

Twist-bend coupling and the statistical mechanics of the twistable wormlike-chain model of DNA: Perturbation theory and beyond

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The simplest model of DNA mechanics describes the double helix as a continuous rod with twist and bend elasticity. Recent work has discussed the relevance of a little-studied coupling G between twisting and bending, known to arise from the groove asymmetry of the DNA double helix. Here the effect of G on the statistical mechanics of long DNA molecules subject to applied forces and torques is investigated. We present a perturbative calculation of the effective torsional stiffness C_{eff} for small twist-bend coupling. We find that the “bare” G is “screened” by thermal fluctuations, in the sense that the low-force, long-molecule effective free energy is that of a model with $G = 0$ but with long-wavelength bending and twisting rigidities that are shifted by G -dependent amounts. Using results for torsional and bending rigidities for freely fluctuating DNA, we show how our perturbative results can be extended to a nonperturbative regime. These results are in excellent agreement with numerical calculations for Monte Carlo “triad” and molecular dynamics “oxDNA” models, characterized by different degrees of coarse graining, validating the perturbative and nonperturbative analyses. While our theory is in generally good quantitative agreement with experiment, the predicted torsional stiffness does systematically deviate from experimental data, suggesting that there are as-yet-uncharacterized aspects of DNA twisting-stretching mechanics relevant to low-force, long-molecule mechanical response, which are not captured by widely used coarse-grained models.

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I. INTRODUCTION

In vivo, double-stranded DNA is typically found in a highly deformed state, which is in part due to the interaction with the many proteins that bend and twist the double helix but in part due to thermally driven deformations. Effort has been devoted to the study of many aspects of DNA mechanics, such as its response to applied twist and bending deformations [1]. These studies often rely on homogeneous elastic models, which, despite their simplicity, describe many aspects of single-molecule experiments [2–6] and are widely used to describe mechanical and statistical-mechanical properties of DNA (see, e.g., Refs. [7–11]).

One of the simplest models describing DNA deformations is the twistable wormlike chain (TWLC), which describes the double helix as an inextensible rod, for which twist and bend deformations are independent. Symmetry arguments suggest that the TWLC is incomplete: The inherent asymmetry of the DNA molecular structure, with its major and minor grooves, gives rise to a coupling G between twisting and bending [12]. Only a limited number of studies have considered the effect of twist-bend coupling on DNA mechanics [13–16]. A systematic analysis of coarse-grained models with and without groove asymmetry has highlighted several effects associated with twist-bend coupling at long [15] and short [16] length scales. Here we aim to clarify the role of G in the statistical mechanics of long DNA molecules, as analyzed in optical and magnetic tweezers.

We focus on analytical and numerical results for the stretching and torsional response of DNA with twist-bend coupling interaction $G \neq 0$. We first present a perturbative expansion for the partition function of the molecule, in which G is treated as the small parameter. The lowest-order results show that twist-bend coupling softens the torsional and bending stiffnesses of the double helix, recovering prior results from entirely different calculations [14]. Our new calculations reveal the existence of a previously unidentified large force scale f_0 ; for forces below this scale, the bare elastic constants, including G , are not directly accessible in stretching and twisting experiments. Instead, for forces below f_0 , only renormalized bending and twisting stiffnesses—which do depend on G —are observed. Because $f_0 \approx 600$ pN, the renormalized elastic model—which is the $G = 0$ TWLC—will be observed in essentially all conceivable single-molecule experiments. Thus, G is “screened,” effectively renormalized to $G = 0$, in single-molecule DNA mechanics experiments.

Prior work [14] suggests a strategy to generalize our results beyond perturbation theory to the regime where DNA is stretched by forces less than f_0 . We validate both the perturbative and nonperturbative results using numerical calculations corresponding to commonly used coarse-grained DNA elasticity models; our results turn out to closely describe results of those numerical models. Given this validation, we turn to experimental data which are reasonably well described by the low-force model but for which there remain discrepancies,

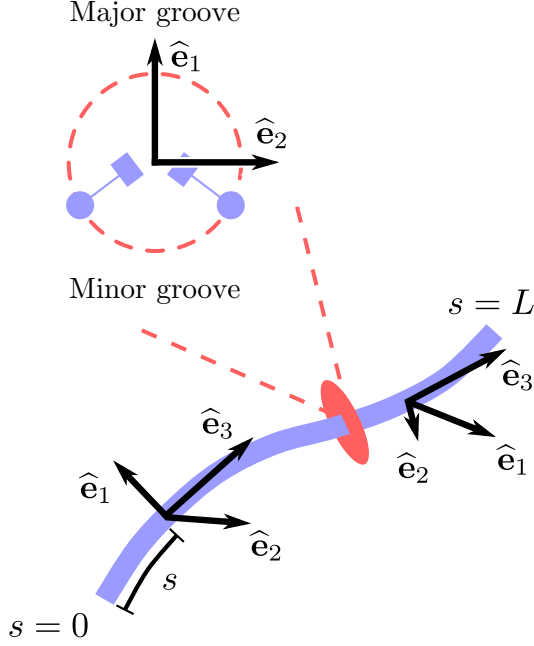


FIG. 1. Bottom: The configuration of a twistable elastic rod can be mathematically described with an orthonormal set of vectors $\{\hat{\mathbf{e}}_1, \hat{\mathbf{e}}_2, \hat{\mathbf{e}}_3\}$ assigned to every point s along the rod. The vector $\hat{\mathbf{e}}_3$ is the tangent to the curve and describes the bending fluctuations along the rod. Top: Cross section of the rod, indicating how the remaining vectors $\hat{\mathbf{e}}_1$ and $\hat{\mathbf{e}}_2$, which describe the torsional state, may be chosen in the particular case of DNA.

suggesting effects beyond simple harmonic elastic models like the TWLC.

II. ELASTICITY MODELS OF DNA

To describe the conformation of a continuous, inextensible, twistable elastic rod, one can associate a local orthonormal frame of three unit vectors $\{\hat{\mathbf{e}}_i\}$ ($i = 1, 2, 3$) with every point along the rod (Fig. 1). In a continuous representation of DNA, the common convention is to choose $\hat{\mathbf{e}}_3$ tangent to the curve and $\hat{\mathbf{e}}_1$ pointing to the DNA major groove. The frame is completed with a third vector, defined as $\hat{\mathbf{e}}_2 = \hat{\mathbf{e}}_3 \times \hat{\mathbf{e}}_1$. An unstressed B-form DNA corresponds to a straight, twisted rod, with the tangent $\hat{\mathbf{e}}_3$ being constant, and with $\hat{\mathbf{e}}_1$ and $\hat{\mathbf{e}}_2$ rotating uniformly about it, with a full helical turn every $l \approx 3.6$ nm or, equivalently, every 10.5 base pairs.

Any deformation from this unstressed configuration can be described by a continuous set of rotation vectors $\boldsymbol{\Omega}$ connecting adjacent local frames $\{\hat{\mathbf{e}}_i\}$ along the rod, using the differential equation

$$\frac{d\hat{\mathbf{e}}_i}{ds} = (\boldsymbol{\Omega} + \omega_0 \hat{\mathbf{e}}_3) \times \hat{\mathbf{e}}_i, \quad (1)$$

where the internal parameter s denotes the arc-length coordinate (Fig. 1) and $\omega_0 = 2\pi/l \approx 1.75 \text{ nm}^{-1}$ is the intrinsic twist of DNA. On setting $\boldsymbol{\Omega} = \mathbf{0}$, one obtains the unstressed configuration mentioned above. Thus, a nonzero rotation vector $\boldsymbol{\Omega}(s) \neq \mathbf{0}$ corresponds to a local deformation at s around this ground state. Defining $\Omega_i \equiv \hat{\mathbf{e}}_i \cdot \boldsymbol{\Omega}$, it follows that $\Omega_1(s)$ and $\Omega_2(s)$ describe local bending deformations, while $\Omega_3(s)$

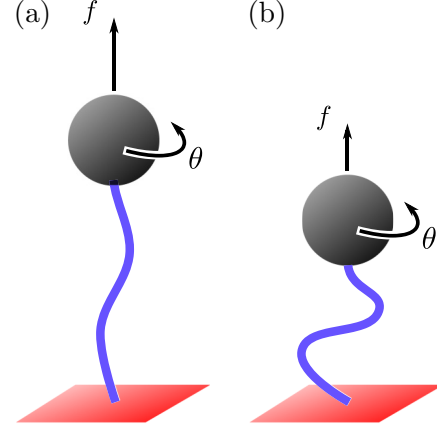


FIG. 2. Typical setup of a magnetic tweezers experiment. A DNA molecule is covalently bound to a substrate at one end and to a paramagnetic bead at the other end. An applied force f stretches the molecule, while an applied rotation θ twists it. The effective torsional stiffness C_{eff} is the proportionality constant connecting the applied rotation with the exerted torque [Eq. (4)]. C_{eff} increases with the force, hence it is higher in (a) than in (b).

describes twist deformations. In the remainder of the paper the s dependence of $\boldsymbol{\Omega}$ will be implicit.

Symmetry analysis of the DNA molecule requires the energy functional E to be invariant under the transformation $\Omega_1 \rightarrow -\Omega_1$, with the consequence that [12]

$$\beta E = \frac{1}{2} \int_0^L ds (A_1 \Omega_1^2 + A_2 \Omega_2^2 + C \Omega_3^2 + 2G \Omega_2 \Omega_3), \quad (2)$$

where $\beta \equiv 1/k_B T$ is the inverse temperature, A_1 and A_2 are the bending stiffnesses, C is the torsional stiffness, and G is the twist-bend coupling constant. These coefficients have dimensions of length and can be interpreted as the contour distance along the double helix over which significant bending and twisting distortions can occur by thermal fluctuations. Our perturbative calculation will use the isotropic-bending version of this model ($A_1 = A_2 = A$), which is described by the following energy functional:

$$\beta E = \frac{1}{2} \int_0^L ds \left[A \left(\frac{d\hat{\mathbf{e}}_3}{ds} \right)^2 + C \Omega_3^2 + 2G \Omega_2 \Omega_3 \right]. \quad (3)$$

Here we have used Eq. (1) to express the sum $\Omega_1^2 + \Omega_2^2$ as the derivative of the tangent vector. The TWLC is obtained by setting $G = 0$ in Eqs. (2) and (3), corresponding to the anisotropic and isotropic cases, respectively.

III. EFFECTIVE TORSIONAL STIFFNESS

In a typical magnetic tweezers experiment, a single DNA molecule of 10^3 – 10^4 bases is attached to a solid substrate and to a paramagnetic bead at its two ends (Fig. 2). The molecule can be stretched by a linear force f and over- or undertwisted by an angle θ . The resulting torque τ exerted by the bead, which can be experimentally measured [17–20], is linear in θ for small θ

$$\tau \approx \frac{k_B T C_{\text{eff}}}{L} \theta. \quad (4)$$

Here C_{eff} is the *effective* torsional stiffness (in contrast to the intrinsic stiffness C) and represents the central quantity of interest here. It expresses the resistance of the DNA to a global torsional deformation, applied at its two ends.

As discussed in more detail below, C_{eff} is in general lower than its intrinsic equivalent C . More specifically, at low stretching forces the bending fluctuations can absorb a significant part of the applied torsional stress, leading to a globally reduced torsional resistance $C_{\text{eff}} < C$. On the other hand, when the applied force is sufficiently large, bending fluctuations are mostly suppressed, and hence the effective torsional stiffness tends to approach the intrinsic one. As a consequence, C_{eff} is going to be a monotonically increasing function of the stretching force.

Moroz and Nelson derived an expression of C_{eff} for the TWLC in the limit of high forces [4,21]. In spite of the good qualitative agreement between the theory and early experiments, more recent studies reported systematic deviations [14,17,20,22]. For completeness we will first present in Sec. III A a short derivation of the TWLC-based theory by Moroz and Nelson. The perturbative calculation in small G is discussed in Sec. III B and generalized beyond perturbative expansion in Sec. III D.

A. The TWLC limit ($G = 0$)

Moroz and Nelson [4,21] mapped the twisted and stretched TWLC onto a quantum mechanical problem of a spinning top, and C_{eff} was obtained from the ground state of the associated Schrödinger equation. Here we present an alternative derivation, following the scheme illustrated in Refs. [1,23], which proves to be more convenient for the perturbative calculation in small G . The starting point is the partition function of a TWLC under applied force f and torque τ . The latter induces a rotation by an angle θ on the end point of the molecule (Fig. 2). The excess linking number, which we will use throughout this work, is $\Delta\text{Lk} = \theta/2\pi$.

To calculate the partition function, we integrate over all possible configurations of the twistable rod, which can be parametrized by the tangent vector $\hat{\mathbf{e}}_3(s)$ and the twist density $\Omega_3(s)$. The resulting path integral takes the form:

$$Z_0 = \int \mathcal{D}[\hat{\mathbf{e}}_3, \Omega_3] e^{-\beta E_0 + \beta \mathbf{f} \cdot \mathbf{R} + 2\pi \beta \tau \Delta\text{Lk}}, \quad (5)$$

where E_0 is the energy of the TWLC, obtained from Eq. (3) by setting $G = 0$, and \mathbf{R} the end-to-end vector

$$\mathbf{R} = \int_0^L \hat{\mathbf{e}}_3 ds. \quad (6)$$

We assume that the force is oriented along the z direction, hence $\mathbf{f} = f\hat{\mathbf{z}}$, with $\hat{\mathbf{z}}$ a unit vector. Using a result due to Fuller [24], originally derived for closed curves, the linking number can be expressed as the sum of twist and writhe, i.e., $\Delta\text{Lk} = \text{Tw} + \text{Wr}$. The excess twist is obtained by integrating over the twist density

$$\text{Tw} = \frac{1}{2\pi} \int_0^L \Omega_3(s) ds, \quad (7)$$

while the writhe is given by [1]

$$\text{Wr} = \frac{1}{2\pi} \int_0^L \frac{\hat{\mathbf{z}} \cdot (\hat{\mathbf{e}}_3 \times d\hat{\mathbf{e}}_3/ds)}{1 + \hat{\mathbf{e}}_3 \cdot \hat{\mathbf{z}}} ds. \quad (8)$$

This representation of the writhe as a single integral (and not as a double, nonlocal integral) is correct modulo 1, with the integer portion equal to zero if the molecule is sufficiently stretched so that it is unlikely to loop back opposite to the direction of the applied force (the case of interest here). Under these conditions, the denominator $1 + \hat{\mathbf{e}}_3 \cdot \hat{\mathbf{z}}$ does not vanish, and the integral in Eq. (8) yields a finite value. The applicability of either Eq. (8) [or the double-integral version of it, which does not require the mod 1 of Eq. (8)] for a highly stretched open chain has been discussed and justified for extended polymers in prior works [4,5,21,25,26].

Next, we insert Eqs. (6), (7), and (8) into Eq. (5), and consider the limit of strong forces and weak torques. The partition function [Eq. (5)] reduces to a Gaussian in this limit and can be easily estimated (details can be found in Appendix A). To lowest order in τ and at large forces, one obtains the following free energy:

$$F_0(f, \tau) = -fL + \sqrt{\frac{fk_B T}{A}} L - \frac{\beta \tau^2 L}{2C_{\text{eff}}} + \dots, \quad (9)$$

where the dots denote constant or higher-order terms in τ . The effective torsional stiffness C_{eff} is given by (see Appendix A)

$$\frac{1}{C_{\text{eff}}} = \frac{1}{C} + \frac{1}{4A} \sqrt{\frac{k_B T}{fA}} + \dots. \quad (10)$$

This equation was originally derived by Moroz and Nelson [4,21] in the fixed-torque ensemble. The same expression can also be obtained in the fixed-linking-number ensemble [5,27]. At high forces, Eq. (10) approaches the twisted-rod limit and $C_{\text{eff}} \rightarrow C$, but, in general, bending fluctuations soften the DNA torsional stiffness, so that $C_{\text{eff}} < C$. The latter originates from a global coupling between torque and writhe (not to be confused with the local twist-bend coupling considered below). Note that the effect of bending fluctuations is governed by the dimensionless parameter $\sqrt{k_B T/fA}$, which is small at low temperatures or high forces.

B. Perturbative (small- G) expansion

We now construct a perturbation expansion for the partition function, using G as the small parameter (the length scale determining whether G is “small” will be made clear below). The full partition function is

$$Z = \int \mathcal{D}[\hat{\mathbf{e}}_3, \Omega_3] e^{-\beta E + \beta \mathbf{f} \cdot \mathbf{R} + \beta \tau \Delta\text{Lk}}, \quad (11)$$

where now βE is given by Eq. (3) and contains a twist-bend coupling term. Assuming that G is small, we can expand the Boltzmann factor in powers of G , which gives to lowest order:

$$Z \approx Z_0 \left[1 + \frac{G^2}{2} \left\langle \left(\int_0^L \Omega_2 \Omega_3 ds \right)^2 \right\rangle + \dots \right], \quad (12)$$

where $\langle \cdot \rangle_0$ denotes the average with respect to the unperturbed (TWLC) partition function [Eq. (5)]. Note that in the perturbative expansion, the term linear in G vanishes by the

$\Omega_2 \rightarrow -\Omega_2$ symmetry of the TWLC. The full calculation of the average in the right-hand side of Eq. (12) is given in Appendix C. The final expression for the free energy is of the form [Eq. (C38)]

$$F(f, \tau) = -fL + \sqrt{\frac{fk_B T}{A^*}}L + \Gamma\tau L - \frac{\beta\tau^2 L}{2C_{\text{eff}}} + \dots, \quad (13)$$

where terms of negligible contribution were omitted (see Appendix C).

In the above, we have introduced the rescaled bending stiffness

$$\frac{1}{A^*} = \frac{1}{A} \left(1 + \frac{G^2}{2AC} \right), \quad (14)$$

together with the parameter

$$\Gamma = \frac{G^2 d^2(f)}{8A^2 C^2 \omega_0}. \quad (15)$$

The dimensionless, force-dependent scale factor $d(f)$ will be discussed below; we note that it appears in the coefficient Γ [Eq. (15)] but not in A^* [Eq. (14)]. Finally, the form of the effective torsional stiffness C_{eff} is

$$\frac{1}{C_{\text{eff}}} = \frac{1}{C} \left[1 + \frac{G^2}{AC} d(f) \right] + \frac{1}{4A} \left(1 + \frac{3}{4} \frac{G^2}{AC} \right) \sqrt{\frac{k_B T}{fA}}. \quad (16)$$

The scale factor $d(f)$ is also present in C_{eff} .

Examination of these formulas indicate that the expansion is in powers of the dimensionless parameter G^2/AC , which, given our current estimates for the stiffnesses ($A \approx 50$ nm, $C \approx 100$ nm, $G \approx 30$ nm), is less than 1, although we note that $G^2/AC < 1$ is a stability requirement for the microscopic energy [12]. Our computation neglects terms beyond first order in G^2/AC .

C. Effective torsional stiffness C_{eff}

Equation (16) is the central result of this paper and extends the TWLC result by Moroz and Nelson [Eq. (10)], which is recovered in the limit $G \rightarrow 0$. The perturbative corrections are governed by the dimensionless parameter G^2/AC and give rise to a further torsional softening of the molecule, i.e., $C_{\text{eff}}(G \neq 0) < C_{\text{eff}}(G = 0)$, as pointed out in Ref. [14]. Equation (16) contains also a force-dependent, crossover function, which can be approximated as (see Appendix C)

$$d(f) \approx \frac{1}{1 + f/f_0}, \quad (17)$$

where $f_0 = A\omega_0^2 k_B T$ is the characteristic force above which $d(f)$ starts to significantly drop below its low-force limit of $d(0) = 1$. To understand this force scale, which has no counterpart in the Moroz and Nelson formula [Eq. (10)], we recall that the correlation length for a stretched wormlike chain is $\xi = \sqrt{Ak_B T/f}$ [1]. Therefore, f_0 is the force associated with a correlation length of the order of the distance between neighboring bases, i.e., $\xi = 1/\omega_0$.

For DNA ($A \approx 50$ nm, $\omega_0 = 1.75$ nm⁻¹, $k_B T = 4$ pN nm) we get $f_0 \approx 600$ pN, which is far above the force where the double helix starts to be itself stretched (≈ 20 pN), force-denatured (≈ 60 pN), and is in fact comparable to where the

covalently bonded backbones will break. Hence, for forces relevant to experiments we are concerned with ($f < 10$ pN), one may simply set $d(f) \approx 1$. We will refer to this limit as the “low-force limit,” but one should keep in mind that our perturbative theory is computed for the “well-stretched” limit, i.e., $f > k_B T/A \approx 0.1$ pN. Therefore our perturbative theory is applicable in the force range of roughly 0.1 to 10 pN.

We emphasize that Eq. (16) can be written as

$$\frac{1}{C_{\text{eff}}} = \frac{1}{C^*} + \frac{1}{4A^*} \sqrt{\frac{k_B T}{fA^*}}, \quad (18)$$

where A^* is defined in (14) and

$$\frac{1}{C^*} \equiv \frac{1}{C} \left[1 + \frac{G^2}{AC} d(f) \right] + \mathcal{O}(G^2/AC). \quad (19)$$

Equation (18) has exactly the same form as the Moroz and Nelson formula [Eq. (10)], with rescaled bending and torsional stiffnesses. The importance of this result is paramount: Since $d(f) = 1$ in the range of experimentally relevant forces, the torsional stiffness (and in fact the partition function itself) depends only on the “renormalized” stiffnesses A^* and C^* , meaning that G by itself cannot be determined from fitting of $C_{\text{eff}}(f)$ (or any other equilibrium quantity versus f); only the effective stiffnesses A^* and C^* can be determined from experiments in the low-force regime.

D. Nonperturbative result for C_{eff} valid for $f < f_0$

Equation (16) has been derived on the basis of a systematic perturbation expansion in G (more formally, in the small parameter $G^2/AC < 1$). When cast in the form of Eq. (18), it is apparent that there is a simple way to extend the results to a more general, nonperturbative case, where G may be large and the bending possibly anisotropic, i.e., $A_1 \neq A_2$ in Eq. (2). The key physical idea here is that for forces below the gigantic force scale f_0 , thermal fluctuations at the helix scale where G correlates bending and twisting fluctuations are unperturbed [$d(f) = 1$], and therefore we might as well just consider DNA to have the effective twisting and bending stiffnesses that it has at *zero* force.

In absence of applied torques and forces ($f, \tau = 0$), the partition function of Eq. (11) can be evaluated exactly [14]. Due to twist-bend coupling, the bending and torsional stiffnesses are renormalized as [14]:

$$\kappa_b = A \frac{1 - \frac{\varepsilon^2}{A^2} - \frac{G^2}{AC} \left(1 + \frac{\varepsilon}{A} \right)}{1 - \frac{G^2}{2AC}}, \quad (20)$$

$$\kappa_t = C \frac{1 - \frac{\varepsilon}{A} - \frac{G^2}{AC}}{1 - \frac{\varepsilon}{A}}, \quad (21)$$

where we have introduced the parameters $A = (A_1 + A_2)/2$ and $\varepsilon = (A_1 - A_2)/2$. Equations (20) and (21) quantify the energetic cost of bending and twisting deformations, respectively, in the same way A and C do within the TWLC. Note that, by setting $G = 0$ in these expressions, one recovers the TWLC limit, $\kappa_t = C$ and $\kappa_b = 2A_1 A_2 / (A_1 + A_2)$, i.e., the renormalized bending stiffness is the harmonic mean of A_1 and A_2 , which is a known result (see, e.g., Refs. [28,29]). If $G \neq 0$, then one has $\kappa_b < 2A_1 A_2 / (A_1 + A_2)$ and $\kappa_t < C$,

i.e., twist-bend coupling “softens” the bending and twist deformations of the DNA molecule as early as $f = 0$, $\tau = 0$. Equation (16) then describes two different effects: One is the thermally induced torsional softening due to bending fluctuations [already present in the TWLC expression (10)] and the other is the G -induced softening, which is captured by the two factors between parentheses in Eq. (16).

Setting $\varepsilon = 0$ and expanding Eqs. (20) and (21), one finds $\kappa_b = A^* + \mathcal{O}(G^4)$ and $\kappa_t = C^* + \mathcal{O}(G^4)$, which suggests the following, more general, nonperturbative result for C_{eff} , valid as long as $f \ll f_0$:

$$\frac{1}{C_{\text{eff}}} = \frac{1}{\kappa_t} + \frac{1}{4\kappa_b} \sqrt{\frac{k_B T}{f \kappa_b}}. \quad (22)$$

This relation, similarly to Eq. (18), has the same form as the Moroz and Nelson formula [Eq. (10)], with A and C replaced by κ_b and κ_t [much as our result for C_{eff} of Eq. (19) has the Moroz-Nelson form with $A \rightarrow A^*$ and $C \rightarrow C^*$]. As we will show in the next section, this new, nonperturbative result for the continuum model is in excellent agreement with numerical Monte Carlo (MC) and molecular dynamics (MD) calculations.

E. Twist-bend-coupling-induced DNA unwinding

An intriguing feature of the perturbative calculation is the appearance of a term linear in τ in the free energy [Eq. (13)], which induces an unwinding of the helix at zero torque. In particular, from Eqs. (11), (13), and (15) it follows that

$$\langle \Delta Lk \rangle|_{\tau=0} = -\frac{1}{2\pi} \frac{\partial F}{\partial \tau} \Big|_{\tau=0} = -\frac{d^2(f)}{16\pi\omega_0} \frac{G^2 L}{A^2 C^2}. \quad (23)$$

The scale for this thermal unwinding is very small: Using typical values of DNA parameters ($A \approx 50$ nm, $C \approx 100$ nm, $G = 30$ nm, $\omega_0 = 1.75$ nm⁻¹) we find an unwinding angle per contour length of $2\pi \langle \Delta Lk \rangle / L = -G^2 / 8\omega_0 A^2 C^2 \approx -2.6 \times 10^{-6}$ rad/nm (about -5×10^{-5} degrees per base pair). This G -generated shift in helix twisting is inconsequential small, but it is worth noting that this term is present in the perturbation theory.

It has been long known that there is a gradual unwinding of the double helix as the temperature is increased [30,31], and this effect has been recently observed at the single-DNA level [32]. Although one might imagine an overall T^2 dependence of this term (from the factors of β in the Boltzmann factor), this dependence can only generate a tiny fraction of the observed temperature-dependent unwinding of $\approx -1 \times 10^{-2}$ degrees/K bp. The experimentally observed unwinding is likely due to temperature-dependence DNA conformational changes [32] and is beyond the scope of being captured by the simple elastic models discussed here; in particular the observed unwinding of DNA with increasing temperature is not attributable to the twist-bend coupling G .

F. “Janus strip” limit ($\omega_0 \rightarrow 0$)

Equation (22) is not generally valid for any arbitrary polymer with twist-bend coupling, but its validity is linked to the physical parameters characterizing DNA elasticity. These conspire to set forces encountered in typical experiments

(below 10 pN) to be far below the characteristic force $f_0 = k_B T A \omega_0^2 \approx 600$ pN at which one starts to see effects at the helix repeat scale, i.e., force-driven unwinding of the double helix due to quenching of thermal fluctuations and the influence of G . In this sense, ω_0 can be regarded as a “large” parameter: combinations of it and the elastic constants give dimensionless constants large compared to unity, e.g. $A\omega_0$, $C\omega_0 \approx 10^2 \gg 1$ and $\sqrt{Ak_B T} / f \omega_0 \gg 1$ for $f < 10$ pN).

For this reason, several ω_0 -dependent terms, which in principle would contribute to C_{eff} at order G^2 , can in practice be neglected in the application of the theory to DNA [see, e.g., Eqs. (C30) and (C31) in Appendix]. The neglect of these terms leads to C_{eff} taking the simple form given by Eq. (18), in which A^* and C^* are the renormalized stiffnesses.

While not relevant to DNA, we might imagine other polymer structures for which ω_0 is not so large, i.e., where ω_0 is closer in size to $1/A$ or $1/C$. In this case one cannot ignore these additional terms, and $d(f) \approx 1/(1 + f/f_0)$ might drop significantly over experimentally relevant force ranges. Chiral proteins, lipid filaments, or even nanofabricated objects might comprise realizations of such situations.

As an example we consider the extreme limit $\omega_0 \rightarrow 0$, corresponding to a “Janus strip,” an elastic strip with inequivalent faces (i.e. inequivalent major and minor “grooves”) and, thus, nonzero G . In this case, using the more complete and complicated results for the perturbative expansion given in the Appendix, we obtain to lowest order in G ,

$$\frac{1}{C_{\text{eff}}} = \frac{1}{C} + \frac{1}{4A} \left[1 + \left(\frac{3}{4} + \frac{2A^2}{C^2} \right) \frac{G^2}{AC} \right] \sqrt{\frac{k_B T}{fA}}. \quad (24)$$

In this limit, compared to the large- ω_0 case relevant to DNA, there is a more gradual shift of C_{eff} up to its high-force limit and an inequivalence of the form of C_{eff} to the Moroz-Nelson form. Physically, this is because the intrinsic chirality of the filament is now gone, eliminating the “screening” of effects of G at low forces and the simple dependence of the low-force thermodynamics on only the coarse-grained stiffnesses κ_t and κ_b . Experiments on such Janus strips, or on “soft-helix” objects where $A\omega_0$, $C\omega_0 < 1$ could provide realizations of this limit of the theory. Reference [14] showed that Eq. (24) fits experimental data for DNA surprisingly well, despite not taking account of double helix chirality.

IV. NUMERICAL CALCULATIONS

To check the validity of the analytical results presented above, we performed numerical simulations of two different models. The first model, referred to as the *triad model*, is obtained from the discretization of the continuum elastic energy (2) and treated using MC computations. The second model is oxDNA, a coarse-grained model of nucleic acids [33], treated using MD calculations.

A. Triad model

The triad model is composed of a series of N orthonormal vectors $\{\hat{\mathbf{e}}_i(k)\}$ with $i = 1, 2, 3$ and $k = 0, 1, 2 \dots N$, each representing a single base pair, interacting with its neighbors according to Eq. (2). The total length of the molecule is $L = Na$, with $a = 0.34$ nm the base-pair distance. The ground state

of this model is a twisted, straight rod, with $\hat{\mathbf{e}}_3$ being aligned with the direction of the stretching force, and the vectors $\hat{\mathbf{e}}_1$, $\hat{\mathbf{e}}_2$ rotating about $\hat{\mathbf{e}}_3$ with an angular frequency ω_0 . A cluster move consisted of a rotation of the whole subsystem beyond a randomly selected triad by a random angle. The new rotation vector $\mathbf{\Omega}$ was calculated based on Rodrigues's rotation formula (see, e.g., Supplementary Material of Ref. [15]), and then the energy was updated from a discretized version of Eq. (2), with the addition of a force term [see Eq. (5)]. The move was accepted or rejected according to the Metropolis algorithm. The stiffness constants A_1 , A_2 , C , and G are input parameters for the model and may, therefore, be arbitrarily chosen, provided the stability condition $G^2 < A_2C$ is met [for which the quadratic form in Eq. (2) is positive definite].

The effective torsional stiffness was calculated at zero torque from linking number fluctuations:

$$C_{\text{eff}} = \frac{L}{4\pi^2 \langle (\Delta Lk - \langle \Delta Lk \rangle)^2 \rangle}. \quad (25)$$

The variance of linking number in the denominator was evaluated from the topological relation $\Delta Lk = \Delta Tw + Wr$, with twist and writhe obtained from the discretization of Eqs. (7) and (8), respectively. To check the validity of our results, the writhe was also evaluated from the double-integral formula, following the method of Ref. [34], and no significant differences were found for forces > 0.25 pN. In all simulations, the size of the system was 600 triads (base pairs), above which the results remained identical within that force range.

1. Isotropic bending

Figure 3 shows the results of Monte Carlo calculations for the isotropic model of Eq. (3), with $A = 50$ nm, $C = 100$ nm, and $G = 0, 20, 40$ nm (top to bottom). The data are plotted as a function of the dimensionless parameter $\sqrt{k_B T}/fA$. The numerical errors are smaller than the symbol sizes and hence not shown.

In absence of twist-bend coupling ($G = 0$, upper panel), the Monte Carlo data are in excellent agreement with the Moroz-Nelson theory. We compare them both to Eq. (10) (dashed line) and the following expression (solid line):

$$C_{\text{eff}} = C \left(1 - \frac{C}{4A} \sqrt{\frac{k_B T}{fA}} \right). \quad (26)$$

The latter is obtained from the lowest-order expansion of Eq. (10) in $\sqrt{k_B T}/fA$ and is a straight line when plotted as a function of the rescaled variable of Fig. 3. Equations (10) and (26) coincide to leading order in $1/\sqrt{f}$, and any differences in the two expressions only occur at low force scales, where higher-order corrections become relevant. Equation (26) fits the Monte Carlo data over the whole range of forces analyzed ($f \geq 0.25$ pN), while Eq. (10) deviates at low forces [35].

The middle panel of Fig. 3 shows Monte Carlo results for $G = 20$ nm (points), which we compare both to the results for the perturbative expansion for $1/C_{\text{eff}}$, Eq. (18) (dashed line), and the following similar expansion result for C_{eff} (solid line):

$$C_{\text{eff}} = \frac{C}{1 + \frac{G^2}{AC} d(f)} \left[1 - \frac{C}{4A} \frac{1 + \frac{3G^2}{4AC}}{1 + \frac{G^2}{AC} d(f)} \sqrt{\frac{k_B T}{fA}} \right], \quad (27)$$

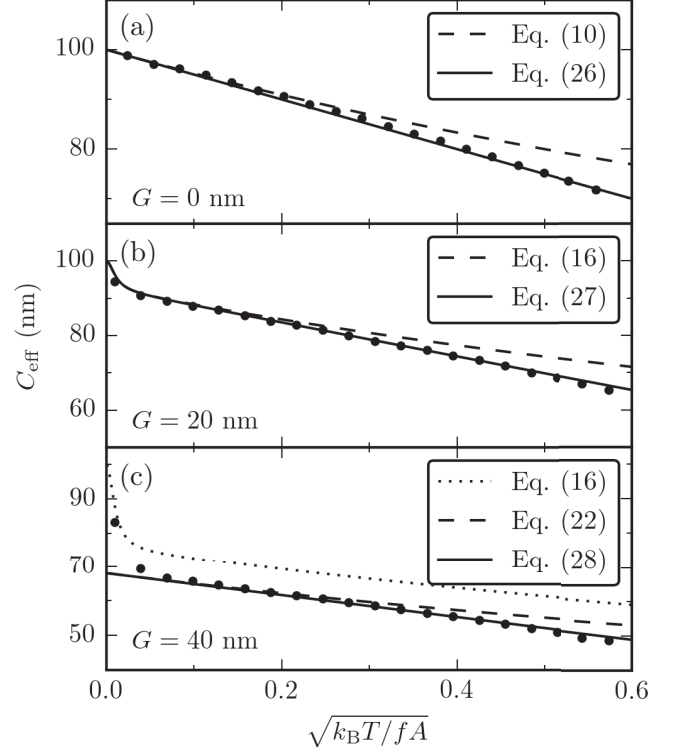


FIG. 3. Comparison of Monte Carlo simulations of the triad model (points) with various analytical expressions of C_{eff} (lines) for $A = 50$ nm, $C = 100$ nm and (a) $G = 0$, (b) $G = 20$ nm, and (c) $G = 40$ nm. The data are plotted as a function of $\sqrt{k_B T}/fA$ and correspond to $f \geq 0.25$ pN. The numerical results are in excellent agreement with the analytical expressions, both in the perturbative and nonperturbative regimes (see text). Error bars of Monte Carlo data are smaller than symbol sizes.

obtained by expanding Eq. (16) to lowest order in $1/f$. The latter is in excellent agreement with Monte Carlo data in the whole range of forces considered, indicating that $G = 20$ nm falls within the range of validity of the perturbative calculation ($G^2/AC = 0.08$ in this case). We note that the two perturbative expansion results converge together at high forces [$k_B T/(Af)$] $^{1/2} \rightarrow 0$ and also show the upturn at the very highest forces associated with the force dependence of $d(f)$.

Finally, the lower panel of Fig. 3 shows the results of Monte Carlo simulations for $G = 40$ nm. The numerical data deviate substantially from Eq. (16) (dotted line), indicating that G is leaving the range of validity of the perturbative calculation ($G^2/AC = 0.32$). The remaining curves show the nonperturbative result for $1/C_{\text{eff}}$ Eq. (22) (dashed line), together with the following nonperturbative expression for C_{eff} (solid line)

$$C_{\text{eff}} = \kappa_t \left(1 - \frac{\kappa_t}{4\kappa_b} \sqrt{\frac{k_B T}{f\kappa_b}} \right), \quad (28)$$

the latter being in excellent agreement with Monte Carlo data for $f < f_0$, validating the nonperturbative result (note that $[k_B T/(f_0 A)]^{1/2} \approx 0.01$). For $f > f_0$, the upturn of C_{eff} toward the bare value of C is apparent; this effect, while not given

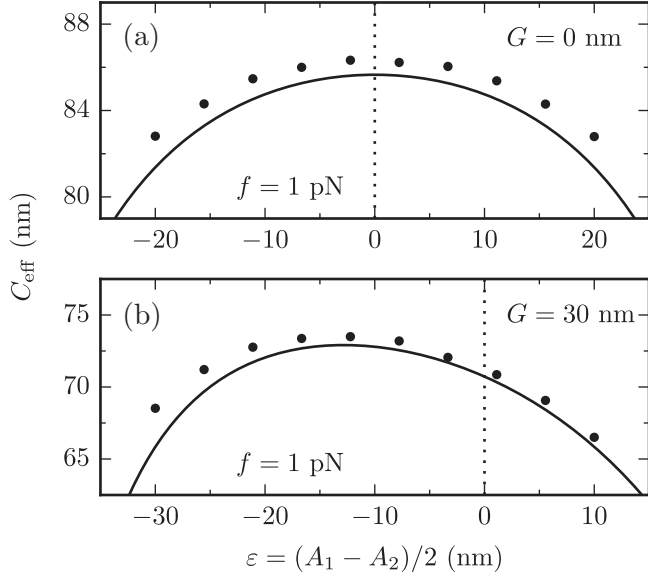


FIG. 4. Effect of bending anisotropy ($A_1 \neq A_2$) on the effective torsional stiffness at a fixed force $f = 1$ pN with (a) $G = 0$ and (b) $G = 30$ nm. Numerical data from Monte Carlo simulations of the triad model (points) are in good agreement with the analytical, nonperturbative predictions of Eq. (28) (solid lines). The vertical dashed lines indicate the isotropic case ($\varepsilon = 0$). A nonvanishing twist-bend coupling (lower panel) induces a $\varepsilon \rightarrow -\varepsilon$ symmetry breaking. Error bars of Monte Carlo data are smaller than symbol sizes.

by the nonperturbative results, is present in the perturbation expansion results. We conclude that our nonperturbative result indeed provides a quantitative account of C_{eff} for $f < f_0$, where we expect it to be valid.

2. Anisotropic bending

While the perturbative calculation [Eq. (16)] was restricted to the isotropic case ($A_1 = A_2$), the nonperturbative result [Eq. (28)] has a broader range of applicability and is able to describe the anisotropic case as well. Figure 4 shows the results of Monte Carlo calculations of C_{eff} for various values of the anisotropy parameter $\varepsilon = (A_1 - A_2)/2$ and a fixed value of the force $f = 1$ pN. The Monte Carlo data are in very good agreement with Eq. (28), plotted with solid lines. The differences are within 5% and are probably due to higher-order corrections in $1/f$ (recall that all analytical results are based on a large-force expansion). In absence of twist-bend coupling ($G = 0$), Eq. (28) is symmetric in ε , as in this case Eqs. (20) and (21) give $\kappa_b = (A^2 - \varepsilon^2)/A$ and $\kappa_t = C$, respectively. A nonzero G induces nonvanishing terms, which are linear in ε both in κ_b and κ_t [Eqs. (20) and (21)], leading to a breaking of the $\varepsilon \rightarrow -\varepsilon$ symmetry.

B. oxDNA

oxDNA is a coarse-grained model describing DNA as two intertwined strings of rigid nucleotides [33]. It has been used for the study of a variety of DNA properties, ranging from single molecules to large-scale complexes [15,16,33,36–38]. To date, two versions of oxDNA exist: one with symmetric

grooves (oxDNA1) [33] and one with asymmetric grooves (oxDNA2) [37]. Comparing the torsional response of the two versions will allow us to infer the effect of the groove asymmetry on C_{eff} . Differently from the triad model, in which the stiffness constants A_1 , A_2 , C , and G are input parameters, in oxDNA they are determined by the molecular force fields used. These force fields were accurately tuned so that the experimental DNA structural, mechanical, and thermodynamic properties (as persistence length, melting temperatures, and torque-induced supercoiling) are well reproduced [33]. As for real DNA, for oxDNA the elastic constants are emergent via coarse graining of fluctuations of smaller-scale, molecular motion degrees of freedom.

	A_1	A_2	C	G	κ_b	κ_t
oxDNA1	84(14)	29(2)	118(1)	<0.3	43	118
oxDNA2	85(10)	35(2)	109(1)	25(1)	44	92

grooves (oxDNA1) [33] and one with asymmetric grooves (oxDNA2) [37]. Comparing the torsional response of the two versions will allow us to infer the effect of the groove asymmetry on C_{eff} . Differently from the triad model, in which the stiffness constants A_1 , A_2 , C , and G are input parameters, in oxDNA they are determined by the molecular force fields used. These force fields were accurately tuned so that the experimental DNA structural, mechanical, and thermodynamic properties (as persistence length, melting temperatures, and torque-induced supercoiling) are well reproduced [33]. As for real DNA, for oxDNA the elastic constants are emergent via coarse graining of fluctuations of smaller-scale, molecular motion degrees of freedom.

The stiffness parameters of oxDNA were recently estimated from the analysis of the equilibrium fluctuations of an unconstrained molecule [15] and are shown in Table I (the values of the elastic constants for oxDNA2 shown are the result of transformation of the values obtained in Ref. [15] for the helical coordinate system used in that paper to the nonhelical coordinate system of this paper; see Appendix D). In line with the symmetry arguments of Ref. [12], twist-bend coupling is absent in oxDNA1 (symmetric grooves), while its magnitude is comparable to that of the elastic constants A_1 , A_2 , and C in oxDNA2 (asymmetric grooves). Table I also reports the values of κ_b and κ_t , which can be obtained in two different, yet consistent, ways [15]: either indirectly from Eqs. (20) and (21), by plugging in A_1 , A_2 , C , and G of Table I, or directly from the analysis of the corresponding correlation functions in simulations (κ_b and $2\kappa_t$ are, respectively, the bending and twist persistence lengths [14]).

Figure 5 shows a plot of the effective torsional stiffness as a function of the applied force for oxDNA1 (circles) and oxDNA2 (triangles). C_{eff} was evaluated using twist fluctuations via Eq. (25). At large forces, and in agreement with the experimental evidence, oxDNA undergoes a structural transition, and hence the simulations were restricted to $f \leq 10$ pN. The solid and dotted lines of Fig. 5 are plots of Eq. (28) using κ_b and κ_t from Table I. For both oxDNA1 and oxDNA2 there is an excellent agreement between the nonperturbative theory and simulations. In the case of oxDNA1 the nonperturbative theory reduces to the Moroz-Nelson result, with $\kappa_t = C$ and $\kappa_b = A(1 - \varepsilon^2/A^2)$; the good account of oxDNA1 C_{eff} by this formula was noted previously (see Ref. [39], Fig. S7). We note that in the light of our present results, this good agreement

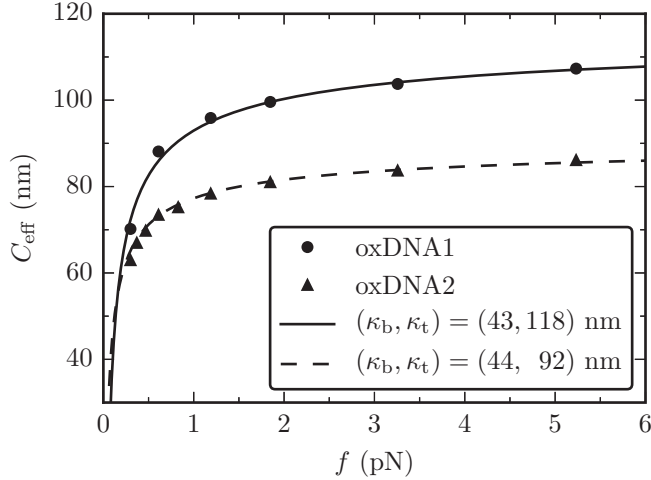


FIG. 5. Comparison of oxDNA simulations to Eq. (28). Solid and dashed lines show the nonperturbative result for C_{eff} for oxDNA1 and oxDNA2 values of Table I, respectively. Error bars for the oxDNA data are smaller than symbol sizes.

validates the use of the values of the stiffness parameters obtained in Ref. [15].

V. DISCUSSION

We have investigated the effect of the twist-bend coupling G on the statistical-mechanical properties of the twistable-wormlike-chain model of a stretched DNA molecule, using analytical and numerical methods. Our major analytical results are based on a perturbative calculation of the effective torsional stiffness C_{eff} , the torsional resistance of a long DNA molecule stretched by an applied force f . The calculation is valid for small values of G and generalizes the expression derived by Moroz and Nelson, which was obtained for $G = 0$ [4].

A. Screening effect for $f < f_0$

A striking feature of our theory is the appearance of a large force scale, $f_0 = k_B T A \omega_0^2 \approx 600$ pN. For forces well below this gargantuan force level (essentially all single-DNA mechanical experiments concern forces far below this value) the effect of G becomes solely renormalization of the bending and twisting stiffnesses κ_b and κ_t ; direct effects of twist-bend coupling are “screened” at lower force scales. Only at forces $f \gg f_0$ do the bare elastic constants start to reveal themselves: In this regime C_{eff} finally approaches its intrinsic value C . Note that the large force regime $f \gg f_0$ is experimentally inaccessible, as it corresponds to forces beyond those where DNA rapidly breaks.

This “screening” feature of the perturbative theory suggested to us that we could consider DNA for $f < f_0$ to be described by a TWLC with persistence lengths set to the zero-force long-molecule stiffnesses κ_b and κ_t . Combining formulas for the stiffnesses for freely fluctuating DNA [14] with the C_{eff} formula of Moroz and Nelson [4] gave us a non-perturbative formula for C_{eff} in terms of the elastic constants A_1, A_2, C , and G . MC calculations for the triad model which

discretizes the continuum elasticity theory (3) were found to be in excellent agreement both with the perturbative (i.e., small G) and nonperturbative (for larger G) expressions of C_{eff} . We note that, despite being inaccessible experimentally, in MC simulations we observed the very high force behavior of the perturbative theory—namely the increase of C_{eff} from its low-force Moroz-Nelson behavior toward its “naked” value of C in the triad MC calculations.

The screening discussed here applies to single-molecule measurements sampling the torsional response of a kilobase-long molecule. Locally, at the distance of a few base pairs, twist-bend coupling has directly observable effects, as discussed recently [16,40]. For instance, in DNA minicircles, the twist oscillates as a response to pure bending deformations, as seen in x-ray structures of nucleosomal DNA [16].

B. oxDNA under moderate forces is described by the TWLC plus twist-bend coupling

To test whether our analytical results describe the coarse-grained behavior of a more realistic molecular model of DNA, we carried out MD simulations of oxDNA, a coarse-grained model describing DNA as two intertwined strings of rigid nucleotides [33]. C_{eff} for oxDNA1, a DNA model with symmetric grooves, was determined in previous work [39] and found to be in agreement with the ($G = 0$) Moroz-Nelson theory.

For the more realistic oxDNA2, which has the asymmetric grooves of real DNA and hence twist-bend coupling ($G \neq 0$) [15], we found that C_{eff} is in excellent agreement with the nonperturbative theory Eq. (28) without any adjustable parameters, as the elastic constants were determined in previous work [15]. oxDNA2 appears to be precisely described by our nonperturbative theory for forces $f \ll f_0$ (recall that oxDNA undergoes internal structural transitions for forces of a few tens of pN, providing a more stringent constraint on force than the giant force scale f_0). Put another way, the “TWLC plus G ” is the “correct” low-force, long-fluctuation wavelength description of oxDNA2.

C. Experimental data

We finally compare the analytical results with experimental magnetic tweezers data of Refs. [14,22]. Figure 6 shows experimental data (symbols) together with plots of Eq. (28) for two sets of parameters κ_b and κ_t (lines). The latter are identical to the solid and dashed lines of Fig. 5, which are numerically precise descriptions of oxDNA1 and oxDNA2, respectively. Since the force fields in oxDNA were carefully tuned to reproduce several mechanical and thermodynamic properties of DNA [33], it is sensible to directly compare our nonperturbative theory to experimental data (Fig. 6).

As reported in previous papers [14,17] the experimental C_{eff} data are systematically lower than the prediction of the Moroz and Nelson theory, which precisely matches the oxDNA1 results (solid line in Fig. 6). The oxDNA2-nonperturbative theory (dashed curve) is closer to the experimental data, especially in the low-force regime $f < 1$ pN. However, some systematic deviations are noticeable at higher forces, where theory appears to underestimate the

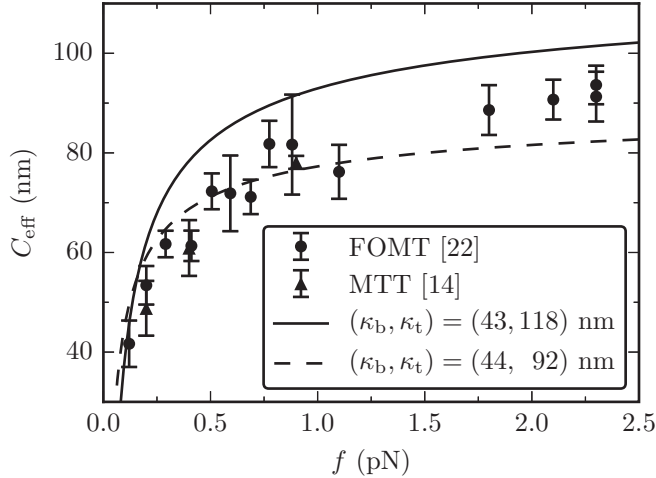


FIG. 6. Comparison of the theory from Eq. (28) (lines) with magnetic tweezer experiments (symbols) for C_{eff} vs. force. The lines have the same parametrization of the solid and dashed lines of Fig. 5, which fit oxDNA and oxDNA2 data, respectively. Two sets of experiments are shown: the freely orbiting magnetic tweezers [22] (circles) and magnetic torque tweezers [14] (triangles).

experimental C_{eff} . In addition, measurements at $f = 15$ pN (albeit for a slightly different assay) yield $C_{\text{eff}} = 110$ nm [41], well above the oxDNA2 value of $C_{\text{eff}} = 92$ nm.

We conclude by noting that oxDNA2—which has the realistic features of groove asymmetry and $G \neq 0$ —produces data in reasonable agreement with experiments. We have also shown that in the force range where we expect that coarse graining of oxDNA2 should agree with our analytical results, it does. In that same force range ($f < 10$ pN), oxDNA2 and our analytical results show some systematic deviations from experiments that suggest that physics beyond simple harmonic elasticity may be in play at intermediate forces (1 to 10 pN), generating torsional stiffening of DNA. A possible mechanism of cooperative structural transition in a two-state model with different base-pair rise (separation) was recently discussed in Ref. [42]. The next generation of coarse-grained DNA models likely will have to consider this kind of additional, internal degree of freedom to properly describe severe distortion of DNA by proteins or similar situations where strong forces are applied at short length scales.

D. The value of the intrinsic torsional stiffness C

Experiments probing torsional properties of DNA, performed since the 1980s, have provided estimates of C ranging from 40 to 120 nm, depending on the technique used [6,43–47]. All these experiments were analyzed within the framework of the standard TWLC, with no twist-bend coupling ($G = 0$). In the model discussed in this paper with $G \neq 0$, the bare torsional stiffness C , being screened by a large force scale, should not be accessible to magnetic tweezers experiments. A main point of this paper is that all the experiments ought to measure κ_t instead. We point out that Eq. (22) holds for measurements at zero force where torsional deformations are governed by the renormalized stiffness κ_t (21) and not by the bare C [14,40]. If DNA were to follow

the model (2), then torsional measurements at a given force should provide a single estimate of the torsional constant κ_t , regardless of whether the DNA is under tension or not (provided that $f \ll f_0$, which is always the case in experiments).

Deviations of experiments from Eq. (22), observed for $f > 1$ pN, indicate that DNA is torsionally stiffer for forces in the 1- to 10-pN range than expected from model (2). One possibility is that there is an additional intrinsic torsional stiffness C' in this regime of forces, as postulated by Schurr [42]. This explains the large spread in the values of the torsional stiffness in earlier experiments: At very weak $f < 1$ pN or zero forces the torsional behavior is governed by κ_t and larger forces by a novel torsional constant C' . Measurements of torsional stiffness from DNA under tension provide systematically higher values compared to the zero-tension data (see Table I of Supplemental of Ref. [14]), suggesting that there are different torsional constants in different force ranges. A similar effect was discussed in Ref. [14], where it was argued that earlier torsional DNA experiments identified two different stiffnesses: κ_t at low or zero tension and C , the intrinsic stiffness, at high tension. The present paper argues against this conclusion of Ref. [14], since the intrinsic torsional stiffness is screened at all experimentally accessible forces ($f < f_0$).

E. Effects beyond the TWLC model

The TWLC with $G \neq 0$ is still a highly simplified model of DNA molecular mechanics. One might argue that there are degrees of freedom or other features of DNA relevant to C_{eff} measurements, which are just not captured by the TWLC. The basic TWLC (with $G \neq 0$) Hamiltonian has been obtained at the single-base-pair level by coarse graining detailed molecular MD simulations [28,48], indicating that the basic symmetry features of the TWLC with $G \neq 0$ are present in real DNA or at least in chemical models of real DNA. In addition to the symmetry properties of base-pair-level deformations, the TWLC model also assumes a straight “zero-temperature” (nonfluctuating) ground state, while real DNA has a sequence-dependent nonstraight intrinsic shape; evidence for this comes from crystallography of DNA crystals and detailed chemical-structural calculations. Recent work of the latter sort suggests that DNA has an appreciable contribution to its effective persistence length by sequence-dependent bends [49].

This leads to the question of whether DNA intrinsic shape might contribute appreciably to experimental discrepancies between C_{eff} and the predictions of TWLC-type models. Prior work argues against this, showing that small-scale random intrinsic bends generate only a simple renormalization of the bending modulus (captured in the TWLC model by shifting the value of A) [50] and no renormalization of the twisting modulus [51].

These theoretical results for small-scale shape disorder notwithstanding, large-scale *nonrandom* chiral shape of the molecule (say a coiled shape at a length scale ℓ_0) could give chiral responses at zero temperature associated with removal of those coils. At finite temperature, effects of such coiling would be relevant for forces $< k_B T A / \ell_0^2$, where the correlation length for bending fluctuations is large enough to allow fluctuations to be affected by ℓ_0 . Taking $\ell_0 \approx 10$ nm (30 bp)

sets this force scale to $\lesssim 2$ pN, not far from the force range where experiment and TWLC disagree, suggesting that this permanent chiral shape might contribute to the discrepancy between TWLC and experimental C_{eff} values. Future oxDNA-like models, which incorporate sequence-shape detail, might be able to observe effects of nonrandom chiral structure. From the experimental side, high-precision measurements using DNA molecules of different sequence composition (perhaps tuned to have nonrandom chiral intrinsic shape) might be able to determine how likely it is that sequence is responsible for the discrepancies in C_{eff} between experiments and the TWLC theory.

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APPENDIX A: TWLC AT STRONG STRETCHING

We will first consider the simple case of the TWLC ($G = 0$), following closely the approach of Ref. [1]. At high forces, the molecule is strongly oriented along the force direction, which is chosen to be parallel to $\hat{\mathbf{z}}$. It proves convenient to decompose the tangent vector as

$$\hat{\mathbf{e}}_3 = t_z \hat{\mathbf{z}} + \mathbf{u}, \quad (\text{A1})$$

where the vector \mathbf{u} is orthogonal to $\hat{\mathbf{z}}$, i.e., $\mathbf{u} = u_x \hat{\mathbf{x}} + u_y \hat{\mathbf{y}}$. Using the identity $|\hat{\mathbf{e}}_3| = 1 = \sqrt{t_z^2 + \mathbf{u}^2}$ and expanding to lowest order in \mathbf{u} we get

$$\hat{\mathbf{e}}_3 = \left[1 - \frac{\mathbf{u}^2}{2} + \mathcal{O}(\mathbf{u}^4) \right] \hat{\mathbf{z}} + \mathbf{u}, \quad (\text{A2})$$

while its derivative is found to be

$$\frac{d\hat{\mathbf{e}}_3}{ds} = \frac{d\mathbf{u}}{ds} + \mathcal{O}(\mathbf{u}^2) \hat{\mathbf{z}}. \quad (\text{A3})$$

Combining this with Eq. (1), we find

$$\Omega_1^2 + \Omega_2^2 = \left(\frac{d\hat{\mathbf{e}}_3}{ds} \right)^2 = \left(\frac{d\mathbf{u}}{ds} \right)^2 + \mathcal{O}(\mathbf{u}^3). \quad (\text{A4})$$

Introducing the Fourier transform $\mathbf{u}_q = \int_0^L ds e^{-iqs} \mathbf{u}(s)$, and neglecting higher-order terms, we write the bending and stretching contribution to the energy as follows:

$$\frac{A}{2} \int_0^L ds \left(\frac{d\hat{\mathbf{e}}_3}{ds} \right)^2 - \beta \mathbf{f} \cdot \mathbf{R} = \frac{1}{2L} \sum_q (Aq^2 + \beta f) |\mathbf{u}_q|^2 - \beta f L, \quad (\text{A5})$$

where we expressed the force as $\mathbf{f} = f \hat{\mathbf{z}}$, while the end-to-end vector $\mathbf{R} = \int_0^L ds \hat{\mathbf{e}}_3$ was approximated based on Eq. (A2).

The torque in Eq. (5) is coupled to the linking number, which is the sum of twist and writhe [Eqs. (7) and (8),

respectively]. In the high-force limit, Eq. (8) becomes

$$\begin{aligned} 2\pi \text{Wr} &= \frac{1}{2} \int_0^L \left(\mathbf{u} \times \frac{d\mathbf{u}}{ds} \right) \cdot \hat{\mathbf{z}} ds + \mathcal{O}(\mathbf{u}^4) \\ &= \frac{1}{2L} \sum_q \mathbf{u}_q^T \begin{pmatrix} 0 & -iq \\ iq & 0 \end{pmatrix} \mathbf{u}_q^* + \mathcal{O}(\mathbf{u}^4), \end{aligned} \quad (\text{A6})$$

where we have rewritten the cross product as a matrix multiplication. Thus, the writhe couples the x and y components of the two-dimensional vector \mathbf{u}_q .

Adding up all terms, and with the help of simple algebraic manipulations, we obtain the following energy for the TWLC to lowest order in \mathbf{u} :

$$\begin{aligned} \beta E_0 - \beta \mathbf{f} \cdot \mathbf{R} - \beta \tau 2\pi \Delta L k \\ = \frac{1}{2L} \sum_q \mathbf{u}_q^T \mathbf{M}_q \mathbf{u}_q^* + \frac{C}{2} \int_0^L ds \omega_3^2 - \frac{\beta^2 \tau^2 L}{2C} - \beta f L, \end{aligned} \quad (\text{A7})$$

where we introduced the matrix

$$\mathbf{M}_q = \begin{pmatrix} Aq^2 + \beta f & -iq\beta\tau \\ iq\beta\tau & Aq^2 + \beta f \end{pmatrix}. \quad (\text{A8})$$

We have also introduced the shifted twist density

$$\omega_3(s) \equiv \Omega_3(s) - \frac{\beta\tau}{C}, \quad (\text{A9})$$

which allowed us to eliminate linear terms in Ω_3 . Thus, in the high-force limit, the TWLC under applied torque reduces, to lowest order, to a Gaussian model, where bending (\mathbf{u}_q) and twist (ω_3) are independent variables. The torque τ couples to the bending degrees of freedom through the off-diagonal terms of the matrix \mathbf{M}_q . The eigenvalues of \mathbf{M}_q are easily found to be

$$\lambda_q^\pm = Aq^2 + \beta f \pm q\beta\tau, \quad (\text{A10})$$

and the corresponding eigenvectors are $(\hat{\mathbf{x}} \pm i\hat{\mathbf{y}})/\sqrt{2}$. Writing Eq. (A7) on this basis allows us to calculate the partition function, from which the free energy is found to be

$$F_0 = -fL - \frac{\beta\tau^2 L}{2C} + \frac{k_B T}{2} \sum_q \log(\lambda_q^+ \lambda_q^-), \quad (\text{A11})$$

where we have neglected additive constants. Expanding to quadratic order in τ

$$\log(\lambda_q^+ \lambda_q^-) \approx \log(Aq^2 + \beta f)^2 - \left(\frac{q\beta\tau}{Aq^2 + \beta f} \right)^2, \quad (\text{A12})$$

and replacing the sum over momenta with an integral $\sum_q \rightarrow (L/2\pi) \int dq$, we obtain

$$\begin{aligned} F_0(f, \tau) &= F_0(f, 0) - \frac{\beta\tau^2 L}{2C} - \frac{\beta\tau^2 L}{8A} \sqrt{\frac{k_B T}{fA}} \\ &= F_0(f, 0) - \frac{\beta\tau^2 L}{2C_{\text{eff}}}. \end{aligned} \quad (\text{A13})$$

Combining the two last terms in the right-hand side, one obtains the Moroz and Nelson relation [Eq. (10)]. $F_0(f, \tau = 0)$ is the zero-torque free energy and is obtained by integrating

the first term in the right-hand side of Eq. (A12). Although the integral is divergent, it can be regularized by introducing a momentum cutoff $\Lambda \approx 2\pi/a$, where $a = 0.34$ nm is the separation between neighboring base pairs. As it turns out, however, this cutoff does not affect any force-dependent terms, and one has

$$F_0(f, 0) = F_0(0, 0) - fL + \sqrt{\frac{fk_B T}{A}}L, \quad (\text{A14})$$

where $F_0(0, 0)$ is cutoff dependent. Interestingly, from Eq. (5) one finds to lowest order in τ

$$2\pi \Delta Lk = -\frac{\partial F_0(f, \tau)}{\partial \tau} = \frac{\beta \tau L}{C_{\text{eff}}}, \quad (\text{A15})$$

which quantifies the induced over- or undertwisting on the application of a torque. Using this expression, one obtains the force-extension relation at fixed linking number [4,21]

$$\frac{z}{L} = -\frac{1}{L} \frac{\partial F_0}{\partial f} = 1 - \sqrt{\frac{k_B T}{4fA}} - \frac{C^2}{2} \left(\frac{k_B T}{4Af} \right)^{3/2} \left(\frac{2\pi \Delta Lk}{L} \right)^2, \quad (\text{A16})$$

which shows a characteristic parabolic profile for the extension of an over- or undertwisted, stretched molecule.

APPENDIX B: Ω_2 AT STRONG STRETCHING

The thermal average in Eq. (12) contains Ω_2 , which needs to be expressed in terms of \mathbf{u}_q and ω_3 , the degrees of freedom of the system [Eq. (A7)]. For this purpose, we use the relation

$$\Omega_2 = \hat{\mathbf{e}}_1 \cdot \frac{d\hat{\mathbf{e}}_3}{ds}, \quad (\text{B1})$$

which can be easily obtained from Eq. (1). In the high-force limit, where the tangent $\hat{\mathbf{e}}_3$ points predominantly along the force direction, $\hat{\mathbf{z}}$, one has

$$\hat{\mathbf{e}}_1 = [\cos \psi + \mathcal{O}(\mathbf{u}^2)]\hat{\mathbf{x}} + [\sin \psi + \mathcal{O}(\mathbf{u}^2)]\hat{\mathbf{y}} + \mathcal{O}(\mathbf{u})\hat{\mathbf{z}}. \quad (\text{B2})$$

Here we have introduced the twist angle

$$\psi(s) = \int_0^s \Omega_3(t) dt + \omega_0 s = \int_0^s \omega_3(t) dt + \left(\omega_0 + \frac{\beta \tau}{C} \right) s \quad (\text{B3})$$

and used Eq. (A9) to express it in terms of the variable ω_3 . Equation (B2) can be obtained by considering an arbitrary rotation that maps a fixed laboratory frame triad, e.g., $\{\hat{\mathbf{x}}, \hat{\mathbf{y}}, \hat{\mathbf{z}}\}$, onto the material frame triad $\{\hat{\mathbf{e}}_1, \hat{\mathbf{e}}_2, \hat{\mathbf{e}}_3\}$ at position s , requiring that $\hat{\mathbf{e}}_3$ remains predominantly oriented along the force direction, as in Eq. (A1). Combining Eqs. (B1) and (B2), it follows that

$$\Omega_2 = \hat{\Psi}(s) \cdot \frac{d\mathbf{u}}{ds} + \mathcal{O}(\mathbf{u}^3), \quad (\text{B4})$$

where we have defined the unit vector

$$\hat{\Psi}(s) \equiv \cos \psi(s) \hat{\mathbf{x}} + \sin \psi(s) \hat{\mathbf{y}}. \quad (\text{B5})$$

Therefore, in the high-force limit, Ω_2 can be written as a scalar product between a unit vector $\hat{\Psi}(s)$, depending exclusively on twist variables, and a vector $d\mathbf{u}/ds$, involving only the

bending degrees of freedom. Finally, from Eq. (B4) it follows that

$$\tilde{\Omega}_{2,q} = \frac{1}{L} \sum_{q'} (-iq') \Psi_{q-q'} \cdot \mathbf{u}_{q'}. \quad (\text{B6})$$

The remainder of the calculation, presented below, will be based on Eqs. (B4) and (B6).

APPENDIX C: DETAILS OF THE PERTURBATIVE CALCULATION

To calculate the average appearing in Eq. (12), it first needs to be rewritten as a function of the integration variable ω_3 [see Eq. (A9)]. This can be performed as follows:

$$\begin{aligned} & \left\langle \left(\int_0^L ds \Omega_2 \Omega_3 \right)^2 \right\rangle_0 \\ &= \frac{\beta^2 \tau^2}{C^2} \left\langle \left(\int_0^L ds \Omega_2 \right)^2 \right\rangle + \left\langle \left(\int_0^L ds \Omega_2 \omega_3 \right)^2 \right\rangle \\ &= \frac{\beta^2 \tau^2}{C^2} \langle \tilde{\Omega}_{2,0}^2 \rangle + \frac{1}{L^2} \sum_{q,k} \langle \tilde{\Omega}_{2,q} \tilde{\Omega}_{2,k} \tilde{\omega}_{3,-q} \tilde{\omega}_{3,-k} \rangle, \end{aligned} \quad (\text{C1})$$

where $\tilde{\Omega}_{2,q}$ and $\tilde{\omega}_{3,q}$ denote the Fourier components of Ω_2 and ω_3 , respectively. Note that we have neglected a linear term in ω_3 , which vanishes due to the symmetry $\omega_3 \leftrightarrow -\omega_3$. Moreover, in order to simplify the notation, we have dropped the subscript from all averages $\langle \cdot \rangle_0$, which will be always calculated within the TWLC model, i.e., for $G = 0$.

Before proceeding to the calculation of Eq. (C1), it will prove useful to first present some properties. In particular, we are going to use the following expressions, obtained from the correlation functions in the TWLC model [Eq. (A7)],

$$\langle \mathbf{u}_q \cdot \mathbf{u}_k \rangle = \frac{2L(Aq^2 + \beta f)}{(Aq^2 + \beta f)^2 - (q\beta\tau)^2} \delta_{q,-k} \quad (\text{C2})$$

and

$$\langle \mathbf{u}_q \otimes \mathbf{u}_k \rangle = \frac{-2iLq\beta\tau}{(Aq^2 + \beta f)^2 - (q\beta\tau)^2} \delta_{q,-k}. \quad (\text{C3})$$

For convenience, we have introduced the shorthand notation

$$\mathbf{a} \otimes \mathbf{b} \equiv \hat{\mathbf{z}} \cdot (\mathbf{a} \times \mathbf{b}) = a_x b_y - a_y b_x, \quad (\text{C4})$$

which is antisymmetric with respect to the interchange of \mathbf{a} and \mathbf{b} . From Eq. (C3) it follows that $\langle \mathbf{u}_q \otimes \mathbf{u}_k \rangle = 0$, when $\tau = 0$ (in this case the matrix \mathbf{M}_q is diagonal, hence the cross correlations $\langle u_q^x u_{-q}^y \rangle = 0$). Moreover, for $\tau = 0$ and $q = -k$, Eq. (C2) reduces to:

$$\langle |\mathbf{u}_q|^2 \rangle_{\tau=0} = \frac{2L}{Aq^2 + \beta f}, \quad (\text{C5})$$

which can be easily obtained from equipartition [12]. We are also going to use the following symmetries:

$$\langle u_q^x u_k^x \rangle = \langle u_q^y u_k^y \rangle, \quad (\text{C6})$$

$$\langle u_q^x u_k^y \rangle = -\langle u_q^y u_k^x \rangle, \quad (\text{C7})$$

which allow us to rearrange scalar products as follows:

$$\begin{aligned}
 & \langle \Psi_{-q} \cdot \mathbf{u}_q \Psi_{-k} \cdot \mathbf{u}_k \rangle \\
 &= \langle u_q^x u_k^x \rangle [\langle \Psi_{-q}^x \Psi_{-k}^x \rangle + \langle \Psi_{-q}^y \Psi_{-k}^y \rangle] \\
 & \quad + \langle u_q^x u_k^y \rangle [\langle \Psi_{-q}^x \Psi_{-k}^y \rangle - \langle \Psi_{-q}^y \Psi_{-k}^x \rangle] \\
 &= \frac{1}{2} [\langle \mathbf{u}_q \cdot \mathbf{u}_k \rangle \langle \Psi_{-q} \cdot \Psi_{-k} \rangle + \langle \mathbf{u}_q \otimes \mathbf{u}_k \rangle \langle \Psi_{-q} \otimes \Psi_{-k} \rangle],
 \end{aligned} \tag{C8}$$

where we have used the fact that the bending (\mathbf{u}) and twisting (Ψ) degrees of freedom are independent within the TWLC. We are now ready to proceed to the calculation of Eq. (C1). We will need to evaluate two distinct terms, which will be treated separately.

1. First term in Eq. (C1)

The first term in Eq. (C1) already contains a factor of order $\mathcal{O}(\tau^2)$, which means that up to quadratic order in τ it is sufficient to evaluate the corresponding average for $\tau = 0$

$$\begin{aligned}
 \langle \tilde{\Omega}_{2,0}^2 \rangle_{\tau=0} &= \frac{1}{L^2} \sum_{qk} (-qk) \langle \Psi_{-q} \cdot \mathbf{u}_q \Psi_{-k} \cdot \mathbf{u}_k \rangle_{\tau=0} \\
 &= \frac{1}{2L^2} \sum_q q^2 \langle |\Psi_q|^2 \rangle_{\tau=0} \langle |\Psi_q|^2 \rangle_{\tau=0},
 \end{aligned} \tag{C9}$$

where we have used Eqs. (B6) and (C8), together with the property $\langle \mathbf{u}_q \otimes \mathbf{u}_k \rangle_{\tau=0} = 0$ [see Eq. (C3)]. Next, we need to calculate the following quantity

$$\langle |\Psi_q|^2 \rangle_{\tau=0} = \int_0^L ds ds' e^{iq(s-s')} \langle \hat{\Psi}(s) \cdot \hat{\Psi}(s') \rangle_{\tau=0}. \tag{C10}$$

From Eqs. (B3) and (B5) one finds

$$\begin{aligned}
 \langle \hat{\Psi}(s) \cdot \hat{\Psi}(s') \rangle_{\tau=0} &= \langle \cos[\psi(s) - \psi(s')] \rangle \\
 &= \frac{e^{i\omega_0(s'-s)}}{2} \left\langle \exp \left(i \int_s^{s'} \omega_3(t) dt \right) \right\rangle + \text{c.c.},
 \end{aligned} \tag{C11}$$

where c.c. denotes the complex conjugate. To proceed, we perform a Fourier transform of the exponent

$$\int_s^{s'} \omega_3(t) dt = \frac{1}{L} \sum_q h_q \tilde{\omega}_{3,q}, \tag{C12}$$

where we have introduced the complex variable

$$h_q = \frac{e^{-iqs'} - e^{-iqs}}{-iq}. \tag{C13}$$

Performing Gaussian integration in $\tilde{\omega}_{3,q}$, one finds

$$\begin{aligned}
 \left\langle \exp \left(\pm \frac{i}{L} \sum_q h_q \tilde{\omega}_{3,q} \right) \right\rangle &= \exp \left(-\frac{1}{LC} \sum_{q>0} h_q h_{-q} \right) \\
 &= \exp \left(-\frac{|s' - s|}{2C} \right).
 \end{aligned} \tag{C14}$$

As expected, the decay of the twist correlation function is governed by $2C$, i.e., the twist persistence length in the TWLC. Combining Eqs. (C11), (C12), and (C14), we obtain

$$\langle \hat{\Psi}(s) \cdot \hat{\Psi}(s') \rangle_{\tau=0} = \cos[\omega_0(s' - s)] e^{-|s' - s|/2C}. \tag{C15}$$

Inserting this into Eq. (C10) yields

$$\begin{aligned}
 \langle |\Psi_q|^2 \rangle_{\tau=0} &= \frac{L}{2} \left[\frac{1}{i(q - \omega_0) + \frac{1}{2C}} + \frac{1}{i(q + \omega_0) + \frac{1}{2C}} \right. \\
 & \quad \left. + \frac{1}{i(-q + \omega_0) + \frac{1}{2C}} + \frac{1}{i(-q - \omega_0) + \frac{1}{2C}} \right] \\
 &= \frac{L}{2C} \left[\frac{1}{(q + \omega_0)^2 + \frac{1}{4C^2}} + \frac{1}{(q - \omega_0)^2 + \frac{1}{4C^2}} \right].
 \end{aligned} \tag{C16}$$

Finally, combining Eqs. (C9), (C5), and (C16), we find

$$\begin{aligned}
 \langle \tilde{\Omega}_{2,0}^2 \rangle_{\tau=0} &= \frac{1}{2\pi} \int_{-\infty}^{+\infty} \frac{dq q^2}{Aq^2 + \beta f} \langle |\Psi_q|^2 \rangle \\
 &= \frac{L}{A} \left[1 - \sqrt{\frac{\beta f}{A}} \frac{\sqrt{\frac{\beta f}{A} + \frac{1}{2C}}}{\left(\sqrt{\frac{\beta f}{A} + \frac{1}{2C}} \right)^2 + \omega_0^2} \right] \\
 &\equiv \frac{L}{A} d(f),
 \end{aligned} \tag{C17}$$

where we introduced a force-dependent scale factor $d(f)$. Note that $0 \leq d(f) \leq 1$, with $d(f) \rightarrow 1$ at small forces and $d(f) \rightarrow 0$ at high forces. Commonly accepted estimates of the DNA elastic constants put them in the vicinity of $C = 100$ nm and $A = 50$ nm, while the applied forces in typical experiments are in the range 0.1 pN $\lesssim f \lesssim 10$ pN. Recalling that room temperature corresponds to $k_B T \approx 4$ pN nm, it follows that $\beta f/A$ is at least one order of magnitude larger than $1/4C^2$. This allows for the following simplification

$$d(f) \approx \frac{1}{1 + f/f_0}, \tag{C18}$$

neglecting higher-order terms in $C^{-1}\sqrt{A/\beta f}$. We have also introduced a characteristic force

$$f_0 = Ak_B T \omega_0^2 \approx 600 \text{ pN}, \tag{C19}$$

whose value greatly exceeds those at which the double helix breaks. Thus, for the force range of interest, we may set $d(f) = 1$ in Eq. (C17). Summarizing, this first term of Eq. (C1) provides the following contribution to the free energy:

$$\Delta F^{(1)} = -\frac{G^2}{2} \frac{\beta \tau^2}{C^2} \langle \tilde{\Omega}_{2,0}^2 \rangle = -\frac{\beta \tau^2 L}{2C} \frac{G^2}{AC} d(f). \tag{C20}$$

As a final remark, we note that we could have obtained Eq. (C18) using the approximation

$$\langle |\Psi_q|^2 \rangle \approx \pi L [\delta(q + \omega_0) + \delta(q - \omega_0)]. \tag{C21}$$

Formally, this corresponds to taking the limit $C \rightarrow \infty$ in Eq. (C16), i.e., approximating the Lorentzian distributions $1/[2C(q \pm \omega_0)^2 + 1/2C]$ with δ functions. This is a valid approximation as long as $\omega_0 \gg 1/2C$, making the Lorentzians sharply peaked at large momenta $q = \pm \omega_0$, where the integrand in Eq. (C17) varies slowly.

2. Second term in Eq. (C1)

Using the same decomposition as in Eq. (C8), the second term in Eq. (C1) can be written as

$$\begin{aligned}
& \sum_{q,k} \langle \tilde{\Omega}_{2,q} \tilde{\omega}_{3,-q} \tilde{\Omega}_{2,k} \tilde{\omega}_{3,-k} \rangle \\
&= \frac{1}{L^2} \sum_{q,k,q',k'} (-q'k') \langle \Psi_{q-q'} \cdot \mathbf{u}_{q'} \Psi_{k-k'} \cdot \mathbf{u}_{k'} \tilde{\omega}_{3,-q} \tilde{\omega}_{3,-k} \rangle \\
&= \frac{1}{2L^2} \sum_{q,k,q',k'} (-q'k') [\langle \mathbf{u}_{q'} \cdot \mathbf{u}_{k'} \rangle \langle \Psi_{q-q'} \cdot \Psi_{k-k'} \tilde{\omega}_{3,-q} \tilde{\omega}_{3,-k} \rangle \\
&\quad + \langle \mathbf{u}_{q'} \otimes \mathbf{u}_{k'} \rangle \langle \Psi_{q-q'} \otimes \Psi_{k-k'} \tilde{\omega}_{3,-q} \tilde{\omega}_{3,-k} \rangle] \\
&= \frac{1}{2L^2} \sum_{q'} q'^2 [\langle |\mathbf{u}_{q'}|^2 \rangle I_s(q', \tau) + \langle \mathbf{u}_{q'} \otimes \mathbf{u}_{-q'} \rangle I_a(q', \tau)],
\end{aligned} \tag{C22}$$

where we used the fact that the \mathbf{u} correlators are diagonal in momentum space, hence $k' = -q'$ [see Eqs. (C2) and (C3)]. We have also introduced the symmetric

$$I_s(q', \tau) = \sum_{q,k} \langle \Psi_{q-q'} \cdot \Psi_{k+q'} \tilde{\omega}_{3,-q} \tilde{\omega}_{3,-k} \rangle \tag{C23}$$

and antisymmetric products

$$I_a(q', \tau) = \sum_{q,k} \langle \Psi_{q-q'} \otimes \Psi_{k+q'} \tilde{\omega}_{3,-q} \tilde{\omega}_{3,-k} \rangle. \tag{C24}$$

In what follows, we are going to compute the contribution of I_s and I_a to the free energy separately.

a. Symmetric products

For the evaluation of Eq. (C23), we will first focus on the average inside the summation, which may be written in the following way:

$$\begin{aligned}
& \langle \Psi_{q-q'} \cdot \Psi_{k+q'} \tilde{\omega}_{3,-q} \tilde{\omega}_{3,-k} \rangle \\
&= \int_0^L ds ds' e^{i(q-q')s + i(k+q')s'} \langle \hat{\Psi}(s) \cdot \hat{\Psi}(s') \tilde{\omega}_{3,-q} \tilde{\omega}_{3,-k} \rangle.
\end{aligned} \tag{C25}$$

We may now use Eqs. (C11)–(C14) so as to obtain

$$\begin{aligned}
& \langle \hat{\Psi}(s) \cdot \hat{\Psi}(s') \tilde{\omega}_{3,-q} \tilde{\omega}_{3,-k} \rangle \\
&= -\frac{L^2}{2} \left[e^{i\bar{\omega}_0(s'-s)} \frac{\partial^2}{\partial h_{-q} \partial h_{-k}} \left\langle \exp \left(\frac{i}{L} \sum_p h_p \tilde{\omega}_{3,p} \right) \right\rangle \right. \\
&\quad \left. + e^{-i\bar{\omega}_0(s'-s)} \frac{\partial^2}{\partial h_{-q} \partial h_{-k}} \left\langle \exp \left(-\frac{i}{L} \sum_p h_p \tilde{\omega}_{3,p} \right) \right\rangle \right] \\
&= -L^2 \cos[\bar{\omega}_0(s'-s)] \frac{\partial^2 e^{-\frac{1}{LC} \sum_{p>0} h_p h_{-p}}}{\partial h_{-q} \partial h_{-k}} \\
&= \frac{L}{C} \cos[\bar{\omega}_0(s'-s)] \left(\delta_{q,-k} - \frac{1}{LC} h_q h_k \right) e^{-|s'-s|/2C},
\end{aligned} \tag{C26}$$

where we have introduced the shifted intrinsic twist

$$\bar{\omega}_0 \equiv \omega_0 + \frac{\beta\tau}{C}. \tag{C27}$$

Differently from the calculation of $\langle \Omega_{2,0}^2 \rangle$ in Eq. (C9), we can no longer ignore the torque dependence of ψ [see Eq. (B3)]. Plugging Eq. (C26) back in Eq. (C25), integrating in s and s' and summing over q and k , we obtain

$$\begin{aligned}
I_s(q', \tau) &= \frac{L^3}{C} - \frac{L^3}{4C^2} \int_{-\infty}^{+\infty} dr \cos(q'r) \cos(\bar{\omega}_0 r) e^{-|r|/2C} \\
&= \frac{L^3}{C} - \frac{L^3}{8C^3} \left[\frac{1}{(q' + \bar{\omega}_0)^2 + \frac{1}{4C^2}} + \frac{1}{(q' - \bar{\omega}_0)^2 + \frac{1}{4C^2}} \right] \\
&\approx \frac{L^3}{C} - \frac{\pi L^3}{4C^2} [\delta(q' + \bar{\omega}_0) + \delta(q' - \bar{\omega}_0)].
\end{aligned} \tag{C28}$$

Throughout the calculation we introduced the variable $r \equiv s' - s$ in the double integral. Similar to Eq. (C21), we also approximated the two Lorentzians with δ functions. Note that I_s depends on the torque τ through $\bar{\omega}_0$, as indicated by Eq. (C27). Combining Eqs. (C2) and (C28), one finds

$$\begin{aligned}
& \frac{1}{2L^2} \sum_q q^2 \langle \mathbf{u}_q \cdot \mathbf{u}_{-q} \rangle I_s(q, \tau) \\
&= \frac{1}{L} \sum_q \frac{Aq^2 + \beta f}{(Aq^2 + \beta f)^2 - (q\beta\tau)^2} q^2 I_s(q, \tau) \\
&= \frac{1}{L} \sum_q \frac{q^2 I_s(q, \tau)}{Aq^2 + \beta f} + \frac{\beta^2 \tau^2}{L} \sum_q \frac{q^4 I_s(q, 0)}{(Aq^2 + \beta f)^3} + \mathcal{O}(\tau^4).
\end{aligned} \tag{C29}$$

We are interested in terms proportional to τ^2 . There are two such contributions, the first one being

$$\begin{aligned}
& \frac{1}{L} \sum_q \frac{q^2 I_s(q, \tau)}{Aq^2 + \beta f} = \frac{-L^3}{AC} \left(\sqrt{\frac{\beta f}{4A}} + \frac{1}{4C} \frac{\bar{\omega}_0^2}{A\bar{\omega}_0^2 + \beta f} + \dots \right) \\
&= -\frac{L^3}{AC} \sqrt{\frac{\beta f}{4A}} - \frac{L^3 d(f)}{4AC^2} \\
&\quad \times \left\{ 1 + \frac{\beta\tau}{C\omega_0} \tilde{d}(f) + \frac{\beta^2 \tau^2}{C^2 \omega_0^2} \tilde{d}(f) [4d(f) - 1] \right\} + \dots \\
&= -\frac{L^3}{AC} \sqrt{\frac{\beta f}{4A}} + \dots,
\end{aligned} \tag{C30}$$

where we defined $\tilde{d}(f) \equiv 1 - d(f)$, with $d(f)$ the scale factor given in Eq. (C18), and where the dots indicate omitted terms, which do not significantly contribute to the result. These terms are either independent of the torque and force or are of higher order than τ^2 . We note that $d(f) \ll 1$, i.e., it is negligibly small for the experimentally accessible forces $f \ll f_0 \approx 600$ pN. The only surviving term in Eq. (C30) is independent of τ and proportional to \sqrt{f} , hence contributing to the force-extension response. The remaining term to evaluate

in Eq. (C29) is

$$\begin{aligned} \frac{\beta^2 \tau^2}{L} \sum_q \frac{q^4 I_s(q, 0)}{(Aq^2 + \beta f)^3} &= \frac{\beta^2 \tau^2}{4\pi} \frac{\partial^2}{\partial A^2} \int \frac{dq}{Aq^2 + \beta f} I_s(q, 0) \\ &= \frac{\beta^2 \tau^2 L^3}{C} \left[\frac{3}{16A^2} \sqrt{\frac{k_B T}{fA}} - \frac{2d^3(f)}{CA^3 \omega_0^2} \right] \\ &\approx \frac{3\beta^2 \tau^2 L^3}{16A^2 C} \sqrt{\frac{k_B T}{fA}}. \end{aligned} \quad (\text{C31})$$

Note that terms containing ω_0^{-2} are always multiplied by A^{-2} or C^{-2} , hence forming dimensionless constants. Typical values for the case of DNA are $(A\omega_0)^{-2} \approx 10^{-4}$ and $(C\omega_0)^{-2} \approx 3 \times 10^{-5}$, which provide negligible contributions to the free energy, compared to other terms of the same order in τ . Therefore, the term proportional to d^3 in Eq. (C31) can be safely neglected. Combining Eqs. (C29)–(C31), we find that the relevant contribution of the symmetric term in Eq. (C22) to the free energy is

$$\Delta F^{(2)} = \frac{G^2 L}{4AC} \sqrt{\frac{fk_B T}{A}} - \frac{\beta \tau^2 L}{2C} \frac{3G^2}{16A^2} \sqrt{\frac{k_B T}{fA}}. \quad (\text{C32})$$

b. Antisymmetric products

The final part of the derivation is devoted to the calculation of the antisymmetric product in Eq. (C22). We start by expanding Eq. (C3) as follows:

$$\langle \mathbf{u}_q \otimes \mathbf{u}_{-q} \rangle = \frac{-2iLq\beta\tau}{(Aq^2 + \beta f)^2} + \mathcal{O}(\tau^3). \quad (\text{C33})$$

The calculation of the twist correlator is performed in a similar fashion as above, which yields

$$\begin{aligned} \langle \widehat{\Psi}(s) \otimes \widehat{\Psi}(s') \tilde{\omega}_{3,-q} \tilde{\omega}_{3,-k} \rangle \\ = \frac{L}{C} \sin[\omega_0(s' - s)] \left(\delta_{q,-k} - \frac{1}{LC} h_q h_k \right) e^{-|s' - s|/2C}. \end{aligned} \quad (\text{C34})$$

We may take the Fourier transform of this expression and plug it back into Eq. (C24) so as to obtain

$$\begin{aligned} I_a(q', \tau) &= -\frac{iL^3}{8C^3} \left[\frac{1}{(q' + \bar{\omega}_0)^2 + \frac{1}{4C^2}} - \frac{1}{(q' - \bar{\omega}_0)^2 + \frac{1}{4C^2}} \right] \\ &\approx -\frac{i\pi L^3}{4C^2} [\delta(q' + \bar{\omega}_0) - \delta(q' - \bar{\omega}_0)]. \end{aligned} \quad (\text{C35})$$

Finally, plugging Eqs. (C33) and (C35) into the second term of Eq. (C22), transforming the sum into an integral and performing the remaining integration, we find

$$\begin{aligned} \frac{1}{2L^2} \sum_q q^2 \langle \mathbf{u}_q \otimes \mathbf{u}_{-q} \rangle I_a(q, \tau) \\ \approx -\frac{i}{L} \sum_q \frac{q\beta\tau}{(Aq^2 + \beta f)^2} q^2 I_a(q, \tau) \\ = \frac{i\beta\tau}{2\pi} \frac{\partial}{\partial A} \int \frac{dq q I_a(q, \tau)}{Aq^2 + \beta f} = \frac{\beta\tau L^3}{4C^2} \frac{\partial}{\partial A} \frac{\bar{\omega}_0}{A\bar{\omega}_0^2 + \beta f} \\ = -\frac{\beta\tau L^3}{4C^2} \frac{\bar{\omega}_0^3}{(A\bar{\omega}_0^2 + \beta f)^2} \approx -\frac{\beta\tau d^2(f)L^3}{4A^2 C^2 \omega_0}, \end{aligned} \quad (\text{C36})$$

where we have omitted terms, which are either higher order in τ , or negligibly small compared to other terms of the same order [recall $(C\omega_0)^{-2} \approx 3 \times 10^{-5}$]. Summarizing, the contribution of the antisymmetric product to the free energy is

$$\Delta F^{(3)} = \frac{G^2 d^2(f) \tau L}{8A^2 C^2 \omega_0}. \quad (\text{C37})$$

3. Collecting the results

Throughout the derivation we found three distinct contributions to the free energy, coming from Eqs. (C20), (C32), and (C37). Adding these to Eq. (A13), i.e., the free energy of the TWLC, we find

$$\frac{1}{L} F(f, \tau) \approx -f + \sqrt{\frac{fk_B T}{A_{\text{eff}}}} + \Gamma \tau - \frac{\beta \tau^2}{2C_{\text{eff}}}, \quad (\text{C38})$$

where we have omitted both terms independent of f and τ and higher-order corrections in τ and G . We have also introduced the effective bending stiffness

$$\frac{1}{A_{\text{eff}}} = \frac{1}{A} \sqrt{1 + \frac{G^2}{4AC}} \approx \frac{1}{A} \left(1 + \frac{G^2}{2AC} \right), \quad (\text{C39})$$

together with the proportionality constant

$$\Gamma = \frac{G^2 d^2(f)}{8A^2 C^2 \omega_0}. \quad (\text{C40})$$

Finally, we reach the following expression for the effective torsional stiffness:

$$\frac{1}{C_{\text{eff}}} = \frac{1}{C} \left[1 + \frac{G^2}{AC} d(f) \right] + \frac{1}{4A} \left(1 + \frac{3G^2}{4AC} \right) \sqrt{\frac{k_B T}{fA}}, \quad (\text{C41})$$

corresponding to Eq. (16) of the main text and the central result of this work.

APPENDIX D: INTRINSIC BENDING

The analysis above is based on description of the ground-state configuration of DNA relative to a straight molecular axis [$\mathbf{\Omega} = \mathbf{0}$ in Eq. (1)], i.e., for a molecular axis which is straight in the ground state. However, one can also choose coordinates where the ground state of the double helix is a helix while still respecting the symmetry of the elastic model. In fact, this is a rather natural outcome for most choices of DNA deformation which are based on molecular modeling, where coordinates are usually chosen relative to the orientations of the base pairs (e.g., using the vector connecting the junctions of the bases to the sugar-phosphate backbone as a reference), due to the groove asymmetry of DNA. Most relevant here, our previous determination of the elastic constants of oxDNA2 [15] analyzed deformations relative to a helical coordinate system. We now show how to transform the elastic constants in such a helical coordinate system to the straight-line coordinates relevant to our calculations.

Intrinsic bending consistent with groove asymmetry, usually reported in the DNA literature as a nonzero value of the average roll [52], can be described using the following

modification of Eq. (1):

$$\frac{d\hat{\mathbf{e}}_i}{ds} = (\boldsymbol{\Omega} + l_2\hat{\mathbf{e}}_2 + l_3\hat{\mathbf{e}}_3) \times \hat{\mathbf{e}}_i, \quad (\text{D1})$$

where l_2 and l_3 correspond to the intrinsic bending and twisting densities, respectively, with $l_2 \ll l_3$. A nonzero l_1 is incompatible with the symmetry of the double helix.

Solving Eq. (D1) for $\boldsymbol{\Omega} = \mathbf{0}$, one finds that the ground-state configuration is a helix, with a linking number equal to $\text{Lk}_0 = \omega_0 L / 2\pi$, where

$$\omega_0 = \sqrt{l_2^2 + l_3^2}. \quad (\text{D2})$$

Furthermore, from the solution of Eq. (D1), it follows that the rotation matrix transforming the helical ground state of Eq. (D1) to the straight one of Eq. (1) is

$$\mathbf{R} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & l_3/\omega_0 & l_2/\omega_0 \\ 0 & -l_2/\omega_0 & l_3/\omega_0 \end{pmatrix}, \quad (\text{D3})$$

expressed on the body frame $\{\hat{\mathbf{e}}_1, \hat{\mathbf{e}}_2, \hat{\mathbf{e}}_3\}$ of the former.

The total elastic free energy should not depend on the coordinate system used to describe it, so the energy in the helical coordinates ($l_2, l_3 \neq 0$) should equal that found in nonhelical coordinates ($l_2 = 0$ and $l_3 = \omega_0$). The deformations in the “straight” model are given by $\boldsymbol{\Omega}' = \mathbf{R}^T \boldsymbol{\Omega}$, where $\boldsymbol{\Omega}$ are the deformation parameters of the helical model. From the condition that the integrand of Eq. (2) has to remain invariant under this transformation, one obtains the following relations

mapping the elastic constants from the helical coordinates to the straight ones:

$$A_{1,s} = A_1, \quad (\text{D4})$$

$$A_{2,s} = A_2 - \frac{2xG - x^2(C - A_2)}{1 + x^2}, \quad (\text{D5})$$

$$C_s = C + \frac{2xG - x^2(C - A_2)}{1 + x^2}, \quad (\text{D6})$$

$$G_s = G - \frac{x(C - A_2) + 2x^2G}{1 + x^2}, \quad (\text{D7})$$

where $x \equiv l_2/l_3 \ll 1$, and where the subscript s indicates the “straight” frame result. The transformation (D3) mixes A_2 , G , and C , changing their values, but conserves the symmetry of the elastic constant matrix. These formulas allow one to measure elastic constants using arbitrarily chosen helical reference coordinates and then convert them to elastic constants suitable for using strains defined relative to a straight-line ground state.

For unconstrained (zero force and torque) oxDNA2 simulations, we measured reference helix parameters $l_2 = 0.1349 \text{ nm}^{-1}$ and $l_3 = 1.774 \text{ nm}^{-1}$, giving $\omega_0 = 1.779 \text{ nm}^{-1}$ and $x = 0.076$. Elastic constants reported in Ref. [15] ($A_1 = 85 \text{ nm}$, $A_2 = 39 \text{ nm}$, $C = 105 \text{ nm}$, and $G = 30 \text{ nm}$) were measured in reference to helical coordinates; for use in our analytical theory we transform them to the straight coordinates using (D4)–(D7) to obtain the oxDNA2 values in Table I.

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$$\frac{1}{C_{\text{eff}}} = \frac{1}{C} + \frac{a_1}{\sqrt{f}} + \frac{a_2}{f}, \quad (\text{D8})$$

one gets

$$\frac{C_{\text{eff}}}{C} = 1 - \frac{a_1}{\sqrt{f}} + (a_1^2 C - a_2) \frac{C}{f}. \quad (\text{D9})$$

Numerics suggests that the term in $1/f$ is very small, implying $a_2 \approx a_1^2 C$.

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