# ONE-STEP PROTEIN ANALYSIS USING SLANTED NANOFILTER ARRAY S. H. Ko<sup>1</sup> and J. Han<sup>1,2,3\*</sup>

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## ABSTRACT

We have designed a novel nanofilter-based, continuous-flow preconcentration and separation chip. Our design relies on angled nanofilters and enables both protein preconcentration and separation with a simple one-step introduction of analytes. Proteins and other biomolecules are first manipulated to form a narrow, concentrated band, followed by size-based separation for analysis. Separation resolution and detection limit issues, which plagued previously published nanofilter-base chips, were improved considerably. With further optimizing device parameters, device could be used for on-site biomolecule analysis, such as biologics (protein drugs) purity and efficacy monitoring.

KEYWORDS: Preconcentration and Separation, Nanofilter Arrays, Protein

## **INTRODUCTION**

Size-based separation of proteins (e.g. SDS-PAGE) is widely used to check the purity of protein drugs in pharmaceutical manufacturing process, and to ensure the lack of toxic impurities (*e.g.* protein aggregates). While liquid gel electrophoresis has been automated in a microfluidic platform, this technique still requires polymeric sieving matrices that can increase technical complexity, preventing implementation of truly portable, on-site drug purity and efficacy tests. As an alternative, our group previously published asymmetric nanofilter array (ANA) [1]. However, the detection sensitivity of the device is low because of short optical path lengths. In addition, a number of electrical connections and a voltage controller are required. In this work, we have designed a single-step, one-inlet-one-outlet protein analysis device, in order to both concentrate and separate proteins based on size. Not only is this system simple and straightforward to operate compared with previous ANA chip, it also enhances the detection sensitivity of ANA chip, matching or exceeding that of the standard SDS-PAGE technique.



Figure 1: (a) Schematics and SEM images of continuous-flow preconcentration and separation device. (b) Illustration of the principle of the proteins preconcentration (focusing) and separation based on their size in the slanted nanochannel arrays device.(c) Illustration of sieving mechanism in the nanochannel.

# EXPERIMENTAL

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Figure 1(a) shows schematics of continuous-flow preconcentration and separation device with slanted nanofilter arrays. The device is fabricated by standard MEMS fabrication process. The preconcentration/separation device has one-inlet-one-outlet and slanted nanochannel arrays with two different depths; shallow region (40 nm  $\sim$  100 nm) and deep region (100 nm and 300 nm). Nanofilters formed by the shallow regions is tilted (by 45° or 135°) from the main channel and field direction, driving molecules toward the edge of the channel [2]. The device consists of two regions (preconcentration and separation regions), each with dissimilar angles (135° for preconcentration region and 45° for separation region).

To demonstrate size-base protein preconcentration and separation, we used three proteins, such as BSA (66 kDa), Ovalbumin (45 kDa) and Trypsin inhibitor (21 kDa). All protein samples used were labeled by Alexa dye with different emission wavelength, and denatured by heat (95 °C) and SDS surfactant. To prevent non-specific adhesion of proteins to the silicon-glass surface and reduce electroosmotic flow, the nanochannel arrays was coated by POP-6 polymer before loading protein samples. Buffer solution is 5X TBE with 0.05% SDS molecule.

#### **RESULTS AND DISCUSSION**

Protein samples are directly loaded from the inlet reservoir continuously (no need to create 'launching band') with a single voltage applied. After loaded proteins are mechanically focused toward the bottom edge of the channel wall in the preconcentration region by slanted nanochannels [2], they are then size-separated in the separation region, as shown in Figure 1(b). The sieving mechanism is Ogston sieving (molecule size < shallow nanochannel depth) in which smaller molecules have less configurational entropic energy barrier by steric hindrance, resulting in greater jump passage probability and therefore low deflection angle, as shown in Figure 1(c). Hence, different size molecules have own distinct trajectories, as shown in Figure 2 (deflection angle : BSA > Trypsin inhibitor).



Figure 2: Demonstration of multiple proteins separation based on their size. (a) 60 nm (shallow region) / 327 nm (deep region), (b) 40 nm /100 nm (c) 40 nm / 100 nm with glycerol. Deflection angles of two proteins in 100 nm deep region (d) and 300 nm deep region (e).

The deflection angle of each protein and the angle difference between proteins are critically affected by both shallow and deep region depths. When the shallow region depth decreases, separation resolution (the angle difference) was improved, because smaller filter size resulted in higher entropic barrier. And the separation resolution also increases with deep region depth. The best size selectivity for a given shallow region occurs in  $K_d\sim1$  and  $K\sim K_s$  (K=K<sub>s</sub>/K<sub>d</sub> : ratio of the partition coefficient, K<sub>d</sub> : deep region partition coefficient, K<sub>s</sub> : shallow region partition coefficient), so that the device with 300 nm deep region has higher selectivity than 100 nm deep region due to the large K<sub>d</sub> (0.992) in 300 nm deep region, compared to K<sub>d</sub> (0.975) in 100 nm deep region [3]. In the case of small molecular weight (below 45 kDa), however, protein diffusion rate is large, leading to low separation resolution when nanochannel arrays as sieving matrix are used. Generally, in SDS-PAGE, glycerol has been used for achieving better separation resolution because it makes solution more viscous, which reduces diffusion rate. When glycerol was added into the protein mixture, baseline separation of 3 proteins was achieved in the same device (40 nm/100 nm) with better separation resolution and low diffusivity than without glycerol, as shown in Figure 2(c).

The key innovation here is the integrated preconcentration region, which not only defines the 'launching band' for protein separation but also carry out significant preconcentration to improve the overall detection sensitivity, even in a thin nanofluidic channel (~100 nm). To check enhancement of detection sensitivity, we measured limit of detection (LOD) and preconcentration factor with different concentration proteins (BSA, red box in Figure 3) and inlet channel width. Figure 3(a) shows that LOD (80ng/mL) and preconcentration factor (~100 fold) achieved. This is higher detection sensitivity than the silver staining method (1~10ng of protein required per ~10 $\mu$ L loading volume), yet LOD and preconcentration factor could be further enhanced simply by increasing the width and collecting more molecules in the preconcentration stage (Figure 3(b)).



#### CONCLUSION

In this paper, we have developed a new continuous-flow protein preconcentration and separation nanofluidic device. Biomolecule preconcentration and separation was achieved successfully with better separation efficiency and lower detection sensitivity than previously developed nanofilter sieving system. In addition, the device performance could be further improved by investigating additional experimental parameters, such as nanochannel dimension, angle and device width etc. We believe the device demonstrated here can be used as a point-of-care drug efficacy monitoring system, due to its operational simplicity, robustness (no degradation of gels) and minimal sample use (~1nL of sample volume). The high detection sensitivity of the system could enable detection of low-level impurities in biologics drug that can lead to significant toxicity in patients.

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