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A light-activatable photosensitizer for photodynamic therapy based on diarylethene derivative

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Materials and methods

Materials and instrumentation

Fluorescence data was measured by a F-4700 fluorescence spectrophotometer (Hitachi) at room temperature (slit: 5 nm, 5 nm). The absorption spectra were measured on a UV-1700 spectrophotometer (Shimadzu, Japan). The mass spectra were obtained by Maxis MHR-TOF ultra-high resolution quadrupole time of flight mass spectrometer (Bruker Germany). The ^{1}H NMR and ^{19}F NMR spectra were acquired on a nuclear magnetic resonance spectrometer (400 MHz, Bruker Co., Ltd., Germany). The δ value represents the shift of the spectrum relative to TMS ($(CH_3)_4Si$ = 0.00 ppm), expressed in ppm. Transmission electron microscopy (TEM) images were taken on a JEM-1011 electron microscope (JEOL, Japan) at an accelerating voltage of 100 kV. Dynamic light scattering (DLS) measurements were performed on a Malvern zeta sizer Nano-ZS90. Confocal imaging data were obtained on TCS SP8 confocal laser scanning microscope (Leica Co., Ltd., Germany). The data of MTT experiment was measured with a microplate reader (TRITURUS). The light sources included 520 nm LED source (CCS Int. 168.3 mW cm^{-2}), 465 nm LED source (CCS Int., 334 mW cm^{-2}), a portable UV lamp and xenon lamp (520 nm) with a filter (CELHXF300, 40 mW cm^{-2}).

Compound 1 was prepared according to the reported literature [1]. Compound 2 was purchased from Energy Chemical. Common solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. The moiety of DAE was synthesized according to the reported literature [2].

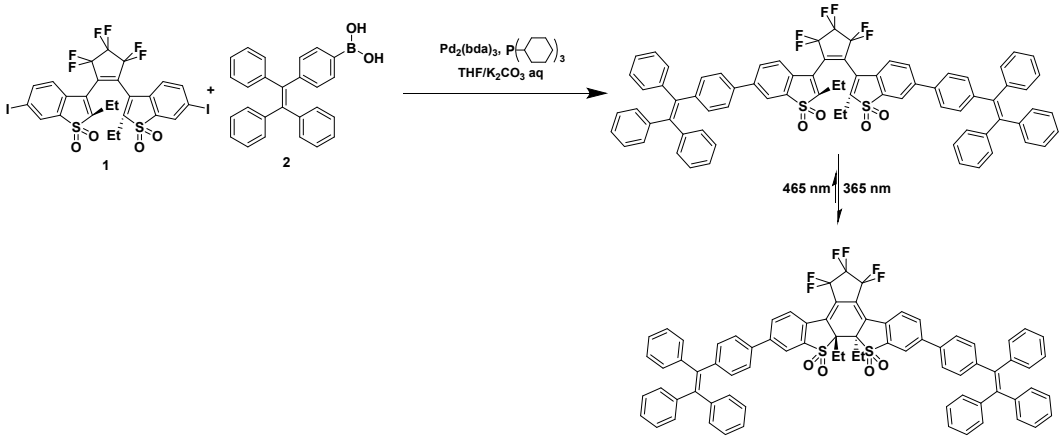

Figure S1 Synthesis of DAE-TPE and its switch between opened form and closed form.

Synthesis of Compound DAE-TPE

A mixture of compound 1 (59 mg, 0.074 mmol), compound 2 (66.6 mg, 0.177 mmol), $Pd_2(bda)_3$ (11.8 mg, 0.013 mmol) and 18% tricyclohexylphosphine toluene solution (0.1 ml) were dissolved in 5mL tetrahydrofuran, then saturated K_2CO_3 solution (5 mL) was added. The mixture was stirred at room temperature for 5.5 h under argon protection. The reaction was quenched with diluted hydrochloric acid. The resulting solution was treated with chloroform three times. The organic layers were combined and washed with saturated brine. After removing organic solvent, the residue was purified by silica gel column (petroleum ether: ethyl acetate=1:1), the orange-red solid compound was obtained (yield: 70.0%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.84-7.91 (m, 2 H), 7.73-7.76 (m, 1 H), 7.52-7.55 (m, 1 H), 7.32-7.34 (m, 3 H), 7.14-7.19 (m, 2 H), 7.04-7.13 (m, 35 H), 2.37-2.56 (m, 4 H), 1.06-1.52 (m, 6 H); ^{19}F (376 MHz, Chloroform-*d*): δ -109.82 and -109.84, -109.85, -132.17. MS：calculated for $[M+Na]^+$ =1243.3, found 1243.8.

Preparation of DAE-TPE NPs

DAE-TPE (1.6 mg) was mixed with pluronic F-127 (20 mg) in 2 mL tetrahydrofuran. After removing the solvent with rotary evaporator, the dried mixture was mixed with

deionized water (4 mL) and then shaken vigorously to afford DAE-TPE NPs (0.3 mM, DAE-TPE is believed to be completely wrapped in F-127) by ultrasound. DAE NPs and TPE NPs were prepared in the same way.

The closed form of DAE-TPE NPs/DAE NPs could be prepared by irradiating ultraviolet light with DAE-TPE NPs/DAE NPs.

$^1O_2$ generation

The ability of $^1O_2$ generation of nanoparticles were evaluated by 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA). ABDA can be oxidized by $^1O_2$, resulting in a decrease in its own absorption. The mixture of nanoparticles (10 μM) and ABDA (100 μM) was irradiated with a 520 nm light source (168.3 mW cm^{-2}) in an aqueous solution. The UV-Vis spectra of ABDA photooxidation were recorded every 5 min for a total of 30 min.

Cell incubation

HeLa cells were seeded in DMEM medium containing 10% FBS, 1% penicillin and 1% streptomycin in confocal cell culture dishes and cultured in an incubator (37 ^OC, 5%CO_2/95% air) for 24 h.

Cell uptake experiment

The uptake of DAE-TPE NPs in the CF by cancer cells was studied in HeLa cells. The DAE-TPE NPs in the CF (5 μM) and cells were co-incubated for 0 h, 1 h, 5 h, 10 h, 12 h and 13 h, respectively. The real-time imaging was performed to monitor cell uptake using a confocal fluorescence microscope (λ_{ex} = 488 nm, collection channel: 600-700 nm, scale: 100 μm).

In vitro cytotoxicity assays

HeLa cells were seeded in a 96-well cell culture plate (the volume is 200 μL/well), followed by incubating in an incubator (37°C, 5% CO_2/95% air) for 24 hours. Then the cells were incubated with different concentrations (0, 4, 6, 8, 10, 12, 14, 16, 18, and 20 μM) of DAE-TPE NPs for 12 h and then irradiated with UV, washed once with PBS and added fresh medium, and then irradiated with a xenon lamp equipped with a 520 nm cut-off filter (52 mW cm^{-2}) for 0, 30, 60, and 90 min. Subsequently, 200 μL MTT solution (5.0 mg mL^{-1}) in PBS was added to each well. After 4 h, the remaining MTT

solution was removed and 200 μL DMSO was added to each well. The TRITURUS microplate reader was used to measure the absorbance of the solution at 490 nm.

Intracellular $^1O_2$ detection

The $^1O_2$ indicator DCFH-DA was used to detect $^1O_2$ produced DAE-TPE NPs after irradiating with UV in HeLa cells. In brief, HeLa cells were seeded in confocal dishes and then incubated with different subsequent treatments: group 1, control; group 2, incubated with 5 μM DAE-TPE NPs for 12 h, then irradiated with UV and washed three times with PBS, incubated with 20 μM DCFH-DA for 20 min (DAE-TPE NPs + UV); group 3, incubated with 20 μM DCFH-DA for 20 min, then washed three times with PBS, and irradiated with a xenon lamp equipped with a 520 nm cut-off filter (40 mW cm^{-2}) for 10 min (520 nm 10 min); group 4, incubated with 20 μM DCFH-DA for 20 min, then washed three times with PBS, and irradiated with a xenon lamp equipped with a 520 nm cut-off filter (40 mW cm^{-2}) for 20 min (520 nm 20 min); group 5, incubated with 5 μM DAE-TPE NPs for 12 h, then irradiated with UV and washed with PBS three times, incubated with 20 μM DCFH-DA for 20 min, then washed with PBS three times, and irradiated with a xenon lamp equipped with a 520 nm cut-off filter (40 mW cm^{-2}) for 10 min (DAE-TPE NPs + UV then 520 nm 10 min); group 6, incubated with 5 μM DAE-TPE NPs for 12 h, then irradiated with UV and washed three times with PBS, incubated with 20 μM DCFH-DA for 20 min, then washed three times with PBS, and irradiated with a xenon lamp equipped with a 520 nm cut-off filter (40 mW cm^{-2}) for 20 min (DAE-TPE NPs + UV then 520 nm 20 min); group 7, pre-incubated with 20 mM NaN_3 for 1.5 h, then incubated with 5 μM DAE-TPE NPs for 12 h, then irradiated with UV and washed three times with PBS, incubated with 20 μM DCFH-DA for 20 min, then washed with PBS three times, and irradiated with a xenon lamp equipped with a 520 nm cut-off filter (40 mW cm^{-2}) for 10 min (DAE-TPE NPs + UV then 520 nm 10 min + NaN_3); group 8, pre-incubated with 20 mM NaN_3 for 1.5 h, incubated with 5 μM DAE-TPE NPs for 12 h, then irradiated with UV and washed with PBS three times, and incubated with 20 μM DCFH-DA for 20 min, then washed three times with PBS, and irradiated with a xenon lamp equipped with a 520 nm cut-off filter (40 mW cm^{-2}) for 20 min (DAE-TPE NPs + UV then 520 nm 20 min + NaN_3). After

treatments, confocal fluorescence imaging was performed to detect the level of singlet oxygen (λ_{ex} = 488 nm, collection channel: 510-600 nm, scale bar: 100 μm).

Apoptosis test

YF488-Annexin V/propidium iodide (PI) Apoptosis Detection Kit was used to perform double-stain imaging of photoinduced cell death of HeLa cells. In brief, HeLa cells were seeded in confocal dishes and then incubated with different subsequent treatments: group 1, control; group 2, incubated with 15 μM DAE-TPE NPs for 12 h then irradiated with UV (DAE-TPE NPs + UV); group 3, irradiated with a xenon lamp equipped with a 520 nm cut-off filter (52 mW cm^{-2}) for 30 min (520 nm 60min); group 4, irradiated with a xenon lamp equipped with a 520 nm cut-off filter (52 mW cm^{-2}) for 60 min (520 nm 60 min); group 5, incubated with 15 μM DAE-TPE NPs for 12 h, then irradiated with UV and washed three times with PBS, and irradiated with a xenon lamp equipped with a 520 nm cut-off filter (52 mW cm^{-2}) for 30 minutes (DAE-TPE NPs + UV then 520 nm 30 min); group 6, incubated with 15 μM DAE-TPE NPs for 12 h, then irradiated with UV and washed three times with PBS, and irradiated with a xenon lamp equipped with a 520 nm cut-off filter (52 mW cm^{-2}) for 60 min (DAE-TPE NPs + UV then 520 nm 60 min). After treatment, cells were stained with YF488-Annexin V/PI Apoptosis Detection Kit according the manual. The apoptosis imaging was observed by confocal microscope (YF488 excitation light source: 488 nm, collection channel: 490-560 nm; PI excitation light source: 561 nm, collection channel: 600-680 nm, scale bar: 100 μm). In addition, the fluorescence intensity of the above treated cells was quantitatively analyzed by flow cytometry.

Live and dead cell staining

Calcein-AM and PI cellular viability kit was used to stain HeLa cells for confocal imaging. In brief, HeLa cells were seeded in confocal dishes and then incubated with different subsequent treatments: group 1, control; group 2, incubated with 15 μM DAE-TPE NPs for 12 h then irradiated with UV (DAE-TPE NPs + UV); group 3, irradiated with a xenon lamp equipped with a 520 nm cut-off filter (52 mW cm^{-2}) for 30 min (520 nm 30 min); group 4, irradiated with a xenon lamp equipped with a 520 nm cut-off filter (52 mW cm^{-2}) for 60 min (520 nm 60 min); group 5, incubated with 15 μM DAE-TPE

NPs for 12 h, then irradiated with UV and washed three times with PBS, and then irradiated with a xenon lamp equipped with a 520 nm cut-off filter (52 mW cm^{-2}) for 30 min (DAE-TPE NPs + UV then 520 nm 30 min); group 6, incubated with 15 μM DAE-TPE NPs for 12 h, then irradiated with UV and washed three times with PBS, and then irradiated with a xenon lamp equipped with a 520 nm cut-off filter (52 mW/cm^2) for 60 min (DAE-TPE NPs + UV then 520 nm 60 min). After treatment, cells were stained with Calcein-AM and PI cellular viability kit according the manual. The live-dead cell imaging was observed by confocal microscope (Calcein-AM excitation source: 488 nm, collection channel: 500-550 nm, PI excitation source: 561 nm, collection channel: 580-650 nm, scale: 100 μm).

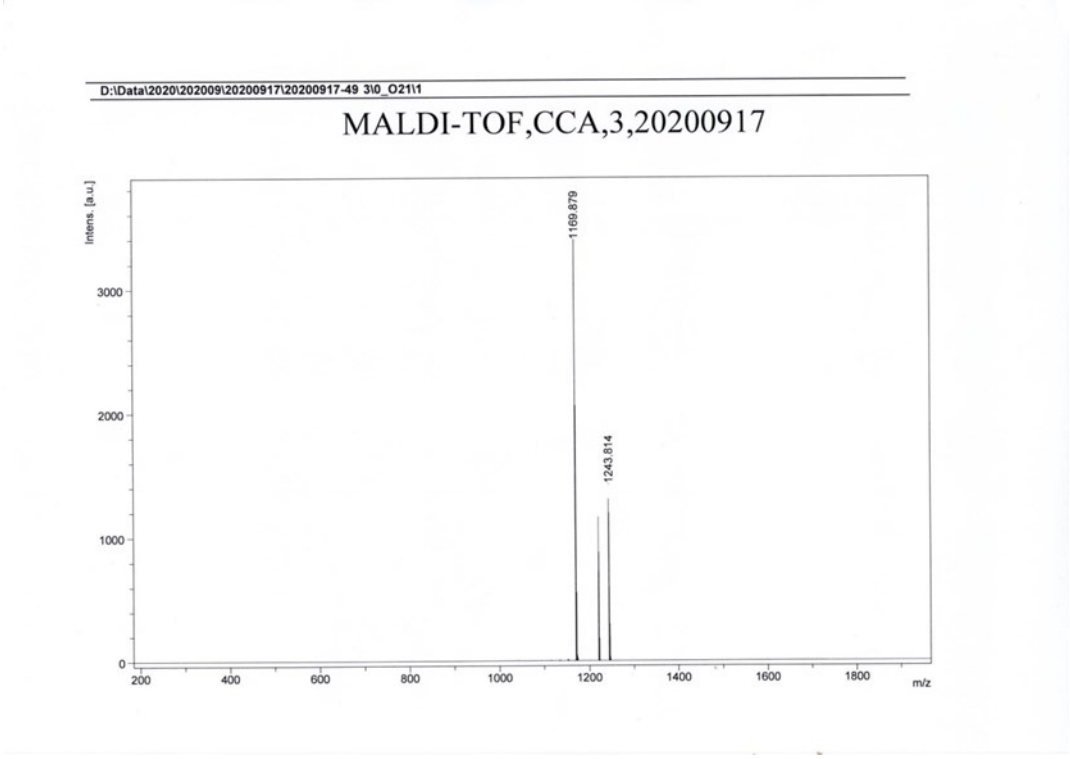

Figure S2 MS of DAE-TPE.

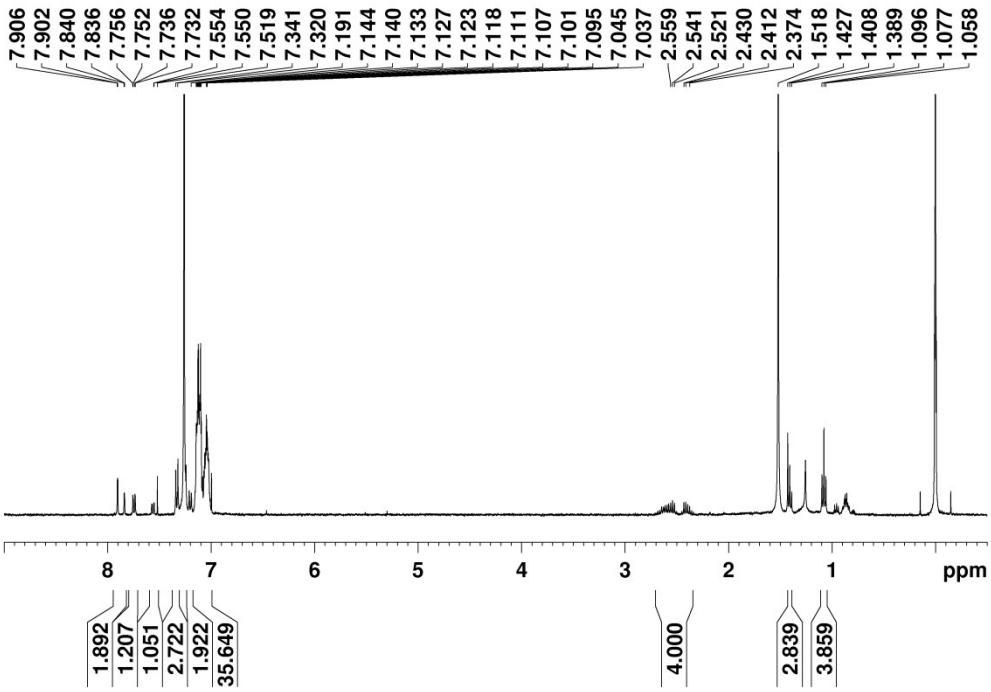

Figure S3 ^{1}H NMR spectrum of DAE-TPE in $CDCl_3$.

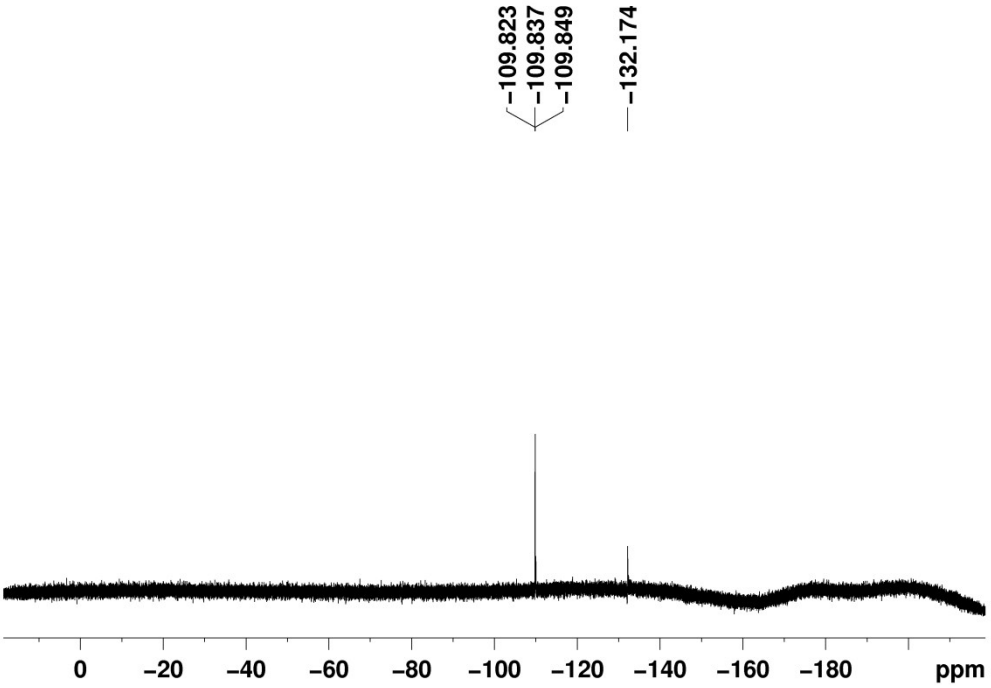

Figure S4 ^{19}F NMR spectrum of DAE-TPE in $CDCl_3$.

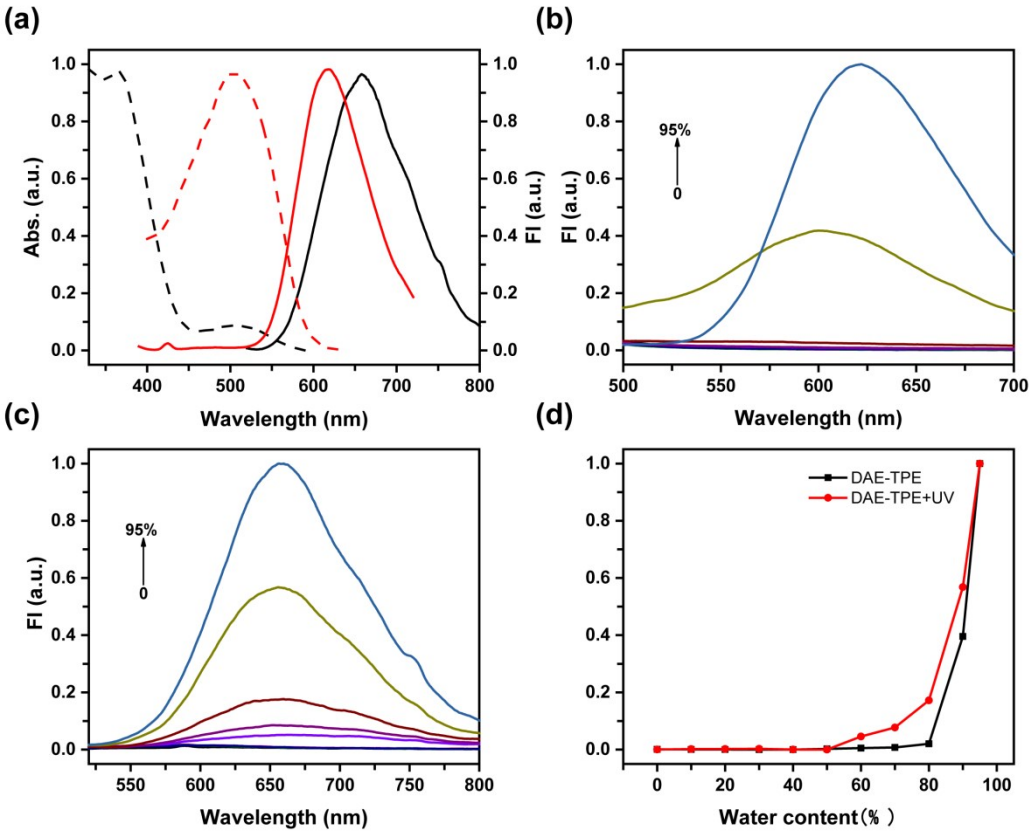

Figure S5 Absorption (dashed line) and emission spectra (solid line) of DAE-TPE NPs (10 μM) before (black line) and after UV irradiation (red line) in aqueous solution (a). Fluorescent intensity of DAE-TPE in H_2O/THF mixtures with different water fraction before (b, λ_{ex} = 365 nm) and after (c, λ_{ex} = 500 nm) UV irradiation. (d) Plot of fluorescence intensity of DAE-TPE before (measured at 620 nm) and after (measured at 660 nm) UV irradiation in H_2O/THF mixtures with different water fraction.

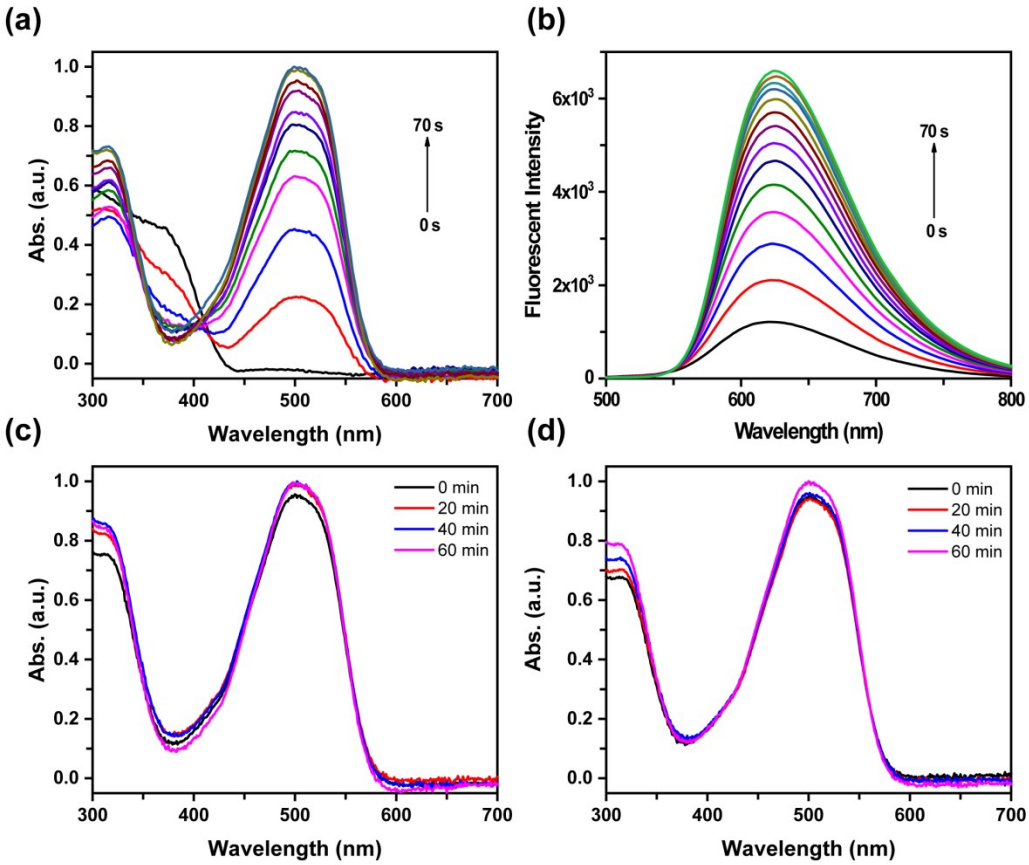

Figure S6 The evolution of absorption (a) and fluorescence (b) spectra (λ_{ex} = 410 nm) of DAE-TPE (5 μM) upon UV irradiation in toluene solution. The evolution of absorption spectra of DAE-TPE with 465 nm irradiation (c) and 520 nm irradiation (d) in toluene solution.

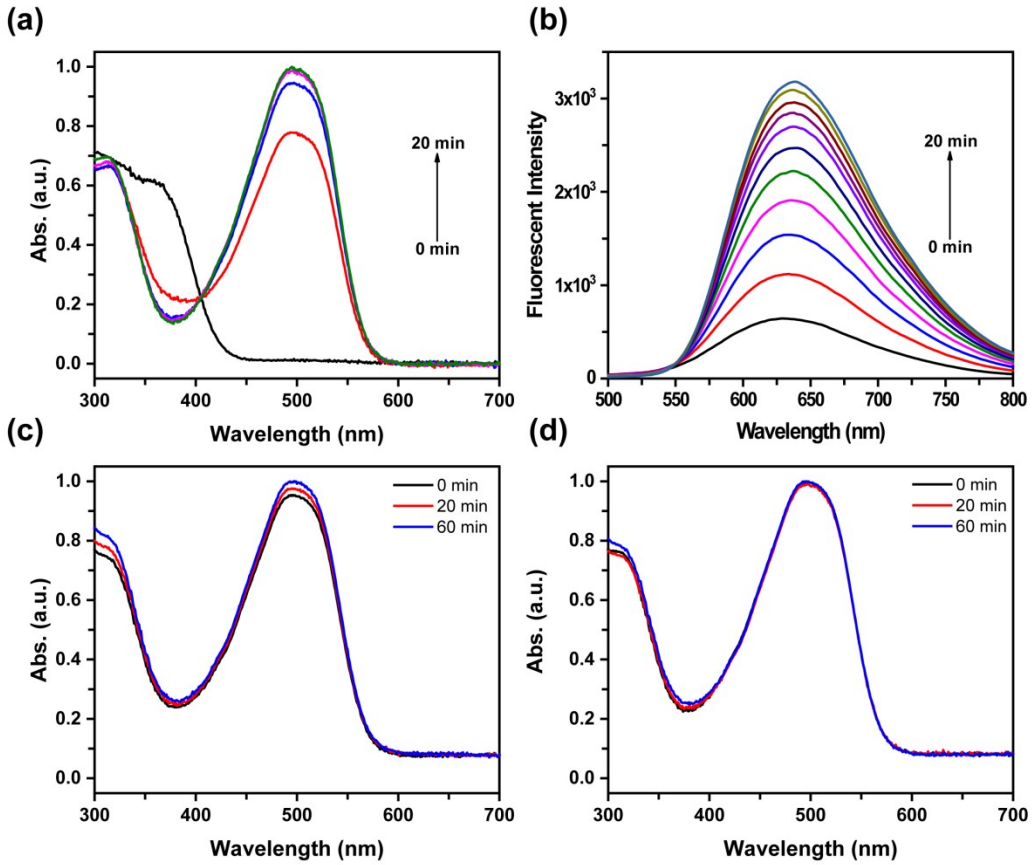

Figure S7. The evolution of absorption (a) and fluorescence (b) spectra (λ_{ex} = 410 nm) of DAE-TPE (5 μM) upon UV irradiation in 1, 4-dioxane solution. The evolution of absorption spectra of DAE-TPE with 465 nm irradiation (c) and 520 nm irradiation (d) in 1, 4-dioxane solution.

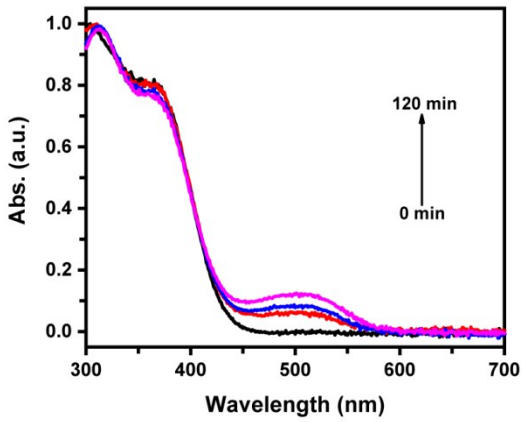

Figure S8. The evolution of absorption spectra of DAE-TPE (5 μM) upon UV irradiation in DMSO solution.

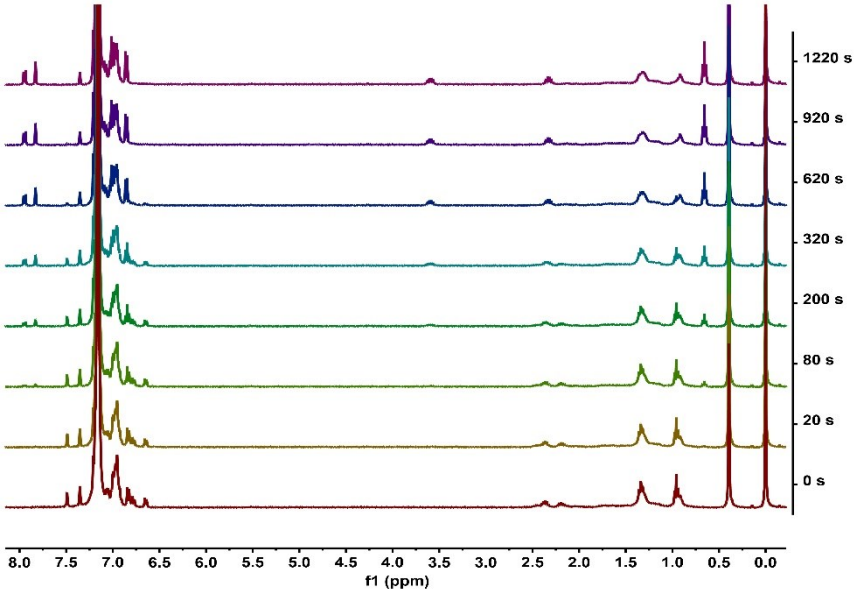

Figure S9. The evolution of ^{1}H NMR spectra of DAE-TPE upon 365 nm irradiation in deuterated benzene.

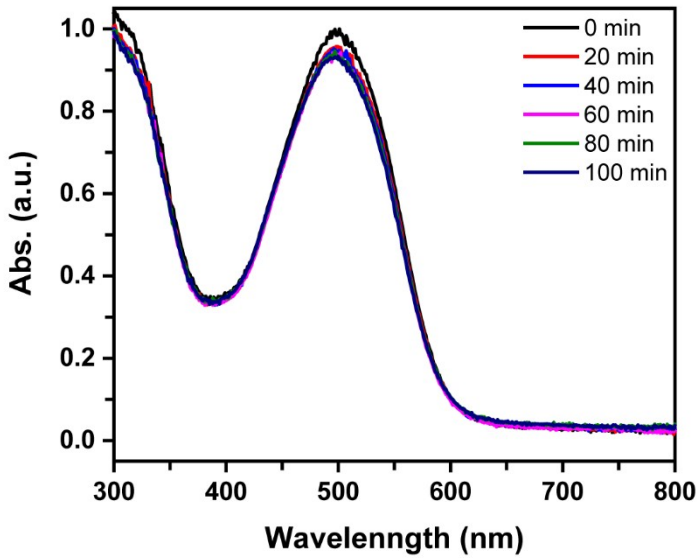

Figure S10 The evolution of absorption spectra of DAE-TPE NPs (5 μM) upon 520 nm irradiation in aqueous solution.

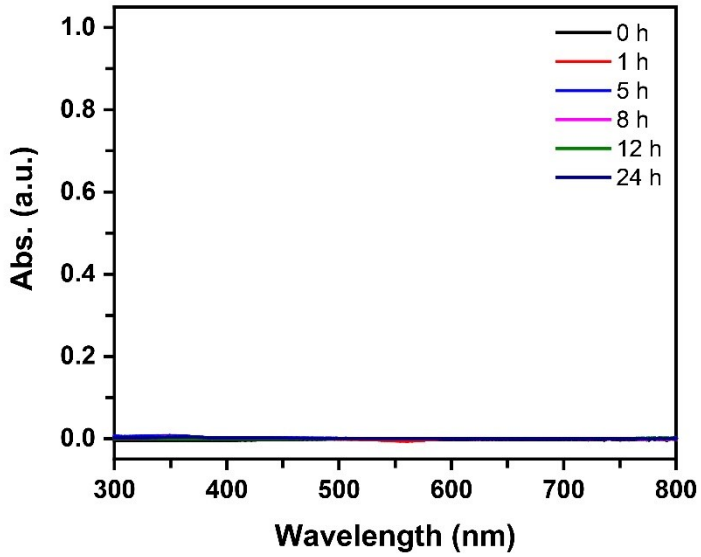

Figure S11 The evolution of absorption spectra of the supernatant containing DAE-TPE NPs within 24 h.

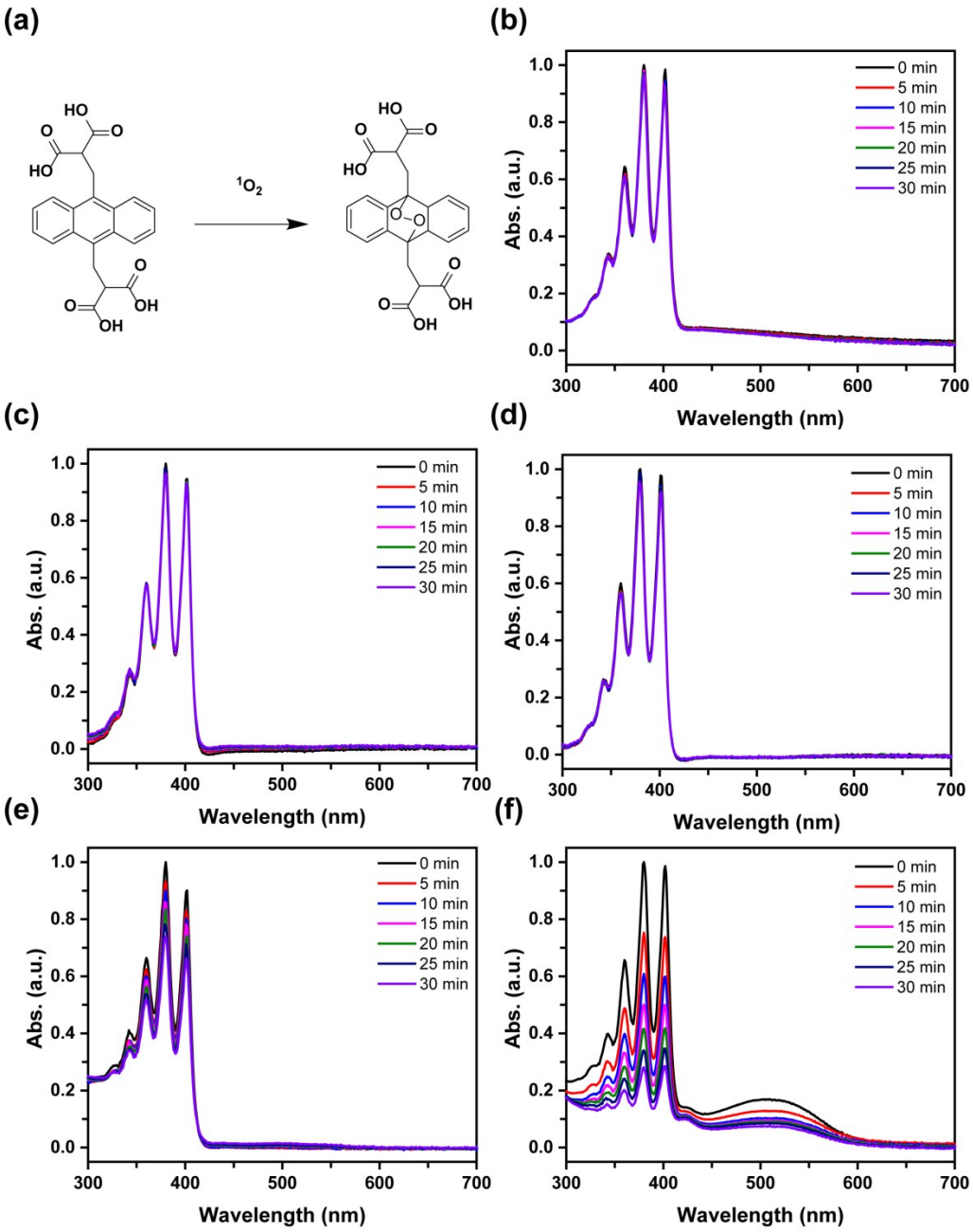

Figure S12. (a) Mechanism diagram of the reaction between ABDA and $^1O_2$; (b) The evolution of UV-Vis absorption spectra of ABDA in TPE NPs solution irradiated for different durations with light irradiation (520 nm, 168.3 mW cm^{-2}); (c) The evolution of UV-Vis absorption spectra of ABDA in DAE NPs (opened form) solution irradiated for different durations with light irradiation (520 nm, 168.3 mW cm^{-2}); (d) The evolution of UV-Vis absorption spectra of ABDA in DAE NPs (closed form) solution irradiated for different durations with light irradiation (520 nm, 168.3 mW cm^{-2}); (e) The evolution of UV-Vis absorption spectra of ABDA in DAE-TPE NPs (opened form

form) solution irradiated for different durations with light irradiation (520 nm, 168.3 mW cm^{-2}); (f) The evolution of UV-Vis absorption spectra of ABDA in DAE-TPE NPs (closed form) solution irradiated for different durations with light irradiation (520 nm, 168.3 mW cm^{-2})

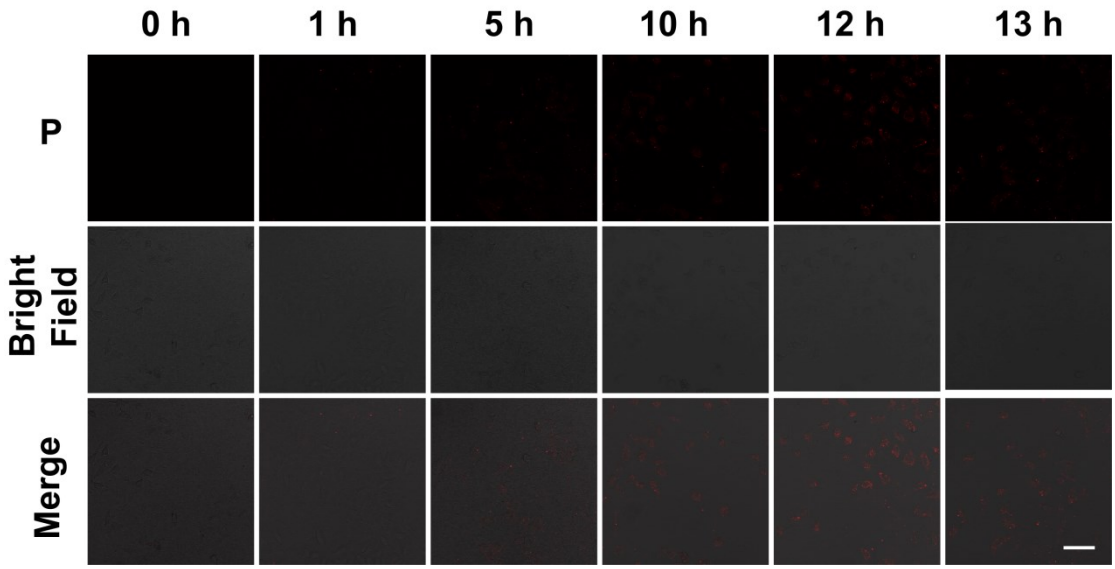

Figure S13 Real-time uptake images of DAE-TPE NPs (5 μM) in HeLa cells. Cells were incubating with DAE-TPE NPs (5 μM), and then confocal fluorescence images were recorded at different time points (0 h, 1 h, 5 h, 10 h, 12 h and 13 h).

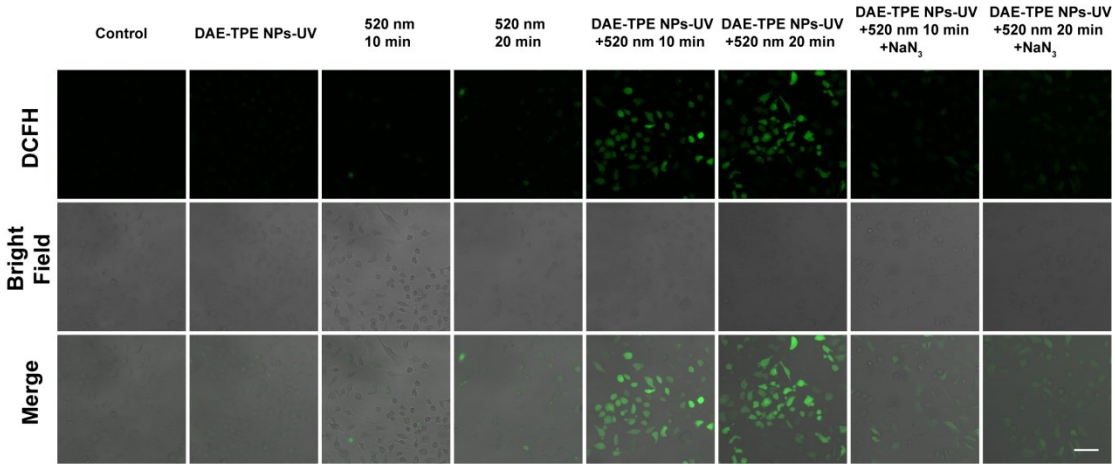

Figure S14 CLSM images of HeLa cells (scale bar: 100 μm) stained with DCFH-DA after different treatments. Different treatments included control, DAE-TPE NPs + UV only, 520 nm only (40 mW cm^{-2}, 10/20 min), DAE-TPE NPs + UV then 520 nm (40 mW cm^{-2}) for 10/20 min +NaN_3 groups.

Reference

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2. Y. C. Jeong, S. I. Yang, K. H. Ahn and E. Kim, *Chem. Commun.*, 2005, **19**, 2503-2505.