equations (4.21, 4.26), this requirement is always satisfied for $l_c$ larger than $D$.

## 5 Discussion

In summary, we have seen that the PB theory reproduces a number of the qualitative features of the experiments of Radler *et al.*: singular behavior in the $R$ spacing at the isoelectric point and saturation of the $R$ spacing at higher lipid concentrations. Apart from the singular behavior, the model also predicts that lipoplexes in the presence of excess DNA are negatively charged and that they are positively charged in the presence of excess liposome (see discussion at the end of Sect. 3), which is indeed observed. This is encouraging but when we compare Figure 2 quantitatively with the data of reference [7] we encounter difficulties. The observed variation of the DNA spacing across the isoelectric point is less sharp than Figure 2 while the magnitude of the observed jump in the DNA spacing is significantly less than the one predicted by equations (1.1, 1.2). A strictly quantitative comparison between our theory and the currently available experiments in fact cannot be done since our key assumption – Chapman lengths must be large compared to the layer spacing – is not obeyed. A typical value for the Chapman length of the lipoplexes is in the range of 5-10 Å while $D$ is of order 20 Å.

There is of course no reason why lipoplexes with lower concentrations of cationic lipids cannot be studied to test the theory. A key experiment would be the following. According to the present description, the spacing $R^*$ does *not* represent a minimum in some effective rod-rod potential (*e.g.* a deformation attraction competing with an electrostatic repulsion of the form $f(R) \approx (k_B T D)/(l_B R^2)$). If such a minimum did exist then $R^*$ would be expected to

be independent of the charged lipid density $\sigma_c$. We would, on the contrary, expect that the spacing *diverges* in the limit of small $\sigma_c$:

$$R^*(\sigma_c) \cong \frac{1}{2b\sigma_c} + \frac{1}{4b}\sqrt{\frac{eDl_B}{\sigma_c}} \tag{5.1}$$

(Eq. (1.2)). Recall that our theory is expected to work better for small $\sigma_c$ and large $R^*$ where the details of the DNA architecture are less important so measurement of $R^*(\sigma_c)$ would constitute a significant test. Alternatively, if we compare equations (1.1, 1.2), we see that the jump in the $R$ spacing at the isoelectric point in this same limit should scale with $R_{iso} = \frac{1}{2b\sigma_c}$ itself.

The electrostatic phenomena, such as counter-ion release, discussed in this paper are certainly going to be encountered as well if $D$ exceeds the Chapman length, but there will be changes at the quantitative level. The analytical complications of the PB theory at higher cationic lipid concentrations – both in terms of having to solve a partial non-linear differential equation and of having to model the architecture of DNA more accurately – necessarily require extensive numerical work in this regime. A second serious limitation of the model is the assumption that we are in the limit of no added salt. Recent simulations on the absorption of DNA on a cationic surface indicate that in the presence of added salt the importance of both counter-ion release and of the isoelectric point appears to be reduced [25], which might explain why the observed jump at the isoelectric point is less singular than expected. Another questionable aspect of the theory is that we assumed that the lipid bilayers were rigid. We already mentioned that deformable lipid layer can mediate attractions [11]. In addition, *repulsion* between two deformable membranes is known to produces attractive pair interaction between "adhesion" molecules connecting the two membranes [26] so for deformable membranes, the electrostatic repulsion between the layers actually could produce an effective long-range attraction between the rods. Next, lipid membranes can adjust their thickness somewhat. It is thus possible to vary the surface charge density of the bilayer by adjusting the bilayer thickness, an effect we did not account for. It should also be recalled that mean-field theories – such as the PB theory – are sensitive to thermal fluctuations at singular points in the phase diagram. Thermal fluctuations thus could alter the dependence of $R^*$ on the volume fraction of DNA or lipid near the isoelectric point. PB theory also does not account well for short-range correlations between the ions.

The apparent success of the theory in accounting for the observed phenomena at the qualitative level, notwithstanding the above mentioned limitations, would indicate that the statistical physics of the counter-ions indeed is of key importance for the structure of a lipoplex and that we should not try to analyze the structure of a lipoplex in terms of the effective rod-rod interaction.

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## Appendix: Free energy

To compute the free energy per unit length per rod, first rewrite equation (2.7) in the limit of $D$ small compared to $R$ and low cationic lipid concentration where we can neglect the variation of potentials and concentrations across the normal of the layer (*i.e.* we assume $D \ll l_c$):

$$\begin{aligned} F \cong\ & Dk_BT[\ln(c_B/c_0) - 1] \\ & \times \int_{-R/2}^{R/2} dx c(x) + 2k_BT[\ln(\sigma_0/\sigma_L) - 1] \int_{-R/2}^{R/2} dx \sigma(x) \\ & - e\int_{-R/2}^{R/2} dx \left[\pm\frac{Dc(x)}{2} + \sigma(x)\right]\phi(x) + \frac{\lambda\phi(R/2)}{2}\cdot \end{aligned} \tag{A.1}$$

Here we have used equation (2.3) to eliminate $c(x)$ and $\sigma(x)$ from the arguments of the logarithms (the plus sign refers to excess DNA, the minus sign to excess cationic liposomes). Using the condition of overall charge neutrality plus the requirement that the average cationic lipid concentration equals $\sigma_c$, we can eliminate the first two integrals:

$$\begin{aligned} F \cong\ & \pm\frac{k_BT}{b}\left(1 - \frac{R}{R_{iso}}\right)[\ln(c_B/c_0) - 1] \\ & + \frac{k_BT}{b}\left(\frac{R}{R_{iso}}\right)[\ln(\sigma_0/\sigma_L) - 1] \\ & - e\int_{-R/2}^{R/2} dx \left[\pm\frac{Dc(x)}{2} + \sigma(x)\right]\phi(x) \\ & + \frac{1}{2}\lambda\phi(R/2). \end{aligned} \tag{A.2}$$

We can simplify equation (A.2) by using the Poisson-Boltzmann equation in the last term, followed by a partial integration and the use of the boundary condition equation (2.14), with the result:

$$\begin{aligned} F \cong\ & \pm\frac{k_BT}{b}\left(1 - \frac{R}{R_{iso}}\right)[\ln(c_B/c_0) - 1] \\ & + \frac{k_BT}{b}\left(\frac{R}{R_{iso}}\right)[\ln(\sigma_0/\sigma_L) - 1] \\ & - \frac{\varepsilon_w D}{8\pi}\int_{-R/2}^{R/2} dx \left(\frac{d\phi(x)}{dx}\right)^2 + \lambda\phi(R/2). \end{aligned} \tag{A.3}$$

The Poisson-Boltzmann equation can be integrated as:

$$\frac{1}{2}\left(\frac{d\phi}{dx}\right)^2 = \frac{4\pi k_BTe\left(c_B + \frac{2\sigma_0}{D}\right)\left(e^{-\frac{e\phi}{k_BT}} - e^{-\frac{e\overline{\phi}}{k_BT}}\right)}{\varepsilon_w}$$

(excess DNA) (A.4)

$$\frac{1}{2}\left(\frac{d\phi}{dx}\right)^2 = \frac{4\pi k_B T e\left\{\left(\frac{2\sigma_0}{D}e^{-\frac{e\phi}{k_BT}} - c_B e^{\frac{e\phi}{k_BT}}\right) - \left(\frac{2\sigma_0}{D}e^{-\frac{e\overline{\phi}}{k_BT}} - c_B e^{\frac{e\overline{\phi}}{k_BT}}\right)\right\}}{\varepsilon_w}$$

(excess liposome) (A.5)

with $\overline{\phi}$ the potential at the midpoint $x = 0$. Using equations (A.4, A.5) in equation (A.3) and the condition of charge neutrality gives:

$$F = \frac{k_BT}{b}\left(1 - \frac{R}{R_{iso}}\right)[\ln(c_B/c_0) - 1] + \frac{k_BT}{b}\left(\frac{R}{R_{iso}}\right)[\ln(\sigma_0/\sigma_L) - 1] - \frac{k_BT}{b} + RDk_BT\left(c_B + \frac{2\sigma_0}{D}\right)e^{-\frac{e\overline{\phi}}{k_BT}} + \lambda\phi(R/2)$$

(excess DNA) (A.6)

$$F = \frac{k_BT}{b}\left(\frac{R}{R_{iso}} - 1\right)[\ln(c_B/c_0) - 1] + \frac{k_BT}{b}\left(\frac{R}{R_{iso}}\right)[\ln(\sigma_0/\sigma_L) - 1] - \frac{k_BT}{b} + RDk_BT\left(\frac{2\sigma_0}{D}e^{-\frac{e\overline{\phi}}{k_BT}} - c_B e^{\frac{e\overline{\phi}}{k_BT}}\right) + \lambda\phi(R/2).$$

(excess liposome) (A.7)

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