Electrophoretic Stretching of DNA Molecules in Cross-Slot Nanoslit Channels

Anthony G. Balducci, Jing Tang, and Patrick S. Doyle

Macromolecules, 2008, 41 (24), 9914-9918 • DOI: 10.1021/ma8015344 • Publication Date (Web): 24 November 2008

Downloaded from http://pubs.acs.org on March 3, 2009

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML
Electrophoretic Stretching of DNA Molecules in Cross-Slot Nanoslit Channels

Anthony G. Balducci,† Jing Tang,† and Patrick S. Doyle* 

Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received July 8, 2008; Revised Manuscript Received September 15, 2008

ABSTRACT: A nanofluidic cross-slot device is designed and fabricated to investigate the effects of slitlike confinement on the electrophoretic stretching of single DNA molecules. The device is capable of trapping and stretching single DNA molecules at the stagnation point of a homogeneous planar elongational electric field. Different from studies of unconfined DNA, the longest relaxation time in slitlike confinement is extension-dependent, and we find the higher extension relaxation time allows better prediction of the drastic increase of extension with applied strain rate in confinement. The low extension relaxation time is important in polymer rotation and small deviations from equilibrium.

I. Introduction

The development of nanofabricated devices capable of confining single DNA molecules creates the potential for altering and control the DNA shape and dynamics. A series of recent single molecule studies have characterized DNA conformation and dynamics at equilibrium in different types of confinement, for instance, tubelike (quasi-one-dimensional) confinement and slitlike (quasi-two-dimensional) confinement. However, in a number of applications ranging from DNA separation to genomic mapping, significantly deformed molecules are important. Recently, nanoslot confinement in one-dimensional (tubelike) channels has been used to create highly extended DNA of interest in particular to direct mapping methods. Here, we report a facile method for dynamically trapping and stretching single molecules in slitlike nanoconfined at a stagnation point. The molecular extensions attained here match those in the most extreme tubelike confinement, but in slitlike channels with photolithographically defined dimensions 3 orders of magnitude larger than the tubelike case. Also, since stretching the polymer can alter the interactions of the polymer with the confining walls, the dynamics of the molecule can vary with extension, making this problem interesting from a fundamental polymer physics standpoint. Even with a recent surge in the research being done in this area (see ref 21 for a recent review), a complete understanding of polymer dynamics in confinement is lacking.

Recently, we found that in slitlike confinement the time scale governing the slowest stress relaxation of single DNA molecules depends on the molecule’s extension. Unlike Rouse or Zimm modes, the relaxation of the molecule very near equilibrium and at higher extensions is best described by two different time constants (see Figure 1); the low extension relaxation time \( \tau_L \) and the higher extension relaxation time \( \tau_H \). In contrast, experiments on unconfined DNA show that a single time constant governs relaxation dynamics in the entire linear force regime (from equilibrium to \( \sim 30\% \) fractional extension). We found that the emergence of the extension-dependent relaxation time is due to the presence of the confining walls. An initially stretched molecule is not sterically confined by the channel walls, but as it relaxes, the lateral dimensions of the molecule grow and the steric confining effects eventually become important. In a simple model which describes the polymer relaxation using a tension-blob framework (see Figure 1), the crossover point where the relaxation time changes occurs when the dimension of the blobs is equivalent to the height of the channel. Before this point, relaxation happens through increasing size of the tension blobs along the chain. During this process, the molecule is no longer sterically confined and can no longer grow. Here, the confining walls also change the spring force of the molecule, in addition to modulating the hydrodynamic drag. The extension at the crossover point, termed as the crossover extension \( X_{ex} \), can be estimated as \( X_{ex} = hN_{blobs} \), where \( h \) is the channel height and \( N_{blobs} \) the number of blobs. By assuming good solvent quality within blobs, a scaling relationship for \( X_{ex} \) was derived as \( X_{ex} \sim h^{-2/3}l_p^{1/3}w^{1/3}L_c \), where \( l_p \), \( w \), and \( L_c \) are the persistence length, effective width, and contour length of the DNA, respectively. The crossover extension depends on the channel height, the size of the DNA, solvent quality, and ionic strength through dependencies in \( w \) and \( l_p \). It is important to note that both relaxation times in confinement are significantly greater than the unconfined relaxation time, pointing to the fact that confinement may allow stretching of DNA molecules at smaller deformation rates, as described below. The purpose of...
this work is to examine the effects of these newly observed relaxation 
processes on the electrophoretic stretching of single 
DNA molecules in slitlike nanoflows.

Polymer deformation in homogeneous extensional 
oflows or fields is a balance of the stretching 
forces applied by the flow or field and the polymer’s 
entropic elasticity tending to recoil 
the molecule.\cite{24} In the unconfined case, a dimensionless group 
termed the Deborah number is typically used to characterize 
this balance. The Deborah number is defined as the product of 
the deformation rate of the field or flow (the strain rate $\dot{\varepsilon}$) 
and the polymer’s longest relaxation time ($\tau$): $De = \dot{\varepsilon} \tau$. A large 
change in extension with $De$ is found\cite{25-27} to occur near the 
theoretically predicted\cite{28} critical value of $De_{crit} \approx 0.5$. The fact 
that the longest relaxation time is extension-dependent in 
slitlike confinement brings some ambiguity to the prediction of where 
this drastic deformation occurs. Therefore, we define two 
Deborah numbers for the current problem and characterize the 
role of each in determining the DNA behavior. $De_1 = \dot{\varepsilon} \tau_1$ is 
defined using the higher extension relaxation time, which 
governs relaxation above the crossover extension $X_{ex}$ to the onset 
of the linear force regime ($\sim$30% fractional extension). $De_2 = \dot{\varepsilon} \tau_2$ is 
defined using the low extension relaxation time, which 
governs dynamics near equilibrium. The correct prediction of 
the required deformation rate to achieve a certain extension is 
important in the design of devices aiming to exploit confinement 
to manipulate DNA molecules.\cite{1,29}

II. Experiments

To investigate confinement-induced changes on stretching DNA, 
we place single DNA molecules in homogeneous extensional 
electric fields under varying degrees of confinement. Electric fields 
are employed to move and stretch DNA because the kinematics 
are purely elongational at length scales larger than the Debye length 
(here $\sim$ 3 nm) and deformation due to shear can be neglected.\cite{29} In 
addition, electric fields are much easier to implement than pressure- 
driven flows for nanoscale devices. In planar elongational 
electrophoretic deformation, the electrophoretic velocity of a point charge 
varies linearly with position:

$$v_x = \mu E_x = \varepsilon_x$$

$$v_y = \mu E_y = -\varepsilon_y$$

where $v_x$ and $v_y$ are the velocities in the $x$ and $y$ directions, 
respectively, $E_x$ and $E_y$ are the electric fields in the $x$ and $y$ 
directions, respectively, $\mu$ is the electrophoretic mobility, and $\varepsilon$ is 
the strain rate. Previous studies have used cross-slit\cite{25,26,31,32} and 
T\cite{27} channels to achieve these kinematic conditions. In tall channels 
the large spans used to create $O(100 \mu m)$ regions of constant strain 
rate are not an issue. However, even slight sagging due to large 
spans in nanochannels affects the strength of the field and may 
cause pinch-off of the channel. Cross-slot nanoslit channels with 
the incorporation of hyperbolically curved sidewalls (see Figure 2) 
are implemented in this study. Since the shape of the sidewalls 
matches exactly the streamlines in homogeneous extensional fields, 
there are no inhomogeneities to disrupt the linear electric field 
profile over the entire intersection region.\cite{33} This development 
minimizes the span needed to create $O(100 \mu m)$ regions of 
homogeneous deformation. Independent control of the potential 
applied to the side reservoirs allows movement of the stagnation 
point via manually providing slight perturbations to the field.\cite{27} 
These small adjustments allow the entrapment of DNA molecules 
at the stagnation point for very high accumulated strains (up to 50 
Hencky strain units $= t_{ex}$, where $t_{ex}$ is the molecule’s residence 
time in the field). Furthermore, confinement of the molecule within 
the focal plane ensures it remains in focus for the entire observation. 
(For experimental demonstration of the trapping ability of the 
device, see movie S1, Supporting Information.)

![Figure 2](image-url)

DNA Molecules in Cross-Slot Nanoslit Channels
Persistence length of the DNA molecules under the experimental buffer condition is \( l_p \approx 53 \text{ nm} \). Our epifluorescence microscopy and detection setup as well as data analysis and extraction of the extension and principal axis of the radius of gyration tensor are described elsewhere.\(^{11,20}\)

**Electric Field Characterization.** The electric field kinematics generated in the intersection region of all cross-slot devices were verified by tracking the center of mass of electrophoresing DNA under conditions in which they do not appreciably deform. \( \lambda \)-DNA was used as the tracer since both relaxation times (\( \tau_1 \) and \( \tau_2 \)) are not large enough to yield significant deformation at the electric fields employed. Figure 2C shows the center of mass position of 189 \( \lambda \)-DNA molecules as they electrophorese through the 2 \( \mu \)m tall channel. The role of the hyperbolically shaped sidewall is easily observed as no disruption to the streamlines occurs even very near the walls. Panels D and E show the experimental determination of the strain rate as the slope of the position versus time plots on semilog scales. The strain rate is indeed uniform in the intersection region of the channel, and experiments at different applied voltages confirmed that the strain rate is linear with applied electric field for all channel heights used (data not shown). The strain rate was calibrated against applied voltage prior to each experiment.

**Relaxation Time Measurements.** Measurement of the longest relaxation time occurred in the same channel used for the stretching experiments. A T4 DNA molecule was stretched to nearly full extension in a high field gradient at the stagnation point, the field was switched off, and the relaxation of the molecule was observed. Two distinct time constants were obtained for the two nanochannels, as expected.\(^{20}\) In the 2 \( \mu \)m tall channel, T4 DNA is not sterically confined,\(^{20}\) and thus only one time constant exists. The relaxation time was fit using the equation

\[
\frac{(X_{\text{ex}}^3) - (X_{\text{eq,ex}}^3)}{L_c} = A \exp \left( \frac{-1}{\tau} \right)
\]

(3)

where \( X_{\text{ex}} \) is the extension of the molecule in the stretched \((x)\) direction, \( X_{\text{eq,ex}} \) is the equilibrium extension in the stretched direction (measured after more than 10 relaxation times after turning off the field), \( L_c \) is the contour length of the T4 DNA molecule (70 \( \mu \)m), and \( \tau \) is time. \( A \) and \( \tau \) are fitted parameters. Fitting regions for the two time constants are the same as those described previously.\(^{20}\) Relaxation times for T4 DNA measured here are summarized in Table 1.

**T4 DNA Stretching Experiments.** T4 DNA was used for the stretching experiments. A typical molecule was moved into the channel intersection and allowed to rest for typically 10 longest relaxation times (\( \tau_2 \) for the nanochannels). The field was then switched on and the molecule observed for 6 min or at least 20 units of strain. The time constraint is to limit photobleaching of and photoinduced damage to the stained DNA molecules. The extension of the molecule in the \( x \)-direction \( (X_{\text{ex}}) \) was measured via a simple threshold. Steady-state averages were obtained by sampling individual traces at time intervals equal to the higher extension relaxation time \( \tau_1 \) after the molecule has experienced a strain of 10 (except for the case of \( De = 0.1 \) in the 150 \( \mu \)m tall channel, a strain of 5 was used because 10 units of strain cannot be attained under this very small applied strain rate due to the observation time constraint). Ensemble averages were taken over at least 10 molecules (at the lowest strain rates) to more than 50 (at the highest strain rates).

III. Results and Discussion

Figure 3A shows the fractional extension (normalized by the contour length) with strain for individual molecules (gray lines) as well as their ensemble-average (bold line) for the 300 nm tall channel at \( De_l = 1 \). Figure 3B shows the ensemble-average extension for four \( De \) in the 300 nm tall channel. It is clearly observed, even for low \( De \), that the molecules reach steady state after an applied strain on order 10. Thus, the trapping ability and residence times afforded by our device is sufficient for experimental observation of the steady-state stretch at these deformation rates. The fact that steady state is reached after \( \sim 10 \) units of strain is interesting in its own right. This is the same order of magnitude as observed in studies of unconfined DNA,\(^{37}\) implying that while confinement may alter the level of stretch that can be attained at a given strain rate, it does not necessarily significantly increase the rate of stretching. More careful studies focusing on the stretching transients are needed to fully characterize these effects.

Figure 4 shows the steady-state extension versus dimensional and nondimensional measures of the strength of the deformation applied. Figure 4A shows very clearly that confinement does indeed aid DNA stretching. The stretch increases at a given strain rate with decreasing channel height, more than \( 7 \)-fold between the 2 \( \mu \)m and 150 \( \mu \)m tall devices at a strain rate of 0.2 s\(^{-1}\). Importantly, at high extensions, the strain rate required to achieve a given extension can be decreased by more than 70\% by exploiting confinement at these scales.

Figure 4B displays the same steady-state average extension versus \( De \), the Deborah number using the low extension relaxation time (\( \tau_2 \)) to normalize the strain rate. The data do not collapse, and the location where the large increase in extension occurs does not agree with the predicted value of \( De_{\text{crit}} \) = 0.5. We conclude that the low extension relaxation time does not govern the coil—stretch transition in slitlike confinement. These results are in accord with our previous data\(^{20}\) where the dynamics of relaxation are governed by the low extension relaxation time only very near equilibrium. We will return to this point below.

Figure 4C displays the steady-state average extension versus \( De_l \), the Deborah number using the higher extension relaxation time to normalize the strain rate. It is clearly seen that this second slow time scale collapses the data quite well, and the drastic increase in extension occurs at approximately \( De_l = 0.5 \). The coil—stretch transition and stretch at higher extensions are better described by the second-longest relaxation time, a phenomenon unique to confinement in polymer physics. This
data collapse is also seen in the inset of Figure 4, where the standard deviation of the average extension (σ/Lc) is plotted against De. Recent studies\textsuperscript{38} have shown that the peak in this plot is a very good indicator of the location of the coil–stretch transition, and here we note that the peak is well-aligned on the abscissa. This alignment confirms that the higher extension relaxation time governs the large increase in the stretch of the molecule with applied strain rate. This finding provides a fundamental basis for the design of devices aiming to utilize slitlike confinement to attain highly extended DNA molecules. In these devices, the higher extension relaxation time (τII) appears to affect stretching at low extensions, below the predicted relative crossover extensions\textsuperscript{20} of Xc/\(L_c\) = 0.17 and 0.27 for the 300 and 150 nm tall channels, respectively. Note that these predicted crossover extensions overestimate the region governed by τII because they predict the center of a gradual transition.\textsuperscript{20} Since the changes of steady-state extensions involved here are small, it is helpful to examine other indicators of behavior departing from equilibrium dynamics.

Figure 5 shows the root-mean-square angle (θRMS) of the principal axis of the in-plane DNA radius of gyration as a function of strain (\(\theta_{\text{RMS}}\), in degrees) versus strain for De = 0.3 for the three channel heights. Green, red, and black solid lines denote the 2 μm, 300 nm, and 150 nm tall channels, respectively; colored dashed lines and markers denote the steady-state ensemble average RMS angle. De = 0.3, 0.2, and 0.4 for the 2 μm, 300 nm, and 150 nm channels, respectively. The horizontal solid line denotes the equilibrium average (θRMS,eq = 52°). (B) θRMS for the three channel heights for De = 0.3, corresponding DeII is 0.3, 0.6, and 1.2 for the 2 μm, 300 nm, and 150 nm channels, respectively.

Figure 4. (A) Ensemble average steady-state extension versus the dimensional strain rate for three channel heights. (B) Ensemble average steady-state extension versus De, the strain rate normalized by the low extension relaxation time. (C) Ensemble average steady-state extension versus De, the strain rate normalized by the higher extension relaxation time. Inset: standard deviation (σ/Lc) of the steady-state stretch versus De.

Figure 5. (A) Root-mean-square angle of the principal axis of the radius of gyration relative to the x-axis (θRMS, in degrees) versus strain for De = 0.3 for the three channel heights. Green, red, and black solid lines denote the 2 μm, 300 nm, and 150 nm tall channels, respectively; colored dashed lines and markers denote the steady-state ensemble average RMS angle. DeII = 0.3, 0.2, and 0.4 for the 2 μm, 300 nm, and 150 nm channels, respectively. The horizontal solid line denotes the equilibrium average (θRMS,eq = 52°).
IV. Conclusions

We have designed a cross-slot device which yields large regions of homogeneous extensional deformation with limited spans amenable to the nanofluidic environment. Thus, we are able to exploit changes to the polymer dynamics induced by nanoslit confinement in order to facilitate dynamic manipulation of single molecules. We are able to easily select, trap, and stretch individual DNA molecules to steady state in this device. The confinement ensures the entire molecule remains in focus during the process, unlike other much taller microfluidic stretching devices.\textsuperscript{25,26,31,32,39} From the stretching results presented here, we conclude that confinement does aid the stretching of single devices.\textsuperscript{25,26,31,32,39} From the stretching results presented here, we conclude that confinement does aid the stretching of single DNA molecules by allowing the use of much smaller strain rates to achieve the same amount of extension. However, the time scale governing the large change in extension with applied strain rate is the higher extension relaxation time (\(\tau_1\)). This finding is important since the prediction of this transition often forms the crux of design specifications for processes involving stretching or deforming DNA molecules, and naive application of unconstrained theory to confined systems would significantly underpredict the strain rates required to deform DNA molecules. The low extension relaxation time (\(\tau_0\)) governs the orientation and small deviations from equilibrium of the molecule. Our results are important for future studies of DNA dynamics in confinement, especially those concerned with the measurement of relaxation times or dynamic manipulation of extended DNA.

Acknowledgment. The authors thank NSF Career Grant CTS-0239012, Singapore-MIT Alliance for Research and Technology (SMART), and U.S. Genomics for funding.

Supporting Information Available: Movie of demonstration of trapping and stretching of T4 DNA molecules; movies comparing steady-state extension of T4 DNA in different channel heights at same \(De\). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes


(15) Han, J.; Craighead, H. Science 2000, 288, 1026.
(23) Balducci et al., A. Macromolecules 2006, 39, 6273.
(35) Mao, P.; Han, J. Lab Chip 2005, 5, 837.
(39) Lee, J. S.; Shaqfeh, E. S. G.; Muller, S. J. Phys. Rev. E 2007, 75, 040802(R)

MA8015344