Inhibition of DNA ejection from bacteriophage by Mg\textsuperscript{+2} counterions

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Experiments showed that MgSO\textsubscript{4} salt inhibits DNA ejection from bacteriophages non-monotonically. There is a MgSO\textsubscript{4} concentration, $N_0$, where the least DNA is ejected. We propose that this is the result of DNA overcharging by Mg\textsuperscript{+2} ions. As Mg\textsuperscript{+2} concentration, $N$, increases, DNA net charge changes from negative to positive. $N_0$ is the concentration where DNA is neutral. For $N \neq N_0$, DNA is charged, prefers to be in solution to lower its electrostatic self-energy, hence more DNA is ejected. Our theory fits experimental data well. Mg\textsuperscript{+2}–mediated DNA–DNA attraction is found to be $-0.004k_BT$ per nucleotide.

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 even 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 considerable bent and strongly confined inside the viral capsid, resulting in a substantially pressurized capsid with internal pressure as high as 50 atm \cite{1-4}. 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 \textit{in vivo} and \textit{in vitro} \cite{2, 3, 5-10}. The \textit{in vitro} experiments additionally revealed possibilities of controlling the ejection of DNA from bacteriophages. One example is the addition of PEG (polyethylene glycol), a large molecule that is 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 negligible effect on the DNA ejection process\cite{2}. In contrast, multivalent counterions such as Mg\textsuperscript{+2}, CoHex\textsuperscript{+3}, Spd\textsuperscript{+3} (spermidine) or Spm\textsuperscript{+4} ( spermine) exert strong effect. One such results is shown in Fig. 1 where the solid circles represent experimental data for the percentage of ejected DNA from bacteriophage $\lambda$ (at 3.5 atm external osmotic pressure) as a function of MgSO\textsubscript{4} concentration\cite{10, 11}. The three different 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\textsuperscript{+2} concentration where the least DNA genome is ejected from the phages. Similar qualitative behavior is observed for other multivalent counterions.

In this paper, we focus on understanding the electrostatics involved in the inhibition of DNA ejection by Mg\textsuperscript{+2} counterions. We propose that the non-monotonic behavior observed in Fig. 1 is the result of Mg\textsuperscript{+2} ions inducing an effective attraction between DNA segments inside the capsid, and the so-called overcharging of DNA by multivalent counterions in free solution. The proposed Mg\textsuperscript{+2}–mediated attraction between neighboring DNA segments inside the capsid is a central argument of this paper and needs to be clarified. It is well-known that Mg\textsuperscript{+2} ions do not or only partially condense free DNA molecules in aqueous solution\cite{12, 13}. However, we argue that due to the entropic confinement of the viral capsid, DNA are strongly bent and thermal fluctuations of DNA molecule is strongly suppressed compared to that
in free solution. 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 are shown experimentally to be able to condense DNA in another confined system: the DNA condensation in two dimension \[14\]. Therefore it is not surprising that Mg$^{+2}$ ions can cause DNA-DNA attractions inside the capsid (a zero-dimensional system).

The overall electrostatics of Mg$^{+2}$ modulated DNA ejection from bacteriophages is following. Due to strong electrostatic interaction between DNA and Mg$^{+2}$ counterions, the counterions condense on the DNA molecule. As a result, the net charge of DNA ($\eta^*$ per unit length) which is the sum of the “bare” DNA charges ($\eta_{\text{bare}} = -1e/1.7A$) and the charges of condensed counterions becomes smaller in magnitude than the “bare” charge. There are strong correlations between the condensed counterions at the DNA surface which cannot be described using standard Poisson-Boltzmann mean-field theory. Strongly correlated counterion theories, various experiments and simulations \[15, 16\] have showed that when these strong correlations are taken into account, $\eta^*$ is not only smaller than $\eta_{\text{bare}}$ in magnitude but can even have opposite sign: this is known as the charge inversion phenomenon. Specifically, the degree of condensation, hence $\eta^*$, depends logarithmically on the concentration of multivalent counterions, $N$. As $N$ increases from zero, $\eta^*$ becomes less negative, neutral and eventually positive. We propose that the multivalent counterion concentration $N_0$, where DNA net charge is neutral, corresponds to the optimal inhibition due to Mg$^{+2}$ induced DNA-DNA attraction inside the capsid. At lower or higher concentrations, $\eta^*$ is either negative or positive. As a charged molecule at these concentrations, DNA prefers to be in free solution than in the bundle inside the capsid. Accordingly, this leads to a higher percentage of ejected viral genome. The dashed line in Fig. 1 is a fit of our theoretical result to the experimental data for MgSO$_4$.

The optimal Mg$^{+2}$ concentration is shown to be $N_0 = 64$ mM. The Mg$^{+2}$-mediated attraction between DNA double helices is found to be $-0.004$ $kBT$/base ($k_B$ is the Boltzmann constant and $T$ is the temperature of the system).

We begin by writing the total energy of the DNA molecule as the sum of the energy of the DNA segment ejected outside the capsid with length $L_o$ and the energy of the DNA segment remaining inside the capsid with length $L_i = L - L_o$, where $L$ is the total length of the viral DNA genome:

$$E_{\text{tot}}(L_o) = E_{\text{in}}(L_i) + E_{\text{out}}(L_o) \tag{1}$$

Because the ejected DNA segment is under no confinement, we neglect contributions from bending energy and approximate $E_{\text{out}}$ by the electrostatic energy of a free DNA of the same length in solution. Treating the DNA molecule as a uniformly charged cylinder with radius $a$ and linear charge density $\eta^*$, one obtains:

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

where $D = 78$ is the dielectric constant of water and $r_s$ is the Debye-Hückel screening length of the solution. The negative sign signifies the fact that the system of the combined DNA and the condensed counterions is equivalent to a cylindrical capacitor under constant charging potential. The net linear charge density of DNA, $\eta^*$, is a function of the counterion concentration $N$ \[13\]:

$$\eta^* = -\left(\eta_c/2Z\right) \ln(N_0/N)/\ln(1 + r_s/a), \tag{3}$$

where $\eta_c = Dk_BT/e$ is Manning critical charge density and $Z$ is the counterion valence. The constant concentration at which DNA is neutral, $N_0$, can be interpreted as the concentration of counterions next to the condensed counterion layer on the DNA surface. A simple derivation for $\eta^*$ can be obtained by dividing the counterion population into two groups, a “bound” (condensed) counterion layer on the DNA and a “free” counterion population in solution. The distribution of the latter is assumed to obey Boltzmann statistics:

$$N(r) = N \exp[-Ze\phi(r)/k_BT] \tag{4}$$

with $\phi(r)$ being the electrostatic potential at radial distance $r$ from DNA central axis. Denoting $N_0 = N(a)$, one immediately gets

$$\phi(a) = -(k_BT/Ze) \ln(N_0/N). \tag{5}$$

On the other hand, the surface potential $\phi(a)$ of a charged cylinder with charge density $\eta^*$ in Debye-Hückel approximation is given by \[17\]:

$$\phi(a) = -2\eta^* \frac{K_0(a/r_s)}{D(a/r_s)K_1(a/r_s)} \simeq 2\eta^*/D \ln(1 + r_s/a) \tag{6}$$

where $K_{0,1}$ are Bessel functions (this expression is twice the value given in Ref. \[17\] because we assume that the screening ion atmosphere does not penetrate the DNA cylinder). Eliminating $\phi(a)$ from Eqs. \[(5)\] and \[(6)\], one gets Eq. \[(6)\].

Obtaining the concentration $N_0$ using first principle calculations is a complicated and non-trivial task \[15, 17\]. In general, it depends on the correlation between “bound” counterions at the DNA surface and its competition with the counterion entropy. However, in practical situations, DNA is almost neutralized ($|\eta_{\text{bare}}/\eta^*| \gg 1$) by the counterions. Therefore, $N_0$ can be very well assumed to be independent of $N$ and $\eta^*$. Within the scope of this paper, we treat it 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:

$$E_{\text{int}}(L_i, d) = E_{\text{bend}}(L_i, d) + E_{\text{int}}(L_i, d).$$ (7)

where $d$ is the average DNA–DNA interaxial distance. To calculate $E_{\text{bend}}$, we employ the viral DNA packaging model used previously [8, 13, 19]. In this model, the genome coils co-axially inward with the neighboring DNA helices forming a hexagonal lattice with lattice constant $d$. A sketch for a cross section of the viral capsid is shown.

FIG. 2. A simplified 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.

$$E_{\text{bend}}(L_i, d) = \frac{4\pi l_p k_B T}{\sqrt{3}d^2} \left\{ -\left( \frac{3\sqrt{3}L_i d^2}{8\pi} \right)^{1/3} \right\}$$

$$+ R \ln \left( \frac{R + (3\sqrt{3}L_i d^2/8\pi)^{1/3}}{[R^2 - (3\sqrt{3}L_i d^2/8\pi)^{2/3}]^{1/2}} \right)$$ (8)

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

To calculate $E_{\text{int}}(L_i, d)$, we notice that multivalent counterions can induce condensation of free DNA in solution [12, 13]. DNA molecules in such a condensate are almost neutralized by the counterions and are arranged in a hexagonal lattice (similar to our viral DNA packaging arrangement) with an equilibrium interaxial distance $d_0$. The value of $d_0$ depends on both the valency and the type of counterions used, but is typically about 2.8 nm. As mentioned earlier we argue that, in the confinement of the viral capsid, Mg$^{2+}$ also induces attraction between DNA segments. Neglecting finite size effect, we approximate the interaction energy the viral DNA coiling inside the capsid as

$$E_{\text{int}}(L_i, d_0) = -L_i \epsilon,$$ (9)

where $-\epsilon$ is the DNA–DNA attraction per unit length. Like the aforementioned parameter $N_0$, we treat $\epsilon$ and $d_0$ as constant fitting parameters of our theory. In total, we have three fitting parameters ($N_0$, $\epsilon$, $d_0$) and three fitting constrains (the two coordinates of the minimum and the curvature of the curve $L_o(N)$ in Fig. 1). Thus our theory does not contain unnecessary degrees of freedom.

Obviously, due to the strong confinement of the viral capsid, the interaxial distance $d$ between neighboring DNA double helices inside the capsid is smaller than the equilibrium distance $d_0$ inside the condensate. The experiments from Ref. [12] 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 $d$:

$$E_{\text{int}}(L_i, d) = L_i \sqrt{3}F_0 \left[ (c^2 + cd) \exp \left( \frac{d_0 - d}{c} \right) \right.$$\n
$$- (c^2 + cd_0) - \frac{1}{2}(d_0^2 - d^2) - L_i \epsilon,$$ (10)

where the empirical values of the constants $F_0$ and $c$ are 0.5 pN/nm$^2$ and 0.14 nm respectively.

Equation (11) together with equations (2), (7), (8) and (10) provide the complete expression for the total energy of the DNA genome of our theory. For a given external osmotic pressure, $\Pi_{\text{osm}}$, and a given multivalent counterion concentration, $N$, 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

$$G(L_o) = E_{\text{tot}}(L_o) + \Pi_{\text{osm}}L_o\pi a^2.$$ (11)

Here, $L_o\pi a^2$ is the volume of the ejected DNA segment in aqueous solution. The specific procedure is following. The energy $E_{\text{in}}(L - L_o, d)$ of the DNA segment inside the capsid is minimized with respect to $d$ to acquire the optimal DNA–DNA interaxial distance for a given DNA ejected length, $d^*(L_o)$. Then, we substitute $E_{\text{int}}(L_o) = E_{\text{in}}(L - L_o, d^*(L_o)) + E_{\text{out}}(L_o)$ into Eq. (11) and optimize $G(L_o)$ with respect to $L_o$ to obtain the equilibrium ejected length $L_o^*(\Pi_{\text{osm}}, N)$. By fitting this $L_o^*$ with experiment data we can obtain the values for the neutralizing counterion concentration, $N_0$, the Mg$^{2+}$ – mediated DNA–DNA attraction, $-\epsilon$, and the equilibrium DNA–DNA distance $d_0$. The result of fitting our theoretical ejected length $L_o^*$ to the experimental data of Ref. [10] is shown in Fig. 1. In this experiment, wild type bacteriophages $\lambda$ was used, so $R = 29$ nm and $L = 16.49$ $\mu$m [20]. $\Pi_{\text{osm}}$ 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 $N_0 = 64$ mM, $\epsilon = 0.004 k_B T$ per nucleotide base, and $d_0 = 2.73$ nm.

The strong influence of the 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 Eq. (9) for DNA–DNA interaction inside the capsid. Firstly, the attraction strength $\epsilon$ appears in the expression for the enthalpy, Eq. (11), with the same sign as $\Pi_{\text{osm}}$. In other words, the attraction between DNA
strands inside capsid acts as an additional “effective” osmotic pressure preventing the ejection of DNA from bacteriophages. This switch from repulsive DNA-DNA interaction for monovalent counterion to attractive DNA-DNA interaction 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 (N = $N_0$). Secondly, the electrostatic energy of the ejected DNA segment given by Eq. 2 is logarithmically symmetrical around the neutralizing concentration $N_0$. This is clearly demonstrated in Fig. 1 where the log-linear scale is used. This symmetry is very similar to the behavior of another system that exhibits charge inversion phenomenon, the non-monotonic swelling of macroring by multivalent counterions [21].

It is very descriptive to compare our fitting values for $\epsilon$ and $N_0$ to those obtained for other multivalent counterions. Fitting done for the DNA condensation experiments by Spm$^{-4}$ and Spd$^{-3}$ shows $\epsilon$ to be 0.07 and 0.02 $k_BT$/base respectively [12, 22]. For our case of Mg$^{+2}$, a divalent counterion, and bacteriophage $\lambda$ experiment, $\epsilon$ is found to be 0.004 $k_BT$/base. This is quite reasonable since Mg$^{+2}$ is a much weaker counterion. Furthermore, $N_0$ was found to be 3.2 mM for the tetravalent counterion, 11 mM for the trivalent counterion. Our fit of $N_0 = 64$ mM for divalent counterions again is in favorable agreement with these independent fits. Note that, in the limit of high counterion valency ($Z \to \infty$), $N_0$ is shown to vary exponentially with $-Z^{3/2}$ [15, 16]. The large increase in $N_0$ from 3.2 mM for tetravalent counterions to 64 mM for divalent counterions is not surprising.

The fitted value $-\epsilon = -0.004 k_BT$ per base explains why Mg$^{+2}$ ions cannot condense DNA in free solution. It corresponds to an attraction of $-1.18k_BT$ per persistence length. Since thermal fluctuation energy of a polymer is about $k_BT$ per persistence length, this attraction is too weak to overcome thermal fluctuations. It therefore can only partially condense free DNA in solution [12]. Only in the confinement of the viral capsid, can this attraction effect appears in the ejection process. It should be mentioned that computer simulation of DNA condensation by idealized divalent counterions [23] does show a weak short-range attraction comparable to our $\epsilon$.

The phenomenological constants $-\epsilon$ and $N_0$ depend very strongly on the strength of the correlations between multivalent counterions on the DNA surface. The stronger the correlations, the greater the value $\epsilon$ and the smaller the concentration $N_0$. In Ref. 10, MgSO$_4$ salt induces strong inhibition effect. Due to this, $N_0$ for MgSO$_4$ falls within the experimental measured concentration range and we use these data to fit our theory. MgCl$_2$ induces weaker inhibition, thus $N_0$ for MgCl$_2$ is larger and apparently lies at higher value than the measured range. More data at higher MgCl$_2$ concentration is needed to obtain reliable fitting parameters for this case. In the future, we plan to complimentary our phenomenological theory with a first principle calculation to understand the “microscopic” quantitative differences between MgSO$_4$ and MgCl$_2$ salts. The authors of Ref. 10 also used non-ideality as an explanation for these differences.

In conclusion, in this letter, it is shown that divalent counterions such as Mg$^{+2}$ can have strong effects on DNA condensation in a confined environment (such as inside bacteriophages capsid) similar to those of counterions with higher valency. This fact should to be incorporated in any electrostatic theories of bacteriophage packaging. The strength of short-range DNA-DNA attraction mediated by MgSO$_4$ salt is first obtained by the authors. It is consistent with the known values for higher valence counterions. It provides a good starting point for future works with DNA-DNA condensation in the presence of divalent counterions.

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