Supporting Information

Soft Particles of Gemini Surfactant/Conjugated Polymer for Enhanced Anticancer Activity of Chemotherapeutics

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EXPERIMENTAL SECTION

Materials. SDHC, SDHC-Cy5 and PMNT (M_w = (2.0-4.0) × 10^4 g/mol, ~100 RU) were synthesized and purified as we reported previously.[1] DCFH-DA was purchased from Sigma Chemical Co.. MCF-7 cells, HeLa cells and A549 cells were obtained from Center for Cell, Institute of Basic Medical Science, Chinese Academy of Sciences. Phosphate buffered saline (PBS), and Dulbecco's modified eagle medium (DMEM) were purchased from Hyclone (Beijing, China). Fetal bovine serum (FBS) was obtained from Sijiqing Biological Engineering Materials (Hangzhou, China).

ζ-Potential Measurement. ζ-Potential measurements for SDHC, PMNT, SDHC/PMNT and MCF-7 cells were carried out with a Nano-ZS instrument (ZEN3600, Malvern Instruments, Worcestershire, U.K.) equipped with a thermostated chamber and employing a 4 mW He-Ne laser (λ = 632.8 nm) at 25.0 ± 0.1 °C. The ζ-potential measurement of each sample was repeated for three times, and the average value was taken.

Dynamic Light Scattering (DLS). DLS measurements for SDHC, PMNT and SDHC/PMNT were studied at a scattering angle of 173° on a Nano-ZS instrument (ZEN3600, Malvern Instruments, Worcestershire, U.K.) equipped with a thermostated chamber and a 4 mW He-Ne laser (λ = 632.8 nm). The temperature was controlled at 25.0 ± 0.1 °C. The DLS measurement of each sample was repeated for three times, and the average value was taken.

Cryogenic Transmission Electron Microscopy (Cryo-TEM). The SDHC and SDHC/PMNT solutions were embedded in a thin layer of vitreous ice on freshly carbon-coated holey TEM grids by blotting the grids with filter paper, and then they were plunged into liquid ethane cooled by liquid nitrogen. Frozen hydrated specimens were imaged by using an FEI Tecnai 20 electron microscope (LaB6) operated at 200 kV with the low dose mode (about 2000 e/nm^2) and the nominal magnification of 50000. For each specimen area, the defocus was set to 1-2 μm. Images were recorded on Kodak SO163 film and then digitized by Nikon 9000 with a scanning step 2000 dpi corresponding to 2.54 Å/pixel.

Scanning Electron Microscopy (SEM). The morphologies of PMNT and SDHC/PMNT were imaged by a field-emission scanning electron microscope (Hitachi S-4300). The samples were prepared by freezing a small drop of PMNT or SDHC/PMNT solutions on a clean silica wafer with liquid nitrogen. Immediately afterward, the frozen samples were lyophilized under vacuum at about -58 °C. Finally, a 1-2 nm Pt coating completed the sample preparation.

Reactive Oxygen Species (ROS) Measurement. 2,7-dichlorofluorescein diacetate (DCFH-DA) was used to probe the generation of ROS. Under alkaline conditions, DCFH-DA was converted into

2,7-dichlorofluorescin (DCFH), which was followed by transforming into highly fluorescent 2,7-dichloro fluorescein (DCF, excitation 488nm, emission at 524 nm, quantum yield: 90%) in the presence of ROS. SDHC (10 μM), PMNT (10 μM), SDHC/PMNT (10 μM) and DOX (10 μM) were added into the solutions of activated DCFH (40 μM), respectively. The solutions were irradiated under white light (400-800nm, 5 mW/cm^2) for 15 min, and the emission intensity of DCF solution at 525nm was recorded every minute with the excitation wavelength of 488 nm.

Isothermal Titration Microcalorimetry (ITC). The ITC experiment was taken in a TAM 2277-201 microcalorimetric system (Thermometric AB, Järfälla, Sweden) with a stainless steel sample cell of 1 mL at 25.00 ± 0.01 °C. Each ITC curve was repeated at least twice with deviation within ±5%. The sample cell was initially loaded with 600 μL MCF-7 cells solution (160000/mL), and then SDHC, PMNT or SDHC/PMNT solution (30 μM) was injected consecutively into the stirred sample cell in portions of 10 μL via a 500 μL Hamilton syringe controlled by a 612 Thermometric Lund pump until the desired range of concentration had been covered. The system was stirred at 60 rpm with a gold propeller.

Cell Culture. Three cancer cells were grown in DMEM with 10% FBS at 37 °C under a humidified atmosphere containing 5% CO_2. The cells were routinely passed by treatment with trypsin.

Defined procedures in cell treatment experiments. (1) SDHC/PMNT treatment: after the cells was seeded and cultured for 12 h, the culture medium was changed for fresh medium with 10 μM SDHC, PMNT or SDHC/PMNT. The cells were incubated for 1 h at 37 ^{o}C, and then the culture medium was discarded carefully. (2) DOX treatment: after SDHC/PMNT treatment, the culture medium with DOX was added quickly. (3) Dark or light treatment: after DOX treatment, the cells were incubated in the dark or under the white light at a fluence rate of 5 mW/cm^2 at room temperature for 1 h.

Cytotoxicity Assay. The cancer cells were seeded in 96-well culture plates at a density of 8×10^3 cells per well, and incubated for another 12 h. After different treatments, the drug was discarded and MTT (5 mg/mL in water, 10 μL per well) in culture medium was added to each well. After incubation for 4 h at 37 °C, the supernatant was abandoned and 100 μL of DMSO was added into each well to dissolve the produced formazan. After shaking the plates for 5 min, absorbance values of the produced purple formazan were recorded with a microplate reader (BIO-TEK Synergy HT, USA) at 570 nm.

Confocal Laser Scanning Microscopy (CLSM). Three cancer cells were seeded in 20 mm confocal dishes at a density of 8×10^4 cells per dish and further cultured in DMEM supplemented with 10% FBS for 12 h. After different treatments, the culture medium was abandoned and the cells were

washed thrice with phosphate buffered saline (PBS, pH 7.4). The confocal images were collected with a confocal laser scanning microscopy (FV1200-IX83, Olympus, Japan). The wavelength of stimulating laser of PMNT is 488 nm and that of SDHC-Cy5 and DOX is 559 nm. The false color of PMNT is green and the false color of SDHC-Cy5 and DOX is red.

Electrospray Ionization Mass Spectrometry (ESI-MS). The DOPC, SDHC/PMNT, and mixture of DOPC and SDHC/PMNT solutions were placed in the dark for 1 h. The mixture of DOPC and SDHC/PMNT solution was irradiated under white light for 1 h. Then all the ESI-MS spectra for the four samples were recorded on a SHIMADZU LCMS-2010 spectrometer.

Reference

(1) Wang, H.; Zhou, L. Y.; Zhou, C. C.; Zhao, W. W.; Wang, J. W.; Liu, L. B.; Wang, S.; Wang, Y. L. Preparation of Gemini Surfactant/Conjugated Polymer Aggregates for Enhanced Fluorescence and Bioimaging Application. *ACS Appl. Mater. Interfaces* 2017, *9*, 23544-23554.

Additional Results.

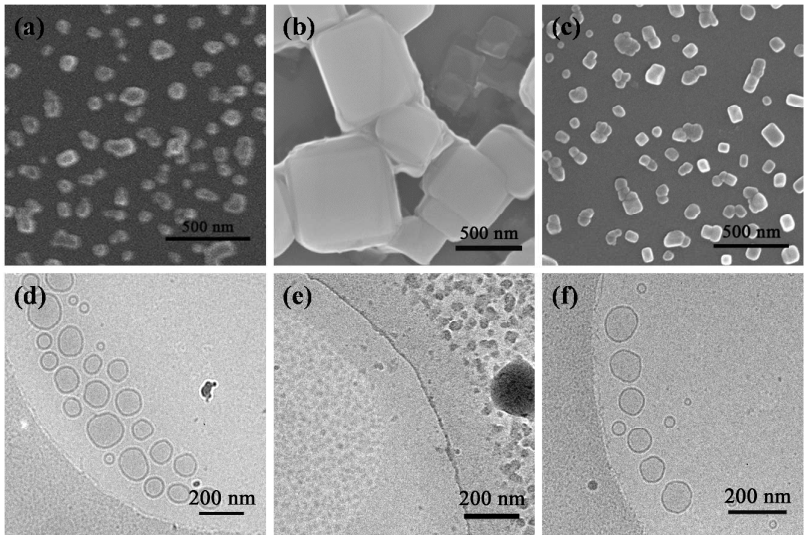

Figure S1. SEM micrographs of (a) SDHC, (b) PMNT and (c) SDHC/PMNT. Cryo-TEM micrographs of (d) SDHC, (e) PMNT and (f) SDHC/PMNT. [SDHC] = 100 μM, [PMNT] = 100 μM.

Table S1. The size and ζ-potential values of SDHC, PMNT and SDHC/PMNT. [SDHC] = 10 μM, [PMNT] = 10 μM.

	d/nm	ζ/mV
SDHC	146.7 ± 14.5	-30.2 ± 0.6
PMNT	1550 ± 54.6	36.8 ± 0.3
SDHC/PMNT	257.5 ± 5.6	-10.7 ± 3.0

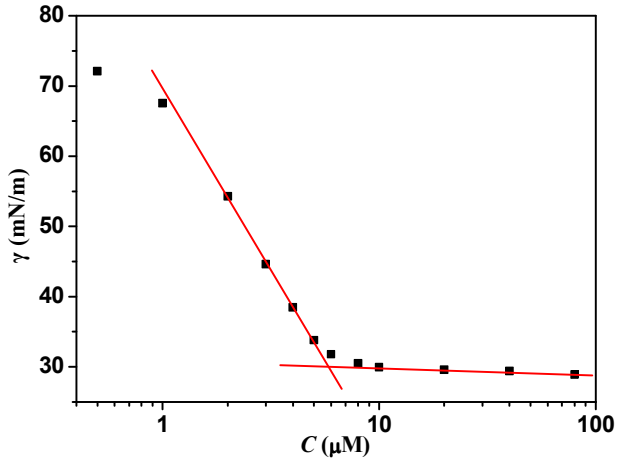

Figure S2. Variations of the surface tension with the SDHC/PMNT concentration at 25.0 °C.

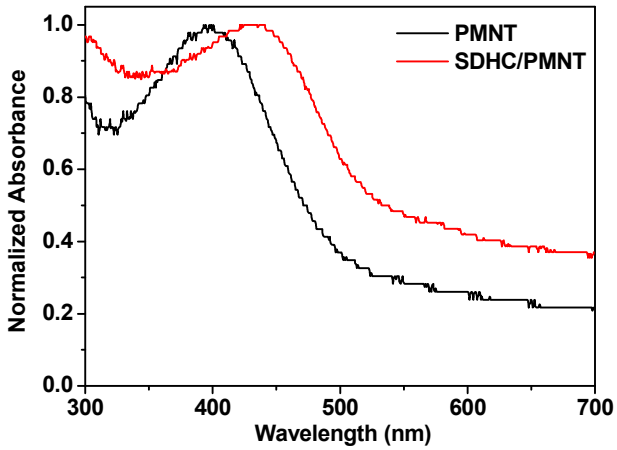

Figure S3. Normalized absorbance spectra of PMNT and SDHC/PMNT. [SDHC] = 10 μM, [PMNT] = 10 μM.

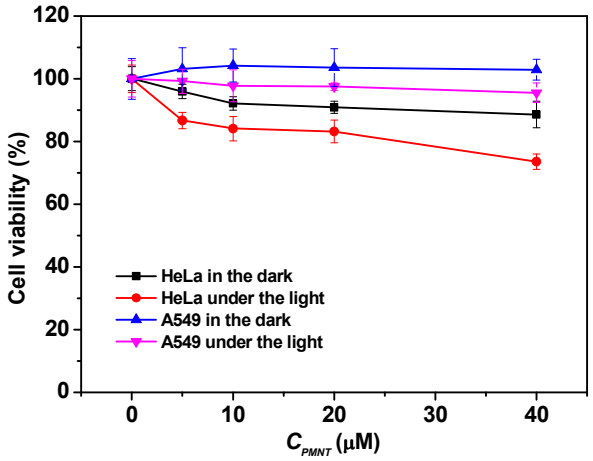

Figure S4. Cell viabilities of HeLa cells and A549 cells as a function of PMNT concentrations with 10 μM SDHC. All the cells were incubated with SDHC/PMNT treatment and dark or light treatment. After that, the cells were further cultured at 37 ^{o}C for 24 h.

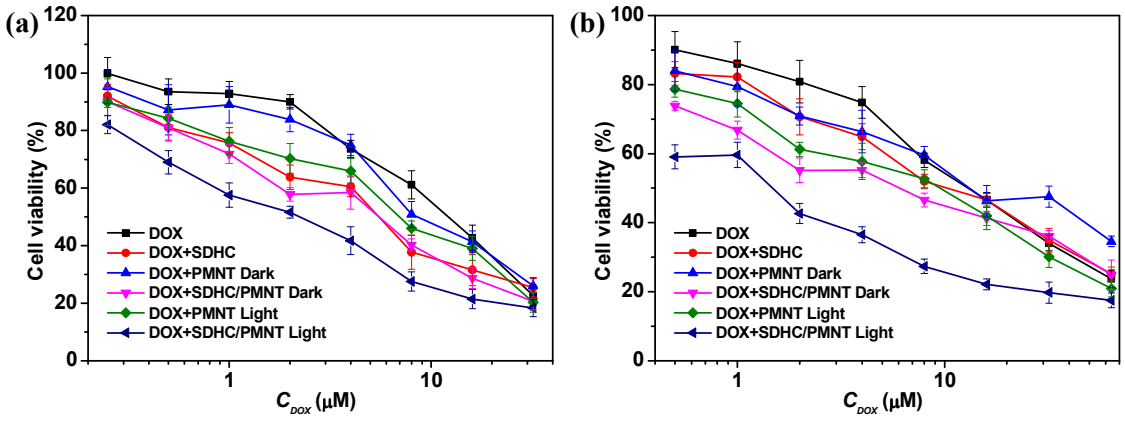

Figure S5. Cell viabilities of (a) HeLa cells and (b) A549 cells as a function of DOX concentrations. All the cells were incubated with three treatments. After that, the cells were further cultured at 37 ^{o}C for 24 h.

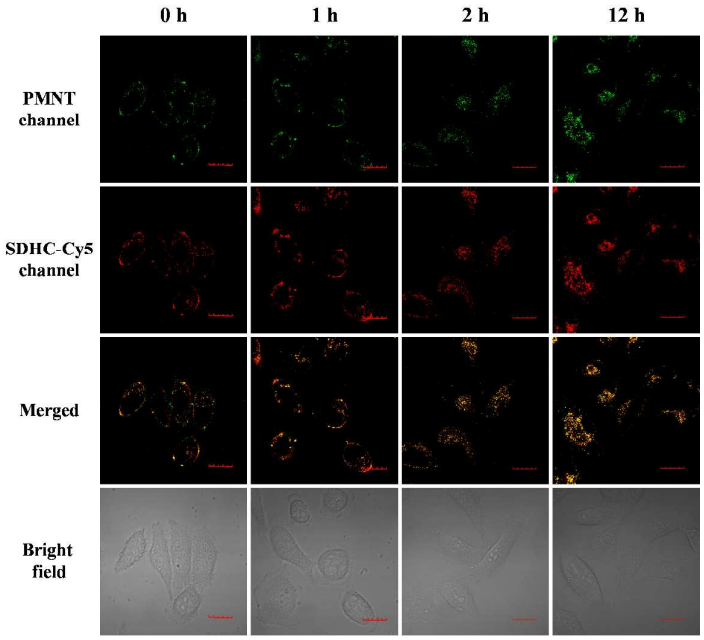

Figure S6. The CLSM images of SDHC/PMNT against uptake time in MCF-7 cells. The sample was cultured in fresh medium under dark from 0 h to 12 h after SDHC/PMNT treatment. The scale bar is 20 μm.

Table S2. Thermodynamic parameters of SDHC, PMNT and SDHC/PMNT with MCF-7 cells derived from ITC curves in Figure 3c.

	SDHC	PMNT	SDHC/PMNT
ΔH (kJ/mol)	-516.5	116.8	-236.8
$T\Delta S$ (kJ/mol)	-474.4	137.9	-202.1

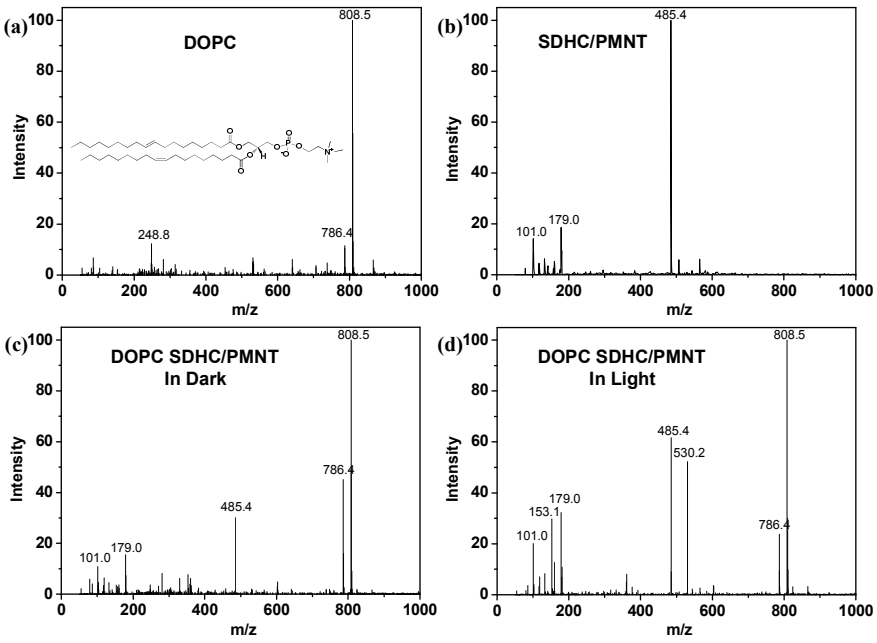

Figure S7. ESI-MS spectra of (a) DOPC, (b) SDHC/PMNT, (c) DOPC/SDHC/PMNT in dark and (d) DOPC/SDHC/PMNT in light.