Supporting Information

Self-catalyzed Tumor Ferroptosis Based on Ferrocene Conjugated Reactive Oxygen Species Generate and Responsive Polymer

Experimental Section

Materials and Methods

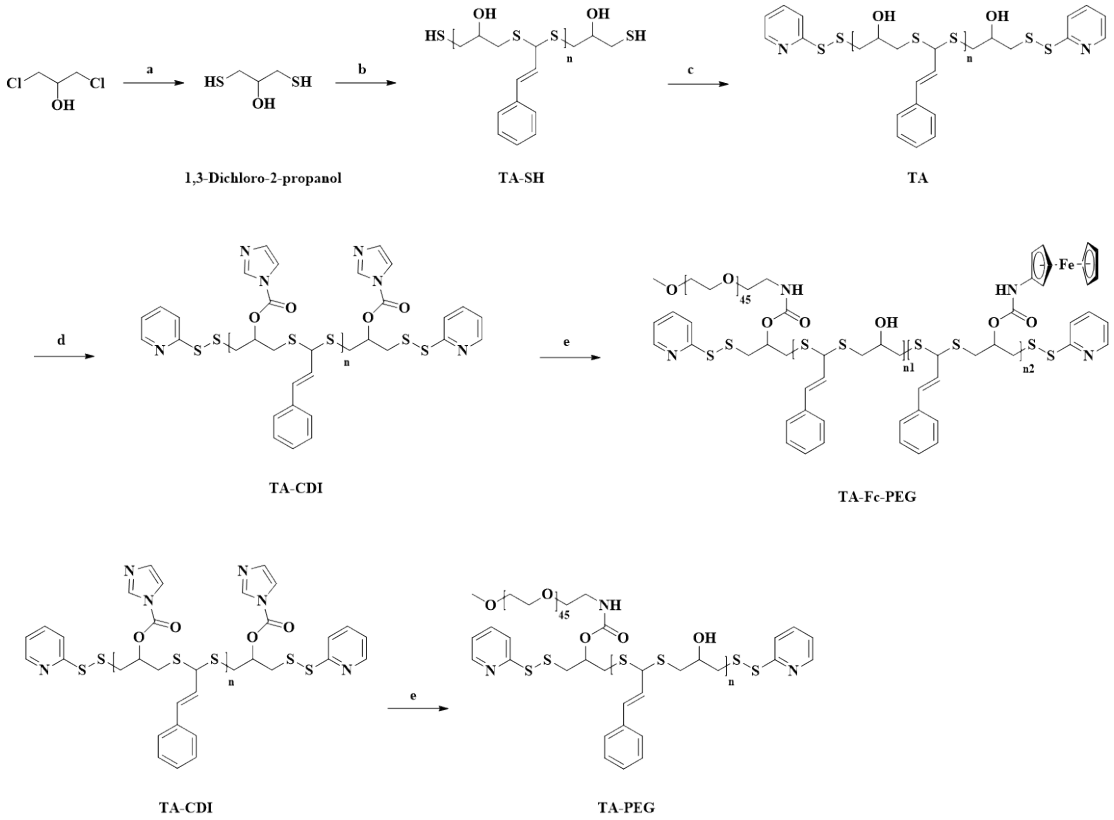
1,3-Dichloro-2-propanol, carbon disulfide (CS_2), cinnamaldehyde (CA), 2,2'-dithiodipyridine, *N, N*'-carbonyldiimidazole (CDI) and acetic acid were purchased from ENERGY (Shanghai, China). Aminoferrocene (Fc) and sodium disulfide nonahydrate ($Na_2S{\cdot}9H_2O$) were purchased from Aladdin Industrial, Inc. (Shanghai, China). Amine terminated methoxy poly (ethylene glycol) (mPEG-NH_2) was purchased from JenKen Co. LTD. (Beijing, China).

Dulbecco's modifed eagle's medium (DMEM), trypsin-EDTA and penicillin-streptomycin were obtained from Gibco BRL (Eggenstein, Germany). Fetal bovine serum (FBS) was obtained from ExCell Biology, Inc (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and glutathione synthase inhibitor DL-buthionine sulfoximine (BSO) was purchased from Sigma-Aldrich. Vitamin C (Vc) and glutathione were purchased from ENERGY (Shanghai, China). Hoechst 33342 was purchased from Life Technologies. Aminophenyl fluorescein (APF) and BODIPY$^{665/676}$ were purchased from Thermo Fisher Scientific. Anti-Glutathione Peroxidase 4 antibody was purchased from Abcam. Horse radish peroxidase (HRP) conjugated goat anti-rabbit IgG antibody was purchased from Biosharp.

NMR spectra were measured on a Bruker ARX 400 NMR spectrometer (Bruker, Billerica, MA). Deuterated chloroform or deuterated dimethyl sulfoxide was used as the solvent for NMR measurements. Nanoparticle hydrodynamic diameters (Dh) measurements were carried out in aqueous solution using a Malvern ZS90 dynamic light scattering instrument (DLS) with a He-Ne laser (633 nm) and 90° collecting optics. The data were analyzed using Malvern Dispersion Technology Software 7.0.2. The polymer dispersity index (PDI) of polymers were determined by

gel permeation chromatography (GPC) measurements on a Waters GPC system (Waters, Milford, MA). The system was equipped with a Waters 2414 refractive index detector and a Waters 1515 HPLC solvent pump. Four Waters styragel high-resolution columns (HR4, HR2, HR1, and HR0.5) were also equipped with molecular weights of 5000−600,000, 500−20,000, 100−5000, and 0−1000 respectively. HPLC grade *N, N*'-dimethylformamide (DMF) was used as eluent at 35 °C with a flow rate of 1.0 mL/min. Monodispersed polystyrene standards with a molecular weight range of 1.31×10^3 to 5.51×10^4 were used to generate the calibration curve. The absorption spectra were measured on a UV-3802 (UNICO, Shanghai, China) spectrophotometer. Confocal images were acquired by confocal microscope (CLSM, Nikon Ti-E A1, Japan). Photoluminescence (PL) spectra were measured on a Shimadzu RF-6000 spectrofluorometer (Shimadzu UV-2600, Japan). Absorbance and fluorescence intensity were measured by a multifunctional micropore detection board analysis system (Biotek Cytation5, BioTek, United States). The mice were imaging by *In-Vivo* Xtreme (Bruker, German).

Synthesis of TA-Fc-PEG and TA-PEG.



Scheme S1. Synthetic procedures of TA-Fc-PEG and TA-PEG. a: CS_2, $Na_2S \cdot 9H_2O$, H_2O. b: CA, hydrochloric acid. c: 2,2'-dipyridyl disulfide, acetic acid, THF. d: CDI, DMF. e: Fc, mPEG-

NH_2, DMF.

Synthesis of 1, 3-dimercapto-2-propanol. 1,3-Dimercapto-2-propanol was synthesized according to the literature reported procedure.[1] CS_2 (13.2 mL, 220 mmol) and $Na_2S{\cdot}9H_2O$ (48 g, 200 mmol) were added to 24 mL water and the reaction mixture was stirred at 40 °C for 5 h. Then, the excess CS_2 was removed under reduced pressure and the remaining solution was diluted with 70 mL water to get a ~ 33% solution of sodium trithiocarbonate. Subsequently, 1,3-dichloro2-propanol (6.72 mL, 70 mmol) was gradually added to the solution at 0 °C and the reaction mixture was stirred at 60 °C for 5 h. Afterwards, heating was stopped and the solution was cooled to room temperature and then washed with ethyl acetate (5 × 100 mL). The aqueous part was collected and acidified by adding concentrated sulphuric acid slowly and then extracted with diethyl ether (3 ×50 mL). The ether extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to get the crude product as a light-brown oil, which was further purified by vacuum distillation at 90 °C. The desired product was obtained as a light-yellow liquid (2.77 g, 32%).

Synthesis of TA-SH. 1,3-Dimercapto-2-propanol (0.26 g, 2.09 mmol) and CA (0.26 g, 2 mmol) were added into a glass Schlenk flask with a magnetic stirrer. Then hydrochloric acid (8 μL, 9.6 mmol) was added and the reaction mixture was stirred at 0 °C for 30 min under argon atmosphere. The viscous solution was washed by water to remove most of the hydrochloric acid and then dissolved in tetrahydrofuran (THF, 2 mL) and precipitated from excess of cold *n*-hexane (Hex). The light-yellow precipitate was resolved in THF and reprecipitated to Hex. The oligomers were removed by gel column (1% crosslinked: separation of M_W 600-14,000 lipophilic polymers) using THF as eluents and dried under vacuum to obtain TA-SH as a light-yellow waxy solid (0.18 g, 35%).

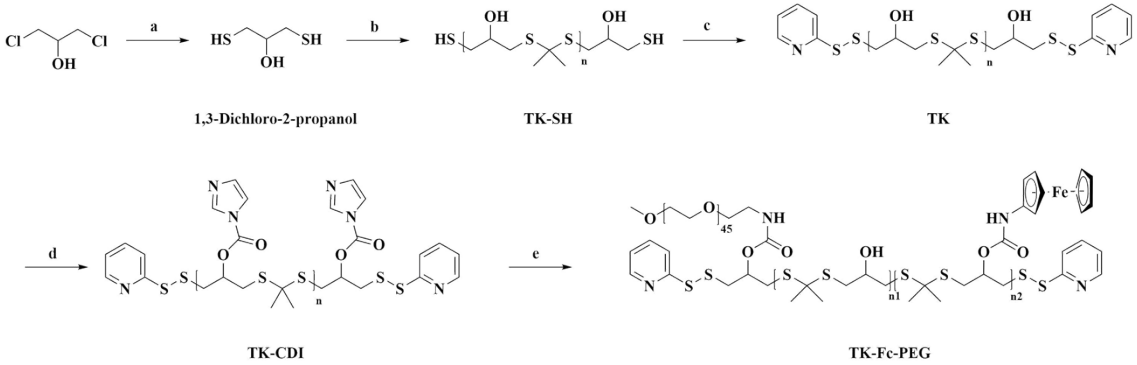
Synthesis of TA. TA-SH (0.54 g, 0.12 mmol) and 2,2'-dithiodipyridine (0.052 g, 0.24 mmol) were dissolved in dry degassed THF (4 mL), then 1 drop of conc. acetic acid was added. The reaction mixture was stirred at 25 °C for 24 h under argon atmosphere. Afterwards, the solution was concentrated under reduced pressure and then purified by a gel column using THF as eluents and dried under vacuum to obtain TA as a light-yellow solid (0.45 g, 76%).

Synthesis of TA-CDI. TA (0.45 g, 0.1 mmol) and CDI (0.32 g, 2 mmol) were dissolved in *N, N*'-dimethylformamide (DMF, 5 mL) and the solution was stirred at 25 °C for 24 h under argon atmosphere. Afterwards, the solution was concentrated under reduced pressure and then purified

by a gel column using DMF as eluents and dried under vacuum to obtain TA-CDI as a light-yellow solid (0.46 g, 59.7%).

Synthesis of TA-Fc-PEG. TA-CDI (0.36 g, 0.06 mmol), Fc (0.18 g, 0.9 mmol) and mPEG-NH_2 (0.18 g, 0.09 mmol) were dissolved in DMF (5 mL) and the reaction mixture was stirred at 25 °C for 24 h under argon atmosphere. Afterwards, the solution was concentrated under reduced pressure and then purified by a gel column using DMF as eluents and dried under vacuum to obtain TA-Fc-PEG as a brown solid (0.29 g, 40.2%).

Synthesis of TA-PEG. TA-PEG was synthesized by a similar procedure just without addition of Fc and obtained as a light-yellow solid (0.21 g, 38.9%).



Scheme S2. Synthetic procedures of TK-Fc-PEG. a: CS_2, Na_2S, H_2O. b: acetone, hydrochloric acid. c: 2,2'-dipyridyl disulfide, acetic acid, THF. d: CDI, DMF. e: Fc, mPEG-NH_2, DMF.

Synthesis of TK-SH. TK-SH was synthesized *via* a similar procedure by replacing CA with acetone and obtained as a white waxy solid (0.51 g, 68%).

Synthesis of TK. TK was synthesized *via* a similar procedure by replacing TA-SH with TK-SH and obtained as a light-yellow solid (0.41 g, 75%).

Synthesis of TK-CDI. TK-CDI was synthesized *via* a similar procedure by replacing TA with TK and obtained as a light-yellow solid (0.43 g, 58.1%).

Synthesis of TK-Fc-PEG. TK-Fc-PEG was synthesized *via* a similar procedure by replacing TA-CDI with TK-CDI and obtained as a brown solid (0.39 g, 54%).

Preparation of TA-Fc-PEG nanoparticles (PTAF)

The PTAF were prepared by nanoprecipitation method from the amphiphilic polymer TA-Fc-PEG. In brief, TA-Fc-PEG (100 mg) were dissolved in 1 mL of DMSO, and then gradually added into 9 mL of ultrapure water during stirring. After additional stirring for 1 h, the solution was transferred into dialysis bag (MWCO 3500) to remove DMSO against ultrapure water for 24 h,

and the solution was filtered through a 0.45 μm filter to obtain PTAF. PTA and PTF were obtained *via* similar preparation method.

***In vitro* CA release**

PTAF (5 mg mL^{-1}, 1 mL) were transferred into the dialysis membrane tubing (MWCO 3500 Da) and then were immersed in the phosphate buffer saline (PBS, pH 7.4, 15 mL) or H_2O_2 (100 mM, 15 mL) with gentle shaking (80 rpm) at 37 °C. At 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48 h, 1mL of the external PBS/H_2O_2 was collected and 1 mL of fresh PBS/H_2O_2 were subsequently replenished. The release studies were repeated in triplicate under the same conditions. The collected solution of CA was lyophilized and analyzed by UV-3802 spectrophotometer.

***In vitro* Fenton reaction detection**

Hydroxyl radicals were detected by using disodium terephthalate as a capture agent, which can react with hydroxyl radicals to generate fluorescent 2-hydroxyterephthalic disodium. In brief, Fenton reaction was performed by mixing 50 μL of H_2O_2 (500 μM) with disodium terephthalate solution (100 μL, 50 mM) and 100 μL PBS, PTA, PTF and PTAF at the Fc-equivalent concentration of 60 μg mL^{-1} and CA-equivalent concentration of 120 μg mL^{-1}. The solution was subjected to fluorescence tracing by using a fluorescence spectrometer at the excitation wavelength at 310 nm and emission wavelength at 425 nm.

Cell culture and tumor model

Mouse breast cancer cell line 4T1 cells were cultured in DMEM medium with 10% FBS and 1% penicillin-streptomycin. Cells were cultured in 5% CO_2 and 21% O_2 incubator at 37 °C. Female BALB/c mice and BALB/c nude mice (20 ± 2 g, 5-6 weeks old) were purchased from Hunan SJA Laboratory Animal Co., Ltd (Hunan, China). 4T1 cells (5×10^6) were injected into the right mammary fat pads to establish an orthotopic 4T1 tumor model. After the tumor volumes reached to 100 mm^3, the mice were used for subsequent experiments. At the end of experiments, all mice were killed by CO_2 inhalation. All animal experiments approved by the Ethics Committee of the South China University of Technology followed the guidelines of "Guangdong Province Experimental Animal Policies and Regulations" and "National Experimental Animal Management Regulations".

Intracellular GSH depletion

The intracellular GSH depletion was monitored by using Thiol Tracker™ Violet as an

indicator and analyzed by CLSM. Briefly, 4T1 cells at a density of 1×10^5 per well were seed into 24-well plates and incubated for 24 h. Subsequently, the cells were treated with PBS, PTA, PTF and PTAF at the Fc-equivalent concentration of 15 μg mL^{-1} and CA-equivalent concentration of 30 μg mL^{-1}, respectively. After incubating for 4 h, the cells were stained with Thiol Tracker™ Violet (20 μM) for 30 min. Then, the cells were washed with PBS and then fixed by 4% paraformaldehyde for 20 min. Finally, the cells were washed with PBS, covered with antifade mounting medium and studied by confocal laser scanning microscope (CLSM).

4T1 cells at a density of 1×10^4 per well were cultured in 96-well black culture plates and incubated for 24 h. The cells were treated with PBS, PTA, PTF, PTAF at the Fc-equivalent concentration of 15 μg mL^{-1} and CA-equivalent concentration of 30 μg mL^{-1}, respectively. After incubating for 4 h, cells were washed by PBS for three times, then stained by Thiol Tracker Violet (20 μM). After staining, the probe was rinsed three times with PBS then placed in PBS. The GSH level was analyzed using a microplate system (Thiol Tracker Violet Ex/Em = 404/526 nm).

Intracellular reactive oxygen species (ROS) production

Intracellular ROS level was monitored by 2′,7′-dichlorofluorescin diacetate (DCFH-DA), which could be oxidized to produce fluorescent compound of dichlorofluorescin (DCF) in the presence of ROS. Briefly, 4T1 cells were seeded into a 35 mm glass-bottom dishes at a density of 2×10^5 per well and incubated overnight. Subsequently, the cells were treated with PBS, PTA, PTF, and PTAF at the Fc-equivalent concentration of 15 μg mL^{-1} and CA-equivalent concentration of 30 μg mL^{-1}, respectively. After incubating for 4 h, the cells were washed with PBS and stained with DCFH-DA (20 μM) for 30 min. Nuclei were stained with Hoechst 33342 for 10 min. Then, the cells were washed with PBS and the DCF fluorescence images were acquired by CLSM.

4T1 cells (1×10^5 cells per well) were cultured in 24-well culture plates and incubated for 24 h. Subsequently, the cells were treated with PBS, PTA, PTF, PTAF at the Fc-equivalent concentration of 15 μg mL^{-1} and CA-equivalent concentration of 30 μg mL^{-1}, respectively. After incubating for 4 h, cells were washed by PBS for three times, then stained by DCFH-DA (20 μM). Cells were rinsed three times with PBS, collected and analyzed by Flow Cytometry.

Intracellular H_2O_2 production

Intracellular H_2O_2 level was monitored by OxiVision Green as the specific fluorescent probe.

Briefly, 4T1 cells were cultured in 24-well culture plates at a density of 2×10^5 per well and incubated overnight. Subsequently, the cells were treated with PBS, PTA, PTF, and PTAF at the Fc-equivalent concentration of 15 μg mL^{-1} and CA-equivalent concentration of 30 μg mL^{-1}, respectively. After incubating for 4 h, the cells were washed with PBS and stained with OxiVision Green (10 μM) for 30 min. Nuclei were stained with Hoechst 33342 for 10 min. Then, the cells were washed with PBS and the OxiVision Green fluorescence images were acquired by Fluorescence microscope.

4T1 cells (1×10^5 cells per well) were cultured in 24-well culture plates and incubated for 24 h. Subsequently, the cells were treated with PBS, PTA, PTF, PTAF at the Fc-equivalent concentration of 15 μg mL^{-1} and CA-equivalent concentration of 30 μg mL^{-1}, respectively. After incubating for 4 h, cells were washed by PBS for three times, then stained by OxiVision Green (10 μM). Cells were rinsed three times with PBS, collected and analyzed by Flow Cytometry.

Intracellular •OH production

Intracellular •OH level was monitored by aminophenyl fluorescein (APF) as the specific fluorescent probe. Briefly, 4T1 cells (1×10^5 cells per well) were seed into 24-well plates and incubated for 24 h. Subsequently, the cells were treated with PBS, PTA, PTF and PTAF at the Fc-equivalent concentration of 15 μg mL^{-1} and CA-equivalent concentration of 30 μg mL^{-1}, respectively. After incubating for 4 h, the cells were washed with PBS and stained with APF (20 μM) for 30 min. Nuclei were stained with Hoechst 33342 for 10 min. Then, the cells were washed with PBS and then fixed by 4% paraformaldehyde for 20 min. Finally, the cells were washed with PBS, covered with antifade mounting medium and studied by CLSM and quantitatively measured by a multifunctional micropore detection board analysis system.

***In vitro* cytotoxicity**

The *in vitro* cell cytotoxicity was evaluated on 4T1 cells using a standard MTT assay. Briefly, 4T1 cells at a density of 1×10^4 per well were seed into 96-well plates and incubated overnight. Subsequently, PTA, PTF and PTAF at different concentrations were added into the wells for 24 h incubation. Afterward, MTT solution (10 μL, 5 mg mL^{-1}) was added to each well for another 4 h. Then, the medium containing MTT solution was removed and dimethyl sulfoxide (DMSO, 200 μL per well) was added to dissolve formazan crystals for 10 min. Consequently, multifunctional micropore detection board analysis system was used to measure the absorbance at 490 nm.

Furthermore, 4T1 cells at a density of 5×10^3 per well were seed into 96-well plates and incubated for 24 h. Subsequently, the cells were treated with PBS, PTA, PTF and PTAF at the Fc-equivalent concentration of 45 μg mL^{-1} and CA-equivalent concentration of 90 μg mL^{-1} and with the addition of DFO (100 μM), GSH (5 mM), catalase (35 U/ml) and thiourea (1 mM) respectively. After 24 h incubation, the cell viability was studied by MTT assay as mentioned above.

Western Blot Analysis.

Western blot was used to detect the change of GPX4 protein expression in 4T1 cells. Briefly, 4T1 cells were seed into 12-well plates at a density of 2×10^5 cells in DMEM medium with 10% FBS and incubated overnight. Subsequently, the cells were treated with PBS, PTA, PTF, PTAF at the Fc-equivalent concentration of 15 μg mL^{-1} and CA-equivalent concentration of 30 μg mL^{-1}, respectively. After 12 h incubation, cells were lysed and protein concentration was measured by BCA Protein Assay Kit. The protein was separated *via* 12% electrophoresis, transferred to nitrocellulose filter membranes (Millipore, U.S.A.), and blocked with 4% bovine serum albumin for 2 h to block the nonspecific binding. Afterward, the membranes were incubated with diluted primary antibodies GPX4 (1:1000) at 4 °C for 12 h. Following washings, the membranes were incubated for 2 h with goat anti-rabbit secondary antibody immunoglobulin G, and GAPDH (1:5000) according to standard protocols. Finally, the expression of GPX4 was measured by GE Amersham Imager 600 with the use of enhanced chemiluminescence working solution.

Intracellular LPO accumulation

Intracellular LPO level was monitored by using $BODIPY^{665/676}$ as an indicator and analyzed by CLSM. Briefly, 4T1 cells at a density of 1×10^5 per well were seed into 24-well plates and incubated for 24 h. Subsequently, the cells were treated with PBS, PTA, PTF, PTAF and PTAF (in the presence of DFO (100 μM)) at the Fc-equivalent concentration of 15 μg mL^{-1} and CA-equivalent concentration of 30 μg mL^{-1}, respectively. After incubating for 4 h, the cells were washed with PBS and stained with $BODIPY^{665/676}$ (20 μM) for 30 min. Nuclei were stained with Hoechst 33342 for 10 min. Then, the cells were washed with PBS and then fixed by 4% paraformaldehyde for 20 min. Finally, the cells were washed with PBS, covered with antifade mounting medium and studied by CLSM.

***In vivo* imaging and bio-distribution study**

The *in vivo* biodistribution of the nanoparticles loaded with 11-chloro-1,1'-di-n-propyl-

3,3,3',3'-tetramethyl-10,12-trimethyleneindatricarbocyanine iodide (IR-780, an NIR dye) were examined using 4T1 tumor bearing Balb/c nude mouse model. Firstly, 4T1 cells (5×10^6) were injected into the right mammary fat pads to establish an orthotopic 4T1 tumor model. After tumor volume reached 200 mm^3, the tumor-bearing mice were randomly divided into 4 groups (n= 3) and intravenously injected with 200 μL of (1) PBS, (2) PTF, (3) PTA and (4) PTAF at IR780 dose of 1 mg kg^{-1}. The body fluorescence images were obtained at 6, 12, 24 h after injection using *in vivo* Xtreme (Bruker, German). Finally, all mice were sacrificed at 24 h after administration for *ex vivo* examining the IR780 distribution in tumor and major organs.

Tumor growth inhibition

When the tumor volume reached to about 100 mm^3, 4T1 tumor-bearing mice were randomly divided into 4 groups (n= 6) and intravenously injected with (1) PBS, (2) PTF, (3) PTA, (4) PTAF (at CA/Fc equivalent dose of 5 mg kg^{-1} and 2.5 mg kg^{-1}) on day 1, 3, 5, 7. The tumor volumes and body weights were measured every other day. The tumor volume was calculated by using the formula: V = L×W×W/2 (L, the longest dimension; W, the shortest dimension). The mice were sacrificed on day 15 for further examination. Tumors of each group were harvested, fixed in 4% paraformaldehyde, embedded in paraffin, sliced and stained with hematoxylin and eosin (H&E). Tumor sections were also stained with TUNEL apoptosis detection kit assay and then observed by CLSM.

***In vivo* ROS detection**

Tumor-bearing mice received the treatment the same as the therapy group on day 14 for 24 h. Afterwards, the mice were intratumorally injected with 50 μL of 2.5 mg kg^{-1} DCFH-DA for 4 h and then the tumors were harvested for cryosection. After stained with Hoechst 33342, the fluorescence of the frozen tumor sections was detected by CLSM.

***In vivo* LPO detection**

Tumor-bearing mice received the treatment the same as the therapy group on day 14 for 24 h. Afterwards, the mice were intratumorally injected with 50 μL of 2 mg kg^{-1} $BODIPY^{665/676}$ for 4 h and then the tumors were harvested for cryosection. After stained with Hoechst 33342, the fluorescence of the frozen tumor sections was detected by CLSM.

***In vivo* GPX4 detection**

Tumor-bearing mice received the treatment the same as the therapy group on day 14 for 24 h.

Two hundred milligram fresh tumor tissues from different groups were homogenized and proteins were extracted for the following western blot analysis. SDS-PAGE (12%) electrophoresis was used to separate the cellular proteins (30 μg). After transferring onto nitrocellulose filter membranes (Millipore, U.S.A.). Bovine serum albumin solution (4%) was applied to block nonspecific binding sites. The samples were then treated by the monoclonal antibody against GAPDH (1:5000), GPX4 (1:1000), following by horse radish peroxidase (HRP) conjugated goat anti-rabbit IgG antibody (1:10000). An enhanced chemiluminescence (ECL) system (Thermo, U.S.A.) was used to detect signals.

Statistical Analysis

All the data were presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to assess the significance of the difference. Statistical P-values: NS $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

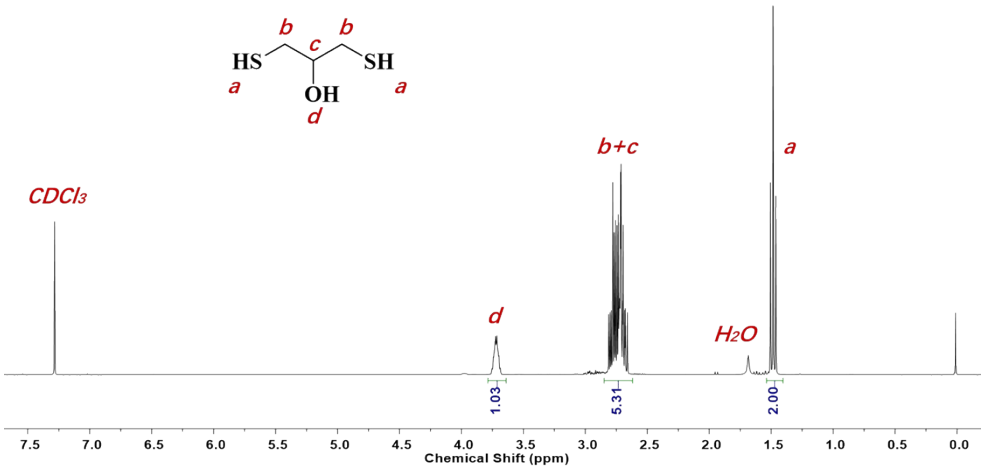


Figure S1. ^{1}H NMR spectrum of 1,3-dimercapto-2-propanol in $CDCl_3$.

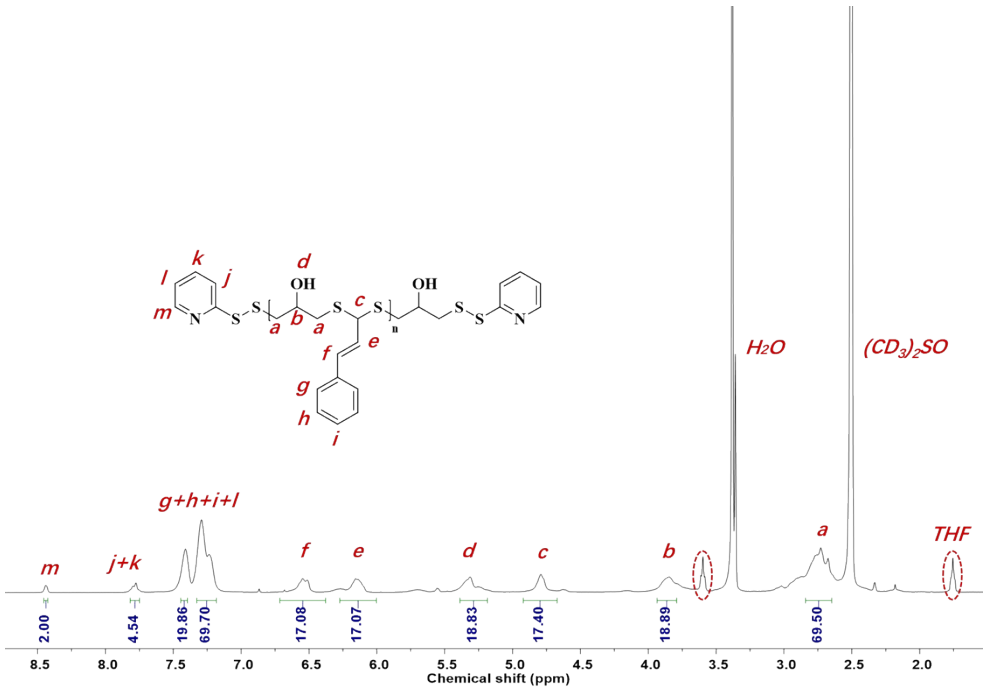


Figure S2. ^{1}H NMR spectrum of TA in DMSO-d_6.

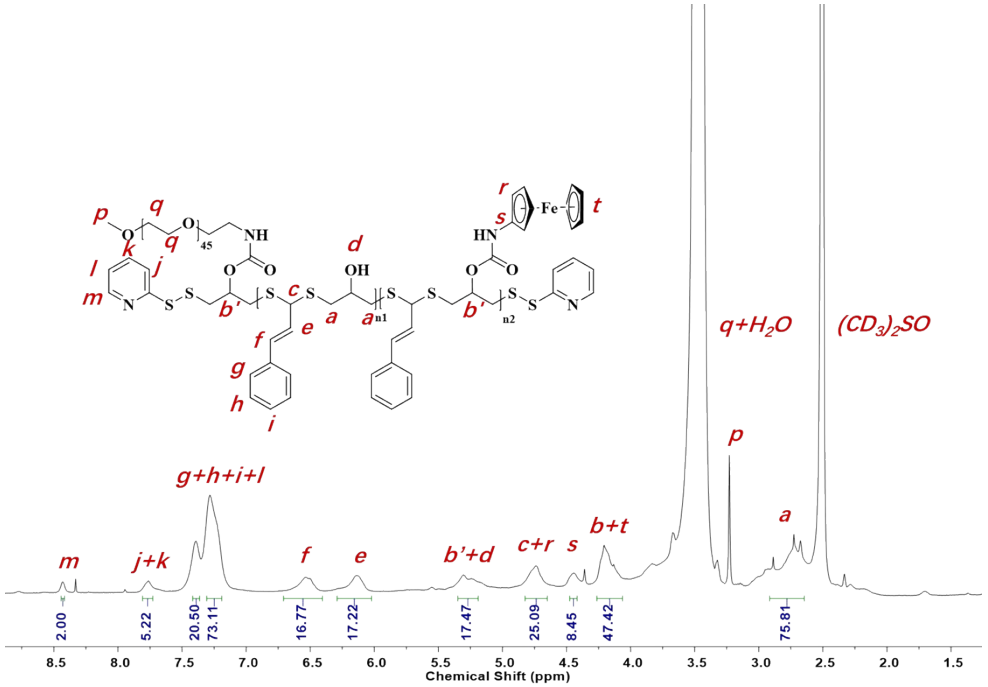


Figure S3. ^{1}H NMR spectrum of TA-Fc-PEG in DMSO-d_6.

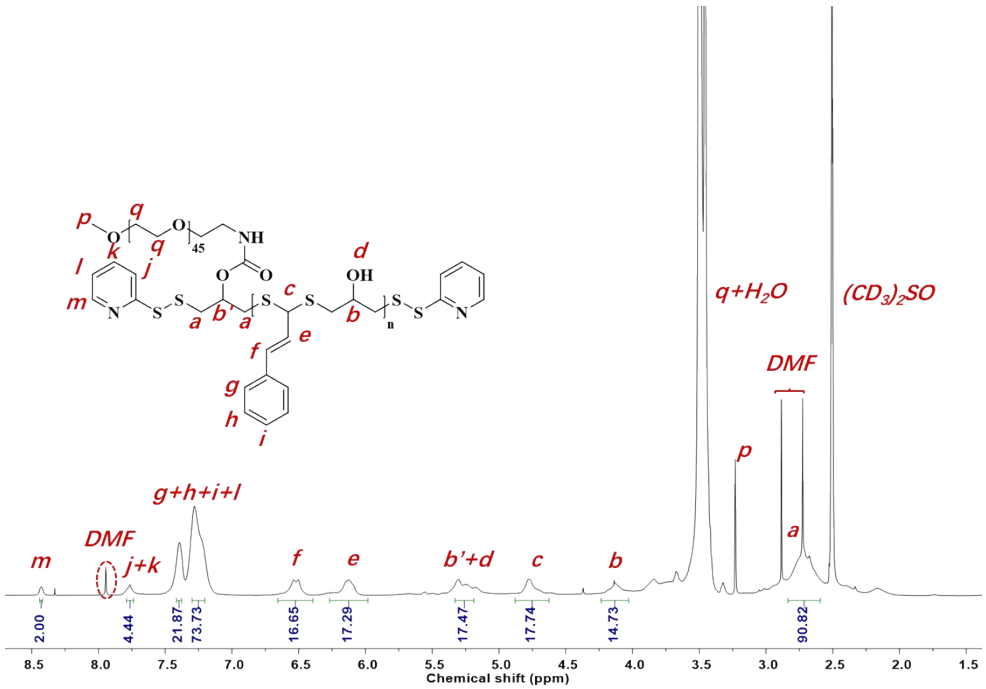


Figure S4. ^{1}H NMR spectrum of TA-PEG in DMSO-d_6.

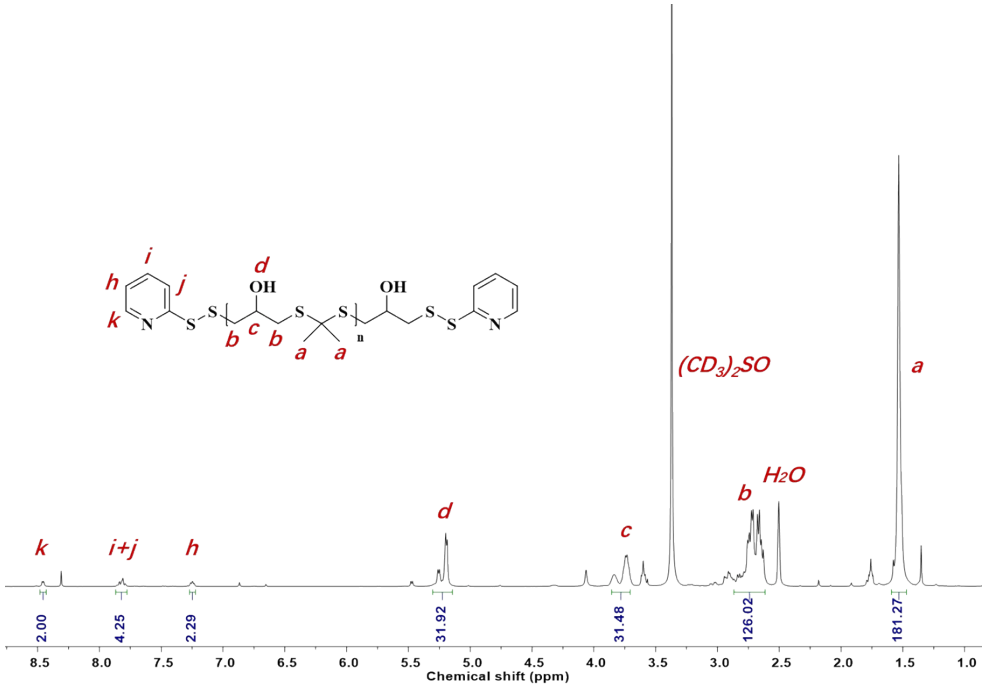


Figure S5. ^{1}H NMR spectrum of TK in DMSO-d_6.

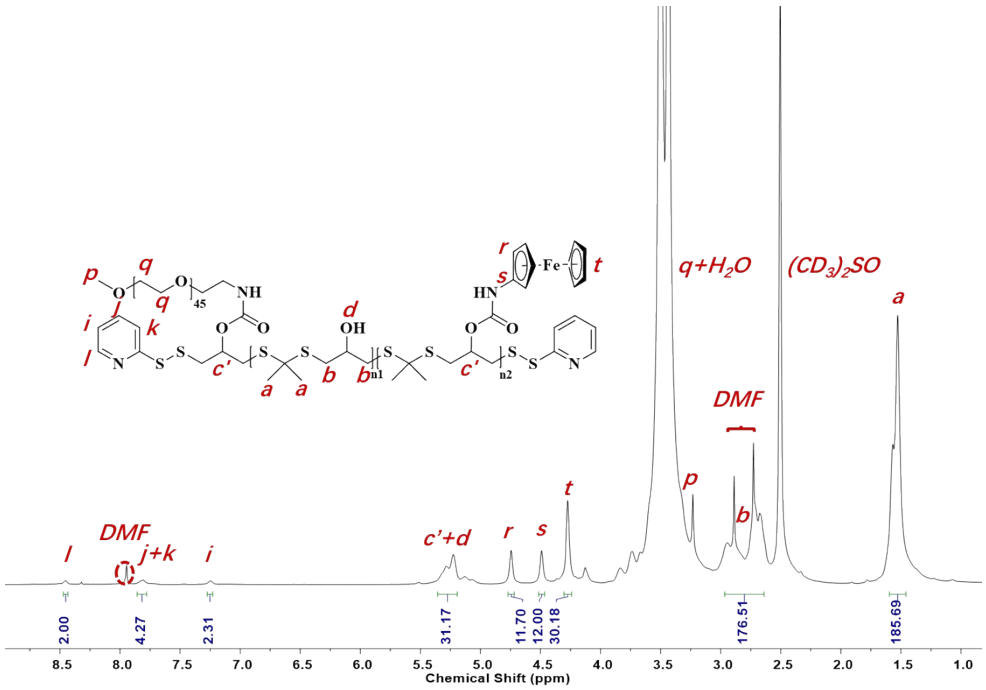


Figure S6. ^{1}H NMR spectrum of TK-Fc-PEG in DMSO-d_6.

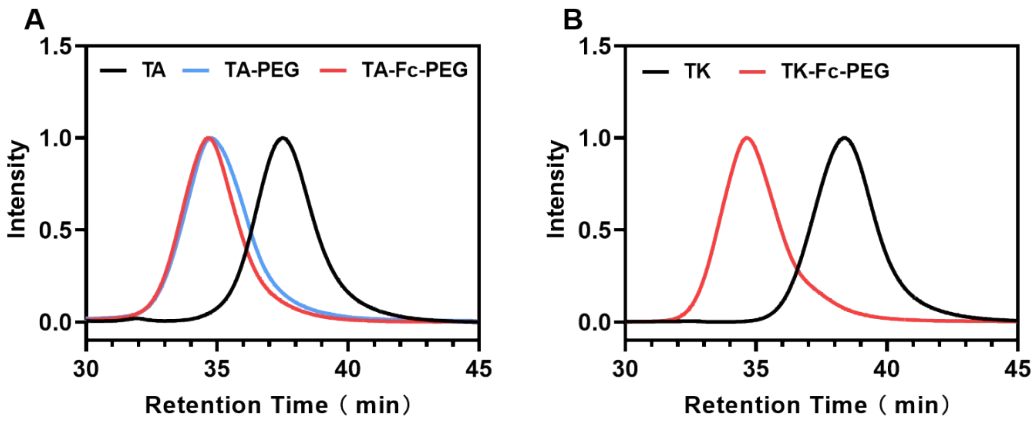


Figure S7. GPC analysis of (A) TA, TA -PEG, TA-Fc-PEG and (B) TK, TK-Fc-PEG.

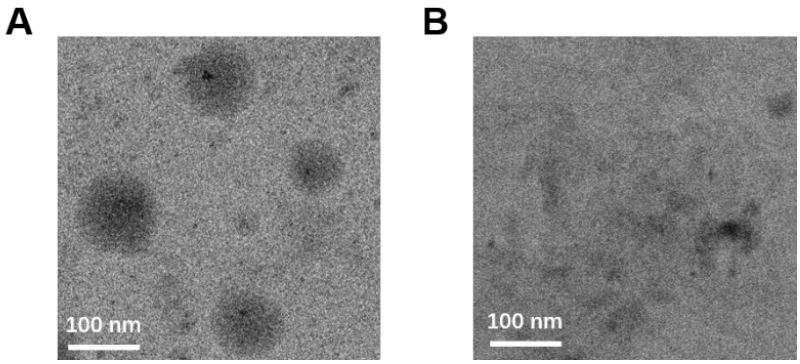


Figure S8. TEM images of (A) PTAF and (B) PTAF+ H_2O_2 (10 mM).

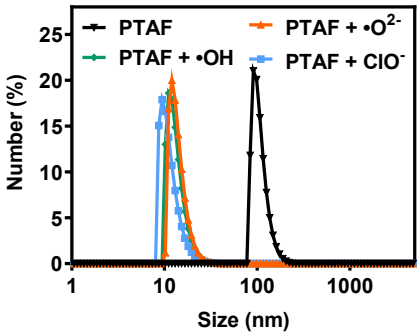


Figure S9. Size changes of TA response to ROS analogues.

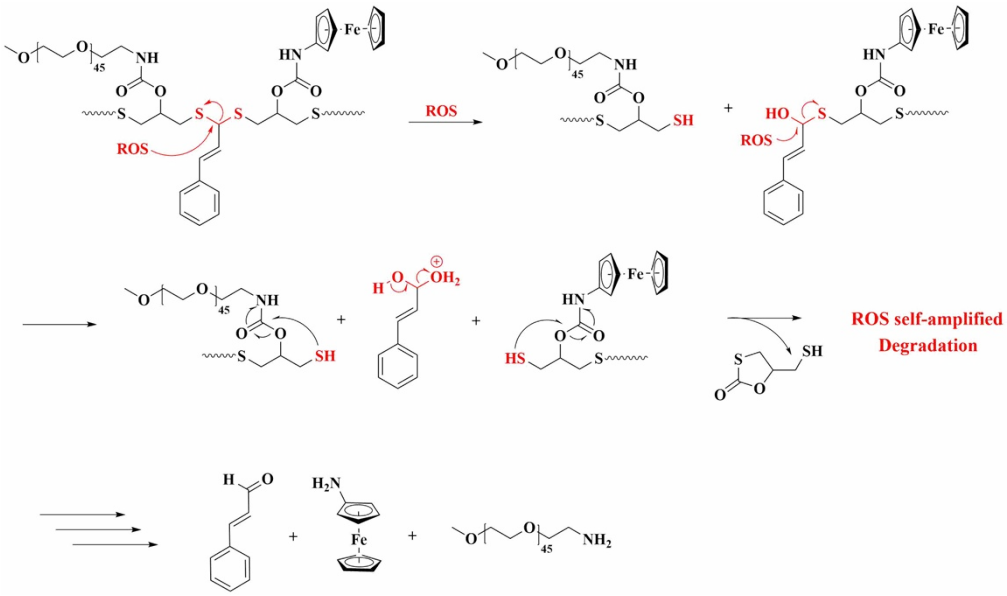


Figure S10. Proposed mechanism for ROS self-catalyzed degradation of TA-Fc-PEG.

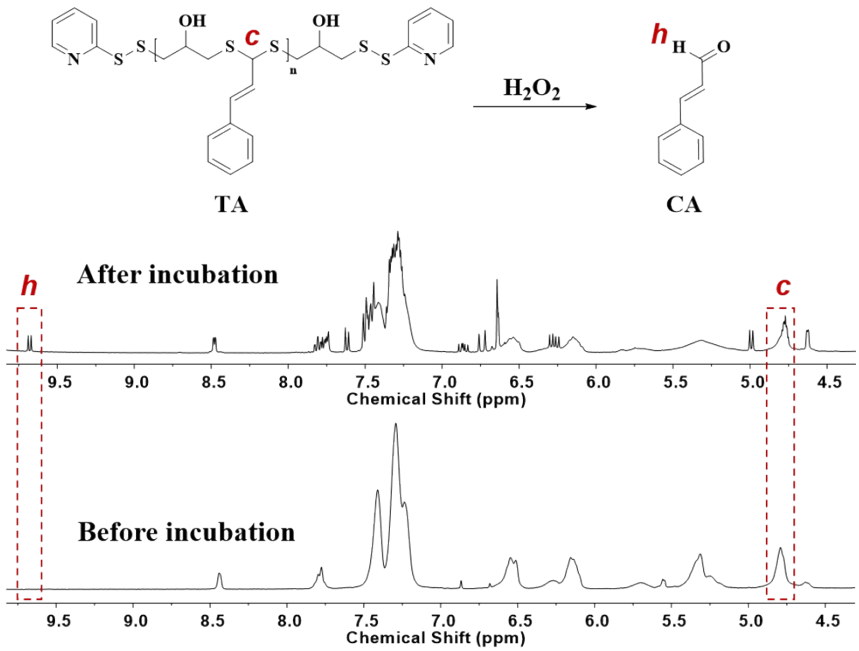


Figure S11. ^{1}H NMR spectra of TA after incubation with H_2O_2 (100 mM) for 48 h.

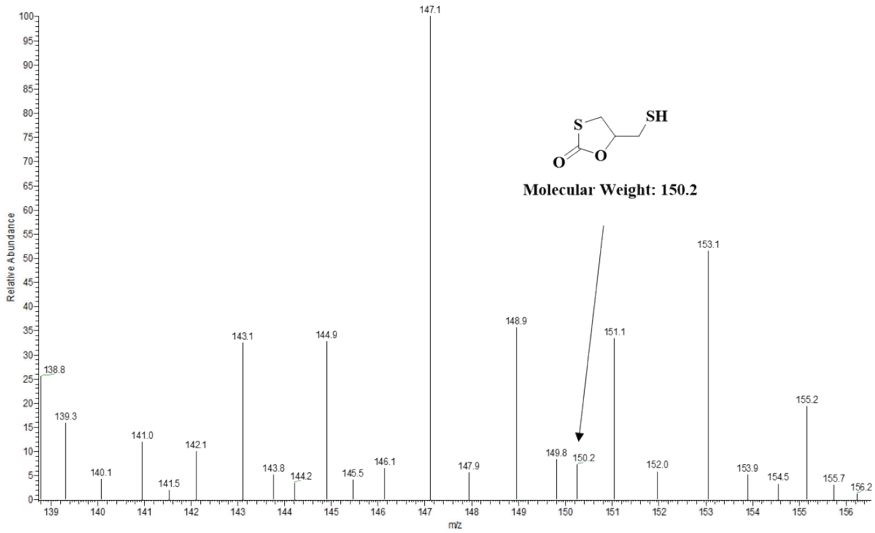


Figure S12. Mass spectrum of degraded products of TA by H_2O_2 treatment.

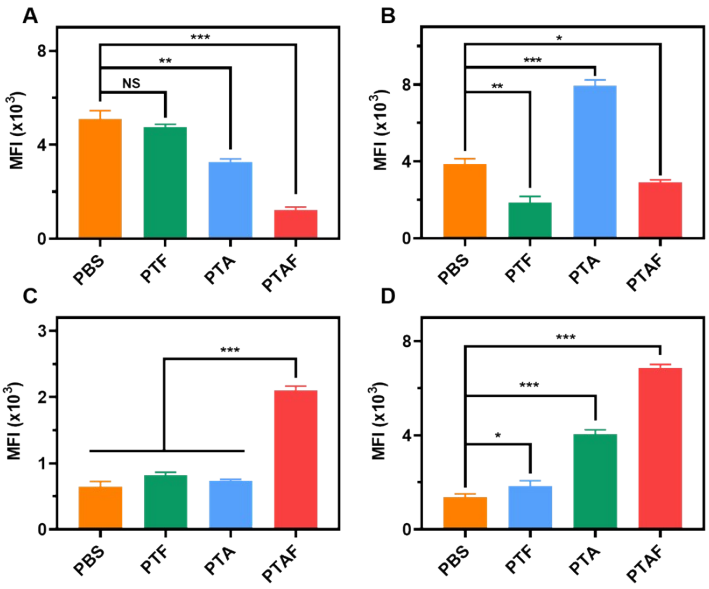


Figure S13. Quantitative analysis of (A) GSH level, (B) H_2O_2 level, (C) •OH level and (D) ROS level in 4T1 cells incubated with various formulations.

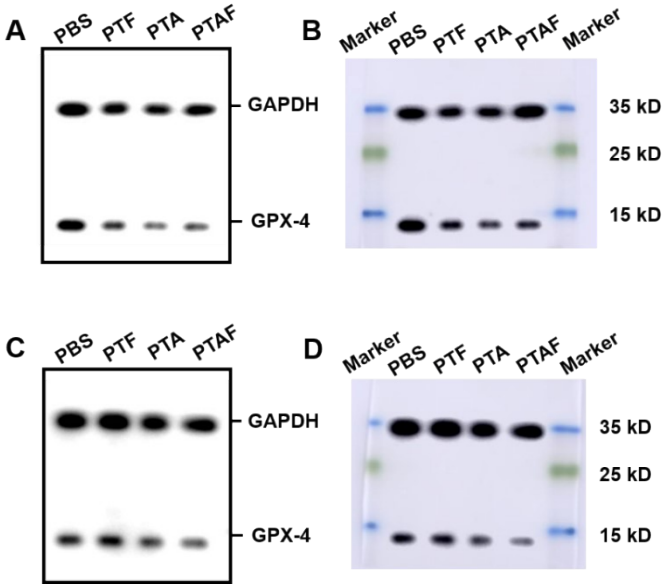


Figure S14. Western blot results for GPX4 expression level in 4T1 cells (A& B) and tumors (C& D) after incubation with various formulations (without/ with protein marker (Blue Plus® IV Protein Marker, 10 kDa-180 kDa)). (Corresponding blots are shown in Figures 2H and 3D)

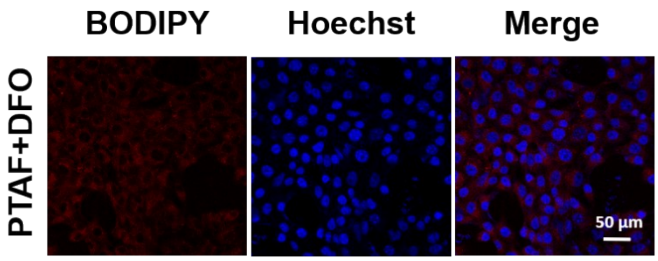


Figure S15. CLSM images of LPO level in 4T1 cells after treatments with PTAF (in the presence of DFO (100 μM)).

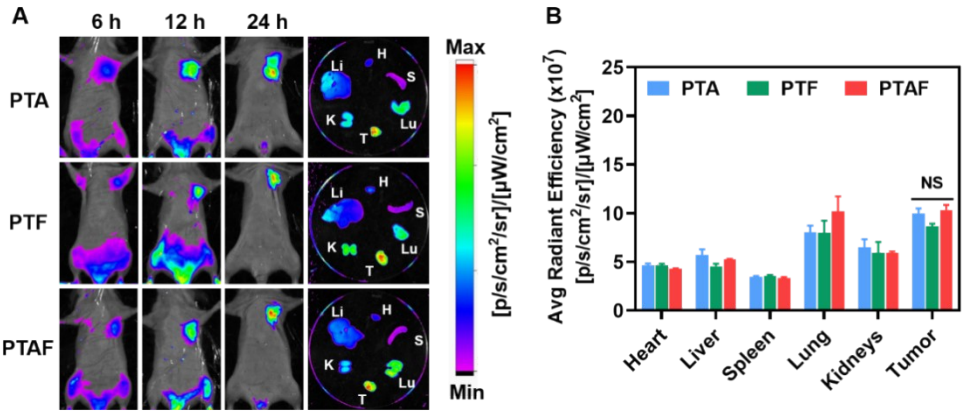


Figure S16. (A) *In vivo* fluorescence imaging of 4T1 tumor-bearing nude mice after intravenous injection of various formulations and ex vivo fluorescence images of the tumor and major organs; (B) Quantitative analysis on the average radiant efficiency of tumor and major organs in Figure A. Statistical P-values: NS $P > 0.05$.

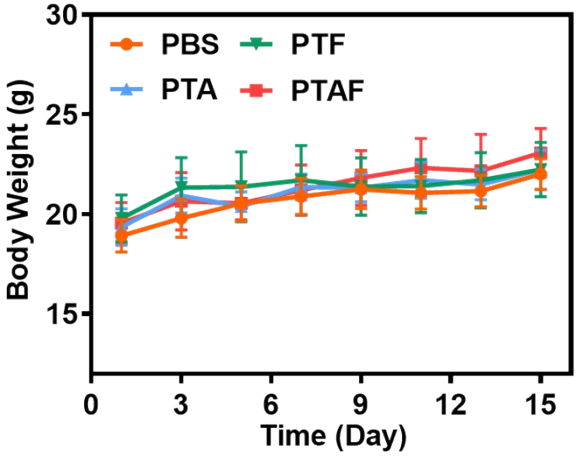


Figure S17. Body weight changes of mice after various treatments.

Reference

1. L. Zhang, D. Duan, Y. Liu, C. Ge, X. Cui, J. Sun and J. Fang, *J. Am. Chem. Soc.* 2014, **136**, 226-233.