Nanofluidic Molecular Filters for Size-Separation of Biomolecules

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Abstract

Micro/nanofluidic molecular sieving structures[1] have great potential for faster molecular separation. However, their use has been largely limited to big molecules such as DNA, since it is rather challenging to fabricate structures with near-molecular dimensions. We present a novel separation method using nanofluidic filters, which is based on the steric hindrance effect of molecules[2-4]. When biomolecules enter nanofluidic gaps with larger but comparable gap thickness, they are hindered from entering the gap due to the confinement entropy changes. Since this effect will be dependent on the relative size of the molecules to the gap thickness, one can achieve size-based separation of biomolecules using an array of nanofluidic filters.

To demonstrate this, we fabricated nanofluidic trap array devices consists of alternating thin (60~120nm) and thick (up to 500nm) regions in a microfabricated channel. The device was fabricated using the standard photolithography and etching techniques, and filled with standard electrophoresis buffers. Double stranded DNA molecules between 100bp (~34nm long) and 2000bp were separated by DC electrophoresis and detected by laser induced fluorescence method. In all the experiments, smaller DNA molecules migrate faster, since they would have more available space within the thin channel, and therefore can enter the filter more easily.

This separation mechanism would be applicable to not only DNA but any biomolecules, and could be useful in developing nanofluidic molecular sieving systems for proteins and other biomolecules, which are generally smaller (1-10nm).

Background

Recently, micro/nanofluidic molecular sieving and separation systems, using MEMS-fabricated regular structures, drew much attention. Compared with nanoporous materials or membranes, regular molecular sieving structures are mechanically and chemically more robust, can be integrated into standard MEMS processes easily, and could be precisely engineered to have better separation efficiencies. Since the first introduction of the idea[5], regular micro/nanofluidic structures have been used for separating large DNA molecules and particles with far greater speed and efficiency[1, 6]. However, separation of smaller biomolecules (such as proteins and small DNA) has not been achieved yet, mainly due to the difficulty in fabricating nanofluidic sieving structures (or filters) with sizes comparable to biomolecules.

In this work, we present the use of nanofluidic molecular filters for separating smaller biomolecules. These molecules are smaller than the gap size of the nanofluidic filters, but they still get separated in the nanofluidic filter devices due to the steric hindrance mechanism (or Ogston sieving)[2-4, 7](Fig. 1). As demonstrated by Beck and Schultz [8, 9] experimentally, biomolecule transport is hindered in a nanopore even when the size of the molecule is smaller than the pore diameter. Indeed, Beck and Schultz's experimental data suggest that ~40% reduction in diffusive transport for a molecule about 10 times smaller than the pore diameter.

This opens up a possibility of nanofluidic size-based separation and filtration of biomolecules that are small (1~5nm in diameter), even with nanofluidic filters as large as 50nm or larger. When the sizes of DNA molecules are comparable to the thin channel depth, they are hindered from entering the trap with steric hindrance effect (see Fig.1), and their mobility becomes size-dependent due to the confinement entropy change. For example, smaller DNA molecules migrate faster and have higher effective mobility since they are favored to enter the trap due to their smaller size.

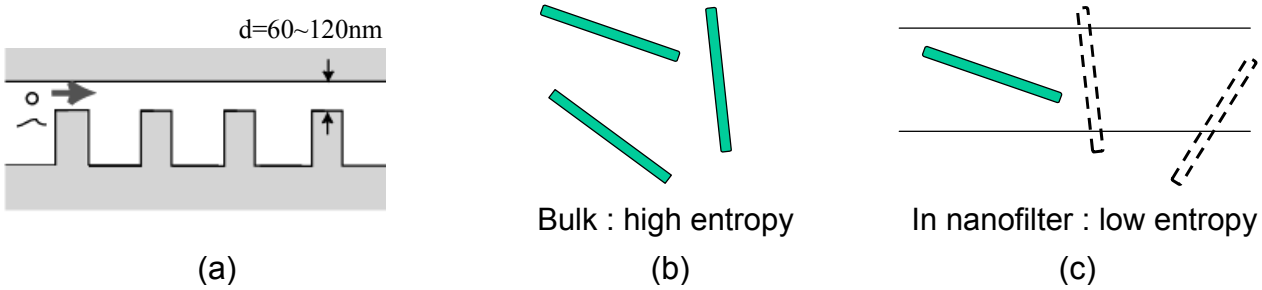

Fig. 1 Sieving of biomolecules by steric hindrance. (a) Biomolecules can be sieved when they move through nanofluidic filters larger than (but comparable to) their size (b) Rod-like biomolecules in bulk solution (c) Rod-like biomolecules in nanofilter

Experimental

The nanofluidic filters were fabricated on Si substrate, by standard photolithography and etching techniques, as described in previous publication[10]. One of the advantage of this fabrication strategy is that no high-resolution lithography technique is necessary to make a nanofluidic channel as thin as 50nm, since we are confining molecules vertically ('thin' channel instead of 'narrow' channel). The final devices used in this work have nanofluidic filters (60-120nm thickness), in between the 'thick" regions (500-1000nm thickness). The period of the structure was 4μm, and each period contains one entropic trap (transition from thick to thin regions). The total length of the device was 3cm (separation channel), but detection was done at different distances from the band-launching site. The loading and launching the biomolecule sample was achieved in the standard double T-junction channel, by controlling the potential at each reservoir.

For DNA experiment, double stranded DNA ladder samples (sized between 100-1500bp) were purchased (New England Biolab), and were labeled by YOYO-1 dye (Molecular Probes) with the dye to basepair ratio of 10:1. As a buffer for DNA electrophoresis, we used 5X Tris-Borate-EDTA (TBE) buffer. For protein separation experiment, several fluorescence-labeled proteins were purchased from Molecular Probes, and were denatured in a buffer solution with 2% sodium dodecyl sulfate (SDS). As a final electrophoresis buffer, we used 5X TBE buffer with 0.1% SDS. Both DNA and SDS-coated proteins carry negative charges, which helped prevent them from adsorbing to the nanofluidic filter walls that is also negatively charged. An inverted microscope was used for detection of the biomolecules, using an Ar-ion laser (488nm) as a light source. Fluorescence light from the biomolecules were detected by a cooled CCD camera (Sensicam QE, Cooke Corp.) and processed using an imaging software (IPLab, Scanalytics).

Result and Discussion

Fig. 2-(a) shows the separation result of 100bp double stranded DNA ladder sample, which were obtained at the 1cm away from the launching position. At the field as low as 26.67V/cm, all 12 peaks were baseline-resolved without ambiguity. (100, 500, and 1000bp DNA bands have higher intensity for easy peak assignment.) The thick region of the device was 500nm, whie the nanofilter gap was 80nm. Some of the smaller DNA molecules in this sample were smaller than 80nm. For example, 100bp double-stranded DNA molecules are only ~34nm long even when they are fully extended. Similar separation results were obtained using the device with 120nm filter gap size.

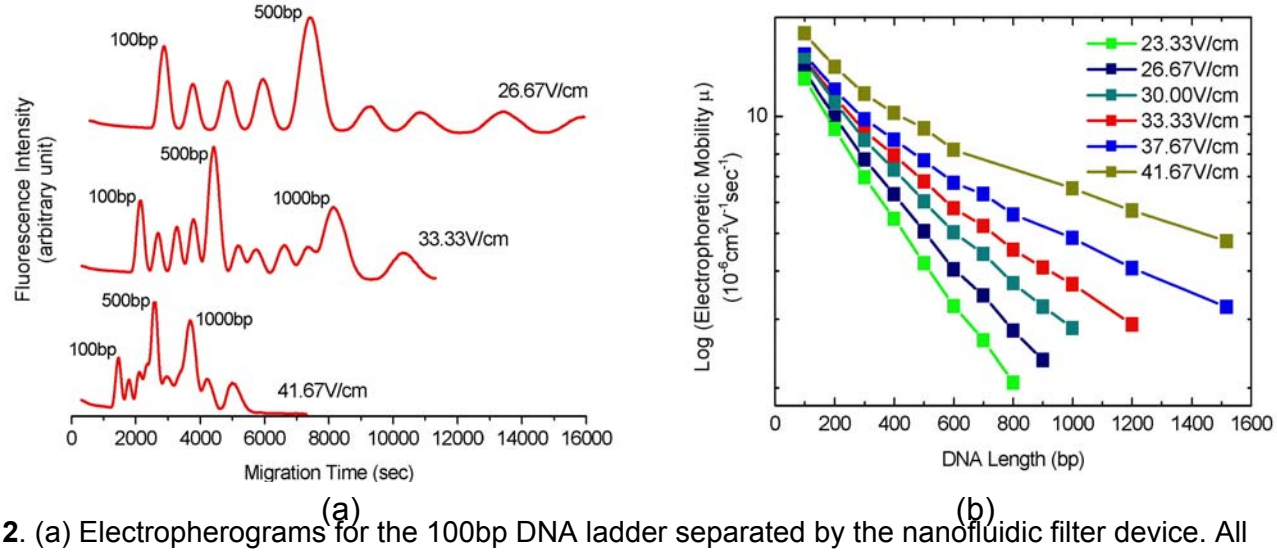

Fig. 2. (a) Electropherograms for the 100bp DNA ladder separated by the nanofluidic filter device. All the peaks existing in the sample were assigned unequivocally, and a baseline separation was achieved in the case of 26.67V/cm. (b) DNA mobility vs length at different field electrophoresis field.

The period of the device (the length of one thick and one thin region) was 4μm. Therefore, there are about 2500 nanofluidic filters (entropic barriers) used for this separation. The separation resolution was increased as the electrophoresis field is decreased. This suggests that the sieving mechanism here is not due to inherent electrophoretic mobility differences or dielectrophoretic effects. We also performed control experiments with the nanofluidic channel of 60nm thickness (no entropic barriers). In such devices, no separation was observed at any similar electric field values. This excludes the possibility of the chromatographic separation due to the non-specific binding of the molecules to the wall.

We conducted experiments with many different DNA molecules and at different fields. The separation result was summarized in Fig. 2-(b). The mobility data of DNA molecules with various lengths at low electric field values fits well into an exponential function, represented as a straight line in Fig. 2-(b). At high fields and for larger molecules, the molecules tend to deviate from the exponential behavior. Interestingly, such behavior is also known in gel electrophoresis as well. DNA molecules longer than the nanofilter size (~120nm, longer than 400bp) would have different interaction with nanofilter, so they are expected to have different sieving characteristics. Also, the original theory[3] of steric hindrance sieving was based on the (quasi) equilibrium partitioning between nanopores and open spaces (which is relevant to size-exclusion gel chromatography), while our experiment is essentially non-equilibrium condition.

Fig. 3 shows the electropherogram of separating protein molecules (SDS-denatured). Two fluoresecently labeled proteins were denatured with SDS, and were separated in the nanofluidic filter devices with 80nm gap size. As in the DNA separation case, the separation resolution improves as the driving electric field is decreased. We also ran a control electrophoresis experiment with two SDS-coated proteins in a flat channel as a control experiment, where we could not observe any separation.

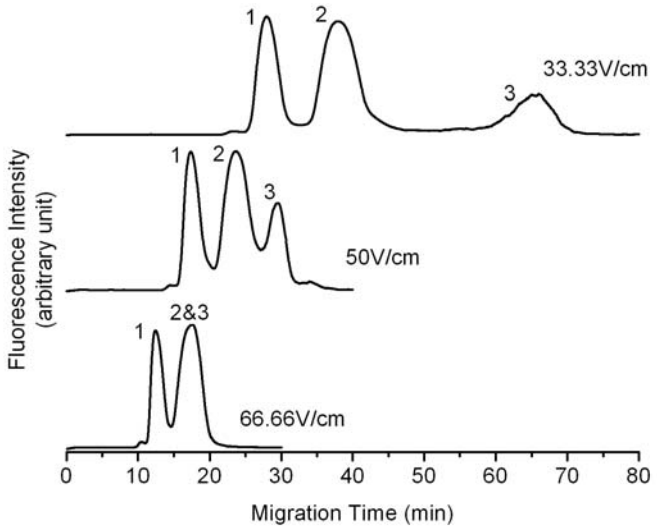

Fig. 3. Separation of cholera toxin subunit B (1, 11.4kD), lectin phytohemagglutinin-L (2, 120kD) and low density human lipoprotein (3, 179kD). Proteins were coated with SDS by standard protocol. The device has 60nm thin regions, 500nm thick regions and 4mm trap period.

The separation speed obtained in this work is comparable to that of gel electrophoresis, and slower than that of capillary electrophoresis using polymeric sieving matrices. It has to be noted that the 80~120nm filter gap size was not optimized in any way for this separation application (especially for proteins), and there is a lot more room to increase separation efficiency and resolution, by carefully designing and modeling the nanofluidic filter structures. Simply decreasing the size of the nanofilters would enhance the separation resolution and selectivity for smaller biomolecules. We recently characterized the fabrication process of the nanofluidic filters as thin as 20nm[11]. More importantly, this device does not use any buffer sieving agents or matrix, which is difficult to be integrated into a complex micro-total-analysis system.

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