Nanofluidic Devices for Rapid Analysis of DNA and Proteins

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***Abstract*-A new class of nanofluidic filter array devices were designed and fabricated to perform rapid separation (both analytical and preparative) of DNA and proteins covering very broad biological size scales. By virtue of its separation efficiency, gel-free operation, ease of sample recovery and high throughput, these nanofluidic filter array devices hold great promise for an integrated biomolecule sample preparation and analysis system.**

I. Introduction

Direct analysis of biologically-relevant entities such as nucleic acids and proteins offers the potential to outperform conventional analysis techniques and diagnostic methods through enhancements in speed, accuracy, and sensitivity. Nanofluidic systems with critical dimensions comparable to the molecular scale open up new possibilities for direct observation, manipulation and analysis of biomolecules (single or ensemble), thus providing a novel basis for ultra-sensitive and high-resolution sensors and medical diagnostic systems. Inspired by this concept, we have developed a new class of nanofluidic filter devices and have implemented them as controllable molecular sieves for rapid analytical separation of various physiologically-relevant macromolecules such as DNA and proteins [1]. In addition, we have conducted theoretical studies of molecular sieving process in the context of periodic free-energy landscapes created by the patterned nanofluidic filter arrays. The kinetic model constructed based upon the equilibrium partitioning theory and the Kramers rate theory properly describes the field-dependent sieving behavior, presenting notable progress beyond the existing equilibrium model in conventional gels [2]. We have further devised a microfabricated anisotropic sieving structure consisting of a two-dimensional periodic nanofluidic filter array (anisotropic nanofilter array, ANA). The designed structural anisotropy in the ANA causes different-sized biomolecules to follow distinct migration trajectories, leading to efficient continuous-flow separation [3]. Continuous-flow separation of both DNA and proteins covering broad biological size ranges were achieved within a few minutes, thus demonstrating the potential of the ANA as a generic molecular sieving structure for an integrated biomolecule sample preparation and analysis system.

II. Ogston Sieving and Entropic Trapping of Biomolecules Across a Nanofilter

We have applied microfabricated regular nanofluidic filters as artificial molecular sieves for size-fractionation of different biomolecules. Molecular crossings of the nanofilter under the influence of electric field can be described as biased thermally activated jumps across free energy barriers at the nanofilter threshold [2]. At high ionic strength where the Debye length λ_D is negligible compared to the nanofilter shallow region depth d_s, the energy barriers are solely determined by the configurational or conformational entropy loss within the constriction due to steric exclusion (a purely steric limit). For biomolecules with diameters smaller than the nanofilter constriction (*i.e.*, Ogston sieving), the steric energy barrier favors DNA and proteins with a smaller size for passage due to their greater retained configurational freedom [1] (Fig. 1a). For macromolecules with diameters greater than the nanofilter constriction size, passage requires the molecules to deform and form hernias at the cost of their internal conformational entropy (*i.e.*, entropic trapping) [2]. In entropic trapping, the steric energy barrier for DNA escape has been shown to depend solely on the inverse of the electric field strength. Further, longer molecules have a larger surface area contacting the constriction and thus have a greater probability to form hernias that initiate the escape process (*i.e.*, a higher escape attempt frequency) (Fig. 1b). Therefore, in entropic trapping, longer molecules assume a greater jump passage rate and advance faster [2].

III. Results and Discussion

A. Separation of Biomolecules in One-Dimensional Nanofilter Array

The efficiency of Ogston sieving for separation of biomolecules was demonstrated in a one-dimensional nanofilter array with a shallow region depth of 55 nm and a pitch number of 1 μm (Fig. 2) [1]. The speed and resolution obtained by the one-dimensional nanofilter array is already comparable to current state of the art systems (i.e. capillary gel electrophoresis). The separation efficiency of the nanofilter array can be further improved, in principle, by simply scaling down the nanofilter period by other advanced sub-100nm lithography tools.

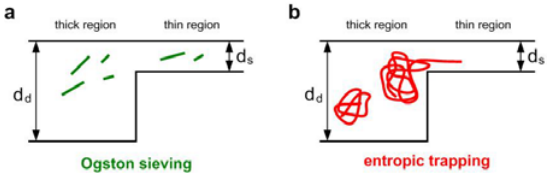

Fig. 1. Ogston sieving (a) and entropic trapping (b) of biomolecules across a nanofilter.

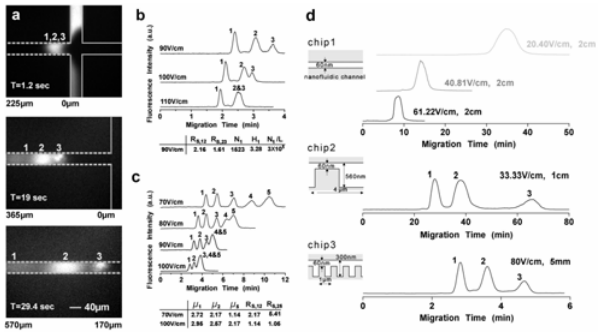

Fig. 2. a, Sequence of fluorescence images showing separation of the SDS-protein complexes (1: cholera toxin subunit B, 11.4 kDa; 2: lectin phytohemagglutinin-L, 120 kDa; 3: low density human lipoprotein, 179 kDa with E_{av}= 100 V/cm. b-c, Separation of SDS-protein complexes (b) and dsDNA molecules (c) under different applied fields. Band assignment for DNA: (1) 50 bp; (2) 150 bp; (3) 300 bp; (4) 500 bp; (5) 766 bp. d, Comparison of three different nanofilter array devices.

B. Continuous-Flow Biomolecule Separation in a Two-Dimensional Nanofilter Array

Continuous-flow fractionation of biomolecules has several highly-desirable benefits: 1) increased sample throughput that is ideal for preparative sample separation; 2) sorted biomolecule streams can be easily collected for downstream analysis or subsequent manipulation; 3) continuous-harvesting of the subset of biomolecules of interest for downstream analysis to enhance the specificity and sensitivity of biomarker detection and biosensing. In this work, we have conceived a two-dimensional nanofilter structure to continuously separate biomolecules covering a broad size range [3]. The separation process in the two-dimensional nanofilter array takes advantage of the differential bidirectional transport of biomolecules of different sizes through periodic nanofilter array (Fig. 3a). Fig. 3b shows a specially designed two-dimensional nanofilter array device incorporating many microfluidic channels connecting to fluid reservoirs. Fig. 3c-d shows clear experimental evidence of high resolution separation of small DNA in the Ogston sieving regime. The stream deflection angle θ depends on the exact field conditions as shown in Fig. 3d. The size selectivity estimated from these small DNA experiments is about 20 bp DNA length, which is by far the best size selectivity demonstrated by artificial molecular sieves. The two-dimensional nanofilter array can also separate long DNA molecules with high resolution based on the entropic trapping mechanism. We have successfully separated λ DNA – Hind III digest (2–20 kbp) within a few minutes [3]. The two-dimensional nanofilter array is also capable of separating flexible proteins under both native and denaturing conditions in the Ogston sieving regime [3]

In summary, we have designed and implemented a new class of nanofluidic sieving structures that can efficiently separate biologically-relevant macromolecules covering broad biological size scales. Because of their precisely characterized environment, the nanofluidic artificial sieving structures are also ideal for the theoretical study of molecular dynamics and stochastic motion in confining spaces. We have successfully demonstrated high-resolution separation (both analytical and preparative) of a wide range of DNA fragments (50–23000 bp) and proteins (11–400 kDa) within a few minutes. By virtue of its separation efficiency and high throughput, the nanofilter array devices holds great promise as an integrated biomolecule sample preparation and analysis system.

ACKNOWLEDGMENT

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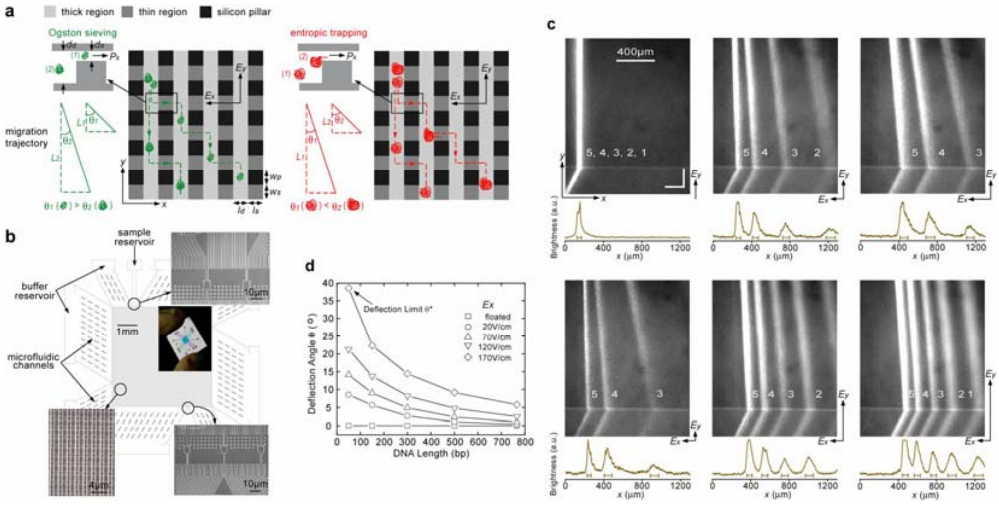

Fig. 3. a, Bidirectional motion of biomolecules in a two-dimensional array of nanofilters. b, Structure of the microfabricated device incorporating the nanofilter array integrated with microfluidic channels. c, Fluorescent photographs of fractionation of the PCR marker under different field conditions as indicated by the horizontal and vertical vectors representing E_x and E_y, respectively. The band assignment: (1)50 bp; (2) 150 bp; (3) 300 bp; (4) 500 bp; (5) 766 bp. d, Measured deflection angle θ as a function of DNA length when E_y=100 V/cm and E_x was varied, as indicated.