Enhanced Molecular Filtering at Nano-channel by using Self-spreading Lipid Bilayer as Molecular Transport and Filtering Medium

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The self-spreading behavior of DMPC bilayer containing TR-DHPE molecule was observed in the presence of channel-type nano-architecture (nano-channel). The concentration of TR-DHPE at the spreading edge region was gradually decreased by passing through the nano-channel. The filtering efficiency was increased by elongating the channel-length, which was explained by considering a formation of a local compressed state of the spreading bilayer in the elongated channel region. In addition to the channel length, the filtering effect was found to be tuned by the channel angle (entrance angle). These two dependencies are important for precise control of filtering efficiency and selectivity.

Key words: self-spreading lipid bilayer, nano-architecture, phase transition, molecular manipulation

1. INTRODUCTION

There is a growing interest in the field of molecular manipulation at low dimension. Especially, the use of small architecture is of great interest for their possibility to offer novel molecular manipulation and separation phenomenon [1-6]. For example, three-dimensional nanofluidic sieves have been fabricated within microfluidic devices by a microfluidic colloidal self-assembly process [5]. By using this fluidic system, fast separation of DNA and proteins of wide size range was achieved by an electrophoresis. Furthermore, the incorporation of asymmetry to the architecture enabled to separate microspheres with different size by applying a fluid flow [6]. Including these two examples, almost previously reported strategies using small architectures relied on an external field, such as the electrophoresis and the fluid flow. Although the working device becomes smaller and smaller by using versatile small architectures, the use of outer bias has inherent disadvantage toward miniaturizing the total device system.

In order to overcome this limitation, the use of spontaneous phenomena can be a breakthrough for future ultra-small devices. For example, the use of spontaneous growth of lipid bilayer enables to transport any molecule in the bilayer toward any direction without applying any external biases [7-10]. This effect is known as the self-spreading behavior of lipid bilayer. The driving energy of the self-spreading is the gain in free energy via bilayer-substrate interaction such as the van der Waals and electrostatic interactions [9]. By using the self-spreading lipid bilayer as the molecular transport medium, we have succeeded in the appearance of novel molecular filtering phenomenon [11, 12]. Our experiment has demonstrated that the doped dye molecule in the self-spreading lipid bilayer was selectively filtered by a nano-architecture array. The origin of the appearance of the filtering effect is thought to be the formation of a chemical potential barrier at the narrowest region between opposing two nano-architectures, which is caused by a local structural change in the self-spreading lipid bilayer. Change in the

chemical potential, i.e., change in the molecular solubility, at the narrowest region acts as a filter for the doped molecules in the self-spreading lipid bilayer. Since the chemical potential is dependent on the nature of dopant molecule such as size, charge, and hydrophilicity, strong dependency of the filtering efficiency on the structure of dopants was clearly appeared [12].

However, the filtering efficiency in the previous study was at most 1 % per single nano-architecture array. Although this system could be applied nanoscopic device that can work under completely nonbiased condition, further increase in filtering efficiency and selectivity is desired. In order to enhance the filtering efficiency, we have investigated the effect of elongation of nano-architecture, i.e., we used channel-type nano-architecture (nano-channel) with different channel length to make clear the effect of channel structure and to enhance the filtering ability.

2. EXPERIMENTAL PROCEDURE

Coverslips were cleaned with piranha solution ($\text{H}_2\text{O}_2/\text{H}_2\text{SO}_4=1/4$ vol% mixture). Au nano-gates were fabricated using a high-resolution EBL system (ELS-7700H, Elionix Co., Ltd., Japan). After development in developer solution, 2 nm chromium and 30 nm gold layers were successively deposited by sputtering (ULVAC, MPS-4000, Japan). Then, the resist layer was removed by immersion in acetone solution for 2 min and in remover solution for 2 min in an ultrasonic

Fig. 1 AFM image of an array of hexagonal architectures. Enlarged image of left-side end of (a) is shown in (b).

Fig. 2 Successive fluorescence microscope images of the self-spreading DMPC bilayer containing TR-DHPE on (a) glass substrate (control experiment) and (b-d) nano-channel substrates. The lower panels show the successive change in the fluorescence intensity line profiles.

bath. The fabricated structures were characterized using atomic force microscopy (AFM; Nanoscope IIIA, Digital Instruments). As shown in Fig. 1, we have fabricated an array of hexagonal architectures. There are three parameters that characterize the nano-channel properties; channel length l , channel angle θ , and channel width w . In the present study, we have fabricated substrates with $l = 0.5, 1, 5, 10, 20 \mu\text{m}$ and $\theta = 30, 60, 90, 120, 150$, and 180° . w was fixed as 100 nm .

The self-spreading lipid bilayer was prepared as follows: 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was dissolved in chloroform with a concentration of 1.0 mg/ml ; then, the dye-labeled lipid molecule Texas Red 1, 2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (TR-DHPE) was mixed to the desired molecular ratio (typically $1 \text{ mol}\%$). A drop of the solution was positioned on the substrate using a clean Teflon rod and then dried under ambient conditions. After evaporation, a lipid lump was left on the substrate. By the immersion of the substrate into $100 \text{ mM Na}_2\text{SO}_4$ aqueous solution, the lipid bilayer spread spontaneously from the lipid lump on the substrate. The self-spreading behavior was monitored by epi-fluorescence microscopy with high-pressure mercury

lamp. The light intensity was tuned to be ca. 1 mW under the objective lens to avoid photobleaching.

3. RESULTS and DISCUSSION

DMPC bilayer showed spreading behavior on both glass (control) and nano-channel substrates. As shown in Fig. 2(a), continuous and bright DMPC bilayer containing TR-DHPE spread with time on the glass substrate. The fluorescence intensity in the self-spreading bilayer reflects the concentration of fluorescent TR-DHPE molecule in the spreading bilayer. The fluorescence intensity profiles shown in the lower panel show gradual increase toward the spreading edge at any time. This indicates that TR-DHPE molecule is likely to accumulate at the spreading edge region. Similar phenomenon has already been found and explained by other group [13].

The self-spreading bilayer on the nano-channel substrate with $l = 0.5, 5, 20 \mu\text{m}$ were shown in Fig. 2 (b), (c), and (d), respectively. Different from the bilayer on the glass substrate, periodic dark bands were observed on the nano-channel substrate. Judging from the width of the dark bands, they can be attributable to the region of nano-channel arrays, i.e., the substrate with nano-channel of $l = 0.5, 5, 20 \mu\text{m}$, the width of the dark

Fig. 3 Change in the normalized fluorescence intensity as the function of the number of gate passed. The channel length is 0.5 μm (blue), 1 μm (light blue), 5 μm (light green), 10 μm (orange), and 20 μm (red).

band are also 0.5, 5, 20 μm , respectively. The width of bright bands corresponds to the bilayer that is present on the region between nano-channel arrays. Since the width of the region between two nano-channel arrays was designed as 2 μm on any nano-channel systems, the width of the bright bands was ca 2 μm . The appearance of periodic dark and bright bands on the nano-channel substrate strongly suggests that the bilayer spread only through the 100-nm-width channel region, without spreading over the architecture. This is owing to the ability of gold surface to inhibit the diffusion of lipid molecule [14].

In order to characterize the filtering ability of these nano-channel substrates, we have analyzed the changes in fluorescence intensity at the spreading edge after fully passing through each nano-channel array. The normalized fluorescence intensity was plotted as the function of the number of passed nano-channel array (Fig. 3). In the case of $\theta = 30^\circ$, the nano-channel with $l = 0.5$ and 1 μm did not show detectable filtering effect, i.e., no decrease in the fluorescence intensity was observed. On the other hand, gradual decrease was found on the nano-channel with $l = 5, 10$ and 20 μm . As the general tendency, the nano-channel with longer l can exhibit higher filtering efficiency at any angle. The highest filtering ability was appeared on the nano-channel with $\theta = 120^\circ$ (Fig. 3(d)). In this case, detectable filtering effect can be seen on the nano-channel even with $l = 0.5 \mu\text{m}$. Further increase in θ to more than 150° reduced the filtering ability. This means that there is an appropriate angle in order to achieve an effective filtering phenomenon. The most significant filtering effect in the present study was observed on the nano-channel with $\theta = 120^\circ$ and $l = 20$

μm (red line in Fig. 3(d)).

The filtering efficiency per single nano-channel array, ε , can be characterized by following equation.

$$I_n^{\text{norm.}} = \left(1 - \frac{\varepsilon}{100}\right)^n \quad (1)$$

where $I_n^{\text{norm.}}$ is the normalized fluorescence intensity after fully passing through n^{th} gate array. By fitting the data in Fig. 3 with eq (1), we obtained ε as the function of θ for nano-channels with different l (Fig. 4). As can be expected from Fig. 3, the highest ε was obtained by using longest nano-channel ($l = 20 \mu\text{m}$). The filtering effect has originally been observed with an array of rhombus nano-architecture, i.e., nano-channel with $l = 0$ (12). The origin of the appearance of the observed filtering effect is thought to be the formation of a chemical potential barrier at the narrowest region

Fig. 4 θ -dependency of the filtering efficiency ε . The channel length is 0.5 μm (+), 1 μm ($\times$), 5 μm ($\square$), 10 μm ($\triangle$), and 20 μm ($\circ$).

between opposing two nano-architectures, caused by a local structural change in the self-spreading lipid bilayer. Change in the chemical potential, i.e., change in the molecular solubility, at the narrowest region acts as a filter for the molecules. By elongating the narrowest region with the use of channel-type nano-architectures, we have succeeded in increasing the filtering efficiency from 1 % to 5 % [12]. In addition to the channel length, we have also found strong dependence on the channel angle θ . Although we need more time to make clear the observed θ dependency, it seems to be necessary to take into account fluid mechanics of the self-spreading lipid bilayer especially at the entrance of the nano-channel to discuss the mechanism of θ dependency. Further investigation with both experimental and theoretical analysis will clarify the importance of channel angle for the filtering phenomenon.

4. CONCLUSION

Filtering ability of nano-channel architectures was found to be strongly dependent on the channel length and channel angle. The highest filtering efficiency was observed on the nano-channel with $\theta = 120^\circ$ and $l = 20 \mu\text{m}$. Although l dependency can be explained by assuming local compression effect at the narrowest region, it would be necessary to take into account fluid mechanics in order to understand the observed θ -dependency. Both l and θ dependency would contribute for the precise control of the filtering phenomenon, which can offer a systematic control of molecular filtering efficiency and selectivity in this novel molecular manipulation system.

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- [1] C. K. Choi, J. D. Fowlkes, S. T. Retterer, P. Siuti, S. Iyer and M. J. Doktycz, *ACS Nano*, 4, 3345-3355 (2010).
- [2] J. Regtmeier, R. Eichhorn, L. Bogunovic, A. Ros and D. Anselmetti, *Anal. Chem.*, 82, 7141-7149 (2010).
- [3] Z. Li and G. Drazer, *Phys. Rev. Lett.*, 98, 050602/1-050602/4 (2007).
- [4] J. Fu, R. B. Schoch, A. L. Stevens, S. R. Tannenbaum and J. Han, *Nature Nanotechnology*, 2, 121-128 (2007).
- [5] Y. Zeng and D. J. Harrison, *Anal. Chem.*, 79, 2289-2295 (2007).
- [6] K. Loutharback, J. Puchalla, R. H. Austin, J. C. Sturm, *Phys. Rev. Lett.*, 102, 045301/1-045301/4 (2009).
- [7] J. Nissen, K. Jacobs and J. O. Radler, *Phys. Rev. Lett.*, 86, 1904-1907 (2001).
- [8] Y. Kashimura, J. Durao, K. Furukawa and K. Torimitsu, *Jpn. J. Appl. Phys.*, 47, 3248-3252 (2008).
- [9] H. Nabika, A. Fukasawa and K. Murakoshi, *Phys. Chem. Chem. Phys.*, 10, 2243-2248 (2008).
- [10] H. Nabika, A. Fukasawa and K. Murakoshi, *J. Langmuir*, 22, 10927-10931 (2006).

- [11] H. Nabika, A. Sasaki, B. Takimoto, Y. Sawai, S. He and K. Murakoshi, *J. Am. Chem. Soc.*, 127, 16786-16787 (2005).
- [12] H. Nabika, N. Iijima, B. Takimoto, K. Ueno, H. Misawa and K. Murakoshi, *Anal. Chem.*, 81, 699-704 (2009).
- [13] J. Nissen, S. Gritsch, G. Wiegand and J. O. Radler, *Eur. Phys. J. B*, 10, 335-344 (1999).
- [14] J. T. Groves, N. Ulman and S. G. Boxer, *Science*, 275, 651 (1997).

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