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DNA kinks and bubbles: Temperature dependence of the elastic energy of sharply bent 10 nm size DNA molecules

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A 10 nm long DNA molecule can bend through large angles reversibly. Past the linear regime, its equilibrium nonlinear bending elasticity is governed by a critical bending torque $\tau_c \approx 30\,\mathrm{pN}\times\mathrm{nm}$ at which the molecule develops a kink. This nonlinearity has long been attributed to the nucleation of a bubble or melted region in the molecule. Here we measure the temperature dependence of the critical bending torque for nicked DNA, and determine that the entropy associated with the kink in the nonlinear regime is negligible. Thus in the case of nicked DNA the kink is not a bubble, but a compact region deformed beyond a yield strain. We further argue that, with our boundary conditions, the same is likely true for intact DNA. The present measurements confirm that the critical bending torque τ_c is a materials parameter of DNA mechanics analogous to the bending modulus $B \approx 200\,\mathrm{pN}\times\mathrm{nm}$.

I. Introduction

Double stranded (ds) DNA, with a diameter of $\sim 2\,\mathrm{nm}$ and a persistence length $l_p \sim 50\,\mathrm{nm}$, is mechanically similar to a semi-flexible rod. Its linear bending elasticity is described by the worm-like-chain (WLC) model [1], where the bending elastic energy for a molecule of contour length $2L$ is written:

$$E = \int_0^{2L} \frac{1}{2}\frac{B}{R^2(s)}\,. \qquad (1)$$

R is the radius of curvature, s the arc length along the rod, and $B = k_B T l_p$ the bending modulus ($B \approx 200\,\mathrm{pN}\times\mathrm{nm}^2$). This form corresponds to the linear elasticity regime of a thin rod [2], and must break down for R sufficiently small in (1). What happens beyond the linear elasticity regime is an interesting problem in general [3] and in particular for DNA, a self-assembling molecule with many "soft" internal degrees of freedom. As a practical matter, understanding the nonlinear bending elasticity of DNA is important because DNA is often very much deformed as it interacts with DNA binding proteins in the cell, is packaged into nucleosomes, viruses, etc. Then there are nanotechnology applications where DNA is used as a "molecular spring" [4], again under large deformations. For these reasons, quite some effort has been devoted to understanding what happens with DNA when deformations are such that eq. (1) breaks down [5–11].

Soon after the cyclization experiments of Widom et al [5] suggested that ds DNA "softens" for large bending compared to (1), a mechanism was proposed by Yan and Marko [6] invoking the nucleation of a localized ss region or "bubble" in the DNA to account for the softening. Subsequent work in the field built on these experiments and this idea [12–18]. However, cyclization experiments are not the only way to explore the softening transition. Wiggins and co-workers [19] exploited direct AFM imaging of surface immobilized DNA molecules to measure the tangent vector autocorrelation function, and found that it is best described by a linear (rather than quadratic) dependence of the energy on the bending angle. We have recently obtained detailed measurements of the elastic energy of highly stressed DNA molecules [9]. Our measurements are obtained with yet a different experimental method, which starts with a molecule with built in stress (Fig. 1) and measures the elastic energy with an equilibrium method [9, 10]. They show the following. If a short (well below one persistence length) DNA molecule is bent with zero torque boundary conditions at the ends (basically by pulling the ends of the molecule towards each other), the internal torque, which is maximum in the middle of the molecule, cannot exceed a critical value $\tau_c \approx 30\,\mathrm{pN}\times\mathrm{nm}$. As the maximum internal torque reaches τ_c, a defect or "kink" is formed, and the local torque at the kink remains equal to τ_c independent of the deformation. τ_c is a materials parameter (e.g. independent of the length of the molecule), and we have shown that with the two parameters τ_c and B a complete description of the DNA bending elastic energy vs end-to-end-distance (EED) is obtained [10]. These results were obtained with DNA molecules 18 to 30 bp long, with a nick in the middle. The presence of the nick allows a direct, equilibrium measurement of the elastic energy, which in turn reveals the softening transition in the bending elsticity of DNA directly in the form of an actual kink in the energy vs EED curve [9, 10]. However, we also showed that for intact (non-nicked) DNA the same constant-torque-kink phenomenology applies [11], although those measurements were obtained with a more indirect method based on melting curve analysis.

Here we measure the temperature dependence of the critical bending torque τ_c in the case of nicked DNA. We extract the entropy S_{kink} associated with the kink in the DNA, which turns out to be essentially zero. Therefore the kink, which controls the bending stiffness in the nonlinear regime, cannot be a bubble, in the sense of a melted region which must carry an entropy of several

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units of k_B. We further point out that the physics of a kinking transition governed by a critical bending torque is actually different from the physics of a transition governed by nucleation of a melted region: these are not two equivalent descriptions. The present experiments however unambiguously support the former description for our equilibrium constructs. We further discuss why the same probably is true also for the non-nicked equilibrium constructs.

II. Results

We measure the critical bending torque τ_c using the method described in [9]. This is an equilibrium method, and provides directly the elastic energy, but is restricted to DNA molecules with one nick. We believe that in the geometry we use (no torsional constraints) the bending elasticity behavior is essentially the same for nicked and non-nicked DNA (the only difference being a slightly lower value for τ_c in the nicked case [11]), as we argue further on. However, here we study the temperature dependence of the elastic energy and τ_c for nicked molecules only. Briefly, we hybridize two linear DNA strands to form the stressed molecule of Fig. 1a , which consists of a bent ds part (N_d base pairs long) and a stretched ss part (N_s bases long). This molecule can relax its internal elastic energy by forming dimers as in Fig. 1b. Since base pairing in one dimer is identical to base pairing in two monomers, the equilibrium concentrations of monomers and dimers result from a balance between the elastic energy of the monomer, E_{el}, and the dissociation entropy of the dimer, according to [9, 10, 20]:

$$E_{el} = \frac{1}{2} k_B T \ln\left(\frac{X_D}{X_M^2}\right) , \tag{2}$$

where X_M, X_D are the mole fractions of monomers and dimers. A small ($< 1\,k_BT$) correction which we apply to this formula to take into account some residual electrostatic energy in the dimer is described in [9]. We measure concentrations of monomers and dimers by gel electrophoresis of the equilibrated samples (Fig. 2), and obtain from (2) the elastic energy of the stressed monomer Fig. 1. This molecule is essentially a system of two coupled springs: the ds part, which is bent, and the ss part, which is stretched. The stretching elasticity of ss DNA is well known [21, 22], so from the measurements of the elastic energy of the whole molecule, E_{tot}, one can calculate the elastic energy E_d in the ds part of the molecule ($E_{tot} = E_d + E_s$, where E_s is the elastic energy in the ss part). Repeating the measurements for a series of molecules with increasing N_s (the number of bases in the ss part) and fixed N_d (number of bp in the ds part) one obtains in effect the bending energy E_d for different values of the EED x (see Fig. 1). It is this energy function $E_d(x)$ which is described by the critical torque τ_c. More in detail, E_d and τ_c are obtained from the experimental measurements of E_{tot} as follows [9].

For the bending energy E_d of the ds part vs EED x we use the analytic expression obtained in [10]:

$$E_d(x) = \begin{cases} \tau_c \arccos\left(\dfrac{x}{2R}\right) & \text{for } 0 < x < x_c\,, \\ \dfrac{5B}{L}\dfrac{x_0 - x}{2L} - k_B T \ln\left(\dfrac{2L-x}{2L-x_0}\right) & \text{for } x_c < x < x_0\,, \end{cases} \tag{3}$$

where $R = L(1 - 2\gamma^2/45)$ and $x_0 = \langle x \rangle_{f=0} = 2L(1 - k_BTL/(5B))$; $\gamma = L\tau_c/(2B)$. The upper form corresponds to the kinked solution, the lower to the smoothly bent one. The critical EED x_c at which the molecule develops a kink is found by equating the upper and lower expressions (or, to order γ^2, from $x_c = 2L[1-(4\gamma^2/15)]$). The contour length of the DNA is $2L = 0.33\,\text{nm} \times N_d$. This formula describes the bending energy vs EED of a rod of contour length $2L$, bending modulus B, which develops a (constant torque) kink at a critical value τ_c of the internal torque. For the stretching energy of the ss part $E_s(x)$ we use a polynomial expansion of the Marko-Siggia expression [23]:

$$E_s(x) = \frac{9k_BT}{4N_s l_s^2}\left[x^2 + \frac{x^3}{N_s l_s} + \frac{3x^4}{(N_s l_s)^2}\right] , \tag{4}$$

where $l_s \approx 0.8\,\text{nm}$ is the persistence length of ss DNA [24].

The elastic energy E_{tot} is then calculated from:

$$E_{tot} = E_d(x_{eq}) + E_s(x_{eq})\,, \tag{5}$$

where x_{eq} is determined from the mechanical equilibrium condition:

$$(\partial E_d/\partial x)_{x_{eq}} + (\partial E_s/\partial x)_{x_{eq}} = 0\,. \tag{6}$$

Finally, τ_c in (3) is adjusted to fit the calculated value of E_{tot} to the measured value.

Fig. 3a displays measurements of the elastic energy E_{tot} of two molecule such as represented in Fig. 1a, at different temperatures from 25 °C to 70 °C. The squares refer to a molecule with $N_d = 30$, $N_s = 15$, while for the circles $N_d = 18$, $N_s = 12$. The samples (in 10 mM Tris buffer with 1 mM EDTA, 5 mM $MgCl_2$, 100 mM NaCl, pH = 7.9) where equilibrated at the corresponding temperature for 50 hours prior to loading into the gels. With these values of N_d, N_s, the ds part of the molecule is kinked in both cases [9, 10]. The elastic energy decreases with temperature, as expected (at the melting transition it must go to zero), but this is quite a small effect. In Fig. 3b we show the corresponding calculated values of the critical bending torque τ_c. These are obtained as explained above, using in (3) the values $B = 50\,k_BT \times \text{nm}$, $2L = 6\,\text{nm}$ (for $N_d = 18$, and $2L = 10$ nm for $N_d = 30$) and in (5) the value $l_s = 0.764\,\text{nm}$ for the ss DNA persistence length (same values as in [10]). Note that we set the persistence length of ds DNA l_d a constant, so the bending modulus B is proportional to the temperature T.

The critical torque decreases with increasing temperature (i.e. the kink becomes "softer"), but this is again a small effect. Extrapolating the linear fit, from 0 °C to 80 °C, τ_c decreases by only $\sim 8\%$. Similarly, the dependence of the bending energy of the ds part, E_d, on temperature is very slight (Fig. 3c).

Finally we show in Fig. 4 measurements of the elastic energy E_{tot} vs N_s, (similar to Fig. 3 of Ref. [10]), obtained at $T = 55\,^{\circ}\mathrm{C}$. They show that the phenomenology at 55 °C is indeed the same as the previously reported phenomenology at 25 °C. Notice that the fit in Fig. 4 is very constrained: both the slope of the roughly linear part to the left of the kink and the absolute value of E_{tot} in that part are given by τ_c. Fig. 4b shows the energy function (3) used to obtain the fit of part (a).

The very slight temperature dependence of the elastic energy E_{tot} and the critical bending torque τ_c qualitatively tell us that the entropy associated with the kink in the ds DNA is small. While the slope of the graphs of Fig. 3b, which is dimensionally an entropy, does not directly give the entropy of the kink ΔS_{kink}, this can be obtained as follows. Consider the expression (3) for the bending free energy (upper branch, i.e. in the kinked state). Note that a free energy is what is actually measured in the experiments (see eq. (2)). The free energy ΔF_{kink} associated with the kink is, in the formulation (3), $E_d(x_c)$; in terms of τ_c this is

$$\Delta F_{kink} = \frac{L\tau_c}{3B}\tau_c\,, \tag{7}$$

because $E_d(x_c) = \tau_c\Delta$, where Δ is the critical bending angle at which the kink forms [10], and $\Delta = 2\gamma/3$, $\gamma = L\tau_c/(2B)$ (see derivation of (3) in [10]). This is valid for $\gamma \ll 1$, which is the case here. If ΔF_{kink} has an entropy component: $\Delta F_{kink} = \Delta E_{kink} - T\Delta S_{kink}$ then $(\partial/\partial T)\Delta F_{kink} = -\Delta S_{kink}$, supposing ΔS_{kink}, ΔE_{kink} are approximately temperature independent; this is consistent with the linear temperature dependence seen in the graphs of Fig. 3. Then:

$$\Delta S_{kink} = -\frac{\partial}{\partial T}\left(\frac{L}{3B}\tau_c^2\right) = -\frac{2L\tau_c}{3B}\frac{\partial}{\partial T}\tau_c\,, \tag{8}$$

ignoring for the moment the temperature dependence of B. For the molecule C30D18 (Fig. 3) we have: $\tau_c \approx 26\,\mathrm{pN\times nm}$, $L = 3\,\mathrm{nm}$, $B = 200\,\mathrm{pN\times nm^2}$, so $2L\tau_c/(3B) \approx 0.24$ while from the slope of the graph in Fig. 3b, $(\partial/\partial T)\tau_c \approx -3\times10^{-2}$ units of $\mathrm{k_B}$ and therefore $\Delta S_{kink} \approx 7\times10^{-3}$ units of $\mathrm{k_B}$. On the other hand, since $\Delta = 2\gamma/3 = L\tau_c/(3B)$, $\Delta F_{kink} = \tau_c\Delta \approx (26/4.2)\,\mathrm{k_B T_{room}}\times 0.12 \approx 0.7\,\mathrm{k_B T_{room}}$, using $1\,\mathrm{pN}\times 1\,\mathrm{nm} \approx (1/4.2)\,\mathrm{k_B T_{room}}$, $T_{room} = 300\,\mathrm{K}$. Therefore, the entropic part ΔS_{kink} is a tiny fraction of the free energy of the kink ΔF_{kink}. Similarly, for the molecule C45D30 (Fig. 3) we find $\Delta S_{kink} \approx 1.5\times10^{-2}$ units of $\mathrm{k_B}$ and $\Delta F_{kink} \approx 1.4\,\mathrm{k_B T_{room}}$. Notice that the quantities ΔF_{kink}, ΔS_{kink} scale with L, whereas τ_c is a materials parameter" independent of L. Correspondingly, the slopes of the energy graphs (Fig. 3a and c) scale roughly with L, while the slopes of the critical torque graphs are roughly independent of L.

III. Conclusion

In summary, we measure the temperature dependence of the critical bending torque τ_c associated with the kink which controls the nonlinear bending elasticity of DNA [9, 10]. The present measurements are restricted to the case of nicked DNA. For this case, we find that the dependence is very slight, which means that the entropy associated with this defect is negligible. Therefore the kink cannot be a bubble, which would carry an entropy of several units of $\mathrm{k_B}$. The atomistic structure of the kink cannot be inferred from these experiments alone, but it must be a defect where the two DNA strands are still close packed. Eventually it may be possible to crystallize a stressed molecule such as the cartoon Fig. 1 and observe the structure directly.

What is the situation for non-nicked DNA? We believe that for non-nicked constructs as in Fig. 1 the phenomenology is the same (no bubble at the kink), for the following reasons. We have already shown that measurements of the elastic energy of the non-nicked equilibrium constructs are consistent with the same phenomenology of the softening transition, i.e. a constant torque kink [11]. While the experimental method used was less direct, it nonetheless gives a very similar value for τ_c for intact DNA: $31\,\mathrm{pN\times nm}$ vs $27\,\mathrm{pN\times nm}$ for nicked DNA. It seems unlikely that the defect which controls this softening transition is of a different nature (bubble vs no bubble) in the two cases, yet gives rise to the same nonlinearity. Furthermore, if the kink for non-nicked DNA was a bubble (i.e. associated with an entropy of several units of $\mathrm{k_B}$), the elastic energy would have a corresponding large temperature dependence, which is absent in the nicked case. Then at high enough temperature the elastic energy of the non-nicked construct of Fig. 1 would actually be lower than for the nicked construct, a paradoxical prediction. Note also that Vologodskii and collegues have examined the activity of a DNA exonuclease on DNA minicircles [8]; this enzyme digests tracts of ss DNA. Their experiments suggest that there are no such tracts in the minicircle.

Finally, note also that our measurements of τ_c for intact DNA [11] were obtined through melting curve analysis of stressed molecules. The different molecules in that study have quite different melting temperatures (up to 10 °C different), yet the fitting procedure we used to extract the elastic energy gives essentially the same value for τ_c for the different molecules. This suggests once again that there is no large temperature dependence of τ_c in the intact DNA case.

While this paper was under review, a study appeared in the journal Science [25] reporting additional evidence for the softening transition of DNA bending, ob-

tained through single molecule cyclization experiments. While this paper fails to mention our prior studies which mapped out the softening transition unambigously and in detail using very short molecules [9–11], its finding that the cyclization rate is only moderately (approximately a factor 2) higher for DNA with one nick compared to the same intact DNA (Fig. S3 in [25]) confirms our measurements of a modest change in the critical torque and the elastic energy in the two cases [11]. In short, the measurements in [25] confirm that the bending elasticity of intact and nicked DNA is quite similar at room temperature.

During the review process of this paper, concern was expressed that the DNA in our construct may be peeling off at the ends, which may affect the entropy measurements. However, this scenario is not consistent with the measurements, because this would lead to a significant entropic part in the free energy (order of 1 unit of k_B per base peeled off: the peeled off bases are not under tension in one of the strands), i.e. a temperature dependence which is just not there in the measurements (compare Fig. 4 of this paper with Fig. 3a of [10]. Also, if a strand was peeling off it would do so at the nick, not at the ends, because the bending torque is zero at the ends and maximum at the center, in our construct. Furthermore, the peeling off at the ends scenario is totally incompatible with the range of the kinked or soft regime in terms of N_s. We show this in Fig. 5, where the soft regime extends at least from $N_s = 48$ to $N_s = 15$ (and smaller N_s too presumably); this is 33 bases which would have peeled off. But there are only 30 bp in the ds tract to begin with! A further control is provided by changing the sequence at the ends of the ds tract to either higher or lower GC contents. The sequence of the ds tract for the $N_d = 18$ molecules of this paper is:
5' − CTC TCA CGT TCG TCG TAT − 3'
i.e. with a low binding energy TAT triplet at one end and a high binding energy triplet CTC at the other end; on the other hand, the sequence for the the $N_d = 30$ molecules is:
5' − CTG CTC TCA CGT GTG GAG TCG TCG TAT GTC − 3'
i.e. with high binding energy triplets at both ends. However, the measured critical torque is the same, in fact even slightly lower (Fig. 3) for the $N_d = 30$ sequence (that the elastic energy for $N_d = 30$ is slightly higher at low temperature compared to $N_d = 18$ is an effect of the scaling of this quantity with L, as explained in the paper: a lower measured critical torque necessarily corresponds, for the same length molecule, to a lower elastic energy). In summary: increasing the stability of the ends of the ds tract does not affect the observed softening transition.

In conclusion, we show that for nicked DNA the temperature dependence of the elastic energy in the kinked state is small, so the kink cannot be a bubble in the ordinary sense of a high entropy melted region. For intact DNA, we propose that the same is true (no bubble at the kink, with our boundary conditions and at equilibrium) and give reasons for this assertion, based on our previous work [11] and on the recent results of the Ha group [25]. However, we do not provide experimental evidence for this assertion in this paper, as the present measurements refer only to nicked DNA. And we also recognize that the nature of the kink, or even its occurrence, may depend on the boundary conditions (e.g. torsion vs no torsion) and the process (e.g. pre-stressed structure vs cyclization process). For instance, the cyclization study [26] reports J-factors which depend on temperature between 23 °C and 42 °C; for one of the three sequences studied, designed to be more prone to forming bubbles, they find a temperature dependence of the J-factors beyond the effect due to the linear temperature increase of the persistence length l_p. In general, measuring temperature dependencies, as in the present study, will help clarify the nature of the defect which controls high curvature softening in the different cases.

For our equilibrium structures (Fig. 1) and boundary conditions (no torsional constraints and zero bending torque at the ends) the physics of this nonlinearity is different from the physics of thermally assisted bubble formation. In our formulation, the materials parameter is the critical bending torque τ_c, whereas the energy or free energy associated with the kink scales with the length of the molecule L. This is reflected, for example, in the measurements of Fig. 3 which show that the slope of the energy vs temperature graph scales with L, while the slope of the τ_c vs T graph is independent of L. It is similarly reflected in the scaling with L of the E_{tot} vs N_s graphs published in [10]. While the atomistic detail of this nonlinearity may be complex, it is striking that the "thermodynamic" description is so simple: a constant torque kink beyond a yield point defined by the critical bending torque τ_c.

It is also remarkable that the two parameters B and τ_c, which describe the bending elasticity of DNA, depend very little on temperature (below the melting temperature). The persistence length l_p varies no more than 30% with temperature in the range 5 °C to 60 °C [27], so that the corresponding decrease of B in this temperature range is less than 20%.

In summary, this paper presents the first measurements vs temperature of the softening transition of DNA bending. While the measurements are restricted to the case of nicked DNA, the result is a surprise: there is essentially no temperature dependence! For the small temperature dependence that we do measure, the slope of the E_{tot} vs T graph increases with L in a manner consistent with the constant torque kink theory; the slope of the τ_c vs T graph is however essentially independent of L, also consistent with the theory. Finally, we provide hard evidence that, for nicked DNA, the kink is not a bubble. This last result is quite unexpected.

Acknowledgments

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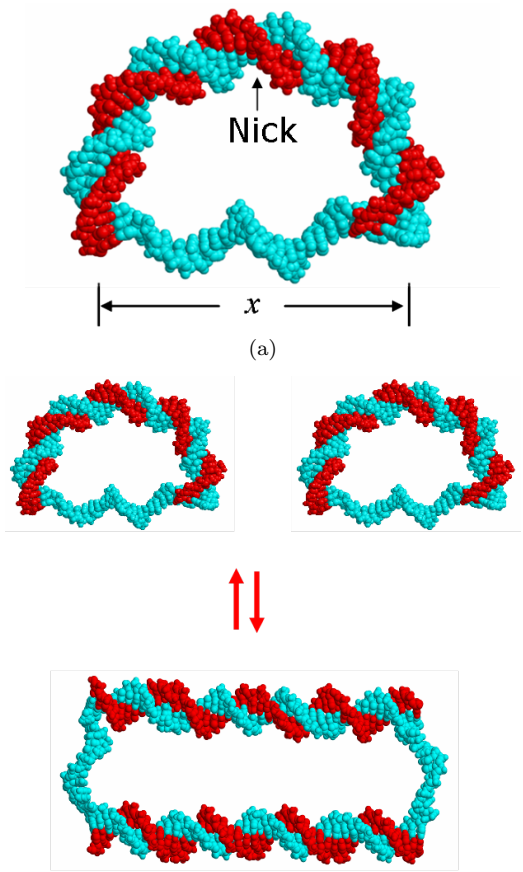
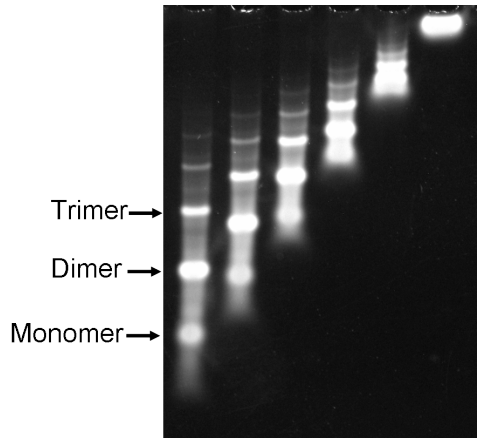
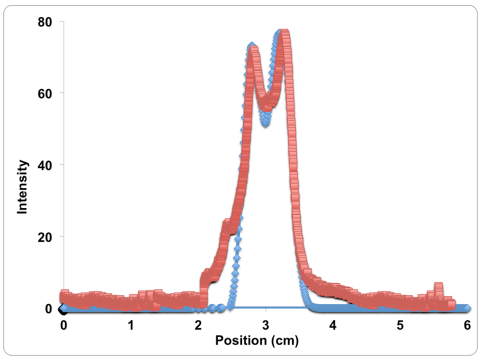


FIG. 1. (Color online) (a) Cartoon of a stressed DNA molecule used in the experiments. The ds DNA is from the PDB structure 1KX5, the ss DNA from PDB 1BNA. The molecule is formed by hybridizing two linear single strands, and has a nick in the middle (i.e. one strand backbone is interrupted). The elastic energy of the molecule is the sum of the bending energy of the ds part and the stretching energy of the ss part. x is the EED of the ds part of the molecule. (b) Two molecules ("monomers") in (a) can form a dimer, where the elastic energy is relaxed. Base pairing (i.e. the binding energy) is identical in two monomers or one dimer, so dimer formation is driven by the elastic energy of the monomer. This elastic energy is measured from the monomer-dimer equilibrium.

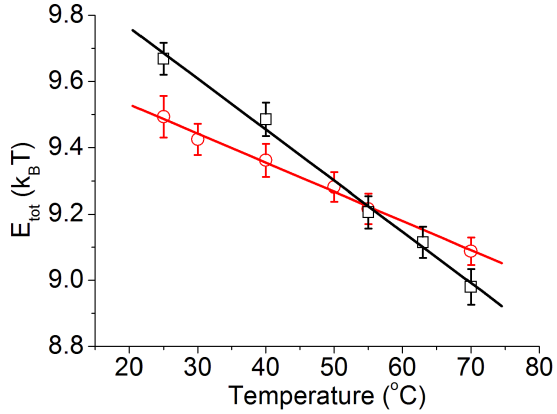


(a)

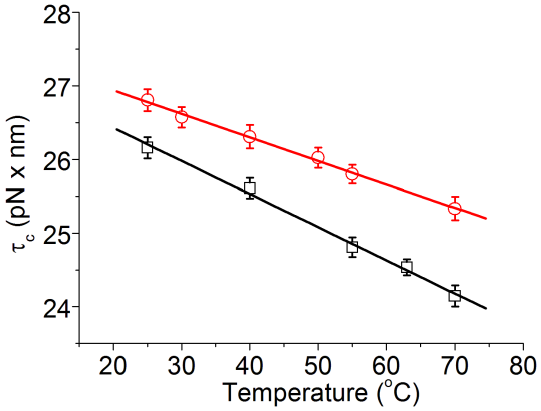


(b)

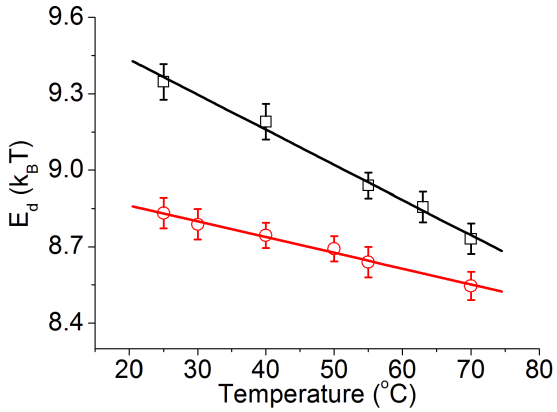
FIG. 2. (Color online) (a) Example of a gel used to determine the equilibrium concentrations of monomers and dimers in the dimerization equilibrium experiments [9]. All lanes were loaded with the same sample, at successive (10 min) intervals. The purpose of this is to extrapolate back in time the initial (equilibrium) concentrations of the two species at "zero" time, since some monomer - dimer inter-conversion occurs in the gel. (b) Intensity profiles of the gel (red squares) in (a) and the model (blue diamonds) used to extrapolate the initial concentrations.



(a)



(b)



(c)

FIG. 3. (Color online) (a) Temperature dependence of the total elastic energy E_{tot} for two different molecules, as in Fig. 1(a). Red circles: $N_d = 18$ (the number of bases in the ds part), $N_s = 12$ (molecule C30D18); black squares: $N_d = 30$, $N_s = 15$ (molecule C45D30). (b) The corresponding critical bending torque τ_c calculated from the measurements in (a) using eqs. (3) - (6) (see text). (c) The elastic energy in the ds part of the molecule, E_d, calculated from the measurements in (a) using eqs. (3) - (6).

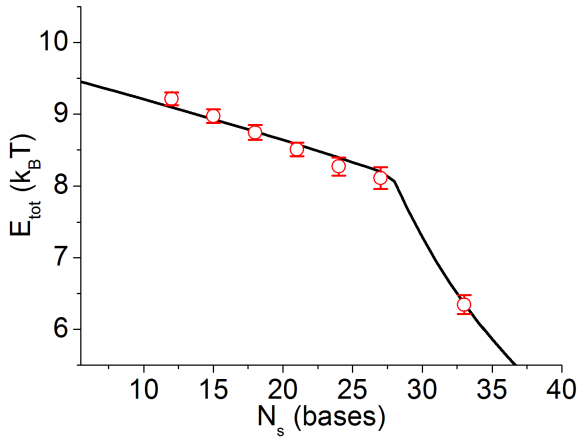


FIG. 4. (Color online) The total elastic energy E_{tot} for a series of molecules with $N_d = 18$ and varying N_s (the number of bases in the ss part), measured at 55 °C. The solid line is a fit using eqs. (3) - (6), as explained in the text, giving $\tau_c = 25.5\,\mathrm{pN} \times \mathrm{nm}$. τ_c is the only fitting parameter, the other parameters being fixed as before ($L = 3\,\mathrm{nm}$; $l_s = 0.764\,\mathrm{nm}$; $B = 50\,\mathrm{k_B T} \times \mathrm{nm}$). The kink in the line corresponds to the value of N_s above which the ds part of the molecule is smoothly bent, and below which the ds part is kinked [10].

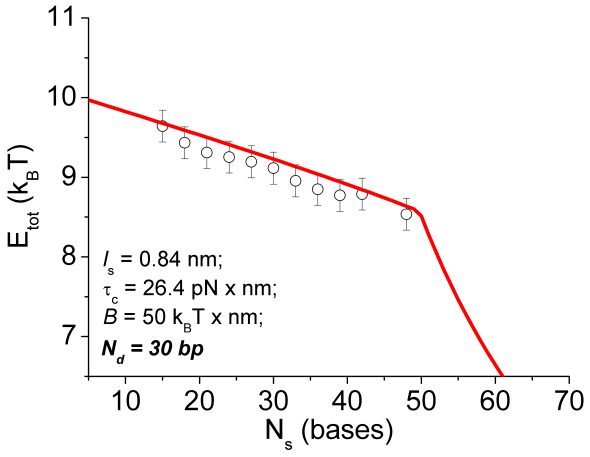


FIG. 5. (Color online) The total elastic energy E_{tot} for a series of molecules with $N_d = 30$ and varying N_s (the number of bases in the ss part). Again, the solid line is a fit using eqs. (3) - (6), with parameters marked in the figure.