Nanofluidic BioMEMS Research

RLE Group
Micro / Nanofluidic BioMEMS Group

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Overview of group

Nanofluidic BioMEMS group in RLE (Han group) is exploring various ways that the micro/nanofabrication techniques can be used for advanced biomolecule manipulation and separation applications. It is now possible to reliably fabricate nanofluidic gaps and filters that have regular, controllable structures, with near-molecular dimensions (10 -100nm). One can take advantage of these nanofluidic structures for advanced separation and manipulation of various biomolecules and bioparticles, including cell, cellular organelles, DNA, protein, and carbohydrates. The research of Nanofluidic BioMEMS group is currently actively designing, fabricating and testing the new kinds of molecular sieves and filters that can be essential for the next-generation biomolecule assays in the new era of genomics, proteomics and glycomics. At the same time, the subject of molecular stochastic motion and molecular interaction with nanostructure is actively studied, in order to provide firm theoretical and scientific ground for the development of novel nanofluidic molecular filters.

1. Biomolecule Separation in Nanofluidic Filters by Steric Hindrance Mechanism

Sponsors
National Science Foundation

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In this project, we have successfully demonstrated the first microfabricated nanofilter array chip that can size-fractionate small biomolecules, such as SDS-protein complexes and small dsDNA molecules based on the Ogston sieving mechanism without using sieving matrices. Nanofilter arrays with a gap size of 40-180nm were fabricated and characterized (Fig. 1). Complete separation of SDS-protein complexes and small DNA molecules were achieved in several minutes (Fig. 2). The separation efficiency of the miniature nanofilter array chip is comparable to current state of the art systems (i.e. capillary gel electrophoresis). The nanofilter array chip is chemically and mechanically robust, and can be used over a long period without degradation of its characteristics. The nanofilter array chip allows the use of different buffer systems, and this opens up possibilities for integrating different biomolecule sensors and separation and reaction chambers in one single chip, without the concern of sieving matrix crosstalk and contamination. Therefore, the nanofilter array chip presented here is an important milestone toward a truly integrated proteomic sample-preparation microsystem that includes fully integrated multiple separation and purification steps.

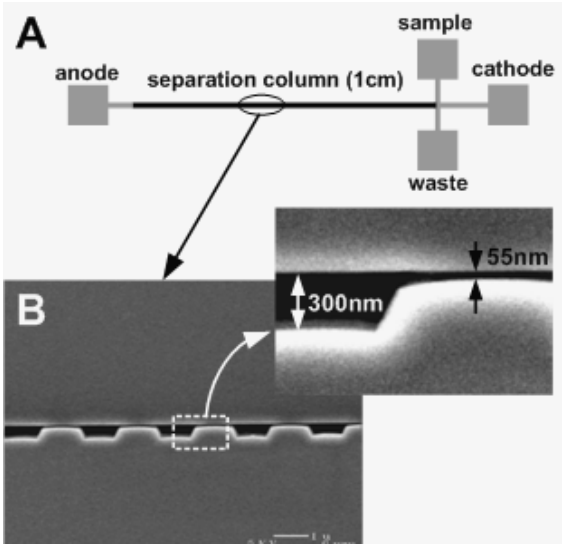

Fig. 1. (A) Layout of the nanofilter array chip. The device includes four buffer access holes (anode, cathode, sample and waste), a 1cm separation column (a periodic array of nanofilters) and a T-Shaped injector. **(B)** SEM images of the cross-section of a periodic nanofilter array along the separation channel. The nanofilter consists of a thin region (d_s=55nm) and a thick region (d_d=300nm) of equal lengths. The period of one nanofilter is L (L=2μm).

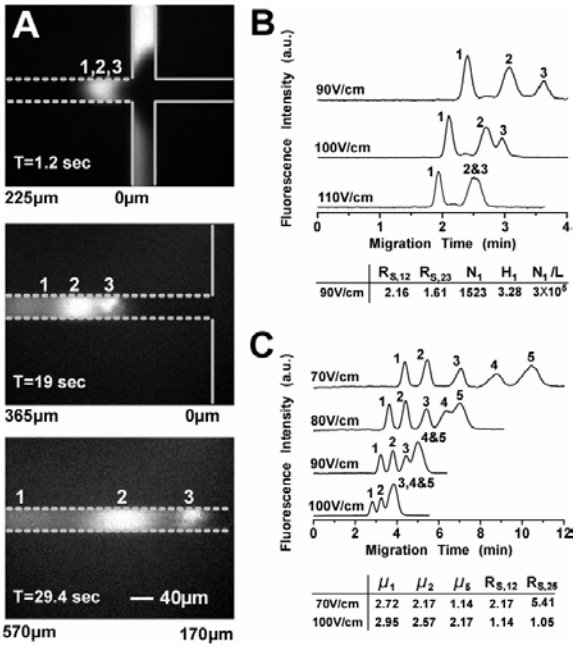

Fig. 2. Separation of SDS-protein complexes and dsDNA molecules in a nanofilter array device (d_s: 60nm, d_d: 300nm, L: 1μm). Band assignment for SDS-protein complexes: (1) cholera toxin subunit B (MW: 11.4kDa); (2) lectin phytohemagglutinin-L (MW: 120kDa); (3) low density human lipoprotein (MW: 179kDa). Band assignment for DNA (PCR marker sample): (1) 50bp; (2) 150bp; (3) 300bp; (4) 500bp; (5) 766bp. **(A)** Sequence of fluorescence images showing separation of the SDS-protein complexes under the electric field of 100V/cm. The solid lines indicate the T-shaped injector and the dashed lines indicate the nanofilter array. The values listed under the images indicate the distance from the injection point. **(B & C)** Separation of SDS-protein complexes and dsDNA molecules under different applied fields. Separation length: 5mm.

2. Nanofluidic Biomolecule Preconcentration

Sponsors
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In all biomolecule sensing technologies, detection becomes increasingly difficult or impossible when the analyte concentration is lower than a certain level (the detection limit). However, in complex blood serum samples, most of the important biomolecule markers are available only in trace amounts (fM to nM). Therefore, the detection (or identification) of these markers after pre-fractionation and separation is extremely difficult. To solve this problem, numerous efforts have been made on developing a pre-concentration process before or after separation. So far, the single pre-concentration method with the highest concentration factor among all the strategies is the micellar electrokinetic sweeping, which can achieve a concentration factor as high as 500-to 7000- fold.

Here, we present a novel way to achieve rapid pre-concentration for a charged biomolecule that can achieve an up to 10 million fold sample pre-concentration within 30 minutes. Ionic charge separation will happen once the electrical field is applied across the nano-filter. It has been reported that a flow several times stronger than general EOF caused by induced-charge layer will present with confined geometry. As a consequence, a barrier that can trap both positively and negatively charged molecules is formed by extending the Debye layer (non-equilibrium charge polarization) into the microfluidic channel with a stronger carrier flow. This device can concentrate

a sample without the complexity of varying the buffer concentration (such as in electrokinetic focusing), any additional additive (such as SDS in micellar sweep techniques) and or any complex structure that will make the downstream analysis difficult. Because of the simple structure of the device, various integrations and applications including sample pre-concentration for advanced blood proteome analysis, sample injection for microchip electrophoresis/ chromatography, and environmental trace analysis are possible.

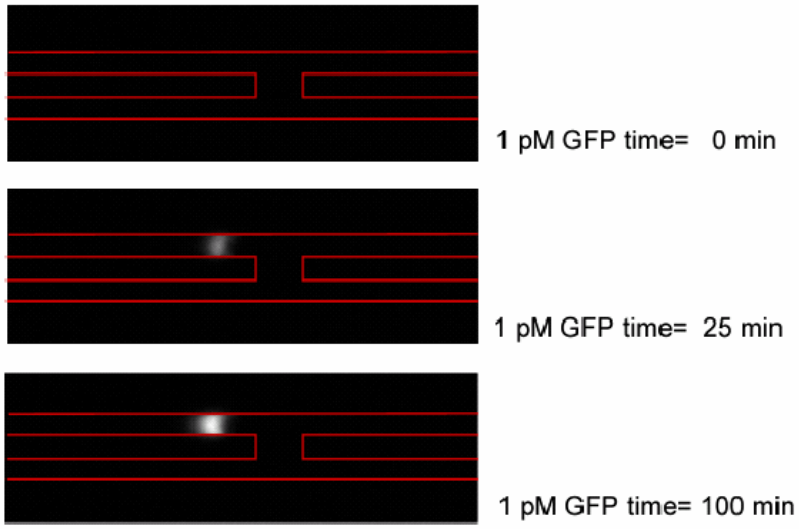

Figure. 1. Pre-concentration phenomena for 100 minutes, starting from highly diluted 33pM (10-12M) GFP solution. The detection condition barely detects the 33μM GFP concentration, which means at 25 min or later, the concentration of the plug exceeds 1μM. Voltage applied across top-down channel is 10 volts, while 4 volts along the top channel (pictures were taken by CCD camera with 1 sec exposure)

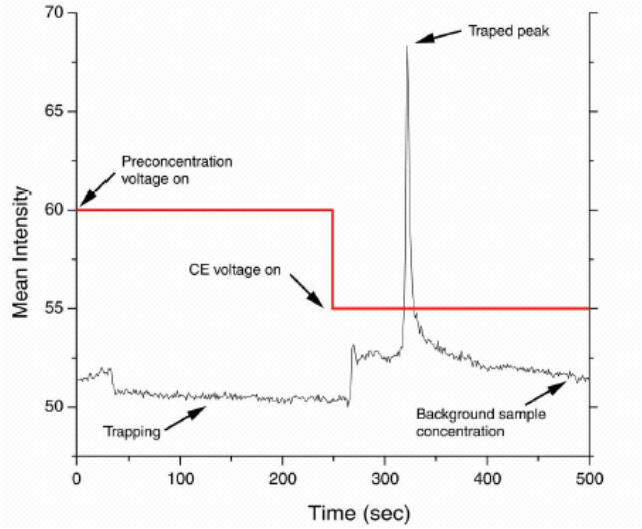

Figure. 2. Picture showing the electrokinetic capture/release profiles. After 250 sec, the waster channel was floated to perform an EOF- driven CE in the top channel. Shown between 300 and 350 sec is the releasing of captured proteins.

3. Hindered Diffusion of Biomolecules Confined in Nanofluidic Channels

Sponsors
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Hindered transport of macromolecules in liquid-filled pores is important to biological membrane processes associated with cell biology and medical physiology, chromatography, separation, and heterogeneous catalysis. It is highly desirable to conduct well-controlled, model-based studies of molecular and fluidic transport process in a confined space. Compared to nanoporous track-etched membranes, micromachined nanofluidic structures offer unique advantages, including well-controlled physical and chemical properties and compatibility with various single molecule detection (SMD) methods as well as easy integration to μTAS.

The objective of this research is to investigate the stochastic motion of biomolecules or small particles confined in a nanofluidic channel. We characterized glass-glass and glass-Si bonding processes for the fabrication of planar nanofluidic channels. We demonstrated that glass-glass nanofluidic channels as thin as 25 nm with aspect ratio of 0.0005 (depth to width) can be achieved with the developed glass-glass bonding technique. We also found that silicon-glass nanofluidic channels, as thin as 20 nm with an aspect ratio of 0.004 can be reliably obtained with anodic bonding technique. Cross-sectional SEM analysis after bonding was performed to prove that there is no significant change of the depth of the nanofluidic channels due to anodic bonding and glass-glass fusion bonding processes.

We have been examining the conformation and diffusion of a single, large DNA molecule confined in a slit glass nanochannel using epifluorescence video microscopy (Fig 2(A)). The Brownian motion of single DNA molecules (λ-DNA, for example) can be observed and the diffusivity is characterized as a function of the degree of chain confinement (depth of the channel) and chain length. In addition, the effects of spatial confinement and surface boundary layer on the diffusivity of small biomolecules within a nanochannel are being investigated by two-photon fluorescence correlation spectroscopy (FCS), shown in Fig 2 (B).). The potential impact of this research would be significant both scientifically and technologically by offering a better understanding of molecular diffusion and transport in confined environments as well as generating new concepts of molecular sorting and manipulation technology.

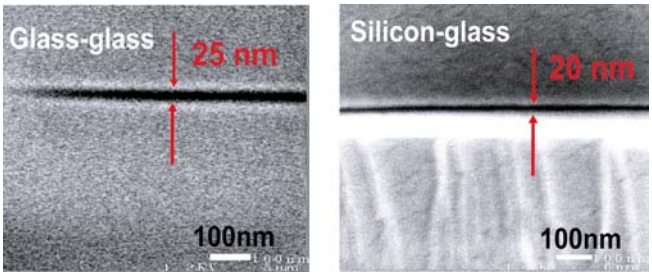

Figure 1: Cross-sectional SEM images of the glass-glass channel with a depth of 25 nm (A) and silicon-glass channel with a depth of 20 nm (B).

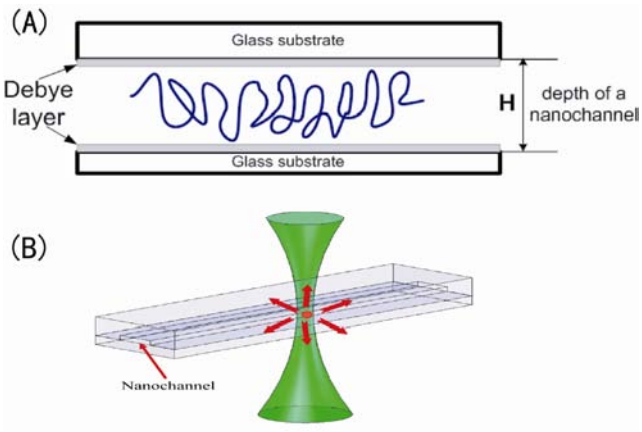

Figure 2: (A) Schematic diagram of a single, large DNA molecule confined to a slit glass nanochannel with a depth of H. (B) Schematic diagram of detecting single, small molecules by two-photo FCS in a slit nanochannel with vertical confinement.

Publications

Journal Articles

Y.-C. Wang, M. H. Choi, and J. Han, "Two-Dimensional Protein Separation with Advanced Sample and Buffer Isolation Using Microfluidic Valves," *Anal. Chem.*,**76**, 4426, (2004).

J. Han and A. K. Singh, "Rapid protein separations in ultra-short microchannels: microchip sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing," *J. Chromatogr. A*, **1049**, 205, (2004).

Y.-C. Wang, A. L. Stevens, and J. Han, "Million-fold Preconcentration of Proteins and Peptides by Nanofluidic Filter", *Anal. Chem.* To be published (2005)

Journal Articles, submitted for publication

J. Fu, P. Mao, and J. Han, "Ogston Sieving of Biomolecules in a Nanofilter Array Chip," submitted to Applied Physics Letters (2005)

P. Mao and J. Han, "Fabrication and Characterization of 20 nm Nanofluidic Channels by Glass-Glass and Glass-Silicon Bonding," submitted to Lab on a Chip (2005)

Meeting Papers, Published

J. Han and J. Fu, "Biomolecule Separation by Steric Hindrance using Nanofluidic Filters," in *Proceedings of the 26th Annual International Conference of the IEEE EMBS*. San Francisco, (2004), 2611.

J. Fu and J. Han, "Biomolecule Separation in Nanofluidic Filters by Molecular Steric Hindrance Mechanism," in *Proceedings of the 2004 Micro Total Analysis Systems International Conference*, vol. 1. Malmo, Sweden, (2004), 285.

Theses

Pan Mao (ME), "Fabrication and Characterization of Nanofluidic Channels for Studying Molecular Dynamics in Confined Environments," January 2005.

Arnaud LeCoquic (EECS), "Gate Potential Control of Nanofluidic Devices," May 2005.