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COMMUNICATION

Spatially controlled DNA unzipping by microfluidic interface positioning on a molecule perpendicular to a multicomponent flow†

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A DNA molecule is rotated under a transversal electric field to be perpendicular to a longitudinal, multicomponent microfluidic flow. Positioning the interface allows us to control DNA unzipping with spatio-temporal resolution. This demonstrates that a microfluidic gradient can be applied at the single molecule level for a dynamic intramolecular stimulation.

Due to the important roles of molecular gradients in biological and synthetic systems, efforts have been devoted for the realization of controlled gradients at a microscale. The development of numerous microfluidic tools^{1,2} has enabled the creation and fine control of molecular gradients via diffusion-based and/or flow-based approaches.³ Microfluidics-based gradient devices have been successfully used to modulate or control various biological processes, such as cell migration,⁴ stem cells differentiation,⁵ axon guidance,⁶ and embryonic development.⁷ Microfluidic gradients have also been applied at a single cell level. For instance, a seminal demonstration was reported by Takayama et al. who succeeded to deliver small molecules into selected domains inside an individual cell by a partial treatment using a laminar multicomponent flow.⁸ However, to our knowledge, microfluidic gradients have never been applied to create a controlled perturbation at a single-molecule level. In this communication, we describe the application of electric field for positioning a single DNA molecule perpendicularly to a longitudinal, multicomponent microfluidic flow in order to control its unzipping, i.e. intramolecular denaturation, with spatio-temporal resolution. The application of electric fields to manipulate DNA molecules in nano- or microfluidic channels has been widely used for applications such as DNA stretching,^{9,10} separation,^{11–15} concentration,¹⁶ mapping^{17–19} or polymer physics investigations.^{20–25} Contrary to these classical approaches, where DNA molecules are usually stretched in the same direction as that of the microfluidic flow, here we applied the electric field to rotate a single DNA molecule, orthogonally to a multicomponent microfluidic laminar flow composed of a buffer phase and a denaturant phase.

Figure 1A shows our experimental set-up. It consisted in a microfluidic device having two inlets for creating a laminar flow with two components : a DNA buffer solution (upper phase) and a denaturing solution (lower phase). Under our experimental conditions, the Peclet number varied between 50

and 100 (S.I., Table 1), which ensured a steep profile of denaturing agent concentration around the interface position. To apply an electric field perpendicular to the flow, two electrodes were inserted in reservoirs situating at both ends of another channel (vertical channel in Fig. 1A) perpendicular to the main channel. Finally, a single DNA molecule was tethered on a 10 μm diameter pillar, placed at the crossing region.

Figure 1. Experimental set-up and concept. A) A microfluidic device, made of PDMS, contains two inlets (one for a DNA buffer solution, another one for a denaturant solution), one main channel and two outlets. The main channel is crossed by another channel, along which an electric field is generated from two electrodes. A single pillar (10 μm in diameter) is at the crossing region. All channels are 7.5 μm high. B) A monobiotinylated doubled-stranded DNA (dsDNA) is attached to the

streptavidinated pillar in the absence of electric field. The electric field is increased to bring the DNA molecule perpendicular to the flow axis (1). Increasing the denaturant flow rate allows to maintain the interface at different positions of the molecule, thus unzipping DNA in a spatio-temporally controlled fashion (2).

Our strategy for intra-molecular control of DNA unzipping is shown in Fig. 1B. First, in the absence of electric field and denaturing phase flow ($Q_D = 0$), a double-stranded DNA molecule (dsDNA) was tethered to the pillar by streptavidin-biotin binding. Then, constant flows of DNA buffer ($Q_B \neq 0$) and denaturing phase ($Q_D \neq 0$) were applied to keep the interface far from the pillar while stretching the DNA molecule along the flow (Fig. 1B, top left). Then, the application of an increasing electric field gradually rotated DNA (Fig. 1B, top middle) until an angle close to 90° when the electrostatic attraction created by the electric field overcame the longitudinal force created by the flow (Fig. 1B, top right). The electric field was then kept constant and the increase in Q_D allowed us to maintain the interface at different positions, thus inducing a partial DNA denaturation, or unzipping, when a steep denaturant concentration gradient was created along the DNA molecule (Fig. 1B, bottom).

Figure 2. DNA unzipping by microfluidic interface positioning. A) Flow rate of the denaturant phase (Q_D) as a function of time. The flow rate of the buffer phase ($Q_B = 50$ nL/min) and the electric field (400

V/cm) are fixed. B) Length of a dsDNA molecule as a function of time under non-denaturing ($T = 25^\circ\text{C}$, green triangles) and denaturing ($T = 37^\circ\text{C}$, blue circles) conditions. C) Fluorescence microscopy images of the DNA molecule in denaturing conditions as a function of time. Scale bar is 10 μm .

The intramolecular unzipping experiment was performed as follows. First, the PDMS surface of the microfluidic channel was covalently covered with streptavidin prior to bonding to a PDMS-coated microscopy glass slide (see S.I., Materials and Methods for details). The device was then filled with a 0.3 μM solution of monobiotinylated λDNA concatemers (3 copies of λDNA per molecule, i.e. 145,500 base pairs, in average) in a buffer composed of 10 $\times$ Tris-EDTA and YOYO-1 DNA fluorescent dye (60 nM). At this step, the conjugation led to one or several concatemers bound to the pillar and the experiment was continued only in the former case. After DNA binding to the pillar, the flow rate of the DNA buffer solution was fixed at $Q_B = 50$ nL/min. Then, the denaturant solution (89% V/V formamide in 10 $\times$ Tris-EDTA) was added into the microfluidic device at a starting flow rate of $Q_D = 5$ nL/min. An electric field of 400 V/cm was applied to rotate DNA. Under these conditions, DNA could be maintained almost perpendicular to the flow in the whole range of investigated flow rates. Lower electric fields led to smaller angles with respect to the flow direction while much higher fields induced DNA breakage or detachment from the pillar.

We applied a stepwise increase in Q_D (Fig. 2A) and DNA was observed by fluorescence microscopy. Since YOYO-1 is an intercalator that specifically dyes double-stranded, non denaturated part of DNA, we characterized the course of local DNA denaturation, or unzipping, by measuring the length of the fluorescent dsDNA part (Figs. 2B-C). A first experiment was performed at 37°C , a temperature at which DNA is fully denaturated in a 89% V/V formamide solution. Figure 2 shows that for each denaturant flow rate, dsDNA reached rapidly a stable length that could be maintained as long as the flow rate was not changed. For the lower flow rates, a slight decrease in the time-average dsDNA length was observed (from 42.2 ± 0.2 μm to 40.4 ± 0.4 μm) with an increase in Q_D from 5 nL/min to 14 nL/min. In this regime of Q_D , the interface remained too far from DNA to induce significant denaturation. Interestingly, a strong and stepwise decrease in dsDNA length (17.0 ± 0.2 μm and 9.4 ± 0.8 μm) was observed with a further increase in Q_D (17 nL/min and 20 nL/min, respectively). Under these conditions, it was thus possible to maintain a single DNA molecule in different partially denaturated states, thanks to the local gradient of formamide along the concatemer. For higher flow rates ($Q_D \geq 23$ nL/min), dsDNA could not be distinguished anymore, which was attributed to full denaturation by formamide. To be sure that the extinction of YOYO-1 fluorescence was due to local denaturation, we performed a control experiment at 25°C , a temperature at which DNA is not denaturated in a 89% V/V formamide solution. Fig. 2B (green triangles) shows that the whole concatemer remained fluorescent for the whole range of flow rates. This shows that DNA can be observed in both formamide and buffer solutions but only when it is double-stranded. Therefore, the decrease in dsDNA length observed at 37°C (denaturing conditions) is unambiguously attributed

to the partial DNA unzipping.

Figure 3. Length of dsDNA molecule as function of the denaturant flow rate (Q_D). The flow rate of the buffer phase ($Q_B = 50$ nL/min) and the electric field (400 V/cm) are fixed. Symbols and error bars show mean values $\pm$ SD from triplicates. Error bars are not shown when they are smaller than the symbol size. $T = 37$ °C.

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We then investigated how DNA unzipping could be spatially controlled by positioning the buffer/denaturant interface. Figure 3 shows the average dsDNA length as a function of Q_D on triplicate experiments. Although a significant variability between experiments was observed, three features were reproducibly observed. At a low Q_D , the formamide flow was localized far from DNA and no denaturation was observed. Conversely, at a high Q_D , full denaturation was always achieved. Interestingly, in the intermediate range of Q_D values, dsDNA length significantly decreased with an increase in Q_D due to the successive positions of buffer/denaturant interface that progressed across the DNA molecule.

We described a microfluidic device in which a single DNA molecule was electrostatically maintained perpendicular to a multicomponent flow composed of buffer and denaturing solutions. We showed for the first time that a simple control of the solution flow rates allowed us to move the buffer/denaturant interface at different positions across the DNA molecule, resulting in successive, stable, partially denaturated states. This concept of intramolecular control by microfluidic interface positioning, demonstrated here with a simple unzipping process, could be extended to other chemical or biochemical intramolecular stimulations (e.g., compaction agents, enzymes) for a broad range of fundamental or practical chemical, biophysical or biotechnological applications.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Materials and methods, Table S1. See DOI: 10.1039/b000000x/

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Supporting information for:

Spatially controlled DNA unzipping by microfluidic interface positioning on a molecule perpendicular to a multicomponent flow

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1. Materials and Methods

Materials

λ DNA (390 $\mu\text{g/mL}$), 100 $\times$ Tris-EDTA buffer solution (pH 8), 3-(Aminopropyl) triethoxy silane (APTES), trimethylchlorosilane (TMCS), glutaraldehyde (8% in water) and formamide were purchased from Sigma. Streptavidin was purchased from Life Technologies. 12 nt single-stranded oligonucleotide 5'-GGGCGGCGACCT-3' biotinylated via a TEG linker at 3' position and complementary to one of the sticky ends of λ DNA was purchased from Eurogentec. T4 DNA ligase solution (350 U/ μL) and 10 $\times$ ligase buffer were purchased from Takara. Unless specified, all other chemicals were from Sigma.

Microfluidic device fabrication

Mask Patterning

The mask pattern was designed using L-edit and generated using μPG 101 Laser writing system (Heidelberg instruments) on the optical masks coated with 1000 Å thick chrome and 1 μm thick AZ 1518 positive photoresist (Nanofilm, CIPEC company). The optical mask was then developed for 1 min in MIF-726 positive developer (Microchemicals Company), and after rinsing with MQ water, the chrome metal layer was etched for 1 min in Chrome-Etch 3144 (Honeywell). The mask was then rinsed again with MQ water and dried.

Soft lithography

To achieve proper spatial resolution, we used an improved soft lithography method by performing the usual photolithography steps (resist coating, insulation, and development) directly on the front surface of mask. First, an adhesion layer (Omnicoat) was coated on the mask and baked at 200°C for 1 min. Negative photoresist SU8 2005 (Clariant) was then spin coated to achieve a thickness of 7.5 μm followed by soft baking at 65°C and 95°C for 1 min and 2 min, respectively. The coated mask was then exposed to UV using MJB4 aligner (Süss MicroTec), and baked again at 65°C and 95°C for 1 min and 2 min, respectively. Finally, after the development of the resist in SU8 developer for 1 min, the obtained mold was rinsed with isopropyl alcohol, dried and treated with trimethylchlorosilane (TMCS) vapor for 5 min.

PDMS chip fabrication

The polydimethylsiloxane PDMS (RTV 615, GE Toshiba Silicones Co., Ltd.) was prepared by mixing base-polymer and cross-linker at 10:1 ratio. It was then poured onto the mold, degassed under the vacuum and cured at 80 °C overnight.

PDMS surface modification

Preparation of PDMS-APTES solid substrate

PDMS chip was removed from the mold, rinsed with absolute ethanol and dried using pressurized air. The PDMS surface was then activated by air plasma at 300 - 400 mTorr for 3 min (Plasma cleaner, Harrick), and treated for 10 min with 10% (v/v) solution of 3-Aminopropyl-triethoxysilane (APTES) in absolute ethanol. The APTES-treated PDMS was then rinsed with 96% ethanol, dried, and baked at 125 °C for 30 min.

Immobilization of streptavidin on PDMS

APTES treated surface was then treated with glutaraldehyde solution (8% in water) for 1 hour at room temperature, carefully rinsed using MQ water and dried by pressurized air. The crosslinking of streptavidin on PDMS surface was then achieved by incubating the glutaraldehyde-treated PDMS surface with streptavidin water solution (0.1 mg/mL) for 2 hours. The streptavidin-treated PDMS surface was then rinsed twice using MQ water and finally dried using pressurized air.

Bonding glass substrate and streptavidin-coated PDMS

The cover slips (Menzel-Glaser, 24 mm wide, 60 mm long, 0.13-0.16 mm thick) were cleaned with absolute ethanol and spin coated with PDMS (10:1). After baking at 125 °C for 10 min, the cover slips were treated with air plasma for 3 min at 300 – 400 mTorr and assembled with streptavidin-coated PDMS chip. Liquid PDMS was then applied all along the contact line between the chip and the PDMS-coated cover slip, prior to baking at 50 °C for 15 min.

Preparation of monobiotinylated λ DNA concatemers

50 μ L of λ DNA (48.5 kbp) stock solution (390 μ g/ mL) was incubated at 65 $^{\circ}$ C for 5 min prior to cooling in ice for 5 min. λ DNA concatemers were prepared by adding to the ice-cooled mix of λ DNA, 4 μ L of T4 DNA ligase (Takara 350 U/ μ L) and 6 μ L of 10 $\times$ ligase buffer (Takara) at 16 $^{\circ}$ C for 5 hours. To bind biotin at one end of thus obtained λ DNA concatemers, 0.6 μ L of diluted 3'-biotinylated single-stranded oligonucleotide (4.48 μ g/mL) complementary to one of the sticky ends of λ DNA was added to the λ DNA concatemers solution (325 μ g/ mL) to give a λ DNA:oligonucleotide molecular ratio of 1:1. After adding an additional amount of 4 μ L of T4 DNA ligase, the mix was incubated again at 16 $^{\circ}$ C for overnight. This procedure led to the preparation of monobiotinylated concatemers having mainly 3 copies of λ DNA.

DNA unzipping experiment

First, the microfluidic device with closed outlets was filled via Tygon tubing using syringe pump (Harvard apparatus) at 3000 nL/ min with DNA-buffer solution containing λ DNA concatemers (0.3 μ M in nucleotides), YOYO-1 fluorescent dye (0.06 μ M) and mercaptoethanol (1% V/V) in Tris-EDTA buffer (10 $\times$). This led to the binding of individual DNA concatemers to the pillar of the device. Two Pt electrodes were then inserted in their respective reservoirs, and DNA-buffer flow rate was decreased to $Q_B = 50$ nL/min. After the equilibration of the buffer flow, the denaturant flow, containing formamide (89% V/V), Tris-EDTA buffer (10 $\times$), rhodamine B (10 μ M) and mercaptoethanol (1% V/V), was set at $Q_D = 5$ nL/min. The electric field (400 V/cm, Labsmith high voltage sequence wizard) was then applied to rotate DNA attached to the pillar perpendicularly to the flow. The denaturant (Q_D) flow rate was then increased stepwise at a fixed DNA-buffer flow rate ($Q_B = 50$ nL/min), and the length of double-stranded DNA was monitored by fluorescence microscopy (YOYO-1 fluorescence). Rhodamine B fluorescence was used to check the position of the interface.

Fluorescence microscopy

Fluorescence microscopy was performed with an AxioObserver D1 inverted microscope (Zeiss), equipped with a 100 $\times$ oil immersion objective lens. Images were acquired with a

highly sensitive EMCCD camera (Photonmax 512B, Princeton Scientific) and Metavue image acquisition software (Molecular Devices).

2. Supplementary Table

Table S1. Estimation of the Peclet (Pe) number range under our experimental conditions. Pe was calculated as $Pe = Q/(hD)$ where Q , h , and D where the flow rate, the channel height and the diffusion coefficient, respectively. Minimal and maximal flow rates (Q) were 50 and 80 nL/min, respectively. The values of the diffusion coefficient of formamide in water (D) at 25 °C and 37°C were taken from literature.¹

Q (nL/min)	T (°C)	D (m ² /s)	Pe
50	25	1.72×10^{-9}	64.6
50	37	2.20×10^{-9}	50.5
80	25	1.72×10^{-9}	103
80	37	2.20×10^{-9}	80.8

3. Supplementary Reference

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