DNA like-charge attraction and overcharging by divalent counterions in the presence of divalent co-ions

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Abstract Strongly correlated electrostatics of DNA systems has drawn the interest of many groups, especially the condensation and overcharging of DNA by multivalent counterions. By adding counterions of different valencies and shapes, one can enhance or reduce DNA overcharging. In this paper, we focus on the effect of multivalent co-ions, specifically divalent co-ions such as SO_4^{2-} . A computational experiment of DNA condensation using Monte Carlo simulation in grand canonical ensemble is carried out where the DNA system is in equilibrium with a bulk solution containing a mixture of salt of different valency of co-ions. Compared to systems with purely monovalent co-ions, the influence of divalent co-ions shows up in multiple aspects. Divalent co-ions lead to an increase of monovalent salt in the DNA condensate. Because monovalent salts mostly participate in linear screening of electrostatic interactions in the system, more monovalent salt molecules enter the condensate leads to screening out of short-range DNA–DNA like charge attraction and weaker DNA condensation free energy. The overcharging of DNA by multivalent counterions is also reduced in the presence of divalent co-ions. Strong repulsions between DNA and divalent co-ions and among divalent co-ions themselves lead to a *depletion* of negative ions near the DNA surface as compared to the case without divalent co-ions. At large distances, the DNA–DNA repulsive interaction is stronger in the presence of divalent co-ions, suggesting that divalent co-ions’ role is not only that of simple stronger linear screening.

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1 Introduction

The problem of DNA condensation in the presence of multivalent counterions has seen a strong revival of interest in recent years. DNA study enables us to find effective ways of gene delivery for the rapidly growing field of genetic therapy. Condensation of DNA inside viruses such as bacteriophages provides excellent study candidates for this purpose (see review [1]). Furthermore, the promising development of DNA-based nanotechnology that can be controlled exquisitely at nanoscale into precise 2D and 3D shapes enables the fabrication of precise nanoscale devices that have already shown great potential for biomedical applications such as: drug delivery, biosensing, and synthetic nanopore formation [2, 3].

In aqueous solution, the phosphate group of DNA nucleotide becomes negatively charged. Hence, DNA is charged negatively, with the linear charge density of about $1e/1.7\text{\AA}$, and surface charge density of about $1e/1\text{nm}^2$. These are among the highest charge densities for biological molecules, therefore, electrostatics and the screening condition of the solution play an important role in the structure and functions of DNA systems. One of the most interesting and important electrostatic aspects of the DNA system is the ability to condense DNA by multivalent counterions by CoHex^{+3} , Spd^{+3} or Spm^{+4} [4–7]. In a restricted environment, such as inside a viral capsid, even divalent counterions such as Mg^{+2} can also cause strong and non-monotonic effects [8]. By varying the salinity of solution, one can vary the amount of DNA ejected from viruses. There is an optimal counterion concentration, $c_{Z,0}$, where the least DNA genome is ejected from the phages. For counterion concentration, c_Z , higher or lower than this optimal concentration, more DNA is ejected from phages.

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 in the presence of counterions of tri-, tetra-, and higher valence [9–13]. This can be understood as follows. At low concentration of the counterions, DNA is undercharged. At high concentration of the counterions, DNA is overcharged (becomes a positively charged molecule). Electrostatic repulsions among DNA molecules keep them from condensation. At intermediate concentration, DNA is almost neutral thus, they are able to condense into bundle under short-range attraction forces. Although, divalent counterions are known to condense DNA only partially in free solution [4, 5], DNA virus provides a unique experimental setup. The constraint of the viral capsid strongly eliminates configurational entropic cost of packaging DNA. This allows divalent counterions to influence DNA condensation similar to that of trivalent/tetravalent counterions. Indeed, DNA condensation by divalent counterions has also been observed in another environment where DNA configuration is constrained, namely the condensation of DNA in two-dimensional systems [14]. For virus systems, theoretical fitting suggests that the DNA is neutralized at $c_{Z,0} \approx 75\text{ mM}$ for divalent counterions, and the short-range DNA attraction at this concentration is $-0.004k_B T$ per nucleotide base [15, 16].

The marginal case of divalent counterions also shows ion specificity effect. For example, in free solution, Mn^{+2} ions are able to condense DNA into disorder bundle but Mg^{+2} ions are not [4]. In DNA ejection from bacteriophages, the non-monotonicity is observed for MgSO_4 salt but not for MgCl_2 salt up to the concentration of 100mM [8]. In a recent paper [17], it is been shown that some of these ion specificity effects can be captured by studying the difference in the hydration radius of different counterions. In this paper, we focus on

the effect of divalent *co-ions* on the overcharging and condensation of DNA by multivalent counterions. The problem is studied using a Monte Carlo simulation of the DNA system in the grand canonical ensemble. It is shown that divalent coions make it easier for monovalent salt to enter the condensate to screen out and weaken DNA–DNA interactions. At the same time, they cause a depletion of negative ions near the DNA surface, which enhance DNA charge inversion by multivalent counterions. At large distance, the repulsive interaction among undercharged or overcharged DNA is stronger in the presence divalent co-ions. Some preliminary result of this work was presented in Ref. [18].

The paper is organized as follows. In Section 2, the simulation model and method are presented. In Section 3, various simulation results on the fugacities of the salts, the ion concentration variation in DNA bundles, the osmotic pressure and DNA packaging free energy are presented and discussed. We conclude in Section 4.

2 The simulation model and method

We assume the DNA molecules in the condensate to arrange in a two-dimensional hexagonal lattice with lattice constant d (see Fig. 1a). The DNA axis is parallel to the z -axis. A periodic simulation cell with $N_{DNA} = 12$ DNA molecules in the horizontal (x, y) plane and three full helix periods in the z direction is used (see Fig. 1b). The individual DNA molecule is modeled as an impenetrable cylinder with negative charges glued on it 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 of 1 nm . The solvent water is treated as a dielectric medium with dielectric constant $\epsilon = 78$ and temperature $T = 300^\circ\text{K}$. The positions of DNA molecules are fixed in space. The mobile ions in solution are modeled as hard spheres with unscreened Coulomb interaction (the *primitive ion* model). For simplicity, all ions have radius of $2\text{\AA}$.

In practical situations, the DNA bundle is in equilibrium with an aqueous solution containing free mobile ions at given concentrations. Many buffer solutions contain 50 mM of NaCl salt (1:1 salt) and 10 mM of MgCl_2 salt (2:1 salt). In this paper, we focus on the

Fig. 1 **a** 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. **b** The periodic simulation box contains 12 DNA cylinders. The box dimension in the (x, y) plane is shown. The length of the box in the z -axis is three helix periods

influence of divalent co-ions, which is the case where there is MgSO_4 salt (2:2 salt) present in solution. Overall, our DNA bundle is in equilibrium with a solution containing a mixture of as many as three different salts. Clearly, due to the presence of DNA charges, the concentration of salts inside the DNA bundle will be different than those in the bulk. Mutual correlations among different ions may favor one particular salt to other. Thus, to properly simulate the DNA bundle for these systems, and to properly capture the electrostatic screening in the system, one needs to go beyond canonical simulation. Here, we simulate the system using grand canonical Monte Carlo method. The detail of this method and parameters of our systems, the chemical potentials of ion species can be found in other works [17, 19, 20]. To study the influence of divalent co-ions, two solutions with different salt mixtures are simulated and compared to each other. Solution A contains a mixture of 50 mM NaCl and a varying concentration of MgCl_2 salt. Solution B contains a mixture of 50 mM NaCl, 10 mM MgCl_2 , and a varying concentration of MgSO_4 salt. For each simulation run, about 500 million MC moves are carried out. To ensure thermalization, about 50 million initial moves are discarded before doing statistical analysis of the result of the simulation. All simulations are done using the physics simulation library SimEngine developed by one of the authors (TTN).

3 Results and discussion

3.1 Monovalent salt differences between the two solutions

In Fig. 2, we plot the scaled fugacity of the monovalent salt, $B_{1:1} = (V^2/\Lambda_+^3 \Lambda_-^3) e^{\beta \mu_{1:1}}$, in a bulk solution containing 50 mM 1:1 salt as a function of divalent counterion concentration in the same solution. Here V is the volume of the system, Λ_i are the thermal wavelengths of each ion species, and the salt chemical potential is an algebraic sum of the chemical potentials of individual ion species $\mu_{1:1} = \mu_+ + \mu_-$. The volume used for these plots is $V \approx 3300 \text{ nm}^3$. With increasing counterion concentrations, c_{2+} , the monovalent salt fugacity

Fig. 2 The chemical potential of a function of divalent counterion concentrations. The *diamond symbols* are for the case of solution A, the *square symbols* are for the case of solution B

$B_{1:1}$ increases rapidly for solution type A (without divalent co-ions), while it decreases as much as 40% for solution type B. The observed increase in solution A can be contributed to the increase in the concentration of the monovalent Cl^- ions that accompanies the divalent counterions, Mg^{+2} , in solution A. As the divalent counterions concentration increases, the Cl^- concentration also increases, leading to a higher chemical potential for the 1:1 salt. The linear dependence of the fugacity on the concentration agrees with this. The observed decrease in solution B can be understood as a consequence of the Gibbs–Duhem theorem where, in a mixture, increasing the concentration of one species (keeping the concentration of the other species constant), leads to a reduction in the chemical potential of the other species.

Figure 2 shows that the fugacity of 1:1 salt for solution B is smaller than that of solution A at the same divalent counterions. This suggests that it is easier to insert a monovalent salt molecule into the DNA condensate in equilibrium with solution A. This is indeed true. In Fig. 3a, the averaged total number of monovalent salt molecules, n_1 , in the DNA condensate as a function of the divalent counterion concentrations is plotted. The data is for the case of inter-DNA distance of $d = 40 \text{ \AA}$. One can see that, in both cases, n_1 decreases with increasing counterion concentrations. This is to be expected. At low concentration c_{2+} , DNA molecules are screened by monovalent counterions; at high concentration c_{2+} , DNA molecules are screened by divalent counterions. This behavior can only be captured in a grand-canonical simulation, not in standard canonical simulation where the number of mobile ions are fixed in advanced. Importantly, Fig. 3a shows that, although the numbers are within the error bars, the number of monovalent salt molecules is systematically higher by as much as 10% in the presence of divalent co-ions. This result agrees with what was stated earlier that divalent co-ions make it easier for monovalent salt to enter the DNA condensate.

To avoid confusion, it should be noted here that in our simulation, the bulk and the DNA condensate are in thermodynamic equilibrium and they have the same chemical potentials for component salts. When we said higher chemical potential, we mean that it is higher than that of the other solution (chemical potential 1:1 salt of solution A is higher than that of solution B). In other words, when the chemical potential is high, it is costlier to insert the salt molecule into either the DNA condensate or the bulk. Vice versa, when the chemical potential is lower, it is easier to insert the salt molecule into either the bulk or the DNA

Fig. 3 The number of 1:1 salt molecules (**a**) and Mg^{+2} counterions (**b**) inside a DNA bundle with $d = 40$ corresponding to a simulation volume of $V_{\text{cell}} \approx 1700 \text{ nm}^3$. Both are plotted as a function of divalent counterion concentrations. The *diamond symbols* are for the case of solution A and the *square symbols* are for the case with solution B

condensate. Figure 3a also supports our claim that more 1:1 salt are present in the DNA condensate in the case of divalent co-ions.

For comparison, in Fig. 3b, the number of Mg^{+2} counterions is also plotted for different concentrations. The number of Mg^{+2} needed to neutralize the DNA is 360 ions. At low concentration, the number of Mg^{+2} is slightly below this number, suggesting that DNA is also screened by monovalent counterions. At high concentration, the number of Mg^{+2} ions is more than needed to neutralize the DNA and the number of 1:1 salt is reduced by 60% at the highest concentration simulated. The system is dominated by the divalent ions. This is in agreement with the grand-canonical picture that we use. Note that we are never in a counterion-only regime even at low divalent salt concentration. In this limit, the DNA should be screened by monovalent salt present in the solution.

3.2 Overcharging of DNA at high divalent counterion concentration

Next, let us look at the overcharging of DNA by multivalent counterions at high concentration, c_{2+} . In Fig. 4a we plot total charge density of the negative ions as a function of distance from a DNA cylinder. For solution A, this is simply the charge density of Cl^- co-ions, $-ec_{\text{Cl}}$. For solution B, this is the sum $-ec_{\text{Cl}} - 2ec_{\text{SO}_4}$. These density profiles are plotted for the divalent counterion concentration of $c_{2+} \approx 500$ mM and for inter-DNA distance of $d = 50\text{\AA}$. One can see that at large distance r from DNA axis, $r \geq 17\text{\AA}$, the negative charge density *decreases* with increasing r . This means, for an observer from far away, the negative ions are accumulated near the DNA or the apparent net charge of DNA is *positive*. Indeed, the charge density should obey the Boltzmann distribution and is higher at the place of lower electrostatic potential energy. In Fig. 4, for negative charges, their concentration *increases* as one approaches the DNA from far away. Since the electrostatic potential at infinity is zero, this increase means that the electrostatic potential is becoming more positive as one approaches the DNA. In other words, DNA apparent charge is positive. This is a clear indication of an overcharging effect.

Another conclusion one can draw from Fig. 4a is that from distance $r = 17\text{\AA}$ down to $r = 10\text{\AA}$, the negative charge density decreases with decreasing r . One can deduce that this

Fig. 4 **a** The total charge density of negative ions as a function of distance from DNA axis, in unit of molar concentration. **b** The accumulated amount of charges per helix DNA from DNA surface in units of elementary charges e . It is $-20e$ at the DNA surface (not shown)

range of r corresponds to the condense layer of counterions around DNA. Our result shows that the length of this counterion condensation layer is approximately $7\text{\AA}$ within the surface of a DNA molecule. This agrees with the fact that if Mg^{2+} counterions were to form a two-dimensional strongly correlated liquid on the DNA surface, the averaged distance between neighbor counterions in this liquid is also about $7\text{\AA}$. Additionally, the discreteness of DNA charges is also about $7\text{\AA}$.

The overcharging effect is more evident if one plots the accumulation of all charges, positive and negative, from the DNA axis. In Fig. 4b, the accumulated charge (DNA and all ions) as function of distance from a DNA molecule's axis is plotted for one helix period. At the DNA surface, the amount of charges per period is $-20e$. As one moves further away from the surface, positive counterions condense on DNA to reduce its charges. At the distance of $r \approx 14 - 16\text{\AA}$, the accumulated charge starts to become positive and it is maximally positive at $r \approx 16\text{\AA}$. This once again confirms the overcharging of DNA.

Figure 4a and b also show that the presence of divalent co-ions has a strong quantitative influence on DNA overcharging degree. Within the condensation layer, the negative divalent co-ions are repelled from DNA much stronger in the presence of divalent co-ions compared to the case without divalent co-ions. This is to be expected. For solution B at high divalent co-ion concentration, the negative ions are mostly made of divalent co-ions. The repulsion from DNA negative charges on the co-ions is larger in solution B than that of solution A. Hence less negative charges in the condensation layer around each DNA.

Outside this layer, $r \geq 17\text{\AA}$, the peak of the total negative ion concentration for solution B (the blue curve) is slightly higher. The slope of this curve is also higher. This means that negative ions are accumulated at the surface of the condensed layer. This leads to a reduction in the correlation among divalent counterions, hence a significant reduction in overcharging degree as shown in Fig. 4b.

3.3 DNA–DNA “effective” interaction mediated by counterions

In the presence of divalent coions, the ionic strength of the solution is larger. Additionally, as Section 3.1 shows, more monovalent salt molecules enter the condensate in the case of solution B. These factors lead to a smaller Debye screening radius, r_s , for solution B. This

Fig. 5 The osmotic pressure of the DNA bundle as function of the interaxial DNA distance d for divalent counterion concentration of **a** 30 mM - low concentration; and **b** 507 mM - high concentration. The *diamond symbols* are for the case of no divalent co-ions (solution A) and the *square symbols* are for the case with divalent co-ions (solution B) in bulk the solution. Explicit values for the concentration of component salts are given in the corresponding figure legend. The *solid lines* are guides to the eye

weakens electrostatic interactions among DNA molecules. This is indeed what we observed in simulation. Figure 5a plots the osmotic pressure of DNA bundle as a function of the inter-axial DNA distance, d , for solution A containing about 30 mM MgCl_2 salt concentration and solution B containing about 20 mM MgSO_4 salt concentration. Both solutions contain divalent counterion concentration of $c_{2+} \simeq 30$ mM. Similarly, Fig. 5b plots the osmotic pressure of DNA bundle as a function of the inter-axial DNA distance for the case of high divalent counterion concentration of $c_{2+} \simeq 500$ mM. Because this osmotic pressure is directly related to the “effective” force between DNA molecules at that inter-axial distance [21, 22], this figure also serves as a plot of DNA–DNA effective interaction mediated by multivalent counterions. In all cases, 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 macro-ions [12, 13, 23]. The maximum attraction occurs at the distance $d \simeq 26 - 27 \text{ \AA}$, in good agreement with various theoretical and experimental results [4, 24]. For smaller d , the DNA–DNA interaction experiences sharp increase due to the hardcore repulsion between the counterions.

From Fig. 5 it can be seen that the DNA–DNA attraction at short range ($r < 30 \text{ \AA}$) is reduced in the presence of divalent co-ions at both low concentration and high concentration of the divalent counterions. The overall reduction can be as much as 40% in the presence of divalent co-ions.

On the other hand, at larger distance, the DNA–DNA repulsive interaction among undercharged (low c_{2+}) and overcharged (high c_{2+}) is stronger in solution B. This means that divalent co-ions do not play the simple role of linear Debye–Hückel screening of the “dressed” DNA charge. Rather, they bind to divalent counterions and reduce the amount of charges available to participate in linear screening as the dressed counterion theory suggested [25].

From the P–V curves such as those in Fig. 5a and b, one can calculate the free energy of packaging DNA into condensate by integrating the pressure with the volume. This free energy is plotted in Fig. 6 as a function of the divalent counterion concentrations. It shows a non-monotonic dependence of the electrostatic contribution to DNA packaging free energy. This is common for DNA condensation by multivalent counterions and is the result of

Fig. 6 The free energy of packaging DNA molecules into condensate as a function of the divalent counterion concentrations

interplay between DNA overcharging effect and DNA–DNA like-charge attraction. It is consistent with the correlation theory of DNA reentrant condensation by multivalent counterions [9, 23, 26] and the experiment results on ejecting DNA from bacteriophage under varying counterion concentrations [8]. At low (high) c_{2+} , DNA molecules is undercharged (overcharged) and repel each other. At medium c_{2+} , DNA like-charge attractions cause condensation into bundles. Figure 6 further shows that the DNA packaging free energy is higher in the presence of divalent co-ions. This can be understood as the consequence of all the physics presented so far: reduction of DNA–DNA like-charge interaction and overcharging. This also agrees with the experimental fact that MgSO_4 cause earlier DNA decondensation, and more DNA ejected from bacteriophages [8] compared to MgCl_2 .

4 Conclusions

In conclusion, a computational study of the influence of multivalent co-ions on strongly correlated electrostatics of DNA condensation by multivalent counterions is presented. Divalent co-ions' influence is multiple folds. First, in a grand canonical equilibrium with particle reservoir at given ion concentrations, divalent co-ions reduce the cost of adding monovalent salt to the DNA bundle system by as much as 40% in terms of fugacity and 10% in terms of concentration. Because monovalent salts mostly participate in screening of electrostatic interaction in the system, more monovalent salt in the bundle leads to stronger screening of short-range DNA–DNA like-charge attraction and weaker DNA condensation free energy. Secondly, the strong repulsion between DNA and divalent co-ions and among divalent co-ions leads to depletion of negative ions near the DNA surface as compared to the case without divalent co-ions. The condensation layer of counterions near DNA surface is shown to be about $7\text{\AA}$. In the presence of divalent co-ions, negative charge concentration is slightly higher at the surface of the condensed layer. This also leads to a significant reduction of DNA overcharging. The overall results of our study is the reduction of DNA strongly correlated electrostatics and agrees well with experimental facts that MgSO_4 solution causes stronger DNA ejection from bacteriophages than MgCl_2 solution.

In our computer simulation, we focus on the specific case of divalent coions to compare with monovalent counterions. We believe most important qualitatively behaviors will not change significantly for higher counterion, co-ion valency. Additionally, there are many other factors that were neglected in our simplified model. One approximation is that in the simulation, the position of the DNA cylinders are straight with infinite bending rigidity. Inside viruses, DNA is bent, and the configuration entropy of the DNA is not necessarily zero, and there is not a perfect hexagonal arrangement of DNA cylinder with fixed inter-DNA distance [24]. The relative orientation of individual DNA cylinder is also fixed in our simulation. Allowing for various orientation of DNA charges can lead to a frustration effect similar to spin glass in a two-dimensional Ising model [7]. The physical parameters of the system, such as ion sizes, can also affect the strength of DNA–DNA short-range attraction [17]. The dielectric discontinuity at the DNA surface is also another important factor that was neglected in this model and can have important consequences on the screening of DNA in different electrolytes [27]. Inclusion of all these factors is beyond the scope of this paper and needs to be considered in more detail in future work. Nevertheless, we believe that the qualitative difference between DNA condensation with monovalent co-ions and with divalent co-ions discussed in this paper will not be affected.

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