Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

for

Facile Fabrication of Cross-linked Vesicle via "Surface Clicking" of Calixarene-based Supra-amphiphile

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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 ^1H NMR spectroscopy in D_2O, 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/Å^2.

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.

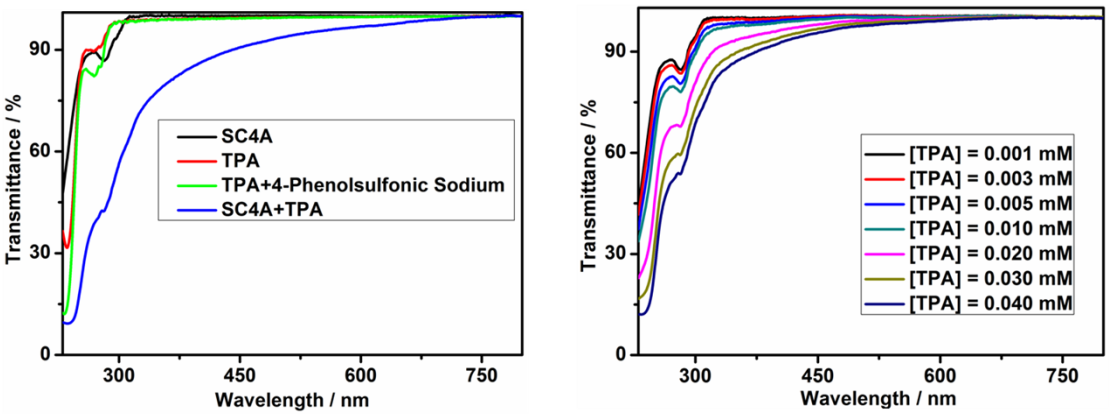


Fig. S1 (a) Optical transmittances of free SC4A, free TPA, the complex of TPA with SC4A, TPA with 4-phenolsulfonic sodium at 25 °C in water. (b) Optical transmittance of SC4A with TPA of different concentrations at 25 °C in water.

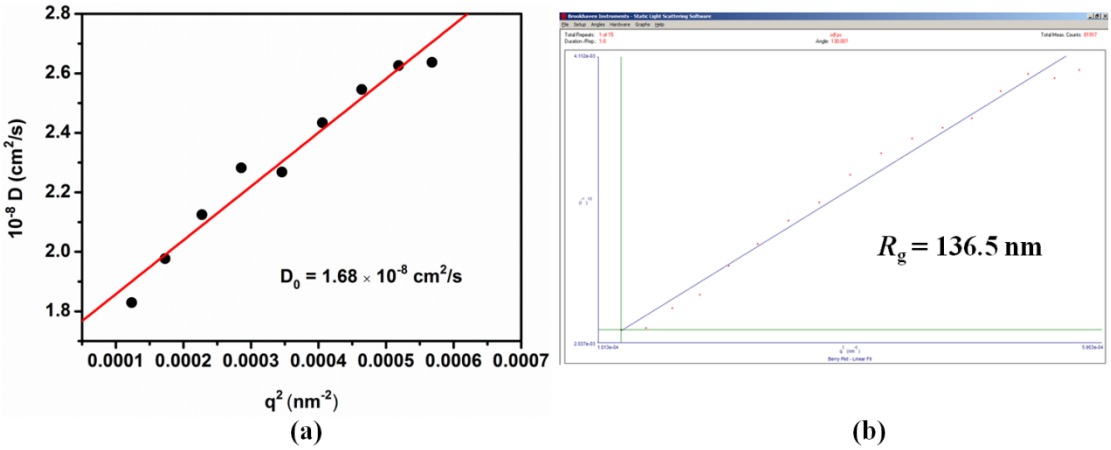
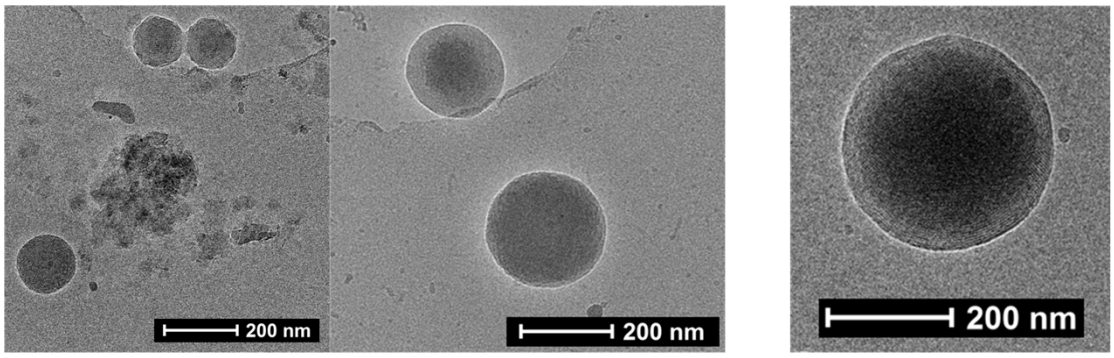


Fig. S2. (a) Dependence of the q^2 on quadratic diffusion coefficient of the SC4A–TPA assembly at different angles ($50 \leq \theta \leq 130°$). (b) SLS data of the SC4A–TPA assembly. [SC4A] = 0.01 mM, [TPA] = 0.04 mM.

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.



(a) (b)

Fig. S3. (a) Cryo-TEM images of the cross-linked vesicle. (b) Enlarged Cryo-TEM images of the cross-linked vesicle.

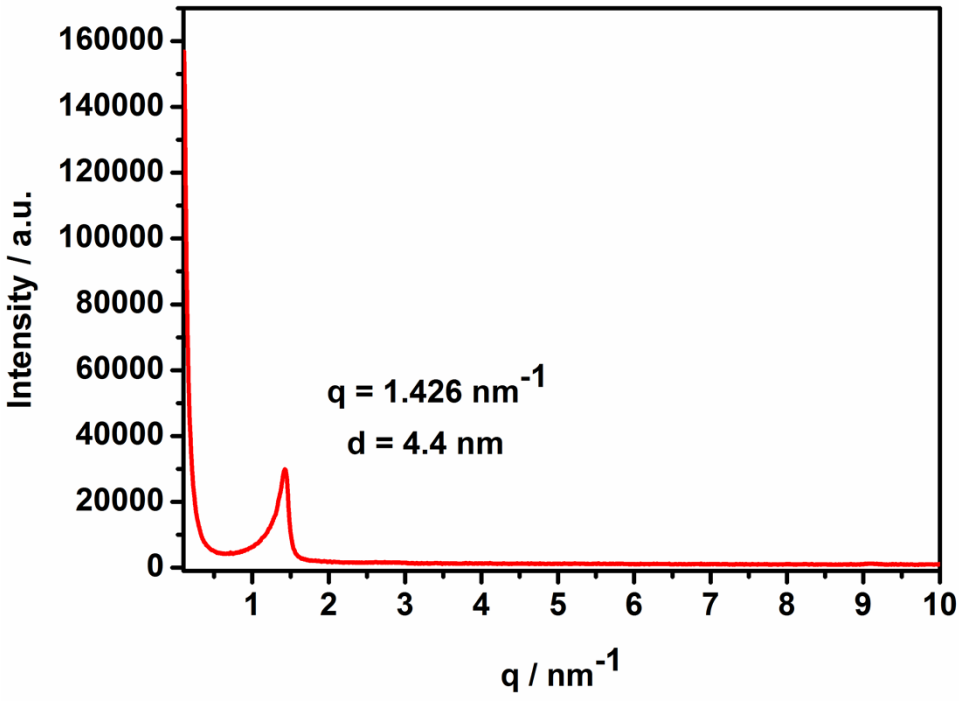


Fig. S4. Small-angle X-ray scattering (SAXS) scan of cross-linked vesicle.

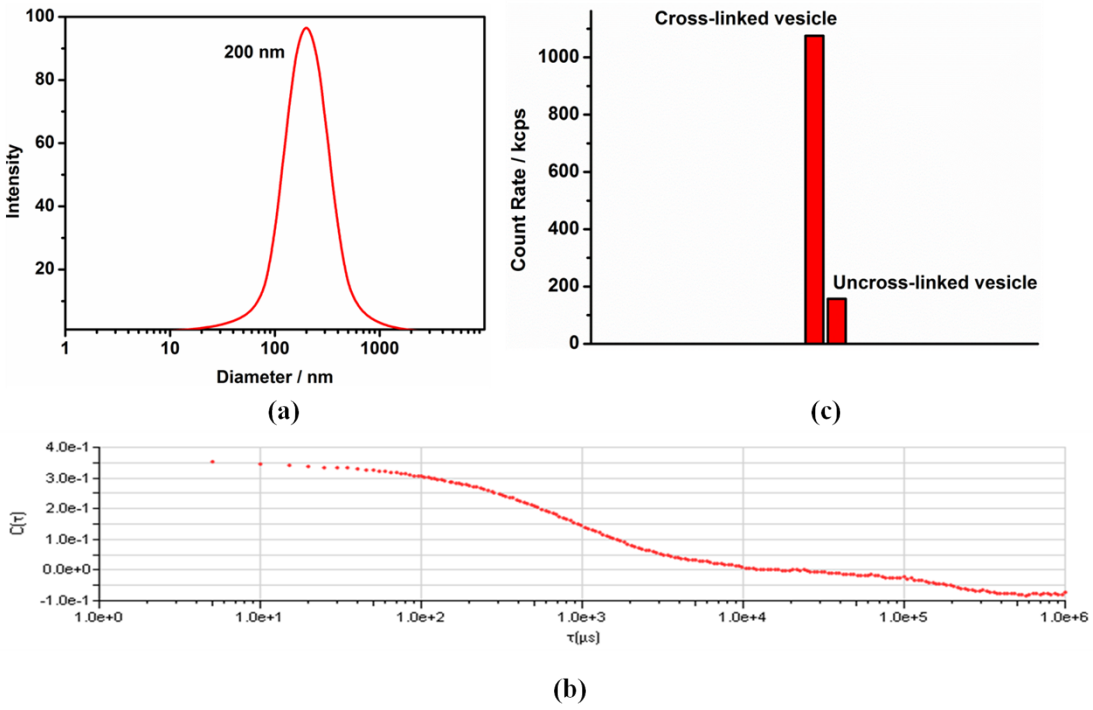


Fig. S5. (a) DLS data of the cross-linked SC4A–TPA vesicle after dialysis for 24 h. (b) Count rates of the dynamic and cross-linked vesicles after dialysis for 24 h. (c) Auto-correlation function of the dynamic vesicle after dialysis for 24 h.

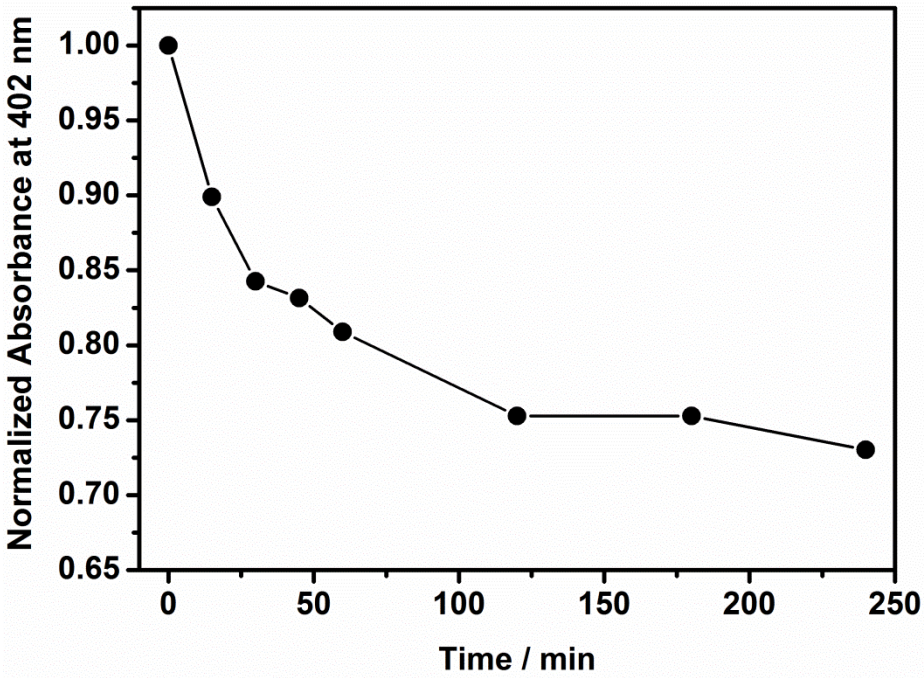


Fig. S6. Dependence of the normalized absorbance at 402 nm on dialyzing time of the HSYA-loaded cross-linked vesicle.

1. G. Arena, A. Contino, G. G. Lombardo and D. Sciotto, *Thermochim. Acta*, 1995, **264**, 1.

2. S. Zhang and Y. Zhao, *Macromolecules*, 2010, **43**, 4020.