RESEARCH ARTICLE

Force-fluctuation physics of confined DNA: probing the breakdown of the Marko-Siggia law

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Abstract

Introduction: The study of polymers in nanofluidic systems such as nanopores and nanochannels is an important avenue of research in the physical and life sciences today. Complex nanofluidic devices containing varying topography are ideal for quantifying the behaviour of polymers under confinement. This study investigates the Marko-Siggia force-extension relationship under confinement. We measure the transverse fluctuations of deoxyribonucleic acid (DNA) confined between two pits in a nanofluidic slit to measure the potential breakdown of this model.

Methods: We took images of fluorescently tagged single DNA molecules in the nanopit array with video-fluorescence microscopy and analyzed the standard deviation of the peak position along the direction of the molecular extension.

Results: We were able to measure the parabolic relation between position and the strength of transverse fluctuations. By examining the peak variance as a function of slit height, our results indicate that the two dimensional version of the force-fluctuation relationship may be appropriate in the limit of strong confinement. However, we could not consistently measure the absolute length of the DNA stretched between two pits, which prevented us from fully exploring the force-extension and force-fluctuation relationships of confined DNA.

Conclusions: These experiments further demonstrate that nanofluidic confinement serves as a useful tool for testing the extreme properties of polymers, and our results suggest further investigation into the breakdown of the Marko-Siggia force law.

Introduction

The development of nanofluidic devices such as nanopores, nanochannels, and nanoslits has led to significant advancements in many areas of research (1). These devices have small volumes and provide constrained dynamics, allowing individual biological molecules to be studied with remarkable spatial and temporal resolution. Nanofluidics also have applications in next-generation genetic mapping technologies (2).The physics of deoxyribonucleic acid (DNA) molecules under confinement is fundamentally different than in bulk form (3). The unique behavior displayed by polymers under confinement is a significant area of study in nanoscience. Understanding it is key for the further development of a diverse array of fields ranging from polymer electronics (4) to polymeric membranes (5).

A polymer is a molecule made up of a number of repeating "monomer" units in a chain. There is no long-range order in orientation along the polymer, and its structure is described by a random walk (6). To maximize entropy, a polymer randomly coils with a characteristic radius. When stretched, the number of possible configurations decreases, and due to thermal fluctuations, the polymer will relax to a more condensed and higher entropy state: the polymer tends to bunch as there are more ways for it to be bunched than stretched. This phenomenon is known as entropic elasticity. Biological polymers such as DNA are often described by the worm-like chain model of entropic elasticity, which correctly predicts rigidity over a short range known as the persistence length. This model was tested by Marko and Siggia (7), who derived the eponymous force law relating the force acting on the ends of a polymer to its relative extension. More recently, Baba *et al* (8) derived a relationship between the

magnitude of transverse fluctuations of a stretched polymer and the force acting on it.

The behaviour of DNA changes when it is confined to regions below its characteristic size. While modifications to DNA's equilibrium structure have been well characterized (3), it is unknown whether the Marko-Siggia force law is modified by confinement. Here, we use a nanopit-nanoslit system to test the effects of confinement on the force-extension and force-fluctuation relationships of DNA. The nanopit-nanoslit system consists of a 100 nanometer slit, which is embedded with a lattice of square pits. This slit is small enough that the molecule behaves as if in two dimensions. The molecules have more ways to orient themselves in the pits than in the slits, so the pits can be said to act as entropic traps. Such a system has previously been used to demonstrate directed molecular organization (9) and nonlinear modifications to molecular diffusion (10). We intend to use this system to measure the transverse fluctuations and compare them to polymer theory.

The traditional worm-like behavior of the Marko and Siggia force-extension curve is described by the following equation (7):

$$\phi = \frac{f l_p}{k_B T} = \frac{l}{L} + \frac{1}{4(1-\frac{l}{L})} - \frac{1}{4} \qquad (1)$$

where Φ is the normalized force, f is the absolute force (typically piconewtons), l_p is the persistence length (typically 50 nanometers), k_B is the Boltzmann constant, T is the temperature, l is the extension of the molecules (the distance between the pits in this experiment), and L is the contour length between the two nanopits of the fluctuating polymer.

The relationship between the maximum standard deviation and the normalized force as described by Baba *et al.* (8) follows as:

$$\hat{\sigma}^2_{max} = \frac{\sigma^2_{max}}{L l_p} = \frac{\phi^{-1}}{4(1+\frac{3\phi^{-1}}{2})} \qquad (2)$$

where σ^2_{max} is the maximum variance of an individual DNA molecule and $\hat{\sigma}^2_{max}$ is the normalized variance. It should be noted that in the limit of two dimensions, the magnitude of transverse fluctuations double because they are now limited to a single spatial dimension, as discussed by Baba *et al.* The statistical behaviour of DNA in the nanopit-nanoslit system was derived by Reisner *et al* (9).

There has been speculation that the Marko-Siggia force law is modified by confinement. Binder (11) suggests that it breaks down in two-dimensional limit, while Chen *et al.* (12) claim it is modified by an increased effective persistence due to confinement, and Lin *et al.* (13) suggest that confinement acts as a bandpass filter and forbids certain fluctuation modes. We hope that the analysis of fluctuations in the wide parameter space provided by the nanopit-nanoslit system will shed light on potential modifications to the well-known force law under confinement.

Apparatus and Procedure

The experiments were performed in glass nanofluidic chips, fabricated using standard clean-room processes as described by Resiner *et al* (9). The chips contained nanofluidic slits with heights on the order of 100 nanometers. The slits were embedded with a lattice of square pits. The size, spacing, and depth of the pits as well as the height of the nanofluidic slit serve as independent variables to dictate the behaviour of the DNA (Figure 1).

Microfluidic channels in the chip served as an interface to the nanoscale system. The chip was fastened in a plastic chuck which was mounted on a Nikon Eclipse Ti-U inverted microscope. A lamp and a dichroic filter was used to stimulate the fluorescent dye molecules. DNA from a lambda phage virus (48,500 base-pairs or 16 microns in length) was stained with YOYO-1 fluorescent dye. The DNA was dissolved in 50 mM Tris buffer and pipetted into the microfluidic reservoirs of the chip. Pressure was applied to flow the DNA into the nanofluidic slits. Under equilibrium, the molecules self-assembled into the pits. We focused on geometries where the molecules occupied two pits, recording movies several minutes in length, observing the molecule fluctuating throughout its environment. After being recorded, the molecules were flown out of the field of view and new molecules were brought in.

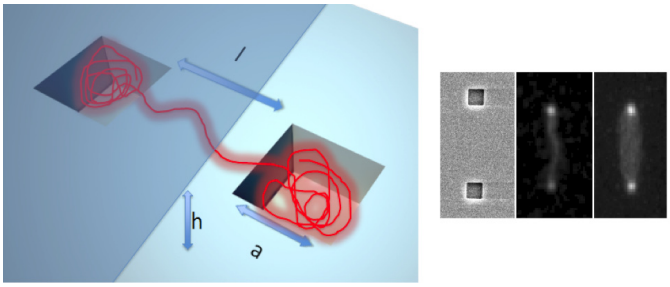

Fig. 1
Overview of the experiment.
Left: A schematic of two nanopits in a nanofluidic slit. The pits have width ***a*** , are separated by a distance ***l*** , and the roof of the system has height ***h*** above the floor. The pits have the same depth as the height of the slit.
Right: A scanning electron micrograph of two pits, an optical fluorescence micrograph of a molecule spanning two pits, and a projection of a movie of that molecule, demonstrating the variation in position. The distance between the pits is 5 microns.

Analysis

A movie of each studied molecule was opened as a three dimensional (two space and one time) matrix in MATLAB. At each frame, the brightest pixel of each row was calculated. Because the DNA is uniformly stained, maxima in recorded intensity serve as proxies for maxima in DNA concentration. For each row, an array of maximal positions over time was generated. The standard deviation of this array served as a measurement for the standard deviation of the position of the molecule at that point along its contour.

A custom built MATLAB program was used to determine the extended contour length (*L*). The first step involved subtracting the background noise in the fluorescence video image. A box was manually aligned to each of the two nanopits that contained the trapped DNA molecule. The summed pixel intensity of the two boxes was determined, as well as the pixel intensity of the entire molecule. The ratio of the intensity in the two pits to the total intensity of the molecule was used as a proxy to calculate the extended contour length between the two pits because the total contour length (L_T) of the molecule is known (19135 nm).

Results

It can be seen from the right half of Figure 2 that the standard deviation along the molecule increases as the position becomes further from the pits, peaking at the mid-point. Baba *et al*. (2012) derived this relationship as parabolic. In order to establish commonality between their optical traps and our entropic traps, we examined this relationship. In Figure 3, the variance is plotted as a function of the longitudinal coordinate and it can be seen that the parabolic relationship described by Baba *et al* (8) does indeed hold. The variance peaks at the middle of the stretched part of the molecule and behaves according to the quadratic law about this position. In our experiments, the DNA molecule was trapped at both ends, so the transverse fluctuation is symmetric about the midpoint. Once again, this result was also obtained by Baba *et al* (8) by using dual trap optical tweezers to trap the DNA molecule.

It was previously mentioned (3) that DNA behaves differently under confinement. We also postulated that the Marko-Siggia force-extension relationship (7) is not ideal to describe a DNA molecule under confinement. We measured the magnitude of transverse fluctuations of DNA in 500 nanometer pits separated by 1000 nanometers across slit heights ranging from 50 to 170 nanometers (Figure 4). We used the statistical theory of Reisner *et al* (9) to calculate the equilibrium conformation of the molecule, and coupled this result to the force-fluctuation relationship of Baba *et al* (8). to generate a theoretical prediction. As a comparison, we examined the two-dimensional limit of this relationship and the effective persistence described by Chen *et al* (12). In Fig.4, the Marko and Siggia 3D force-fluctuation curve is shown in red and the curve in blue is derived from the two dimensional limit of the force-fluctuation model. It can be seen in Fig.4, that the data points are bounded by the 3D and 2D force fluctuation curves and neither model adequately describes the behavior of the data across all regimes. The fact that the 3D force law does not ad

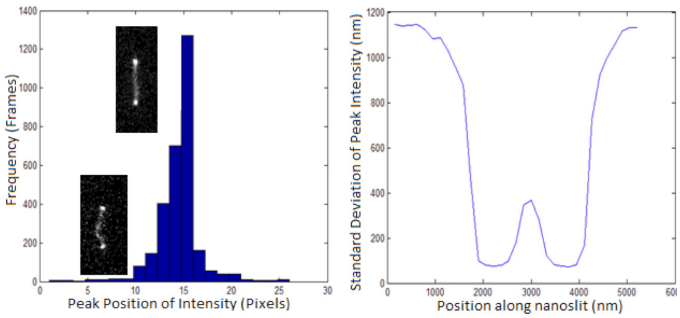

Fig. 2
Analysis to determine standard deviation. Left: A histogram of peak positions in a 3000 frame movie. The micrograph in the center of the histogram corresponds to a straight molecular configuration, while the other micrograph corresponds to a molecule fluctuation to the left. Right: The standard deviation of peak intensities, as a function of position along the nanoslit. The plateaus at the edges correspond to random noise, the two valleys correspond to the molecular contour trapped in the pits, and the peak between them represents the actual transverse fluctuations of the molecule.

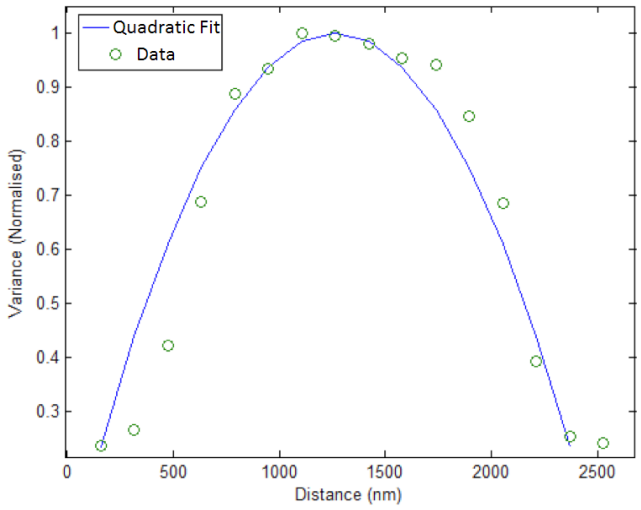

Fig. 3
Variance along molecular position for a DNA molecule. The molecule is trapped in a nanopit-nanoslit sytem with a slit height of 110 nm and with pits separated by 2000 nm. We find agreement with Baba et al.'s [6] assertion that variance behaves parabolically with distance from the end points.

equately describe the data points in the limit of strong confinement suggests that the bulk description of the Marko-Siggia force law may indeed break down under confinement. The modification of Chen *et al* (12) did not significantly affect the theoretical prediction.

Discussions

We sought to replicate the findings of Baba *et al* (8) in our solid-state nanofluidic system. We observed a parabolic relationship in transverse fluctuations with respect to the position along the molecule, which is an identical result to Baba *et al* (8). However, our observation of the force-extension relationship under confinement (Figure 4) suggests that we may have observed a breakdown of the accepted theoretical model. The behavior of the data in Figure 4 is best described as an interpolation of the 3D and 2D force fluctuation models. While these results suggest new confined polymer physics, it is regrettable that we were unable to fully explore the force-fluctuation and force-extension relationships due to difficulties in measuring the extended contour length of the molecule (the total length of the DNA stretched between the pits).

Initially, this study also expected to explore the relationship between the variance and the inverse of the normalized force, as was done by Baba *et al* (8) in the nanopit-nanoslit system. A challenge that presents itself in nanopit experiments that is not present in optical tweezer experiments is the fluctuating length of the stretched polymer, as contour moves in and out of each pit. This required a method to determine the extended contour length (L) of the DNA molecule trapped between nanopits.

In Section IV, the method used to determine the extended contour length *L* was described. This method was found to be imprecise and unstable as it was very sensitive to the positioning of the rectangular box. Even a small amount of residual background noise made a significant difference in the final computation. Overall we were unable to measure the length and instead, we had to use the statistical theory (9) as a proxy for an actual measurement of the length. We desire an objective and automatic way of calculating the extended contour length, in order to better compare our measurements to other work.

Our initial results suggest that this is potentially a fruitful avenue of polymer physics research, but to fully explore it we must develop a better image analysis method that is not as sensitive to background noise as the current approach.

Conclusions

Transverse fluctuations of single DNA molecules trapped in the nanopit-nanoslit system were studied in this paper. The findings in this paper indicate that the Marko-Siggia force-extension and force-fluctuation relationships are not the best model to describe DNA molecules under strong confinement (small slit heights). We have also demonstrated that this nanofluidic system can serve as a powerful alternative to optical trapping systems for advanced polymer physics research. Future work will further explore these relationships using improved image analysis algorithms.

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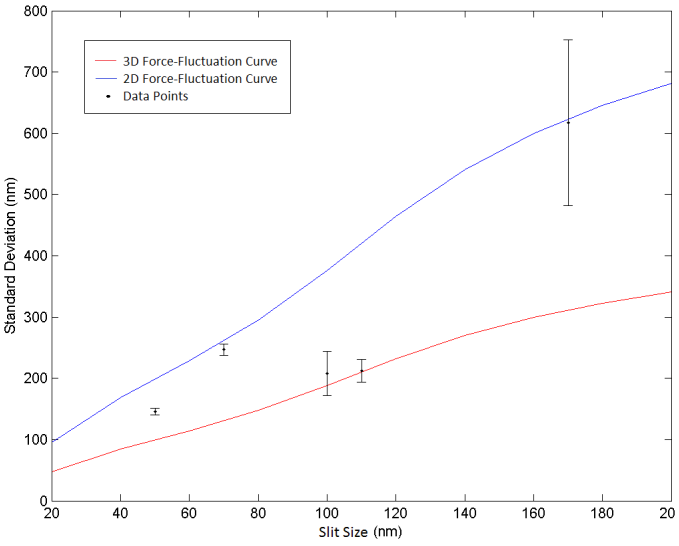

Fig. 4
Peak standard deviations of DNA in 500 nanometer pits separated by 1000 nm are measured. Theoretical curves from a combination of the statistical nanopit model and the force-fluctuation relationship are shown.

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