Nanofluidic BioMEMS

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 Confinement and Detection in Nanofluidic Filters

Sponsors
Dupont-MIT Alliances
National Institute of Health (EB005743)

Project Staff
Reto Schoch (past member), Lih Feng Cheow

Nanofluidic channels can be used to enhance surface binding reactions, since the target molecules are closely confined to the surfaces which are coated with specific binding partners. Moreover, diffusion-limited binding can be significantly enhanced if the molecules are steered into the nanochannels via either pressure-driven or electrokinetic flow. By monitoring the nanochannel impedance, which is sensitive to surface binding, low analyte concentrations have been detected electrically in nanofluidic channels within response times of 1-2 hours(1). This represents a $\sim$54 fold reduction in the response time using convective flow compared to diffusion-limited binding. At high flow velocities the presented method of reaction kinetics enhancement is potentially limited by force-induced dissociations of the receptor-ligand bonds. Optimization of this scheme could be useful for label-free, electrical detection of biomolecule binding reactions within nanochannels on a chip.
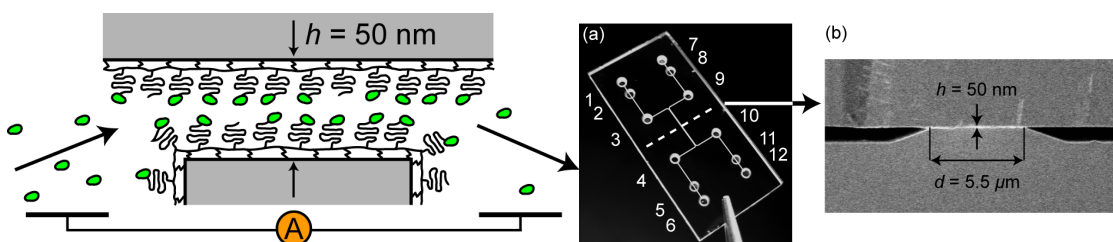

Figure 1: Enhanced binding in nanochannels.(Left) Target molecules bind to the surface antibodies with better binding kinetics, due to nanochannel confinement (Right) Nanofluidic devices used for detection.

2. Nanofluidic Biomolecule Preconcentration for Enhancing Biomolecule Detection

Sponsors
NIH CDP Center grant (GM68762)
National Institute of Health (EB005743)

Project Staff
Jeong Hoon Li, Leon Li, Vincent Liu, Phillip Dextras, Sung Jae Kim

During the last project year, we made significant progress in the engineering of better, larger protein concentration systems. We developed several fabrication strategies for making protein concentration devices in a PDMS microfluidics format(2-4). PDMS is a widely-used material among bioengineers to build advanced biosystems and microfluidic devices. With these new fabrication strategies, one can now build protein concentration devices without the need for sophisticated fabrication facilities. For example, one can pattern ion permselective polymers such as Nafion® (liquid form) on the surface(3), instead of nanochannels to make a protein concentration device.. This method shows better reliability and repeatability, and simple surface patterning technique allows one to quickly parallelize multiple concentrator devices for even higher throughput. By utilizing electrokinetic trapping in PDMS preconcentrator, we achieved a concentration factor as high as $\sim10^5$ within $\sim$5 mins, and the total plug volume as large as

200pL(3). Alternatively, a self-sealed vertical Nafion junction can be made, which has a better mass transfer between the microchannel and nanojunction(4).
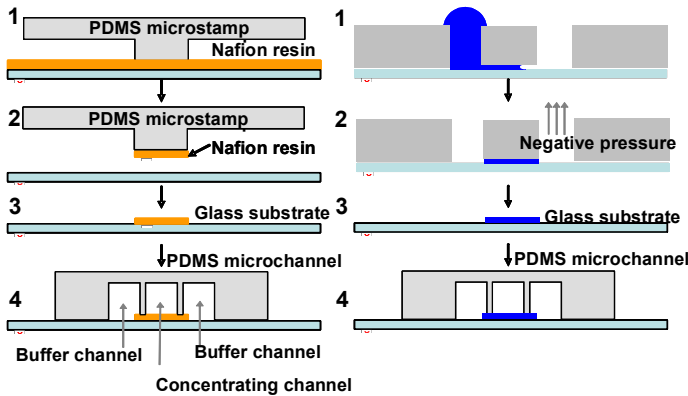
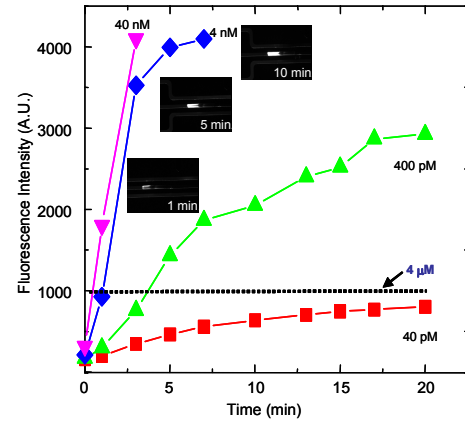**(a) Micro-stamping technique**   **(b) Capillary lithography technique**

Figure 2: PDMS-protein concentration system (**left**) Fabrication of via surface-patterned ion-selective membrane using **(a)** micro-stamping technique and **(b)** micropatterning in capillaries. (Ref.(3)) (**right)** Preconcentration of β-phycoerythrin protein versus electrokinetic trapping time. This result shows that we can achieve a preconcentration factor of ~10^4 in 5 min. Fluorescence images of 4nM protein shown next to the graph indicate an increase of the concentrated plug in size and concentration with trapping time.

3. Fabrication of Nanofluidic Membrane for Biomolecule Separation

Sponsors
KIST (Korean Institute of Science and Technology) - IMC (Intelligent Microsystems Center)
National Institute of Health (EB005743)

Project Staff
Pan Mao, Aniruddh Sarkar, Masumi Yamada

In order to increase the overall throughput of nanofluidic systems in general, we have devised a novel fabrication technique for massively-parallel, vertical nanochannels(5). By combining photolithography and anisotropic etching of Si, we have made a massively parallel (5mm×5mm) array of nanofilters for biomolecule separation (Figure 3). Compared with previous planar nanofilters, this method can increase the throughput of nanofluidic systems significantly (10~100 fold). Efficient DNA separation was demonstrated at much higher throughput(5), and protein sorting experiments are under way currently. With this novel fabrication technology, we expect to drastically increase the sample throughput of nanofilter protein separation systems.
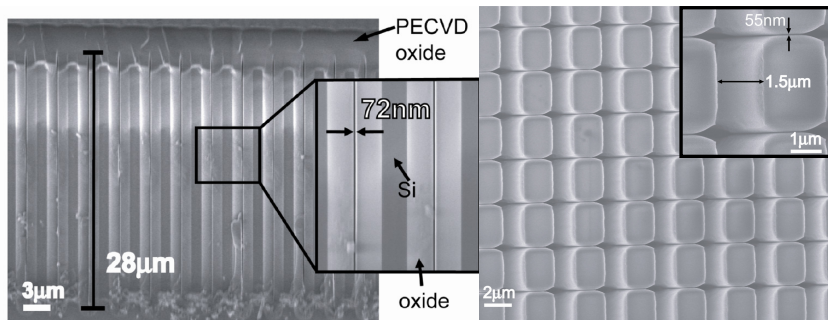
Figure 3 Nanofilter membrane structures. A&B: Cross section (A) and top view (B) of the nanofilter membrane structures. Typical nanofilter gap sizes can be tuned down to 30~60nm, with the depth of the membrane to be ~50µm. In (B), parallel membrane filters (each ~2µm thick) are separated by 1.5µm gap. Courtesy of Mao and Han (MIT)

4. Continuous-flow pI-based separation of biomolecules using parallel electrophoresis

Sponsors
KIST (Korean Institute of Science and Technology)-IMC (Intelligent Microsystems Center)
National Science Foundation (CBET-0347348)

Project Staff
Dr. Yong-Ak Song

In this project we develop an ampholyte-free pI-based fractionation of peptides and proteins as a sample preparation step for mass spectrometry. The continuous-flow sorting approach is based on the free-flow zone electrophoresis which allows a high-throughput separation of molecules based on the mass-to-charge ratio. After collecting the binary sorted samples at an initial pH value, the sorting step is repeated for each collected fraction at a different pH value, as shown in Figure 4. This serial combination of two fractionation steps at two different pH values enables to isolate molecules within a specific pI range from complex sample mixtures. The pI information of the isolated molecules which is currently not provided by the standard ion-exchange chromatography will lead to a substantial reduction of peptide sequencing time in shotgun proteomics. The electrical junction for the fractionation was created by the submicron thick hydrophobic layers patterned on glass substrate prior plasma bonding with the PDMS chip.

We validated the two-step sorting result of a peptide mixture pI 9.7, pI 7.2 and pI 5.1 with the MALDI-MS. Peptide with pI 9.7 was successfully removed in the first sorting step, while pI 5.1 was removed in the second sorting step. After this two-step sorting, only pI 7.2 falling between pH 6-8 was isolated. This approach can be used to collect the molecules within any specific pI range, say pI 6-7 or even narrower with pI 6-6.5. We applied the two-step fractionation approach to a more complex sample such as trypsin-digested BSA and isolated peptides with pI values falling into a pI range of 6-7. The test of the device with more complex samples (such as human serum), as well as fractionation in extreme pH ranges will ultimately demonstrate its potential in sample preparation for mass spectrometry.
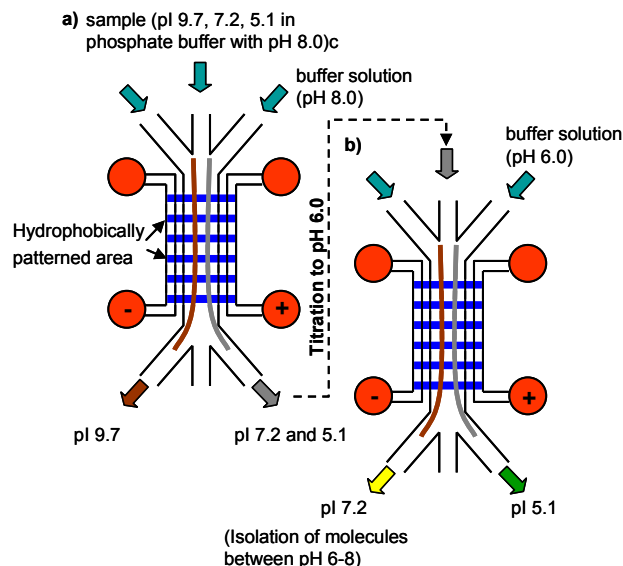
Figure 4. a) Schematic of the continuous-flow binary sorting in single fractionation step. Depending on the mass-to-charge ratio, the molecules can be separated into positively and negatively charged groups. b) Through a combination of the two sorting steps via titration to a lower pH value of the buffer, for instance, molecules falling into a specific pI range can be isolated from a mixture, as exemplarily shown for a mixture of 3 peptides

5. Enhanced Enzyme Assay using nanofluidic Preconcentration

Sponsors
NIH CEHS (ES002109)
National Institute of Health (EB005743)
NIH CDP Center grant (GM68762)

Project Staff
Dr. Jeong Hoon Lee

We recently developed a novel method of increasing both the reaction rate and the sensitivity of low-abundance enzyme assay using a micro/nanofluidic preconcentration chip(6). The disposable preconcentration device made out of PDMS with a surface-patterned ion-selective membrane increases local enzyme/substrate concentrations for rapid monitoring of enzyme activity. As a model system, we used trypsin as the enzyme and BODIPY FL casein as the fluorogenic substrate. We demonstrated that the reaction rate of trypsin-BODIPY FL was significantly enhanced by increasing the local concentrations of both trypsin and BODIPY FL casein in the preconcentration chip. The reaction time required to turn over substrates at 1 ng/mL was only ~10 min compared to ~1 h without preconcentration, which demonstrates significantly higher reaction rate through the increase of the concentrations of both the enzyme and substrate. Furthermore, trypsin activity can be measured down to a concentration level of 10 pg/mL, which is ~100 fold enhancement in sensitivity compared to the result without the preconcentration step. This micro/nanofluidic preconcentrator chip could be used as a generic micro reaction platform to study any enzyme-substrate systems, or other biochemical reaction systems in low concentration ranges.
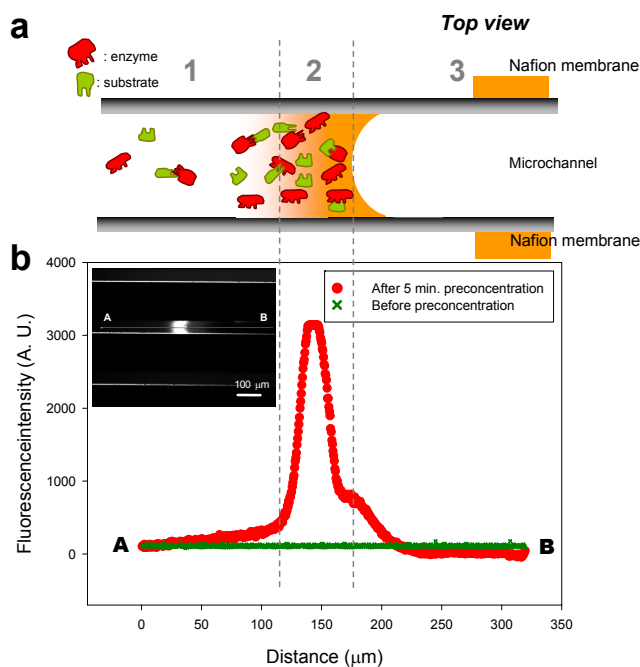
Figure 5. (a) Schematic of electrokinetic trapping of enzyme and substrate and (b) intensity profile along the microchannel as a measure of the enzyme-substrate reaction product after 5 minutes of preconcentration. It shows an increased concentration of the enzyme-substrate reaction product in the concentrated reaction zone generated by electrokinetic trapping (zone 2). Zone 1 contains the mixture of trypsin and BODIPY FL casein outside of the concentrated reaction zone. The difference of the fluorescence signals between zone 1 and 2 indicates an enhanced enzyme-substrate reaction through the preconcentration. The increase of the intensity was partly due to the stacking of the reaction products flowing from zone 1. The Zone 3 illustrates the depletion zone, allowing a control experiment to estimate the background noise that can be generated from the adsorption of the reaction products on the side wall of the microchannel.

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