Supplemental material for Discontinuities at the DNA supercoiling transition

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Behavior of extended DNA with fluctuations

The behavior of extended DNA is appreciably affected by thermal fluctuations. For the applied forces in the range considered in this experiment, we can use the following fixed-torque free energy:

$$\frac{\mathcal{G}(\tau)}{L} = -F - \frac{\tau^2}{2C_{bare}} + \frac{kT}{B}\sqrt{BF - \frac{\tau^2}{4}}, \tag{S1}$$

where the last term is the lowest-order correction due to fluctuations [1].

The fluctuations decrease the extension:

$$-\frac{\partial \mathcal{G}}{\partial F} = L\left[1 - \frac{kT}{2}\left(BF - \frac{\tau^2}{4}\right)^{-1/2}\right]. \tag{S2}$$

(The $-1/32$ in Eq. (1) comes from an approximation to a higher-order correction [1].)

Expanding the last term of Eq. (S1) to match the form of a "zero-temperature" chain, we can instead write

$$\frac{\mathcal{G}(\tau)}{L} = -F_{\text{eff}} - \frac{\tau^2}{2C_{\text{eff}}}, \tag{S3}$$

where the effective force and twist elastic constant are given by

$$F_{\text{eff}} = F - kT\sqrt{\frac{F}{B}} \tag{S4}$$

$$C_{\text{eff}} = C_{bare}\left(1 + kT\frac{C_{bare}}{4B\sqrt{BF}}\right)^{-1}. \tag{S5}$$

Note that C_{eff} is a function of force: there is less "softening" at higher forces. In the experiments of Forth *et al.*, the renormalized C_{eff} was measured directly via the torque. However, the range of applied forces was small enough that C_{eff} did not change appreciably, and a single value of $C = (89\text{ nm})kT$ was quoted. Here, we also use the same renormalized but force-independent value for C.

Changing Eq. (S3) to a fixed-linking-number expression via a Legendre transformation, we arrive at our expression for the straight state free energy (also found in Ref. [2]):

$$\mathcal{F}_s(K, L) = \frac{C}{2}\left(2\pi\frac{K}{L}\right)^2 L - F_{\text{eff}}L. \tag{S6}$$

Derivation of linear expressions for $\mathcal{F}_{\text{CS}}$ and z_{CS}

We first write down the linear scaling of the free energy and extension with linking number. For any δK that does not take the system out of the CS,

$$\mathcal{F}_{\text{CS}}(K + \delta K, L) = \mathcal{F}_{\text{CS}}(K, L) + 2\pi\tau\delta K; \tag{S7}$$

$$z_{\text{CS}}(K + \delta K, L) = z_{\text{CS}}(K, L) - q\delta K, \tag{S8}$$

where q is the slope of extension versus linking number and τ is the CS torque. Next, to find the scaling with increasing L, we imagine adding a piece of stretched DNA of length δL at the coexisting torque (keeping the system in a stable CS). This also adds an amount of linking number that scales with δL, $\delta K[\delta L] = \tau\delta L/(2\pi C)$, which we

will have to unwind to get back to the original K. First adding the piece of stretched DNA, and then unwinding to find the dependence on L only, we find

$$\mathcal{F}_{\rm CS}(K, L+\delta L) = \mathcal{F}_{\rm CS}(K, L) - \left(\frac{\tau^2}{2C} + F_{\rm eff}\right)\delta L. \tag{S9}$$

Similarly for the extension, [using $\xi(\tau)$ from Eq. (1)]

$$z_{\rm CS}(K, L+\delta L) = z_{\rm CS}(K, L) + \left(\xi(\tau) + \frac{\tau}{2\pi C}q\right)\delta L. \tag{S10}$$

Combining Eqs. (S7) and (S8) with Eqs. (S9) and (S10), we can write the free energy and extension of the CS as linear in K and L, each with a slope and an intercept:

$$\mathcal{F}_{\rm CS}(K, L) = \mathcal{F}_0 + 2\pi\tau K - \left(\frac{\tau^2}{2C} + F_{\rm eff}\right)L; \tag{S11}$$

$$z_{\rm CS}(K, L) = -z_0 - qK + \left(\xi(\tau) + \frac{\tau}{2\pi C}q\right)L. \tag{S12}$$

Note that C and $\xi(\tau)$ are known from experiments on stretched DNA, leaving the four anticipated force-dependent quantities to be described by a theory of supercoiling: τ, q, $\mathcal{F}_0$, and z_0.

Self-repulsion

It is essential to include a repulsive force between sections of the DNA that come near each other; without it, the rod can pass through itself, unphysically removing linking number in the process and preventing the formation of plectonemes. The physical origins of repulsive forces in DNA include both electrostatic and entropic effects. We use discretized versions of the repulsive interactions described in Ref. [3].

Electrostatic forces are modeled using a Debye-Huckel screened Coulomb interaction:

$$E_{\rm SC}(r) = \frac{|e_-\nu d|^2}{\epsilon}\frac{e^{-r/\lambda_D}}{r}, \tag{S13}$$

where $\nu = 8.4$ nm^{-1} is the effective number of electron charges per unit length, $\lambda_D = 0.8$ nm is the Debye screening length, and $e_-^2/\epsilon = 2.9$ pN nm^2. (These values are dependent on the ionic concentration of the buffer, and were picked to match with ≈ 150 mM NaCl.)

The entropic free energy of a helical structure is calculated in Ref. [3], coming from the increasing confinement of fluctuations in more tightly coiled structures. We use the same free energy, written as a pairwise interaction between segments:

$$E_{\rm ent}(r) = \frac{2^{5/3}\sqrt{\pi}\Gamma(1/3)}{\Gamma(5/6)}\frac{kTd^2}{(B/kT)^{1/3}r^{5/3}}. \tag{S14}$$

Since we also include straight parts of the DNA that should not have the same entropic interaction, we cut off the entropic potential at a distance of $2B/kT$, where the argument for the form of the potential breaks down [3].

Extra terms in the circular end-loop model

Extra terms in the free energy that we have not considered would change the predictions of the circular end-loop model — these could include electrostatic interactions, entropic effects, etc. In fact, we can solve for the properties that such an extra free energy term (call it $\mathcal{F}_{\rm extra}$) would need to have in order to make the model match the experimental data.

Adding this unknown term, we have

$$\mathcal{F}_l(K_l, L_l) = \frac{C}{2L_l}\left[2\pi(K_l - {\rm Wr}_{\rm loop})\right]^2 + (2\pi)^2\frac{B}{2L_l} + \mathcal{F}_{\rm extra}(K_l, L_l). \tag{S15}$$

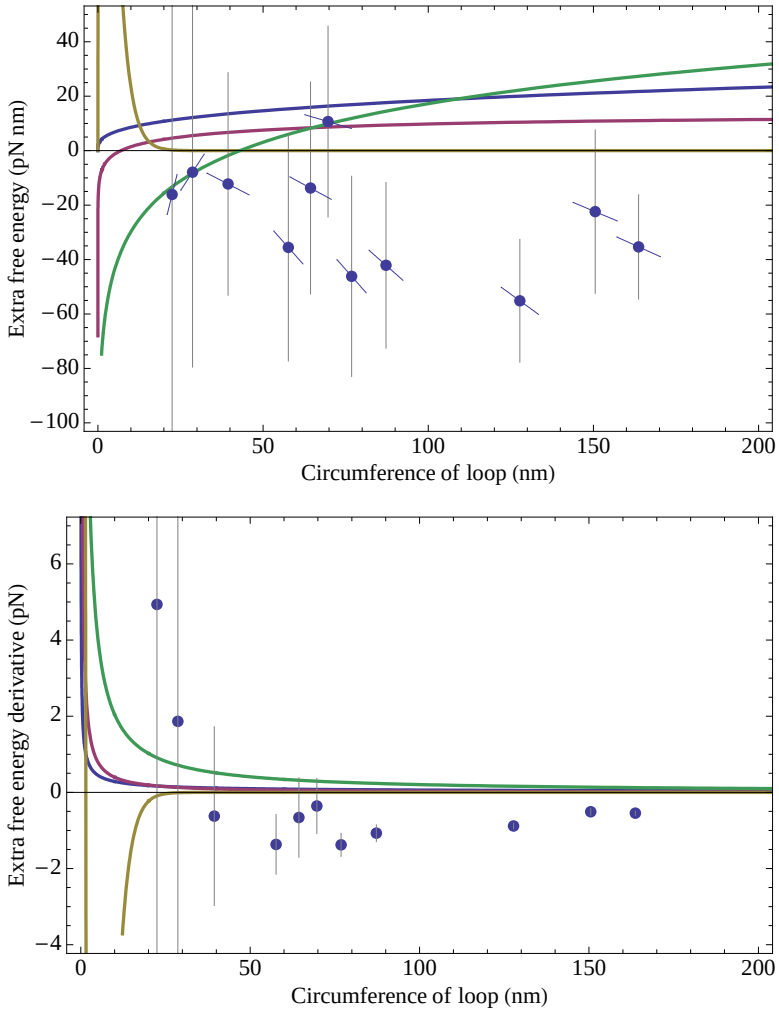

FIG. S1: Entropic corrections from the literature do not help the circular end-loop model fit the data. The dots show the required free energy contribution $\mathcal{F}_{\text{extra}}$ (top plot) and its derivative with respect to end-loop circumference $d\mathcal{F}_{\text{extra}}/dL_l$ (bottom plot) that would produce an $\mathcal{F}_0$ and z_0 that match with the experiment (with $\text{Wr}_{\text{loop}} = 0.8$). Bars on the top plot show the required derivative, the value of which is shown on the bottom plot. Vertical grey lines show one standard deviation error bars. Note especially the inability of any of the proposed entropic terms to match the well-constrained negative derivative at large end-loop circumferences (which happen at low force in the experiment); this produces L_l (and thus z_0) that are too small at low forces. A lessening of the effective force felt by the end-loop of about 0.5 pN would help agreement, but none of the proposed corrections provides this.

Since the terms we will imagine adding will not depend on K_l, we will assume that $\mathcal{F}_{\text{extra}}$ is only a function of L_l. Setting the force and torque equal to the coexisting state values ($d\mathcal{F}_l/dL_l = -(F_{\text{eff}} + \tau^2/(2C))$; $d\mathcal{F}_l/dK_l = 2\pi\tau$) then gives

$$L_l^* = 2\pi\sqrt{\frac{B}{2(F_{\text{eff}} + d\mathcal{F}_{\text{extra}}/dL_l)}} \tag{S16}$$

$$K_l^* = \frac{\tau L_l^*}{2\pi C} + \text{Wr}_{\text{loop}}. \tag{S17}$$

We now use the fact that

$$\mathcal{F}_0 = \mathcal{F}_l^* + (F_{\text{eff}} + \frac{\tau^2}{2C})L_l^* - 2\pi\tau K_l^* \tag{S18}$$

$$z_0 = \xi(\tau)L_l^* - q\left(K_l^* - \frac{\tau L_l^*}{2\pi C}\right) \tag{S19}$$

to solve for the necessary values of $\mathcal{F}_{\text{extra}}$ and $d\mathcal{F}_{\text{extra}}/dL_l$ in order to match with the experimental $\mathcal{F}_0$ and z_0. We find

$$\mathcal{F}_{\text{extra}} = \mathcal{F}_0 + 2\pi\tau \text{Wr}_{\text{loop}} - F_{\text{eff}} L_l^* - \frac{2\pi^2 B}{L_l^*} \tag{S20}$$

$$\frac{d\mathcal{F}_{\text{extra}}}{dL_l} = \frac{2\pi^2 B}{L_l^{*2}} - F_{\text{eff}}, \tag{S21}$$

where

$$L_l^* = \frac{z_0 + q\text{Wr}_{\text{loop}}}{\xi(\tau)}. \tag{S22}$$

These required properties of the added free energy term are plotted in FIG. S1 for $\text{Wr}_{\text{loop}} = 0.8$.

We can then test whether different possible extra free energy terms would match the requirements. Here we try four possibilities taken from the literature. First, there is electrostatic repulsion coming from like charges on opposite sides of the DNA circle. This looks like (using the Debye-Huckel formulation from Ref. [3])

$$\mathcal{F}_{\text{extra}}^{\text{electrostatic}} = kT l_B \nu^2 K_0 \left(\frac{L_l}{\pi \lambda_D} \right) L_l \tag{S23}$$

and is plotted in yellow in FIG. S1. Second, Odijk calculates the free energy for a circular DNA loop and finds terms in the free energy [4] [Eq. (2.13)]

$$\mathcal{F}_{\text{extra}}^{\text{Odijk}} = kT \log \frac{2\pi L}{B/(kT)} - \frac{(kT)^2}{8B} L; \tag{S24}$$

this is plotted in purple in FIG. S1. Third, a similar term is found by Tkachenko in solving for the J-factor for unconstrained DNA cyclization [5] [Eq. (4)]:

$$\mathcal{F}_{\text{extra}}^{\text{Tkachenko}} = 5kT \log \frac{L}{B/(kT)}; \tag{S25}$$

this is plotted in green in FIG. S1. Finally, we could imagine that entropic contributions from confinement similar to the one used by us for our elastic simulation could be important. Although the form was derived for a different configuration (superhelical DNA), we could try it to see if something similar might help. Integrating the confinement entropy from Marko and Siggia [3] over a circle gives

$$\mathcal{F}_{\text{extra}}^{\text{confinement}} = \frac{kT}{(B/kT)^{1/3}(L/(2\pi))^{2/3}} L, \tag{S26}$$

which is plotted in blue in FIG. S1.

Although these possible terms are only initial guesses at the possible corrections due to entropic and other effects, we see that they are all qualitatively unable to help, especially at long loop lengths, which is where the circular loop model fares worst at fitting the data.

Calculating entropic contributions from fluctuations in plectoneme location, length, and linking number

To investigate entropic effects, we would like to find the free energy of states with multiple plectonemes [6], including fluctuations of linking number and length both within individual plectonemes and moving among different plectonemes. We can achieve this by calculating the partition function for a state with n plectonemes, identifying unique states by the plectoneme positions s_i, the plectoneme lengths L_{pi}, and the plectoneme linking numbers K_{pi}:

$$\begin{aligned} Z_n(K, L) = & \frac{1}{L_0^n} \int_0^L ds_1 \int_{s_1}^L ds_2 ... \int_{s_{n-1}}^L ds_n \\ & \frac{1}{L_0^n} \int_0^L dL_{p1} \int_0^L dL_{p2} ... \int_0^L dL_{pn} \\ & \frac{1}{K_0^n} \int_{-\infty}^{\infty} dK_{p1} \int_{-\infty}^{\infty} dK_{p2} ... \int_{-\infty}^{\infty} dL_{Kn} \\ & \exp\left[-\mathcal{F}_n(L, K, L_{pi}, K_{pi})/kT\right], \end{aligned} \tag{S27}$$

where we have neglected the complications coming from the possibility that plectonemes could overlap. The constants L_0 and K_0 set the length change and linking number change, respectively, that produce an independent state. Since we are only concerned with the free energy difference between the straight state and coexisting state, these constants would be set by the change in entropy of the degrees of freedom in the straight state that are lost to the collective modes we are integrating over in the coexisting state.

The first line of integrals represents the choice of where to put each plectoneme, which does not change the free energy ($\mathcal{F}_n$ does not depend on s_i). We therefore simply get a factor of L^n, divided by $n!$ since plectonemes are indistinguishable:

$$Z_n(K,L) = \frac{(L/L_0)^n}{n!}\frac{1}{L_0^n K_0^n}\int_0^L \prod_i dL_{pi} \int_{-\infty}^{\infty}\prod_i dK_{pi} \exp\big[-\mathcal{F}_n(L,K,\{L_{pi}\},\{K_{pi}\})/kT\big]. \tag{S28}$$

Next we need to know the free energy of coexisting states that are away from the equilibrium plectoneme length and linking number. Assuming that the plectoneme free energy density is quadratic in linking number density (as in Marko's model [2]), this turns out to be

$$\begin{aligned}\mathcal{F}_n(L,K,\{L_{pi}\},\{K_{pi}\}) = &\sum_{i=1}^{n}\frac{C}{2}\left(\frac{1}{1+v}\right)\left(2\pi\frac{K_{pi}}{L_{pi}}\right)^2 L_{pi} \\ &+\frac{C}{2}\left(2\pi\frac{K-\sum K_{pi}}{L-\sum L_{pi}}\right)^2 (L-\sum L_{pi}) - F_{\rm eff}(L-\sum L_{pi}) + n\mu,\end{aligned} \tag{S29}$$

where μ is the chemical potential for plectoneme ends and $v \equiv 2CF_{\rm eff}/\tau^2$.

We first evaluate the integrals over K_{pi}, which amount to n Gaussian integrals; this gives

$$\begin{aligned}Z_n(K,L) = \frac{(L/L_0)^n}{n!}\frac{1}{L_0^n K_0^n}\pi^{n/2}\int_0^L \prod_i dL_{pi}\left(\frac{\prod_i L_{pi}/c_1}{1+(1+v)\frac{\sum L_{pi}}{L-\sum L_{pi}}}\right)^{1/2} \\ \exp\Big(-\frac{1}{kT}\left[\frac{\frac{C}{2}(2\pi K)^2}{L-\sum L_{pi}+(1+v)(\sum L_{pi})} - F_{\rm eff}(L-\sum L_{pi})+n\mu\right]\Big).\end{aligned} \tag{S30}$$

Now changing to unitless variables $x_i = L_{pi}/L_p$ and $y = L_p/L$, and rearranging to move all the factors that depend on the sum of the plectoneme lengths y into the exponent, the term in the exponent becomes

$$f(y) = \frac{1}{kT}\left(\frac{\frac{C}{2}(2\pi K)^2/L}{1+vy} - F_{\rm eff}L(1-y)+n\mu\right) + \frac{1}{2}\log\left(\frac{1+vy}{1-y}\right), \tag{S31}$$

and we have

$$\begin{aligned}Z_n(K,L) &= \frac{(L/L_0)^n}{n!}\frac{1}{L_0^n K_0^n}\pi^{n/2}\int_0^L \prod_i dx_i \sqrt{\prod_i L_{pi}/c_1}\exp\left[-f(\textstyle\sum L_{pi}/L)\right] \\ &= \frac{(L/L_0)^n}{n!}\frac{1}{L_0^n K_0^n}\pi^{n/2}\int_0^L dL_p\ \delta\left(\textstyle\sum L_{pi} - L_p\right)\int_0^{L_p}\prod_i dL_{pi}\sqrt{\prod_i L_{pi}/c_1}\exp\left[-f(L_p/L)\right] \\ &= \frac{(L/L_0)^n}{n!}\frac{1}{L_0^n K_0^n}\pi^{n/2}\int_0^L dL_p\frac{L_p^n}{L_p}\left(\frac{L_p}{c_1}\right)^{n/2}\left[\int_0^1\prod_i dx_i\sqrt{\prod_i x_i}\ \delta\left(\textstyle\sum x_i - 1\right)\right]\exp\left[-f(L_p/L)\right] \\ &= \frac{(L/L_0)^{2n}(L/c_1)^{n/2}}{K_0^n}\ \frac{\pi^{n/2}\ \gamma_n}{n!}\int_0^1 dy\ \exp\left[-(f(y)-\frac{3n-2}{2}\log y)\right].\end{aligned} \tag{S32}$$

The integral in large square brackets (characterizing fluctuations in the individual plectoneme lengths that do not change the total plectoneme length) gives a numerical constant $\gamma_n = \pi^{n/2}/(2^n\Gamma(3n/2)) = 2^{\lfloor\frac{n-1}{2}\rfloor}\pi^{\lfloor\frac{n}{2}\rfloor}/(3n-2)!!$. To evaluate the y integral over total plectoneme length, we make a Gaussian approximation [noting that the total length is well-constrained by $f(y)$]. Then the fluctuations in the (fractional) total length of plectonemic DNA are of size

$$\sigma_y = \left(\frac{d^2}{dy^2}\left[f(y)-\frac{3n-2}{2}\log y\right]\Big|_{y^*}\right)^{-1/2}, \tag{S33}$$

where y^* is the equilibrium value of y, and the derivative is

$$\frac{d^2}{dy^2}\left[f(y)-\frac{3n-2}{2}\log y\right]=\frac{1}{2}\left(\frac{1}{(1-y)^2}+\frac{3n-2}{y^2}-\frac{v^2\left(1-\frac{8\pi^2CK^2}{LkT(1+vy)}\right)}{(1+vy)^2}\right). \tag{S34}$$

Without the entropic corrections, the equilibrium length is $y^*=(u-1)/v$, where $u=2\pi CK/(\tau L)$. We can safely use this value if we are far from $y^*=0$ and $y^*=1$, and get

$$\sigma_y=\frac{\sqrt{2}}{v}\left(\frac{1}{u}\frac{2\tau^2L}{kTC}-\frac{1}{u^2}+\frac{1}{(v-u+1)^2}+\frac{3n-2}{(u-1)^2}\right)^{-1/2}. \tag{S35}$$

[Since we are usually near $y^*=0$ at the transition, to calculate the length-dependence shown in Fig. 4 (left), we approximate y^* numerically and use Eq. (S33) instead of Eq. (S35).] In the end, we have

$$Z_n(K,L)=\frac{(L/L_0)^{2n}(L/c_1)^{n/2}}{K_0^n}\;\frac{\pi^{n/2}\,\gamma_n}{n!}\sqrt{2\pi}\sigma_y\left(\frac{u-1}{v}\right)^{(3n-2)/2}\left(\frac{v-u+1}{uv}\right)^{1/2}\exp\left[-\mathcal{F}(K,L)/kT\right]. \tag{S36}$$

The full partition function for all plectonemic states is then

$$Z(K,L)=\sum_{n=1}^{\infty}Z_n(K,L) \tag{S37}$$

(which we can numerically approximate by truncating the series at a reasonable n), such that the coexisting state free energy is given by $\mathcal{F}_{\mathrm{CS}}(K,L)=-kT\log Z(K,L)$. For the experimental values, we find that only the single plectoneme $n=1$ state contributes significantly near the transition.

Independence of results on entropic effects

In the paper, we have set the entropy from the previous section to zero ($S=0$) for most of the calculations. How would we expect that including S would change any of the results?

First, S would create a shift between the experimental $\mathcal{F}_0$ and the predictions from models that do not include fluctuations. We find that this shift is largely independent of force, and is mostly dependent on L_0. We do not currently have a way of calculating L_0, but we expect that it should be on the order of the persistence length of DNA, about 50 nm. We find that setting L_0 to about 100 nm makes the prefactor equal to 1, or equivalently sets $S=0$. If we assume that L_0 is about equal to the persistence length of DNA, we expect that we would need to shift the model predictions by at most about $kT\log 2\approx 5$ pN nm.

Second, we find that S has a logarithmic dependence on L. This means that we expect $\mathcal{F}_0$ to decrease by something on the order of $kT\log(L_2/L_1)$ when we increase the length from L_1 to L_2. For the experimental lengths (with $L_2\approx 2L_1$), this again corresponds to a shift of about 5 pN nm.

Shifting $\mathcal{F}_0$ by these amounts would slightly change only the theory curves for $\mathcal{F}_0$ (about 5 pN nm), Δz (about 10 nm), and $\Delta\tau$ (about 1 pN nm).

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[4] T. Odijk, J. Chem. Phys. **105**, 1270 (1996).
[5] A. V. Tkachenko, q-bio/0703026 (2007).
[6] If the free energy necessary to nucleate a plectoneme is large compared to kT, then the coexisting state will contain a single plectoneme. If this is not the case, however (for example, when L becomes large), we will need to consider equilibrium states in which multiple plectonemes coexist.