Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Cross-Linked Small-Molecule Capsules with Excitation Wavelength-Dependent Photoluminescence and High Loading Capacity: Design, Synthesis and Application in Imaging-Guided Drug Delivery

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General Method. Routine ^{1}H NMR spectra were obtained on a Bruker AV II-400. The ^{1}H NMR chemical shifts were measured relative to D_2O as the internal reference (D_2O: δ 4.79 ppm). The particle sizes were measured with a dynamic light scattering (DLS) analyzer (Malvern ZetasizerNano ZS90). The fluorescence emission intensity of Nile Red at a wavelength of 630 nm (excited at 587 nm) was measured using a RF5301-pc fluorescence spectrometer. The fluorescence emission intensity of cSCs at the different excitation wavelengths was measured using a RF5301-pc fluorescence spectrometer. The absolute quantum yields were collected on a Horiba Jobin Yvon-Edison Fluoromax-4 fluorescence spectrometer with a calibrated integrating sphere system. To reduce the fluctuation in the excitation intensity, the xenon lamp was kept on for 1 hour prior to the experiment. UV-Vis spectrometry was monitored on a UV-2600 (Shimadzu, Japan) instrument. The fluorescence lifetime was determined on a Horiba tempro-01 instrument. Suitable narrow bandpass filters were used to eliminate contributions from the scattering of the incident beam from the NR@cSCs. Picosecond time-resolved fluorescence spectra were measured by the time-correlated single-photon-counting (TCSPC) method on a Nano-Log spectrofluorometer (Horiba JobinYvon), by using a laser diode as an excitation source (NanoLED, 405 nm) and a UV−vis detector TBX-PMT series (250−850 nm) by Horiba JobinYvon. Lifetimes were evaluated with the DAS6 Fluorescence Decay Analysis Software. TEM studies were carried out using a Tecnai G2F20S-TWIN instrument, operating at 120 kV. The TEM specimens were prepared by gently placing a carbon-coated copper grid on the surface of the sample. The TEM grid was then removed, stained with an aqueous solution of 2% phosphotungstic acid, dried for 0.5 h at room temperature, and then subjected to TEM observation. The DMF content of diluent for the cSCs (cross-linked small-molecule capsules) and T-cSCs (cSCs with templated NPs) was measured at 205 nm by HPLC (Agilent 1260 LC, Zorbax C18 column 4.6 ×150 mm) with mixed eluents ($V_{methanol}/V_{water}$ = 20/80).

The package load capacity and *in vitro* release of Nile Red (NR), camptothecin (CPT) or doxorubicin (DOX) were performed in sink (AHYQ SHA-C) at 37 ^{o}C. The content of NR, CPT or DOX were analyzed at 254 nm by HPLC (Agilent 1260 LC, Zorbax C18 column 4.6 ×150 mm) with mixed eluents ($V_{water}/V_{methanol}$ = 35/65 for Nile Red, $V_{water}/V_{methanol}$ = 55/45 for CPT and $V_{methanol}/V_{NaAc\ solution}/V_{acetic\ acid}$= 70/29/1 for DOX) within 10 min and flow rate 1.0 mL/min at 25 ^{o}C. The cell line was grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 U mL^{-1} penicillin and 100 U mL^{-1} streptomycin in 37 ^{o}C, 5% CO_2. Cell toxicity was evaluated by measuring the percentage of cell viability via the Thiazolyl Blue Tetrazolium Bromide (MTT). The absorbance at 570 nm was then measured using a microplatereader Varioscan Flash (Thermo Fisher SCIENTIFIC). The cell viability (%) was obtained according to the manufacturer's instructions. The cellular uptake of HeLa cells incubated with cSCs, DOX and DOX@cSCs was observed under confocal laser scanning microscopy (CLSM, ZEISS, LSM 780).

Chemicals. The amphiphilic molecule **1** was synthesized according to the previous report.[1] Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. All solvents for reactions were freshly distilled prior to use. Deionized water was used in all aqueous experiments.

Preparation of Cross-linked Small-molecule Micelles (cSMs).[2, 3] Cross-linker **2** (19.07 mg, 0.08 mmol) and photoinitiator PI2959 (0.44 mg, 2 μmol) in DMF (800 mg) were added into a micellar solution of **1** (24.63 mg, 0.05 mmol) in deionized water (16.0 mL). The reaction mixture was dispersed by ultrasonic processing and was deoxygenated with flowing nitrogen. The mixture was irradiated in a rayonet photoreactor for 4 h to make sure all alkyne groups were consumed. The

mixture was dialyzed against deionized water for 24 h (Spectra/Pore, MWCO 2 KDa) to obtain the cSMs as a pale yellow solution.

Determination of the Critical Micells Concentration (CMC) of 1.[4] A known amount of Nile Red in CH_2Cl_2 was added to a series of vials and the CH_2Cl_2 was evaporated. The amount was chosen to give a Nile Red concentration of 1×10^{-4} M in the final solution. A measured amount of **1** solution was added to each vial and the deionized water was added to the vials to make the concentrations of **1** ranging from 2.4×10^{-4} to 1.28 mM. The vials were vibrated at room temperature overnight, and then the fluorescence emission intensity at the wavelengths of 630 nm (excited at 587 nm) was measured. The critical micelle concentration (CMC) was obtained as the intersection of the tangents to the two linear portions of the graph of the fluorescence intensity as a function of **1**. From Figure S3, the CMC of compound **1** was 94.6 μM.

***In Vitro* Stability Assay.**[5] The stability of cSCs in water was evaluated by diluting the concentrations of **1** below its CMC (94.6 μM). Briefly, the cSMs and cSCs ([**1**] = 200 μM) was diluted to the concentrations of 200 μM, 50 μM, 12.5 μM, 3.13 μM, 0.78 μM, respectively. Afterwards, the particle sizes of above solutions were recorded by DLS to evaluate its stability.

The FBS stabilities of cSCs were investigated by incubation with 10% (V/V) FBS. Briefly, 4.5 mL of cSCs ([amphiphilic small-molecule] = 20 mM) were mixed with 0.5 mL FBS. The particle sizes at 0 and after 6, 12 and 24 h of incubation at 37 °C were recorded to evaluate their stability.

Preparation of Hydrophobic DOX. Doxorubicin hydrochloride (DOX·HCl, 1.0 equiv) was added with excess triethylamine (3.0 equiv) in aqueous solution to produce hydrophobic DOX. After the aqueous solution was centrifuged and lyophilized, DOX was afforded as a red powder.

***In Vitro* Release Assay.** Each 1.0 mL of the aliquot sample, CPT@cSCs or DOX@cSCs (containing CPT or DOX 100.0 μg/mL), was added into a dialysis bag (MWCO 6000 − 8000) and dialyzed against 20.0 mL of different buffers (4-(2-hydroxyethyl)-1-piperazineethanesulfonicaci (HEPES) buffer 7.4 and ABS buffer 5.0, with 1.0% tween 80) with gentle shaking (100 rpm) at 37 °C. At predetermined periods, 1.0 mL of the solution was collected from the corresponding different reservoirs and the samples were detected by fluorescence spectra. To keep a constant volume after each sampling, 1.0 mL of corresponding buffer solution was added to the reservoir. The data of the release experiment were averaged over three times. The content of CPT and DOX presented in the samples was determined HPLC.

Cytotoxicity Assay. Briefly, cytotoxicity assay of cSMs was measured by seeding cells in 96-well plates with culture medium plus 10% (v/v) FBS and 1% (v/v) penicillin/streptomycin at 37 °C with 5% CO_2 for 24 h. Then fresh culture medium containing different concentrations of cSMs were added to plates, respectively. After incubation for 24 h, culture media were removed and fresh media (200 μL) containing MTT solution (40 μL, 5.0 mg mL^{-1}) were added to each well. The medium was removed after 2 h of incubation and dimethyl sulfoxide DMSO (100 μL) was added to each well, the absorption of each well was determined at 570 nm using a micro-plate reader. In addition, the cytotoxicity of cSCs, free DOX and DOX@cSCs against different type of cells was also determined by similar way.

Cellular Uptake Evaluation by Confocal Laser Scanning Microscopy (CLSM). HeLa cells (5×10^4 cells/mL) were seeded in an Φ = 35 mm glass Petri dish and incubated for about 24 h. Subsequently, the culture medium was changed with an equal concentration of cSCs, DOX·HCl, and DOX@cSCs ([DOX] = 2.5 μg/mL). After incubation for different time, the cells were washed three

times with HEPES solution. The cellular uptake was evaluated by CLSM (blue channel : λ_{ex} = 408 nm; green channel : λ_{ex} = 488 nm; red channel : λ_{ex} = 543 nm).

References

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3. X. Dai, X. Zhou, C. Liao, Y. Yao, Y. Yu and S. Zhang, *Chem. Commun.*, 2019, **55**, 7199-7202.
4. C. Li, S. Zhang, J. Pang, Y. Wu and Z. Gu, *Adv. Funct. Mater.*, 2015, **25**, 3764-3774.
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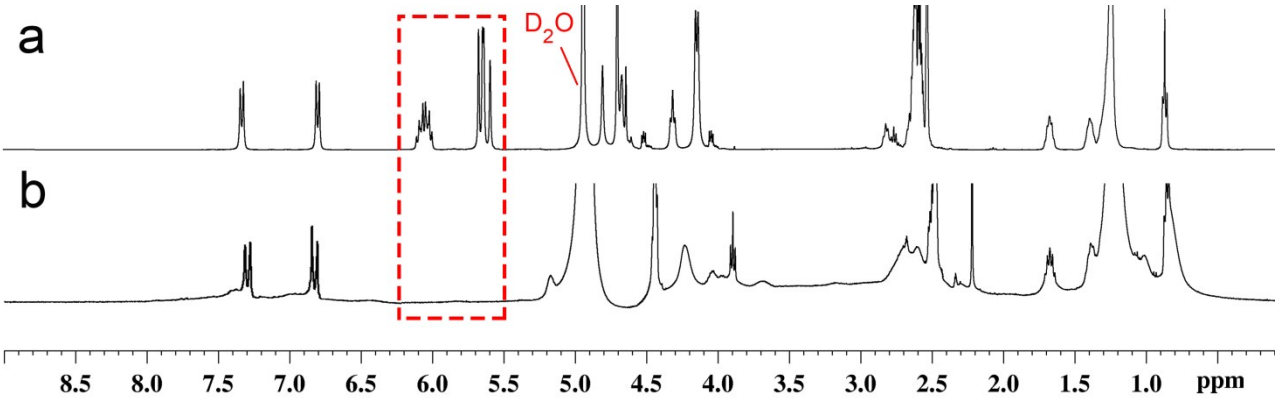

Figure S1. ^{1}H NMR spectra of (a) the 2:3 mixtures of **1** and **2** before cross-linking and (b) the cSCs.

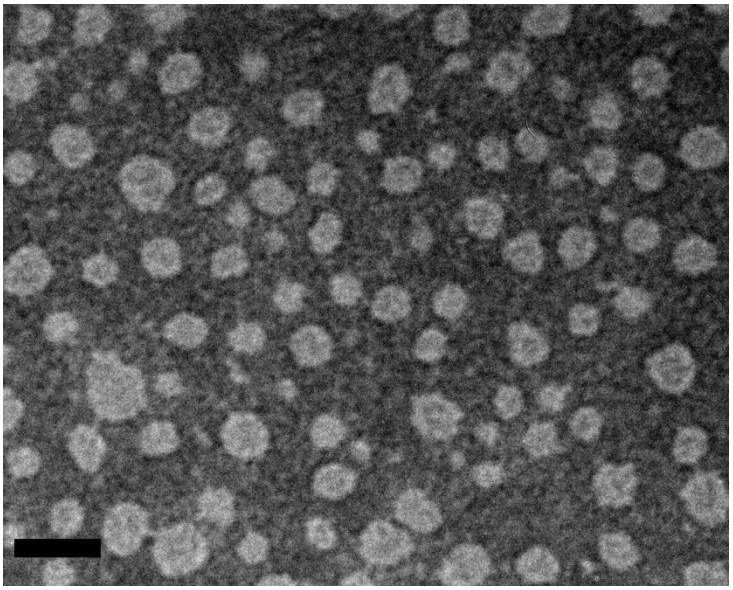

Figure S2. TEM micrographs of T-cSCs. The scale bar is 100 nm.

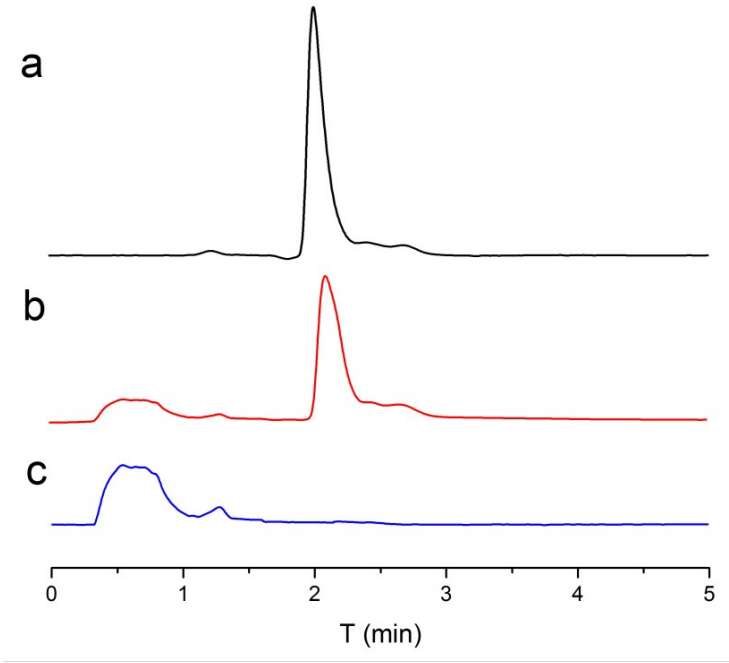

Figure S3. The HPLC spectrogram of (a) 20 μg/mL standard DMF aqueous solution, acid-hydrolyzed diluent of (b) T-cSCs and (c) cSCs.

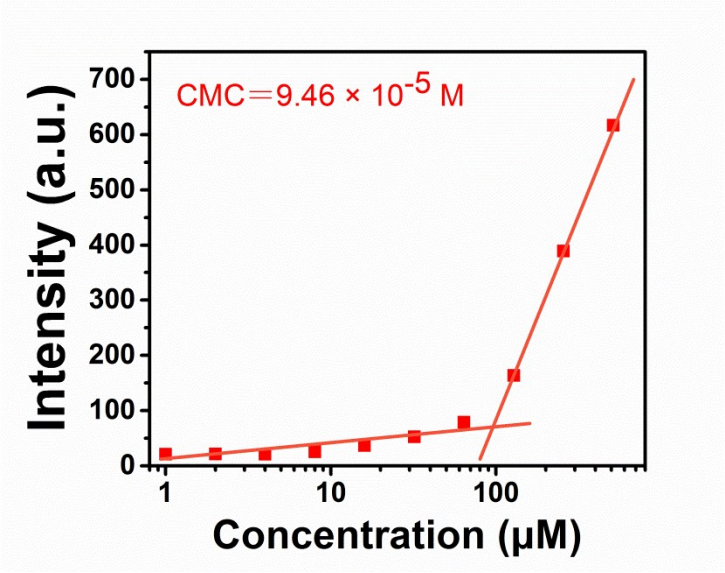

Figure S4. Emission intensity at 630 nm (F_{630}) of Nile Red as a function of concentrations of **1**. [Nile Red] = 1.0×10^{-4} mol/L, λ_{ex} = 587 nm.

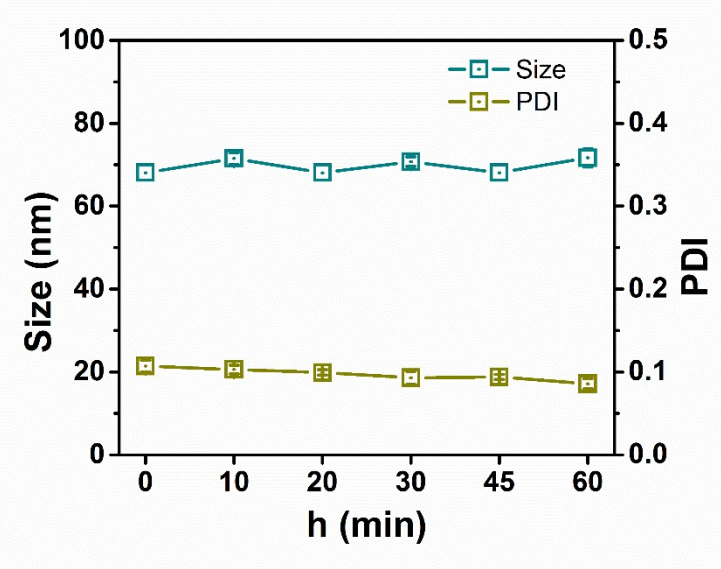

Figure S5. The nanoparticle size and polydispersity index (PDI) of cSCs with treatment of sonication under 40 °C. [cSCs] = 1.2 mg/mL.

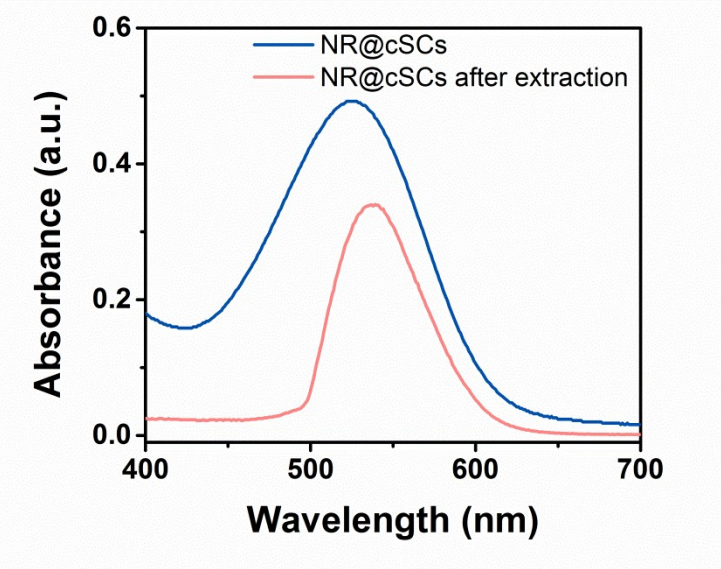

Figure S6. UV-Vis absorption spectra of NR@cSCs and after extraction with chloroform.

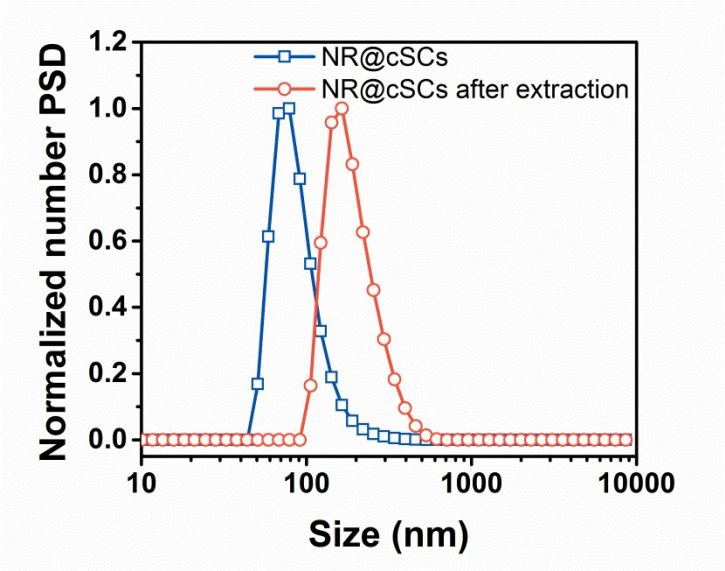

Figure S7. DLS of NR@cSCs and after extraction with chloroform.

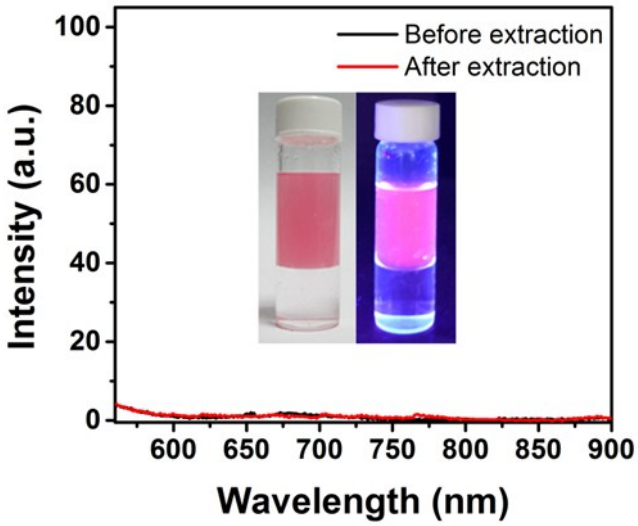

Figure S8. Fluorescence (FL) intensity of Nile Red chloroform solution pre- and post- extraction in chloroform, λ_{ex} = 540 nm. Inside: the photographs of bright field and under 365 nm UV-light for aqueous solution of NR@cSCs in the presence of chloroform after shaking and standing overnight.

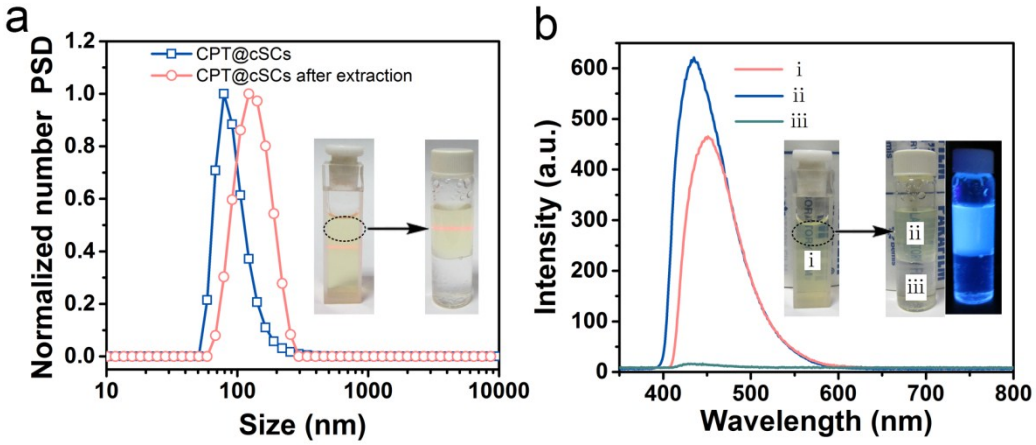

Figure S9. (a) DLS of CPT@cSCs before and after extraction with chloroform. (b) Fluorescence (FL) intensity of CPT@cSCs NPs aqueous solution (i), the aqueous phase (ii) and chloroform phase (iii) of the CPT@cSCs after extraction with chloroform, λ_{ex} = 380 nm. Narrow bandpass filter L-42 was used to eliminate contributions from the scattering of the incident beam from the CPT@cSCs. Inside: the photographs of bright field and under 365 nm UV-light for aqueous solution of CPT@cSCs and CPT@cSCs in the presence of chloroform after shaking and standing overnight.

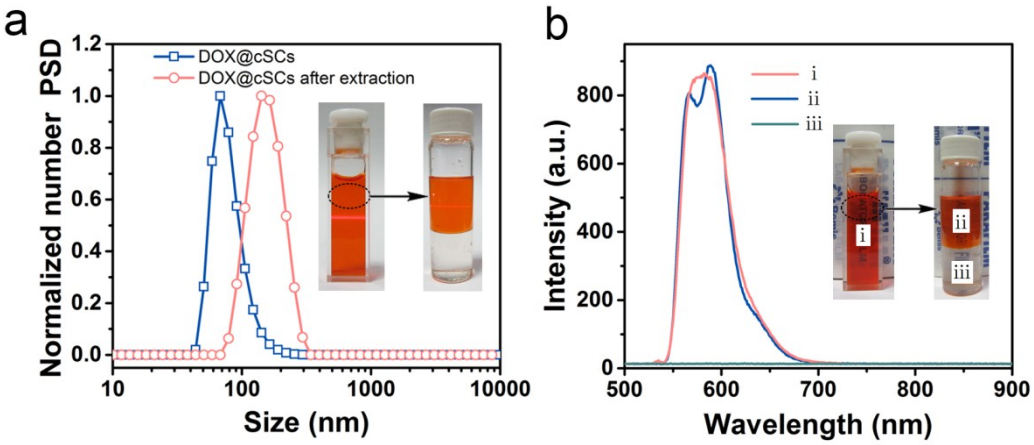

Figure S10. (a) DLS of DOX@cSCs before and after extraction with chloroform. (b) Fluorescence (FL) intensity of DOX@cSCs NPs aqueous solution (i), the aqueous phase (ii) and chloroform phase (iii) of the DOX@cSCs after extraction with chloroform, λ_{ex} = 530 nm. Narrow bandpass filter O-56 was used to eliminate contributions from the scattering of the incident beam from the DOX@cSCs. Inside: the photographs of bright field for aqueous solution of DOX@cSCs and DOX@cSCs in the presence of chloroform after shaking and standing overnight.

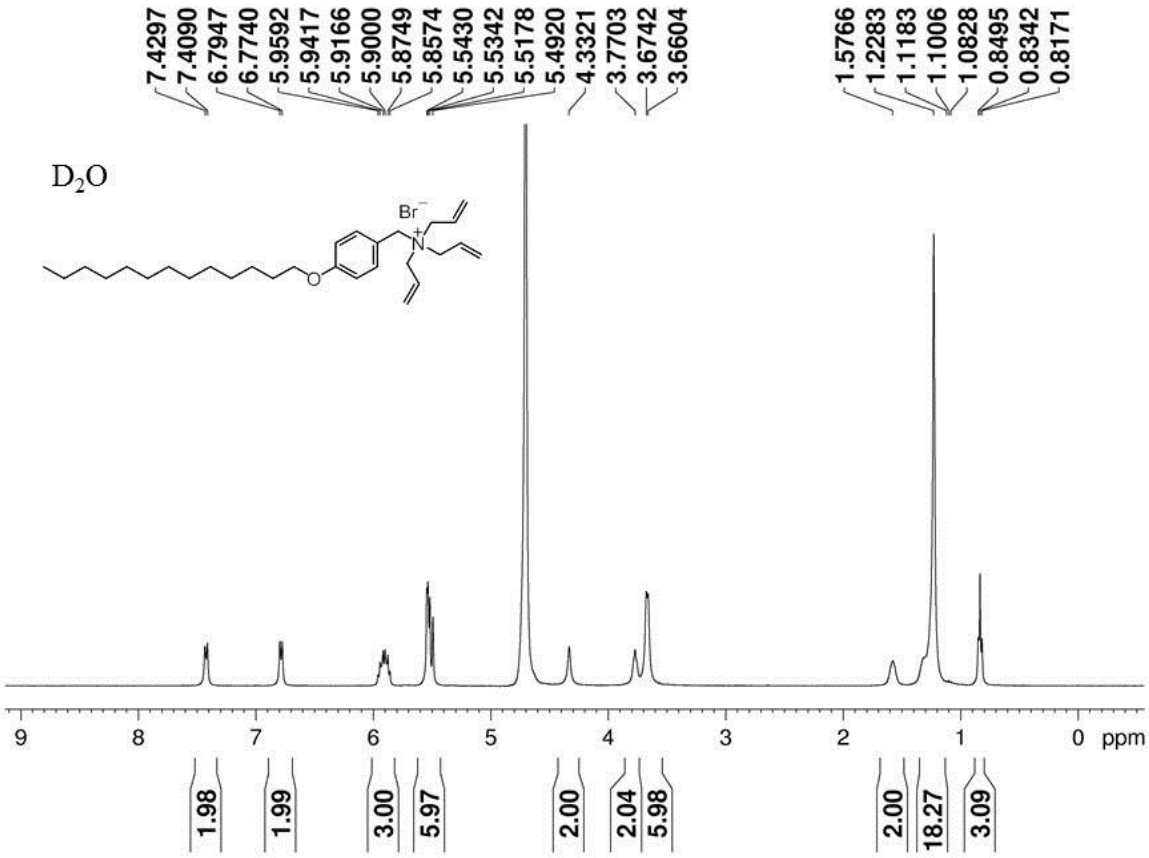

Figure S11. ^{1}H NMR spectra of amphiphile molecule **1**.

Table S1. PL emission time-resolved photoluminescence (TRPL) decays measurements fitting results of cSCs aqueous solution (0.02 mg/mL) at different excitation wavelength.

λ_{em} (nm)	480	500	520	540	560	580
τ_A (ns)	0.53	0.55	0.73	0.76	0.81	0.86
τ_1 (ns)	1.52	1.62	1.77	1.75	1.83	1.92
	41.10%	44.56%	45.34%	45.55%	47.58%	48.16%
τ_2 (ns)	6.10	6.46	7.00	6.95	7.23	7.50
	26.69%	28.15%	28.23%	30.13%	28.37%	27.91%
τ_3 (ns)	0.21	0.22	0.24	0.24	0.26	0.28
	32.21%	27.28%	26.43%	24.31%	24.05%	23.93%
χ^2	1.18	1.16	1.16	1.18	1.17	1.16

τ_A: average life time; χ^2: fitting constant.

Table S2. Fluorescence quantum yield (Φ_F) of cSCs aqueous solution (0.02 mg/mL) at different excitation wavelength.

λ_{ex} (nm)	340	360	380	400	440	460	480	520
λ_{em} (nm)	474	473	474	482	501	517	542	574
Φ_F (%)	2.09	3.48	5.10	7.73	11.95	9.51	6.84	5.18