Confinement-Induced Entropic Recoil of Single DNA Molecules in a Nanofluidic Structure

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The behavior of DNA molecules is observed in a nanofluidic device near the interface of two regions that produce different configuration entropies. An electric field is applied to drive the molecules partway across the interface. Upon removal of the field, the molecules recoil to the higher-entropy region with a profile characteristic of a force localized to the interface and independent of length. This is consistent with a confinement-mediated entropic force, distinct from the well-known entropic elasticity common to all polymers. An estimate of the hydrodynamic drag is used to produce a lower bound for the force. The phenomenon can be exploited to separate long-strand polyelectrolytes according to length.

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Nanofabrication and nanometer-scale fluidic structures have recently provided new tools for the exploration of molecular behavior at the single-molecule level. Micro- and nanofluidics are expected [1] to find significant applications in biotechnology and medicine, so the study of molecular dynamics in confined environments is relevant. When the dimensions of a vessel are comparable with the size of an enclosed molecule, confinement is expected to introduce new behavior due to the prohibition of conformations that would otherwise be available to a molecule free in solution. When the number of available conformations varies with position, the consequent gradient of entropy, and thus free energy, produces a force. Here we report the first direct observations of polymer motion caused by a confinement-mediated entropic force. Individual DNA molecules stained with fluorescent dye were observed in a nanofabricated structure while recoiling from a region constrained by 35 nm pillars densely spaced between a floor and ceiling 60 nm apart. Velocity measurements closely fit the behavior expected for a confinement recoil force, and produce a lower bound for the force of 5.7 fN for these conditions.

The existence of entropic forces was suggested first by Meyer, Susich, and Valkó [2] in 1932 to explain the elasticity of polymer strands in rubber, and developed shortly after by Kuhn [3]. Since then numerous theoretical [4,5] and experimental treatments of entropic elasticity have appeared in the literature, including observation of entropic elasticity in single DNA molecules [6–8] and even single strands of nonbiological polymers [9]. Early theoretical treatments of confinement and its effect on entropy came from Flory [10], Casassa [11], and Edwards [12] but had to wait for experimental support until the idea of entropic trapping [13–17] was invoked to explain anomalous mobilities observed for DNA electrophoresis in certain regimes. More recently, entropic trapping has been demonstrated with bulk methods [18], and observed on the microscopic scale with DNA in a nanofluidic device [19]. These observations led to a revised understanding of how polymers escape from entropic traps [20], wherein thermal fluctuations drive a small portion of the chain over the entropic barrier and this extension subsequently pulls over the rest of the molecule.

If a DNA molecule is driven to overcome an entropic barrier by electric field and arrested during its passage, so that part of the molecule remains in the more energetically favorable high-entropy region, this part is expected to create an impetus for the molecule to evacuate the low-entropy region and return to the high-entropy region. To observe this motion a nanofluidic device (Figs. 1 and 2) was employed with an entropic barrier formed by an abrupt interface between regions of high and low entropy for a DNA molecule. To achieve the desired entropy modulation the two regions must differ in the degree of confinement they impose. This can be accomplished by varying the width of a tube or height of a slit, or by introducing a mesoporous material. In this work the entropy difference is established by populating some regions of a quasi-two-dimensional nanofluidic structure with densely spaced nanopillars between the floor and ceiling. The dense pillars act as

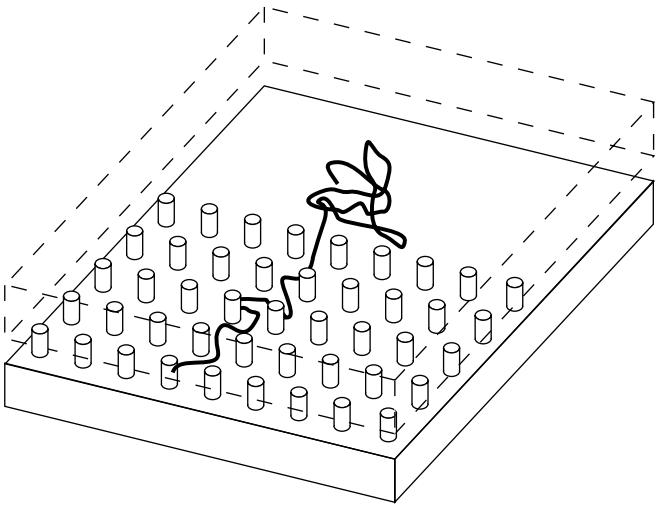

FIG. 1. The fluidic device consists of a quasi-two-dimensional gap between a floor and ceiling approximately 60 nm in height. Some regions of the device are populated with nanopillars.

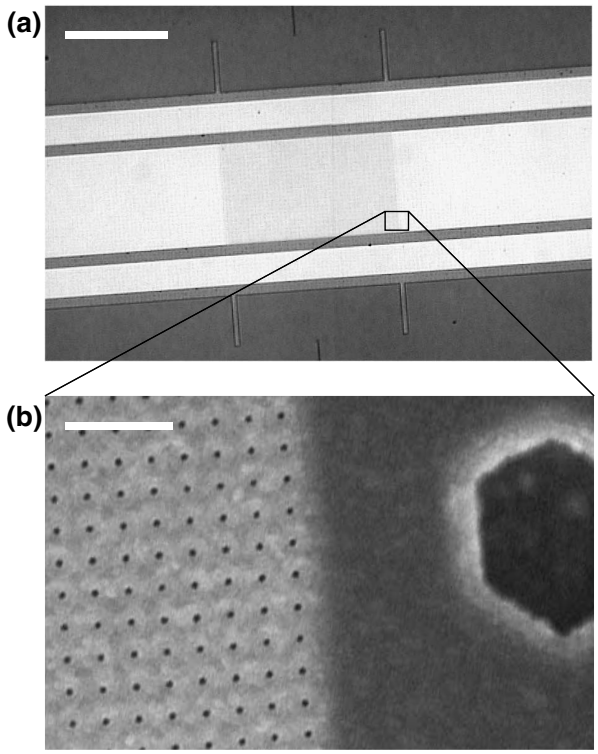

FIG. 2. (a) An optical micrograph of the device. Though the pillars are too densely spaced to scatter light, they change the effective index of the region, so it is visible in slight contrast to the neighboring pillar-free region. The scale bar represents 0.5 mm. Note the inset box is not drawn to scale. (b) A scanning electron micrograph of the abrupt interface between the high and low entropy regions. The pillars are approximately 35 nm in diameter with center-to-center spacing of 160 nm. The large hexagonal structure is one of many ceiling supports. They are spaced such that they do not affect the confinement entropy. The scale bar represents 500 nm.

an artificial gel or mesoporous solid. Because of the regularity afforded by the nanofabrication techniques [21] used to make these structures the entropy in each of the regions can be made sufficiently uniform that only molecules that straddle the abrupt interface will have an impetus to move. This situation can be compared with a slippery chain falling from a table. If the chain is either entirely on the table or entirely on the floor, it will not move. However, if it is placed with part on the table and part on the floor, it will eventually all spill onto the floor.

The development of a model to predict the motion of a recoiling molecule benefits from several simplifying conditions in these experiments. First, entropy is approximately proportional to length. Self-avoidance of the molecule tends to reduce the entropy from its ideal chain value, but for stiff molecules this effect is small until the molecule is very long, and even then the correction is logarithmic only in length [22]. The point where the molecule emerges from the dense-pillar region acts as a tether for the free end of the molecule. This creates a further reduction in entropy that also depends logarithmically on chain length, so for long strands this reduction is independent of length and can thus be neglected in computing forces [23]. The part of the molecule in the dense pillar region can also be treated as having constant entropy per unit length, though the confining effect of the pillars requires that the value for this region be regarded as an *effective entropy* rather than the true equilibrium entropy. This is due to the fact that many of the accessible states of the molecule do not effectively communicate with each other on the time scale of the measurement. These approximations lead to an expression for the entropy of

$$S = L_1 s_1 + L_2 s_2 , \tag{1}$$

where L_1 and L_2 are the lengths occupying regions 1 and 2, respectively, and s_1 and s_2 are the characteristic entropies per unit length of the two regions. The free energy $F = U - TS$ and the force f is dF/dL_1, where region 1 is taken to be the dense-pillar region. An immediate consequence of this is that the entropic force is approximately constant, irrespective of the length of DNA on either side of the interface.

The simplest model for how the molecule should recoil relates the velocity to the force and viscous drag coefficient by $f = g\upsilon$. When, as in these experiments, the molecule is long compared with the hydrodynamic screening length (which in this case is dominated by vertical confinement) the drag coefficient is approximately proportional to the molecule length [8]. Thus we can write the drag coefficient in terms of the specific drag, ρ, as $g = \rho L_1$. Combining these equations leads to a first-order differential equation,

$$\frac{dL_1}{dt} = -\frac{f}{\rho L_1} . \tag{2}$$

This can be integrated to give

$$L_1 = \sqrt{-\frac{2f(t - t_0)}{\rho}} , \tag{3}$$

where t_0 is the time at which the molecule completes its recoil. For all times before that $(t - t_0)$ is negative.

The fluidic devices were manufactured using silicon nitride chemical vapor deposition and electron beam lithography using a sacrificial layer removal method which allows flexible and precise control over the interior geometry of the device [21]. Silicon nitride is transparent for visible light and allows direct visualization of fluorescent molecules through the walls of the device. T2 phage DNA molecules, with a fully stretched length of nearly 51 μm, were suspended in Tris-borate EDTA buffer and stained with YOYO-1 dye according to methods described elsewhere [20]. With the buffer concentration used in these experiments, the Debye screening length is about 0.7 nm. Since YOYO-1 is an intercalating dye, it allows measurement of not just the position but the entire contour of the molecule.

T2 molecules were accumulated at the boundary of the dense-pillar region using a weak electric field (0.25 V/cm). Then, pulses of field at 5 V/cm were

applied for intervals between 2 and 5 sec, driving the assembly of molecules partially into the unfavorable region. The recoil of the molecules into the pillar-free region was recorded using fluorescence videomicroscopy and an intensified CCD video camera. A selection of images (Fig. 3) taken from a video sequence acquired this way shows the recoil [24]. Position data for the ends of the recoiling molecules were extracted from images as in Fig. 3 for analysis. Figure 4 shows the end-point positions as a function of time for 56 recoil events. Also shown superimposed is the average of the 56 trajectories and a fit to it using the model in Eq. (3). Entropic elasticity has been shown both theoretically [25] and experimentally [26,27] to produce recoil, but these observations have shown uniformly that elastic recoil is initially rapid followed by a gradual slowing, whereas here the trend is opposite; the recoil is initially slow and gradually increases in speed. This suggests the presence of a confinement-entropic force, distinct from entropic elasticity. Interestingly, both of these exhibit square-root time dependence, but with opposite sign on the time variable. Entropic elasticity can be further ruled out as the motive force here by noting that molecules entirely in the dense-pillar region contract only slightly during the time the nearby molecules recoil completely (note the molecule at the left of the panel showing $t = 76.5$ s in Fig. 3), and that elastic recoil could not produce a center-of-mass motion as is observed here. Electrostatic interactions could produce similar effects, but the Debye screening length at these buffer concentrations is too short to allow significant electrostatic interactions with charges on the pillars. Chain-chain interactions can also be rejected because the negative backbone charges are mutually repulsive, tending to elongate rather than coil the molecule.

Because the t_0 values for the various curves have been set to zero, the constant-force fit has just one free parameter, the ratio f/ρ, having a value from the fit of $3.1 \pm 0.1\ \mu\text{m}^2/\text{s}$. This determines the magnitude of the entropic force if the hydrodynamic drag of the molecule is known. To estimate the hydrodynamic drag, the DNA molecule can be modeled as a cylinder moving longitudinally in the center of a slit. The pillars are neglected in this approximation, as are deviations of the molecule from the center of the slit. In addition, during the recoil process, the DNA will not be fully stretched. Hence, bunching of a molecule will cause it to have an effective hydrodynamic radius larger than the hard-cylinder radius of a DNA strand. Because all three of these effects tend to make this model underestimate the drag force, the resulting figure represents a lower bound for the entropic force.

A useful result from the mechanics of viscous flow is that the longitudinal motion of a cylinder-in-slit has the same force-velocity relation as a cylinder-in-cylinder,

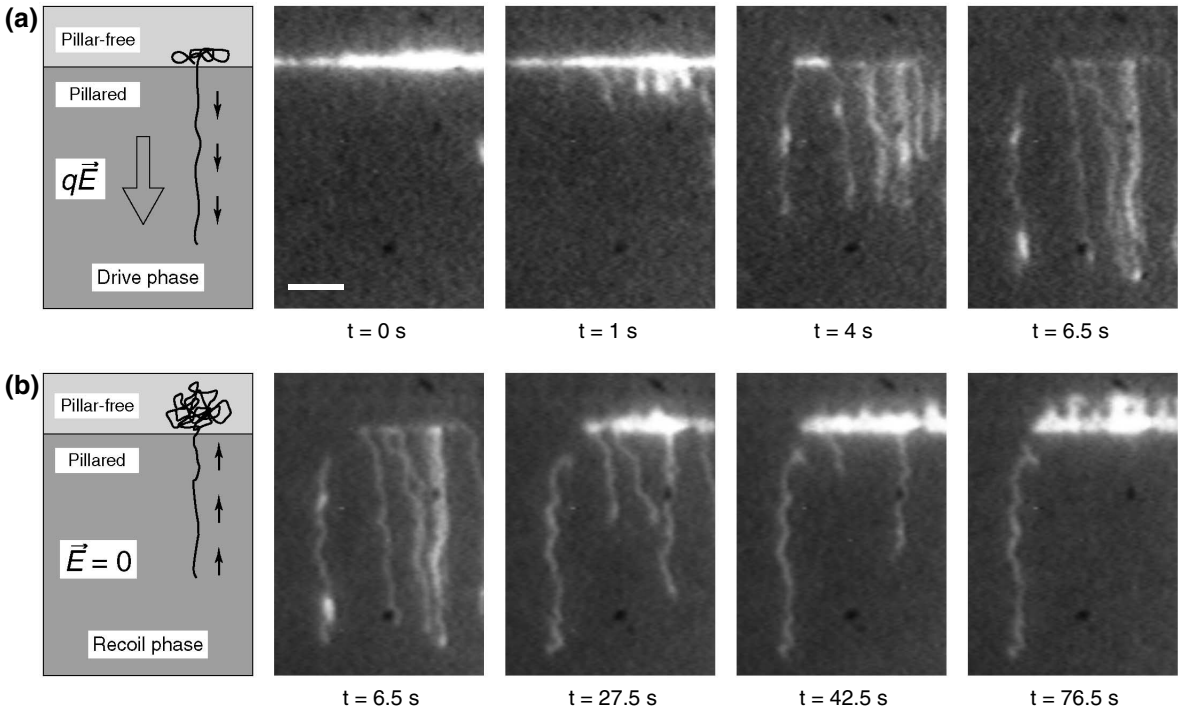

FIG. 3. (a) DNA molecules are driven into the dense-pillar region, which occupies the bottom 80% of each frame. Six or seven molecules are shown, one (on the left) having been completely lodged in the unfavorable region. Occasionally molecules enter the dense-pillar region by herniation. However, these hernias quickly unfold in the electric field once an end of the molecule has been incorporated. The scale bar represents 5 μm. (b) Entropic recoil at various stages ending when all the molecules have recoiled except the leftmost. Without the interaction between the two regions, this molecule experiences no center-of-mass motion and only slight contraction. This effect shows the contribution from entropic elasticity is negligible and immediately suggests a method for separation of long-strand polymers in accordance to length.

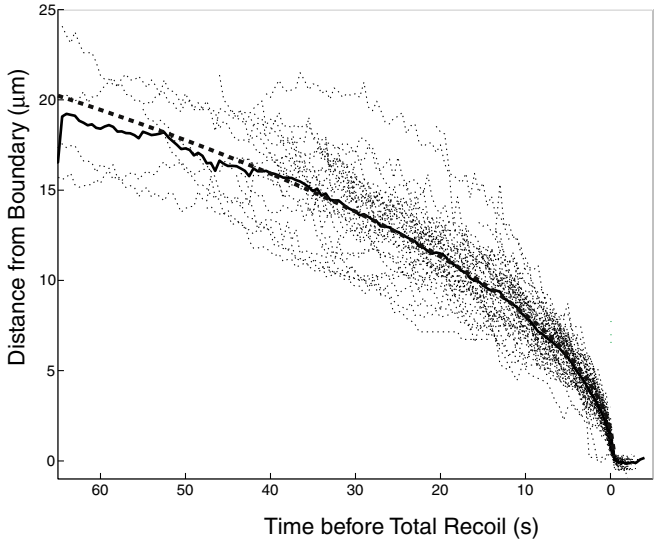

FIG. 4. The distal-end position for 56 recoil events as a function of time. The t values have been shifted so that $t_0 = 0$ for all events. The thin dashed lines show the position data for the individual events. The solid black line is the average of these traces. The heavy dotted line is a fit to the data using Eq. (3).

except with an effective diameter 27% larger than the floor-to-ceiling height of the slit. Using this, the hard-cylinder diameter of DNA, and the floor-to-ceiling height of the device, we estimate the specific drag, ρ, of a DNA chain in this environment to be 1.82 fN s/μm^2. This leads to a lower bound of the entropic force, $f = 5.7$ fN. The actual hydrodynamic drag is significantly higher than this. From Fig. 3 it is evident that the molecules are extended to only approximately 35% of their fully stretched length. This will increase the drag, and hence our figure for the entropic force, by a similar ratio. A rough theoretical estimate of the expected force can be made by observing that the entropy difference between the two regions will be on the order of k_b per Kuhn length, or about 40 fN.

All available evidence indicates that the motion is entropic in nature, and due to confinement rather than stretching. This mechanism of polymer motion is expected to apply to all linear polymers, and may play a role in translocation of polymers in biological processes. The complete lack of recoil for molecules that fully clear the pillar-free region allows for the separation of long-strand polyelectrolytes on the basis of length. When molecules of dissimilar length are present, shorter strands will clear the interface in a shorter time than longer strands. When the field is removed, those molecules with some portion still in the pillar-free region will recoil, effecting a spatial separation from the shorter molecules that clear the interface. A careful study of recoil velocity as a function of array dimensions and other parameters will provide a means to test our understanding of how entropy decreases with confinement.

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