

DNA Spools under Tension

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DNA spools, structures in which DNA is wrapped and helically coiled onto itself or onto a protein core, are ubiquitous in nature. We develop a general theory describing the nonequilibrium behavior of DNA spools under linear tension. Two puzzling and seemingly unrelated recent experimental findings, the sudden quantized unwrapping of nucleosomes and that of DNA toroidal condensates under tension, are theoretically explained and shown to be of the same origin. The study provides new insights into nucleosome and chromatin fiber stability and dynamics.

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Introduction.—Wrapped DNA-protein complexes are ubiquitous in nature [1] and play key roles in many fundamental life processes. Prominent examples of DNA wrapping proteins are the LacI repressor [2] participating in the bacterial gene regulation, the DNA gyrase [3] directing changes in DNA topology, RNA polymerase [4] copying DNA to RNA, and the histone octamer [5] performing DNA packaging into nucleosomes leading in each cell to the enormous condensation of meters of DNA into micron sized chromosomes. Besides the natural wrapped architectures there are attempts to design nanoparticles imitating that motive [6] as a means to efficiently pack and transport DNA into cells. In most of these ligand-DNA complexes the geometry and chemistry of the ligand surface enforces the DNA to follow a superhelical wrapping path with one or more tight turns. Remarkably, upon addition of multivalent condensing agents (as in sperm cells) or under high crowding conditions (as in virus capsids or during ψ condensation) DNA also shows an intrinsic ability to self-organize into large toroidal spools [7].

In the past decade single molecule experiments have become available allowing one to apply tension to individual polymers in order to probe their mechanical properties [8] as well as their interaction with ligands [9–11] and molecular motors [12]. Static and dynamic force spectroscopy [13] developed into a powerful tool for measuring equilibrium as well as kinetic characteristics of single molecules, going far beyond the information accessible by classical bulk experiments. Application of these methods to DNA-spool geometries has been awaited for long and was reported only recently for single nucleosomes [14] and single DNA toroidal condensates [10,11]. These experiments—at first glance completely unrelated—reside on different length and energy scales and ground on different mechanisms of wrapping. Despite that, they both reveal the same surprising result apparently contradicting all the available bulk data: the unfolding of wrapped DNA from the spools is a catastrophic event; i.e., it is sudden and quantized and happens one DNA turn at a time. The aim of this Letter is to theoret-

cally explain this unusual nonequilibrium effect and to demonstrate the universality behind it. Our theory is then applied to nucleosomes and DNA toroids allowing to extract from experiments the relevant energetic parameters and to resolve apparent “oddities” in the dynamics of these systems.

General model.—The DNA is assumed to be adsorbed on the protein spool surface along a predefined helical path with radius R and pitch height H . This path accounts for the typical chemical structure of such a protein spool surface (e.g., distribution of charges, hydrogen donors/acceptors, etc.) along which the DNA is adsorbed with a net adsorption energy density ϵ_{ads} given by the difference of the total DNA-protein binding energy and the stored DNA bending energy per length along the helical path. The degree of DNA adsorption is described by the desorption angle α which is defined to be zero for one full turn wrapped (cf. Fig. 1). After a short inspection it becomes clear that the unwrapping problem is nonplanar and that the spool needs to rotate transiently out of the plane while performing a full turn, an effect already pointed out by Cui and Bustamante [15]. Therefore a second angle, β , is introduced to describe the out-of-plane tilting of the spool. When a tension F (along the Y axis) acts on the two outgoing DNA “arms” the system (i.e., the wrapped spool together with the free DNA ends) will simultaneously respond with (i) *DNA deformation*, with (ii) *spool tilting*, and with (iii) *DNA desorption* from the spool. In the following we assume that the DNA has freely rotating ends (as in the experiment [14]) that allow us to neglect the twist degree of freedom. Then the total energy of the system as a function of α and β reads $E_{\text{tot}} = 2R\epsilon_{\text{ads}}\alpha + 2E_{\text{bend}} - 2F\Delta y$. Here the first term is the adsorption energy, the second term is the bending deformation energy of the two free DNA portions, and the third term describes the gain in potential energy by pulling out the DNA ends by a distance $2\Delta y$. Applying linear elasticity theory and elementary geometry E_{tot} can be redistributed into the following three terms:

$$E_{\text{tot}}(\alpha, \beta) = E_{\text{comp}}(\alpha) + E_{\text{geom}}(\alpha, \beta) + E_{\text{stiff}}(\alpha, \beta). \quad (1)$$

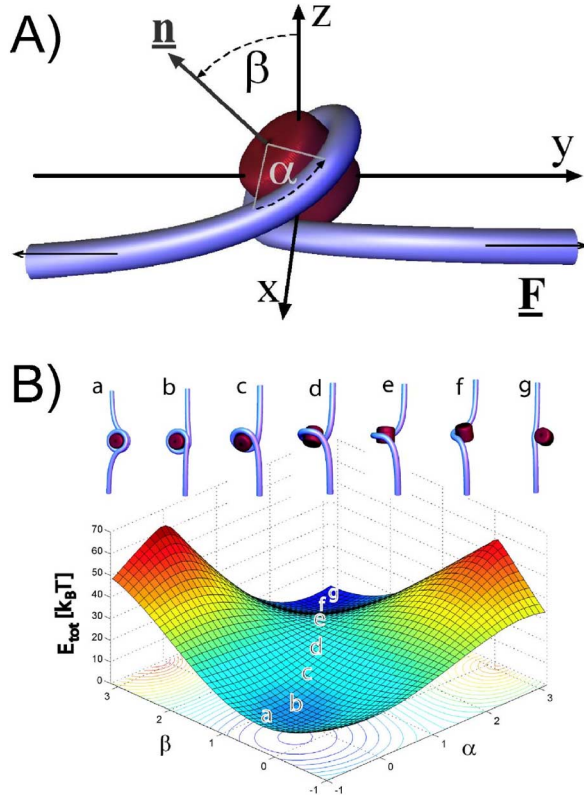


FIG. 1 (color online). (a) An unfolding DNA-protein spool under tension is characterized by two angles: the desorption angle α and the tilt angle β . (b) The energy landscape of a DNA spool [as given by Eq. (1) for $A = 50$ nm, $F = 4$ pN, and $\epsilon_{\text{ads}} = 0.7k_B T/\text{nm}$] and its unfolding pathway. Note that only states b and g correspond to (meta)stable states.

The first term $E_{\text{comp}} = 2R(\epsilon_{\text{ads}} - F)\alpha$ describes the competition of the adsorption and the applied force. The “geometrical” energy term $E_{\text{geom}} = 2FR[\cos\beta\sin\alpha - (H/2\pi R) \times (\pi - \alpha)\sin\beta]$ stems from the gain or loss of potential energy by spool opening (change of α) and rotation (change of β). Finally, the last and most remarkable term $E_{\text{stiff}} = 8\sqrt{AF}\{1 - \sqrt{[1 + (R/\bar{R})\cos\beta\cos\alpha + (H/2\pi\bar{R})\sin\beta]}/2\}$ accounts for the stiffness of the nonadsorbed DNA portions. Here $A \approx 50k_B T$ nm is the DNA bending stiffness at room temperature and $\bar{R}^2 = R^2 + H^2/4\pi^2$. Two effects contribute equally to E_{stiff} : (i) the bending energy of the deformed DNA arms and (ii) the loss of potential energy by “wasting” length due to DNA deformation. Entropic contributions to Eq. (1) can be neglected if the tension length $\lambda = \sqrt{A/F}$ [16] is smaller than the DNA persistence length $A/k_B T$, which is here always the case. To understand the implications of Eq. (1) on the kinetics of unwrapping we consider two limiting cases. First let us look at the case of a large thin spool, i.e., $R \gg A/k_B T$ (or, equivalently, an infinitely flexible polymer) and $R \gg H$, where we may neglect E_{stiff} . In that case and for $F > \epsilon_{\text{ads}}$ the spool moves from the (thermodynamically) metastable state M_1 with $\alpha = \alpha_0 = -\arccos(1 - \epsilon_{\text{ads}}/F)$ and $\beta = 0$ via a saddle point S at $\alpha = 0$ and $\beta = \alpha_0$

into a more favorable minimum M_2 at $\alpha = \pi + \alpha_0$ and $\beta = \pi$. Remarkably S constitutes a significant energetic barrier between M_1 and M_2 given by $\Delta E_{\text{tot}} = 2FR(\alpha_0 \cos\alpha_0 - \sin\alpha_0)$. For hypothetical yet reasonable parameter values, say $R = 50$ nm, $\epsilon_{\text{ads}} = 1k_B T/\text{nm}$, and $F = 2\epsilon_{\text{ads}}$ we obtain a huge barrier of $\Delta E_{\text{tot}} \approx 70k_B T$. A second interesting limit of Eq. (1) is given by a flat spool and high polymer stiffness, i.e., $A \gg Rk_B T$ and $R \gg H$. For not too large forces ($F \lesssim A/R^2$) and $\epsilon_{\text{ads}} \lesssim F$ the kinetic behavior is roughly dominated by the term E_{stiff} . In this case we find a transition path from $(\alpha, \beta) = (0, 0)$ over the saddle point $(\pi/2, \pi/2)$ to the state (π, π) with a barrier height $\Delta E_{\text{tot}} = 8\sqrt{AF}(1 - 1/\sqrt{2})$. Note that in this limit the DNA actively participates in the suppression of unwrapping ($\Delta E_{\text{tot}} \sim A^{1/2}F^{1/2}$), which can even give rise to negative resistance effects [17] for small forces. In preliminary conclusion, in both limiting cases the unwrapping meets significant kinetic barriers but for different reasons: because of unfavorable projection of the force in terms of the (α, β) configurational space in the first limit and due to significant transient bending of the DNA arms during the transition in the second limit. For realistic DNA spools we are somewhere in between these two cases.

Nucleosome unwrapping.—The most abundant DNA spool in nature is the nucleosome where 1 and 3/4 turns of DNA, 147 bp, are wrapped around a protein core on a left-handed superhelical path with diameter 4.2 and 2.5 nm pitch. The question about the equilibrium and kinetic stability of nucleosomes is one of the important experimentally unsettled questions in present molecular biology. How can nucleosomes be highly stable with its wrapped DNA being highly accessible at the same time [18]? A recently performed experiment [14] measuring the critical force required to unwrap single nucleosomes reveals an interesting and unexpected behavior [19]. When small forces ($F < 10$ pN) are applied for short times (~ 1 – 10 s) the nucleosome unwraps only partially by releasing the outer 60–70 bp of wrapped DNA [moving from state a to state b in Figure 1(b)] in a gradual and equilibrium fashion. For higher forces ($F \gtrsim 20$ pN) nucleosomes show a pronounced sudden nonequilibrium release behavior of the remaining 80 bp [cf. c – g in Fig. 1(b)]—the latter force being much larger than expected from equilibrium arguments [20]. In fact, experiments [18] measuring spontaneous partial unwrapping of nucleosomal DNA suggest $30k_B T$ per 147 bp leading to an unpeeling force of ~ 2.5 pN. To explain this peculiar finding Brower-Toland *et al.* [14] conjectured that there must be a barrier in the adsorption energy located after the first 70–80 bp which reflects some biochemical specificity of the nucleosome structure at that position. Their analysis of the dynamical force spectroscopy measurements revealed an apparent barrier of $\sim 38k_B T$ smeared out over not more than 10 bp. However, there is no experimental indication of such a huge specific barrier, neither from the crystal structure [5] nor from the

equilibrium accessibility to nucleosomal DNA [18]. Consequently, the question arises if the barrier is really caused by biochemistry of the nucleosome or, as we show below, by its underlying geometry and physics.

To see that the effect is mainly physical we apply Eq. (1) to compute the barrier. For this purpose we model the nucleosomal adsorption energy density as $\varepsilon_{\text{ads}}(\alpha) = \varepsilon_{\text{ads}}^0 + \theta(\alpha)\varepsilon_{\text{es}}$, where $\varepsilon_{\text{ads}}^0 \approx 0.7k_B T/\text{nm}$ is taken from the reversible part (for the first 60–70 bp) of the measurement in Ref. [14]. The introduction of the step function ($\theta = 0$ for $\alpha < 0$ and $\theta = 1$ for $\alpha \geq 0$) together with a new parameter ε_{es} , the electrostatic interaction energy density, accounts for the DNA-DNA repulsion of the two adjacent helical gyres which acts only for $\alpha < 0$ (more than one turn present) reducing the net ε_{ads} to the smaller effective value $\varepsilon_{\text{ads}}^0$. Using $\varepsilon_{\text{ads}}^0$ from above we can compute the barrier height for nucleosome unfolding for various values of ε_{es} as done in Fig. 2(a). To relate the barrier heights from Fig. 2(a) to the dynamical force spectroscopy (DFS) measurements in Ref. [14] we generalize the classical relation between the loading rate r_F and the most probable rupture force F^* [13] to the case of

nonlinear force-barrier dependence and obtain $\ln(r_F/r_0) = \ln[-\nu_{\text{att}}k_B T/(r_0\Delta E')] - \Delta E/k_B T$ [21]. Here r_F and ΔE are functions of F^* and $\Delta E' = \partial(\Delta E)/\partial F^*$. $r_0 = 1 \text{ pN s}^{-1}$ is an arbitrary scale on the r_F axis and ν_{att} is the typical attempt frequency of the nucleosome. Assuming ν_{att} in the range 10^5 – 10^8 s^{-1} , we can fit the experimental data from [14] to obtain the corresponding values of ε_{es} (cf. Fig. 3). Keeping in mind that ν_{att} is dominated by the slowest process involved in the unfolding event, we estimate $\nu_{\text{att}} \lesssim 10^6 \text{ s}^{-1}$ [22]. The latter implies $\varepsilon_{\text{es}} \approx 1.4$ – $1.7k_B T/\text{nm}$ [23]. This indicates that the second turn is much more weakly adsorbed than the first one. This explains why under equilibrium conditions (at $F = 0$) the DNA deeply inside the nucleosomes (almost the whole bound DNA) can be rather easily accessed by proteins [18], but the nucleosome is still highly stable: The line $\alpha = 0$ can be moved to each position inside the nucleosome if the left and the right DNA arms are adsorbed or desorbed in a consistent manner. Beyond that ($\alpha > 0$) the assisting electrostatics switches off and the nucleosome is suddenly strongly stabilized [by $(60$ – $70)k_B T$ in total].

Toroid unspooling.—When long DNA molecules condensed with multivalent counterions were stretched in a single molecule experiment [10] Baumann *et al.* found a surprising behavior. When a critical force (typically $F \approx 4$ – 12 pN) is reached large portions of DNA are released in packets in a discontinuous manner (“stick release pattern”). When the same experiment was redone recently by another group [11] a pronounced quantization in the DNA release length of $\approx 300 \text{ nm}$ was clearly demonstrated. It was noted in Refs. [10,11] that the latter correlates exactly with the typical size ($R \approx 50 \text{ nm}$) of toroidal condensates formed in solution and led those authors to the conclusion that a single turn of DNA unwraps from the toroid spool at a time. Despite that interesting finding the mechanism behind this nonequilibrium effect remained unexplained. However, in the light of our theory the explanation is again straightforward as a DNA toroid exhibits a spool geometry with $R \approx 50 \text{ nm}$ and $H \ll R$. The “first limit”

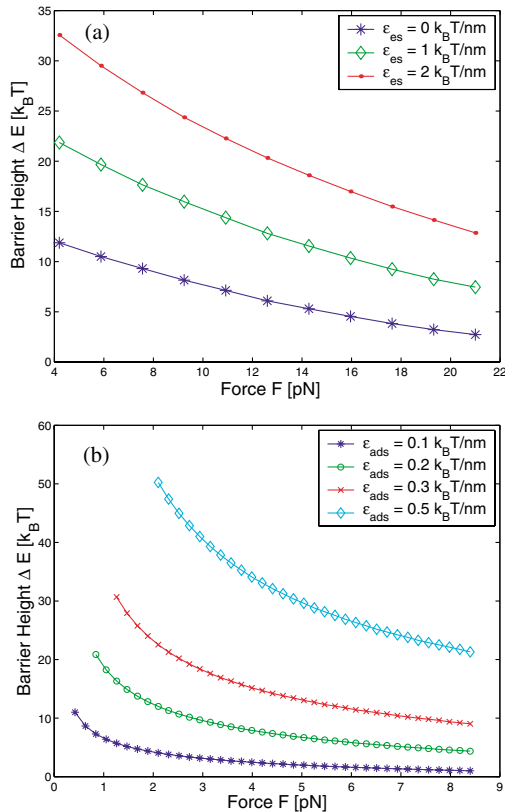


FIG. 2 (color online). Kinetic barriers opposing DNA-spool unfolding as a function of applied tension computed for (a) the nucleosome ($R = 4.2 \text{ nm}$, $H = 2.4 \text{ nm}$, $\varepsilon_{\text{ads}}^0 = 0.7k_B T/\text{nm}$, and $A = 50k_B T/\text{nm}$; cf. the text) for various interstrand repulsion energy densities ε_{es} , and, for (b) the DNA toroid ($R = 50 \text{ nm}$, $H = 2.4 \text{ nm}$, and $A = 40k_B T/\text{nm}$) for various adsorption energy densities ε_{ads} .

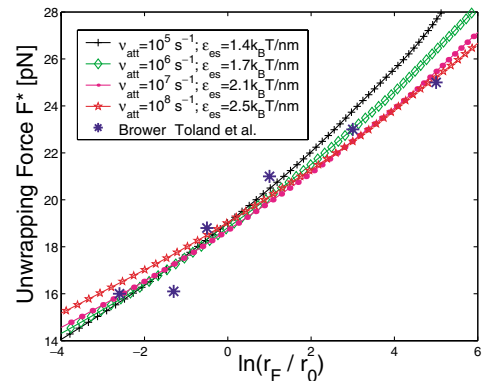


FIG. 3 (color online). Optimal fits of the DFS data from Ref. [14] for various attempt frequencies give the corresponding electrostatic DNA-DNA repulsion ε_{es} .

considered above gives a good approximation here. The barrier heights for different values of ε_{ads} as a function of force are computed in Fig. 2(b). Similar to the case of the nucleosome, the attempt frequency ν_{att} is dominated by the rotational friction—here of the 50 nm sized toroid object—leading to $\nu_{\text{att}} \approx 3 \times (10^2-10^3) \text{ s}^{-1}$. For high concentrations of the condensing agent spermidine one finds $\varepsilon_{\text{ads}} \approx 0.2-0.3k_B T/\text{nm}$ (cf. [10,11], and the references therein). In the case of equilibrium this means a very small peeling-off force of $F \approx 1-1.5 \text{ pN}$. Our model together with Fig. 2(b) allows us now to predict the activated nonequilibrium behavior to have very low unfolding frequencies $\nu_{\text{unf}} = \nu_{\text{att}} \exp[-\Delta E(F)/k_B T]$, for instance, $10^{-6}-10^{-2} \text{ s}^{-1}$ for $F = 2 \text{ pN}$, $10^{-3}-1 \text{ s}^{-1}$ for $F = 4 \text{ pN}$, and $0.3-50 \text{ s}^{-1}$ for $F = 8 \text{ pN}$, consistent with experimental findings [10,11].

Conclusion.—We have shown that DNA spools ranging from protein-DNA complexes to DNA toroids share a universal feature inherited by their geometry: They are strongly kinetically protected from mechanical disruption upon applied tension. In the case of chromatin fibers consisting of large arrays of nucleosomes and other DNA spooling proteins this effect provides a great biological advantage. Strong molecular motors like RNA polymerase and helicase or microtubuli during cell division are known to act on the fiber with significant transient tensions of the order of 20 pN or even more. While a hypothetical “fiber A” consisting of DNA and nonspooling proteins (say only DNA bending proteins) would immediately lose most of its protein content a “fiber B” constituted of DNA spools would survive long time periods (up to 10^6-10^8 times longer than “fiber A”). We can speculate that this obvious advantage was not overlooked by nature and has flown into the chromatin fiber design and the nucleosome-spool shape. The remarkable universality of the “kinetic protection” also shows up in the case of DNA toroids which are roughly ~ 10 times larger while the DNA is ~ 10 times more weakly adsorbed than for typical DNA-protein spools. While the biological implications of this finding still have to be fully figured out, it seems that this might play a role in the injection/ejection process of DNA from viral capsids inducing similar quantization effects as found here. Looking at the wealth of peculiar effects revealed by the single molecule experiments [10,11] we feel that the present understanding of DNA condensation kinetics is still incomplete, yet one partial mystery seems resolved.

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- [23] Note that ε_{es} contributes to Eq. (1) through E_{comp} . Thus it modulates the height of the barrier but does not account for its presence. In the absence of that barrier one expects an unwrapping of the second turn at a critical force $\varepsilon_{\text{ads}}^0 + \varepsilon_{\text{el}}$ that is on the order of 8 to 10 pN—still much smaller than the measured unwrapping forces.