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Biocompatible CdSe Quantum Dot-Based Photosensitizer under Two-Photon Excitation for Photodynamic Therapy

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1. Materials and Methods

All chemicals used were obtained from Aldrich Chemical Company. Electronic absorption spectra in the UV/Vis absorption were recorded on a Varian Cary 100 UV-visible spectrophotometer. Steady-state visible photoluminescence (PL) and excitation spectra were recorded on a Photon Technology International (PTI) Alphascan spectrofluorimeter. ^{1}H NMR spectra were recorded on a Varian INOVA 400 MHz spectrometer. High-resolution mass spectra, reported as *m/z*, were obtained on a Autoflex Bruker MALDI-TOF system. The hydrogen diameters of the mean size and distribution of the biocompatible QDs were determined by dynamic light scattering (DLS) at a scattering angle of 173^{0} (Zetasizer Nano-ZS, Malvern, UK) at 25℃, employing an argon laser (λ = 500 nm).

2. Synthesis and Measurement.

Quantum Dots. Core shell CdSe/ZnS protected by tri-*n*-octylphosphine oxide (TOPO) with a 4-5 nm average diameter were synthesized according to published methods but with modifications to the shell growth temperatures. For this work, we used 200℃ for ZnS shell growth. All the QDs were purified and stored in hexane before further treatment. QDs concentrations were determined using the extinction coefficients, and the error in these measurements was estimated by comparing the wide range of reported extinction coefficients in the literature.

N_3-PEG-N_3 (n=8, 12). Poly(ethylene glycol) (average MW 400, 600) (0.175 mol), tetrahydrofuran (THF) (200 mL), and methanesulfonyl chloride (46.1 g, 0.402 mol) were placed in a 500mL round-bottom flask and cooled to ~0 °C. Triethylamine (60.0 mL, 0.430 mol) was added dropwise over 30 min. The reaction mixture was gradually warmed to room temperature and

stirred overnight. The mixture was then diluted with H_2O (200 mL) and $NaHCO_3$ (12.5 g, 0.149 mol). Sodium azide (31.0 g, 0.477 mol) was added, and the biphasic reaction mixture was heated to distill off the THF and then refluxed overnight. After cooling, the reaction mixture was extracted five times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give pale brown oil. The crude product was chromatographed on silica gel with 20:1 CH_2Cl_2:MeOH as the eluent; the reaction yielded 73.13 g (~90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.65-3.69 (m), 3.40 (t, 4H, J_1=5.16 Hz, J_2=4.96 Hz). IR (neat): 2869.7, 2108.3, 1452.5, 1350.3, 1300.2, 1117.4 cm^{-1}.

H_2N-PEG-NH_2 (n=8, 12). 48.3 mmol of N_3-PEG-N_3 was dissolved in 300 mL of THF, and triphenylphosphine (27.9 g, 106 mmol) was added. The solution was stirred at 25 °C for 4 h before adding 4 mL of water and stirring overnight. The THF was removed in vacuo, and 100 mL of water was added. The precipitate was removed by vacuum filtration and the filtrate washed with toluene (3 ×50 mL). The water was removed in vacuo to yield the pure product as light-yellow oil (~70%).^{1}H NMR (400 MHz, $CDCl_3$): δ 3.6-3.8 (m), 3.24 (t, J =4.92 Hz, 4H). IR (neat): 3416.7, 2871.4, 1677.2, 1471.5, 1350.7, 1301.2, 1108.2, 950.9 cm^{-1}.

H_2N-PEG-NH_2 (n=8, 12) modified Amphiphilic Polymers. Poly(maleic anhydride-alt-1-octadecene) (PMAO, M_n) 30 000-50 000, Sigma-Aldrich, St. Louis, MO) reacted with diamine poly(ethylene glycol) (n=12) in chloroform overnight (at room temperature) to form amphiphilic polymers (PMAO-PEG) (molar ratio of PMAO/PEG ranged from 1:10 to 1:30).

Water-Soluble Quantum Dots. The monodisperse QDs (purified and dispersed in chloroform) and the amphiphilic polymer (PMAO-PEG) were mixed in chloroform and stirred overnight (room temperature) (molar ratio of QD/PMAO-PEG ranged from 1:5 to 1:30). After that, chloroform was

then gradually removed by rotary evaporation at room temperature. The PBS buffer solution of water-soluble QDs was sonicated for 2 h resulting in a clear solution. This transfer process had a 100% efficiency, and no residue was observed; however, to remove possible larger contaminants the solutions were passed through a 0.2 μM Nylon syringe filter. An ultracentrifuge (Beckman Coulter Optima L-80XP) was used to further concentrate and purify (remove excess amphiphilic polymer) the materials (typically at 200000-250000*g* for 1.5 h).

Water soluble porphyrin (TrisMPyP-COOH**).** Preparation of water soluble porphyrin (TrisMPyP-COOH) was shown in Scheme SI2, which was according to Ref. [1].

Two photon measurement In the cavityless upconversion lasing experiment, the pump source was an Optical Parametric Amplifier (Topas, Coherent) which is pumped by self mode-locked Tisapphire laser with Spitfire Regenerative Amplifier (Spectra Physics–Tsunami; 800nm, pulsed width 100fs, repetition rate 1 kHz). The laser passed through a polarizer, a focusing lens and IR filter (Hot Filter) was used to block the 800nm light. The incident laser was focused into the center of the sample solution inside the cuvette. A second lens focused transmitted signal into the spectrometer (Ocean Optics, USB4000). The same laser system was used for PL experiments.

Determination of the two-photon action cross-section The two-photon action cross-section ($\square_{\sigma 2P}$) was determined by comparison to a standard. Rhodamine 6G with a reported 3.6 GM at 1064 nm excitation was used as the standard material for the present set of experiments [2]. Within this framework, the two-photon action cross-section of a QD sample is given by:

$$\phi\sigma_{2P(QD)} = \frac{PL_{QD} \times c_{Rh} \times QY_{Rh} \times \eta_{Rh} \times n_{Rh}}{PL_{Rh} \times c_{QD} \times QY_{QD} \times \eta_{QD} \times n_{QD}} \times \phi\sigma_{2P(Rh)}$$

where: PL_{QD} and PL_{Rh} designate the photoluminescence intensities measured for QD and Rhodamine 6G, respectively; c is the concentration and QY is the quantum yield of the

fluorophore (Rhodamine 6G or QDs); η_{QD} and η_{Rh} designate the CCD detector efficiencies at the QD and Rhodamine 6G emission wavelengths, respectively; n_{QD} and n_{Rh} are the refractive indices of the solvents used for the QD sample and the Rhodamine 6G standard.

Measurement of FRET efficiency and donor–acceptor distances *r* The FRET efficiency, *E*, can be measured experimentally and is commonly defined as

$$E = 1 - \frac{F_{DA}}{F_D}$$

where F_{DA} is the integrated fluorescence intensity of the donor in the presence of the acceptor(s) and F_D is the integrated fluorescence intensity of the donor alone (no acceptors present). So the distance *r* is defined as an expression for the donor-acceptor separation distance for each number *n*

$$r_n = [\frac{nR_0{}^6(1-E)}{E}]^{1/6} = R_0[\frac{n(1-E)}{E}]^{1/6}$$

where *n* is the average number of acceptor molecules interacting with one donor; R_0 is the Förster radius [3].

References:

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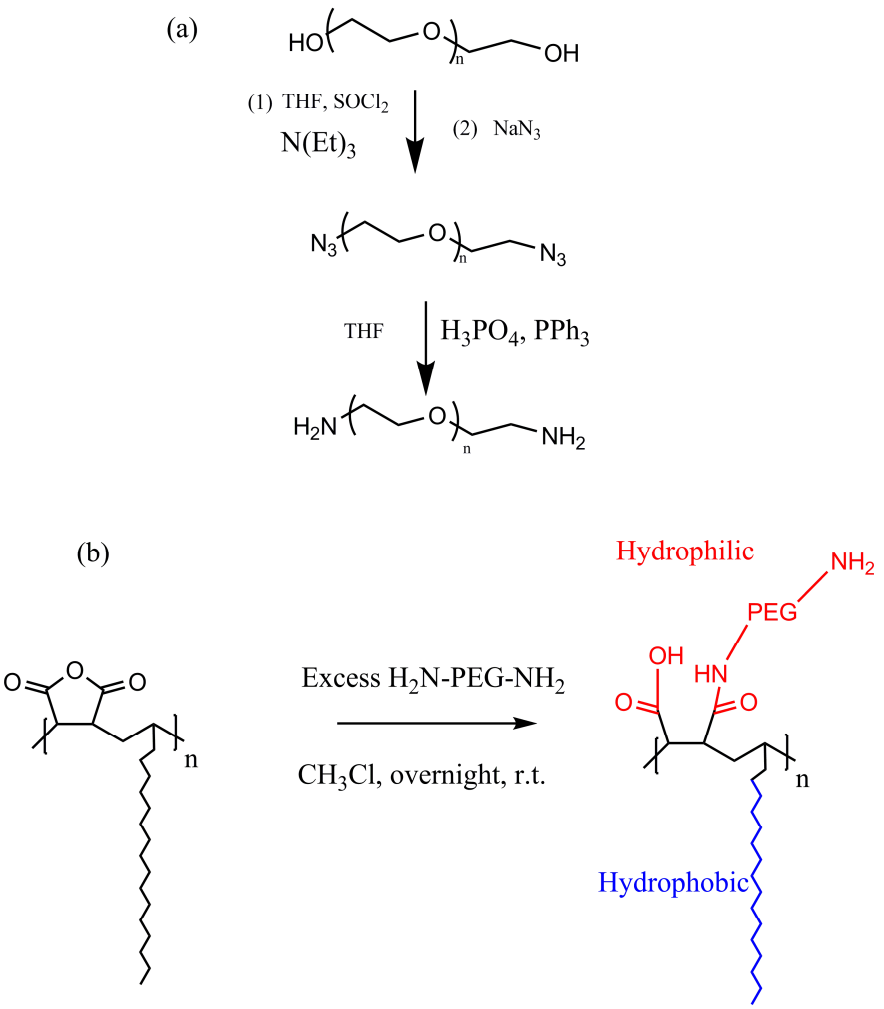
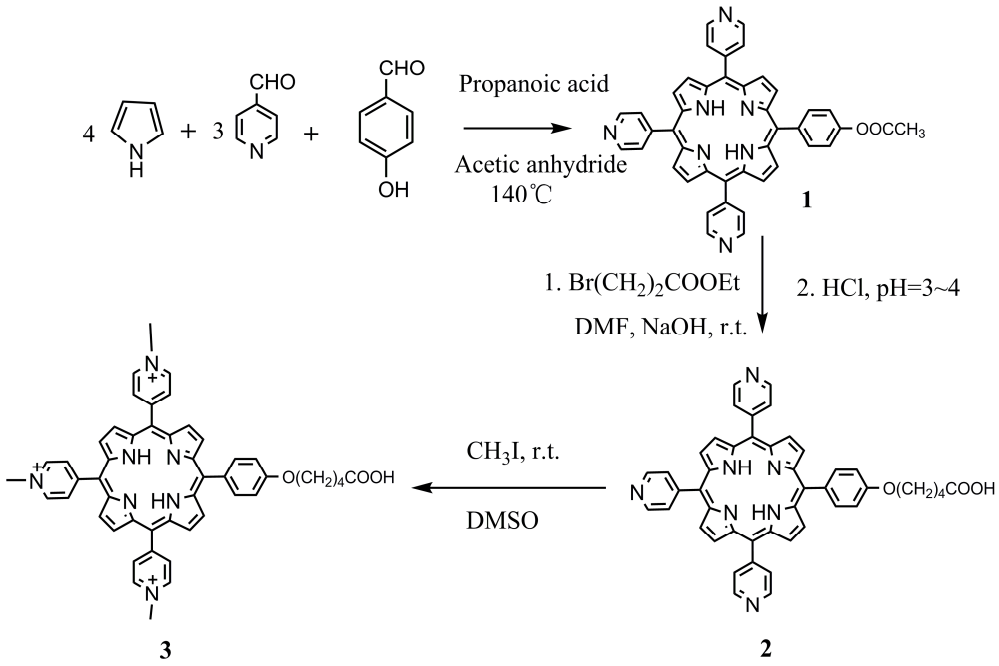
[2] Kaatz, P.; Shelton, D. P.; *J. Opt. Soc. Am. B.* **1999**, *16*, 998.

[3] Clapp, A. R.; Medintz, I. L.; Mauro, J. M.; Fisher, B. R.; Bawendi, M. G.; Mattoussi, H.; *J. Am. Chem. Soc.* **2004**, *126*, 301.



Scheme SI1. (a) Preparation of diamine-functioned poly(ethylene glycol) (n=8, 12) (b) Chemical modification of a block copolymer with an 16-carbon side chain. This hydrophobic side chain is directly attached to the hydrophilic acrylic acid segment and interacts strongly with the hydrophobic tails of TOPO. Dynamic light scattering shows a compact QD-polymer structure, indicating that QDs are tightly wrapped by the hydrophobic segments and hydrocarbon side chains.



Scheme SI2. Preparation of water soluble porphyrin (TrisMPyP-COOH).

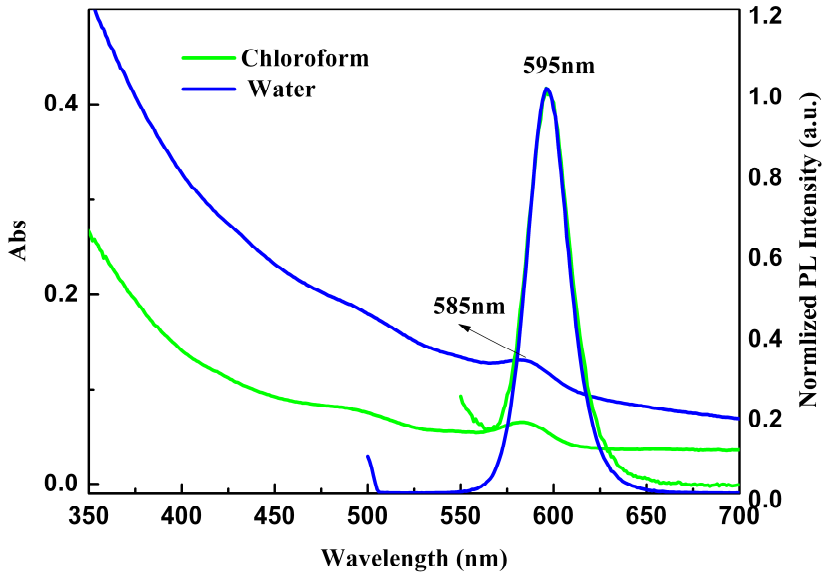


Figure SI1. UV-vis absorption and fluorescence spectra of QDs in organic solvent and in the water environment. The emission maximum was not changed with the change of environment.

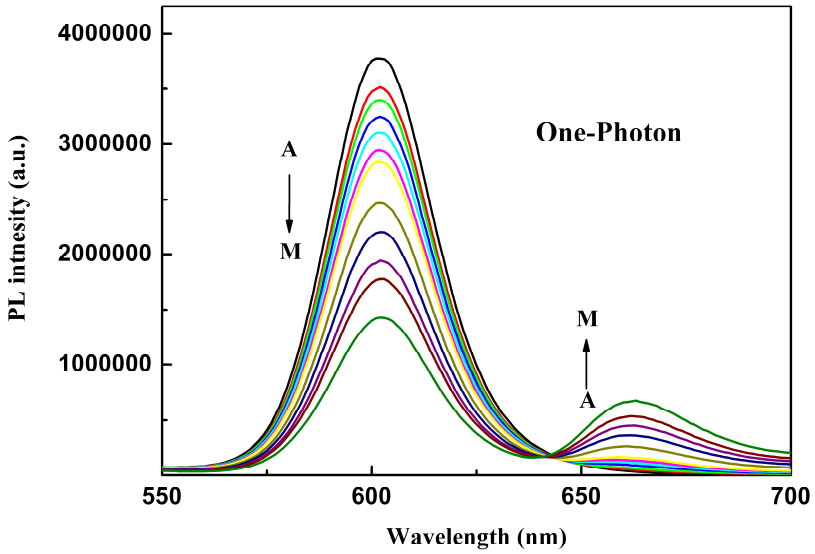


Figure SI2. PL spectra of QDs titrated by porphyrin using one-photon excitation (λ_{ex}=480 nm) [QDs]=0.25 μM, A-M, [porphyrin] =0, 0.45, 0.90, 1.82, 2.73, 3.63, 4.54, 9.10, 13.6, 18.17, 22.71, 31.80 μM.

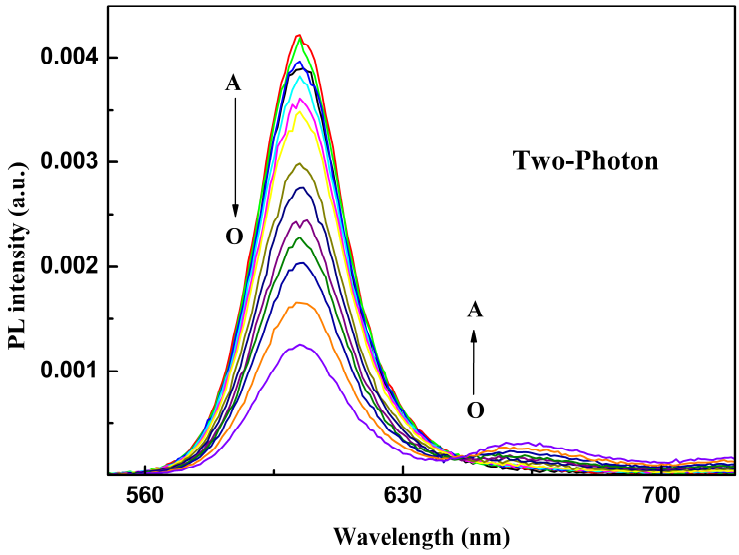


Figure SI3. PL spectra of QDs titrated by porphyrin using two-photon excitation (λ_{ex}=960 nm) [QDs]=0.25 μM, A-O, [porphyrin]=0, 0.45, 0.90, 1.82, 2.73, 3.63, 4.54, 9.10, 13.6, 18.17, 22.71, 31.80, 45.43, 68.14 μM.

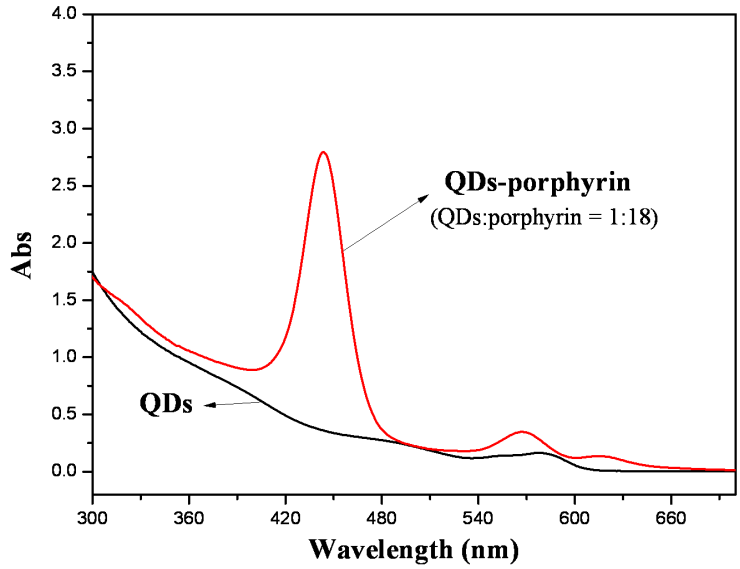


Figure SI4. The UV-vis absorption spectra of QDs and QDs-porphyrin.[QDs]=0.745 μM; [porphyrin]=13.5 μM; the ratio of QDs to porphyrin is 1:18.