Supporting Information

Supramolecular Polymeric Micelles by the Host-Guest Interaction of Star-like Calix[4]arene and Chlorin e6 for Photodynamic Therapy**

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1. Materials

p-Tert-butylphenol, formaldehyde solution (40%), potassium hydroxide, potassium carbonate, anhydrous aluminum chloride and *p*-toluenesulfonyl chloride were from Sinopharm Chemical Reagent Company. Methoxy poly(ethylene glycol) (PEG, Mn=1000) and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), HOECHST 33342 were purchased from Sigma-Aldrich. Chlorin e6 (Ce6) was supplied by the Chlorophyll Factory of Hangzhou Electrochemical Group Co., Ltd. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and phosphate buffered solution (PBS) were purchased from PAA Laboratories GmbH. Toluene and acetonitrile were dried under calcium hydride and distilled prior to use. All the other chemicals were used as received without any further purification. Clear polystyrene tissue culture treated 6-well, and 96-well plates were obtained from Corning Costar.

2. Synthesis of 5,11,17,23-De-tert-butyl-25,26,27,28-tetra-poly(ethylene glycol)$_{1000}$ calix[4]arene (DC4-PEG)

Starting material, p-tert-butylcalix[4]arene was synthesized from cyclization of p-tert-butylphenol and formaldehyde [1]. In order to enlarge the cavity of p-tert-butylcalix[4]arene for the encapsulation of guest molecules, the tert-butyl groups were removed as described previously [2]. Then four hydrophilic PEG chains were grafted onto the lower rim of 5,11,17,23-De-tert-butyl calix[4]arene (DC4) to

obtain a star PEGylated calix[4]arene (DC4-PEG). The synthetic route of DC4-PEG is described in Figure S1.

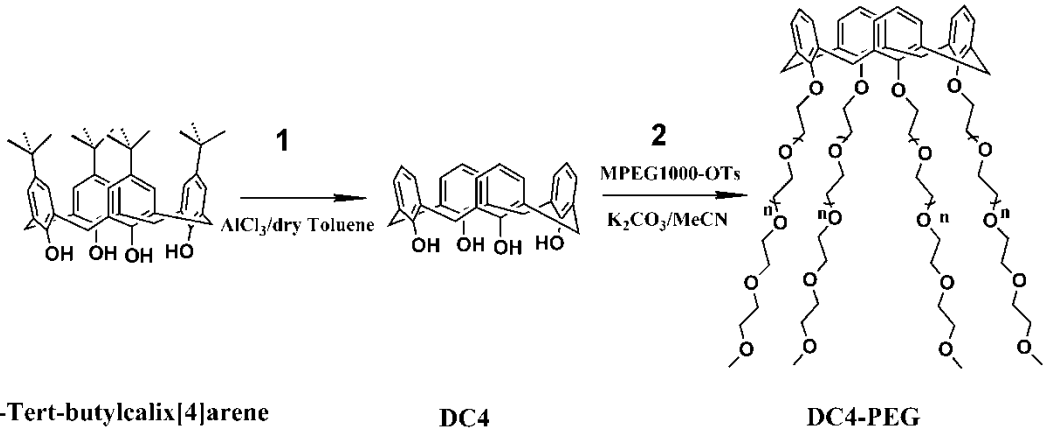

Fig. S1. Synthetic route of 5,11,17,23-De-tert-butyl-25,26,27,28-tetra-poly(ethylene glycol)$_{1000}$ calix[4]arene (DC4-PEG).

A slurry of 5,11,17,23-De-tert-butyl calix[4]arene (DC4, 0.636 g, 1.5 mmol), tosylated PEG (PEG-OTs, 7.2 g, 6.3 mmol) [2] and K_2CO_3 (1.1 g, 8 mmol) was refluxed in dry acetonitrile (150 mL) for 48 h under a nitrogen atmosphere. After the reaction, the solvent was removed by rotatory evaporator. Then the mixture was dissolved in deionized water (20 mL) and extracted with dichloromethane (2 x 100 mL). The combined organic extracts were evaporated under reduced pressure to afford yellowish paste, and then purified with silicon column TLC. The mixture solvent of CH_2Cl_2/CH_3OH=15:1 (v/v) was taken as eluent. The purified product was precipitated in ice-cold diethyl ether to obtain white powder (2.1 g, yield: 48%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ ppm: 3.20 (s, 12H, O*CH$_3$*), 3.38-4.33 (m, 330H, O*CH$_2$*CH$_2$O, ArC*H$_2$*Ar), 6.61 (t, 4H, *p*-phenol), 7.03 (d, 8H, *m*-phenol). ^{13}C NMR (400 MHz, $CDCl_3$, 298 K) δ ppm: 31.3, 32.2, 35.3 (Ar-*C*H$_2$-Ar), 59.1 (O*C*H$_3$), 69.4,

70.8, 72.0, 73.1, 75.2 (OCH_2CH_2O), 121.9, 130.0, 133.5, 155.9 (phenol). IR (cm^{-1}): 2947 ($\nu_{as\ CH2}$), 2873 ($\nu_{s\ CH2}$), 1638, 1447, 1348, 1344, 1301, 1241, 1106 ($\nu_{as\ C\text{-}O\text{-}C}$), 946, 833 ($\nu_{C\text{-}C\text{-}O}$).

3. Characterization of DC4-PEG and DC4-PEG/Ce6

Both ^{1}H NMR and ^{13}C NMR measurements were carried out on Varian Mercury plus 400 NMR spectrometer (America) using $CDCl_3$ or d_6-DMSO as the solvents at 25 ^{o}C. Fourier transformed infrared (FTIR) measurements were performed on a Bruker Equinox-55 FTIR spectrometer (KBr pellets).

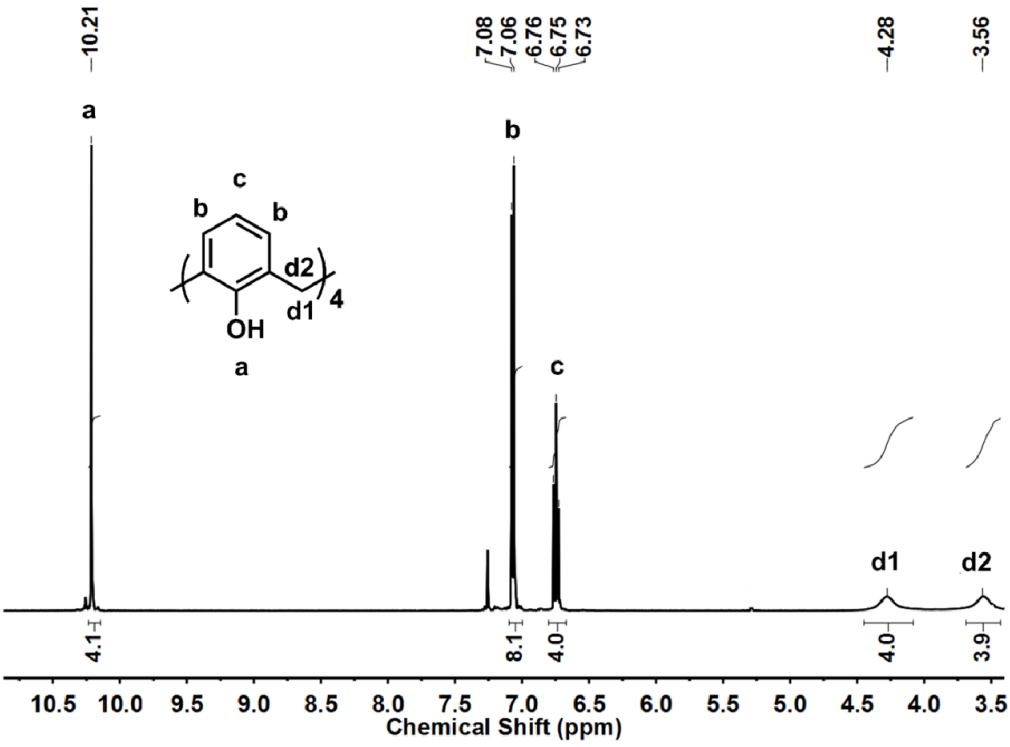

Fig. S2. ^{1}H NMR spectrum of 5,11,17,23-De-butyl calix[4]arene (DC4).

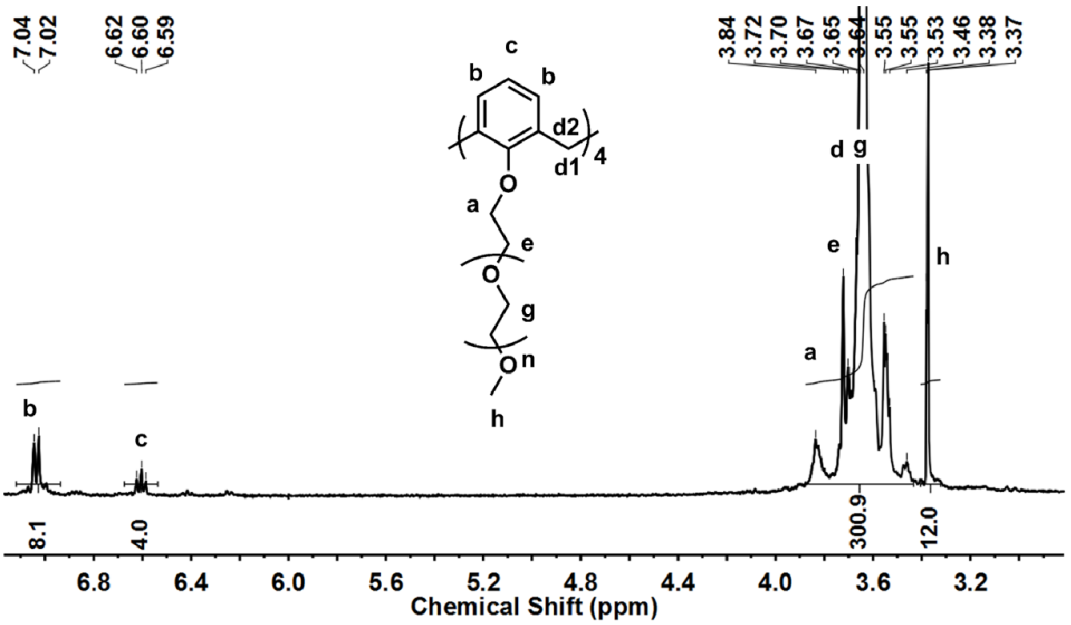

Fig. S3. ^{1}H NMR spectrum of 5,11,17,23-De-tert-butyl-25,26,27,28-tetra-poly(ethylene glycol)$_{1000}$ calix[4]arene (DC4-PEG).

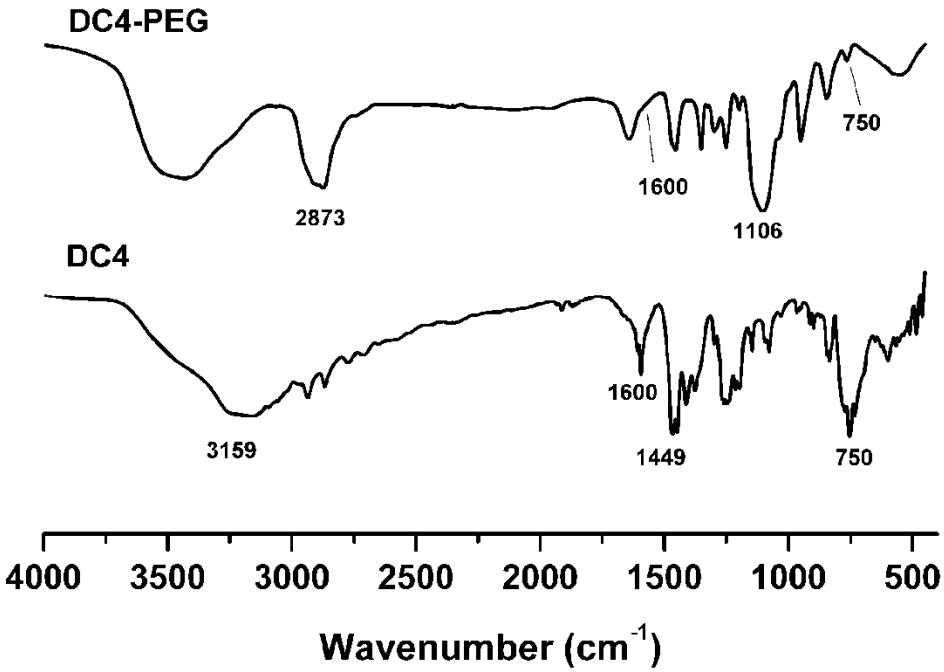

Figure S4. FTIR spectra of DC4 and DC4-PEG.

4. ^{1}H NMR titration

The equal amount of DC4-PEG (8.8 mg, 2 μmol) was dissolved in d_6-DMSO (0.5 mL) in seven NMR tubes, and then different amount of Ce6 was added with DC4-PEG/Ce6 mole ratio from 8:1 to 1:8. Subsequently, 50 μL D_2O was injected by pipettor. The complexes were treated under ultrasonic oscillation for 5 minutes.

5. Self-assembly of Ce6 and DC4-PEG

Both Ce6 (5 mg, 8.3 μmol) and DC4-PEG (36.5 mg, 8.3 μmol) were dissolved in d_6-DMSO (0.5 mL). ^{1}H NMR spectra were recorded by adding different amount of D_2O into d_6-DMSO in the same tube.

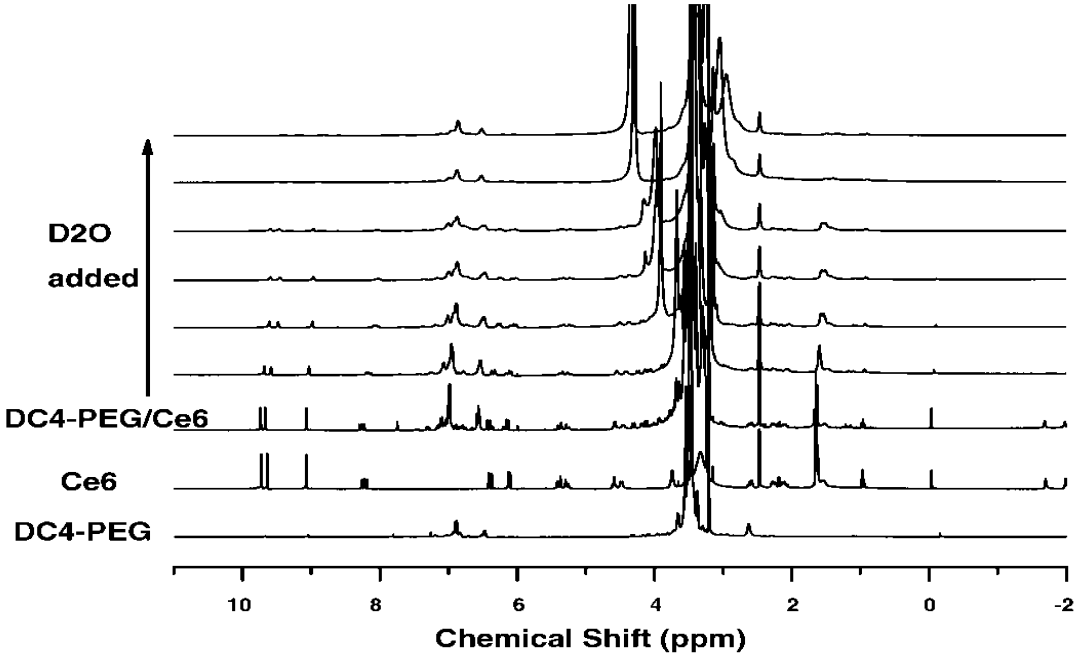

Fig. S5. ^{1}H NMR spectra of Ce6, DC4-PEG/Ce6 complexes in d_6-DMSO and D_2O mixture with different D_2O content (vol%): From buttom to top is 0, 9.1, 16.7, 23.1, 28.6, 37.5, 44.4, respectively.

6. Characterization of micelle size and morphology

A combination of dynamic light scattering (DLS) and transmission electron microscopy (TEM) was used to characterize the size and morphology of micelles. The hydrodynamic diameter of the DC4-PEG/Ce6 micelles was determined by DLS at an angle of 135° and temperature of 25 °C (Zetasizer Nano-S, Malvern Instrument Ltd., England). The micelle morphology was observed by TEM on a JEM 2010 microscope at an accelerating voltage of 200 kV (JEOL, Japan). In details, a drop of DC4-PEG/Ce6 solution (1 mg/mL) was spreaded onto an amorphous holey-carbon film supported by a copper grid (XinXingBaiRui, Beijing, China), then lyophilized by a freeze dryer (Christ Alpha 1-4 LD plus, Germany) to observe morphology.

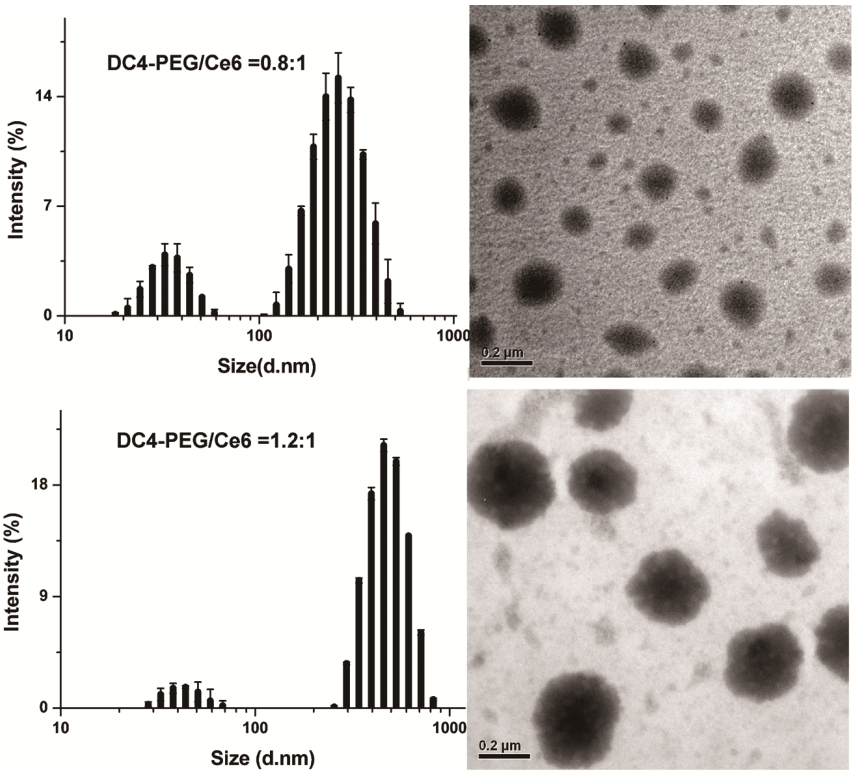

Fig. S6. DLS plots and representative TEM images of DC4-PEG/Ce6 micelles (0.8:1) and (1.2:1).

7. Cell culture and dark cytotoxicity assay

HeLa cells (a human uterine cervix carcinoma cell line) were cultured in DMEM supplied with 10% FBS, and antibiotics (50 units/mL penicillin and 50 units/mL streptomycin) in a humidified atmosphere containing 5% CO_2 at 37 ^{o}C. Dark cytotoxicity of free Ce6, DC4-PEG and DC4-PEG/Ce6 against HeLa cells was assessed in vitro with the MTT assay. HeLa cells were seeded into 96-well plates at an initial seeding density of 8000 cells/well in 200 μL medium and incubated for 24 h. The culture medium was replaced with 200 μL serum-free culture medium containing serial dilutions of free Ce6 or DC4-PEG/Ce6. The cells were grown for another 24 h. Then 20 μL of 5 mg/mL MTT assays stock solution in PBS was added to each well. After incubation for 4 h, the medium containing unreacted dye was removed carefully. The obtained blue formazan crystals were dissolved in 200 μL per well DMSO and the absorbance was measured in a BioTek Elx800 at a wavelength of 490 nm.

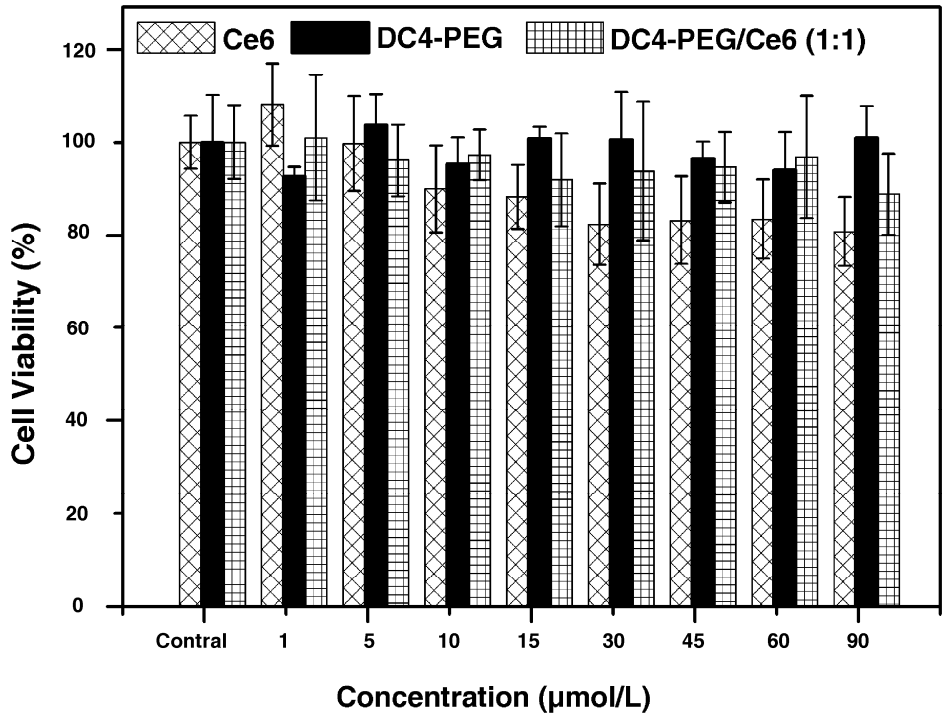

Fig. S7. Cell viability of HeLa cells against Ce6, DC4-PEG and DC4-PEG/Ce6 micelles after incubation for 24 h with different concentrations.

8. Cellular uptake of free Ce6 and DC4-PEG/Ce6

Cellular uptake experiments were performed using flow cytometry, which was used to provide statistics on the uptake of free Ce6 and DC4-PEG/Ce6 micelles into HeLa cells. HeLa cells (5.0×10^5 cells per well) were seeded on 6-well culture plates and grown overnight. Then the free Ce6 and DC4-PEG/Ce6 micelles dissolved in DMEM culture medium with a Ce6 concentration of 75 μmol/L were added to different wells, and the cells were incubated at 37 ^{o}C for 5, 30, 120 and 240 min. After washing with PBS, medium replacement, and trypsin treatment, samples were prepared for flow cytometry analysis. Data for 1.0×10^4 gated events were collected and analysis was

performed by means of a flow cytometer (FACSCalibur, BD Co., America). The interpretation of flow cytometry analysis was carried out using the Cellquest software.

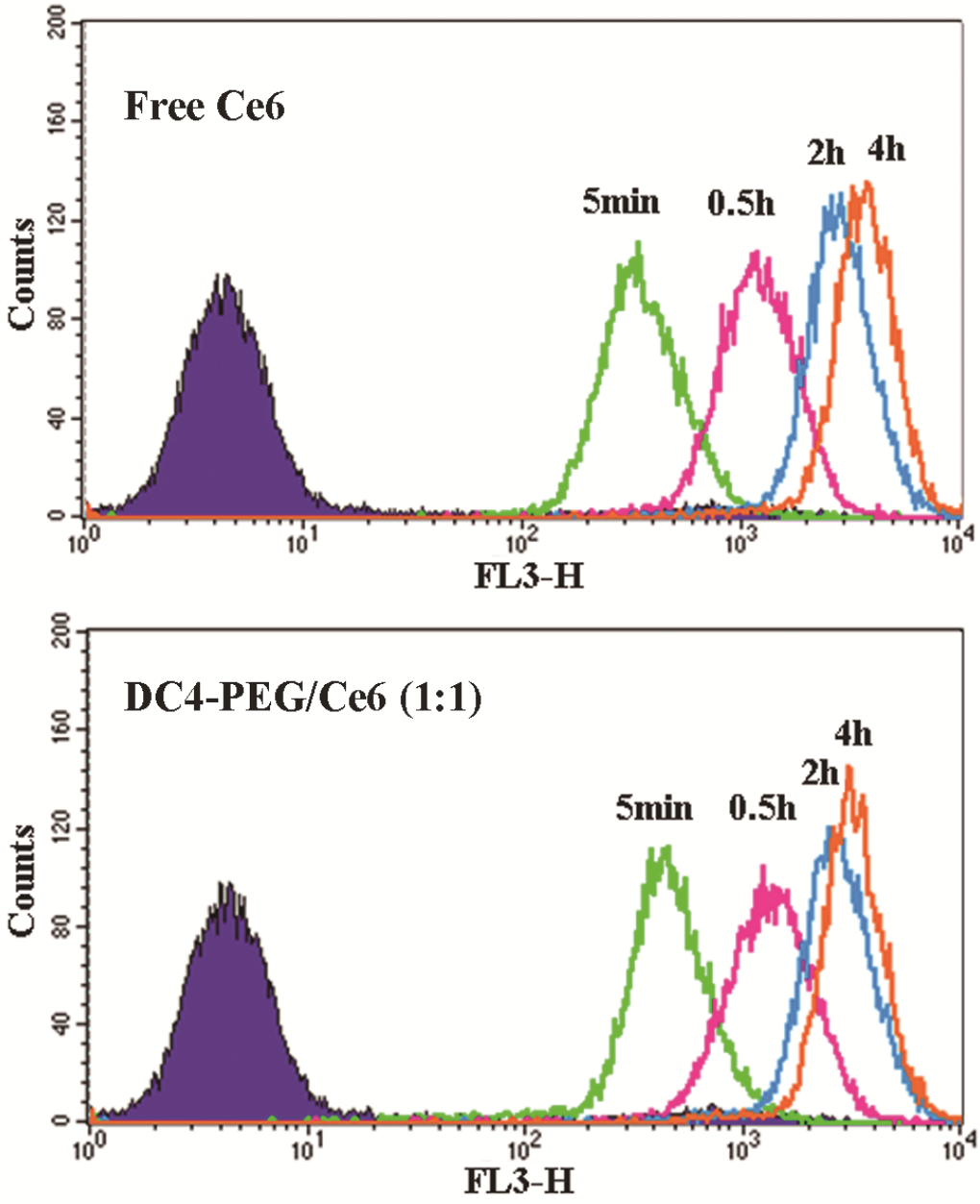

Fig. S8. Flow cytometry histogram profiles of HeLa cells that were incubated with free Ce6 and DC4-PEG/Ce6 micelles for 5 min, 0.5 h, 2 h and 4 h at 37 ^{o}C.

For confocal laser scanning microscopy (CLSM) studies, HeLa cells (2.0×10^5) were seeded on cell culture coverslips in a 6-well tissue culture plate. After 24 h incubation, the free Ce6 and DC4-PEG/Ce6 (1:1) dissolved in DMEM culture medium with a Ce6 concentration of 75 μmol/L were added to different wells and the cells were incubated at 37 ^{o}C for predetermined time intervals. Then the cells were washed with PBS and fixed with 4% paraformaldehyde for 30 min at room temperature, and the slides were rinsed with PBS for three times. Finally, the cells were stained with HEOCHST 33342 for 10 min and the slides were rinsed with PBS for three times. The slides were mounted and observed by a confocal laser scanning microscopy (BX61W1, OLYMPUS Co., Japan) with a 40x oil-immersion objective lens. Fluorescence images of HEOCHEST-loaded nucleus were excitated at 488 nm, and Ce6 excitation was at 633 nm.

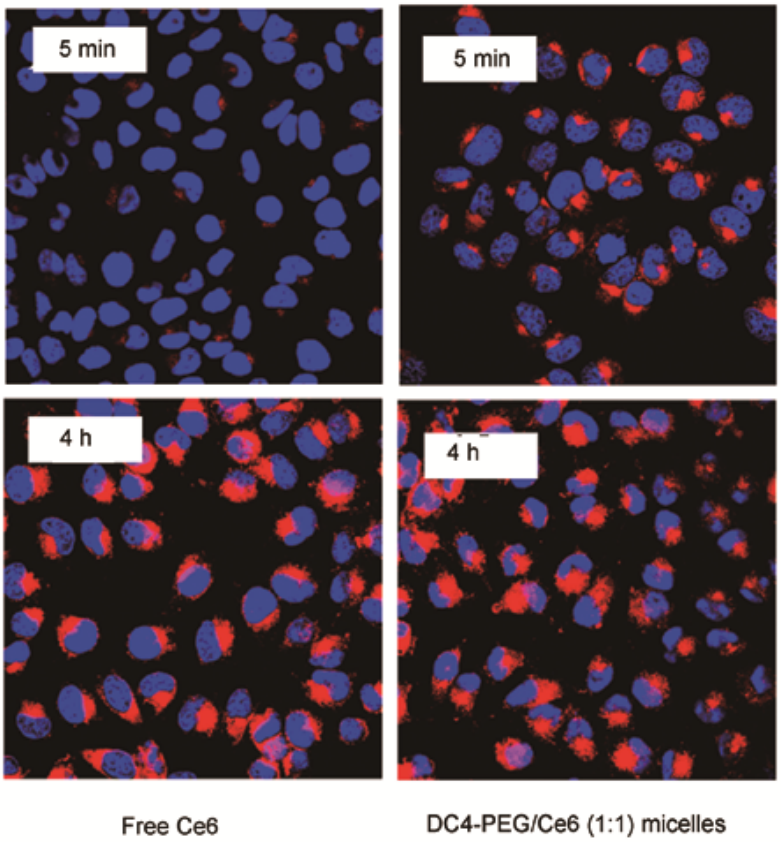

Fig. S9. CLSM images of HeLa cells for incubation with free Ce6 (left) and DC4-PEG/Ce6 micelles (right) for 5 min, 2 h and 4 h at 37 ^{o}C. (Cell nuclei were stained with Heochest 33342 in blue and Ce6 was in red.)

9. In vitro phototoxicity effect

To investigate the effect of photoirradiated cell death, HeLa cells were also chosen for in vitro assay. The cells culture and seeding are the same as dark toxicity assay. Incubated for 4 h after addition of PSs, each well was photoirradiated by a 630 ± 5 nm, continuous wave diode laser (PDT630 Diode Laser Devices, XingDa Photo & Electrics Medical Instrument Co., Ltd, China) (fluence rates: 120 mW/cm^2, irradiation time: 500 s, fluence: 20 J/cm^2). The next operation was the same to dark toxicity

assay. After photoirradiation, the cells were incubated for another 24 h, and then the cell viability was evaluated by MTT assay.

References

[1] C. D. Gutsche, M. Iqbal and D. Stewart, *J. Org. Chem*., **1986**, *51*, 742-745.

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[3] L. J. Zhu, Y. F. Shi, C. L. Tu, R. B. Wang, Y. Pang, F. Qiu, X. Y. Zhu, D. Y. Yan, L. He, C. Y. Jin and B. S. Zhu, *Langmuir*, **2010**, *26*, 8875-8881.