Apparent persistence length renormalization of bent DNA

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We derive the single molecule equation of state (force-extension relation) for DNA molecules bearing sliding loops and deflection defects. Analytical results are obtained in the large force limit by employing an analogy with instantons in quantum mechanical tunneling problems. The results reveal a remarkable feature of sliding loops—an apparent strong reduction of the persistence length. We generalize these results to several other experimentally interesting situations ranging from rigid DNA-protein loops to the problem of anchoring deflections in atomic force microscopy stretching of semiflexible polymers. Expressions relating the force-extension measurements to the underlying loop or boundary deflection geometry are provided and applied to the case of the gal repressor dimer protein. The theoretical predictions are complemented and quantitatively confirmed by molecular dynamics simulations.

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In nature, DNA is rarely found in its straight "naked" state as usually depicted in the introductory pages of elementary textbooks. In most *in vivo* situations, an overwhelming fraction of DNA is rather strongly configurationally constrained by binding proteins causing loops, bends, and deflections. The advent of single molecule stretching techniques [1] has opened the possibility of measuring the "equation of state" of single tethered DNA molecules in a variety of different conditions [2]. While the statistical mechanics of unconstrained DNA under tension is theoretically well understood in the framework of the wormlike chain (WLC) model [3] the presence of topological constraints such as supercoiling [4,5] or geometrical constraints such as protein-induced kinks and bends [6–9] renders analytical results more difficult. In this paper we expand the repertoire of analytically solvable equations of state by deriving the force-extension relation for a DNA molecule featuring loops and large deflections in the limit of strong stretching forces (for the small forces case see [7]). The computation is performed by evaluating quadratic fluctuations around the looped solution—a nonconstant saddle point of the DNA elastic energy. The method is essentially analogous to the semiclassical treatment of tunneling amplitudes in quantum mechanics and instantons in quantum field theory [10]. After deriving the general result [that we accompany with molecular dynamics (MD) simulations] we focus on two interesting experimental applications: the stretching of the gal repressor (GalR) loop complex [11], and of tangentially anchored semiflexible polymers from a surface in atomic force microscopy (AFM) experiments.

Stretching a sliding loop. In the following we neglect the DNA twist degree of freedom for it is not constrained from outside and no external torsional torques are acting on it. In this case, DNA of length L is described in the continuum limit by specifying only the unit vector tangent $\underline{\mathbf{t}}(s)$ to the chain. Here s is the contour length along the DNA with $-L/2<s<L/2$. The chain is submitted to an external constant force so that the kinetic plus potential energy of such a chain reads

$$E_0=\int_{-L/2}^{L/2}\left[\frac{A}{2}\left(\frac{d\underline{\mathbf{t}}}{ds}\right)^2-\mathbf{F}\cdot\underline{\mathbf{t}}\right]ds$$

with the bending stiffness $A=l_P k_B T$ where l_P is the orientational persistence length and $k_B T$ the thermal energy; for DNA at room temperature $l_P\approx 50$ nm [12].

In the following we parametrize the tangent as $\underline{\mathbf{t}}=(\cos\phi\cos\vartheta,\sin\phi\cos\vartheta,\sin\vartheta)$ and put the force along the x axis so that the potential energy part writes $-F\cos\phi\cos\vartheta$. Note that the angle ϑ is measured with respect to the equatorial plane (as on a globe). This parametrization is necessary to take properly into account the inextensibility of the chain imposed by the condition $\underline{\mathbf{t}}^2=1$. In the following it is convenient to introduce the dimensionless contour length $t=s/\lambda$ with the *deflection length* $\lambda=\sqrt{A/F}$ [6,13]. The latter becomes the relevant length scale characterizing the loss of orientational correlation in the case of DNA under large tension (replacing the usual tension-free persistence length $l_P=A/k_B T$). In these coordinates the chain energy writes

$$E_0=\sqrt{AF}\int_{-L/2\lambda}^{L/2\lambda}\left[\frac{1}{2}(\dot{\phi}^2\cos^2\vartheta+\dot{\vartheta}^2)-\cos\phi\cos\vartheta\right]dt. \tag{1}$$

The relevant saddle point—the loop in the x-y plane shown in Fig. 1(a)—satisfies $\delta E_0=0$ with the boundary conditions $\vartheta_{loop}(\pm L/2\lambda)=0$, $\phi_{loop}(\pm L/2\lambda)=0$, and 2π. In the limit of large lengths $L/\lambda\to\infty$ the loop solution is the well-known "kink" solution from theory of quantum tunneling [10] $\cos\phi_{loop}=1-2\cosh^{-2}(t)$ and $\vartheta_{loop}=0$ with the loop energy given by $E_{loop}=8\sqrt{FA}-LF$. For large values of $\sqrt{AF}$ we can expand E_0 in terms of quadratic fluctuations $(\delta\vartheta,\delta\phi)$ around the looped saddle point $(\vartheta_{loop},\phi_{loop})$

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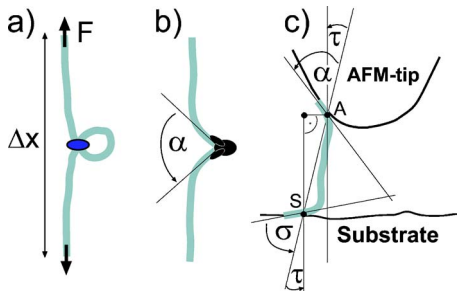

FIG. 1. (Color online) Examples for large deflections in a stretched DNA molecules. (a) A freely sliding linker protein stabilizes a DNA loop. (b) A rigid ligand with opening angle α causes a kink in the DNA. (c) Tangentially anchored DNA stretched from a surface by an AFM tip. The tilting angle τ as well as anchoring angles α and σ can strongly affect the elastic response.

$$E_0 = E_{loop} + \frac{\sqrt{AF}}{2}\int \delta\phi \hat{\mathbf{T}}_{\parallel}\delta\phi dt + \frac{\sqrt{AF}}{2}\int \delta\vartheta \hat{\mathbf{T}}_{\perp}\delta\vartheta dt, \tag{2}$$

with the in- and out-of-plane fluctuation operators $\hat{\mathbf{T}}_{\parallel} = -\partial^2/\partial t^2 + (1 - 2\cosh^{-2} t)$ and $\hat{\mathbf{T}}_{\perp} = -\partial^2/\partial t^2 + (1 - 6\cosh^{-2} t)$, respectively. A closer inspection of the discrete spectrum of the two operators reveals the physics behind. $\hat{\mathbf{T}}_{\parallel}$ has a zero eigenvalue resulting from the translational shift invariance of the loop along the chain that costs no energy for $L/\lambda \to \infty$. The absence of negative eigenvalues is in agreement with intuition, as in two dimensions, the loop is a (topologically) stable saddle point. The out-of-plane fluctuation operator $\hat{\mathbf{T}}_{\perp}$ shows a richer behavior. Again it possesses a zero mode, this time resulting from rotational invariance of the problem. More remarkably, in contrast to $\hat{\mathbf{T}}_{\parallel}$ the out-of-plane fluctuation operator $\hat{\mathbf{T}}_{\perp}$ has a negative eigenvalue -3. The latter underlines the intrinsic instability of the loop in three dimensions as described by the elastic energy expressions (1) and (2). This brings us to the question how to describe the obviously stable physical situation shown in Fig. 1(a). In order to model the action of a sliding linker [7] we introduce a term accounting for the DNA self-interaction. In lowest order it can be written as a function of the perpendicular distance $\Delta z_c = \lambda \int_{-t_c}^{t_c} \sin \delta\vartheta(t) dt \approx \lambda \int_{-t_c}^{t_c} \delta\vartheta(t) dt$ of the two overcrossing DNA arms at the contact point $t_c \approx 1.915$ (resulting from the crossing condition $t_c = 2 \tanh t_c$). The total energy of the chain can now be written as

$$E_{tot} = E_0 + V\left(\lambda \int_{-t_c}^{t_c} \delta\vartheta(t) dt\right), \tag{3}$$

with a short-ranged, but otherwise arbitrary, attractive interaction potential V acting at the crossing point. Note that if $V(\Delta z_c)$ has a minimum at $\Delta z_c = 0$ the loop saddle point stays unaffected by the self-interaction. DNA is known to effectively attract itself in many solvents despite its strong negative bare charge. Typical situations inducing DNA self-attraction are poor solvents (such as alcohol, and small neutral polymers such as polyethylene glycol) [14], the presence of multivalent counterions (like hexaammine cobalt (111) and Spermidine) or small cationic proteins acting as linkers between two DNA surfaces. Single molecule stretching experiments on DNA condensed with multivalent counterions performed by several groups [15] might bear loops or related structures such as DNA toroids [16].

The partition function of the system in Fig. 1(a) can generally be written as $Q_{loop} = \int_{loop} \delta(\underline{\mathbf{t}}^2 - 1)\mathcal{D}^3[\underline{\mathbf{t}}] e^{-\beta E_{tot}[\underline{\mathbf{t}}]}$ where $\int_{loop}$ represents the path integral over the functional neighborhood of the loop solution and the δ function enforces the chain inextensibility. This partition function is nothing but the Euclidean path integral of a quantum particle moving on an unit sphere under the influence of an external constant force. Using the ϕ, ϑ parametrization, the partition function can be rewritten as $Q_{loop} = \int \mathcal{D}[\phi]\mathcal{D}[\vartheta] e^{-\beta E_{tot}[\vartheta,\phi] - \ln C_m}$ with a metric term $C_m = \exp\{\int \delta(0) ds \ln[\cos(\vartheta)]\}$ resulting from the inextensibility constraint. It can be shown that this term does not contribute at the quadratic level of the approximation because we expand around $\vartheta_{loop} = 0$. By virtue of the decomposition of the in- and out-of-plane fluctuations at the quadratic level [Eqs. (2) and (3)] the partition function can be conveniently factorized,

$$Q_{loop} = e^{-\beta E_{loop}} Q_{\parallel} Q_{\perp}^{V}, \tag{4}$$

with

$$Q_{\perp}^{V} = \int \frac{d(\Delta z_c)}{\lambda} e^{-\beta V(\Delta z_c)} Q_{\perp}(\Delta z_c), \tag{5}$$

and with the in- and out-of-plane plane partition functions

$$Q_{\parallel} = \int^{*} \mathcal{D}[\delta\phi] e^{-(\beta\sqrt{AF}/2)\int \delta\phi \hat{\mathbf{T}}_{\parallel}\delta\phi dt}, \tag{6}$$

$$Q_{\perp} = \int^{*} \mathcal{D}[\delta\vartheta]\delta\left(\frac{\Delta z_c}{\lambda} - \int_{-t_c}^{t_c} \delta\vartheta dt\right) e^{-\beta\sqrt{AF}/2\int \delta\vartheta \hat{\mathbf{T}}_{\perp}\delta\vartheta dt}. \tag{7}$$

The notation $\int^{*}$ reminds us that the (translational and rotational) zero modes of $\hat{\mathbf{T}}_{\parallel}$ and $\hat{\mathbf{T}}_{\perp}$, respectively, have to be handled with care. A naive Gaussian integration would lead to a formal divergence in both cases. After dealing with this problem by introducing collective coordinates, a method well known from tunneling theory [10], and applying the Gelfand-Yaglom method for the computation of the fluctuation determinant we obtain the in-plane partition function $Q_{\parallel} = (4/\pi)\beta L F e^{-(L/2)\sqrt{F/A}}$.

The computation of $Q_{\perp}$ which follows similar lines of reasoning, requires rewriting the δ function in Eq. (7) in its Fourier representation. After introducing Π_c, the characteristic function of the interval $[-t_c, t_c]$ (i.e., $\Pi_c(t) = 1$ for $t \in [-t_c, t_c]$ and $=0$ otherwise), we rewrite $\int_{-t_c}^{t_c} \delta\vartheta dt = \int_{-\infty}^{\infty} \Pi_c(t)\delta\vartheta(t) dt$ as a scalar product. The path integral in Eq. (7) becomes a Gaussian with a source term $ik\Pi_c$ and can be solved by constructing the Green's function for $\hat{\mathbf{T}}_{\perp}$. This leads to the result

$$Q_\perp = 8\sqrt{\frac{\Gamma}{\pi}}\left(\frac{l_P}{\lambda}\right)^{3/2} e^{-(L/2\lambda)} e^{\Gamma(l_P/\lambda^3)z^2},$$

with $\Gamma = 2/(9t_c^3 - 30t_c) \approx 0.35$ a numeric constant. Combining those results we obtain

$$Q_\perp^V = \frac{8}{\lambda}\sqrt{\frac{\Gamma}{\pi}}\left(\frac{l_P}{\lambda}\right)^{3/2} e^{-L/2\lambda} \int e^{\Gamma(l_P/\lambda^3)z^2 - \beta V(z)} dz. \quad (8)$$

The resulting force extension relation $\langle \Delta x \rangle = k_B T(\partial/\partial F) \ln Q_{loop}$ writes in leading order

$$\frac{\langle \Delta x \rangle}{L} = 1 - \frac{1}{2\sqrt{\beta l_P}}\left(1 + 8\frac{l_P}{L}\right)\frac{1}{\sqrt{F}} + \frac{9}{4\beta L F} + \frac{3\Gamma}{2}\frac{1}{L\lambda}\frac{\int_{-\infty}^{\infty} z^2 e^{\beta\Gamma A^{-1/2}F^{3/2}z^2 - \beta V(z)} dz}{\int_{-\infty}^{\infty} e^{\beta\Gamma A^{-1/2}F^{3/2}z^2 - \beta V(z)} dz}. \quad (9)$$

In the limiting case of a very deep potential $V(z)$ strongly localized around $z=0$, the last term is negligible and the force-extension relation becomes independent of the detailed nature of the contact interaction. If we in addition consider large forces, the $O(1/\beta LF)$ term can be neglected and the relation (9) can be cast in a more illuminating form

$$\frac{\langle \Delta x \rangle}{L} \approx 1 - \frac{1}{2\sqrt{\beta l_P^{app}}\sqrt{F}} \quad (10)$$

that resembles the well-known loop-free WLC response $\langle \Delta x \rangle / L \approx 1 - (2\sqrt{\beta l_P}\sqrt{F})^{-1}$ [3], but with a strongly renormalized apparent persistence length l_P^{app},

$$l_P^{app} = l_p\left(1 + 8\frac{l_p}{L}\right)^{-2}. \quad (11)$$

Equations (10) and (11) show that one has to be very cautious when interpreting experimental stretching data in terms of persistence length and stiffness. The presence of a loop modifies the elastic response of the chain in such a manner that the persistence length can appear effectively reduced as stated in Eq. (11). For a single loop this is obviously a finite-size effect involving the scaled total length L/l_p. But the effect remains significant over a large range of parameters: for $l_P/L = 0.1$ one finds $l_P^{app} \approx 0.31 l_P$ whereas for $l_P/L = 0.02$ there is still a remarkable effect, namely $l_P^{app} \approx 0.74 l_P$. We mention that an apparent reduction of stiffness has also been found in Ref. [17] for a WLC with folding elements but there only in the low force regime.

To check the prediction of Eqs. (10) and (11) we performed MD simulations [18]. As seen from Fig. 2(a) the theoretical predictions quantitatively agree with the simulation results.

Stretching kinked DNA. The same approach can be used to calculate the elastic response of DNA with deflections as depicted in Figs. 1(b) and 1(c) [19]. However, having solved the sliding loop problem we can obtain the results much more directly. To see this we rewrite Eq. (10) in terms of the three relevant lengths L, l_P, and λ,

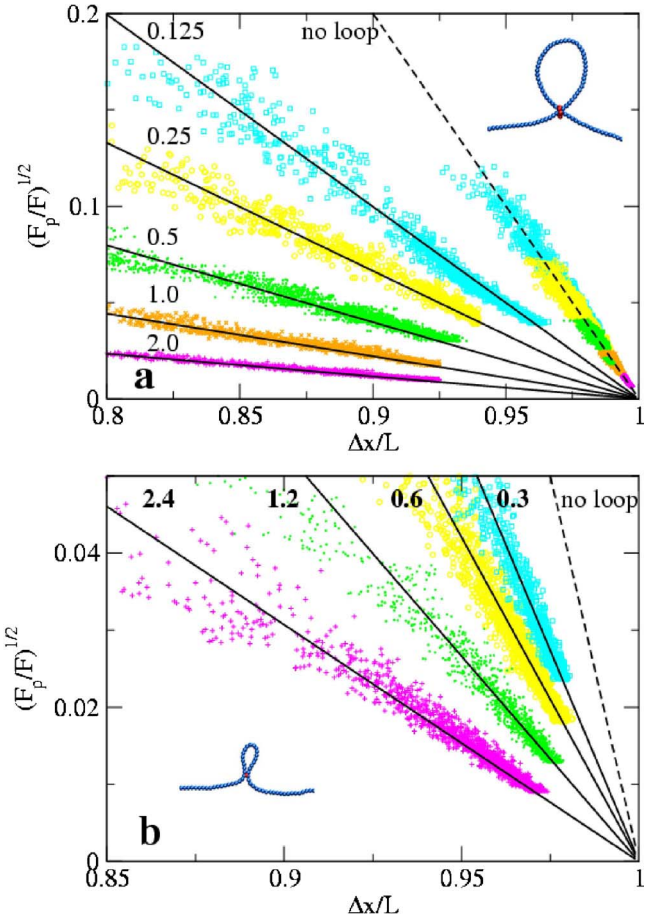

FIG. 2. (Color online) (a) Stretching DNA looped with a sliding linker: Comparison of MD simulation results (data points) with theoretical prediction (solid lines) for the force-extension relation as given by Eq. (10). The force is measured in units of $F_P = k_B T/l_P$. The different slopes correspond to different values of l_P/L. The dashed line shows free chain (no loop) behavior. (b) The stretching of a GalR loop complex: MD simulation (dots) vs theory, Eq. (13) (solid lines) for various values of l_P/L.

$$\frac{\langle \Delta x \rangle}{L} \approx 1 - \left(\frac{\lambda}{2l_P} + \frac{4\lambda}{L}\right). \quad (12)$$

The first term in the brackets is the length loss by thermal fluctuations of a straight DNA chain-with the fluctuation contribution of the loop being negligible in the large F limit ($\lambda \ll L$). The second term in the bracket, $4\lambda/L$, describes the loss of length due to the (loop-induced) elastic deflection. This asymptotic decomposition of the two contributions in 12 leads to immediate generalizations. After short inspection it is easy to see that any strongly localized [20] DNA deflection [as in Figs. 1(b) and 1(c)] can be appropriately continued and mapped piecewise onto fractions of the full loop solution [Fig. 1(a)]. By this reasoning, any length loss induced by a given deflection angle α (between 0 and 2π) is given by $\int_{s_0(\alpha)}^{\infty}[1 - \cos\phi_{loop}(s)]ds$.

As a first example consider the stretching of rigid DNA-protein complexes that come as large kinks or fixed angle loops [cf. Fig. 1(b)]. A prominent example is the GalR re-

pressor complex that was studied in single molecule experiments [11]. Following the upper reasoning one obtains the same force-extension relation as in Eq. (10) but with an apparent persistence length given as a function of the opening angle α of the complex,

$$l_P^{app} = \frac{l_p}{\left\{1 + 8\frac{l_p}{L}\left[1 - \cos\left(\frac{\pi - \alpha}{4}\right)\right]\right\}^2} \tag{13}$$

We note that a previous numerical study of a discretised version of the WLC with local kinks showed a rescaling of the force-extension curve that can be described by our formula (cf. Fig. 5 in Ref. [8] for $\alpha = 90°$). For the GalR complex the opening angle is not known but there are some indications for the antiparallel configuration, i.e., $\alpha \approx 0$ [11,21]. With Eqs. (10) and (13) at hand, one can predict the loss of length due to the presence of a GalR complex with the conjectured angle $\alpha = 0$. For the force $F = 0.88$ pN applied in the experiment [11] with a loop size of 38 nm [11], Eqs. (10) and (13) predict a loss of length of 56 nm (with the DNA hidden inside the loop included). Remarkably, the experimental Lia *et al.* [11] is 55 nm $\pm$ 5 nm. The latter result together with (10) and (13) gives convincing evidence for the antiparallel loop model. An independent check of (10) and (13) is provided by MD simulations [shown in Fig. 2(b)], which were performed for various ratios of l_p/L. In conclusion, Eq. (10) enables one to directly measure the angle α—an important feature of the DNA-protein complex geometry.

A second application concerns AFM stretching experiments of semiflexible polymers [22]. A stable anchoring can be achieved when the polymer is tangentially attached at its two ends as in Fig. 1(c). Force extension data in such a setup have to be interpreted with care. The boundary anchoring angles at the AFM tip as well as at the surface—α and σ, respectively—can significantly alter the measured apparent persistence length, a fact that has been overlooked before. A more trivial effect of a tilting angle τ in Fig. 1(c) (between the line of contact points and the force direction) can alter the result in addition [23]. A simple calculation for large forces F again gives the same functional relation as in (10) with an "apparent persistence length" given by

$$l_P^{app} = \frac{l_p \cos(\tau)}{\left\{1 + 8\frac{l_p}{L}\left[1 - \frac{1}{2}\left(\cos\frac{\alpha}{2} + \cos\frac{\sigma}{2}\right)\right]\right\}^2}. \tag{14}$$

While the angle τ can be completely canceled by shifting the tip in the lateral direction, the influence of α and σ cannot be fully eliminated by this procedure. This means that for short- to intermediate-sized semiflexible polymers ($l_P/L \sim 1 - 1/30$) the influence of boundary conditions has to be taken into account through Eq. (14).

In conclusion, the force-extension relation of looped DNA that we derived in the limit of a strong stretching force can be generalized to DNA featuring large deflections. In particular our approach provides an analytical expression for the force-extension response of DNA-bending proteins that can give important information with regard to the DNA-protein complex geometry. Large deflections due to the anchoring at the AFM tip and at the substrate also affect the measured persistence length for not too long molecules. Finally these effects might be related to the strong reduction of the persistence length found in condensed DNA stretching experiments by Baumann *et al.* [15].

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[18] Langevin MD simulation of a semiflexible bead-spring chain

with up to 200 beads; the stretching force applied at the end beads; the contact potential was realized by a flexible sliding linker, i.e., closed bead-spring chain [7].

[19] The exact force-extension relations (satisfying boundary conditions at finite intervals) involving elliptic integrals are omitted here. The contributions of the boundary conditions and the breaking of translational symmetry both involve $O(1/\beta LF)$ terms that are negligible in the large force limit leading immediately to Eqs. (13) and (14).

[20] By strongly localized we mean a deflection that decays to zero on a length scale much shorter than the whole molecule.

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