# Control of Supported Lipid Bilayer Self-Spreading through Nanogap by Local Electric Field

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## 1. Introduction

A lipid bilayer supported on a solid substrate is regarded as a promising candidate for a model cell membrane [1]. The supported lipid bilayer is separated from the substrate by a thin lubricating water layer (10-20 Å) [2] and retains many of the properties of cell membranes such as lateral fluidity [1]. Although there has been a lot of research on supported lipid bilayers, the spatial and temporal control of the membrane itself and of the molecules in the membrane undoubtedly constitute the key technologies for a variety of future applications.

The application of an external electric field is a promising way to control the specific lipid molecules embedded in the membrane. If the external electric field (10-100 V/cm) is applied tangentially to the membrane, charged lipid molecules [3-5] and membrane associated proteins [5] are reorganized by electrophoretic and electroosmotic forces leading to steady-state concentration gradients. These concentration gradients result from the competition between the diffusive mixing and field-induced motion of the charged molecules. Although some interesting work, such as the Brownian ratchet [6] has been reported using this technique, the molecules or membranes to be controlled should have net charges.

We have demonstrated some interesting features of supported lipid bilayers that derive from the self-spreading nature of the lipid bilayer, including the observation of fluorescence resonance energy transfer [7] and the effect of a nanogap structure on the membrane [8,9]. In this study, we investigated the way that an electric field applied to a single nanogap affects the dynamics of lipid bilayers passing through the nanogap. For this purpose, we fabricated patterned surfaces with a nanogap structure in a microchannel. Using these devices, we observed the self-spreading behavior of a lipid bilayer passing through a nanogap while applying a direct current (DC) field.

# 2. Experimental

Figure 1(a) shows a schematic view of the device structure used in this study. A pair of electrodes with a separation of less than 100 nm was fabricated by electron beam lithography and the liftoff technique using Au/Ti (30 nm/1 nm) on a silicon wafer with a thermally oxidized SiO<sub>2</sub> layer. Microchannel that was a 10  $\mu$ m wide and that had wells at both ends was fabricated on this nanogap structure using an organic photoresist.

We prepared a 7:3 mixture of L- $\alpha$ -phosphatidylcholine and L- $\alpha$ -phosphatidylglycerol (both extracted from egg yolk) containing 1 mol% of headgroup-labeled Texas Red-DHPE. A small amount of the solid was attached to the well. The self-spreading of the lipid bilayer was initiated by immersing the device in a buffer solution [100 mM NaCl + 10 mM Tris-HCl (pH = 7.6)]. Fluorescence from the lipid bilayer was observed using an Olympus BX51-FV300 confocal laser scanning microscope. All the observations were performed in a buffer solution at room temperature.

# 3. Results and Discussion

Figure 2 shows the typical time evolution of a self-spreading lipid bilayer before and after passage through a nanogap with an applied voltage (V) = 0 V as a control, where  $t = t_0$  is the time at which the advancing lipid bilayer reaches the nanogap. A red fluorescent single lipid bilayer developed along a microchannel from the left side. We confirmed that the lipid bilayer did not develop on the photoresist or gold pattern but only on the hydrophilic SiO<sub>2</sub> surface [8]. In all the present experiments, the lipid bilayer passed through a nanogap with a semicircular shape without electric field applied on the nanogap.

Figure 3 shows the time evolution of a self-spreading lipid bilayer before and after passage through a nanogap as a result of the temporal switching of the applied voltage. Before the lipid bilayer passed through the nanogap, no voltage dependent change was observed [Figs. 3(a) and (b)]. However, when the lipid bilayer reached the nanogap, the self-spreading was stopped by the application of 50 mV DC [Fig. 3(c)]. This continued for about 300 s corresponding to the application of voltage [Fig. 3(d)]. Interestingly, the lipid bilayer started to develop again immediately after the applied voltage was returned to 0 V [Figs. 3(e)-(g)]. We confirmed that this phenomenon could be repeatedly observed depending on the temporal switching of the applied voltage [Figs. 3(h)-(l)]. It should be mentioned that this phenomenon was only observed for less than 10 nm nanogap that means the resolution limit of our scanning probe microscope (SEM). Although the origin of this phenomenon remains unclear, we assume that it is related to the electric double layer formed between narrow nanogaps. The width of the electric double layer, namely the Debye length, is roughly estimated by a few nanometers under our experimental condition. Therefore, the electric field can be effectively applied between nanogaps without being shielded by counter ions if we use a narrow nanogap with a separation of less than 10 nm. In such a situation, the electric field between nanogaps becomes large ( $\sim 10^5$  V/cm) when the applied voltage is 50 mV, which is at least three to four orders of magnitude higher than in previous reports [5]. In such a strong electric field, molecules should be trapped between the nanogaps because of the

intramolecular charge separation even if they have no net charge [10]. This strong trapping force overcomes the self-spreading and diffusion of lipid molecules. It should be mentioned that the temporal switching response was fairly fast. For example, Fig. 3(e) is a fluorescent image obtained just after the applied voltage was set at 0 V, in which a small percolation of the lipid bilayer can be observed. This is an evidence for the electric trapping of the lipid bilayer.

## 4. Conclusion

We investigated the effect of a DC electric field applied to a nanogap on a lipid bilayer passing through the nanogap structure. We demonstrated that the development of the self-spreading lipid bilayers could be controlled by the temporal switching of the applied electric field. This is because the electric field was effectively applied between nanogaps by the fact that the width of the nanogap is close to the that of the electric double layer.

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Fig. 1 (a) Schematic diagram of the device. A microchannel and wells are formed on a gold nanogap structure using a photoresist. At the beginning of the experiments, a lipid source is fixed inside the well. (b) Magnified view of the device around a nanogap. (c) SEM image of a 15 nm nanogap. (d) Chemical structure of Texas Red-DHPE.

Lipid bilayer t = t <sub>0</sub> 300 Microchannel = t <sub>0</sub> 300 t <sub>0</sub> :150 t <sub>0</sub> :50 t <sub>0</sub> +200 t <sub>0</sub> +400 t <sub>0</sub> +700	11di	подар
L t <sub>0</sub> -150 t <sub>0</sub> t <sub>0</sub> +50 t <sub>0</sub> +200 t <sub>0</sub> +400 t <sub>0</sub> +700	Lipid bilayer	t = t <sub>o</sub> -300 Microchannel
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t <sub>0</sub> +700		t <sub>o</sub> +400
	78928) SYABA	t <sub>0</sub> +700

Fig. 2 Typical time evolution of a self-spreading lipid bilayer before and after passing through a nanogap. The red areas are the fluorescence from Texas Red-DHPE. The lipid bilayer grows from left to right along the microchannel. The time at which the advancing lipid bilayer reaches the nanogap is set at  $t = t_0$ .

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Fig. 3 Time evolution of a self-spreading lipid bilayer before and after passing through a nanogap. The red areas are the fluorescence from Texas Red-DHPE. The lipid bilayer grows from left to right along the microchannel. The time at which the advancing lipid bilayer reaches the nanogap is set at  $t = t_0$ .