Electronic Supplementary Material (ESI) for Chemical Communications

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

λDNA (390 µg/mL), 100× 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 λDNA was purchased from Eurogentec. T4 DNA ligase solution (350 U/µL) and 10x 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 200ºC 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 65ºC and 95ºC for 1 min and 2 min, respectively. The coated mask was then exposed to UV using MJB4 aligner (Süss MicroTec), and baked again at 65ºC and 95ºC 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 polydimethysiloxane 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 ºC 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 ºC 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 ºC 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 ºC for 15 min.

Preparation of monobiotinylated λDNA concatemers

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

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 λDNA concatemers (0.3 µM in nucleotides), YOYO-1 fluorescent dye (0.06 µM) and mercaptoethanol (1% V/V) in Tris-EDTA buffer (10×). 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×), rhodamine B (10 µM) 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× 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

1. C. M. Gary-Bobo and H. W. Weber, *J. Phys. Chem.*, 1969, **73**, 1155-1156