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The elastic energy of sharply bent nicked DNA

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Abstract – We obtain measurements of the elastic energy of short (18–30 bp) molecules of ds DNA constrained into a sharply bent conformation, using a thermodynamic method with the DNA in solution. We consider the case where there is one nick in the ds DNA, and find that the system develops a kink at a critical torque $\tau_c \approx 27\,\mathrm{pN} \times \mathrm{nm}$. In this regime the elastic energy is linear in the end-to-end distance (EED). For smaller torques the DNA is smoothly bent and described by the worm-like–chain energy, which is also approximately linear in the EED, but with a different slope. Thus we access both the high and low elastic energy regimes, and the transition between the two.

Introduction. – The elastic energy of constrained configurations plays an important role in the behavior of proteins and DNA, both for natural and artificial systems. We are interested in the elastic behavior of "DNA springs", which have been recently used to mechanically perturb the conformation of proteins, ribozymes, and peptides, with a view of achieving mechanical control over a variety of chemical reactions [1–6]. In the protein-DNA chimeras [7], a ds DNA molecule of contour length $L \sim 10$–$20\,\mathrm{nm}$ (30–60 bp) is attached by the ends to two spots on the surface of the protein typically 3–4 nm apart, so that if the protein is not greatly deformed then the DNA is sharply bent, *i.e.* $x \ll L \ll l_d$, where x is the end-to-end distance (EED) of the DNA, L the contour length, $l_d \approx 50\,\mathrm{nm}$ the persistence length of ds DNA.

The use of DNA oligomers in these highly bent configurations to generate known forces (acting on the protein) requires an accurate model of DNA mechanics at length scales well below the thermal persistence length. While the elastic behavior of DNA in the opposite limit ($L \gg l_d$) is well known to agree with the worm-like–chain model, as shown by single-molecule pulling experiments [8], the behavior for sharp bending has proven more difficult to characterize and understand. Understanding this regime of large deformations, however, has broad biophysical implications for physiologically relevant protein-DNA interactions [9] and for DNA packaging in, *e.g.*, viruses. In general, biomolecules deform when they bind each other and their substrates; it has been proposed recently that such induced fit [10] mechanisms may optimize the specificity of molecular recognition, even though the elastic energy cost of the deformation in effect lowers the binding affinity [11,12]. Thus it is becoming increasingly interesting to determine the elastic energy cost of equilibrium deformations of bio-molecules, DNA in this case. Finally, the regime of large deformations points to new problems in polymer physics; for all of these reasons the short length scale mechanics of DNA has received considerable attention recently.

Deviations from the WLC elastic energy $E/L = B/(2R^2)$ are expected for $R \ll l_d$, where R is the radius of curvature and B the bending modulus (related to l_d by $B = k_B T l_d$), because of bubble formation in the DNA [13]. Theoretical models have been developed that couple long length scale conformational degrees of freedom to such local-structural modifications [14]. Generically, these allow for the generation of localized defects under applied stress and the localization of strain at these points along the polymer chain. The result is a nonlinear strain softening of the polymer under sufficiently large imposed bends.

Some results from cyclization experiments seemed to exhibit this nonlinearity [15]; however, these measurements were later re-interpreted as in agreement with the WLC [16]. A recent AFM study of the conformations of

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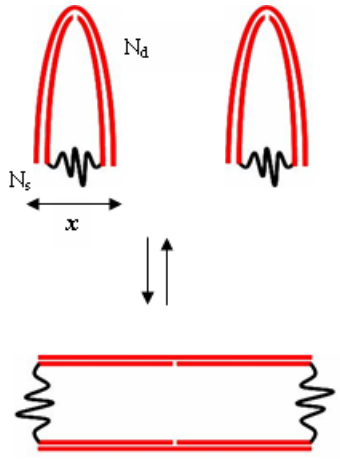

Fig. 1: (Colour on-line) Cartoon of the monomer-dimer equilibrium used to measure the elastic energy of the monomers. N_d is the number of base pairs in the ds part of the monomer (which contains a nick); N_s the number of bases in the ss part. x is the EED of the ss part (and ds part).

DNA adsorbed on a surface finds, on the other hand, that the statistics is best described by a linear (rather than quadratic) dependence of the energy on the bending angle [17,18]; however the proportionality constant then depends on the length scale chosen to coarse-grain the AFM images [17]. In these experiments the mechanical boundary conditions are not specified: one measures thermal fluctuations.

Experiment. – We approach the problem with the system shown in fig. 1. The sequence of two synthetic DNA oligomers is designed to produce, upon hybridization, a stressed molecule where the ds DNA part (which contains one nick in the middle) is bent and the ss part is stretched. This is mechanically similar to the protein-DNA chimeras [4,19–21], with the protein replaced by a short ss DNA element. Unlike the protein case, the stretching elasticity of ss DNA is quantitatively known. We measure the total elastic energy E_{tot} of the molecule, as a function of N_s (the number of bases in the ss part, fig. 1) at fixed N_d (the number of bp in the ds part). This energy is the sum $E_{tot} = E_s + E_d$, where E_s (E_d) is the elastic energy in the ss (ds) part of the molecule. Using the condition for mechanical equilibrium

$$-\left.\frac{\partial E_s}{\partial x}\right|_{x_{eq}} = \left.\frac{\partial E_d}{\partial x}\right|_{x_{eq}}, \qquad (1)$$

where x is the EED of the ss (and the ds) part (x_{eq} is its equilibrium value), the known form of $E_s(x)$, and a polynomial expansion for the energy of the ds DNA part, we extract from the measurements $E_d(x)$, *i.e.* the mechanical response of the DNA spring.

The energy $E_{tot}(N_s)$ is measured using a thermodynamic method which we introduced previously for the protein-DNA chimeras [20,21]. Referring to fig. 1, internal stresses in these DNA molecules can be relaxed by forming dimers, where the ds DNA is not bent and the ss DNA is not (much) stretched. Because the possible base pairings are the same for two monomers and one dimer, the free-energy difference between the two species is essentially the elastic energy of the monomers. Writing the chemical potentials for monomers and dimers:

$$\mu_M = E_{el} + k_B T \ln X_M; \qquad \mu_D = k_B T \ln X_D, \qquad (2)$$

where X_M, X_D are the mole fractions of monomers and dimers and E_{el} the elastic energy of the monomer, at equilibrium ($2\mu_M - \mu_D = 0$) we have

$$E_{el} = \frac{1}{2} k_B T \frac{X_D}{X_M^2}. \qquad (3)$$

The concentrations of monomers and dimers are measured from the intensities of the corresponding gel electrophoresis bands (fig. 2), using suitable calibrations. As the sample runs through the gel, a certain amount of monomer dimer interconversion occurs, visible as interband smear in fig. 2(a). To extract the equilibrium (*i.e.* initial) monomer and dimer amounts we use a simple reaction-diffusion model where monomers and dimers have different mobilities in the gel and given rates of inter-conversion; we adjust the model parameters to fit, with fixed parameters, the gel profiles at the different times, and can thus extrapolate the monomer and dimer concentrations at zero time, *i.e.* the equilibrium concentrations. An example of these fits is shown in fig. 2(b). The mobilities are measured from the time-lapse gels (fig. 2); the interconversion rate is essentially fixed by the requirement of reproducing the interband intensity at the different times (fig. 2(b)). Finally, the results are rather insensitive to the model's parameters: even setting the interconversion rates to zero (which does not fit the gel profiles well) makes a difference of typically ∼15% in the extrapolated zero time concentrations, which after taking the log in eq. (3) changes the energy by only $\sim 0.2 k_B T$.

The elastic energy of the monomer is obtained from the measured equilibrium concentrations using (3). Figure 3(a) shows this elastic energy for a series of molecules with fixed $N_d = 30$ and increasing N_s. We applied a correction to the energy given by (3), reflecting the electrostatic and strain energy in the dimer (fig. 1) due to the electrostatic repulsion between the ds DNA parts, which stretches the ss parts. Specifically, we minimized the energy $E_{correction} = (E_{Electro} + E_{Strain})/2$, made up of the sum of the screened electrostatic interaction between discrete charges distributed on the two ds DNA backbones using a Debye length of 1 nm and the elastic energy of two stretched ss DNA strands each of n_k Kuhn lengths $l_k \sim 1.5$ nm, treating each as a Hookean spring with spring constant $3k_BT/2n_k l_k^2$. We assume the simple planar geometry of fig. 1, so the EED determines all the distances. The minimum of this energy (*i.e.* for the equilibrium value of the EED) corrects the bare energy (squares, fig. 3(a)) to give the full energy of the dimers

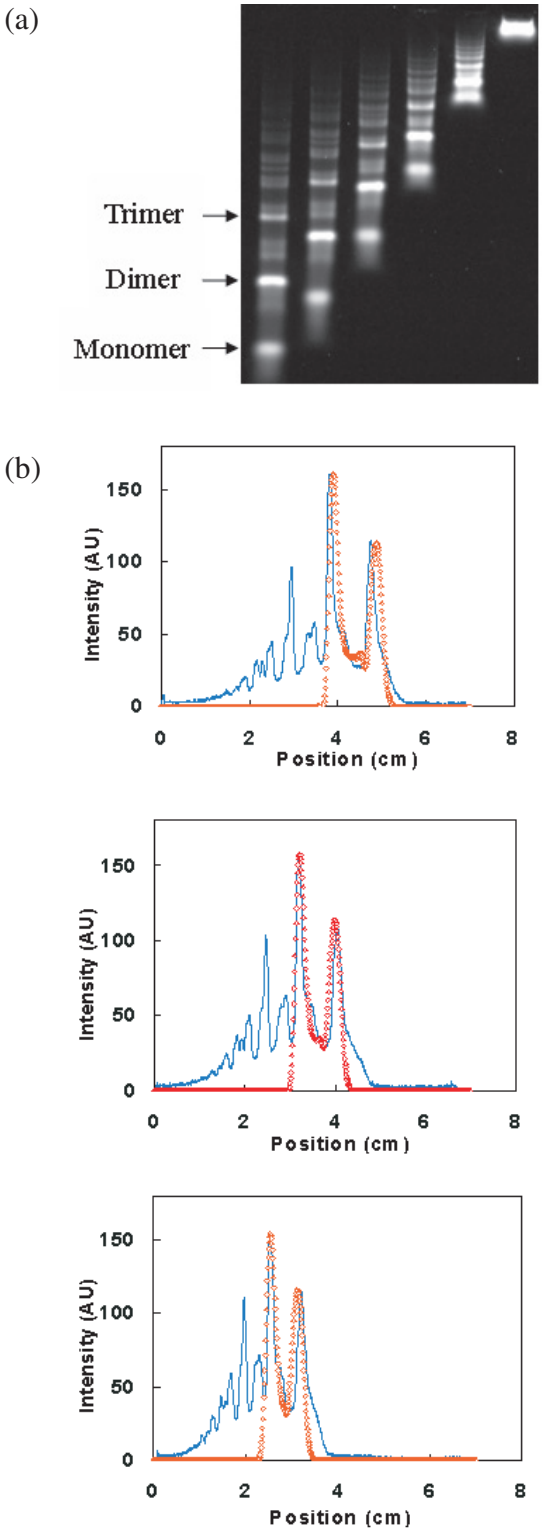

Fig. 2: (Colour on-line) a) Gel electrophoresis of one sample ($N_d = 30$, $N_s = 15$); all lanes contain the same sample, but different lanes were loaded at successive times from left to right in order to follow the evolution of the bands as they move through the gel. b) Intensity profiles of the left three lanes in a), and the fit with the reaction-diffusion model used to extract the equilibrium values (initial values) of the concentrations of monomers and dimers.

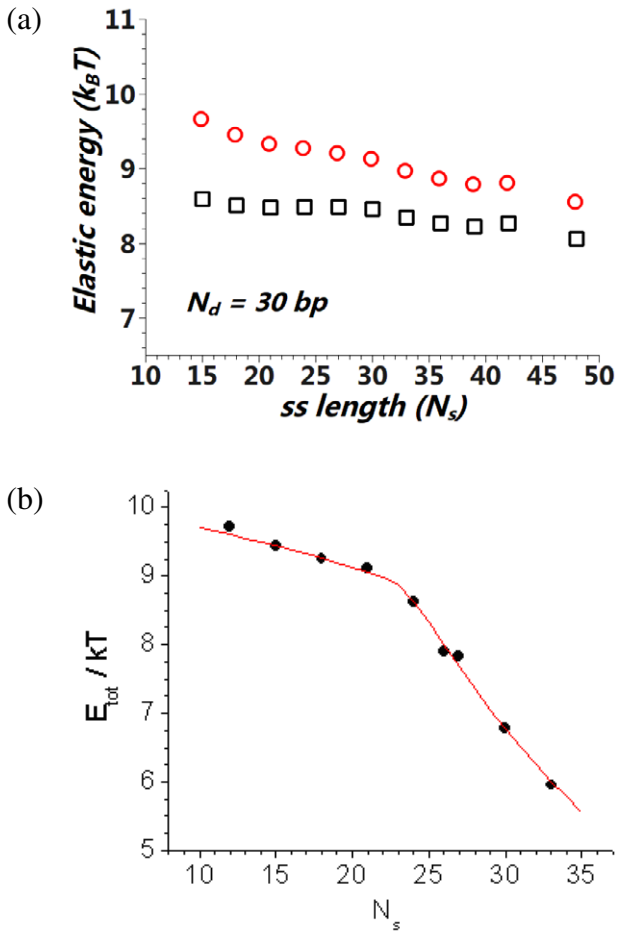

Fig. 3: (Colour on-line) a) The total elastic energy of the monomer of fig. 1, E_{tot}, *vs.* N_s, for $N_d = 30$. The squares are the values obtained from eq. (3) using the monomer and dimer concentrations measured from the gels. The circles are the same values corrected to take into account the electrostatic energy of the dimer (see text). Thus the circles represent the actual elastic energy of the monomer. b) E_{tot} *vs.* N_s, for $N_d = 18$ (including the correction mentioned in a). The change of behavior for $N_s \approx 23$ is evident to the meanest intellect.

(circles, fig. 3(a)). This correction is also applied to the data of fig. 3(b).

Discussion. – Referring to fig. 1, the monomer can be viewed as two (nonlinear) springs, representing the ds and ss part, constrained to have the same EED. Increasing N_s means softening the ss spring. The experiment shows (fig. 3(a)) that the elastic energy E_{tot} is linear with N_s and in fact almost constant in the regime explored. This means that, in this regime, the elastic energy *vs.* EED of the bent ds DNA, $E_d(x)$, is linear in x (*i.e.* this regime is constant force), as follows. We write the energy of the (stretched) ss part of the molecule as

$$E_s(x) = \frac{9k_BT}{4N_s l_s^2}[x^2 + O(x^3) + \cdots], \qquad (4)$$

where $l_s \approx 0.75\,\mathrm{nm}$ is the persistence length of ss DNA [22] and we take the number of persistence lengths equal to $N_s/3$. The terms beyond the first can be obtained for example from the Marko-Siggia interpolation formula [23], but we neglect them here because we are in the regime (verified *a posteriori*) $x \ll N_s\, l_s$. For the energy of the ds part of the molecule we make the Ansatz

$$E_d(x) = E_0 - ax - \cdots, \tag{5}$$

$a > 0$, again valid for small x. From the condition of mechanical equilibrium (1) and (4), (5) we find the equilibrium total energy

$$E_{tot} = E_d(x_{eq}) + E_s(x_{eq}) = E_0 - \frac{a^2 l_s^2}{9 k_B T} N_s \tag{6}$$

which is linear in N_s as seen in the experiments (fig. 3(a)). From the experimental measurements of E_{tot} *vs.* N_s (fig. 2), using (6), we determine E_0 and a, *i.e.* the form of $E_d(x)$ (see (5)) in this regime. This is a regime where, in contrast to the WLC regime of small bending, the energy is linear in the EED, *i.e.* the force is constant. It must correspond to the formation of a kink in the middle of the ds DNA (at the position of the nick), characterized by a critical (constant) torque

$$\tau_c \approx aL, \tag{7}$$

where $L = N_d \times 0.33\,\mathrm{nm}$ is the contour length of the ds DNA. From the data of fig. 3(a), using (6) and (7) we find

$$a \approx 2.73 \pm 0.06\,\mathrm{pN}; \quad \tau_c \approx 27.0 \pm 0.6\,\mathrm{pN} \times \mathrm{nm}. \tag{8}$$

Eventually (for large N_s) the energy of fig. 3(a) must go over to the WLC form and drop to zero; to observe the cross-over we reduced N_d to 18 and obtained the data of fig. 3(b). It is evident that there is a transition for $N_s \approx 23$, which we associate with the formation of the kink: to the left of the transition region the equilibrium conformation has a kink while to the right the ds DNA is smoothly bent. Because $E_s(x)$ is a smooth function, it is evident from fig. 3 that the force $|\partial E_d/\partial x|$ actually decreases for decreasing x as the kink develops. In fact, in the region $22 \leqslant N_s \leqslant 30$ (fig. 3(b)) we observe in the gels a splitting of the monomer band (fig. 4), which we associate with the coexistence of two different conformations of the monomer: ds DNA kinked (and ss DNA compact) *vs.* ds DNA smoothly bent (and ss DNA stretched). The ratio of the free energies of the two conformations goes from <1 to >1 as N_s moves through the transition, as seen from the relative brightness of the two monomer bands. In this region we calculate E_{tot} (fig. 3(b)) as the statistical average $E_{tot} = (X_1 E_1 + X_2 E_2)/(X_1 + X_2)$; where E_1 is calculated from (3) using for X_M the mole fraction X_1 corresponding to one of the monomer bands, and similarly for E_2 . From the slope of the linear part of the graph of fig. 3(b) (*i.e.* for $N_s \leqslant 21$) we extract the parameter a for this case, and find $a \approx 4.0 \pm 0.2\,\mathrm{pN}$, which using (7) gives $\tau_c \approx 23.6 \pm 1.4\,\mathrm{pN} \times \mathrm{nm}$, essentially consistent with (8) (considering that we are neglecting differences in the kink angle for the two cases). In conclusion, in the transition region there are two possible mechanical states (kinked and un-kinked) for this system of two nonlinear springs. By tuning N_s the two states can be made almost degenerate (as seen in fig. 4 where we have nearly the same equilibrium amounts of the two different monomers).

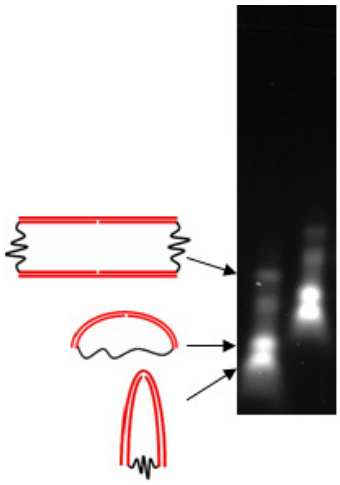

Fig. 4: (Colour on-line) Gel electrophoresis of a sample with $N_d = 18$, $N_s = 26$, showing the splitting of the monomer band, with cartoons depicting the conformations attributed to the different bands. The two lanes contain the same sample loaded at different times. The gel runs downwards.

We now extract the curve $E_d(x)$ for $0 \leqslant x \leqslant L$ using the measurements of fig. 3(b). Our procedure is to guess a form of $E_d(x)$ and verify that in conjunction with the known form of $E_s(x)$ it produces the measured $E_{tot}(N_s)$. For $E_d(x)$ we take a polynomial expression that behaves like (5) for $x \ll L$ and goes over to the WLC energy for $x \approx L$:

$$E_d(x) = E_0 - ax - bx^5 + cx^7; \quad b, c > 0. \tag{9}$$

The boundary conditions to ensure the latter property are

$$E_d(x = L) = 0; \qquad \left.\frac{\partial E_d}{\partial x}\right|_{x=L} = -\frac{5}{4}\frac{B}{L^2}, \tag{10}$$

as explained below. The choice of the powers 5 and 7 is somewhat arbitrary but these are the smallest exponents which produce a good fit for the data of fig. 3(b). Once these exponents are fixed, b and c are calculated from the boundary conditions (10). For $E_s(x)$ we take (see (4)) the first three terms in a polynomial expansion of the Marko-Siggia formula [23]:

$$E_s(x) = \frac{9 k_B T}{4 N_s l_s^2}\left[x^2 + \frac{x^3}{N_s l_s} + \frac{3x^4}{(N_s l_s)^2}\right]. \tag{11}$$

From the condition of mechanical equilibrium (1) we calculate the equilibrium EED x_{eq}, and thus $E_{tot}(x_{eq}) = E_s(x_{eq}) + E_d(x_{eq})$. Figure 5 shows the energy of the ds

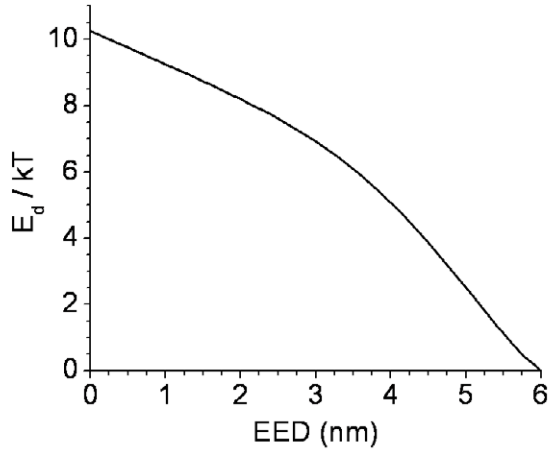

Fig. 5: The elastic energy of the ds part of the molecule, $E_d(x)$, *vs.* the EED x. This energy (see (9)) gives rise to the calculated $E_{tot}(N_s)$ plotted as a line in fig. 3(b). For reference, we give the polynomial expression for this particular curve: $E_d(x) = 10.25 - 1.0x - 1.63 \times 10^{-3}x^5 + 3.01 \times 10^{-5}x^7$, where x is the EED in nm and E_d is in units of k_BT.

DNA which gives rise to the total energy E_{tot} plotted in fig. 3(b) together with the experimental measurements.

Two comments on this result. 1) The nonlinearity of the coupled system (9), (11), (1) makes the location of the knee in the graph of E_{tot} *vs.* N_s (fig. 3(b)) depend sensitively on the value of l_s, offering a new way to measure the persistence length of ss DNA, for short synthetic sequences. We find that $l_s = 0.755\,\text{nm}$ gives a good fit (fig. 3(b)), exactly consistent with the literature value of 0.75 nm for this ionic strength [22]. 2) The second boundary condition (10) comes from a simple, approximate, analytical calculation of the energy of a slightly bent rod with zero torque boundary condition at the ends, approximating the rods shape with a 3d order polynomial, and agrees within a few percent with the energy of the bent rod calculated numerically from the known formulas [24]. The main point is, the appropriate effective boundary condition (10) (effective because when E_d is of order $1\,k_BT$ or smaller, thermal fluctuations are important and the system is not purely mechanical) is the one corresponding to infinitesimal bending of the rod. For a purely mechanical rod the minimum energy solution would correspond instead to infinitesimal compression, giving the boundary condition $(\partial E_d/\partial x)(x = L) = 0$ instead of (10), but we cannot fit the data of fig. 3(b) with this choice. In the future, it would be interesting to investigate this point with a finite-temperature model.

Conclusion. – In summary, we show experimentally that short, nicked, ds DNA constrained to bend (fig. 1) develops a (constant torque) kink at a critical torque $\tau_c \approx 27\,\text{pN} \times \text{nm}$ (for the present sequence). For $\tau > \tau_c$ (the regime $x \ll L$) the kinked solution is stable; for $\tau < \tau_c$ (the regime $x \approx L$) the bent solution is stable. Both regimes, and the transition region (fig. 3(b)) can be described using a polynomial approximation to the energy $E_d(x)$, which allows to obtain, from the measured total elastic energy E_{tot} (fig. 3) the elastic energy of the bent (or kinked) ds DNA as a function of EED (fig. 5). Thus we can now measure experimentally the elastic energy and force which can be obtained with a DNA molecular spring of given sequence.

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