Electronic Supplementary Information

Tubulation of Liposomes via the Interaction of Supramolecular Nanofibers

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MATERIALS AND METHODS

Materials

1,2-dioleoyl-*sn*-glycero-phosphatidylcholine (DOPC) was purchased from NOF Corp. (Tokyo, Japan), and 5, 10, 15, and 20-tetrakis(4-sulfonatephenyl)porphyrins were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Sodium hydroxide and hydrochloric acid were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). All the reagents were used as received.

Ultraviolet–visible (UV–Vis) Absorption Measurements

All the samples were thoroughly dispersed using a vortex mixer before collecting their UV–vis absorption spectra. Absorption spectra in the range of 300–800 nm were recorded at 25 °C using a spectrophotometer (Shimadzu, UV-2550, Kyoto, Japan).

Dynamic Light Scattering and ζ-potential Measurements

The hydrodynamic diameter and ζ-potential of the DOPC liposomes were measured using an instrument for electrophoretic light scattering with a laser Doppler system (Zetasizer Nano ZS, Malvern Instruments Ltd. Malvern, UK).

Cryogenic-Transmission Electron Microscopy (Cryo-TEM) Measurements

Cryo-TEM samples were prepared using a universal cryofixation and cryopreparation system (Leica EM CPC, Wetzlar, Germany). To prevent water evaporation from the sample, the isolated chamber was humidified to near saturation before introduction of the sample. Sample droplets (2–3 μL) were placed on a microperforated cryo-TEM grid and then absorbed using filter paper, thus resulting in the formation of a thin liquid film (10–300-nm thick) that freely spanned the micropores in a carbon-coated lace-like polymer layer supported by a metal mesh grid. After a minimum holding time of 30 s, the sample grid assembly was rapidly vitrified with liquid ethane at its melting temperature (from −163 to −170 °C). The holding time was adopted to relax any possible flow deformation that may have resulted from the blotting process. The vitreous specimen was stored in liquid nitrogen until it was loaded into a cryogenic sample holder (Gatan 626-DH). Imaging was performed using a JEOL JEM-3100 FEF instrument operating at 300 kV (Tokyo, Japan). The use of a minimal dose system was necessitated by the electron radiation sensitivity of the sample probe. Images were recorded using a Gatan 794 multiscan digital camera and processed using DigitalMicrographs version 3.8.1 software. The optical density gradients in the background, which are normally ramp-shaped, were digitally corrected using a custom-made subroutine compatible with DigitalMicrographs.

Preparation of Liposomes and Porphyrin–Liposome Composites

Liposomes comprising DOPC were prepared using the well-known extrusion method. A 130 mM solution of DOPC was dissolved in chloroform in a glass vial and then evaporated to dryness under a flow of nitrogen gas. The dried lipid film was then hydrated with MilliQ water (1 mL), followed by vortexing for 1 min. The suspension was subsequently frozen and melted eight times using liquid nitrogen and a water bath, sequentially, and then extruded eleven times at a temperature above the phase transition temperature (T_m) through a 50-nm pore-sized polycarbonate membrane. Separately, H_4TPPS^{2-} was dissolved in water to obtain a 1.0 mM H_4TPPS^{2-} aqueous solution. The H_4TPPS^{2-} aqueous solution was then mixed with the liposomes at desired molar ratios at temperatures below T_m of the mixture. The pH values of the mixed solution were adjusted by adding 1 M of aqueous NaOH or HCl solution.

Calculation of coherence length of each porphyrin J-aggregates (L)[1]

The coherence length $L = (N+1) \times$ the radius of the porphyrin, where N is the spectroscopic aggregation number calculated using the following equation:

$$\frac{\Delta\nu_{1/2}(\mathrm{M})}{\Delta\nu_{1/2}(\mathrm{J})} = N^{1/2}$$

,

where $\Delta\nu_{1/2}$(M) and $\Delta\nu_{1/2}$(J) are the full-width at half-maximum values of the absorption peaks of the porphyrin monomer (431 nm) and J-aggregate (490 nm), respectively. The radius of the porphyrin was calculated to be ~1 nm.

Calculation of pulling force (Φ_{pull}) ***supplied from porphyrin J-aggregates***[2,3]

The relationship between the pulling force (Φ_{pull}) and radius (r) of the membrane curvature can be calculated based on considerations of the membrane mechanical equilibrium and is given by

$$\Phi_{pull} = 2\pi \cdot \kappa_B \cdot \left(\frac{1}{r} - J_S^B\right),$$

where k_B is the bilayer bending modulus and J_S^B is the spontaneous curvature of the bilayer. For symmetrical bilayers with zero spontaneous curvature, J_S^B=0, the required pulling force is inversely proportional to the cylinder radius,

$$\Phi_{pull} = 2\pi \cdot \kappa_B \cdot \frac{1}{r}.$$

This relationship has been verified experimentally and has been used to measure k_B. Using the value k_B=6.2 × 10^{-20} J, it can be readily estimated that for a tube with a radius of ~17 nm, the pulling force must equal ~23 pN.

FIGURES

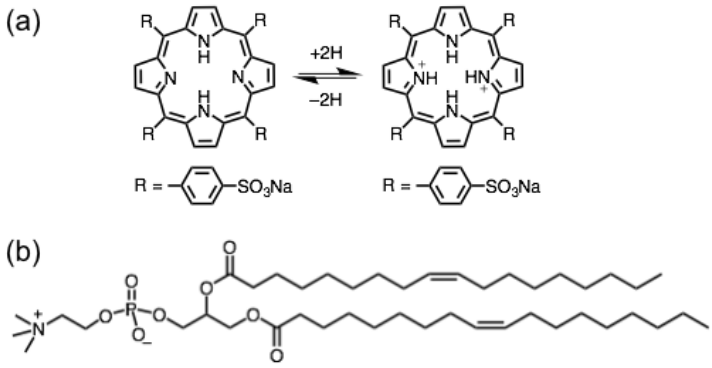

Fig. S1 Structures of (a) the free-base (H_2TPPS^{4-}) and diacid (H_4TPPS^{2-}) forms of anionic porphyrins and (b) DOPC.

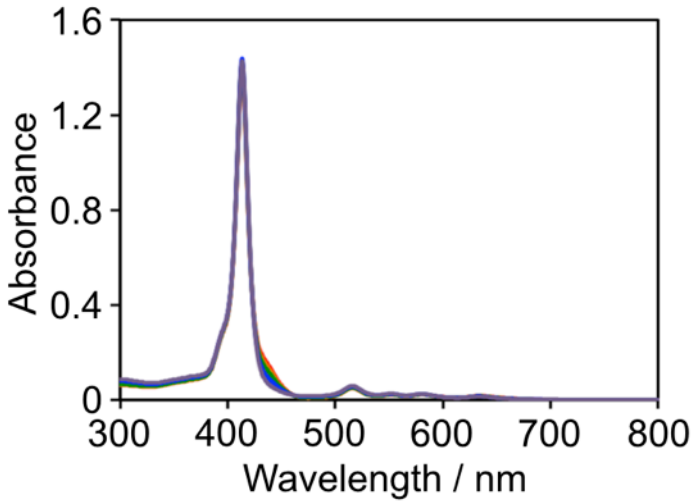

Fig. S2 UV–vis absorption spectral changes of H_2TPPS^{4-} upon addition of DOPC liposomes. [H_4TPPS^{2-}] = 50 μM, [DOPC] = 0–1000 μM, pH = 6.0.

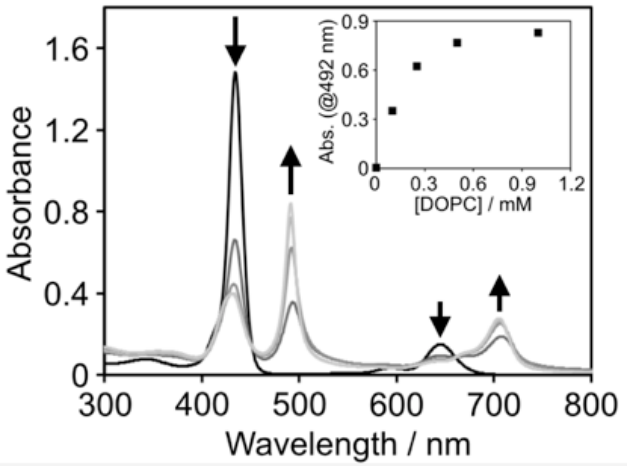

Fig. S3 UV–vis absorption spectral changes of H_4TPPS^{2-} upon addition of DOPC liposomes. [H_4TPPS^{2-}] = 50 μM, [DOPC] = 0–1000 μM, pH = 2.0. Inset: plots of absorbance at 492 nm *vs*. DOPC concentration.

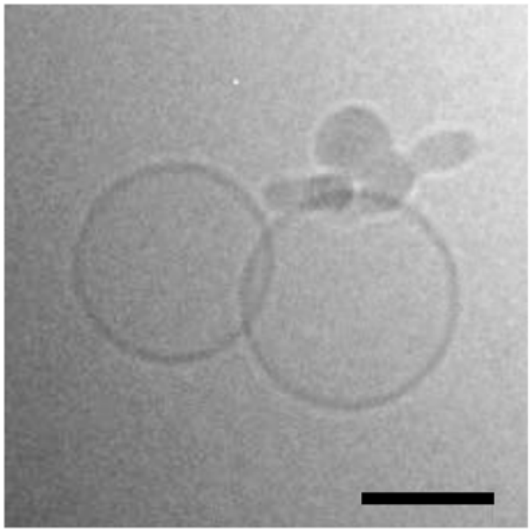

Fig. S4 Cryo-TEM image of DOPC liposomes in the presence of H_2TPPS^{4-} at pH 6.0. [DOPC]=500 μM, [porphyrin]=500 μM. The scale bar is 100 nm.

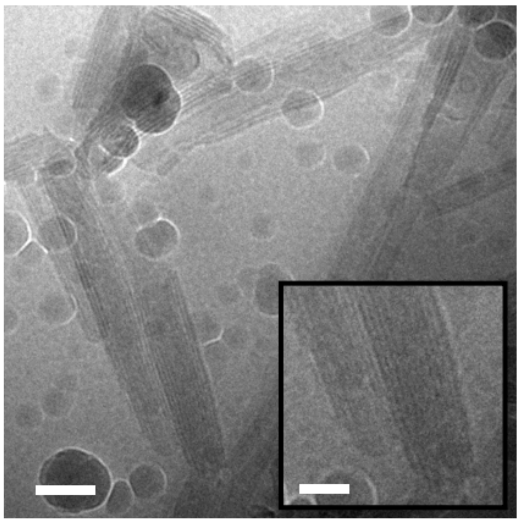

Fig. S5 Cryo-TEM image of DOPC liposomes in the presence of H_4TPPS^{2-} at pH 2.0. [DOPC]=500 μM, [porphyrin]=50 μM, *R*=10.

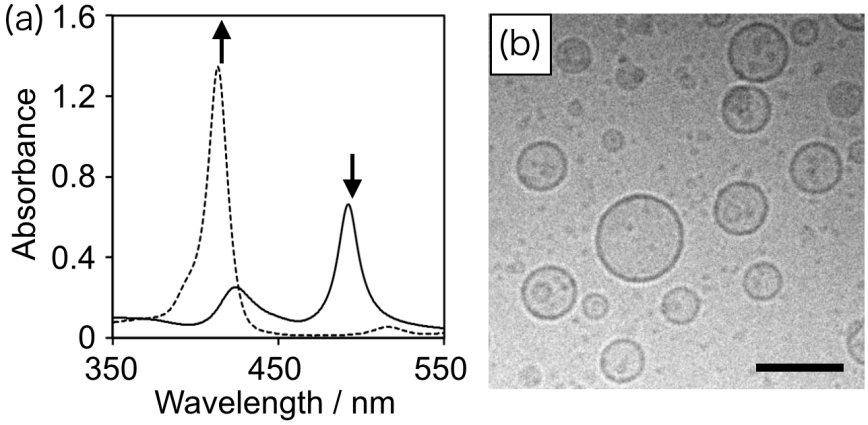

Fig. S6 (a) UV–vis spectral changes of H_2TPPS^{4-} or H_4TPPS^{2-} aqueous solutions at various pH values in the presence of DOPC liposomes: pH 2.0 (solid line) and after restoration of the pH to 6.0 (dashed line). [DOPC]=500 mM], [porphyrin]=500 mM. (b) Cryo-TEM image of DOPC liposomes in the presence of H_2TPPS^{4-} after restoring the pH to 6.0 from 2.0.

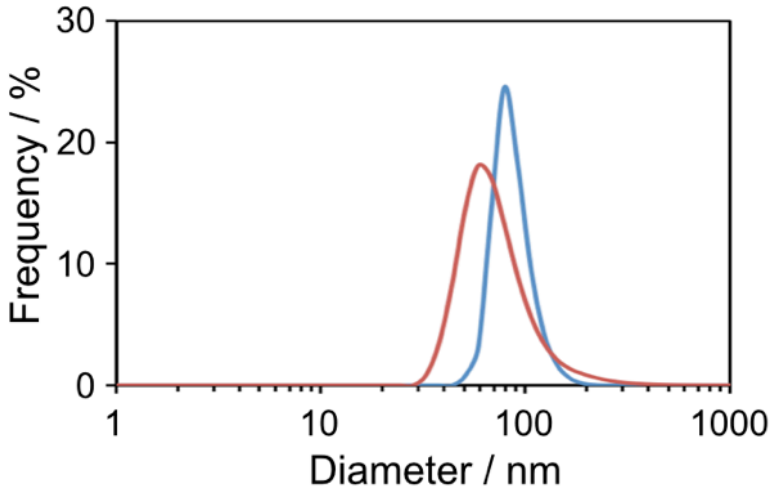

Fig. S7 DLS size distribution of as-prepared DOPC liposomes (blue line) and reconstructed DOPC liposomes (red line) at pH 6.0.

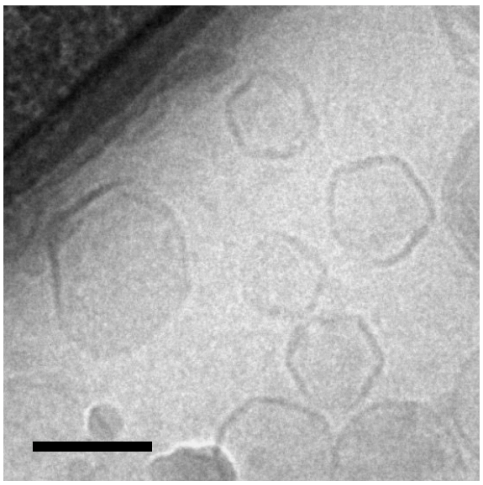

Fig. S8 Cryo-TEM image of DOPC liposomes in the presence of PSS at pH=2.0. [DOPC]=500 mM, [porphyrin]=500 mM, [PSS]=2.9 mM. Scale bar is 100 nm.

Table S1 ζ-Potential of DOPC liposomes in the presence of various concentrations of PSS. [DOPC]=500 μM, pH=2.0.

[PSS] / μM	0	0.059	0.29	2.9
ζ-potential	+24	+23	– 41	– 67

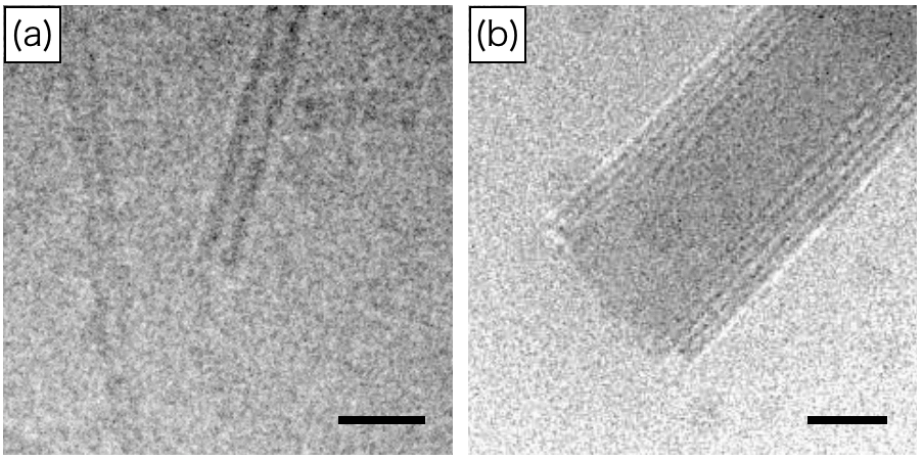

Fig. S10 Magnified cryo-TEM images of H_4TPPS^{2-}/DOPC tubes: (a) *R*=1 and (b) *R*=50. The scale bars are 50 nm.

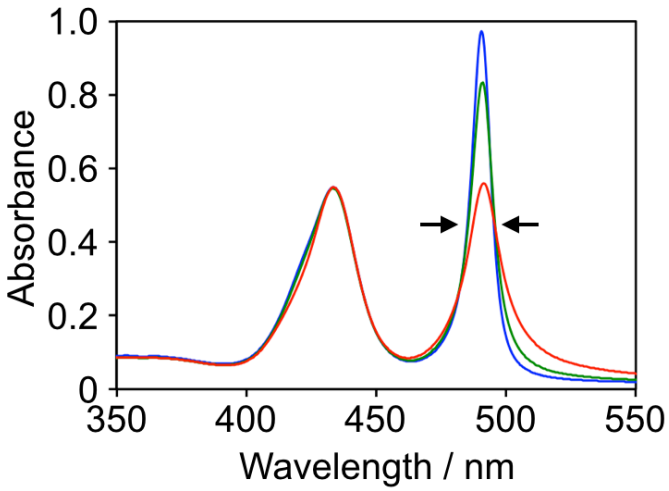

Fig. S11 Time-dependent UV-vis absorption spectra of the mixture of DOPC liposomes and H_4TPPS^{2-} ([porphyrin]=500 mM, [DOPC]=500 mM, *R*=1) incubated for 10 min (red solid line), 1 day (green solid line), and 3 days (blue solid line) after adjusting the pH to 2.0.

REFERENCES

(1) A. S. R. Koti, J. Taneja and N. Periasamy, *Chem. Phys. Lett.*, 2003, **375**, 171-176.

(2) I. Tsafrir, D. Sagi, T. Arzi, M. A. Guedeau-Boudeville, V. Frette, D. Kandel and J. Stavans, *Phys. Rev. Lett.*, 2001, **86**, 1138-1141.

(3) R. Barziv and E. Moses, *Phys. Rev. Lett.*, 1994, **73**, 1392-1395.