

Nanofluidic BioMEMS Research

RLE Group

Micro / Nanofluidic BioMEMS Group

Academic and Research Staff

Professor Jongyoon Han

Research Affiliates

Dr. Yong-Ak Song

Dr. Masumi Yamada

Dr. Jeong Hoon Lee

Dr. Sung Jae Kim

Dr. Ali Bhagat

Graduate Students

Pan Mao

Hansen Bow

Vincent Liu

Leon Li

Lih Feng Cheow

Aniruddh Sarkar

Pilnam Kim (Seoul National University, visiting student)

Sung Hee Ko (PostECH, visiting student)

Myung Ji Kim (Korea University, visiting student)

Jun Min Lee (Korea University, visiting student)

Undergraduate Students

Mahati Chintapalli (Course 3)

Ara Tan (Summer student, 09)

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

Sponsors

National Institute of Health (EB005743)
SMA-II FRP-1

Project Staff

Aniruddh Sarkar
Lih Feng Cheow
Masumi Yamada

Most sensitive immunoassay techniques are achieved by immobilizing antibodies or targets, on a solid substrate, which enables washing of unbound species and background molecules. However it is known[1] that such solid-phase immunoassays bring additional complexities such as: a) Antigens/antibodies may lose part of their activity during the surface adsorption/binding reactions or just due to their proximity to a surface. b) Transport (kinetics) of the analyte from liquid phase to the surface limits the speed of the assay. Antibodies are relatively large proteins (~150kD), and antigen-antibody complexes could be separated easily from unbound antibody due to the size changes incurred by the immunobinding. Such an immunoseparation strategy has been successfully used for detecting biomarkers[2] and even counting low abundance proteins from a single cell lysate[3].

We have developed a new assay strategy to use the ANA continuous flow size-sorter to separate the bound and unbound species while in suspension, based on their size[4]. **Fig. 1** shows the preliminary results of detecting C-Reactive Protein (CRP) [5] using this technique. Sample solution was mixed with 50 μ g/ml anti-CRP antibodies (FITC labeled), and then loaded into the ANA separation device. CRP reacted with antibodies and formed complexes, which were then separated away into separate stream from unbound labeled antibodies. This would yield a sensitive yet completely homogeneous assay that is also simpler to perform as it does not involve any surface immobilization or washing steps. To this end, we are currently building an integrated microdevice that combines ANA separation and on-line preconcentration in a single device. The “bound” stream in **Fig. 1** is believed to be from multi-antibody complex to a single CRP, therefore yielding big size differences. *An evolved version of this idea will be realized in the specific aim #3 of this renewed project.*

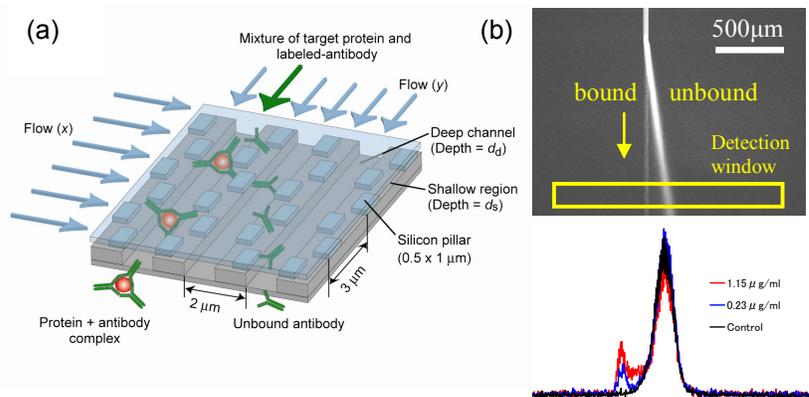


Fig. 1 Immunoseparation assay. (A) Overall strategy. Primary immunobinding can be done on chip or ex situ, and the bound and unbound species are separated. Only bounded species are collected and concentrated to obtain higher sensitivity (B) Fluorescence image of separating C-Reactive Protein (CRP) with labeled antibodies (50 μ g/ml). Detection was done in the ANA device by measuring the fluorescence intensity of bound stream. (Adapted from Yamada et al. Anal. Chem.[4])

2. Nanofluidic Biomolecule Preconcentration for Enhancing Biomolecule Detection

Sponsors

NIH CDP Center grant (GM68762)
National Institute of Health (EB005743, CA119402)

Project Staff

Jeong Hoon Li
Leon Li
Sung Jae Kim
Sung Hee Ko

We recently applied concentration-enhanced biochemical assay idea to enzyme activity assay[6, 7], where both low-abundance enzyme and substrate (usually the fluorogenic sensor molecule) can be co-concentrated, and therefore the substrates get turned over with significantly enhanced kinetics for higher-sensitivity activity measurement. Recently, we applied the concentration-enhanced enzyme assay to two important cellular kinases, i.e, MK2 and PKA, directly from human HepG2 cells [7].

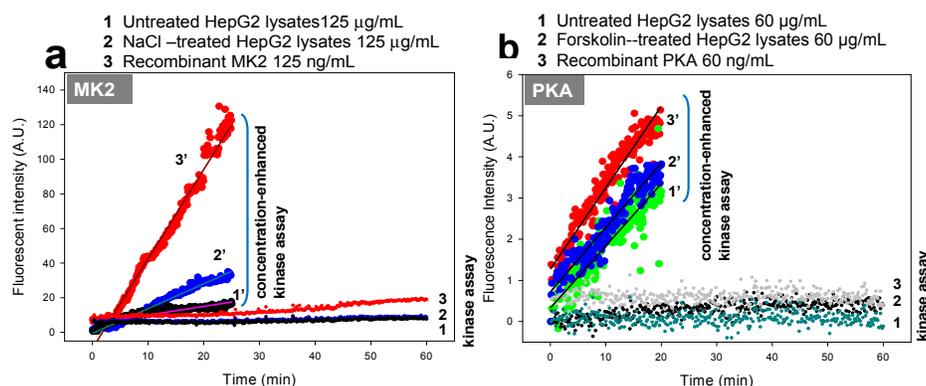


Fig. 2. Performance of concentration-enhanced kinase activity assay (a) Fluorescence signal (from converted SOX substrate) from the untreated HepG2 lysates (1&1'), NaCl-stimulated HepG2 lysates (2&2'), and recombinant active MK2 enzyme (3&3') in MK2 kinase assay. Significant increases in MK2 phosphorylation of the SOX substrate and resultant fluorescence signal are observed using the 20-min concentration within the microfluidic device (1', 2', 3') compared with un-concentrated assay conditions (1, 2, 3). (b) PKA activity assay, either with (1', 2', 3') or without (1, 2, 3) concentration enhancement. (c) MK2-SOX reaction velocity as a function of lysate concentration (or recombinant enzyme concentration) demonstrating the increase in the reaction velocity (25-fold increase) and the sensitivity of low-abundance kinase activity assay (65 fold increase) with concentration enhancement. (unit: AU/min) (From Lee et al. *JACS*, 2009 [7])

HepG2 cell lysate was prepared to have the overall protein concentration of $\sim 125\mu\text{g}/\text{mL}$, equivalent to 125 cells/ μL . We diluted 0.5 μL of such lysates (by 1:10 ratio) in the premixed biosensor cocktail (with ATP, inhibitor cocktails, kinase buffer and 10mM Sox substrates, which is a standard buffer for Omnia[®] assay[8]). The resulting 5 μL lysate-SOX substrate mix (which contains cellular proteins from approximately 65 cells) was then loaded into the sample reservoir for concentration-enhanced assay microchip. (Sample #1(1') and 2(2') in **Fig. 2A**) Also as a positive control, recombinant enzyme was mixed in 1X PBS buffer and used in the assay (Sample #3 (3') in **Fig. 2A**). Then, reaction to turn over SOX substrates (to render them fluorescent) by kinases were monitored, both with (conditions 1'~3') or without (conditions 1~3) concentration enhancement. We assessed changes in activity as a function of time from HepG2 cell samples with known MK2 activators (data 2 and 2'; stimulated with NaCl for 30 mins), compared with untreated MK2 from HepG2 cell lysates (data 1 and 1'). As a negative control, we confirmed that

no changes in fluorescence signal occur from the samples only with lysate buffer (without HepG2 cell lysate) in MK2 biosensor mixtures, with or without concentration enhancement. It was shown, both for MK2 and PKA tested, that the sensitivity of the activity measurement has been improved significantly due to the concentration enhancement (**Fig. 2A**). Furthermore, with concentration, NaCl-treated MK2 activities can be measured even when the original cell lysate was diluted further down to well below $\sim 10\mu\text{g/mL}$ level. This means that proteins from only ~ 5 cells were loaded into the reservoir of the microfluidic device and successfully detected. This clearly demonstrates the potential of detecting kinase activities from a single cell, directly from the cell lysates, if the cell growth / lysis device is properly integrated with the concentration-enhanced kinase assay device. Considering that the concentrated enzyme assay uses just a very small fraction of all the kinases contained within the $5\mu\text{L}$ sample loaded into the microchip, further improvement of sensitivity is certainly possible via further optimization in concentration device and/or chemistry of SOX substrates.

3. Fabrication of Nanofluidic Membrane for Biomolecule Separation

Sponsors

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

Project Staff

Pan Mao

Yong-Ak Song

One of the critical problems in the nanochannel separation system is its relatively low throughput. Recently, we have developed a novel fabrication method[9] for building ultra-high aspect ratio (vertical) nanochannels, for building high-throughput ANA structures. Using this, we have demonstrated relatively fast sample processing rate of $\sim 1\mu\text{L/hr/channel}$, in electrophoretic separation. (**Fig. 3**)

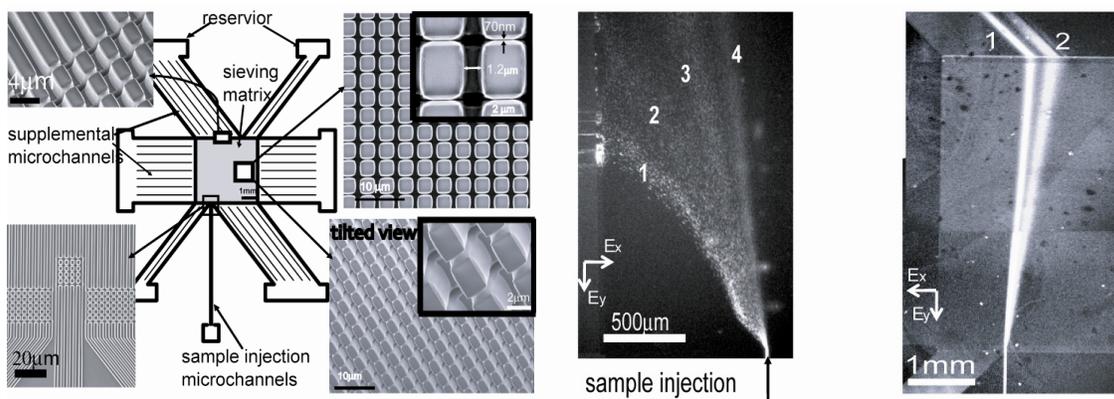


Fig. 3 Continuous fractionation of biomolecules through the two-dimensional anisotropic pillar array device. (LEFT) The device includes a sieving matrix and surrounding microfluidic channels. The pillar array consists of horizontal nanochannels with a width of 70 nm and longitudinal microchannels with a width of 1.2 μm . Supplemental microchannels connecting sieving matrix and reservoirs are 1.5 μm in width. They are all 15 μm deep. (MIDDLE) Fluorescence micrographs show separation of the mixture of λ -DNA Hind III digest. Electric fields E_x and E_y applied both in horizontal and longitudinal directions in the sieving matrix are 80 V cm^{-1} and 30 V cm^{-1} , respectively. Band assignment: (1) 23.13 kbp; (2) 9.4 kbp; (3) 6.58 kbp; (4) 4.36 kbp. (RIGHT) Fluorescence micrographs showing separation of the mixture of FITC (2) and R-phycoerythrin (1). The nanofilter size is ~ 40 nm. $E_x=250 \text{ V cm}^{-1}$ and $E_y=40 \text{ V cm}^{-1}$. It is obtained by combining two fluorescence micrographs taken in the same run but with two different filter sets since they have a different spectrum. (Ref. [9])

4. Continuous-flow cell sorting based on mechanical flexibility

Sponsors

SMART center

Project Staff

Hansen Bow

Dr. Ali Bhagat

Sha Huang

Han Wei Hou (National University of Singapore / SMART center)

Wong Cheng Lee (National University of Singapore / SMART center)

Currently there are three main methods to quantify deformability of single cells: atomic force microscopy (AFM), optical tweezers, and fluid-driven movement through pores[10-12]. In the past, movement through pores has been accomplished by micropipette aspiration experiments, which are serial, labor intensive, and imprecise. Microfabricated devices enable the creation of custom structures similar to micropipettes, so that parallel and repeated measurements can be made on the same cell. Flow-based cell deformability measurement has previously been done[13] in microfluidic devices. However, often these studies used a single constriction, which limits both the sensitivity and specificity of detection, as well as sample throughput. Another challenge is making the sorting / detection specific enough to overcome stochastic noise due to the cells' natural variation in size and other properties. In this work, we have demonstrated that one can overcome these issues by employing many (~1000) filters in series, as well as implementing an anisotropic filter array design. We demonstrated the operation of the device using glutaraldehyde(GA)-treated and normal red blood cells (RBCs).

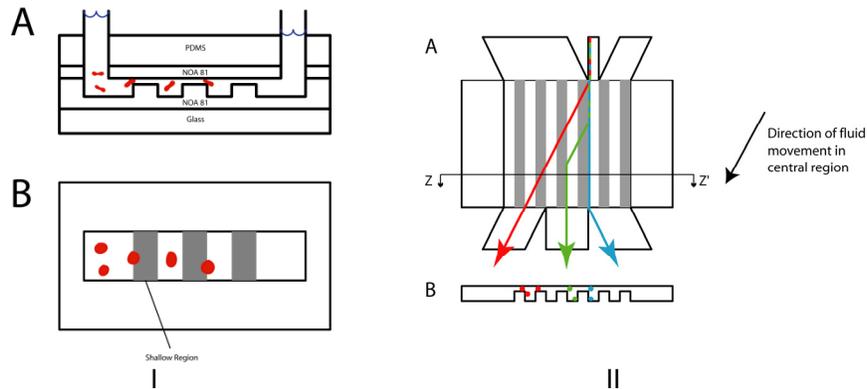


Fig. 4 IA. Side-view of device with slits in series. IB. Top-view of the device. IIA. Top view of continuous-flow deformability-based separation device. B. Side-view of cross-section A-A'.

The first device used in this study is depicted in **Fig. 4.I**. The height of red blood cells is greater than the widths of the slits. Therefore, the cells must deform to get through. Less deformable cells are expected to travel slower through the slits, leading to a separation according to the deformability. The principle of the continuous flow separation device is depicted in **Fig. 4.II**. The red (left) line indicates the trajectory more deformable cells will take, as they are not hindered by the shallow regions. The blue (right) line indicates the trajectory of rigid cells, which slide parallel to the constriction posed by the slit. The cells pass through multiple slits before escaping the device, which would increase the selectivity of the sorting. Also, clogging is potentially reduced due to the parallel filtration system design.

References

1. Davies, C., *Introduction to immunoassay principles*, in *The Immunoassay Handbook*, D. Wild, Editor. 2005, Elsevier Science Ltd. p. 3-40.
2. Herr, A.E., A.V. Hatch, D.J. Throckmorton, H.M. Tran, J.S. Brennan, W.V. Giannobile, and A.K. Singh, *Microfluidic immunoassays as rapid saliva-based clinical diagnostics*. Proc. Natl. Acad. Sci. U. S. A., 2007. 104(13): p. 5268-5273.
3. Huang, B., H.K. Wu, D. Bhaya, A. Grossman, S. Granier, B.K. Kobilka, and R.N. Zare, *Counting low-copy number proteins in a single cell* Science, 2007. 315: p. 81-84.
4. Yamada, M., P. Mao, J. Fu, and J. Han, *Rapid quantification of disease-marker proteins using continuous-flow immunoseparation in a nano-sieve fluidic device*. Anal. Chem., 2009. Published on the web (10.1021/ac901226z).
5. Zethelius, B., L. Berglund, J. Sundstrom, E. Ingelsson, S. Basu, A. Larsson, P. Venge, and J. Ärnlöv, *Use of multiple biomarkers to improve the prediction of death from cardiovascular causes*. New Engl. J. Med., 2008. 358(20): p. 2107-2116.
6. Lee, J.H., Y.-A. Song, S.R. Tannenbaum, and J. Han, *Increase of Reaction Rate and Sensitivity of Low-Abundance Enzyme Assay using Micro/Nanofluidic Preconcentration Chip*. Anal. Chem., 2008. 80 (9): p. 3198-3204.
7. Lee, J.H., B. Cosgrove, A.D. Lauffenburger, and J. Han, *Microfluidic concentration-enhanced cellular kinase activity assay*. J. Am. Chem. Soc., 2009. Published on the web (DOI:10.1021/ja902594f).
8. Shults, M.D., K.A. Janes, D.A. Lauffenburger, and B. Imperiali, *A multiplexed homogeneous fluorescence-based assay for protein kinase activity in cell lysates*. Nature Methods, 2005. 2: p. 277-283.
9. Mao, P. and J. Han, *Massively-Parallel Ultra-High-Aspect-Ratio Nanochannels as Mesoporous Membranes*. Lab Chip, 2009. 9(4): p. 586-591.
10. Shelby, J.P., J. White, K. Ganesan, P.K. Rathod, and D. Chiu, *A microfluidic model for single-cell capillary obstruction by Plasmodium falciparum-infected erythrocytes*. PNAS, 2003. 100(25): p. 14618-14622.
11. Tracey, M.C., R.S. Greenaway, A. Das, P.H. Kaye, and A.J. Barnes, *A silicon micromachined device for use in blood cell deformability studies*. IEEE Transactions on Biomedical Engineering, 1995. 42(8): p. 741-761.
12. Brody, J.P., Y. Han, R.H. Austin, and M. Bitensky, *Deformation and flow of red blood cells in a synthetic lattice: evidence for an active cytoskeleton*. Biophysical Journal, 1995. 68: p. 2224-2232.
13. Brody, J.P., Y. Han, R.H. Austin, and M. Bitensky, *Deformation and flow of red blood cells in a synthetic lattice: evidence for an active cytoskeleton*. Biophys. J., 1995. 68(6): p. 2224-2232.

Publications

Journal Articles

Yong-Ak Song, Candy Batista, Raul Sarpeshkar and Jongyoon Han, "Rapid fabrication of microfluidic polymer electrolyte membrane fuel cell in PDMS by surface patterning of perfluorinated ion-exchange resin," *Journal of Power Sources*, 183, 674-677, 2008.

Jianping Fu, Pan Mao, and Jongyoon Han, "Artificial molecular sieves and filters: a new paradigm for biomolecule separation," *Trends in Biotechnology* 26, 311-320, 2008

Duong-Hong, D., J. Han, J.-S. Wang, N. G. Hadjiconstantinou, Y. Z. Chen, and G.-R. Liu, "Realistic Simulations of Combined DNA Electrophoretic and Electroosmotic Flows in Nano-Fluidic Devices," *Electrophoresis*, 29, 4880-4886, 2008.

Hansen Bow, Jianping Fu, and Jongyoon Han, "Decreasing effective nanofilter size by modulating electrical double layers: Separation enhancement in microfabricated nanofilters," *Electrophoresis*, 29, 1-6, 2008.

Pan Mao and Jongyoon Han, "Massively-Parallel Ultra-High-Aspect-Ratio Nanochannels as Mesoporous Membranes," *Lab on a Chip*, 9, 586-591, 2009.

Zi Rui Li, Gui Rong Liu, Jongyoon Han, Yu Zong Chen, Jian-Sheng Wang, Nicolas Hadjiconstantinou, "Transport of biomolecules in asymmetric nanofilter arrays," *Analytical and Bioanalytical Chemistry*, 394, 427-435, 2009.

Sung Jae Kim, Leon Li, and Jongyoon Han, "Amplified Electrokinetic Response by Concentration Polarization near Nanofluidic Channel," *Langmuir*, 25, 7759-7765, 2009.

Lee, J.H., B. Cosgrove, A.D. Lauffenburger, and J. Han, "Microfluidic concentration-enhanced cellular kinase activity assay." *Journal of the American Chemical Society*, 2009. Published on the web (DOI:10.1021/ja902594f).

Yamada, M., P. Mao, J. Fu, and J. Han, "Rapid quantification of disease-marker proteins using continuous-flow immunoseparation in a nano-sieve fluidic device." *Anal. Chem.*, 2009. Published on the web (10.1021/ac901226z).

Zi Rui Li, G. R. Liu, J. Han, Y. Cheng, Y. Z. Chen, J.-S. Wang, N. G. Hadjiconstantinou, "Analytical description of Ogston-regime biomolecule separation using nanofilters and nanopores" *Physical Review E* **2009**, accepted for publication.

Meeting Papers, Published

Yong-Ak Song and Jongyoon Han, "High-throughput Sample Preparation for Mass Spectrometry by Continuous-Flow pI-based Fractionation of Peptides and Proteins," (oral) Proceedings of the MicroTAS 2008 Symposium, San Diego, CA, Vol. 2, pp. 1323-1325, October 2008.

Sung Jae Kim and Jongyoon Han, "Self-Sealed Vertical Nanoporous Junctions for Integrating Various Nanomaterials in PDMS Microfluidic System," (poster), Proceedings of the MicroTAS 2008 Symposium, San Diego, CA, Vol. 2, pp. 1042-1044, October 2008.

Sung Jae Kim and Jongyoon Han, "Amplified Electrokinetic Fluid Pumping and Switching by Concentration Polarization Near Nanofluidic Channel," (poster), Proceedings of the MicroTAS 2008 Symposium, San Diego, CA, Vol. 1, pp. 197-199, October 2008.

Hansen Bow, Patrick Abgrall, and Jongyoon Han, "Microfabricated Slits in Series: A Simple Platform to Probe Differences in Cell Deformability," (poster), Proceedings of the MicroTAS 2008 Symposium, San Diego, CA, Vol. 2, pp.1199-1201, October 2008.

Yong-Ak Song, Aniruddh Sarkar, and Jongyoon Han, "Encapsulation of Biomolecules with Programmable Concentrations in Microdroplets using Electrokinetic Concentrator," (poster), Proceedings of the MicroTAS 2008 Symposium, San Diego, CA, Vol. 2, pp. 1420-1422, October 2008.

Pan Mao and Jongyoon Han, "Fabrication of Massively-Parallel Regular Nanofluidic Filters for High-Throughput Biomolecule Separation," (poster), Proceedings of the MicroTAS 2008 Symposium, San Diego, CA, Vol. 1, pp. 853-855, October 2008.

Theses

Pan Mao (ME), "Ultra-High-Aspect-Ratio Nanochannels for High-Throughput Biological Applications," Ph.D. degree, July 2009.

Vincent Liu (EECS), "Development of an Integrated Capillary valve-based Preconcentrator and a Surface-based Immunoassay," M.S. degree, January 2009

Leon Li (HST/EECS), "Immunoassay Sensitivity and Kinetic Enhancement in Cell Culture Media using Electrokinetic Preconcentration," M. S. degree, January 2009

Lih Feng Cheow (EECS), "Development of an Enzyme-Linked Immunosorbent Assay (ELISA) with Enhanced Sensitivity in a Nanofluidic System," June 2009