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Using DNA to probe nonlinear localized excitations?

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Abstract. – We propose an experiment using micro-mechanical stretching of DNA to probe nonlinear energy localization in a lattice. Using numerical simulations and kinetics calculations we estimate the order of magnitude of the expected force fluctuations. They appear to be at the border of present experimental possibilities.

In the last few years, numerous studies have been devoted to localized modes in nonlinear lattices because they provide examples of localization in the absence of any disorder. Approximate solutions have been obtained for one-dimensional or multidimensional lattices [1] and a proof of existence of time-periodic, spatially localized, solutions, or breathers, has been given for a broad range of Hamiltonian coupled oscillator lattices [2, 3]. Moreover, a *spontaneous* localization of energy in such lattices has been found [4]. However, these studies are theoretical and numerical and, to our knowledge, an experimental demonstration of nonlinear energy localization in a lattice has yet to be given. The detection of nonlinear localized excitations with scattering techniques (neutrons, Raman, infra-red spectroscopy) is difficult because their signature in the dynamical structure factor is hardly separated from the signals given by static defects, slowly moving domain walls and dislocations or nonlocalized phonon and multi-phonon modes [5]. *An unambiguous observation would require a detection in real space* rather than in Fourier space. The remarkable progress in micro-mechanical techniques raises the hope that this is now possible.

DNA is a very good candidate for such a direct experimental observation for two reasons. First, as shown by the recent experiment of Bockelmann *et al.* [6], the progress in biochemistry and micro-mechanics can be efficiently combined to manipulate a single molecule. Second, DNA is known to undergo large-amplitude nonlinear breathing motions which can be detected indirectly via proton-deuterium exchange when the hydrogen bonds connecting base pairs are broken [7] and could act as precursors of DNA thermal denaturation [8,9]. The determination of the DNA sequence by mechanical opening was first proposed and analyzed by Viovy *et al.* [10] and then investigated by Thompson *et al.* [11]. Both studies concluded that individual base

pairs cannot be resolved by present techniques. What made the experiment of Bockelmann *et al.* possible is that the separation of DNA strands occurs in a cooperative manner, by domains that involve tens or hundreds of base pairs, and therefore, rather than detecting a single base-pair breaking, the experiment is sensitive to the fast opening of these domains. This cooperative effect is well known in the context of DNA thermal denaturation and gives rise to fine structures in DNA "melting" curves [12], related to the base sequence. However large amplitude breathing motions of DNA are also detected in homopolymers [13] and model studies indicate that they give rise to local denaturation bubbles, due to nonlinear energy localization [8,9]. In this letter we show that, if the experiment of Bockelmann *et al.* [6] were performed on a segment of *homopolymer* instead of natural DNA, a breaking by domains would also be observed. This *apparent inhomogeneity* of the molecule would be due to the *dynamical disorder* associated to intrinsic localized modes. The experiment *would therefore allow the first direct observation of nonlinear energy localization in a homogeneous lattice.*

Our approach is based on a model of DNA which has been introduced to describe its thermal denaturation [8,9]. Only one degree of freedom y_j is introduced for the j-th base pair. It corresponds to the distance between the two bases and the Hamiltonian of a molecule with N base pairs is simply written as the sum of a kinetic term, a coupling energy W along the strands and the binding energy of a base pair $V(y_j) = D\left[\exp[-\alpha y_j] - 1\right]^2$ written as a Morse potential

$$H_0 = \sum_{j=1}^{N} \frac{1}{2} m \left(\frac{\mathrm{d}y_j}{\mathrm{d}t}\right)^2 + W(y_j, y_{j-1}) + V(y_j) \ .$$

The choice of the coupling term W is particularly important to ensure that such a simple model can give a correct description of the mechanics and statistical mechanics of DNA denaturation [9]. It is written as

$$W(y_j, y_{j-1}) = \frac{1}{2} K \left[1 + \rho \exp[-\delta(y_j + y_{j-1})]\right] (y_j - y_{j-1})^2$$

and it can be viewed as describing a link of variable stiffness. When the base pairs are stacked (y_j and $y_{j-1} \approx 0$), the effective coupling constant along the strands is $K(1+\rho)$, while when either one of the coupled base pairs is open, the coupling constant drops to K. This gives more freedom to the system, thus increasing its entropy, and, as shown by the statistical mechanics of the model, this results in a sharp denaturation transition in this one-dimensional system [14]. It may seem bold to propose an experiment on DNA on the basis of such a simplified model of the molecule, but investigations of this model [15] have shown that it is able to reproduce the multi-step melting curves and even provide a quantitative agreement with experiments [16], which indicates that it should be able to describe the features of DNA involved in the proposed mechanical denaturation. And moreover its simplicity allows numerical simulations over long time intervals which are necessary to assess the feasibility of the experiment. We have chosen a system of units which is appropriate for the scale of the experiment. Length is expressed in Å, mass in atomic mass units (a.m.u.) and energy in eV. This defines a time unit (t.u.) equal to $1.021 \cdot 10^{-14}$ and a force unit of 1602 pN. With these units, the parameters of the model have been chosen to be $m = 300$, $k = 0.04$, $\rho = 0.5$, $\delta = 0.35$, $D = 0.04$, $\alpha = 4.45$ which gives a thermal denaturation temperature of 360 K. The parameters take into account the average effect of the solvent. Moreover the internal oscillations that are responsible for the breathing of DNA are several orders of magnitude faster than the slow bending oscillations of a polymer chain. Therefore, we cannot argue from the presence of the solvant to restrict our study to overdamped motions and the kinetic term in the Hamiltonian cannot be ignored.

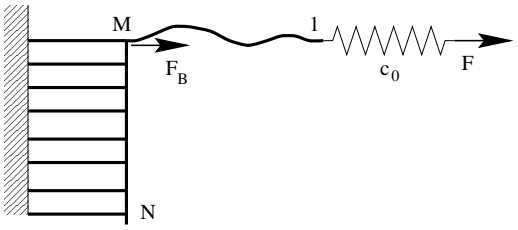

Fig. 1. – Schematic picture of the micro-mechanical denaturation of DNA. The actual experiment is symmetrical with respect to the hatched plane. The thick lines schematize the DNA molecule (undenaturated base pairs and denaturated strand) and the spring corresponds to the force lever in the actual experiment.

In a mechanical denaturation, the end of one strand is attached to a fixed point while the second strand is connected to an elastic lever. Moving apart the two points with a constant speed v tears apart the two strands while the deflection of the lever provides a measurement of the force F at the end of the denaturated strand. A schematic picture of the experiment is shown in fig. 1. The mechanical device can be included in the theory by introducing an extra term into the Hamiltonian which becomes $H = H_0 + (c_0/2)(y_0 - y_1)^2$, where c_0 is the elastic constant of the force lever and $y_0 = vt$ is constrained to move at a prescribed velocity v. The force F is given by $F = c_0(y_0 - y_1)$. A correct simulation of the actual experiment must include the thermal bath at temperature T which is coupled to all the molecular degrees of freedom y_1 to y_N while the thermal fluctuations of the displacement y_0 of the force lever, which is connected to a macroscopic apparatus, can be neglected. In our calculations, the thermal bath is simulated with a five-thermostat extended Nose-Hoover scheme [17].

Figure 2 shows the time evolution of the stretching force F deduced from a numerical simulation of a micro-mechanical denaturation experiment at temperature $T = 250$ K and a

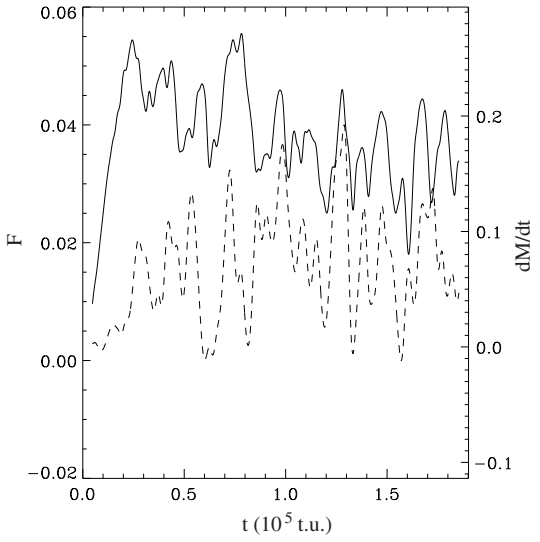

Fig. 2. – Time evolutions of the stretching force F (full line) and of the time derivative $\mathrm{d}M/\mathrm{d}t$ of the number of broken base pairs (dashed line) obtained from a molecular-dynamics simulation at temperature $T = 250$ K and stretching velocity $v = 0.0025$ Å/t.u.

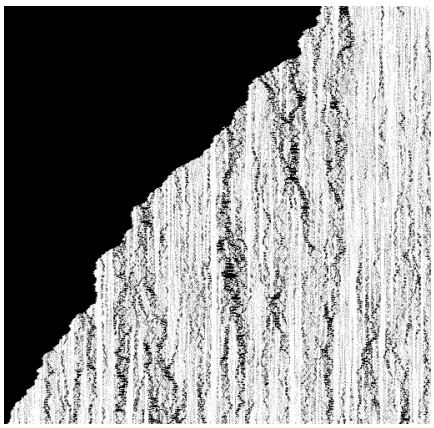

Fig. 3. – Gray scale picture of the stretching of the base pairs observed by molecular-dynamics simulation of the DNA model. The horizontal axis extends along the 512 base pairs investigated in the simulation. The vertical axis is the time axis. The figure shows a time interval of $1.25 \cdot 10^5$ t.u. The gray scale extends from white ($y = 0$), corresponding to base pairs which are closed, to black ($y > 1.5$) corresponding to base pairs which are highly stretched or broken. The black region at the left of the figure is the denaturated part of the molecule.

stretching velocity of $v = 0.0025$. Some very long simulations with stretching velocities 10 times smaller have been performed. They do not show significantly different results because, although the time scale is still very short compared to a real experiment, on a scale of 10 ns the system has reached a steady state. The force F exhibits large fluctuations similar to the ones which are observed in an actual experiment with natural DNA [6]. As shown in fig. 2, the origin of these fluctuations lies in large variations in the rate at which the number M of denaturated base pairs increases with time. Such fluctuations may seem surprising since the simulation is performed with a *homogeneous* DNA model where all base pairs are the same. In order to explain their origin, it is important to understand that the breaking of a given base pair is not purely mechanical. It is assisted by thermal fluctuations. This is why the measured force is always well below the critical force $F_c = D\alpha/2 = 0.089$ which corresponds to the mechanical breaking of a bond linked by the Morse potential $V(y)$, *i.e.* the slope of the Morse potential at the inflexion point. This is confirmed by a direct observation of the propagation of the breaking in the numerical simulation (fig. 3). In the part of the molecule which is not yet denaturated, this figure shows the typical pattern of nonlinear localization of thermal energy [5,8]. Moving along the molecule, one crosses regions where the amplitude of the base pair motions is very small (light grey) and darker regions where the base pairs undergo large fluctuations corresponding to the "breathing of DNA" observed experimentally [7,13]. The important point for the experiment is that these "cold" and "warm" regions persist over thousands of periods of the small-amplitude vibrations due to the stability of discrete breathers in a nonlinear lattice. As shown in fig. 3, when the front separating the open part of the molecule from the undenaturated part reaches a "cold" region it is temporarily blocked, and F increases. Then the build-up of elastic energy caused by the constant-speed motion of the lever that pulls on the denaturated strand becomes sufficiently high to induce a fast breaking of the base pair at the front, restarting the propagation of the breaking front, which releases the stress and F drops. It is important to notice that, contrary to the case of natural DNA [6], this result cannot be explained by an equilibrium theory that would lead to uniform breaking due to the translational invariance of the homopolymer. Therefore we must analyze

the kinetics of the breaking.

For a quantitative analysis, it is convenient to consider two aspects separately, the breaking of the base pair which is at the front (denoted by its index M along the chain) under the force $F_{\rm B}$ exerted by the denaturated strand on this base pair itself (fig. 1), and the way this local force is transmitted to the lever by the denaturated strand, giving rise to the force F which is measured.

The breaking of the M-th base pair under the stretching force $F_{\rm B}$ can be viewed as a chemical dissociation along the reactive coordinate y_M for a system that comprises the $N-M$ bases that have not been broken. It is described by the Hamiltonian

$$H_1 = \sum_{j=M+1}^{N} \frac{1}{2} m \left(\frac{{\rm d}y_j}{{\rm d}t}\right)^2 + W(y_j, y_{j-1}) + V(y_j) + \left[\frac{1}{2} m \left(\frac{{\rm d}y_M}{{\rm d}t}\right)^2 + V_{\rm eff}(y_M)\right],$$

where $V_{\rm eff}(y_M) = D\left[\exp[-\alpha y_M] - 1\right]^2 = -F_{\rm B} y_M$ is an effective potential which has a metastable minimum for $y_M \approx 0$, corresponding to the closed state of the M-th base pair. The breaking of the pair is obtained when y_M overcomes the barrier of $V_{\rm eff}$ under the effect of fluctuations coming both from the thermal bath and from the rest of the molecule due to the coupling potential $W(y_M, y_{M-1})$. The fluctuations transmitted by the molecule have a complex spectrum and, as shown in fig. 3, their amplitude may depend very significantly on the position along the chain, which explains why the denaturation does not proceed at uniform pace.

A quantitative calculation of the lifetime τ of base pair M amounts to solving the Kramers problem in the presence of a colored noise resulting from the nonlinear dynamics of the molecule, but an estimation of τ can be obtained by the multidimensional transition state theory [18] which gives

$$k_{\rm TST} = \frac{1}{\tau} = \frac{\langle \delta(y_M - Y_1) \dot{y}_M \theta(\dot{y}_M) \rangle}{\langle 1 - \theta(y_M - Y_1) \rangle},$$

where Y_1 is the base-pair stretching corresponding to the maximum of $V_{\rm eff}$, θ is the step function, and the averages $\langle \ldots \rangle$ have to be calculated with the Hamiltonian H_1. Introducing the transfer integral operator associated to the homogeneous part of H_1 ($j = M+1 \ldots N$), in the limit of large N where only the lowest eigenvalue contributes, we get

$$k_{\rm TST} = \frac{1}{\tau} = \frac{1}{\sqrt{2\pi m \beta}} \frac{\exp[-\beta V_{\rm eff}(Y_1)] \phi_0(Y_1)}{\displaystyle\int_{-\infty}^{Y_1} \exp[-\beta V_{\rm eff}(y_M)] \phi_0(y_M) {\rm d}y_M},$$

where ϕ_0 is the eigenfunction of the transfer integral operator that corresponds to its lowest eigenvalue. Although the limit of large N is formally taken in the calculation, actually the results only depend on the base pairs which are within the correlation length of the dynamics of the fluctuations, *i.e.* less than 10 base pairs at 250 K. For the model parameters of DNA a continuum limit approximation for the transfer integral operator is not valid and ϕ_0 has to be obtained numerically [8]. The calculation is the same as the one that has been performed to study the statistical mechanics of DNA denaturation. The expression of τ explains why the micro-mechanical denaturation of DNA can be very sensitive to energy localization because, in addition to the usual Arrhenius factor which already generates a fast dependence of τ upon temperature, the temperature dependence is magnified by the very fast dependence of $\phi_0(Y_1)$ upon T. The function ϕ_0 is highly peaked, and Y_1 corresponds to a point in its tail where the function is small. As the width of ϕ_0, which determines the mean stretching $\langle y \rangle$ of the base pairs, varies significantly with T, the relative value of $\phi_0(Y_1)$ can exhibit large variations for

small variations of T. The nonlinear energy localization, which creates the "warm" and "cold" regions seen in fig. 3, induces rapid changes in the propagation speed of the breaking, which are reflected by the force fluctuations. The TST underestimates the lifetime of the base pair so that, for a given mean value of F deduced from the numerical experiment, the theoretical value of τ is smaller than the actual mean value of the base pair lifetime $\overline{\tau} = 1/v$ imposed by the speed of the lever. The method can however be used to evaluate rather accurately the amplitude of the variations of the stretching force. Simulation data give extrema $\langle y \rangle_{\min}$ and $\langle y \rangle_{\max}$ for the mean value of the base stretching averaged over time and over the correlation length. From the transfer integral calculation of $\langle y \rangle$ one can associate an effective ϕ_0 eigenfunction of the transfer operator (corresponding to an effective temperature) to each of these mean values. From these functions we can calculate the values $F_{\min}$ and $F_{\max}$ corresponding to $\overline{\tau} = 1/v$. This calculation leads to $0.024 < F < 0.050$, in good agreement with the simulation results of fig. 2.

As the experiment is performed by slow stretching, the inertia of the denaturated strand can be neglected so that the mean values $\overline{F}$ and $\overline{F_{\mathrm{B}}}$ are equal. But the fluctuations of F include both the variations δF_{B} due to nonlinear energy localization and the thermal fluctuations ΔF of the elastic stress of the denaturated strand. A meaningful experiment can only be performed when $\Delta F \ll \delta F_{\mathrm{B}}$. This point was examined by Thompson *et al.* [11] who concluded that individual base pairs cannot readily be resolved but the stem-loop structure of RNA should be. The situation of the experiment that we discuss here is intermediate between these two cases, because, as explained above, the force fluctuations that we are considering *are not due to single base pair openings, but to collective denaturation of patches of 10 or 20 pairs.* Treating the denaturated strand as a harmonic chain with a coupling constant K, corresponding to the large-amplitude limit of the coupling potential W, ΔF can be estimated to $\Delta F = c_0[M k_{\mathrm{B}} T/K]^{1/2}$, *i.e.* $\Delta F = 0.012 \approx \delta F_{\mathrm{B}}/3$ at $T = 250$ K with the parameters of our simulation for a denaturated strand of 200 base pairs.

In conclusion, our results show that the mechanical denaturation of DNA is not only controlled by base sequence but also by nonlinear energy localization. This effect should be considered even when investigating inhomogeneous DNA because such experiments do not give a direct picture of base-pair binding energies. The pattern is modified by temporary localization of thermal energy. Our estimates suggest that a micro-mechanical experiment with homogeneous DNA would be feasible, although harder to perform than the recent studies of inhomogeneous DNA because the domains that break collectively are smaller. The length of the DNA segment which is required for an experiment is on the micron scale which forbids the use of a pure homopolymer. Using biological techniques it is however possible to insert a homopolymeric sequence in natural DNA molecules. The numerical simulations performed with 256 or 512 base pairs suggest that a sequence of a few hundreds of base pairs would be enough to detect the effect of the localized modes. The stretching velocity is the factor that would probably pose the highest challenge to experimentalists. In current experiments performed by pulling on the molecule with a glass needle [6] the stretching velocity is low and the lifetime of a base pair exceeds the lifetime of localized modes so that their effects are averaged out. This explains why a fairly good correlation between the stretching force and the base sequence can be observed in spite of the thermal fluctuations. We have used much higher stretching velocities obtained with stretching forces of about 60 pN instead of 3 to 10 pN in the experiment of Bockelman *et al.* The limitation imposed by the time scale of molecular-dynamics simulations which can hardly exceeds a few tens of ns does not allow us to extrapolate our results with a sufficient accuracy to provide a precise value for the optimal stretching velocity, but it is likely that the most appropriate technique to probe nonlinear energy localization in DNA will be atomic force microscopy rather than micro-manipulations

with a glass needle. This experiment would certainly be at the boarder of present experimental abilities but it would be of high interest because it would provide the first direct experimental evidence of nonlinear energy localization in a lattice.

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