# Dielectrophoresis-based Liposome Delivery to a Planar Lipid Membrane for Efficient Membrane Protein Reconstitution

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## ABSTRACT

This work presents a strategy that realizes efficient incorporation of membrane proteins in liposomes into a planar bilayer lipid membrane by using dielectrophoretic (DEP) force. The device consists of a chamber and a channel separated by a μm-sized aperture that suspends a planar bilayer membrane. Applying an AC electric field across the membrane, the DEP force concentrates at the bilayer area therefore attracts the liposomes. We also observed fusion of liposomes autonomously occurs into the lipid membrane under the DEP effect. This DEP-based fusion method will be highly applicable to membrane protein study.

## INTRODUCTION

Recently several research groups have been developing the system using a planar bilayer membrane for membrane protein study, especially electrophysiological analyses on ionchannel proteins. This artificial bilayer-membrane platform aims at improvement of data-throughput and reproducibility as well as robotizing the conventional patch-clamp method. In the previous works, we succeeded in multiple ionchannel monitoring on microfluidic devices [1], yet faced difficulty on inefficient reconstitution of the proteins to the planar membranes. In this study we therefore focus on exploring a methodology to control the process of the reconstitution. Inspired by the electro cell-fusion with DEP [2] and the common reconstitution technique of proteoliposome fusion [3], we integrate the functionality of DEP at a planar membrane device (Figure 1). Our strategy of installing the DEP feature in the device is to enforce the proteoliposomes to migrate toward the planar membrane surface and to vigorously induce fusion of the liposome with the membrane.

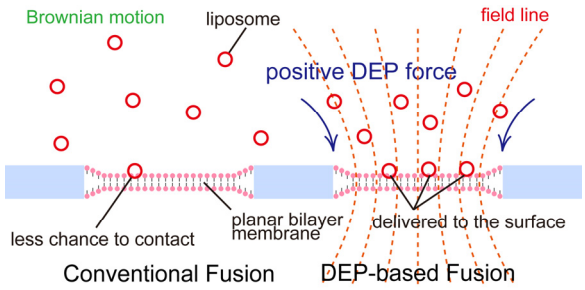


*Figure 1 Conceptual diagram of the fusion process of liposomes and a planar lipid bilayer membrane with a conventional method (left) and the DEP-based method (right). The applied DEP force attracts liposomes to the bilayer and enhances their fusion.*

## EXPERIMENTAL

Figure 2 represents the schematics of the device: an aperture (diameter of 10 – 70 μm) separates an upper chamber and a bottom channel. The PMMA main frame is micromachined with precision drills. An ITO-glass electrode is glued beneath the channel and a silver-wire electrode is placed above the aperture. A planar bilayer lipid membrane is obtained by filling the upper chamber with aqueous solution followed by a sequential infusion of DPhPC lipid-decane solution and an aqueous buffer at the channel [1]. In this paper we use liposome as a model of proteoliposome.

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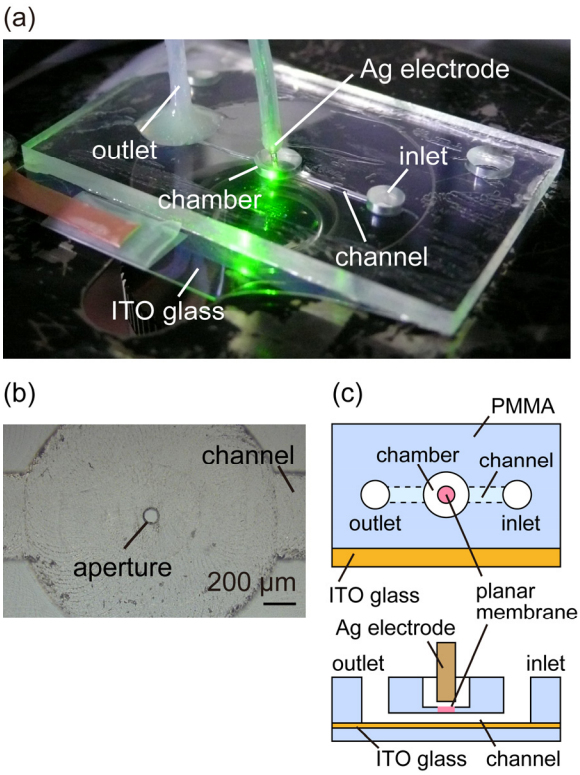


*Figure 2 DEP-based device. (a) Overview of the device. (b) Microscopic image of the aperture. (c) Top-view and Side-view of the device. The chamber volume is 20 µL. The channel width and height are 500 µm and 200 µm, respectively. The thickness of the aperture wall is ca. 100 µm.*

Giant liposomes (Egg PC with rhodamine-labeled PE) are formed by electroformation [4], and infused at the bottom channel after the formation of the planar membrane. Applied AC electric field between the electrodes generates DEP phenomena across the membrane formed at the aperture. We applied the field of 0.2 – 1.0 $kV_p$/cm and the frequency of 0.1 – 1.0 MHz. Images are taken through the ITO-glass by a confocal inverted microscopy.

## RESULTS & DISCUSSION

First we confirmed if DEP phenomena occur at the device. Figure 3 shows giant liposomes attracted to the narrow aperture by the DEP force, forming a pearl chain (Note that a bilayer membrane is not formed in this case). The pearl chain formation

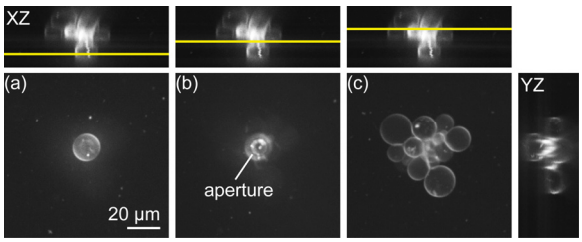


*Figure 3 Pearl chain formation with giant liposomes induced by DEP. The z-planes for the respective confocal xy-images are indicated with the yellow lines on the xz-plane images. (a) Image beneath the aperture, (b) on the aperture plane, and (c) above the aperture. The aperture diameter: 10 µm. Applied field: 0.5 $kV_p$/cm, frequency: 300 kHz.*

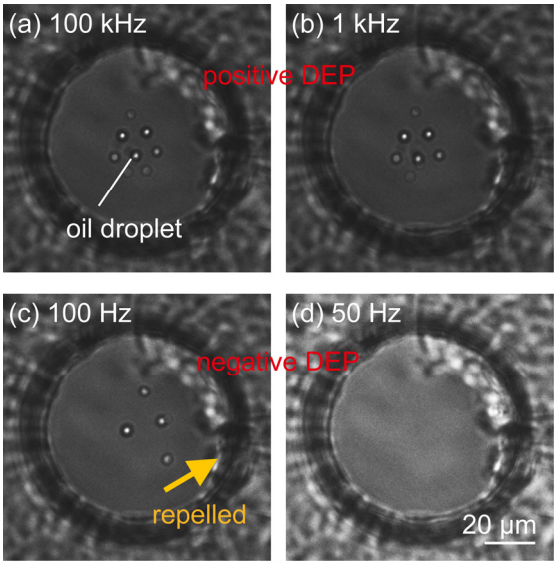


*Figure 4 DEP effect on oil droplets at a thick lipid-decane film, depending on applied frequency. At 100 Hz the direction of the DEP force changes from positive to negative, resulting in repelling the droplets outside the aperture. Applied field: 0.5 $kV_p$/cm, blight field images.*

occurs immediately by the application of the AC field. Unless otherwise applied the field, the liposomes merely pass through and never block the aperture. This result ensures that the layout of the electrodes and the aperture enhances the electric field at the aperture effectively.

On the other hand, if a thick lipid-decane layer exists at the aperture, no liposomes are influenced by the field but only oil droplets weakly respond (Figure 4).

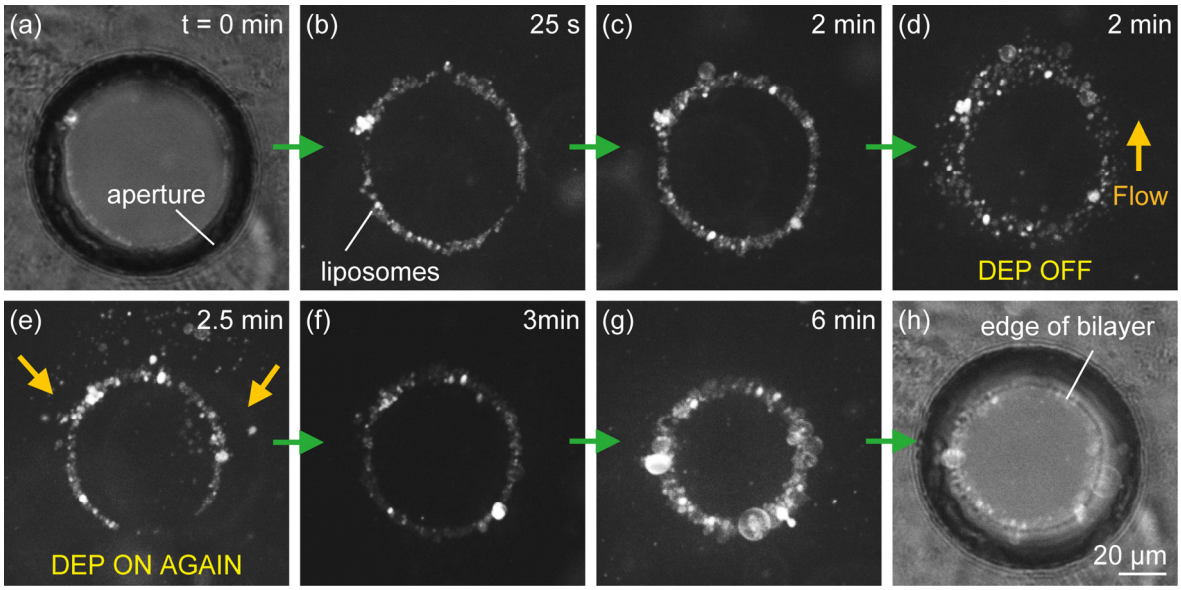


*Figure 5 DEP effect on liposomes at the surface of a planar bilayer lipid membrane. Liposomes gather at the edge of the bilayer membrane under the DEP force (at image d, liposomes are released by turning off the field). The number of liposomes increases over time (compare b and g). Applied field: 1 $kV_p$/cm, frequency: 100 kHz. Blight field images: a and h, red fluorescent images: b to g (by rhodamine-tagged lipid).*

This is because the lipid-decane layer hinders the electric field at the aperture and weakens the DEP effect. Besides, the electrical properties of liposomes are very close to aqueous solution; therefore the DEP force against the liposomes is weaker than the oil droplets.

However, the DEP force enhances together with thinning the membrane thickness, and once a planar bilayer formed, the force becomes strong enough to draw the liposomes to the bilayer surface. Figure 5 shows a series of images representing the attractive force of DEP on the liposomes. With applying the AC field, the liposomes start gathering at the edge of the bilayer and the number increases over the course of time. As clearly seen at the panels 5c to 5e, the liposomes do not autonomously migrate to the membrane surface but they are strongly captured by the DEP force. Note that it is not possible to realize such a high surface concentration of liposomes at a planar membrane without DEP effect. On rare occasions, even larger liposomes are trapped by the DEP force and delivered to the bilayer (data not shown).

In order to realize the reconstitution of membrane proteins to the bilayer, it is necessary to induce fusion of liposomes with a planar membrane. With our system we found that fusion of liposomes voluntary occurs at the membrane when contacting each other. Figure 6 represents an example of the liposome fusion on a planar membrane; the liposome with fluorescent-labeled lipid molecules that fused with the membrane is indicated by the white arrow at the panel 6c (seen as a blight spot). When the fusion occurred, the blight spot burst and the diffusion of the labeled molecules are observed to the right hand side (see the images 6d – 6g and the line profile of the fluorescence intensity 6b).

## CONCLUSIONS

In this paper we aimed at providing a system that enables to control the reconstitution of membrane proteins to a planar membrane. Focused on the DEP

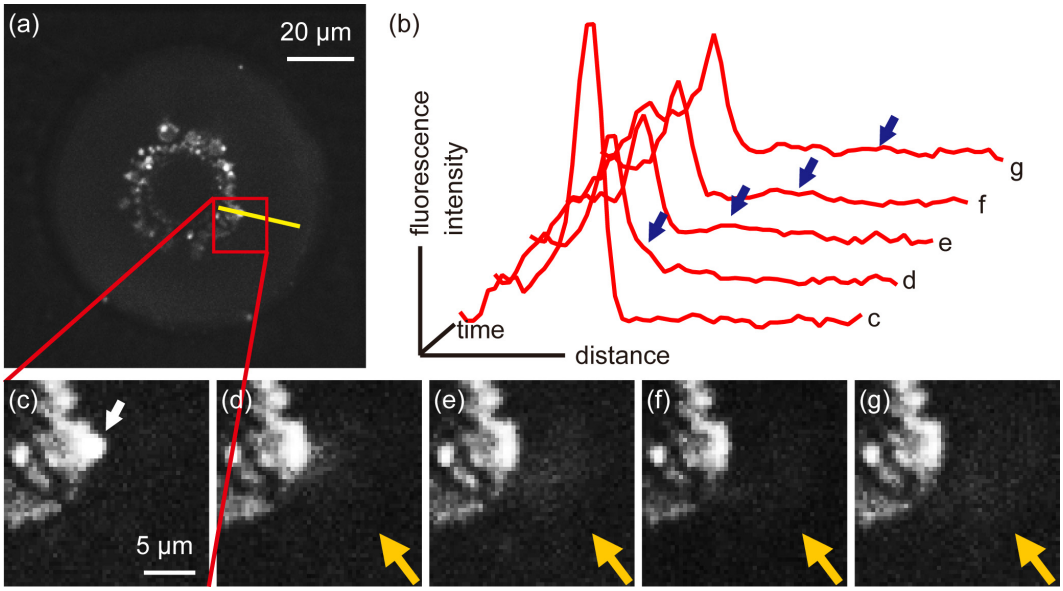


*Figure 6 Liposome fusion on a planar lipid membrane. The liposome indicated by the white arrow on image c fused on the membrane. The diffusion of fluorescent-labeled lipid molecules was shown on a series of images (d to g). The fluorescent intensity profiles along the yellow line (on image a) over the time of the images c to g are shown in the panel b. The diffusion of the labeled lipids is clearly detected (see blue arrows). The time interval between each image is 100 ms. Applied field: 0.4 $kV_p$/cm, frequency: 100 kHz.*

phenomena we succeeded in delivering liposomes to a planar membrane by integrating the DEP function within a common planar membrane device. Since this DEP device and the previously-developed devices share the design of their main frames, for instance, the pair of electrodes and the device geometry, we believe that our DEP-based methodology for the protein reconstitution will be easily integrated to the planar-membrane platform for ionchannel analyses.

## ACKNOWLEDGEMENT

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