Softening of DNA near Melting

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Near the melting transition the bending elastic constant, $\kappa$, an emergent property of double-stranded DNA (dsDNA), is shown not to follow the rod-like scaling for small length $N$. The reduction in $\kappa$ with temperature is determined by the denatured bubbles for a continuous transition, e.g., when the two strands are gaussian, but by the broken bonds near the open end in a Y-like configuration for a first-order transition as for strands with excluded volume interactions. In the latter case, a lever rule is operational implying a phase coexistence although dsDNA is known to be a single phase. The results are obtained by pruned and enriched Rosenbluth algorithm (PERM) for interacting strands on a cubic lattice with additional semi-flexibility when bound.

Double-stranded DNA (dsDNA) is a semiflexible polymer which behaves like a rod for lengths less than the persistence length $l_p \sim 50\text{nm}$. In contrast, the individual strands of DNA (ssDNA) are highly flexible with $l_p \sim 2\text{nm}$ \cite{1,3}. Recent experiments showed that the bending elastic constant, $\kappa$, and associated $l_p$, of dsDNA decreases over a temperature range of 20°C near melting \cite{4}. The melting is caused by the breaking of the base pairing, with the fraction $n_c$ of unbroken bonds as a measure for the transition, viz., $n_c \neq 0$ ($n_c = 0$) in the dsDNA (ssDNA) phase. Why is dsDNA rigid? This question resembles a similar one about solids \cite{5}, but there are fundamental differences between the two cases. For a solid, rigidity is an emergent phenomenon \cite{6}, where the shear modulus is a consequence of continuous symmetry-breaking. There is no such scenario for $\kappa$, which, incidentally, is not the response function associated with $n_c$. Instead, topological arguments associate two elastic constants to dsDNA, the twist that changes the helical nature, and the bending elastic constant (and $l_p$) related to the entropy (and energetics) of a long chain \cite{7,8}. Both the elastic constants are emergent as they occur only in the bound state \cite{9}, and therefore linked to the DNA phase transition. Of the two, the bending elastic constant is a large scale property that, like many other polymeric properties, should be insensitive to microscopic details. Our purpose in this paper is to study, via simulations, the softening of dsDNA, especially, how the bending elastic constant, an emergent property, changes as the melting point is approached.

Bending of DNA on short length-scales is associated with several vital genome-based processes like transcription, DNA repair, replication etc. \cite{10}. DNA needs to bend or fold substantially to fit into the volume of a cell nucleus. It is understood that such a sharp bending for a rigid structure like DNA, on short length scales, is facilitated by the formation of small flexible bubbles \cite{11,12}. Multiple bubbles provide a more efficient way of bending DNA than a single large bubble \cite{13}, as small bubbles act as hinges for the rigid segments. This coupling has important consequences for the chemical reactivity of sterically hindered base sites implying that the local curvature should facilitate reaction \cite{14}.

As the base-pair energy $\sim 6-9\text{ kcal/mol}$, thermal fluctuations can lead to a cooperative breaking of the hydrogen bonds in the long length limit. This is the melting of DNA\cite{7}. The dichotomy of the problem can be recognized from the two different approaches for the phases. The melting is generally modeled by two polymers (length $N \to \infty$)\cite{20}, with native base-pairing, where only monomers at the same position along the two strands pair on contact with an energy gain $-\epsilon$ ($\epsilon > 0$). In contrast, dsDNA at room temperature, especially in the analysis of various single molecular experiments, is treated as a semiflexible chain devoid of any inner details, involving an elastic constant for the curvature of the chain or, equivalently, an energy cost for bends \cite{21,22}. This change in Hamiltonian of DNA around the melting point is a signal of the difficulty of the problem \cite{23}.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{Schematic diagram of renormalization of the elastic behaviour of dsDNA due to bubbles. For Gaussian chains, tangents $t_i$, $t_j$ can be used to define a persistence length (see text). Can one define a persistence length for configurations with bubbles?}
\end{figure}

For semiflexible dsDNA to melt, we allow it to be disrupted by broken H-bonds either in the interior with the formation of bubbles or by a Y-like fork near the open end of the chains. The strands in these open regions are taken as flexible polymers. In a coarse-grained picture, the bubbles introduce flexibility allowing dsDNA to bend, leading to a renormalization of the elastic constant as shown schematically in Fig. 1. Two different models are considered here, viz., I and II, on a cubic lattice. In model I, the strands are Gaussian random walkers, while in model II, we incorporate both self and
mutual avoidance. In each case, we consider two varieties of polymers, viz., (i) flexible polymers where both the single and double strands are flexible, and (ii) semiflexible dsDNA where the bound part is semiflexible but the bubbles consist of flexible chains. The semi-flexibility in dsDNA is incorporated by penalizing a bend with an energy cost when the strands are overlapped i.e. with three successive contact points, or equivalently, two successive paired bonds. The energy for bending is taken to be $E_b = -\eta \cos \theta$ where $\theta$ is the angle between the two successive overlapped segments and $\eta(>0)$ is the bending energy constant [24]; see Fig. 2. Whereas a bent ds configuration as in Fig. 2; is energetically favourable compared to Fig. 2b, the latter is a source of additional entropy. Consequently, bubbles (Fig. 2d) are to be expected at higher temperatures vis-à-vis bent ds-chains at lower temperatures.

To explore the elastic behaviour, a force $f$ is applied at the end point $r_1(N)$ of each strand $i = 1, 2$, keeping the other ends fixed. The additional force-term in the Hamiltonian is $H_f = -f \cdot x$, where $x = r_1(N) + r_2(N)$. In the linear response regime, the bending elastic constant $\kappa$ can be related to the zero-force fluctuations of $x$ as $\bar{\kappa} \equiv k_B T \kappa = \langle (x^2) - \langle x \rangle^2 \rangle$. As we see, the bending elastic constant is not the response function associated with $\eta$ and so the conventional critical behaviour of response functions in phase transition problems are not applicable here.

Naively, one may interpret $\bar{\kappa}$ as the variance of the end-to-end distance of the center-of-mass (CM) chain $X(i) = [r_1(i) + r_2(i)]/2$. If the CM chain behaves as a single polymer, then the expected $N$ dependence of $\bar{\kappa}$ is given by the root-mean-square end-to-end distance $R$, with a scaling behaviour $R \sim N^\nu$, where the size exponent $\nu = 1/2$ for a Gaussian chain and $\approx 0.588$ for a self-avoiding walk (polymers in good solvent). For a semiflexible gaussian chain, the crossover from $R \sim N$ for $N \sim l_p$ to $R \sim \sqrt{N}$ for $N \gg l_p$, is given by

$$R^2 = 2l_p N \left\{ 1 - \frac{l_p}{N} \left( 1 - e^{-N/l_p} \right) \right\},$$

with $l_p$ as the persistence length. Here both $l_p$ and length $N$ are measured in units of bond lengths ($=1$). In general, the temperature dependence of $\bar{\kappa} / N^{2\nu}$ would show us how DNA softens as the melting point $T_c$ is reached.

In terms of the individual coordinates,

$$\bar{\kappa} = 2 \langle r_1(N)^2 \rangle_c \left( 1 + \frac{\langle r_1(N) \cdot r_2(N) \rangle_c}{\langle r_1(N)^2 \rangle_c} \right),$$

is determined by the inter-chain correlation. In the perfect bound state with no bubbles, $r_1 = r_2$, and we get $\bar{\kappa} / \langle r_1(N)^2 \rangle_c = 4$, while in the high-temperature phase, if the two chains remain uncorrelated, then $\bar{\kappa}$ is equal to the sum of the individual modulus. Then, $\bar{\kappa} / \langle r_1(N)^2 \rangle_c = 2$, for gaussian chains. The ratio is expected to be $>2$ for strands with excluded volume interactions, because there will be inter-strand correlations for long chains as dictated by the second virial coefficient (or overlap concentration $c^*$) [27]. Moreover, in the bound phase, individual strands also acquire the stiffness of the state, punctuated by bubbles and Y-fork. Therefore, the microscopic stiffness would no longer be the sole parameter determining the overall elasticity of the chain (Fig. 1).

For Gaussian semiflexible chains, the tangent-tangent correlation or bond-bond correlation (Fig. 1) decays exponentially for large $|i-j|$, $\langle t_i \cdot t_j \rangle \sim \exp(-|i-j|/l_p)$ providing a definition for persistence length $l_p$. This definition is not applicable for cases with excluded volume interaction, as SAWs are critical objects [28]. We show here that Eq. (11) fails to be useful even for the Gaussian case, with bubbles, so that $l_p$ as a crossover length from a rod-like behaviour at short length is questionable.

For simulation, we have used the zero parameter version of the flatPERM (Pruned and Enriched Rosenbluth Algorithm) which generates equilibrium configurations through cloning and pruning [29, 30]. The weighted atmosphere at each step serves as the weight of that step $w_i$ and the weight of a configuration is the successive multiplication of the weights of the previous steps $W_N = \prod_{i=1}^{N} w_i$. For example, for the very first step, each of the chain has 6 different possibilities to step into. Thus, a total of 36 possibilities, of which there are 6 possible ways of making a contact, thus the local weighted atmosphere becomes $w_1 = 30 + 6 \exp(\epsilon/k_BT)$. Similarly, the weight for the second step including a bend and excluded volume interaction is $w_2 = 4 \exp(\epsilon/k_BT) + \exp(\epsilon/k_BT) \exp(\eta/k_BT) + 20$. For gaussian strands, reverse steps in the ds mode with $E_b = \eta$ are considered with appropriate change in $w_2$. The partition function for chain length $N$ is estimated by averaging over the weights of configurations of length $N$ with respect to the number of started tours. Pruning and enrichment is done continuously depending on whether the ratio of the weight of the particular configuration and the partition function estimate ($Z_N$) for length $N$, ratio $W_N/Z_N$ is greater or smaller than 1 respectively. Error bars of fluctuations are estimated on the fly. Throughout the simulation, we
chose $\epsilon = k_B = 1$.

A bubble is defined to be a continuous section of broken bonds embedded within bound segments, thus a $Y$ fork at the end of the DNA is not considered to be a bubble here; see Fig. 2(d). To characterize the transition, we computed the fraction $f_b$ of broken bonds that goes into forming bubbles and $Y$, the fraction in the $Y$-fork like region. The transition temperature is determined from the specific heat curves, as the intersection of the curves for different lengths for model I which undergoes a continuous type transition and from the peak of the curves for model II which undergoes a first-order transition. In all cases the order of transition is found to be independent of the value of stiffness $\eta$.

If dsDNA behaves as a semiflexible chain, then the elastic modulus is expected to scale as $\bar{\kappa}/N^{2\nu} \sim N^{2-2\nu}$ for small chain lengths, which is the signature of a rod-like behavior, and then show, for larger $N$, a cross-over to a gaussian or saw-like behaviour. This is expected only if $\eta \neq 0$. No rod-like behaviour is seen for the $\eta = 0$ case. Fig. 3(a) shows that for model I, a tightly bound DNA (without bubbles) at $\epsilon/T = 10, \eta/T = 3$ ($k_B = 1$) satisfies Eq. (1) with $l_p = 5.2$, consistent with the estimate of $l_p$ from a transfer matrix calculation. For model II also, at low temperatures, it is possible to define a rod-like behaviour. However, the crossover description fails near the transition where we have a substantial contribution from $f_b$ and/or $Y$. In the log-log plot, the slope for small lengths is not consistent with the rigid rod expectations. For $T$ close to $T_c$, DNA is neither rod-like nor completely flexible for small chain lengths. We call this region as soft DNA. It follows that though an effective elastic constant can be defined, persistence length may not have any special significance.

Model I: For Gaussian chains, the melting transition is continuous at $T_c = 1.336$ for $\eta = 3$ and $T_c = 0.928$ for $\eta = 0$. Below melting, bubbles develop, and the fraction of broken bonds, $1 - n_c$, increases with temperature continuously to 1 as $T \rightarrow T_c$ for $N \rightarrow \infty$. For finite chains, there are also broken bonds at the open end, but $Y$ vs $T$ curve sharpens into a step function for $N \rightarrow \infty$. Stiffness on the ds segments has the effect of suppressing the bubble formation at lower temperatures but the continuous transition remains intact. Fig. 4(a) shows the fractions for $\eta = 0$ and $\eta = 3$. As the bubbles act as hinges, the decrease of the elastic constant with $T$ can be attributed to the broken bonds. An effective chain description à la Fig. 1 requires $\kappa$ to be renormalized by the bubbles as

$$\frac{1}{N} \kappa = -\Delta_{ll}[(1-n_c)^a - 1] + 2,$$  \hspace{1cm} (model I)  \hspace{1cm} (3)

where the exponent $a$ takes care of the softening by the bubbles and $\Delta_{ll} = (\bar{\kappa}/N)^{\text{bound}} - (\bar{\kappa}/N)^{\text{unbound}}$. A value of $a = 0.1$ is found to give a good agreement of $(\bar{\kappa}/N)$ of Fig. 3(b) with $n_c$ data taken from Fig. 4(a). Although the value of the exponent ($a = 0.1$) remains a puzzle, our results validate the overall picture of Fig 1 in case of a continuous melting.

Model II: For self and mutually-avoiding chains the melting transition is first-order at $T_c = 1.536$ for $\eta = 3$ and $T_c = 0.745$ for $\eta = 0$. The temperature dependence of $f_b$ and $Y$ are shown in Fig. 5(a), while that of $(\bar{\kappa}/N^{2\nu})$ in Fig. 5(b), where $\nu$ takes into account the effect of excluded volume interaction (see Eq. 2). There are significant differences from model I. Close to melting, most of the broken bonds are in the $Y$-fork, the fraction in the bubbles remains more or less the same. Consequently, Eq. (4), encoding Fig. 1 is not meaningful; but instead an empirical equation, reminiscent of the lever rule in phase coexistence, is found to describe the data. Taking $$(\bar{\kappa}/N^{2\nu}) - \Delta_{ll} Y^{2\nu} + (\bar{\kappa}/N^{2\nu})_{b}, \hspace{1cm} (model \hspace{0.2cm} II)$$

where $f_b$ of the bound phase for $Y \rightarrow 0$, and $(\bar{\kappa}/N^{2\nu})_{b}$ of the unbound phase for $Y \rightarrow 1$ with $\Delta_{ll} = (\bar{\kappa}/N^{2\nu})_{b} - (\bar{\kappa}/N^{2\nu})_{u}$, the two limiting values were adjusted to get a good fit with the values of $Y$ taken from Fig. 5(a). These points are also shown in the figure. Eq. 4.

![FIG. 3. (Color online) Log-log plot of $\bar{\kappa}/N^{2\nu}$ vs. $N$. (a) Model I for flexible $\eta = 0$ and semi-flexible chain $\eta = 3$, and different $\Delta T = T - T_c$. The curve $f(x)$ is a fit to the data points for $\epsilon/T = 10, \eta/T = 3$ using Eq. (1) with $l_p$ as a parameter. (b) Model II for flexible $\eta = 0$ and semi-flexible chain $\eta = 3$, and different $\Delta T = T - T_c$. $f(x)$ is a straight line of slope $(2 - 2\nu)$ representing the rod-like scaling regime. No other data sets show the initial slope of $f(x)$.](image-url)
suggestions coexistence of the bound and the unbound state, although dsDNA is a single phase. The bubbles in the interior do not play any significant role in the softening.

We note that the bubble fraction $f_b$ is lower for the semi-flexible models [Figs. 4(a) and 5(a)], and is a manifestation of the coupling between bubble formation and DNA bending energetics (see Fig. 2). For stiffer bonds, it becomes energetically favorable to maintain a bound state and make a straight move than to form a bubble to become flexible; in other words, bending energy of a semi-flexible DNA reduces the possibility of bubble formation. This tendency to maintain the bound state decreases the entropy of the system compared to the $\eta = 0$ case, thereby providing thermal stability to the bound phase. Thus the melting temperature is higher for nonzero $\eta$, and the transition becomes sharper. However, the bubble size distribution and thus the average bubble length near the transition remain unaltered by stiffness.

To conclude, we showed that dsDNA softens as the melting point is approached though the origin of softening depends on the nature of the transition. For a continuous transition, as in the case of gaussian strands, the thermally-generated denatured bubbles reduce the elastic constant, though the characteristic exponent is not properly understood. For a first-order transition, as in the case of strands with excluded-volume interaction, the melting is dominated by Y-like configurations of single stands at the open end. The softening is controlled by this Y-region, in a way reminiscent of the lever rule of phase coexistence, though dsDNA remains a single phase. In none of the cases, we could find any small length region with a rod-like behaviour where Eq. 11 can be used. A persistence length as a crossover length is unambiguously only for very tightly bound dsDNA. We feel that, till melting, the elastic constant is a meaningful quantity rather than the persistence length. As based on directly measurable quantities, our results should be easily testable through experiments.
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In our study, it is assumed that the bending rigidity is isotropic i.e. the bending energy only depends on the angle by which the polymer is bent locally, and do not depend on the direction of bending, although, it has been shown that the bending rigidity in the direction of the grooves is essentially smaller than in the perpendicular direction [22, 20].