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Direct Determination of DNA Twist-Stretch Coupling

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The symmetries of the DNA double helix require a new term in its linear response to stress: the coupling between twist and stretch. Recent experiments with torsionally-constrained single molecules give the first direct measurement of this important material parameter. We extract its value from a recent experiment of Strick *et al.* [Science **271** (1996) 1835] and find agreement with an independent experimental estimate recently given by Marko. We also present a very simple microscopic theory predicting a value comparable to the one observed.

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1. Introduction: The idea of studying the response of DNA to mechanical stress is as old as the discovery of the double helix structure itself [1]. While many elements of DNA function require detailed understanding of specific chemical bonds (for example the binding of small ligands), still others are quite nonspecific and reflect overall mechanical properties. Moreover, since the helix repeat distance of $\ell_0 \approx 3.4\,\mathrm{nm}$ involves dozens of atoms, it is reasonable to hope that this length-scale regime would be long enough so that the cooperative response of many atoms would justify the use of a continuum, classical theory, yet short enough that the spatial structure of DNA matters. In this Letter we will argue that this expectation is indeed fulfilled.

Early work showed that a simple model of DNA as a cylindrical elastic rod gives a reasonable account of many features of its long-scale behavior, for example supercoiling [2]. Some authors sought to justify this picture by invoking a shell of structured water around the DNA [2]. The model contained two elastic constants, the resistance to bending and twisting, and a number of elegant experiments yielded fair agreement on their values[3]. More recently, techniques of micromanipulation via optical tweezers and magnetic beads have yielded improved values for the bend stiffness from the phenomenon of thermally-induced entropic elasticity [4][5], as well as the direct measurement of a third elastic constant, the stretch modulus, by exploring the force range 10–50 pN [6][7]. Significantly, the relation between bending stiffness, stretch modulus, and the diameter of DNA turned out to be roughly as predicted from the classical theory of beam elasticity [6][7][8], supporting the expectations mentioned above.

Still missing, however, has been any direct measurement of the elastic constants reflecting the *chiral* (*i.e.* helical) character of DNA. One such constant, a twist-bend coupling, was investigated by Marko and Siggia [9], but no direct experimental measurement has yet been devised. In this Letter we introduce a new chiral coupling, the twist-stretch energy. We will explain why our term is needed, extract its value from the experiment of Strick *et al.* [10], and compare it to the prediction of a simple microscopic model to see that its magnitude is in line with the expectations of classical elasticity theory. J. Marko has independently introduced the same coupling and estimated its value from different experiments[11]; our values are in rough agreement.

2. Experiment: DNA differs from simpler polymers in that it can resist twisting, but it is not easy to measure this effect directly due to the difficulty of applying external torques to a single molecule. Early investigations of DNA twist were either limited to passive, fluorescence-depolarization measurements [3], or else to studying global shape changes in circular DNA of varying linkage [2]. The first single-molecule stretching experiments constrained only the locations of the two ends of the DNA strand. The unique feature of the experiment of Strick *et al.* was the added ability to constrain the *orientation* of each end of the molecule.

We will study Fig. 3a of ref. [10]. In this experiment, a constant force of 8pN was applied to the molecule and the end-to-end length z_{tot} monitored as the terminal end was rotated through $\Delta\mathrm{Lk}$ turns from its relaxed state (which has Lk_0 turns). In this way the helix could be over- or undertwisted by as much as $\pm 10\%$. Over this range of imposed linkage z_{tot} was found to be a linear function of σ:

$$\varepsilon = \text{const.} - 0.15\sigma \quad \text{where} \quad \sigma \equiv \Delta\mathrm{Lk}/\mathrm{Lk}_0 \quad \text{and} \quad \varepsilon \equiv (z_{\mathrm{tot}}/z_{\mathrm{tot},0}) - 1 \ . \tag{1}$$

Thus σ is the fractional excess link and ε is the extension relative to the relaxed state. Eqn. (1) is the experimentally observed twist-stretch coupling.

The existence of a linear term in (1) is direct evidence of the chiral character of the molecule, and its sign is as expected on geometrical grounds: untwisting the molecule tends to lengthen it. Still geometry alone cannot explain this result [12]; we must seek an explanation of the experimental result not in terms of a geometrical ball-and-stick model but in the context of an elastic response theory.

3. *Simple Model:* We will begin by neglecting bend fluctuations (see below). A straight rod under tension and torque will stretch and twist. We can describe it by the reduced elastic free energy

$$f_1(\sigma,\varepsilon) \equiv \frac{F_1(\sigma,\varepsilon)}{k_BT z_{\mathrm{tot},0}} = \frac{{\omega_0}^2}{2}\left[\bar{C}\sigma^2 + \bar{B}\varepsilon^2 + 2\bar{D}\varepsilon\sigma\right] - \tau\varepsilon \ . \tag{2}$$

The twist persistence length is $\bar{C} \approx 75\,\mathrm{nm}$ [3], while the helix parameter $\omega_0 = 2\pi/\ell_0 = 1.85/\mathrm{nm}$. We will take[1] $\bar{B} \approx 1100\,\mathrm{pN}/{\omega_0}^2 k_BT \approx 78\,\mathrm{nm}$ [7]. In the experiment under study the reduced force is $\tau = 8\,\mathrm{pN}/k_BT \approx 1.95/\mathrm{nm}$. For a circular beam made of isotropic material the cross-term $\bar{D}$ is absent [8], since twisting is odd under spatial inversion while stretching is even. For a helical beam, however, we must expect to find this term.

[1] $\bar{B}$ reflects the intrinsic stretchiness of DNA, since electrostatic self-repulsion simply shifts the equilibrium length without affecting the spring constant. Indeed experiments show little or no dependence of $\bar{B}$ on salt, unlike the situation with the effective bend persistence length [7]. We also expect $\bar{C}$ to reflect intrinsic elasticity, since twisting does not affect the average charge distribution.

We now minimize f_1 with respect to ε, σ at fixed tension with an imposed constraint on the overtwist σ. Minimizing at fixed σ and τ gives $\Lambda = {\omega_0}^2(\bar{D}\varepsilon + \bar{C}\sigma)$ and hence $\varepsilon = \varepsilon_{\sigma=0} - (\bar{D}/\bar{B})\sigma$. Comparing to (1), we obtain the desired result: $\bar{D} = 12\,\mathrm{nm}$. To compare this to Marko's analysis, we note that his dimensionless g equals our $\bar{D}\omega_0$, so that we get $g = 22$. Marko's result was $g = 35$ [11]; the difference comes mainly from the more recent value of $\bar{B}$ which we have used here. This agreement indicates that the data show a real material parameter of DNA and not some artifact.

4. Bend Fluctuations: To arrive at (2) we listed the variables which were constrained, coupled to external forces, and/or observed in the experiment, namely ε and σ, then wrote the most general quadratic function allowed by symmetry. Thus (2) is a phenomenological model; its coefficients $\bar{C}$, $\bar{B}$, $\bar{D}$ reflect both intrinsic elasticity and the effects of thermal fluctuations. Indeed it is well known that thermal bend fluctuations reduce the effective stretch modulus at modest tension via the "entropic elasticity" effect [4][5]. Our procedure may seem inconsistent, since we arrived at our value of $\bar{D}$ by using the *intrinsic* stretch modulus in sect. 3. In this section we will justify the procedure by sketching a more elaborate model with bend fluctuations and again comparing to (1). Details of this calculation will appear elsewhere [12].

We begin by defining local variables (Fig. 1a) (see [9]). DNA is a stack of base pairs. We neglect sequence effects and so regard all base pairs as copies of one standard slice. The standard slice contains a reference point with the property that the locus of these is the helix axis, a straight line of length L in the relaxed state. Through this reference point we next draw a fixed vector; a convenient choice is the "dyad" pointing into the minor groove and perpendicular to the helix axis.

To describe stressed states, we simply specify the locus of reference points as a parameterized curve in space (dotted line in Fig. 1a) and the dyad as a field of vectors $\hat{E}_1$ normal to this curve. We let $\hat{E}_3$ be the unit tangent to the axis and complete to an orthonormal triad by defining $\hat{E}_2 = \hat{E}_3 \times \hat{E}_1$. Next we introduce a parameter s to label each slice; s corresponds to arc length along the original, unstressed helix axis and so always runs from 0 to L. The actual arc length along the distorted axis will not be $\mathrm{d}s$ but rather $(1 + \alpha(s))\,\mathrm{d}s$; α is the axial strain.

Thus our local variables are $\hat{E}_i(s)$ and $\alpha(s)$. Our program consists of four steps: *i)* Find the strains in terms of the local variables. *ii)* Write the general linear elasticity theory of these strains with a force coupling to the extension ε and a torque coupling to the twist σ. *iii)* Compute and minimize the free energy to find the end-to-end length

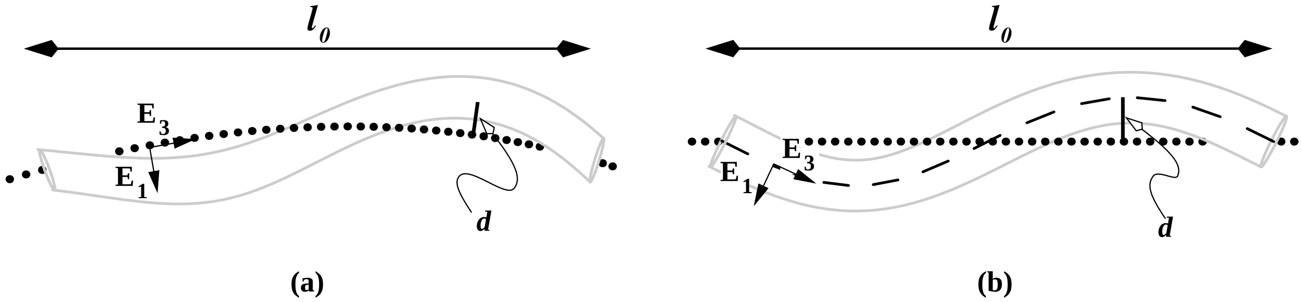

Fig. 1: Schematic diagrams defining variables used in the text. The offset from the helix axis has been exaggerated for clarity. a) Notation used in §4. We describe the DNA by the helix axis (dotted curve) and the axis $\hat{E}_1$, which is a fixed vector in each base pair. b) Notation used in §5. The helix axis (dotted line) is now supposed straight. We describe the DNA by the dashed curve and the axis $\hat{E}_1$ as before.

$\langle z_{\rm tot}\rangle$ in terms of the constrained σ and the applied force τ. *iv)* We can then relate the experimental result to intrinsic elastic constants.

Steps i,ii: In the relaxed state each slice bears a constant relation to its predecessor. Thus while $\hat{E}_{10}$, $\hat{E}_{20}$, and $\hat{E}_{30}$ all vary in space, the derivatives with respect to s are of the form $\mathrm{d}\hat{E}_{i0}/\mathrm{d}s = -\varepsilon^{ijk}\Omega_{j0}\hat{E}_{k0}$, where Ω_{j0} are the constants $(0, 0, \omega_0)$. For the deformed state, the same formula defines the functions $\Omega_i(s)$. Our strain variables are then $\Omega_1(s)$, $\Omega_2(s)$, $\Omega_3(s) - \omega_0$, and $\alpha(s)$. The end-for-end symmetry of DNA implies that the elastic matrix is unchanged upon changing the sign of Ω_1 [9]. Thus we generalize the model of [9] to

$$f_2 = \frac{1}{2L}\int_0^L \mathrm{d}s\Big[A'{\Omega_1}^2 + A{\Omega_2}^2 + C(\Omega_3-\omega_0)^2 + B{\omega_0}^2\alpha^2 + 2D\omega_0(\Omega_3-\omega_0)\alpha + 2G(\Omega_3-\omega_0)\Omega_2 + 2K\omega_0\Omega_2\alpha\Big]\,. \qquad (3)$$

Here G is the twist-bend coupling of [9], while K is an allowed coupling between stretch and bend [12]. K reflects the possibility that under extension the helix axis can move away from the chosen reference point, so that the latter no longer follows a straight line.

We may apply a perturbative treatment to (3). Such an approximation is valid since in the experiment we are analyzing the applied force is large enough to keep the end-to-end distance over 90% the full relaxed contour length, but not large enough to create large intrinsic stretch α. In addition, the applied overtwist σ is at most $\pm 10\%$ [10]; indeed the slope reported in (1) can be found from an even smaller range of σ than this.

Steps iii, iv: It proves useful to refer the frame $\{\hat{E}_1,\ \hat{E}_2,\ \hat{E}_3\}$ to a standard frame $\{\hat{e}_1,\ \hat{e}_2,\ \hat{z}\}$ rotating at spatial frequency ω_0. We then write the deformed frame in terms of three small quantities: two deviations of the tangent vector $t_{1,2}(s)$ and an angle $\varphi(s)$. To first order in these we have $\hat{E}_1 = \hat{e}_1 + \varphi\hat{e}_2 - t_1\hat{z}$, $\hat{E}_2 = -\varphi\hat{e}_1 + \hat{e}_2 - t_2\hat{z}$, $\hat{E}_3 = t_1\hat{e}_1 + t_2\hat{e}_2 + \hat{z}$. Substituting into (3) and adding external tension τ and torque Λ then gives a linear elastic theory. The measured twist-stretch coupling $\bar{D}$ can then be read off as the combination $\bar{D} = D - \frac{GK}{A}$ of intrinsic elastic parameters. (Parenthetically we note that $\bar{C} = C - \frac{G^2}{A}$ is nearly equal to C because we expect G to be small, and similarly for $\bar{B} = B - \frac{K^2}{A}$. The corrections are small because they reflect the small deviation of DNA from a straight circular rod.) Thus we have found the interpretation of the experimentally-determined twist-stretch coupling found in section 2: in terms of the intrinsic elasticity of DNA the slope in (1) fixes the combination $(DA - GK)/AB$ in (3) to be 0.15. The low-force stretching experiments give bend stiffness $A = 40\,\mathrm{nm}$ [7]. Other experiments fix $B,\ C$ to the approximate values quoted earlier. The remaining combinations of the couplings in (3) do not appear to be relevant for existing experiments.

We can now address the concern mentioned at the start of this section. The entropic elasticity phenomenon is a breakdown of linear elasticity when the applied force $\tau \to 0$; it arises because some Fourier modes of t_i get large fluctuations in this limit. Inspecting our elastic theory shows that these dangerous modes have spatial frequency near ω_0; they decouple completely from σ, which couples linearly to the modes of wavenumber 0. Thus the entropic contribution to $\langle z_{\mathrm{tot}}\rangle$ can simply be absorbed into the constant term of (1), and does not affect the slope used in our calculation.

5. Microscopic Model: The elastic theory in the previous section was very general, but it gave no indication of the expected magnitudes of the various couplings. To gain further confidence in our result, we will now see how to *estimate* the expected twist-stretch coupling based on the measured values of the other elastic constants and geometrical information about DNA. We will use a simple, intuitive microscopic picture of DNA as a helical rod to show how twist-stretch coupling can arise and get its general scaling with the geometric parameters. While the model is unrealistic it captures the underlying symmetries and shows that the value of $\bar{D}$ calculated above is reasonable.

Our picture will be a beam of isotropic elastic material of circular cross-section, initially bent into a helix of pitch ℓ_0, with the beam center slightly displaced from the helix axis by $d_0 \ll \ell_0$ [13]. Fig. 2b defines our notation. In this section we will consider only uniform deformations of the helix; in particular the helix axis will always be straight. It proves convenient to define slightly different variables from the previous section: instead of following the helix axis, now our curve follows the centerline of the beam. We again call the tangent to this curve $\hat{E}_{30}(s)$, where the arc length s runs from 0 to $\widetilde{L}$, but now $\widetilde{L}$ is slightly longer than the end-to-end length of the relaxed beam. Next we draw a second curve, the locus of points farthest from the helix axis. Let $\hat{E}_{10}(s)$ be the field of vectors perpendicular to the tangent $\hat{E}_{30}(s)$ and pointing from the first to the second curve. Finally complete $\hat{E}_{30}$, $\hat{E}_{10}$ to an orthonormal triad by defining $\hat{E}_{20} = \hat{E}_{30} \times \hat{E}_{10}$.

The distorted beam will then have its modified frame $\{\hat{E}_1(s),\ \hat{E}_2(s),\ \hat{E}_3(s)\}$, where now s is the arc length rescaled by $(1+\alpha)^{-1}$ to again run from 0 to $\widetilde{L}$ and α is the axial strain as before. We also define strain variables Ω_i as before; for the uniform deformations considered these are constants independent of s. For a nearly-straight, circular rod the elastic energy is then [8]$f_3 = \frac{1}{2}\left[A(\Omega_2-\Omega_{20})^2 + C(\Omega_3-\Omega_{30})^2 + B{\omega_0}^2\alpha^2\right]$. We have rotated our reference frames about the tangent vector to eliminate Ω_1. The constants A, C, and B can in turn be expressed in terms of the effective Young modulus, shear modulus, and diameter of the rod, but we instead use the measured values quoted earlier.

To use f_3 we need to find Ω_2 and Ω_3 in terms of the helix parameters: helix axis offset d, end-to-end length $z_{\rm tot}$, and total rotation of the cross-section. The latter quantity plays the role of linking number for open DNA, and so we will call it Lk. To get the required relations it is helpful to use the physical image of a gyroscope rotating at "angular frequency" $|\vec{\Omega}|$ about an axis parallel to $\vec{\Omega}$ while moving at constant "speed" along an axis $\hat{E}_3$ fixed in the body. We take "time" to run from 0 to $\widetilde{L}$, the original relaxed arc length; to allow for intrinsic stretching we then take the "speed" to be $1+\alpha$. One then finds that

$$d = \Omega_2(1+\alpha)/|\vec{\Omega}|^2\ , \quad z_{\rm tot} = \widetilde{L}\Omega_3(1+\alpha)/|\vec{\Omega}|\ , \quad {\rm Lk} = \widetilde{L}|\vec{\Omega}|/2\pi\ . \tag{4}$$

To fix $\vec{\Omega}_0$ we impose the values $\alpha_0 = 0$, $\omega_0 = 2\pi{\rm Lk}_0/z_{\rm tot,0} = 1.85/{\rm nm}$, and a helix offset d_0. We will choose d_0 to get the observed value of $\bar{D}$ and see that it is reasonable. Working to second order in d_0 eqn. (4) gives $\Omega_{30} = \omega_0(1-{d_0}^2{\omega_0}^2)$, $\Omega_{20} = d_0{\omega_0}^2$.

We must now minimize f_3 with the constraint of fixed $z_{\rm tot}$ and Lk. Let $z_{\rm tot} \equiv (1+\varepsilon)z_{\rm tot,0}$, so that ε again measures changes in end-to-end distance, and ${\rm Lk} = (1+\sigma){\rm Lk}_0$ as usual. Again using (4), one finds $\Omega_2 - \Omega_{20} = \beta\omega_0$, $\Omega_3 - \Omega_{30} = \omega_0\sigma - {\omega_0}^2 d_0\beta$, and $\alpha = \varepsilon + \omega_0 d_0\beta - (\omega_0 d_0)^2\sigma$, where β is free. Substituting into f_3 and minimizing over β reveals a $\sigma\varepsilon$ coupling corresponding to $\bar{D} = (\omega_0 d_0)^2(C-A)B/A$. This formula fits our measured value of $\bar{D}$ if we choose $d_0 = 0.2\,{\rm nm}$.

The value of d_0 is not known *a priori,* since of course DNA is not really an elastic continuum with circular cross-section. Nevertheless, inspection of the known molecular structure indeed suggests an elastic center offset from the helix axis by a couple of Ångstroms [14]. In any case we have shown that the measured value of $\bar{D}$ is of the order of magnitude expected from simple elasticity theory.[2]

6. Conclusion: We have pointed out a strong twist-stretch coupling in torsionally-constrained DNA stretching experiments, evaluated it, argued that it reflects intrinsic elasticity of the DNA duplex, and shown that the value we obtained is consistent with elementary considerations from classical elasticity theory. A greater challenge remains to predict this coupling from the wealth of available crystallographic information on the conformation of short oligomers.

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[2] Actually a helical beam can have a twist-stretch coupling even if its axis is on center, $d_0 = 0$, provided its cross-section is not circular [13]. To explain the observed coupling in this way would require a rather large eccentricity of 70%. However for molecules such as actin, for which $d_0 = 0$, this second mechanism may be important.

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