A Patterned Anisotropic Nanofilter Array for Continuous-flow Separation of DNA and Proteins

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Microfabricated regular sieving structures hold great promise as an alternative to gels to improve biomolecule separation speed and resolution. In contrast to the disordered gel porous network, these regular structures also provide well-defined environments ideal for study of molecular dynamics in confining spaces. However, previous regular sieving structures have been limited for separation of long DNA molecules, and separation of smaller, physiologically-relevant macromolecules, such as proteins, still remain as a challenge. Here we report a microfabricated anisotropic sieving structure consisting of a two-dimensional periodic nanofluidic filter array (an Anisotropic Nanofilter Array, or ANA). The designed structural anisotropy in the ANA causes differently-sized molecules 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 structure for an integrated biomolecule sample preparation and analysis system.

Figure 1: Ogston sieving of the PCR marker through the ANA. For A, only $E_y$ applied and $E_x=25$ V/cm; for B, $E_x=35$ V/cm, $E_y=25$ V/cm; for C, $E_x=60$ V/cm, $E_y=25$ V/cm; for D, $E_x=35$ V/cm, $E_y=12.5$ V/cm; for E, $E_x=35$ V/cm, $E_y=50$ V/cm; for F, $E_x=35$ V/cm, $E_y=75$ V/cm. Band assignment: (1) 50-bp; (2) 150-bp; (3) 300-bp; (4) 500-bp; (5) 766-bp.

Figure 2: Entropic trapping of long DNA (the $\lambda$ DNA–Hind III digest) through the ANA. Fluorescent photographs show separation of $\lambda$ DNA–Hind III digest with different electric field conditions. In A, B, and F, $E_x=185$ V/cm and $E_y=100$ V/cm. In C, $E_x=50$ V/cm and $E_y=100$ V/cm. In D, $E_x=145$ V/cm and $E_y=100$ V/cm. In E, $E_x=170$ V/cm and $E_y=100$ V/cm. Band assignments are 2,322 bp (1), 4,361 bp (2), 6,557 bp (3), 9,416 bp (4), and 23,130 bp (5).

REFERENCES
High-throughput, Continuous-flow Separation of Biomolecules in a High-aspect-ratio Nanofilter Array

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We have developed a novel fabrication approach to generate massively-parallel, high-aspect-ratio vertical nanofluidic channels with smooth, vertical sidewalls and precise control of uniform gap sizes (lateral trench width) down to 50 nm (Figure 1) [1]. The aspect ratio can be as high as 400 and the channel depths are more than 20 μm. This technique enables us to fabricate a large area of solid membrane structures with well-defined pore size and geometries, which can be very useful for membrane-based application such as filtration, separation and fuel cells. Also, using such systems as molecular sieving filters, we demonstrated efficient continuous-flow size-fractionation of large DNA molecules in a two-dimensional (2D) vertical nanofilter array device fabricated by this method (Figure 2). Our device allows much higher sample volume processing rate (1μL/hour), compared with the planar nanofilter array chip previously reported [2]. We believe that these devices could be a key to the efficient proteomic sample preparation microsystems as well as useful in purifying and separating various bioparticles and nanoparticles.

REFERENCE