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Electrostatically induced undulations of lamellar DNA-lipid complexes

H. Schiessel[1,a] and H. Aranda-Espinoza[2]

[1] Max-Planck-Institute for Polymer Research, Theory Group, PO Box 3148, 55021 Mainz, Germany
[2] Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, PA 19104, USA

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Abstract. We consider DNA-cationic lipid complexes that form lamellar stacks of lipid bilayers with parallel DNA strands intercalated in between. We calculate the electrostatically induced elastic deformations of the lipid bilayers. It is found that the membranes undulate with a periodicity that is set by the DNA interaxial distance. As a consequence the lamellar repeat distance changes resulting in a swelling or compression of the lamellar stack. Such undulations may be responsible for the intermembrane coupling between DNA strands in different layers as it is observed experimentally.

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1 Introduction

Electrostatic adsorption of polyelectrolytes onto oppositely charged surfaces, such as lipid membranes, has been the subject of intense experimental and theoretical research in the last decade. Of particular interest is the spontaneous complexation of DNA with both cationic and neutral lipids due to their possible application to gene therapy [1,2]. These so-called "lipoplexes" show a diversity of equilibrium and metastable structures [3–13]. For example, it has been shown through X-ray diffraction analysis [5,7,13] that DNA molecules and lipids can form lamellar complexes with DNA intercalated in between lipid bilayers.

Several theoretical studies help to understand many of the phenomena observed for lipoplexes [14–28], however many more remain to be elucidated. An important problem is the dependence of the interaxial spacing of DNA rods in lamellar complexes on the DNA/lipid composition. Bruinsma [19] presented an analytical approach that is applicable to lipoplexes with weakly charged bilayers. The numerical study of Harries *et al.* [22] predicts the interaxial spacing also for higher charge densities. According to both studies the isoelectric point of the lipoplex (the point at which the anionic charges of the DNA balance the cationic charges of the lipids) is unstable to further adsorption of DNA or lipids. The formation of say an isoelectric complex is driven by the release of the small counterions that were "condensed" on the highly charged DNA and on the charged bilayer before complexation. A lipoplex close to the isoelectric point is very susceptible to the uptake of further cationic lipids or DNA – if available – since this will be accompanied by the release of the corresponding counterions into the lipoplex[1]. The theoretical predictions show good agreement with a recent experimental study [13].

A different approach to the problem of the distance between DNA strands was given by Dan [14]. In this study the preferred distance was predicted to be the result of two competitive mechanisms, electrostatic repulsion between the strands and their membrane-induced attraction due to the perturbation of the lipid packing in the membrane close to the adsorbed DNA. The model assumes DNA strands adsorbed on a single membrane as it was investigated experimentally by Fang and Yang [6] using atomic force microscopy experiments. They found that the distance between DNA strands was about 5 nm, a distance that was predicted by Dan within her model [14].

In that model it is assumed that the membrane (that is supported by a solid surface) is locally perturbed close to the DNA in such a way that the monolayer thickness is slightly increased [14]. It should be expected that for lamellar lipoplexes one also has perturbations. Since the membranes are allowed to undergo shape changes freely (no supporting layer) one might expect undulations leading to a compression or swelling of the whole lamellar stack

[a] e-mail: heli@mpip-mainz.mpg.de

[1] A similar instability is also expected for the complexation of charged spheres and oppositely charged polyelectrolytes. A single highly charged chain will wrap around a single sphere forming a complex that is beyond the isoelectric point ("overcharging") and this effect is driven by the release of counterions from the wrapped chain [29]. On the other hand, a chain in a solution of highly charged spheres will complex more spheres than necessary to be isoelectric, and this effect is driven by the release of counterions of the complexed spheres [30].

as depicted in Figure 2. Such undulations might lead to an intermembrane coupling between DNA rods in different layers – resulting in a 3D ordering of the DNA rods. Lipoplexes with a 3D rectangular ordering of the DNA molecules were indeed observed experimentally [8,10].

Most experiments on lamellar lipoplexes indicate that such a type of perturbation of the membranes around the DNA molecules – if present – is small [5,7]. Undulations of the membranes should lead to a lamellar repeat distance that is larger or smaller than the sum of the bilayer thickness and the diameter of the DNA molecule (including a hydration shell). Considering complexes at the isoelectric point and changing the ratio of charged to neutral lipids it was observed that the lamellar repeat distance stays always close to a value that indicates flat membranes [5,7]. Thus even though the lipid dilution experiments lead to a considerable increase of the interaxial spacing between DNA rods, the undulations remain too small to be non-ambiguously detected. On the other hand, for more flexible membranes where detectable membrane undulations could be expected the system switches to the inverse hexagonal phase instead [9].

Recently Subramanian *et al.* [31] studied the complexation of the anionic polypeptide poly-glutamic acid with a mixture of cationic (DDAB) and neutral (DLPC) lipids. It was observed that the lipid organizes in a multilamellar phase with the polypeptide chains intercalated in between the membranes. Compared to the DNA complexes discussed above, the polypeptides do not show any in-plane ordering even though it is assumed that they are in the α-helical state. As for the DNA lipoplexes a "lipid dilution" experiment was performed for isoelectric polypeptide lipoplexes. Contrary to the outcome for the DNA complexes, a considerable increase of the lamellar spacing was found when the cationic lipids were diluted by neutral ones. For high lipid dilution the spacing saturated at a constant value of 60 Å which coincides with the equilibrium value of pure DLPC membranes. Subramanian *et al.* [31] suggested that this behavior could be due to a "pinching mechanism" including membrane undulations similar to the ones depicted in Figure 2 (case $h > 0$). The pinching sites are formed due to the electrostatic interaction between the negatively charged poly-glutamic acid and the cationic DDAB lipids. Away from the pinched regions the properties of the lipoplex are dominated by the properties of the pure DLPC membranes.

Whether it is possible to have pinches in a lipoplex was studied by one of the authors [23]. By comparing the gain in electrostatic free energy with the bending energy of forming a pinch, the parameter range was estimated at which pinching can be expected. It was shown that this effect should occur if the line charge density of the rods is sufficiently high and the membranes are sufficiently flexible, a situation that might be fulfilled for the polypeptide lipoplex considered in reference [31].

A different approach to the pinching problem is taken in the present study. We start out with a perfectly flat lamellar lipoplex as depicted in Figure 1. The DNA rods are assumed to be ordered within a 3D rectangular lattice as it was observed by Battersby *et al.* [8] and by Artzner *et al.* [10]. Our goal is to calculate how the electrostatic interaction between the negatively charged "rods" and the positively charged membranes modifies the conformation of the membranes. We calculate the electrostatic free energy within the Debye-Hückel approximation which is the linearized version of the Poisson-Boltzmann theory (as it is used in Refs. [19,22]); this allows us to calculate this complicated geometry on an analytical level. We show that there are in principle two possibilities, namely a compression of the lamellar stack as depicted in Figure 2 ($h < 0$) or an expansion as depicted in the same figure (case $h > 0$).

In the next section we introduce the model system and calculate its electrostatic and bending free energies for arbitrary but small periodic undulations of the membranes. By minimizing the free energies of the undulation with respect to its Fourier components we show in Section 3 that the electrostatic interaction usually favors a compression of the lamellar complex – at least if the underlying assumptions of our model are fulfilled. These assumptions are discussed in Section 4 where we also present some conclusions.

2 Free energy of model lipoplex

The aim of the following calculation is to determine the electrostatic contribution to the undulations of a lamellar stack of membranes with DNA molecules intercalated in between. Our model system consists of two constituents, the membranes and the DNA molecules. The membranes have a uniform thickness t and carry positive charges on both sides. The surface charge density is given by $\sigma/2$ on each side of the bilayer and is assumed to be uniform. In our model the membranes are perfectly transparent for the electric field lines, *i.e.*, we have a homogeneous dielectric constant throughout the lipoplex. The bilayers are flexible with a bending rigidity k_c. The DNA molecules are modeled as infinitely long rigid rods of radius r. For simplicity, we assume the negative charges of the DNA molecules to be located along their middle axis with the linear charge density $-\rho$. Following the experimental observation of a lamellar stack with DNA forming smectic arrays we arrange the components of our model in the following way (*cf.* Fig. 1). All membranes are parallel to the XY-plane with their midplanes at the positions $z = 0, \pm 2\,(r + t/2)\,, \pm 4\,(r + t/2)\,...$ The DNA rods are aligned in the Y-direction. The interhelical spacing between neighboring DNA molecules is constant and is denoted by $d = 2\pi/q$. Excluded volume requires that $d \geq 2r$. The rods in one layer are located at $x = 0, \pm d, \pm 2d...$, in the neighboring layers they are displaced by $d/2$, *i.e.*, they are at the positions $x = \pm d/2, \pm 3d/2, ...$ etc. Furthermore, the rods are assumed to be always attached to the two neighboring membranes.

The electrostatic interaction between the charges is calculated within the Debye-Hückel approximation. In this approximation the potential Φ is determined by $\Delta\Phi = \kappa^2\Phi$ with the appropriate boundary conditions. Here κ^{-1} denotes the Debye screening length that is given by

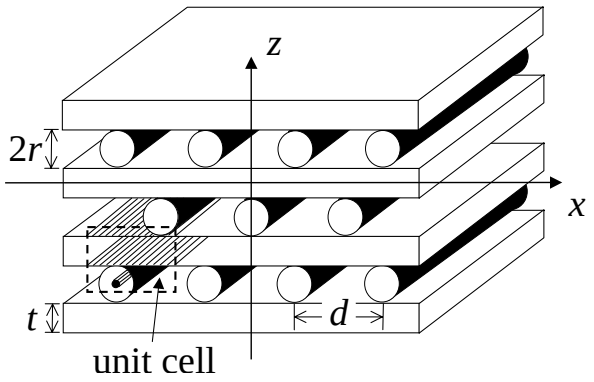

Fig. 1. Schematic view of the model lipoplex for the case of flat membranes (see text for detail).

$\kappa^{-1} = (8\pi n_{\rm s} l_{\rm B})^{-1/2}$ where $n_{\rm s}$ is the bulk salt concentration and $l_{\rm B} = e^2/\varepsilon k_{\rm B}T$ is the Bjerrum length (e is the unit charge, $k_{\rm B}T$ is the thermal energy and ε the dielectric constant; $\varepsilon \approx 80$ in an aqueous solution). The total electrostatic contribution $F_{\rm el}$ to the free energy of the system is given by the sum of the (screened) electrostatic interactions and the translational entropy of the small mobile ions (electrolyte ions and counterions) [32,33]:

$$F_{\rm el} = \frac{1}{2}\int {\rm d}S\sigma'\Phi. \tag{1}$$

The integration extends over all charged surfaces of the system with σ' being the corresponding charge densities.

We ask the following question: How are the membranes deformed by the electrostatic interaction? In order to answer this question we will calculate the induced undulations of the membrane up to the first order in the deformation amplitude.

Consider the membrane at $z = 0$. Electrostatics induces a deformation $z = u(x)$ around the flat state, $z \equiv 0$. Due to the symmetry the deformation profile is of the form

$$u(x) = \widehat{\sum_n} a_n \cos(nqx) \tag{2}$$

where the hat denotes summation over odd n only. This undulation leads to the following curvature energy $f_{\rm bend} = (k_{\rm c}/2)\left(\nabla^2 u\right)^2$ (per area A):

$$f_{\rm bend} = \frac{k_{\rm c}}{2}\widehat{\sum}_n a_n^2 n^4 q^4. \tag{3}$$

In order to calculate the electrical free energy, equation (1), we compute first the electrical potential $\Phi_{\rm M}^{(u)}(x,z)$ induced by the charges on the upper surface of the membrane (note that $\Phi_{\rm M}^{(u)}$ is translational invariant in Y-direction). At that charged surface, *i.e.*, at $z = t/2 + u(x)$, we have the boundary condition $\partial\Phi_{\rm M}^{(u)}/\partial n = -2\pi\sigma/\varepsilon$ which is here of the form

$$-\frac{\partial\Phi_{\rm M}^{(u)}}{\partial x}\widehat{\sum_n} a_n nq\sin(nqx) + \frac{\partial\Phi_{\rm M}^{(u)}}{\partial z} = -\frac{2\pi\sigma}{\varepsilon} \tag{4}$$

(up to terms of the order a_n^2). By expanding $\Phi_{\rm M}^{(u)}$ up to first order in the amplitudes a_n we find the following form of the potential above the membrane ($z > t/2 + u(x)$)

$$\Phi_{\rm M}^{(u)}(x,z) = \varphi^{(0)}(x,z) + \widehat{\sum_n} a_n \varphi^{(n)}(x,z). \tag{5}$$

Each $\varphi^{(n)}$ fulfills the Debye-Hückel equation separately. They can be expanded in Fourier series $\varphi^{(n)} = \sum_m B_m^{(n)}(z)\cos(mnqx)$ where $B_m^{(n)}(z) = b_m^{(n)}\exp(-\kappa_{nm}z)$ with $\kappa_{nm} = \sqrt{\kappa^2 + (nmq)^2}$. The coefficients $b_m^{(n)}$ follow from the boundary condition at the membrane together with the fact that due to symmetry $\Phi_{\rm M}^{(u)}(x, z < t/2) \equiv \Phi_{\rm M}^{(u)}((\pi/q) - x, t - z)$. We find that only the coefficients $b_1^{(n)}$ are non-vanishing and are given by $b_1^{(n)} = \pi\sigma\kappa/\varepsilon\kappa_n$. This leads to (for $z > t/2$):

$$\Phi_{\rm M}^{(u)}(x,z) = \frac{\pi\sigma}{\varepsilon\kappa}\left[{\rm e}^{-\kappa(z-t/2)} + \widehat{\sum_n} a_n \frac{\kappa^2}{\kappa_n}{\rm e}^{-\kappa_n(z-t/2)}\cos(nqx)\right]. \tag{6}$$

The total potential $\Phi_{\rm M}$ induced by the membrane at $z = u(x)$ is the sum of the contributions of the upper charged boundary, $\Phi_{\rm M}^{(u)}$, *cf.* equation (6), and of the lower one, $\Phi_{\rm M}^{(l)}$: $\Phi_{\rm M} = \Phi_{\rm M}^{(u)} + \Phi_{\rm M}^{(l)}$. Using $\Phi_{\rm M}^{(l)}(x,z) = \Phi_{\rm M}^{(u)}(x, z+t)$ we find:

$$\Phi_{\rm M}(x,z) = \frac{2\pi\sigma}{\varepsilon\kappa}\left[\cosh\left(\frac{\kappa t}{2}\right){\rm e}^{-\kappa z} + \widehat{\sum_n} a_n \frac{\kappa^2}{\kappa_n}\cosh\left(\frac{\kappa_n t}{2}\right){\rm e}^{-\kappa_n z}\cos(nqx)\right]. \tag{7}$$

Furthermore, the potential induced by the line charge of the rod has the form

$$\Phi_{\rm R}(R) = -\frac{2\rho}{\varepsilon}K_0(\kappa R) \tag{8}$$

where R is the distance from the line and K_0 is a modified Bessel function with $K_0(x) \simeq -\ln x$ for $x \ll 1$ and $K_0(x) \simeq (\pi/2x)^{1/2}\exp(-x)$ for $x \gg 1$. The total electrical potential Φ is the sum of the potential Φ_1 that follows from all membranes and the potential Φ_2 that is due to all the rods: $\Phi = \Phi_1 + \Phi_2$.

We calculate now the total electrostatic contribution to the free energy per unit cell. A unit cell has the width d (in X-direction) and a height that corresponds to the (average) distance between neighboring layers (for the case of a flat membrane – depicted in Figure 1 – this height equals $2r + t$). According to equation (1), we obtain the total electrostatic energy (per unit cell) by integrating the total potential over all charged surfaces that lie within this cell. The unit cell in Figure 1 contains three charged surfaces, $S_{\rm M}^{(u)}$, $S_{\rm M}^{(l)}$ and $S_{\rm R}$. $S_{\rm M}^{(u)}$ is a stripe of the upper

surface of one bilayer that is uniformly charged with the density $\sigma/2$. $S_{\rm M}^{(l)}$ is the corresponding lower charged surface. $S_{\rm R}$ is the surface carrying the charges of one rod; we assume this to be the surface of a cylinder with radius $\delta r \ll r$ and charge density $-\sigma_{\rm R} = -\rho/2\pi\delta r$. It follows that the electrostatic energy (per area) has three contributions: The inter-(and intra-) membrane interaction $f_{\rm M}$, the membrane-rod interaction $f_{\rm MR}$ and the interaction between the rods $f_{\rm R}$. Thus

$$f_{\rm el} = f_{\rm M} + f_{\rm MR} + f_{\rm R} = \frac{\sigma/2}{2A}\int_{S_{\rm M}} {\rm d}S\Phi_1 - \frac{\sigma_{\rm R}}{A}\int_{S_{\rm R}} {\rm d}S\Phi_1 - \frac{\sigma_{\rm R}}{2A}\int_{S_{\rm R}} {\rm d}S\Phi_2. \quad (9)$$

Here we made use of the identity $-\int_{S_{\rm R}} {\rm d}S\sigma_{\rm R}\Phi_1 = \int_{S_{\rm M}} {\rm d}S\frac{\sigma}{2}\Phi_2$.

We start by calculating the change of the membrane-membrane interactions $F_{\rm M}$ induced by their undulations (up to first order in a_n). The position of the midplane of the kth membrane is given by

$$u_k(x) = 2k(r + t/2 + h) + (-1)^k \widehat{\sum_n} a_n \cos(nqx) \quad (10)$$

with $k = 0, \pm1, \pm2, ...$ and $h = -\widehat{\sum}_n a_n$. Figure 2 shows schematic views of lamellar structures that are compressed – case (a) with $h < 0$ – and swollen – case (b) with $h > 0$. Denote the contribution of kth membrane to the potential by $\Phi_{\rm M}^{(k)}$. Then

$$f_{\rm M} = \frac{q\sigma}{4\pi}\sum_{k=-\infty}^{\infty}\int_0^{2\pi/q} {\rm d}x\Phi_{\rm M}^{(k)}\left(x, \frac{t}{2} + \widehat{\sum_n} a_n\cos(nqx)\right) \simeq \frac{\pi\sigma^2}{\varepsilon\kappa}\frac{\cosh\left(\frac{\kappa t}{2}\right)\cosh(\kappa r)}{\sinh(\kappa(r + t/2))} + \frac{\pi\sigma^2\cosh^2\left(\frac{\kappa t}{2}\right)\widehat{\sum}_n a_n}{\varepsilon\sinh^2\left(\kappa\left(r + \frac{t}{2}\right)\right)}. \quad (11)$$

Equation (11) shows that a swelling of the system ($h = -\widehat{\sum}_n a_n > 0$) decreases the membrane-membrane interaction whereas a compression ($h < 0$) is unfavorable. Note that we neglected terms of second order in the a_n. As can be seen from equation (3) terms of the form a_n^2 lead to a renormalization of the bending constant, $k_{\rm c}' = k_{\rm c} + \delta k|_{\rm el}$. It can be shown that $\delta k|_{\rm el} = 3\pi\sigma^2/8\varepsilon\kappa^3 \approx T/\kappa^3 l_{\rm B}\lambda_{\rm GC}^2$ ($\lambda_{\rm GC} = e/2\pi l_{\rm B}\sigma$ is the Gouy-Chapman length)[35–43]. For a wide range of parameters one has $\delta k|_{\rm el} \ll k_{\rm c}$. In the following we use the bare bending rigidity $k_{\rm c}$, keeping in mind that it has to be replaced by $k_{\rm c}'$ when $\delta k|_{\rm el}$ is comparable to $k_{\rm c}$.

We estimate now the contribution of the membrane-rod attraction. Consider the rod at the position $x = 0$ and $z = -r - t/2 - h$. The rod is located within an infinite stack of membranes. This can be accounted for by simply summing *twice* over the contributions of all the membranes that are located *above* the rod, *i.e.* $f_{\rm MR} = -(2\rho/d)\sum_{k=0}^{\infty}\Phi_{\rm M}^{(k)}(0, -r - t/2 - h)$. From equation (7) follows that $\Phi_{\rm M}^{(k)}(0, z)$ is given by (note that $z < 0$)

$$\Phi_{\rm M}^{(k)}(0, z) \simeq \frac{2\pi\sigma}{\varepsilon\kappa}\cosh\left(\frac{\kappa t}{2}\right){\rm e}^{\kappa(z - 2k(r + t/2 + h))} + (-1)^{k+1} \times \frac{2\pi\sigma\kappa}{\varepsilon}\widehat{\sum_n}\frac{a_n}{\kappa_n}\cosh\left(\frac{\kappa_n t}{2}\right){\rm e}^{\kappa_n\left(z - 2k\left(r + \frac{t}{2}\right)\right)}. \quad (12)$$

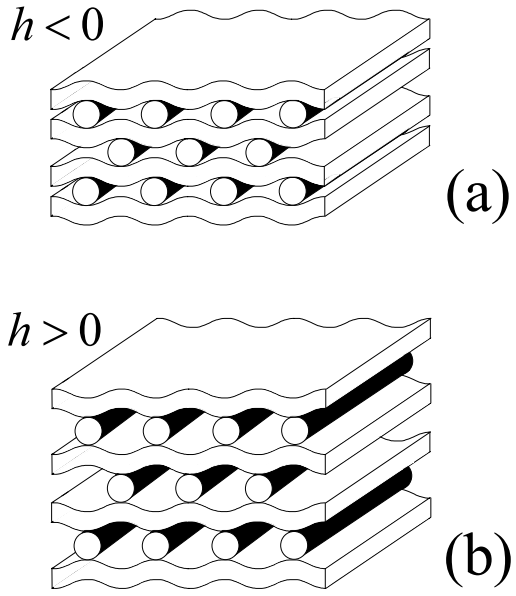

Fig. 2. Membrane undulations in lipoplexes. Shown are the two cases (a) $h < 0$: compression and (b) $h > 0$: swelling of the lamellar stack.

The contribution of the first term of equation (12) to $F_{\rm MR}$ is of the form:

$$f_{\rm MR}^{(1)} \simeq -\frac{\sigma\rho q}{\varepsilon\kappa}\frac{\cosh(\kappa t/2)}{\sinh(\kappa(r + t/2))} - \frac{\sigma\rho q}{\varepsilon}\frac{\coth\left(\kappa\left(r + \frac{t}{2}\right)\right)}{\sinh\left(\kappa\left(r + \frac{t}{2}\right)\right)}\cosh\left(\frac{\kappa t}{2}\right)\widehat{\sum_n} a_n. \quad (13)$$

The second term of equation (12) leads to the following expression:

$$f_{\rm MR}^{(2)} = \frac{\sigma\rho q\kappa}{\varepsilon}\widehat{\sum_n}\frac{\cosh(\kappa_n t/2)}{\kappa_n\cosh(\kappa_n(r + t/2))}a_n. \quad (14)$$

The total membrane-DNA contribution $f_{\rm MR} = f_{\rm MR}^{(1)} + f_{\rm MR}^{(2)}$ favors a compression of the lamellar stack – thus constituting a competing mechanism to the membrane-membrane repulsion.

We are left with the calculation of the interaction energy between the rods. We focus here one two important cases. *Case 1*: $\kappa t \gg 1$ and $\kappa d \ll 1$ ("vertical screening"): In this case the interaction between rods in different layers is negligible compared to the rod-rod interaction within

the same layer. Then it is sufficient to sum over the contributions of all rods to the left and to the right of the given rod:

$$f_{\mathrm{R}} = \frac{\rho^2 q}{\pi\varepsilon}\sum_{k=1}^{\infty} K_0\left(\kappa d k\right) \simeq \frac{\rho^2 q}{\pi\varepsilon}\int_0^{\infty} \mathrm{d}k K_0\left(\kappa d k\right) = \frac{\rho^2 q^2}{4\pi\varepsilon\kappa}\cdot \tag{15}$$

Case 2: $\kappa t \ll 1$ and $\kappa d \ll 1$ (weak screening): In this case all rods contribute to the interaction energy. After some algebra we arrive at

$$f_{\mathrm{R}} \simeq \frac{\rho^2 q^2}{4\pi\varepsilon\kappa^2\left(r+t/2\right)} + \frac{\rho^2 q^2}{4\pi\varepsilon\kappa^2\left(r+t/2\right)^2}\widehat{\sum}_n a_n. \tag{16}$$

As expected, in both cases the repulsive rod-rod interaction favors swelling.

3 Undulations in isoelectric complexes

We consider first the lamellar complex in equilibrium with a solution of free DNA strands. We ask: What is the interaxial distance d between the DNA strands in the lipoplex that minimizes the electrostatic free energy of the complex? As pointed out by Bruinsma [19] and in the introduction to this paper counterion release will control d in complexes that are assembled from highly charged components. This effect is not included in a simple Debye-Hückel theory. Therefore we consider first weakly charged rods with a linear charge density below the Manning threshold. We then argue below that our model can also be applied to highly charged rods.

We neglect entropic changes due to the adsorption of free DNA strands into the lipoplex. We will show that, as a result of the geometry, such a system will equilibrate at the isoelectric point – if the electrostatic interaction is sufficiently long-ranged. In the following we only account for the contributions independent of the a_n's and treat the contribution of membrane bending afterwards as a perturbation.

Let us first consider the case of high ionic strength where $\kappa r \gg 1$ (strong screening). Then the free energy per area is given by

$$f_{\mathrm{tot}} \simeq -\frac{4\pi\sigma\rho \mathrm{e}^{-\kappa r}}{\varepsilon\kappa d} \tag{17}$$

i.e., by the membrane-rod attraction, equation (13); other terms are negligible. It follows that the minimum is at $d \to 0$. Excluded volume interaction between the rods will lead to $d = 2r$. Clearly, in the case of strong screening as a result of the short range of the electrostatic interaction the lipoplex is equilibrated far from the isoelectric point. The resulting complex is "overcharged" by the DNA rods. (A similar situation occurs for the adsorption of rods on an oppositely charged surface, *cf.* Refs. [44] and [45].)

We discuss next the two cases introduced above. *Case 1*: $\kappa t \gg 1$ and $\kappa d \ll 1$ (vertical screening): From equations (13) and (15) we find (up to terms of the order κd)

$$f_{\mathrm{tot}} \simeq -\frac{2\pi\sigma\rho}{\varepsilon\kappa d} + \frac{\pi\rho^2}{\varepsilon\kappa d^2} \tag{18}$$

f_{tot} is minimized for $d = d_{\mathrm{iso}} = \rho/\sigma$ which corresponds to the isoelectric point of the complex, *i.e.*, the point at which the charges of the cationic lipids and of the DNA are exactly balanced. *Case 2*: $\kappa t \ll 1$ and $\kappa d \ll 1$ (weak screening): From equations (13) and (16) follows

$$f_{\mathrm{tot}} \simeq -\frac{2\pi\sigma\rho}{\varepsilon\kappa^2\left(r+t/2\right)d} + \frac{\pi\rho^2}{\varepsilon\kappa^2\left(r+t/2\right)d^2}\cdot \tag{19}$$

Again the free energy is minimized at the isoelectric interhelical spacing $d = d_{\mathrm{iso}} = \rho/\sigma$.

Thus in the limiting case $\kappa \to 0$ (no salt, no screening) the lipoplex is forced to be at the isoelectric point. We assumed above that the rods are weakly charged. DNA molecules are beyond the Manning threshold and thus the release of condensed counterions upon adsorption of DNA rods modifies the situation. A discussion of such effects is given in reference [45] for the case of adsorption of DNA on a planar, oppositely charged surface; the authors find that with increasing screening length more and more counterions are released and that the interhelical spacing approaches the isoelectric one. This limiting case should carry over to lipoplexes as well. In the following we will restrict ourselves, for simplicity, to isoelectric complexes and assume a complete release of all condensed counterions into the bulk salt solution, the later assumption being asymptotically correct for weak screening. At finite salt concentrations a partial counterion release should be expected (*cf.* also the discussion on the end of this paper).

We consider now the undulations occuring in lipoplexes in general and then focus again on the isoelectric point. The change of the total electrostatic free energy as a function of the deformation follows from the equations (11, 13–16):

$$\Delta f_{\mathrm{el}} \simeq \begin{cases} \left(\dfrac{\pi\sigma^2}{\varepsilon} - \dfrac{\sigma\rho q}{\varepsilon}\right)\widehat{\sum}_n a_n & \text{for } \kappa t \gg 1, \kappa d \ll 1 \\ \left(\frac{\pi\sigma^2}{\varepsilon} - \frac{\sigma\rho q}{\varepsilon} + \frac{\rho^2 q^2}{4\pi\varepsilon}\right)\frac{\widehat{\sum}_n a_n}{\kappa^2\left(r+\frac{t}{2}\right)^2} & \text{for } \kappa t \ll 1, \kappa d \ll 1. \end{cases} \tag{20}$$

The change of the total free energy due to bending is given by the sum of the electrical contribution Δf_{el} and the bending energy f_{bend}, equation (3). Minimizing f_{tot} with respect to the amplitudes a_n leads to $a_n \simeq A/n^4$ with

$$A \simeq \begin{cases} \frac{\sigma\rho q - \pi\sigma^2}{\varepsilon k_{\mathrm{c}} q^4} & \text{for } \kappa t \gg 1,\ \kappa d \ll 1 \\ \frac{\sigma\rho q - \pi\sigma^2 - \rho^2 q^2/4\pi}{\varepsilon k_{\mathrm{c}} q^4 \kappa^2 (r+t/2)^2} & \text{for } \kappa t \ll 1,\ \kappa d \ll 1. \end{cases} \tag{21}$$

Thus the deformation modes decrease rapidly with increasing n. Now we are in the position to calculate the

deformation of the membranes. Inserting $a_n = A/n^4$ into equation (2) we find the following deformation profile

$$u(x) = A\widehat{\sum}_n \frac{\cos(nqx)}{n^4} = A\left(\frac{\pi^4}{90} - \frac{\pi^2 x^2}{12} + \frac{\pi x^3}{12} - \frac{x^4}{48}\right) \tag{22}$$

where the polynomial expression is valid for $0 \leq qx \leq \pi$ (the continuation outside this interval follows from the symmetry of the configuration). A good approximation for all values of x (relative error smaller than 1.5%) is given by $u(x) = A\cos(qx)$.

The coefficient A at the isoelectric point of the complex $2\pi\sigma/q = \rho$ is given by

$$A \simeq \begin{cases} \frac{\pi\sigma^2}{\varepsilon k_c q^4} \text{ for } \kappa t \gg 1,\ \kappa d \ll 1 \\ 0 \quad\quad \text{for } \kappa t \ll 1,\ \kappa d \ll 1. \end{cases} \tag{23}$$

In the case of vertical screening we find a positive (and κ-independent) value of A and thus a negative value of h, $h = -\pi^4 A/90$, corresponding to a compression of the isoelectric lamellar stack. Interestingly, in the case of weak screening the undulations disappear. In fact, as long as the vertical screening is operative the membrane–membrane repulsion is smaller than the membrane-rod attraction in the isoelectric lipoplex and therefore we find a compression of the lamellar stack. For weak screening, the rod-rod repulsion between different layers cancels this net attraction, *cf.* equation (20). Let us consider typical values for ρ, σ, ε and k_c, say $\rho = e/1.7$ Å (DNA), $\sigma = e/100$ Å^2, $\varepsilon = 80$ (water) and $k_c = 20 k_B T$. For these values we find (in the case of vertical screening) $h \approx -1$ Å, a minute account compared to the other dimensions in the system. This indicates that it might be difficult to experimentally detect these electrostatically induced undulations. However, since severe approximations were involved to arrive at our model system these undulations might be detectable in certain experimental situations (*cf.* also the discussion at the end of the paper).

Finally, we estimate how the undulations of the isoelectric complex disturb the interaxial spacing between the rods and in turn move the complex away from its isoelectric point. We consider the case of vertical screening (*Case 1*, $\kappa t \gg 1$, $\kappa d \ll 1$). In this case the amplitudes of the undulations depend strongly on the interhelical distance, namely $A \sim d^4$, *cf.* equation (23). Inserting $a_n = A/n^4$ into equations (3) and (20) we find two correction terms to equation (18), namely $\pi^2\sigma^4 d^4/\left(2880\varepsilon^2 k_c\right)$ from the membrane bending and $-\pi^2\sigma^4 d^4/\left(1440\varepsilon^2 k_c\right)$ from the electrostatics. Evidently, the bending contribution favors smaller values of d that lead to smaller undulations, whereas the electrostatic free energy is lowered for larger undulations, *i.e.*, larger values of d are favorable. Since the electrostatic contribution is larger than the bending term the complex is slightly moved away from the isoelectric point towards a larger value of the interhelical distance $d = d_{iso} + \Delta d$, with

$$\Delta d = \frac{\pi}{1440}\frac{\kappa\rho^5}{\varepsilon k_c \sigma^3}\,. \tag{24}$$

Note that for $\kappa \to 0$ this effect vanishes and the complex is forced to be at the isoelectric point (as expected). For the above given typical values we find $\Delta d \approx 50$ Å$/\kappa^{-1}$. In that sense the undulations lead to an effective repulsion between the DNA strands proportional to d^3 (as long as one is close enough to the isoelectric point). Undulations are one of several mechanisms that might be responsible for the increase of the interhelical spacing with increasing salt concentration as it is observed experimentally (*cf.* Ref. [13] for details).

4 Discussion

The main idea of the preceding analysis is to give a simple estimate of the role of the electrostatic interactions in a lamellar lipoplex. In many instances some of the underlying assumptions are not fulfilled. But even in this case our model might give an idea about what the contributions of the electrostatics to the overall conformation might be.

One severe approximation is the assumption of a transparent membrane. The lipid bilayer represents a low dielectric slab that – depending on its thickness – might screen most of the electrical field so that charges (say of phosphate group on a DNA molecule) are not "seen" on the other side of the membrane. As a rule of thumb, a membrane that is much thinner than the distance D of a charge from the membrane might be considered to be transparent for this charge whereas a thicker membrane (of thickness $t > D$) is opaque and can be approximated by an infinitely thick slab. In that case the effect of the low dielectric lipid can be accounted for by the use of an appropriate image charge – which is a simple task for a flat membrane but difficult to handle for an undulating one. The two cases $t < D$ and $t > D$ are elaborated in some detail in Footnote 2 in reference [23]. The typical thickness of a lipid membrane is 24 Å which is of the order of the diameter of the DNA rod (20 Å), *i.e.*, one is in the crossover regime between the two cases. In any case, the presence of the low-dielectric lipid will lead to a modification of the simple situation discussed in this paper. It should be expected that the partial confinement of electrical field lines emanating from the DNA rods by the neighboring membranes favors a swelling of the lamellar stack.

Another feature not considered in this study is the demixing of neutral and charged lipids within the bilayers. There might be at least three effects. (i) The electrostatic attraction drives the cationic lipids towards the DNA rods, resulting in a depletion of charged lipids in the membrane parts in between two neighboring rods in the same layer. This reduces the membrane-membrane repulsion between neighboring membranes resulting in an increase of the compression of the lipoplex. This effect should be important if the average mole fraction of cationic lipids in the bilayers is low. (ii) For the opposite limit of highly charged membranes their surface charge density σ might exceed the surface charge density $\rho/2\pi r$ of the rods. In this case an enhancement of neutral lipids close to the DNA is expected that allows for a better matching of the two

charge densities. These two effects were indeed observed in the numerical study by Harries *et al.* [22] (*cf.* Fig. 7 in that paper). (iii) Finally, membrane undulations may also affect the charge densities on each side of the bilayer and *vice versa*. A depletion of cationic lipids on one side of the membrane will be accompanied by an enhancement on the other side due to the symmetry of the arrangement of DNA rods, *cf.* Figure 1. This might lead to a spontaneous curvature of the bilayer of either sign which in turn affects the membrane undulations discussed in our study. It is clear that the competition of these three effects can lead to a rather complex behavior of the charge density profile of the lipids along the X-direction. In our model we do not account for these effects. We expect that our approach has to be modified especially when a large fraction of the lipids is neutral. In that case the description of local, highly charged pinches [23] might be more appropriate (*cf.* the discussion of the polypeptide lipoplex [31] given in the Introduction of our paper).

The charges of the DNA molecule in our model were arranged along a single line instead of being located on the surface of a finite-size cylinder. This is an excellent approximation as long as the screening length is much larger than the radius of the cylinder, *i.e.*, as long as $\kappa r \ll 1$. For the opposite limiting case (not further discussed in this paper) the potential around the cylinder is different for the two arrangements of charges, *cf.* the discussion in reference [44].

We used in this study the Debye-Hückel theory to describe the electrostatic interaction in the lipoplex. This theory should be reasonable for weakly charged systems where it follows from a linearization of the Poisson-Boltzmann equation. This equation itself is a mean-field equation and is therefore not able to describe ion-correlation effects that become important at short distances, especially in the presence of multivalent salt (*cf.* Ref. [46] and references therein). For monovalent salt it is generally believed to provide a reasonable description of the electrostatics. The use of its linearized form relies on the assumption that the electrostatic potential acting on an elementary charge does not exceed the thermal energy at any point in the system. At high charge densities as encountered in a typical lipoplex the use of the Debye-Hückel approximation is therefore questionable. However, we expect it to be a reasonable description when one accounts also consistently for counterion condensation [47,48]: A fraction of the counterions is assumed to condense on the polyions and reduces its effective charge density, the remaining non-condensed counterions form a Debye-Hückel cloud around it. The amount of condensed counterions changes upon adsorption in the lipoplex. How many counterion will be released depends on the charge density of the cationic membrane, the demixing of lipids close to the adsorbed DNA molecules, the dielectric contrast between the membrane and the water gap etc. When we gave numerical examples in this paper we assumed complete counterion release; the numerical values, especially for ρ, have to be modified accordingly if there is only partial counterion release. This should, however, not change the qualitative behavior of the system. A more detailed study of the role of counterion release in such systems is in progress.

Recently it has been possible to non-ambiguously detect undulations in a lipoplex [49]. From a careful analysis of the data on the lipoplex presented in reference [10] if was possible to construct an electron density map revealing its high resolution structure. The structure shows undulations with an amplitude of a few Angstrom leading to a compression of the lamellar structure as depicted in Figure 2a. Furthermore, the amplitudes show a sharp increase for larger interhelical spacings. Both observations are in qualitative and semi-quantitative agreement with the results of our model calculation, *cf.* equation (23). It is worth noting that the lipids of these lipoplexes form a lipid-gel phase [10]. In this phase the lipid bilayers show a high compressibility allowing the DNA-induced deformations to cross nearly unperturbed through the bilayer (as implicitely assumed in our model). The induced undulations might be the prevailing mechanism for the interlayer coupling that leads to the rectangular columnar superlattice of the DNA strands observed for this class of lipoplexes.

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