The pulling force of a single DNA molecule condensed by spermidine

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In a recent experiment, a single DNA double helix is stretched and relaxed in the presence of spermidine, a short positive polyelectrolyte, and the pulling force is measured as a function of DNA extension. In a certain range of spermidine concentration, a force plateau appears whose value shows maximum as a function of spermidine concentration. We present a quantitative theory of this plateau force based on the theory of reentrant condensation and derive almost parabolic behavior of the plateau force as a function of the logarithm of the spermidine concentration in the range of condensation. Our result is in good agreement with experimental data.

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DNA condensation by strongly positively charged proteins, histones, is used by nature for compaction of DNA in cell nucleus (DNA is strongly negatively charged). Positive proteins, protamines, are used for additional compaction of DNA in the sperm [1]. Gene therapy uses complexes of DNA with long positive polyelectrolytes or other macroroations. The net charge of these complexes can be positive and therefore they are not repelled by negative cell membrane in the course of gene delivery. Thus, condensation of DNA by positive macroroations is an extremely important physical phenomenon. It is intensively studied in the model system of double helix DNA with spermidine, a flexible polymer with length 15 Å and charge +3. It is known that in a dilute DNA solution at some concentration of spermidine, \( s = s_c \), each long DNA molecule self-condenses into a toroid [2]. When \( s \) grows farther, at a much larger concentration, \( s = s_d \), DNA dissolves back [3]. This phenomenon is called reentrant condensation and has got theoretical explanations [4, 5].

Recently, a new single molecule technique has been used to measure the force necessary to pull a single DNA double helix from a toroidal condensate of DNA [6] (Fig. 1). In the experiment, extremely small concentration of DNA (2 nM of nucleotides) is dissolved in water with certain concentrations of spermidine (varying from 200 μM to 200 mM). Dual-trap tweezers are used to stretch a single DNA molecule tethered between two protein-coated polystyrene beads. DNA is first stretched and then relaxed. During these processes, the pulling force \( f \) is measured. Experiments are repeated at various spermidine concentrations. It is observed that in the interval \( s_c < s < s_d \), there is a fairly large range of DNA extension, \( x \), where force \( f \) is significantly larger than the force described in the wormlike chain model [7]. Moreover, in this range of \( x \), \( f \) is almost a constant (a force plateau, see Fig. 2). The plateau value of the force, \( f_p \), is plotted as a function of \( s \) in Fig. 3.

![FIG. 1: Schematic illustration of the experimental setup. Partly condensed long DNA is pulled by two beads.](image1)

![FIG. 2: The pulling force \( f \) measured as a function of extension \( x \) is schematically shown by the solid line for \( s_c < s < s_d \), when DNA is condensed by spermidine. At \( x < x_0 \), the force \( f_p \) is a constant whose value depends on \( s \). The dashed line shows the wormlike chain behavior of \( f \) in the absence of DNA condensation (\( s < s_c \) or \( s > s_d \)). At \( x > x_0 \), the condensate is completely eliminated by the pulling force and the wormlike chain behavior is recovered.](image2)

In this paper, we suggest a quantitative theory of the \( f_p(s) \) curve based on the theory of reentrant condensation [5]. We assume that the whole process is slow enough so that the system is always in equilibrium. Then in zero order approximation, the force needed to detach DNA from the condensate is just the free energy difference per unit length between the free coil state and the condensed state of the DNA-spermidine complex. We call it \( f_0 \).
The pulling force $f_p$ measured in the experiment is somewhat larger than $f_0$ since the detached part of the DNA molecule is stretched, and therefore loses entropy with respect of its free coil state. In the end of the paper, we show that this effect adds a small correction to the pulling force,

$$f_p = f_0 \left( 1 + \sqrt{\frac{k_B T}{lf_0}} \right)$$

when $k_B T/lf_0 \ll 1$. Here $l$ is the persistence length of DNA. In this paper, we first calculate $f_0$ and then $f_p$ using Eq. 1. Our result is shown in Fig 3. It demonstrates reasonably good agreement with experimental data.

![Figure 3](image-url)

**FIG. 3:** Comparison between the theoretical result (the solid line) and experimental data (points with error bars taken from Fig. 2 of Ref. [2]) for the pulling force $f_p$ as a function of spermidine concentration $s$. $f_0$ is shown by the dashed line.

Let us start from the theory of reentrant condensation of DNA with spermidine without any external forces. In the solution, positively charged spermidine molecules are absorbed on the negatively charged surface of double helix DNA. Since spermidine molecules are strongly charged (+3), they form a two dimensional strongly correlated liquid on the surface of the DNA molecule. When a new spermidine molecule approaches the DNA helix, it repels already absorbed spermidine molecules and creates an electrostatic image of itself, similar to the image on a conventional metallic surface. Attraction to the image leads to an additional negative chemical potential $\mu_c$ for spermidine molecules on the surface of DNA. As a result, if $s$ exceeds some concentration, $s_0$, charge of the DNA-spermidine complex changes sign and becomes positive, i.e., spermidine molecules overcharge DNA (see review of the theory of charge inversion in Ref. [3]).

Adsorbed layers of the correlated spermidine liquid also lead to self-attraction of DNA. In the spot where two turns of DNA touch each other, the surface density of the correlated liquid is doubled and the correlation energy per spermidine molecule is reduced [4]. This short range force of self-attraction leads to condensation of DNA if the macroscopic Coulomb repulsion is not very strong. As a result, in the vicinity of the neutralizing concentration $s_0$ ($s_c < s_0 < s_d$), DNA-spermidine complex is condensed into a toroid.

The condition of equilibrium between spermidine molecules in the bulk and on the surface of DNA has a form

$$\mu_c + Z e \phi = k_B T \ln(s/v_0),$$

where $Z = +3$ is the valence of spermidine, $\phi$ is the electric potential on the surface of the DNA-spermidine complex, and $v_0$ is the normalizing volume of spermidine. The left hand side is the chemical potential of a spermidine molecule in the complex, the right hand side is its chemical potential in the bulk of the solution. Notice that the entropy of spermidine can be expressed through the total concentration $s$ because absorption of spermidine by DNA practically does not change $s$ (the concentration of DNA nucleotides is at least $10^5$ times smaller than $s$). In the vicinity of $s_0$ ($s_c < s < s_d$), the net charge of the complex is small and the spermidine concentration on the DNA surface does not change much. Since $\mu_c$ is determined by this concentration, it is approximately a constant. According to Eq. (2), when $\phi = 0$, i.e., the complex is neutral, there is a simple relation between $\mu_c$ and $s_0$,

$$s_0 = \frac{1}{v_0} \exp \left( \frac{|\mu_c|}{k_B T} \right).$$

Now we calculate the free energy of the charged complex in its coil state. For this purpose, we treat the DNA-spermidine complex as a capacitor with the capacitance per unit length $C$. Suppose $s$ is such that the complex is overcharged ($s_0 < s < s_d$). Overcharging enhances the Coulomb self-energy and reduces both the correlation energy and the entropy of spermidine molecules. Taking the free energy of a neutral coil as zero, and using Eq. (2), we get the free energy of the complex per unit length

$$f_1 = \frac{1}{2} C \phi^2 + \frac{C \phi}{Ze} \mu_c - \frac{C \phi}{Ze} k_B T \ln(s/v_0) = -\frac{1}{2} C \phi^2,$$

where the three terms allow for the three parts of the free energy mentioned above, and $C \phi/Ze$ is the number of spermidine molecules overcharging the complex. The final expression is the same as the free energy of a capacitor kept under a constant voltage $\phi$. It is easy to see that this expression is also true for an undercharged complex. Since the DNA-spermidine complex can be considered as a long cylinder, $C$ is given by expression

$$C = \frac{D}{2 \ln(1 + r_s/R)},$$
where $D = 80$ is the dielectric constant of water, $r_s$ is the Debye-Hückel screening radius, and $R = 10 \, \text{Å}$ is the radius of the double helix cross section.

We first assume that $r_s \gg R$. In this case, the complex in the condensed state is practically neutral. Indeed, if the condensed complex were charged, the Coulomb-self energy of the macroscopic condensate would be too large to hold it. We define phenomenological parameter $\varepsilon < 0$ as the free energy per unit length of the condensed complex calculated from the free energy of a neutral coil. It includes the gain of correlation energy in the spots where two turns of DNA touch each other, and also the loss of certain entropic elasticity of the coil in the condensate.

When the complex goes from the neutral condensed state to the charged coil state, the free energy increment per unit length is

$$f_0 = -\frac{1}{2}C\phi^2 - \varepsilon.$$  \hspace{1cm} (6)

![FIG. 4](image)

**FIG. 4:** The zero order approximation to the pulling force, $f_0$, as a function of $\ln s$ given by an inverted parabola. The maximum value of $f_0$, $|\varepsilon|$, is achieved at $s = s_0$ where the free DNA-spermidine complex is neutral. $s_{c,d}$ are the concentrations of spermidine at which the condensate dissolves.

If $r_s$ is comparable with $R$ (as in experiment [8]), the approximation used above that the condensate is neutral has to be revised and the Coulomb energy of the condensate should be taken into account. If we introduce $C'$ as the effective capacitance per unit length of the complex in the condensate, similarly to Eq. (11), the free energy per unit length is just $-C'\phi^2/2$. Accordingly, in Eq. (6), $C$ should be replaced by $C - C'$. Using Eqs. (2) and (3), we rewrite Eq. (6) as

$$f_0 = |\varepsilon| - \frac{(C - C')k_B^2T^2}{2Z^2e^2} \ln^2 \frac{s}{s_0}.$$  \hspace{1cm} (7)

The function $f_0(\ln s)$ is shown in Fig. 4 by an inverted parabola. According to Eq. (4), the maximum value $f_0 = |\varepsilon|$ is achieved at $s = s_0$ where the free complex is neutral. Also, we get the two concentrations $s_c$ and $s_d$ at which $f_0$ goes to zero,

$$s_{c,d} = s_0 \exp\left(\frac{1}{k_BT} \sqrt{\frac{2|\varepsilon|Z^2e^2}{C - C'}}\right),$$  \hspace{1cm} (8)

where the upper (lower) sign corresponds to the first (second) subscript of $s_{c,d}$. Exactly at these two concentrations, one can see transition from the coil state to the condensed state or vice versa in light scattering experiments with solutions of DNA and spermidine [5].

In order to calculate $C'$, we assume that the condensate is macroscopic and densely packed. Then

$$\phi = \int_0^\infty \frac{\rho e^{-r/r_s}}{Dr}4\pi r^2dr = \frac{4\pi r_s^2\rho}{D},$$  \hspace{1cm} (9)

where $\rho$ is the charge density of the condensate. The charge of the complex per unit length is $\pi R^2\rho/\alpha$ where $\alpha = 0.91$ is the filling factor for the hexagonal dense packing of cylinders. This gives

$$C' = \frac{\rho \pi R^2}{\phi} = \frac{DR^2}{4\alpha r_s^2}.$$  \hspace{1cm} (10)

We see that when $r_s \gg R$, we can drop $C'$ in $C - C'$ because $C' \ll C$, i.e., the condensate is almost neutral.

As we mentioned before, $f_0$ is only the zero order approximation to the pulling force. The pulling force $f_p$ is given by Eq. (11). We need four experimental parameters to calculate it. The spermidine concentrations $s_{c,d}$ at which the condensate dissolves, the average DNA persistence length $l$, and the average Debye-Hückel screening radius $r_s$. Following Ref. [11], we take $s_c = 0.35 \, \text{mM}$, $s_d = 150 \, \text{mM}$. Therefore $s_0 = 7.2 \, \text{mM}$ (Using Eq. (8)).

We use $l = 500 \, \text{Å}$ corresponding to the persistence length of a neutral DNA coil. At $s_0 = 7.2 \, \text{mM}$, spermidine and its counter ion contribute to $r_s$ even more than monovalent salt (10 mM). Treating every spermidine molecule as a point-like trivalent ion, we get $r_s = 13 \, \text{Å}$ from the standard Debye-Hückel expression [10]. Calculating $C$ and $C'$ according to Eqs. (5) and (10), we finally get $|\varepsilon| = 0.11$ $k_BT/\text{bp}$ (1bp = 3.4 Å) from Eq. (8).

For these parameters, $f_0$ and $f_p$ are shown together with the experimental data in Fig. 4. We see that our result for $f_p$ agrees pretty well with the experimental data. Notice that correction $f_p - f_0$ is much smaller than $f_0$ in the range of experimental data. This justifies the use of the perturbative result Eq. (11).

Finally, let us derive Eq. (11). For this purpose, we consider the detached part of DNA as a wormlike chain with the contour length $L$ and end-to-end distance $x$ (see Fig. 1), while the contour length of the whole DNA molecule is $L_0$. We also assume that the system is always in equilibrium during the whole process. The free energy of the system is

$$F(x, L) = -(L_0 - L)f_0 + F_e$$
\[-(L_0 - L)f_0 + \frac{k_BT}{l} \left[ \frac{x^2}{2L} + \frac{L}{4(1-x/L)} - \frac{x + L}{4} \right], \tag{11}\]

where \(-(L_0 - L)f_0\) is the free energy of the condensed part of DNA, and \(F_e\) is related to the entropic elasticity of the detached part of DNA. The expression for \(F_e\) is obtained for a free wormlike chain with fixed contour length \(L\) and end-to-end distance \(x\): \(F_e(x) = f_0^2 \int_0^x f_e(x')dx' + \text{const.}\). Here \(f_e\) is the force needed to keep the end-to-end distance \(x\) for a free wormlike chain with length \(L\) and is given by an interpolation formula \[f_e = \frac{k_BT}{l} \left[ \frac{x}{L} - \frac{1}{4} + \frac{1}{4(1-x/L)^2} \right]. \tag{12}\]

The energy zero point has been chosen at \(x = 0, L = L_0\), i.e., at the free energy of a DNA-spermidine complex in its coil state.

For a given \(x\), the contour length \(L\) of detached DNA can be found from the condition of minimal free energy, namely, \(\partial F/\partial L|x = 0\). If we define \(a \equiv x/L < 1\), this minimum condition can be written as

\[2a^2(1-a)^2 + a^2 - \frac{4lf_0}{k_BT} (1-a)^2 = 0. \tag{13}\]

Finding \(a\) from this equation and putting \(L = x/a\) back to Eq. (11), we calculate the magnitude of the pulling force

\[f_p = \frac{f_0}{a} + \frac{k_BT}{l} \left[ \frac{a}{2} - \frac{1}{4} + \frac{1}{4(1-a)} \right]. \tag{14}\]

Notice that \(a\) does not depend on \(x\) and \(L\) (see Eq. (13)). Therefore, according to the definition \(x = aL\), the two lengths \(x\) and \(L\) are always proportional to each other. This result was also obtained in Ref. [11] using a different approach. From Eq. (14) it is easy to see that \(f_p > f_0\) since \(a < 1\). There are two contributions to \(f_p - f_0\). The first is a geometrical effect (the term \(f_0/a\) in Eq. (14)). Namely, to change the end-to-end distance by \(\Delta x\), a larger length \(\Delta L = \Delta x/a\) must be pulled out of the condensate. The second contribution is the free energy increment of the detached part of DNA, \(F_e\). These two contributions are equal in the limiting case of \(k_BT \ll f_0\) (see Eqs. (15), (16) and (1)). Since \(f_p\) does not depend on \(x\), we get a force plateau with increasing \(x\), as observed in the experiment (see Fig. 2). This plateau ends when \(x = aL_0\) and all DNA is pulled out of the condensate. At \(x > x_0\), the pulling force starts to increase with \(x\) according to Eq. (12).

When \(k_BT/f_0 \ll 1\), we have \(1 - a \ll 1\), and Eq. (14) becomes

\[f_p = f_0 + \frac{k_BT}{4f_0(1-a)}. \tag{15}\]

Solving Eq. (13) in the same limit, we get

\[a = 1 - \frac{1}{2} \sqrt{\frac{k_BT}{lf_0}}. \tag{16}\]

Substituting Eq. (16) in Eq. (15), we arrive at our final result Eq. (11). It gives the pulling force \(f_p\) in the first order perturbation theory in the small parameter \(\sqrt{k_BT/f_0}\). In experiment [6], we have \(k_BT/lf_0 = 0.06\) at \(s = s_0\). Therefore, Eq. (11) can be used in almost all the range \(s_c < s < s_d\). At \(s\) close to \(s_{c,d}\), where \(f_0 \to 0\), the perturbation theory fails. In principle, we can calculate \(f_p\) numerically using Eqs. (13) and (14) in the whole interval \(s_c < s < s_d\).

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