BIOMOLECULE SEPARATION IN NANOFLUIDIC FILTERS BY STERIC HINDRANCE MECHANISM

Jianping Fu[1] **and Jongyoon Han**[2, 3]

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

Abstract

A novel biomolecule size-fractionation strategy is explored in this work by using nanofluidic filters. The separation of biomolecules is based on the steric hindrance induced entropic barrier effect, which is size-dependent. For demonstration, DNA sample (100bp DNA ladder) and SDS denatured protein mixture (FITC-conjugated ovalbumin (MW~45K) and low density human lipopropein (MW~179K)) are completely separated by dc electrophoresis in an 80nm nanofluidic filter device. In the low field region, the electrophoretic mobility μ of DNA fragments can be nicely fit into the exponential function, $\mu \sim \exp(-sL)$, where L is the total length of short rigid DNA molecule and s is the proportionality constant.

Keywords: biomolecule separation, nanofluidics, steric hindrance, entropic barrier

Micro/nanofluidic molecular sieving structures fabricated with microfabrication technology have been applied for biomolecular separation with great speed and efficiency [1-3]. However, their uses have been largely limited to large biomolecules, since it is generally difficult to fabricate structures with comparable molecular dimension to serve as efficient sieving matrix. In this paper, we present a novel separation method using nanofluidic filters to fractionate small biomolecules based on their sizes. The separation is based on the entropic free energy barrier caused by the steric hindrance of biomolecules in nanofilters. When biomolecules are moving into a nanofluidic filter from the thick region, they get confined and lose some degree of freedom in the molecular conformation (chain configuration) (Fig. 1). This entropy loss will pose a free energy barrier for the molecules, as suggested originally by Giddings [4,5]. This could be a basis for sieving biomolecules whose sizes are even smaller than the gap size of the nanofilter.

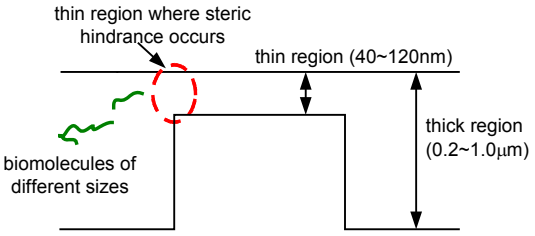

Figure 1. Cross-sectional schematic picture of one nanofluidic trap. When molecules travel through the thin region, certain configuration entropy will be lost due to steric constrains imposed from the walls. The entropic free energy barrier caused by this will slow down the motion of molecules and induce the size dependence of electrophoretic mobility in the nanofluidic filter.

The nanofluidic filter device is fabricated with microfabrication technology in cleanroom environment. Detailed information about the fabrication process is available in previous publications [2,6]. Before all electrophoresis experiments, the nanofluidic filter device is filled with buffer solution through the buffer access holes. As a buffer solution, Tris-Borate-EDTA (TBE) buffer at 5X (445mM) concentration is used in all our experiments to efficiently quench the electroosmotic flow. Under this buffer concentration, the thickness of electrical double (Debye) layer is negligible as an affecting factor for separation. A T-shaped injector for electrophoretic sample injection is integrated in our nanofluidic filter device (Fig. 2(a)). During each separation experiment, a band of sample with the length equal to the channel overlap at the T intersection

(about 200μm) is delivered into the separation column by manipulating the potentials applied at the four buffer access holes (Fig. 2(b)).

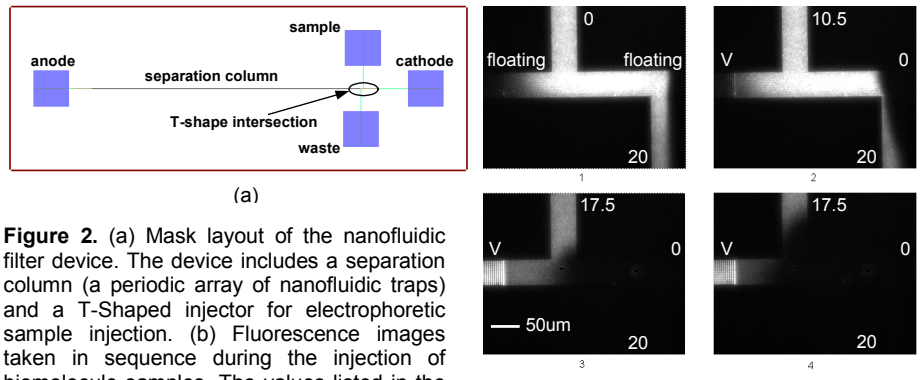

Figure 2. (a) Mask layout of the nanofluidic filter device. The device includes a separation column (a periodic array of nanofluidic traps) and a T-Shaped injector for electrophoretic sample injection. (b) Fluorescence images taken in sequence during the injection of biomolecule samples. The values listed in the figures are the voltage applied during the injection in all our experiments.

In our experiment, 100bp DNA ladder samples (New England BioLabs) are labeled with YOYO-1 dye (Molecular Probes) at a dye/base pair ratio of 1:10. For protein separation, FITC-conjugated protein mixture (ovalbumin (MW~45K, Molecular Probes) and low density human lipoprotein (MW~179K, Molecular Probes)) is denatured with 2% sodium dodecyl sulfate (SDS) to impose uniform hydrodynamic and charge characteristics. To get an electrophoregram, an inverted fluorescence microscope is used to detect the Ar-ion laser-induced fluorescence signal at various separation distances (1-3cm) along the separation column. The microscope images are recorded by a CCD camera (Sensicam QE, Cooke Corp.) and the fluorescence signals from the laser detection point are averaged for data analysis.

DNA molecules (100bp DNA ladder sample) are first separated in a nanofluidic filter device with 80nm thin regions, 500nm thick regions and 4um trap period (Fig. 3). As the applied field lowered down, 100bp DNA ladder is completely separated into individual bands (Fig. 3(a)). This shows that the molecules as small as 100bp double stranded DNA (34nm extended length) can be sieved in the filter as large as 80nm. The log plot of electrophoresis mobility μ verses DNA fragment size is shown in Fig. 3(b). As shown in the figure, an exponential function $\mu \sim \exp(-sL)$, from the free volume model of gel electrophoresis[7] can fit the experimental data well in the low field region. Here, L is the total length of DNA molecule and is proportional to DNA base pair number N. At higher fields, the data deviate from the exponential behavior, suggesting other transport mechanisms in effect in those cases. Figure 4 shows the electrophoregram of SDS denatured protein mixture of ovalbumin and low-density human lipoprotein from the same nanofluidic filter device. Before the experiment, TBE 5X buffer with 0.1% SDS is flushed into the separation column by running electroosmotic flow for several hours. Complete separation of these two proteins can be achieved in 40mins at field strength of 50V/cm. This shows that the molecules as small as proteins can be indeed separated in a nanofluidic filters as large as 80nm.

In conclusion, we have demonstrated a novel separation method by using nanofluidic filter device to separate small biomolecules (small DNA, proteins). This novel separation mechanism is applicable to any biomolecules for size-based separation, and could be useful in developing

nanofluidic molecular sieving systems for various biomolecules, including proteins and carbohydrates. The nanofluidic filters presented here can replace liquid or polymeric gel for biomolecule separation and can be fabricated and integrated easily into complex μ-TAS systems. The regularity and well-defined geometry of nanofluidic filters also provide a good platform for theoretical study of electrophoresis or hindered transport of flexible polyelectrolytes in gels.

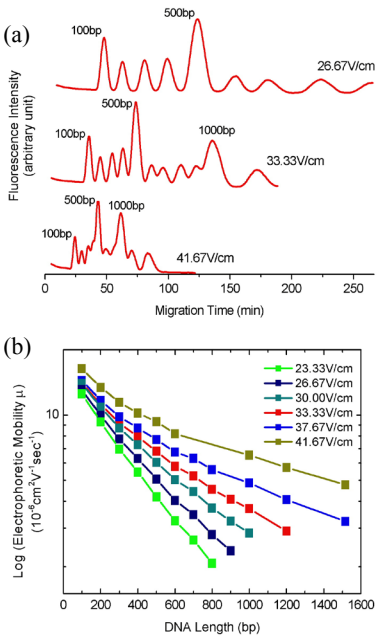

Figure 3. Separation of 100bp DNA ladder in the nanofluidic filter device with 80nm thin regions, 500nm thick regions and 4um trap period. (a) electropherograms of 100bp ladder sample taken at 1cm downstream from the injection point under different electrical fields. Smaller DNA fragments elude first from the channel; 500bp and 1000bp bands have increased intensity to serve as reference peaks as shown in figure. Apparently there is a trade-off between the resolution and the speed of separation; with a lower electrical field, more resolved separation is obtained, but the speed of the separation is compromised. Separation resolution becomes poorer when the driving electric field is increased. (b) Logarithmic plot of electrophoresis mobilities verses DNA length under different fields. In the low electrical field region, an exponential function $\mu \sim \exp(-sL)$ can nicely fit the mobility data. In the high field regions, the mobility data depart from the exponential behavior, especially for the longer DNA molecules.

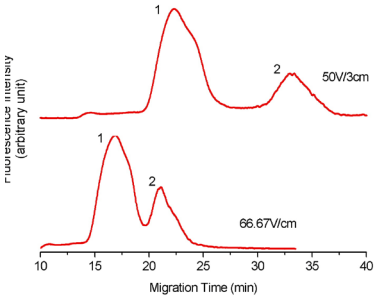

Figure 4. Separation of ovalbumin and low density human lipopropein in the nanofluidic filter device with 80nm thin regions, 500nm thick regions and 4um trap period. Electropherograms are taken 1cm downstream from the injection point. Band assignment: ovalbumin (1), human lipoproperin (2). Ovalbumin band has increased intensity to serve as reference peak. In 40 mins, these two proteins are completely separated into two different bands.

References

[1] W. D. Volkmuth, R. H. Austin, *Nature*, **358**, 600-602 (1992).

[2] J. Han, H. G. Craighead, *Science*, **288**, 1026-1029 (2000).

[3] L. R. Huang, *et al. Nature Biotechnology*, **20**, 1048-1051 (2002); L. R. Huang, *et al. Science*, **304**, 987-990 (2004).

[4] J. C. Giddings, E. Kucera, C. P. Russell, M. N. Myers, *J. Phys. Chem.*, **72**, 4397-4408 (1968).

[5] J. C. Giddings, *Unified Separation Science* (John Wiley & Sons, New York, 1991).

[6] J. Han, H. G. Craighead, *J. Vac. Sci. Technol. A,* **17**, 2142-2147 (1999).

[7] J. L. Viovy, *Rev. Mod. Phys.*, **72**, 813-872 (2000)