Electronic Supplementary Information

Water-Soluble Chiral CdSe/CdS Dot/Rod Nanocrystals for Two-Photon Fluorescence Lifetime Imaging and Photodynamic Therapy

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Cell Culture and Cell Viability Assay. The Hela cells were cultured in Dullbecco's modified Eagle medium (DMEM), containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C in a 5% CO_2/95% air incubator. The methyl thiazolyl tetrazolium (MTT) assay was used to determine the cellular viability. About 6000 cells per well were seeded in 96-well plates and cultured overnight for 70–80% cell confluence. The cells were then cultured in the medium supplemented with indicated doses of target samples for 24 h. After that, 10 μL MTT (0.5 mg/mL) solution was added into each well. After 3 h incubation at 37 °C, the supernatant was removed and 200 μL of dimethyl sulfoxide (DMSO) was added. A PowerWave XS/XS2 microplate spectrophotometer was used to record the absorbance intensity at 490 nm. The absorbance of the untreated cells was used as a control and its absorbance was set as the reference value for calculating 100% cellular viability.

Two-photon FLIM of living cells. The Hela cells were seeded in a single dish plates (4000 cells per well) for 24 h in a humidified, 5% CO_2 atmosphere at 37°C. Then L-cysteine-functionalized CdSe/CdS dot/rod NCs (5 nM) was added for 4 hours. Washed once with PBS buffer. Images of CdSe/CdS dot/rod NCs stained cells were collected at 600-650 nm under 800 nm excitation with Zeiss LSM 880 meta NLO inverting-typed laser scanning confocal microscope.

Measurement of the singlet oxygen generation ($^1O_2$) efficiency. Water-soluble anthracene-9,10-diyl-bis-methylmalonate (ADMA) was used as an $^1O_2$-trapping agent, and $TMPyP_4$ was used as a standard photosensitizer, whose $^1O_2$ quantum yield is Φ_r = 74% in water.[2,3] In the experiment, 20 μL ADMA in a PBS solution (1 mg/mL) was added to CdSe/CdS NCs in a PBS solution, and a white light emitting diode was employed as the irradiation source. The absorption of ADMA at 261 nm was recorded after various irradiation times to obtain the decay rate of the photosensitizing process. The $^1O_2$ quantum yield of the photosensitizer (Φ_Δ) for CdSe/CdS dot/rod NCs can be calculated according to the following formula:

$$\phi_\Delta = \phi_r \frac{K_{NCs} A_r}{K_r A_{NCs}}$$

where K_{NCs} and K_r are the decomposition rate constants of ADMA for the CdSe/CdS dot/rod NCs and $TMPyP_4$, respectively, and A_{NCs} and A_r represent the light absorbed by the CdSe/CdS dot/rod NCs and $TMPyP_4$, respectively, which were determined by integration of the optical absorption bands in the wavelength range of 200-700 nm.

Two-photon PDT. The Hela cells were seeded in the culture plates (4000 cells per well) at 37°C for 24 h, and then the cells were washed once with PBS. DMEM medium (2 mL) with L-cysteine-functionalized CdSe/CdS dot/rod NCs (5 nM) was added. After the cells were further cultured for 4 h, the cells were stained with Calcein-AM and PI, the plates were irradiated under 1 kHz femtosecond 800 nm light (1 W cm^{-2}) for 10 min, and the cells were inculcated another 4 h for apoptosis. Then the cell death imaging was visualized with inverting-typed laser scanning confocal microscope (Zeiss LSM 880 meta NLO) at time-lapse model. Under the excitation at 488 nm, collection ranges were 500-550 nm for Calcein-AM emission and 600-650 nm for PI emission, respectively.

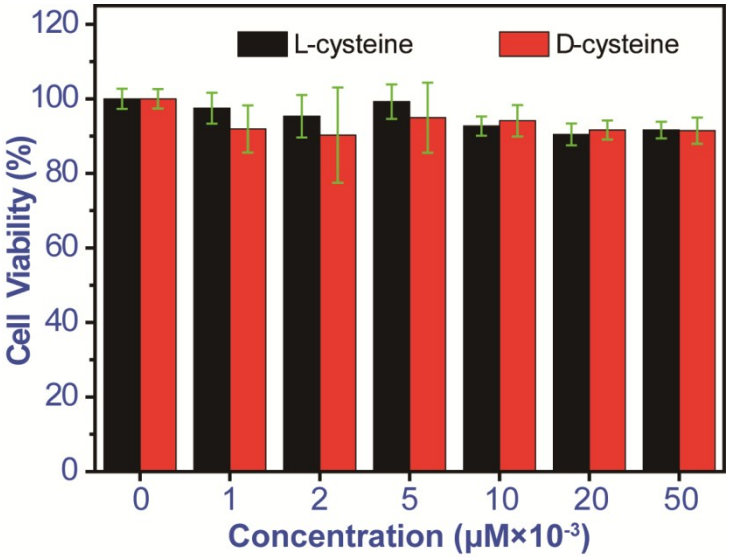

Figure S1 Viabilities of HeLa cells treated with D- and L-cysteine at different concentrations.