Electrophoretic stretching of DNA molecules using microscale T junctions

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(Received 30 March 2007; accepted 9 May 2007; published online 30 May 2007)

Controlled trapping and stretching of DNA molecules are critical for single molecule genomic and polymer physics studies. The authors present a microfabricated T junction which can trap and stretch single *free* DNA molecules using electrophoretic forces. The device does not require special end functionalization of the DNA. They show that two physical mechanisms of stretching can occur depending on the length of the DNA relative to the channel width in the junction region. Stable trapping and stretching of DNA molecules up to lengths of 485 kbp are demonstrated. © 2007 American Institute of Physics. [DOI: 10.1063/1.2745650]

The ability to trap and stretch biopolymers is important for a number of applications ranging from single molecule DNA mapping¹ to fundamental studies of polymer physics.² Optical or magnetic tweezers can be used to trap and stretch single DNA molecules, but they rely on specific modification of the DNA ends.³ Alternatively, one end of the DNA can be held fixed and the molecule stretched with an electric field⁴ or hydrodynamic flow.⁵ Untethered *free* DNA can be driven into nanochannels to partially stretch molecules.^{6,7} Hydrodynamic planar elongational flow generated in a cross-slot geometry has been used to stretch free DNA⁸ but trapping a molecule for a long time at the stagnation point is not trivial.⁹ Electric fields have been used to either confine molecules in a small region in a fluidic channel¹⁰ or to partially stretch molecules as they electrophorese past obstacles,^{11–13} into contractions¹⁴ or through cross-slot devices.¹⁵ Partial stretching occurs in these aforementioned electrophoresis devices because the molecule has a finite residence time¹⁴. Currently, simple methods do not exist to trap and stretch DNA or other charged biomolecules.

DNA can be physically envisioned as a series of charges distributed along a semiflexible Brownian string. Molecules can be electrophoretically stretched due to field gradients that vary over the length scale of the DNA. Deformation of a DNA will depend on the details of the kinematics of the electric field.^{12,16} Electric fields are quite unusual in that they are purely elongational.^{12,15,16} Here, we present a microfluidic device which is able to trap and stretch biomolecules using electric field gradients.

We investigate the stretching of DNA molecules in a symmetric channel consisting of a narrow T-shape part in the center and three identical wide parts outside, as shown in Fig. 1(a). The vertical part and horizontal part of the T junction have the same length l_2 while the width of the vertical part is twice the width of the horizontal part: $w_2=2w_3$. Hence the T junction is equivalent to half of a cross-slot channel. The dimensions used in this study were $l_1=1$ mm, $l_2=3$ mm, $w_1=80 \ \mu$ m, $w_2=40 \ \mu$ m, and $w_3=20 \ \mu$ m. In order to suppress the local electric field strength maximum, the two corners of the T junction were rounded using an arc with radius $R=5 \ \mu$ m [Fig. 1(c)]. When symmetric potentials are applied to the channel in a manner, as shown in Fig. 1(b) a

Because $l_1, l_2 \gg w_3$, a simple circuit, as shown in Fig. 1(d), can be used to analogize this channel. The center T-junction region is neglected and each straight part of the channel is represented with a resistor with resistance proportional to l/w. The potential at each point indicated in Fig. 1(d) can be solved analytically. The resulting field strengths in uniform regions 1 and 2 are given by

$$|\mathbf{E}_1| = |\mathbf{E}_2| = \frac{\Phi}{3l_1(w_3/w_1) + 2l_2}.$$
(1)

As a result, the resulting extensional field in the T junction is nearly homogeneous. The electrophoretic strain rate is approximately given by $\dot{\epsilon} \approx \mu |\mathbf{E}_1|/w_3$, where μ is the electrophoretic mobility. For the remaining analysis, we nondimensionalize the variables:

$$\hat{x} = \frac{x}{w_3},$$

$$\hat{y} = \frac{y}{w_3},$$

$$\hat{\mathbf{E}} = \frac{\mathbf{E}}{|\mathbf{E}_1|},$$

$$\hat{\epsilon} = \dot{\epsilon} \frac{w_3}{\mu |\mathbf{E}_1|}.$$
(2)

In Fig. 2(a) we show a finite element calculation of the dimensionless electric field strength $|\hat{\mathbf{E}}|$ in the region around the T junction. We assume insulating boundary conditions for the channel walls. Although the corners have been rounded, there is still a small local maximum in field strength at the corners. Figure 2(b) shows the dimensionless field strength and strain rate in the junction. Due to symmetry, the data along $\hat{y}=0$ and $\hat{x}=0$ overlap. The electric field and strain rate for an idealized T channel without any end effects are indicated by the dotted lines. The entrance (or exit) region starts at about 30% of the length w_3 before the entrance (or

0003-6951/2007/90(22)/224103/3/\$23.00

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local planar elongational electric field with a stagnation point can be obtained within the T junction and uniform fields in the three straight arms. We use \mathbf{E}_1 and \mathbf{E}_2 to represent the uniform electric field obtained in uniform region 1 and uniform region 2, respectively.

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FIG. 1. (Color online) Schematic diagrams of (a) channel geometry, (b) location of uniform/elongational fields and stagnation point, (c) expanded view of T junction, and (d) circuit analogy of the channel.

exit) of the T junction and extends a full length of w_3 into the uniform straight region. Within the T junction, there is a homogeneous elongational field, but the strain rate is $\approx 0.74 \mu |\mathbf{E}_1|/w_3$ due to entrance/exit effects. The field kinematics was experimentally verified using particle tracking.¹⁷

We use soft lithography¹⁸ to construct 2 μ m high polydimethylsiloxane microchannels. T4 DNA [165.6 kbp (kilobasepairs), Nippon Gene] and λ -DNA concatomers (integer multiples of 48.5 kbp from end-to-end ligation, New England Biolabs) were used in this study. DNA were stained with YOYO-1 (Molecular Probes) at 4:1 bp:dye molecule and diluted in 5×TBE (0.45*M* tris borate, 10 mM EDTA) with 4 vol % β -mercaptoethanol to a final concentration of 0.07 μ g/ml. The stained contour lengths are 70 μ m for T4 DNA and integer multiples of 21 μ m for λ -DNA concatomers. The bottom two electrodes were connected to two separate dc power supplies and the top electrode was grounded. Molecules were observed using fluorescent video microscopy.¹³

In a typical experiment, we first applied symmetric potentials to electrophoretically drive DNA molecules into the T-junction region and then trapped one molecule of interest at the stagnation point of the local extensional field [Fig. 3(a)]. With the application of two power supplies we were able to adjust the two potentials individually and therefore freely move the position of the stagnation point. This capability of stagnation point control allowed us to trap any DNA molecule in the field of view even if it initially did not move toward the stagnation point. Furthermore, we could also overcome fluctuations of a trapped molecule. For example, if a trapped DNA begins to drift toward the right reservoir, the



FIG. 2. (Color online) (a) Dimensionless electric field strength in the T-junction region from finite element calculation. The white lines are the electric field lines. (b) Dimensionless electric field strength and strain rate along the $\hat{y}=0$ or $\hat{x}=0$ trajectory. The dotted lines are for an ideal T junction without entrance or exit effects.



FIG. 3. (a) Stretching of a T4 DNA trapped at the stagnation point in the T channel (0.17 s between images) at De=2.0. (b) The steady state behavior of a T4 DNA (0.33 s between images). The molecule began to drift towards the left, then was pulled back by stagnation point control. (c) The mean steady state fractional extension of T4 DNA vs De. Each point represents the average of 15-30 molecules.

potential applied in the left reservoir can be increased so that the position of the stagnation point would reverse the direction of the drifting molecule [Fig. 3(b)]. The DNA solution was sufficiently dilute such that only one molecule entered the T junction at a time. Even with manual control of the power supplies, we found it was quite facile to capture a selected DNA.

The T4-DNA in Fig. 3 has a maximum stretch of $\approx 50 \ \mu$ m and extends just slightly beyond the region in the T junction where homogeneous electrophoretic elongation is generated. The dimensionless group which determines the extent of stretching in this region is the Deborah number $De=\tau \dot{\epsilon}$ where τ is the longest relaxation time of the DNA (measured¹⁷ to be 1.3 ± 0.2 s). In Fig. 3(c) we see that strong stretching occurs once De>0.5, similar to what is observed in hydrodynamic flows.⁸

We next tried to stretch molecules which have contour lengths much larger than $2 \times w_3$ (40 μ m). In Fig. 4 we show the stretching of a concatomer of λ -DNA which has a contour length of 210 μ m (10-mer, 485 kbp). As the molecule enters the T junction it is in a coiled stated with mean radius of gyration $\approx 2.7 \ \mu m.^{19}$ Initially the stretching is governed by De due to the small coil size. However, as the arms of the DNA begin to extend into regions of constant electric field, stretching occurs due to a different mechanism. For stretched lengths $\geq 2 \times w_3$, the chain resembles a set of symmetrically tethered chains (with contour lengths one-half that of the original chain) in a homogeneous electric field. Stretching still occurs but is now governed by the $Pe = \mu E l_p / D_{1/2}$, where is the electrophoretic mobility $[(1.35 \pm 0.14)]$ μ $\times 10^{-4}$ cm²/s V], l_n is the persistence length (\approx 53nm), and

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FIG. 4. Stretching of a λ -DNA 10-mer in the T channel (0.33 s between images) at $|\mathbf{E}_1|$ =45 V/cm (Pe \approx 52). For ease of presentation, each frame was centered at *x*=0.

 $D_{1/2}$ is the diffusivity of a chain with a contour length half that of the original chain [$\approx 0.062 \ \mu m^2/s$ for this 10-mer (Ref. 19)]. The molecule in Fig. 4 reaches a final steady state extension which is 94% of the full contour length.

Our DNA trapping and stretching device has several advantages over other methods. Electric fields are much easier to apply and control and their connections have smaller lagtimes than hydrodynamic fields in micro-/nanochannels. Further, the purely elongational kinematics of electric fields are advantageous for molecular stretching. The field boundary conditions also allow for the use of only three connecting channels to generate a homogeneous elongational region and straightforward capture of a molecule by adjusting the stagnation point. Stretching can occur even beyond the elongational region due to a molecule straddling the T junction and feeling a tug-of-war on the arms by opposing fields. The fabrication is also quite simple compared to nanochannels and the design allows for facile capture, stretch, and release of a desired molecule. In the future, devices will be designed to feed one molecule at a time into the T junction.

The authors acknowledge funding from the MIT Center for Environmental Health Sciences (NIEHS P30 ES002109).

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