EFFICIENT BIOMOLECULE PRE-CONCENTRATION BY NANOFILTER-TRIGGERED ELECTROKINETIC TRAPPING

Ying-Chih Wang[1], Christine H. Tsau[2], Thomas P. Burg[3], Scott Manalis[2] and Jongyoon Han[2,3]

[1]Dept. of Mechanical Engineering, [2]Biological Engineering Division
[3]Dept. of Electrical Engineering and Computer Science,
Massachusetts Institute of Technology, Cambridge, MA 02139, USA

ABSTRACT

This paper describes a novel nanofluidic device that can pre-concentrate dilute protein and peptide solution by electrokinetic trapping mechanism—up to 10^7 fold in concentration. By applying an electrical field across a 40 nm deep nanofluidic filter, one can generate an induced space charge layer as an energy barrier for charged biomolecules in the bulk. Coupling with a tangential field along the microchannel, this device can be used as either a preconcentrator or an injector for various applications.

Keywords: nanofilter, preconcentration, electrokinetic trapping

1. INTRODUCTION

Since there is no PCR-equivalent amplification technique in proteomics, sample preconcentration coupled with advanced sample separation steps is a must for detecting low-abundance protein species from common samples. Also, μTAS needs efficient preconcentration because of the mismatch in typical sample volumes (~1μl or larger) and the microchip internal volume (~1nL or smaller). The novel electrokinetic trapping mechanism can achieve more than a million fold biomolecule pre-concentration, enabled by nanofluidic channel, which is the best chip-based pre-concentrator reported so far.[1] Moreover, this work first successfully used a nonlinear electroosmotic flow for a stable electrokinetic trapping. Unlike other electrokinetic trapping pre-concentrators,[2, 3] where an electrical field was applied across either porous silica particles or Nafion® membrane as a means to create ion depletion, we used planar nanofluidic channel of a known size to achieve a stable operation over several hours.

2. EXPERIMENTAL

In this work, we use devices made of silicon nitride (by sacrificial layer etching) / PDMS or Si / SiO2 / glass in various dimensions to demonstrate the preconcentration mechanism triggered by ion-selective nanochannels. Unlike the 20 μm wide, 1.5 μm deep microchannels reported,[1] this paper presents new devices with larger internal volume, made of different substrate materials such as silicon-nitride for nano channels and PDMS for micro channels or wet-etched microchannels in Pyrex wafers instead of dry etched silicon substrate. Compared with the previous device, these new devices have ~1000 times larger internal volume (50μm x50μm cross section microchannel), which will lead to faster preconcentration with higher sample throughput.

0-9743611-1-9/μTAS2005/$20©2005TRF

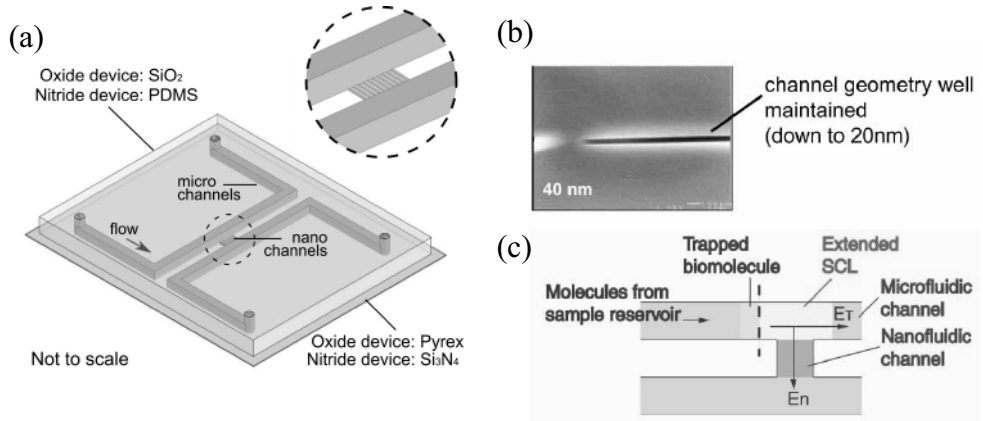

Figure 1. (a) Perspective view of nanofluidic pre-concentrator. Both oxide and nitride devices have 40 nm deep nano channels while 1.5 μm deep μ-channel for oxide device, 45 μm for wet-etched device, and 50 μm deep for nitride device. (b) SEM image of the nanochannels. (c) The trapping mechanism.

The key to achieve electrokinetic trapping is a set of nanofilters that is small enough to have electrical double layer overlapping (Figure 1). The nanofilter devices can be made of silicon nitride (by sacrificial layer etching) / PDMS or Si / SiO2 / glass. These nanofilters work as ion selective membranes that can generate concentration polarization or space charge extension, depending on the applied field strength. Once the extended space charge layer is formed, it will work as a barrier to charged molecules, as well as a source to generate electroosmosis flow of the second kind (Figure 2, 3). As a result, the device can achieve more than a million fold pre-concentration within thirty minutes. Figure 1 shows two parallel micro channels that are connected by ten 40 nm deep nano channels.

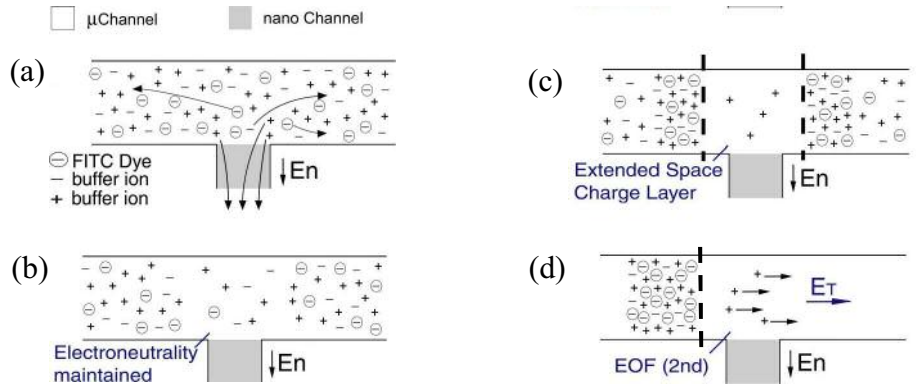

Figure 2. Mechanism of pre-concentration: (a) ion-selective property of the nano channel under small En; (b) concentration polarization under diffusion-limited condition; (c) at higher field, electroneutrality is no longer maintained, generating an extended space charge layer, (d) With proper E_T and En, the trapping region and depletion region will be formed as indicated; therefore, samples will be collected in front of the virtual barrier driven by nonlinear electrokinetic flow.

3. RESULTS AND DISSCUSSION

Efficient preconcentration of proteins and peptides in various devices are shown in Figure 4(a). This device will work with various buffers and additives, at a buffer ionic strength as high as 10mM. We were also able to increase the volume of preconcentrator for more efficient preconcentration, as shown in Figure 4(b & c), using a nanochannel made from Si_xN and PDMS microchannel. For an even larger channel, 100 μm x 45 μm in this case, it takes voltage higher than the device's passivation can handle (500V) to generate the extended space charge layer across the microchannel, therefore, a stable barrier can not be well maintained. However, the preconcentration can still be observed with a small pressure driven flow replacing the tangential field E_T.

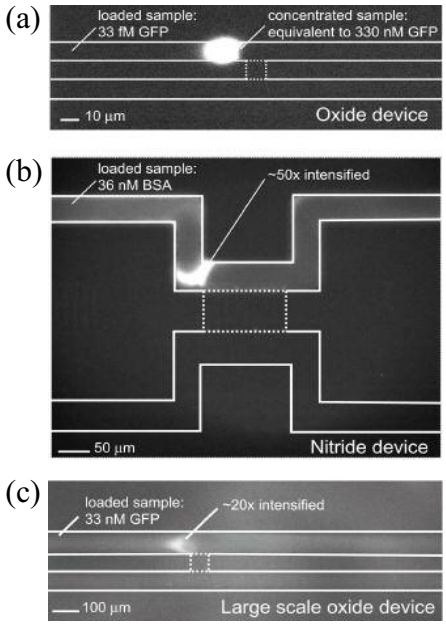

Figure 3. Preconcentrations in various devices: (a) Collection of 33 fM GFP sample in silicon oxide based device, where En= 10V/cm and E_T =.5V/cm. This demonstrates a preconcentration with a factor of ten million. (b) Collection of 36 nM BSA in nitride-PDMS device, where En= 50V/cm and E_T= 22 V/cm. (c) collection of 33 nM GFP in deep wet-etched microchannel. En= 0V/cm, and E_T= 200 V/cm. Samples were driven by pressure gradient.

4. CONCLUSIONS

This electrokinetic trapping mechanism can be integrated with many different systems with different surface and buffer requirements. Moreover, because it is easy to fabricate and requires only single buffer system, this device can be used as a preconcentrator for various downstream detection / separation tools. With its larger scale, robust mechanical properties, and high efficiency of preconcentration, this system is expected to play a key role in both conventional and microfluidic biomolecule analysis.

ACKNOWLEDGEMENT

This work was supported by CSBi (MIT), NSF CTS-0347348, -0304106, and NIH CDP center.

REFERENCES

[1] Wang, Y.-C., Stevens, A. L., and J. Han. *Million-fold Preconcentration of Proteins and Peptides by Nanofluidic Filter.* Anal. Chem., 2005. **77**: p. 4293-4299

[2]. Singh, A.K., et al. *A Novel Miniaturized Protein Preconcentrator Based on Electric Field- addressable Retention and Release*. Proc. μTAS2002, Nara, Japan, 2002: p. 347-349

[3]. Astorga-Wells, J. and H. Swerdlow, *Fluidic Preconcentrator Device for Capillary Electrophoresis of Proteins.* Anal. Chem., 2003. **75**: p. 5207-5212.