Melting a stretched DNA

D. Marenduzzo1, A. Maritan2,3, E. Orlandini2,3, F. Seno2,3, A. Trovato2

1 SUPA, School of Physics, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JZ, Scotland
2 Dipartimento di Fisica, Universita’ di Padova, and CNISM, Unità di Padova, Via Marzolo 8, 35131 Padova, Italy
3 INFN, Sezione di Padova, Via Marzolo 8, 35131 Padova, Italy

We study the melting of a double stranded DNA in the presence of stretching forces, via 3D Monte-Carlo simulations, exactly solvable models and heuristic arguments. The resulting force-temperature phase diagram is dramatically different for the cases where the force is applied to only one strand or to both. Different assumptions on the monomer size of single and double stranded DNA lead to opposite conclusions as to whether DNA melts or not as it overstretches.

PACS numbers: 82.35.Lr, 87.14.G-,05.70.Fh,82.37.Rs

Single molecule experiments have by now enriched our knowledge of biopolymer physics at the nanoscale [1, 2, 3]. DNA may be grabbed, twisted and pulled with atomic force microscopes, laser tweezers etc., and in this way a number of its physical, chemical and elastic properties may be measured. Arguably the best characterised single molecule experiment is the adiabatic stretching of single stranded DNA (ssDNA) and of double stranded DNA (dsDNA), the double helical B-form. In most cases, the resulting force-elongation curves may be described by simple statistical mechanics models, e.g. the worm-like or the freely-jointed chain. These inextensible polymer models work at relatively low force, and for chain length larger than the persistence length, ~ 50 nm (150 base-pairs (bp)) for dsDNA and ~ 2 nm (4 bp) for ssDNA. Still, this classic experiments has prompted several other questions, some still outstanding. Most relevant for us is the fact that, at about ~ 65 pN, dsDNA elongates to about 1.7 times the B-DNA contour length: this regime is usually referred to as overstretching. Is overstretched DNA melted, ssDNA, or is it a different form of dsDNA, named S-DNA [2, 3]? These two competing pictures are not easily discriminated, since the contour lengths of S-DNA and ssDNA at the overstretching transition are similar (0.56-0.58 nm per bp). A further complication is given by the possibility of pulling one or both strands – experiments with laser tweezers may often not be able to tell what is happening [2, 3], as one strand may fall off the other due to the presence of nicks breaking the DNA backbone, unpeeling [2]. Are the two ensembles equivalent, or any similar?

Theories of DNA overstretching are often phenomenological, determining parameters from fitting to data [2, 3]. On the other hand, a lot of theoretical work focused on simpler models to find the temperature-force \((T - f)\) phase diagram of biopolymers perturbed by external forces – see e.g. [4, 5, 10, 11, 12]. DNA overstretching was only recently tackled within similarly simplified models, by assuming that it amounts to melting ds to ssDNA [13, 14]. On the basis of a continuum model [13], it was proposed that when one strand is being pulled, there should be a “reentrance” at low \(T\), similar to that found for DNA unzipping [4]. Another interesting debated question is whether the ds phase is destabilised or not by a stretching force [2, 13]. A final prediction is that the melting transition, which is first order at \(f = 0\), should become second order at \(f \neq 0\) [13, 14].

Here we will be concerned with the melting transition of a model of dsDNA anchored on one side and subject to external stretching forces, \(f_1\) and \(f_2\) on the two strands on the other side (Fig. 1). At variance with previous studies, we introduce two distinct pulling modes: (A) \(f_1 = f\) and \(f_2 = 0\), i.e. one of the two strands is stretched and the other one is left free, DNA unpeeling; (B) \(f_1 = f_2 = f/2\), i.e. both strands are stretched by independent, but equal, forces, stretched DNA denaturation. Each strand will be considered at some coarse grained level and modeled as a self-avoiding polymer. Strand complementarity is taken into account by allowing a pair of monomers in different strands to be bound with an energy \(-\epsilon < 0\) only if their monomer index along the two strands is equal [13]. Monomer sizes (length of 1 bp) and persistence lengths are here the only parameters controlling the microscopic properties of dsDNA and ssDNA. We choose monomer sizes in two fundamentally distinct ways. If we assume dsDNA exists also in the stretched S-form, monomer sizes are then equal for both ssDNA and dsDNA (cases \(A_0 B_0\)). If not, dsDNA monomer size, from the B-form, is smaller than for ssDNA (cases \(A_1 B_1\)). The values of monomer sizes and persistence lengths mostly impact on the large and small force behaviour of the system respectively.

From now on \(T\) will be measured in units of \(k_B / \epsilon\) (\(k_B\) is the Boltzmann constant). Unlike all previous work, we perform numerical simulations finding a phase diagram valid in the whole \(T\) range. We can thus discover that models A and B behave amazingly differently, both qualitatively and quantitatively.

We begin with heuristic arguments to determine the critical force, \(f_c(T)\), separating the zipped phase from the unzipped/melted one. At \(f = 0\) we have the standard melting phase transition occurring [13, 16] at \(T = T_m\), i.e. \(f_c(T_m) = 0\). For case \(A_0\), unpeeling with same
monomer size for ss and dsDNA, at large \( f \) the (almost) completely stretched strand acts as a 1D substrate for the adsorption of the free (un-stretched) strand. In this case the only allowed conformation are Y-like configuration with no bubbles. An energy-entropy argument leads to a vertical asymptote for \( f_c(T) \) at \( T_o = \epsilon/s_s \), where \( s_s \) is the entropy per monomer of a single DNA strand \([17]\). This argument is fully confirmed by numerical results coming from 3D Monte-Carlo simulation of model (A), i.e. two paired DNA strands, one of which is under a stretching force of modulus \( f \) (see Fig. 2, with typical snapshots from the simulations shown as well). Each strand is modelled by a self-avoiding chain, made up by \( N \) beads of size \( \sigma \) (\( \sim 1 \) nm, the size of ssDNA), connected by springs. To model homologous base pairing, we chose a truncated Lennard-Jones potential, with minimum value \(-\epsilon\), attained when the corresponding beads are at a mutual separation \( \sigma \). The bonds between successive beads are harmonic springs with minimal and maximal elongations equal to 0.7 \( \sigma \) and \( \sigma \) respectively (the maximum elongation is then \((N-1)\sigma \)). The simulations were performed by proposing local deformations of the chain. We used multiple Markov chains \([18]\) to improve sampling efficiency at small \( T \) or large \( f \), and reweighted the simulation data prior to analysis according to the Ferrenberg-Swedsen algorithm \([19]\). The critical points were estimated via the peaks of the specific heat. Our data suggest a first order transition at all \( f \neq 0 \).

The phase diagram found numerically (Fig. 2) shows that there is as expected a vertical asymptote. At small forces, the unpeeling pulling mode stabilises the zipped dsDNA, so that the critical temperature, \( T_c(f) \), increases with \( f \) (this agrees with a thermodynamic analysis of DNA overstretching data \([3, 14]\)).

To study model \( B_0 \), we have performed our simulations in the case in which both DNA strands are under the action of the same force \( f_1 = f_2 = f/2 \). As bubbles (see Fig. 1) are stretched out, the two strands are on average closer to each other with respect to the \( f = 0 \) case and one may argue that the dsDNA should be stabilised by \( f \). Another expectation is that at very large \( f \), both strands become straight in the same direction and the zipped phase is again stabilised since base pairing does not lead to further entropy cost. Both these expectations are confirmed by the Monte-Carlo simulations (see Fig. 2, dotted line): the region of stability of the zipped phase, in the \((T, f)\) plane, increases, and the denaturation temperature appears to be a monotonically increasing function of \( f \). The phase diagrams for both models \( A_0 \) and \( B_0 \) are somewhat surprising as at low \( T \) (and indeed at any \( T \) for model \( B_0 \)) DNA can stay zipped even at large \( f \). This would however be compatible with the interpretation of overstretched DNA as a ds form different from B-DNA \([1]\). The key point is that monomer sizes of S-DNA and ssDNA are roughly the same. The overstretching transition (B-DNA to S-DNA) takes place in Fig. 2 at 65pN (the energy scale \( \epsilon \) was set to 2.7 Kcal/mol, by assuming \( T_m = 70 \) °C). For unpeeling, overstretching can be followed by further unzipping to ssDNA, whereas this is not possible when pulling both strands. A second elongation transition is indeed observed in some cases \([3, 4]\). Our model also suggests that at higher \( T \), and when pulling just one strands, overstretching should change to a melting transition, even assuming the existence of S-DNA. The picture emerging from our results might thus reconcile both interpretations of overstretched DNA.

It is instructive to compare the phase diagrams found with the Monte-Carlo simulations to those of an exactly solvable model for DNA melting under a stretching force. In these simplified models the two strands are mutually avoiding directed walks along the [11] diagonal of a 2D lattice (Fig. 3a). When two beads are in contact they gain a pairing potential \( \epsilon \), and one end of the chain is fixed at the origin for both strands, while at the other end there are stretching forces, \( f_1 \) and \( f_2 \) (Fig. 3b). The case with \( f_2 = 0 \) corresponds to our model \( A_0 \).

One can write down a recursion for the partition function of a system with only one degree of freedom, the open end distance \( x \). If the strands are subjected to two forces \( f_1 \) and \( f_2 \) (e.g. identified by their projection along
the positive [-1 1] vector, Fig. 2a), then these are:
\[ Z_{N+1}(x) = Z_N(x + 1)y_2/y_1 + Z_N(x - 1)y_1/y_2 + Z_N(x)(y_1y_2 + 1/(y_1y_2)) \]
for \( x > 0 \)
\[ Z_{N+1}(0)e^{-1/T} = Z_N(1)y_2/y_1 + pZ_N(0)(y_1y_2 + 1/(y_1y_2)) \]  

In Eqs. (1) and (2) \( Z_N(x) \) indicates the partition function of two walks with open end distance \( x, y_1 = e^{\beta f_1}, y_2 = e^{\beta f_2} \), and \( p \) is a parameter which controls the weight of dsDNA segments and which we temporarily set to 1, implying that both monomer sizes and persistence lengths are equal in both ss and ds phases. The phase diagrams for models \( A_0 \) and \( B_0 \) can be found with e.g. a generalisation of the methods used in Ref. [3] and are plotted in Fig. 3b.

Fig. 3b shows that the phase diagram is qualitatively similar to the one already found in 3D Monte Carlo simulations in the continuum. The presence of a vertical asymptote for unpeeling, at \( T = T_a = \frac{1}{\log 2} \), the transition temperature for polymer adsorption on a wall, is confirmed. Interestingly, while unpeeling, at \( f \neq 0 \) is first order, stretched denaturation is second order, as can be seen from the plots of average percentage of zipped bases \( \langle x/N \rangle \), and of the average open end distance per monomer \( \langle x/N \rangle \), Fig. 3d. (The unpeeling phase diagram has been found, in another context, in 3D.) Simulation data show consistently a much smoother transition when pulling both strands. On the other hand, in the case of DNA unpeeling there are some differences between Monte Carlo simulations and exact results, most notably the shape of the phase diagram close to \( T_m \), as the critical temperature decreases with \( f \).

Interestingly, an expected difference concerns the order of the melting transition at zero force, which is thought to be first order in 3D [10], and is second order in the directed model. Another realistic feature that the simulated model reproduces is the larger effective persistence length of the ds chain with respect to the ss one, at variance with the lattice model.

To gain more insight, we present a Flory-like argument to rationalise the \( f \to 0 \) behaviour of the phase diagram for DNA unpeeling. We consider both cases \( A_0 \) and \( A_1 \), in which the effective length of dsDNA segments is larger (\( \ell_d \sim 300 \) bp \( \sim 100 \) nm for B-DNA) with respect to that of ssDNA ones (\( \ell_s \sim 8 \) bp \( \sim 4 \) nm). The monomer sizes are \( a_d \) for dsDNA and \( a_s \approx 0.56 \) nm for ssDNA, and may differ. Let us consider \( T \approx T_m \) and a small force \( f \ll 1 \). If we disregard denaturation bubbles (considering only Y-shaped configurations), the energy of two DNA strands with \( m = 6N \) open bp, under the action of an unpeeling force \( f \), is \( E = -c(N - m) - f \cdot r \), where \( r \) is the position of the un-anchored end of the stretched strand. We may expect the probability density, \( P(r|N,m) \), for the stretched strand to be described by a Gaussian approximation, in D-dimension,
\[ P(r|N,m) = \frac{1}{\sqrt{2\pi \sigma^2(\theta)}} e^{-\frac{(r - \theta \ell_d a_d)^2}{2\sigma^2(\theta)}} \]
\[ \sigma^2(\theta) \propto \frac{1}{\alpha^2(\theta) + \ell_s a_s^2} \]

The total number of Y-like configurations is given by \( N \) modulo power law corrections, \( C(N|m) \propto \exp((N-m)/\ell_d) \). Minimizing the free energy \( F = E - TS \) with respect to \( r \) and \( \theta \), we obtain the following form for the critical force, \( f_c(T) \),
\[ f_c^2(T) = \frac{T - T_m}{T_m} \frac{2T}{\ell_d a_d^2 - \ell_s a_s^2}, \quad T_m = \frac{\ell_d a_d^2}{s(2\ell_d - \ell_s)} \]

Thus if \( \ell_d a_d^2 > \ell_s a_s^2 \), as in reality, \( T_c \) increases with \( f \). Notice that \( f_c(T) \sim (T - T_m)^{1/2} \) at \( T \sim T_m \) (self-avoidance would change the exponent from 1/2 to \( 1/2 \) to \( \nu \approx 0.588 \), although this trend is not discernible in Fig. 2 due, possibly, to the distance from the thermodynamic limit). The behavior of \( f_c \) given by Eq. (5) is only valid for \( T < T_m \). Away from \( T_m \) this transition line has to turn left and e.g. approach a vertical asymptote for \( a_s = a_d \). This is indeed the shape of the phase diagram obtained with Monte-Carlo simulations for model \( A_0 \) with \( a_s = a_d \), as \( \ell_d > \ell_s \) due to the interaction. Why is the dsDNA phase instead destabilised by \( f \) in the exact model (fig. 3b)? A plausible reason is that the argument we have just proposed neglects denaturation bubbles, and thus predicts \( T_m = T_a \) for all forces, when \( \ell_d = \ell_s, a_d = a_s \). Bubbles are unimportant for the continuum simulations, as the melting is first order for self-avoiding chains, but are
crucial for the exact model, as the transition is second order and there are \( N^{1/2} \) bubbles at the transition \( [3] \). Bubbles increase \( T_m \) and the stability of the zipped phase at \( f = 0 \), and might indeed cause the small force reentrance to disappear.

We now turn to models \( A_1 \) and \( B_1 \), assuming that dsDNA can only be in the B-form. It is then possible to generalize our exact results by introducing different values \( \xi = \ell_d/\ell_s = 25 \) and \( a = a_d/a_s = 0.6 \) for Kuhn lengths and monomer sizes of B-DNA and ssDNA. A second difference is that close to the denaturation transition the shape of the critical line is affected by denaturation bubbles as well. As a result for directed walks dsDNA is stabilised by a force only when both strands are stretched. When the transition is first order (DNA unpeeling), then we can derive \( df_c(T)/dT = -\delta S_{u,z}/\delta x_{u,z} \), where \( \delta S_{u,z} \) and \( \delta x_{u,z} \) are the changes in entropy and elongation (along the direction of \( f \)) between the zipped and the unzipped phases [3]. Close to \( T = 0 \) for DNA unpeeling both these quantities are positive, hence there is no reentrance, whereas close to the melting temperature \( \delta S_{u,z} > 0 \) but the sign of \( \delta x_{u,z} \) may vary.

To conclude, we presented Monte-Carlo simulations and exact results to determine the phase diagram of dsDNA melting in the presence of an external force, stretching one or both strands – DNA unpeeling or stretched DNA denaturation. Our results show that these two cases, which may often be difficult to distinguish in single molecule experiments, lead to strikingly different results, both qualitatively and quantitatively. Contrarily to previous claims, our results suggest that DNA unpeeling is a first order phase transition. We have discussed how to introduce in our models the realistic persistence lengths and monomer sizes of ds and ssDNA. This can be done in different ways according to what is assumed about the nature of the overstretched DNA state. Even if we assume dsDNA can exist in the S-DNA form we have shown that overstretcing transition to a melted ssDNA is possible when one strand is being pulled. According to our calculations, we may infer the phase diagram of a realistic model, taking these effects as well as self-avoidance, fully into account. We predict that it should (i) display no “reentrance” for low \( T \), and (ii) show dsDNA stabilisation by force, close to \( T_m \).[21]

\[ p(\beta, f, \xi, a) = \left[2\cosh(\beta f a \xi)\right]^{1/\xi}/2\cosh(\beta f) \]

The resulting phase diagrams are shown in Fig. 4. The most striking difference with respect to the phase diagrams in Figs. 2 and 3 is that there is now a bound region for the zipped phase, and at large force the DNA is always ss and denatured, in agreement with the interpretation of overstretched DNA as melted DNA. A second difference is that close to the denaturation transition the shape of the critical line is affected by denaturation bubbles as well. As a result for directed walks dsDNA is stabilised by a force only when both strands are stretched. When the transition is first order (DNA unpeeling), then we can derive \( df_c(T)/dT = -\delta S_{u,z}/\delta x_{u,z} \), where \( \delta S_{u,z} \) and \( \delta x_{u,z} \) are the changes in entropy and elongation (along the direction of \( f \)) between the zipped and the unzipped phases [3]. Close to \( T = 0 \) for DNA unpeeling both these quantities are positive, hence there is no reentrance, whereas close to the melting temperature \( \delta S_{u,z} > 0 \) but the sign of \( \delta x_{u,z} \) may vary.

To conclude, we presented Monte-Carlo simulations and exact results to determine the phase diagram of dsDNA melting in the presence of an external force, stretching one or both strands – DNA unpeeling or stretched DNA denaturation. Our results show that these two cases, which may often be difficult to distinguish in single molecule experiments, lead to strikingly different results, both qualitatively and quantitatively. Contrarily to previous claims, our results suggest that DNA unpeeling is a first order phase transition. We have discussed how to introduce in our models the realistic persistence lengths and monomer sizes of ds and ssDNA. This can be done in different ways according to what is assumed about the nature of the overstretched DNA state. Even if we assume dsDNA can exist in the S-DNA form we have shown that overstretcing transition to a melted ssDNA is possible when one strand is being pulled. According to our calculations, we may infer the phase diagram of a realistic model, taking these effects as well as self-avoidance, fully into account. We predict that it should (i) display no “reentrance” for low \( T \), and (ii) show dsDNA stabilisation by force, close to \( T_m \).[21]

\[ p(\beta, f, \xi, a) = \left[2\cosh(\beta f a \xi)\right]^{1/\xi}/2\cosh(\beta f) \]

The resulting phase diagrams are shown in Fig. 4. The most striking difference with respect to the phase diagrams in Figs. 2 and 3 is that there is now a bound region for the zipped phase, and at large force the DNA is always ss and denatured, in agreement with the interpretation of overstretched DNA as melted DNA. A second difference is that close to the denaturation transition the shape of the critical line is affected by denaturation bubbles as well. As a result for directed walks dsDNA is stabilised by a force only when both strands are stretched. When the transition is first order (DNA unpeeling), then we can derive \( df_c(T)/dT = -\delta S_{u,z}/\delta x_{u,z} \), where \( \delta S_{u,z} \) and \( \delta x_{u,z} \) are the changes in entropy and elongation (along the direction of \( f \)) between the zipped and the unzipped phases [3]. Close to \( T = 0 \) for DNA unpeeling both these quantities are positive, hence there is no reentrance, whereas close to the melting temperature \( \delta S_{u,z} > 0 \) but the sign of \( \delta x_{u,z} \) may vary.

To conclude, we presented Monte-Carlo simulations and exact results to determine the phase diagram of dsDNA melting in the presence of an external force, stretching one or both strands – DNA unpeeling or stretched DNA denaturation. Our results show that these two cases, which may often be difficult to distinguish in single molecule experiments, lead to strikingly different results, both qualitatively and quantitatively. Contrarily to previous claims, our results suggest that DNA unpeeling is a first order phase transition. We have discussed how to introduce in our models the realistic persistence lengths and monomer sizes of ds and ssDNA. This can be done in different ways according to what is assumed about the nature of the overstretched DNA state. Even if we assume dsDNA can exist in the S-DNA form we have shown that overstretcing transition to a melted ssDNA is possible when one strand is being pulled. According to our calculations, we may infer the phase diagram of a realistic model, taking these effects as well as self-avoidance, fully into account. We predict that it should (i) display no “reentrance” for low \( T \), and (ii) show dsDNA stabilisation by force, close to \( T_m \).[21]
values of $\ell_s$, $a_s$, $\ell_d$ and $a_d$, holds. Stretched denaturation enhances stabilisation (Figs. 2-4).