Bending and Twisting Elasticity of DNA

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ABSTRACT: We derive the elastic theory suitable for describing the free energy required to deform a stiff helical molecule with the symmetry of DNA. At quadratic levels in the strains we find, in addition to the independent bending and torsional energies incorporated in previous theories, a previously unknown coupling between twist and bend. If the backbone is given constant curvature, minimization of the free energy with respect to the twist degrees of freedom indicates that this coupling drives a decrease in the molecular twist, or an unwinding of the helix. New experiments are proposed to bring out the symmetry-breaking effects of the twist–bend coupling: (i) ring closure experiments will indicate a helix repeat that becomes progressively more underwound for smaller rings, and (ii) gel mobilities of supercoiled rings of integral-helix-repeat length, with equal and opposite added linking numbers, will differ.

I. Introduction

DNA, in addition to being the information storage element for biological protein synthesis,[1,2] is the molecule most like the theorist's notion of a polymer. In living organisms, single linear chains of as many as 10^8 elementary units (nucleotide "base pairs") not only are precisely controlled in length but also have precisely controlled sequences of interchangeable base pairs. The ability to control such a long chain with such precision is impossible using today's synthesis methods applied to the molecules usually associated with polymer science, e.g. polystyrene, poly(dimethylsiloxane), poly(ethylene–propylene).

In water, at moderate salt concentrations, a linear DNA molecule (removed from an organism and from contact with proteins) behaves as a flexible, self-avoiding polymer at long length scales, with statistics identical to those expected for any linear chain in a good solvent. However, at short length scales, DNA is a stiff polymer with, in addition to bending persistence, persistence of internal twisting degrees of freedom.[3,4] In this paper, we focus on the description of the free energy of deformation at these shorter length scales. Interactions of DNA with proteins which regulate its expression and control its conformation frequently occur on these scales.

Our major result is that for a molecule with the symmetry of DNA, the bending and twisting degrees of freedom are not independent. At the lowest (quadratic) order in deformations from the undistorted state, there is a coupling of twist and bend. To our knowledge, all previous researchers[3-8,14] have used models with decoupled twist and bend degrees of freedom. Intriguingly, Fuller[3] commented that left- and right-handed twists are distinct for a chiral structure such as B-DNA; no effort was subsequently made to understand the nature of this asymmetry. We show quantitatively how our twist–bend coupling gives rise to the effect that Fuller suggested.

This work is organized as follows. To begin, in section II.A we must introduce appropriate strain degrees of freedom. We ignore microscopic stretching degrees of freedom, which corresponds to the fact that, over a persistence length of DNA (the length of chain which needs about kT of energy to be deformed into a circuit, about 500 Å),[4] a compression or extension requiring a similar energy is a miniscule fraction of that persistence length. Ignoring the stretching degrees of freedom allows any

deformation to be written in terms of the sequence of rotations relating the successive base-pair orientations, a prescription followed by previous researchers.[3-8,14] We then expand the free energy in these strains and identify what couplings remain after taking into account the symmetries of the molecule.

What remains at quadratic order, in addition to decoupled twisting and bending energies, is a coupling of twist to bend. In section II.B, we express the resulting quadratic free energy in terms of more useful quantities than our strains, namely the (Frenet–Serret) curvature and torsion of the backbone and the rate of change of the molecule twist. This allows simple computation of the response of the twist degrees of freedom to backbone deformations later in the paper. In section II.C, we make some brief comments about thermal fluctuations in our model.

In section III.A we carry out a simple perturbative calculation using minimization of the quadratic free energy, deriving the effect of the twist–bend coupling for a chain constrained to follow a path with constant curvature and torsion. We find that the twist–bend coupling causes an unwinding of the helix, under conditions of constant backbone curvature. The shift in twist is nonlinear (quadratic) in the twist–bend coupling, and thus always of the same sign. In section III.B we verify the perturbative result in a limit that allows an exact solution of the functional minimization problem.

In section IV, we discuss several consequences of our results. First, in section IV.A, we discuss the effective free energy after averaging over the helical repeat length, which now has a term that breaks chiral symmetry (as does the molecule itself), due to the nonzero coupling of twist and bend. In section IV.B, we discuss the twist state of small DNA rings, along with recent experiments. In section IV.C, we note that the twist–bend coupling by itself cannot increase the twist of DNA adsorbed onto a helical path on a cylindrical surface. This suggests that the increased twist reported for DNA on nucleosomes is due to details of the adsorption potential. In section IV.C, we note that the breaking of chiral symmetry by the quadratic twist–bend coupling (and by higher-order couplings) should lead to differences in certain properties of topoisomeric rings of DNA with excess linking numbers $+n$ and $-n$. This suggests new experiments that could be done to test our ideas. Finally, in section IV.E, we discuss the additional questions raised by this work.
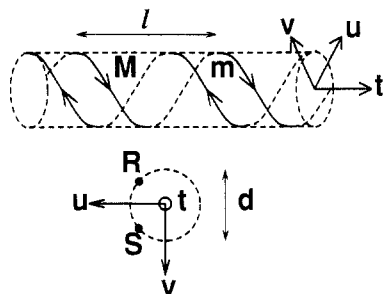
Figure 1. Schematic diagram of the B-DNA molecule. The molecular diameter is $d \approx 20$ Å, the helical repeat length is $l = 2\pi/\omega_0 = 34$ Å, corresponding to a stack of about 10.5 nucleic acid bases. The nucleotides are bound between the sugar–phosphate backbone helices: we note the arrows on the side view (upper portion of figure), which indicate the opposite directedness of the two helices. The wide major groove is marked "M", while the narrower minor groove is marked "m". The lower part of the figure shows the end view of B-DNA, with tangent t directed out of the page.

II. Elastic Free Energy of DNA

A. Symmetry Analysis. B-DNA molecules[2] are right-handed chiral rods of cross-sectional diameter $d = 21$ Å. As schematically shown in Figure 1, the pairs of nucleotides (occupying the major groove region, denoted M in Figure 1) are arranged in a helix with a pitch of about $l = 34$ Å corresponding to a helical repeat every 10.5 base pairs (bp). We define the molecular axis (the center of the molecule) to be described by the space curve $\mathbf{r}(s)$, with s being arclength. The tangent $\mathbf{t} \equiv d\mathbf{r}/ds$ thus has unit length.

At any s, consider the plane perpendicular to $\mathbf{t}$. The two sugar–phosphate backbones (the two helices, drawn with opposing arrows in Figure 1) intersect this plane at two points R and S. We define $\mathbf{u}$ to be the unit vector in this plane that points from the molecular axis to the midpoint of $\overline{RS}$. A final unit vector $\mathbf{v}$ is defined by $\mathbf{v} = \mathbf{t} \times \mathbf{u}$ so that the set $\{\mathbf{u}, \mathbf{v}, \mathbf{t}\}$ forms a right-handed coordinate system at each point s. It will be helpful to temporarily use indexed vectors $\mathbf{e}^{(1)} \equiv \mathbf{u}$, $\mathbf{e}^{(2)} \equiv \mathbf{v}$, and $\mathbf{e}^{(3)} \equiv \mathbf{t}$.

A general deformation of the molecule that maintains $\mathbf{t}^2 = 1$ may be described by infinitesimal rotations $\Omega(s)$ of the coordinate axes:[3,9]

$$\frac{d\mathbf{e}^{(i)}}{ds} = (\omega_0 \mathbf{e}^{(3)} + \Omega) \times \mathbf{e}^{(i)} \tag{1}$$

where $\omega_0 = 2\pi/l = 0.185$ Å^{-1} determines the helical repeat length in the absence of deformations. We may think of the components $\Omega_i \equiv \Omega \cdot \mathbf{e}^{(i)}$ as "strains" which locally generate rotations of the coordinates around $\mathbf{e}^{(i)}$. If $\Omega = 0$, the molecule takes its undistorted configuration shown in Figure 1. The molecular axis $\mathbf{r}(s)$ is obtained for general Ω by integrating the tangent equation $d\mathbf{r}/ds = \mathbf{e}^{(3)}$.

The integral $\mathrm{Tw} = L/l + \int ds\, \Omega_3/(2\pi)$ is defined to be the double helix "twist"[3] where L is the molecule length, and where the integral is from $s = 0$ to $s = L$. For an undistorted molecule, $\mathrm{Tw} = L/l$, and Tw just counts the number of helical turns of length l along the chain. For a distorted chain, the *excess* twist per helix repeat is $(\mathrm{Tw} - L/l)/(L/l) = \langle\Omega_3\rangle/\omega_0$, where we use the notation $\langle\Omega_3\rangle \equiv L^{-1}\int_0^L ds\, \Omega_3(s)$ to denote an average along the chain of length $L \gg l$.

Since we assume that the $\Omega = 0$ state is equilibrium, we may write the free energy for small strains as a Taylor expansion in Ω and its s derivatives.[10] The lowest order

terms are

$$\frac{F}{kT} = \int_0^L ds\, \left[A_0 + \frac{1}{2}\sum_{i,j} A_{ij}\Omega_i\Omega_j + \frac{1}{6}\sum_{i,j,k} A_{ijk}\Omega_i\Omega_j\Omega_k + ... \right] \tag{2}$$

where we have introduced the matrices A_{ij} and A_{ijk}, which are symmetric under all permutations of their indices, and where the integral runs over the molecular axis of length L. If we ignore nucleotide-sequence dependence of the elastic properties of the molecule (or if we restrict our attention to symmetric repeats such as $\binom{AT}{TA}_N$ then the A matrices have no s dependence, since in these coordinates, every point along the molecule in the undistorted state is equivalent. We will refer to these matrices as the "elastic constants": they may depend on environmental factors (temperature, ionic strength, pH, etc.). We will ignore the constant free energy A_0 for the remainder of this paper.

The second-order matrix has six independent components: A_{11}, A_{22}, A_{33}, A_{12}, A_{13}, and A_{23}. We now show how symmetries make some of these components vanish. Note that rotation by 180° around the vector $\mathbf{u}$ is a symmetry of the undistorted molecule (see Figure 1). Now consider an infinitesimal segment of length ds from $s = -ds/2$ to $ds/2$, with uniform strain $\Omega = (\Omega_1, \Omega_2, \Omega_3)$. Rotation of this segment by 180° around $\mathbf{u}(s = 0)$ yields precisely the segment configuration with uniform strain $\Omega' = (-\Omega_1, \Omega_2, \Omega_3)$. Therefore configurations Ω and Ω' have the same free energy, indicating that $A_{12} = A_{13} = 0$.[11]

We note that rotations by 180° around either $\mathbf{t}(0)$ or $\mathbf{v}(0)$ do not take our infinitesimal distorted segment to a configuration with transformed Ω because these operations are not symmetries of the undistorted molecule. The lack of symmetry under these operations is due to the existence of two distinct regions of the DNA surface bounded by the two helices, marked "M" and "m" in Figure 1. These two regions are referred to as the "major groove" and the "minor groove", respectively. The minor groove is narrow, while the major groove is wider, filled up by the nucleotides which are bound between the backbones. The two grooves are also distinguished by the opposite directedness of the sugar–phosphate backbones on their boundaries. A rotation of the undistorted molecule around either $\mathbf{t}$ or $\mathbf{v}$ by 180° exchanges the major and minor grooves.

Chiral polymers without this particular broken symmetry (e.g. a double-helix polymer with indistinguishable backbones, and therefore invariant under rotation by 180° about $\mathbf{t}$) will have $A_{23} = 0$. Finally, one should note that reflections (useful in derivation of the elastic energy of thin rods with reflection symmetries[10] cannot be used to analyze a chiral rod such as DNA.

Thus, the nonzero elements of the $n = 2$ elastic constant matrix are A_{11}, A_{22}, A_{33}, and A_{23}. Physically, A_{11} and A_{22} are (distinct) bending constants associated with bends *locally* in the planes perpendicular to $\mathbf{u}$ and $\mathbf{v}$, respectively. We expect these constants to be approximately equal to the bend persistence length ≈ 500 Å.[4] The constant A_{33} is just the twist rigidity, and is roughly equal to the twist persistence length ≈ 500 Å.[4]

In addition to the bending rigidities and twist rigidity, we have a coupling A_{23} of bends about the local $\mathbf{v}$ axis and the twist in the quadratic ($\mathcal{O}(\Omega^2)$) elastic theory. Its overall magnitude should also be controlled by the degree by which rotations of the molecule by 180° about $\mathbf{v}$ and $\mathbf{t}$ are not symmetries. For DNA, these operations essentially exchange the major and minor grooves of the molecule, which are rather different in structure: we thus expect $A_{23} \approx A_{ii}$.

The above analysis applied to A_{ijk} indicates that only A_{111}, A_{123}, A_{133}, and A_{122} are nonzero. Extension of this to higher-order terms is straightforward.

B. Free Energy in Terms of Axis Curvature, Torsion, and Twist. For the remainder of this paper, we study the free energy of section II.A, truncated at quadratic order in the strains Ω:

$$\frac{F}{kT} = \frac{1}{2} \int_0^L ds \; [A_{11}\Omega_1^2 + A_{22}\Omega_2^2 + A_{33}\Omega_3^2 + 2A_{23}\Omega_2\Omega_3] \quad (3)$$

Our aim is to re-express this free energy in terms of variables such as the molecular curvature which are more intuitive than Ω_i. In what follows, s subscripts denote s derivatives.

The molecular axis $\mathbf{r}(s)$ may be expressed in terms of an ordered set of orthonormal vectors: the tangent unit vector $\mathbf{t} = \mathbf{r}_s$, the normal $\mathbf{n}$, and the binormal $\mathbf{b}$, where the last two are defined through the Frenet–Serret equations:[12]

$$\mathbf{t}_s = \kappa\mathbf{n} \qquad \mathbf{n}_s = -\kappa\mathbf{t} + \tau\mathbf{b} \qquad \mathbf{b}_s = -\tau\mathbf{n} \quad (4)$$

where we have introduced the curvature $\kappa(s)$ and torsion $\tau(s)$. We can expect $\mathbf{n}$, $\mathbf{b}$, and $\mathbf{t}$ to vary on a scale comparable with the bend persistence length.

Since the vectors $(\mathbf{u}, \mathbf{v})$ and $(\mathbf{n}, \mathbf{b})$ are coplanar and with $\mathbf{t}$ each form right-handed triads, we may write

$$\mathbf{u} + i\mathbf{v} = e^{-i\phi(s)}(\mathbf{n} + i\mathbf{b}) \quad (5)$$

where the winding angle $\phi(s)$ rotates the slowly varying $(\mathbf{n}, \mathbf{b})$ into the rapidly rotating (about one cycle per helix length l) $(\mathbf{u}, \mathbf{v})$. The three variables κ, τ, and ϕ may be used to eliminate the three Ω_i, using the relations

$$\Omega_1 = -\mathbf{v}\cdot\mathbf{t}_s = \kappa \sin\phi$$

$$\Omega_2 = \mathbf{u}\cdot\mathbf{t}_s = \kappa \cos\phi$$

$$\Omega_3 = \mathbf{v}\cdot\mathbf{u}_s - \omega_0 = \phi_s - \bar{\omega} \quad (6)$$

where $\bar{\omega} = \omega_0 - \tau$.

Using notation $A \equiv (A_{11} + A_{22})/2$, $B \equiv (A_{11} - A_{22})/2$, $C \equiv A_{33}$, and $D \equiv A_{23}$, we write (3) as

$$\frac{F}{kT} = \frac{1}{2} \int_0^L ds \; [A\kappa^2 - B\kappa^2 \cos 2\phi + C(\phi_s - \bar{\omega})^2 +$$

$$2D\kappa(\phi_s - \bar{\omega}) \cos\phi] \quad (7)$$

We see that A is the usual bending rigidity of the Kratky–Porod model of a stiff polymer,[13] B is an asymmetric bending constant,[14] C is the twist rigidity,[3,4] and D is the twist-bend coupling. We note that B and D multiply oscillating functions with zero mean, the former making about two cycles per helix repeat and the latter making about one cycle per helix repeat. The effects of B, and even the existence of D, have not been mentioned in the previous work on DNA conformation energetics.[3-8,14]

C. Thermal Fluctuations. The model (3) looks like a Gaussian model, being quadratic in the variables Ω_i. However, Euler angles[9] describing the orientation of the local coordinates $\mathbf{e}^{(i)}(s)$ should be integrated over, rather than Ω_i, in the calculation of statistical-mechanical averages. The free energy in terms of Euler angles is nonlinear, complicating evaluation of the partition function. However, there are some basic comments that can be made about thermal averages using energy (3).

Consider rotated coordinates $\mathbf{f}^{(i)}$ where $\mathbf{f}^{(1)} + i\mathbf{f}^{(2)} \equiv e^{i\omega_0 s}(\mathbf{e}^{(1)} + i\mathbf{e}^{(2)})$ and $\mathbf{f}^{(3)} = \mathbf{e}^{(3)}$. The new unit vectors satisfy $\mathbf{f}_s^{(i)} = \Omega \times \mathbf{f}^{(i)}$. This transformation removes the uniform rotation of the $\mathbf{e}^{(i)}$ around the tangent at rate ω_0.

We suppose that space-fixed axes $\{\mathbf{x}, \mathbf{y}, \mathbf{z}\}$ are rotated into $\{\mathbf{f}^{(i)}(s)\}$ by successive rotations around the z, $x'(s)$, and $z''(s)$ axes by angles $\alpha(s)$, $\beta(s)$, and $\gamma(s)$. For a chain of length L, it is straightforward to show that $\Omega_i(s) \rightarrow -\Omega_i(L - s)$ under the transformation $\{\alpha(s) \rightarrow \alpha(L - s), \beta(s) \rightarrow \beta(L - s), \gamma(s) \rightarrow \gamma(L - s)\}$.

The quadratic portion of the free energy (2) and the integration measure for statistical-mechanical averages over the Euler angles (a product of $d\alpha$ $d[\cos\beta]$ $d\gamma$ factors) are unchanged by this transformation. Therefore, $\langle \int ds \; \Omega_i \rangle_T = 0$, where $\langle \cdot \rangle_T$ indicates that a thermal average is taken.

This means that for a freely fluctuating open chain of length L, the mean twist is just that of the undistorted state: $\langle \text{Tw} \rangle_T = L/l$. Of course, there will be correlations between the bending strain $\Omega_2(s)$ and the twisting strain $\Omega_3(s)$ introduced by A_{23}. At higher order, there are terms in the energy that change when $\Omega_i \rightarrow -\Omega_i$ (e.g. the cubic terms of (2)): these will lead to small nonzero $\langle \Omega_i \rangle_T$.

For a ring polymer of length L, we require that the molecular axis be closed ($\mathbf{r}(L) = \mathbf{r}(0)$) and that the local coordinate axes come back to themselves ($\mathbf{e}^i(L) = \mathbf{e}^i(0)$). Only configurations of the polymer satisfying these constraints should be included in the partition sum, a further complication beyond the nonlinearity of the energy function. It is easy to see that with the ring constraints, the above argument for $\langle \Omega_i \rangle = 0$ will fail. This is because generally, the two configurations related by $\Omega_i(s) \rightarrow -\Omega_i(L - s)$ are not both closed. We demonstrate this with a simple example. Suppose that $\Omega_1(s) = \kappa \sin \omega s$, $\Omega_2(s) = \kappa \cos \omega s$, and $\Omega_3(s) = \omega - \omega_0$, for constant κ and ω. Referring to (6), we see that $\mathbf{r}(s)$ is a circle of radius $1/\kappa$, and that there is excess twist proportional to $\omega - \omega_0$. The torsion of the molecule axis is zero. For smooth closure of the circular molecule, we require $\omega L = 2\pi n$ for integer n.

Now consider the new configuration $\Omega_i'(s) = -\Omega_i(L - s)$: it is easily seen that the curvature is again constant $\kappa' = \kappa$, but that the torsion of this new configuration is $\tau' = 2\omega_0$. This nonzero torsion and curvature means that the axis is not a right helix, and is certainly not closed. The loop constraint thus breaks the symmetry of the statistical weight under $\Omega_i(s) \rightarrow -\Omega_i(L - s)$. An important consequence of this is that a relaxed but circular DNA molecule will not have zero average excess twist. In the next section we will explore this phenomenon by computing the lowest energy twist configuration for a DNA segment with prescribed, constant backbone curvature and torsion.

III. Minimum Free Energy for Constant Curvature and Torsion

In this section we calculate the configuration of minimum free energy of a DNA molecule, assuming that the (Frenet–Serret) curvature and torsion of the molecule axis $\mathbf{r}(s)$ do not depend on s. This will allow us to understand what the effective free energy is, in terms of curvature, torsion, and the twist of the molecule after taking into account the short-length-scale twist response. We will find that a bend of the molecule induces a change in the equilibrium twist and that a twist of the molecule changes the effective bending rigidity. Essential to the following is the physical fact that there is a separation of length scales corresponding to (a) typical bends of radius comparable to the bend persistence length ($\approx A \approx 500$ Å[4]) or twists over lengths comparable to the twist persistence length ($\approx C \approx 500$ Å[4]) and (b) the helix repeat length ($l \approx 34$ Å) at which the twist-bend coupling oscillates. We seek $\phi(s)$ minimizing (7) for the case where κ and τ are just constants. This will allow us to compute the twist response

to axis curvature and torsion, useful for, e.g., computing the twist response of DNA wrapped around a cylinder to form a superhelix, as occurs in adsorption onto nucleosomes.

For κ and τ independent of s, and for $L \gg l$, (7) becomes

$$\frac{F}{LkT} = \frac{A\kappa^2}{2} + \frac{C}{2} \int_0^L \frac{ds}{L} \left[(\phi_s - \bar{\omega})^2 - b \cos 2\phi - 2d \cos \phi \right] \quad (8)$$

where $b \equiv B\kappa^2/C$ and $d \equiv D\bar{\omega}/C$ are just constants. Since κ and τ will be much smaller than ω_0, we see that $d/b \approx \omega_0/\kappa \gg 1$. We have thrown away a term of the form $\phi_s \cos \phi$ which can be integrated, giving rise to a surface term of order $1/L$ which can be ignored in favor of the remaining terms.

The $\phi(s)$ minimizing (8) satisfies the Euler–Lagrange equation $\partial_s(\delta F/\delta\phi_s) = \delta F/\delta\phi$, or

$$\phi_{ss} = b \sin 2\phi + d \sin \phi \quad (9)$$

which may be integrated to yield

$$\phi_s^2 = \omega^2 - b \cos 2\phi - 2d \cos \phi \quad (10)$$

where ω is an integration constant that we will see is related to the mean twist, and therefore will be comparable to ω_0.

A. Effect of Twist–Bend Coupling for Small κ/ω_0.

In this subsection, we perturbatively calculate the free energy (8) at second order in the small parameters b/ω_0^2 and d/ω_0^2, using $\phi(s)$ satisfying (10). We first note the results at "zeroth order" in b and d. Setting $b = d = 0$ allows (10) to be integrated to obtain $\phi(s) = \omega s + \phi(0)$, corresponding to a uniform winding rate. The free energy per length is $F/LkT = A\kappa^2/2 + C(\omega - \bar{\omega})^2/2$. The constant ω is then set by minimizing F/LkT, giving $\omega = \bar{\omega}$, or $\Omega_3 = 0$. The equilibrium twist is thus just $Tw = L/l$, *independent* of the axis curvature and torsion.

For nonzero b and d, we may compute the relation between the molecule length L and $\Phi \equiv \phi(L) - \phi(0)$, the total winding angle, by simply integrating (10):

$$L = \int_0^\Phi \frac{d\phi}{\phi_s} = \frac{\Phi}{\omega} \left(1 + \frac{3}{16} \frac{b^2}{\omega^4} + \frac{3}{4} \frac{d^2}{\omega^4} + \mathcal{O}(3) \right) \quad (11)$$

where terms of higher order than b^2 and d^2 (denoted $\mathcal{O}(3)$) are ignored. The mean rate of change of ϕ along the molecule (averaged over many helix repeats) is therefore

$$\langle \phi_s \rangle = \frac{\Phi}{L} = \omega \left(1 - \frac{3}{16} \frac{b^2}{\omega^4} - \frac{3}{4} \frac{d^2}{\omega^4} + \mathcal{O}(3) \right) \quad (12)$$

We note the absence of terms linear in b and d which, since they multiply $\cos 2\phi$ and $\cos \phi$, vanish apart from a small boundary contribution.

The free energy per length is thus

$$\frac{F}{LkT} = \frac{A\kappa^2}{2} +$$
$$\frac{C}{2} \frac{\langle \phi_s \rangle}{2\pi} \int_0^{2\pi} d\phi \frac{(\phi_s - \bar{\omega})^2 - b \cos 2\phi - 2d \cos \phi}{\phi_s} \quad (13)$$

up to boundary corrections of size $1/L$. Since there are no $\mathcal{O}(1)$ terms in $\langle \phi_s \rangle$, and since we know that at equilibrium $\phi_s - \bar{\omega}$ will be at most $\mathcal{O}(1)$, we may replace $\langle \phi_s \rangle = \omega$ without making $\mathcal{O}(2)$ errors in F/L.

At this point we aim to write the free energy as an expansion in $\omega - \bar{\omega}$. Plugging in ϕ_s, obtained from (10) (noting that $\phi_s > 0$ for a right-handed molecule) allows us

to write the integrand of (13) as

$$-\frac{1}{\omega^3} \left(\frac{b^2}{4} \cos^2 2\phi + d^2 \cos^2 \phi \right) + \frac{(\omega - \bar{\omega})^2}{\omega} \quad (14)$$

where we have ignored terms linear in $\cos \phi$ and $\cos 2\phi$ that vanish after the ϕ integrations and where we have kept all terms to $\mathcal{O}(2)$ in b and d. Integration yields the free energy per length:

$$\frac{F(\omega)}{LkT} = \frac{A\kappa^2}{2} + \frac{C(\omega - \bar{\omega})^2}{2} - \frac{C\omega}{\omega^3} \left(\frac{d^2}{4} + \frac{b^2}{16} \right) + \mathcal{O}(3) \quad (15)$$

which we see has a nontrivial term at $\mathcal{O}(2)$ in b and d.

If we imagine that we constrain the molecular axis to have constant curvature and torsion, then we can compute the equilibrium twist response by minimizing the free energy with respect to ω, giving

$$\omega = \bar{\omega} + \frac{1}{\omega^3} \left(\frac{d^2}{4} + \frac{b^2}{16} \right) + \mathcal{O}(3) \quad (16)$$

and equilibrium free energy

$$\frac{F}{LkT} = \frac{A\kappa^2}{2} - \frac{C}{\omega^2} \left(\frac{d^2}{4} + \frac{b^2}{16} \right) \quad (17)$$

We observe that as $d^2 \propto \kappa^2$, there is a softening of the bending rigidity due to the twist–bend coupling. The equilibrium winding rate is obtained from (12) to be

$$\langle \phi_s \rangle = \bar{\omega} - \frac{1}{2} \frac{d^2}{\omega^3} - \frac{1}{8} \frac{b^2}{\omega^3} + \mathcal{O}(3) \quad (18)$$

We see that the equilibrium winding angle is *reduced* by the presence of nonzero b and d: the helix tends to be unwound by the relaxation of the twist degrees of freedom, if the axis is given curvature. The corresponding twist is

$$Tw = \frac{L}{2\pi} \left(\omega_0 - \frac{1}{2} \frac{d^2}{\omega^3} - \frac{1}{8} \frac{b^2}{\omega^3} \right) + \mathcal{O}(3) \quad (19)$$

again showing an unwinding of the helix for nonzero b and d.

B. Exact Solution to the Euler–Lagrange Equation for $b = 0$.

In the case where we set $b = 0$ (a reasonable approximation since we will usually have $\kappa/\omega_0 \ll 1$ and thus $b/d \ll 1$) the Euler–Lagrange equation (10) may be explicitly integrated. The length of molecule l over which ϕ changes by 2π is

$$l = \int_0^{2\pi} \frac{d\phi}{\phi_s} = \frac{4}{(\omega^2 + 2d)^{1/2}} \mathbf{F}(k) \quad (20)$$

for $k^2 = 4d/(\omega^2 + 2d)$, where $\mathbf{F}$ is the elliptic integral $\mathbf{F}(k) \equiv \int_0^{\pi/2} dx \, (1 - k^2 \sin^2 x)^{-1/2}$.

The free energy of a segment of length l may be explicitly computed by plugging (10) into the free energy (7) and then integrating over ϕ from 0 to 2π. Per unit length, the resulting free energy is:

$$\frac{F}{kTL} = \frac{A\kappa^2}{2} + \frac{C(\omega - \bar{\omega})^2}{2} +$$
$$C\left[\omega(\bar{\omega} - \omega) - \frac{\pi\bar{\omega}(\omega^2 + 2d)^{1/2}}{2\mathbf{F}(k)} + (\omega^2 + 2d)\frac{\mathbf{E}(k)}{\mathbf{F}(k)} \right] \quad (21)$$

where $\mathbf{E}$ is another elliptic function: $\mathbf{E}(k) \equiv \int_0^{\pi/2} dx \, (1 - k^2 \sin^2 x)^{1/2}$. Equation 21 is easily numerically minimized
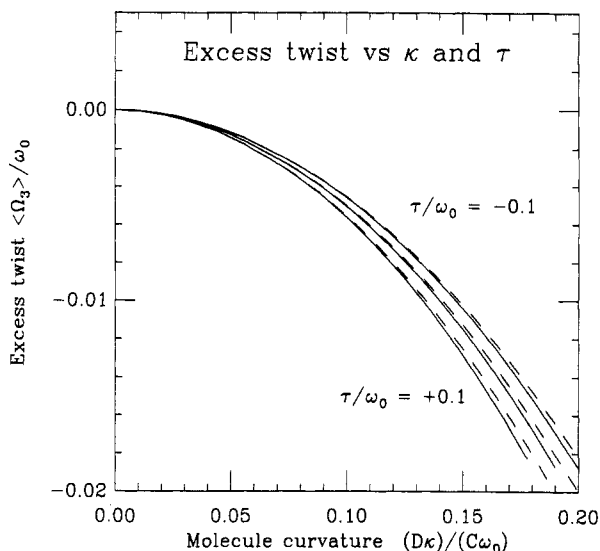
Figure 2. Equilibrium excess twist $\langle\Omega_3\rangle/\omega_0$ per unperturbed helix repeat for $b = 0$, versus dimensionless curvature $D\kappa/(C\omega_0)$. The solid curves are obtained from the exact solutions of the Euler–Lagrange equation, while the dashed curves are the $\mathcal{O}(d^2)$ perturbative result. Results for three values of axis torsion are shown, from top to bottom, $\tau/\omega_0 = -0.1, 0$, and $+0.1$, respectively.

to find the equilibrium value of ω. Since the average winding rate is just $\langle\phi_s\rangle = 2\pi/\bar{l}$, we have $\langle\Omega_3\rangle \equiv 2\pi/\bar{l} - \omega$.

In Figure 2 we display the exact result for the excess twist $\langle\Omega_3\rangle/\omega_0$ as a function of the dimensionless curvature $D\kappa/(C\omega_0) = d/(\bar{\omega}\omega_0)$, for torsions $\tau/\omega_0 = 0$ and $\tau/\omega_0 = \pm0.1$. The dashed lines show the $\mathcal{O}(d^2)$ excess twist from the previous subsection for $b = 0$, namely $\langle\Omega_3\rangle/\omega_0 = -d^2\omega_0/(2\bar{\omega}^3)$. As discussed above, the response to constant curvature is an untwisting of the helix. The exact and perturbative results are asymptotically the same as $D\kappa/(C\omega_0) \rightarrow 0$ and do not substantially differ until $D\kappa/(C\omega_0) > 0.2$. Positive torsion gives rise to a slight further unwinding of the helix, while negative torsion slightly reduces the twist response. Torsions beyond about $\tau^{-1} \approx 50$ Å, or $\tau/\omega_0 \approx 0.1$, are not likely in experiment, so we conclude that our perturbative results will apply in most experimental situations.

IV. Discussion

In this section we first consider the consequences of eq 19, which implies that a gradual bend of the DNA molecule gives rise to an "unwinding" or reduction in average twist. The effects that we will discuss are (a) the effective free energy for a DNA segment averaged over one period of twist includes a term that couples twist to the square of curvature, reflecting the broken chiral symmetry of the molecule; (b) DNA rings with large curvatures formed in cyclization experiments will have twists lower than that of a straight segment; (c) a chain constrained to follow a helical path with constant curvature and torsion (a solenoidal supercoil as found in nucleosomes), but without constraint of its twist, will be underwound; (d) rings of DNA with linking number excesses ΔLk of $+n$ and $-n$ will have different average structures. Finally, we will discuss the shortcomings of the present work and describe how one might overcome them.

A. Effective Free Energy Averaged over the Helix Repeat. The free energy used to describe distortions of DNA after averaging over the helix repeat length is usually taken to be the sum of a bending energy proportional to the square of curvature and a twisting energy proportional to the square of the deviation of the twist from ω_0. For constant molecular axis curvature and torsion, we find

these contributions to the elastic energy from the degrees of freedom at subhelical scales. However, we also find a coupling of the square of the curvature to the twist, which arises from the constraint that the axis follows a path with mean curvature and torsion.

We imagine a segment of DNA with an imposed twist and curvature: its free energy per unit length is given by (15). Using the relations (12) between ω and $\langle\phi_s\rangle$ and (6) between ϕ_s and Ω_3 and keeping only lowest-order terms, we may express the free energy in terms of the average excess twist $\langle\Omega_3\rangle$:

$$F = \frac{1}{2}\int ds \left[\left(A - \frac{D^2}{2C}\right)\kappa^2 + C\langle\Omega_3\rangle^2 + \frac{D^2}{C}\frac{\langle\Omega_3\rangle}{\omega_0}\kappa^2\right] \quad (22)$$

There is no term of type $\tau\kappa^2$ in the effective free energy to this order of approximation: τ would first appear in a term proportional to $(D^2/C)\tau\langle\Omega_3\rangle\kappa^2/\omega_0^2$. We note that B does not appear at this order since it first occurs in a term proportional to κ^4. For fixed κ, minimization with respect to $\langle\Omega_3\rangle$ gives the excess twist (19) for $\tau = 0$: $\langle\Omega_3\rangle/\omega_0 = -(D/C)^2(\kappa/\omega_0)^2/2$.

The free energy (22) differs from the usual curvature–twist model (the first two terms)[3–8,14] by having an additional term of the form $\langle\Omega_3\rangle\kappa^2$. This term, being odd in $\langle\Omega_3\rangle$, discriminates between positive (right-handed) and negative (left-handed) twists and is due to the right-handed double-helix structure of DNA. For left-handed DNA, the same analysis would lead to this free energy with a $\langle\Omega_3\rangle\kappa^2$ term of opposite sign. In either case, the response to curvature is an unwinding of the molecular helix. Alternately, we observe that an imposed twist changes the effective persistence length: overwinding the helix on the molecule ($\langle\Omega_3\rangle > 0$) stiffens the molecule, while underwinding the helix ($\langle\Omega_3\rangle < 0$) makes the resulting structure easier to bend.

The original quadratic elastic constant matrix A_{ij} must have positive eigenvalues for stability of the unstressed state: $A_{11} > 0$ and $A_{22}A_{33} > A_{23}^2$. Assuming that $A_{22} > 0$ and $A_{33} > 0$ (the latter is required for twist stability in (22)), we see that $(A_{11} + A_{22})A_{33} > A_{23}^2$ holds. This is just the stability condition that the bending (κ^2) term in (22) be positive.

Equation 22 is suitable for describing the twist response when the molecular axis is constrained to have slowly varying (compared to the helix repeat) curvature and torsion. As mentioned in section II.C, any constraint on the curvature, torsion, or topology will drive the excess twist to a nonzero equilibrium value. These constrained situations are distinct from an open chain: recall from section II.C that the equilibrium excess twist vanishes for the open chain.

The first correction for $D \neq 0$ to the effective free energy is of order $\langle\Omega_3\rangle\kappa^2$, cubic in the strains. One might think that cubic terms in the free energy (A_{111}, A_{122}, A_{123}, and A_{133}) will lead to corrections at this order. However, the contribution of these terms may be easily seen to vanish at cubic order by considering their contribution to the free energy (8) as a perturbation:

$$\frac{\delta F}{kT} = \int\frac{ds}{3!}[A_{111}\kappa^3\sin^3\phi + 3A_{122}\kappa^3\sin\phi\cos^2\phi +$$
$$6A_{123}\kappa^2(\phi_s - \bar{\omega})\sin\phi\cos\phi + 3A_{133}\kappa(\phi_s - \bar{\omega})^2\sin\phi] \quad (23)$$

To obtain the third-order contribution of these terms to the free energy, we need only plug into (23) the lowest-order approximation to the solution of the Euler–Lagrange equation,[15] $\phi(s) = \omega s + \phi(0)$. The higher-order corrections to ϕ that we calculated in section III.A introduce terms

of higher than third order. The oscillating functions of ϕ cause each of the third-order contributions to (23) to vanish. This is analogous to the vanishing of the contribution of A_{23} to the free energy, at second order in the strains.

B. Twist State of Small DNA Rings. Circular DNA molecules can be made with precisely controlled circumferences.[16-18] Molecules with circumferences of as little as 200 bp may be constructed, corresponding to circles of radius $r = 100$ Å. The radii of such rings are less than the persistence length, and thus they are circular, as fluctuation away from this shape will require many kT. The formation of such rings by cyclization will require fluctuations that bend the rings into essentially circular shape.

The duration of such a bend fluctuation will be of the order of the relaxation time of a segment of the persistence length ($\approx A$) subject to hydrodynamic effects, or $A^3\eta/kT$, where η is the solvent viscosity. Using $A \approx 500$ Å and $\eta \approx 0.01$ P, we find a lifetime of around 3×10^{-5} s. The twist degrees of freedom will equilibrate rather quickly, since one only needs to wait for twist to diffuse down the helix,[4] requiring a time of order $Ad^2\eta/kT$ (here d is the DNA diameter of 20 Å), or about 5×10^{-8} s. The separation of these two time scales indicates that the twist state equilibrates during the bending fluctuations. When cyclization occurs, we therefore expect the twist state to be described by the free energy minimum calculated in section III.

We thus use eq 19 for the twist response for a DNA circle with $\kappa = 1/r$ and $\tau = 0$ to find $\langle\Omega_3\rangle/\omega_0 = -0.5(D/C)^2/(r\omega_0)^2$. For $r = 100$ Å, we find $\langle\Omega_3\rangle/\omega_0 \approx -1.6 \times 10^{-3}$-$(D/C)^2$. This is a small, but measurable, effect: accuracies of 0.01 base pairs in determination of helix repeats have been obtained in recent cyclization experiments.[16-18] In fact, ≈ 200 bp rings[17] were measured to have a helix repeat of 10.54 bp, while longer 250 bp[16] rings showed a helix repeat of 10.45 ± 0.02 bp, and 366 bp rings[18] were found to have a helix repeat of 10.44 bp. The variation in helix repeat in the correct direction (an unwinding) to be described by the twist–bend coupling and the experimentally observed effect could be produced by a twist–bend coupling of $D/C \approx 2.4$.

It is essential to note the solvent conditions and the precise sequence in these experiments differed so that this explanation of the variation of the helix repeat is not at all conclusive. However, an unambiguous experiment could clearly be designed to test our predictions.

C. Twist State of the Helical Supercoil on the Nucleosome. In eukaryotic cells, DNA is stored adsorbed onto disk-shaped protein complexes called nucleosomes: the DNA is wrapped around nucleosomes in a left-handed helical configuration[2] similar to that drawn in Figure 3a. This fact motivates consideration of the twist response for a chain whose molecular axis is constrained to follow a helix.

The radius of this nucleosome "superhelix" is about $r = 45$ Å, and the superhelical repeat is about $h = 27$ Å. The curvature and torsion of a helix are both constant, with $\kappa = r/(r^2 + p^2)$ where $h = 2\pi p$. For the nucleosome superhelix, $\kappa = 0.022$ Å^{-1} ($\approx 1/r$ because $r \gg p$). If we suppose that the twisting degrees of freedom may relax once the molecule is adsorbed onto this superhelix, we would expect a twist decrease of $\langle\Omega_3\rangle/\omega_0 \approx -8 \times 10^{-3}(D/$
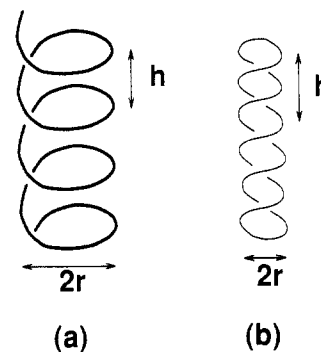
Figure 3. Supercoil structures: solid line represents an entire DNA molecule. (a) left-handed "solenoidal" supercoil as found in chromatin; (b) right-handed "plectonemic" interwound supercoil assumed by a free ring with negative excess linking number.

$C)^2$ (equivalent to an increase in helix repeat length for a straight segment of about $+0.08(D/C)^2$ bp).

The twist of DNA adsorbed on nucleosomes has been measured.[19] This can be done with the following experiment: (a) adsorption of a linear molecule onto nucleosomes; (b) ligation of the linear chain into a ring while it is nucleosome-adsorbed; (c) release of the ring from the nucleosomes. The ring is observed to be supercoiled, and the linking number, which is a topological invariant, may be measured. Per unit length of molecule, it is found that $\Delta Lk/L = -2.0 \times 10^{-3}$ Å^{-1}: about one link is removed per 146 bp of DNA.

By White's formula,[20] $\Delta Lk = \Delta Tw + Wr$, where the "writhing number" Wr is a function of the molecule axis geometry. For a left-handed superhelix, $Wr/L = -s(1 - s)/(2\pi p)$ with $s = p/(r^2 + p^2)^{1/2}$.[8] For the nucleosome-adsorbed state we have $Wr/L = -3.2 \times 10^{-3}$ Å^{-1}, giving $\Delta Tw/L = \langle\Omega_3\rangle/(2\pi) = +1.2 \times 10^{-3}$ Å^{-1}, or $\langle\Omega_3\rangle/\omega_0 = +4 \times 10^{-2}$. The DNA is thus inferred to be overwound by 4% in the nucleosome-adsorbed state.[19] We caution that there exist alternative explanations of this effect that propose writhing of the internucleosomal, or linker, DNA.[21]

Recent experiments that chemically "mark" exposed regions of the 146 bp DNA segment on a nucleosome[22] indicate that $\Delta Tw = +0.23$, giving $\Delta Tw/L = 4.6 \times 10^{-4}$ Å^{-1} or $\langle\Omega_3\rangle/\omega_0 = 1.6 \times 10^{-2}$, which is smaller than the result of the supercoiling studies, but still positive (overwound molecule).[23] It was further found that about two-thirds of the DNA segment bound to the nucleosome had a helix repeat of 10.05 bp, while the other one-third had a helix repeat of about 10.7 bp, suggesting that the twist on the nucleosome is not uniform.

Comparing these experimental results with our theoretical estimate of the twist change due to the twist–bend coupling for a helically bound chain, we see that it is unlikely that the presence of the twist–bend interaction is responsible for the twist of DNA on nucleosomes. In fact, supposing that the twist can freely relax after the chain is adsorbed predicts a negative excess twist, while a positive excess twist is actually observed. This suggests that details of the adsorption potential favor a twist increase for DNA present on the nucleosomes: in other words, the adsorption potential constrains twist as well as the molecular axis curvature and torsion. Further support for this conclusion comes from the result that the helix repeat changes from place to place on the adsorbed molecule.[22]

D. Structural Asymmetry of $\Delta Lk = \pm n$ Topoisomers. Rings of DNA may be placed in states of different helix topology, states indexed by the integer linking number Lk of the two phosphate–sugar backbones. Chains

with identical chemical content but with differing linking numbers (different topologies) are referred to as topoisomers, and they will have different geometrical structures due to the different amounts of stress energy stored during the addition or removal of units of linking. Typical supercoiled rings are "interwound superhelices" with the basic structure shown in Figure 3b. If we suppose that the twisting number of the rod configuration $Tw_0 = L/l$ is an integer (experimentally obtained by the use of a chain that is a near-integer multiple of l in length), then the excess linking $\Delta Lk = Lk - L/l$ is a positive or a negative integer.

If the energy is just a sum of κ^2 and $\langle \Omega_3 \rangle^2$ contributions (i.e. if $D = 0$), we would expect the geometrical structures of molecules with excess linking numbers $\Delta Lk = +n$ to be related to those with $\Delta Lk = -n$ by mirror reflection or "parity" (the operation $\mathbf{r} \rightarrow -\mathbf{r}$). Thus, geometrical properties whose definitions are invariant under parity reversal, such as the average radius of gyration of the molecules, will be the same for topoisomers with $\Delta Lk = \pm n$. Likewise, properties of free supercoils in a nonchiral (parity-symmetric) environment, such as (a) diffusion rate across a thin porous membrane, (b) sedimentation coefficient in a nonchiral solvent, (c) electrophoresis mobility in a nonchiral medium, or (d) scattering properties in a nonchiral solvent, should be identical for chains with $\Delta Lk = \pm n$, for vanishing twist–bend coupling $D = 0$.

However, DNA rings with a twist–bend coupling $D \neq 0$ will have a free energy term $\langle \Omega_3 \rangle \kappa^2$ coupling bend and twist. Such a term breaks the parity symmetry of the free energy, and will cause topoisomers with linking $+n$ and $-n$ to have *different* structures, and thus, e.g., different mobilities in gel electrophoresis experiments.

E. Further Questions. The twist–bend coupling discussed in this paper suggests some immediate theoretical investigations. First, it should be feasible to compute the coupling using models for the mechanical energy of DNA developed by several research groups.[24] Furthermore, the higher-order elastic constants might be at least estimated in magnitude using such calculations. Finally, it would be very interesting to estimate the scale of variations in the elastic free energy that arise from variations in the base-pair sequence.

Real DNA often is intrinsically bent.[25] It would be useful to apply the approach of this paper to a helical polymer with intrinsic curvature and torsion. A recent paper by Irwin and Olson examines this problem.[26]

Once the scale of variations due to base-pair sequence is approximately known, it may be interesting to repeat the computations of this paper, with the modification that there are arclength-dependent elastic constants, as a way to take into account the effect of variations in sequence. In our formalism, arclength is treated analogously to time in a Lagrangian dynamics problem; thus sequence-dependent elastic constants will add "time" dependence to the elastic constants. If the sequence statistics can be modeled by a reasonably simple ensemble, there may be interesting problems involving the forcing of the twist by correlated stochastic forces. The nature of the twist states may include spatial twist chaos.[27] We note that arclength dependence of the curvature and torsion of the backbone will also translate into time-dependent forcing of the twist.

Now that we have ruled out the twist–bend coupling as being responsible for the overtwisting of DNA condensed on nucleosomes, we plan to focus on understanding what sort of adsorption potentials can give rise to the observed twist in chromatin. Clearly, it will be necessary to use a potential that is sensitive to where the minor and major

grooves are exposed to the protein: this introduces the notion that the state of DNA on nucleosomes may be described by the dynamics of a nonlinear oscillator in a periodic potential. Mode-locking and chaos are both possible in such a system. There may be even more interesting twist states arising from the alternation of adsorbed DNA and the connecting 'linker' segments.

The calculations in this paper consider the backbone path to be prescribed. For chains not adsorbed to surfaces, it would be interesting to study the more difficult problem of the response of the axis curvature and torsion as well as that of the twist. For example, for rings, the curvature and torsion will have some small nonuniformity in the minimum free energy state; the twist response will be slightly altered from that calculated in this paper for small rings.

Thermal fluctuations should be studied for the basic model introduced in section II. We need a more complete picture of the fluctuations of an open chain, especially those that lead to ring formation, and also of fluctuations that occur after rings with a certain linking number are formed. As mentioned above, the exact form of the free energy of a ring as a function of excess twist would be interesting, and not too hard to calculate for small fluctuations. Finally, we are now at work on a model for the plectonemic supercoiling of rings of DNA which have excess linking: we hope to be able to estimate the amount of asymmetry between chains with opposite excess linking.

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