Supplementary Material (ESI) for Chemical Communications

Synthesis of Organic-Inorganic Hybrid Bicelles - Lipid Bilayer Nanodiscs Encompassed by Siloxane Surfaces

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Electronic Supporting Information

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Experimental

Materials

1,2-dipalmitoyl-*sn*-glycero-3-phosphochonine (DPPC), 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (DPPG) and 1,2-dihexanoyl-*sn*-glycero-3-phosphochonine (**2**) were purchased from Avanti Polar Lipids, Inc. (AL, USA). All other chemicals were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and were used without further purification. Organoalkoxysilane lipid **1** was synthesized in five steps according to previously reported route (details in reference 9d on main text).

Characterization of *N*,*N*-dihexadecyl-N^{α}-6-[(3-triethoxysilylpropyl)dimethylammonio]hexanoyl] alaninamide bromide (**1**).

^{1}H NMR (600 MHz, $CDCl_3$): δ 0.67 (2H, t, *J* = 7.7 Hz, $SiCH_2$), 0.88 (6H, t, *J* = 6.9 Hz, $N((CH_2)_{15}CH_3)_2$), 1.23 (9H, t, *J* = 7.1 Hz, $(CH_3CH_2O)_3Si$), 1.21-1.33 (54H, m, $N(CH_2CH_2(CH_2)_{13}CH_3)_2$, $N^+CH_2CH_2CH_2CH_2$), 1.33 (3H, d, *J* = 6.9 Hz, $CONHCH(CH_3)CO$), 1.40-1.53 (4H, m, $N(CH_2CH_2(CH_2)_{13}CH_3)_2$), 1.67-1.88 (6H, m, $CH_2CH_2CH_2N^+CH_2CH_2CH_2CH_2CO$), 2.22 (1H, dt, *J* = 7.1 and 14.8 Hz, $N^+CH_2CH_2CH_2CH_2CHHCO$), 2.35 (1H, dt, *J* = 7.1 and 14.8 Hz, $N^+CH_2CH_2CH_2CH_2CHHCO$), 3.09 (1H, m, $NCHH(CH_2)_{14}CH_3$), 3.25 (2H, m, $NCH_2(CH_2)14CH_3$), 3.33 (3H, s, CH_2N^+ $(CH_3)(CH_3)CH_2$), 3.34 (3H, s, $CH_2N^+(CH_3)(CH_3)CH_2$), 3.45-3.57 (5H, m, $NCHH(CH_2)_{14}CH_3$, $CH_2CH_2N^+CH_2CH_2$), 3.83 (6H, q, *J* = 7.1 Hz, $(CH_3CH_2)_3OSi$), 4.76 (1H, quin, *J* = 6.9 Hz, $CONHCH(CH_3)CO$), 6.84 (1H, d, *J* = 6.9 Hz, $CONHCH(CH_3)CO$). Anal. Calcd for $C_{52}H_{108}BrN_3O_5Si{\cdot}0.5H_2O$: C, 64.23; H, 11.30; N, 4.32. Found: C, 64.36; H, 11.39; N, 4.39. HRMS (FAB^+, *m*/*z*) calcd for $C_{52}H_{108}N_3O_5Si$ ($[M-Br]^+$), 882.8058; found, 882.8052.

FT-IR measurement

FT-IR spectra of hybrid bicelles were recorded using a Fourier transform infrared spectrometer (JASCO FTIR-420, Japan). Lyophilized sample of hybrid bicelles was subjected to prepare the potassium bromide (KBr) disk as a specimen.

DLS measurement

Hydrodynamic diameter of bicelles was measured by a dynamic light scattering spectrometer equipped with a He-Ne laser at 633 nm (Zetasizer Nano-ZS, Malvern). Number distribution of bicelles in dispersion was obtained by analyzing a time course of scattering light intensity at an angle of 173° from the incident light with the Cumulant method. The sample temperature was maintained at 25 ºC by the thermostat temperature controller installed in the equipment. The sample dispersion was filtrated using

commercially available hydrophilic syringe filter with 450 nm pores prior to the observation.

SAXS measurement

SAXS measurements were carried out using a Rigaku NANOViewer SAXS system equipped with a X-ray generator (Cu Kα radiation, λ = 1.5418 Å) operated at 30 kV and 40 mA. Camera length was set to 700 mm. The scattered pattern was acquired on a blue imaging plate for 1 hour. The obtained pattern was analyzed with a Rigaku NANO-Solver program. Prior to SAXS measurement, the bicellar suspension was centrifuged at 7,000 rpm for 30 minutes to obtain a precipitate in a capillary tube.

Cryo-TEM observeation

The specimen for Cryo-TEM was prepared by rapid freezing of vesicular dispersion. A copper microgrid with 200 mesh was used and was pre-treated with glow-discharger (HDT-400, JEOL) to obtain hydrophilic surface. An aliquot (3 μL) of bicelle dispersion was placed on the mesh and immediately plunged into liquid propane using a specimen preparation machine (EM CPC, Leica). The temperature of specimen was maintained by cryotransfer holder (Model 626.DH, Gatan) at lower than -140 ^{o}C during the observation. Microscopic observation was carried out using a transmission electron microscope (JEM-3100FEF, JEOL) at an acceleration voltage of 300 kV with zero-loss imaging mode. The concentration of lipid was set to 500 μM for all of the Cryo-TEM observation.

AFM observation

AFM specimen was prepared by casting an aliquot (1.5 μL) of the dispersion of bicelles to the freshly-cleaved mica surface. The sample was dried for 3 hours in a silica gel containing dessicator at room temperature. The microscopic image of dried bicelles in air was acquired by tapping mode AFM using scanning probe microscope (SPI3800N, Seiko Instruments Inc.) with a cantilever (SI-DF20) at room temperature (25 ^{o}C).

Surfactant solubilization assay

An aqueous suspension of phospholipid or hybrid bicelles was prepared as described in main text. To the 0.9 ml of bicelle dispersion, 0.1 ml of the stock solution with various concenration of Triton X-100 (TX-100) was added and well mixed. The final concentrations of TX-100 were set to 0, 0.2, 0.5, 1, 2 and 3 equivalents to the total concentration of lipids (38 mM). The sample cuvettes were incubated at 25 ^{o}C prior to the measurement. A series of the size distribution of the bicellar mixture were measured using DLS to evaluate the effect of the TX-100 on the bicellar structure

Additional Figures

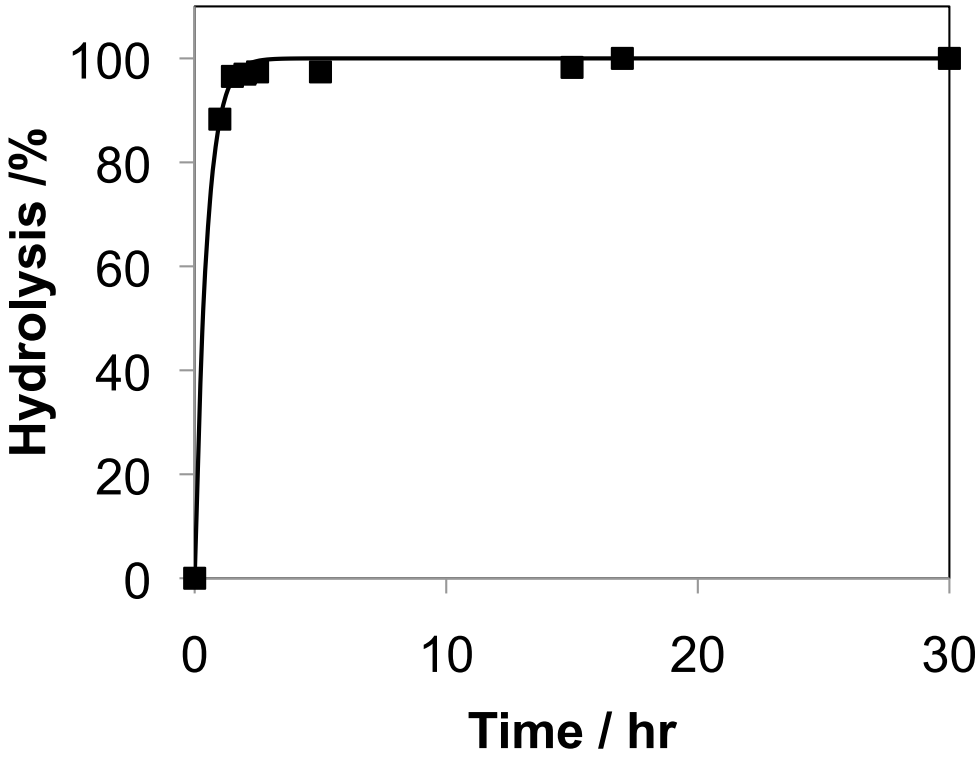

Figure S1. Time-course of the hydrolysis of lipid **1** in D_2O.

q = [**1**] / [**2**] = 3.5, at 25 ^{o}C.

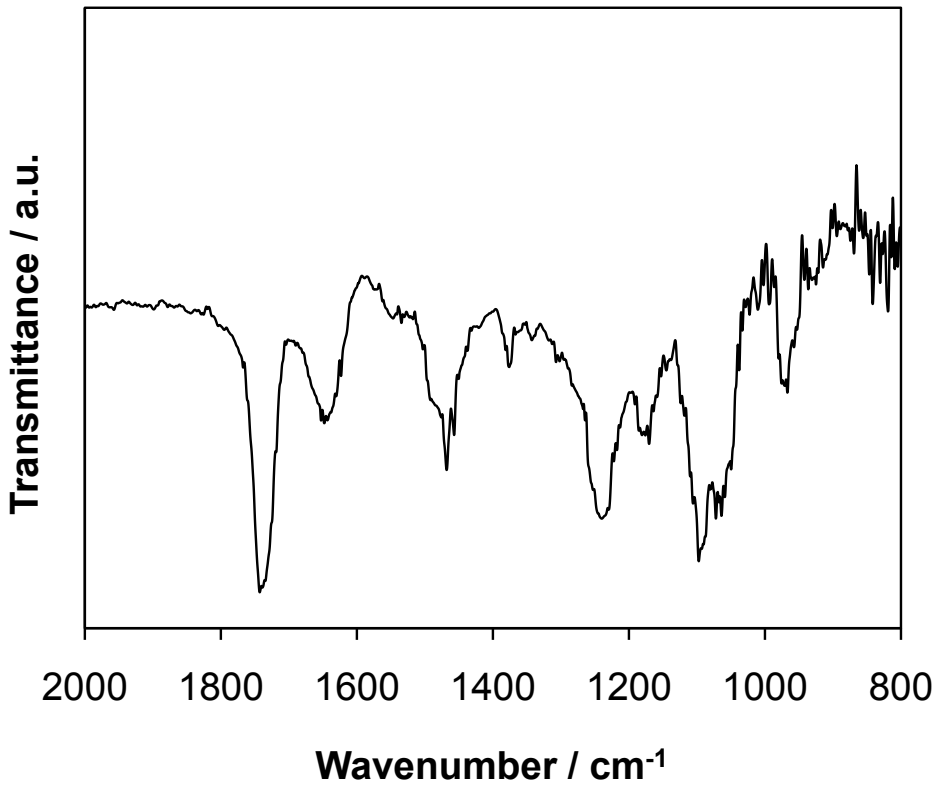

Figure S2. FT-IR spectrum of hybrid bicelles.

q = [**1**] / [**2**] = 3.5 at 25 ^{o}C.

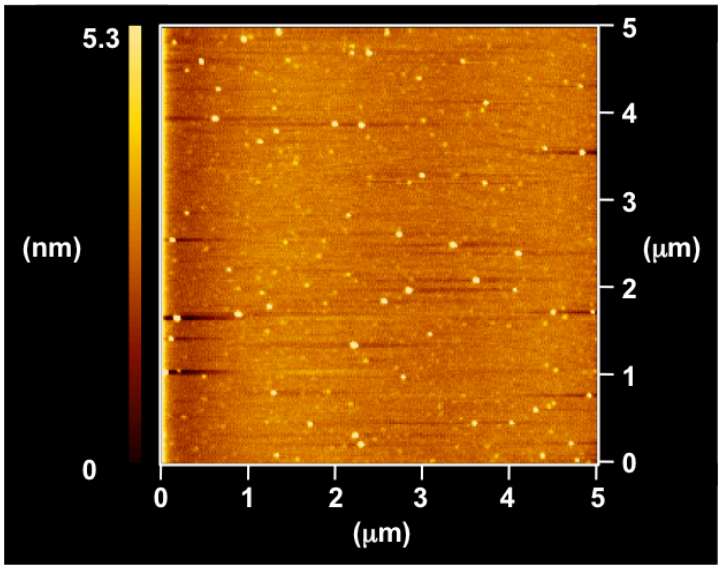

Figure S3. Wide angle AFM image of hybrid bicelles on mica substrate observed in an air. q = [**1**] / [**2**] = 3.5, at 25 ^{o}C.

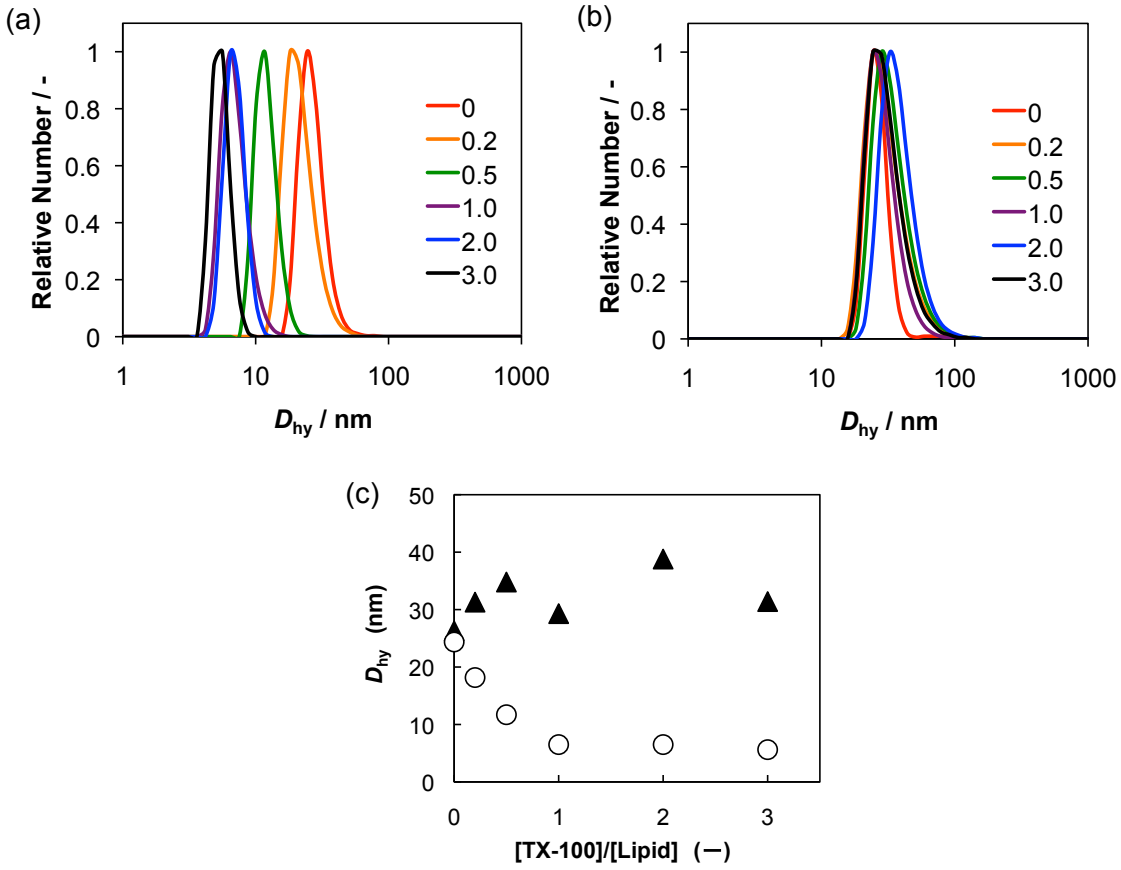

Figure S4. Effect of the TX-100 concentration on the size distribution of hybrid bicelles (a) and phospholipid bicelles (b). The peak hydrodynamic diameters were plotted against TX-100 concentration in (c). Closed triangle: hybrid bicelle, Opened circle: phospholipid bicelle.
[Lipid] = 3.8 mM, q = [**1** or DPPC] / [**2**] = 3.5, at 25 ^{o}C.

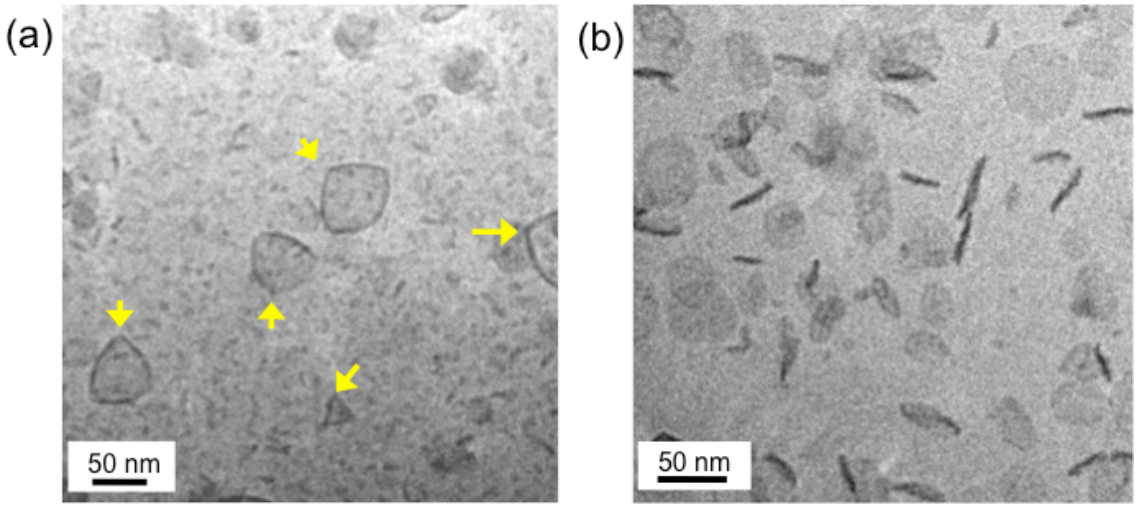

Figure S5. Mechanical disruption of hybrid (a) or phospholipid (b) bicelles by ultrasonic irradiation. Yellow arrows in panel (a) indicate the appeared edges on bicelles upon the ultrasonic irradiation. [Lipid] = 3.8 mM, at 25 ^{o}C.

(a): q = [**1**] / [**2**] = 3.5, (b): [DPPC] / [**2**] / [DPPG] = 3.4 : 1.0 : 0.21.