NANOFuidic SINGLE-mOLECULE SORTer CONCEPTuALLY PROVEN BY SORTING OF DNA

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ABSTRACT

We have proposed and experimentally demonstrated the concept of a single-molecule sorting that identifies individual single-molecules, realizing the ultimate level resolution for any separation-based technology. The single-molecule sorting was realized using a nanofluidic network consisting of a single inlet channel that branches off into multiple outlet channels. It includes two major functional elements such as a single-molecule detection and identification element and a flow path switching element to independently separate single-molecules. With this system we have successfully demonstrated the world’s first single-molecule sorting using DNA as a test sample.

KEYWORDS: Single-molecule, Nanofluidics, Single-molecule manipulation, Single-molecule sensing

INTRODUCTION

Separation and separation-based analysis of biomolecules such as chromatography, electrophoresis, etc. are important in the field of biotechnology. With these techniques, though, issues concerning resolution and sensitivity regularly arise which have been and will continue to be addressed through development of new technology. Theoretically, then, it can be surmised that maximum resolution and sensitivity should be realized using single-molecule technology that detects and manipulates only a single molecule at a time. If other technical difficulties presented by single-molecule technology can be overcome, such as the need for ultra high sensitive detection, extreme noise reduction, how to manipulate single-molecule, detection errors, etc., then 100% determinacy should be theoretically obtainable.

Herein we propose a single-molecule sorter that identifies and separates single biomolecules one by one. The physical size exclusion effect of the nanochannel serves as the underlying principle for the single-molecule sorter. Because the cross-section of the nanochannel can be as small as the size of a biomolecule, only a single molecule can pass into the nanochannel at one time. Thus, a one-dimensional single molecular stream is automatically created in the nanochannel. To take advantage of the characteristics of nanofluidics, we have developed and experimentally demonstrated a single-molecule sorter based on a nanofluidic system that is functionalized with electrodes, for single-molecule sensing and manipulation. For the first evaluation of the concept, we have experimentally demonstrated sorting of three lengths of DNA, as the first demonstration of single-molecule sorting.

Experimental

The nanofluidic single-molecule sorter was fabricated on a quartz substrate by depositing and patterning Ti (1 nm)/Au (100 nm) electrode strips using electron beam evaporation (EBX-6D, ULVAC), followed by conventional photolithography. Nanochannel structures (15 μm long, 50 to 100 nm depth and 50 to 500 nm width) were created using focused ion beam (FIB) etching. During this process, the Ti/Au strips were cut to form a pair of nano-gap electrodes whose gap exactly equals the width of the nanochannel. All patterned electrical leads on the substrate were sandwiched with guard electrodes to minimise leakage of current. A 100-nm Cr layer as a wet etching mask was deposited and patterned, followed by wet etching of the quartz with a buffered HF solution (BHF 6300F, Daikin Chemical) to fabricate the microchannels. Finally, the substrate was sealed with a thin ultra-pure, soft grade of polydimethylsiloxane (PDMS) (SIM 240, Shinetsu Silicone) and then coated by vacuum ultraviolet (VUV) light bonding [1] with a second 100-μm-thick quartz plate having contact holes that were aligned with the microchannel ends on the substrate. Because quartz is transparent at the VUV wavelength of 172 nm and PDMS strongly absorbs at that wavelength, the substrate was illuminated from the bottom for 10 s, corresponding to a 150 mJ dose, to bond the interface between the PDMS and the quartz. An additional 60 s or more of illumination changed the surface from PDMS to SiO2, so that the internal surface of the nanofluidic device was fully hydrophilic. Although the top of the Au/Ti electrodes was not bonded, no leakage was observed. If necessary, the hydrophilic surface could be repeatedly recovered with additional exposure of VUV light. The electric field distribution resulting from application of voltage between the electrodes was simulated using a commercially available finite element solver (COMSOL ver. 3.5, COMSOL).

Electrical measurements were made using a Sub-Femtoamp Remote SourceMeter (Model 6430, Keithley). The sampling time was maximally 1 ms, and the corresponding bandwidth was about 1 kHz, eliminating high frequency noise. The root-mean-square value of the background noise was less than 10 fA to 1 fA under open-circuit conditions and less than 40 fA under ultrapure water. We applied an excitation voltage of 0.1 V or less to suppress electrical breakdown and electrolysis at the electrodes. To reduce electrical noise, the device was placed on a homemade peltier-cooling plate that maintained the temperature at 4 ± 0.1°C.

Ultra-pure water was refined by UL-Pure (Komatsu Electronics), passed through a centrifuge filter (Microcon YM-3, Millipore) to remove nanometer sized particles and pre-vacuumed to remove dissolved air. DNA samples included λ -DNA (48.5 kbp) and restriction fragments of λ -DNA cut by Xho I (15.0 and 33.5 kbp). Both fragments were separated by electrophoresis and purified using SUPREC-EZ (9140, TAKARA). The density and purity of the DNA was confirmed by gel electrophoresis and UV absorbance (Gene Spec III, Hitachi). All DNA samples were dissolved at a final concen-
tration of 1 fM in 0.1 × TBE buffer (0.009 M tris, 0.2 mM EDTA, 0.009 M boric acid, pH 7.5) and labelled with a fluorescent dye (SYBR GOLD: S11494, Invitrogen) at 1 μM final concentration.

All experiments were conducted under an epi-fluorescent microscope (BX-50, Olympus) equipped with an EM-CCD camera (MC681SPD, Texas Instruments) to visualise the motion of DNA during experiments. The entire system, except for the mercury lamp and its power source, was placed in a homemade shield box. The nanofluidic device was covered with a second small shield box with an aperture to allow access to the device by the microscope objective.

RESULTS AND DISCUSSION

As schematically shown in Figure 1A, the device conceptually consists of three main functional elements. The first component is the nanochannel network, which has a single inlet channel branching off to multiple outlet channels to separate single molecules. The sensing element detects flow speed and identifies a flowing single molecule. The switching element switches the flow path to an appropriate outlet channel via feedback from the sensing element. With this format, each flowing single molecule should theoretically be separated and collected deterministically.

Figure 1B shows the scanning ion microscopy of the single-molecule sorter observed using a focused ion beam (FIB) system (JFIB 2300, JEOL). Several electrodes are fabricated on the substrate: 1) to detect the speed of a flowing single molecule; 2) to identify a single molecule; and 3) to switch the flow path. All sensing and manipulation are designed to be conducted by electrically. The inserted figure shows the magnified cross-section of the nanochannel. Because the nanochannel is fabricated by FIB etching and the beam profile has a Gaussian distribution, the cross section is V-shaped.

We first investigated electrical single molecule detection and identification in the nanochannel of the single-molecule sorter. For this purpose, we used DNA as a sample molecule, because DNA is easy to be prepared to any size by PCR or restriction enzyme cutting. When the DNA solution was fed into the inlet channel, capillary action automatically drew the DNA solution into the nanochannel and toward the outlet channel through the electrode gap. The outgoing solution was continuously evaporated at the outlet boundary, causing the DNA solution to continuously flow through the nanochannel. The flow speed was therefore passively regulated by the balance between the shape and hydrophilicity of the nanochannel and the evaporation rate. As a result, we did not require an external pumping system. Figure 2 shows the snapshots taken at 3.3-ms intervals of a 15.0-kbp sized DNA molecule flowing through the electrode gap. The relative position of the DNA to the electrodes is clearly visualised in Figure 2B, the electric current measured as the single DNA molecule passed through the electrode gap (as shown in figure. 2A) is presented. We expected, based on previous reports [2], that the presence of DNA in the electrode gap would reduce the ionic current path between the electrodes. Our studies showed that there is a certain baseline current level in the absence of DNA that decreases when DNA is present. In the figure, the baseline ionic current is about 1 pA, which dropped mostly to about 0.3 pA and minimally 0.07 pA when the DNA molecule was in the electrode gap.

We next demonstrated single molecule sorting. We used 3 sizes of DNA as sample molecules: 15.0, 33.5 and 48.5 kbp. Each DNA solution was independently fed into the single-molecule sorter to test a series of automatic operations including flow speed detection, single molecule identification and flow path switching to an appropriate outlet channel. The information of the length of DNA determined the outlet channel while the velocity of the flowing molecule determined the appropriate switch timing. In the identification process, the size of the DNA molecule was calculated based on the retention time of the electrical measurements, which ranged from 2 ± 3 ms for 15 kbp to 4 ± 2 ms for 33 kbp and 6 ± 2 ms for 48.5 kbp, respectively. The current values themselves were not largely different for each size of DNA. Thus the identification of each single DNA was conducted only based on the retention time. Figure 3 shows the measured electric current of each size of DNA and the fluorescent image taken during switching at the branching of the main channel. We programmed the system to cause the 15.0-kbp DNA to flow to the left channel A, the 33.5-kbp DNA to the middle channel B and the 48.5-kbp DNA to the right channel C. The switching was conducted by electrophoresis, which was generated by several electrodes fabricated...
CONCLUSION

We have proposed and experimentally demonstrated the concept of a “single-molecule sorter” that identifies and separates individual single-molecules, realizing the future ultimate level of resolution and sensitivity for any separation-based technology. The single-molecule sorter was created using a nanofluidic network with electrodes fabricated by FIB etching and photolithography. With this device, we have successfully demonstrated the world’s first single-molecule sorting by separating DNA molecule as a sample.

We have not investigated the sorting of a single base of a DNA, proteins and other smaller molecules yet, because of the difficulty of visualizations of such small molecules with the present setup. One of our tentative goals is the comprehensive sorting of proteins. For this purpose, the sensitivity of the electrical measurement should be improved through further miniaturization of the nanochannel to enhance the ion blockage effect by a single molecule, at the same time of improving the visualization. In addition, alternative current (AC) analysis can evaluate permittivity, which would reflect the conformational information of a molecule; the macroscopic value of permittivity is determined by a conglomerate of numerous internal polarizations, which in turn are determined by the composition and conformation of the molecule. Higher frequency measurements are plagued with increasing noise, but high-sensitive AC impedance analysis may be able to identify and separate the similar sized molecules by their conformational differences.

Ultimately we hope to realize comprehensive single molecule sorting in all of our endeavors, and we believe that the concept of a single-molecule sorter or more generally of single-molecule processing will find a great number of applications in the future.

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Figure 3. Fluorescent images and measured electric current during sorting of three sizes of single DNA molecules. Top column is differentiated signals, while the middle column is raw data. The bottom column is fluorescent images.