The melting or denaturation of DNA refers to the separation of the two complementary DNA strands, a process which can be induced either thermally or mechanically. The associated phase transition is well understood by means of theoretical models, e.g., by Poland and Scheraga, and their various extensions. Thermal melting of DNA forms the basis of the PCR technique, while statistical and dynamical properties of denatured loops may turn out to be relevant for understanding DNA-protein interactions and gene expression initiation.

Interestingly, it is a common practice to use plasmid (circular) DNAs during PCR, since most bacteria come with circular DNA as a means of protection against degradation. The resulting entanglement of the two strands due to the natural twist of the DNA molecule imposes an obvious obstacle for the denaturation process (as well as replication, protein synthesis, etc.), which is overcome in nature by means of special DNA manipulating proteins. Nevertheless, the thermal behavior of a circular DNA chain in absence of such helper proteins proves to be a nontrivial problem and has been addressed recently.

The circular geometry entails the presence of a new topological invariant in the system: the number of times two chains of the DNA wind around each other, namely, the linking number (LK). The thermodynamics of the system should therefore be investigated within the corresponding, restricted phase space. This framework is also relevant to single-molecule experiments on DNA in which the chain ends are rotationally constrained. We here discuss the implications of LK conservation on thermal melting characteristics within the framework of the Poland-Scheraga (PS) model.

A consequence of fixing the linking number is that, the denatured loops form in expense of (right-handed) torsional stress on surrounding DNA duplex segments. As with any elastic ribbon with finite bending and twist modulus, dsDNA responds to torsion by “supercoiling” (bending the backbone as in coiling telephone cords) and/or by “overtwisting” (modifying the stacking angle). Thermodynamics of a fixed-LK DNA chain whose bound segments are unbendable but have finite twist rigidity was investigated by Rudnick & Bruinsma. The alternative extension of the PS model considering the possibility of supercoil formation, but not overtwisting, has also been discussed recently. It has been shown that the transition is a kind of Bose Einstein condensation (BEC) where the macroscopic loop formed above the melting temperature plays the role of the condensate.

We show below that a BEC-like transition takes place in the “overtwisting” scenario, too, which is the main contribution of this article. We next compare this phenomenon with a similar observation we made earlier for the supercoiling response and with the denaturation of DNA with free ends. We conclude that, the birth of a nontrivial macroscopic loop at the melting point is the defining characteristics of the thermal denaturation of DNA under fixed linking number, irrespective of how the molecule responds to torsional stress.

Ref. gives a detailed account of the melting transition in the PS model. The partition function of the model can be expressed in closed form and its singular behavior, with proper treatment of the loop entropy, yields a first-order melting transition. A similar analysis is given in Ref. for the case of nonzero supercoil density under the constraint that the total length of the denatured loops is proportional to that of supercoils (mimicking LK conservation). This system, unlike the PS model, displays a continuous melting transition, accompanied by a loop “condensate” that appears at $T_c$ and grows gradually with temperature.
Overtwisting, i.e., increasing the angle between the successive base pairs, is the alternative to supercoiling by which a partially denatured circular DNA chain can accommodate the resulting torsional stress on duplex regions. An extension of the PS model with overtwist has been investigated earlier in \cite{[15]}, which we extend below. In particular, we show here that the melting transition in this scenario, too, is accompanied by the formation of a macroscopic loop.

To this end, following \cite{[15]}, let us consider an arbitrary configuration of a DNA chain of a total length \( L \) composed of denatured segments with total length \( L_l \) and bound segments with \( L_b = L - L_l \). We assume that the linking number expelled by the loops is uniformly distributed along the chain and results in a uniform increase in \( \frac{\omega}{L} \) percoiling by which a partially denatured circular DNA chain can accommodate the resulting torsional stress on unit length of bound and denatured DNA, respectively. With

\[
\mathcal{H} = \kappa \frac{L_b^2}{L_b} + \epsilon_b L_b,
\]

where \( \kappa > 0 \) is a measure of twist stiffness in units of energy and \( \epsilon_b < 0 \) is the binding energy per unit length. The canonical partition function for the DNA chain can then be expressed as \cite{[13]}

\[
Z^n(L_b, L_l) = \int \frac{dz_l dz_l}{(2\pi i)^2} \frac{Q^{\kappa=0}(z_l, z_l)}{z_l^{L_l+1} z_l^{L_l+1}} e^{-\beta \kappa \frac{L_b^2}{L_b}},
\]

where the grand sum \( Q^{\kappa=0} = \frac{1}{1 + A \Phi_c(z_l)} \) follows from the usual PS model with the Boltzmann weight \( \omega \) for a unit bound segment and fugacities \( \{z_b, z_l\} \) per unit length of bound and denatured DNA, respectively. The contour integral has a simple pole for \( z_b \) which by Cauchy formula yields

\[
Z^n(L_b, L_l) = \frac{1}{2\pi i} \int \frac{dz_l}{z_l^{L_l+1}} e^{-\beta \kappa \frac{L_b^2}{L_b}} \omega^{L_b} \times \left[ 1 + A \Phi_c(z_l) \right]^{L_l-1} = \int dz_l e^{-L_F(z_l, m_l)},
\]

with

\[
F(z_l, m_l) = -(1 - m_l) \log \left[ \omega(1 + A \Phi_c(z_l)) \right] + m_l \log z_l + \beta \kappa \frac{m_l^2}{1 - m_l} + O(L^{-1}),
\]

where \( m_{l,b} = L_{l,b}/L \) and \( m_b + m_l = 1 \). In the thermodynamic limit, the partition function can be evaluated using the saddle-point condition \( \partial F = 0 \). Therefore, \( F(z_l, m_l) \) serves as a free energy functional for the DNA chain. Minimization yields a continuous phase transition for \( c > 2 \) governed by the singularity of the polypoly function at \( sz = 1 \) \cite{[15]}. It is straightforward to show the critical temperature \( T_c \) shifts linearly with the ratio of the overtwist penalty \( \kappa \) and the binding energy \( \epsilon_b = -k_B T \log \omega \):

\[
T_c = \frac{T_c^{PS}}{1 - \kappa \epsilon_b \left( \frac{1}{1 - m_l^2} - 1 \right)}.
\]

Here \( T_c^{PS} \) is the critical temperature of the original PS model and \( m_l^2 \) is the critical loop fraction which we find to be independent of the twist stiffness (therefore equal to the corresponding value in the PS model):

\[
m_l^2 = \frac{A \zeta_c}{1 + A(\zeta_c + \zeta_{c-1})}.
\]

Here \( \zeta_c = \Phi_c(1) \) is the Riemann zeta function.

Our goal is to investigate the existence of a macroscopic loop for \( T > T_c \) in the above picture. Let us assume that such a loop exists with size \( L_0 \equiv m_0 L \) and calculate \( m_0 \). As for a Bose gas below the condensation temperature, the estimated amount of denatured DNA, when calculated as a sum over microscopic loops, is now short of the actual value by \( L_0 \). Therefore we set \( L_l = L_l^{\text{micro}} + L_0 \) and substitute in Eq. \[2\]

\[
Z^n(L_l, L_b) \to Z^n(L_l - L_0, L_b) \frac{L_0}{L_0},
\]

which, following the same steps, now yields an additive macroscopic loop correction to Eq. \[3\]

\[
F(z_l, m_l, m_0) = F(z_l, m_l) - m_0 \log(sz_l) + \frac{A \zeta_c}{1 + A(\zeta_c + \zeta_{c-1})}.
\]

Note that \( m_l \) is the total loop density, including microscopic and macroscopic contributions.

For \( T < T_c \) (\( z_l < 1/s \)), the free energy is minimized \( \text{wrt} \ m_0 \) at the extremal value \( m_0 = 0 \). Therefore no macroscopic loop exists below \( T_c \). The total fraction of denatured bases (all due to microscopic loops) can be calculated by setting \( \partial_m F = \partial z_l F = 0 \). It increases with temperature slower than in the PS model, due to the additional (overtwist) energy penalty of denaturability. The loop-size distribution essentially decays exponentially with a power-law correction as in the PS model.

In the high-temperature phase where the loop fugacity is fixed at its upper bound \( z_l = 1/s \), the above picture is no longer valid. Setting now \( \partial F(z_l, m_l, m_0) = 0 \) yields a unique solution with \( m_0 \neq 0 \) (the extremal value \( m_0 = 0 \) does not yield a minimum.) Therefore, a finite fraction of the base pairs is located in a macroscopic loop. Setting \( \partial F/\partial m_0 = 0 \) yields \( z_l = 1/s \), as expected in the high-temperature phase. Accordingly, the probability distribution function for the microscopic loop sizes \( p(l) \sim l^{-c} \) is now scale invariant. This picture is in contrast with the PS model, since no microscopic loop survives for \( T > T_c \). An analytical expression for the mass fraction in microloops and the macroloop can be obtained.
FIG. 1: The fraction of the denatured DNA (solid) and the contribution from the microscopic loops (dashed) for the supercoiling (left) and overtwisting (right) scenarios, as a function of temperature. The parameters $s = 5$, $\epsilon_b = 3$, $c = 3.5$, $A = 0.1$ are the same for both figures, while the stiffness parameters for overtwisting ($\kappa = 1.0$) and supercoiling (see Ref. [19]) are comparable.

from the remaining two minimization conditions wrt $m_l$ and $z_l$:

$$0 = \frac{\partial F}{\partial m_l} \Rightarrow \frac{\omega}{s} (1 + A\zeta_c) = e^{-\beta\kappa(1+1/(1-m_l)^2)}$$

$$0 = \frac{\partial F}{\partial z_l} \Rightarrow m_l = \frac{m_0 + R}{1 + R},$$

where $R \equiv \frac{A\zeta_c}{1 + A\zeta_c}$ is a temperature-independent constant.

The transition is continuous, since setting $m_0 = 0$ ($T \to T_c^-$) recovers the critical value of $m_l$ in Eq. (5) found from the low-temperature limit $T \to T_c^-$. Solving for $m_0$ in Eqs. (7)-8, then substituting $\ln \omega = -\epsilon_b/k_BT$ and using Eq. (4) one finds that the macroscopic loop fraction has the exact form

$$m_0 = 1 - \frac{1}{\sqrt{1 + B(T - T_c)}}$$

where $B = -\epsilon_b/(1 + R)^{-2}$. $m_0$ grows linearly with temperature in the vicinity of $T_c$ and approaches unity only in the limit $T \to \infty$. The total fraction of microscopic loops decreases with temperature while their distribution remains a power law. In Fig. 1 we present a comparison of the “overtwist” and the “supercoil” dominated scenarios in terms of macroscopic and microscopic loop fractions, for a generic set of parameters at which both display a second-order melting transition. Note that, the discontinuity in $dm_l/dT$ at $T = T_c$ (inset) in the supercoiling model is hardly visible. However, the cusp in the microscopic loop fraction is clear and exists even for $c < 3$ where the discontinuity at $T_c$ shifts to higher derivatives of the free energy (see Ref. [19] for more on PS model with supercoiling). The cusp in $m_l$ is more prominent in the “overtwist” picture with a comparable set of parameters.

The presence of the macroloop above $T_c$ does not depend on the precise value of $c$ (as long as there’s a melting transition), while its size relative to the total amount of denatured DNA, $m_0/m_l$, does (Fig. 2). For $c \leq 2$, the macroscopic loop vanishes together with the transition itself. Otherwise, the relative size of the macroloop grows faster with temperature as $c$ gets larger. This trend as a function of $c$ converges to a limiting curve which is indistinguishable from that obtained for $c = 3.5$ and shown in Fig. 2.

Generalization of our results to $\sigma \equiv (L_s - L_l)/L \neq 0$ is straightforward after substituting $\beta\kappa(m_l + \sigma)^2/(1 - m_l)$ for the twist penalty in Eq. (3). The melting picture for nonzero $\sigma$ remains qualitatively unaltered, except when

FIG. 2: The weight of the macroloop among the total amount of denatured bases is shown as a function of temperature, for various values of $c$. The uppermost curve for $c = 3.5$ is indistinguishable from those obtained for higher values of $c$. 
\[ \sigma \leq -m_f^\dagger, \] where the critical point itself disappears and a macroscopic loop exists at all temperatures.

Finally, noting that the limit \( \kappa \to 0 \) recovers the first-order transition, it is interesting to see how the sharp denaturation in the PS model smoothen out with the introduction of twist penalty. This crossover is shown in Fig. 3 where the total loop fraction given in Fig. 1 is extended into the \( \kappa \)-dimension. The region between the two sheets that join at the critical line \( t = 0 \) is the macroscopic loop fraction which smoothly approaches unity as \( \kappa \to 0 \).

To summarize, we reconsidered the melting thermodynamics of a DNA chain with fixed linking number. We assumed that the denatured loops appear by transferring LK to duplex regions through overtwisting. We showed that, despite the different melting scenarios observed in supercoiling and overtwisting pictures, a feature common to both is the appearance of a macroscopic loop which grows monotonously with temperature. While the total fraction of denatured pairs increases with temperature, the DNA mass in the microscopic loops decreases above \( T_c \). This condensation phenomenon is analogous to BEC, except it takes place at high temperature. Whether it is dynamically accessible is an interesting question, since merging microscopic loops towards a macroloop entails diffusing denaturation bubbles across torsionally strained duplex regions. Investigations in this direction, as well as towards a joint theoretical framework that incorporates both overtwist and supercoiling are in progress.

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