An intermediate phase in DNA melting

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We predict a novel temperature-driven phase transition of DNA below the melting transition. The additional, intermediate phase exists for repetitive sequences, when the two strands have different lengths. In this phase, the excess bases of the longer strand are completely absorbed as bulge loops inside the helical region. When the temperature is lowered, the excess bases desorb into overhanging ends, resulting in a contour length change. This continuous transition is in many aspects analogous to Bose Einstein condensation. Weak sequence disorder renders the transition discontinuous.

The base-pairing interaction between the two strands of DNA is not only pivotal to its biological function [1], but also leads to intriguing applications in nanotechnology [2]. One approach to probe this interaction is to monitor the DNA conformation as a function of temperature. Experimentally, one can observe the number of basepairs formed (using UV absorption [3, 4]), as well as changes of intra-molecular distances on the nanometer scale (using modern single-molecule techniques [5]). On the theoretical side, the temperature dependence of DNA conformations has been studied for almost fifty years, using models of various degrees of complexity [6, 7, 8, 9, 10, 11, 12, 13]. Particular attention has been paid to the characteristics of the melting transition, where the two strands separate completely. Whereas early models yielded only a crossover [12], the Poland-Scheraga (PS) model [7] was the first to display a phase transition, albeit a continuous one, which appeared to be at variance with the experimentally observed sharp jump in the fraction of bound basepairs [4]. Only recently have mechanisms been proposed [10, 11] which yield an abrupt, first order transition. So far, however, most analyses of DNA melting have incorporated only native interactions, i.e. base pairs that occur in the ground state of the molecule (see [6, 13] for notable exceptions). It is our aim here to show that such non-native interactions can introduce an intermediate phase in the melting behavior of DNA, associated with an additional conformational transition before strand separation.

Non-native interactions are particularly relevant for repetitive DNA sequences, which are common in genomes [14]. Periodic DNA, with e.g. a single base repeat such as TTT... or a higher order repeat such as CAGCAG..., can take on basepairing patterns with asymmetric loops and the two complementary strands can be shifted relative to each other. Here, we consider the general situation where the two strands can have arbitrary lengths $N, M$. We describe the DNA using a generalized PS model [13] and calculate its equilibrium behavior analytically. We find that for $N \neq M$, the bound phase splits into two separate phases. The low temperature phase is characterized by an extensive length of the unbound end on the longer strand, whereas in the new intermediate phase these overhanging bases are absorbed into the helical region. Mathematically, and also conceptually, many aspects of this transition are analogous to Bose-Einstein condensation (BEC), as “particles” (bases) condense into a single “state” (the overhanging end), which thereby acquires macroscopic “occupation” (length). Obviously, the analogy extends only to the behavior of the partition function, as there is no quantum coherence in the DNA problem. Effectively, the transition amounts to a temperature sensitive change in the contour length of the DNA molecule, which should be observable with optical or single molecule methods. While the transition is continuous for perfectly periodic sequences, we find that it becomes a first order transition once (weak) sequence disorder is introduced. We also show that the non-native interactions can change the order of the melting transition, as has been conjectured previously [13].

*DNA model.*— We consider two DNA strands with lengths $N$ and $M \geq N$, respectively, and describe their interaction with a generalized PS model [13, 16]. Specifically, a base $i \leq N$ of the lower strand can form a base pair $(i, j)$ with every complementary base $j \leq M$ of the upper strand, whereas the formation of base pairs within a strand can be neglected (since we are interested only in sequences with a high degree of complementarity and a low degree of self-complementarity). Due to geometrical constraints, we may neglect the ‘crossing’ of base pairs, e.g. two base pairs $(i_1, j_1)$ and $(i_2, j_2)$ with $i_1 < i_2$ but $j_1 > j_2$. The basepairing pattern $\mathcal{S}$, i.e. the set of

![FIG. 1: A possible configuration of two complementary DNA strands with a repetitive sequence (a bead represents one repeat unit). Note that repetitive sequences can form basepairing patterns with asymmetric loops. In general we allow for different strand lengths $N, M$. The last repeat units (squares) are permanently bound.](image-url)
all formed base pairs, then creates a DNA conformation consisting of bound segments alternating with (possibly asymmetric) loops, see Fig. 1. To simplify the discussion, we enforce the base pair \((N, M)\) at the right end, so that we need to consider only one overhanging end. Experimentally, this boundary condition would be realized e.g. by a few particularly strong basepairs at one end.

To each basepairing pattern \(S\), we assign a statistical weight \(Q(S)\), which takes the form of a product with factors of four different types: (i) a Boltzmann factor \(q = e^{\varepsilon_b/k_BT}\) for every basepair with binding energy \(\varepsilon_b < 0\), (ii) a Boltzmann factor \(g^2 = e^{-\varepsilon_c/k_BT}\) for every loop with loop initiation cost \(\varepsilon_c > 0\), (iii) an entropic factor \(B_l(m) = s^m m^{-c}\) for each loop, which is the increase in the number of polymer configurations when \(m\) bases form a (floppy) loop instead of being in a (rigid) double helical conformation, (iv) and a similar entropic factor \(A(n) = s^n n^{-c}\) for a single-stranded end of \(n\) bases. Here, the exponents \(c, \bar{c}\) in the entropic factors are universal in that they are independent of the detailed polymer properties, but are sensitive to excluded volume interactions. For interacting self-avoiding loops one has \(c \approx 2.15\), while \(\bar{c} \approx 0.1\) [13]. Whereas the value of \(c\) determines the critical behavior at the melting transition [13], the non-universal constant \(s\) has no qualitative effect on the melting behavior (we use \(s = 10\) in all numerical examples). In the following, we apply the DNA model to perfectly periodic sequences, where each repeat unit can be treated as an effective base with renormalized parameters (we use \(\varepsilon_b = 6\) and \(\varepsilon_c = 3\) in temperature units, \(k_B = 1\)). We emphasize that our simplistic model for the involved energies and entropies is meant to illustrate the physical phenomena in a transparent way, but leads to an unrealistic temperature scale. With a more detailed description [13], we find that all of the interesting behavior happens at accessible temperatures [13].

**Free energy of periodic DNA.**—To obtain the equilibrium properties of the DNA model, we calculate the partition sum over all basepairing patterns, \(Z_N^M = \sum_S Q(S)\). By separating the single stranded ends from the double stranded part, see Fig. 1, we write \(Z_N^M\) as

\[
Z_N^M = \sum_{i=0}^{N-1} \sum_{j=0}^{M-1} A(i) A(j) W_{N-i}^{-j}.
\]

Here, \(W_r^t\) is the partition function of two complementary and periodic strands of length \(r\) and \(t\) with the first and last base pair formed. \(W_r^t\) obeys the recursion relation

\[
W_{r+1}^{t+1} = q W_r^t + g^2 q \sum_{k+r,m,t} B_z(k+m) W_{r-m}^{t-m} - k.
\]

with the initial conditions \(W_1^1 = q\) and \(W_1^t = W_t^1 = 0\) for \(i > 1\) [13]. Eqs. 1. and 2. can be used to calculate \(Z_N^M\) for finite lengths \(N, M\). To extract the thermodynamic behavior in the limit of long strands, we take the \(z\)-transform \(\mathcal{Z}(x, y) = \sum_{N,M=0}^{\infty} Z_N^M x^N y^M\). Compared to the related case of a single self-complementary RNA strand folding back onto itself [13, 16], we need two instead of one transformation variables here, due to the second strand of DNA. One obtains

\[
\mathcal{Z}(x, y) = \frac{\hat{A}(x)\hat{A}(y)q_{xy}}{1 - q_{xy} + q_{xy}^2 y B(y) - x \hat{B}(x)},
\]

where the transforms of the entropic factors are given by \(\hat{A}(z) = \phi_c(sz) + 1\) and \(\hat{B}(z) = \phi_c(sz)\), with the polylogarithm \(\phi_c(z) = \sum_{n=1}^{\infty} z^n n^{-c}\).

The \(z\)-transformation carried out above amounts to a change from the canonical to the grand canonical ensemble. The transformation variables \(x, y\) play the role of fugacities for bases in the lower and upper strand, respectively. However, for the ensuing discussion, it is advantageous to keep the length \(N\) of the shorter strand fixed as a reference. Hence, we perform the inverse transformation for the lower strand by contour integration in \(x\), see Fig. 2, to obtain the partition sum \(\mathcal{Z}_N(y_0)\) for \(N\) bases on the lower strand and the upper strand coupled to a “nucleotide reservoir” with fixed fugacity \(y_0\). Whenever both strands are bound, \(\mathcal{Z}(x, y)\) has a singularity at \(x^+(y_0) < x^\ast\), see Fig. 2. For large \(N\), the contour integration is dominated by the residue at \(x^+(y_0)\), leading to \(\mathcal{Z}_N(y_0) = \hat{A}(y_0) x^+(y_0)^{-N}\). Hence, the free energy of the bound phase is given by \(N f_b(y_0) - T \ln \hat{A}(y_0)\), where the first term is the contribution of the helical region with a free energy per length \(f_b(y_0) = T \ln x^+(y_0)\), and the second term is the contribution from the unbound end of the longer strand. The free energy for given \(N\) and \(M\) is then obtained by saddle point integration,

\[
\frac{F(T, N, M)}{T} = -\ln \hat{A}(y_0) + N f_b(y_0) + M \ln(y_0),
\]

where the fugacity \(y_0\) is determined by

\[
\langle M \rangle_{y_0} = y_0 \frac{\partial \ln \hat{A}(y_0)}{\partial y_0} - N y_0 \frac{\partial f_b(y_0)}{\partial y_0}.
\]

**Phase diagram.**—To extract the physical behavior of the DNA model from Eqs. 4. and 5., we focus on two observables, the total number of base pairs, \(N\), and the
the overhang length diverges below $c N$ (tem- 
ture is approached. Monte Carlo simulation data (circles) 
a minimal value and increases again, as the melting temper-

FIG. 3: Top: The length of the unbound end, normalized 
by the total length $N$ of the shorter strand. For finite sys-
tems ($N = 1000$, dashed line), the unbound end shrinks to 
a minimal value and increases again, as the melting temper-

length of the single-stranded overhang. The fraction $\theta$ of 
bound base pairs is calculated from the free energy per 
length of the helical region as

$$\theta = -\frac{q}{T} \frac{\partial f_0(y_0)}{\partial q}. \quad (6)$$

To obtain the overhang length, we note that the right
hand side of Eq. (4) decomposes the total length $M$ of the 
upper strand into two contributions, where the first term
is the expected overhang length and the second term corre-

At low temperatures, the two DNA strands are com-
dered to be completely aligned and excess bases of 
the longer strand form an overhanging end. However, we ob-
serve that the overhang length decreases with increasing

This transition is in fact completely analogous to BEC, 
as Eq. (5) parallels the equation of state for an ideal Bose gas: If we divide Eq. (4) by our system
size $N$ and introduce the “particle density” $\alpha = M/N$,

we obtain

$$\alpha = \frac{1}{N} \frac{\phi_0^{-1}(s y_0)}{\phi_0(s y_0) + 1} + \bar{\alpha}(y_0), \quad (7)$$

where $\bar{\alpha}(y_0) = -\frac{y_0}{T} \frac{\partial f_0(y_0)}{\partial y_0} \geq 1$ is the density inside the
helical region. In Eq. (7), the first term on the right
hand side corresponds to the occupation of the ground
state of an ideal Bose gas, whereas $\bar{\alpha}(y_0)$ is analogous to the occupation of the excited states. The fugacities of a
Bose gas and our DNA are bounded: for the former, by the
energy of the ground state, and for the DNA by the
weight of an unbound monomer, i.e. $y_0 \leq s^{-1}$. The
population of the excited states increases monotonically with
the fugacity, and attains a finite maximal value, in our

FIG. 4: Left: The fugacity $y_0$ vs. $T$ for different system sizes $N$. In the thermodynamic limit, $y_0 = s^{-1}$ for $T < T_c$. As for
BEC, $y_0$ approaches its limiting value as $s^{-1} - y_0 \sim 1/N$. Right: Phase diagram of periodic DNA. At low tempera-
tures, both strands are completely aligned and excess bases of
the longer strand form an unbound end. In the intermediate
phase, all excess bases are absorbed into the helical region.

It is easily shown that $\bar{\alpha}_{max}$ approaches 1 at low

temperatures, and consequently all excess bases of the longer
strand are condensed in the overhang, as illustrated in
Fig. 4 (right). As $T$ increases, more and more bases are
absorbed in the helical region ($\bar{\alpha}_{max}$ increases), and
the system enters the intermediate phase at $T = T_c$, where
$\bar{\alpha}_{max} = \alpha$. At $T_c$ the condensate fraction vanishes,
as the solid line shows in Fig. 3 (top). If $T$ is raised to the
melting temperature $T_m$, which is independent of $\alpha$, the
strands separate and $\theta$ vanishes (denatured phase). Note
that the intermediate phase exists only when $\alpha$ is not too

It has been previously predicted (15) that the loop ex-
component $c$ is effectively reduced by one for periodic sequences compared to the standard PS-model with native base pairs only. This prediction is explicitly confirmed by our exact calculation of the free energy. We find [17], that there is no melting transition if $c \leq 2$, that the transition is continuous if $2 < c \leq 3$ and of first order if $c > 3$. For $2 < c \leq 3$, we obtain $\theta \sim (T - T_m)^{1/2}$, using the same method as [21] for the standard PS-model. To illustrate this, we plot $\theta$ for periodic sequences and for the standard PS-model in Fig. 3 (bottom). Whereas for the latter $\theta$ drops discontinuously to zero for $c = 2.15$, $\theta$ of periodic DNA vanishes with zero slope. Only after increasing $c$ artificially to 3.15 does periodic DNA exhibits a similar first order transition.

Weak sequence disorder.— Is the intermediate phase identified above robust against sequence disorder? To address this question, we replace a small fraction of base pairs by bases that can pair with each other, but not with other bases in the sequence. Fig. 5 shows the average length of the overhanging end, obtained by Monte Carlo simulation, for evenly spaced mutations with densities 0.005 and 0.01 and mutation strength $\bar{\varepsilon}_b = 2$. The plot suggests that in the presence of weak sequence disorder the transition described above remains, but is of first order instead of being continuous. The unbound end keeps its ground state length up to certain temperature, and then shortens rapidly. This is readily understood, when comparing the energy barriers for forming bulgeloops with and without mutations. The formation of a bulge loop on the longer strand of a perfectly periodic molecule requires only the initiation energy $\varepsilon_b$. In the presence of mutation, however, shifting both strands breaks mutated basepairs. Hence, to form a bulge loop, all mutations to the left of the loop have to be broken and the energy barrier for loop formation grows with the distance from the end. Due to this extensive energy barrier for loop formation, mutated basepairs stay bound in a finite temperature range. For a sufficiently low density of mutations, there is a temperature $T_c$, at which the entropy gained by distributing excess bases in loops along the molecule outweighs the energetic costs [21]. Below $T_c$ all mutations are bound, if $T > T_c$, as many mutations open, as are necessary to absorb all excess bases. On increasing the mutation density, $T_c$ approaches the melting temperature and the intermediate phase vanishes.

Discussion.— We have identified a BEC-like conformational transition in periodic and nearly periodic DNA, which occurs below the melting transition. This transition leads to a change in the contour length of the DNA molecule, which is roughly proportional to $M - N$. We also expect an effect on the persistence length of the helical region due to the increased density of bulgeloops. The hallmark of the transition, i.e. the shortening of the unbound end, could be directly observed by resonant energy transfer between fluorescent dyes located at the ends of the two strands. We expect the existence of the intermediate phase to be independent of the details of our model. Furthermore, we have shown that the additional conformations possible for repetitive sequences change the critical behavior at the melting transition.

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