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Manipulating a single adsorbed DNA for a critical endpoint

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Abstract – We show the existence of a critical endpoint in the phase diagram of unzipping of an adsorbed double-stranded (ds) polymer like DNA. The competition of base pairing, adsorption and stretching by an external force leads to the critical end point. From exact results, the location of the critical end point is determined and its classical nature established.

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A critical end point (CEP) occurs when a continuous transition line terminates on a first-order transition line. Its specialty: even though it is surrounded by three phases, still there is no three phase coexistence, but, instead, a scale-free critical phase coexists with a noncritical phase [1,2]. For comparison, a triple point, also surrounded by three phases, would show three phase coexistence. A CEP is expected to occur in various mixtures and ferroelectrics and in vortex lattice [1–3]. We show here that a different way of locating and studying a CEP is through single molecular manipulations of an adsorbed DNA.

The melting of DNA is known to be a crucial step in many biological processes [4]. The double-stranded DNA (dsDNA) is a bound state of two polymers or strands held together by hydrogen bonds of base pairs. The phenomenon of cooperative breaking of the base-pairings thermally or otherwise is melting. The recognition of force as a thermodynamic variable for this process has helped in completing the phase transition picture of dsDNA [5]. Even though the nature of the thermal denaturation of DNA remains a puzzle, the force induced unzipping transition is theoretically well-settled [5–18]. In addition, the force-induced unzipping transition has emerged as a possible scenario for opening of DNA [19] with the replication Y-fork as the junction of two-phase coexistence, the zipped and the unzipped DNA. A thermodynamic description of DNA would entail two conjugate ensembles of fixed force and fixed distance. These two ensembles are important in both theoretical and experimental situations [8,20–22].

The melting and unzipping of DNA are generally considered in the free environment of bulk solutions, but often the presence of interacting surfaces cannot be ignored. *In vivo*, during replication, DNA gets attached to the membrane but otherwise it remains away (“desorbed”) from the membrane. The protein-induced membrane-DNA attachment is used in the replication process and cell division [23]. In gene therapy, targeted delivery is achieved by taking advantage of adsorption-desorption of DNA on cationic liposomes [24,25]. That metallic (*e.g.*, gold), semiconducting (*e.g.*, silicon) or insulating (*e.g.*, mica) surfaces can also adsorb DNA has opened up the possibility of biosensors for fast and precise detection of DNA in samples like hair, blood etc. In all these cases, the surface-DNA interaction depends (and hence tunable) on the nature of the surface, fluctuation of the surface as for fluid membranes, ionic concentration of the environment, nature of hydrophobicity and van der Waals interactions. A well-studied system in recent years is DNA on gold where the DNA can be attached to the surface with a thiol group and a small linker [26–28]. The model setup we are considering is similar to the case of DNA on a gold plate and is shown in fig. 1. A force pulls only one of the two strands of a DNA which can adsorb on an attractive surface. The DNA as usual has the base pairing energy. We here treat the surface-DNA interaction as an additional parameter in the problem. It transpires that for certain ranges of the attraction with surface there would be a competition between adsorption, unzipping, and melting. These three processes can lead to a CEP. The essential feature that holds the key for the CEP is that the force-induced unzipping is strictly first order but

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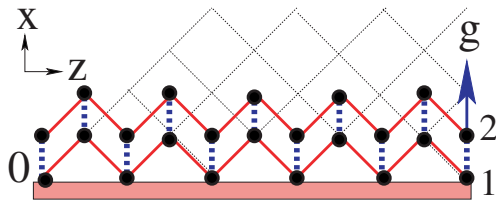


Fig. 1: Schematic diagram of DNA adsorbed on the surface (shaded region). One end of the DNA is always kept anchored on the surface at the origin. The free strand (denoted by 1) can gain energy $-\epsilon_w$ for every contact with the surface (*i.e.* $x_1 = 0$). An external force g (shown by arrow) is applied at the free end of the pulled strand (denoted by 2 and shifted by a unit distance to make it visible). The bold dotted lines denote the base pairing (energy $-\epsilon_b$) between the two strands of the DNA. For all figures, we take $k_B = 1$ and $\epsilon_b = 1$.

the adsorption-desorption transition of a polymer from a surface is continuous [29–31].

Previous studies of DNA unzipping showed that the lattice model preserves, even in two dimensions, the basic results of DNA unzipping including the first-order nature of the phase transition and the existence of a re-entrant region [7]. The generic arguments of these studies also showed that the choice of the lattice is not crucial. For the problem at hand, we have also done Monte Carlo simulations in $2+1$ dimensions (on a cubic lattice) and find that the force-distance isotherms are qualitatively similar to the isotherms obtained in $1+1$ dimensions (see below). Consequently we focus mostly on the results obtained from analysis of exact results in $1+1$ dimensions.

We model the DNA by two directed self-avoiding walks on a $(D=1+1)$ -dimensional square lattice. See fig. 1. The walks, labeled 1 and 2, starting from the origin, are directed along the diagonal of the square (z -direction). The walks are not allowed to *cross each other* but whenever they meet (*i.e.* $x_1(z) = x_2(z)$) there is a gain in energy $-\epsilon_b$ ($\epsilon_b > 0$) for every contact. This is the base pairing. At the diagonal ($x=0$) there is an impenetrable attractive surface, with an energy $-\epsilon_w$ ($\epsilon_w > 0$), which favours the adsorption of the DNA. In $1+1$ dimensions, the surface is a line passing through the diagonal of a square lattice, and only one of the strands can get adsorbed on it (*i.e.* $x_1 = 0$), since the two strands of the DNA cannot cross each other. One end of the DNA is always kept anchored at the origin. We apply an external force g , along the transverse direction (x -direction) on the free end of one of the strands of the DNA. The other strand is left free. Henceforth, the strand which is left free is called the “free strand” and the strand, on which the external force acts is called the “pulled strand”. The endpoint positions $x_i(N)$ is to be shortened to x_i . In $2+1$ dimensions, the surface is a plane passing through the diagonal of a cubic lattice [31]. Unlike the $(1+1)$ -dimensional case, both the strands of the DNA can get adsorbed on the surface and still satisfy the non-crossing constraint on the plane (y -direction).

The two energies independently give us two special temperatures: i) T_w the temperature for desorption of the DNA from the surface, and ii) T_m the melting temperature of dsDNA. In the absence of a surface, the melting temperature is given by $k_B T_m = \epsilon_b / \ln(4/3)$ [7–9]. For ssDNA, $k_B T_w = \epsilon_w / \ln 2$ [29]. Thermal fluctuations create bubbles in the dsDNA changing its effective elastic behaviour with concomitant rise in the desorption temperature. We consider energies such that $T_w < T_m$ and for numerical results we choose units so that $k_B = 1$, and $\epsilon_b = 1$. This convention is also adopted in the following discussion, unless we want to show a general formula or show the dependence on ϵ_b .

There are four distinct phases differentiated by $\langle x_i \rangle$ for $i=1,2$ as $N \rightarrow \infty$, ($\langle \dots \rangle$ denotes thermal averaging). i) Z_a : zipped DNA adsorbed on the surface with $\langle x_i \rangle / N \rightarrow 0$ for $i=1,2$. ii) Z_d : zipped DNA desorbed from the surface with $\langle x_i \rangle / N = O(1)$, for $i=1,2$. iii) U_{ad} : unzipped DNA with the free strand adsorbed on the surface and the pulled strand is stretched in the direction of the force. This phase is characterized by $\langle x_1 \rangle / N \rightarrow 0$ but $\langle x_2 \rangle / N = O(1)$. iv) U_{dd} : unzipped DNA with both the strands desorbed from the surface¹ with $\langle x_2 \rangle / N = O(1)$ and $\langle x_1 \rangle / \sqrt{N} = O(1)$. The adsorbed or the zipped phases may also be characterized by the fraction of monomers in contact, *e.g.*, Φ_w the fraction of polymers in contact with the wall and Φ_b the fraction of bound base pairs. In a zipped phase $\Phi_b \neq 0$ while for the adsorbed phase $\Phi_w \neq 0$.

One can do a zero-temperature ($T=0$) analysis of the problem, keeping ϵ_b constant. The energies of the three phases, namely Z_a , Z_d , and U_{ad} are, respectively, given by $E_{Z_a} = -N(\epsilon_w/2 + \epsilon_b)$, $E_{Z_d} = -N(g + \epsilon_b)$, and $E_{U_{ad}} = -N(\epsilon_w/2 + g)$. For $\epsilon_b < \epsilon_w < 2\epsilon_b$, the phase U_{ad} is always unstable (*i.e.* it has higher energy). The transition from phase Z_a to phase Z_d occurs at $g = \epsilon_w/2$. On the other hand, for $\epsilon_w = 2\epsilon_b$, there is a degeneracy for Z_d and U_{ad} which occurs at $g = \epsilon_w/2$. But, for $\epsilon_w > 2\epsilon_b$, the Z_d -phase is no longer favourable. The change in stability of phases Z_d and U_{ad} as ϵ_w is tuned is an indication that these phases could be stabilized by entropy at intermediate temperatures under appropriate conditions. For $\epsilon_w = 0$, we can only have Z_d and U_{dd} , while, for $\epsilon_w > 0$, as the $T=0$ analysis shows, phase Z_a must exist. For very high temperatures $T \gg T_w, T_m$, the stable phase is U_{dd} . When $\epsilon_w = \infty$, the free strand, which remains adsorbed on the surface at all temperatures, acts like a zig-zag hard-wall. In this case, only phases Z_a and U_{ad} survive in the phase diagram. Consequently, there is a continuous evolution of the phase diagram for DNA as ϵ_w is changed. These phases can therefore be represented in a 3-dimensional g - T - ϵ_w phase diagram. We show the cross-sections (g - T plane) of this phase diagram for various ϵ_w in fig. 2.

We like to point out the generic nature of the results, especially the existence of the phases and the nature of

¹There is a fifth phase U_{aa} in $2+1$ dimensions where DNA melts but both strands remain adsorbed on the surface. See, *e.g.*, [32] for experiments on such melting of adsorbed oligomers on gold.

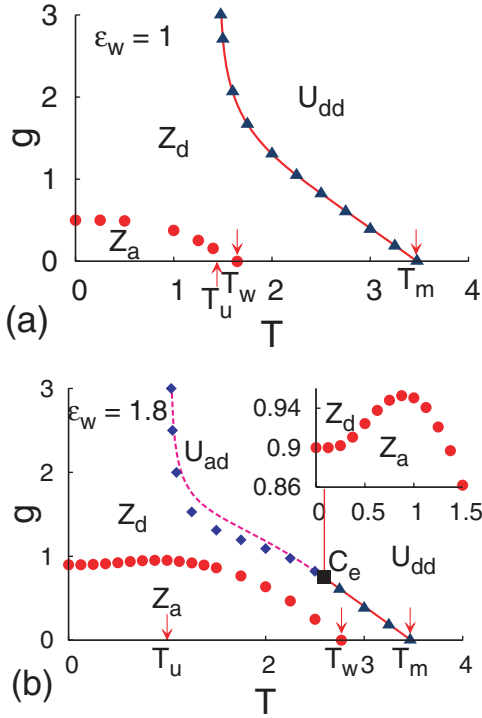


Fig. 2: g vs. T phase diagrams (a) for $\epsilon_w = 1$ and (b) for $\epsilon_w = 1.8$. C_e represents the critical end point. The re-entrance on the phase boundary separating phases Z_a and Z_d is shown in the inset. The points are from the transfer matrix and lines are eqs. (4) and (5).

the phase transitions. Similarly, the choice of a straight wall is not a restriction; a zig-zag wall with all monomers getting absorbed also shows similar behaviour [33]. For an experimental realization, DNA tethered to a gold or mica surface looks promising. For example, mica-DNA interaction can be fine-tuned by NaCl and MgCl₂ over a broad range of 0.02 eV per bp to 0.35 eV per bp [28]. For gold 1 kbp DNA adsorption has also been studied [26]. Unzipping force measurements in single-molecule experiments, like atomic force microscopy, for finite chains with proper finite size analysis [12] could verify the theoretical results presented here.

Let $D_n(x_1, x_2)$ be the partition function (temperature dependence not shown explicitly) of a dsDNA in the fixed distance ensemble where n -th monomers of the strands are at positions x_1 (free strand) and x_2 ($x_2 \geq x_1$) (pulled strand), respectively, from the wall. $D_n(x_1, x_2)$ satisfies the recursion relation ($x_2 \geq x_1 \geq 0$)

$$D_{n+1}(x_1, x_2) = \sum_{i,j=\pm 1} D_n(x_1+i, x_2+j) \times [1 + \mathcal{W}\delta_{x_1,0}] [1 + \mathcal{B}\delta_{x_1,x_2}], \quad (1a)$$

where,

$$\mathcal{W} = (e^{\beta\epsilon_w} - 1), \quad \mathcal{B} = (e^{\beta\epsilon_b} - 1), \quad \text{and } \beta = 1/k_B T. \quad (1b)$$

The initial condition $D_0(x_1, x_2) = \delta_{x_1,0}\delta_{x_2,0}$. The canonical partition function with an external force g at the end

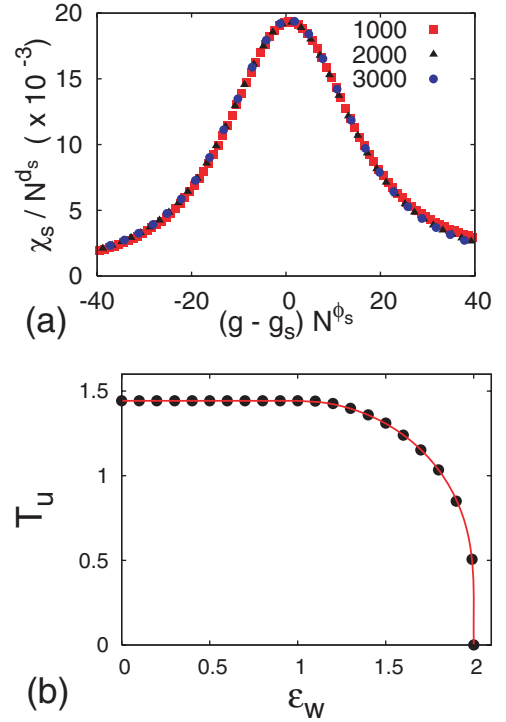


Fig. 3: (a) The nature of data collapse of the extensibility at $T = 1.5$ for $N = 1000, 2000$ and 3000 at transition Sz. (b) T_u vs. ϵ_w curve from data collapse (points) and the exact curve (solid line) from eq. (6).

of the pulled strand is then obtained by summing over all the allowed configurations of the DNA of length N on the lattice:

$$Z_N(\beta, g) = \sum_{x_2 \geq x_1 \geq 0} D_N(x_1, x_2) e^{\beta g x_2}. \quad (2)$$

From the partition function we calculate the endpoint averages $\langle x_1 \rangle$ and $\langle x_2 \rangle$. The appropriate response function is the isothermal extensibility, which can be expressed in terms of fluctuations of the position of the end monomer

$$\chi = \left. \frac{\partial \langle x_2 \rangle}{\partial g} \right|_T = \frac{1}{k_B T} [\langle x_2^2 \rangle - \langle x_2 \rangle^2]. \quad (3)$$

The N (length) dependence and finite size scaling of these quantities would be utilized to identify the phases and the phase transitions with the help of the Bhattacharjee-Seno data collapse program [34]. (See fig. 3(a) as an example of the data collapse of $\chi(g, T)$.) The nature of the transition (first or second order) is inferred from the values of the relevant exponents [12]. The phase diagrams are obtained by the repeated use of finite size scaling.

In fig. 4(a), we have shown the force-distance isotherms for $\epsilon_w = 1.8$ at two different temperatures $T = 0.5$ and 1.5 for the chain of length $N = 1000$. In both cases, we start from the ground state at $g = 0$, an adsorbed DNA on the surface. The phases can be identified by the extensibility

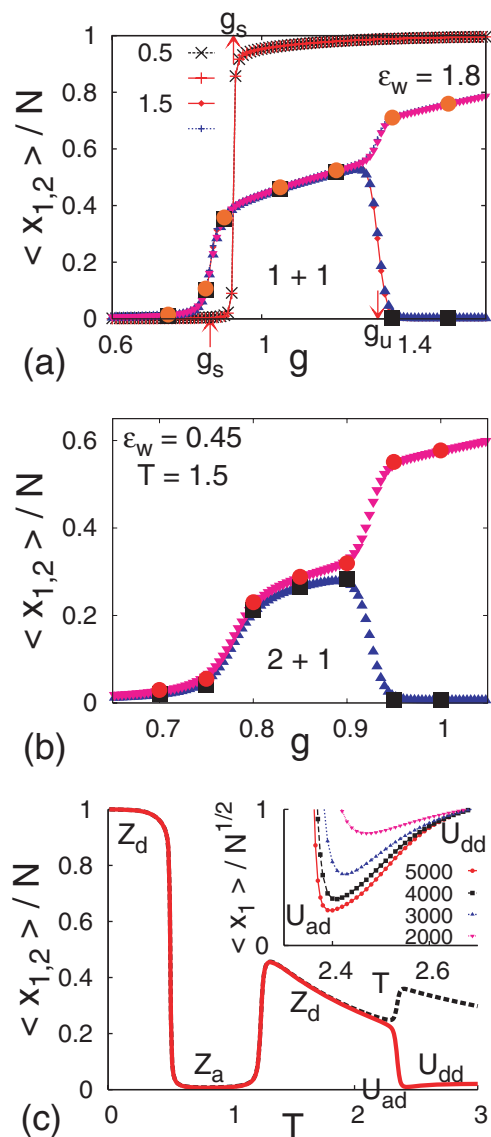


Fig. 4: The $\langle x_i \rangle / N$ vs. g ($i = 1, 2$) isotherms for $N = 1000$ (a) for the (1+1)-dimensional case at temperatures, $T = 0.5$ and 1.5 for $\epsilon_w = 1.8$; (b) for the (2+1)-dimensional case. In both (a) and (b), the big squares (for x_1) and circles (for x_2) show the averages obtained by Monte Carlo simulations and the upper and the lower triangles are the estimates given by the multiple histogram technique at various g . (c) $\langle x_{1,2} \rangle / N$ vs. T for the DNA of length $N = 3000$ at $g = 0.925$. The solid (dashed) line represents the free (pulled) strand. The inset shows $\langle x_1 \rangle / \sqrt{N}$ vs. T for various chain lengths.

of $\langle x_1 \rangle$ and/or $\langle x_2 \rangle$. At $T = 0.5$, there is a critical force, g_s , at which the DNA gets unzipped from the surface but remains double stranded. We call this as ‘‘transition Sz’’. But at $T = 1.5$, we see the sequence $Z_a \rightleftharpoons Z_d \rightleftharpoons U_{ad}$, an additional transition (to be called ‘‘transition Uz’’) at $g = g_u$. The isotherms are obtained at $T = 1.5$ by two different methods with results comparing nicely. The lines are from the exact transfer matrix based on eq. (1a), whereas the bigger symbols (squares and circles)

are obtained by performing Monte Carlo simulations for longer chains using the multiple histogram technique [35]. The details of Monte Carlo simulation will be discussed elsewhere. The estimates, so obtained, are shown by the upper and the lower triangles for the free and the pulled strand respectively in fig. 4(a). An isotherm for a 2+1 dimensional case is also shown in the fig. 4(b) and is similar to the 2-dimensional case, as already mentioned.

Figure 2(a) shows the phase diagram for $\epsilon_w = 1$ as a representative in $0 < \epsilon_w \leq \epsilon_b = 1$. It contains three phases, namely Z_a , Z_d and U_{dd} . The phase boundary separating phase Z_a from phase Z_d is shown by circles. The minimum temperature, T_u , above which the unzipping of the dsDNA to two single strands takes place is at $T_u = \epsilon_b / \ln 2$ same as for $\epsilon_w = 0$ case. For low values of g , the pulled strand is not necessarily straight. If we ignore the effect of the wall the phase boundary can be calculated exactly from the above recursion relation as

$$g_u(T) = \frac{k_B T}{2} \ln \left(\frac{2e^{-\beta \epsilon_b} - 2}{1 - 2e^{-\beta \epsilon_b}} \right). \quad (4)$$

Below T_u , the DNA remains double stranded for any value of force g . That the effect of the wall is negligible is borne out by the excellent agreement [33] of the phase boundary for $\epsilon_w \leq 1$ (fig. 2(a)).

The situation of interest is $1 < \epsilon_w < 2$. In this case, we have all the four phases in the phase diagram, including the $T = 0$ unstable phase U_{ad} . With $\epsilon_w > \epsilon_b = 1$, pair breaking, as opposed to desorption, would play an important role at low temperatures. As a result, the phase boundary separating phase Z_a from phase Z_d , loses its monotonicity seen in the $\epsilon_w = 1$ case and a thin slice of re-entrance starts appearing in the phase diagram at intermediate temperatures. Such an intermediate re-entrance was also found in ref. [8], in a different context of a dsDNA with a force at an interior point. We have shown the phase diagram for $\epsilon_w = 1.8$ in fig. 2(b), as a representative of this regime with the re-entrance shown in the inset. Apart from this feature, there is a region in the phase diagram which involves three phases, namely Z_a , Z_d and U_{ad} . The transitions from phase Z_d to phases U_{ad} , and U_{dd} are of first order, whereas, the transition from phase U_{ad} to phase U_{dd} is second order. The critical line is at $T_e = \epsilon_w / \ln 2$ for all g . This is the temperature at which the adsorbed free strand in phase U_{ad} desorbs from the surface. If we ignore the effect of attraction of the wall on phase Z_d , the first-order phase boundary can be obtained by equating the free energies of the two phases obtained from eq. (1a). For the Z_d -to- U_{ad} transition we then get

$$g_\ell(T) = \frac{k_B T}{2} \ln \left[\frac{(e^{-\beta \epsilon_w} - e^{-2\beta \epsilon_w})^{1/2}}{e^{\beta \epsilon_b} - (e^{-\beta \epsilon_w} - e^{-2\beta \epsilon_w})^{1/2}} - 1 \right], \quad (5)$$

while the Z_d -to- U_{dd} transition is still given by eq. (4). Of course eq. (5) will not be appropriate in the temperature

region where there is re-entrance in the desorption boundary. This is seen when compared with the numerical results (fig. 2(b)). However, the large force asymptote is given correctly, especially the dependence of T_u on ϵ_w as

$$\epsilon_w = k_B T_u \ln \left[\frac{2}{1 - \sqrt{1 - 4 \exp(-\epsilon_b/k_B T_u)}} \right]. \quad (6)$$

This expression has the correct limits and matches with the numerics as shown in fig. 3(b), justifying *a posteriori* the neglect of the effect of the attractive wall in these conditions.

The free-strand desorption critical line terminates on the first-order boundary for phase Z_d separating it from phases U_{ad} and U_{dd} . This point of intersection is the CEP. Note that $g_\ell(T)$ and $g_u(T)$ meet at $T_e = \epsilon_w/\ln 2$ with same slope as they should for a CEP. This point is shown in fig. 2(b), by C_e (filled square). For $\epsilon_w = 1.8$, C_e is at $g_e = 0.742618\dots$, $T_e = 2.59685\dots$ (with $\epsilon_b = 1$). The CEP appears in the phase diagram only for $\epsilon_w > 1$, and shifts towards the melting point as ϵ_w is increased. That the two curves meet at CEP with the same slope is a confirmation of its nature. At a triple point the angle between any two phase boundaries is strictly less than 2π .

The phase diagram for $\epsilon_w = 2$ has a new feature that Z_d just becomes unstable at $T = 0$. For $\epsilon_w > 2$, a triple point appears in the phase diagram where Z_a , Z_d and U_{ad} coexist. The CEP still persists but Z_d is now stabilized by entropy. With the increase of ϵ_w beyond 2, the region representing phase Z_d shrinks rapidly and both the triple point and the CEP shift towards higher temperatures and disappear independently from the phase diagram. The crossing of the thermal desorption and thermal melting temperature introduce new complications. These will be discussed elsewhere.

In fig. 4(c), we have plotted the scaled distances $\langle x_{1,2} \rangle/N$, of the end monomers of both the strands from the surface as a function of temperature T at an external applied force $g = 0.925$. This particular value of the force lies in a small region which allows us to see all the possible phases, including the re-entrance between the phase Z_a and the phase Z_d (see fig. 2(b)). Just by increasing the temperature, the DNA can be made to go through the sequence

$$Z_d \iff Z_a \iff Z_d \iff U_{ad} \iff U_{dd}.$$

In the last phase, the free strand of the DNA desorbs from the surface and stays at a distance of \sqrt{N} from the surface (not visible in this scale). To make it visible, we have plotted, in the inset, the scaled separation, $\langle x_1 \rangle/\sqrt{N}$, of the end monomer of free strand from the surface as a function of T for DNA of various lengths. The plot confirms the existence of such a transition.

The adsorption-desorption transition in the model is a classical second-order transition. One therefore expects a Landau-type theory to be applicable for the CEP.

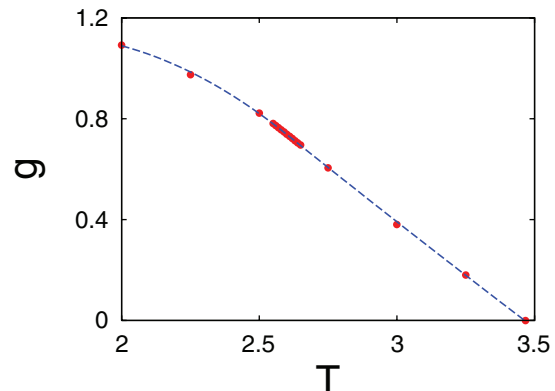


Fig. 5: Phase diagram in the neighbourhood of C_e to show the difference in curvature of the two phase boundaries. The lines are the fits of eq. (7) and the points are from the transfer matrix calculations.

A CEP is described by an eighth-order Landau function $F = t\phi^2 + \phi^4 + w\phi^6 + \phi^8$ in terms of a suitable order parameter [1]. The first-order transition takes place between two ordered phases ($\phi \neq 0$) with $w > 0$, $t < 0$ or between an ordered phase ($\phi \neq 0$) and a disordered phase for $w < 0$, $t > 0$. A second-order transition takes place between the other ordered phase and the disordered phase at $t = 0$. The CEP is at $t = 0$, $w = -3/\sqrt{2}$. The first-order line develops a singularity associated with the behaviour of the specific heat across the critical line. Even though the first-order lines are continuous with the same slope, the curvatures are different because the specific heat has a jump discontinuity across the critical line.

By fitting the first-order boundaries near the CEP at (T_e, g_e) (for $\epsilon_w = 1.8$), we find

$$g_{\ell,u}(T) = a(T - T_e) + b_{\ell,u}(T - T_e)^2 + \dots, \quad (7)$$

with $a = -0.87 \pm 0.01$, $b_\ell = -0.49 \pm 0.03$, $b_u = 0.024 \pm 0.019$, where the subscript u is for $T > T_e$ and ℓ for $T < T_e$. This jump in the second-order is consistent with the prediction of the Landau theory. Figure 5 shows the fits near the critical end point.

To summarize, we established the possibility of four different phases in a set up to unzip an adsorbed dsDNA by pulling a single strand. We find that depending upon the relative strengths of the binding on the surface ϵ_w and the pairing energy ϵ_b , either all the four phases or a few of them, are present in the phase diagram. For a wide range of ϵ_w/ϵ_b , we find that a critical end point is present in the phase diagram. Furthermore, for a narrow range of ϵ_w , we also have a triple point in the phase diagram. It seems that the unzipping of an adsorbed dsDNA by pulling a single strand can be a potential candidate to explore the critical end point and this will open up a new vista for single molecular spectroscopy.

Note Added in Proofs: In a recent paper Marenduzzo *et al.* [36] also obtained eq. (4) in a different context of melting of a stretched DNA.

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