Supplementary Information

A GSH-activatable theranostic prodrug based on photoinduced electron transfer for cancer fluorescence imaging and therapy

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1. Synthesis

1.1 Synthesis of HCy

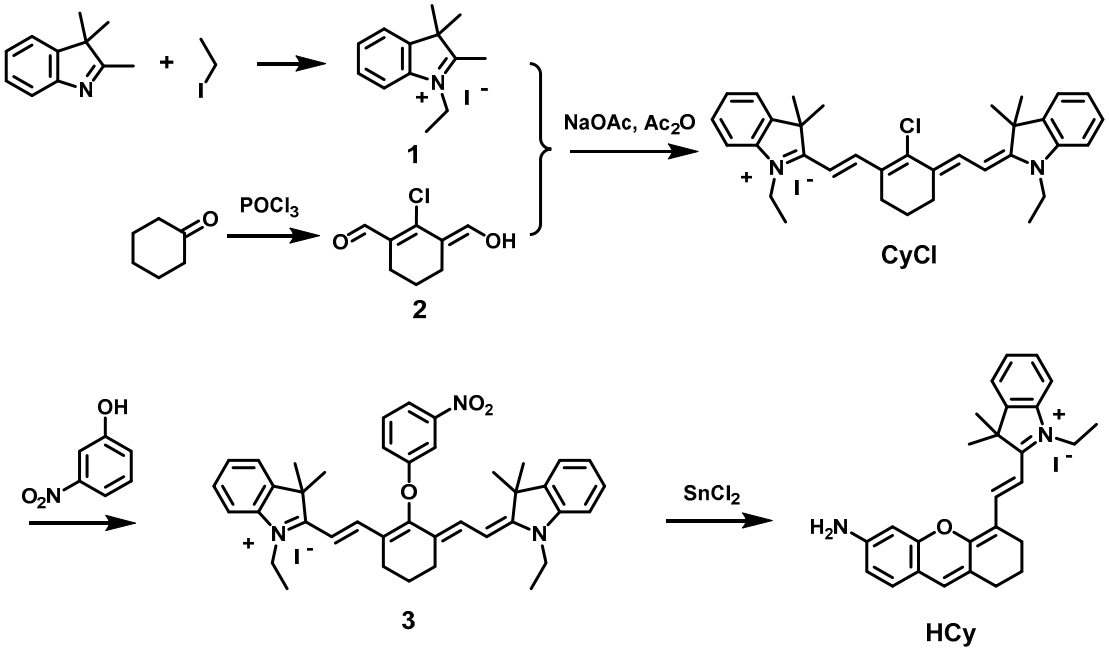

Figure S1. Synthetic route of HCy.

Synthesis of compound 1

2,3,3-trimethylindolenine (3.18 g, 20 mmol) was dissolved in toluene (5 mL), iodoethane (3.12 g, 20 mmol) was added dropwise under argon atmosphere. The mixture was stirred at 80 °C for 24 h, cooling down to room temperature and filtered. Compound 1 was obtained as a pink solid without further purification (5.62 g, 89%).

Synthesis of compound 2

Dry dimethylformamide (DMF) (10 mL, 135 mmol) was placed into a 100 mL two-neck round flask under argon atmosphere. Dichloromethane (DCM) (10 mL) containing phosphorus oxychloride (10 mL, 65 mmol) was added dropwise over 30 min at 0 °C.

The solution was stirred at room temperature for 30 min, followed by adding cyclohexanone (2.5 g, 25 mmol) dropwise. The resulting solution was then reflux for 3 h. After cooling down to room temperature, the mixture was poured into cold water and kept in refrigerator overnight. Compound 2 was filtered and washed with water, which was obtained as a yellow solid (3.10 g, 68%).

Synthesis of compound CyCl

Compound 1 (1.83 g, 5.8 mmol), compound 2 (0.5 g, 2.9 mmol) and sodium acetate (0.48 g, 18 mmol) were dissolved in acetic anhydride (10 mL) under argon atmosphere. The mixture was stirred at 130 °C for 3 h. After cooling down to room temperature, the solution was dropped into cold diethyl ether. The formed precipitate was filtered and re-dissolved in DCM, followed by washing with diethyl ether and aqueous $NaHCO_3$. CyCl was obtained as a metallic green solid (1.80 g, 91%).

Synthesis of compound 3

3-Nitrophenol (347 mg, 2.5 mmol), CyCl (667 mg, 1 mmol) and K_2CO_3 (345 mg, 2.5 mmol) were dissolved in acetonitrile (15 mL). The mixture was stirred at room temperature under argon atmosphere for 12 h. The solvent was evaporated and the precipitate was dissolved in DCM, followed by washing with aqueous $NaHCO_3$ for three times and dried over Na_2SO_4. The crude product was purified via silica chromatography (eluent: methanol/DCM =1/10 to 1/8). Compound 3 was obtained as a

golden solid (423 mg, 57%).

Synthesis of compound HCy

Compound 3 (254 mg, 0.34 mmol) was dissolved in CH_3OH (10 mL). $SnCl_2$ (1.3 g, 6.86 mmol) in concentrated HCl (1.4 mL) was added to above solution under argon atmosphere. The mixture was refluxed overnight. After reaction, CH_3OH was evaporated. The mixture was diluted with DCM, washed with water for three times, followed by neutralizing with $NaHCO_3$. The crude product was purified via silica chromatography (eluent: methanol/DCM =1/10 to 1/5). HCy was obtained as a blue solid (85 mg, 48%).

1.2 Synthesis of Fe-SS-HCy

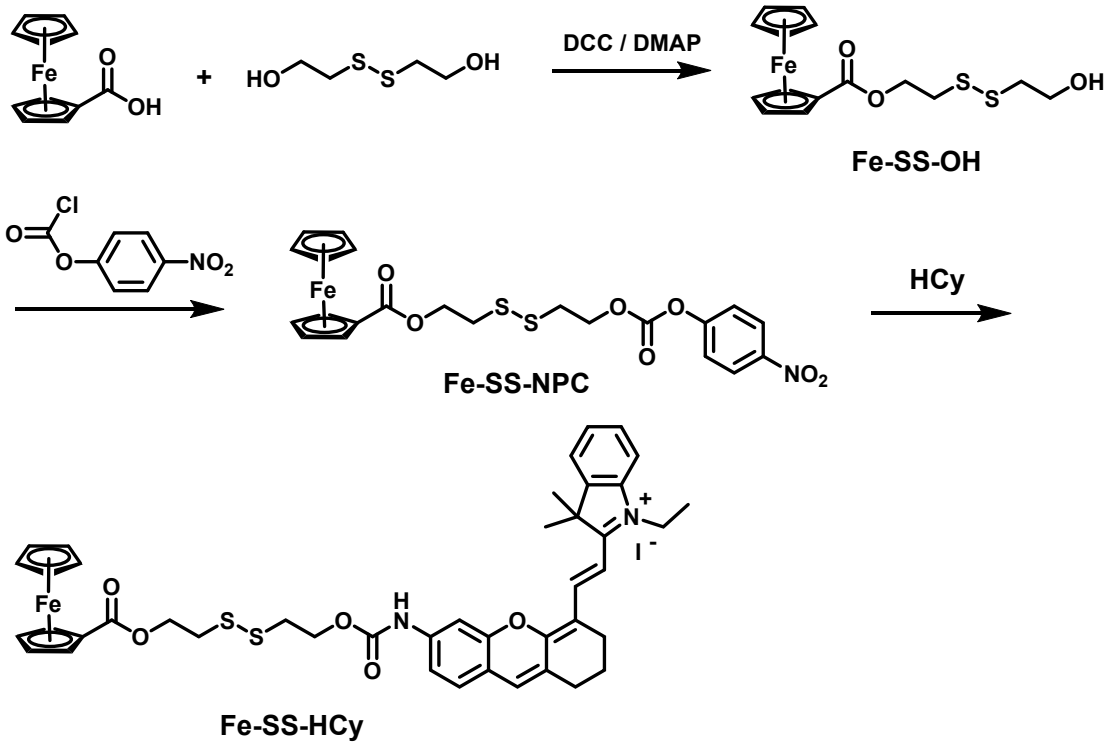

Figure S2. Synthetic route of Fe-SS-HCy.

Synthesis of compound Fe-SS-OH

Ferrocenecarboxylic acid (922 mg, 4 mmol), 2,2′-dithiodiethanol (618 mg, 4.01 mmol) and DMAP (976 mg, 8 mmol) were dissolved in anhydrous DCM under argon atmosphere. DCC (1.03 g, 5 mmol) was added dropwise into above solution at 0°C. The mixture was stirred at room temperature for 24 h. After reaction, the resulting DCU was filtered. The crude product was purified via silica chromatography (eluent: DCM/acetone = 5/1). Fe-SS-OH was obtained as a yellow oil (976 mg, 67%).

Synthesis of Fe-SS-NPC

Fe-SS-OH (73.2 mg, 0.2 mmol) and triethylamine (61 mg, 0.6 mmol) were dissolved in DCM (5 mL) and cooled to 0 ℃. p-Nitrophenyl chloroformate (120 mg, 0.6 mmol) in DCM (2 mL) was added dropwise. Subsequently, the solution was stirred at room temperature overnight. Then the mixture was washed with $NaHCO_3$ and extracted with DCM three times. The organic layer was combined, dried over Na_2SO_4. The crude product was purified via silica chromatography (eluent: DCM). Fe-SS-NPC was obtained as a yellow oil (89 mg, 84%).

Synthesis of Fe-SS-HCy

Fe-SS-NPC (53.1 mg, 0.1 mmol) was dissolved in dry DMF (5 mL) which contained triethylamine (1 mL). HCy (52.4 mg, 0.1 mmol) in DMF (3 mL) was added dropwise and stirred at room temperature for 18 h. Subsequently, DCM was added into the solution and washed with water three times, followed by saturated $NaHCO_3$. The crude

product was purified via silica chromatography (eluent: methanol/DCM = 1/10 to 1/8). Fe-SS-HCy was obtained as blue solid (64.5 mg, 82%).

2. Computational details

The energies of the optimized geometries of all compounds were determined by using the density functional theory (DFT) method with the Becke, three-parameter, Lee−Yang−Parr (B3LYP) hybrid functional for exchange correlation and LanL2DZ in the form of a basis set. The optimized geometries of all compounds are shown in Fig S15. The calculations were performed using the Gaussian 09 and GaussView software.

3. Cell experiments

Cell culture

MCF-7, BEL-7404 cancer cells were cultured in RPMI-1640 medium. A549 cancer cells and HUVEC normal cells were cultured in DMEM medium. The medium was complemented with 10% fetal bovine serum (FBS, Invitrogen, USA) and 1% penicillin/streptomycin (Life Technologies, USA). The cell lines were maintained in humidified atmosphere at 37 °C and 5% of CO_2.

Cell viability assay

All cell viability tests were evaluated using a standard Cell Counting Kit-8 (CCK-8) assay kit (Solarbio, China). Typically, cancer cells were incubated with DMEM/ RPMI-1640 medium in 96-well plates at a density of 5.0×10^3 cells per well overnight. The

medium was then replaced by DMEM/ RPMI-1640 containing different concentration of Fe-SS-HCy. Then, cells were incubated for 24 h or 48 h. To investigate the cell viability influenced by NEM, cancer cells were pre-treated with 0.5 mM NEM, followed by incubation with Fe-SS-HCy for 24 h. Subsequently, CCK-8 working solution was added as 10 μL per well, followed by another incubation for 2 h. Cell viability was assessed by the measurement of the absorbance at the wavelength of 450 nm on the microplate reader (EnSpire, Perkin Elmer). Treatment procedures were replicated five times for each cell line.

Cell imaging by confocal laser scanning microscopy (CLSM)

1×10^5 cells per milliliter were seeded in 35 mm diameter μ-dishes and cultured overnight in supplemented medium. The medium was replaced by fresh medium containing 0.3 mg/mL Fe-SS-HCy. The cells were incubated with different time periods. Subsequently, the lysosomes, nucleus, mitochondria and lipid droplet were stained with LysoTracker, Hoechst 33342, MitoTracker and Nile Red, respectively. Fe-SS-HCy was further removed by washing with PBS three times. Live cells images were taken using CLSM (LSM710, Carl Zeiss). HCy was excited with a 635 nm laser and detected in the range from 680 to 760 nm. The cell lysosomes were excited with a 561 nm laser and detected in the range from 600 to 650 nm. The cell nuclei were excited with a 405 nm laser and detected in the range from 425 to 475 nm. The cell mitochondria were excited with a 561 nm laser and detected in the range from 600 to 650 nm. The lipid droplets

were excited with a 561 nm laser and detected in the range from 600 to 650 nm.

For the intracellular ROS detection assay, the fluorescent probe DCFH-DA (Solarbio, China) was used according to the manufacture's instruction. Specifically, 1×10^5 cells per milliliter were seeded in 35 mm diameter μ-dishes and cultured overnight in supplemented medium. The cells were divided into four groups: (1) Control group; (2) probe group; (3) Fe-SS-HCy group; (4) + Fe-SS-HCy + probe group. Then live cells were imaged using CLSM (LSM710, Carl Zeiss). DCFH-DA was excited with a 488 nm laser, detected in the range from 500 to 550 nm.

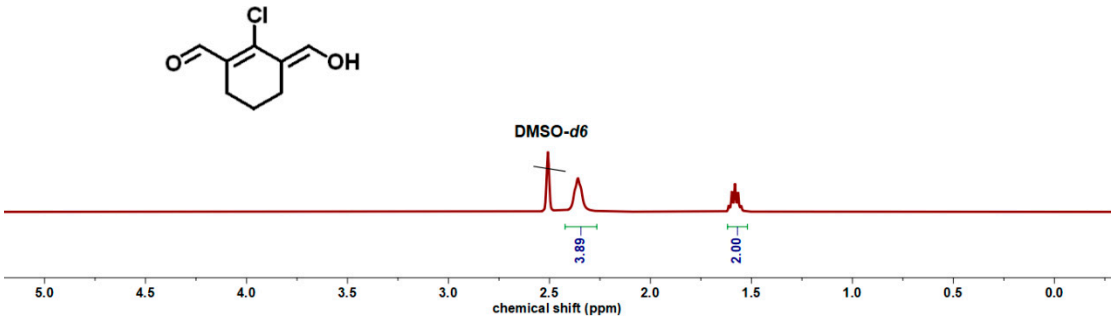

Figure S3. ^{1}H NMR of compound 2 (400 MHz, DMSO-d_6).

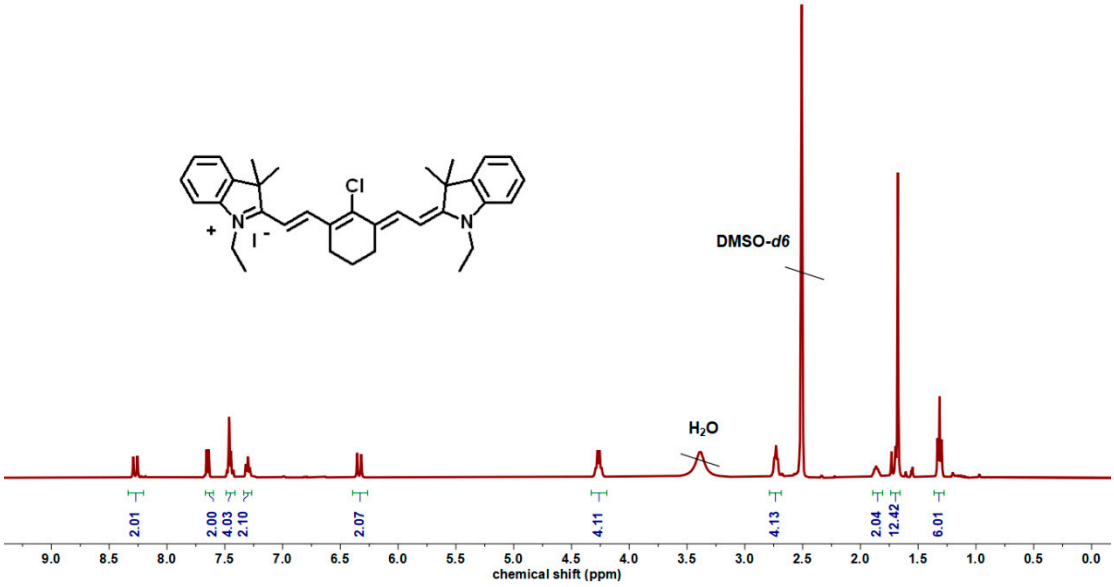

Figure S4. ^{1}H NMR of CyCl (400 MHz, DMSO-d_6).

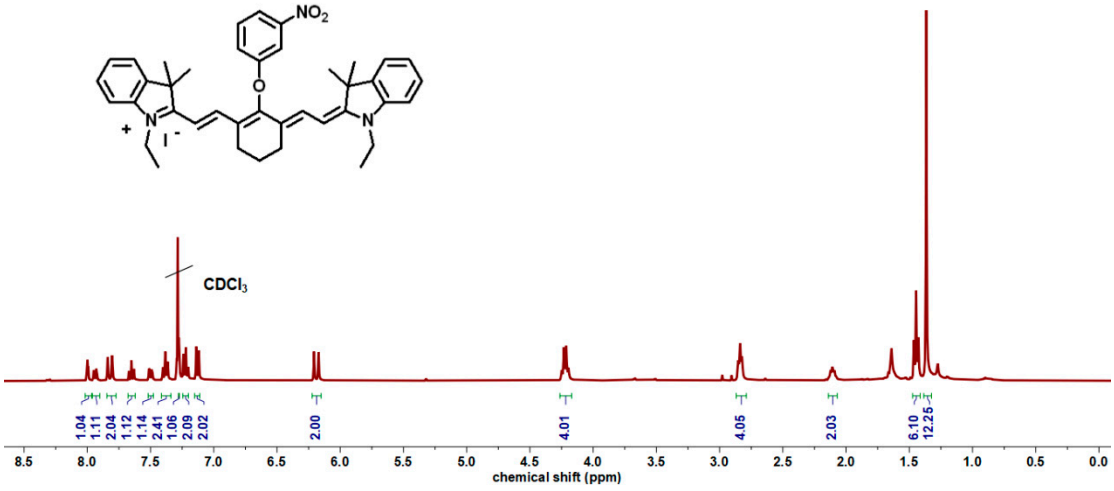

Figure S5. ^{1}H NMR of compound 3 (400 MHz, $CDCl_3$).

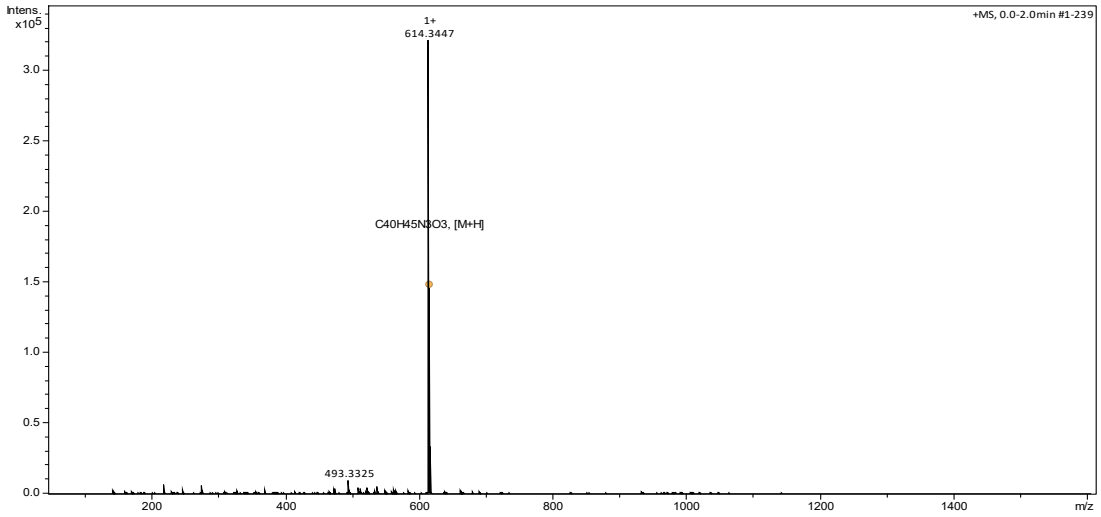

Figure S6. HRMS of compound 3, m/z = 614.3447 is assigned to $[M-I]^+$.

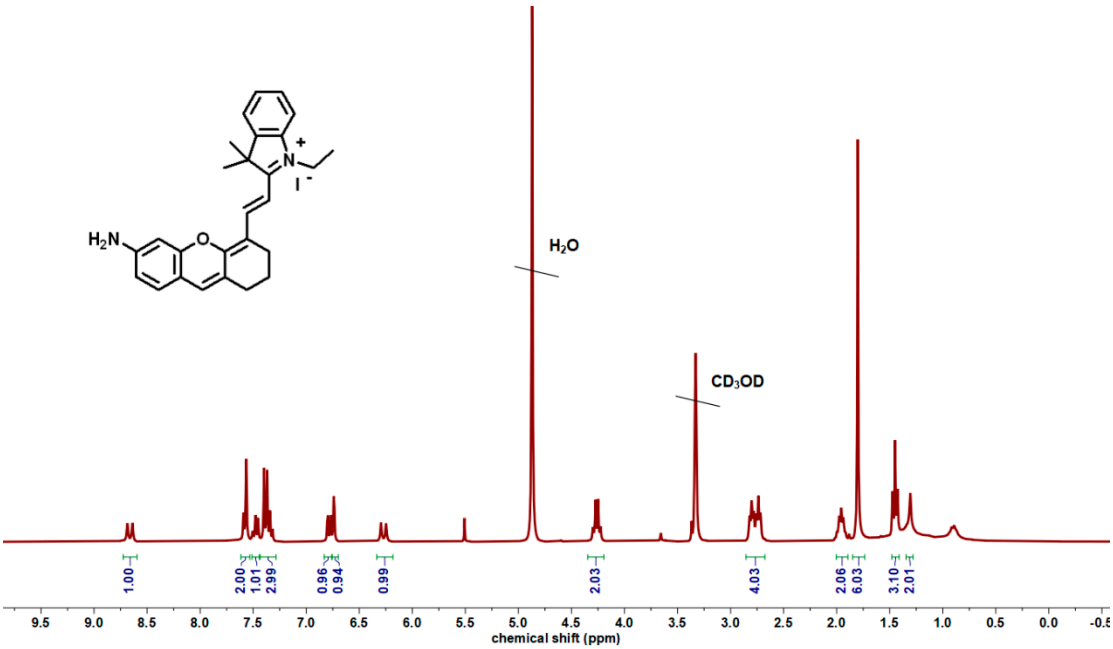

Figure S7. ^{1}H NMR of HCy (400 MHz, CD_3OD).

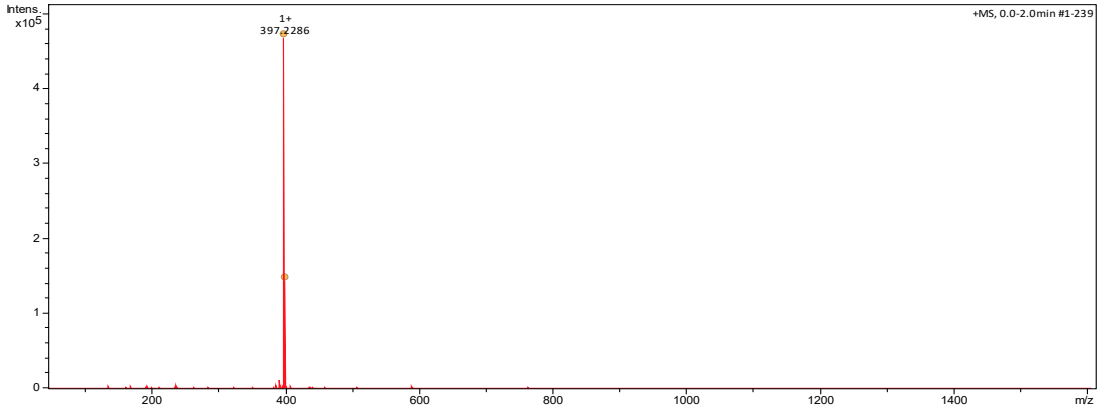

Figure S8. HRMS of compound HCy, m/z = 397.2286 is assigned to $[M-I]^+$.

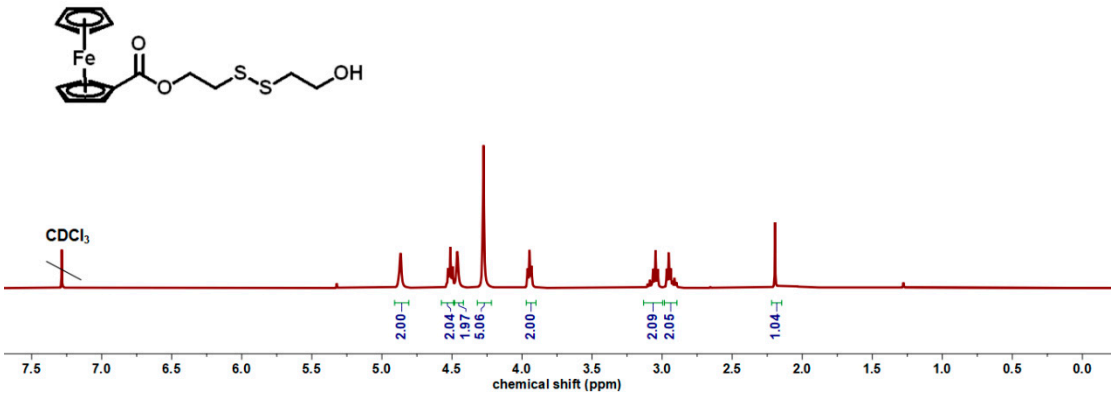

Figure S9. ^{1}H NMR of Fe-SS-OH (400 MHz, $CDCl_3$).

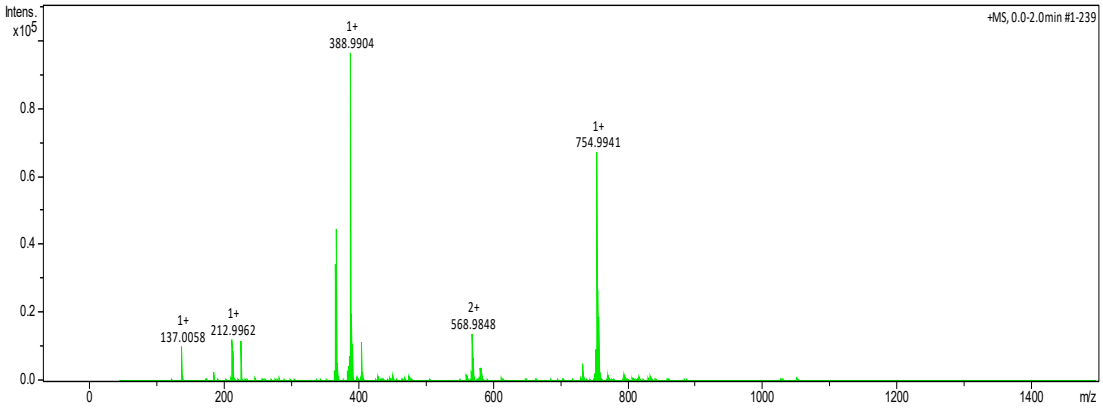

Figure S10. HRMS of compound Fe-SS-OH, m/z = 388.9904 is assigned to $[M+Na]^+$.

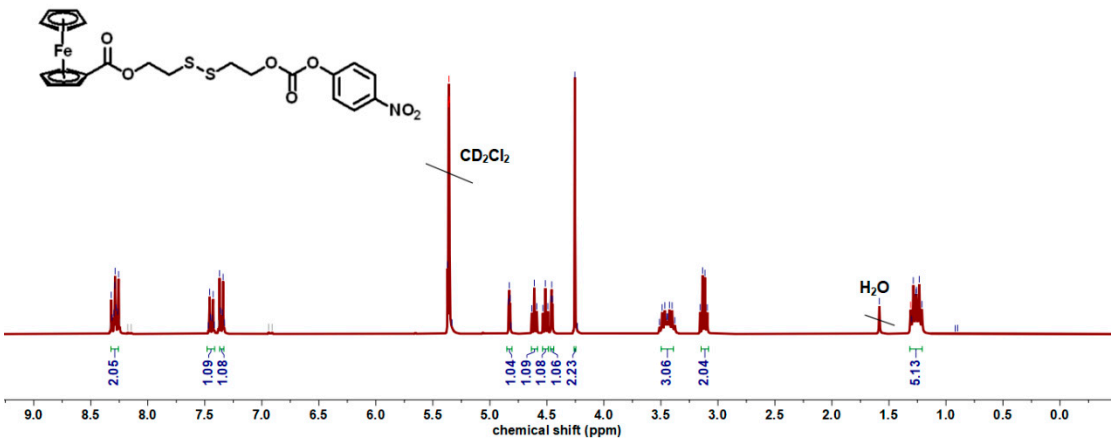

Figure S11. ^{1}H NMR of Fe-SS-NPC (400 MHz, CD_2Cl_2).

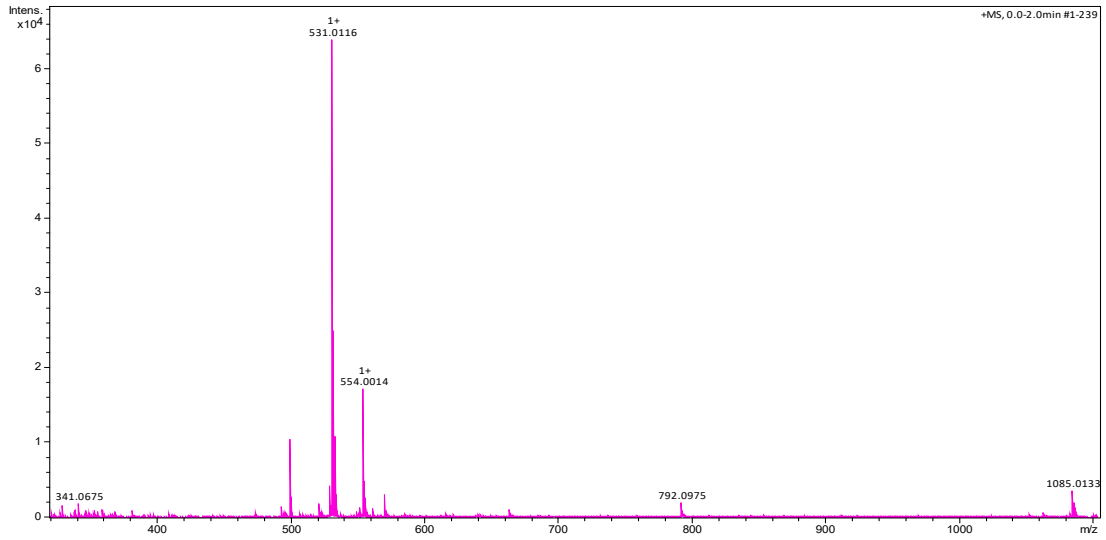

Figure S12. HRMS of compound Fe-SS-NPC, m/z = 531.0116 is assigned to $[M+H]^{+}$.

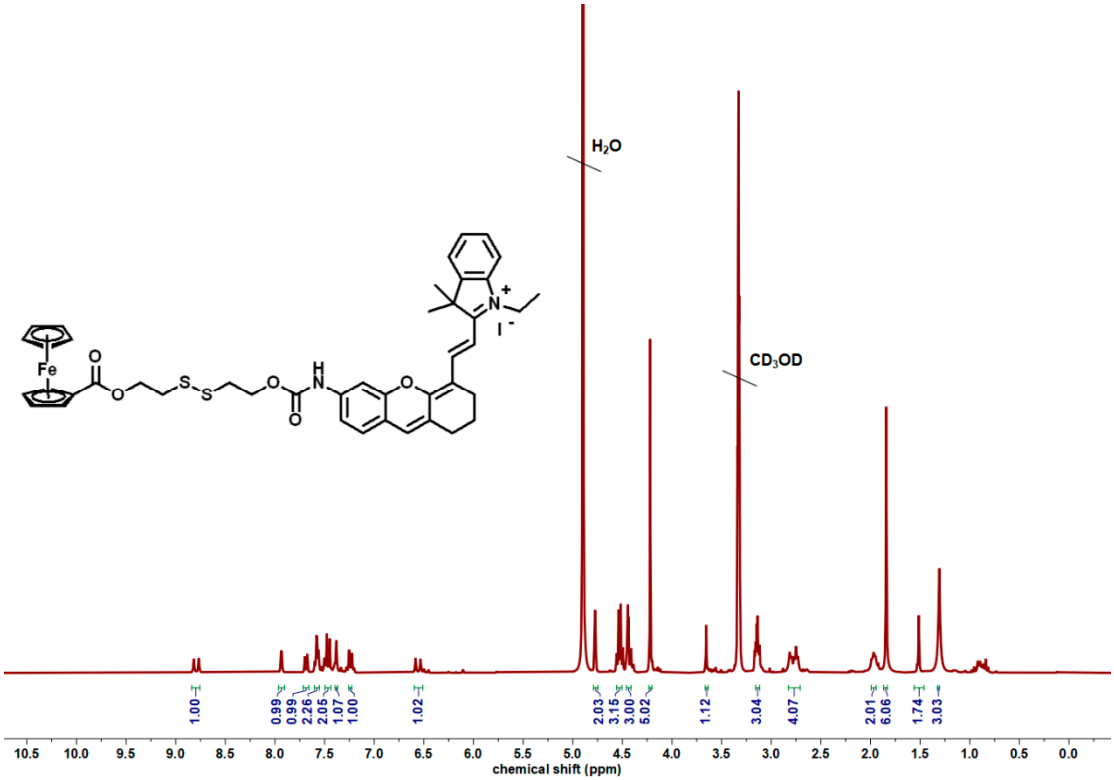

Figure S13. ^{1}H NMR of Fe-SS-HCy (400 MHz, CD_3OD).

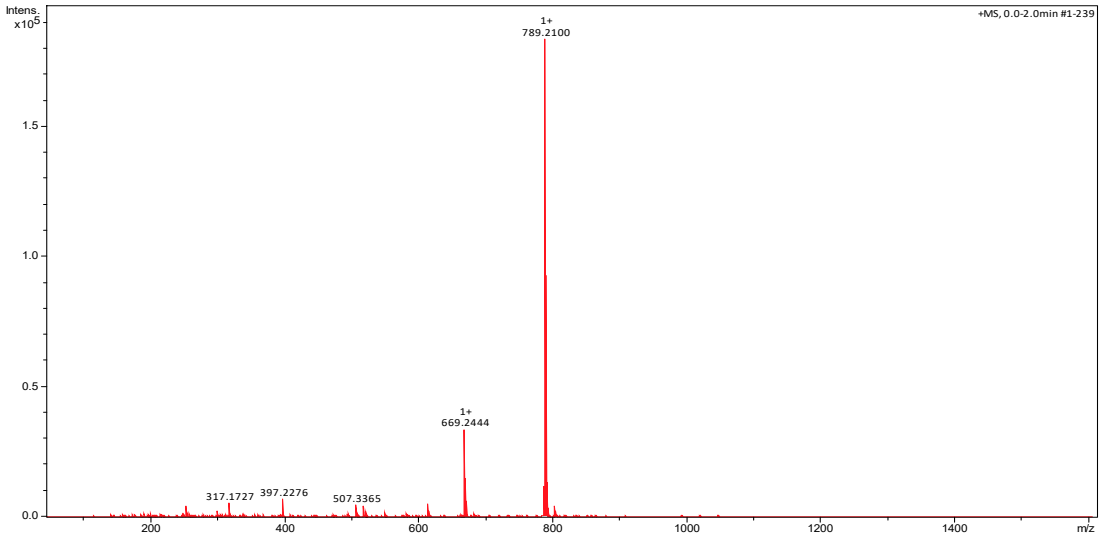

Figure S14. HRMS of compound Fe-SS-HCy, m/z = 789.2100 is assigned to $[M-I]^{+}$.

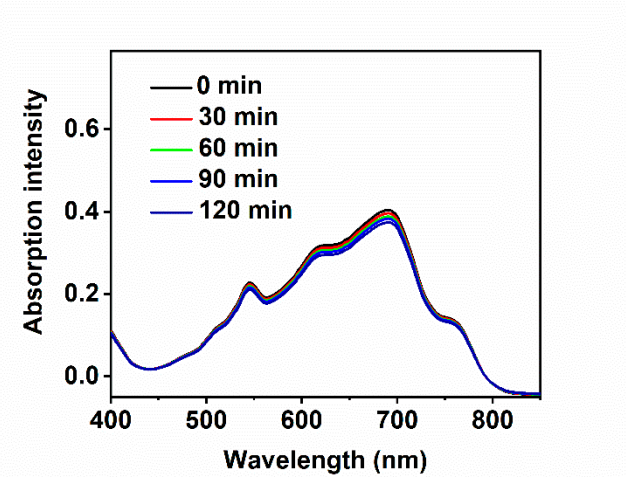

Figure S15. The stability of Fe-SS-HCy irradiated upon red light laser for 2 hours. The UV-vis absorption spectra of Fe-SS-HCy were recorded every 30 min. Negligible change was observed of Fe-SS-HCy spectra, suggesting a good molecular stability during irradiation.

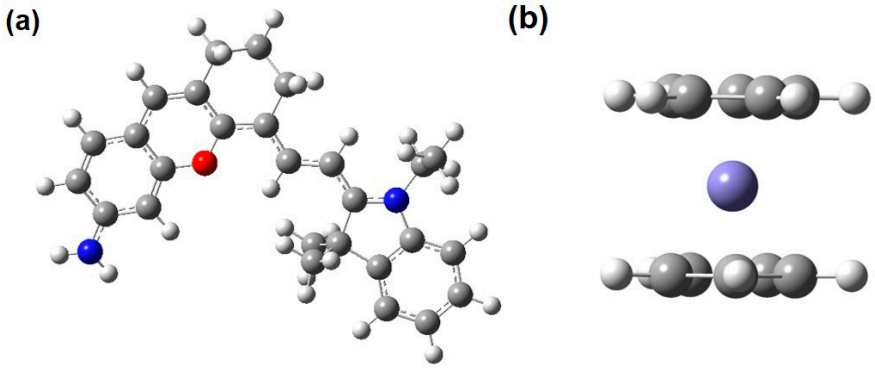

Figure S16. Optimized structures of HCy and ferrocene calculated by density functional theory (DFT).

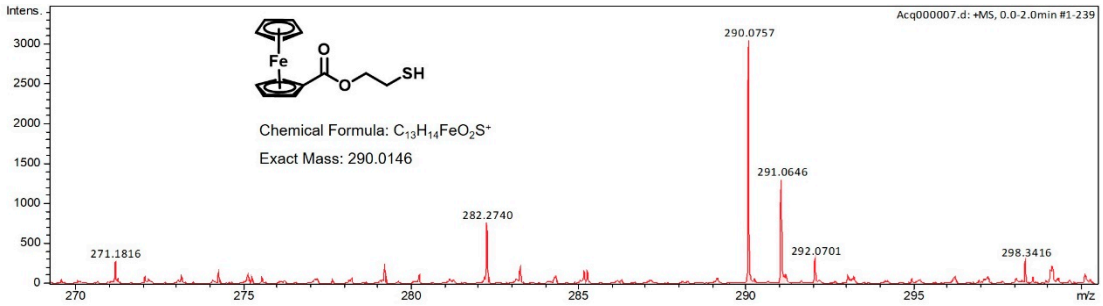

Figure S17. The HRMS spectra of Fe-SS-HCy after GSH treatment.

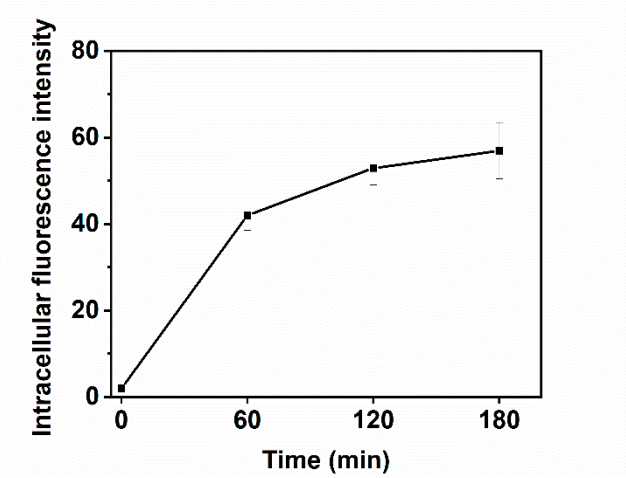

Figure S18. Intracellular fluorescence of MCF-7 cells after incubation with Fe-SS-HCy for different time period.

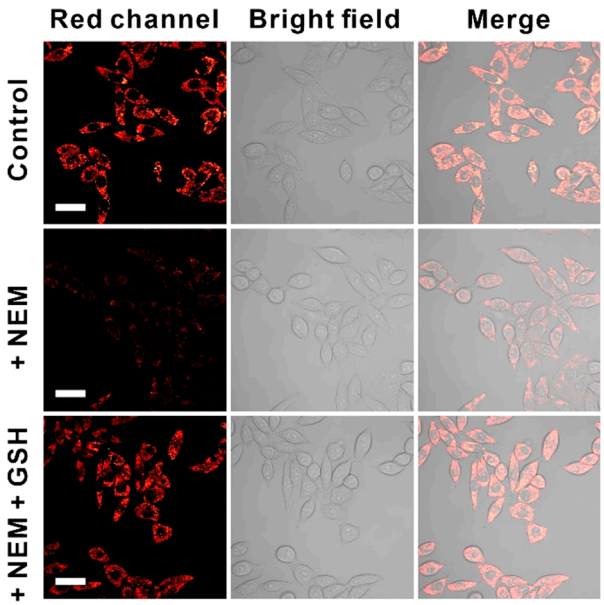

Figure S19. CLSM images of MCF-7 cancer cells treated with only Fe-SS-HCy (up), Fe-SS-HCy + NME (middle) and Fe-SS-HCy + NEM + GSH (bottom).

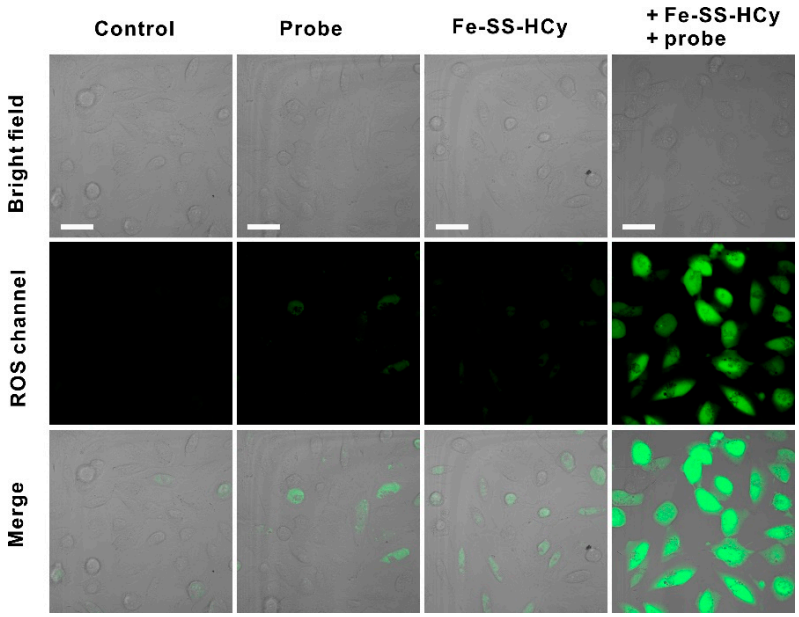

Figure S20. Generation of intracellular ROS in MCF-7 cells as observed by CLSM.

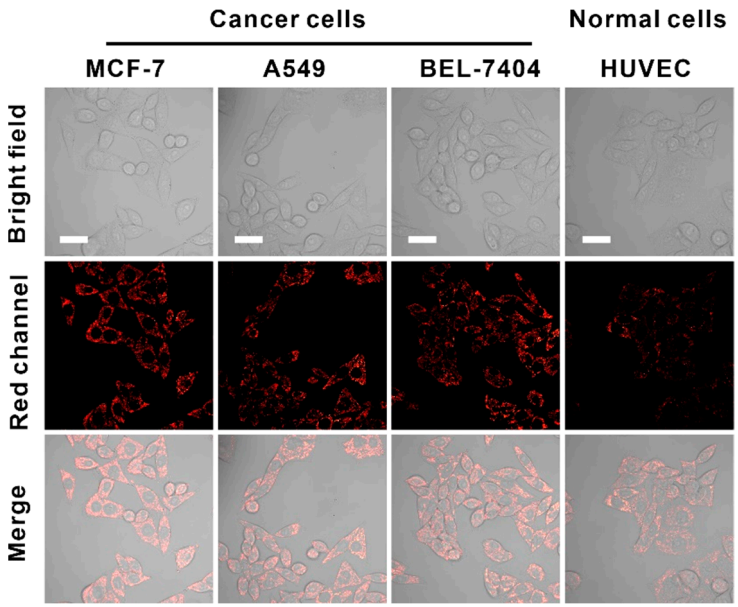

Figure S21. Fluorescence imaging of endogenous GSH for cancer cell recognition. Fluorescence imaging of different cancer cell lines (MCF-7, A549 and BEL-7404) and normal cell lines (HUVEC) after being incubated with 10 μM Fe-SS-HCy for 120 min.

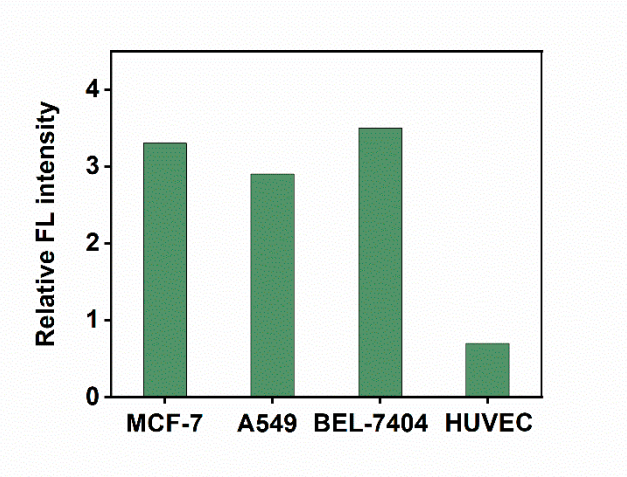

Figure S22. The relative fluorescence intensities between different cells in Fig. S21 calculated by ImageJ software.