Supporting Information
for
Facile Fabrication of Cross-linked Vesicle via “Surface Clicking” of Calixarene-based Supra-amphiphile

Shu Peng, Jie Gao, Yu Liu* and Dong-Sheng Guo*

Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Collaborative Innovation Center of Chemical Science and Engineering, Nankai University, Tianjin 300071, China
Experimental Section

Materials Preparation. Hydroxysafflor yellow A (HSYA), sodium ascorbate, 4-phenolsulfonic sodium, and CuCl$_2$ were commercially available and used without further purification. $p$-Sulfonatocalix[4]arene (SC4A), (dodecyloxybenzyl) tripropargylammonium (TPA) and water-soluble azido cross-linker were synthesized and purified according to the procedures reported previously$^{[1,2]}$ and identified by $^1$H NMR spectroscopy in D$_2$O, performed on a Bruker AV400 spectrometer.

Preparation of the dynamic SC4A–TPA vesicle: SC4A was first dissolved in double-distilled water, and then TPA solution was dropwise mingled with the SC4A solution to get the dynamic SC4A–TPA vesicle. The formation of the SC4A–TPA vesicle would achieve balance in about 3 h ([SC4A] = 0.01 mM, [TPA] = 0.04 mM).

Preparation of the cross-linked SC4A–TPA vesicle: water-soluble azido cross-linker (300 μL of 10 mM aqueous solution), CuCl$_2$ (10 μL of 10 mM aqueous solution), and sodium ascorbate (100 μL of 10 mM aqueous solution) were added to the dynamic SC4A–TPA vesicle solution ([SC4A] = 0.1 mM, [TPA] = 0.4 mM) in double-distilled water (5.0 mL). The reaction mixture was stirred slowly at room temperature for 24 h under N$_2$ atmosphere. The resulting cross-linked vesicle (2.0 mL) was separated from unreacted starting materials by size-exclusion chromatography (G-50 column, purchased from Sigma-Aldrich) while maintaining the water. Finally, the purified fraction was diluted to a volume of 20 mL to yield a cross-linked vesicle solution ([SC4A] = 0.01 mM, [TPA] = 0.04 mM).

Preparation of the HSYA-loaded cross-linked vesicle: SC4A, HSYA were first dissolved in double-distilled water. Then TPA solution was dropwise mingled with stable mixing solution to get the HSYA-loaded dynamic vesicle. The SC4A–TPA vesicle would achieve balance in about 3 h ([SC4A] = 0.1 mM, [TPA] = 0.4 mM, [HSYA] = 0.1 mM). Then water-soluble azido cross-linker (300 μL of 10 mM aqueous solution), CuCl$_2$ (10 μL of 10 mM aqueous solution), and sodium ascorbate (100 μL of 10 mM aqueous solution) were added to the dynamic vesicle solution in double-distilled water (5.0 mL). The reaction mixture was stirred slowly at room temperature for 24 h under N$_2$ atmosphere. The resulting cross-linked vesicle (2.0 mL) was separated from unreacted starting materials by size-exclusion chromatography (G-50 column) while maintaining the water. Finally, the purified fraction was diluted to a volume of 20 mL to yield a cross-linked vesicle solution.
UV–Vis Spectroscopy
The optical transmittance of the aqueous solution was measured in a quartz cell (light path 10 mm) on a Shimadzu UV-3600 spectrophotometer equipped with a PTC-348WI temperature controller.

High-Resolution TEM and SEM Experiments
High-resolution TEM images were acquired using a Tecnai G2 F20 high-resolution transmission electron microscope equipped with a CCD camera (Orius 832, Gatan) operating at an accelerating voltage of 200 kV. The sample for high-resolution TEM measurements was prepared by dropping the solution onto a copper grid. The grid was then air-dried. SEM images were recorded on a Shimadzu SS-550 scanning electron microscope. The sample for SEM measurements was prepared by dropping the solution onto a coverslip, followed by evaporating the liquid in air.

Cryo-TEM Experiments
Aliquots of 3.5 μL of sample were applied to glow-discharged Quantifoil grids, blotted for 6s in a room temperature and 100% humidity chamber, and plunged into liquid ethane cooled by liquid nitrogen in the automated EFI Vitrobot device. We performed structural analysis by cryo-electron microscopy, on the FEI Talos F200C with constant-power C-Twin objective lens which was equipped with a Gatan Model 626 cryo-transfer specimen holder, operated at 200 kV. Images were recorded on the FEI 16Megapixel Ceta CMOS camera. The electron dose for each micrograph was approximately 30 e/Å².

Small-angle X-ray scattering (SAXS)
SAXS experiments were mainly performed with the high-flux small-angle X-ray scattering instrument (SAXSess, Anton Paar) equipped with a Kratky block collimation system and a Philips PW3830 sealed-tube X-ray generator (Cu Kα).

DLS and SLS Measurements
Angle-dependent DLS experiments and SLS experiments were examined on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (TurboCorr) at 636 nm. Others were measured by NanoBrook 173 Plus at scattering angle of 90°.

Zeta Potential Measurement
Zeta potential of the SC4A–TPA vesicles was measured by NanoBrook 173.
Fig. S1 (a) Optical transmittances of free SC4A, free TPA, the complex of TPA with SC4A, TPA with 4-phenolsulfonic sodium at 25 °C in water. (b) Optical transmittance of SC4A with TPA of different concentrations at 25 °C in water.

Fig. S2. (a) Dependence of the $q^2$ on quadratic diffusion coefficient of the SC4A–TPA assembly at different angles ($50 \leq \theta \leq 130^\circ$). (b) SLS data of the SC4A–TPA assembly. [SC4A] = 0.01 mM, [TPA] = 0.04 mM.

Particles in solution move under Brownian motion and their diffusion coefficient $D$ can be related to their (hydrodynamic) size by the Stokes–Einstein equation ($R_h = k_B T / 6 \pi \eta D$). In Fig. S1a, the diffusion coefficient $D$ was obtained. According to Stokes–Einstein equation, the averaged hydrodynamic radius ($R_h$) of the SC4A–TPA assembly was 145 nm. SLS data show a radius of gyration ($R_g$) of 137 nm. The ratio $R_g/R_h$ is 0.94.
Fig. S3. (a) Cryo-TEM images of the cross-linked vesicle. (b) Enlarged Cryo-TEM images of the cross-linked vesicle.

Fig. S4. Small-angle X-ray scattering (SAXS) scan of cross-linked vesicle.
Fig. S5. (a) DLS data of the cross-linked SC4A–TPA vesicle after dialysis for 24 h. (b) Count rates of the dynamic and cross-linked vesicles after dialysis for 24 h. (c) Autocorrelation function of the dynamic vesicle after dialysis for 24 h.

Fig. S6. Dependence of the normalized absorbance at 402 nm on dialyzing time of the HSYA-loaded cross-linked vesicle.