Supporting Information

Repetitive formation of optically-observable planar lipid bilayers by rotating chambers on a microaperture

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Section S1. Chemicals

A synthetic lipid, diphytanoyl phosphatidylcholine (DPhPC), was purchased from Avanti Polar Lipids (USA). Oil solvent, n-decane, and α-hemolysin were purchased from Sigma-Aldrich (USA). All aqueous solutions were prepared with ultrapure water from a Milli-Q system (Merck, Germany). Potassium chloride, potassium phosphate, and 1-hexanol were purchased from Wako Pure Chemical Industries (Japan). Poly(methyl methacrylate) (PMMA) plates were purchased from Nitto Jushi Kogyo (Japan). Parylene C (poly(chloro-p-xylylene)) was purchased from Specialty Coating Systems (USA). NMD3 was purchased from Tokyo Ohka Kogyo (Japan). Aluminum wire was purchased from Nilaco (Japan). Aluminum etchant was purchased from Wako Pure Chemical Industries (Japan).

Section S2. Device fabrication

The designed device for the repetitive formation of an optically-observable bilayer is shown in Figure 2. The fluidic channel in the basal part and the chambers of the rotating part were manufactured on a PMMA plate via micro-machining (MM-100, Modia systems, Japan). The basal part was assembled using a channel part, a thin film with 100-μm aperture, and a PMMA frame, using acrysunday solvent adhesive (Acrysunday, Japan). The thin film was composed of a 3-μm-thick parylene sheet. The micro-apertures (diameter, 100 μm) in the parylene sheet were fabricated using standard photolithographic techniques. In detail, parylene C was deposited on a No. 1 glass substrate (Matsunami, Japan) via chemical vapor deposition (CVD) using a parylene deposition machine (LABCOTER PDS2010, Specialty Coating Systems, USA). Positive photoresist, S1818, was coated on an aluminum layer on the parylene substrate, by using spin-coating. The positive photoresist layer was patterned by exposure to ultra-violet light through a glass mask and developed by the NMD3 developer. Aluminum layer was patterned by Al-etchant. The parylene sheet was then etched with O2 plasma (20 mL/min of oxygen flow rate, 50W; RIE-10NR, SAMCO, Japan) in the defined region, using the aluminum pattern. After etching of the parylene, the residual S1818 and aluminum were removed by acetone and Al-etchant respectively. The basal part containing the parylene sheet was enveloped within 2-μm-parylene C by using CVD to seal and avoid leakage of liquids.
**Section S3. Optical and electrical characteristic measurements**

The lipid-bilayer formation was observed using IX71 inverted microscope (Olympus, Japan) with a ×20 objective lens and a digital CCD camera (Orca R2, Hamamatsu, Japan) via transmitted light. The stage of the microscope was covered with a Faraday cage to reduce electrical noise. The area of formed bilayer was determined by imaging analysis using the ImageJ 1.48 (NIH, USA). Ag/AgCl electrodes attached to a patch clamp amplifier (CEZ2400, Nihon Koden, Japan) and held using a manipulator were used to record the electrical characteristics. The current across the planar lipid bilayer was digitized by a Digidata 1440A digitizer (Molecular Devices, USA). The current was analyzed using Clampfit Ver. 10.3.2 (Molecular Devices, USA). When the chamber was rotated, the single-electrode at the chamber was moved to the next chamber by a manipulator to follow the electrical signal at the particular bilayer.

**Section S4. Determination of specific capacitance**

The initial capacitance value \(C_0\) is defined as the capacitance value before lipid bilayer formation. The area of the lipid bilayer \(A_{\text{bilayer}}\) and the area of the annulus surrounding the bilayer \(A_{\text{annulus}}\) were measured from the optical image. When the thickness of the annulus is constant, the capacitance value of lipid bilayer region \(C_{\text{bilayer}}\) was calculated by a following equation.

\[
C_{\text{bilayer}} = C_{\text{measured}} - \frac{A_{\text{annulus}}}{A_{\text{bilayer}} + A_{\text{annulus}}} C_0
\]  

(eq. 1)

\(C_{\text{measured}}\) is the measured capacitance value. The capacitance value of lipid bilayer region was obtained by subtracting the capacitance of annulus from the measured capacitance value; the second term of eq. 1 is the estimated capacitance of annulus by using \(C_0\). The specific capacitance was obtained by dividing \(C_{\text{bilayer}}\) by \(A_{\text{bilayer}}\).

The thickness of lipid bilayer was estimated by following equation.

\[
d_{\text{bilayer}} = \frac{A_{\text{bilayer}}}{\varepsilon_0 \varepsilon C_{\text{bilayer}}}
\]  

(eq. 2)

The values, \(\varepsilon_0\) and \(\varepsilon\) indicate the dielectric constant of free space and lipid bilayer, respectively. When \(\varepsilon\) is 2, which is the normally taken as the value of the dielectric constant of lipid bilayer, the thickness of formed lipid bilayer was calculated to be 2.41 ± 0.02 nm.
Figure S1. Emulsions in a microaperture

Fig. S1 Emulsions trapped in microaperture.

Note for Movie S1 Reformation of a planar lipid bilayer

Reformation of a planar lipid bilayer by the developed device. The movie was reconstructed from the sequential images taken by a microscope.