Electronic Supplementary Material (ESI) for Chemical Communications

SUPPLEMENTARY INFORMATION

Native and fluorescent dye-dependent single-DNA molecule microchip dynamics as measured by differential interference contrast microscoy

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The AVI movie files show the dynamics of a single DNA molecule intercalated with YOYO-1 (Fig. 1A(a), movie M1) and a single native-DNA molecule (Fig. 1A(b), movie M2) under an applied electric field along the microchannel as described in Fig. 1.

Experimental

Chemicals and reagents

A 10.0 mM Gly-Gly (Sigma, St. Louis, MO, USA) buffer solution was prepared by dissolving in deionized water (pH 8.2). The microchip dynamic coating was made by dissolving 1.0% (w/v) of polyvinylpyrrolidone (PVP, M_r = 1 000 000; Polysciences Inc., Warrington, England) in Gly-Gly buffer. The mixture was vigorously shaken for 2 min and then allowed to stand for 2 h to remove bubbles. λ-DNA (M_w = 48 502 bp) was obtained from Promega (Madison, WI, USA). YOYO-1 intercalating dye was purchased from Molecular Probes (Eugene, OR, USA). All chemicals were A.C.S. grade. All solutions were filtered through a 0.2 μm membrane filter and photobleached overnight using a UV-B lamp.

DNA sample preparation

For fluorescence detection, the λ-DNA sample was labeled with the YOYO-1 intercalator at a 1:5 molar ratio of dye to nucleotide pairs (bp) in 10.0 mM Gly-Gly buffer solution. For DIC detection, λ-DNA samples were prepared in 10.0 mM Gly-Gly buffer without YOYO-1. The DNA sample was further diluted to 100.0 fM using 10.0 mM Gly-Gly buffer solution prior to the start of single molecule imaging experiments.

Preparation of microchips for DNA detection by DIC

DIC system experiments were performed using customized glass (quartz) microfluidic chips designed with a 610.0 μm thickness manufactured by DBT (Digital Bio Technology Co., Seoul, Korea) for this research. The 70.0 × 18.0 mm microchip consisted of a 60.0 mm long separation channel and side arms 4.0 mm long (ESI†, Fig. S2B). The injection design was a double-T channel with a 300.0 μm offset (ESI†, Fig. S2A). The channel was 100.0 μm in width and 5.0 μm in depth. The reservoirs were 2.0 mm in diameter and 1.0 mm deep (ESI†, Fig. S2C). The injection and separation channel length was 60.0 mm from reservoir 1 to reservoir 3. DNA injection was conducted by capillary force after dynamically coating or filling the inner wall of channel with 1.0% PVP to suppress adsorption of the DNA sample and electroosmotic flow (EOF). Detection was measured 30.0 mm from the injection reservoir (ESI†, Fig. S2D).

Differential interference contrast microscopic system with a temperature controller

The DIC system consisted of an upright Olympus BX51 microscope (Olympus Optical Co., Ltd., Tokyo, Japan)

equipped with a DIC slider (U-DICT, Olympus) and a 40× objective lens (Olympus UPlanFl 40×/0.75 N.A., W.D. 0.51). For real-time single-molecule detection, a CCD camera (QuantEM: 512SC, Photometrics, Tucson, AZ, USA) was installed to the top of the microscope. Camera exposure time was 10-50 ms. Temperature was applied from 20 °C to 40 °C using a temperature controller (CU-201, Live Cell Instruments, Seoul, Korea) placed on the stage of the upright microscope. Electrophoresis and manipulation of DNA molecules were performed with a high-voltage power supply (DBHV-100, Digital Bio Technology Co., Ltd., Seoul, South Korea). DNA samples were driven at 16.7-166.7 V/cm. MetaMorph 7.0 software (Universal Imaging Co., Downingtown, PA, USA) was used for image collection and data processing.

Table S1 Velocity of native DNA molecules in microchannels based on changing temperature (°C) and electric field (V/cm)

Electric field (V/cm)	Velocity (μm/s)[a]				
	20 °C	25 °C	30 °C	35 °C	40 °C
16.7	8.6±0.4	25.5±2.0	31.0±4.2	36.2±3.0	40.6±3.5
33.3	14.3±4.2	45.3±5.5	53.8±2.2	77.5±3.8	87.2±4.5
50.0	32.5±3.5	64.4±6.0	89.3±7.9	129.1±12.1	131.4±0.0
66.7	45.8±9.2	67.1±0.0	130.6±14.1	171.0±18.1	184.3±0.8
83.3	70.7±5.2	71.5±10.8	167.7±12.7	202.6±11.3	234.2±8.9
100.0	76.2±8.7	91.7±6.3	219.0±11.7	222.6±0.0	278.0±16.1
116.7	93.4±6.2	126.8 ±14.1	259.0±23.9	269.6±16.1	399.8±19.8
133.3	108.0±12.7	162.8 ±2.2	319.1±23.7	359.9±20.7	537.3±24.8
150.0	116.2±3.6	184.4 ±9.6	339.5±21.8	412.8±22.4	573.4±26.0
166.7	116.2±8.1	193.9±0.8	381.1±14.8	526.2±15.3	674.1±19.1

[a]Velocity of the mean±standard deviation of 5 measurements.
*DNA flow direction is in the same direction of EOF.

Table S2 The ratio of net velocity of DNA in microchannels based on changing temperature (°C) and electric field (V/cm)

Electric field (V/cm)	Ratio of net velocity (Native DNA/YOYO-DNA)				
	20 °C	25 °C	30 °C	35 °C	40 °C
16.7	1.3	2.7	1.5	1.4	0.9
33.3	0.9	1.6	1.4	1.8	1.7
50.0	1.5	1.6	1.6	1.9	1.8
66.7	1.7	1.4	1.7	1.8	1.9
83.3	1.4	1.3	1.7	2.0	1.6
100.0	1.0	1.2	2.1	1.8	1.6
116.7	1.0	1.1	1.8	1.5	1.9
133.3	1.1	1.1	1.9	1.9	2.0
150.0	0.8	1.3	1.7	1.8	2.0
166.7	0.7	1.0	1.5	1.8	2.2

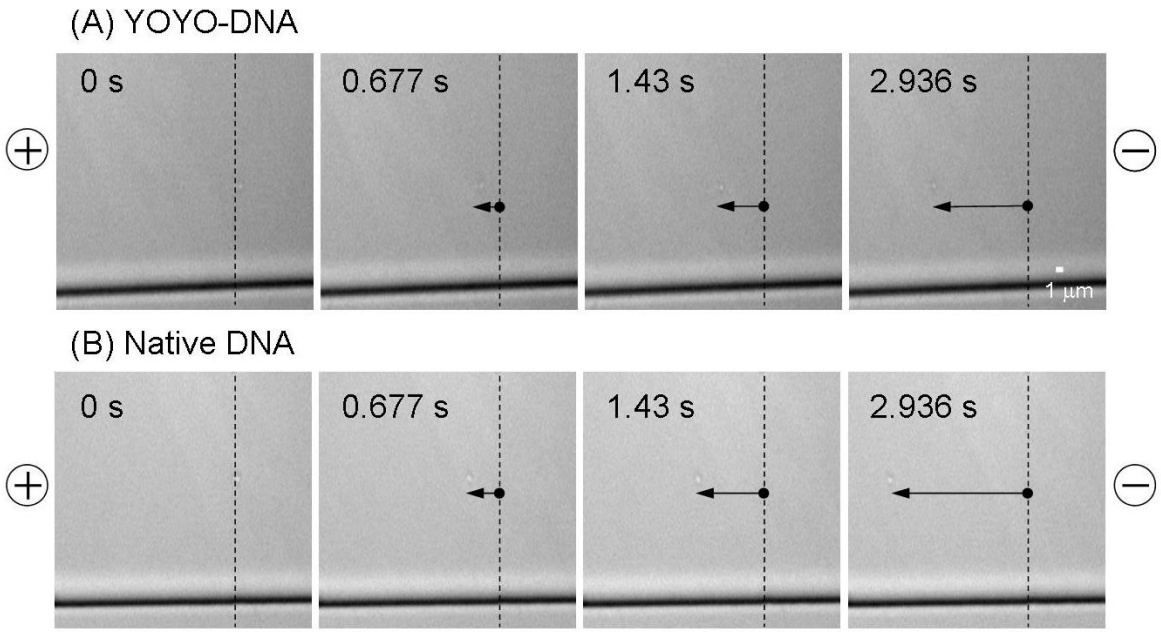

Fig. S1 DIC images of a single flowing (A) DNA molecule intercalated with YOYO-1 (YOYO-DNA) and (B) a native-DNA molecule under an applied electric field strength of 16.7 V/cm in a glass microchip filled with a 1.0% PVP (M_r = 1 000 000) sieving gel matrix. DIC conditions: camera, QuantEM:512SC; exposure time, 50 ms; objective lens, Olympus UPlanFl 40×/0.75 N.A., W.D. 0.51; temperature, 25 °C, sieving gel, 1.0% PVP (M_r = 1 000 000); microchip, 100.0 μm width by 5.0 μm depth. Black arrow, flow direction of λ-DNA; dotted line, λ-DNA position at steady state.

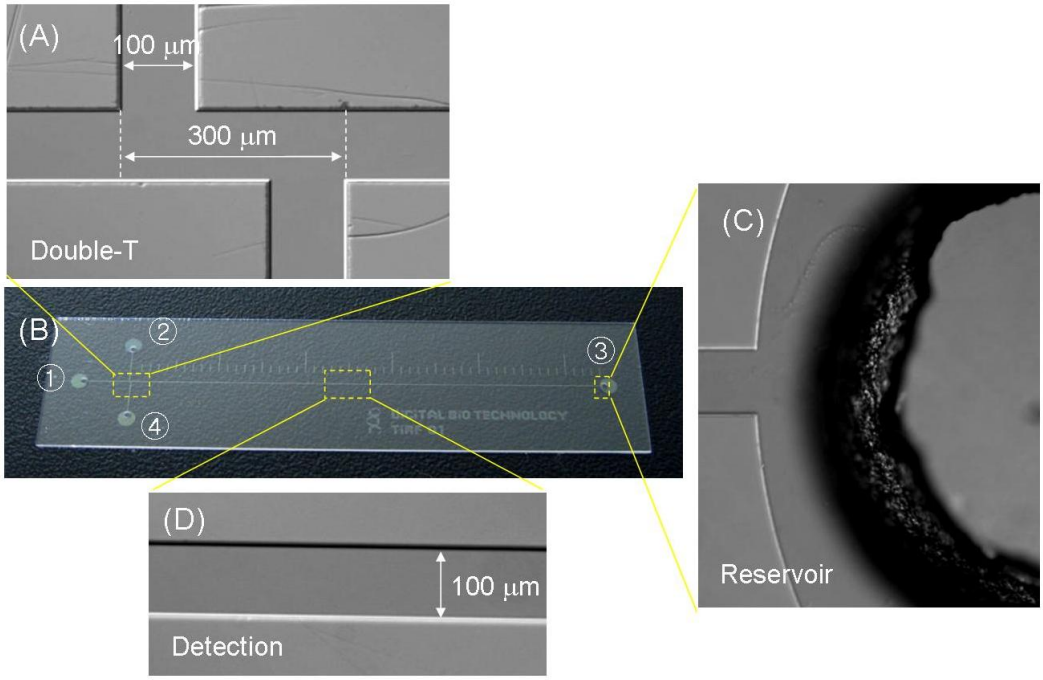

Fig. S2 Microchip for the detection of single native-DNA and YOYO-DNA molecules by DIC microscopy. (A) DIC image of the double-T section of the microchip, (B) photograph of the entire glass microchip, and DIC images of the (C) reservoir and (D) detection section at the microchip.