

Denaturation of Heterogeneous DNA

Dinko Cule and Terence Hwa

Department of Physics, University of California at San Diego, La Jolla, California 92093-0319

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The effect of heterogeneous sequence composition on the denaturation of double-stranded DNA is investigated. The resulting pair-binding energy variation is found to have a negligible effect on the critical properties of the smooth second-order melting transition in the simplest (Peyrard-Bishop) model. However, sequence heterogeneity is dramatically amplified upon adopting a more realistic treatment of the backbone stiffness. The model yields features of “multistep melting” similar to those observed in experiments. [S0031-9007(97)04133-1]

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The denaturation or melting of double-stranded DNA molecules upon changes in ambient temperatures or solvent conditions is a subject which has had a long history [1,2]. In early theoretical studies [1,3], DNA melting was described by the nearest-neighbor 1D Ising model which yielded only a sharp *crossover* but not a thermodynamic phase transition [4]. A smooth second-order transition was later demonstrated in a modified version of the 1D Ising model [5], after including an effective *long-range* interaction arising from the configurational entropy gain of the denatured segments. Experimentally, a purified DNA sample containing a unique sequence and length is found to exhibit distinct multistep melting, where the “melting curves” (to be specified below) exhibit sharp features consisting of plateaus of variable sizes separated by steps [2,6]. These fine features have been attributed to the melting of individual “domains” associated with variations in the composition of the nucleotide sequences, since the binding energies of the two kinds of base pairs, adenine-thymine (AT) and guanine-cytosine (GC), are significantly different [7]. The effect of binding energy variation has been studied previously using the nearest-neighbor 1D Ising model [1,8]. In this paper, we investigate this effect in detail by incorporating the important configurational entropy of the denatured strands in a systematic way. We find that sequence heterogeneity itself is not sufficient to produce multistep melting, with the phase transition remaining to be of the second order. Nevertheless, heterogeneity can be dramatically amplified by small changes in the detailed form of the configurational entropy, leading to the apparent multistep melting behavior for finite length sequences.

A simple way of incorporating the entropy of the single-stranded segments is to model the two single strands $\mathbf{r}_n^{(1)}$ and $\mathbf{r}_n^{(2)}$ by random walks [9], with the index n denoting the n th base pair. The binding of complementary base pairs is described by a potential function $V_n(\mathbf{r}_n^{(1)} - \mathbf{r}_n^{(2)})$, where V_n has a hard core (reflecting the repulsion of the phosphate backbone) and an attractive (short-ranged) tail mimicking the hydrogen bond between each base pair. Further taking into account the directional specificity of the hydrogen bonds, one obtains the follow-

ing Hamiltonian for a double strand of N base pairs:

$$\beta\mathcal{H} = \frac{1}{T} \sum_{n=1}^N \left\{ \frac{K}{2} (y_{n+1} - y_n)^2 + V_n(y_n) \right\}. \quad (1)$$

In Eq. (1), y_n is the component of the relative displacement field $\mathbf{r}_n^{(1)} - \mathbf{r}_n^{(2)}$ along the direction of hydrogen bond and is the important degree of freedom we will focus on. The quadratic coupling describes the stiffness of the backbone.

The model (1) without the n dependence in the interaction V is known as the Peyrard-Bishop (PB) model [10]. Although PB specifically used the Morse potential for V , the qualitative behavior of the system is well known for a large class of short-ranged potentials, via a mapping to a fictitious quantum mechanics problem [11]. Let the depth of the (asymmetric) “potential well” $V(y)$ be U_0 and the range of attraction be a ; then a continuous phase transition [12] occurs at a critical temperature T_m , given by the condition $T_m^2/(2Ka^2) \sim U_0$. This phase transition is characterized by a discontinuity in the specific heat C and an algebraic divergence in the average separation distance ℓ between the base pairs, $\ell \sim (T_m - T)^{-\nu}$ with $\nu = 1$. The inverse of the pair distance ℓ^{-1} is the order parameter of this transition and can be directly related (see below) to the fraction of unbroken base pairs, a key experimental observable [2].

Next, we describe the effect of a variable interaction $V_n(y)$ which is fixed by the DNA sequence. For simplicity, we restrict y to the positive real axis, and model the interaction as a product of a short-ranged function $\delta_a(y)$ [with $\delta_a(0 < y \leq a) \approx 1$ and $\delta_a(y > a) \rightarrow 0$] and a variable strength V_n [13]. We assume the sequence to be random and short-range correlated, with an average pairing energy $\overline{V_n} = -U_0$, and the fluctuation $\delta V_n = V_n - \overline{V_n}$ described by its variance $\overline{\delta V_m \delta V_n} = \Delta \delta_{m,n}$. (Here, the overbar denotes average over the ensemble of random sequences.) Some important conceptual issues to understand are whether the melting transition survives in the presence of the quenched-in variable interaction, and if so, what is the nature of the transition.

These issues have been studied in the past decade in the context of some closely related systems, e.g., one describing the adsorption of a Gaussian random heteropolymer by a solid surface [14–18] (with y_n being the distance of the n th monomer from the surface and $V_n(y)$ giving the interaction of that monomer with the surface), and another describing the wetting of a 1D interface from a random substrate [19–21]. It is known that the melting transition still exists and the effect of quench-in randomness is *marginal* in the renormalization group sense. This conclusion is straightforwardly reached, for instance, by considering the perturbative effect of a weak disorder on the melting temperature: Assuming that the effect of randomness is negligible in the small Δ limit, then fluctuation in base separation y_n is correlated along the backbone up to the length $\xi \sim \ell^2$. At a temperature T slightly below the melting temperature T_m of the pure system, $\xi \sim T_m^2/(T_m - T)^2$ becomes very long. Variation in the interaction energy U_0 averaged over the scale ξ is $\delta U \sim \sqrt{\Delta \xi}/\xi \approx \sqrt{\Delta}(T_m - T)/T_m$, which leads to a *shift* in the melting temperature of the order

$$\delta T_m \propto (T_m - T)\sqrt{\Delta}/U_0. \quad (2)$$

The effect of randomness is revealed by comparing δT_m with $\delta T \equiv T_m - T$ in the limit $\delta T \rightarrow 0$. Randomness is irrelevant if $\delta T_m \ll \delta T$, but is non-negligible if otherwise. The problem at hand is “marginal” since $\delta T_m \sim \delta T$.

Much effort has been devoted to resolving whether the randomness is marginally relevant or marginally irrelevant. Early studies [14,19] suggest that it is marginally irrelevant, such that critical properties of the melting transition are the same as those of the pure case (up to logarithmic corrections). However, more recent renormalization-group studies [20,21] find the randomness to be marginally relevant, indicating that the scaling properties should be different from the pure case beyond a crossover length $\xi_\times \sim \exp[c U_0^2/\Delta]$ with $c \sim O(1)$, or equivalently if the reduced temperature is within the Ginzburg temperature $\delta T_\times \sim \xi_\times^{-1/2}$. The actual scaling behavior in the asymptotic “strong coupling” regime is however not accessible from these studies.

The theoretical results have so far not been carefully tested numerically: In Ref. [19], evidence in support of the irrelevancy of randomness was reported, while the contrary was claimed in Ref. [20]. The numerics in the latter work was in fact rather qualitative; no attempt in characterizing the alleged strong coupling regime was made. The numerics in Ref. [19] was flawed on the other hand by assuming a particular value for the melting temperature which was later shown to be incorrect [20]. We have thus reinvestigated the nature of the melting transition numerically.

As in Refs. [19,20], we use a transfer matrix calculation [24]. We start with the transfer integral solution of the “wave function” $\phi_n(y)$ [10],

$$\phi_{n+1}(y) = \int_{y'} \exp\left[-\frac{K}{2T}(y - y')^2 - \frac{V_n(y)}{T}\right] \phi_n(y'). \quad (3)$$

To speed up the numerics, we placed the recursion relation (3) on a lattice such that y takes on only non-negative integer values, from 0 to L . We further restricted $y_{n+1} - y_n \in [0, \pm 1]$. The potential function $V_n(y)$ has the simple form $V_n(y = 0) = -U_0 \pm \sqrt{\Delta}$ with equal probability, and $V_n(y > 0) = 0$. To characterize the system, we compute (for each configuration of the random potential V_n) the “steady-state” probability distribution $P_n(y)$ of finding y_n at y . This is obtained (up to normalization) as the product of the forward- and backward-propagated wave functions (see also [20]). Sufficiently long segments ($\sim 10^4$) close to the two ends of the sequence are truncated to ensure that the results are independent of the choice of boundary conditions. From the steady-state distribution, we compute the moments $\langle y_n^m \rangle_L \equiv \sum_{y=0}^L y^m P_n(y)$, and then average over different realization of the randomness (or average over n for very long sequences). For example, the average pair distance ℓ is given by $\ell = \langle y_n \rangle_L$.

We first identify the critical point by monitoring the L dependence of the dimensionless variance, $\delta y = [\langle y_n^2 \rangle_L - \langle y_n \rangle_L^2]^{1/2}/\ell$, which should be L independent at the critical point. This is shown in the inset in Fig. 1(a) for systems with $U_0 = -1$, $\Delta = 1$, $K = 1$, and $N = 10^5$, averaged over ten independent realizations of random sequences. The parameters are chosen such that $\xi_\times \sim O(1)$ and the system is readily in the strong coupling regime. (Note also that the unit of n in our simplified discrete model is no longer one base pair. With the above parameters, the length of our system corresponds to a sequence of several thousand base pairs of the PB model [10], with $\pm 20\%$ pair-to-pair variation in the binding energy.) Using the empirically obtained value of T_m , we plot the pair distance ℓ vs the reduced temperature in Fig. 1(a). An exponent $\nu = 1$ is

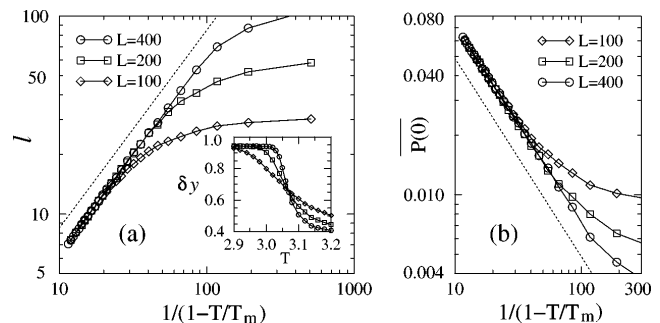


FIG. 1. (a) The average pair distance ℓ for various transverse system sizes. The melting temperature $T_m \approx 3.07$ is determined from the plot in the inset. (b) The melting curves, showing the fraction of unbroken pairs $P(0)$. Scaling laws with $\nu = 1$ are shown by the dotted straight lines.

obtained, indicating the absence of anomalous scaling. To further test the relevancy of the randomness, we directly measure the average fraction of unbroken pairs, $\overline{P_n(0)}$, which should scale as $1/\ell \sim (T_m - T)$ if the randomness is irrelevant. However, as pointed out in Ref. [22], relevancy of the randomness would imply additional singularity in the distribution $\overline{P_n(y \rightarrow 0)}$, resulting in the anomalous scaling of $\overline{P_n(0)}$. This quantity is plotted against the reduced temperature in Fig. 1(b). Again, we find no evidence of anomalous scaling. Since our calculations are performed in the strong-coupling regime, we conclude that either the randomness is irrelevant or the asymptotic scaling in the strong-coupling regime is almost indistinguishable from the pure problem. Similar results have been obtained for a variety of different parameter choices.

While the ultimate resolution to the issue of the relevancy of sequence heterogeneity may require yet larger systems with good statistics, it is clear from Fig. 1 that the melting transition encountered here is rather smooth. Indeed, the numerically obtained melting curve for a *single* sample is very smooth [see Fig. 3(b) below], without any noticeable fine structures. The smoothness of the transition is irrefutable even in the specific heat curves used to support the relevancy of randomness in Ref. [20]. This makes the experimentally observed multistep melting behavior rather puzzling. To investigate the possible cause of multistep melting, we recall a recent numerical finding [25] that fluctuation effect in DNA melting is much enhanced upon adopting a more realistic form of the backbone stiffness, to reflect the fact that the DNA is significantly more rigid in the double-stranded conformation. An explicitly y -dependent stiffness

$$K(y_n, y_{n+1}) = K_1 + (K_2 - K_1) e^{-(y_n + y_{n+1})/2b} \quad (4)$$

was used in Ref. [25] to match the stiffness of the double strand K_2 (for $y = 0$) and the single strand $K_1 < K_2$ (for $y \rightarrow \infty$). Numerical solution [25] of the homogeneous version of (3) using the modified stiffness $K(y', y)$ yields what appears to be a *first-order* melting transition.

Let us examine the effect of the variable stiffness in some detail. To facilitate the analysis, we modify the exponential factor in (4) to $e^{-y_n/b}$. This does not cause any significant differences since $y_n \approx y_{n+1}$. With the modified form of $K(y)$, one can straightforwardly perform the transfer integral (3), which in the continuum limit yields a Schrödinger-like equation for $\phi_n(y)$, with an effective potential $\tilde{V}_n(y) = V_n(y) + \frac{T}{2} \ln[K(y)/K_1] + \text{const}$, as well as an effective diffusion coefficient $T/2K(y)$. Let us focus on the form of \tilde{V} : In addition to the original attractive potential $V_n(y)$ of range a , there is now a *repulsive* term of the order $U_1 = (T/2) \ln(K_2/K_1) > 0$ of range b . The latter plays the role of an (entropic) *barrier* and is the main effect introduced by the variable stiffness $K(y)$. Qualitative features of the homogeneous system can be

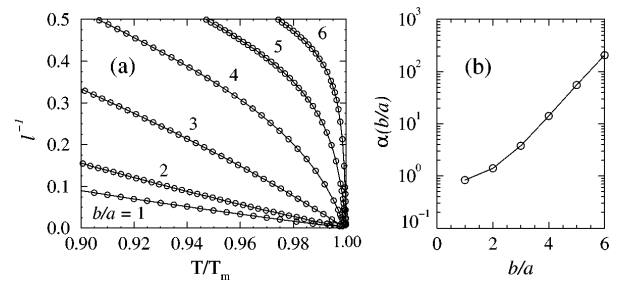


FIG. 2. (a) Solution of the homogeneous PB model with a variable backbone stiffness. The order parameter ℓ^{-1} still vanishes linearly with the reduced temperature. (b) The slope α has an exponential dependence on the ratio b/a . T_m itself changed by $\sim 50\%$ over the range of b/a studied.

obtained by solving the Schrödinger equation using a constant stiffness K and a toy potential function $\tilde{V}(y)$ on the half space $y > 0$, with $\tilde{V} = -U_0 + U_1$ for $y < a$, $\tilde{V} = +U_1$ for $a < y < b$, and $\tilde{V} = 0$ for $y > b$. We find the order parameter ℓ^{-1} of the homogeneous problem still to have the form $\ell^{-1} = \alpha \cdot (T_m - T)/T_m$ near T_m , although the amplitude α increases *exponentially* for increasing U_1 or b/a . Our finding is verified numerically (Fig. 2) by exactly diagonalizing the transfer integral (3) for the homogeneous problem, using the Morse potential for $V(y)$ and Eq. (4) for $K(y, y')$ as in Ref. [25]. Note that the width of the transition region scales as $1/\alpha$. Thus, for the parameter value $K_2/K_1 = 1.5$ and $b/a \approx 5$ used in Ref. [25] [corresponding to $\alpha \approx 100$ according to Fig. 2(b)], the transition region is extremely narrow, making it very much first-order-like in appearance.

The heterogeneous system is studied next using the lattice transfer matrix algorithm, incorporating the toy potential \tilde{V} as described above, with $U_0 \rightarrow U_0 \pm \sqrt{\Delta}$. We used $U_1 = 0.2$ and $b/a = 3$ such that the α value of the homogeneous system is ~ 100 as in Ref. [25]. For such large values of α 's, the melting curves for *individual* samples display drastic multistep behavior even for rather long sequences of $N = 10^5$; a typical example is shown in Fig. 3(a). The very smooth melting curve for the original heterogeneous model without barrier ($\alpha \sim 1$) is shown in Fig. 3(b) for comparison. As in the pure PB model with entropic barrier, we expect that the heterogeneous

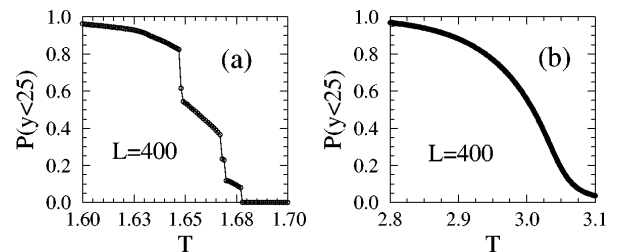


FIG. 3. Melting curves for a *single* random sequence: (a) with and (b) without the entropic barrier (see text).

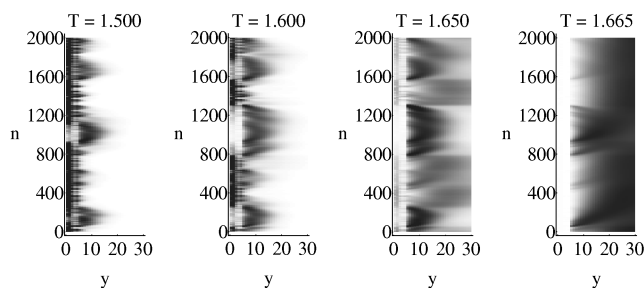


FIG. 4. Density plot of the probability distribution $P_n(y)$ for a system with entropic barrier as in Fig. 3(a). Darker shades correspond to larger probability.

model still undergoes a second-order melting transition with self-averaging melting curve at sufficiently large scales. We can understand the sharp steps in Fig. 3(a) as resulting from the first-order-like transition of various *domains* with different local transition temperatures T_m , shifted by variations in the average local U_0 along the sequence: Because of the small width of the transition region ($\sim 1/\alpha$), a sequence length of $O(\alpha^2)$ is necessary just to reduce the typical shift in T_m down to the size of the transition region. Thus, the crossover length for the onset of self-averaging is expected to be a factor of $O(\alpha^2)$ longer for the system with barrier. The exponential dependence of α on the barrier makes the multistep feature easily observable for realistic sequence lengths.

The domain structures are readily visualized by plotting the full probability distribution $P_n(y)$ at various temperatures close to the melting point (Fig. 4). It is seen that isolated segments of the sequence unbind already at as much as 10% below the nominal transition temperature, indicating that the equilibrium configuration of the DNA consists of localized bubbles of denature regions. To test whether the experimentally observed multistep features are indeed the result of an effective barrier induced by the variable stiffness, one needs to determine very accurately the form of $K(y)$.

To summarize, we have shown that variations in base-pair binding is by itself insufficient to generate the multistep melting behavior for heterogeneous DNA strands. However, the inclusion of a variable backbone stiffness results in an entropic barrier which yields a sharp, first-order-like transition for the homogeneous system, and multistep melting for the heterogeneous system. Asymptotically, the transition is still expected to be second order; however, the crossover length is exponentially long.

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