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 Separation in Nanofluidic Filters by Steric Hindrance Mechanism

Sponsors

National Science Foundation (CTS-0347348)
National Institute of Health (EB005743)
SMA-II FRP-1

Project Staff

Jianping Fu

Manipulation of charged biomolecules through confining environments has broad applications in life science. Recent progress in fabricating well-defined spatial constraints allows direct observation of novel molecular dynamic behavior in molecular-size confining structures. Further, it shows exceptional promise for providing regular sieving media with superior separation performance. Here we demonstrate a continuous-flow, biomolecule-separation device that makes use of a patterned anisotropic sieving matrix consisting of a two-dimensional periodic array of nanofilters. The electrophoretic drift of biomolecules in the sieving medium involves a differential bidirectional motion through two-dimensional, periodically modulated, free-energy landscapes that results in a vectorial apparent electrophoretic mobility that directs molecules of different sizes to follow radically different paths. This method provides a novel basis for dispersing small fluid-borne biomolecules into distinct fractions. A fluorescently labeled dsDNA mixture (50-766 bp) used to characterize the device was separated in 1 minute with a resolution of about 10%. The patterned anisotropic sieve was also used for size-fractionation of SDS-protein complexes of size ranging from 11 to 200 kDa in 1 minute. By virtue of its gel-free and continuous-flow operation, this device suggests itself as a key component of an integrated microsystem that prepares and analyzes biomolecule samples.

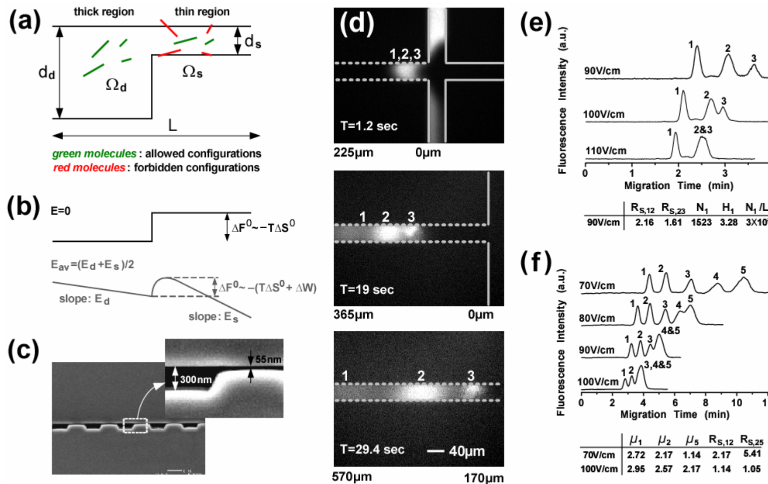

Figure 1: (a) Partitioning of rigid, rod-like molecules in a slit-like nanofilter. (b) Free energy landscape of a nanofilter. (c) An SEM images of a periodic array of nanofilters with alternating thin and thick regions. (d-f) Separation of SDS-protein complexes (d & e) and dsDNA molecules (f) in a one-dimensional nanofilter array chip (d_s: 55nm, d_d: 300nm, L: 1µm). Band assignment for SDS-protein complexes: (1) cholera toxin subunit B (MW: 11.4kDa); (2) lectin phyto-hemaglutinin-L (MW: 120kDa); (3) low-density human lipoprotein (MW: 179kDa), for DNA: (1) 50bp; (2) 150bp; (3) 300bp; (4) 500bp; (5) 766bp.

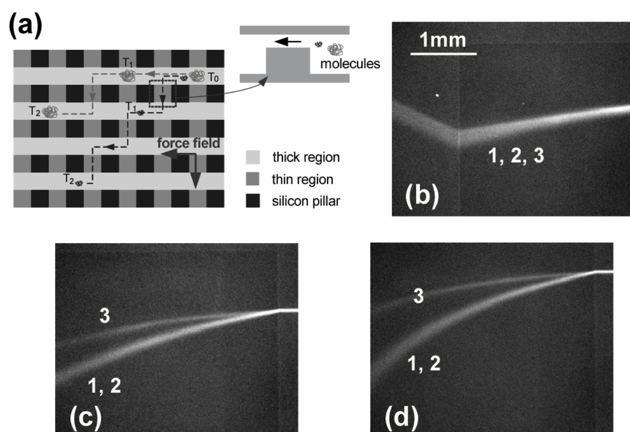

Figure 2: (a) Bidirectional transport of biomolecules in the 2-D nanofilter arrays. (b) Fluorescence images of pulse-field separation of SDS complexes inside the 2-D nanofilter arrays. Different values of vertical and horizontal fields can be applied with different durations. Band assignments for SDS-protein are the same as in Figure 1.

2. Modeling of Separation Dispersion in Nanofluidic molecular filters

Sponsors
SMA-II FRP-1
NIH CDP Center (GM68762)
Dupont Fellewship

Project Staff
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In the past decade, microfabricated devices have been developed that can separate, detect, and analyze various biomolecules. In contrast to the sieving gels that are historically used in these studies, microfabricated devices are precisely designed and constructed. The deterministic structure of these devices facilitates experiment design and theory testing. Periodic nanofilter arrays have been shown to separate DNA from 100 bp to 10 kbp (figure 1 and 2). These nanofilters consist of a regular sequence of free and constricted regions, with 50-100 nm being the characteristic dimension of the constricted region. In this context, the DNA is smaller than the constriction size, suggesting the applicability of the Ogston sieving mechanism. Movement is characterized by the partitioning between the free and constricted regions due to steric constraints. DNA has a persistence length of 50 nm (150 bp) and can be approximated as semi-rigid rods in this size range, facilitating theoretical analysis.

We investigated the effects on separation efficiency and resolution of changing various device and experiment parameters. These parameters include the strength of the electric field; depth of the deep region; depth of thin and deep regions, while maintaining their ratio; silicon substrate bias; buffer strength; and period of the nanofilter array.

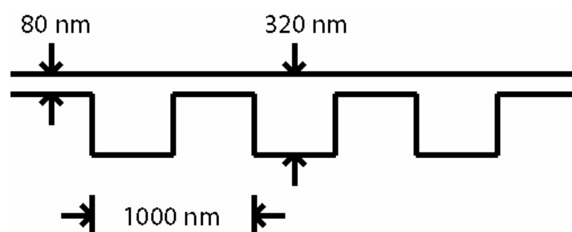

Figure 1: Cross-section of basic device. The typical separation region length is 1 cm.

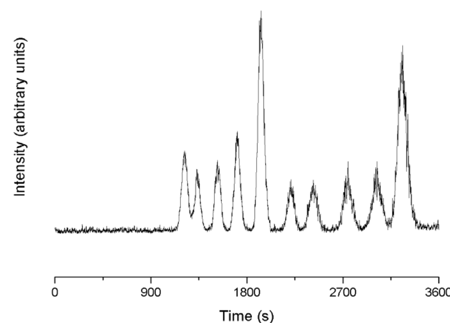

Figure 2: Fluorescence intensity as a function of time. Separation of 100 bp to 1000 bp (100-bp interval) is achieved in less than 1 hr at 20 v/cm in an 80nm thin-gap device.

3. Nanofluidic Biomolecule Preconcentration

Sponsors
NIH CDP Center grant (GM68762)

Project Staff
Ying-Chih Wang

The overall goal of this project is to integrate bead-based ELISA assay with advanced microfluidic sample preparation tools, to achieve higher detection sensitivity and selectivity. The Han group has recently demonstrated several basic tools for advanced protein sample separation and concentration, which would be critical in the integration of proteomic sample preparation microdevices. Combination of these tools will allow advanced, automatic proteomic sample preparation, which is equivalent to conventional 2D protein gel electrophoresis. However, the availability of efficient protein preconcentration process will allow orders-of- magnitude higher detection sensitivity for low-abundance protein species.

We developed a nanofluidic biomolecule concentrator, which can achieve concentration of proteins or peptide up to 10^6~10^8 fold or more [1,2]. This is several order of magnitude better in terms of concentration factors compared with other preconcentration strategies (Figure 1).

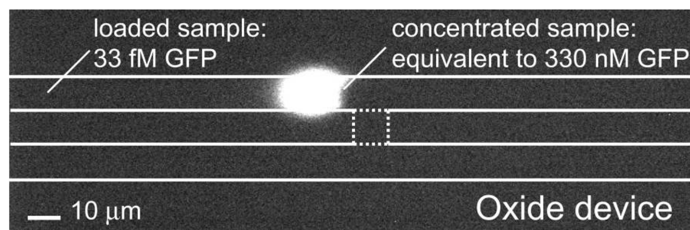

Figure 1:
Pre-concentration of dilute protein solution using Nanofluidic preconcentrator. (From Wang et al. MicroTAS 2005)

As a first step to implement the above integration, we will integrate these tools with a simple, bead-based ELISA assay (Figure 2) within the microfluidic device, as a tool for molecular analysis of complex biomolecule samples such as cell extract or serum.

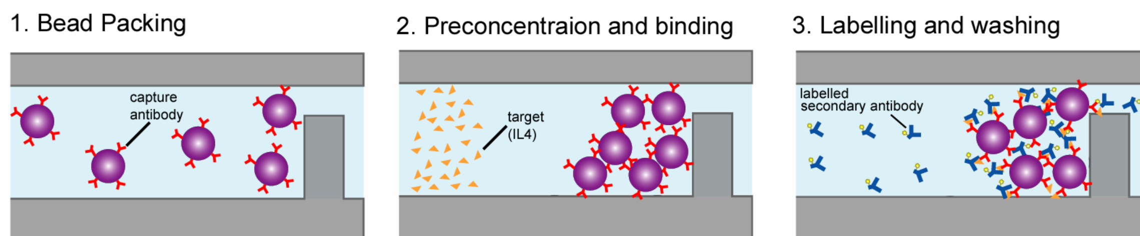

Figure 2: Bead-based ELISA assay in a microfluidic channel

4. Nanochannels for Transport Studies of Ions/Molecules/Fluids

Sponsors
Dupont-MIT Alliance

Project Staff
Pan Mao
Reto Schoch
Hongchul Jang
Patrick Doyle (co-PI, ChemE, MIT)
Anthony Balducci (ChemE, MIT)

Synthetic polymer membranes, especially perfluorinated membranes such as Nafion® have many important engineering applications. Its ion-permselectivitiy, nanoporosity, and chemical / physical stability promises applications not only in batteries and fuel cells, but also in other applications such as molecular and cellular separation and biosensing. However, in order to produce better membranes and filters, it would be essential to have a detailed engineering model for molecular and ionic transport processes in the system. While theoretical research has been pursued in the area of membrane transport processes, it is still challenging to characterize the general transport data quantitatively based on theoretical models, and theoretical discussions on the membrane processes are often macroscopic and phenomenological.

Regular nanofluidic channels and filters (Figure 1), in addition to their numerous applications, are ideal model systems for studying molecular and ionic transport processes in nanoporous membranes, with a good control on physical dimension of nanochannel and channel wall surface chemistry. In this project, we specifically focus on the ion and molecular transport processes in thin (20-40nm) nanofluidic channel, and perform experimental studies on transport using such regular nanochannels (Figure 2&3). Such scientific studies have many implications and opportunities in the membrane science and engineering. 1) With a well-defined geometry of nanofluidic channel, one can test the validity of macroscopic membrane transport models (such as Donnan potential) and bridge them with microscopic models of ion transport and hindered molecular (particle) transport. 2) Transport studies in nanochannel do not suffer from mechanical uncertainties of polymeric membrane (such as water / methanol content in Nafion®), which will allow “clean” transport data. 3) In the over-limiting current regime of ion-selective membrane, there are many opportunities in using Nafion® or nanofluidic channels for sample preparation[1] and desalination. This study will identify the opportunities for broader applications of perfluorinated membranes, which can be modeled by nanofluidic channels.

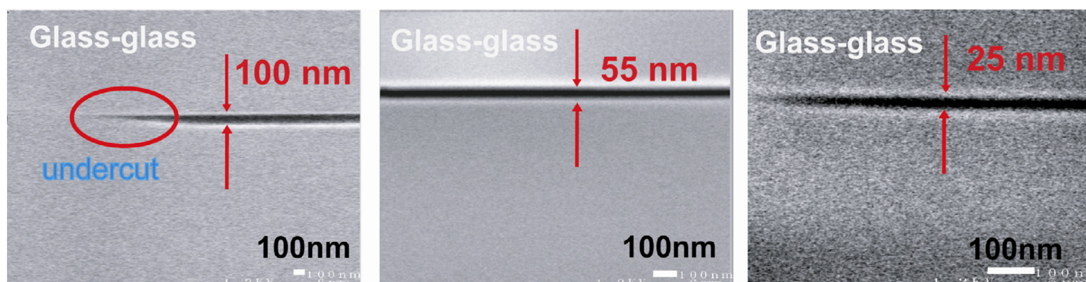

Figure 1: Cross-section SEM micrograph of planar nanochannels

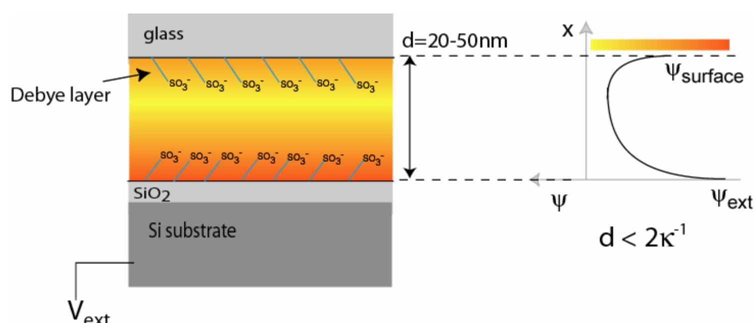

Figure 2: Nanofluidic channel as models for perm-selective membrane. Surface of nanofluidic channels can be coated to have high negative charge density. Surface potential can be controlled by applying an external field from the Si substrate of the device.

5. Fabrication of Nanofluidic Membrane for Biomolecule Separation

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

Project Staff
Pan Mao
Hongchul Jang

Nanofluidics have gained tremendous successes in the last few years because they provide unique capability in biomolecular manipulation and control. For nanofluidic applications, one critical issue is the availability of reliable, reproducible fabrication strategies for nanometer-sized structures. A simple technique, without nanolithography or special tools, has been developed to generate planar nanochannels with precise control of depths to the nanometer scale for many applications including separation and preconcentration. However, two big issues with these planar nanofluidic channels are the limited fluidic conductance that results and low throughput.

Here we describe a novel fabrication approach to generate massively-parallel vertical nanochannels with the well-controlled gap size down to 100 nm. We use anisotropic wet etching (KOH) to make deep, vertical trenches on Si (110) substrate (Figure 1A). Alternatively, conventional deep reactive ion etching (DRIE) can be performed to produce very deep trenches, and then the sidewalls can be smoothed by a short KOH etching. Then the width of the trench channel is further decreased to a desired thickness even below 50 nm (Figure 1B) by growing thermal oxide. Also, backside etching of the Si wafer can yield thin membranes over a wide area (~ 6-inch wafer) with well-defined membrane thickness, if needed. Our method requires neither expensive nanolithography expertise nor other tools and allows the integration of a large number

of narrow, vertical nanofluidic filters with fluidic conductance 10~100 times higher than planar nanochannels. Furthermore, we have demonstrated efficient, high-throughput separation of large DNA molecules in our vertical nanofilter-array device based on an entropic trapping mechanism (Figure 2). We believe that these membrane devices could be a key to the high-throughput Microsystems for sample preparation.

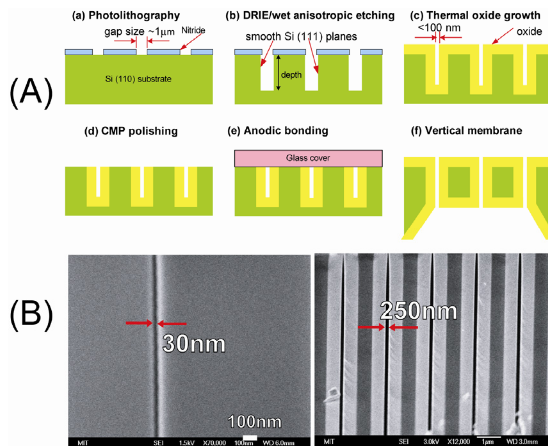

Figure 1: (**A**) Schematic diagram of fabricating massively-parallel vertical nanofluidic membranes. (**B**) Cross-sectional SEM micrograph of vertical nanochannels with lateral gap sizes (widths) of 250 nm (left) and 30 nm (right).

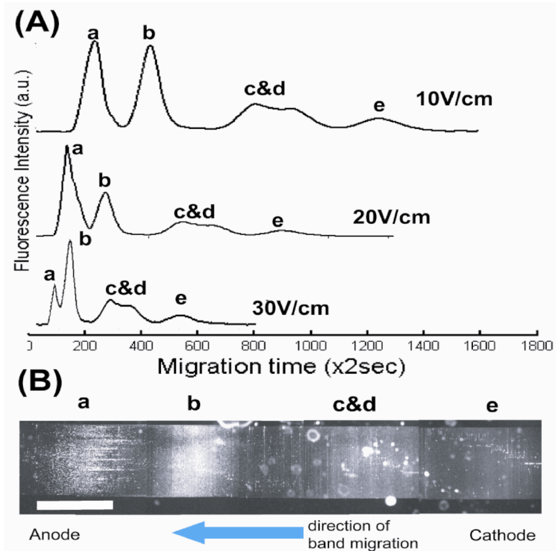

Figure 2: (**A**) Separation of the mixture of λ-DNA and λ-DNA digested by Hind-III in a lateral nanofilter array chip under different electrical fields. Peak assignment: (a) 48.5kbp ,(b) 23kbp, (c) 9.4kbp, (d) 6.5kbp, and (e) 4.4kbp. (**B**) Direct observation of DNA bands separated at 10 V/cm. The scale bar is 1 mm.

6. Continuous-flow pI-based separation of biomolecules using diffusion potential

Sponsors

KIST (Korean Institute of Science and Technology)-IMC (Intelligent Microsystems Center)
National Science Foundation (CTS-0347348)
Merck-MIT postdoctoral fellowship

Project Staff

Dr. Yong-Ak Song
Tiffany Chen

In this work, we have developed a simple microfluidic chip that can sort biomolecules based on their isoelectric point (pI) values in a simple buffer system. The new method differs from previous approaches such as transverse isoelectric focusing [1] or free-flow electrophoresis [2] in that this process involves no external power supply and no special ampholyte. Instead, we utilize the diffusion potential generated by the diffusion of different buffer ionic species in-situ at the laminar flow junction. The use of diffusion potential in microfluidics was previously demonstrated with the mass transport of dye molecules between the two streams in [3]. However, they did not explicitly demonstrate a separation of two species. In our device, we establish a laminar flow junction between two buffers with different pH and concentrations. A potential gradient is developed

across the liquid junction, generating a high-enough electric field to mobilize and to collect biomolecules at the boundary when their pI values fall between the two buffer pH values. The computational modeling shows a decreasing potential gradient from 17.1V/cm to 6.9V/cm along the 2-mm-long micro-channel (20µm deep, 100µm wide), as the concentration gradient becomes shallower toward the end of the channel due to mixing (Figure 1). In our initial experiment, two pI-markers (Figure 2) as well as two proteins were successfully sorted in this device, with a flow rate of 5~10 µL/min. To characterize the accuracy of this pI-based sorting process, we tested sorting behavior of the device by changing the pH value of the sample buffer in 0.1pH step. It was shown that a peptide can be sorted into a different output stream with a ~0.1pH unit resolution. We are currently working on the development of new buffer systems as well as on the hybrid approach with a superimposed external electric field to increase the sorting efficiency and resolution. Once fully developed, it can potentially be a pI-based sample fractionation tool for proteomic analysis of complex biomolecule samples.

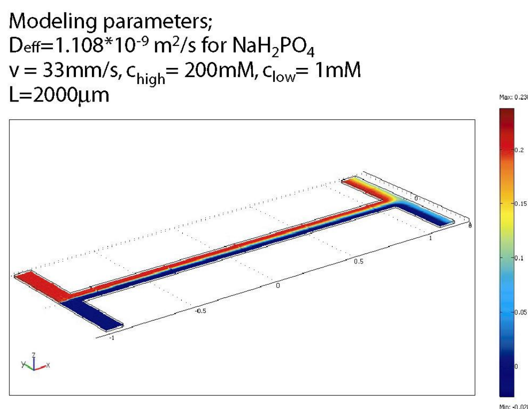

Figure 1. A 3D steady-state concentration distribution in a 2-mm-long microfluidic channel with a concentration ratio of 200. Based on this concentration distribution, the diffusion potential as well as the potential gradient can be calculated.

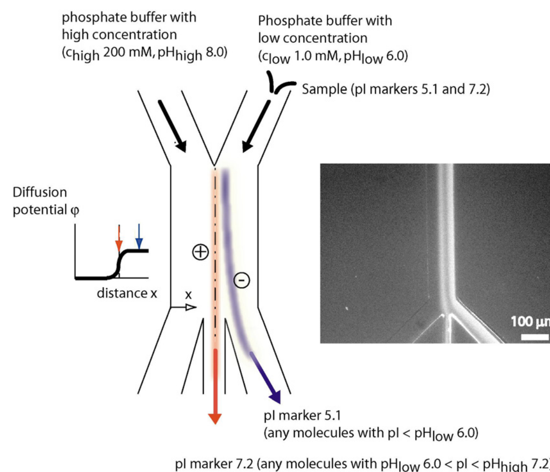

Figure 2. Schematic view of the pI-based sorting process and separation of two pI markers with pI values of 5.1 and 7.2 using diffusion potential at a concentration difference of 200 and at a flow rate of 10 µl/min.

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7. Integrated Microfluidic Device for preconcentration and detection of multiple biomarkers

Sponsors

NIH-NCI (CA119402)

Project Staff
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Scott R. Manalis

We are currently developing an integrated nano- and microfluidic system for the parallel detection of multiple cancer biomarkers. The system will be based on the nanofluidic concentrator developed by the Han lab and is expected to achieve a detection limit for biomarker concentration that is significantly lower than what is possible with existing immunoassays such as ELISA and radioimmunoassays. A key attribute of the nanofluidic concentrator design is that the region where biomolecules are concentrated must not be larger than about 10-100 picoliters. In order to measure the concentration of specific biomarkers within this region, we propose to integrate the concentrator with a detector of similar volume in order to avoid dilution. The detection method is conceptually similar to ELISA; however, the readout of the biomarker/antibody binding is based on the direct detection of mass that absorbs to the surface of a sensor known as the suspended microchannel resonator (SMR). The SMR detector has a volume of 10 picoliters and a mass resolution of near 100 femtograms. Thus, we anticipate that the combined concentrator and SMR detection system will allow a specific biomarker to be detected at a resolution near 1 pg/mL. Since the SMR provides a real-time readout and the concentrator is easily controlled by an applied voltage, the integrated system can operate in a closed-loop mode in which the sample is amplified until the biomarker concentration reaches the vicinity of the disassociation constant of the biomarker/antibody complex. Thus, the linear range of the detector can be dynamically tuned over a wide range of biomarker concentrations.

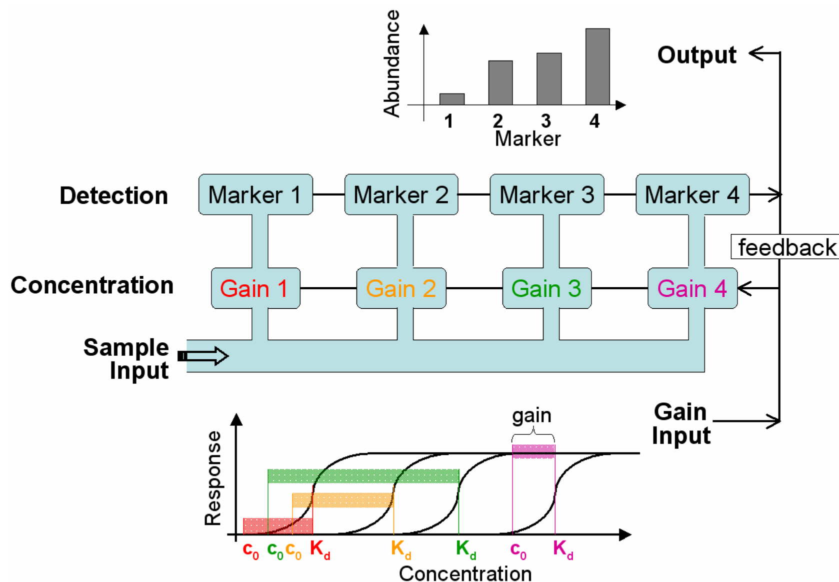

Figure 1: Schematic of parallel closed-loop operation of four devices. Sample is alternately concentrated and detected multiple times for different concentration periods until a signal is read out. The time needed to concentrate the sample to half-maximal readout is indicative of both the initial concentration and the dissociation constant.

Publications

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Noel I. Reyes-Gonzalez (EECS), “Pulsed Field Separation of Biomolecules in a Nanofluidic Filter Array,” M.S. degree, January 2006.

Yin Ren (EECS), “Preconcentration of Biomolecules by Microfluidic channels and the Nafion Membrane,” AUP, June 2006.

Hansen Bow (EECS), “Characterization of Nanofilter Arrays for Small Molecule Separation,” M.S. degree, June 2006