Supporting Information

Morphology and curvature controls of hybrid liposomes using metal complex lipids and viscosities for photo-chemical reaction in hydrophobic fields

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Experimental sections

Materials

Chemicals were purchased from commercial sources and used without further purification.

Synthesis

Hybrid liposomes DMPC/1(*x*) and DPPC/2(*x*) (*x* = 0.125, 0.25, 0.5)

1 and DMPC were mixed from $CHCl_3$ stock solutions at a known but arbitrary molar ratio and dried under a flow of nitrogen. They were placed under vacuum for 12 h to remove traces of $CHCl_3$. The mixtures were hydrated in ultrapure water and allowed to stand at over the phase transition temperature for 3 h. The suspensions were freeze-thawed three times (liquid N_2 and 55 °C water) and then extruded 11 times at 55 °C through 1 μm polycarbonate filters. The samples were conserved in the dark at 7 °C and used within 5 days. **DPPC/2(*x*)** was prepared by the same procedure using **2** and DPPC instead of **1** and DMPC.

Physical measurements

Cryogenic transmission electron microscopy (Cryo-TEM) images were collected by means of a JEOL/JEM-2100F(G5) instrument. After a small amount (3–5 μL) of the sample solution was placed on a TEM copper grid covered by a porous carbon film, the excess solution on the grid was immediately plunged into liquid propane (–180°C) and cooled by liquid nitrogen in a cryofixation apparatus (Reichert KF-80; Leica) to fix liquid water as vitreous ice. Then, the ice was transferred into the specimen stage of a Cryo-TEM operated at an acceleration voltage of 200 kV using its cryotransfer apparatus cooled by liquid nitrogen. The specimen stage was cooled by liquid helium, and the specimen was kept at –269°C during observation. Dynamic light scattering (DLS) and Zeta potential values were recorded on a Malvern Instruments/Zetasizer Nano ZS. Differential scanning calorimeteric (DSC) analyses were carried out with a Seiko Instruments/DSC 6220 under a N_2 atmosphere using a sealed pan. The concentration of the sample in water was 7.5 mM in water.

Variable temperature wide angle X-ray diffraction (VT-WAXD)

Samples were prepared by a conventional method as follows. DMPC and DMPC/**1(0.18)** were dissolved in chloroform/methanol (4:1 v/v) and dried under a N_2 flow. Then, the lipid film was stored in vacuo for 12 hours to remove organic solvent completely. The dry lipid films were dispersed in distilled (Wako Pure Chemicals Industries, Ltd. Osaka, Japan) and deionized water (Simplicity UV, Merck Millipore, Billerica, MA) with intermittent voltexing at 50ºC (Sahara 310, Rocker Scientific Co., Ltd., Taipei, Taiwan). The final concentration of DMPC was 25 wt%. The lipid dispersions were then enclosed in a glass capillary (Hilgenberg GmbH, Malsfeld, Germany) for subsequent WAXD measurements. VT-WAXD was carried out at the beam line BL-07 in the Kyushu synchrotron

radiation source (SAGA-LS, Tosu city, Saga, Japan) with a monochromatized beam of wavelength λ of 0.1 nm. The sample-to-detector distance (275 mm) was calibrated with Silicon. The samples were heated from 291 K to 308 K at the scanning rate of 1 K/min using temperature controlled N_2 flow. A 2-dimensional CCD camera (Saturn A200; Rigaku Co., Ltd, Matsumoto, Japan) was used for acquisition of the WAXD data and the exposure time was 29s. The Debye-Scherrer ring obtained was linearized using the 1- and 2- dimensional analysis program Fit2D. The peak position and halfwidth was estimated by fitted the WAXD profile to a single Lorentz function (OriginPro 2015J, Light Stone, Tokyo, Japan). The modulus of the scattering vector is defined as $S = 2\sin\theta/\lambda$, where 2θ is the scattering angle.

Viscosity evaluation using the photo-isomerization of trans-stilbene in liposomes

Trans-stilbene was solubilized as a viscosity probe in the non-polar portion of the liposomes. For preparing pristine liposomes formed by DMPC or DPPC, trans-stilbene and the phospholipid were dissolved together in a mixed solvent of methanol and chloroform. The molar ratio of trans-stilbene to DMPC or DPPC was 1 to 500. A thin film of the mixture of trans-stilbene and the phospholipid, formed after the evaporation of the mixed solvent, was dispersed in an aqueous solution and was filtered with Extruder(R). The diameter of the liposomes had been adjusted to 1 μm by the filtering. Fluorescence decay curves of trans-stilbene solubilized in the liposomes were recorded with a picosecond time-resolved fluorescence spectrometer formed by an excitation light source, a spectrograph, and a streak camera. The sample for the time-resolved fluorescence measurement was irradiated with the forth harmonic (305 nm, 1 kHz) of the optical parametric amplifier (OPerA Solo, Coherent, Santa Clara, CA, USA) output. The optical parametric amplifier was pumped by the output from a Ti:sapphire regenerative amplifier (35 fs, Legend Elite USP, 800 nm, 1 kHz, Coherent, Santa Clara, CA, USA) seeded by a cw mode-locked Ti:sapphire oscillator (Micra, Coherent, Santa Clara, CA, USA). The fluorescence light emitted from the sample on 300 nm excitation was dispersed by an imaging spectrograph (Acton SpectraPro 2300i (SP-2358), Princeton Instruments, Trenton, NJ, USA) and was detected by a streak camera (Hamamatsu C10627, Hamamatsu Photonics K. K., Hamamatsu, Japan). The fluorescence polarization at the magic angle (54.7 degrees) from the pump polarization was selected by a polarizer. The streak camera was pre-triggered by the output from the Ti:sapphire oscillator. A typical instrumental response time of the time-resolved fluorescence measurement for this study was 20 ps. Observed 2D images of time- and wavelength-resolved fluorescence intensity were analyzed by commercial software (Igor Pro 6.3, Wavemetric, Inc., Lake Oswego, OR, USA). The rate constant of the photoisomerization of trans-stilbene was calculated from the observed fluorescence lifetime and the radiative rate constant [1]. The viscosity of the liposome was then estimated from the photoisomerization rate constant, by using the relation $k_{iso} = -1.6 + 12.2\eta^{-0.244}$ [2] derived from the observed dependence of the rate constant on viscosity in alkane solvents [3].

[1] Sumitani M.; Nakashima, N.; Yoshihara K.; Nagakura S., Tempreature Dependence of Fluorescence Lifetimes of trans-Stilbene. *Chem. Phys. Lett.* **1977**, *51*, 183-185.

[2] Nojima, Y.; Iwata, K., Viscosity Heterogeneity Inside Lipid Bilayers of Single-Component Phosphatidylcholine Liposomes Observed with Picosecond Time-Resolved Fluorescence Spectroscopy. *J. Phys. Chem. B* **2014**, *118*, 8631-8641.

[3] Courtney, S. H.; Kim, S. K.; Canonica, S.; Fleming, G. R., Rotational Diffusion of Stilbene in Alkane and Alcohol-Solutions. *J. Chem. Soc. Faraday Trans. 2* **1986**, *82*, 2065-2072.

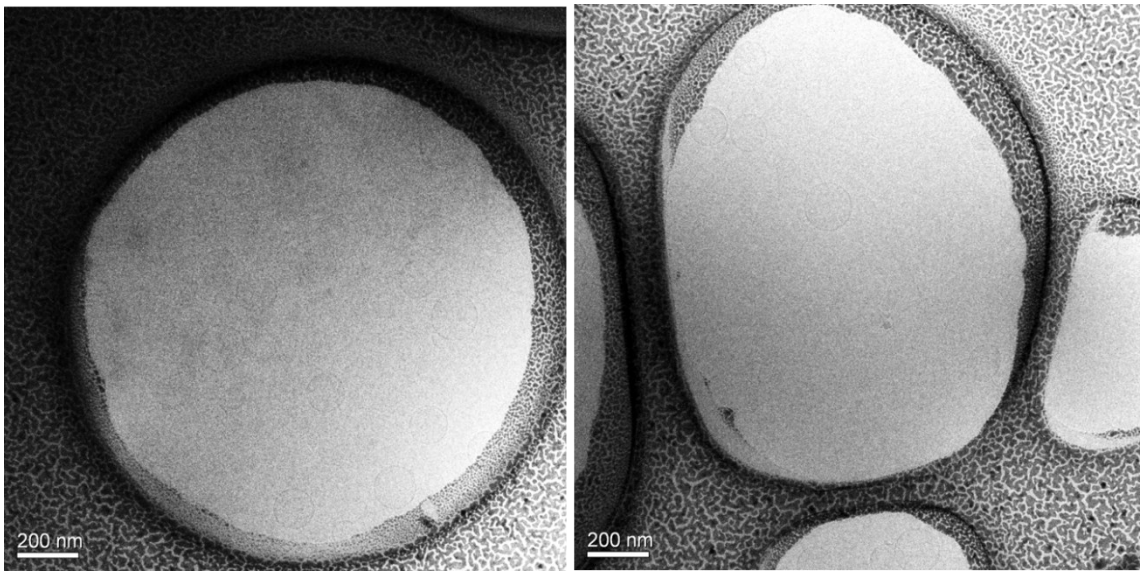

Figure S1. Cryo-TEM images of **DMPC/1(0.25)**.

Figure S2. Cryo-TEM images of **DMPC/1(0.5)**.

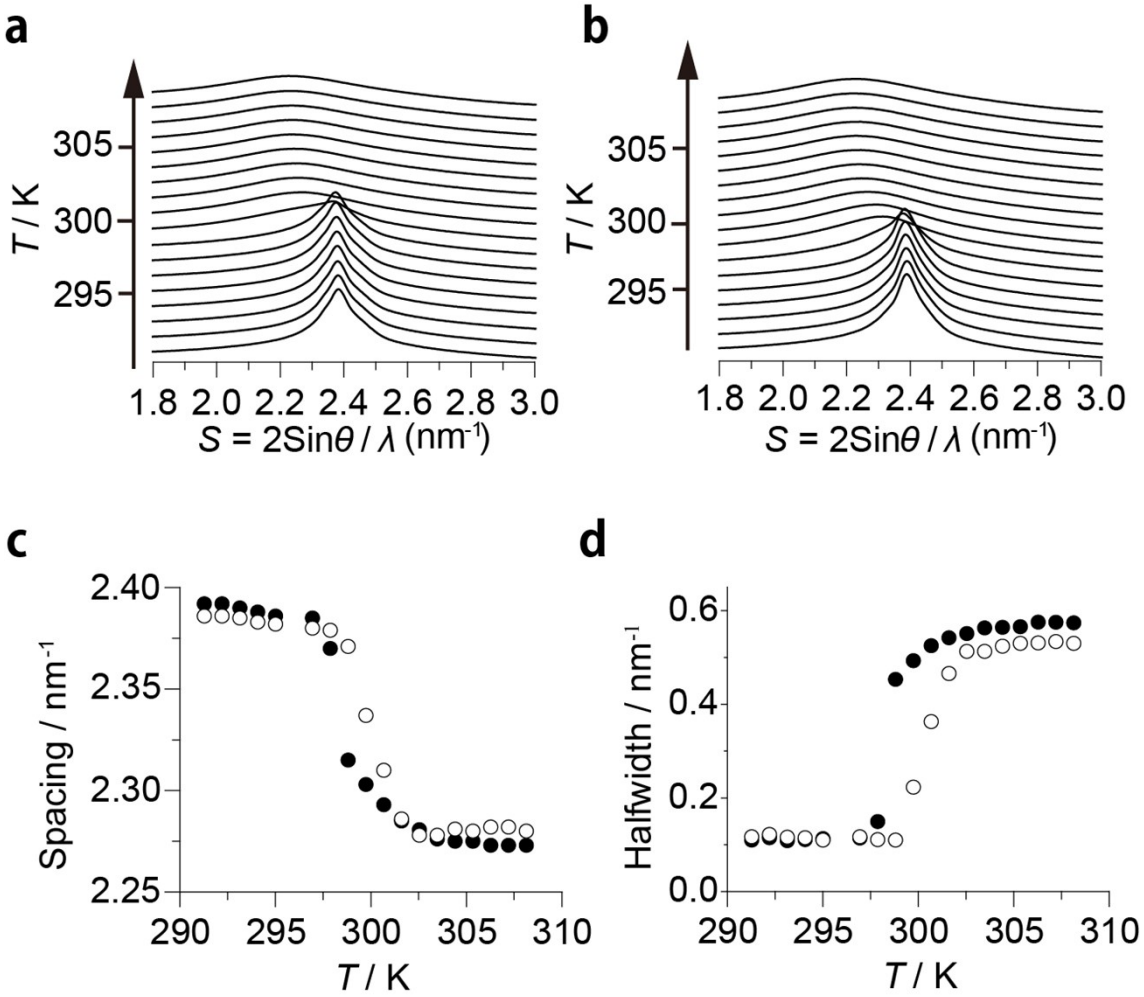

Figure S3. VT-WAXD profiles of (a) **DMPC/1(0.18)** and (b) DMPC bilayers. The samples were heated from 291 K to 308 K at the rate of 1 K/min. (c) Peak positions and (d) halfwidths of the WAXD peak were plotted as a function of temperature. The open and closed circles correspond to **DMPC/1(0.18)** and DMPC bilayers, respectively.

Table S1. Decay times of the photo-isomerization of trans-stilbene in DMPC/1(x)

	288 K			313 K		
DMPC:1(16)	τ_1/ps	τ_2/ps	$I_2/(I_1+I_2)$	τ_1/ps	τ_2/ps	$I_2/(I_1+I_2)$
1:0	310±30	1100±30	0.47±0.03	160±9	1300±200	0.05±0.01
1:0.25	130±15	630±25	0.37±0.01	90±8	530±20	0.17±0.06
1:0.5	90±10	270±50	0.30±0.01	60±6	200±13	0.23±0.03

Table S2. Decay times of the photo-isomerization of trans-stilbene in DPPC/2(x)

	300 K			330 K		
DPPC:1(18)	τ_1/ps	τ_2/ps	$I_2/(I_1+I_2)$	τ_1/ps	τ_2/ps	$I_2/(I_1+I_2)$
1:0	300±20	960±30	0.48±0.03	90±2	1600±100	0.040±0.005
1:0.25	130±20	710±30	0.39±0.01	70±2	380±60	0.056±0.007
1:0.5	60±4	460±20	0.188±0.007	40±9	160±30	0.09±0.01

Table S3. Decay times and viscosity parameters for DPPC at 288 K

	288 K				
DPPC	τ_1/ps	τ_2/ps	η_1/mPa·s	η_2/mPa·s	$I_2/(I_1+I_2)$
	270±40	1300±40	27±9	241±5	0.47±0.02