Electronic Supplementary Material (ESI) for RSC Advances.

A Near IR Photosensitizer Based-on Self-Assembled CdSe Quantum Dot-aza-BODIPY Conjugate Coated with Poly(ethylene glycol) and Folic Acid for Concurrent Fluorescence imaging and Photodynamic Therapy

Yu Yan[a], Jiangwei Tian[c], Fengrui Hu[b], Xiaoyong Wang[b] and Zhen Shen[a]*

Table of contents

1. Experimental details

1.1 Materials and methods

MALDI-TOF MS spectra were measured on a Bruker Daltonics autoflex^{II}. The ^{1}H NMR spectra were measured on a Bruker 500 MHz spectrometer. Fluorescence spectral measurements were carried out on a Hitachi F–4600 fluorometer. UV-visible absorption spectra were recorded on a SHIMADZU UV–2550 spectrometer. Confocal fluorescence imaging studies were performed on an LSM 710 confocal laser-scanning microscope (Carl Zeiss Co., Ltd.).

2.2 Cell culture, MTT assay and confocal fluorescence imaging

Cell Culture. HeLa cells were maintained following protocols provided by the American Type Tissue Culture Collection. Cells were seeded at a density of 1×10^{6} cells per well for confocal imaging in RPMI 1640 Medium supplemented with 10 % fetal bovine serum (FBS), $NaHCO_3$ (2 g/L), and 1% antibiotics (penicillin/streptomycin, 100 U/ml). Cultures were maintained at 37 ℃ under a humidified atmosphere containing 5 % CO_2.

MTT Assay. MTT assay was carried out to investigate the dark toxicity and phototoxicity of QD-**aza-a**@PEG conjugate. HeLa cells were first seeded to two 96-well plates at a seeding density of 1×10^{4} cells per well in 200 μL complete medium, which were incubated at 37 ℃ for 24 h. After rinsing with PBS, HeLa cells were incubated with 200 μL culture media containing QD-**aza-a** @ PEG conjugate to allow for final concentrations of 0, 0.025, 0.05, 0.1, 0.25, 0.5 μM. One plate was kept in the dark as controls for studying dark toxicity. The cells were incubated in the dark for a further 24 h, the medium was removed and each well was washed twice with 50 μL PBS. Fresh medium was added to each well and the selected wells were irradiated using a 635 nm laser at power of 20 W cm^{-2} for 180 s. Afterward, the cells were grown for another 24 h. Then, 20 μL of 5 mg mL^{-1} MTT solution in PBS was added to each well. After 4 h incubation, the medium containing unreacted MTT was removed carefully, and 200 μL DMSO was added to each well to dissolve the produced blue formazan. After 1 h the optical density (OD) at a wavelength of 490 nm was measured with Bio-Rad microplate reader. The IC_{50} value was calculated to be 0.05 μM.

Fluorescence imaging. HeLa cells (1×10^{5} cells per well) were seeded on 6-well plates and incubated in complete medium for 24 h at 37 ℃. The medium was then replaced with fresh culture

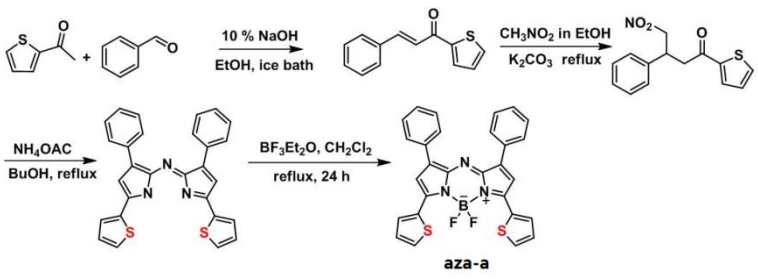
medium containing 0.25 μM QD-**aza-a** @ PEG conjugate to incubate for 24 h at 37 ℃. Wells were also spiked with similar concentration of PEGylated QD-**aza-a** conjugate without FA as control. The medium was removed and each well was washed twice with 50 μL PBS. Prior to imaging, the medium was removed. Cell imaging was carried out after washing cells with PBS for three times. Confocal fluorescence imaging was performed with a Zeiss LSM 710 laser scanning microscope. The conjugate incubated cells were excited at 635 nm, and the emission was collected from 650 to 750 nm.

Target PDT on Hela Cells. HeLa and HaCaT cells were incubated with 0.25 μM QD-**aza-a** @ PEG conjugate for 24 h at 37 ℃. The following procedures are the same as use for bioimaging.

Phototoxicity Assay. HeLa cells (1×10^5 cells per well) were seeded on 6-well plates and incubated in complete medium for 24 h at 37 ℃. The medium was then replaced with fresh culture medium containing 0.25 μM QD-**aza-a** @ PEG conjugate to incubate for 24 at 37 ℃. The cells were irradiated with a 635 nm laser at a power of 20 mW cm^{-2} for 180 s. Afterwards the cells were stained with Annexin V-FITC according to the manufacturer's instruction, harvested, rinsed with PBS, suspended and subjected to visualize the apoptotic cells with confocal laser scanning microscope. Annexin V-FITC was excited at 488 nm, and the emission was collected from 495 to 535 nm.

1.3 Synthesis section

Procedure for the preparation of aza-a [1]**:**



All reagents were obtained from commercial suppliers and used without further purification unless otherwise indicated. Air and moisture-sensitive reactions were carried out under an argon atmosphere.

Synthesis of the thienyl-substituted chalcone: phenyl-3-thienylprop-2-en-1-one:

Benzaldehyde (30 mmol) and 2-ancethylthiophene (30 mmol) were dissolved in 15 mL ethanol and cooled in an ice bath. 10 % NaOH solution was then added dropwise over 30 min and the mixture was stirred for 3 h. During the course of the reaction, the product precipitated from the solution. After filtration and washed with cold ethanol, the product was obtained as white solid by rescrystallization from ethanol.

Synthesis of the 4-Nitro-3-phenylprop-1-one:

A mixture of thienyl-substituted chalcone (30 mmol), nitromethane (8.1 mL, 150 mmol) and diethylamine (15.48 mL, 150 mmol) were dissolved in ethanol (20 mL) and refluxed for 24 h under Ar. After cooling at room temperature, the reaction mixture was extracted with ethyl acetate and washed with water for 3 times. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and concentrated to give the target compound as a yellowish oily residue in nearly quantitative yield, which was used in the next step without further purification.

Synthesis of the dipyrroazamethene:

A solution of 4-Nitro-3-phenylprop-1-one (10 mmol) and ammonium acetate (350 mmol) was refluxed in *n*-butanol (30 mL) for 24 h under Ar. After the solution was cooled to room temperature, the solution was washed with water and extracted three times with dichloromethane and dried with sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using petroleum ether / ethyl acetate (4/1; v/v) as the eluent to obtain a dark blue-black solid.

Synthesis of the aza-a:

A dried flask was charged with dipyrroazamethenes (3 mmol) and flushed with nitrogen. Dry dichloromethane (100 mL) and dry diisopropylethylamine (about 5 mL) were added. The solution was stirred at room temperature for 15 min, borontrifluoride diethyletherate (about 5 mL) was added. After stirred at room temperature for 48 h, the mixture was washed with water and brine, and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuum. The residue was chromatographed on silica gel using petroleum ether / ethyl acetate (3/2; v/v) as eluent to provide the product as copper shiny green-black crystals.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.21 (s, 2H), 7.29 (t, J = 5.0 Hz, 2H), 7.47 (m, 6H), 7.76 (d, J = 5.0 Hz, 2H), 8.08 (d, J = 10.0 Hz, 4H), 8.40(d, J = 5.0Hz, 2H), 8.0-8.07 (m, 8H), 8.78 (s, 8H).

MALDI-TOF-MS m/z: calcd for $C_{28}H_{18}BF_2N_3S_2$: 509.10, found: 510.360 $[M+H]^+$.

Aza-a Conjugate to Qdots [2]**:**

A mixture of CdSe QDs (10 μM) and **aza-a** (400 μM) in $CHCl_3$ was stirred overnight at room temperature to prepare QDs-**aza-a** conjugate.

The preparation of FA-PEGylated QD-**aza-a** conjugate is shown in Scheme 1. FADSPE-PEG_{2000}(1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N(methoxy(polyethylenegly col)) copolymers: PEG-phospholipids and DSPE-PEG_{2000}-FA (8.5:1.5, mol/mol) was dissolved in chloroform (5 mL). CdSe QD-**aza-a** conjugate (dispersed in chloroform) and DSPE-PEG_{2000}/DSPE-PEG_{2000}-FA were mixed at 1:2 (w/w) ratio in a 25 mL round-bottom flask, and then 5 mL deionized water was added gradually to the mixture. After removing chloroform by slow evaporation (70 ℃, 15 min), the conjugate became water soluble. The exceeds empty lipid micelles (PEG micells and PEG capped **aza-a** molecules) were purified from solubilized Qdot-**aza-a** @ PEG conjugate with repeated ultracen-trifugation at 100,000 g. Finally, the conjugate was centrifuged at 3,000 g and the large aggregates were discarded.

I.4 Calculation of QDs:aza-a ratios

QD and **aza-a** ratio in Qdot-**aza-a** @ PEG conjugate was calculated using the Beer-Lambert law: A = εbc, where A = absorbance, ε is the absorption coefficient, b is the path length (1 cm), and c is the concentration. The absorption coefficient at 400 nm for QD was used to determine the concentration of QD while the absorption coefficient at 725 nm for **aza-a** was used to determine the concentration of the photosensitizer.

I.5 FRET parameters measurements

The experimental FRET efficiency E is defined as Eq. (1) [3a]:

$$E = \frac{I_D - I_{DA}}{I_D} \qquad (1)$$

where I_D and I_{DA} are the fluorescence intensity of the donor (QD) alone and the donor in the presence of acceptor (**aza-a**), respectively.

Förster distance R_0 designating the donor-acceptor separation at 50 % energy transfer efficiency is expressed as Eq. (2) [3b]:

$$R_0 = 9.78\times10^3\left[\kappa^2 n_D^{-4}\phi_D J(\lambda)\right]^{1/6} \quad (2)$$

where n_D is the refractive index of the solvent, Φ_D is the donor quantum yield in the absence of acceptor, J (λ) is the spectral overlap integral, and κ^2 is the dipole orientation factor. κ^2=2/3, a value for randomly oriented dipole, which was assumed for FRET in QD-dye conjugate.

$$J = \int f_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \quad (3)$$

The overlap integral J is conveniently expressed in OLIs, λ is the wavelength of the light, most often expressed in nanometers, $\varepsilon_A(\lambda)$ is the molar extinction coefficient of the acceptor, usually in M^{-1} cm^{-1}, and f_D (λ) is the fluorescence spectrum of the donor normalized on the wavelength scale [3c]:

$$f_D(\lambda) = \frac{F_{D_\lambda}(\lambda)}{\int F_{D_\lambda}(\lambda)d\lambda} \quad (4)$$

where $F_{D\lambda}(\lambda)$ is the donor fluorescence per unit of wavelength interval and the integral extends over the relevant donor emission band(s). Here, J (λ) value was obtained through origin 8.0 software.

The Φ_D value was calculated according to the following Eq. (5) [3d]:

$$\phi_D = \phi_s \times \frac{A_S}{A_X} \times \frac{I_X}{I_S} \times \left(\frac{n_X}{n_S}\right)^2 \quad (5)$$

where A is the absorbance, F is the area under the emission curve, n is the refractive index of the solvent used in the measurement, and the subscripts s and x represent the standard and sample, respectively. Here, zinc phthalocyanine was used as the standard.

Estimate of the QD-**aza-a** @ PEG conjugate donor—dye acceptor separation distance (r) is calculated using the Eq. (6) [3e]:

$$r = \left(\frac{n(1-E)}{E}\right)^{1/6} R_0 \quad (6)$$

where n is the number of **aza-a** acceptor attached to the QD donor.

2. Supplementary spectra

2.1

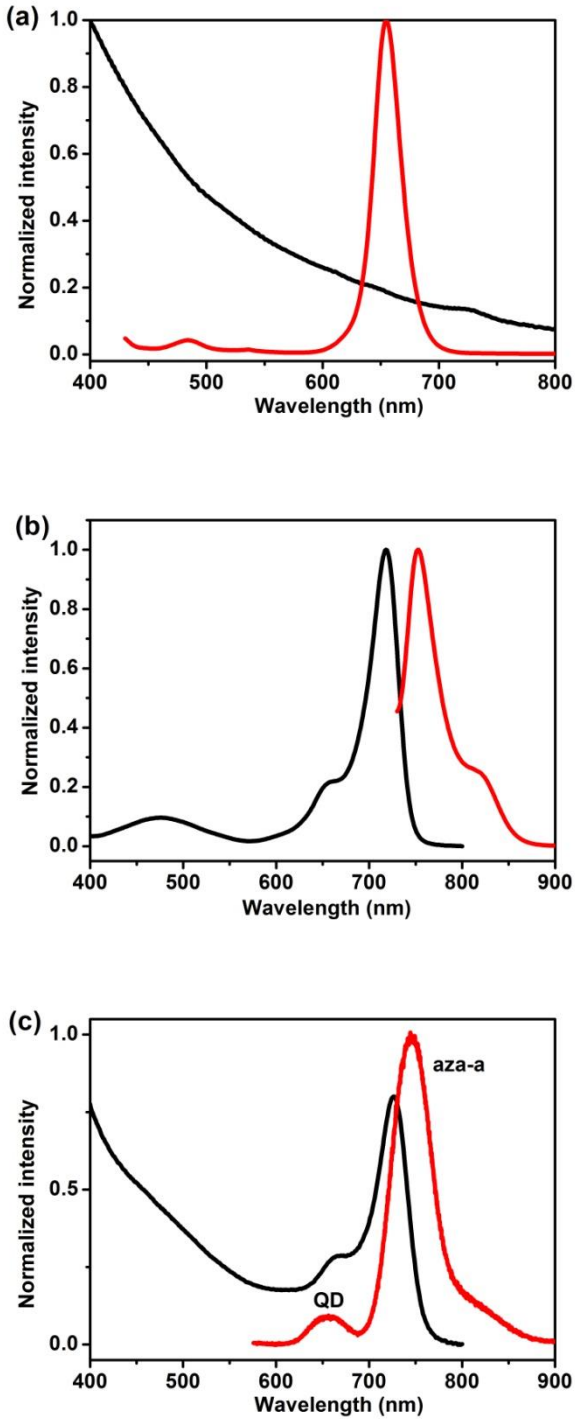


Figure S1. Normalized absorption (black) and emission (red) spectra of (a) CdSe QDs (λ_{ex}= 420 nm, [QDs]= 1×10^{-6} M). (b) **aza-a** (λ_{ex}= 655 nm, [**aza-a**]= 1×10^{-6} M) and (c) QD-**aza-a** @ PEG conjugate (λ_{ex}= 420 nm, [QDs]= 1×10^{-6} M, [**aza-a**]= 1.7×10^{-5} M).

2.2

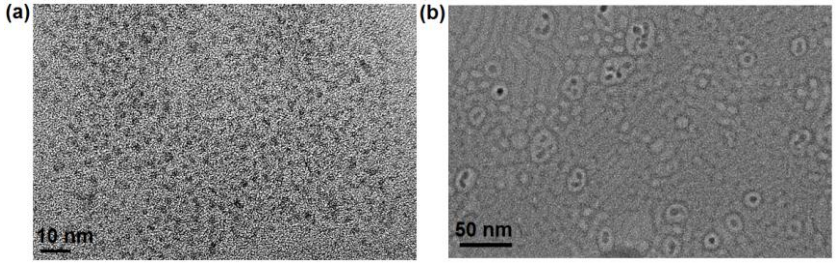


Fig S2. TEM images of (a) CdSe QD; (b) QD-**aza-a** @ PEG conjugate with core-shell structure negatively stained 1% Phosphotungstic acid to give a black layer surrounding the CdSe QDs indicating the DSPE-PEG_{2000} coating layer.

2.3

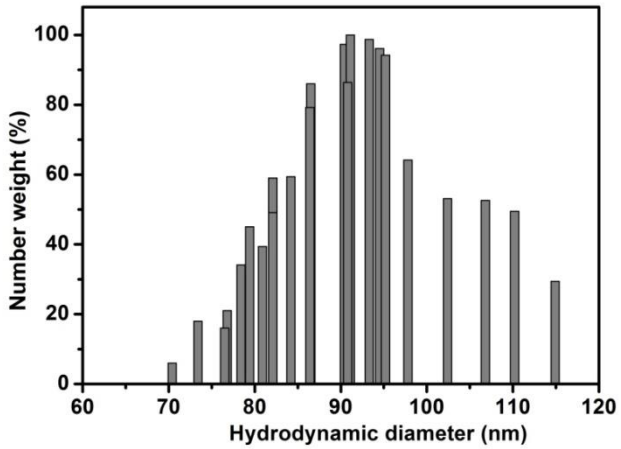


Figure S3. Size distribution of QD-**aza-a** @ PEG conjugate in water.

2.4

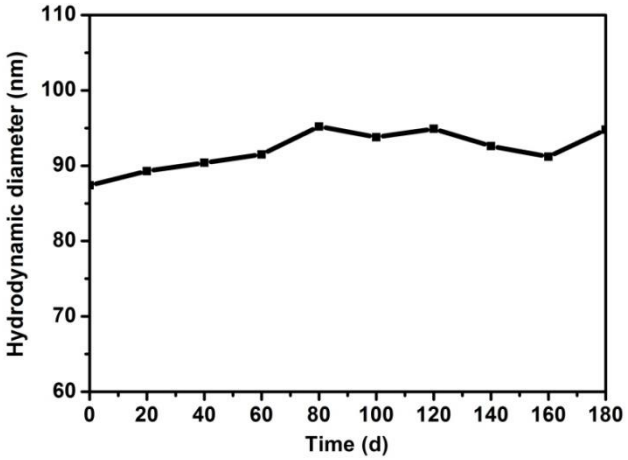


Figure S4. Size stability test of QD-**aza-a** @ PEG conjugate in water.

2.5

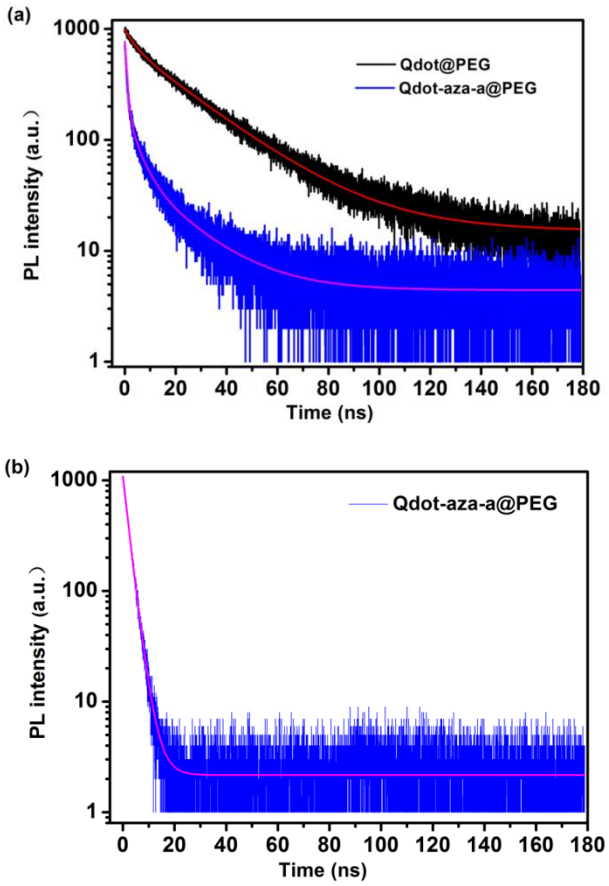


Fig S5. Luminescence lifetime decay curves of QD-**aza-a** @ PEG conjugate (a) lifetime of QDs at 655 nm and (b) lifetime of **aza-a** at 755 nm.

2.6

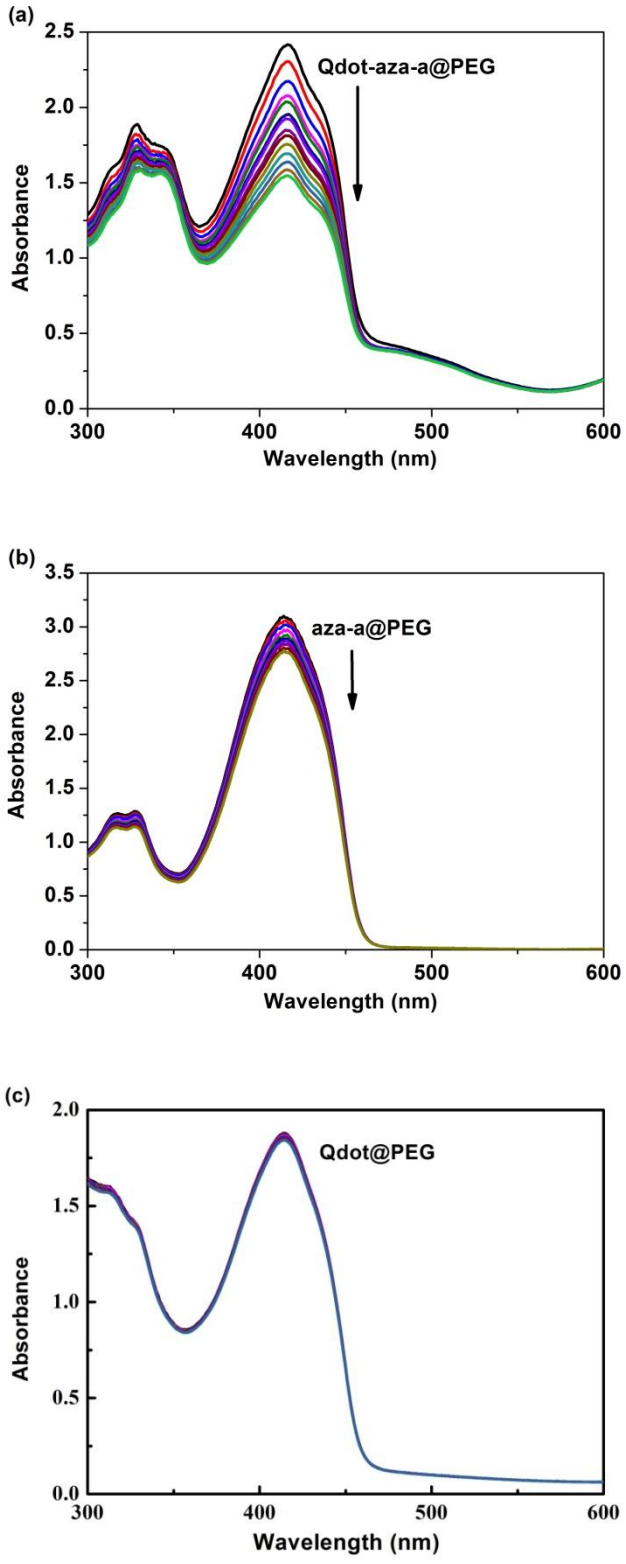
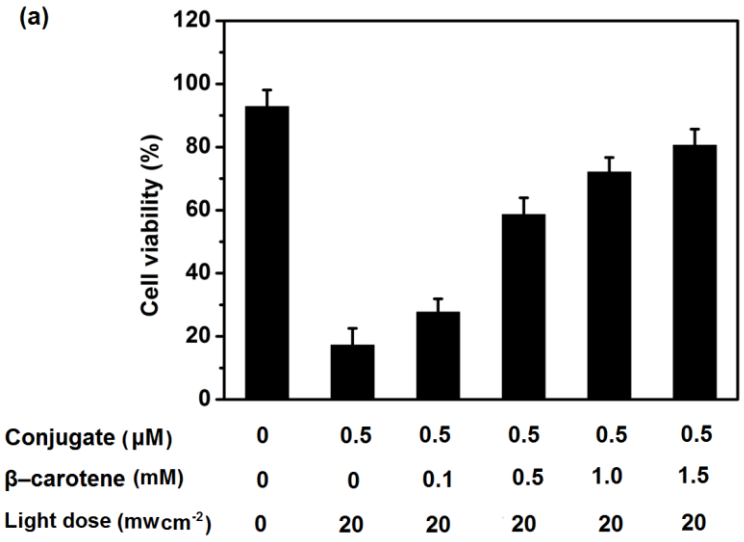
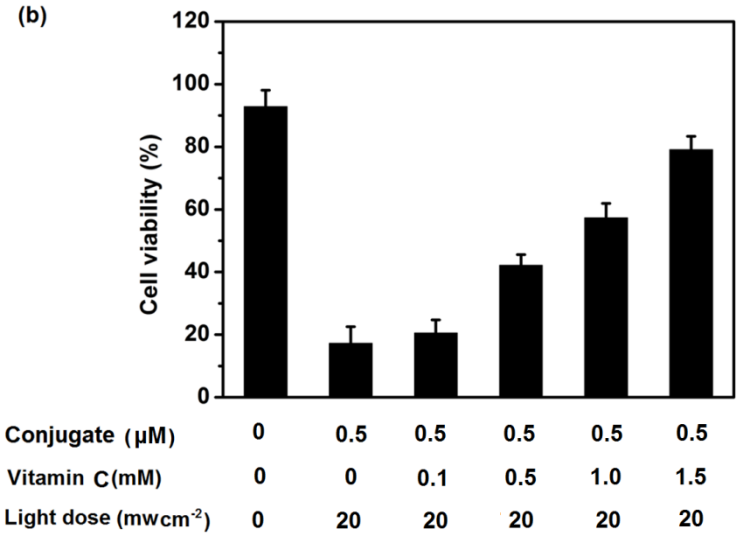
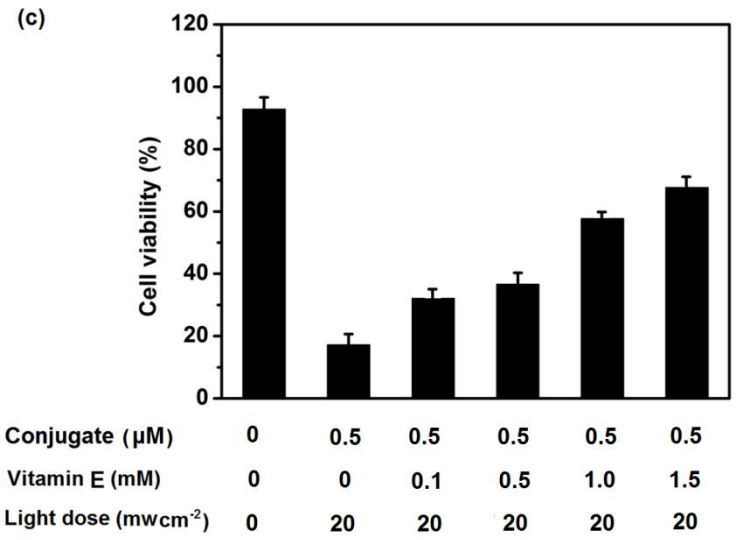


Fig S6. Changes in the absorption spectra of DPBF upon irradiation (λ_{irr} = 635 nm) with 3 min interval in the presence of (a) QD-**aza-a** @ PEG conjugate; (b) PEGylated-**aza-a**; (c) PEGylated-QD. ([QDs]= 1×10^{-6} M, [**aza-a**]= 1.7×10^{-5} M).

2.7


(a)
Cell viability (%)
120
100
80
60
40
20
0
Conjugate (μM) 0 0.5 0.5 0.5 0.5 0.5
β–carotene (mM) 0 0 0.1 0.5 1.0 1.5
Light dose (mwcm-2) 0 20 20 20 20 20


(b)
Cell viability (%)
120
100
80
60
40
20
0
Conjugate (μM) 0 0.5 0.5 0.5 0.5 0.5
Vitamin C(mM) 0 0 0.1 0.5 1.0 1.5
Light dose (mwcm-2) 0 20 20 20 20 20


(c)
Cell viability (%)
120
100
80
60
40
20
0
Conjugate (μM) 0 0.5 0.5 0.5 0.5 0.5
Vitamin E (mM) 0 0 0.1 0.5 1.0 1.5
Light dose (mwcm-2) 0 20 20 20 20 20

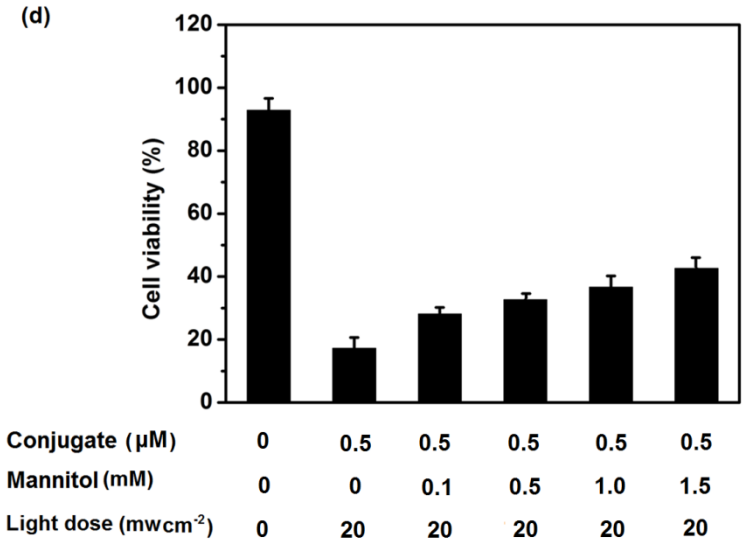


Figure S7. (a) *β*–carotene, (b) vitamin C, (c) vitamin E and (d) mannitol concentration-dependent inhibition against HeLa cell death induced by QD-**aza-a**@PEG conjugate-mediated PDT. Data are means ± SEM (n = 4, $^{**}P < 0.01$ and $^{***}P < 0.001$ for treatment with QD-**aza-a** @PEG ([QDs]= 0.1 mM, aza-a= 1.7 mM), 20 mW cm^{-2} irradiation for 3 min in the presence compared to absence of ROS scavenger using a Student's t test.

2.8

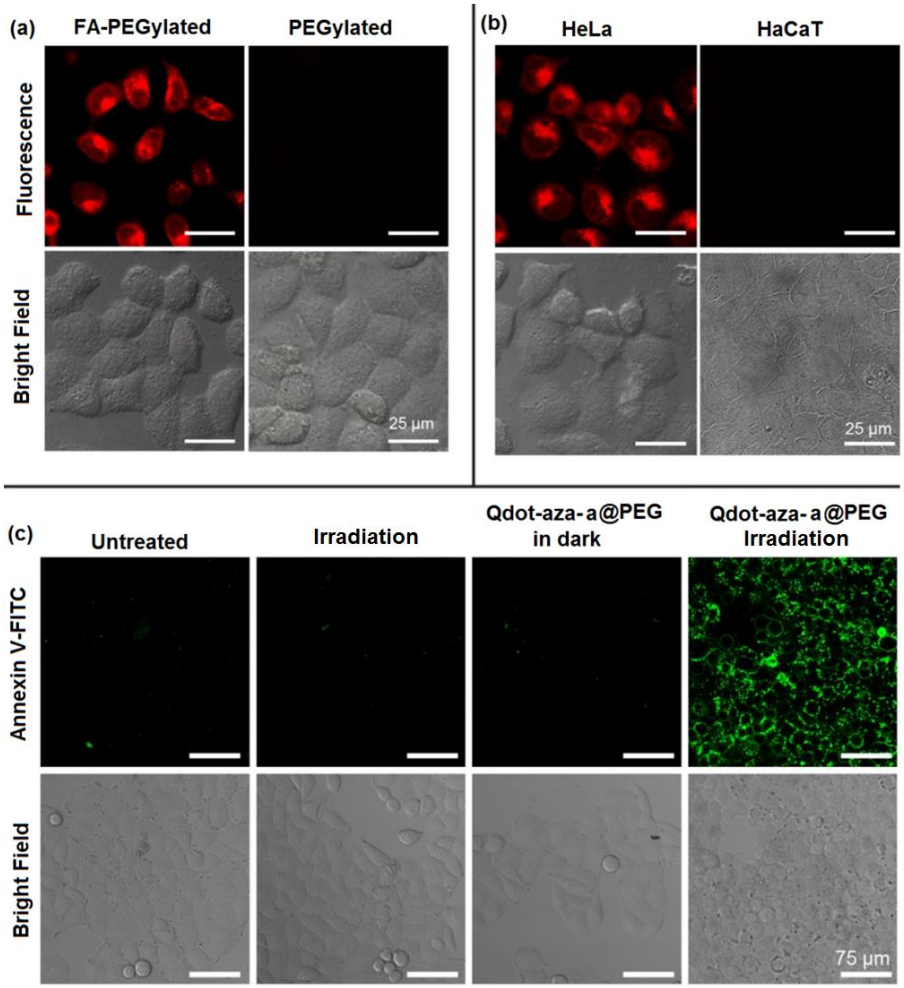


Fig S8. (a) Confocal fluorescence and bright field images of HeLa cells incubated with QD-**aza-a**@PEG conjugate and PEGylated QD-**aza-a** without FA irradiated with a 635 nm laser. (b) Confocal fluorescence images of HeLa and HaCaT cells incubated with QD-**aza-a**@PEG conjugate irradiated with a 635 nm laser. (c) Confocal fluorescence images of Annexin V-FITC stained HeLa cells with different treatments to study the cell death induced by QD-**aza-a** @PEG conjugate mediated PDT. For PDT, HeLa cells irradiated with a 635 nm laser for 3 min at a power density of 20 mW cm^{-2}. ([QDs]= 5×10^{-7} M, [aza-a]= 8.5×10^{-6} M).

2.9

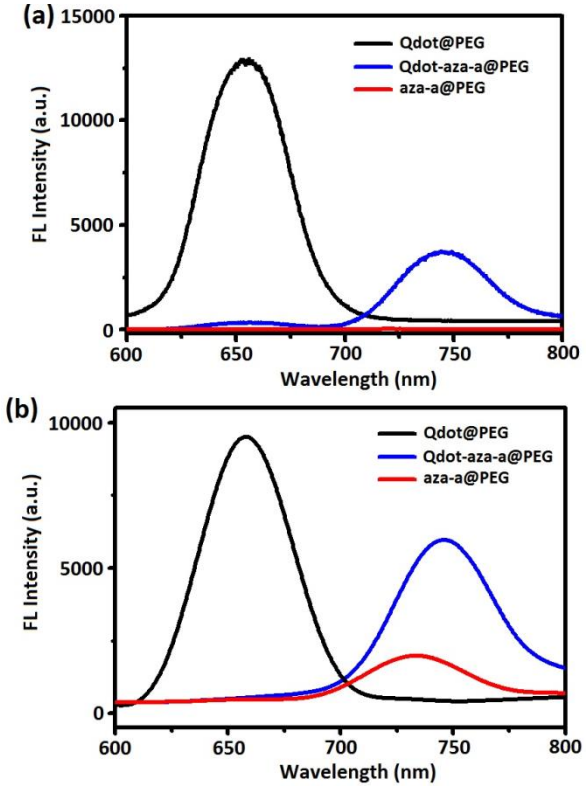


Figure S9. Photoluminescence spectra of CdSe QD-aza-a@PEG conjugate (blue), PEGylated QD (black) and PEGylated aza-a (red) at the same concentration in water (a) λ_{ex}= 420 nm and (b) λ_{ex}= 635 nm. ([QDs] = 1×10^{-6} M, [aza-a]= 1.7×10^{-5} M)

References

[1] (a) A. M. Brouwer, *Pure Appl. Chem.*, 2011,**83**, 2213 – 2228. (b) U. Resch-Genger and P. C. DeRose, *Pure Appl. Chem.*, 2010, **82**, 2315. (c) J. R. Lakowicz, Principles of Fluorescence Spectroscopy, 3rd ed., Springer, New York, 2006. (d) J. S. Hsiao, B. P. Krueger, R. W. Wagner, T. E. Johnson, J. K. Delaney, D. C. Mauzerall, G. R. Fleming, J. S. Lindsey, D. F and Bocian, R. J. Donohoe, *J. Am. Chem. Soc.*, 1996, **118**, 11181-11193.

[2] (a) W. W. William , L. H. Qu, W. Z. Guo and X. G. Peng, *Chem. Mater.*, 2003, **15**, 2854-2860. (b) H. Z. Zhong and G. D. Scholes, *J. Am. Chem. Soc.*, 2009, **131**, 9170-9171. (c) X. H. Zhong, Y. Y. Feng, Y. L. Zhang, *J. Phys. Chem.*, 2007, **111**, 526-531.

[3] (a) A. R. Clapp, I. L. Medintz, J. M. Mauro, B. R. Fisher, M. G. Bawendi and H. Mattoussi, *J. Am. Chem. Soc.*, 2003, **126**, 301-310. (b) M. C. Chirio-Lebrun and M. *Prats, Biochem. Educ.*, 1998, **26**, 320-323. (c) Igor Medintz I and Hildebrandt N. FRET- Förster Resonance Energy Transfer From Theory to Applications. Wiley-VCH. Chapter 3. (d) G. A. Crosby and J. N. Demas, *J. Phys. Chem.*, 1971, **75**, 991-1024. (e) I. L. Medintz, A. R. Clapp, H. Mattoussi, E. R. Goldman, B. Fisher and J. M. Mauro, *Nat. Mater.*, 2003, **2**, 630-638.