Effect of Fluorescent Tags on Translocation through Nanochannels

Swati Goyal, Young-tae Kim and Samir M. Iqbal, Senior Member, IEEE

Abstract—The fluorescent tagging of biomolecules can have substantial effects on the charge distribution and translocation of the molecules through nanochannels. We show that the diffusion and calculated flux of translocating protein molecules through nanochannels are strong functions of the tags used. The size of the nanopore channels of a membrane also affect whether the channels provide facilitated transport or act just like affinity chromatography. These findings have important implications on micro/nanofluidic based biophysical studies that greatly discount the effects of tagged dyes on molecular transport and their mechanics.

I. INTRODUCTION

Nanochannels and microchannels provide elegant means to study biophysical properties of important biological molecules like nucleic acids and proteins. Recent years have seen a lot of growth in experiments with micromachined or synthetically fabricated membranes for selective permeability, active filtration, molecular separation, etc. [1,2]. Synthetic membranes have been shown viable in widely varying solution conditions of salinity, pH, and temperatures for transport analysis of molecules like DNA and proteins [3,4].

The miniaturization of devices provides many advantages like faster analysis, smaller volumes and quantities of analytes required, reduced cost and optimal chemical footprint. Organic dyes and synthesized entities like quantum dots are used to measure in situ optical data from interactions and reactions in real time. However, the thermodynamic, mechanical and energy effects of tagging biological molecules are often discounted, while pushing the limits to single molecule measurements. A simple dye tagging of DNA sample can result in molecular conformation changes, and hence can alter duplex stabilities and melting temperatures. Fluorescent dyes photobleach, quench statically or can interact with each other [5,6].

Proteins and DNA tagged with organic dyes have been used for separation studies through nanochannels, where diffusion was putatively different between different molecules due to size, or biological and chemical interactions [2,4,7-9]. The effects of charge, size and the presence of complementary molecule is shown to enhance the flux of corresponding molecules across the nanochannels [2,10,11].

In this work, we contend and demonstrate that tagged dyes can have non-negligible effects on molecular transport through nanochannels. We report protein diffusion through polycarbonate membrane nanochannels and show that dyes have a direct effect on protein diffusion through nanochannels. The phenomena of selective molecule transport, also known as facilitated transport, breaks down because of specific charge contributions of fluorescent tags on protein diffusion through nanochannels of specific sizes.

II. MATERIALS AND METHODS

For device fabrication, polycarbonate membranes with nanochannel diameters of 200 nm, 100 nm, 50 nm and 15 nm were used. Surface carboxyl groups were generated using UV/Ozone plasma for 220 seconds with shiny side facing up as previously described [12]. For PDMS casting, Sylgard 184 was mixed in 10:1 ratio and polymerized at 110 °C for 4 hours. The holes of 6 mm diameter were made using biopsy punch. Two layers of PDMS were aligned and membrane was sealed in between using Sylgard 184 in ratio 20:1 and left overnight at room temperature to dry (Fig. 1). The membranes were 6 μm thick with nanochannel density of 6x10^8 channels/cm². The effective filtration area was 110 mm². For antibody immobilization on surface and inside the nanochannels, Rabbit IgG antibody was diluted 1:500 in

Manuscript received March 30, 2010. This work was supported by the National Science Foundations CAREER Grant ECCS-0845669 to S. M. Iqbal.

Swati Goyal was with the Department of Bioengineering at the University of Texas at Arlington, TX, USA. She is now with Life Technologies Corporation, CA, USA (e-mail: Swati.Goyal@lifetech.com).

Young-tae Kim is with the Department of Bioengineering at the University of Texas at Arlington, TX, USA (e-mail: ykim@uta.edu).

S. M. Iqbal is with the Department of Electrical Engineering at the University of Texas at Arlington, TX, USA. He is affiliated with the Nanotechnology Research and Teaching Facility and serves on the Joint Graduate Studies Committee of the Biomedical Engineering Program between University of Texas at Arlington and University of Texas Southwestern Medical Center at Dallas. (phone: 817-272-0228; fax: 817-272-7458; e-mail: smiqbal@uta.edu).

Fig. 1. Polycarbonate membranes were sandwiched between two PDMS castings. The solution was fed from the shiny side and collected from a similar hole in the lower casting.
PBS with 20 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 10 mM N-Hydroxysuccinimide (NHS) (Sigma, Saint Louis). The antibody solution was applied on the upper side of the membrane (UV activated surface) and incubated at 4 °C for overnight. The devices were then washed with copious amount of PBS, and incubated with 4% goat serum in PBS for one hour to block unreacted carboxyl groups and decrease the non-specific protein adsorption. It was then washed vigorously and used for filtration. The mixture of two secondary antibodies was used as sample: goat anti-rabbit IgG tagged with Alexa 488 and goat anti mouse IgG1 tagged with Alexa 546. The antibody concentration across the membrane was measured as a function of the fluorescence of respective tagged dyes. Another experiment was conducted to see if the presence of specific fluorescent molecule had any effect on molecule mobility through nanochannels. Here, the fluorescent tags of antibodies in sample mixture were exchanged (i.e., goat anti-rabbit IgG was now tagged with Alexa 546 and goat anti mouse IgG1 was tagged with Alexa 488), where all other parameters and molecular concentrations were kept the same. The mixture was then filtered again through 15 nm membranes immobilized with rabbit IgG antibodies.

III. RESULTS AND DISCUSSION

The flux of molecule was measured as slope of the plot of normalized fluorescence versus time. For 200 and 100 nm nanochannels (Fig. 2(a),(b)), the flux of goat anti-mouse IgG1 Alexa 546 (non-complementary) was significantly higher than that for goat anti-rabbit IgG Alexa 488 (complementary). These devices behaved like affinity chromatography column [13]. Such columns hold the complementary protein and available space is open for movement of free non-interacting proteins. However, when the membrane pores size was decreased to 50 nm (Fig. 3(a)); there was a transition in flux. The flux of complementary antibody, i.e. goat anti-rabbit IgG tagged with Alexa 488, increased than that for non-complementary antibody. For complementary molecules, in 15 nm nanochannels, the attractive potential created by surface antibody was too high, and there was no free space for non-interacting proteins to pass through. This has been indeed reported before, where increase in pore size has been shown to reduce the selectivity of the functionalized membranes [9], but effect of fluorescent dyes has not been explored yet.
The specific interactions of surface bound antibodies with unlabelled proteins have been recently shown to alter the diffusive flow of specific proteins. In line with the understanding of facilitated transport [14], the flux of BSA through anti-BSA antibody functionalized membrane nanochannels was seen to increase than non-interacting human hemoglobin [1]. The experiments elucidating facilitated transport theory have shown the effects of molecule-nanochannel interactions where binding events can overcome the Debye length effects and increase the flux of interacting molecules than that for non-interacting ones [15]. These and other experiments have shown that the membrane selectivity stems from the interactions of the permeating molecules with surface immobilized molecules [10]. However, in experiments reported here, interchanging the fluorescent tags on target antibodies did not reverse the fluorescent signal through membranes functionalized with specific antibodies. Counter-intuitively, interchanging the dye on two antibodies in feed solution didn’t alter the membrane selectivity. The protein tagged with Alexa 488 always had higher flux irrespective of binding selectivity with the nanochannel functionalized antibodies (Fig. 4).

This work unambiguously shows that the size, charge and chemical properties of the fluorescent dyes have non-negligible effects on molecular separation studies in micro/nanochannels. Antigen-antibody interaction, thus, cannot be only defined in terms of ligand-probe interactions in micro/nano-confinements.

ACKNOWLEDGMENT

The authors would like to acknowledge P. P. Ramachandaran, Shawn Christensen and Digan Dave for experimental help. We also acknowledge the use of Genome Biology Group facilities at the UT-Arlington.

REFERENCES


