AIE based GSH activatable photosensitizer for imaging-guided photodynamic therapy

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Experimental section

General apparatus and reagents

Unless otherwise stated, all the starting materials for synthesis were commercially available and used without further purification. The solvents used for emission measurements were purified by standard procedures. The compounds **2** and **4** were prepared by the literature method [S1-S2]. The compound **TPEPY** used for Cyclic voltammograms experiment was prepared by reference S1. The synthetic procedure is summarized in Scheme S1.

Emission spectra were recorded on a Hitach F-4500 spectrophotometer. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded using a Varian instrument. HRMS were recorded using LC/MSD TRAP XCT Plus (1200 Agilent). Cellular imaging was recorded on a Zeiss LSM 710 laser scanning confocal microscopy. Fluorescence quantum yield was measured using an Edinburgh-steady-state/transient fluorescence spectrometer FLS 1000.

Cell Culture

The CT-26 cells were seeded in DMEM medium containing 10% (v/v) fetal calf serum and (100 units/mL) penicillin-streptomycin (100 μg/mL), and were maintained in a humidified incubator with 5% CO_2 at 37 ^{0}C.

Confocal imaging

In order to study exogenous biothiol fluorescence imaging, CT-26 cells were cultured in 96-well microtiter plates, each cell was treated with 25 μM **TPEPy-S-Fc** for 2 h, then processed according to different imaging requirements. Prior to confocal fluorescence imaging, cells were washed three times with 10 mM PBS buffer.

Cytotoxicity studies of cells by MTT method

The CT-26 cells were seeded in a 96-well microtiter plate, each well planted approximately 20,000 cells. After incubation overnight, the medium was removed, cells were washed with 10 mM PBS buffer, and then the cells were treated with various concentrations of the **TPEPy-S-Fc** assembly (0-60 μM) at 37 ^{0}C for 24 h. Afterwards the growth medium was removed and added 100 uL MTT solution (0.5 mg/mL) to each well. The cells were incubated for a further 3 h. Then the MTT solution was removed and add DMSO (100 μL) to each well. The absorbance of MTT at 616 nm was measured by a microplate reader (Genios Tecan). The cells without any treatment were used as control.

Calculated the HOMO/LUMO levels of TPEPY and ferrocence (Fc)

The HOMO/LUMO levels of **TPEPY** and **Fc** were estimated from the respective absorption spectra and oxidation potentials of cyclic voltammograms. The initial oxidation potentials of **Fc** and **TPEPY** were 0.32 V and 0.97 V by cyclic voltammetry (Fig. S2b and S2c) in CH_3CN solution. $\mathbf{E_g = hc/\lambda_{Eg}}$. calculating optical band gaps are 2.55eV and 2.71eV. Where **h** is Planck constant (4,135 x 10^{-15} eV.s), **c** is light speed (3 x 10^8 m.s^{-1}) and λ_{Eg} is the band gap wavelength (m). The optical band gap wavelengths (λ_{Eg}) for all compounds in study were taken from derivative of UV-vis curves.Then calculated separately the HOMO/LUMO levels of **TPEPY** and **Fc**.

The optical band gaps (E_g) were obtained from absorption spectra of **Fc** and **TPEPY** in CH_3CN solution (Fig. S2a), using the following equation:

$$E_g = hc/\lambda_{Eg}$$

Where h is Planck constant (4.135×10^{-15} eV.s), c is light speed (3×10^8 m.s^{-1}) and λ_{Eg} is the band gap wavelength.

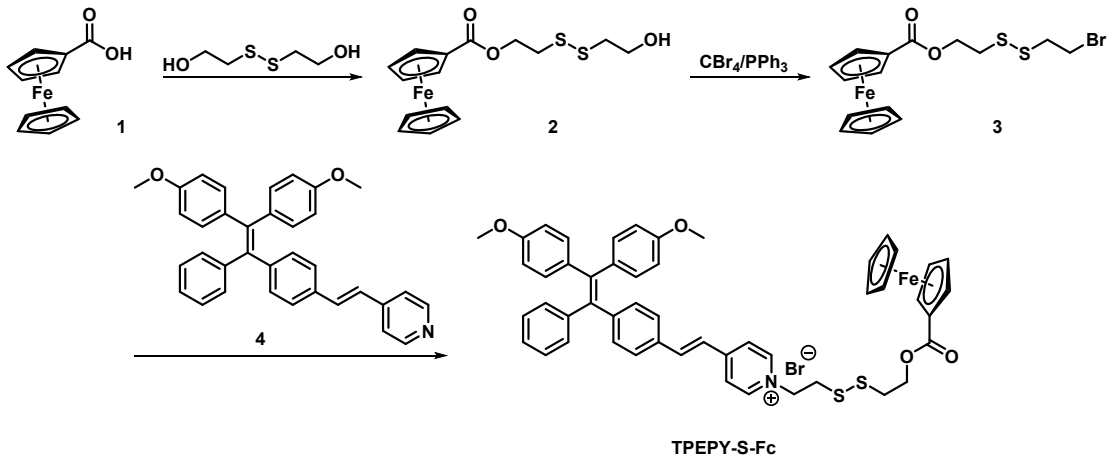
$^1O_2$ quantum yield Measurement.

The $^1O_2$ quantum yield of TPEPY-S-Fc before and after being cut by GSH was quantified using 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA) as the indicator and Rose Bengal (RB) as the standard photosensitizer (the $^1O_2$ quantum yield for Φ_{RB} is 75% in water). In the presence of $^1O_2$, ABDA could undergo oxidation to yield endoperoxide, resulting in a decrease of ABDA absorption. The $^1O_2$ quantum yield was measured using Rose Bengal (RB) as the reference photosensitizer and calculated using the following formula:

$$\phi_{TPE} = \phi_{RB} \frac{K_{TPE} A_{RB}}{K_{RB} K_{TPE}}$$

where K_{TPE} and K_{RB} are the decomposition rate constants of ABDA in the presence of TPEPY-S-Fc before and after being cut by GSH and RB respectively. Φ_{RB} is the $^1O_2$ quantum yield of RB ($\Phi_{RB} = 0.75$ in water). A_{TPE} and A_{RB} represent the integration area of absorption bands ranging from 400 to 800 nm of the probe and RB, respectively.

Synthesis



Scheme S1. Synthesis route of **TPEPY-S-Fc**

Synthesis of compound 3

Compound **2** (200 mg, 0.55 mmol) and triphenylphosphine (143 mg, 0.55 mmol) were dissolved in dichloromethane (30 mL) and stirred for 15 min at 0 ^{o}C. Then carbon tetrabromide (180 mg, 0.54 mmol) was added in portions over 30 min. The mixture was stirred at room temperature for 10 h. After the reaction was completed, the mixture was washed with water (2×15 mL) and brine (150 mL) three times. The organic layer was dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure. The crude product was separated and purified by silica gel column chromatography (EA/PE, 80:20, v/v) to obtain a red oily liquid. The solid product was obtained after freezing. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.80 (s, 2H), 4.46 (t, J = 6.2 Hz, 2H), 4.39 (s, 2H), 4.20 (s, 5H), 3.67–3.58 (m, 2H), 3.15–3.08 (m, 2H), 3.02 (t, J = 6.1 Hz, 2H).

Synthesis of compound TPEPY-S-Fc

Compounds **3** (65 mg, 0.015 mmol), **4** (50 mg, 0.010 mmol) and KI (200 mg, 0.120 mmol) were dissolved in acetonitrile (50 mL), stirred and refluxed overnight. After the reaction was completed, the solvent was removed under reduced pressure, and the obtained solid was dissolved with 20 mL dichloromethane. Then the organic solution washed with water (3×10 mL). The organic phase was dried with Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified with chromatography (dichloromethane/methanol = 100/5, v/v) to give the desired product **TPEPY-S-Fc** as a red solid (32 mg, yield 35%). ^{1}H NMR (400 MHz, DMSO-d_6): 8.92 (d, J = 6.3 Hz, 2H), 8.21 (d, J = 6.7 Hz, 2H), 7.93 (d, J = 16.4 Hz, 1H), 7.52 (d, J = 8.5 Hz, 2H), 7.43 (d, J = 16.4 Hz, 1H), 7.21–7.10 (m, 3H), 7.05 (d, J = 8.2 Hz, 2H), 6.98 (d, J = 6.6 Hz, 2H), 6.89 (d, J = 15.8, 8.5 Hz, 4H), 6.71 (t, J = 9.3 Hz, 4H), 4.81 (t, J = 5.7 Hz, 2H), 4.73 (s, 2H), 4.48 (s, 2H), 4.39 (t, J = 6.2 Hz, 2H), 4.23 (s, 4H), 3.68 (s, 6H), 3.43 (t, J = 7.0 Hz, 2H), 3.09 (t, J = 5.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 158.4 ,158.3, 154.1, 148.1, 144.5, 144.4, 143.6, 142.5, 141.9, 138.5, 138.1, 135.8, 135.7, 132.6, 132.5, 132.2, 131.8, 131.3, 128.0 , 127.9, 126.4, 123.5, 121.2 , 113.2, 113.0, 109.9, 71.6, 70.2, 69.8, 61.5, 58.6, 55.1, 55.0, 38.6, 37.3, 31.4, 30.1, 29.6,

29.3. HRMS (MADAL-TOF), m/z: calcd. for $C_{50}H_{46}FeBrNO_4S_2$ [M-Br$^-$] 844.2212, found: 844.2200。

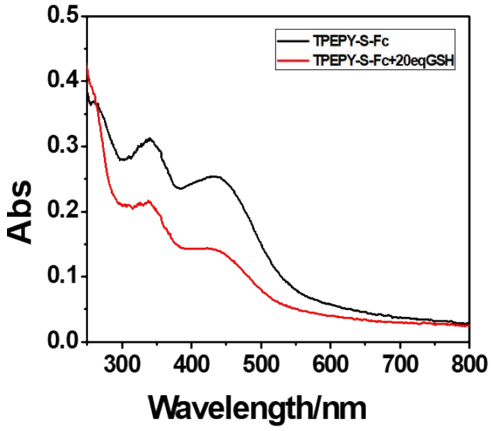


Fig. S1. The absorption of **TPEPY-S-Fc** (20 μM) before and after addition of 20 equiv of GSH in PBS (10 mM, pH = 7.4 with 0.5% DMSO) buffer solution. The spectra were recorded after incubation for 80 min at 37 °C, λ_{ex} = 430 nm.

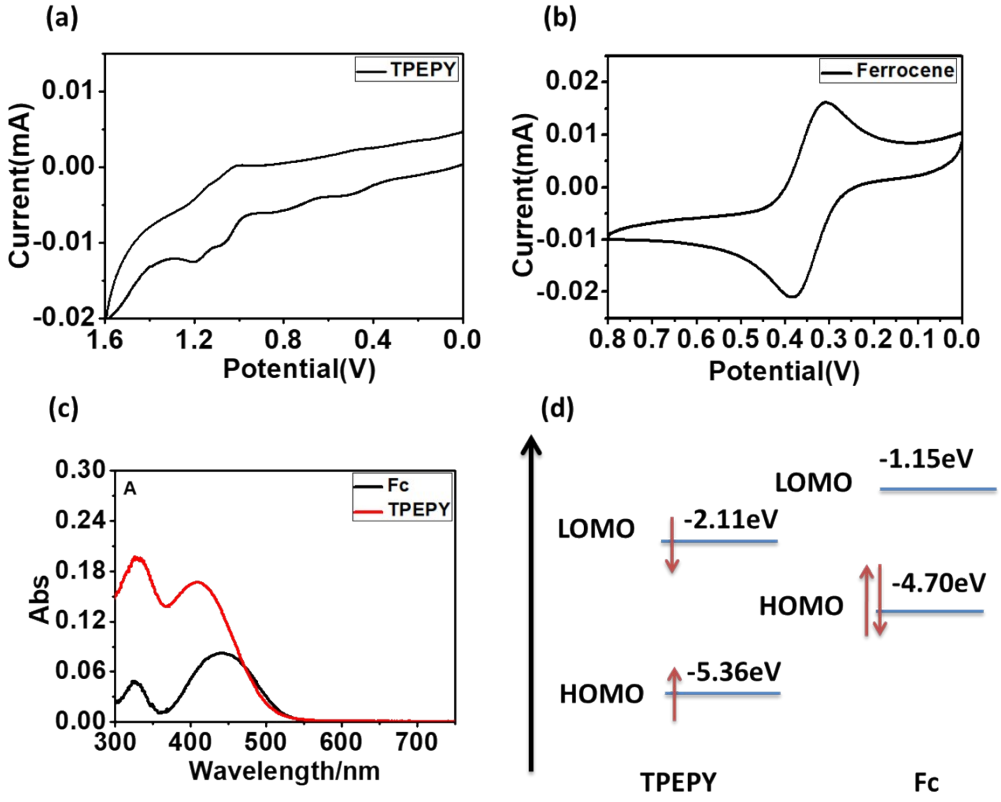


Fig. S2. (a) Absorption spectra of **Fc** and **TPEPY** in acetonitrile solution. The Cyclic voltammograms of (b) **Fc** and (c) **TPEPY**. Pt was used as working electrode and counter electrode; Ag/AgCl in saturated KCl was used as reference electrode; *n*-

Bu4NPF6 (0.1 M) in acetonitrile was used as supporting electrolyte. (d) The calculated HOMO/LUMO levels of **Fc** and **TPEPY**

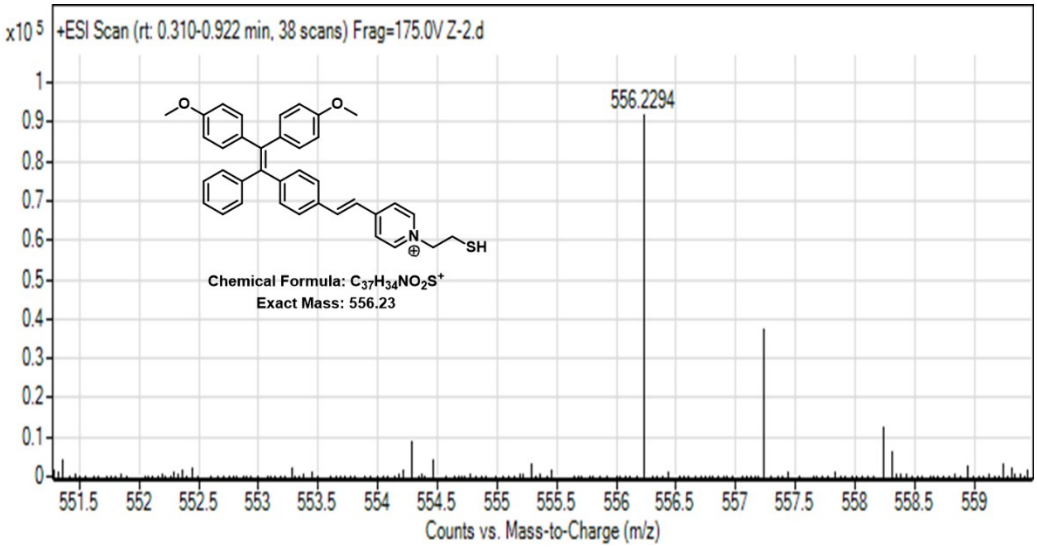


Fig. S3. The HRMS spectra of **TPEPY-SH**

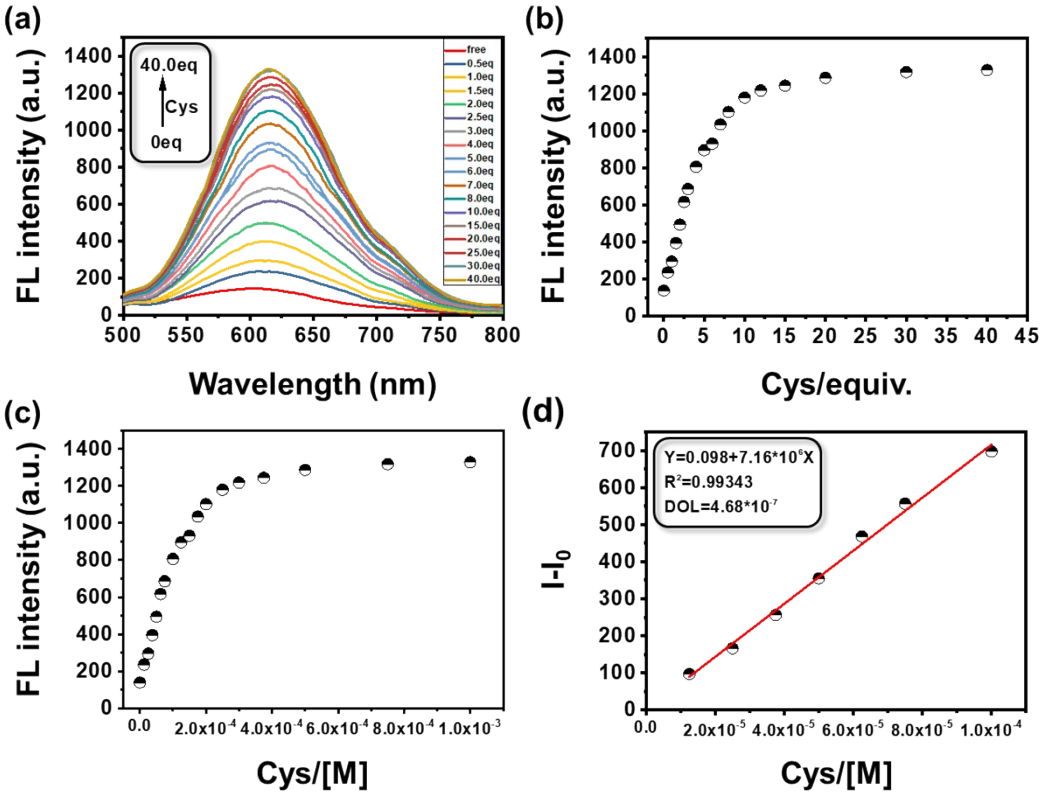


Fig. S4. The fluorescence spectra of **TPEPY-S-Fc** (20 μM) upon addition of various amount of Cys (0 to 40 equivalents) in PBS (10 mM, pH = 7.4 with 0.5% DMSO) buffer solution. The spectra were recorded after incubation for 80 min at 37 °C, λ_{ex} = 430 nm.

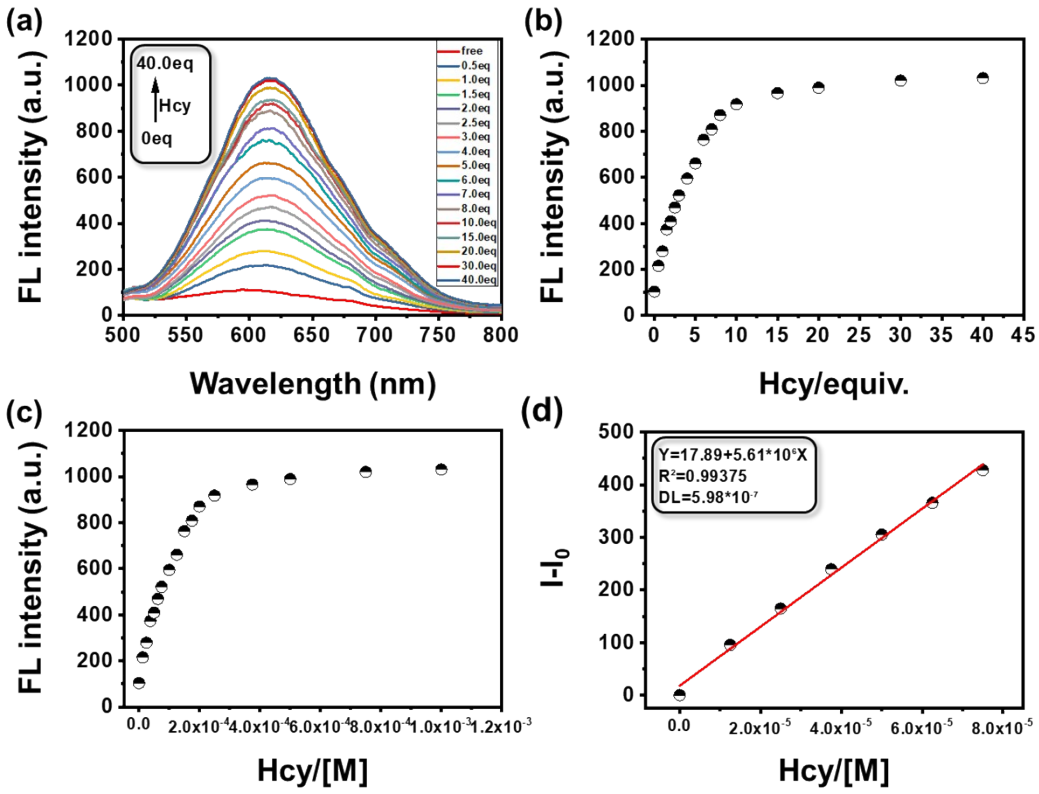


Fig. S5. The emission spectra of **TPEPY-S-Fc** (20 μM) upon addition of various amount of Hcy (0 to 40 equivalents) in PBS (10 mM, pH = 7.4 with 0.5% DMSO) buffer solution. The spectra were recorded after incubation for 1 h at 37 °C, λ_{ex} = 430 nm.

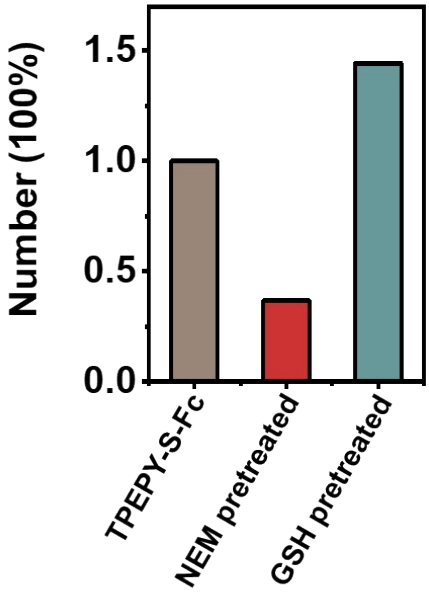


Fig. S6. Quantitative comparisons of the intracellular red fluorescent intensity via different treated methods.

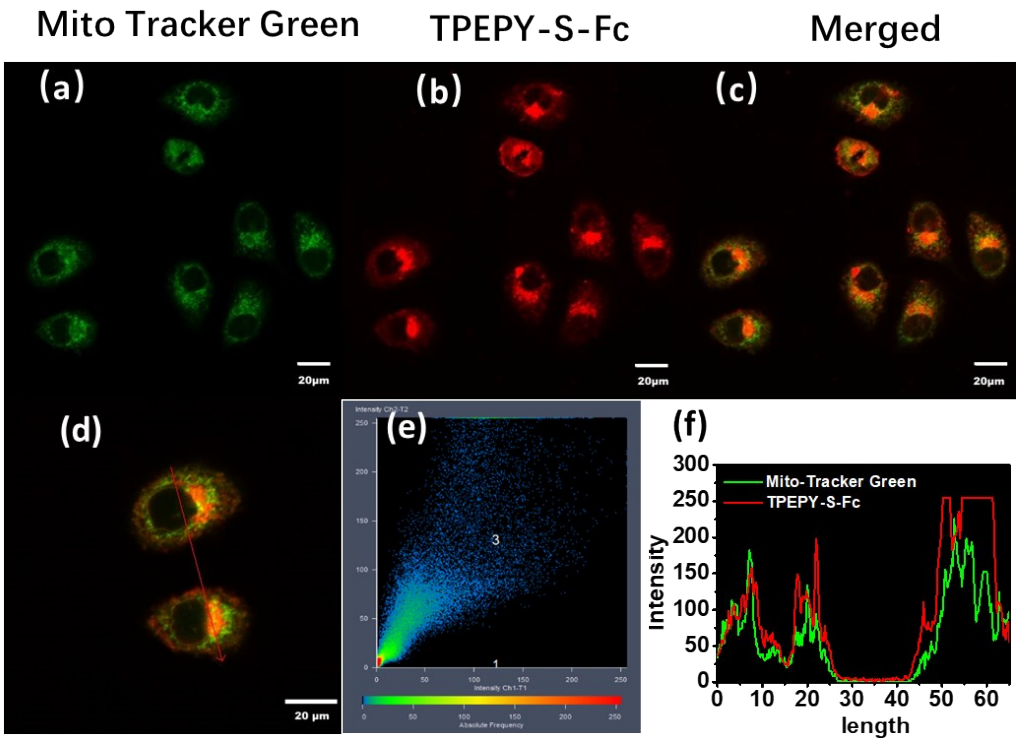


Fig. S7. Confocal fluorescence images of SMMC cells stained with (a) 100 nM Mito Tracker green (green channel, λex = 488 nm, collection: 495-545 nm), and (b) 10μM **TPEPY-S-Fc** (red channel, λex = 405 nm, collection: 560-740 nm). (c) Merged images of (a and b). (d) Enlarged picture of the selected region in (c). (e) The fluorescence intensity correlation plot of **TPEPY-S-Fc** and Mito Tracker Green. Pearson's correlation coefficient: 0.89 (f) Intensity profile within the regions of interest (ROIs; white line in (d)) of **TPEPY-S-Fc** and Mito Tracker Green across the cells.

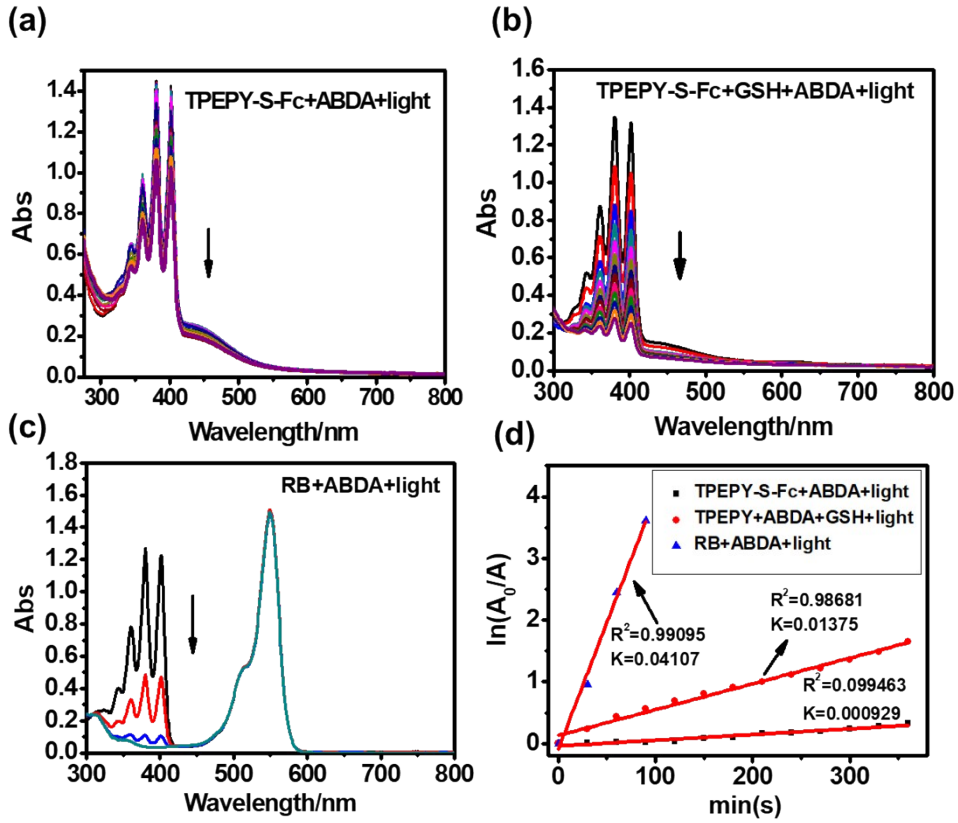


Figure S8. (a) **TPEPY-S-Fc** (20 μM) and ABDA (120 μM) were mixed for illumination. (b) **TPEPY-S-Fc** (20 μM) after cut by GSH and mixed with ABDA (120 μM) for illumination. (c) RB (20 μM) and ABDA (120 μM) were mixed and illuminated with a Xenon lamp irradiation (400-800 nm) with a power density of 400 mW/cm^2.

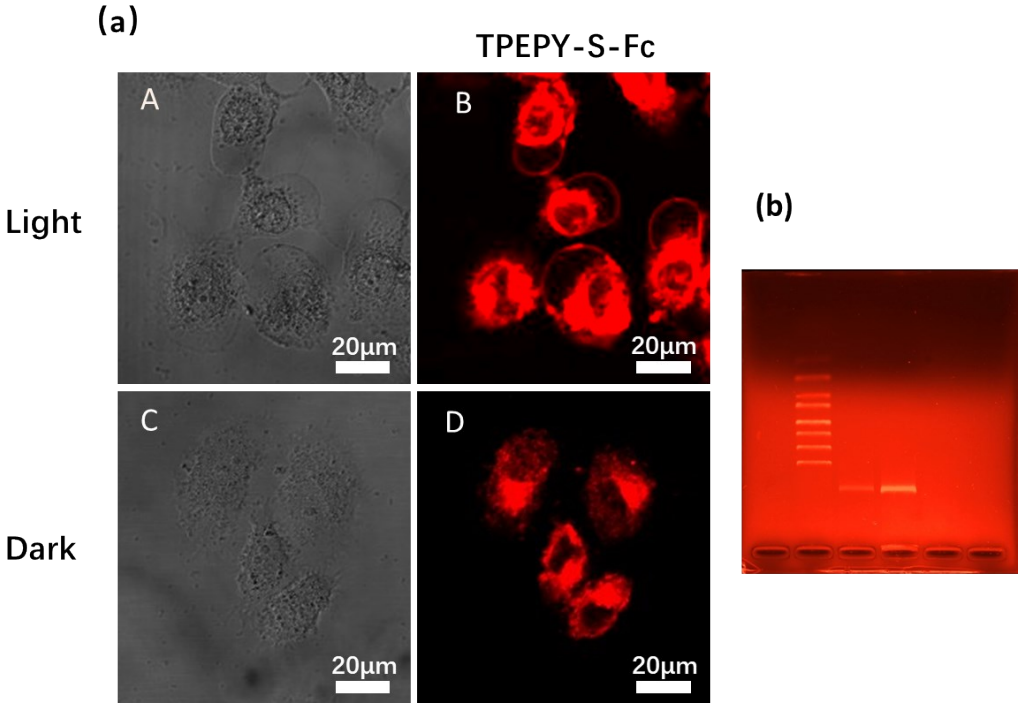


Fig. S9. (a) Confocal images of SMMC cells stained after the PDT treatment (20 μM of **TPEPY-S-Fc** incubation and light irradiation). SMMC cells with the incubation of **TPEPY-S-Fc** and light irradiation (A, B). SMMC cells just treated with **TPEPY-S-Fc** incubation (C, D). The scale bar is 20 μm. λ_{ex}: 405 nm; λ_{em}: 560-740 nm. (b) DNA analysis by agarose gel electrophoresis. In gel electrophoresis, no DNA laddering were detected (A, from left to right: lane1, marker; lane2, -light **TPE-PY-S-Fc**; lane3, -light **TPE-PY-S-Fc**; indicating that apoptosis did not happen.

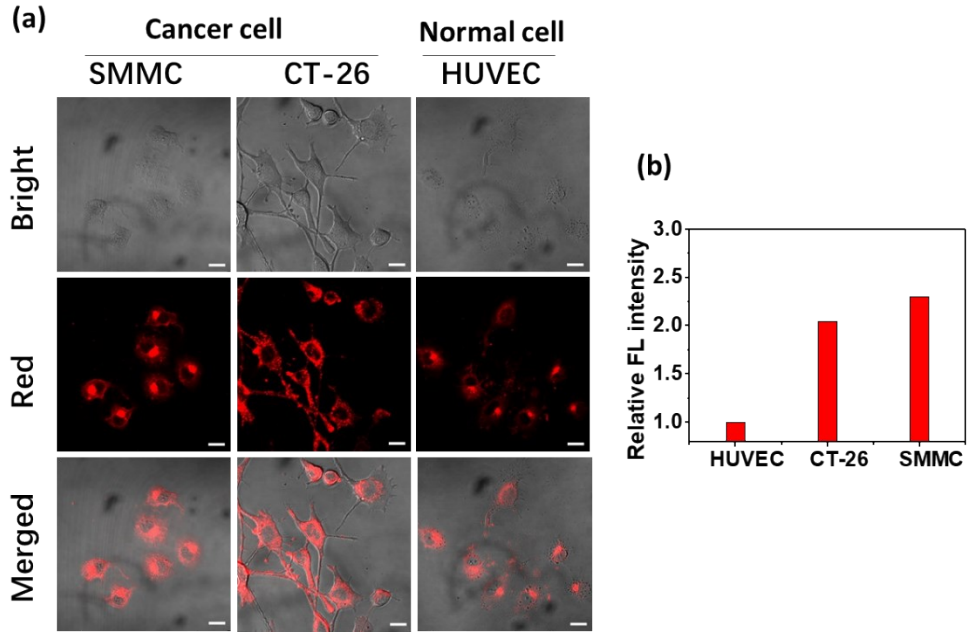


Fig. S10. Fluorescence imaging of endogenous biothiols for cancer cell recognition. (a) Fluorescence imaging of different cancer cell lines (SMMC and CT-26) and normal cell lines (SUVEC) after being incubated with 10 μM **TPYPY-S-Fc** for 120 min. (b) Thebar chart shows the relative fluorescence intensity between different cells. λ_{ex}: 405 nm; λ_{em}: 560-740 nm.

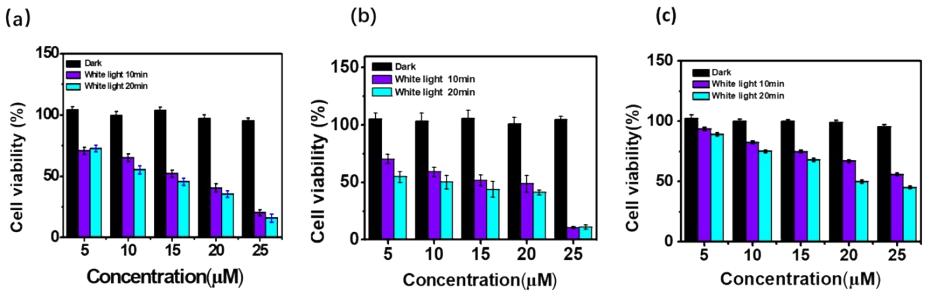


Fig. S11. Cytotoxicity **TPEPY-S-Fc** with or without light illumination. (a) SMMC cells, (b) CT-26, and (c) SUVEC cells.

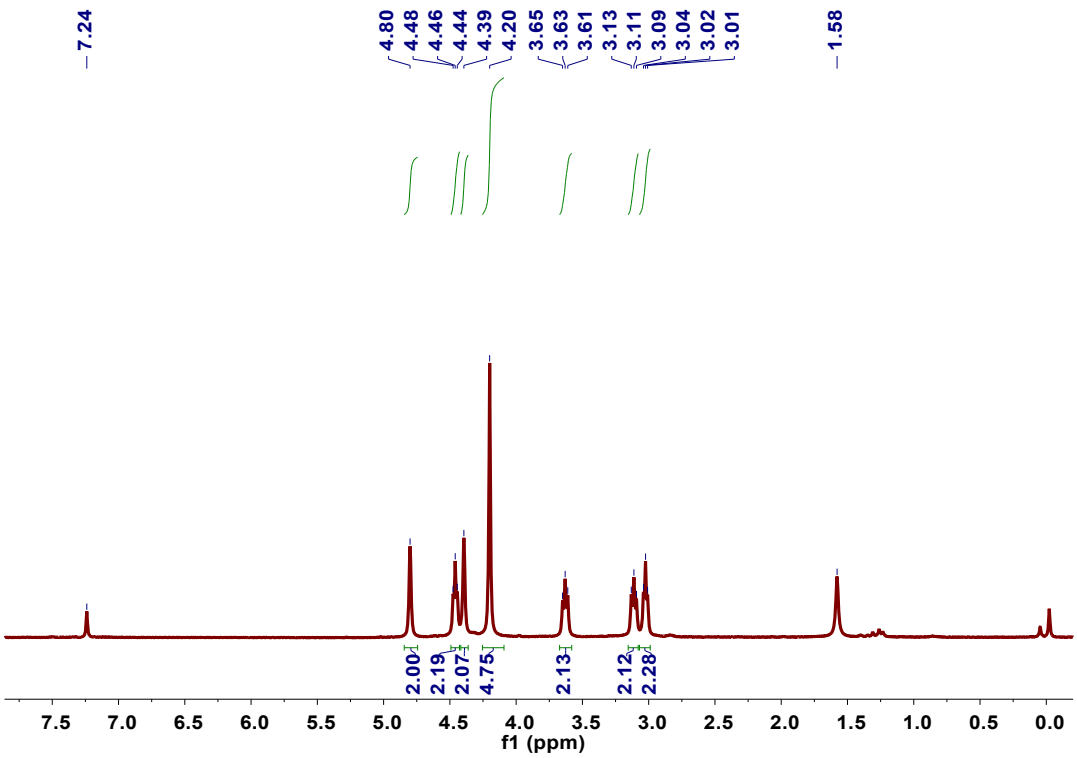


Fig. S12. The ^{1}H NMR spectrum of 3 in $CDCl_3$ solution

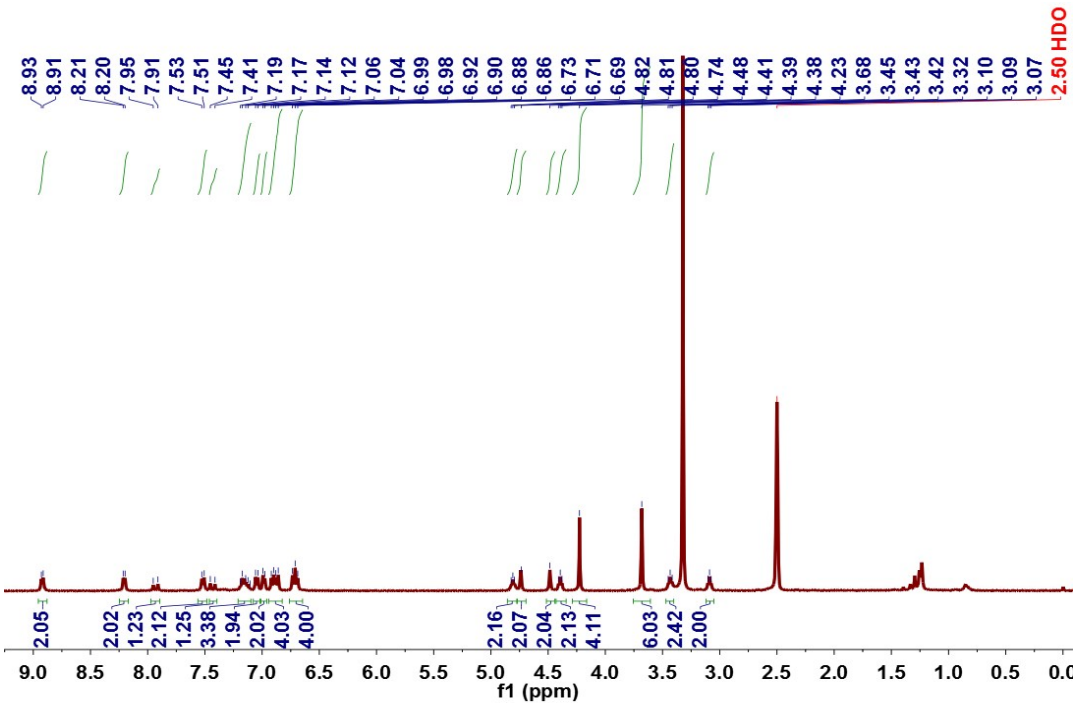


Fig. S13. The ^{1}H NMR spectrum of **TPEPY-S-Fc** in $CDCl_3$ solution.

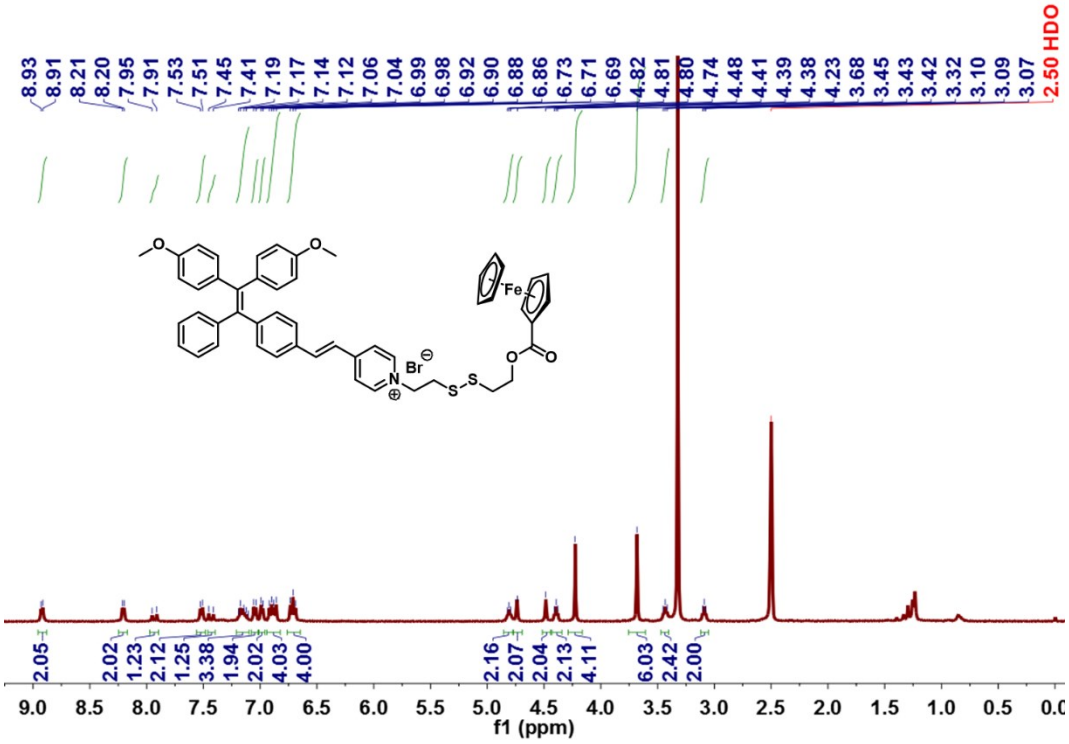


Fig. S14. The ^{13}C NMR spectrum of TPEPY-S-Fc in $CDCl_3$ solution.

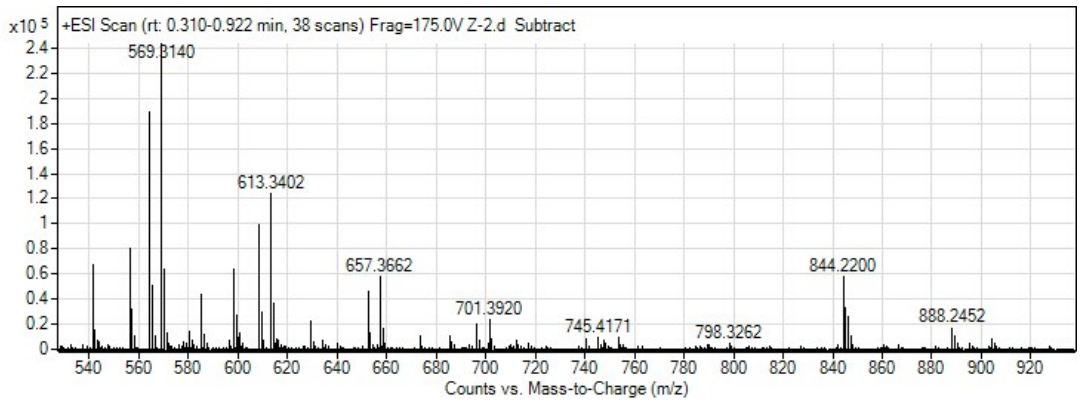


Fig. S15. The HR MS spectrum of TPEPy-S-Fc.

References:

S1: F. Hu, Y. Yuan, D. Mao, W. Wu, B. Liu, *Biomaterials*, 2017, **144**, 53−59;

S2: Y. Kang, X. Ju, L.-S. Ding, S. Zhang and B.-J. Li, *ACS Appl. Mater. Interfaces*, 2017, **9**, 4475−4484.