CONTINUOUS-FLOW BIOMOLECULE SEPARATION THROUGH PATTERNED ANISOTROPIC NANOFLUIDIC SIEVING STRUCTURE

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Abstract

We report a microfabricated anisotropic sieving structure (Anisotropic Nanofilter Array: ANA) for continuous-flow biomolecule separation. The designed structural anisotropy in the ANA causes different-sized biomolecules to follow different trajectories, leading to efficient separation. Continuous-flow Ogston sieving-based separation of short DNA and proteins as well as entropic trapping-based separation of long DNA were achieved, thus demonstrating the potential of the ANA as a generic sieving system for biomolecules of broad size scales.

Keywords: Nanofluidics, anisotropy, biomolecule separation, DNA, protein

1. Introduction

Efficient methods of separating and purifying biomolecules from a complex mixture are essential in biology and biomedical engineering. Recently, there has been great interest in switching from conventional porous gel media to patterned regular sieving structures, either by colloidal templating of self-assembled bead arrays or by various microfabrication techniques. However, the regular sieving structures reported in the literature so far have proven efficacious only for long DNA separation and their applicability to smaller, physiologically-relevant macromolecules remains questionable. Here we report a unique anisotropic sieving structure design and its implementation for continuous-flow separation of biomolecules of very broad biological size scales, based on two distinct sieving mechanisms: Ogston sieving [1] and entropic trapping [2].

2. Anisotropic Nanofilter Array (ANA)

The design of the ANA consists of a two-dimensional periodic nanofilter array (**Fig. 1**). The separation mechanism of the ANA relies on different sieving characteristics along two orthogonal directions within the ANA, which are perpendicular and parallel to the nanofilter rows. Upon application of two orthogonal electric fields E_x and E_y, negative-charged molecules assuming a drift motion in deep channels can be selectively driven to jump across the nanofilter to the adjacent deep channel. Molecular crossings of the nanofilter under the influence of E_x can be described as biased thermally activated jumps across free energy barriers at the nanofilter threshold [3]. For Ogston sieving, this energy barrier originates from the configurational entropy loss within the constriction

due to the steric constraints of the nanofilter wall, and this barrier favors molecules with a smaller size for passage (**Fig. 1A**) [1], resulting in a greater jump passage rate P_x and thus a larger stream deflection angle θ for shorter molecules. For molecules 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). Longer molecules can assume a greater jump passage rate P_x due to their larger surface area contacting the constriction and thus have a greater probability to form hernias that initiate the escape process, resulting in a larger deflection angle θ (**Fig. 1B**) [2].

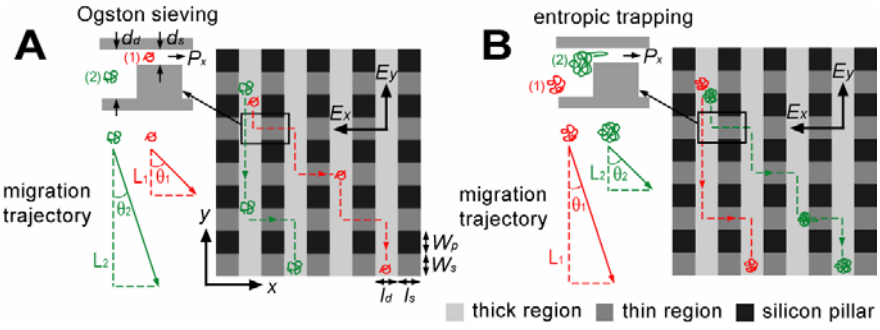

Figure 1. Schematic showing negatively charged molecules assuming bidirectional motion in the ANA under two orthogonal electric fields E_x and E_y. Dashed lines and arrows indicate migration trajectories projected onto the *x*-*y* plane for both the Ogston sieving (A) and the entropic trapping regimes (B).

3. Results and discussion

Figure 2 and Fig. 3 show separation results of a PCR marker sample (50─766 bp, rod-like rigid molecules with end-to-end distance of 16─150 nm, Ogston sieving) and λ DNA–Hind III digest (2.027─23.13 kbp, radii of gyration R_g: 140─520 nm, entropic trapping) through the ANA (with d_s=55 nm), respectively. A close look at the fluorescence images revealed that, as expected, for Ogston sieving of the PCR marker, shorter DNA fragments followed more deflected migration trajectories than longer ones, while for entropic trapping of λ DNA–Hind III digest, longer ones followed more deflected trajectories. From the fluorescence intensity profiles in Fig. 2, the size selectivity of the ANA in the Ogston sieving regime can be estimated to be about 5 nm [4]. The ANA is also capable of separating mixtures of proteins that have different molecular weights based on the Ogston sieving mechanism [1, 4].

Continuous-flow separation through the ANA should be applicable to other molecular properties (*e.g.*, charge density (pI) or hydrophobicity) that can lead to differential transport across the nanofilters. We believe the ANA can be used as a generic sieving structure to separate other particles of interest with nanoscale dimensions, including nanoparticles and nanowires, viruses and cell organelles.

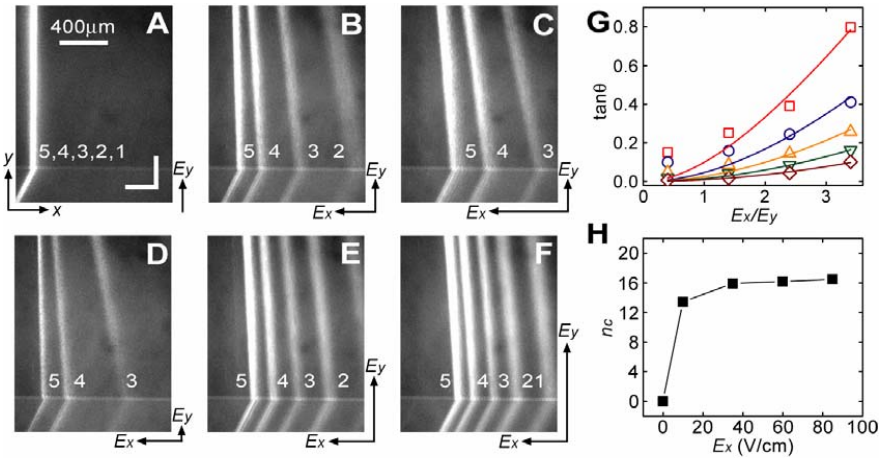

Figure 2. Ogston sieving of the PCR marker through the ANA. (A to F) Fluorescent photographs of the PCR marker stream pattern under different field conditions as indicated. White scale bar for electric field: 25 V/cm. Band assignment: 1, 50 bp; 2, 150 bp; 3, 300 bp; 4, 500 bp; 5, 766 bp. (G) tanθ as a function of E_x/E_y at fixed E_y= 25 V/cm (50 bp (□), 150 bp (○), 300 bp (△), 500 bp (▽), 766 bp (◇)). The solid lines are calculated theoretical curves [4]. (H) The effective peak capacity n_c on E_x when E_y=25 V/cm.

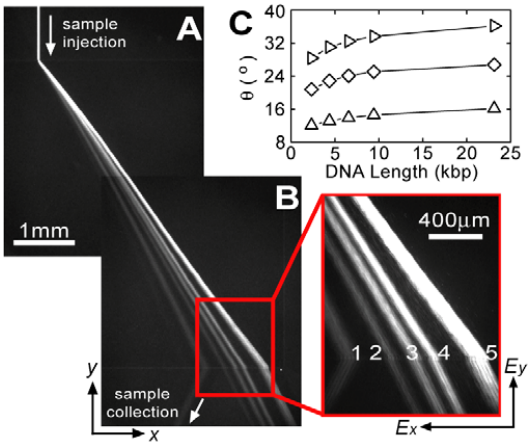

Figure 3. Entropic trapping of λ DNA–Hind III digest through the ANA. (A to B) Fluorescent photographs showing separation of λ DNA–Hind III digest with E_x=185 V/cm and E_y=100 V/cm. Band assignment: 1, 2.322 kbp; 2, 4.361 kbp; 3, 6.557 kbp; 4, 9.416 kbp; 5, 23.130 kbp. (C) θ as a function of DNA length with E_y=100 V/cm and E_x varied (E_x: 80 V/cm (△), E_x: 145 V/cm (◇),E_x: 185 V/cm (▷).

4. Reference

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