The bend stiffness of S-DNA

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Abstract. –
We formulate and solve a two-state model for the elasticity of nicked, double-stranded DNA that borrows features from both the Worm Like Chain and the Bragg–Zimm model. Our model is computationally simple, and gives an excellent fit to recent experimental data through the entire overstretching transition. The fit gives the first value for the bending stiffness of the overstretched state as about 10 nm · k_B T, a value quite different from either B-form or single-stranded DNA.

Introduction and Summary. – When double-stranded DNA is subjected to longitudinal forces greater than about 65 pN it undergoes a radical conformational change, marked by a sudden, almost twofold increase in contour length. The structural characterization of the resulting overstretched or (“S-state”) DNA is complicated by the fact that techniques such as X-ray crystallography are not applicable to single molecules. In this Letter, we instead characterize overstretched DNA by examining its elastic constants, and to this end formulate and solve a model that synthesizes features of both the Worm Like Chain (WLC) and the Bragg–Zimm model of the helix–coil transition in peptides. Thus we model DNA as consisting of two different, coexisting conformations, each with its own elastic constants. We solve this model and show that it gives a good fit to recent data on the overstretching transition in nicked, double-stranded DNA. From these fits, we conclude that the bend stiffness of S-DNA is intermediate between the known values for single stranded and double stranded DNA. Our result supports the work of Léger et al. [3, 4], who argued that S-DNA has a definite helical pitch and hence is a new duplex conformation of DNA.

Our model and solution method differ from those offered by Marko [5], who assumes the bend stiffnesses of the two conformational states to be identical; our analysis will show that on the contrary the stiffnesses are markedly different. The analysis of Viovy and Cizeau [6] is essentially a mean-field approximation to the model we study here; in addition, the authors did not quote any value for the S-DNA bend stiffness, presumably because the experimental data available at that time did not permit such a determination.

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The model studied here is a continuum limit of a more general class of discrete persistent-chain models. Such models give better fits to the stretching curves of single-stranded DNA than either the continuum WLC or the freely jointed chain models. Details will appear elsewhere.

Our model and method are also of some general interest beyond DNA. For example, both can be adapted to the study of the stretching of polypeptides with a helix-coil transition.

**Model.** We begin by formulating a discretized form of the WLC, which we call the “Discrete Persistent Chain.” Later we will introduce an Ising-like variable on each chain link describing a cooperative transition from B- to S-form.

The DPC models the polymer as a chain of \( N \) segments of length \( b \), whose conformation is fully described by the collection of orientation vectors \( \{ \hat{t}_i \} \) for each segment. Thus the relaxed total contour length is \( L_{\text{tot}} \equiv N \cdot b \). Bend resistance is taken into account by including an energy penalty at each link proportional to the square of the angle \( \Theta_{i,i+1} = \arccos(\hat{t}_i \cdot \hat{t}_{i+1}) \) between two adjacent links. The energy functional describing this model is thus given by

\[
\mathcal{E}[\{ \hat{t}_i \}] = -\sum_{i=1}^{N} \frac{fb}{k_B T} \hat{t}_i \cdot \hat{z} + \sum_{i=1}^{N-1} \frac{A}{2b}(\Theta_{i,i+1})^2. \tag{1}
\]

The partition function for this energy functional is \( Z = \prod_{i=1}^{N} \int_{S^2} d^2\hat{t}_i e^{\mathcal{E}[\{ \hat{t}_i \}]/k_B T} \), where \( S^2 \) is the two-dimensional unit sphere.

To compute \( Z \) we use the transfer matrix technique, interpreting each integral above as a generalized matrix product among matrices with continuous indices: \( Z = \vec{v} \cdot \vec{T}^{N-1} \cdot \vec{w} \). In this formula \( \vec{v} \) and \( \vec{w} \) are vectors indexed by \( \hat{t} \), or in other words functions \( v(\hat{t}) \), \( w(\hat{t}) \). The matrix product \( \vec{T} \cdot \vec{v} \) is a new vector, defined by the convolution:

\[
(\vec{T} \cdot \vec{v})(\hat{t}_i) = \int_{S^2} d^2\hat{t}_j \vec{T}(\hat{t}_i, \hat{t}_j)v(\hat{t}_j). \tag{2}
\]

Here the matrix elements of \( \vec{T} \) are given by \( T(\hat{t}_i, \hat{t}_j) = e^{-\mathcal{E}_i(\hat{t}_i, \hat{t}_j)/k_B T} \); we will not need the explicit forms of \( \vec{v} \) and \( \vec{w} \) below.

The force-extension relation can be obtained from \( Z \) by differentiating with respect to the force. It is here that the transfer matrix formulation can be used to greatly simplify the calculation of the force-extension relation, since all that is needed to compute the logarithmic derivative of \( Z \) in the limit of long chains is the largest eigenvalue of \( \vec{T} \), which we will call \( \lambda_{\text{max}} \):

\[
\left( \frac{z}{L_{\text{tot}}} \right)_{\text{large \( N \)}} \frac{d}{df} \ln(\lambda_{\text{max}})^N = \left( \frac{k_B T}{b} \right) \frac{d}{df} \ln \lambda_{\text{max}}. \tag{3}
\]

Analogously to ref., it is straightforward to add an intrinsic stretch modulus to the calculation outlined above, obtaining an “Extensible DPC” model.

To study overstretching, we now extend the extensible DPC by giving each link a discrete variable \( \sigma \), which takes the values \( \pm 1 \). We will take \( \sigma = +1 \) to mean the segment is in the B-state and \( \sigma = -1 \) for the S-state. The factor by which a segment elongates when going from B to S will be called \( \zeta \), i.e. \( b^{(S)} = \zeta b \) (with \( \zeta > 1 \)). We assign a bend stiffness parameter \( A \) to B-DNA, and a different \( A^{(S)} = \beta \zeta A \) to S-DNA; \( \beta \) is a dimensionless parameter with \( \beta \zeta < 1 \). Similarly we assign a bend stiffness \( \eta_A \) to a hinge joining a B and an S segment.
The full energy functional for the Ising–DPC model is thus:

$$\frac{\mathcal{E}[\{\hat{t}_i, \sigma_i\}]}{k_B T} = -\sum_{i=1}^{N-1} \left( \frac{\alpha_0}{2} (\sigma_i + \sigma_{i+1}) + \gamma (\sigma_i \sigma_{i+1} - 1) + \frac{f b}{2k_B T} \left[ \left( \frac{1+\sigma_i}{2} - \frac{1+\sigma_{i+1}}{2} \right) \hat{t}_i \cdot \hat{z} + \left( \frac{1+\sigma_{i+1}}{2} - \frac{1+\sigma_i}{2} \right) \hat{t}_{i+1} \cdot \hat{z} \right] - \frac{A}{2b} \left[ (1-\sigma_i)(1-\sigma_{i+1}) \beta + |\sigma_i - \sigma_{i+1}| \eta + (1+\sigma_i)(1+\sigma_{i+1}) \right] \right) \right). \tag{4}$$

The first line is the pure-Ising part, with \(2\alpha_0 k_B T\) the intrinsic free energy cost of converting a single segment from B to S and \(2\gamma k_B T\) the energy cost of creating a B→S interface. Note that we ignore a contribution to the energy functional from the first and last segments. In the long-chain limit this does not affect the outcome of our calculation.

The partition function for the energy functional (4) is given by

$$Z = \prod_{i=1}^{N-1} \sum_{\sigma_i = \pm 1} \int d^2 \hat{t}_i \prod_{i=1}^{N-1} e^{-\mathcal{E}_i(\hat{t}_i, \sigma_i, \hat{t}_{i+1}, \sigma_{i+1})/k_B T}, \tag{5}$$

where now \(\mathcal{E}[\{\hat{t}_i, \sigma_i\}] = \sum_{i=1}^{N-1} \mathcal{E}_i(\hat{t}_i, \sigma_i, \hat{t}_{i+1}, \sigma_{i+1})\). We again calculate \(Z\) with the aid of the transfer matrix technique, writing eq. (3) as \(Z = \langle \hat{v}^T N - 1, \hat{v} \rangle\), with \(T\) now the transfer matrix for our Ising-DPC model, which carries an additional 2-by-2 structure due to the Ising variables. The dot products are thus defined as

$$(T \cdot \hat{v})_{\sigma_i \sigma_j} = \sum_{\sigma_f = \pm 1} \int d^2 \hat{t}_j T_{\sigma_i \sigma_j} (\hat{t}_i, \hat{t}_j) v_{\sigma_f} (\hat{t}_j). \tag{6}$$

The individual matrix elements \(T_{\sigma_i \sigma_j}\) are given explicitly by

$$T_{-1, -1} (\hat{t}_i, \hat{t}_{i+1}) = \exp \left[ \frac{1}{2} \zeta \left( \hat{t}_i + \hat{t}_{i+1} \right) \cdot \hat{z} - \frac{\beta A}{b} \left( 1 - \hat{t}_i \cdot \hat{t}_{i+1} \right) - \alpha_0 \right],$$

and related expressions for \(T_{1, 1}, T_{1, -1}\), and \(T_{-1, 1}\), where \(\hat{f} \equiv \frac{f b}{k_B T}\).

We approximate the largest eigenvalue of the transfer matrix \(T\) using a variational approach \(\hat{v}\). We choose a three-parameter family of trial eigenfunctions with azimuthal symmetry, peaked in the direction of the force \(\hat{z}\):

$$V_{\omega_1, \omega_{-1}, \varphi} (\hat{t}) = \begin{pmatrix} e^{i \omega_1 \hat{t} \cdot \hat{z} \cos \varphi} \\ e^{i \omega_{-1} \hat{t} \cdot \hat{z} \sin \varphi} \end{pmatrix}. \tag{7}$$

These trial functions were chosen such that their squared norm is independent of all parameters: \(\|V_{\omega_1, \omega_{-1}, \varphi}\|^2 = 2\pi\). Eq. (7) shows that the \(\omega\)'s give the degree of alignment of the monomers (how forward-peaked their probability distribution is), whereas \(\varphi\) describes the relative probability of a monomer to be in the two states. The variational estimate for the maximal eigenvalue is thus

$$\lambda_{\text{max}} \equiv \max_{\omega_1, \omega_{-1}, \varphi} y(\omega_1, \omega_{-1}, \varphi) \equiv \max_{\omega_1, \omega_{-1}, \varphi} \frac{\langle \hat{v}_{\omega_1, \omega_{-1}, \varphi}, T, \hat{v}_{\omega_1, \omega_{-1}, \varphi} \rangle}{\|\hat{v}_{\omega_1, \omega_{-1}, \varphi}\|^2}, \tag{8}$$
The maximization over $\varphi$ can be done analytically: defining the $2 \times 2$ matrix $\hat{T}(\omega_1, \omega_{-1})$ by
\[
\hat{v}_{\omega_1, \omega_{-1}, \varphi} \cdot \hat{T} \cdot \hat{v}_{\omega_1, \omega_{-1}, \varphi} = (\cos \varphi, \sin \varphi) \cdot \hat{T}(\omega_1, \omega_{-1}) \cdot (\cos \varphi, \sin \varphi),
\]
gives that
\[
\lambda^*_{\text{max}} = \max_{\omega_1, \omega_{-1}} \frac{\hat{g}(\omega_1, \omega_{-1})}{\|\hat{v}_{\omega_1, \omega_{-1}, \varphi}\|^2},
\]
(10)
where $\hat{g}(\omega_1, \omega_{-1})$ is the maximal eigenvalue of the $2 \times 2$ matrix $\hat{T}(\omega_1, \omega_{-1})$. The following section will calculate this eigenvalue in a continuum approximation to $\hat{T}(\omega_1, \omega_{-1})$, illustrating the procedure by considering in some detail the matrix element $\hat{T}_{1,1}(\omega_1, \omega_{-1})$. The other matrix elements can be obtained analogously. Writing out the integrals explicitly, we have
\[
\hat{T}_{1,1}(\omega_1) = \frac{\omega_1 e^{\alpha_0} \beta}{\sinh(2\omega_1)} \int_{S^2} q^2 \hat{t}_i e^{\hat{a}_i \hat{v}} \int_{S^2} q^2 \hat{t}_{i+1} \left[ e^{(\hat{a}_i + \hat{v}) \cdot \hat{a}_{i+1}} \right],
\]
(11)
where we have introduced $\hat{a} \equiv \omega_1 + \hat{v}$. Condensing notation even further we define $\mu^2 = \hat{a}^2 + (\frac{\hat{a}}{\hat{a}})^2 + 2\hat{a} \cdot \hat{v} = \tilde{\omega}_1$, which allows us to write
\[
\hat{T}_{1,1}(\omega_1) = (2\pi)^2 \omega_1 e^{\alpha_0 - A/b} \frac{\beta}{\sinh(2\omega_1)} \int_{\hat{a}} \frac{d^4 \hat{a}}{\hat{a} A} \beta e^{(\mu^2 - \hat{a}^2 + 2 \hat{a} \cdot \hat{v})/(2 A)} \left[ e^{\mu} - e^{-\mu} \right].
\]
(12)

Continuum Limit. – We could now proceed to evaluate the force-extension relation of the Ising-DPC model, by evaluating eq. (10) numerically and using eq. (6). To simplify the calculations, however, we first pass to a continuum limit. To justify this step, we note that the continuum (WLC) approximation gives a good account of single-stranded DNA stretching out to forces beyond those probed in overstretching experiments (about 90 pN) [11]. As mentioned earlier, the continuum approximation is also quite good for double-stranded DNA, because the latter’s persistence length is so much longer than its monomer size.

In the continuum limit $b$ is sent to zero holding $L_{\text{tot}}$ fixed; hence $N \to \infty$. The bookkeeping is more manageable after a shift in $\mu$: $x \equiv \mu - (A/b)$. Eq. (12) then reduces to
\[
\hat{T}_{1,1}(\omega_1) = \frac{\omega_1 e^{\alpha_0}}{\sinh(2\omega_1)} \frac{(2\pi)^2 b}{A} \int_{-\tilde{\omega}_1}^{+\tilde{\omega}_1} dx \exp \left[ -\frac{b}{2 A} x^2 + 2 x - \frac{\hat{a}^2}{2 A} \right]
\approx \frac{\omega_1 e^{\alpha_0}}{\sinh(2\omega_1)} \frac{(2\pi)^2 b}{A} \int_{-\tilde{\omega}_1}^{+\tilde{\omega}_1} dx e^{2x (1 + \frac{x^2}{2 A})} e^{-\frac{\hat{a}^2}{2 A}}.
\]
(13)
The last integral can be worked out exactly, and expanding the result to second order in $b$ we end up with
\[
\frac{A}{2 \pi b} \frac{1}{\hat{v}_{\omega_1, \omega_{-1}, \varphi}} \hat{T}_{1,1}(\omega_1) = e^{\alpha_0} \left[ 1 + b \left( \frac{\beta}{k_B T} - \frac{\omega_1}{2 A} \right) \left( \coth(2\omega_1) - \frac{1}{2\omega_1} \right) \right].
\]
(14)

In similar fashion, we can obtain the following expressions for the other matrix elements.
\[
\frac{A}{2 \pi b} \frac{1}{\hat{v}_{\omega_1, \omega_{-1}, \varphi}} \hat{T}_{1,1}(\omega_1, \omega_{-1}) = \frac{1}{\eta} \left( \frac{\omega_1}{\sinh(2\omega_1)} \frac{1}{\sinh(2\omega_{-1})} \right)^{1/2} \frac{2 \sinh(\omega_1 + \omega_{-1})}{\omega_1 + \omega_{-1}}
\]
(15)
To obtain a nontrivial continuum limit we must now specify how the parameters $A$, $\alpha_0$, and $\gamma$ depend on $b$ as $b \to 0$. The choices

$$\alpha_0 = -\frac{1}{2} \ln \beta + b\bar{\alpha}, \quad \gamma = -\frac{1}{2} \ln (\bar{\gamma} b)$$

(16)

give a well-defined limit, where we hold $A$, $\bar{\alpha}$, $\beta$ and $\bar{\gamma}$ fixed as $b \to 0$. With these choices, the matrix $\frac{1}{\|\vec{v}_{\omega_1, \omega_{-1}}\|^2} \tilde{T}(\omega_1, \omega_{-1})$ takes the form

$$\frac{1}{\|\vec{v}_{\omega_1, \omega_{-1}, \phi}\|^2} \tilde{T}(\omega_1, \omega_{-1}) = \frac{2\pi b}{A\sqrt{\beta}} \left( 1 + b \left( \frac{P}{Q} \frac{Q}{R} \right) \right),$$

(17)

with

$$P = \bar{\alpha} + \left( f \frac{\omega_1}{k_B T} - \frac{\omega_1}{2A} \right) \left( \coth(2\omega_1) - \frac{1}{2\omega_1} \right),$$

$$R = -\bar{\alpha} + \left( \frac{\zeta f}{k_B T} - \frac{\omega_{-1}}{2A\beta} \right) \left( \coth(2\omega_{-1}) - \frac{1}{2\omega_{-1}} \right),$$

$$Q = \frac{\bar{\gamma} \sqrt{\beta}}{\eta} \left( \frac{\omega_1 \omega_{-1}}{\sinh(2\omega_1) \sinh(2\omega_{-1})} \right)^{\frac{1}{2}} \left( \frac{2 \sinh(\omega_1 + \omega_{-1})}{\omega_1 + \omega_{-1}} \right).$$

(18)

Note that the prefactor $\frac{2\pi b}{A\sqrt{\beta}}$ in eq. 17 does not contribute to the force-extension result eq. 3, because it does not depend on the force. In terms of the individual matrix entries, the quantity to be maximized now reads (see eq. 8):

$$\ln \tilde{y}(\omega_1, \omega_{-1}) = \frac{b}{2} \left( P + R + \sqrt{(P - R)^2 + 4Q^2} \right).$$

(19)

Writing $\Omega = b^{-1} \ln \lambda^*_{\text{max}} = b^{-1} \times \max \ln \tilde{y}(\omega_1, \omega_{-1})$, the force-extension in the continuum limit is finally given by

$$\langle \frac{z}{L_{\text{tot}, b}} \rangle = k_B T \frac{d\Omega}{df}.$$ 

(20)

We evaluate $\Omega$ by numerically maximizing eq. 19.

So far, we have not included stretch moduli for the B- and S-DNA. This is easily implemented to first order in $f/E$ by replacing $f$ with $f(1 + f^2 k_{\text{strain}})$ in the matrix elements for the two states respectively (eq. 7). This procedure yields theoretical force-extension curves like the one plotted in Fig. 1.

In summary, our model contains the following seven parameters. $2\bar{\alpha} k_B T$ is the free energy per unit length required to flip B-DNA into the S-state, and is measured in [J/nm]. $Q$ measures the cooperativity of the transition and has units [1/nm]. $A$ is the bend stiffness parameter of B-DNA, with units [nm]. The dimensionless parameter $\beta$ is the ratio of the B- and S-DNA bend stiffnesses. $E^{(B)}$ and $E^{(S)}$ are the stretch stiffnesses of B and S-DNA, and are measured in pN. Finally, $\zeta$ is the dimensionless elongation factor associated with the B→S transition.

Discussion of fit. – Fig. (1) shows a fit to some recent experimental data (similar data appear in [12]). Our model reproduces the experimental data rather well, but with so many fit parameters one may ask whether it actually makes any falsifiable predictions. To answer this question we note that the data below the transition suffice to fix $A$ and $E^{(B)}$ as usual, roughly speaking from the curvature and slope of the curve below the transition. Similarly, the data above the transition fix $A^{(S)} = \zeta \beta A$ and $E^{(S)}$. The vertical jump in the curve at
the transition fixes $\zeta$. The horizontal location of the jump fixes $\bar{\alpha}$, and the steepness of the jump fixes the cooperativity $Q$. Thus all of the model’s parameters are fixed by specific features of the data. Two additional, independent features of the data now remain, namely the rounding of the curve at the start and end of the transition. Our model predicts these features fairly successfully.

The fit recovers the known values for the effective persistence length of B-DNA of around 50 nm and its stretch modulus of about 1000 pN. Our first result is that the bend stiffness of S-DNA from our fit as $A^S = \beta \zeta A = 12.32$ nm. Similar results were obtained using the older data of Cluzel et al.\(^1\). If S-DNA consisted of two unbound, single strands, we might have expected $A^S$ to be twice as large as the value $A^\infty \approx 0.75$ nm appropriate to single-stranded DNA (as obtained from stretching experiments on ssDNA, restricted to forces above those required to pull out secondary structure\(^2\)). On the contrary, we find that the bend stiffness of S-DNA is intermediate between that of B-DNA and that of two single strands.

Finally, our fit gives the stretch modulus of S-DNA is substantially higher than that of B-DNA. This conclusion is consistent with the idea that the contour length of S-DNA is determined by its covalently bonded sugar-phosphate backbones, which are much straighter than in B-DNA; the contour length of B-DNA is instead determined by weaker, base-stacking interactions.

Relation to prior work. – Several authors have studied the entropic elasticity of two-state chains. As soon as the overstretching transition was discovered, Cluzel proposed a pure Ising model by analogy to the helix-coil transition.\(^4\) Others then introduced entropic elasticity, but required that both states have the same bending stiffness as B-DNA\(^5\) or took one of the two states to be infinitely stiff\(^6\), or to be a FJC\(^7\). Also several earlier works made a mean-field approximation instead of diagonalizing the full transfer matrix. We believe our Ising-DPC model to be the first consistent formulation incorporating the coexistence of two different states with arbitrary elastic constants. Our approach also is calculationally more straightforward than some, and minimal in the sense that no unknown potential function needs to be chosen.

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\(^1\)The fit value of $\bar{\alpha}$ should be regarded as an average of the two different costs to convert AT or GC pairs. The fit value of $Q$ has no direct microscopic significance, as the apparent cooperativity of the transition will be reduced by the sequence disorder.

\(^2\)Hagerman’s result that the persistence length of a single strand of poly(dT) DNA is between 2 nm and 3 nm\(^8\) does not come from a stretching experiment and should not be compared directly.
Fig. 1 – Least-squares fit of the Ising-DPC model to an overstretching dataset (48.5 kbp $\lambda$ DNA construct; buffer 500 mM NaCl, 20 mM Tris, pH 8). Data kindly supplied by C. Bustamante and S. Smith. Fit parameters: $A = 43.75$ nm, $\bar{\alpha} = 5.45$ nm$^{-1}$, $\bar{\beta} = 0.16$, $\bar{Q} = 0.13$ nm$^{-1}$, $\bar{\zeta} = 1.76$, $E^{(B)} = 1.2 \times 10^3$ pN and $E^{(S)} = 1.0 \times 10^4$ pN. $\chi^2 = 9.22$ at $N = 825$; points with $1.11 < \langle f \rangle < 1.55$ were excluded from the fit.