Strongly correlated electrostatics of viral genome packaging

Toan T. Nguyen

Received: 10 December 2012 / Accepted: 16 January 2013 / Published online: 2 April 2013
© Springer Science+Business Media Dordrecht 2013

Abstract The problem of viral packaging (condensation) and ejection from viral capsid in the presence of multivalent counterions is considered. Experiments show divalent counterions strongly influence the amount of DNA ejected from bacteriophage. In this paper, the strong electrostatic interactions between DNA molecules in the presence of multivalent counterions is investigated. It is shown that experiment results agree reasonably well with the phenomenon of DNA reentrant condensation. This phenomenon is known to cause DNA condensation in the presence of tri- or tetra-valent counterions. For divalent counterions, the viral capsid confinement strongly suppresses DNA configurational entropy, therefore the correlation between divalent counterions is strongly enhanced causing similar effect. Computational studies also agree well with theoretical calculations.

Keywords DNA virus • DNA overcharging • Multivalent counterions • Strongly correlated electrostatics

1 Introduction

The problem of DNA condensation in the presence of multivalent counterions has seen a strong revival of interest in recent years. This is because of the need to develop effective ways of gene delivery for the rapidly growing field of genetic therapy. DNA viruses such as bacteriophages provide excellent study candidates for this purpose. One can package genomic DNA into viruses, then deliver and release the molecule into targeted individual

T. T. Nguyen
Nano and Energy Center, Vietnam National University-Hanoi,
144 Xuan Thuy, Cau Giay Street, Hanoi, Vietnam

T. T. Nguyen (✉)
School of Physics, Georgia Institute of Technology, 837 State Street,
Atlanta, GA 30332-0430, USA
e-mail: toan.nguyen@physics.gatech.edu

cells. Recently there is a large biophysics literature dedicated to the problem of DNA condensation (packaging and ejection) inside bacteriophages (for a review, see for example, [1]).

Most bacteriophages, or viruses that infect bacteria, are composed of a DNA genome coiling inside a rigid, protective capsid. It is well-known that the persistence length, l_p , of DNA is about 50 nm, comparable to or larger than the inner diameter of the viral capsid. The genome of a typical bacteriophage is about 10 microns or 200 persistence lengths. Thus the DNA molecule is considerably bent and strongly confined inside the viral capsid resulting in a substantially pressurized capsid with internal pressure as high as 50 atm [2–5]. It has been suggested that this pressure is the main driving force for the ejection of the viral genome into the host cell when the capsid tail binds to the receptor in the cell membrane, and subsequently opens the capsid. This idea is supported by various experiments both *in vivo* and *in vitro* [3, 4, 6–11]. The *in vitro* experiments additionally revealed possibilities of controlling the ejection of DNA from bacteriophages. One example is the addition of PEG (polyethyleneglycol), a large molecule incapable of penetrating the viral capsid. A finite PEG concentration in solution produces an apparent osmotic pressure on the capsid. This in turn leads to a reduction or even complete inhibition of the ejection of DNA.

Since DNA is a strongly charged molecule in aqueous solution, the screening condition of the solution also affects the ejection process. At a given external osmotic pressure, by varying the salinity of solution, one can also vary the amount of DNA ejected. Interestingly, it has been shown that monovalent counterions such as NaCl have a negligible effect on the DNA ejection process [3, 12, 13]. In contrast, multivalent counterions such as Mg^{+2} , CoHex $^{+3}$ (Co-hexamine), Spd $^{+3}$ (spermidine) or Spm $^{+4}$ (spermine) exert strong effect, both qualitatively and quantitatively different from that of monovalent counterions. In this paper, we focus on the role of Mg^{+2} divalent counterion on DNA ejection. In Fig. 1, the percentage of ejected DNA from bacteriophage λ (at 3.5 atm external osmotic pressure) from

Fig. 1 (Color online) Inhibition of DNA ejection depends on $MgSO_4$ concentration for bacteriophage λ at 3.5 atm external osmotic pressure. Solid circles represent experimental data from [11, Fang 2009, personal communication], where different colors corresponds to different experimental batch. The dashed line is a theoretical fit of our theory. See Section 4

[11, Fang 2009, personal communication] are plotted as a function of MgSO_4 concentration (solid circles). The three colors correspond to three different sets of data. Evidently, the effect of multivalent counterions on the DNA ejection is non-monotonic. There is an optimal Mg^{+2} concentration where the minimum amount of DNA genome is ejected from the phages.

The general problem of understanding DNA condensation and interaction in the presence of divalent counterions is rather complex because many physical factors involved are energetically comparable to each other. In this paper, we focus on understanding the non-specific electrostatic interactions involved in the inhibition of DNA ejection by divalent counterions. We show that some aspects of the DNA ejection experiments can be explained within this framework. Specifically, we propose that the non-monotonic influence of multivalent counterions on DNA ejection from viruses is expected to have the same physical origin as the phenomenon of reentrant DNA condensation in free solution [14–16].

Due to strong electrostatic interaction between DNA and Mg^{+2} counterions, the counterions condense on the DNA molecule. As a result, a DNA molecule behaves electrostatically as a charged polymer with the effective net charge, η^* per unit length, equal to the sum of the “bare” DNA charges, $\eta_0 = -1e/1.7\text{\AA}$, and the charges of condensed counterions. There are strong correlations between the condensed counterions at the DNA surface which cannot be described using the standard Poisson-Boltzmann mean-field theory. Strongly correlated counterion theories, various experiments and simulations [17–22] have showed that when these strong correlations are taken into account, η^* is not only smaller than η_0 in magnitude but can even have opposite sign: this is known as the *charge inversion* phenomenon. The degree of counterion condensation, and correspondingly the value of η^* , depends logarithmically on the concentration of multivalent counterions, c_Z . As c_Z increases from zero, η^* becomes less negative, neutral and eventually positive. We propose that the multivalent counterion (Z -ions for short) concentration, $c_{Z,0}$, where DNA’s net charge is neutral corresponds to the optimal inhibition due to Mg^{+2} -induced DNA–DNA attraction inside the capsid. At counterion concentration c_Z lower or higher than $c_{Z,0}$, η^* is either negative or positive. As a charged molecule at these concentrations, DNA prefers to be in solution to lower its electrostatic self-energy (due to the geometry involved, the capacitance of DNA molecule is higher in free solution than in the bundle inside the capsid). Accordingly, this leads to a higher percentage of ejected viral genome.

The fact that Mg^{+2} counterions can have such strong influence on DNA ejection is highly non-trivial. It is well-known that Mg^{+2} ions do not condense or only condense partially free DNA molecules in aqueous solution [23, 24]. Yet, they exert strong effects on DNA ejection from bacteriophages. We argue that this is due to the entropic confinement of the viral capsid. Unlike free DNA molecules in solution, DNA packaged inside capsid are strongly bent and the thermal fluctuations of DNA molecule is strongly suppressed. It is due to this unique setup of the bacteriophage where DNA is pre-packaged by a motor protein during virus assembly that Mg^{+2} ions can induce attractions between DNA. It should be mentioned that Mg^{+2} counterions have been shown *experimentally* to condense DNA in another confined system: the DNA condensation in two dimension [25]. Results from our computer simulations in Section 5 (see also [26, 27]), also show that if the lateral motion of DNA is restricted, divalent counterions can induced DNA condensation. The strength of DNA–DNA attraction energy mediated by divalent counterions is comparable to the theoretical results.

The organization of the paper as follows: In Section 2, a brief review of the phenomenon of overcharging DNA by multivalent counterions, and the reentrant condensation phenomenon are presented. In Section 3, these strongly correlated electrostatic phenomena are used to setup a theoretical study of DNA ejection from bacteriophages. In Section 4, the

semi-empirical theory is fit to the experimental data of DNA ejection from bacteriophages. The fitting results are discussed in the context of various other experimental and simulation studies of DNA condensation by divalent counterions. In Section 5, an Expanded Ensemble Grand Canonical Monte-Carlo simulation of a DNA hexagonal bundle is presented. It is shown that the simulation results reaffirm our theoretical understanding. We conclude in Section 6.

2 Overcharging of DNA by multivalent counterions

In this section, let us briefly visit the phenomenal of overcharging of DNA by multivalent counterions to introduce various physical parameters involved in our theory. More detail descriptions, and other aspect of strongly correlated electrostatics can be found in [18]. Standard linearized mean field theories of electrolyte solution states that in solutions with mobile ions, the Coulomb potential of a point charge, q , is screened exponentially beyond a Debye-Hückel (DH) screening radius, r_s :

$$V_{DH}(r) = \frac{q}{r} \exp(-r/r_s). \quad (1)$$

The DH screening radius r_s depends on the concentrations of mobile ions in solution and is given by

$$r_s = \sqrt{\frac{Dk_B T}{4\pi e^2 \sum_i c_i z_i^2}} \quad (2)$$

where c_i and z_i are the concentration and the valence of mobile ions of species i , e is the charge of a proton, and $D \approx 78$ is the dielectric constant of water.

Because DNA is a strongly charged molecule in solution, linear approximation breaks down near the DNA surface because the potential energy, $eV_{DH}(r)$, would be greater than $k_B T$ in this region. It has been shown that, within the general non-linear mean-field Poisson-Boltzmann theory, the counterions would condense on the DNA surface to reduce its surface potential to be about $k_B T$. This so-called Manning counterion condensation effect leads to an “effective” DNA linear charge density:

$$\eta_c = -Dk_B T/e. \quad (3)$$

In these mean field theories, the charge of a DNA remains negative at all ranges of ionic strength of the solution. The situation is completely different when DNA is screened by multivalent counterions such as Mg^{2+} , Spd^{3+} or Spm^{4+} . These counterions also condense on DNA surface due to their strong attraction to DNA negative surface charges. However, unlike their monovalent counterparts, the electrostatic interactions among condensed counterions are very strong due to their high valency. These interactions are even stronger than $k_B T$ and mean field approximation is no longer valid in this case. Counterintuitive phenomena emerge when DNA molecules are screened by multivalent counterions. For example, beyond a threshold counterion concentration, the multivalent counterions can even over-condense on a DNA molecule making its net charge *positive*. Furthermore, near the threshold concentration, DNA molecules are neutral and they can attract each other causing condensation of DNA into macroscopic bundles (the so-called *like-charged attraction* phenomenon).

To understand how multivalent counterions overcharge DNA molecules, let us write down the balance of the electro-chemical potentials of a counterion at the DNA surface and in the bulk solution.

$$\mu_{cor} + Ze\phi(a) + k_B T \ln[c_Z(a)v_o] = k_B T \ln[c_Z v_o]. \quad (4)$$

Here v_o is the molecular volume of the counterion, Z is the counterion valency. $\phi(a)$ is the electrostatic surface potential at the dressed DNA. Approximating the dressed DNA as a uniformly charged cylinder with linear charged density η^* and radius a , $\phi(a)$ can be written as:

$$\phi(a) = \frac{2\eta^*}{D} \frac{K_0(a/r_s)}{(a/r_s)K_1(a/r_s)} \simeq \frac{2\eta^*}{D} \ln\left(1 + \frac{r_s}{a}\right) \quad (5)$$

where K_0 and K_1 are Bessel functions (this expression is twice the value given in [28] because we assume that the screening ion atmosphere does not penetrate the DNA cylinder). In (4), $c_Z(a)$ is the local concentration of the counterion at the DNA surface:

$$c_Z(a) \approx \sigma_0 / (Ze\lambda) = \eta_0 / (2\pi a Ze\lambda) \quad (6)$$

where $\sigma_0 = \eta_0 / 2\pi a$ is the bare surface charge density of a DNA molecule and the Gouy-Chapman length $\lambda = Dk_B T / 2\pi \sigma_0 Ze$ is the distance at which the potential energy of a counterion due to the DNA bare surface charge is one thermal energy $k_B T$. The term μ_{cor} in (4) is due to the correlation energies of the counterions at the DNA surface. It is this term which is *neglected* in mean-field theories. Several approximate, complementary theories, such as strongly correlated liquid [17, 18, 29], strong coupling [19, 21] or counterion release [30, 31] have been proposed to calculate this term. Although with varying degree of analytical complexity, they have similar physical origins. In this paper, we followed the theory presented in [18]. In this theory, the strongly interacting counterions in the condensed layer are assumed to form a two-dimensional strongly correlated liquid on the surface of the DNA (see Fig. 2). In the limit of very strong correlation, the liquid form a two-dimensional Wigner crystal (with lattice constant A) and μ_{cor} is proportional to the interaction energy of the counterion with background charges of its Wigner-Seitz cell. Exact calculation of this limit gives [18]:

$$\mu_{cor} \approx -1.65 \frac{(Ze)^2}{Dr_{WS}} = -1.17 \frac{1}{D} (Ze)^{3/2} \left(\frac{\eta_0}{a}\right)^{1/2}. \quad (7)$$

Here $r_{WS} = \sqrt{\sqrt{3}A^2/2\pi}$ is the radius of a disc with the same area as that of a Wigner-Seitz cell of the Wigner crystal (see. Fig. 2). It is easy to show that for multivalent counterions, the so-called Coulomb coupling (or plasma) parameter, $\Gamma = (Ze)^2 / Dr_{WS} k_B T$, is greater than one. Therefore, $|\mu_{cor}| > k_B T$, and thus cannot be neglected in the balance of chemical potential, (4).

Knowing μ_{cor} , one can easily solve (4) to obtain the net charge of a DNA for a given counterion concentration:

$$\eta^* = -\frac{Dk_B T}{2Ze} \frac{\ln(c_{Z,0}/c_Z)}{\ln(1 + r_s/a)}, \quad (8)$$

where the concentration $c_{Z,0}$ is given by:

$$c_{Z,0} = c_Z(a) e^{-|\mu_{cor}|/k_B T} \quad (9)$$

Fig. 2 (Color online) Strong electrostatic interactions among condensed counterions lead to the formation of a strongly correlated liquid on the surface of the DNA molecule. In the limit of very strong interaction, this liquid forms a two-dimensional Wigner crystal with lattice constant A . The shaded hexagon is a Wigner-Seitz cell of the background charge. It can be approximated as a disc of radius r_{WS}

Equation (8) clearly shows that for counterion concentrations higher than $c_{Z,0}$, the DNA net charge η^* is positive, indicating the over-condensation of the counterions on DNA. In other words, DNA is overcharged by multivalent counterions at these concentrations. Notice (7) shows that, for multivalent counterions $Z \gg 1$, μ_{cor} is strongly negative for multivalent counterions, $|\mu_{cor}| \gg k_B T$. Therefore, $c_{Z,0}$ is exponentially smaller than $c_Z(a)$ and a realistic concentration obtainable in experiments.

Besides the overcharging phenomenon, DNA molecules screened by multivalent counterions also experience the counterintuitive like-charge attraction effect. This short range attraction between DNA molecules can also be explained within the framework of the strong correlated liquid theory. Indeed, in the area where DNA molecules touch each other, each counterion charge is compensated by the “bare” background charge of *two* DNA molecules instead of one (see Fig. 3). Due to this doubling of background charge, each counterion condensed in this region gains an energy of:

$$\delta\mu_{cor} \approx \mu_{cor}(2\eta_0) - \mu_{cor}(\eta_0) \simeq -0.46 \frac{1}{D} (Ze)^{3/2} \left(\frac{\eta_0}{a}\right)^{1/2}. \quad (10)$$

Fig. 3 (Color online) Cross section of two touching DNA molecules (large yellow circles) with condensed counterions (blue circles). At the place where DNA touches each other (the shaded region of width A shown), the density of the condensed counterion layer doubles and additional correlation energy is gained. This leads to a short range attraction between the DNA molecules

As a result, DNA molecules experience a short range correlation-induced attraction. Approximating the width of this region to be on the order of the Wigner crystal lattice constant A , the DNA–DNA attraction per unit length can be calculated:

$$\mu_{\text{DNA}} \simeq -\frac{2\sqrt{2aA}\sigma_0}{Ze}|\delta\mu_{\text{cor}}| \simeq -0.34\frac{1}{D}\eta_0^{5/4}\left(\frac{Ze}{a}\right)^{3/4}. \quad (11)$$

The combination of the overcharging of DNA molecules and the like charged attraction phenomena (both induced by multivalent counterions) leads to the so-called reentrant condensation of DNA. At small counterion concentrations, c_Z , DNA molecules are undercharged. At high counterion concentrations, c_Z , DNA molecules are overcharged. The Coulomb repulsion between charged DNA molecules keeps individual DNA molecules apart in solution. At an intermediate range of c_Z around $c_{Z,0}$, DNA molecules are mostly neutral. The short range attraction forces are able to overcome weak Coulomb repulsion leading to their condensation. In this paper, we proposed that this reentrant behavior of DNA condensation as function of counterion concentration is the main physical mechanism behind the non-monotonic dependence of DNA ejection from bacteriophages as a function of the Mg^{+2} concentrations.

3 Theoretical calculation of DNA ejection from bacteriophage

We are now in the position to obtain a theoretical description of the problem of DNA ejection from bacteriophages in the presence of multivalent counterions. We begin by writing the total energy of a viral DNA molecule as the sum of the energy of DNA segments ejected outside the capsid with length L_o and the energy of DNA segments remaining inside the capsid with length $L_i = L - L_o$, where L is the total length of the viral DNA genome:

$$E_{\text{tot}}(L_o) = E_{\text{in}}(L_i) + E_{\text{out}}(L_o). \quad (12)$$

Because the ejected DNA segment is under no entropic confinement, we neglect contributions from bending energy and approximate E_{out} by the electrostatic energy of a free DNA of the same length in solution:

$$E_{\text{out}}(L_o) = -L_o \left(\eta^{*2}/D \right) \ln(1 + r_s/a), \quad (13)$$

where the DNA net charge, η^* , for a given counterion concentration is given by (8). The negative sign in (13) signifies the fact that the system of the combined DNA and the condensed counterions is equivalent to a cylindrical capacitor under constant charging potential. As shown in previous section, we expect the η^* to be a function of the Z -ions concentration c_Z and can be positive when $c_Z > c_{Z,0}$. In the limit of strongly correlated liquid, $c_{Z,0}$ is given in (9). However, the exponential factor in this equation shows that an accurate evaluation of $c_{Z,0}$ is very sensitive to an accurate calculation of the correlation chemical potential μ_{cor} . For practical purposes, the accurate calculation of μ_{cor} is a highly non-trivial task. One would need to go beyond the flat two-dimensional Wigner crystal approximation and takes into account not only the non-zero thickness of the condensed

counterion layer but also the complexity of DNA geometry. Therefore, within the scope of this paper, we are going to consider $c_{Z,0}$ as a phenomenological constant concentration whose value is obtained by fitting the result of our theory to the experimental data.

The energy of the DNA segment inside the viral capsid comes from the bending energy of the DNA coil and the interaction between neighboring DNA double helices:

$$E_{in}(L_i, d) = E_{bend}(L_i, d) + E_{int}(L_i, d). \quad (14)$$

where d is the average DNA–DNA interaxial distance.

There exists different models to calculate the bending energy of a packaged DNA molecules in literature [5, 9, 32–34]. In this paper, for simplicity, we employ the viral DNA packaging model used previously in [9, 32, 33]. In this model, the DNA viral genome are assumed to simply coil co-axially inward with the neighboring DNA helices forming a hexagonal lattice with lattice constant d (Fig. 4). For a spherical capsid, this model gives:

$$E_{bend}(L_i, d) = \frac{4\pi l_p k_B T}{\sqrt{3}d^2} \left\{ - \left(\frac{3\sqrt{3}L_i d^2}{8\pi} \right)^{1/3} + R \ln \frac{R + \left(3\sqrt{3}L_i d^2 / 8\pi \right)^{1/3}}{\left[\left(R^2 - (3\sqrt{3}L_i d^2 / 8\pi)^{2/3} \right)^{1/2} \right]} \right\}, \quad (15)$$

where R is the radius of the inner surface of the viral capsid.

To calculate the interaction energy between neighboring DNA segments inside the capsid, $E_{int}(L_i, d)$, we assume that DNA molecules are almost neutralized by the counterions (the net charge, η^* of the DNA segment inside the capsid is much smaller than that of the ejected segment because the latter has higher capacitance). In the previous section, we have shown that for almost neutral DNA, their interaction is dominated by short range attraction forces. Hence, one can approximate:

$$E_{int}(L_i, d_0) = -L_i |\mu_{DNA}|. \quad (16)$$

Here, d_0 is the equilibrium interaxial distance of DNA bundle condensed by multivalent counterions. Due to the strongly pressurized viral capsid, the actual interaxial distance, d ,

Fig. 4 A model of bacteriophage genome packaging. The viral capsid is modeled as a rigid spherical cavity. The DNA inside coils co-axially inward. Neighboring DNA helices form a hexagonal lattice with lattice constant d . A sketch for a cross section of the viral capsid is shown

between neighboring DNA double helices inside the capsid is smaller than the equilibrium distance, d_0 , inside the condensate. The experiments from [23] provided an empirical formula that relates the restoring force to the difference $d_0 - d$. Integrating this restoring force with d , one obtains an expression for the interaction energy between DNA helices for a given interaxial distance d :

$$E_{int}(L_i, d) = L_i \sqrt{3} F_0 \left[(c^2 + cd) \exp\left(\frac{d_0 - d}{c}\right) - (c^2 + cd_0) - \frac{1}{2} (d_0^2 - d^2) \right] - L_i |\mu_{DNA}|, \quad (17)$$

where the empirical values of the constants F_0 and c are 0.5 pN/nm² and 0.14 nm respectively.

As we showed in the previous section, like the parameter $c_{Z,0}$, accurate calculation of μ_{DNA} is also very sensitive to an accurate determination of the counterion correlation energy, μ_{cor} . Adopting the same point of view, instead of using the analytical approximation (11), we treat μ_{DNA} and d_0 as additional fitting parameters. In total, our semi-empirical theory has three fitting parameters ($c_{Z,0}$, μ_{DNA} , d_0).

4 Fitting of experiment of DNA ejection from bacteriophages and discussion

Equation (12) together with (13), (14), (15) and (17) provide the complete expression for the total energy of the DNA genome of our semi-empirical theory. For a given external osmotic pressure, Π_{osm} , and a given Z -ion concentration, c_Z , the equilibrium value for the ejected DNA genome length, L_o^* , is the length that minimizes the total free energy $G(L_o)$ of the system, where

$$G(L_o) = E_{tot}(L_o) + \Pi_{osm} L_o \pi a^2. \quad (18)$$

Here, $L_o \pi a^2$ is the volume of ejected DNA segments in aqueous solution. The result of fitting our theoretical ejected length L_o^* to the experimental data of [11] is shown in Fig. 1. In the experiment, wild type bacteriophages λ was used, so $R = 29$ nm and $L = 16.49$ μ m [35]. Π_{osm} is held fixed at 3.5 atm and the Mg^{+2} counterion concentration is varied from 10 mM to 200 mM. The fitted values are found to be $c_{Z,0} = 64$ mM, $\mu_{DNA} = -0.004 k_B T$ per nucleotide base, and $d_0 = 2.73$ nm.

The strong influence of multivalent counterions on the process of DNA ejection from bacteriophage appears in several aspects of our theory and is easily seen by setting $d = d_0$, thus neglecting the weak dependence of d on L_i and using (16) for DNA–DNA interactions inside the capsid. Firstly, the attraction strength $|\mu_{DNA}|$ appears in the expression for the free energy, (18), with the *same* sign as Π_{osm} (recall that $L_i = L - L_o$). In other words, the attraction between DNA strands inside capsid acts as an additional “effective” osmotic pressure preventing the ejection of DNA from bacteriophage. This switch from repulsive DNA–DNA interactions for monovalent counterion to attractive DNA–DNA interactions for Mg^{+2} leads to an experimentally observed decrease in the percentage of DNA ejected from 50% for monovalent counterions to 20% for Mg^{+2} counterions at optimal inhibition ($c_Z = c_{Z,0}$). Secondly, the electrostatic energy of the ejected DNA segment given by (13) is logarithmically symmetrical around the neutralizing concentration $c_{Z,0}$. This is well

demonstrated in Fig. 1 where the log-linear scale is used. This symmetry is also similar to the behavior of another system which exhibits a charge inversion phenomenon, the non-monotonic swelling of macroion by multivalent counterions [36].

It is very instructive to compare our fitting values for μ_{DNA} and $c_{Z,0}$ to those obtained for other multivalent counterions. Fitting done for the experiments of DNA condensation with Spm^{+4} and Spd^{+3} shows μ_{DNA} to be -0.07 and $-0.02 k_B T/\text{base}$ respectively [14, 23]. For our case of Mg^{+2} , a divalent counterion, and bacteriophage λ experiment, μ_{DNA} is found to be $-0.004 k_B T/\text{base}$. This is quite reasonable since Mg^{+2} is a much weaker counterion leading to much lower counterion correlation energy. Furthermore, $c_{Z,0}$ was found to be 3.2 mM for the tetravalent counterion, 11 mM for the trivalent counterion. Our fit of $c_{Z,0} = 64$ mM for divalent counterions again is in favorable agreement with these independent fits. Note that in the limit of high counterion valency ($Z \rightarrow \infty$), (9) shows that $c_{Z,0}$ varies exponentially with $-Z^{3/2}$ [17–19]. The large increase in $c_{Z,0}$ from 3.2 mM for tetravalent counterions to 11 mM for trivalent counterions, and to 64 mM for divalent counterions is not surprising.

It is quantitatively significant to point out that our fitted value $\mu_{DNA} = -0.004 k_B T$ per base explains why Mg^{+2} ions cannot condense DNA in free solution. This energy corresponds to an attraction of $-1.18 k_B T$ per persistence length. Since the thermal fluctuation energy of a polymer is about $k_B T$ per persistence length, this attraction is too weak to overcome thermal fluctuations. It therefore can only partially condense free DNA in solution [24]. Only in the confinement of the viral capsid can this attraction effect appear in the ejection process. It should be mentioned that computer simulations of DNA condensation by idealized divalent counterions [26, 27] show a weak short-range attraction comparable to our μ_{DNA} . This suggests that in the presence of divalent counterions, electrostatic interaction are an important (if not dominant) contribution to DNA–DNA short range interactions inside viral capsid.

The phenomenological constants μ_{DNA} and $c_{Z,0}$ depend strongly on the strength of the correlations between multivalent counterions on the DNA surface. The stronger the correlations, the greater the DNA–DNA attraction energy $|\mu_{DNA}|$ and the smaller the concentration $c_{Z,0}$. In [11], MgSO_4 salt induces a strong inhibition effect. Due to this, $c_{Z,0}$ for MgSO_4 falls within the experimental measured concentration range and we use these data to fit our theory. Experiment data suggests MgCl_2 induces weaker inhibition, thus $c_{Z,0}$ for MgCl_2 is larger and apparently lies at higher value than the measured range. More data at higher MgCl_2 concentrations is needed to obtain reliable fitting parameters for this case. In fact, the value $c_{Z,0} \simeq 104$ mM obtained from the computer simulation of [26] is nearly twice as large as our semi-empirical results. This demonstrates again that this concentration is very sensitive to the exact calculation of the counterion correlation energy μ_{cor} . The authors of [11] used non-ideality and ion specificity as an explanation for these differences. From our point of view, they can lead to the difference in μ_{cor} , hence in the value $c_{Z,0}$.

Lastly, we would like to point out that the fitted value for the equilibrium distance between neighboring DNA in a bundle, $d_0 \simeq 27.3 \text{ \AA}$ is well within the range of various known distances from experiments [9, 23].

5 Simulation of DNA hexagonal bundles in the presence of divalent counterions

To verify the strongly correlated physics of DNA–DNA interaction in the presence of divalent counterions inside viral capsids, we perform simulation of a system of DNA

hexagonal bundle in the presence of difference concentrations of divalent counterions. Detailed description of the simulation model can be found in [26, Nguyen 2013, manuscript in preparation]. The DNA bundle in hexagonal packing is modeled as a number of DNA molecules arranged in parallel along the z -axis. In the horizontal plane, the DNA molecules form a two dimensional hexagonal lattice with lattice constant d (the DNA–DNA interaxial distance) (Fig. 5).

Individual DNA molecule is modeled as an impenetrable cylinder with negative charges glued on it. The charges are positioned in accordance with the locations of nucleotide groups along the double-helix structure of a B-DNA. The hardcore cylinder has radius of $7\text{\AA}$. The negative charges are hard spheres of radius $2\text{\AA}$, charge $-e$ and lie at a distance of $9\text{\AA}$ from the DNA axis. This gives an averaged DNA radius, r_{DNA} of 1nm . The solvent water is treated as a dielectric medium with dielectric constant $D = 78$ and temperature $T = 300\text{ }^\circ\text{K}$. The positions of DNA molecules are fixed in space. This mimics the constrain on DNA configurational entropy inside viruses and other experiments of DNA condensation using divalent counterions. The mobile ions in solution are modeled as hard spheres with unscreened Coulomb interaction (the *primitive ion* model). The coions have radius of $2\text{\AA}$ and charge $-e$. The divalent counterions have radius of $2.5\text{\AA}$ and charge $+2e$.

In practical situation, the DNA bundle is in equilibrium with a water solution containing free mobile ions at a given concentration. Therefore we simulate the system using Grand Canonical Monte-Carlo (GCMC) simulation. The number of ions are not constant during the simulation. Instead their chemical potentials are fixed. The chemical potentials are chosen in advance by simulating a DNA-free salt solution and adjusting them so that the solution has the correct ion concentrations. Another factor that complicates the simulation of reentrant condensation phenomenon arises from the fact that there are both monovalent and divalent salts in solution in experiments. At very low concentration of divalent counterions, DNA is screened mostly by monovalent counterions. To properly simulate the DNA bundle at this low c_z limit, we need to include both salts in our simulations. the standard GCMC method

Fig. 5 (Color online) A DNA bundle is modeled as a hexagonal lattice with lattice constant d . Individual DNA molecule is modeled as a hard-core cylinder with negative charges glued on it according to the positions of nucleotides of a B-DNA structure

for ionic solution [37] is generalized to simulate of a system containing a mixture of both multivalent and monovalent salts. To mimic mixtures used in experiments, we maintain c_1 at about 50 mM and varies c_Z from 10 mM to about 300 mM.

As the goal of the computer simulation, we are concerned with calculating the “effective” DNA–DNA interaction, and correspondingly the free energy of assembling DNA bundle at different Z -ion concentrations. To do this, the Expanded Ensemble method [27] is used. This scheme allows us to calculate the osmotic pressure of the DNA bundle by sampling the system free energies at slightly different volumes. These osmotic pressure would directly proportional to the “effective” DNA–DNA interaction in the system.

5.1 Overcharging of DNA by multivalent counterions

As aforementioned in the introduction and Section 2, and suggested by our theoretical fit of bacteriophage DNA ejection experiments earlier, one of the striking consequence of the strong correlation among condensed Z -ions is the possibility of DNA molecules being overcharged by at high concentration c_Z . Our simulations shown that this is indeed the case for $c_Z \geq 104$ mM. In Fig. 6, the local coion concentration, $c_1(r)$, as functions of the distance from a DNA central axis for the bundle with the interaxial DNA distance, d , is chosen to be $50\text{\AA}$. They are the largest systems simulated in this work. The value $d = 50\text{\AA}$ is reasonably large, so that the influence from neighboring DNA helices in the bundle is minimal and each DNA behaves almost as if it were isolated.

As one can see, for low c_Z , $c_1(r)$ decreases monotonically as r decreases from ∞ to $10\text{\AA}$ (the DNA cylinder radius). This suggests that the DNA net charge, η^* , is negative at these concentrations. However, for $c_Z \geq 100$ mM, $c_1(r)$ increases as r decreases from ∞ . This accumulation of coions clearly suggests that η^* is positive at these concentrations. In other words, DNA molecules are overcharged.

Note that, at high Z -ion concentrations, $c_1(r)$ only starts to decrease again when r decreases below $17\text{\AA}$. This is easily understood if we approximate the thickness of the condensed counterion layer to be the same as the average distance between Z -ions along the DNA surface, or about $14\text{\AA}$. Then, the value $r = 14\text{\AA}$ corresponds to an approach

Fig. 6 (Color online) Local concentration of coions as a function of distance from the axis of a DNA in the bundle for different divalent counterion concentrations, for $d = 50\text{\AA}$

distance of $7\text{\AA}$ to the surface of the DNA, and is roughly half the thickness of the condensed counterion layer. Clearly, at such small approach, DNA “bare” charges are not screened by Z -ions and η^* is not a meaningful physical quantity.

5.2 Counterion mediated DNA–DNA interactions and the DNA packaging free energy

In Fig. 7, the osmotic pressure of DNA bundle at different c_Z is plotted as a function of the interaxial DNA distance, d . Because this osmotic pressure is directly related to the “effective” force between DNA molecules at that interaxial distance [27, 38], Fig. 7 also serves as a plot of DNA–DNA interaction. As one can see, when c_Z is greater than a value around 20 mM, there is a short-range attraction between two DNA molecules as they approach each other. This is the well-known phenomenon of like-charge attraction between macroions mentioned in Section 2 [31, 39]. The attraction appears when the distance between these surfaces is of the order of the lateral separation between counterions (about $14\text{\AA}$ for divalent counterions). The maximal attraction occurs at the distance $d \simeq 28\text{\AA}$, in good agreement with various theoretical and experimental results [9, 23].

It is also very illustrative to look at the DNA–DNA “effective” interaction at larger d . At these separations, the distribution of counterions in the bundle can be considered to be composed of two populations: condensed layers of counterions near the surfaces of the DNA molecules and diffuse layers of counterions further away. Of course, there is no definite distance that separates condensed from diffused counterions. Nevertheless, it is reasonable to expect the thickness of the condensed counterion layer to be of the order of the average lateral distance between counterions on the DNA surface. So for $d > 35\text{\AA}$, both counterion populations are present and one expects DNA–DNA interaction to be the standard screened Coulomb interaction between two charged cylinders with charge density η^* . As evident from Fig. 7, at small c_Z , such as for $c_Z = 14\text{ mM}$, DNA–DNA interaction is repulsive. As c_Z increases, DNA–DNA interaction becomes less repulsive and reach a minimum around 75 mM. As c_Z increases further, DNA–DNA repulsion starts to increase again. This is the same behavior as that of the phenomenon of reentrant DNA condensation by multivalent counterions [14, 17, 18].

Fig. 7 (Color online) The osmotic pressure of the DNA bundle as a function of the interaxial DNA distance d for different divalent counterion concentration c_Z shown in the inset. The solid lines are guides to the eye

The non-monotonic dependence of DNA–DNA “effective” interaction on the counterion concentration is even more clear if one calculates the free energy, μ_{DNA} , of packaging DNA into bundles. This free energy is the difference between the free energy of a DNA molecule in a bundle and that of an individual DNA molecule in the bulk solution ($d = \infty$). It can be calculated by integrating the pressure with the volume of the bundle. Per DNA nucleotide base, the packaging free energy is given by:

$$\begin{aligned}\mu_{\text{DNA}}(d) &= \frac{l}{L_z N_{\text{DNA}}} \int_{\infty}^d P_{\text{osm}}(d') dV \\ &= \frac{l}{N_{\text{DNA}}} \int_{\infty}^d P_{\text{osm}}(d') \frac{2L_x L_y}{d'} dd'\end{aligned}\quad (19)$$

where $l = 1.7\text{\AA}$ is the distance between DNA nucleotides along the axis of the DNA. The numerical result for $\mu_{\text{DNA}}(d)$ at the optimal bundle lattice constant $d = 28\text{\AA}$ is plotted in Fig. 8 as function of the c_z . Due to the limitation of computer simulations, the numerical integration is performed from $d = 28\text{\AA}$ to $d = 50\text{\AA}$ only. However, this will not change the conclusion of this paper because the omitted integration from $d = 50\text{\AA}$ to $d = \infty$ only gives an almost constant shift to μ_{DNA} .

Once again, the non-monotonic dependence of the electrostatic contribution to DNA packaging free energy is clearly shown. There is an optimal concentration, $c_{z,0}$, where the free energy cost of packaging DNA is lowest. It is even negative indicating the tendency of the divalent counterions to condense the DNA. At smaller or larger concentrations of the counterions, the free energy cost of DNA packaging is higher and positive. These results are consistent with the correlation theory of DNA reentrant condensation by multivalent counterions [14, 17, 18]. For small c_z , DNA molecules are undercharged ($\eta^* < 0$). For large c_z , DNA molecules are overcharged ($\eta^* > 0$). To condense the DNA molecules, one has to overcome the Coulomb repulsion between them. Therefore, the free energy cost of packaging is positive. For $c_z \approx c_{z,0}$, the DNA molecules are almost neutral, $\eta^* \approx 0$. The Coulomb repulsion is negligible and the free energy cost of condensing DNA molecules is lowest. Furthermore, the like-charge attraction among DNA molecules mediated by the

Fig. 8 (Color online) The free energy of packaging DNA molecules into hexagonal bundles as a function of the divalent counterion concentrations. The points are results of numerical integration of P_{osm} from Fig. 7. The solid line is a simple cubic spline interpolation

counterions [39] is dominant in this concentration range, causing the electrostatic packaging free energy to become negative. Figure 8 gives the short-range attraction among DNA molecules to be $-0.008k_B T/\text{base}$. This is about twice as large as the fitted value obtained from the viral DNA ejection experiments in Section 4 [40]. There are many factors that leads to this quantitative discrepancy. Our main approximation is that in the simulation, the position of the DNA cylinders are straight with infinite bending rigidity. Inside viruses, DNA are bent, and the configuration entropy of the DNA are not necessary zero, and there is not a perfect hexagonal arrangement of DNA cylinder with fixed inter-DNA distance. The physical parameters of the system such as ion sizes, DNA orientations, etc. [41, 42] can also affect the strength of DNA–DNA short range attraction. Nevertheless, the non-monotonic electrostatic influence of divalent counterions on DNA–DNA “effective” interaction is clearly demonstrated in our idealized simulation.

Another important point to note is that, for simplicity, we simulate the system with monovalent coions. The neutralizing concentration $c_{Z,0}$ is about 100 mM from our simulation in this case. In the experiment setup, the MgSO_4 salt (divalent coions) shows a minimum in the amount of DNA ejection from viral capsid at about 64 mM. The data for MgCl_2 salt (monovalent coions) seems to suggest a minimum in DNA ejection at about 100 mM, about the same as our simulation, although this behavior needs more systematic experimental study.

5.3 Role of finite size of counterions

In all the systems simulated so far, we set the radius of the divalent counterion to $2.5\text{\AA}$. The results agrees qualitatively and semi-quantitatively with some of the experimental results of DNA ejection from capsid with MgSO_4 salt. However, experimental results shows that there is a strong ion specific effects among MgSO_4 salt, MgCl_2 , or Mn salt counterions. This shows that the role of the hydration effect, and the entropy of the hydrated water molecules are significant and need to be properly taken into account when one deals with the problem of DNA confinement inside viral capsids. In a recent study (Nguyen 2013, manuscript in preparation), a first step is taken to study this ion specific effect. Specifically, the problem of how DNA–DNA interaction is affected by changing the radius of the counterions is studied.

In Fig. 9, the osmotic pressure (which is proportional to the effective DNA–DNA interaction) of the hexagonal DNA bundle is plotted as a function of the inter DNA distance for three counterion sizes, $2\text{\AA}$, $2.5\text{\AA}$, and $3\text{\AA}$, respectively. The counterion concentration is chosen to be approximately 100mM in each simulation. As one can see, the first consequence of changing counterion size is obviously the equilibrium distance of the DNA bundle. The optimal inter-DNA distance, d^* , where the short-range DNA attraction is strongest increases with the counterion radius. As the counterion radius is increased from $2.0\text{\AA}$ to $2.5\text{\AA}$ to $3.0\text{\AA}$, d^* increases from $26\text{\AA}$ to $27\text{\AA}$ then $29\text{\AA}$ respectively. This is not suprising. Entropic costs of confining larger counterions inside DNA bundle leads to a higher value for the optimal interaxial DNA distance d^* .

Inside viral capsids, the DNA–DNA electrostatic interactions have to compete with the bending energy. Depending on the percentage of DNA packaged, the equilibrium interaxial DNA distance can be far from these optimal values. It is expected that the effect of ion sizes will be more pronounced. At the highest packaging length, where $d \simeq 28\text{\AA}$, smaller ion hydration radius will condense DNA better. This effect will be studied in more detail in a future work.

Fig. 9 (Color online) The osmotic pressure of the DNA hexagonal bundle as function of the lattice constant, d , for three values of the counterion radius. Equilibrium positions of DNA molecules increases roughly by 2σ as the counterion radius increases. The attractive electrostatic interaction is also shifted as well

In Fig. 10, the free energy of packaging DNA into an hexagonal bundle with the optimal inter-DNA distance, d^* , is plotted as a function of the counterion concentrations for the three different counterion radii.

Again, for all values of a counterion's radius, the behavior of the free energy of packaging on the concentration c_Z is non-monotonic, and all the minima of the free energy are negative. This shows that the finite size of ion does not change the fact that the DNA molecules can condensate to form a bundle due to correlation attraction. Within this limited simulation scope, it is found that the counterion sizes have weak influence on the depth and position of the maximal DNA condensation bundle. However, it is clear from Fig. 10 that the width of the region where DNA is condensed (packaging free energy is negative) is broadened with increasing ion sizes. It can be suggested from these results that, in experiments,

Fig. 10 Free energy per nucleotide base of packaging DNA molecules into bundles as a function of counterion concentration c_Z for different kinds of ions

counterions with bigger hydration radius would condense DNA more easily and have a bigger range where DNA ejection from viral capsid is inhibited.

6 Conclusion

In conclusion, this paper has shown that divalent counterions such as Mg^{+2} have strong effects on DNA condensation in a confined environment (such as inside the bacteriophage capsid) similar to those of counterions with higher valency. We propose that the non-monotonic dependence of the amount of DNA ejected from bacteriophages has the same physical origin as the reentrant condensation phenomenon of DNA molecules by multivalent counterions. Fitting our semi-empirical theory to available experimental data, we obtain the strength of DNA–DNA short-range attraction mediated by divalent counterions. The fitted values agree quantitatively and qualitatively with experimental values from other DNA system and computer simulations. This shows that in the problem of viral DNA package where DNA lateral motion is restricted, divalent counterions can play an important role similar to that of counterions with higher valency. This fact should be incorporated in any electrostatic theories of bacteriophage packaging. Results from our theoretical and simulation can provide a starting point for future works with DNA–DNA condensation in the presence of divalent counterions.

Acknowledgements We would like to thank Lyubartsev, Nordenskiöld, Tung Le, Seil Lee, Shklovskii, Evilevitch, Fang, Gelbart, Phillips, Rau, and Parsegian for valuable discussions. The author acknowledges the financial support of the Vietnam National Foundation for Science and Technology NAFOSTED Contract 103.02-2012.75, and US National Science Foundation grant CBET-1134398. We also acknowledge the hospitality of the Fine Theoretical Physics Institute (Minnesota, USA), the Aspen Center of Physics (Colorado, USA), and the International Center for Theoretical Physics (Trieste, Italy) where parts of this work was done. The authors are indebted to Dr. Lyubartsev for providing us with the source code of their Expanded Ensemble Method. This code forms the basis to develop the simulation program used in this work.

References

1. Knobler, C.M., Gelbart, W.M.: Physical chemistry of DNA viruses. *Annu. Rev. Phys. Chem.* **60**, 367 (2009)
2. Smith, D.E., Trans, S.J., Smith, S.B., Grimes, S., Anderson, D.L., Bustamante, C.: The bacteriophage straight phi29 portal motor can package DNA against a large internal force. *Nature* **413**, 748 (2001)
3. Evilevitch, A., Lavelle, L., Knobler, C.M., Raspaud, E., Gelbart, W.M.: Osmotic pressure inhibition of DNA ejection from phage. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 9292 (2003)
4. Castelnovo, M., Bowles, R.K., Reiss, H., Gelbart, W.M.: Osmotic force resisting chain insertion in a colloidal suspension. *Eur. Phys. J. E* **10**, 191 (2003)
5. Petrov, A.S., Lim-Hing, K., Harvey, S.C.: Packaging of DNA by bacteriophage epsilon15: structure, forces, and thermodynamics. *Structure* **15**, 807 (2007)
6. Letellier, L., Boulanger, P., Plancon, L., Jacquot, P., Santamaria, M.: Main features on tailed phage, host recognition and DNA uptake. *Front. Biosci.* **9**, 1228 (2004)
7. Black, L.W.: DNA packaging in dsDNA bacteriophages. *Annu. Rev. Microbiol.* **43**, 267 (1989)
8. Murialdo, H.: Bacteriophage lambda DNA maturation and packaging. *Annu. Rev. Biochem.* **60**, 125 (1991)
9. Purohit, P.K., Inamdar, M.M., Grayson, P.D., Squires, T.M., Kondev, J., Phillips, R.: Forces during bacteriophage DNA packaging and ejection. *Biophys. J.* **88**, 851 (2005)
10. Evilevitch, A., Castelnovo, M., Knobler, C.M., Gelbart, W.M.: Measuring the force ejecting DNA from phage. *J. Phys. Chem. B* **108**, 6838 (2004)

11. Evilevitch, A., Fang, L.T., Yoffe, A.M., Castelnovo, M., Rau, D.C., Parsegian, V.A., Gelbart, W.M., Knobler, C.M.: Effects of salt concentrations and bending energy on the extent of ejection of phage genomes. *Biophys. J.* **94**, 1110 (2008)
12. Fuller, D.N., Rickgauer, J.P., Jardine, P.J., Grimes, S., Anderson, D.L., Smith, D.E.: Ionic effects on viral DNA packaging and portal motor function in bacteriophage $\phi 29$. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 11245 (2007)
13. Smith, D.E.: Single-molecule studies of viral DNA packaging. *Cur. Opin. in Virol.* **1**, 134 (2011)
14. Nguyen, T.T., Rouzina, I., Shklovskii, B.I.: Reentrant condensation of DNA induced by multivalent counterions. *J. Chem. Phys.* **112**, 2562 (2000)
15. Saminathan, M., Antony, T., Shirahata, A., Sigal, L.H., Thomas, T., Thomas, T.J.: Ionic and structural specificity effects of natural and synthetic polyamines on the aggregation and resolubilization of single-, double-, and triple-stranded DNA. *Biochemistry* **38**, 38213830 (1999)
16. Pelta, J., Durand, D., Doucet, J., Livolant, F.: DNA mesophases induced by spermidine: structural properties and biological implications. *Biophys. J.* **71**, 48 (1996)
17. Shklovskii, B.I.: Screening of a macroion by multivalent ions: correlation-induced inversion of charge. *Phys. Rev. E* **60**, 5802 (1999)
18. Grosberg, A.Y., Nguyen, T.T., Shklovskii, B.: Low temperature physics at room temperature in water: charge inversion in chemical and biological systems. *Rev. Mod. Phys.* **74**, 329 (2002)
19. Moreira, A.G., Netz, R.R.: Simulations of counterions at charged plates. *Eur. Phys. J. E* **8**, 33 (2002)
20. Besteman, K., Eijk, K.V., Lemay, S.G.: Charge inversion accompanies DNA condensation by multivalent ions. *Nat. Phys.* **3**, 641 (2007)
21. Kanduć, M., Naji, A., Podgornik, R.: Counterion-mediated weak and strong coupling electrostatic interaction between like-charged cylindrical dielectrics. *J. Chem. Phys.* **132**, 224703 (2010)
22. Hsiao, P.-Y., Luijten, E.: Salt-induced collapse and reexpansion of highly charged flexible polyelectrolytes. *Phys. Rev. Lett.* **97**, 148301 (2006)
23. Rau, D.C., Parsegian, V.A.: Direct measurement of the intermolecular forces between counterion-condensed DNA double helices. Evidence for long range attractive hydration forces. *Biophys. J.* **61**, 246 (1992)
24. Hud, N.V., Downing, K.H.: Cryoelectron microscopy of λ phage DNA condensates in vitreous ice: the fine structure of DNA toroids. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 14925 (2001)
25. Koltover, I., Wagner, K., Safinya, C.R.: DNA condensation in two dimensions. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 14046 (2000)
26. Lee, S., Le, T.T., Nguyen, T.T.: Reentrant behavior of divalent-counterion-mediated DNA–DNA electrostatic interaction. *Phys. Rev. Lett.* **105**, 248101 (2010)
27. Lyubartsev, A.P., Nordenskiöld, L.: Monte Carlo simulation study of ion distribution and osmotic pressure in hexagonally oriented DNA. *J. Phys. Chem.* **99**, 10373 (1995)
28. Winterhalter, M., Helfrich, W.: Effect of surface charge on the curvature elasticity of membranes. *J. Phys. Chem.* **92**, 6865 (1988)
29. Perel, V.I., Shklovskii, B.I.: Screening of a macroion by multivalent ions: a new boundary condition for the Poisson-Boltzmann equation and charge inversion. *Physica A* **274**, 446 (1999)
30. Bruinsma, R.: Electrostatics of DNA-cationic lipid complexes: isoelectric instability. *Eur. Phys. J. B* **4**, 75 (1998)
31. Gelbart, W.M., Bruinsma, R.F., Pincus, P.A., Parsegian, A.V.: DNA-inspired electrostatics. *Phys. Today* **53**, 38 (2000)
32. Riemer, S.C., Bloomfield, V.A.: Packaging of DNA in bacteriophage heads: some considerations on energetics. *Biopolymers* **17**, 785 (1978)
33. Kindt, J., Tzili, S., Ben-Shaul, A., Gelbart, W.M.: DNA packaging and ejection forces in bacteriophages. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 13671 (2001)
34. Petrov, A.S., Harvey, S.C.: Packaging double-helical DNA into viral capsids: structures, forces, and energetics. *Biophys. J.* **95**, 497 (2008)
35. Baker, T.S., Olson, N.H., Fuller, S.D.: Adding the third dimension to virus life cycles: three-dimensional reconstruction of icosahedral viruses from cryo-electron micrographs. *Microbiol. Mol. Biol. Rev.* **63**, 862 (1999)
36. Skinner, B., Shklovskii, B.I.: Non-monotonic swelling of a macroion due to correlation-induced charge inversion. *Physica A* **388**, 1 (2009)
37. Valleau, J.P., Cohen, L.K.: Primitive model electrolytes. I. Grand canonical Monte-Carlo computations. *J. Chem. Phys.* **72**, 5935 (1980)
38. Guldbrand, L., Nilsson, L.G., Nordenskiöld, L.: A Monte Carlo simulation study of electrostatic forces between hexagonally packed DNA double helices. *J. Chem. Phys.* **85**, 6686 (1986)

39. Naji, A., Arnold, A., Holm, C., Netz, R.R.: Attraction and unbinding of like-charged rods. *Eur. Phys. Lett.* **67**, 130 (2004)
40. Lee, S., Tran, C.V., Nguyen, T.T.: Inhibition of DNA ejection from bacteriophage by Mg^{+2} counterions. *J. Chem Phys.* **134**, 125104 (2011). [arXiv:cond-mat/0811.1296](https://arxiv.org/abs/cond-mat/0811.1296)
41. Grønbech-Jensen, N., Mashl, R.J., Bruinsma, R.F., Gelbart, W.M.: Counterion-induced attraction between rigid polyelectrolytes. *Phys. Rev. Lett.* **78**, 24772480 (1997)
42. Lyubartsev, A.P., Tang, J.X., Janmey, P.A., Nordenskiöld, L.: Electrostatically induced polyelectrolyte association of rodlike virus particles. *Phys. Rev. Lett.* **81**, 5465 (1998)