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Discontinuities at the DNA supercoiling transition

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While slowly turning the ends of a single molecule of DNA at constant applied force, a discontinuity was recently observed at the supercoiling transition when a small plectoneme is suddenly formed. This can be understood as an abrupt transition into a state in which stretched and plectonemic DNA coexist. We argue that there should be discontinuities in both the extension and the torque at the transition and provide experimental evidence for both. To predict the sizes of these discontinuities and how they change with the overall length of DNA, we organize a phenomenological theory for the coexisting plectonemic state in terms of four parameters. We also test supercoiling theories, including our own elastic rod simulation, finding discrepancies with experiment that can be understood in terms of the four coexisting state parameters.

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A DNA molecule when overtwisted can form a *plectoneme* [1,2] (inset of Fig. 1), a twisted supercoil structure familiar from phone cords and water hoses, which stores added turns (linking number) as "writhe." The plectoneme is not formed when the twisted DNA goes unstable (as in water hoses [3]), but in equilibrium when the free energies cross—this was vividly illustrated by a recent experiment [4] (Fig. 2), which showed repeated transitions between the straight "stretched state" (SS) [described by the wormlike chain (WLC) model [5]] and a coexisting state (CS) of stretched DNA and plectoneme [6]. This transition, in addition to being both appealing and biologically important, provides an unusual opportunity for testing continuum theories of coexisting states. Can we use the well-established continuum theories of DNA elasticity to explain the observed [4] jumps in behavior at the transition?

The recent experiment measures the extension (end-to-end distance) and torque of a single molecule of DNA held at constant force as it is slowly twisted [4]. A straightforward numerical implementation of the elastic rod model [7–9] for DNA in these conditions (with fluctuations incorporated via entropic repulsion [7]) leads to two quantitative predictions that are at variance with the experiment. First, the experiment showed a jump Δz in the extension as the plectoneme formed (Fig. 1) that appeared unchanged for each applied force as the overall DNA length was varied from 2.2 to 4.2 kbp, whereas the simulation showed a significant increase in Δz at the longer DNA length. Second, no discontinuity was observed in the (directly measured) filtered torque data (Fig. 1), yet the simulation predicted a small jump.

Simulation is not understanding. Here we analyze the system theoretically, focusing on the physical causes of the behavior at the transition. We use as our framework Marko's two-phase coexistence model [6,10], which we generalize to incorporate extra terms that represent the interfacial energy between the plectoneme and straight regions of the DNA. We show that any model of the supercoiling transition in this parameter regime can be summarized by four force-dependent parameters. After extracting these parameters directly from the experiments, we use them to predict the torque jump (which we then measure) and to explain why the extension jump appears length independent. Finally, we use our formulation to test various models of plectonemes, finding discrepancies mainly at small applied force.

The transition occurs at the critical linking number K^* when the two states have the same free energy $\mathcal{F}$, where $\mathcal{F}$ is defined by the ensemble with constant applied force and linking number. We therefore need models for the free energy $\mathcal{F}$ and extension z of the SS and CS.

The properties of stretched unsupercoiled DNA are well established. At small enough forces and torques that avoid both melting and supercoiling, DNA acts as a torsional spring with twist elastic constant C [6,18]: $\mathcal{F}_{SS}(K,L)=\frac{C}{2}(2\pi\frac{K}{L})^2L-F_{\text{eff}}L$, where K is the added linking number, L is the overall (basepair) length of DNA, the effective force $F_{\text{eff}}=F-kT\sqrt{F/B}$ [6] (see supplemental material [19]), F is the force applied to the ends of the DNA,

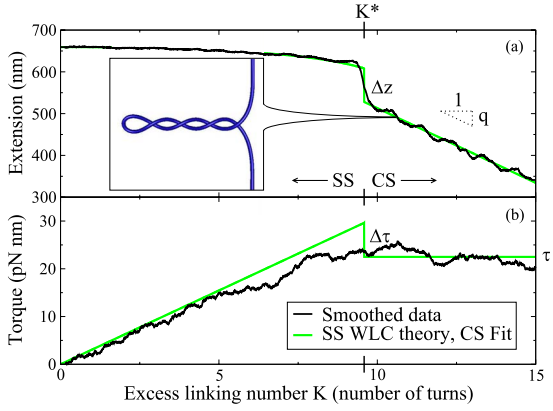

FIG. 1. (Color online) Extension and torque as a function of linking number K, for $L=2.2$ kbp at $F=2$ pN. Black lines show data from Ref. [4], smoothed using a "boxcar" average of nearby points. The green (gray) lines show WLC predictions below the transition (in the unsupercoiled SS) and fits to the data after the transition (in the CS), linear for the extension and constant for the torque. The size of the torque jump, not visible in the smoothed data, is implied by the coexisting torque τ, the CS fit, and the transition linking number K^* in the extension data. Inset: simulated DNA showing the CS of a plectoneme and straight DNA, ignoring thermal fluctuations. The ends are held with fixed orientation and pulled with a constant force F, here 2 pN.

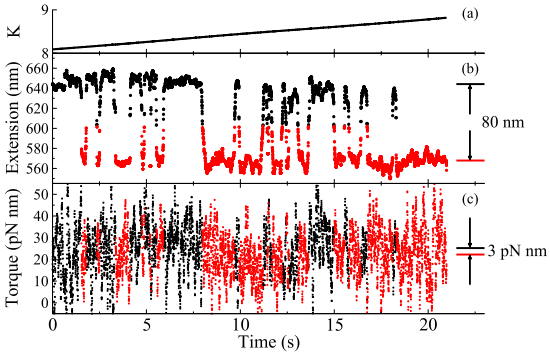

FIG. 2. (Color online) Directly measuring the torque jump by observing thermal hopping, for the same conditions as Fig. 1. As linking number K is slowly increased near K^*, thermal fluctuations induce hopping between states with (CS) and without (SS) a plectoneme. Averaging over these two states gives a direct way of measuring the torque jump: analogously to a lock-in amplifier, we set a threshold in the extension signal to separately average the SS (black) and CS [red (gray)] data near the transition. Using multiple traces, we find an average torque jump of $\Delta\tau=2.9\pm0.7$ pN nm for $L=2.2$ kbp at $F=2$ pN. Additionally, this value of $\Delta\tau$ implies (see text) that the transition should happen over a range of linking number K (a) of about 0.9 turns, agreeing with the observed range.

$B=43\pm3$ nm$\times kT$ is the DNA's bending elastic constant, $C=89\pm3$ nm$\times kT$, and the thermal energy $kT=4.09$ pN nm for this experiment (at 23.5 °C). Differentiating with respect to K gives the torque: $\tau_{SS}=(d\mathcal{F}_{SS}/dK)/2\pi=2\pi CK/L$. The extension of unsupercoiled DNA is shortened by thermal fluctuations, and in the relevant force regime is approximately given by $z_{SS}=\xi(\tau_{SS})L$, where [11]

$$\xi(\tau)=1-\frac{1}{2}\left[\frac{BF}{(kT)^2}-\left(\frac{\tau}{2kT}\right)^2-\frac{1}{32}\right]^{-1/2}. \quad (1)$$

Since supercoiling theories must include contact forces, they are less amenable to traditional theoretical methods. Even so, many theories have been successful in predicting properties of the CS; such methods have included detailed Monte Carlo simulations [12], descriptions of the plectoneme as a simple helix [7,8,13], and a more phenomenological approach [6]. However, none of these theories has yet been used to predict discontinuities at the SS-CS transition. Here we connect the free energy and extension predictions from any given model to the corresponding predictions for discontinuities at the transition.

We will use the framework of two-phase coexistence adopted by Marko [6,10] to describe the CS as consisting of two phases, each with constant free energy and extension per unit length of DNA [20]. Since phase coexistence leads to a linear dependence on K of the fraction of plectonemic DNA (keeping the torque fixed), in this model both $\mathcal{F}_{CS}$ and z_{CS} are linear functions of added linking number K and length L (just as the free energy of an ice-water mixture is linear in the total energy, and the temperature remains fixed as the ice melts). This linearity, along with the known properties of the SS, allows us to write $\mathcal{F}_{CS}$ and z_{CS} as (see supplemental material [19])

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

$$z_{CS}(K,L)=-z_0-qK+\left(\xi(\tau)+\frac{\tau}{2\pi C}q\right)L, \quad (3)$$

where q is the slope of extension versus linking number and τ is the CS torque. That is, $\mathcal{F}_{CS}$ and z_{CS} are specified by four force-dependent values: their slopes with respect to K (τ and q), which describe how the plectonemic phase coexists with the stretched phase; and $K=L=0$ offsets ($\mathcal{F}_0$ and z_0), which describe the extra free energy and extension necessary to form the interface between the phases—the end loop and tails of the plectoneme.

The experimental observables can then be written in terms of these four values. Easiest are τ and q, which are directly measured. Next, the linking number K^* at the transition is found by equating the CS free energy with that of the SS: $\mathcal{F}_{CS}(K^*,L)=\mathcal{F}_{SS}(K^*,L)$ implies

$$K^*=\frac{L}{2\pi C}(\tau+\Delta\tau), \quad \text{with } \Delta\tau=\sqrt{\frac{2C}{L}\mathcal{F}_0}, \quad (4)$$

where $\Delta\tau$ is the jump in the torque at the transition. Lastly, inserting K^* from Eq. (4) into Eq. (3), we find the change in extension at the transition:

$$\Delta z=z_0+q\sqrt{\frac{L\mathcal{F}_0}{2\pi^2C}}-L[\xi(\tau)-\xi(\tau+\sqrt{2C\mathcal{F}_0/L})]. \quad (5)$$

To additionally include entropic effects, we can write $\mathcal{F}_0=\mu-TS$, where μ is the energy cost for the end loop and tails, and S is the entropy coming from fluctuations in the location, length, and linking number of the plectoneme. Using an initial calculation of S that includes these effects (in preparation; see supplemental material [19]), we find that S varies logarithmically with L, and that setting $S=0$ is a good approximation except when L changes by large factors.

Given experimental data (τ, q, K^*, and Δz), we can solve for the four CS parameters. The results from Ref. [4] are shown as circles in Fig. 3 for the two overall DNA lengths tested. If we assume that the DNA is homogeneous, we expect the results to be independent of L (except for a logarithmic entropic correction to $\mathcal{F}_0$ that would reduce it at the longer L by about $kT\log 2\approx5$ pN nm ; see supplemental material [19]). We do expect $\mathcal{F}_0$ and z_0 to be sensitive to the local properties of the DNA in the end loop of the plectoneme, so we suspect that the difference in z_0 between the two measured lengths could be due to sequence dependence. With this data, we can also predict the length dependence of the discontinuities, as shown in Fig. 4 (left). Here we included entropic corrections to $\mathcal{F}_0$ (see supplemental material [19]), and we find that entropic effects significantly decrease the length dependence of the extension jump.

Note that here we are solving for the experimental size of the torque jump using the observed K^* and τ in Eq. (4). We also find direct evidence of $\Delta\tau$ in the data by averaging over the torque separately in the SS and CS near the transition (Fig. 2). With data taken at $F=2$ pN and $L=2.2$ kbp, we find $\Delta\tau=2.9\pm0.7$ pN nm, in good agreement with the pre-

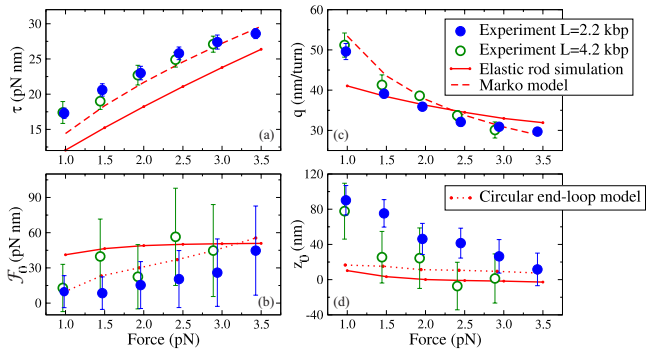

FIG. 3. (Color online) The four parameters describing the CS (coexisting torque τ, extension versus linking number slope q, and the extra free energy $\mathcal{F}_0$ and extension z_0 necessary to form the end loop and tails of the plectoneme) as a function of applied force. The circles show values calculated from experimental data taken at two different overall DNA lengths L. Model predictions for our simulation [21] and Marko's model [6] are shown as solid and dashed lines, respectively (using $S=0$ for $\mathcal{F}_0$ predictions). The circular end-loop model uses average τ and q values from the experiment to predict $\mathcal{F}_0$ and z_0, shown as dotted lines.

diction from K^* (3.9 ± 2.6 pN nm; see Fig. 4). We can also predict the width of the range of linking numbers around K^* in which hopping between the two states is likely (where $|\Delta\mathcal{F}| < kT$): expanding to first order in $K-K^*$ gives a width

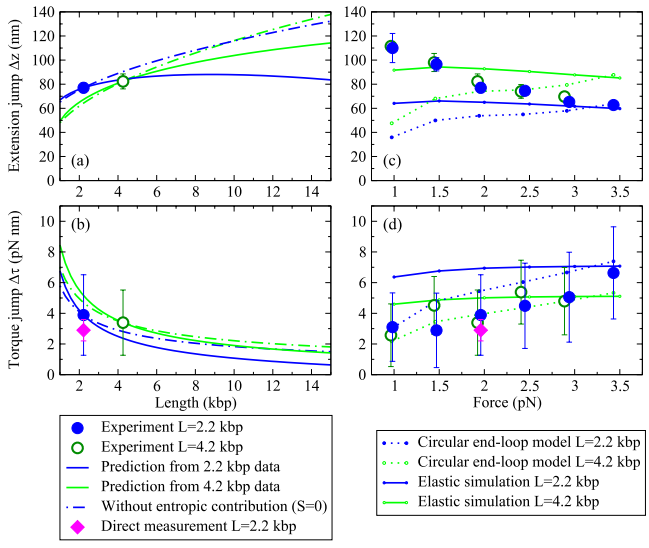

FIG. 4. (Color online) [(a) and (b)] Predicted length dependence of the extension and torque jumps at $F=2$ pN. Using the CS parameters extracted from the experiment at two different lengths, Eqs. (4) and (5) predict the L dependence of Δz and $\Delta\tau$. The circles show experimentally measured values [with the torque jump here calculated from K^* using Eq. (4)]. Without entropic corrections to $\mathcal{F}_0$ ($S=0$; dot-dashed lines) Δz depends noticeably on L but including an initial estimate of S (solid lines) shows that entropic effects can significantly reduce this length dependence. [(c) and (d)] Force dependence of the extension and torque jumps and predictions from two models. Disagreements with experimental data can be understood in terms of the four CS parameters in Fig. 3. Also plotted as a diamond is $\Delta\tau$ measured using the direct method depicted in Fig. 2.

of $2kT/(\pi\Delta\tau)$. This predicts a transition region width of about 0.9 turns for the conditions in Fig. 2, agreeing well with the data.

We can now use various plectoneme models to calculate the four CS parameters, which in turn give predictions for the experimental observables. The results are shown as lines in Figs. 3 and 4 (right). As we expect entropic corrections to be small (changing $\mathcal{F}_0$ by at most about 5 pN nm), we set $S=0$ for these comparisons.

First, we test Marko's phase coexistence model [6]. The plectoneme is modeled as a phase with zero extension and an effective twist stiffness $P<C$. Shown as dashed lines in Fig. 3, the Marko model predicts the coexisting torque and extension slope well, with P as the only fit parameter (we use $P=26$ nm). However, the Marko model (and any model that includes only terms in the free energy proportional to L) produces $\mathcal{F}_0=0$ and $z_0=0$.

In order to have a discontinuous transition, we must include the effects of the end loop and tails of the plectoneme. The simplest model assumes that the coexistence of stretched and plectonemic DNA requires one additional circular loop of DNA. Minimizing the total free energy for this circular end-loop model gives

$$\mathcal{F}_0 = 2\pi\sqrt{2BF_{\text{eff}}} - 2\pi\tau \text{Wr}_{\text{loop}}; \tag{6}$$

$$z_0 = 2\pi\xi(\tau)\sqrt{B/(2F_{\text{eff}})} - q\text{Wr}_{\text{loop}}, \tag{7}$$

where Wr_{loop} is the writhe taken up by the loop. For a perfect circle $\text{Wr}_{\text{loop}}=1$, and $\text{Wr}_{\text{loop}}<1$ for a loop with two ends not at the same location. We chose $\text{Wr}_{\text{loop}}=0.8$ as a reasonable best fit to the data. Using the experimentally measured τ and q, the predictions are shown as solid lines in Figs. 3 and 4; $\mathcal{F}_0$ is fit fairly well, but z_0 is underestimated, especially at small applied forces.

In an attempt to more accurately model the shape of the plectoneme, we use an explicit simulation of an elastic rod, with elastic constants set to the known values for DNA. We must also include repulsion between nearby segments to keep the rod from passing through itself. Physically, this repulsion has two causes: screened Coulomb interaction of the charged strands and the loss of entropy due to limited fluctuations in the plectoneme. We use the repulsion free energy derived for the helical part of a plectoneme in Ref. [7], modified to a pairwise potential form (see supplemental material [19]). We find that the simulation does form plectonemes (inset of Fig. 1), and we can extract the four CS parameters, shown as solid lines in Fig. 3 [21]. Since $\mathcal{F}_0$ and z_0 are nonzero, we find discontinuities in the extension and torque at the transition; their magnitudes are plotted in Fig. 4.

Both the circular loop model and the simulation produce torque and extension jumps of the correct magnitude, but in both cases Δz has an incorrect dependence on force and too much dependence on length. Our approach provides intuition about the causes of the discrepancies by singling out the four values (connected to different physical effects) that combine to produce the observed behavior. Specifically, we can better understand why the models' predictions are length-dependent: as displayed in Fig. 4 (top left), the negligible

length-dependence observed in experiment is caused by a subtle cancellation of a positive length dependence [smaller than either model and described by Eq. (5)] combined with a negative contribution coming from entropic effects. One would expect, then, that any plectoneme model (even one that explicitly includes entropic fluctuations) might easily miss this cancellation. In general, without this intuition, it is difficult to know where to start in improving the DNA models.

The largest uniform discrepancy happens at small applied forces, where both models underestimate z_0 [22], leading to an underestimate of Δz. We have examined various effects that could alter z_0, but none have caused better agreement (see also supplemental material [19]). Adding to the circular end-loop model softening or kinking [14] at the plectoneme tip or entropic terms from DNA cyclization theories [15,16] uniformly *decreases* z_0. Increasing B in Eq. (6) by a factor of four (perhaps due to sequence dependence) does raise z_0 into the correct range, but it also raises $\mathcal{F}_0$ from Eq. (7) to values well outside the experimental ranges. Finally, z_0 would be increased if multiple plectonemes form at the transition, but we find that the measured values of $\mathcal{F}_0$ are too large to allow for more than one plectoneme in this experiment.

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[18] As described in Ref. [17] (see also supplemental material [19]), C is renormalized to a smaller value by bending fluctuations. We use C calculated from the torque measured in the experiment, which gives its renormalized value.
[19] See EPAPS Document No. E-PLEEE8-80-R06910 for supplemental material. For more information on EPAPS, see http://www.aip.org/pubservs/epaps.html.
[20] The language of phase coexistence is approximate in that the finite barrier to nucleation in one-dimensional systems precludes a true (sharp) phase transition.
[21] We have also explored increasing the entropic repulsion by a constant factor of up to 3. Though this does bring the torques closer to the experiment, the only other significant change is a decrease in $\mathcal{F}_0$ (data not shown)—specifically, this does not change the discussed discrepancies between the simulation and experiment.
[22] Though the 4.2 kbp z_0 data alone would be arguably consistent with the model predictions, the 2.2 kbp data highlights the discrepancy at small applied forces.