The torque transfer coefficient in DNA under torsional stress

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In recent years, significant progress in understanding the properties of supercoiled DNA has been obtained due to nanotechniques that make stretching and twisting of single molecules possible. Quantitative interpretation of such experiments requires accurate knowledge of torques inside manipulated DNA. This paper argues that it is not possible to transfer the entire magnitudes of external torques to the twisting stress of the double helix, and that a reducing torque transfer coefficient (TTC < 1) should always be assumed. This assertion agrees with simple physical intuition and is supported by the results of all-atom molecular dynamics (MD) simulations. According to MD, the TTCs around 0.8 are observed in nearly optimal conditions. Reaching higher values requires special efforts and it should be difficult in practice. The TTC can be partially responsible for the persistent discrepancies between the twisting rigidity of DNA measured by different methods.

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The twisting stiffness is a unique property of DNA that makes possible its supercoiling, which is essential for genome compaction and regulation [1]. At present, the mechanics of supercoiled DNA is frequently studied by using nanotechniques that offer a means to stretch and twist single molecules [2–4]. Due to its remarkable conceptual simplicity this method has gained broad recognition. The effects of external twisting depend upon the torsional rigidity of DNA and the values of applied torques. Accurate knowledge of these quantities is necessary for interpretation of experimental data. The recent methodological advances made twisting of single DNA with external torques of known magnitude [5, 6] possible. However, one question has never been asked: Is it evident that the twisting stress created inside DNA always corresponds to the external torque measured from outside?

A simple example shown in Fig. 1 demonstrates that the answer to the above question can be negative. Consider the left panel. An elastic rod is fixed on a solid base, with a constant torque $\tau$ applied to its top, which causes a deviation of the equilibrium twist angle $\Delta \Phi = \Phi - \Phi_0$. For simplicity, assume that the bending rigidity is very high and bending deformations can be neglected. If the torque axis is deviated by angle $\theta$ only the vertical torque projection works and the twisting stress inside the cylinder as well as $\Delta \Phi_s$ decrease as $\cos \theta$. The above effect persists with the addition of other degrees of freedom. Fig. 1 illustrates this for Brownian dynamics (BD) of a discrete wormlike rod (WLR) model where bending, twisting and translational motions of DNA are reproduced in agreement with experiments [3]. In the harmonic approximation the torsional part of the free energy is

$$U_s(\Phi) = \frac{l_t}{2L} (\Phi - \Phi_s)^2$$

(1)

where $L$ is the DNA length, $k$ is Boltzmann’s constant, and $T$ is the temperature. The twisting rigidity of DNA is characterized by parameter $l_t$ called the torsional persistence length. In this case

$$\Delta \Phi_s = \frac{\tau L}{kT l_t}$$

(2)

and the probability distribution of torsional fluctuations is Gaussian

$$P_\Phi \sim \exp \left[ -\frac{l_t}{2L} (\Phi - \Phi_s)^2 \right].$$

(3)

The right panel of Fig. 1 shows a coarse-grained WLR representation of a DNA trimer. Each base pair is modeled by a rigid composite bead of four particles $O$, $X$, $Y$, and $Z$. The torque transfer coefficient in DNA under torsional stress

$$\tau(\Phi)_n = \frac{\tau L}{kT l_t}$$

(4)

and the probability distribution of torsional fluctuations is Gaussian

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Y, and Z that define a local Cartesian frame. The OZ axis is constrained to pass through the center of the following bead in the chain. Other degrees of freedom are restrained by harmonic nearest-neighbor potentials adjusted to provide the desired macroscopic rigidity for bending and twisting [3]. The twisting torques are applied to terminal composite beads by using the earlier algorithm [4], with a small modification to deviate the torque vector by a fixed angle θ with respect to the local OZ axes. The external torque at one end is compensated by reactions at the opposite end so that the integral external force and torque on the molecule are always zero. This can be viewed as if a demon in the moving global frame twists one end of the molecule and holds the opposite end so that the overall translation and rotation are not perturbed. The results of BD simulations of a 14-mer DNA fragment are shown in the middle panel. As expected, the effective torque decreases approximately as cos θ. The analytical linear dependence corresponding to the true $l_t$ value is reproduced in BD only with θ=0.

FIG. 2: (Color online) The normalized probability distributions of twisting fluctuations in DNA obtained by all-atom MD simulations [7]. The Φ angles are measured directly between the terminal base pair frames; therefore, Φ = 0 for one helical turn. The CG dodecamer was considered in relaxed state ($τ = 0$) and under steady torsional stress induced by external torques of $τ = -80$ and $+60$ pN nm, respectively. The dashed lines show the corresponding Gaussian distributions [Eq. (3)] for the computed values of $Φ_{τ}$ and $l_τ$. The solid lines display the analytical distributions for the best-fit anharmonic torsional potential

It might seem that the above example is irrelevant because in experiments the external torques are always applied along the stretched DNA double helix. The problem is that local twisting axes in real DNA may be inclined with respect to the helix direction. This issue surfaced unexpectedly in the recent study of elastic properties of strained DNA by all-atom molecular dynamics (MD) [4,10]. In these computations steady twisting torques were applied to two short DNA fragments that I denote here as TA and CG (because of the AT- and GC-alternating sequences, respectively). The classical all-atom MD produce the probability distribution of twisting $P_Φ$. The three distributions shown in Fig. 2 were obtained for dodecamer CG (one helical turn) with contrasting values of the external torque. From these data, the $l_t$ value can be extracted by using either Eq. (2) or (3) and the resulting two values should be identical. However, according to Eq. (3) the variance of the distribution should be constant whereas in Fig. 2 it evidently changes with $τ$: that is, the harmonic approximation breaks down. Therefore, a broader issue should be considered, namely, whether or not these MD results agree with statistical mechanics of an anharmonic elastic rod. To answer this question we look for an anharmonic potential that would consistently describe the MD data. Assuming that $Φ_{τ} = 0$ for zero load the free energy is

$$U_τ(Φ) = -τΦ + U(Φ),$$

where $U(Φ)$ is the torsional potential of bare DNA, therefore,

$$τ = \frac{dU}{dΦ}|_{Φ_{τ}}. \quad (4)$$

According to Fig. 2 all the probability distributions are nearly Gaussian; that is, in the vicinity of $Φ_{τ}$ the harmonic approximation is valid and one can use the Taylor series to obtain

$$l_τ(τ) = \frac{L}{kT} U''(Φ_{τ}). \quad (5)$$

Let us look for a polynomial form of $U(Φ)$. We choose $U(0)=0$; in this case the first term is quadratic. For $l_τ(τ)$ not to be constant the polynomial should be of the third degree or higher, for instance,

$$U(Φ) = q_1 \frac{1}{2} Φ^2 + q_2 \frac{1}{6} Φ^3 + q_3 \frac{1}{24} Φ^4. \quad (6)$$

In this case Eq. (4) and (5) give

$$τ = q_1 Φ_{τ} + q_2 \frac{1}{2} Φ_{τ}^2 + q_3 \frac{1}{6} Φ_{τ}^3 \quad (7)$$

$$l_τ = \frac{L}{kT} \left( q_1 + q_2 Φ_{τ} + q_3 \frac{1}{2} Φ_{τ}^2 \right). \quad (8)$$

MD provides $Φ_{τ}$ and $l_τ$ measured for several values of $τ$. Coefficients $q_i$ are found by the least squares minimization of the discrepancy between the left- and right-hand parts of Eq. (7) and (8). The results of this fitting are shown in Fig. 3 by dashed red lines. It is seen that they significantly deviate from the MD points. The deviations are clearly systematic and they go far beyond the error ranges. This situation does not change with increased degree of the polynomial. Note that in all plots the deviations grow with $|τ|$. It turned out that the quality of fitting is radically improved if a torque transfer coefficient (TTC) $q_0$ is added to Eq. (4) as follows:

$$q_0 τ = q_1 Φ_{τ} + q_2 \frac{1}{2} Φ_{τ}^2 + q_3 \frac{1}{6} Φ_{τ}^3, \quad (9)$$

with $q_0$ optimized together with other $q_i$. In this case, the agreement within the range of statistical errors is easily
reached for polynomials of the fourth degree and higher. Interestingly, the optimized TTC values are similar for CG and TA (0.81 and 0.79, respectively) and they vary by only a few percent when the degree of the polynomial is increased up to six. The quality of fitting is illustrated by the solid traces in Fig. 2 and 3. The agreement is quite good, notably, small deviations of \( P_\delta \) from Gaussians in Fig. 2 are well reproduced. These subtleties have low statistical weight and actually do not affect the values of \( \Phi \) directly. The dashed red lines show the results of fitting for a fourth order polynomial torsional potential without a TTC applied. The solid black lines show best fit dependences for a fourth order polynomial with optimized TTC.

The difference between Eq. (7), and (9) indicates that \( \delta \) in Eq. (9) can be reasonably explained. The possible origin of incomplete torque transfer was shown in Fig. 3 above. The DNA can respond to external torques by either twisting or bending or both. The torque fraction that causes twisting varies with angle \( \theta \) whereas the remaining part is largely canceled by auxiliary reaction forces. Such forces are always necessary. In the left panel of Fig. 1 and in single molecule experiments the reaction forces maintain DNA straight and fixed in place. In computer simulations, they are used to zero the integral external force and torque that otherwise would accelerate the overall translation and rotation. All these reactions do not affect torsional fluctuations, but effectively damp all other deformations, which is where a part of the external torque goes.

The orientation of the twisting axis in real DNA is not known. The external torques in MD are applied to terminal bases, with the torque axes orthogonal to the base plane. On closer inspection, however, one notices that this choice may not be optimal. There are other distinguished directions, for instance, the helical axis or the perpendicular to the base-pair plane. However, these directions are well defined and coincident only in the ideal B-DNA model. In reality, bases are not perpendicular to the helix direction and paired bases are not co-planar, therefore, the definition of the base pair plane as well as the helical axis is a matter of convention. Moreover, local twisting occurs due to many rotatable covalent bonds and it is possible that the transfer of the torsional stress to DNA depends upon specific orientations of these bonds near the point where the torque is applied.

To get an idea of the situation in real DNA, I tried to vary the \( \theta \) angle in MD similarly to the BD simulations presented in Fig. 1. The protocols of MD simulations were the same as before. The external torque is applied by using a Cartesian frame rigidly attached to the base. The axes of this frame approximately correspond to the standard convention (see Fig. 4), and by default the torque is directed along the Z axis. Unlike the WLR model, the all-atom DNA does not have cylindrical symmetry, therefore with \( \theta \neq 0 \), we should also check different torque azimuths. The natural qualitatively distinct directions of deviation correspond to \( XZ \) and \( YZ \) planes. With \( \theta \neq 0 \) in the \( XZ \) plane, the torque vector is inclined toward the major (\( \theta < 0 \)) or minor (\( \theta > 0 \)) groove. In the YZ plane, the torque can be inclined toward the backbone of the same (\( \theta < 0 \)) or the opposite (\( \theta > 0 \)) base. The magnitude of the external torque was fixed at \( \pm 40 \) pN nm. A relatively large value is required to obtain variations beyond the range of statistical errors. Earlier studies showed that, in MD, the properties of short DNA fragments change smoothly in this range of torques.

The results shown in Fig. 4 confirm that the effect is also significant in the all-atom MD. Computed points are compared with theoretical dependences of the form \( \delta \cos(\theta - \theta_s) \), with \( \delta \) and \( \theta_s \) adjusted manually. Angle \( \theta_s \) specifies the softest local twisting axis. Parameter \( \delta \) affects simultaneously the vertical displacement and the
inclination of the curves, i.e., the fitting is constrained. The agreement is relatively good for twisting and less so for untwisting. In both planes the softest axes are found close to, but not exactly perpendicular to the base. For deviations in the $YZ$ plane the optimal $\theta_s$ value is about $-5^\circ$ for both signs of the torque. In the $XZ$ plane the softest twisting direction is observed at $\theta_s = +10^\circ$ and $+20^\circ$ for twisting and untwisting, respectively. Therefore, the all-azimuth softest axis may be inclined by $20$-$30\%$ lower than in direct measurements using forced DNA twisting by calibrated external torques [5, 6]. Significant discrepancies in the $C$ value compared to recent single-molecule studies, $C \approx 310$ pN nm$^2$ was considered as a consensus value for random sequence DNA. This value reasonably agrees with some indirect estimates that used single-molecule data [13, 16], but it is at least $20$-$30\%$ lower than in direct measurements using forced DNA twisting by calibrated external torques [3, 4]. Several pitfalls possibly responsible for such a difference have been discussed in the literature [2, 17]. Assuming the TTC values around 0.8, which may slightly vary between different experimental installations, all these data can be accounted for without additional assumptions. Only one observation cannot be explained, namely, the very high apparent torsional rigidity ($C = 440$ pN nm$^2$) reported for equilibrium thermal torsional fluctuations of single stretched DNA [2]. I believe that some other factors could affect this result and it requires further consideration.

In summary, the present paper shows that because of the structural details of the double helix, it is not possible to transfer the entire magnitudes of external torques to the internal twisting stress of DNA, and that a reducing torque transfer coefficient should always be assumed. This assertion agrees with simple physical intuition and it is supported by the results of all-atom MD simulations.
Theoretical estimates indicate that the TTCs around 0.8 should be observed for torques nearly parallel to the double helix. Reaching higher values requires special efforts that should take into account the details of the DNA structure and it should be very difficult for experiments with long molecules. The TTC explains some controversies in the experimental values of DNA twisting rigidity measured by different methods.

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