## **Supporting Information**

# Multifunctional Vehicle of Amphiphilic Calix[4]arene Mediated by Liposome

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#### **Experimental Section**

#### Materials:

Cholesterol, 1,2-dihexadecanoyl-sn-glycero-3-phosphocholine (DPPC), and 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), and fluorescein isothiocyanate (FITC) were purchased from commercial resources and used without further purification. The amphiphilic p-sulfonatocalix[4]arenes bearing tetrabutyl and tetrahexyl chains (SC4AH and SC4AB), biotinylated pyridinium (BtPy), FITC-conjugated pyridinium (FITCPy) and bispyridinium salts of methyl viologen (MV and bis-MV) were synthesized according to the reported procedures.<sup>1–5</sup>

**Preparation of DPPC–SC4AH and DPPC–SC4AB Liposomes.** Vesicular solutions were prepared by using the previously reported film hydration method. In brief, DPPC (7.34 mg) and cholesterol (1.29 mg) were dissolved in 2 mL chloroform. Chloroform was evaporated using a rotary evaporator to create a thin lipid film. This film was re-suspended in 2 mL SC4AH (0.25 mM) or SC4AB (0.5 mM) aqueous solution after 0.5 h of stirring at 55 °C and sonicated for another 0.5 h at the same temperature. The resulting suspension was extruded through a 200 nm membrane while hot to create the lipid vesicles. Giant mixed vesicles prepared from a similar procedure but without sonication and extrution to reduce the diameter.

**Preparation of Guest-Decorated Liposomes.** FITCPy or BtPy were added to vesicular solutions and the resulting solution was gently stirred for 2 h at room temperature to introduce tags to the surface of vesicles.

#### **Measurements:**

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. Steady-state fluorescence spectra were recorded in a conventional quartz cell (light path 10 mm) on a Varian Cary Eclipse equipped with a Varian Cary single-cell Peltier accessory to control the temperature. <sup>1</sup>H NMR spectra were recorded on a Bruker AV400 spectrometer in D<sub>2</sub>O at 25 °C. DSS is used as internal reference.

**Isothermal Titration Calorimetry (ITC)**. A thermostated and fully computer-operated isothermal calorimetry (VP-ITC) instrument, purchased from Microcal Inc., Northampton, MA, was used for all microcalorimetric experiments. All microcalorimetric titrations were performed in aqueous solution at atmospheric pressure and 25 °C. Each solution was degassed and thermostatted by a ThermoVac accessory before the titration experiment. Twenty-nine successive injections were made for titration experiment. A constant volume (10  $\mu$ L/injection) of BtPy solution in a 0.250 mL syringe was injected into the reaction cell (1.4227 mL) charged with SC4AB aqueous solution.

**High-Resolution TEM measurements.** High-resolution TEM images were acquired using a Tecnai 20 high-resolution transmission electron microscope operating at an accelerating voltage of 200 kV. The sample for TEM measurements was prepared by dropping the solution onto a copper grid. The grid was then air-dried.

**DLS measurements.** The samples were examined on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (TurboCorr) at 636 nm at

a scattering angle of 90°. The hydrodynamic diameter was determined by dynamic light scattering experiments.

**Zeta potential measurements.** Zeta potential values were determined on a Brookheaven ZetaPALS (Brookheaven Instrument, USA) at 25 °C. The instrument utilizes phase analysis light scattering to provide an average over multiple particles. Doubly distilled water was used as the background electrolyte for zeta potential measurements.

**Confocal laser scanning microscopy.** For the purpose of giant vesicle imaging, a drop of giant mixed vesicular solution (0.5 mM) after incubation with FITCPy (0.005 mM) on a slide glass was observed by a confocal laser scanning microscope (Olympus FV1000) with excitation at 490 nm for FITCPy. For the purpose of biotin receptor-mediated cancer cell targeting, MCF7 cells were firstly cultured in Roswell Park Memorial Institute's medium (RPMI 1640) supplemented with 10% FBS under a humidified air with 5% CO<sub>2</sub> at 37 °C. Then, MCF7 cells were seeded in a 4-well plate in 1.0 mL complete RPMI 1640 medium. After incubating for 24 h, the cells were treated with corresponding sample. After 6 h, the culture media were removed and cells were washed with PBS twice times, fixed in 4% paraformaldehyde (PFA). The cover slip was removed from the 4-well plate and a glass slide was mounted on the cover slip and fixed. Images were obtained on confocal laser scanning microscope. For competition experiments, MCF7 cells were pre-incubated at 37 °C for 1 h in the presence of free biotin at the concentration of 1 mM.





**Figure S1.** Dependence of the fluorescence intensity of nile red (1  $\mu$ M) on the concentration of SC4AH and SC4AB in water at 25 °C ( $\lambda_{ex} = 540$  nm,  $\lambda_{em} = 647$  nm).



**Figure S2.** Changes in turbidity of lipid vesicle solution (5 mM) upon increasing the concentration of (a) SC4AH from 0 to 40 mol% and (b) SC4AB from 0 to 20 mol% in water at 25 °C.

**Table S1.** Hydrodynamic diameter ( $D_h$ ) of mixed liposomes (0.5 mM) with and without targeting and imaging agents, and after stored at room temperature for 6

months.

Liposome	D <sub>h</sub> /nm	D <sub>h</sub> /nm (with guests)	D <sub>h</sub> /nm (after 6 months)
DPPC-SC4AH	105.1	104.8	121.0
DPPC-SC4AB	87.4	83.1	91.9



**Figure S3.** $\zeta$  potential of DPPC–SC4AB, DPPC–SC4AB + BtPy ([BtPy] = 0.05 mM), DPPC–SC4AB + BtPy + FITCPy ([BtPy] = 0.015 mM, [FITCPy] = 0.01 mM) and DPPC–SC4AB + MV ([MV] = 0.05 mM). The lipid concentration of DPPC was fixed at 0.5 mM.



**Figure S4.** Optical transmittance of pure DPPC (0.5 mM) with and without addition of *bis*-MV (0.025 mM). Inset: Optical transmittance recorded at 450 nm.



**Figure S5.** <sup>1</sup>H NMR spectra of FITCPy, SC4AH–FITCPy complex, and SC4AH in  $D_2O$  at 25 °C ([SC4AH] = [FITCPy] = 1 mM, 400 MHz). DSS was added as an internal reference. The peaks for solvent and DSS are denoted as symbols • and  $\blacktriangle$ , respectively.



**Figure S6.** (a) Fluorescence emission spectra of free FITCPy (0.01 mM), FITCPy with SC4AH (0.025 mM), and FITCPy with SC4AB (0.05 mM). The excitation wavelength is 490 nm. (b) UV/vis spectra of free FITCPy (0.01 mM), FITCPy with SC4AH (0.025 mM), and FITCPy with SC4AB (0.05 mM) in water at 25 °C.



Figure S7. Confocal laser scanning microscopic images of (a) DPPC–SC4AH and (b)

DPPC-SC4AB giant vesicles.



**Figure S8.** TEM images of (a) DPPC–SC4AH and (b) DPPC–SC4AB after incubation with BtPy and FITCPy.



**Figure S9.** Microcalorimetric titration of SC4AB with BtPy in aqueous solution at 25.00 °C. (a) Raw data for 25 sequential injections (10  $\mu$ L per injection) of a BtPy solution (1.99 mM) into a SC4AB solution (0.10 mM). Apparent reaction heat obtained from the integration of the calorimetric traces. (b) "Net" heat effects of complexation of BtPy with SC4AB for each injection, obtained by subtracting the dilution heat from the reaction heat, which was fitted by computer simulation using

the "one set of binding sites" model.

**Table S2.** Complex stability constant  $(K_S/M^{-1})$ , enthalpy  $(\Delta H^{\circ}/(kJ \cdot mol^{-1}))$ , and entropy changes  $(T\Delta S^{\circ}/(kJ \cdot mol^{-1}))$  for 1:1 intermolecular complexation of BtPy with SC4AB in aqueous solution at 25.00 °C.

Complex	Ks	$\Delta H^{\circ}$	$T\Delta S^{\circ}$
SC4AB + BtPy	$(7.37 \pm 0.05) \times 10^4$	$-32.07\pm0.08$	$-4.27\pm0.06$



**Figure S10.** Confocal laser scanning microscopy images of MCF7 cells incubated with (a) free FITCPy, (b) 1-methylpyridinium (Py)-functionalized DPPC–SC4AH liposome and (c) Py-functionalized DPPC–SC4AB liposome for 6 h at 37 °C. [DPPC] = 0.5 mM, [FITCPy] = 0.01 mM and [Py] = 0.015 mM. The scale bar is 60  $\mu$ m.



Figure S11. Cell viability of MCF7 cells after treatment with SC4AH and SC4AB at

different concentrations for 48 h at 37 °C.



**Figure S12.** CLSM images of (a, b) DPPC–SC4AH and (c, d) DPPC–SC4AB giant vesicles in PBS (10 mM, pH 7.2). The scale bar is  $10 \,\mu$ m.

**Table S3.** Hydrodynamic diameter ( $D_h$ ) of mixed liposomes (0.5 mM) in PBS (10 mM, pH 7.2).

	DPPC-SC4AH liposome	DPPC-SC4AB liposome		
D <sub>h</sub> in PBS (nm)	121.3	107.0		
S11 / S12				



**Figure S13.** CLSM images of MCF7 cells incubated with biotin-functionalized (a) DPPC–SC4AH and (b) DPPC–SC4AB liposomes in PBS for 6 h at 37 °C: [DPPC] = 0.5 mM, [FITCPy] = 0.01 mM, and [BtPy] = 0.015 mM. The scale bar is 60  $\mu$ m.

### Reference

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