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# Analytical description of extension, torque and supercoiling radius of a stretched twisted DNA

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We study the mixture of extended and supercoiled DNA that occurs in a twisted DNA molecule under tension. Closed-form asymptotic solutions for the supercoiling radius, extension and torque of the molecule are obtained in the high-force limit where electrostatic and elastic effects dominate. We demonstrate that experimental data obey the extension and torque scaling laws apparent in our formulae, in the regime where thermal fluctuation effects are quenched by applied force.

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Experiments on stretched, supercoiled DNA primarily measure the extension of the molecule as a function of its twisting, or more precisely as a function of change in double helix linking number  $Lk$ . [1]. Past the threshold for buckling of the molecule, one enters a mixed-phase regime where part of the molecule is extended and part of the molecule is plectonemically supercoiled; in this regime the extension depends linearly on linking number precisely because one is in a regime of two-phase coexistence [2, 3].

Although the origin of the linear dependence is understood [2, 4], the slopes of the extension versus linking number curves are not understood analytically. It is of broad interest to understand the slopes since in addition to being practically useful to predict the amount of length absorbed into plectonemic supercoiling, they contain information about the interplay of external force applied to the molecule with interactions between the tightly juxtaposed DNA double helices in the plectonemic region (primarily electrostatic in the regime of interest here) [5]. Below we analyze the parts of the “standard model” of DNA supercoiling relevant to the high-force limit. We obtain asymptotic formulae describing that limit, for the dependence of the extension versus linking number slopes and DNA torque on force and salt concentration, and we show that experimental data obey the scaling behavior implicit in our results in the high-force limit. Analytical formulae for the plectonemic supercoil radius and angle are predicted for future experiments.

We write the free energy of a DNA molecule subject to applied torque and force in the regime where extended and plectonemically supercoiled DNA are in mechanical coexistence [3, 4, 6, 7]. The total molecule length  $L$  is partitioned between the two “phases”: (i) a plectonemic phase of length  $\ell$ , where the filament has bending rigidity  $A$ , torsional rigidity  $C$  and adopts a superhelical shape of radius  $r$  and angle  $\alpha$ ; (ii) an extended worm-like-chain phase of length  $L - \ell$ . The free energy of the extended phase is described in terms of the free energy per length of the untwisted molecule  $g(f) = f - k_B T (f/A)^{1/2} + \dots$  [8], plus a twist energy using a twist modulus that includes effects of writhing fluctuations,  $C_s(f) = C [1 - (C/4A)k_B T / (Af)^{1/2}]$  [6]. The free energy of the plectonemic phase is that of two superhelices wrapped around each other with electrostatic interactions. The interaction energy  $U(r)$  is that of two straight charged cylinders with center axis separated by a distance of  $2r$ , in the Debye-Huckel approximation of the Poisson-Boltzmann equation. For the double helix where two negative charges appear for each base pair this suggests the use of a linear charge density (in electron charge units) of  $\nu = 1/b$ , where  $b = 0.17$  is half of the 0.34 nm spacing of successive base pairs along DNA. However an effective charge is introduced to cope with two effects: (i) the fact that this charge is distributed on the surface of the cylindrical double helix of radius  $a = 1$  nm rather than on its center axis; (ii) the asymptotics of the linear and nonlinear solutions of the Poisson-Boltzmann equation have to match for large separation distances. The effective charge is:

$$\nu = \frac{1}{b} \frac{1}{\gamma(L_B, b, \kappa_D a)} \frac{1}{\kappa_D a K_1(\kappa_D a)}, \quad (1)$$

where  $L_B$  is the Bjerrum length in water,  $\kappa_D^{-1}$  the Debye length, and  $K_n(x)$  the  $n^{\text{th}}$  modified Bessel function of

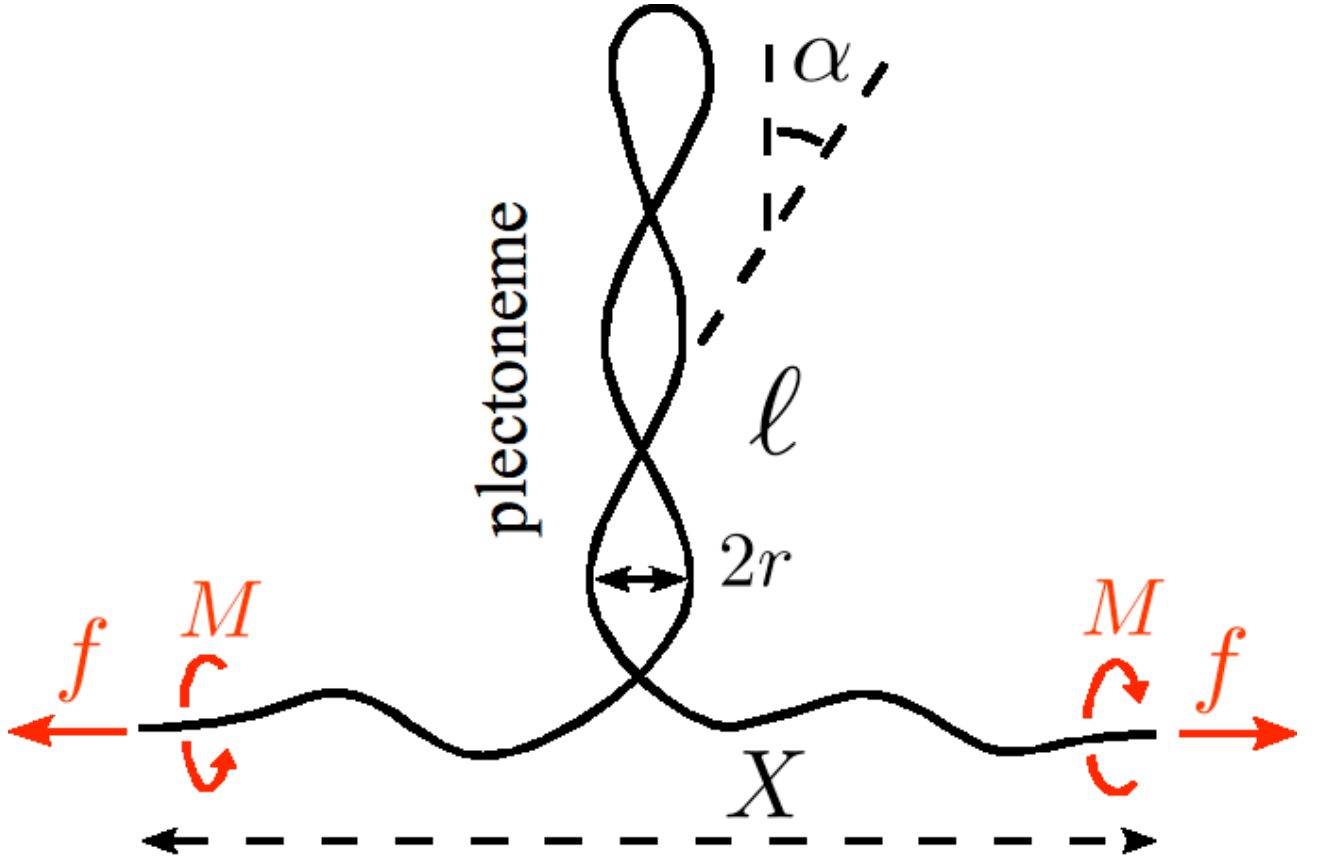


FIG. 1: Supercoiled DNA under force and torque. Molecule length is partitioned between two “phases”: an extended phase, and a plectonemic phase where strong self-interaction occurs.

the second kind [9, 10]. From Table III of [9], the parameter  $\gamma$  is computed to be  $\gamma = (1.64, 1.44, 1.27, 1.14)$  at salt concentrations (50, 100, 200, 500) mM and for  $T = 296.5$  K and  $L_B = 0.7$  nm. The interaction potential in the plectoneme is [3, 11]:

$$U(r) = k_B T \nu^2 L_B K_0(2\kappa_D r), \quad (2)$$

where both  $\kappa_D$  and  $\nu$  depend on the salt concentration. We neglect confinement entropy [3] in the interaction potential for two reasons: (i) it is important for low forces and we presently focus on the moderate to high force regime; (ii) our focus is on an analytical solution so we consider a simple one-term  $U(r)$  (similarly no dependence of  $U$  on superhelical angle  $\alpha$  is considered here).

The bending energy, given by the integral of the curvature squared of the molecule center line in the superhelical configuration, is a function of the superhelical radius  $r$  and angle  $\alpha$ .

Adding together electrostatic, bending and twisting energy terms, the total free energy is:

$$\mathcal{F}(\alpha, r, \lambda_s, \tau_p, \ell) = -g(L - \ell) + \frac{1}{2} C_s \lambda_s^2 (L - \ell) + \left[ \frac{1}{2} C \tau_p^2 + \frac{1}{2} A \frac{\sin^4 \alpha}{r^2} + U(r) \right] \ell \quad (3)$$

where  $\lambda_s = 2\pi \Delta Lk_s / (L - \ell)$  is the linking angle density in the stretched part of the DNA ( $\Delta Lk_s$  is the excess linking number of the extended region), and  $\tau_p = 2\pi \Delta Tw_p / \ell$  is the twist angle density in the plectonemic DNA ( $\Delta Tw_p$  is the excess twist in the plectoneme region).

Once force and  $\Delta Lk$  are specified, the remaining variables are determined by minimization of Eq. 3 subject to the constraint:

$$\Delta Lk = \Delta Tw + Wr = \frac{1}{2\pi} \left( \lambda_s (L - \ell) + \tau_p \ell + \frac{\sin 2\alpha}{2r} \ell \right) \quad (4)$$

where  $\Delta Lk$  is the number of turns introduced into the DNA relative to the relaxed double helix linking number  $Lk_0$  (i.e.,  $\Delta Lk = Lk - Lk_0$ ). The constraint on  $\Delta Lk$  is handled using a Lagrange multiplier  $M$ , i.e. by minimizing

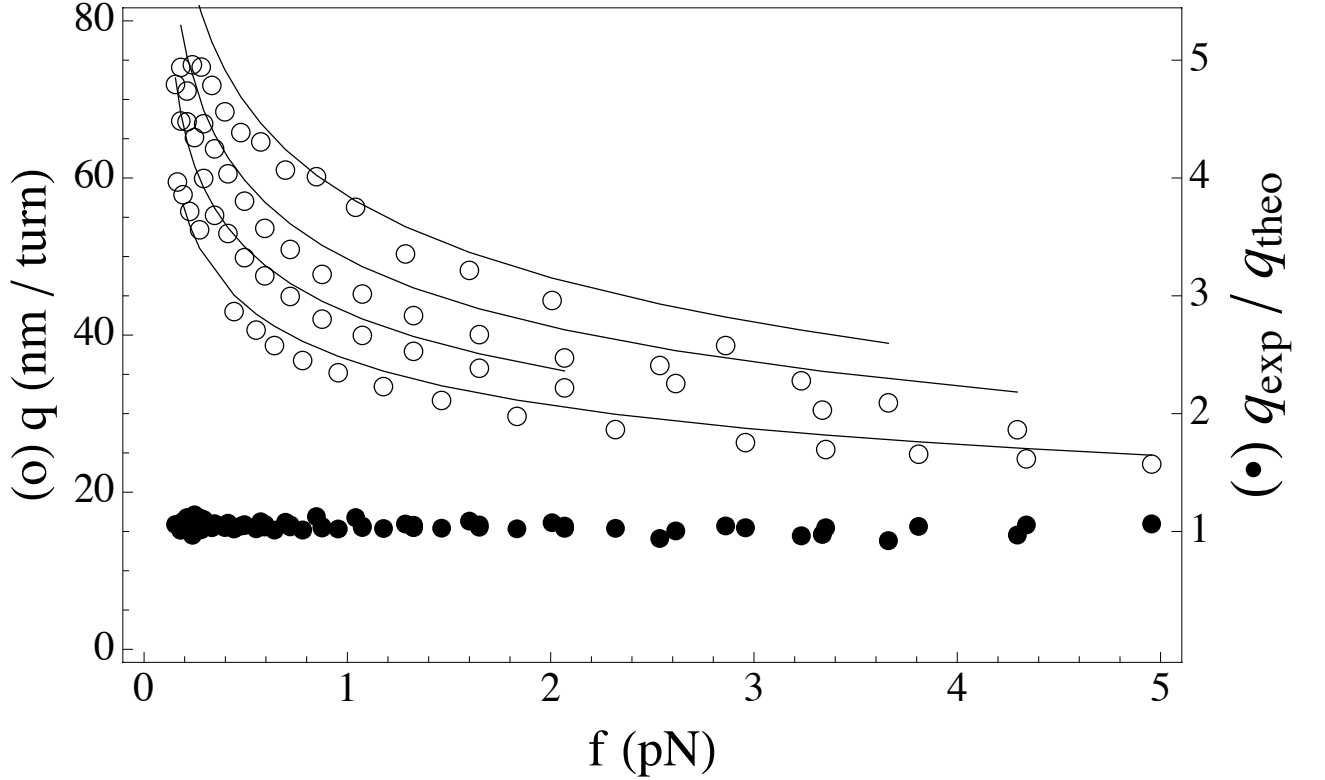


FIG. 2: Comparison of experimental and theoretical slopes as function of the applied force, for 50, 100, 200, and 500 mM salt (top to bottom): (a) experimental data from Ref. [1] (circles); (b) theoretical solution of the full equations  $\nabla\mathcal{G} = \mathbf{0}$  (continuous lines); (c) ratio of the experimental slopes to the formula in Eq. (16) (filled circles). Experimentally given values of  $A = 46, 47, 44, 45$  nm and  $C = 94$  nm were used.

$\mathcal{G} = \mathcal{F} - 2\pi M(\Delta Tw + Wr - \Delta Lk)$ . Equilibrium values of the six variables  $\lambda_s$ ,  $\tau_p$ ,  $\alpha$ ,  $\ell$ ,  $r$ , and  $M$  follow from solving  $\nabla\mathcal{G} = \mathbf{0}$  where  $\nabla = (\partial/\partial\lambda_s, \partial/\partial\tau_p, \partial/\partial\alpha, \partial/\partial\ell, \partial/\partial r, \partial/\partial M)$ . The equilibrium value of  $M$  is the torque in the DNA.

These nonlinear equations, when solved numerically, yield multiple solutions with either  $\ell = 0$ , or  $\ell \neq 0$ . We here focus on the stable solution having  $\ell > 0$ , which exists as soon as the added  $\Delta Lk$  is large enough [7]. The values of  $\alpha$ ,  $r$ ,  $\lambda_s$ ,  $\tau_p$ , and  $M$  in this solution do not depend on  $\Delta Lk$ , and the value of  $\ell$  varies linearly with  $\Delta Lk$  [7]. A consequence is that the mean extension  $\langle X \rangle = -\partial\mathcal{G}/\partial f$  decreases linearly with  $\Delta Lk$ : as linking is added more and more contour length passes from the extended phase to the plectonemic phase of the molecule. Fig. 2 shows that the slopes  $q := \partial\langle X \rangle/\partial\Delta Lk$  from experiment (data from Fig. 3 inset of Ref. [1]) are described well by this theory for four different salt concentrations (50, 100, 200, and 500 mM). Similarly, experimental and theoretical torques are in good accord (Fig. 3).

We now extract the leading scaling behavior for the slope  $q$  and the torque  $M$  from the full equations, in the high-force limit. We first approximate  $C_s(f) \simeq C$ , which reduces the equilibrium equations  $\nabla\mathcal{G} = \mathbf{0}$  to:

$$-A \frac{\sin^4 \alpha}{r^3} + U'(r) + M \frac{\sin 2\alpha}{2r^2} = 0, \quad (5a)$$

$$2A \frac{\sin^3 \alpha \cos \alpha}{r^2} - M \frac{\cos 2\alpha}{r} = 0, \quad (5b)$$

$$g + \frac{1}{2}A \frac{\sin^4 \alpha}{r^2} + U(r) - M \frac{\sin 2\alpha}{2r} = 0. \quad (5c)$$

Eqs. (5a) and (5c) are used to eliminate  $M$ , yielding:

$$\sin^4 \alpha = 2 \left( \frac{gr^2}{A} + \frac{Ur^2}{A} + \frac{U'r^3}{A} \right). \quad (6)$$

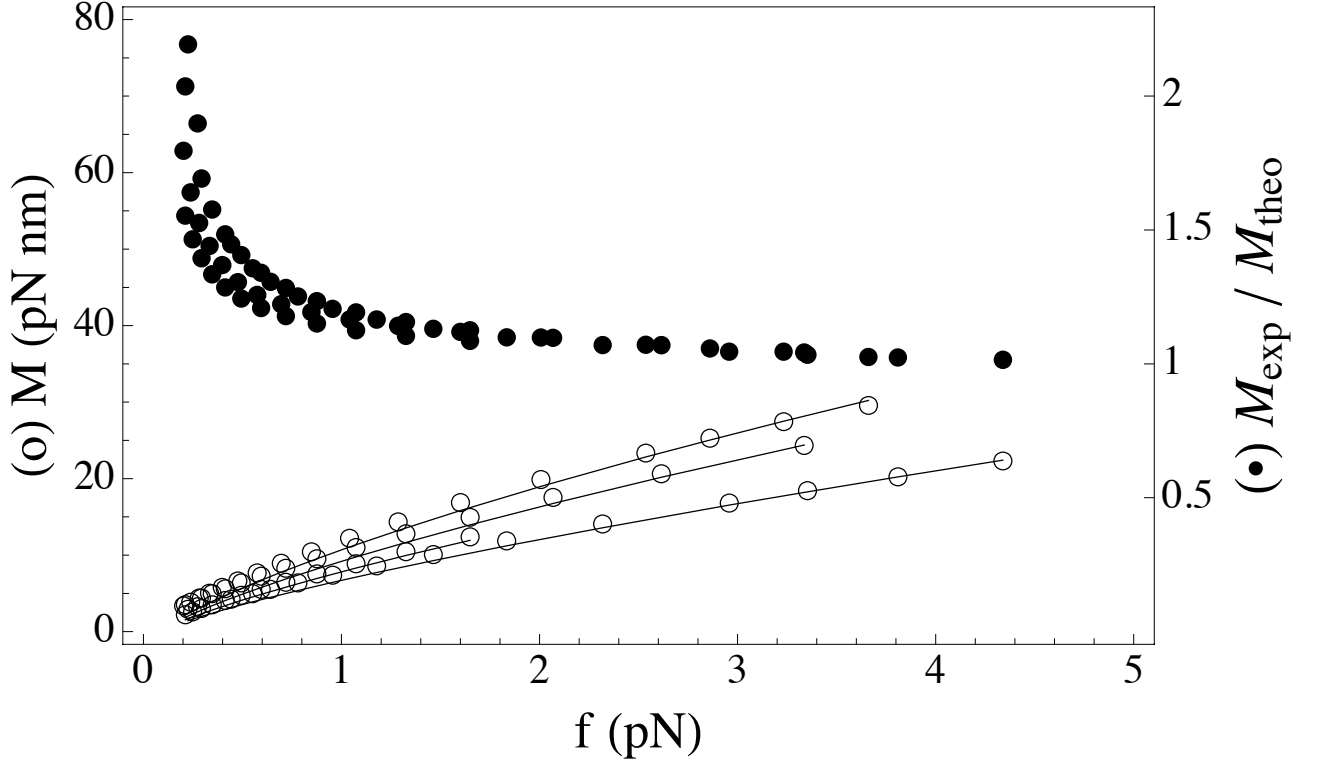


FIG. 3: Comparison of experimental and theoretical torques as function of the applied force for 50, 100, 200, and 500 mM salt: (a) experimental data from Ref. [1] (circles); (b) theoretical solution of the full equations  $\nabla \mathcal{G} = \mathbf{0}$  (continuous lines); (c) ratio of the experimental torque to the formula in Eq. (17) (filled circles). Experimentally given values of  $A = 46, 47, 44, 45$  nm and  $C = 94$  nm were used.

Then multiplying Eq. (5b) by  $\sin 2\alpha$  and Eq. (5a) by  $\cos 2\alpha$  and summing, we obtain:

$$\frac{U'r^3}{A} = -\frac{\sin^4 \alpha}{1 - 2\sin^2 \alpha}. \quad (7)$$

The variable  $\alpha$  can then be eliminated from Eqs. (6) and (7) to yield one equation for the one variable  $r$ :

$$\frac{U'r^3}{A} \left( 1 - 2\sqrt{2 \left( \frac{gr^2}{A} + \frac{Ur^2}{A} + \frac{U'r^3}{A} \right)} \right) + 2 \left( \frac{gr^2}{A} + \frac{Ur^2}{A} + \frac{U'r^3}{A} \right) = 0. \quad (8)$$

We introduce the dimensionless variable  $x := 2\kappa_D r$ . In the regime of moderate to large  $x$  and of low to moderate  $\alpha$ , the leading order of Eq. (8) is  $\frac{gr^2}{A} + \frac{Ur^2}{A} + \frac{3}{2} \frac{U'r^3}{A} = 0$ , or :

$$\sqrt{x} e^{-x} (1 - 1/(6x)) = 1/K \text{ with } K := \sqrt{9\pi/8} \nu^2 L_B k_B T / g(f) \quad (9)$$

$$\text{or } f(x) = x - \frac{1}{2} \text{Log } x - \text{Log}(1 - 1/(6x)) - \text{Log } K = 0 \quad (10)$$

where we have used  $K_0(x) \simeq (\pi/(2x))^{1/2} e^{-x}$  for large  $x$ . The approximate solution is  $x_0 = \text{Log } K$ . Taking the first-order Newton-Raphson estimate of the root gives  $x \simeq x_0 - \frac{f(x_0)}{f'(x_0)}$  yields:

$$x = 2\kappa_D r \simeq \text{Log } K + \frac{\text{Log}(\text{Log } K)}{2 - 1/\text{Log } K}. \quad (11)$$

Once  $r$  is known,  $\alpha$  is computed from Eq. (7):

$$\alpha = \arcsin \sqrt{\frac{U'r^3}{A} + \sqrt{\left( \frac{U'r^3}{A} \right)^2 - \frac{U'r^3}{A}}}. \quad (12)$$

And  $M$  from Eq. (5b):

$$M = \frac{2A}{r} \frac{\sin^3 \alpha \cos \alpha}{\cos 2\alpha}. \quad (13)$$

Eqs. (11) and (12) yield, at lowest order:

$$r \simeq \bar{r} := (2\kappa_D)^{-1} \text{Log } K \text{ and } \alpha \simeq \bar{\alpha} := (2\bar{r}^2 g(f)/(3A))^{1/4}. \quad (14)$$

We note that although our approximation always leads to a solution, this is not the case for the full equations  $\nabla \mathcal{G} = 0$ . For the full equations, there are two  $r$  solutions at low forces, and no solutions beyond a force threshold (we only plot the solution with largest  $r$  value, which can be shown to be stable). The force threshold for disappearance of the stability of the large- $r$  solution is salt-dependent, and can be as low as 3 pN at low salt (5 mM Na+); at forces beyond this threshold the electrostatic interaction cannot support the plectoneme and it will collapse in radius down to  $r \approx 1$  nm, with the two DNAs in close contact. This could explain the observations of plectoneme collapse of Ref. [12] and suggests that collapse transitions might be observable in single-DNA experiments. We note that once in the collapsed configuration, if one were to decrease the force, hysteresis of the collapsed state would be observed. We are analyzing this collapse phenomenon in more detail at present and will discuss it in a forthcoming paper.

Our formulae provide insight into the dependence of the slope  $q$  on force and salt concentration observed experimentally [1]. We compute  $q := \partial \langle X \rangle / \partial \Delta \text{Lk} = -\partial^2 \mathcal{G} / (\partial f \partial \Delta \text{Lk})$  neglecting  $C'_s(f)$  (valid for large force), and taking  $\lambda_s \simeq \tau_p$ :

$$q \simeq g'(f) \frac{4\pi r}{\sin 2\alpha} \simeq 2\pi g'(f) \frac{r}{\alpha} \left(1 + \frac{2}{3}\alpha^2\right). \quad (15)$$

Using Eq. (14), we arrive at:

$$q \simeq 6^{1/4} \pi A^{1/4} \kappa_D^{-1/2} \frac{g'(f)}{g(f)^{1/4}} \sqrt{\text{Log} \left( \sqrt{9\pi/8} \nu^2 L_B k_B T / g(f) \right)} \left(1 + \frac{2}{3}\bar{\alpha}^2\right), \quad (16)$$

which gives the salt and force dependence of the slope of a hat-curve in a supercoiling experiment. Fig. 2 shows experimental slopes divided by this theoretical prediction; the ratio is nearly 1 with only small variation with force and salt concentration, indicating that the experimental data closely follows this functional form.

We now examine the DNA torque  $M$ ; Taylor expansion of (13) for small  $\alpha$  yields  $M \simeq \frac{2A}{r} \alpha^3 (1 + \frac{3}{2}\alpha^2)$ . Using (14), we obtain the following approximate formula for  $M$ :

$$M \simeq (32/27)^{1/4} A^{1/4} \kappa_D^{-1/2} g(f)^{3/4} \sqrt{\text{Log} \left( \sqrt{9\pi/8} \nu^2 L_B k_B T / g(f) \right)} \left(1 + \frac{3}{2}\bar{\alpha}^2\right). \quad (17)$$

In Fig. 3 experimental torque values are divided by this theoretical prediction and we see that, when  $f$  is large enough, the ratio is near 1, with only small variation with  $f$  or salt concentration. As for the slopes  $q$ , experimental data for  $M$  closely follow this functional form, for sufficiently large force.

For forces in the range  $0.25 \text{ pN} \leq f \leq 5 \text{ pN}$  and salt concentrations between 50mM and 500mM, we checked the accuracy of formulae (14), (16), and (17) against the solution of the full equations  $\nabla \mathcal{G} = \mathbf{0}$  and found that the relative error was always below 13% (see Supplementary Materials [13]). Similarly, predicted results for the radius of the supercoils (Fig. 4) show only a small difference between the asymptotic result (Eq. 11) and the exact result of solving  $\nabla \mathcal{G} = \mathbf{0}$ . Finally, comparing Eqs. (16) and (17), one sees that

$$M \simeq \frac{2g(f)}{3\pi g'(f)} q. \quad (18)$$

This recovers a formula first discussed in Ref. [14] and which has been noted to describe experimental data for DNA torque [1].

We have described an analytical treatment of a free energy model for coexisting extended and plectonemically supercoiled DNA, where we have taken the contributions which dominate for moderate to large forces, namely the elasticity and electrostatic self-repulsion of the DNA double helix. This model is simple enough that one can extract an analytical solution asymptotically valid for large forces. Our result for the slope of the extension as a function of linking number matches experimental data strikingly well, with all parameters set by experiment or previous theoretical work (*i.e.*, for the electrostatic interaction). Our results provide a framework for analyzing the scaling

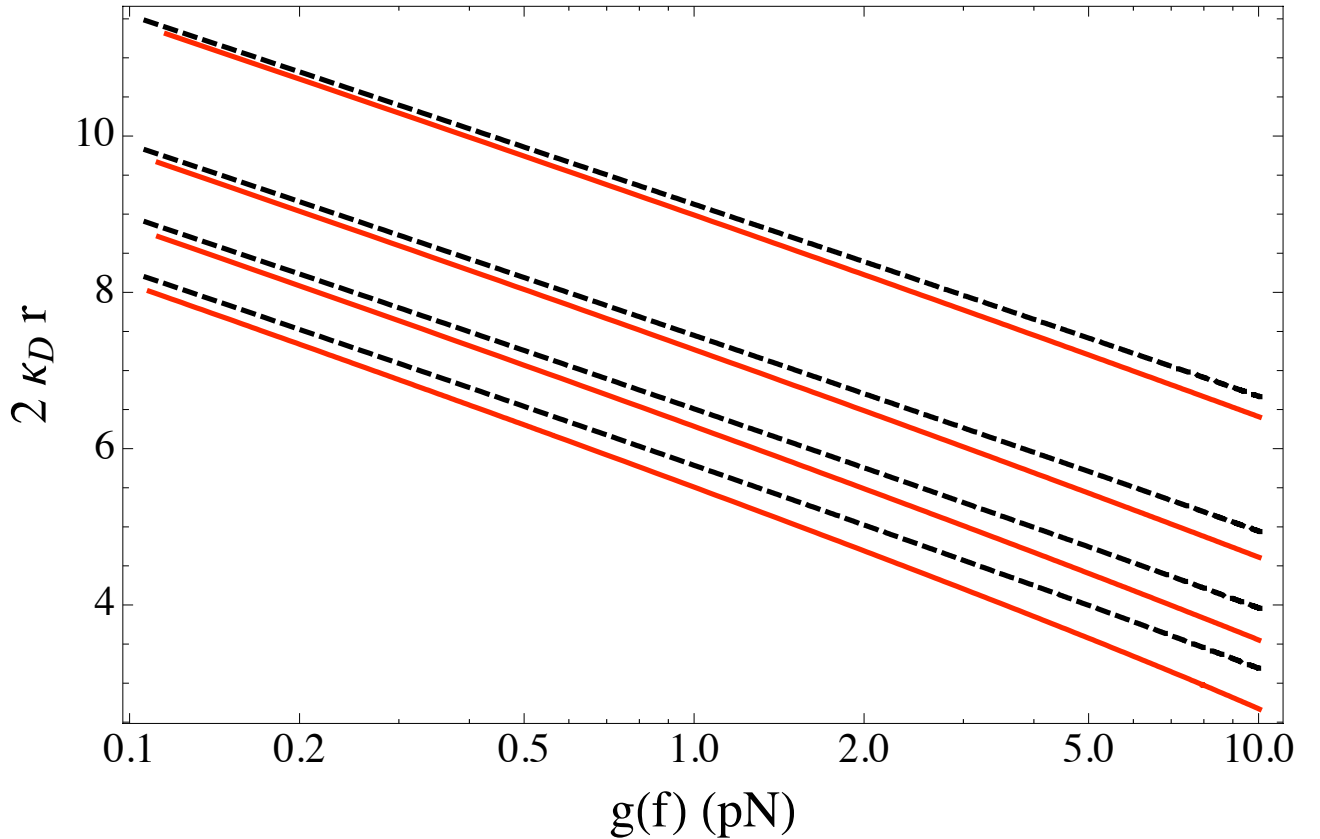


FIG. 4: Supercoiling radius as function of the force function  $g(f)$ , computed with: (a) the full non-linear equations  $\nabla\mathcal{G} = \mathbf{0}$  (plain lines, red); (b) formula in Eq. (11) (dashed lines, black) for the four salt concentration 50mM, 100mM, 200mM, and 500mM (top to bottom). The separation of the curves at high force is due to the non-linearities of  $\nabla\mathcal{G} = \mathbf{0}$ , which are omitted in Eq. (11).

behavior of DNA stretching-twisting experiments. We furthermore give theoretical predictions for the super-helical radius, super-helical angle, and torque in the molecule as functions of salt concentration and applied force.

In the computations presented in [5], a scaling factor of  $\chi = 0.42$  in the effective charge  $\nu$  (thereby using  $\nu^* = \chi\nu(a)$ ), plus a value  $a = 1.2$  nm were used in order to obtain good agreement with experiments. We note that in our model, we have not had to resort to a nonstandard value of the DNA effective charge. We agree with the assertion of [7] and [5] that single-DNA twisting-pulling experiments provide a means to analyze DNA-DNA interactions. The electrostatic potentials in the literature are Debye-Huckel-like, *i.e.*, decaying as  $(e^{-\rho})/\rho$ , where  $\rho = |\mathbf{r} - \mathbf{r}'|$  is the distance between interacting charges at  $\mathbf{r}$  and  $\mathbf{r}'$ , and they involve an effective charge that in turn depends on salt concentration. For example, Ubbink & Odijk [11] have used an effective charge  $\nu^* = \xi/L_B$  with  $\xi$  given in their Table 7 (see also [15]). In counter-ion condensation theory of polyelectrolytes [16], the interaction energy also takes the same form as in Eq. (2), but with a salt-concentration-independent effective charge  $\nu^* = (1/(bL_B) - 1/(2L_B^2))^{1/2} = 0.46$  for DNA in water solution [7, 17]. The amplitude of the DNA-DNA interaction potential is sensitive to precisely where the charges are placed relative to the center of the double helix. This might explain the low values of effective charge inferred in [5]. Effective charges used by different authors are compared in the Supplementary Materials [13].

Finally we note that if the electrostatic-elastic theory were complete, then the rescaled slopes and torques in Figs. 2 and 3 would take on the value 1. While this limit is approached at high forces, at lower forces, there is disagreement, particularly in the case of the torques (Fig. 3). This effect is in part due to polymer confinement entropy [3], neglected here but which becomes important at low forces. There are also uncertainties about the measured low-force torque; the analysis of [1] required an estimate of torque as a boundary condition for integration of a thermodynamic “Maxwell relation” [18]. A more complete discussion of the model of this paper including confinement entropy, finite-size plectonemic and small-loop domain effects, and the collapse transition, is in preparation.

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