Thermal Denaturation of Fluctuating DNA Driven by Bending Entropy

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A statistical model of homopolymer DNA, coupling internal base pair states (unbroken or broken) and external thermal chain fluctuations, is exactly solved using transfer kernel techniques. The dependence on temperature and DNA length of the fraction of denaturation bubbles and their correlation length is deduced. The thermal denaturation transition emerges naturally when the chain fluctuations are integrated out and is driven by the difference in bending (entropy dominated) free energy between broken and unbroken segments. Conformational properties of DNA, such as persistence length and mean-square-radius, are also explicitly calculated, leading, e.g., to a coherent explanation for the experimentally observed thermal viscosity transition.

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Double-stranded DNA (dsDNA) is made up of two intertwined interacting semi-flexible single-strand DNA (ssDNA) chains. Over fifty years ago it was recognized that the intramolecular unwinding of DNA at physiological temperature has as counterpart the thermally induced denaturation above physiological temperature of purified DNA solutions where dsDNA completely separates into two ssDNA. Despite the differences between purified DNA solutions where dsDNA completely separated denaturation above physiological temperature of physiological temperature, to local and transitory unzipping energy) and thermal fluctuations can lead, even at physiological temperature, the intracellular unwinding of DNA at physiological temperature, depending on sequence, chain length, and ionic strength. Experiments show, for example, that there exist a bubble initiation barrier of ~10k_BT and free energy cost of ~0.1k_BT for breaking an additional base pair in an existing A-T bubble. A detailed understanding of equilibrium and dynamical properties of DNA in solution is still being sought and a consensus concerning the physical mechanism behind the denaturation transition has not yet been reached.

A variety of mesoscopic models have been proposed to account for the thermodynamical properties of denaturation bubbles in DNA. They range from i) simple effective Ising-like two-state models to more detailed ones such as ii) loop entropy models (with or without chain self-avoidance), and iii) non-linear phonon models, where the shape of the interaction potential between base pairs is more precisely taken into account. To get a transition in models i) and ii), an effective temperature dependent base-pair chemical potential must be inserted by hand. For finite chains, type (ii) models simply refine the sharpness of the transition, but do not attempt to provide a deeper explanation of the physical mechanism – our aim here. For type (iii) models it has been shown that there can be a denaturation transition analogous to interface unbinding, due to a gain in translational entropy. If, however, physically reasonable values for the model parameters are used, T_m appears to be much too high and the transition width much too large.

It has been shown experimentally that dsDNA is two orders of magnitude stiffer than ssDNA at normal salt concentration. We show in this Letter that taking into account this difference in bending rigidity provides a novel physical explanation for the denaturation mechanism and leads to realistic values for transition temperatures and widths. Despite important recent advances in understanding the crucial role played by DNA bending rigidity in explaining force-extension and cyclization experiments, its importance has not yet been clearly elucidated in the context of denaturation. We show, via a well-defined coupled Ising-Heisenberg statistical model, that an entropy driven denaturation transition emerges by integrating out chain fluctuations, due to the entropic lowering of the energetic barrier for bubble nucleation. This minimal model neglects all other (residual) interactions between bases arising from, e.g., electrostatics, self-avoidance, and helical twist.

We begin by considering a worm like chain (WLC) Hamiltonian H[r_1, r_2] for two interacting ssDNA homopolymers of length L:

\[
H = \frac{1}{2} \sum_{i=1}^{2} \int_0^L ds \left[ \frac{3}{2} \kappa_b(\rho(s)) \dot{r}_i^2(s) + \frac{3}{2} \kappa_s(\rho(s)) \dot{r}_i^2(s) \right] + \int_0^L ds V(\rho(s)),
\]

where s is the curvilinear index, r_i(s) is the position of chain i at base position s, and \rho(s) ≡ r_1(s) − r_2(s) is the relative (internal) coordinate (β = 1/k_BT and σ_0 ≡ 2/\kappa_b). The coefficient \kappa_b is a bending elastic modulus that is proportional to the short distance cut-off \ell_0 ≈ 0.34 nm
(the monomer length) and \( \kappa_s(\rho) = 1/[\beta^2 \kappa_b(\rho)] \). In order to account for the enhanced stiffness of dsDNA, \( \kappa_b \) must depend on \( \rho(s) \), e.g., \( \kappa_b(\rho) = \kappa_b^{ss} + \Delta \kappa_b e^{-(\rho - \rho_0)/\lambda} \), where \( \Delta \kappa_b \equiv \kappa_b^{ds} / 2 - \kappa_b^{ss} > 0 \) and \( \lambda \sim 1 \AA \) is the range [10].

The persistence length of ssDNA is \( \ell_p^{ss} = \beta \kappa_b^{ss} \approx 1 \) nm and that of dsDNA is \( \rho_0^{ds} = \beta \kappa_b^{ds} \approx 50 \) nm (at 300 K and physiological ionic strength). The potential \( V(\rho) \) accounts for both the effective short range hydrogen bonding interaction between complementary bases at the same \( s \) and part of the stacking interaction between neighboring bases; a convenient form is the Morse potential: 

\[
V(\rho) = (D/\ell_0)\ln(\rho - \rho_0)/\lambda
\]

leading to a well depth of \( D/\ell_0 \) at \( \rho_0 \) [8].

At high temperature, \( \rho(s) - \rho_0 \gg \lambda \), and therefore the system decouples into two semi-flexible non-interacting ssDNA chains. After introducing the center-of-mass (external) coordinate, \( X(s) \equiv [r_1(s) + r_2(s)]/2 \), the partition function can be rewritten as a sum over center-of-mass and relative configurations: 

\[
Q = \int \mathcal{D}\rho \mathcal{D}X \exp\{-\beta[H_{ext}[X,\rho] + H_{int}[\rho]]\}
\]

using the effective action 

\[
H_{int} = \frac{3}{8}\int_0^L ds \left[ \kappa_b(\rho)\dot{\rho}^2 + \kappa_s(\rho)\dot{\rho}^2 + V(\rho) \right] \quad (2)
\]

\[
H_{ext} = \frac{3}{2}\int_0^L ds \left[ \kappa_b(\rho)\dot{X}^2 + \kappa_s(\rho)\dot{X}^2 \right].
\]

We integrate over \( X \) to obtain an effective model for \( \rho \). 

\[
Q = \int \mathcal{D}\rho \exp\{-\beta H_{eff}[\rho]\}
\]

where \( H_{eff}[\rho] = H_{int}[\rho] + F_{ext}[\rho] \) with \( F_{ext}[\rho] = -kB T \ln \left[ \int \mathcal{D}X \exp\{-\beta H_{ext}[X,\rho]\} \right] \). The external free energy \( F_{ext}[\rho] \) at frozen \( \rho \) can be evaluated by introducing the local center-of-mass tangent vectors \( t = \dot{X} \) and then changing variables to \( t = t/\sqrt{\kappa_b(\rho)} \). There are two contributions to \( F_{ext} \): one will renormalize the second term in \( H_{int} \) and the other will renormalize the potential to \( V_f \). The latter mainly gives rise to a purely entropic barrier favoring bubbles,

\[
V_f(\rho) = V(\rho) + \frac{3}{2}\kappa_b T}{\ell_0} \ln \left( \frac{\kappa_b(\rho)}{\kappa_b^{ss}} \right),
\]

which lowers the well depth from \( D \) to \( D_R \approx D - (3/2)k_B T \ln [\kappa_b^{ss} / (2\kappa_b^{ds})] \). The entropic bending contribution can be extremely important when \( D \) is in the commonly accepted range of \( 1 \leq D/k_B T < 5 \) at \( T = 350 \) K and \( \kappa_b^{ds} / (2\kappa_b^{ss}) \approx 25 \). Scaling arguments then show that the melting temperature gets reduced by a factor \( \sim 2 \) down to experimental values [11, 12, 13].

In order to illustrate the above mechanism in more detail, we introduce a discretized, exactly soluble, version of the above model, which captures the essential physics. We map the external tangent vector, \( t(s) \), to \( t_i \) \((s = \ell_0)\) and an internal variable \( 1 - 2\Theta \rho(s) - \rho_0 - \lambda \) (with \( \Theta \) the step function) to an Ising variable: \( \sigma_i = 1 \) for an unbroken bond (state A) and \( \sigma_i = -1 \) for a broken one (B). Each link vector can be denoted by the solid angle \( \Omega_i = (\theta_i, \phi_i) \). The energy \( H_{\sigma_i, t_i} \) of a state is

\[
H = \sum_{i=1}^{N-1} \left( \tilde{k}_{i,i+1}(1 - t_i \cdot t_{i+1}) + H_i(J, \tilde{K}, \tilde{\mu}) \right) \quad (5)
\]

\[
H_i = \sum_{i=1}^{N-1} \left( J\sigma_i\sigma_{i+1} + \frac{K}{2}(\sigma_i + \sigma_{i+1}) \right) - \tilde{\mu} \sum_{i=1}^N \sigma_i
\]

Thus \( \beta H \) contains only the dimensionless parameters \( \kappa_i, \beta_i, J, \beta, \mu, \tilde{\mu}, \tilde{K} \). This type of model was introduced in the context of helix-coil transitions in 2D [18] and later used in various forms to study DNA force-extension and cyclization [11, 12, 13]. The first term in \( H \), corresponding to \( H_{ext} \) in the continuum model, is the bending energy of a discrete WLC with a local rigidity \( \kappa_i = \kappa_A = \beta \kappa_b^{ds} / \ell_0 \) for a nearest neighbor link of type A-A, \( \kappa_B = 2\beta \kappa_b^{ss} / \ell_0 \) for B-B and \( \kappa_{AB} \) for A-B. In the Ising part, \( \beta H_i \), the first term mimics the gradient terms in Eq. (4) and accounts for the local destacking energy [8]. The second term accounts for the difference in stacking energy between a segment of dsDNA and a denaturation bubble. The third term corresponds to the energy \( 2\beta \mu \) required to break a base pair, contributing to \( D \) in the continuum model. There is evidence that \( \tilde{K} \ll \tilde{\mu} \), which justifies the choice of \( \tilde{K} = 0 \) adopted below [10]. The bare parameters of the internal (Ising) system \( J, \tilde{\mu}, \tilde{K} \) are taken to be independent of temperature and bubble loop entropy is not explicitly included; the melting transition is therefore driven only by the difference in bending rigidity. The partition function is

\[
Z = \sum_{\{\sigma_i\}} \prod_{i=1}^{N-1} \int \frac{d\Omega_i}{4\pi} \langle \sigma_i | \sigma_i \rangle \hat{P}(\Omega_1, \Omega_2) |\sigma_i\rangle \langle \sigma_i | V \rangle,
\]

where \( |V\rangle = (e^{\mu/2}, e^{-\mu/2}) \) is the end vector, which enters to account for the free chain boundary conditions, and \( \hat{P}(\Omega_i, \Omega_{i+1}) \) is the transfer kernel given by

\[
\hat{P} = \left( \begin{array}{cc}
\left( e^{\kappa_A[\cos \gamma_i + J + \gamma_i \mu + K]} e^{\kappa_A[\cos \gamma_i + J + \gamma_i \mu + K]}
\right)
\end{array} \right)
\]

with \( \cos \gamma_i = t_i \cdot t_{i+1} \). The A and B states form the canonical base, \( |A\rangle = |+1\rangle = (1, 0) \) and \( |B\rangle = |0\rangle = (0, 1) \). Thanks to the rotational symmetry of the bending energy we can again integrate out the chain, leading to an effective Ising model with a free energy, \( H_{1,0} \), containing renormalized parameters:

\[
Z = e^{-(N-1)\Gamma_0} \sum_{\{\sigma_i\}} e^{-\beta H_{1,0}[\sigma_i]} \text{ where } H_{1,0} \equiv H_0(\tilde{J}_0, \tilde{K}_0, \tilde{\mu})
\]

with \( \tilde{J}_0 \equiv J - [G_0(\kappa_A) + G_0(\kappa_B) - 2G_0(\kappa_{AB})]/4 \) and \( \tilde{K}_0 \equiv K - [G_0(\kappa_A) - G_0(\kappa_B)]/2 \equiv K - \Delta \tilde{G}_0^{AB}/2 \) the renormalized Ising parameters, and \( \Gamma_0 \equiv [G_0(\kappa_A) + G_0(\kappa_B) + 2G_0(\kappa_{AB})]/4 \). These parameters depend on chain rigidities through \( G_0(\kappa) \) which is the free energy of a single
joint (two monomer) subsystem with rigidity $\kappa$: $G_0(\kappa) = -\ln(\int \frac{d\ell}{\ell^2} \exp[\kappa(\cos(\theta) - 1)]) = \kappa - \ln(\sinh(\kappa)/\kappa)$. The renormalized quantity $2L_0 = (2 + K_0)$ corresponds to $\beta D_R$ in the continuum model. If the bending free energy gain in opening one link, $\Delta G_{0AB}$, is greater than the intrinsic energy cost, $2(\mu + K_0)$, of opening an interior bond, then $L_0$ becomes negative, signaling a change in stability of the A and B states. In the limit of high $\kappa_A$ and $\kappa_B$, the entropic contribution dominates: $G_0(\kappa) \approx -S_0(\kappa)/k_B \approx \ln(2\kappa)$. The discrete model then reduces to the continuum one and $\Delta G_{0AB} \approx \ln(\kappa_A/\kappa_B)$ corresponds to the correction appearing in $\beta D_R$. The difference between $L_0$ and $\mu$ when $K_0 \neq 0$ creates an end-interior asymmetry that plays an important role in finite size effects.

The Ising partition and correlation functions are obtained using transfer matrix techniques. The eigenvalues, $|0,\pm\rangle$, and the eigenvalues, $\lambda_{0,\pm} = e^{\kappa L_0} [\cosh(L_0) \pm (\sinh(L_0) + e^{4L_0})^{1/2}]$, of the effective Ising transfer matrix allow us to calculate the (dimensionless) free energy per Ising spin of the coupled system, $F = -\ln Z/N$, where $Z = \sum_{r=\pm} \langle V [0,\tau] \rangle^2 \delta_{0,\tau}$. The average of the internal state variable is $\langle c \rangle = \langle \sum_{i=1}^{N} \delta_{r}(N) \rangle = -\partial F/\partial \mu$, from which the fractions of A and B links, $\varphi_A = (1 + \langle c \rangle)/2$ and $\varphi_B = 1 - \varphi_A$, can be derived. The melting temperature $T_m$ is then defined by $\varphi_B(T_m) = 1/2$. When $N \rightarrow \infty$, $\langle c \rangle$ gets simplified to

$$\langle c \rangle_{\infty} = \frac{\partial \ln \lambda_{0,\pm}}{\partial \mu} = \frac{\sinh(L_0)}{[\sinh^2(L_0) + e^{4L_0}]^{1/2}}.$$  

If $L_0$ vanishes at a temperature, $T_m^{\infty}$, sufficiently low for the cooperativity, or loop initiation, factor, $\sigma = e^{4L_0}$, in the denominator to be small, then the system will undergo a melting transition: $\langle c \rangle_{\infty}$ will sharply cross-over from $+1$ for $T < T_m^{\infty}$ (pure A state) to $-1$ for $T > T_m^{\infty}$ (pure B state). Contrary to previous Ising-type models, the melting temperature is not put in by hand but emerges naturally from $L_0 = 0$. In Eq. (3) $e^{-4L_0}$ determines the width of the transition region: $\Delta T_m^{\infty} = 2\partial \langle c \rangle_{\infty}/\partial T |_{T_m^{\infty}} \approx (2 \kappa_B(T_m^{\infty})^2/\mu) \exp[-2J_0(T_m^{\infty})]$. In the limit $N, i \rightarrow \infty$, the influence of end-monomers disappears and the Ising correlation function reduces to $\langle (\sigma_{l,-}(c) \sigma_{l+r,-}(c) \rangle_{\infty} \rightarrow (1-\langle c \rangle_{\infty})^2 \exp(-r/\xi_l)$, where $\xi_l = -\ln^{-1}(\lambda_{0,-}/\lambda_{0,+})$ is the Ising correlation length, the typical size of B (A) domains below (above) $T_m^{\infty}$.

The tangent-tangent correlation function, $\langle t_i \cdot t_{i+r} \rangle_{\infty}$, is obtained using the full transfer kernel method [17], which requires solving a spinor eigenvalue problem: $\hat{P} \hat{\psi} = \lambda \hat{\psi}$, where $\langle \hat{\psi}(\Omega) \rangle = (\hat{\psi}_{+1}(\Omega), \hat{\psi}_{-1}(\Omega))$. We find eigenvalues, $\lambda_{l+r}$, labeled by $l = 0, \ldots, \infty$ and $r = \pm$ with the same form as for $l = 0$ given above, but now where $G_0$ in the renormalized parameters is replaced by $G_1(\kappa) = \kappa - \ln(\sinh(\kappa)\kappa)/\kappa)^{20}$. The eigenspinors are $\langle \hat{\psi}_{l,m,r}(\Omega) \rangle \equiv v_{l,m}Y_{l,m}(\Omega)(l, \tau)$ with $Y_{l,m}(\Omega)$ the spherical harmonics. The transfer kernel can be expanded in terms of the eigenspinors $\hat{P} = \sum_{l,m,r} \lambda_{l+r} \langle \hat{\psi}_{l,m,r} \rangle \langle \hat{\psi}_{l,m,r} \rangle$ and then be used to calculate the correlation functions. In the limit $N, i \rightarrow \infty$, the expression for the tangent-tangent correlation function simplifies to

$$(t_i \cdot t_{i+r})_{N,i \rightarrow \infty} \sum_{r=\pm} (1,\tau)[0,+,2] \exp(-r/\xi_{l,+}) (9)$$

which reveals the importance of the two persistence lengths, $\xi_{l,+} = -\ln^{-1}(\lambda_{l,1,}/\lambda_{0,+})$ (units of $\ell_0$). It is not possible to extract from $(t_i \cdot t_{i+r})$ one unique length for the whole range of $T$, because the weights associated with $\xi_{l,+}^2$ strongly vary with $T$.

We now compare the discrete model predictions with experiment [1] by focusing on the melting profile, $\varphi_B(T)$, of a synthetic nonalternating homopolymer, polydA-polydT. A typical profile for a homopolymer has a sigmoidal shape characterized by $T_m$ and $\Delta T_m$. Of the five independent parameters that appear in the theory when $K = 0$, three are determined experimentally (polymerization index $N$ and bending moduli assuming $\kappa_{AB} = \kappa_A [17]$) and two ($\mu$ and $\bar{J}$) are determined by fitting the model to experiment, hence $T_m = T_m(\mu, \bar{J}; N, \kappa_A, \kappa_B)$. Figure 1a shows $\varphi_B(T)$ for DNA of molecular weight 1180 kDa [1]. From the known persistence lengths we obtain $\kappa_A = 147$ and $\kappa_B = 5.54$ at 300 K. The solid line corresponds to our model fit with $\mu = 4.46$ kJ/mol and $\bar{J} = 9.13$ kJ/mol, leading to $T_m = 326.4$ K. The fitted value for $2\mu$ is close to the experimental energy of 10.5 kJ/mol needed to break an A-T link [2].

The value for $\bar{J} \sim 2\mu$ is also consistent with the idea that destacking energy makes the dominant contribution to DNA stability [1]. The renormalized cooperativity parameter at $T_m = J_0 = 11.5$ kJ/mol $> \bar{J}$. The model fit thus leads to parameter values in accord with experiment (in reality, the fitted values of $\mu$ and $\bar{J}$ implicitly compensate for effects like loop entropy explicitly left out of the model [17]). In Fig. 1b, the curve corresponding to $N \rightarrow \infty$ is shown for the same parameter values. In this case, $\varphi_B(T)$ is given by Eq. (3) and $T_m^{\infty}$ is obtained from $L_0 = 0: k_B T_m^{\infty} \approx 2(\mu + \bar{K})/\ln(\kappa_A/\kappa_B)$. In this limit the transition width is non-zero, due to the finite cooperativity parameter: $\Delta T_m^{\infty} \propto \xi_{l,+}^{-1}(T_m^{\infty}) = 2exp(-2J_0(T_m^{\infty}))$. Since $\xi_{l}(T_m^{\infty}) \sim 2000$, typical helix and bubble domains are flexible within a small window about $T_m^{\infty}$. When $N$ decreases, the width increases (Fig. 1a) roughly as $\Delta T_m(N) \approx \Delta T_m^{\infty} \sim N^{-1}$. Hence even for a long polymer ($N \sim 10^3$), finite size effects are non-negligible, in agreement with experiments [21]. Then the nature of end monomers becomes important, as confirmed in Fig. 1a, which shows how short chains begin to melt by end-unwinding at lower temperatures (the trend that $T_m$ increases with decreasing $N$ will likely be reversed when loop entropy is included [2, 17]). Our model predictions for experimentally accessible A-T pair quantities are in agreement with accepted values [4, 6]: i) $\sigma \approx 10^{-7}$ at $T_m$; and at physiological temperature ii) an interior single base-pair opening probability of $10^{-6}$ with a bub-
homopolynucleotide solutions [23] in qualitative agreement with Fig. 1b. Incorporating bending rigidity into DNA denaturation models thus allows us to make explicit predictions for both melting profiles and DNA mean-size dependent quantities. It will be of great interest to both probe such effects by carrying out experiments on DNA homopolymers and other biopolymers undergoing helix-coil transitions and extend our theory to heteropolymers, mechanical denaturation, and DNA dynamics.

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