Statistical Mechanics of Torque Induced Denaturation of DNA

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A unifying theory of the denaturation transition of DNA, driven by temperature T or induced by an external mechanical torque Γ is presented. Our model couples the hydrogen-bond opening and the untwisting of the helicoidal molecular structure. We describe denaturation as a first order transition from B-DNA to d-DNA phases. The coexistence region is naturally parametrized by the degree of supercoiling σ. The denaturation free energy, the temperature dependence of the twist angle, the phase diagram in the T, Γ plane, and isotherms in the σ, Γ plane are calculated and show a good agreement with experimental data.

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Denaturation of the DNA, due to its essential relevance to transcription processes has been the object of intensive works in the last decades. Experiments on dilute DNA solutions have provided evidence for the existence of a *thermally driven* melting transition corresponding to the sudden opening of base pairs at a critical temperature T_m [1]. Later, following the work of Smith *et al.* [2], micromanipulation techniques have been developed to study single-molecule behavior under stress conditions and how structural transitions of DNA can be *mechanically* induced. While most single-molecule experiments have focused on stretching properties so far, the response of a DNA molecule to an external torsional stress has been studied very recently [3,4], shedding some new light on denaturation [3]. From a biological point of view, torsional stress is indeed not unusual in the living cell and may strongly influence DNA functioning [5,6].

For a straight DNA molecule with fixed ends, the degree of supercoiling $\sigma = (Tw - Tw_0)/Tw_0$ measures the twist Tw (i.e., the number of times the two strands of the DNA double helix are intertwined) with respect to its counterpart Tw_0 for an unconstrained linear molecule. In Strick *et al.* experiment [3], a λ-DNA molecule, in 10 mM PB, is attached at one end to a surface and pulled and rotated by a magnetic bead at the other end. At stretching forces of $\simeq 0.5$ pN, sufficient to eliminate plectonems by keeping the molecule straight, a torque induced transition to a partially denaturated DNA is observed. Beyond a critical supercoiling $\sigma_c \simeq -0.015$ and an associated critical torque $\Gamma_c \simeq -0.05$ eV/rad the twisted molecule separates into a pure B-DNA phase with $\sigma = \sigma_c$ and denaturated regions with $\sigma = -1$. Extra turns applied to the molecule increase the relative fraction of d-DNA with respect to B-DNA.

In this Letter, we provide a unifying understanding of both thermally and mechanically induced denaturation transitions. We show that denaturation can be described in the framework of first order phase transitions with control parameters being the temperature and the external torque. This is in close analogy to the liquid-gas transition, where control parameters are the temperature and the pressure. Our theory gives a natural explanation to the BDNA-dDNA phases coexistence observed in single-molecule experiments [3]. We give quantitative estimates for the denaturation free energy ΔG, the temperature dependence of the average twist angle $\Delta\langle\theta\rangle/\Delta T$, the critical supercoiling σ_c, and torque Γ_c at room temperature in good agreement with the experimental data. Furthermore the dependence of the critical torque as a function of the temperature is predicted.

Presentation of the model.—Our model reproduces the Watson-Crick double helix (B-DNA) as schematized in Fig. 1. For each base pair ($n = 1, \ldots, N$), we consider a polar coordinate system in the plane perpendicular to

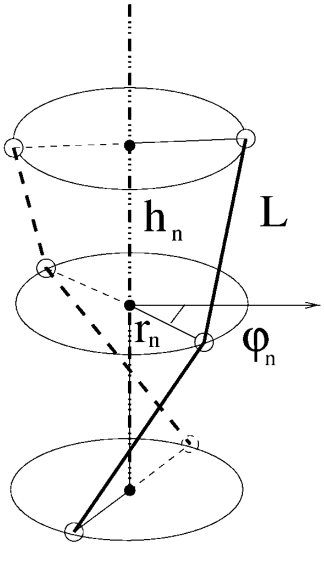

FIG. 1. The helicoidal DNA model: each base pair is modelized through its radius r_n and angle φ_n. The axial distance h_n between successive base pair planes varies while the backbone length along the strands is fixed to L.

the helical axis and introduce the radius r_n and the angle φ_n of the base pair [7]. The sugar phosphate backbone is made of rigid rods of fixed length L while the distance h_n between base planes $n - 1$ and n may fluctuate. The potential energy V associated with a configuration of the degrees of freedom $\{r_n, \varphi_n\}$ reads $V = \sum_n [V_m(r_n) + V_s(r_n, r_{n-1}) + V_b(r_n, r_{n-1}, \theta_n) + V_\Gamma(\theta_n)]$ where $\theta_n = \varphi_n - \varphi_{n-1}$ is the twist angle.

The first contribution to V, the short-range Morse potential $V_m(r_n) = D(e^{-a(r_n - R)} - 1)^2$ represents the hydrogen bonds inside a given pair n [8,9]. Fixing $a = 6.3$ Å^{-1} [10,11], the width of the well amounts to $3a^{-1} \simeq 0.5$ Å in agreement with the order of magnitude of the relative motion of the hydrogen-bonded bases around the rest diameter $R = 10$ Å [12]. A base pair with diameter $r > r_d = R + 6/a$ may be considered as open. The potential depth D, typically of the order of 0.1 eV [8,13] depends on the base pair type [adenine-thymine (AT) or guanine-citosine (GC)] as well as on the ionic strength.

Secondly, the shear force that opposes sliding motion of one base over another in the B-DNA conformation is accounted for by the stacking potential [1] $V_s(r_n, r_{n-1}) = Ee^{-b(r_n + r_{n-1} - 2R)}(r_n - r_{n-1})^2$. Because of the decrease of molecular packing with base pair opening, the shear prefactor is exponentially attenuated and becomes negligible beyond a distance $\simeq 5b^{-1} = 10$ Å, which coincides with the diameter of a base pair [10,11,13,14].

Thirdly, the fluctuations of the axial distance h_n between successive base pair planes are described by an elastic energy

$$\begin{aligned} V_b(r_n, r_{n-1}, \theta_n) &= K[h_n - H]^2 \\ &= K\left[\sqrt{L^2 - r_n^2 - r_{n-1}^2 + 2r_n r_{n-1}\cos\theta_n} - H\right]^2. \end{aligned} \tag{1}$$

The helicoidal structure arises from $H < L$: in the B conformation ($r < r_d$) the minimum of V_b ($= 0$) is reached for a nonzero twist angle; see Fig. 1. The above definition of V_b holds as long as the argument of the square root in (1) is positive. Values of r_n, r_{n-1}, θ_n incompatible with rigid rods having length L are excluded by imposing $V_b = \infty$ for negative arguments. The behavior of a single strand in the denaturated ($r > r_d$) conformation is uniquely governed by this rigid rod condition.

Last of all when the molecule is fixed at one end and subject to a torque Γ on the other extremity, a torsional energy $V_\Gamma(\theta_n) = -\Gamma\,\theta_n$ has to be included. A torque $\Gamma > 0$ overtwists the molecule, while $\Gamma < 0$ undertwists it.

Choice of force and geometrical constants.—The resolution of the model exposed below gives access to statistical and thermodynamical properties as the mean twist angle $\langle\theta_n\rangle$ and axial distance $\langle h_n\rangle$ at ambient temperature, the denaturation free energy ΔG, the melting temperature T_m, and the torsional modulus C. Comparison of these results with experimental data allows us to determine the values of the parameters H, L, D, E, and K entering the potential energy.

Choosing $H = 3$ and $L = 6.95$ Å, we have recovered at room temperature $T = 298$ K the thermal averages $\langle h_n\rangle \simeq 3.4$ Å and $\langle\theta_n\rangle \simeq 2\pi/10.4$ [1]. We have then set the force constants D and E to fit the melting temperature $T_m = 350$ K of the homogeneous Poly(dGdT)-Poly(dAdC)-DNA at 20 mM Na$^+$ [1]; see inset of Fig. 3. This sequence has a GC/AT ratio equal to unity, as the λ-DNA in the experimental conditions of [3]. Among all possible pairs of parameters (D, E) that correctly fit T_m, we have selected the pair ($D = 0.16$ eV, $E = 4$ eV/Å^2) giving the largest prediction for ΔG (see inset of Fig. 2) that is in closest agreement with thermodynamical estimates of the denaturation free energy. Finally, the elastic constant $K = 0.014$ eV/Å^2 has been tuned to give back at ambient temperature the torsional modulus C of B-DNA estimated to $C = 860 \pm 100$ Å [15,16].

Transfer matrix formalism.—The configurational partition function at inverse temperature β can be calculated using the transfer integral method:

$$Z_\Gamma = \int_{-\infty}^{\infty} d\varphi_N \, \langle R, \varphi_N | T^N | R, 0\rangle . \tag{2}$$

As in the experimental conditions, the radii of the first and last base pairs are fixed to $r_1 = r_N = R$. The angle of the fixed extremity of the molecule is set to $\varphi_1 = 0$ with no restriction whereas the last one φ_N is not constrained. The transfer operator entries read $\langle r, \varphi | T | r', \varphi'\rangle \equiv X(r, r') \times \exp\{-\beta[V_b(r, r', \theta) + V_\Gamma(\theta)]\}\chi(\theta)$ with $X(r, r') = \sqrt{rr'}\exp\{-\beta[V_m(r)/2 + V_m(r')/2 + V_s(r, r')]\}$. The $\sqrt{rr'}$ factor in X comes from the integration of the kinetic term; $\chi(\theta) = 1$ if $0 \leq \theta = \varphi - \varphi' \leq \pi$ and 0 otherwise to prevent any clockwise twist of the chain. At fixed r, r', the angular part of the transfer matrix T is translationally invariant in the angle variables φ, φ' and can be diagonalized through a Fourier transform. Thus, for each Fourier mode k we are left with an effective transfer matrix on the radius variables $T_k(r, r') = X(r, r')Y_k(r, r')$ with

$$Y_k(r, r') = \int_0^{\pi} d\theta \, e^{-\beta[V_b(r, r', \theta) + V_\Gamma(\theta)]} e^{-ik\theta}. \tag{3}$$

The only mode contributing to Z_Γ is $k = 0$ once φ_N has been integrated out in (2). The eigenvalues and eigenvectors of T_0 will be denoted by $\lambda_q^{(\Gamma)}$ and $\psi_q^{(\Gamma)}(r)$, respectively, with $\lambda_0^{(\Gamma)} \geq \lambda_1^{(\Gamma)} \geq \ldots$. In the $N \to \infty$ limit, the free-energy density $f^{(\Gamma)}$ is given by $-k_B T \ln \lambda_0^{(\Gamma)}$.

Note that the above result can be straightforwardly extended to the case of a molecule with a fixed twist number $Tw = N\ell$, e.g., for circular DNA. Indeed, the twist density ℓ and the torque Γ are thermodynamical conjugated variables and the free energy at fixed twist number ℓ is the Legendre transform of $f^{(\Gamma)}$.

We have resorted to a Gauss-Legendre quadrature for numerical integrations over the range $r_{\min} = 9.7$ Å $< r < r_{\max}$. The Morse potential V_m increases exponentially with decreasing $r < R$ and may be considered as infinite for $r < 9.7$ Å [11]. The extrapolation procedure to $r_{\max} \rightarrow \infty$ depends on the torque value Γ and will be discussed below. The eigenvalues $\lambda_q^{(\Gamma)}$ and associated eigenvectors $\psi_q^{(\Gamma)}(r)$ are computed using Kellog's iterative method [10]. As in quantum mechanics, $|\psi_0^{(\Gamma)}(r)|^2$ gives the probability of a base pair to be of radius r and can be used to compute thermal averages of microscopic variables.

Bound and delocalized wave functions.— At low temperature, $\psi_0^{(\Gamma)}$ is entirely confined in the Morse potential well and describes a closed molecule. Conversely the following eigenfunctions $\psi_1^{(\Gamma)}, \psi_2^{(\Gamma)}, \ldots$ correspond to an open molecule: they extend up to $r_{\max}$ and vanish for $r < r_d$. They are indeed orthogonal to another family of excited states that are quasiconfined in the Morse potential with much lower eigenvalues. In the absence of torque, the shapes of the open states are strongly reminiscent of purely diffusive eigenfunctions, $\psi_q^{(\Gamma=0)}(r) \simeq \sin[q\pi(r - r_d)/(r_{\max} - r_d)]$ leading to a continuous spectrum in the limit $r_{\max} \rightarrow \infty$. The existence of a torque modifies the shape of these wave functions: $\psi_1^{(\Gamma)}$ is centered around $r < r_{\max}/2$ (respectively, $r > r_{\max}/2$) for $\Gamma > 0$ (respectively, $\Gamma < 0$).

The diffusive nature of the open state wave functions can be understood as follows. For $r, r' > r_d$, the transfer operator $T_0(r, r')$ is compared in Fig. 2 to the exact conditional probability $\rho(r, r')$ that the endpoint of a backbone rod of length L be located at distance r' from the vertical reference axis knowing that the other extremity lies at distance r [17]. For fixed r, T_0 and ρ both diverge in $r' = r \pm L$ and are essentially flat in between. The flatness of T_0 derives from the expression of V_b: a rigid rod with extremities lying in r, r' may always be oriented with some angle θ^* ($\rightarrow 0$ at large distances) at zero energetic cost $V_b(r, r', \theta^*) = 0$. Consequently, $T_0(r, r') \simeq \lambda_1^{(\Gamma)} \rho(r, r')$ and our model can reproduce the purely entropic denaturated phase.

Denaturation transition.—For zero torque, $\lambda_0^{(\Gamma=0)}$ reaches the second largest eigenvalue at a critical temperature $T_m = 350$ K as shown in Fig. 2. For $T > T_m$, the bound state disappears and $\psi_1^{(\Gamma=0)}$ in Fig. 2 becomes the eigenmode with the largest eigenvalue [18]. The percentage of opened base pairs exhibits an abrupt jump from 0 to 1 at T_m, reproducing the UV absorbance vs temperature experimental curve for Poly(dGdT)-Poly(dAdC)-DNA [1]. The difference ΔG between the free energy $f_d^{(\Gamma=0)}$ of the open state ($q = 1$) and the free energy $f_B^{(\Gamma=0)}$ of the close state ($q = 0$) gives the denaturation free energy at temperature T; see Fig. 2. At $T = 298$ K, we obtain $\Delta G = 0.022$ eV in good agreement with the free energy of the denaturation bubble formation $\Delta G \simeq 0.025$ eV estimated in AT rich regions [1,5].

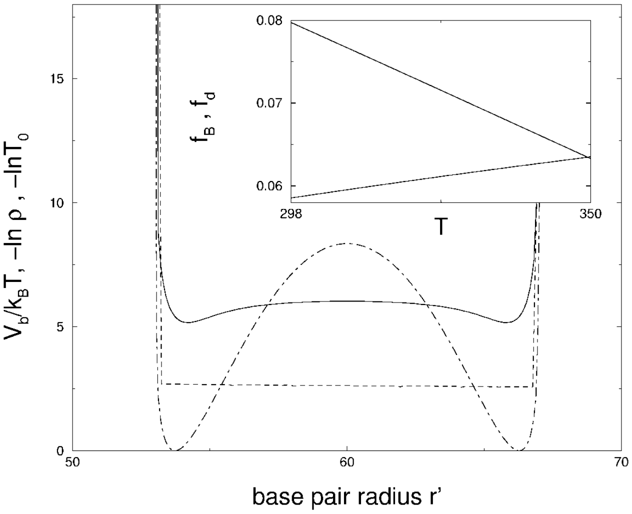

FIG. 2. With respect to $V_b(r = 60$ Å$, r', \theta = 0)$ (dash-dotted line, plotted as a function of r' in units of k_BT for $T = 298$ K), $-\ln T_0(r = 60$ Å$, r')$ (full line) is flat and comparable to $-\ln\rho(r = 60$ Å$, r') + f_d/k_BT$ (dashed line) with $f_d = 3.2k_BT = 0.08$ eV. Similar curves are obtained for any $r > r_d$. Inset: Free energies $f_B^{(\Gamma=0)}$ (lower curve) and $f_d^{(\Gamma=0)}$ (upper curve) of B-DNA and d-DNA in units of eV. ΔG equals 0.022 eV at $T = 298$ K and vanishes at $T_m = 350$ K.

The presence of an overtwisting (respectively, undertwisting) torque $\Gamma > 0$ (respectively, $\Gamma < 0$) strongly affects $f_B^{(\Gamma)}$, leaving almost unchanged the single strand free energy $f_d^{(\Gamma)}$. The denaturation transition takes place at $T_m(\Gamma)$ [19]; see the phase diagram shown in the inset of Fig. 3. We expect a critical point at a high temperature and large positive torque such that $\psi_1^{(\Gamma)}$ is centered on R [19].

The supercoiling, induced by a torque at a given temperature smaller than $T_m(\Gamma = 0) = 350$ K, is the relative change of twist with respect to the value at zero torque in the B-DNA state, $\sigma(\Gamma) = (\langle\theta\rangle_\Gamma - \langle\theta\rangle_{\Gamma=0})/\langle\theta\rangle_{\Gamma=0}$. In Fig. 3, we have plotted the isotherms in the σ, Γ plane. Horizontal lines are critical coexistence regions between the B-DNA phase, on the left of the diagram and the denaturated phase on the right (with $\sigma = -1$). At room temperature, critical coexistence between B-DNA and d-DNA arises at torque $\Gamma_c = -0.035$ eV/rad and supercoiling $\sigma_c = -0.01$. These theoretical results are in good agreement with the values $\Gamma_c = -0.05$ eV/rad, $\sigma_c = -0.015$ obtained experimentally [3].

Twist fluctuations in the B-DNA phase.—For a closed molecule, the average twist angle depends linearly on the applied torque as can be deduced from the left steep line in Fig. 3. The slope $K_\theta = \Gamma/(\langle\theta\rangle_\Gamma - \langle\theta\rangle_{\Gamma=0})$ does not vary with temperature over the range $298 < T < 350$ K

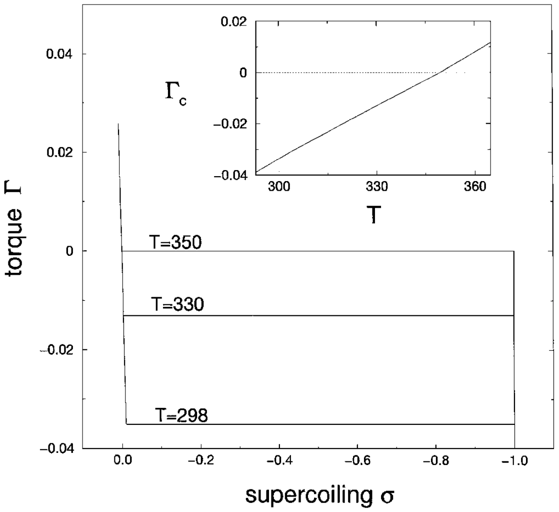

FIG. 3. Isotherms in the σ, Γ plane with Γ measured in units of eV/rad. For $T = 298$ K, the critical supercoiling and torque are $\sigma_c = -0.01$, $\Gamma_c = -0.035$ eV/rad, respectively. Inset: phase diagram in the T, Γ plane. At $T = 298$ K, $\Gamma_c = -0.035$ eV/rad while in the absence of torque (dotted line), $T_m = 350$ K.

and gives the torsional modulus $C \simeq 840$ Å of B-DNA through $C = K_\theta \langle h_n \rangle / (k_B T)$ [15].

As for thermal fluctuations, they lead to an undertwisting $\Delta\langle\theta\rangle/\Delta T \simeq -1.4\,10^{-4}$ rad/K at $\Gamma = 0$. This closely agrees with experimental measures $\Delta\langle\theta\rangle/\Delta T \simeq -1.7\,10^{-4}$ rad/K [20].

We plan to combine the present model with existing elasticity theories of DNA [2,16] to understand the influence of an external stretching force on the structural transition studied in this paper. It would also be interesting to see how the above results are modified in the presence of a heterogeneous sequence.

The present model originates from a previous collaboration [7] of one of us (S.C.) with M. Barbi and M. Peyrard which we are particularly grateful to. We also thank B. Berge, D. Bensimon, C. Bouchiat, E. Bucci, A. Campa, A. Colosimo, V. Croquette, A. Giansanti, and C. Laroche for useful discussions.

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