Structural thermodynamics of lamellar cationic lipid-DNA complex: DNA compressibility modulus

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We have studied theoretically the compressibility modulus $B$ of DNA and complexation adsorption isotherms of DNA and lipids, as a function of DNA spacing $d_{DNA}$ and NaCl electrolyte concentration, respectively, in isoelectric states of lamellar DNA/cationic lipid (CL) self-assemblies. The electrostatic free energy derived from the Poisson-Boltzmann theory predicts partial agreement with measured $B$ values for interhelical separations $d_{DNA} > 33$ Å when use is made of a fit of hydration repulsion from bulk DNA hexagonal phases in solution. For lower interchain separations the prediction worsens due to the hydration interaction that overcomes the electrostatic contribution. An exact match of the system’s counterion electrochemical potentials and the coions of salt in aqueous phase leads to the electrostatic part of the free energy that renders isotherms of $d_{DNA}$ versus ionic strength in qualitative consistency with general trends of available experimental data of CL-DNA complexes. © 2005 American Institute of Physics. [DOI: 10.1063/1.2137697]

I. INTRODUCTION

Cationic liposomes serve as useful vehicles to transport extracellular DNA into cell cytoplasm. In a comprehensive series of experiments, Salditt and co-workers have determined with synchrotron x-ray scattering that DNA chains form two-dimensional (2D) lattice arrangements in the carrier liposome interior. Such self-assembled systems are being studied with the aim of using them in gene therapy. However, recent in vitro experiments show a low rate of DNA transfection through cell and nuclear membranes. Therefore, one expects that a precise knowledge of the system’s microstructure will help to enhance the efficiency of the DNA delivery process.

In their experiments Salditt et al. measured the DNA compressibility modulus $B(d_{DNA})$ as a function of interhelical spacing $d_{DNA}$ in isoelectric systems, that is, stoichiometrically charge neutral cationic lipid (CL)-DNA complexes, where there is the same amount of cationic lipid of the liposomes and anionic DNA charges, without added salt in solution. Since cationic liposomes are made of neutral and positively charged lipids, one such system is obtained by keeping its total lipid composition constant, while the molecular weight ratio of the DNA to the cationic lipid species is kept fixed at a constant value of 2.2 to ensure an overall charge neutrality of the system. At thermal equilibrium this isoelectric complex shows an average DNA-DNA separation $d_{DNA}$. The compressibility modulus relates to the force per unit length of the DNA in such 2D crystalline condensed state. Salditt et al. demonstrated that several forces are implicated in the stability of DNA condensation. These include hydration and electrostatic forces, which are repulsive, and the attractive, but negligibly weak van der Waals force. Until now, no theoretical explanation is available on the specific way these intermolecular forces drive the cationic lipid-DNA complex stability. Salditt et al. discussed several possible mechanisms for the observed DNA effective repulsive force, and considered different microscopic model interactions: In order to be consistent with their observation of the line-shape analysis of scattered light, Helfrich’s steric type of interaction of 2D ordered DNA strands was ruled out since it does not correspond to the lateral strand fluctuations. Also, pure hard-core chain-chain interactions cannot explain the data for $B(d_{DNA})$. They found that ordering is not just an effect of close packing. On the other hand, the three-dimensional (3D) hydration interaction between DNA pairs that was found in bulk solutions to produce repulsion among DNA strands cannot alone account for the experimentally measured $B(d_{DNA})$. Indeed Salditt et al. observed that long-ranged electrostatic interactions of exponential form, as found in hexagonal concentrated DNA phases, do not conform to their data of $B(d_{DNA})$. For this type of interaction to be consistent with $B$, a fitting in the electrolyte concentration up to 14.4 mM is required. However, this concentration of counterions is much larger than the estimated of 1 mM in the interior of actual isoelectric systems. Therefore, a complete comparison between the theory and the available experimental measurements of the compressibility modulus $B$ and its dependence on the interchain distance $d_{DNA}$ remains yet to be performed.

From the theoretical viewpoint, Bruinsma and Mash10 developed, at the level of mean-field Poisson-Boltzmann (PB), the first theory which leads to the long-range electrostatic contribution to $B$. A comparison of their PB analytical solution with the experimental data of Salditt et al. showed a large predicted value of $B$ at small $d_{DNA}$ that, however, decays faster than the data. In their model of a CL-DNA complex, Bruinsma and Mash used an electroneutral system made of two flat positively charged membranes, each one

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having a very low surface density of cationic lipids with an intervening embedded lattice of negatively charged rods. Therefore, the rods are separated by large distances. In this model system the finite size of the lipid is neglected and all counterions are released from the system’s interior.

The observed two-dimensional DNA ordering has been successfully analyzed with the help of the theory developed by Harries et al.\textsuperscript{11} This theory has been used previously to predict the complexation isotherms of the DNA interchain separation as a function of charge on the lipid membranes and fixed the overall molecular weight ratio of the lipid to DNA charges.\textsuperscript{11,12} It was found to reproduce the general trends of the experimentally measured isotherms.\textsuperscript{12} Based on this theory, all possible lipid-DNA composite phases were determined,\textsuperscript{13} and the interplay of spatial corrugations and charge lipid modulations in these complexes was demonstrated.\textsuperscript{14}

In this paper we used the theoretical approach of Harries et al. to obtain numerical solutions of the full nonlinear PB equation and we made a fit of a parametrized form of a hydration repulsion, including the van der Waals interaction and negligible ion size at $c^* = 2$ mM electrolyte concentration. We found good agreement with the measured data of $B(d_{\text{DNA}})$ to a fit of hydration repulsion at $d_{\text{DNA}} > 33$ Å where the electrostatic effective interaction dominates. The effective electrostatic force contains the main contribution due to the large ionic entropy force arising from the extreme counterion release upon lipid-DNA self-assembly.

However, for low values of $d_{\text{DNA}}$, the prediction worsens due to the high strength of the hydration repulsion that overwhelms the electrostatic contribution.

For another set of experimental measurements reported by Koltower et al.,\textsuperscript{8} an exact match of the electrochemical potentials of the different ionic species leads to PB solutions that agree with the major tendency of $d_{\text{DNA}}$ vs $c^*$ experimental curves, whereas complete disagreement remains if this condition is not imposed. These differences are also found on another structural property, namely, the profile distribution of mobile cationic lipids on charged membranes. The magnitude of this profile turned out to be lower than for the cases where the exact ion’s chemical equilibrium was neglected.

II. MEAN-FIELD THEORY

We consider the system’s unit cell shown in Fig. 1 formed by two flat lipid bilayers separated by the mean distance $d_{\text{w}}$. Each membrane has a thickness $d_{\text{m}}$, and it is formed by a mixture of cationic lipid dioleoyl trimethylammonium propane (TAP) at molar fraction $\phi_{\text{TAP}}$ and neutral colipid dioleoyl phosphatidylcholin. The total surface density $\sigma_T$ of lipids on the membrane is given by the surface density $\sigma$ of positive ones, with the average area available to each of the lipid’s head group being $a = 70$ Å$^2$, roughly the same for the neutral component.\textsuperscript{8} The water phase in-between the lipid bilayers has a dielectric constant $\varepsilon = 78$ and contains a univalent electrolyte at concentration $c^*$. In the aqueous region there are two DNA macromolecules whose center to center distance $d_{\text{DNA}}$ defines the lateral size of the cell of depth $h$.\textsuperscript{224906-2}

At the mean-field level the free energy of the system’s formation is, according to the theory of Harries et al.,\textsuperscript{11,13}

$$F_c = (f_{\text{field}} + f_{\text{ion}} + f_{\text{lipid}})h,$$

where

$$f_{\text{field}} = \frac{e}{8\pi} \int_0^{d_{\text{DNA}}} dx \int_0^{d_{\text{w}}} dy (\nabla \psi)^2,$$

is Maxwell’s electric-field stress contribution. The second term in $F_c$ is the entropy of the mixing of pointlike ions and water molecules,

$$f_{\text{ion}} = k_B T \int_0^{d_{\text{DNA}}} dx \int_0^{d_{\text{w}}} dy \left( c^+ \ln \frac{c^+}{c} + c^- \ln \frac{c^-}{c} \right. - \left. (c^+ + c^- - 2c) \right).$$

In the approach of Harries et al., the lipid size and the mobility on the lipid bilayers are taken into account through a corresponding entropy term,

$$f_{\text{lipid}} = 2k_B T \int_0^{d_{\text{DNA}}} dx \left[ \sigma \ln \frac{\sigma}{\sigma_T} + (\sigma_T - \sigma) \ln \frac{\sigma_T - \sigma}{\sigma_T} \right],$$

with $k_B$ being Boltzmann’s constant and $T$ being the temperature. Extremization of the free energy, Eq. (1), yields following Boltzmann’s factors for the distribution of ions:

$$c^* = e^{\frac{-\sigma}{\sigma_T} - \frac{\sigma_T}{\sigma} k_B T},$$

which satisfies the following PB equation:

$$\nabla^2 \psi = -\frac{4\pi}{e} [z e c^+ - z e c^-].$$

The potential $\psi$ fulfills the following boundary conditions:

$$\nabla \psi \cdot \hat{n} = \begin{cases} 4\lambda \varepsilon r_D, & \text{on DNA} \\ 4\pi \sigma(\chi)e, & \text{on membrane}, \end{cases}$$

with $\hat{n}$ being the unit normal to the dielectric boundaries and pointing into the aqueous phase. $r_D = 10$ Å is the radius of the
DNA, which has bare linear charge density $\lambda = -e/1.7 \, \text{Å}$, and $e$ is the elementary charge. Further minimization of Eq. (1) with $\sigma(x)$ subject to the local charge constraint on bilayers, second part of Eq. (7), yields $^{11}$

$$\sigma(x) = \frac{e^{-\phi a + \lambda \lambda}}{a[(1 - \phi a + \phi a + e^{-\phi a}]/a]}. \quad (8)$$

In this equation the value of Lagrange’s ($L$) multiplier $\lambda$, is fixed by the requirement that, at equilibrium, for a given liposomal membrane charge density $e \phi a_i$, the local density $\sigma(x)$ on the corresponding membrane in the CL-DNA system must satisfy the following electroneutrality condition:

$$e \int_0^{d_{DNA}} d x \sigma(x) - e \phi a_i/a = 0. \quad (9)$$

III. RESULTS AND DISCUSSION

A. Compressibility modulus of DNA

The recent x-ray synchrotron experiments by Salditt et al. $^{5,6}$ revealed that DNA strands in the CL-DNA complex experience an effective repulsive force whose magnitude is given in Fig. 2 by the compressibility modulus $B$ as a function of interhelical separation $d_{DNA}$. Such data were taken for eight different isoelectric systems where the cationic lipids are exactly neutralized by the anionic phosphate groups of the DNA. This amounts to a constant (DOTAP)/DNA weight ratio of 2.2 in all cases. Meanwhile the total lipid composition for each system was kept constant. Thus, the weight ratios of both types of lipids forming the lipid bilayers were varied eight times leading to a sequence of cationic lipid molar fractions whose values $\phi a_i = 1, 0.7407, 0.598, 0.5, 0.4, 0.3401, 0.3$, and 0.25, and correspond to different measured equilibrium interhelical separations $d_{DNA} = 27.6, 28.7, 38.1, 38.9, 47.9, 52.4, 53.0$, and 54.7 Å associated to each of those isoelectric complexes, respectively. Wagner et al. $^{16}$ demonstrated that the formation of the lamellar CL-DNA phase is driven by counterion release both from the cationic lipids and from the DNA. Yet, it is maximal at the isoelectric point, where the estimated concentration of counterions was about 1 mM. Moreover, their study emphasized the importance of the very large entropic free-energy gain by released counterions that leads to the effective entropic force driving the association between the DNA and the lipids. In our calculations described below this force of entropic origin is embodied and taken implicitly into account in the electrostatic free energy, Eq. (1). Hence, the equilibrium spacing between DNA chains results from three forces: the attractive van der Waals force that tends to decrease DNA interhelical distance and the two repulsive forces that increase such separation, namely, the hydration and the electrostatic repulsion. The van der Waals interaction between two DNA molecules (modeled as two dielectric cylinders with a dielectric constant of 4) with a distance $d_{DNA}$ apart is given through the following compressibility modulus:

$$B_{vdw}(d_{DNA}) = \frac{S A \sqrt{r_D}}{32} \left(\frac{d_{DNA}}{2d_D}\right)^{7/2}, \quad (10)$$

with the Hamaker constant $A = 5.2 \times 10^{-21} \, \text{J}$. $^{5}$ While the hydration contribution to the compressibility was derived in Ref. 5 from the empirically parametrized expression of the hydration force (having a spatial range of 30 Å) for bulk hexagonal DNA phases,

$$B_{hyd} = d_{DNA} f_{b_{DNA}} e^{-\left(d_{DNA}^2 r_D^2\right)/\lambda_H}, \quad (11)$$

where the observed exponential decay of this repulsive interaction, the force coefficient $f_{b_{DNA}} = 0.2174 k_B T/\text{Å}^2$, and decay length $\lambda_H = 3.1–3.5 \, \text{Å}$ were measured with osmotic stress techniques by Podgornik et al. $^{17}$ For the condensed DNA inside the complex, it was found in Ref. 5 that in highly screened isoelectric complexes by NaCl electrolyte in solution, hydration interaction prevails over the electrostatic one producing DNA separations of the order of 30 Å, similar to what it is observed in bulk 3D DNA hexatic phases, $^{17}$ in spite of confinement and geometrical restrictions prevailing inside.
the complex. Therefore, we use in this paper the above phenomenological parametrization of $B_{\text{pd}}$ with $f_{\text{iso}}$ and $\lambda_H$ as fitting force parameters. Finally, the long-range contribution to the compressibility arising from electrostatic interactions (with its main contribution resulting from the ion’s entropic force) becomes important at separations larger than 30 Å. We calculated it numerically from the free energy $F_c$ of Eq. (1) per unit area of the complex using the following thermodynamic relationship:

$$B_{\text{pd}} = d_{\text{DNA}}^2 \left( \frac{\partial^2 (F_c/I)/\text{area}}{(dI)^2} \right)_{I=b}$$ \hspace{1cm} (12)

When using Eq. (12), we first determined the complex’s free energy $F_c$, Eq. (1), as a function of several DNA spacings $I$ at constant $\phi_{\text{TAP}}$ and electrolyte concentration $c^\ast$. The minimum of $F_c$ sets in the thermally equilibrated DNA separation $I_{\text{DNA}}$. Thereafter, $B_{\text{pd}}$ is evaluated from Eq. (12) at this constant $d_{\text{DNA}}$, which comes about from the given $\phi_{\text{TAP}}$ corresponding to one isoelectric complex. In this manner, the mean-field prediction of the compressibility was obtained for each of the eight different molar fractions corresponding to the various isoelectric systems quoted above. We considered the system’s unit-cell sizes given in Table I. Thus, the total compressibility modulus is $B = B_{\text{pd}} + B_{\text{ys}} + B_{\text{zw}}$, which we plotted and compared with the experimental data in Fig. 2(a).

The comparison is made using three constant force parameters $f_{\text{iso}} = (0.2174, 0.081, 0.02108)k_B T/\AA^2$; and a fixed space decay length $\lambda_H = 3.23 \AA$ in all cases. $B_{\text{pd}}$ was calculated using salt concentrations $c^\ast = 2$ and $4$ mM (with $f_{\text{iso}} = 0.2174k_B T/\AA^2$ and $\lambda_H = 3.23 \AA$ for the latter concentration). It can be observed in Fig. 2(a) that the prediction for $B$ agrees with the experiment for interchain separations $d_{\text{DNA}} > 33 \AA$ using a fit of hydration $B_{\text{yd}}$ with $f_{\text{iso}} = 0.2174k_B T/\AA^2$, $\lambda_H = 3.23 \AA$, and $c^\ast = 2$ mM. However, it deviates substantially from the experimental value for $d_{\text{DNA}} < 33 \AA$. In Fig. 2(b) all components of $B$ for $c^\ast = 2$ mM are shown as a function of $d_{\text{DNA}}$. It can be noted that the case of the best fit to the experimental data has a hydration contribution $B_{\text{yd}}$ that overweights the electrostatic part $B_{\text{pd}}$ in the range $d_{\text{DNA}} < 33 \AA$. Yet, with a selection of different values of $f_{\text{iso}}$, $\lambda_H$ does not even improve the prediction of $B$. The availability of more complete sets of experimental measurements of the hydration force in this self-assembled system would be desirable, as has been done already in a very accurate and exhaustive form for biomembrane bilayers at physiological salt concentrations in solution.

### B. Adsorption isotherms of DNA and lipids

In our calculations outlined above, we assumed that both the chemical potentials of negative $\mu^-$ and positive $\mu^+$ ions are equal, which imply that their bulk concentration are the same, $c^\ast \Lambda^3 = \exp[\beta \mu^+] = \exp[\beta \mu^-]$, with $\Lambda = \Lambda^+ = \Lambda^-$ the thermal wavelength. In what follows we relax this approximation and take into account their real chemical potentials, which are in fact different, $\mu^+ = \mu^*_+ \pm \chi$ and $\mu^- = \mu^*_- \mp \chi$ where at chemical equilibrium $\exp[\beta \mu^*_\pm] = c^\ast \Lambda^3 \gamma_\pm$, with $\gamma_\pm$ being the mean activity coefficient. Then, the profile counterion distributions are $c^\ast = \exp[\beta \mu^*_\pm / \Lambda^3 \gamma^\ast]$ with $\nu = \pm$ and $\beta = 1/k_B T$. $\gamma^\ast$ and $\gamma^\prime$ are the activity coefficients of each ionic species. The overall electroneutrality condition of the system determines $\chi$ according to

$$\int_0^{d_{\text{DNA}}} dx \int_0^{d_{\text{DNA}}} dy [+ zee^\ast(x,y) - zee^\prime(-x,y)] + 2e \int_0^{d_{\text{DNA}}} dx [\sigma(x) = |\lambda|].$$ \hspace{1cm} (13)

Koltover et al.\textsuperscript{8} reported a series of measurements performed on isoelectric CL-DNA complexes. They determined the dependence of DNA spacing $d_{\text{DNA}}$ with added univalent salt NaCl at concentration $c^\ast$ for different charged membranes as given by their cationic molar fraction $\phi_{\text{TAP}}$. Koltover et al. found, at low $\phi_{\text{TAP}} < 0.6$, a nonmonotonous variation of $d_{\text{DNA}}$ for increasing ionic strength $c^\ast$. They observed that DNA separation $d_{\text{DNA}}$ increases slowly starting from small $c^\ast$, with a sudden drop in the magnitude of the DNA interhelical separations at high salt concentration (which value depends on $\phi_{\text{TAP}}$). Such a sharp change of the $d_{\text{DNA}}$ adsorption isotherm is ascribed to the CL-DNA system instability that occurs because both DNA molecules and cationic lipids are released from the system due to the high salt screening of the counterion release mechanism.\textsuperscript{9} At higher $\phi_{\text{TAP}} \approx 0.6$ of the membrane charge, the effect of $c^\ast$ on $d_{\text{DNA}}$ manifests through an observed swelling of the system’s stack of bilayers, with a simultaneous monotonous increase of DNA average separations for increasing $c^\ast$. At these high salt contents direct DNA-DNA electrostatic interaction is screened and hydration repulsion was found to prevail between DNA strands, which contributes to the set an equilibrium $d_{\text{DNA}}$ distance of separation of a range of 30 Å.\textsuperscript{8} In Fig. 3 we show our calculated complexation isotherms of $d_{\text{DNA}}$ vs $c^\ast$ and their comparison with the experimental data of Koltover et al. (black-filled circles joined by line) for $\phi_{\text{TAP}} \approx 0.6$. In the approximate case of equal ion’s chemical potentials $\mu^+ = \mu^_- = \mu^*_\pm (\chi = 0)$, black-filled squares joined by line, and the case with an exact match of real chemical potentials of ions inside the complex $\mu^\pm$ with that for ions in bulk solution $\mu^\pm = \mu^*_\pm = \pm \chi$, black-filled triangle up symbols, $\chi \neq 0$. In order to determine correctly in each case $\mu^\pm$ we use Ref. 20, that contains measured mean activity coefficients $\gamma_{\pm}$ as a function of concentrations $c^\ast$ of the electrolyte NaCl. The
system’s unit-cell sizes $d$ and $d_m$ used in the numerical calculations of $F_c$ were taken from Table I. Additionally, useful values of $d_{\text{DNA}}$ and $d$ were read off from Fig. 6(d) of Ref. 8. We used a cell depth $h = 1 \text{ Å}$. The main features of the calculated $d_{\text{DNA}}$ vs $c^*$ curves is that they generally follow the trends of the corresponding experimental curves when the correct ion’s chemical potentials $(\mu^\pm = \mu^\text{bulk}_\pm + \chi)$ are taken into account. However, the prediction disagrees with the experiment when the approximation $\mu^+ = \mu^-$, that is, Eq. (5) is used. These results show that it is not realistic to assume as a chemical equilibrium condition equal chemical potentials, $\mu^+ = \mu^-$, for the different kinds of ions that reside in the inner side of the complex, with those of ions in the exterior aqueous phase. Rather, the equilibrium value of $\mu^\pm = \mu^\text{bulk}_\pm + \chi$ for each ion’s species should be used. It should be noted that for the calculated curves in Fig. 3 we did not consider the hydration and the van der Waals contributions to the free energy $F_c$ but only the long-range electrostatic part, that is, Eqs. (1)–(9) together with Eq. (13). We also performed numerical calculations of $d_{\text{DNA}}$ curves as a function of $c^*$ including the DNA-DNA phenomenological bulk hydration (hyd) interaction energy $F_{\text{hyd}} = f_{\text{hyd}} \lambda \exp[-(d_{\text{DNA}} - 2r_D)/\lambda]\exp[-(d_{\text{DNA}} - 2r_D)/\lambda]$. Since $F_{\text{hyd}}$ is $c^*$’s independent, the overall effect of $F_{\text{hyd}}$ is to shift the magnitude of $F_c$, but does not change the location of its minimum localized at $l = d_{\text{DNA}}$. Therefore, the inclusion of bulk hydration repulsion into $F_c$ leads to the same results as in the case when it is neglected. Thus, the same curves of Fig. 3 are obtained. However, we believe that $F_{\text{hyd}}$ depends on the confining geometry as presented in the CL-DNA complex. Yet, there remains its comprehensive experimental determination in order to be incorporated in a theoretical framework as discussed here. A similar study for the low charged membrane cases $\phi_{\text{TAP}} < 0.6$ can be made.
Effects in $F_{c}$ do not include neither the hydration nor the van der Waals effects, so we reduce its strength by three orders of magnitude to $0.004 = 10^{-3}$ M. This corresponds to an isoelectric CL-DNA system at $\phi_{TAP} = 0.23$. For their determination we do not include neither the hydration nor the van der Waals effects in $F_{c}$. This corresponds to an isoelectric CL-DNA system at $\phi_{TAP} = 0.9$ of cationic lipids (highly charged membrane). Note that $n(x)$ is symmetrical around the midplane between DNA molecules. The most important feature is the accumulation of the cationic lipids close to and on top of the DNA cylindrical surface centered at the end sides of the cell located at $x = 0.0 d_DNA$ and $x = 0.4 d_DNA$ with a large depletion of lipids at the midplane. The segregation of lipids observed in Fig. 4 results from the high salt content. Thus, for instance, if we reduce its strength by three orders of magnitude to $c^+ = 0.004 M$ and consider a less charged membrane case $\phi_{TAP} = 0.78$, then, the profile $n(x)$ displays very little segregation of lipids, as can be noted in the inset of Fig. 4(a).

When we made use of $\mu^+ = \mu^-$ as the chemical equilibrium of ions and $c^+ = 0.23 M$ salt concentration [Fig. 4(a), dash line], the profile $n(x)$ is larger than the profile of positive lipids at the higher ionic strength $c^+ = 0.5 M$ [Fig. 4(b), dash line], and both profiles obtain their maximum magnitudes in the spatial regimes $0 \leq x \leq 0.1 d_{DNA}$ and $0.9 d_{DNA} \leq x \leq d_{DNA}$ close to the DNA molecules (recall $\phi_{TAP} = 0.9$). This phenomenon results from the more effective lipid's screening to the DNA electric field close to the membrane (for $c^+ = 0.23 M$), where cationic lipids see an imperfectly charge-neutralized DNA molecule by the salt ions. Positive ions form a void in the region of closest approach of the DNA surface to the membrane, while negative ions deflect and get concentrated more in the middle of the cell (see Fig. 6 below). Therefore, at $c^+ = 0.23 M$, the DNA electric field makes the cationic lipids to be crowded where the DNA field is higher, implying a better charge matching on the DNA and membrane. A more realistic determination of the trends of lipid membrane modulations turns out when we use $\mu^\pm = \mu^\pm_{bulk} \pm \chi$ for the ion's chemical equilibrium (see curves in Fig. 4 with black continuous lines for $c^+ = 0.23 M$ and $c^+ = 0.5 M$). Small quantitative deviations are obtained with respect to the profiles derived with $\mu^\pm = \mu^-$ as a condition. This only indicates that both models are not equivalent, with the

**C. Profile distribution of cationic lipids**

The exact match condition on the chemical potentials of the ions also shows qualitative effects on another relevant structural property: the charge modulation of positive lipids on membranes, $n(x) = \sigma(x)/a$. In Fig. 4 we give examples of nondimensionalized cationic lipid density profiles $n(x)$ at two electrolyte concentrations $c^+ = 0.23 M$ and $0.5 M$ for unit-cell sizes $d_{DNA} = 6.802 d_D$ $d_{DNA}$ (taken from Table I), and $h = 1$ Å. For their determination we do not include neither the hydration nor the van der Waals effects in $F_{c}$. This corresponds to an isoelectric CL-DNA system at $\phi_{TAP} = 0.9$ of cationic lipids (highly charged membrane). Note that $n(x)$ is symmetrical around the midplane between DNA molecules. The most important feature is the accumulation of the cationic lipids close to and on top of the DNA cylindrical surface centered at the end sides of the cell located at $x = 0.0 d_DNA$ and $x = 0.4 d_DNA$ with a large depletion of lipids at the midplane. The segregation of lipids observed in Fig. 4 results from the high salt content. Thus, for instance, if we reduce its strength by three orders of magnitude to $c^+ = 0.004 M$ and consider a less charged membrane case $\phi_{TAP} = 0.78$, then, the profile $n(x)$ displays very little segregation of lipids, as can be noted in the inset of Fig. 4(a).

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former ($\chi \neq 0$) being more accurate. We found, however, that the expression $\mu^+ = \mu_{\text{bulk}}^+ \pm \chi^+$ leads to similar predictions as with $\chi = 0$.

D. Local-density functions of ions

The ion's distribution functions $c^\pm(x,y)$ are shown as perspective plots in Figs. 5(a) and 5(b), together with their complementary contour plots in Figs. 5(c) and 5(d), respectively. These profile distributions were obtained for a system with unit-cell sizes $d_{\text{DNA}} = 6.802 \text{lD}$, $d_w$ from Table I, and with $h = 1 \text{ Å}$ and $c^\pm = 0.23M \text{ NaCl}$ salt, and using $\mu^\pm = \mu_{\text{bulk}}^\pm \pm \chi^+$ for the case of an almost neutral membrane $\phi_{\text{TAP}} = 0.3$. Figure 5 refers only to one quarter cell of Fig. 1. Note in Fig. 5(a) that positive ions $c^+$ form a rather compact double layer around the DNA surface, and they are pushed away from the cationic lipid bilayer located at $y = 0$ [front face of the cubic cell of Fig. 5(a)], meanwhile they are more concentrated at the side $y = 2.3$ ($= d_w/2 \text{lD}$ distance away from front face of the cell) where $c^+$ shows a maximum. The contour plot of $c^+$ in Fig. 5(c) depicts the compactness of the positive ion's double layer about the DNA with a void of positive ions in any other region in the cell. If the molar fraction is increased up to $\phi_{\text{TAP}} = 0.9$, the maximum of $c^+$ appearing at $y = 2.3 \text{lD}$ diminishes with the positive ions spreading out mainly between DNA molecules, while the profile strength $c^+$ at $(x/\text{lD}, y/\text{lD}) = (0, 1.5)$, that is, towards the membrane side, gets lower showing a vacuum of ions in the region $0 < x/\text{lD} < 1.0$ [see Fig. 6(a)]. The distribution function $c^-(x,y)$ takes on quite a different appearance as shown in Fig. 5(b). Negative ions have a more widespread distribution in all of the aqueous phase available in the cell and tend to occupy all the space in an inhomogeneous manner, showing their highest condensation close to the membrane for $\phi_{\text{TAP}} = 0.3$ [Figs. 5(b) and 5(d)]. In this case $c^-$ is high near the lipid membrane located at $y/\text{lD} = 0.0$ where there is better electrostatic matching, but with a depletion of ions about $x/\text{lD} = 0.0$ due to electrostatic repulsion with the smeared negative charge on the DNA. In the case of $\phi_{\text{TAP}} = 0.9$ and $c^\pm = 0.23M$, both the profile distribution $c^-(x,y)$ of Fig. 6(b) and its contour plot in Fig. 6(d) show that negative ions are tightly condensed onto the liposomal membrane with a depletion of ions close to the DNA surface while they accumulate more at the middle of

FIG. 6. Same as Fig. 5 for ion’s distribution functions $c^+$ and $c^-$ with $\phi_{\text{TAP}} = 0.9$ and $c^\pm = 0.23M$. Inside (c) there is a region of vacuum, $0 < x/\text{lD} < 0.5$, formed by positive ions on the surface of the DNA, while they are concentrated more in the space between DNA strands. (d) depicts the large accumulation of negative ions onto the lipid bilayer. They form a narrow cloud that shows the depletion of ions close to the DNA surface.
the cell. Thus, they present a strong inhomogeneous screening to the electric field emanating from the cationic lipids, which in turn get more concentrated near the DNA molecule. This fact was already noted also in Sec. III C, where a large spatial segregation of lipids in the unit cell was observed [see Figs. 4(a) and 4(b)], which is more emphasized than in the case of small salt content $c^*=0.004M$ at the same $\phi_{T/AP} =0.9$. Its corresponding function $n(x)$ of charge modulation of lipids is almost flat, reflecting its constant spatial variation as depicted in the inset of Fig. 4(a).

IV. CONCLUSION

In this paper we have made a comparison between experiments and mean-field PB theory results, of the DNA’s compressibility modulus of lamellar cationic lipid-DNA systems at the isoelectric state. Our numerical results emphasized the importance of hydration repulsion on the compressibility modulus of DNA molecules. Hydration interaction becomes the main effect on this thermodynamic property at short-range interhelical separations, whereas at larger separations the long-range electrostatic contribution dominates. Since the DNA interhelical spacing dependence of hydration repulsion has not been measured systematically at different system’s conditions of membrane charge, added salt, DNA/lipid charge ratio, etc., we used the experimentally parametrized form of this interaction, which is valid only for 3D bulk DNA phases in solution. A fit of the hydration force contribution into the theoretical compressibility modulus of the CL-DNA system leads to partial agreement with the experimental data at large DNA separations ($d_{DNA}>33\,\text{Å}$). However, at shorter separations ($d_{DNA}<33\,\text{Å}$) the predicted $B$ deviates substantially from observations.

We also showed the importance of taking correctly into account within the PB theory the exact match of the electrochemical potentials of the different ionic species. The resulting chemical equilibrium condition leads to qualitative agreement between the theory and the observed trends of complexation isotherms of DNA plus lipid’s adsorption in these self-assemblies.

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