

# Self-spreading Lipid Bilayer on Nano-structured Surface with Periodic Array of Metallic and Organic Nano-architectures

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We investigated the lipid bilayer self-spreading behavior on glass substrates modified with complex array of nano-architectures. Simple way of constructing a binary array of metallic / organic nano-architectures was established. The metallic nano-architecture was prepared by nano-sphere lithography (NSL) method. By controlling the drying process in NSL, periodic array of donut-shaped organic nano-architectures, 200 nm in diameter and 4 nm in height, was also constructed on the substrate. In the presence of metallic nano-architectures, the self-spreading velocity was significantly reduced due to the energy cost imposed on passing through the nano-architectures. The self-spreading phenomenon was found to be intact even on the metallic / organic binary array substrate. Fluorescence microscope revealed that the organic nano-architectures kept their initial structures during the self-spreading around the architectures.

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

## 1. INTRODUCTION

Lipid bilayers supported on solid substrate has long been utilized as a model system of cell membrane. Recently, dynamic nature of lipid bilayer has been also paid attention because of the interest for various applications on molecular manipulation system. Spontaneous growth of lipid bilayer from lipid aggregate on hydrophilic surface is induced simply by immersing in water [1-5]. This phenomenon is called as lipid bilayer self-spreading. Recent report demonstrated that the self-spreading lipid bilayer can be used as the medium for molecular transport in micro-fluidic devices [5]. Most prominent aspect of the self-spreading phenomenon is that any kind of molecules in the bilayer, even non-charged molecules, can be transported toward desired direction without any external field. This is an advantage against other molecular transport method such as electrophoresis [6-8], because electrode, leads, or power suppliers are no more needed. Recently, we have developed molecular filtering system based on the self-spreading lipid bilayer [9]. In this system, periodic array of metallic nano-architecture was found to play an important role for the molecular filtering phenomenon. By controlling the substrate functionality, further intriguing aspects would be derived in the self-spreading system. In the present study, we prepared binary array of metallic and organic nano-architectures and the effect for the self-spreading behavior was investigated.

## 2. EXPERIMENTAL

Glass substrates with periodic array of nano-architectures were prepared by modified NSL method. [10-12] Briefly, hexagonal array of polystyrene (PS) beads (Polysciences Inc.) with a diameter of 3  $\mu\text{m}$  was self-assembled on glass or Si substrate. Then gold with a thickness of 30 nm was vapor deposited under

$10^{-3}$  Pa. After the deposition, PS beads were removed by ultrasonication in water or ethanol.

Spreading of the lipid bilayer was conducted according to the reported protocol by using of egg-yolk phosphatidylcholine (egg-PC) doped with 1 mol% of Texas-Red labeled dihexadecanoyl-phosphatidylethanolamine (TR-DHPE). [9] The spreading behavior was observed in-situ by fluorescence microscope in 100 mM  $\text{Na}_2\text{SO}_4$  solution. The spreading behavior was monitored by epi-fluorescence microscope (BX-51, Olympus, Japan) with a  $40 \times 0.80$  N.A. water immersion objective at room temperature. High-pressure mercury lamp was used as light source. To avoid significant photo-bleaching, the light intensity was attenuated to 1 mW using ND filters.

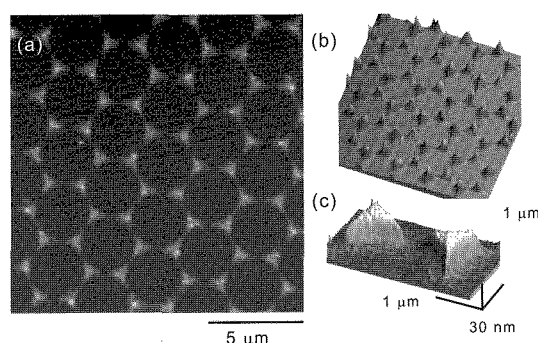


Fig. 1. (a) Two-dimensional and (b) three-dimensional AFM image of periodic array of metallic nano-architecture prepared via ultrasonication in water. (c) Enlarged image of metallic nano-architectures.

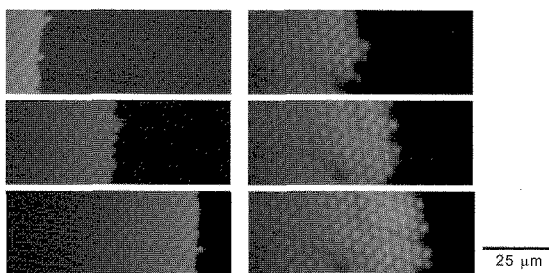


Fig. 2. Time sequences of fluorescence microscopic images of the self-spreading lipid bilayer on (left) glass and (right) NSL substrates at time interval of 2 min.

### 3. RESULTS AND DISCUSSION

Formation of well-organized periodic array of gold nano-triangles was confirmed by AFM images as shown in Fig. 1. No other feature was observed on the substrate surface. The size, height, and inter-architecture distance can be tuned by changing PS diameter and the deposition thickness. Fluorescence microscopic images of the self-spreading bilayers on bare glass and NSL substrate were shown in Figure 2. The lipid bilayer spreads from left to right in each system. On the glass substrate, any cracks or defects can not be seen in the spreading bilayer. Gradual decrease in the fluorescence intensity from the spreading edge to inside is due to the inhomogeneous lipid density distribution [2]. Although the bilayer spread also on NSL substrate, dark spots were observed at the position of the gold architectures. This phenomenon is reasonable because the surface of gold acts as a barrier for the lipid lateral diffusion [13, 14]. Strong physical interaction between the lipid molecules and gold surface prevent free diffusion of the lipid molecule on gold surface. Therefore, it is reasonable to assume that the lipid bilayer cannot spread on the metallic architectures in the present system. This result suggests that the lipid bilayer spreads only through the gaps between gold nano-architectures.

The difference in the spreading dynamics between on bare glass and on NSL substrates was shown in Figure 3. The result on surface-oxidized silicon substrate was also shown. Double-logarithmic plot of the spreading

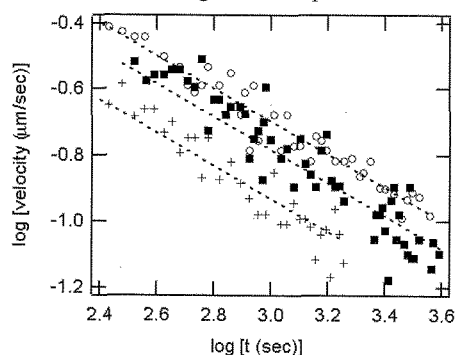


Fig. 3. Double-logarithmic plots between the spreading velocity and the time for (○) Si, (■) glass, and (+) NSL substrate. Dashed line in each plot indicates the least-square fitting of eq 1.

Table 1. Spreading coefficient  $\beta$  on each substrate.

substrate	spreading coefficient ( $\mu\text{m}^2/\text{s}$ )
bare Si	40.5
bare glass	27.3
NSL/glass	13.8

velocity ( $v$ ) and the time ( $t$ ) is known to be useful for the quantitative analysis of the spreading dynamics. [1, 2] Linear relationship between them has been expected by the following equation.

$$\log v = \frac{1}{2} \log \beta - \frac{1}{2} \log t \quad (1)$$

$$\beta = \frac{Wd_w}{2\eta_w} \quad (2)$$

Where,  $\beta$ ,  $W$ ,  $d_w$ , and  $\eta_w$  are the spreading coefficient, the spreading driving energy, the thickness of hydration layer between the spreading bilayer and the substrate ( $\sim 2$  nm), and the viscosity of water ( $\sim 10^{-3}$  Ns/m<sup>2</sup>), respectively.  $W$  is thought to be the gain in free energy by the formation of bilayer - substrate contact [2]. It was found that the linear-law in the double logarithmic plot was intact even on the NSL substrates. Fitting the data to eq 1 (dashed lines in Figure 3) yields  $\beta$  value for each system. The results were listed in Table 1. The difference in  $\beta$  between bare Si and bare glass substrates would be due to the difference in surface roughness or surface charge mainly determined by surface OH density. Further decrease in  $\beta$  at the spreading on NSL substrate suggest the presence of energetic cost for the bilayer when spreads through the metallic gate. The observed energy cost on NSL substrate is tentatively assumed to be due to the formation of local bilayer compressed state at the gate region. Quantitative analysis between the velocity lowering and the gap size has been described elsewhere [9].

When the NSL substrate was prepared via ultrasonication in ethanol, characteristic architectures other than gold were left on the substrates. As shown in Figure 4, donut-shaped architecture was found in each circular compartment surrounded by gold architectures. The line profile of this architecture revealed that the diameter and the height were ca. 300 nm and 4 nm, respectively (Fig. 4c). This architecture would be

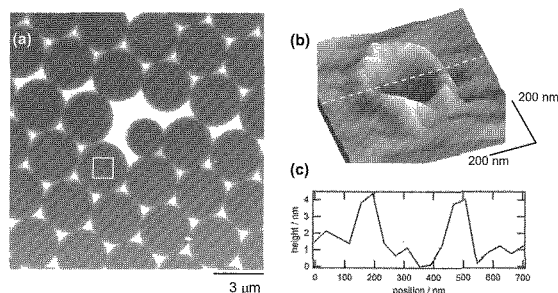


Fig. 4. (a) AFM image of periodic array of metallic and organic nano-architecture prepared via ultrasonication in ethanol. (b) Three dimensional AFM image of the donut-shaped structure indicated by white square in (a). (c) Line profile of the donut structure indicated by dashed line in (c).

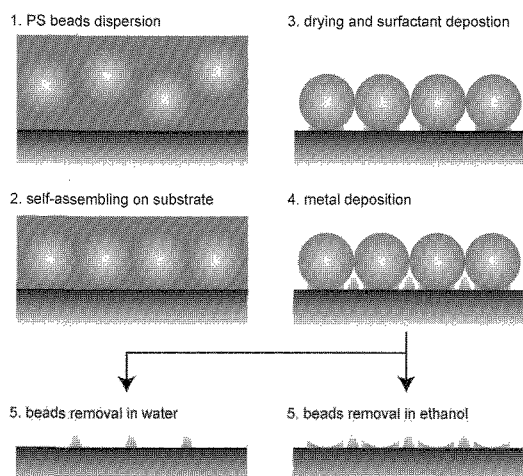


Fig. 5. Schematic diagram for the preparation of nano-architectures substrate. First, closed-packed monolayer of PS beads was prepared by immersing the substrate in PS beads dispersion solution (1 and 2). During drying process, meniscus is formed around the bottom of PS beads. Any component dissolved in water solution is concentrated at the meniscus (3). Metallic component is deposited on the substrate via vacuum evaporation (4). After the evaporation, PS beads were removed by ultrasonication in water or ethanol (5).

consisted of a surfactant that has been included as stabilizing agent in water dispersion of PS beads. The suggested formation process was illustrated in Fig. 5. During drying process of hexagonal array of PS beads, excess amount of surfactant dissolved in the bulk water is concentrated and deposited at the meniscus formed at the bottom of PS beads. This drying process can form donut-shaped architecture by the presence of PS beads as templates. Since the surfactant dissolved in the present system is highly soluble in water, additional ultrasonication in water easily removed these architectures. However, they were not removed by just immersing in water. This enables to carry out self-spreading experiment in water phase.

Fluorescence microscopic images of the self-spreading bilayer on glass and NSL substrate with donut-shaped architectures were shown in Fig. 6. For the preparation of glass substrate only with donut architectures, glass substrates covered with the hexagonal array of PS beads were dried under vacuum

( $\sim 10^{-3}$  Pa; same condition as the metal evaporation). After holding in vacuum for 2 h, the PS beads were removed in ethanol without metal evaporation. Although details in the self-spreading dynamics on these substrates have not been clarified at the present stage, the self-spreading nature was found to be intact. However, it was found that the donut-shaped architecture was not covered with the self-spreading lipid bilayer because dark spots were clearly observed in the spreading bilayer (Fig. 6a). The bilayer spreads only on hydrophilic surfaces and does not spread on hydrophobic surfaces. Our observation implies that the surface of the donut-shaped architecture is not fully hydrophilic. The hydrophobic alkyl chain of the surfactant seems to be partly exposed to the aqueous phase.

On NSL substrate, the dark spot was also observed at almost the center of each compartment (Fig. 6b, c). The dark spot does not disappear during the self-spreading for 1 h. This result indicates that the constructed donut-shaped architecture was very stable even under the condition that the lipid bilayer continuously spreads around them.

Several reports on the characteristic nature of the self-spreading lipid bilayer have been reported. Especially, the use as the medium for molecular transportation, mixing, and chemical reaction within the spreading bilayer are attracted much attention in order for constructing future micro-fluidic devices [5]. For this purpose, it is necessary to design the substrate surface to exhibit requisite chemical and physical properties. The present system has advantage for this purpose because it enables us to introduce both metallic and organic materials on the substrate. By changing the surfactant composition, it is also possible to introduce other kinds of functional materials. For example, binding a specific nucleic acid at the donut architectures will enable micro-fluidic DNA hybridization assay or DNA size separation within the self-spreading lipid bilayer. Although water soluble surfactant was deposited in the present system, appropriate design of microsphere / surfactant system will offer diverse possibility of constructing functional surfaces.

#### 4. CONCLUSION

In the present study, we have investigated the self-spreading behavior of lipid bilayer on the substrate with and without organic/metallic nano-architectures. In the presence of metallic architectures, the bilayer spreads with being compressed at the small gate between the metallic architectures. This brought energetic cost

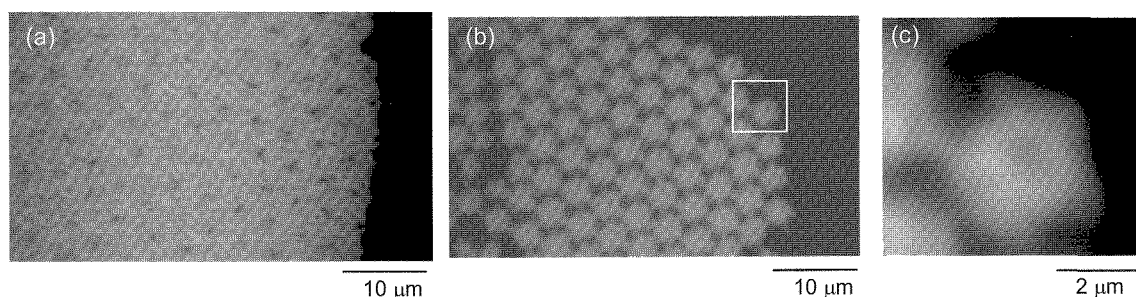


Fig. 6. Fluorescence microscopic images of the self-spreading lipid bilayer on (a) glass and (b) NSL substrate with periodic array of organic nano-architecture. (c) Enlarged image of a single compartment indicated by white square in (b).

for the self-spreading dynamics. Additionally, we presented simple way to construct donut-shaped organic nano-architectures on glass substrate with and without metallic architectures. The self-spreading nature of lipid bilayer was found to be held on these substrates. Also, the constructed organic architectures retained their initial structures during the spreading experiment. The present system offers functional substrate combining chemical and physical properties for the future micro-fluidic devices.

## 5. ACKNOWLEDGEMENT

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