Supplementary Information

Droplet based lipid bilayer system integrated with microfluidic channels for solution exchange

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Supplementary Figures



Figure S1 Design of solution exchange device.

a) b) Top and side view of a double-well chip. These two circular wells were 4 mm in diameter. A bottom of this well had two microfluidic channels that had square cross-section lengths of 500 μ m. The channels were positioned as shown in this figure. c) These channels were prepared by thermocompression bonding of a flat base and a milling base.



Figure S2 Overall view of an experimental system.

Solution transport was conducted by micro syringe pumps. In our experiments, we used just two pumps for the solution exchange. Furthermore, if three-way stop-cocks are used, we can inject various kinds of solution in a droplet as shown in this photograph. The device was covered with a faraday gauge, which we could conduct the electrical measurements during the solution exchanging.

single well



Figure S3 Photograph of a single well chip used in a fluorescent experiment.

This single-well chip was used in order to obtain Figure 3c of main text. The diameter and depth of the circular well was 4.0 mm and 3.0 mm, respectively. Two microfluidic channels were same shape and position as those of the double-well chip.



Figure S4 Probability of bilayer rupture.

We experimentally determined the probabilities of bilayer rupture as 43 % at 20 μ l/s (n=7), 33 % at 30 μ l/s (n=3) and 50 % at 40 μ l/s (n=6). According to this experiment, the lipid membrane formed this system became unstable over the flow rate of 20 μ L/s.



Figure S5 Solution exchange experiment: BLMs stability during the solution exchange. We investigated that whether the formed a BLM can be maintained during solution exchange using alamethicine. In this experiment, we could successfully observe stable conductance between the two droplets via nanopores formed by alamethicine while buffer solution was perfused. Alamethicin is polypeptide of 20 amino acid. When the monomers are incorporated in the BLM, it forms voltage-gated channels and repeats channel open-close activity.^{1,2} First, BLM was formed with alamethicin solution (10 nmol protein/L in 1.0 M KCl buffer solution) with an applied voltage of 50 mV. A few minutes later, BLMs containing alamethicin were formed at the interface of two droplets inside the parylene micro pores. Then, one of the two droplets was perfused with buffer solution while monitoring the current that flew through the proteins. The multiple oligomeric pores of up to three conductance states were clearly observed during perfusing the droplet (enlarged view of Figure S3). These results verified that the solution exchange process did not harm a BLM.

1 J. E. Hall and I.Vodyanoy, T. M. Balasubramanian, G. R. Marshall, ALAMETHICIN: A Rich Model for Channel Behavior, *Biophys. J.*, 1984, **45**, 233-247.

2 D. S. Cafiso, ALAMETHICIN: A Peptide Model for Voltage Gating and Protein-Membrane Interactions, *Annu. Rev. Biophys. Biomol. Struct.*, 1994, **23**, 141-156.