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Dynamics of Individual Polymers using Microfluidic based Microcurvilinear Flow

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Abstract

Polymer dynamics play an important role in a diversity of fields including materials science, physics, biology and medicine. The spatiotemporal responses of individual molecules such as biopolymers have been critical to the development of new materials, the expanded understanding of cell structures including cytoskeletal dynamics, and DNA replication. The ability to probe single molecule dynamics however is often limited by the availability of small-scale technologies that can manipulate these systems to uncover highly intricate behaviors. Advances in micro- and nano-scale technologies have simultaneously provided us with valuable tools that can interface with these systems including methods such as microfluidics. Here, we report on the creation of micro curvilinear flow through a small-scale fluidic approach, which we have used to impose a flow-based high radial acceleration ($\sim 10^3g$) on individual flexible polymers. We were able to employ this microfluidic-based approach to adjust and control flow velocity and acceleration to observe real-time dynamics of fluorescently labeled λ -phage DNA molecules in our device. This allowed us to impose mechanical stimulation including stretching and bending on single molecules in localized regimes through a simple and straightforward technology-based method. We found that the flexible DNA molecules exhibited multimodal responses including distinct conformations and controllable curvatures; these characteristics were directly related to both the elongation and bending dynamics dictated by their locations within the curvilinear flow. We analyzed the dynamics of these individual molecules to determine their elongation strain rates and curvatures ($\sim 0.09 \mu m^{-1}$) at different locations in this system to probe the individual polymer structural response. These results demonstrate our ability to create high radial acceleration flow and observe real-time dynamic responses applied directly to individual DNA molecules. This approach may also be useful for studying other biologically based polymers including additional nucleic acids, actin filaments, and microtubules and provide a platform to understand the material properties of flexible polymers at a small scale.

1. Introduction

Developing new approaches to study the static and dynamic properties of individual molecules has significantly expanded our fundamental understanding of polymer physics^{1–3}. Common methods to the study of macromolecular solutions, including DNA molecules, in flow

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conditions have embraced bulk approaches such as light and neutron scattering, and birefringence⁴. Although these experimental methodologies have provided a tremendous amount of insight regarding the properties of polymer chains, one inherent significant disadvantage is in the macroscopic nature of many studies, as many properties of a single polymer chain must be inferred from indirect measurements in these approaches using averaging assumptions from a large number of polymer chain samples. Due to these limitations, a number of techniques have been developed to enable researchers to observe, manipulate, and measure single polymer chains as a means of studying their dynamics^{5,6}. In addition, the dynamic measurements and detection of DNA molecules based on additional methods such as nanopore and nanofluidic channels have been investigated^{7–10}. When studying individual polymers, DNA molecules have proven to be a particularly interesting model^{11,12} because of their high molecular weight, large contour lengths, mono-dispersity, and their ability to be individually and locally visualized to provide detailed measurements via fluorescence microscopy. While using DNA molecules provides unique advantages, coupling it with unique experimental approaches—especially those with similar small-scale dimensions such as microfabricated technologies—can provide expanded novel capabilities to investigate individual polymer dynamics.

To pursue this experimental avenue, we leveraged bioanalysis tool advancements in soft lithography techniques in the microfluidic domain^{13,14} to build devices with various geometries as a means of providing a platform to achieve specific stimulation and manipulation profiles including single molecule chain stretching and separation^{15,16}. Such individual molecule conformations and dynamics are highly relevant to the environment in living cells, which is known to be highly confined and complicated by structural interactions, which can be directly linked to numerous mechanical deformation effects¹⁷ as well as being important to the mechanical properties of polymer thin films¹⁸. In addition, the protein machinery involved in copying, transcribing and packing DNA molecules have been adapted to exploit the unique physical properties of DNA molecules. For example, RNA polymerases and helicases are motors capable of moving along torsionally constrained DNA molecules. This biologically based machinery is essential from a biomechanics as well as biochemistry perspective as it requires one to understand the micromechanical properties of single DNA molecules. This multi-disciplinary work is a combination of biochemical and physical concepts along with instrumentation, which will continue to open new avenues to understand how DNA molecules are organized and processed inside cells. In this work, we built a microfluidic device that induces high radial acceleration through flow to generate a maximum fluid velocity of up to 0.27 m/s ($z=1\text{ }\mu\text{m}$), which is then applied to mechanically manipulate individual DNA molecules. The DNA molecules in our study were first constrained by attachment at one end to a glass substrate via a digoxigenin-antidigoxigenin linkage^{19,20}. Micro curvilinear flow, which can be induced in the circular side chamber along the side wall at lower flow rates than the flow rate where microvortex^{21–23} fully develop. The response in a circular side chamber that exhibited flow detachment at the opening of the side chamber, was established when flow was applied into our microfluidic channel. The DNA molecules in the device experienced elongation and bending, which were directly related to the high radial acceleration. This system allows us to probe conformation and dynamic responses of single DNA molecules as they experienced stretching, coiling, and bending in response to a balance of internal energy and external stimulation.

2. Results and Discussion

To observe and measure real-time dynamics of individual flexible polymers under a curvilinear flow in the circular side chamber, we first fabricated our microfluidic channel to create a high radial acceleration; an illustration of this experimental setup is shown in Figure 1a. This microfluidic device was fabricated using standard soft-lithography methods²⁴ with poly

(dimethylsiloxane) (PDMS; Dow Corning Corp., MI, USA; No.: Sylgard 184). The polymer was poured over a mold, hardened, removed, treated with oxygen plasma, and bonded to a glass coverslip²⁵ (as described in the **Methods**). All channels used in this study have a rectangular cross section 30 μm wide $\times$ 20 μm high. Micro curvilinear flow was generated through controlling the dimensions of the circular side chamber and the flow velocity, which allowed for curvilinear streamlines in the chamber; this is schematically represented in Figure 1b. Figures 1c and 1d are optical images of the microfluidic device and a microscope image of the circular side chamber of the device, respectively. The syringe pump (Harvard Apparatus, MA, USA; No.: PHD 22/2000), which controlled the fluid flow, was integrated with the microfluidic device through polyethylene tubing (Becton Dickinson, NJ, USA; Intramedic tubing with an internal diameter of 0.78mm, No.: 427416). By controlling the flow rate within the channel, we could dictate the streamlines within the side-chamber, and a single molecule could be deformed in the chamber, as shown in Figure 1e. To control the flow velocity in the device for imposing defined radial accelerations on the DNA, we first characterized the flow profile by imaging 2 μm diameter fluorescent beads (Spherotech Inc., IL, USA; No.: CFP-2052-2) with epi-fluorescent microscopy. We used extended exposure times to map out the path traced by the beads through the channels, thus demonstrating our ability to create flow patterns with defined flow speeds (Fig. 2a). Further examination found that the average linear velocity of the pressure-driven flow in the side chamber increased proportionately to the higher applied syringe pump flow rate. The computational rotational velocities and radial accelerations are listed in Table 1 and were determined using computational fluid dynamics (CFD) (as described in **Methods**). Rotational velocities were obtained in defined locations on the plane next to the bottom of the rectangular cross-section through the velocity profiles in the CFD results. Radial accelerations were calculated in the same locations through user-defined derivative functions (in Fluent) from the rotational velocities obtained above. The flow response in this environment was a combination of a tight rotation radius ($r < 50 \mu\text{m}$) and a high rotational velocity ($v \sim 0.27 \text{ m/s}$) for flow in the constricted channel, which generated a radial acceleration as high as 10^4 m/s^2 (or $\sim 10^3 \text{ g}$) when the flow rate was $75 \mu\text{L/min}$ (center panel in Fig. 2a). The rotational velocity and radial acceleration at the “inner regime” (a distance of $10 \mu\text{m}$ from the main channel measured from the point of inlet into the side chamber) of the side chamber increased by approximately 4 to 6 times for a flow rate for $75 \mu\text{L/min}$ when compared to the velocity and acceleration at the “outer regime” (a distance of $35 \mu\text{m}$ from the main channel) of our circular side chamber. The Reynolds numbers (Re) at the chamber’s opening are 0.4, 0.6, and 0.9 at the flow rates of $50 \mu\text{L/min}$, $75 \mu\text{L/min}$ and $100 \mu\text{L/min}$, respectively, which indicates that the flow is laminar in this system. Interestingly, flow rate limits at both ends of the spectrum restricted our experimental range. At lower flow rates, curvilinear flow could not be fully established deep into the circular side chamber due to a lack of entry of the beads into the side chamber. At $100 \mu\text{L/min}$ flow rates, we could not see the curvilinear flow inside the chamber since micro curvilinear flow did not fully develop (right panel in Fig. 2a). However, for flow rates between these limits, we could relate the distance into the circular side chamber of the flow to the velocity profile for imposing radial accelerations high enough to apply shear force to individual molecules (Fig. 2b and 2c). The distance of the flow into the circular side chamber with a flow rate of $75 \mu\text{L/min}$ was 1.3 times greater than that observed with a flow rate of $50 \mu\text{L/min}$ (Fig. 2c). Furthermore, the lowest flow rate for the creation of a microvortex is at $100 \mu\text{L/min}$ ²¹. Figure 2d shows streamlines for micro curvilinear flow at flow rates corresponding to the experiment results (Fig. 2a), which are in good agreement. At the flow rates of $50 \mu\text{L/min}$ and $75 \mu\text{L/min}$, there are curvilinear flows (Fig. 2a and 2d). At $100 \mu\text{L/min}$, we can see the microvortex flow developed in the chamber based on the computational results (Fig. 2d) or a fully developed vortex formation from the experimental results (Fig. 2a). This could be because there are not enough fluorescent beads in the flow inside the chamber to visualize the distribution, or flow detachment issues at the side chamber.

We then focused on visualizing single molecular polymers as a means of understanding their dynamics as observed in our system. We prepared λ -phage DNA (48,502 base pairs with a contour length of $16.2 \mu\text{m}^{20}$; as described in **Methods**) so that the individual molecules would attach at one end to the bottom of the channel on our glass substrate via anti-digoxigenin binding (as described in the **Methods**; Roche Diagnostics GmbH; No.: 11-333-089-001). DNA molecules were stained with a fluorescent dye (YOYO-1; Molecular Probes, CA, USA; No.: Y3601) for one hour and then introduced into the microchannel. The molecule we studied, λ -phage DNA, has the capacity to transition between a coiled native state and a stretched conformation following mechanical stimulation. In our high radial acceleration approach, the dynamic response can be interplay between the transitioning elongation and the bending imposed by the micro curvilinear flow. After introducing the DNA molecules into the channel, the inlet and outlet pressures were initially equalized to inhibit further flow and allow the DNA molecules to attach to the anti-digoxigenin surface coating over the course of at least one hour. Within these channels, the conformations of single polymer chains under flow in the curvilinear flow were then observed using high-resolution epi-fluorescence microscopy with an Axiovert inverted Zeiss microscope. The DNA was imaged through the bottom surface of the channel, which was #1 borosilicate glass. Images collected were then characterized according to the individual DNA molecule shapes and curvatures observed when compared to flow velocities and the respective locations within the side chamber.

With this approach, we were able to observe the response of single molecules within a micro curved flow with high radial acceleration and analyze their conformational changes due to simultaneous elongation and bending. In addition, a double-stranded DNA molecule in solution will locally bend and exhibit curvature due to thermal fluctuations. Such fluctuations shorten the end-to-end distance of the molecule, even when the decreasing distance is in the opposite direction of an externally applied force. Before addressing these micromechanical issues for the DNA molecules, it is essential to understand the force-extension (in our study, the stretching-elongation under microfluidic flow) behavior of DNA molecules. Figure 3a represents a schematic of predominantly observed locations for DNA molecules within the main channel and the circular side chamber in our fluidics device, although other locations were also noted. We first analyzed individual DNA molecules in the main channel of our microfluidic system, which enabled us to visualize pure elongation with a pressure-driven flow (Fig. 3b; at location (1) in Fig. 3a). Overall, we applied a pressure gradient in our rectangular channel and generated a parabolic fluid velocity profile that is highest in the channel center and zero at the wall²⁶. The fluid shear stress is related to the strain rate in the fluid under the assumption that the viscosity is constant. The strain rate, which is determined by the derivative of the velocity to the width (perpendicular to the main stream), is highest at the wall and zero at the center so the DNA molecules at the bottom of this channel were under the highest shear stress²⁷. Images of two representative DNA molecules elongating in the direction parallel to the flow direction with a flow rate of $50 \mu\text{L}/\text{min}$ are shown in Figure 3b. While there was a slight delay between the initiation of the pumping from the syringe that was likely due to compliance in the elastomer materials, during a three second period, the biopolymer stretched with an elongation strain rate of 0.217 s^{-1} (Fig. 3b; upper molecule) and 0.391 s^{-1} (Fig. 3b; lower molecule), respectively. This was compared to DNA molecules at the 15th second in Figure 3b (left panel). While these were representative molecules, overall, the individual DNA molecules elongated by 65% (Fig. 3b; upper molecule). In addition, we have found instances where 2 DNA molecules are found adjacent to each other from an end-to-end perspective. This provides the ability to analyze DNA within the same experiment simultaneously. These individual DNA molecules are found to elongate by 58% (Fig. 3b; lower molecule). This difference in DNA length could be due to out-of-plane focusing issues, as the DNA was tethered to the bottom surface, yet was allowed to freely move (was unattached) at its opposite end. In addition, the DNA molecules could have been in a non-uniform fluid velocity profile (e.g. a parabolic velocity profile in a rectangular channel). If the free end moved out of the plane of

focus, which was usually near the glass surface where the anti-digoxigenin binding occurred, it may appear to demonstrate false shortening as it would no longer be imaged in its entirety at the free end. This is supported by the changes in fluorescent intensity in the upper molecule in Figure 3b, which appears to display this change in fluorescence in the middle of the molecule between 18 and 21 seconds as the middle portion of the DNA molecule may have moved slightly out of the focal plane. To examine the relaxation response of the λ -phage DNA, which is known to slowly recoil after stretching, we turned off the syringe pump at the 18th second. In this, the elongation strain and elongation strain rate of these two single molecules of DNA were 0.015 and $5.1 \times 10^{-3} \text{ s}^{-1}$ (upper DNA molecule in Fig. 3b), and 3.3×10^{-3} and $1.1 \times 10^{-3} \text{ s}^{-1}$ (lower DNA molecule in Fig. 3b) at the time period between the 18th and 21st second, respectively. We note though that stabilizing the flow after turning on or off the pump is essential to our experiments. Based on our transient simulations for the on-off process, this would take approximately 0.7 seconds thus it should have a minimal effect on our results. This change in length is much smaller for the same time period than when compared to the elongation after an extended conformation was initially imposed. Such overall response of the DNA molecules coiling at a slower rate and thus maintaining longer length is likely related to the fact that λ -phage DNA is known to have a faster elongation rate than a relaxation rate^{5,28}. The upper DNA molecule in Figure 3b started relaxing, but the lower DNA molecule in the same figure was observed to remain elongated after the 18th second despite the cessation of the syringe pump. Additionally, the lower DNA molecule in Figure 3b appears to have still been stretched to its known length²⁰ at the 21st second. Overall, we found that the flow shear within the pressure-driven micro curvilinear flow influenced the dynamic responses of single DNA molecules and dictated the elongation response in the main channel. Although the elongation strain rate of these two DNA molecules was measured in this study, DNA molecules in the main channel were stimulated by fluidic shear stress; therefore the Weissenberg number (shear strain rate $\times$ relation time; in this study, elongation strain rate $\times$ relation time) is obtained as well. Classic polymer theories³ predict that a flexible polymer becomes stretched when the Weissenberg number is equal to or larger than unity. The Weissenberg number for our flexible polymers under shear-induced deformation in the main channel was approximately 3, which agrees with³.

Through systematic observations of individual DNA molecules in a curvilinear flow under well-defined flow rates, we have found that DNA molecules adopt conformations and elongations that are related directly to their location within the channel and the circular side chamber. At the inner regimes, the conformation of single DNA molecules appears to be elongated due to the curvilinear flow with only slight bending. The DNA molecules were stretched by the fluid flow, which only had a slight curvature due to the arcing flow as it moved through the side chamber as shown in Figures 3c (location (2) in Fig. 3a) and 3d (location (3) in Fig. 3a). Thus the tangential direction to the fluid flow only had slight changes through the chamber due to the small curvature for the flow path traced within the chamber for this case. In Figure 3c, the individual DNA molecules extended in an angled direction toward the exit of the circular side chamber. This followed the path that was traced by the beads in Figure 2. For this region during the time between the 1st and 4th second, the applied flow rate was 100 $\mu\text{L}/\text{min}$, and the average strain rate of DNA molecules was 0.15 s^{-1} . When the flow rate was stopped at the 4th second, the DNA appeared to shorten. Again, part of this observed decrease in the shortening of the DNA molecule might be related to issues with the focusing plane when using fluorescent microscopy, as this was a mechanical perturbation within the channel. In addition, a similar effect occurred during the time period between 2 and 3 seconds as shown in Figure 3d. We also characterized the effects of flow on the DNA molecules by examining the DNA elongation strain rate versus the flow rate as shown in Figure 4a. Due to limitations in image capturing, we were not able to specifically consider the location of DNA in the microfluidic channel. When comparing the strain rate of DNA molecules at distinct flow rates, the elongation strain rate at a flow of 75 $\mu\text{L}/\text{min}$ is significantly higher than the rate at 100

$\mu\text{L}/\text{min}$ within the side chamber. This is likely due to the micro curvilinear flow pattern observed with a flow rate of $100 \mu\text{L}/\text{min}$ as displayed in Figure 2. The flow rate of $75 \mu\text{L}/\text{min}$ appeared to be the most stable, while at $50 \mu\text{L}/\text{min}$, the flow rate still produced a micro curvilinear flow pattern. Unlike other methods, which stretch single DNA molecules such as optical and magnetic tweezers, our system can be used to understand dynamic behaviors of several DNA molecules at numerous locations, either in the main channel or in the side chamber, simultaneously. Each of these locations has defined stimuli for the DNA including controls with no stimulation. This enables us to be able to examine and compare a wide range of biopolymer responses including stretching, bending, and elongation in each experiment. When comparing different flow rates, the elongation strain rate at a flow rate of $75 \mu\text{L}/\text{min}$ is 2.66 times higher than $100 \mu\text{L}/\text{min}$ at location (3) (Fig. 4b). This is a result of the flow rate of $75 \mu\text{L}/\text{min}$ creating a greater penetration into the side chamber, which results in a more fully developed micro curvilinear flow. Interestingly, the elongation strain rate at location (3) is 2.51 times higher than location (2) in micro curvilinear flow at the same flow rate (Fig. 4c). Even though the DNA molecules were examined with the same initial conditions to control the microchannel flow (e.g. flow rates in the chamber), the resulting conditions at these two locations could be different. First, each λ -phage DNA molecule might have its own inherent properties including the conformation in a coiled state. Although it would be homogeneous from a molecular viewpoint in large scale averaging experiments, it would be challenging to distinguish for single molecule response⁴. This could result in different elongation and relaxation times in individual molecules thus the imaged DNA could exhibit different responses under similar initial conditions.

Our system can also be used to observe the bending of individual DNA molecules. Figure 5a illustrates the process where a single DNA molecule aligns with the streamlines in micro curvilinear flow, which would induce a bending conformation under flow-stretching. We use this unique approach to observe and measure the curvature of DNA molecules through flow-stretching. We then observed that individual DNA molecules at the outer regime under a flow rate of $50 \mu\text{L}/\text{min}$, as shown in Figure 5b (location (4) in Fig. 3a) responded conformationally so that the DNA molecules were not only elongated, but also had significant bending as well. We found that the elongation and bending was directly related to the location of the molecules within the circular side chamber (Fig. 5b), and that at 1.5 seconds, λ -phage DNA molecules were found to be extended to their full length at location (4) in Figure 3a. In a simple shear

flow (where $v_y = 0$, $v_x = 0$, and $\dot{\gamma} = \frac{dv_x}{dy}$), the magnitude of the elongational and rotational components are equal, and, in such instances, it has been suggested that the polymer chains do not attain a stable, strongly stretched state^{29,30}. The flow in the locations at the outer regimes in the side chamber as shown in Figure 5b appear to have resulted in a stretched conformation that follows the length of the curved path of the fluid. The ability for individual DNA molecules to undergo elongation and bending has been shown at lower shear stresses but with less stability in the conformational form³¹. To examine the conformational response at different locations with respect to bending for this high radial acceleration system, the curvature of the DNA was characterized. The DNA in locations (1), (2), and (3) were found to have curvatures of approximately $0 \mu\text{m}^{-1}$ (Fig. 5c). For location (4) at the outer regime of the micro curvilinear flow, the curvature of the DNA molecules increased to $0.09 \mu\text{m}^{-1}$ as well as having an elongated form. One biologically relevant area where this is important is examining the bending of DNA molecules under flow-stretching, which can be related to the enhancement of DNA recombination and gene transcription that is observed when specific protein-binding sites for activators are replaced by intrinsically bent DNA sequences³² or in binding sequences of unrelated DNA-bending proteins³³. Additionally, when using this micro curvilinear flow system, we must control the flow rate to balance the elongation strain rate and the attachment of DNA where the DNA may be detached even with anti-digoxigenin binding due to the flow. Our approach is capable of creating a multimodal stimulation and examining the dynamic

response for elongation and bending simultaneously of single biopolymers through epi-fluorescence microscopy.

3. Conclusions

Research in single molecule dynamics has provided insight on many levels ranging from polymer physics to probing living cell responses. Additionally, advances in micro- and nano-scale technologies such as microfluidics have been increasingly used to provide experimental interfaces to probe molecules in new ways. By using a curvilinear flow generating microfluidic system, we were able to observe real-time dynamics of individual flexible polymers (fluorescently labeled DNA molecules) through a pressure-driven flow approach capable of producing high rotational velocity (~ 0.27 m/s) and high radial acceleration ($\sim 10^4$ m/s² or $\sim 10^3$ g) on the plane ($z=1$ μ m). This approach enabled us to create and examine the effects of a micro curvilinear flow on a single molecule that could be simultaneously imaged to determine the polymeric structural response. As DNA molecules exhibit distinct conformations that are spatiotemporally linked, the ability to directly control their position within the micro curvilinear flow allowed us to create a coupled elongation and bending stimulation profile. This molecular level approach to non-equilibrium polymer physics could be used in a variety of other areas including examinations of entangled solutions of flexible polymers and cytoskeletal polymers including actin filaments and microtubules.

Methods

DNA Hybridization—We used 10 μ L λ -phage DNA (48,502 base pairs, 31.5×10^6 g/mol) at a concentration of 16 nM and 1.6 μ L oligos (lambAGdig, 5'-AGG TCG CCG CCC-3' digoxigenin) at a concentration of 100 μ M. The buffers and solutions included 1X annealing buffer, 50 μ M NaCl, 10 μ M Tris-HCl with a pH value of 7 to 8, and 1 μ M EDTA (or 10 μ M MgCl₂). We mixed the λ -phage DNA and oligos and then added 31.8 μ L H₂O into a 200 μ L microcentrifuge tube. We then introduced 5 μ L of 10X annealing buffer, 1.6 μ L oligo lambAGdig, and 10 μ L λ -phage DNA into a microcentrifuge tube. The buffer, oligos, and DNA were maintained at 4°C during the experiment. Following this, we heated water on a hot plate to 100°C and immediately immersed the tube with the DNA solution into the water. Our resulting DNA solution was stored in a 4°C refrigerator for at least one hour before use.

Ligation process—Our DNA solution was added to 6 μ L ligation buffer followed by the addition of 4 μ L of ligase. Ligation buffer and ligase were both purchased from Roche (T4 DNA ligase high concentration with 5 units/ μ L; No. 799009). Next, we used a temperature control stage set to 16°C for at least eight hours in a tube rack. To deactivate the ligation process, we heated the DNA solution to 75°C for 10 minutes and then stored the DNA at 4°C for future use. After this process, the DNA solution could be used at room temperature.

Soft Lithography—Microfluidic channels were fabricated through soft lithography. SU-8 photoresist (MicroChem Corp., Newton, MA, USA; Formulations 50–100) was used to create patterns on a silicon wafer, which was implemented as our mold. Polydimethylsiloxane (PDMS; Dow Corning Corp., Midland, MI, USA; No.: Sylgard 184) was then cast against this mold to make a microfluidic device that was 30 μ m wide and 20 μ m deep in the main channel with a circular side chamber. The microfluidic device was then removed from the mold, and treated with oxygen plasma for at least one minute for bonding to a glass coverslip.

Microscopy—Epi-fluorescence and differential interference contrast microscopy were performed using an Axiovert inverted Zeiss microscope with a fluorescein filter set and a 63X (1.4 numerical aperture) oil objective. We used a digital camera (Diagnostic Instruments Inc., MI, USA; No.: 4.1) and an image processor to capture two-dimensional digital images.

Computational Fluid Dynamics—The CFD analysis was performed using Fluent (ANSYS, Inc., Lebanon, NH) along with the three dimensional models being created using Gambit, which is the geometry and mesh software component of Fluent. The computational domain is discretized using hexahedral mesh of approximately 300,000 grids. Mesh independence was verified by examining higher density meshes. We assumed Newtonian fluid using water inside the channel and no-slip boundary conditions. The SIMPLE algorithm was implemented for pressure-velocity coupling and all spatial discretizations were performed using the Second Order Upwind scheme in Fluent. Flow rates (50, 75, 100 $\mu\text{L}/\text{min}$) are specified at the inlet and atmosphere pressure (gauge pressure = 0) at the outlet.

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Fig. 1. Pressure-driven micro curvilinear flow design for application of high radial acceleration on individual DNA molecules. **(a)** Schematic of the pressure-driven fluidic system designed to observe the response dynamics of individual DNA molecules. The microfluidic device was fabricated through soft lithography and the flow rate was controlled via a syringe pump. **(b)** Diagram of the microfluidic configuration used to create the micro curvilinear flow. The surface of the glass substrate at the bottom of this micro curvilinear flow system was treated with anti-digoxigenin for at least one hour to attach the DNA molecules to the surface of the glass. **(c)** An optical image of the poly(dimethylsiloxane) microfluidic device along with, **(d)** a differential interference contrast image of the circular side chamber. Scale bar= 30 μm . **(e)**

Fluorescent image of a YOYO-1 labeled DNA molecule within the channel of this micro curvilinear flow system. Scale bar=4 μm .

Fig. 2.

Effect of velocity on flow profile for the circular side chamber of the micro curvilinear flow device. **(a)** Fluorescent images of beads moving from the main channel into the side chamber under flow rates of 50 $\mu\text{L}/\text{min}$, 75 $\mu\text{L}/\text{min}$, and 100 $\mu\text{L}/\text{min}$. At flow rates that were higher and lower than these, the formation of a vortex flow pattern was inhibited. The rotational velocity and radial acceleration at the inner regime of this side chamber were 6.2 and 4.6 times higher than those observed at the outer regime. Scale bar = 30 μm . **(b)** Schematic of the micro curvilinear flow flow depth penetration compared with the designed vortex depth (the size of the side chamber). **(c)** Percentage of the average depth of penetration or distance into the side chamber when compared to the entire distance across the entire circular chamber. The flow rates were compared to the bead flow profile captured from these increased time exposure images; this was used to characterize the flow profile based on the velocity of the fluid (error

bars represent the standard deviation). **(d)** Streamlines with green scale velocity magnitudes from the computational fluid dynamics simulation in the micro curvilinear flow system at different flow rates.

Fig. 3. Spatial locations within the micro curvilinear flow to examine DNA response. (a) Schematic of specific spatial locations of the DNA molecules within the micro curvilinear flow side chamber. (b) Time-lapse snapshots of two individual DNA molecules in the main channel under a pressure-driven flow at a flow rate of 50 $\mu\text{L}/\text{min}$ at location (1). Before introducing the DNA, we treated the surface of the glass substrate with anti-digoxigenin for at least one hour to induce the attachment of the DNA to the surface. Scale bar= 2 μm . (c) Time-lapse snapshots of an individual DNA molecule at location (2) in the micro curvilinear flow at a flow rate of 100 $\mu\text{L}/\text{min}$. Scale bar= 2 μm . (d) Time-lapse snapshots of an individual DNA molecule at location (3) under a flow rate of 75 $\mu\text{L}/\text{min}$. Scale bar= 4 μm . We measured the change in DNA length between two images with **ImageJ** (a public software from National Institutes of Health; <http://rsbweb.nih.gov/ij/>). Through using the elapsed time, we estimated the elongation strain and elongation strain rate of the DNA molecules.

Fig. 4.

(a) Elongation strain rates versus flow rates within the micro curvilinear flow system (error bars represent the standard deviation; 24 images from 5 samples). (b) Elongation strain rates versus flow rates at location (3) in Figure 3(a). The elongation strain rate at a flow rate of 75 $\mu L/min$ is 2.66 times higher than for 100 $\mu L/min$ (error bars represent the standard deviation). (c) Elongation strain rates versus a constant flow rate of 100 $\mu L/min$ at different locations (location (2) and (3) in Figure 3(a)). The elongation strain rate at location (3) is 2.51 times greater than at location (2) (error bars represent the standard deviation).

Fig. 5.

(a) Schematic of conformation of a single DNA molecule at location (4) in the micro curvilinear flow. (b) Conformation of single DNA molecules in the micro curvilinear flow system under high radial acceleration. Fluorescent images of individual DNA molecules at location (4) and at locations within the side chamber away from the main channel with a flow rate of $50 \mu\text{L}/\text{min}$. DNA molecules were stimulated with a combination of elongation and bending through high radial acceleration. Scale bar = $4 \mu\text{m}$. (c) The curvature of the DNA molecules was characterized in comparison to the location of the molecules within the micro curvilinear flow fluidic system. The curvature of the DNA molecules was approximately $0 \mu\text{m}^{-1}$ at locations 1, 2, and 3 and $0.09 \mu\text{m}^{-1}$ at location (4).

The computational rotational velocities and radial accelerations at specific locations in micro curvilinear flow system through the computational fluid dynamics (CFD) method. (1), (3) and (5) are approximately 10 μm from the main channel for flow rates of 50, 75 and 100 $\mu\text{m}/\text{min}$, respectively. (2) and (4) are approximately 35 μm from the main channel for flow rates of 50 and 75 $\mu\text{m}/\text{min}$, respectively.

Table 1

	50 $\mu\text{L}/\text{min}$		75 $\mu\text{L}/\text{min}$		100 $\mu\text{L}/\text{min}$	
	(x)	(y)	(x)	(y)	(x)	(y)
rotational velocity (m/s)	0.178	0.032	0.220	0.035	0.27	
radial acceleration (m/s^2)	2150	450	17400	3700	6200	