

Theoretical models for tension-dependent DNA looping time

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The influence of tension on DNA looping has been studied both experimentally and theoretically in the past. However, different theoretical models have yielded different predictions, leaving uncertainty about their validity. These currently available models rely on approximations, which we here show to be valid only for short molecules. We briefly review the predictions of those models and propose a model that demonstrates exceptional agreement with simulations for long semiflexible chains. Additionally, we elucidate the relationship between our result and that of the previously proposed two-state model, highlighting the distinct interpretative approach that underpins our framework. Our findings offer predictive insights that pave the way for future experimental validation.

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Introduction. DNA looping is a topic of high interest in the field of biology, as it is important for gene regulation [1–3], as well as DNA recombination, packaging, and many more [4]. These DNA loops can vary considerably in size. In *Escherichia coli*, typical loop lengths range from 100 to 600 base pairs (bp) [5], although significantly longer loops have also been observed. For instance, the *glpFKX* operon, regulated by the transcription factor *GlpR*, has been reported to form loops as long as 1122 bp [6,7]. Even larger loops, up to 2300 bp, have been observed in the case of DNA looping mediated by the *CI* repressor protein of bacteriophage λ [8]. Protein-mediated DNA loops are mainly driven by thermal fluctuations with forces around 80 fN [9]. However, within the highly dynamic and out-of-equilibrium environment of a biological cell, DNA is continually subjected to piconewton-scale forces from its intracellular surroundings, which can exceed those that stem from typical thermal fluctuations by an order of magnitude [9–11]. This effect of tension on the looping time was measured using optical trapping, and it became clear that forces less than a piconewton can increase the looping time by an order of magnitude [12] (see Fig. 1 for an illustration).

This dependence of the looping time on the tension in the DNA has been investigated theoretically in the past by analyzing the cyclization process of a semiflexible polymer. Blumberg *et al.* [13] started this exploration, studying protein-mediated DNA looping as a two-state system under the assumption of detailed balance. Later, Shin and Sung [14] investigated the same topic as a barrier escape problem.

In the low-force regime ($f < 80$ fN), which is particularly relevant in biological contexts, the theories disagree in their predictions for the force dependence of the loop formation time. The first theory suggests that the looping time increases exponentially in f^2 , while the second theory indicates an almost exponential increase in f . Up to this day, it remains unclear which—if either—is correct. Moreover, in this low-force regime, there are no experimental data available to put these theories to the test. At higher forces, one can show that the barrier escape approach of Shin and Sung [14] is in very good agreement with the available experimental data of Chen *et al.* [12], while the two-state model of Blumberg *et al.* [13] is not, as we discuss in Sec. II.

In this Letter, we demonstrate that both the two-state model of Blumberg *et al.* [13] and the barrier escape approach of Shin and Sung [14] are applicable only to short DNA chains under high tension. The latter theory shows excellent agreement with the experimental data of Ref. [12]. Furthermore, we show that the failure of this theory at low tension stems from approximations in the free energy used by Shin and coworkers, rather than from inherent limitations of the barrier escape formalism. When the free energy is instead corrected, we find the barrier escape approach to accurately describe the looping of short chains across the entire force range. However, no existing model reliably describes the force-dependent looping dynamics of long DNA chains. To address this, we introduce a different approach based on the inverse scaling with the equilibrium looping probability, which we find to be in excellent agreement with our simulation results across a broad force range. Furthermore, we provide experimentally verifiable predictions and thus pave the way for future experimental investigations.

Review of prior theories. Theoretical models for protein-mediated DNA looping approximate the system simply as the cyclization of a semiflexible chain, with the ends representing the binding sites. Shin and Sung [14] studied the looping process as a barrier escape problem: Imagining one of the ends

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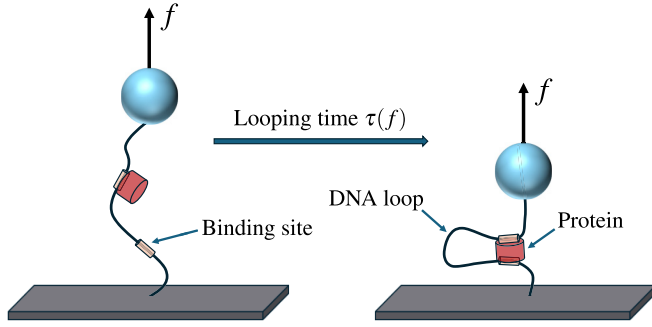


FIG. 1. Protein-mediated DNA looping occurs when two distal binding sites come into close contact and become bound by a protein or protein complex. Using a tethered bead, tension can be applied resulting in a force-dependent looping time $\tau(f)$. Such experiments have been performed in Ref. [12] and show that forces below a piconewton can alter the looping time by an order of magnitude.

of the polymer as fixed, the other can be seen as diffusing in an effective free energy landscape $F(r)$, with r the end-to-end distance. The looping time then becomes the mean first passage time for this diffusing end to reach a certain capture radius r_c (distance between the binding sites when bound by protein) and reads [14,15]

$$\tau_{\text{Shin}}(f) \sim \frac{1}{Z} \int_{r_c}^L dr \int_{r_c}^r dr' \int_{r'}^L dr'' e^{-\beta[F(r)-F(r')+F(r'')]}, \quad (1)$$

with $Z = \int_{r_c}^L e^{-\beta F(r)} dr$ the partition function, $\beta = (k_B T)^{-1}$ the inverse temperature, and L the total DNA length between the two binding sites. Although in an optical tweezer experiment, the force is applied in a specific direction, one can assume the end-to-end distance to align with the force at sufficiently high tension, resulting in a free energy of the form $\beta F_{\text{Shin}}(r) = -\ln[P(r, f=0)] - \beta f r$. For $P(r, f=0)$, Shin and Sung [14] used the mean-field result for a semiflexible chain [14,16]:

$$P_{\text{MF}}(r, f=0) \sim r^2 \left[1 - \left(\frac{r}{L} \right)^2 \right]^{-9/2} e^{\frac{-3L}{4l_p} \left[1 - \left(\frac{r}{L} \right)^2 \right]}. \quad (2)$$

By contrast, Blumberg *et al.* [13] approached the looping process as a two-state system: Either the polymer is looped or it is not. Denoting the energy difference between the looped and unlooped states as $\Delta G(f) = F(z_c) - F(\langle z(f) \rangle)$, with z the projection of the end-to-end distance along the force, $\langle z \rangle$ its average value, and z_c the capture distance such that a loop obeys $z \leq z_c$, they argued that [13]

$$\tau_{\text{Blum.}}(f) \sim e^{\beta \Delta G(f)}. \quad (3)$$

By integration of the Marko-Siggia force-extension relation of a semiflexible chain [17], they found the following free energy [13,18]:

$$\beta F_{\text{Blum.}}(z) = \frac{1}{l_p} \left[-\frac{L^2}{4(z-L)} + \frac{z^2}{2L} - \frac{z}{4} \right] - \beta f z, \quad (4)$$

where the force is chosen to be in the z direction. Although both approaches are different, one could in fact see the two-state model as an approximation of the barrier escape approach. In a two-state model [13], one neglects the

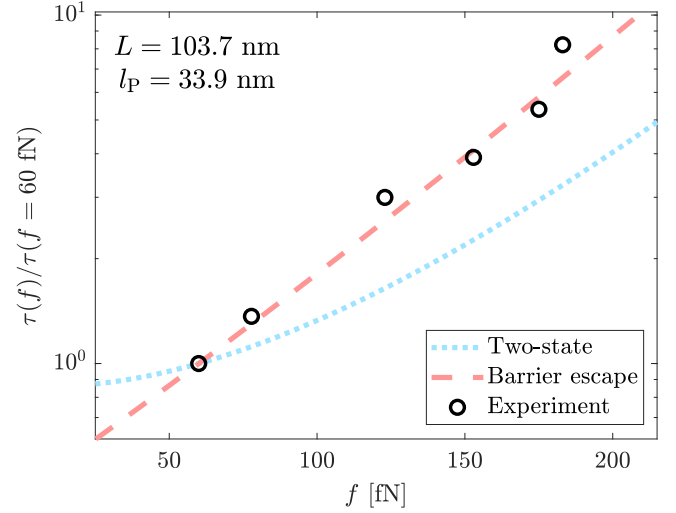


FIG. 2. Experimental data of Chen *et al.* [12] measured using an optical tweezer setup, compared to the theories of Blumberg *et al.* [13] (two-state) and of Shin and Sung [14] (barrier escape). We used $L = 103.7$ nm, $r_c = z_c = 5$ nm, $\beta = 4114^{-1}$ fN nm and $l_p = 33.9$ nm. (The data were extracted from Ref. [12] using Plot-Digitizer. See Appendix A for additional information regarding the choice of parameters.)

transition path, which is integrated over in the approach of Shin and Sung [14]. Mathematically, this makes Eq. (3) a first-order saddle-point approximation of Eq. (1) using the same free energy. In Fig. 2, we show as black circles the experimental data of Chen *et al.* [12], together with the prediction of Blumberg *et al.* [13] (two-state) and of Shin and Sung [14] (barrier escape). As is clear, the latter is in excellent agreement with the experimental data, while the former is not. We note, however, that it is difficult to judge a theoretical model based on one experiment only. Therefore, we will compare to simulation data for varying polymer lengths and over a more extended force regime, once we have introduced our model.

Theoretical model. In an ergodic Markov chain with a stationary distribution, the mean recurrence time of a state is inversely proportional to the long-term probability of occupying that state [19]. This relationship arises naturally, as the stationary probabilities represent the average fraction of time the system spends in each state. Without tension, the looping time was also shown by Jun *et al.* to be inversely proportional to the equilibrium probability to form a loop [20]. We confirm these findings in Appendix C. Recent work reached the same conclusion for a freely jointed chain under tension [18]. There, it was also demonstrated that the barrier escape approach of Eq. (1) fails when local equilibrium is not satisfied, even when the exact free energy landscape is used. In contrast, the inverse scaling with the equilibrium looping probability remained accurate, suggesting that this approach can remain valid even in the presence of some non-Markovian effects. Here, we extend these recent findings to semiflexible chains, connecting them to the work of Jun *et al.* We start by demonstrating that the inverse relationship between looping time and looping probability reproduces the same scaling in f as the two-state model of Blumberg *et al.* [13] for $z_c \rightarrow 0$.

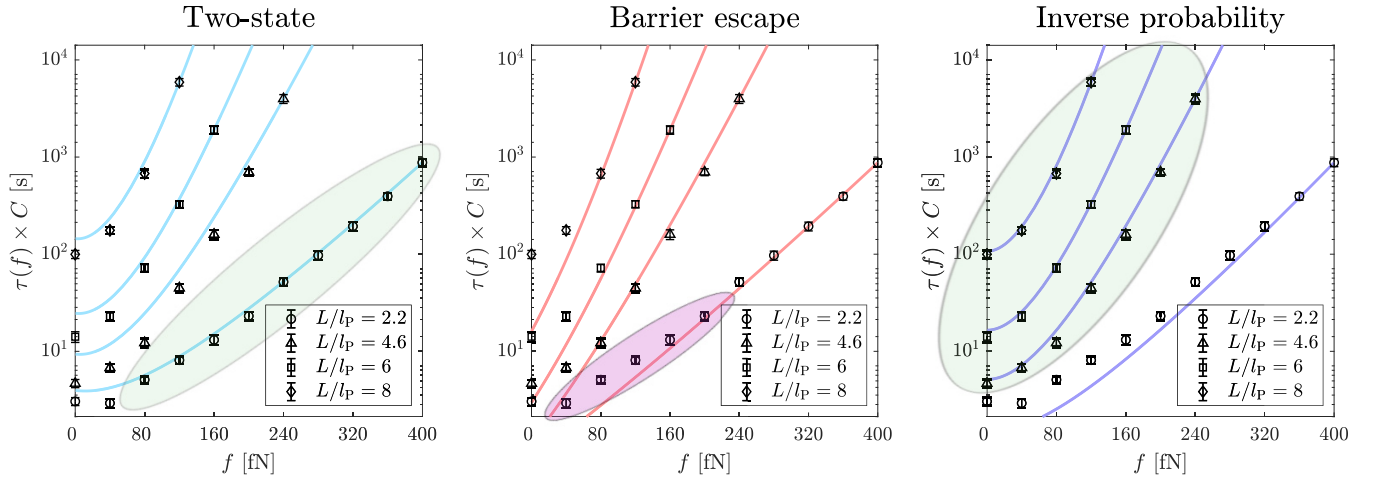


FIG. 3. Three different theories (from left to right) are shown: Blumberg *et al.* [13] (two-state model), Shin and Sung [14] (barrier escape approach), and our model, using the inverse scaling with the looping probability. These are compared with simulations of a semiflexible chain. To reduce visual clutter near $f = 0$, a force-independent prefactor $C = 1, 2, 5, 20$ was introduced for $L/l_p = 2.2, 4.6, 6, 8$, respectively. The protein size was set to $r_c = 10$ nm. Since the theories predict only the scaling with f , their curves were set to match the simulation data at the highest force data point for each chain length. One can observe that for a short chain ($L/l_p = 2.2$) at high forces ($f > 80$ fN), the two-state model makes a good prediction. For long chains, both prior theories fail, while our model on the right makes consistently a very good prediction on the whole force regime. The green areas highlight the region where good correspondence is found between theory and simulation. The pink area roughly corresponds to the region probed in the experiment of Chen *et al.* [12] shown in Fig. 2.

In the process, we recover the prediction that the looping time grows exponentially in f^2 for small values of f .

The derivation in this section does not make any assumptions on the specific polymer model and therefore holds for both flexible and semiflexible chains. Very generally, we denote the free energy for a polymer under tension F in function of the end-to-end vector $\vec{r}$ and applied force f as $F(\vec{r}; f)$ and note that the probability of observing a specific $\vec{r}$ in equilibrium can be written as [14,18]

$$P(\vec{r}; f) = \frac{e^{-\beta F(\vec{r}; f)}}{Z(f)} = \frac{e^{-\beta F(\vec{r}; f=0) + \beta f z}}{Z(f)}, \quad (5)$$

where we again take the force as applied in the z direction. As we are interested in the scaling in f , we can write down

$$P(\vec{r}; f) \sim \frac{e^{\beta f z}}{Z(f)}, \quad (6)$$

as $F(\vec{r}; f=0)$ does not depend on f . When a loop is formed, z is close to zero, by which the exponential factor can be neglected, resulting in

$$P_{\text{loop}}(f) \sim \frac{1}{Z(f)}. \quad (7)$$

That is, for long DNA molecules, $r_c \ll L$, such that the size of the protein can be neglected [18,21]. We also confirm this in Appendix D, where we compare simulation results of $\tau(f)$ for different r_c . Moreover, we can use the thermodynamic relation $G(f) = -k_B T \ln[Z(f)]$ and note that $G(f) = F(\langle z(f) \rangle)$. This last statement explains that the free energy $F(z)$ in function of the extension z yields the free energy $G(f)$ in function of the force f , when evaluated in the average extension $z = \langle z \rangle$. Hence, when the looping time is inversionally proportional to

the looping probability, we have

$$\tau(f) \sim e^{-\beta F(\langle z(f) \rangle)}, \quad (8)$$

which can be evaluated fully analytically (see Appendix D). That is, we recover the result of Blumberg *et al.* [13] [Eq. (3)] for $z_c \rightarrow 0$. One should, however, note that we got this result from a different interpretation, namely, as an inverse scaling with a looping probability, not as a two-state system. The validity of Eq. (8) has been confirmed previously for a freely jointed chain in Ref. [18], and below, we will show it holds for long semiflexible chains as well.

Results. In order to validate the theoretical predictions, we modeled the semiflexible chain as a bead-spring model undergoing Langevin dynamics, of which the details are provided in Appendix B. In Fig. 3, we compare all three theories to simulation data, where the areas in green highlight good correspondence and the pink area marks the region that roughly corresponds to what is currently probed in optical tweezer experiments [12], as shown in Fig. 2. Note, however, that the curves have been rescaled by a length-dependent factor C to improve readability. As a result, the actual timescales for longer loops are expected to be lower than they appear in the figure. For short chains at high tension, the two-state model makes a very good prediction (green area, left panel). This can be understood as follows: The two-state model is a first-order saddle-point approximation of the barrier escape approach. Hence, (1) it has to fulfill local equilibrium, because all the degrees of freedom are mapped to a single reaction coordinate [18,20,22], and (2) the barrier has to be high, as it is a saddle-point approximation. The former condition is satisfied for short chains as l_p/L is large, making the end-to-end distance a slow variable and thus enabling local equilibrium. The latter condition is satisfied under high tension. The barrier escape approach makes a very similar prediction at the high-

est forces but becomes less accurate more quickly when the force is lowered. It is important to note that the barrier escape approach of Shin and Sung [14] underperforms not because of the method itself [Eq. (1)], but due to the approximation of the free energy. While the two-state model remains valid for short chains under high tension, Eq. (1) should generally be applicable to short chains at any force, as local equilibrium is typically satisfied in this regime. This is demonstrated in Appendix E, where we apply the barrier escape approach using the correct effective free energy. There, we show that Eq. (1) can indeed reproduce the correct scaling with f for short chains at any force. For longer chains, however, both the two-state model and the barrier escape approach fail, as the assumption of local equilibrium breaks down and a single reaction coordinate theory is no longer valid.

In Fig. 2, we did, however, observe that it was the barrier escape approach using the free energy of Shin and Sung [14] that made the best prediction. The pink region in Fig. 3 approximately corresponds to the parameters probed in the experiment, as the length $L = 103.7$ nm closely matches the simulated case of $L/l_p = 2.2$. We must therefore conclude that the strong agreement between theory and experiment reported in previous work is not reproduced in our simulations and is thus likely coincidental. Several factors could potentially explain the apparent agreement between theory and experiment. For example, in short DNA constructs, effects such as DNA twist and the phasing of binding sites may play a significant role. These factors could alter the force dependence of the looping time in a way that fortuitously matches the theoretical prediction presented by Shin and Sung [14]. However, our simulation results suggest that this agreement is accidental rather than indicative of a general physical mechanism.

Finally, we show that our model using the inverse scaling with the looping probability consistently makes an excellent prediction for long chains on the whole force regime (green area, right panel). For reasonable chain lengths ($L \gtrsim 4l_p$), our model really complements to prior descriptions of protein-mediated DNA looping under tension. Note as well that our model correctly predicts the quadratic-exponential increase in the looping time with respect to the force in the low-force regime, similar to the two-state model of Blumberg *et al.* [13]. This is because, for low forces ($\beta f l_p \ll 1$) and long chains ($L/l_p \gg 1$), a semiflexible chain becomes an entropic spring [23]; hence, the free energy is quadratic in f . This is not at all present in the barrier escape approach of Shin and Sung [14], which predicts an almost exponential increase instead due to the approximation for the free energy used. We now resolve this debate, by showing that the quadratic-exponential scaling in f is indeed correct for long semiflexible chains under low tension. Nevertheless, we stress again that our model is based on a different interpretation for why this is the case, than the two-state model of Blumberg *et al.* [13].

Conclusion. In this work, we studied protein-mediated DNA looping under tension as a cyclization process of a semiflexible polymer. We first compared two existing theories, namely, the barrier escape approach from Shin and Sung [14] and the two-state model from Blumberg *et al.* [13], with the experimental data of Ref. [12]. From this, we found that only the former approach seems to agree.

Next, we introduced a different theory, which starts from the idea that the looping time inversely scales with the equilibrium looping probability under an applied force f . From this, we were able to show that this reduces to the two-state model of Blumberg *et al.* [13] in the limit of a vanishing capture radius. Therefore, our findings support the prediction of Ref. [13] that in the smallest force regime, the looping time grows with the quadratic-exponential scaling in f . Nevertheless, we emphasize that the reduction of our model to a two-state framework arises purely as a mathematical coincidence. This highlights the potential risk of misinterpreting experimental data as fitting a two-state model when neglecting the size of the protein.

Comparing to simulations for a semiflexible chain, we found that for short chains under relatively high tension, the two-state model makes a good prediction, while for long chains, our inverse scaling with the looping probability performs well over the whole force range. We explained this by noticing that the two-state model is valid only under a local equilibrium assumption, which is valid for short chains, and as it is a saddle-point approximation, it is valid only at high tension. In contrast, our model assumes $r_c \ll L$ and is therefore applicable only for long chains. Whether refining the looping probability for short chains renders the inverse scaling valid for describing the force dependence of $\tau(f)$ remains an open question. Nevertheless, the barrier escape approach [Eq. (1)] is shown in Appendix E to correctly predict the scaling of the looping time with force f for short chains, provided that an accurate effective free energy is used. This is because the approach, like the two-state model, assumes local equilibrium and thus is valid only for short chains. However, unlike the two-state model, it does not require the presence of a high barrier, which allows it to function across a wider range of forces. When the barrier escape theory is applied using the approximate free energy of Shin and Sung [14], as shown in Fig. 3, its performance degrades significantly due to inaccuracies in the free energy landscape. Nevertheless, the barrier escape approach using the approximate free energy of Shin and Sung [14] showed very good agreement with the experimental data of Ref. [12], as illustrated in Fig. 2. This could not be confirmed by our simulations, as no good agreement between theory and simulation was observed in the regime relevant to the experiment (indicated by the pink area in Fig. 3). One possible explanation is that, particularly for short DNA lengths as studied in Ref. [12], DNA twist may affect how the looping time depends on the applied force, since the binding sites must be in phase [8]. As a result, the approximate free energy expression proposed by Shin and Sung [14] may inadvertently capture some of these twist-related effects. Constructing theoretical models is further complicated by the fact that, in longer DNA loops, supercoiling might modulate the looping dynamics [24]. Additional experimental evidence would be necessary to draw definitive conclusions on this.

In summary, we have shown that in order to describe the looping time of long semiflexible chains over a wide range of forces, our proposed method using the inverse of the looping probability performs best. This is relevant for kbp loops that can be found *in vivo* [6–8]. The more specific case of short chains is well described by the barrier escape approach

[Eq. (1)] when the correct effective free energy is used. Nevertheless, when the tension is sufficiently high, the two-state model of Blumberg *et al.* [13] provides a useful analytical expression. Our simulations also resolve the discussion regarding the force dependence of the looping time in the lowest force regime, showing that it does increase exponentially in f^2 in case of long semiflexible chains. We stress that our predictions should be within reach of experimental validation and are looking forward to the additional insights such experiments will yield.

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Data availability. The data that support the findings of this article are openly available [25].

Appendix A: Parameters for the optical tweezer experiment. In Ref. [12], the looping time was measured experimentally using optical trapping. A 1316 bp double-stranded DNA (ds-DNA) molecule with two primary lac operators was used, with one end attached to a polystyrene microsphere held in an optical trap [26]. The lac operators were spaced 305 bp apart, corresponding to a contour length of $L = 305 \times 0.34 \text{ nm} = 103.7 \text{ nm}$. We assume room temperature for all calculations, yielding an inverse thermal energy of $\beta = 4114^{-1} \text{ fN nm}$. For the capture radius, we adopt the value reported by Shin and Sung [14], namely, $r_c = 5 \text{ nm}$.

The final parameter to consider is the persistence length l_p . While the commonly accepted value is $l_p = 45 \pm 5 \text{ nm}$ [27], Shin and Sung [14] used $l_p = 50 \text{ nm}$ to compare with the experimental data of Ref. [12]. However, as noted by the authors of the experimental work reported in Ref. [26], fitting their measured force-extension curve to the semiflexible chain model with l_p as a free parameter yielded a significantly lower value of $l_p = 33.9 \text{ nm}$. This discrepancy may be due to the relatively short DNA length used in the experiment, as previous studies have observed a reduction in the apparent persistence length for short dsDNA [28].

From a theoretical perspective, this observation aligns with the idea that the persistence length may be length-scale dependent, a topic of ongoing research in DNA mechanics [29–33]. Since our theoretical framework is based on the semiflexible chain model, we adopted the experimentally fitted value of $l_p = 33.9 \text{ nm}$ in Fig. 2, as it provides the best match to the experimental data [26].

Appendix B: Simulation details. In order to validate the predictions of all the theoretical models, we performed molecular dynamics (MD) simulations of a semiflexible chain undergoing Langevin dynamics in the program LAMMPS [34]. The polymer is modeled as a bead-spring chain with $N + 1$ beads, connected by harmonic bonds with a bending potential between neighboring bonds. The spring energy is defined as

$$U_s = \frac{K}{2} \sum_{i=0}^{N-1} (|\vec{r}_{i+1} - \vec{r}_i| - b)^2, \quad (\text{B1})$$

where $\vec{r}_i$ is the position vector of bead i , b is the rest length of the bond (set to $b = 1$), and $K = 100$ is the spring constant. A

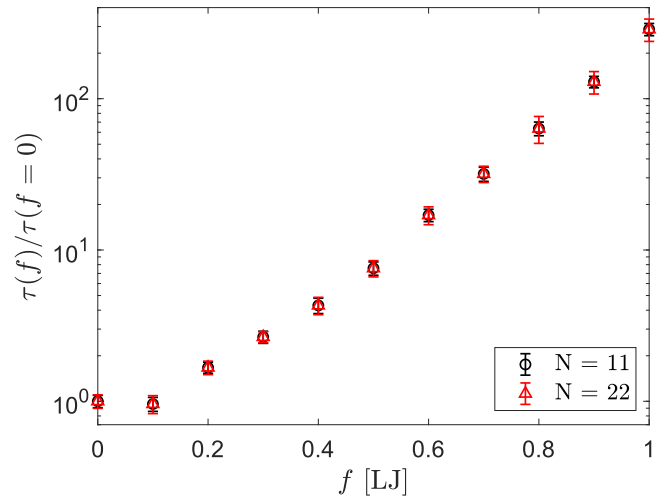


FIG. 4. Normalized looping time $\tau(f)/\tau(f=0)$ for $L = 11$ (corresponding to $L/l_p = 2.2$ in the main text), using two different levels of coarse graining. The first case (black circles) uses $N = 11$, $b = 1$, and $\kappa = 5$, while the second (red triangle) uses $N = 22$, $b = 0.5$, and $\kappa = 10$, such that $l_p = \beta\kappa b = 5$ remains unchanged. The two results are almost indistinguishable, indicating the absence of finite-size effects.

bending potential with stiffness $\kappa = 5$ is applied as

$$U_B = \frac{\kappa}{2} \sum_{i=1}^{N-1} \theta_i^2, \quad (\text{B2})$$

where θ_i is the angle between bond vectors $i - 1$ and i . To simulate tension, we applied equal and opposite forces on the two terminal beads along the z direction.

We use Lennard-Jones (dimensionless) units. The unit of energy is set by inverse temperature $\beta = (k_B T)^{-1} = 1$, and the friction coefficient is $\gamma = 1$. Bead masses are set to $m = 1$. The polymer is initialized in a straight configuration and equilibrated for 10^5 timesteps using LAMMPS's *fix langevin* command, with initial velocities sampled from a Gaussian distribution of variance 1. During equilibration, the timestep was $\Delta t = 0.05$. After equilibration, we switched to a smaller timestep $\Delta t = 0.005$ and used *fix ffl* [35] for the dynamics instead. Similar to Ref. [18], loop formation events were tracked using the REACTOR package [36]. Loop formation was defined as the moment when the two end beads came within the capture distance, taken to be $r_c = 1$.

Finite-size effects. To check for finite-size effects in the shortest simulated chain ($L/l_p = 2.2$), we repeated the simulation with finer coarse graining: $b = 0.5$, $N = 22$, and adjusted the stiffness to $\kappa = 10$ to maintain $l_p = \beta\kappa b = 5$. As shown in Fig. 4, this yielded consistent results, indicating negligible finite-size effects.

Error estimation. The looping time was averaged over 100 independent simulations per data point. The standard error is given by

$$\Delta\tau(f) = \frac{\sigma[\tau(f)]}{\sqrt{100}}, \quad (\text{B3})$$

with σ the sample standard deviation. In Fig. 3, the data are rescaled as $\tau(f)/\tau(f=0) \times C$, and the corresponding uncertainty is propagated as

$$\Delta \left[\frac{C \times \tau(f)}{\tau(f=0)} \right] \approx \frac{C \Delta \tau(f)}{\tau(f=0)}. \quad (\text{B4})$$

Unit conversion. To relate simulation units to physical quantities, we used the persistence length $l_p = \beta \kappa b$ [37], a commonly used quantifier for the bending stiffness of thermally fluctuating chains. By choosing a segment length of $b = 10$ nm (such that a unit length in the simulation corresponds to 10 nm), and given that dsDNA has $l_p \approx 50$ nm, we obtain a force scale of $k_B T/l_p \approx 80$ fN. With $l_p = 50$ nm and $\kappa = 5$, we find that a dimensionless force of $f = 1$ corresponds to approximately 400 fN [14].

Since the dynamics in biological systems includes additional slow steps, such as protein binding and orientation constraints, simulation times cannot be directly mapped to real units. However, these effects are expected to weakly depend on force and act as a constant rescaling factor. To estimate physical timescales, we align the simulation data with experimental results from Ref. [12], where the looping time for a short chain ($L/l_p \approx 2.2$) under $f \approx 80$ fN was measured as $\tau \approx 5.1$ s. This allows us to map dimensionless times to seconds via a global prefactor. For the capture radius r_c of the looping protein, we use $r_c \approx 10$ nm [21,38].

The simulated loop sizes span the range $L/l_p = 2.2$ to $L/l_p = 8$, capturing both short and long loops relevant to biological systems. Both types of loops have been observed in *E. coli* [8] and are relevant for gene regulation scenarios discussed in the main text.

Appendix C: Looping time without tension. An additional advantage of the inverse relationship between looping time and looping probability is that it also enables the exploration of how the looping time depends on other parameters, such as the DNA length, as noted previously in Ref. [20]. In Fig. 5, we illustrate this by comparing simulation results for $\tau(f=0)$ with the inverse looping probability across a range of polymer lengths L . We again find very good agreement between theory and simulation for sufficiently long chains.

For a stiff semiflexible chain in the continuum limit, the end-to-end probability distribution $P(\vec{r})$ is given by [39]

$$P(\vec{r}) = \frac{1}{Z} \int_0^L \mathcal{D}[\hat{t}(s)] e^{-\frac{\beta l_p}{2} \int_0^L \left(\frac{d\hat{t}}{ds} \right)^2 ds} \delta^3 \left(\vec{r} - \int_0^L \hat{t}(s) ds \right), \quad (\text{C1})$$

where $L = Nb$ is the total contour length of the polymer in the discrete model and $\hat{t}(s)$ is the unit tangent vector as a function of the curvilinear distance s . Using the numerical scheme proposed in Ref. [39], one can find the looping probability and hence predict the looping time as $\tau(f=0) \sim 1/P_{\text{loop}}(f=0)$ [18,20], shown as the full purple line in Fig. 5.

For short chains, we also include the analytical approximation from Ref. [40] (gray dashed line), which reads [20,40,41]

$$P_{\text{loop}}(f=0) \Big|_{L \text{ small}} \sim \frac{1}{L^5} e^{-14.055 l_p/L + 0.246 L/l_p}. \quad (\text{C2})$$

For long chains, the semiflexible polymer behaves effectively as a freely jointed chain, resulting in a looping probability that

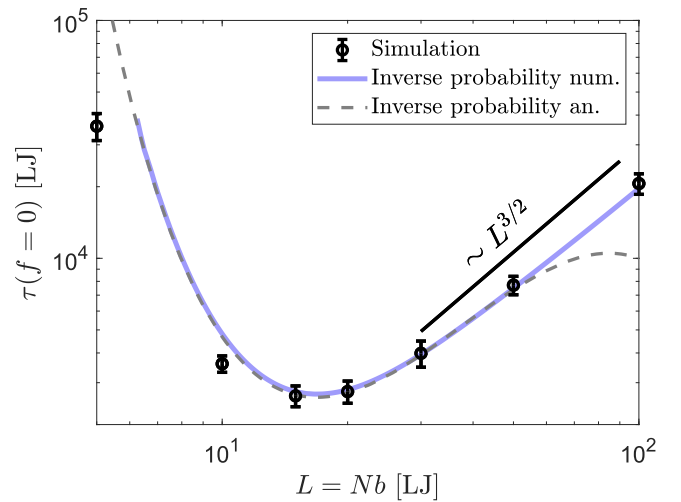


FIG. 5. Looping time at zero force as measured in simulations for a semiflexible chain (symbols), compared to the inverse scaling with the looping probability, $\sim 1/P_{\text{loop}}(f=0)$. The solid purple line shows the numerical result obtained using the algorithm of Ref. [39], while the dashed gray line corresponds to the analytical approximation from Ref. [40] [Eq. (C2)], valid for short chains. Both lines have been vertically shifted to align with the simulation data, as they predict only the scaling behavior. For long chains, the expected scaling matches that of a freely jointed chain, i.e., $\tau(f=0) \sim L^{3/2}$ [18,20].

scales as [18,20]

$$P_{\text{loop}}(f=0) \Big|_{L \text{ large}} \sim L^{-3/2}. \quad (\text{C3})$$

As shown in Fig. 5, these theoretical predictions agree well with the simulation data. In particular, for large L (i.e., $L \geq 15$ or $L/l_p \geq 3$), we confirm that the relation $\tau(f=0) \sim 1/P_{\text{loop}}(f=0)$ holds accurately.

Appendix D: Looping probability with tension. In the main text, the looping probability [the inverse of Eq. (8)] was approximated to scale in f as

$$P_{\text{loop}}(f) \sim e^{\beta F \langle z(f) \rangle}, \quad (\text{D1})$$

which can be evaluated analytically. The quantity $\langle z(f) \rangle$, the mean extension that minimizes the free energy as defined in Eq. (4), is central to both the two-state model [Eq. (3)] and the inverse-scaling approximation [Eq. (8)]. To compute $\langle z(f) \rangle$, we used the analytical force-extension approximation from Ref. [42]:

$$\begin{aligned} \frac{\langle z(f) \rangle}{L} &= \frac{4}{3} - \frac{4}{3\sqrt{\beta f l_p} + 1} - \frac{10e^{\sqrt{\frac{900}{\beta f l_p}}}}{\sqrt{\beta f l_p} \left(e^{\sqrt{\frac{900}{\beta f l_p}}} - 1 \right)^2} \\ &\quad + \frac{(\beta f l_p)^{1.62}}{3.55 + 3.8(\beta f l_p)^{2.2}}. \end{aligned} \quad (\text{D2})$$

Hence, both the two-state model and our inverse scaling with the looping probability can be evaluated fully analytically.

The approximation in Eq. (D1) relies on the assumption that the protein size r_c is small. To assess the validity of

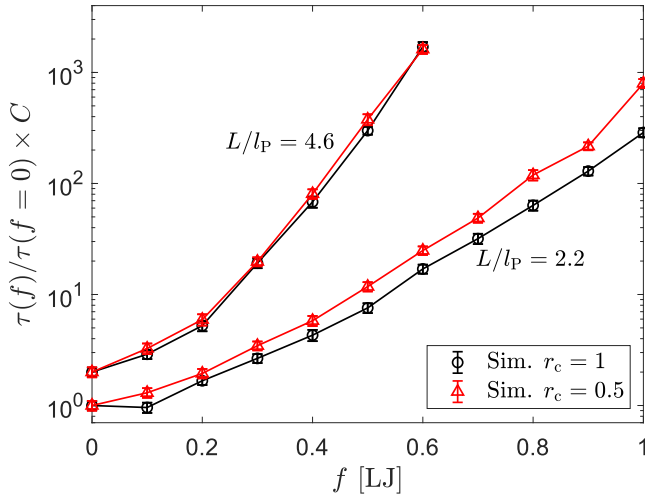


FIG. 6. Normalized looping time $\tau(f)/\tau(f=0) \times C$ (with $C = 1, 2$ for $L/l_p = 2.2, 4.6$, respectively) for two different capture radii. In black circles is $r_c = 1$, while in red triangles is $r_c = 0.5$. For $L/l_p = 4.6$, the two curves are not significantly different, showing that the size of the protein is negligible for long looping lengths.

this approximation, we repeated simulations for $L/l_p = 2.2$ and $L/l_p = 4.6$ using a smaller capture radius of $r_c = 0.5$, compared to $r_c = 1$ used in the main text. The results, shown in Fig. 6, demonstrate that for $L/l_p = 4.6$, the curves corresponding to the two capture radii are nearly indistinguishable. This confirms that in the regime where the looping time exhibits the inverse scaling with the looping probability, the small r_c approximation is indeed valid. Therefore, even if a more precise calculation of the looping probability accounting for the finite protein size were carried out, it would not yield observable differences in the regime of interest. Simulations for even longer chain lengths were not pursued, as reducing the capture radius increases the computational time substantially. However, it is reasonable to assume that for longer chains, the difference between the two curves would be even smaller, since the relative size of the protein becomes less significant compared to the overall looping length.

Appendix E: Barrier escape with correct free energy. In the main text, the barrier escape approach [Eq. (1)] was consistently evaluated using the free energy of Shin and Sung [14], that is, $\beta F(r) = -\ln[P(r; f=0)] - \beta f r$, using for $P(r; f=0)$ the mean-field result [Eq. (2)]. Interestingly, it was found that this approach agrees most closely with experimental data, as shown in Fig. 2. However, Fig. 3 indicates that this theory agrees with simulation data only for short chains under high tension, while significant discrepancies arise at low tension. Whether this disagreement stems from the approach itself [Eq. (1)] or from the approximated free energy cannot be definitively concluded from the findings in the main text. To further investigate, we derive the correct expression for the effective free energy of a semiflexible chain subjected to an external force f applied at its endpoints along a specified direction. This theoretical result will be compared with the free energies obtained from additional simulations conducted for $L/l_p = 2.2$ and 4.6 .

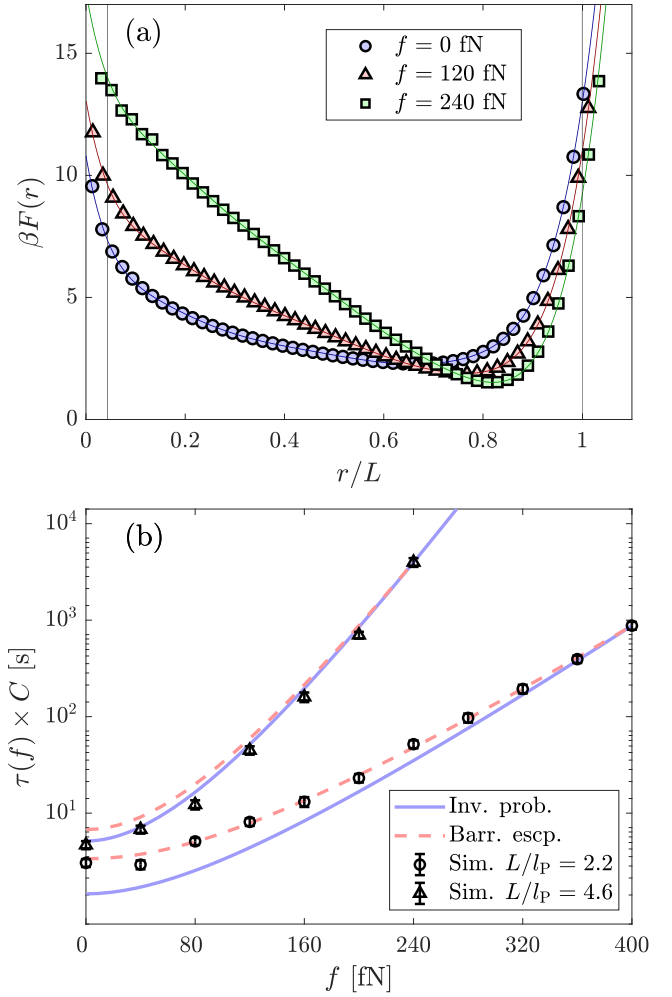


FIG. 7. (a) Free energy $F(r)$ for $L/l_p = 4.6$ at $f = 0, 120$, and 240 fN, extracted from MD simulations as represented by the symbols. The solid lines represent the prediction of Eq. (E6), where only $F(r; f=0)$ was obtained by a fit. (b) Looping time data from Fig. 3 for $L/l_p = 2.2$ and 4.6 , compared to the inverse scaling with the equilibrium looping probability (solid purple line) and the barrier escape approach (pink dotted line), evaluated using the solid line free energies. The theories are again rescaled to the highest data point for every chain length. For short chains ($L/l_p = 2.2$), the barrier escape theory provides an accurate prediction for the looping time across all force regimes. In contrast, for longer chains ($L/l_p = 4.6$), the accuracy of the barrier escape theory diminishes due to the breakdown of the local equilibrium assumption. Nonetheless, in this long-length-scale regime, the inverse scaling with the looping probability serves as a reliable alternative predictor.

In what follows, a tilde above a quantity indicates that it is expressed in terms of three spatial variables, while the absence of a tilde denotes its reduced or effective counterpart. To construct the correct effective free energy, we assume that the free energy at zero applied force, $F(r; f=0)$, is known. This case is typically the most straightforward, since in the absence of force the full free energy $\tilde{F}(x, y, z; f=0)$ is symmetric in all three Cartesian components. Consequently, it reduces to $F(r; f=0)$ by simply substituting $r = \sqrt{x^2 + y^2 + z^2}$.

When a force is applied in the z direction, the full free energy becomes

$$\tilde{F}(x, y, z; f) = F(r; f = 0) - fz. \quad (\text{E1})$$

The corresponding probability density is then given by

$$\tilde{P}(x, y, z; f) \propto e^{-\beta[F(r; f=0) - fz]}. \quad (\text{E2})$$

To obtain the reduced probability density $P(r; f)$, and hence the effective free energy via $\beta F(r; f) = -\ln P(r; f)$, we integrate $\tilde{P}$ over the solid angle:

$$P(r; f) \propto \int_0^\pi d\theta \int_0^{2\pi} d\phi \sin(\theta) \tilde{P}(r, \theta, \phi; f). \quad (\text{E3})$$

Having $x = r \sin(\theta) \cos(\phi)$, $y = r \sin(\theta) \sin(\phi)$, and $z = r \cos(\theta)$, this then becomes

$$P(r; f) \propto \int_0^\pi d\theta \int_0^{2\pi} d\phi \sin(\theta) e^{-\beta[F(r; f=0) - fr \cos(\theta)]} \quad (\text{E4})$$

$$\propto e^{-\beta F(r; f=0)} \frac{\sinh(\beta fr)}{\beta fr}. \quad (\text{E5})$$

So, we obtain as a final expression for the effective free energy

$$F(r; f) = F(r; f = 0) - \frac{1}{\beta} \ln \left[\frac{\sinh(\beta fr)}{\beta fr} \right]. \quad (\text{E6})$$

Equation (E6) was compared to free energies extracted from simulation. To achieve this, for each force, 10^2 simulations were performed, each consisting of 10^9 timesteps, with the exact same simulation protocol as described in Appendix A. Every 10^3 timesteps, the end-to-end distance r was sampled. Afterward, the probability distribution $P(r)$ was then

determined by dividing the observed data into 50 bins and normalizing. Subsequently, the free energy was calculated using the relation $\beta F(r) = -\ln[P(r)]$. For $L/l_p = 4.6$, the free energies at $f = 0, 120$, and 240 fN are presented in Fig. 7(a) by the symbols. The vertical lines indicate the capture radius and the maximum extension for an inextensible polymer $r/L = 1$. As shown in the figure, the data extend slightly beyond this limit due to the finite spring constant used in the simulations. Consequently, the mean-field free energy given by Eq. (2), which assumes an inextensible polymer, becomes slightly inaccurate in this regime. To account for this, the zero-force free energy $F(r; f = 0)$ was fitted using a ninth-order polynomial, represented by the solid blue line in the figure. For the cases with nonzero force, the free energy expression in Eq. (E6) was used, where $F(r; f = 0)$ is given by the polynomial fit. The results for $f = 120$ fN and $f = 240$ fN are shown as solid red and green lines in Fig. 7(a), respectively. As illustrated, Eq. (E6) yields excellent agreement with the free energies obtained directly from simulation.

Next, these effective free energies were used to evaluate the barrier escape approach [Eq. (1)], and the resulting predictions were compared to the simulation data from Fig. 3. The outcomes are shown in Fig. 7(b). For short chains ($L/l_p = 2.2$), the barrier escape approach yields accurate predictions. However, for longer chains ($L/l_p = 4.6$), even when the correct free energies are used, the inverse scaling with the equilibrium looping probability still provides the most accurate description for the force dependence of the looping time. This discrepancy arises because local equilibrium no longer holds for longer chains, as discussed in the main text. Consequently, these findings suggest that for long chains, efforts to refine looping time predictions using single-coordinate barrier escape theories are unlikely to succeed, and the inverse scaling with the equilibrium looping probability remains the more robust approach.

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