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 non-equilibrium 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 and play key roles in many fundamental life processes. Prominent examples of DNA wrapping proteins are: the Lac1 repressor participating in the bacterial gene regulation, the DNA-gyrase directing changes in DNA topology, RNA polymerase copying DNA to RNA, and the histone octamer 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 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 (like in sperm cells) or under high crowding conditions (like in virus capsids or during $\psi$-condensation) DNA also shows an intrinsic ability to self-organize into large toroidal spools.

In the past decade single molecule experiments have become available allowing to apply tension to individual polymers in order to probe their mechanical properties as well as their interaction with ligands and molecular motors. Static and dynamic force spectroscopy 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 and single DNA toroidal condensates. 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 theoretically explain this unusual nonequilibrium effect and to demonstrate the universality behind it. Our theory is then applied to nucleosomes and DNA toroids allowing us 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 $\varepsilon_{ads}$ 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 $\alpha$ which is defined to be zero for one full turn wrapped (cf. Fig. 1). After short inspection it becomes clear that the unwrapping problem is non-planar 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. Therefore a second angle, $\beta$, 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) that allows us to neglect the twist degree of freedom. Then the total energy of the system as a function of $\alpha$ and $\beta$ writes $E_{tot} = 2R\varepsilon_{ads}^0 + 2E_{bend} - 2F\Delta y$. Here the first term is the adsorption energy, the second 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:

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

The first term $E_{comp} = 2R(\varepsilon_{ads} - F)\alpha$ describes the competition of the adsorption and the applied force. The "geometrical" energy term $E_{geom} = 2FR(\cos \beta \sin \alpha - (H/2\pi R)(\pi - \alpha)\sin \beta)$ stems from the gain/loss of potential energy by spool opening (change of $\alpha$) and rotation (change of $\beta$). Finally, the last and most remarkable term $E_{stiff} = 8\sqrt{AF} \left( 1 - \sqrt{1 + (R/\sqrt{R}) \cos \beta \cos \alpha + (H/2\pi R) \sin \beta} / 2 \right)$ accounts for the stiffness of the non-adsorbed DNA portions. Here $A \approx 50k_BTnM$ is the DNA bending stiffness at room temperature and $\overline{R}^2 = R^2 + H^2/4\pi^2$. Two effects contribute equally to $E_{stiff}$: 1) the bending energy of the deformed DNA arms and 2) the loss of potential energy by "wasting" length due to DNA deformation. 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., $R \gg A/k_BT$ (or, equivalently, an infinitely flexible polymer) and $R \gg H$, where we may neglect $E_{stiff}$. In that case and for $F > \varepsilon_{ads}$ the spool moves from the (thermodynamically) metastable state $M_1$ with $\alpha = \alpha_0 = -\arccos(1 - \varepsilon_{ads}/F)$ 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 $\Delta E_{tot} = 2FR(\alpha_0 \cos \alpha_0 - \sin \alpha_0)$. For hypothetical yet reasonable parameter values, say $R = 50nm$, $\varepsilon_{ads} = 1k_BT/nm$ and $F = 2\varepsilon_{ads}$ we obtain a huge barrier of $\Delta E_{tot} \approx 70k_BT$. A second interesting limit of Eq. (1) is given by a flat spool and high polymer stiffness, i.e., $A \gg Rk_BT$ and $R \gg H$. For not too large forces ($F \lesssim A/R^2$) and $\varepsilon_{ads} \lesssim F$ 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 $(\pi,\pi)$ with a barrier height $\Delta E_{tot} = 8\sqrt{AF}(1 - 1/\sqrt{2})$. Note that in this limit the DNA actively participates in the suppression of unwrapping ($\Delta E_{tot} \sim A^{1/2}F^{1/2}$) which can even give rise to negative resistance effects 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 $(\alpha,\beta)$ 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-spool 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 lefthanded superhelical path with diameter 4.2$nm$ 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? A recently performed experiment measuring the critical force required to unwrap single nucleosomes reveals an interesting and unexpected behavior. When small forces ($F < 10pN$) are applied for short times ($\sim 1-10s$) the nucleosome unwraps only partially by releasing the outer 60-70 bp of wrapped DNA (moving from state a to b in Fig. 1B) in a gradual and equilibrium fashion. For higher forces ($F \gtrsim 20pN$) nucleosomes show a pronounced sudden non-equilibrium release behavior of the remaining 80 bp (cf. c-g in Fig. 1B) – the latter force being much larger than expected from equilibrium arguments. In fact, experimental measuring spontaneous partial unwrapping of nucleosomal DNA suggest 30$k_BT$ per 147bp leading to an unequal force of $\approx 2.5pN$. To explain this peculiar finding Brower-Toland et al. 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 $\approx 38k_BT$ 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 \[17\]. 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 physically 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 \( \varepsilon_{\text{ads}}^0 \approx 0.7k_B T/nm \) is taken from the reversible part (for the first 60-70bp) 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 \( \varepsilon_{\text{es}} \), the electrostatic interaction energy density, accounts for the DNA-DNA repulsion of the two adjacent helical grooves which acts only for \( \alpha < 0 \) reducing the net \( \varepsilon_{\text{ads}} \) (cf. Fig. 1A). Using \( \varepsilon_{\text{ads}}^0 \) from above we can compute the barrier height for nucleosome unfolding for various values of \( \varepsilon_{\text{es}} \) as done in Fig. 2A. To relate the barrier heights from Fig. 2A 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

\[
\ln \left( \frac{r_F}{r_0} \right) = \ln \left[ -\nu_{\text{att}}k_B T / (r_0 \Delta E') \right] - \Delta E / k_B T.
\]

Here \( r_F \) and \( \Delta E' \) are functions of \( F^* \) and \( \Delta E' = \partial (\Delta E) / \partial F^* \). \( r_0 = 1pNs^{-1} \) is an arbitrary scale on the \( r_F \) axis and \( \nu_{\text{att}} \) is the typical attempt frequency of the nucleosome. Assuming \( \nu_{\text{att}} \) in the range \( 10^5 \) to \( 10^8 s^{-1} \) we can fit the experimental data from \[14\] to obtain the corresponding values of \( \varepsilon_{\text{es}} \), cf. Fig. 3. Keeping in mind that \( \nu_{\text{att}} \) is dominated by the slowest process involved in the unfolding event we estimate \( \nu_{\text{att}} \lesssim 10^8 s^{-1} \[20\]. The latter implies \( \varepsilon_{\text{es}} \approx 1.4 - 1.7k_B T/nm \). So indeed, at the line \( \alpha = 0 \) there is clear jump in adsorption energy density as we would naively expect from repulsive DNA-DNA electrostatics under these conditions \[21\]. 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 \[17\] but the nucleosome is still highly stable: The line \( \alpha = 0 \) can be moved to each position inside the nucleosome if the left and right DNA arms are adsorbed/desorbed in a consistent manner. Beyond that interesting finding the mechanism behind that single turn of DNA unwraps from the toroid spool at a time. Despite that interesting finding the mechanism behind
this non-equilibrium 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" considered above gives here a good approximation. The barrier heights for different values of $\varepsilon_{\text{ads}}$ as a function of force are computed in Fig. 2B. Similar as in the case of the nucleosome the attempt frequency $\nu_{\text{att}}$ is dominated by the rotational friction – here of the $50 \text{nm}$ 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 $\varepsilon_{\text{ads}} \approx 0.2 - 0.3 k_B T / \text{nm}$ (cf. [10, 11] and the references therein). In case of equilibrium this means a very small peeling-off force of $F \approx 1 - 1.5 \text{pN}$. Our model together with Fig. 2B allows us now to predict the activated non-equilibrium behavior to have very low unfolding frequencies $\nu_{\text{unf}} = \nu_{\text{att}} \exp (\Delta E (F) / k_B T)$, for instance $10^{-6}$ to $10^{-2} \text{s}^{-1}$ for $F = 2pN$, $10^{-3}$ to $1 \text{s}^{-1}$ for $F = 4pN$ and 0.3 to $50 \text{s}^{-1}$ for $F = 8pN$ 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 advantage. Strong molecular motors like RNA polymerase and helicase or microtubuli during cell division are strongly kinetically protected from mechanical disruption due to the fiber geometry. By nature and has flown into the chromatin fiber design.

For the force length relation $\lambda \sim \sqrt{A/F}$, cf. R. Bruinsma and J. Rudnick, Biophys. J. 76, 1725 (1999).

The experiments were performed on DNA chains with up to 17 nucleosomes complexed at well-defined positions. In the force range of interest their coupling can be savely neglected since the intranucleosomal distance $d \sim 40 \text{nm}$ exceeds the DNA-linker induced interaction length $\lambda \sim \sqrt{A/F}$. Birch and J. Rudnick, Biophys. J. 76, 1725 (1999).

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[21] Note that the latter alone would not explain the sudden catastrophic behaviour and the slow kinetics of unfolding if the effects described by Eq. were not included explicitly.
[22] The upper/lower estimate correspond to $\varepsilon_{\text{ads}} = 0.2 / 0.3 k_B T / \text{nm}$; note the strong dependence of $\nu_{\text{att}}$ on $\varepsilon_{\text{ads}}$. 


