Impact of DNA Twist Accumulation on Progressive Helical Wrapping of Torsionally Constrained DNA

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DNA wrapping is an important mechanism for chromosomal DNA packaging in cells and viruses. Previous studies of DNA wrapping have been performed mostly on torsionally unconstrained DNA, while *in vivo* DNA is often under torsional constraint. In this study, we extend a previously proposed theoretical model for wrapping of torsionally unconstrained DNA to a new model including the contribution of DNA twist energy, which influences DNA wrapping drastically. In particular, due to accumulation of twist energy during DNA wrapping, it predicts a finite amount of DNA that can be wrapped on a helical spool. The predictions of the new model are tested by single-molecule study of DNA wrapping under torsional constraint using magnetic tweezers. The theoretical predictions and the experimental results are consistent with each other and their implications are discussed.

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DNA is highly compact in cells and viruses. In eukaryotic cells, DNA is organized into chromatins whose basic units are nucleosomes. On each nucleosome, approximately 147 bp DNA are wrapped left-handed around a histone octamer surface by 1.7 turns [1]. In mammalian sperms, DNA is condensed by spermidine and experiments have shown that spermidine organizes DNA into a compact toroidal conformation [2,3]. In many double-stranded DNA viruses, such as baculoviruses and white spot syndrome virus that replicate in the nucleus of host cells, highly basic polyamines are found in the viruses that are responsible for packaging of the viral DNA in the capsids [4,5]. *In vitro*, polyamines and multivalent cations are reported to be able to organize DNA into toroid structures which can be approximated as helical wrapping [6,7]. As such, a deep understanding of the physics of DNA helical wrapping will provide important insights to understand DNA packaging *in vivo*.

DNA wrapping by multivalent cations and polyamines has been studied by many experiments performed in bulk in the past four decades [8–11]. Recently, single-molecule techniques that allow direct observation of the wrapping and unwrapping dynamics were also added to the pool of methods [7,12–18]. In a typical single-DNA manipulation experiment, force can be applied to a DNA tether and the extension of the tether as a function of force can be determined accurately. During DNA wrapping or unwrapping, decrease or increase in DNA extension is measured. The balance between wrapping and unwrapping depends on the inter-DNA adsorption interaction, the DNA elastic properties, and the force. When DNA is torsionally unconstrained, the relevant DNA elastic property is the DNA bending stiffness. When it is torsionally constrained, the twist stiffness of DNA will also play a significant role. The DNA bending stiffness and twist stiffness are often described by the bending persistence length A and the twist persistence length C in the wormlike-chain (WLC) model [19,20].

DNA helical wrapping under torsionally unconstrained conditions has been discussed by Kulić and Schiessel [6]. In their model, DNA helical unwrapping from a spool is described by two angles: α describing the desorption of DNA from the helical spool surface and β describing the out-of-plane tilting of the spool in the Z-Y plane (see illustration in Fig. 1). The wrapping process can also be described by the two angles with opposite signs, as shown in Fig. 1. The energy (E_{KS}) of a spool of N loops includes contributions from (1) competition between tension and adsorption of DNA to the spool surface (E_{comp}), (2) bending energy of nonadsorbed DNA (E_{stiff}) and (3) a geometric energy term resulting from spool rotation (E_{geom}):

$$\begin{aligned} E_{\mathrm{KS}}(\alpha,\beta) &= E_{\mathrm{comp}} + E_{\mathrm{stiff}} + E_{\mathrm{geom}} \\ &= (F-\varepsilon_{\mathrm{ads}})(N2\pi R + 2R\alpha) + 8\sqrt{k_BT}\sqrt{AF} \\ &\quad\times\left\{1-\sqrt{\left[1+\left(\frac{R}{R'}\right)\cos\beta\cos\alpha-\left(\frac{H}{2\pi R'}\right)\sin\beta\right]/2}\right\} \\ &\quad+2FR\left[-\cos\beta\sin\alpha+\frac{H}{2\pi R}(\pi+\alpha)\sin\beta\right]. \end{aligned} \tag{1}$$

Here, F is the tension applied to DNA, R the radius of the toroid, A the DNA bending persistence length, $R'^2 = R^2 + H^2/4\pi^2$ with pitch height H and $\varepsilon_{\mathrm{ads}}$ the adsorption energy

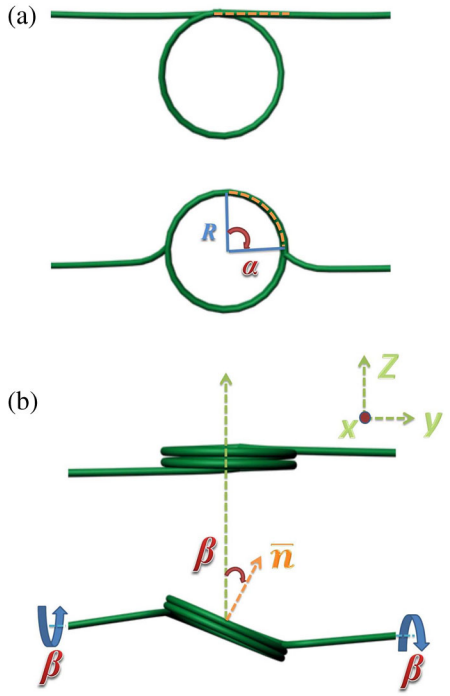

FIG. 1 (color online). The revised KS model. The state of the spool is defined by two angles α and β, which have opposite signs from the original KS model that describe DNA unwrapping. (a) Illustration of the adsorption angle α. The green rod indicates DNA. The dashed yellow line indicates a segment of DNA to be wrapped, which equals the adsorption angle α times the spool radius R. (b) DNA wrapping around the spool by one round results in a twist by approximately 2π assuming negligible helical pitch (see text). Under large enough tension, the two DNA arms are assumed in a plane (X-Y plane). The X axis coincides with the dyad axis of the spool. Out-of-plane tilting of the spool in the Z-Y plane by an angle β leads to additional DNA twist by 2β.

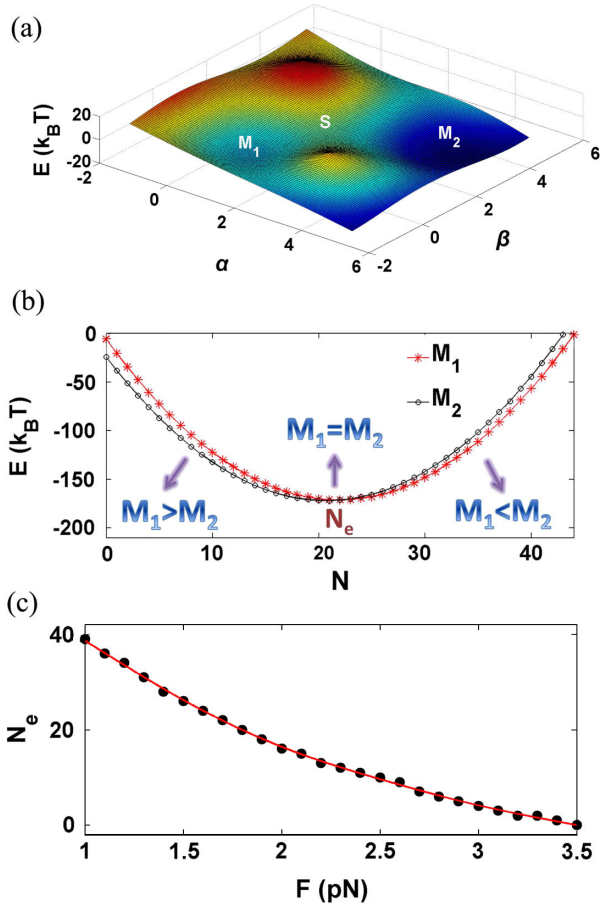

FIG. 2 (color online). Predictions of the revised KS model. (a) The two-dimensional energy landscape for the torsionally constrained 10 102 bp DNA. (b) The energies of the two states M_1 (red asterisk) and M_2 (black circles) as functions of wrapping number N at $F = 1.7$ pN and $\varepsilon_{\text{ads}} = 3.5$ pN. (c) Dependence of the equilibrium wrapping number N_e on the tension F at $\varepsilon_{\text{ads}} = 3.5$ pN. The solid curves were obtained by cubic spline interpolation of the data.

per DNA length adsorbed on the surface. In this model, the states of DNA wrapping are represented in (α, β) space, where DNA wrapping by one round is understood as a transition from one energy minimum near (0,0) to another minimum near (π, π) through an energy barrier around ($\frac{\pi}{2}$, $\frac{\pi}{2}$) in the two dimensional state space [see points M_1, M_2 and S, respectively, in Fig. 2(a)].

The KS (Kulić and Schiessel) model has been applied to explain many previous experiments including DNA unwrapping around nucleosomes [21,22] and DNA wrapping induced by multivalent cations [14,23]. However, most of the previous experiments are performed under conditions where DNA is torsionally unconstrained, while *in vivo* DNA is often torsionally constrained [24,25]. In order to provide further insights into DNA wrapping *in vivo*, we study DNA wrapping under torsional constraint, including developing a new energy model by incorporating the DNA twist energy and performing single-molecule wrapping experiments in 1 mM cobalt hexamine on the torsionally constrained DNA to test the predictions.

Helical wrapping of DNA around a spool will result in DNA twisting (see Fig. 1). Assuming left-handed wrapping as does DNA in nucleosomes, wrapping by one round contributes to the DNA writhe by $\Delta Wr = -[1 - \sin(\gamma)]$, where γ is the pitch angle [26]. For a condensed DNA toroid in 1 mM cobalt hexamine, the spool radius is much greater than the pitch height [10]. One can therefore make an approximation that DNA is wrapped into a flat spool with zero pitch such that $\Delta Wr = -1$ for one round. As the DNA is torsionally constrained, the change in writhe by -1 will result in DNA twist by 2π to maintain a zero linking number difference $\Delta Lk = 0$. Further, a nonintegral round of wrapping, i.e., an out-of-plane tilting of the spool by an angle β will cause an additional twist by 2β (see illustration in Fig. 1). Taken together, the helical wrapping of a torsionally constrained DNA around a spool by N wraps plus an additional out-of-plane tilting will result in a total twist angle of $2\pi N + 2\beta$. It can be accounted for in

the (α, β) space by incorporating the DNA twist energy to the KS model:

$$E(\alpha, \beta) = E_{\mathrm{KS}}(\alpha, \beta) + \frac{1}{2}\left(\frac{C}{L_0}\right)(2\pi N + 2\beta)^2, \quad (2)$$

where C is the twist rigidity and L_0 the contour length of DNA. It is noteworthy that we have ignored the writhe of DNA in the nonwrapped DNA region. It is valid provided that the twist constraint does not significantly affect the planar conformation of the DNA arms as the KS model assumes. The condition can be easily satisfied so long as the tension exerted on DNA is not small. For example, according to discussions by Moroz and Nelson [27] and Marko [28], the reduction in DNA extension due to chiral fluctuations is less than 10% as compared to the prediction by the WLC model for a twistless 3 μm DNA if the accumulated twist number N is smaller than 40 at a tension 1 pN.

The two elastic parameters A and C for DNA have been determined in monovalent salt concentrations ($\sim$150 mM NaCl) to be $A \sim 50$ nm and $C \sim 100$ nm, respectively [27,29]. But they are significantly reduced at higher salt concentration or in the presence of multivalent cations [30,31]. According to Ref. [30], in 1 mM concentration of cobalt hexamine, the DNA bending persistence length A is reduced to $\sim$25 nm. Although the twist persistence length C in 1 mM concentration of cobalt hexamine is not known, previous experiments suggested that its value should significantly decrease in multivalent cation solution [31]. As its exact value is not known, C is treated as a free parameter in this work and a value of 60 nm is chosen to well fit the experimental data. Figure 2(a) shows a two-dimensional energy landscape for the wrapping from N to $N + 1$ using the following parameters: $F = 1.7$ pN, $\varepsilon_{\mathrm{ads}} = 3.5$ pN, $A = 25$ nm, and $C = 60$ nm.

DNA wrapping occurs at conditions where $F < \varepsilon_{\mathrm{ads}}$. An important prediction of the current model is that accumulation of the DNA twist energy will eventually stop DNA wrapping, leading to an equilibrium wrapping number N_e of the spool. The effect of twist is to tilt the energy landscape. To see it, Fig. 2(b) shows the energies of the two states at M_1 and M_2 respectively, as functions of wrapping number N calculated using the same parameters as used in Fig. 2(a). The calculation starts from a nonbalanced state at which $F < \varepsilon_{\mathrm{ads}}$ and $E_{M_1} > E_{M_2}$. DNA prefers to condense to reduce the system's energy. As the condensation proceeds, the twist energy is accumulated to compensate the energy reduction due to the first term in Eq. (1). When the wrapping number N increases to a particular value N_e, the system's energy arrives at a global minimum and at the same time, the spool achieves its equilibrium state ($E_{M_1} = E_{M_2}$). If the wrapping number N is increased further, the energy difference between M_1 and M_2 becomes negative ($E_{M_1} < E_{M_2}$) and the accumulated twist energy counters the adsorption energy. As a result, DNA wrapping will not proceed beyond N_e and fluctuation of the wrapping number N around N_e is expected. This is contrary to the condensation of a torsionally unconstrained DNA where wrapping is always progressive independent of the wrapping number when $F < \varepsilon_{\mathrm{ads}}$. Under our experimental conditions, the adsorption energy density $\varepsilon_{\mathrm{ads}}$ is determined to be around 3.5 pN, as described later.

For a torsionally constrained DNA, the equilibrium wrapping number is located at the intersection of the energy functions of the two states smoothed by cubic spline interpolation [Fig. 2(b)]. Using this method, a value of $N_e \sim 22$ is identified. At $N = N_e$, wrapping and unwrapping are balanced, and as a result the DNA extension reaches equilibrium. Apparently, the equilibrium wrapping number depends on tension, which is larger at smaller tension. To show it, Fig. 2(c) plots N_e as a function of F over a range of 1.0–3.5 pN.

The original KS model predicts progressive DNA wrapping when $F < \varepsilon_{\mathrm{ads}}$. In contrast, a main prediction of the revised KS model is that DNA wrapping is progressive until it reaches a final equilibrium wrapping number. To test this, we performed single-molecule DNA wrapping experiments using a magnetic tweezers setup for both torsionally unconstrained and constrained DNA (Fig. 3). The torsionally constrained DNA was connected to the surfaces via both strands, while the torsionally unconstrained DNA was connected to the surfaces via single strands. Two small permanent magnets were used to apply a force on the DNA which can be adjusted by the position of the magnets. Helical wrapping of DNA is achieved by incubating the DNA in 1 mM cobalt hexamine [7,14,23,32]. To avoid the effects of salt conditions, all the measurements were performed in the same phosphate buffer (10 mM phosphate, pH 7.5). The DNA was originally stretched with a large force ($\sim$15 pN) to avoid any possible looping before the measurement and then reduced to an intended value while its extension z was monitored.

To determine the adsorption energy density $\varepsilon_{\mathrm{ads}}$ under our experimental conditions, we used the torsionally unconstrained DNA (48 502 bp phage λ-DNA) to determine the critical force that balance the wrapping and unwrapping transition. This was done by carefully tuning the force to a point at which the DNA does not condense after half an hour, but starts to condense if the force is reduced by a small amount. Using this method, the critical force, which is approximately the adsorption energy density $\varepsilon_{\mathrm{ads}}$, was determined to be around 3.5 pN.

Figure 3(a) shows progressive wrapping of a torsionally unconstrained λ-DNA at three forces $\sim$0.9, $\sim$1.3, and $\sim$1.7 pN, respectively. The wrapping is stepwise, and is faster at smaller forces. These observations are in general consistent with previously reported results [7,14,23]. Figure 3(b) shows wrapping of a torsionally constrained DNA (10 102 bp) at three similar forces $\sim$0.8, $\sim$1.7, and $\sim$1.9 pN, respectively. In sharp contrast to Fig. 3(a), the

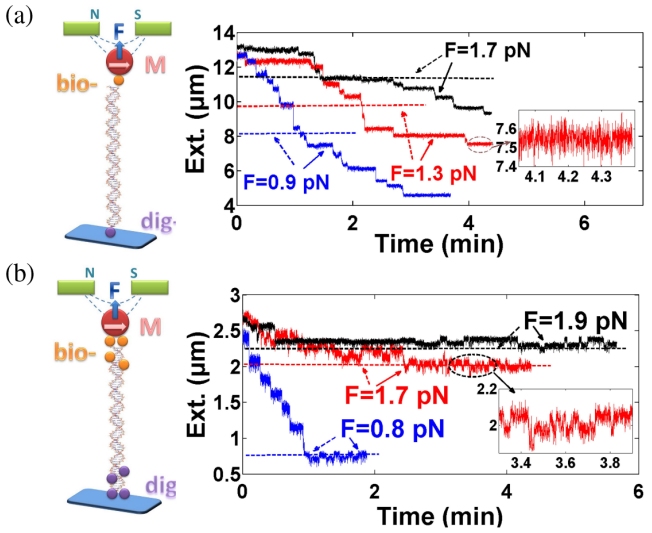

FIG. 3 (color online). Wrapping of torsionally unconstrained 48 502 bp λ-DNA (a) and constrained 10 102 bp DNA (b). (a) Left panel shows a torsionally unconstrained DNA between a streptavidin-coated paramagnetic bead and a digoxigenin-coated cover glass. Right panel shows time courses of the DNA extension under wrapping condition ($F < \varepsilon_{\mathrm{ads}}$). Inset shows a zoomed-in time course of a plateau during which DNA wrapping pauses. (b) Left panel shows a torsionally constrained DNA tether. Right panel shows time courses of the DNA extension at similar forces. Inset shows a zoomed-in time course of the final steady extension when the dynamic fluctuations between wrapping and unwrapping occurs. The horizontal dashed lines in both panels indicate the predicted z_{pred} at the respective forces (indicated by different colors and arrows) assuming that the DNA molecules are torsionally constrained.

torsionally constrained DNA wraps progressively until a steady extension is reached, where the DNA extension fluctuates stepwise around an average value over a long time, suggesting that the equilibrium between wrapping and unwrapping is reached [inset in Fig. 3(b)]. For torsionally unconstrained DNA, the DNA extension could also remain steady over a similar time scale due to the large energy barrier for the wrapping transition. However, dynamic fluctuation between wrapping and unwrapping is not observed at any of the plateaus where the DNA wrapping is paused [inset in Fig. 3(a)]. Overall, this observation is consistent with our theoretical prediction for the existence of a final equilibrium wrapping number for torsionally constrained DNA. The model also predicts that at a smaller force, the equilibrium wrapping number would increase. Experimentally, we find that the final steady extension of the same DNA has a shorter steady extension at a smaller force, consistent with more DNA wrapped on the spool.

In order to compare the experimental results and the theoretical predictions more quantitatively, we calculate the final equilibrium extension of torsionally constrained DNA after wrapping by $N_e(F)$ loops. The amount of DNA adsorbed by the spool is $2\pi R(F)N_e(F)$. For DNA length comparable to DNA persistence length, the looping energy under force can be approximated as $E_{\mathrm{loop}}/k_BT = \frac{1}{2}\frac{A}{l} \times (2\pi)^2 + \frac{FL}{k_BT} = 2\pi(A/2R + FR/k_BT)$, where $l = 2\pi R$. The most probable radius is determined by minimizing the energy with respect to R: $R(F) = \sqrt{k_BTA/2F}$ [33]. Here, the radius of the spool is approximated by the radius of the first DNA loop under a parallel boundary condition, which is demonstrated to serve as the initial scaffold for the following DNA wrapping into the helical spool [7]. The initial DNA looping size is determined by the DNA bending rigidity and tension but independent of the adsorption energy [7]. The nonadsorbed DNA contour length L_{free} is therefore $L_{\mathrm{free}} = L_0 - 2\pi R(F)N_e(F)$, where L_0 is the contour length of the whole DNA. This leads to a prediction of the force-extension curve z_{pred} that should be observed in experiments according to the WLC model: $FA/k_BT = z_{\mathrm{pred}}/L_{\mathrm{free}} - 1/4 + 1/4(1 - z_{\mathrm{pred}}/L_{\mathrm{free}})^2$ [19,34]. To compare with experiments, the predicted z_{pred} (horizontal dashed lines) assuming that the DNA molecules are torsionally constrained are plotted in both panels in Fig. 3. As shown in Fig. 3(a), the torsionally unconstrained DNA can wrap much below the predicted lines, while the torsionally constrained DNA approaches the lines when the dynamic equilibrium between wrapping and unwrapping occurs.

In summary, we have extended the previously proposed KS energy model to describe helical wrapping of torsionally constrained DNA by incorporating a twist energy term. Its applications are limited to cases where the tension exerted on DNA is large enough so that the chiral fluctuation of the nonwrapped DNA is negligible. This revised energy model predicts an equilibrium wrapping number, which is confirmed by single-DNA wrapping experiments in 1 mM cobalt hexamine solution using magnetic tweezers. The theoretical predictions are consistent with the experimental results. Although the DNA wrapping is induced by cobalt hexamine solution in the current work, the model can be applied to study DNA wrapping induced by other multivalent cations, polyamines, and nucleosome assembly in general.

It has been reported that, depending on experimental conditions, multivalent cations can organize DNA into various conformations such as globular, rodlike, and toroids. In this Letter, the buffer conditions and concentration of cobalt hexamine were carefully chosen to be similar to previous experiments in which toroidal DNA organization was reported [7,14]. Although it is unclear whether DNA was organized into a perfect toroidal structure in our experiments, the agreement between the experiments and the predictions based on the helical wrapping model suggests that it likely formed a helical-like structure. To the best of our knowledge, this is the first research that has studied the effects of the twist accumulation on progressive DNA wrapping. Before us, there was a study that investigated the role of tension and twist in single-DNA condensation [7]. However, that study focused on how the

condensation force depends on a preimposed twist, which is different from our studies.

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[1] K. Luger, K. Luger, A. W. Mäder, R. K. Richmond, and D. F. Sargent, Nature (London) **389**, 251 (1997).
[2] L. C. Gosule and J. A. Schellman, Nature (London) **259**, 333 (1976).
[3] D. K. Chattoraj, L. C. Gosule, and A. Schellman, J. Mol. Biol. **121**, 327 (1978).
[4] M. E. Wilson and L. K. Miller, Virology **151**, 315 (1986).
[5] Y. Liu, J. Wu, H. Chen, C. L. Hew, and J. Yan, Virology **408**, 197 (2010).
[6] I. M. Kulić and H. Schiessel, Phys. Rev. Lett. **92**, 228101 (2004).
[7] K. Besteman, S. Hage, N. Dekker, and S. Lemay, Phys. Rev. Lett. **98**, 058103 (2007).
[8] A. V. Slita, N. A. Kasyanenko, O. V. Nazarova, I. I. Gavrilova, E. M. Eropkina, A. K. Sirotkin, T. D. Smirnova, O. I. Kiselev, and E. F. Panarin, J. Biotechnol. **127**, 679 (2007).
[9] V. A. Parsegian, R. P. Rand, and D. C. Rau, Proc. Natl. Acad. Sci. U.S.A. **97**, 3987 (2000).
[10] N. V. Hud and I. D. Vilfan, Annu. Rev. Biophys. Biomol. Struct. **34**, 295 (2005).
[11] U. F. Keyser, S. van Dorp, and S. G. Lemay, Chem. Soc. Rev. **39**, 939 (2010).
[12] Y. Yoshikawa, K. Yoshikawa, and T. Kanbe, Langmuir **15**, 4085 (1999).
[13] C. G. Baumann, V. A. Bloomfield, S. B. Smith, C. Bustamante, M. D. Wang, and S. M. Block, Biophys. J. **78**, 1965 (2000).
[14] W. B. Fu, X.-L. Wang, X.-H. Zhang, S.-Y. Ran, J. Yan, and M. Li, J. Am. Chem. Soc. **128**, 15 040 (2006).
[15] F. T. Chien, S. Lin, P. Lai, and C. Chan, Phys. Rev. E **75**, 041922 (2007).
[16] B. A. Todd and D. C. Rau, Nucleic Acids Res. **36**, 501 (2008).
[17] P. Ranjith, J. Yan, and J. F. Marko, Proc. Natl. Acad. Sci. U.S.A. **104**, 13649 (2007).
[18] B. van den Broek, M. C. Noom, J. van Mameren, C. Battle, F. C. MacKintosh, and G. J. L. Wuite, Biophys. J. **98**, 1902 (2010).
[19] J. F. Marko and E. D. Siggia, Macromolecules **28**, 8759 (1995).
[20] M. Doi and S. F. Edwards, *Theory of Polymer Dynamics* (Oxford University, New York, 1986).
[21] M. A. Hall, A. Shundrovsky, L. Bai, R. M. Fulbright, J. T. Lis, and M. D Wang, Nat. Struct. Mol. Biol. **16**, 124 (2009).
[22] N. Korolev, Y. Fan, A. P. Lyubartsev, and L. Nordenskiöld, Curr. Opin. Struct. Biol. **22**, 151 (2012).
[23] W. B. Fu, H. Chen, M. Li, and J. Yan, J. Comput. Theor. Nanosci. **7**, 213 (2010).
[24] R. R. Sinden, J. O. Carlson, and D. E. Pettijohn, Cell **21**, 773 (1980).
[25] P. Nelson, Proc. Natl. Acad. Sci. U.S.A. **96**, 14342 (1999).
[26] F. B. Fuller, Proc. Natl. Acad. Sci. U.S.A. **75**, 3557 (1978).
[27] J. D. Moroz and P. Nelson, Proc. Natl. Acad. Sci. U.S.A. **94**, 14418 (1997).
[28] J. F. Marko, *Introduction to Single-DNA Micromechanics* (Elsevier, New York, 2005).
[29] Z. Bryant, M. D. Stone, J. Gore, S. B. Smith, N. R. Cozzarelli, and C. Bustamante, Nature (London) **424**, 338 (2003).
[30] C. G. Baumann, S. B. Smith, V. A. Bloomfield, and C. Bustamante, Proc. Natl. Acad. Sci. U.S.A. **94**, 6185 (1997).
[31] F. C. Oberstrass, L. E. Fernandes, and Z. Bryant, Proc. Natl. Acad. Sci. U.S.A. **109**, 6106 (2012).
[32] N. V. Hud and K. H. Downing, Proc. Natl. Acad. Sci. U.S.A. **98**, 14925 (2001).
[33] T. Strick, J.-F. Allemand, V. Croquettea, and D. Bensimona, Prog. Biophys. Molec. Biol. **74**, 115 (2000).
[34] T. Odijk, Macromolecules **28**, 7016 (1995).