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Supplementary information

# Rational Design of Fluorescent Light-Up Probes Based on an AIE Luminogen for Targeted Intracellular Thiol Imaging

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## Experimental Section

**General Information.** Dithiobis(succinimidyl propionate) (DSP), disuccinimidyl suberate (DSS), 3-(2-pyridyldithio)propionic acid *N*-hydroxysuccinimide ester, *N*,*N*-diisopropylethylamine (DIEA), acetic acid, triphenylphosphine, copper (II) sulfate, sodium ascorbate, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), glutathione (GSH), glutamic acid (Glu), cysteine (Cys), glycine (Gly), dithiothreitol (DTT), anhydrous dimethyl sulfate (DMSO), triethylamine, buthionine-sulfoximine (BSO) were purchased from Sigma-Aldrich and used without further purification. Hexane and tetrahydrofuran (THF) purchased from Fisher Scientific were distilled from sodium benzophenoneketyl immediately prior to use. Methanol was dried and distilled from calcium oxide. Deuterated dimethyl sulfoxide ($d_6$-DMSO) and chloroform ($CDCl_3$) with tetramethylsilane (TMS) as internal reference were purchased from Cambridge Isotope Laboratories, Inc.. Peptide with sequences of Asp-Asp-Asp-Asp-Asp (D5) and Asp-Asp-Asp-Asp-Asp-cyclic(Arg-Gly-Asp-D-Phe-Lys) (D5-cRGD) were customized from GL Biochem Ltd.

Dulbecco's modified essential medium (DMEM) was a commercial product of National University Medical Institutes (Singapore). 10 × phosphate-buffer saline (PBS) buffer with pH = 7.4 (ultrapure grade) is a commercial product of 1st BASE Singapore. MilliQ water (18.2 MΩ ) was used to prepare the buffer solutions from the 10 × PBS stock buffer. 1× PBS contains NaCl (137 mM), KCl (2.7 mM), $Na_2HPO_4$ (10 mM), and $KH_2PO_4$ (1.8 mM). Fetal bovine serum (FBS) and trypsin-EDTA solution were purchased from Gibco. Milli-Q water was supplied by a Milli-Q Plus System (Millipore Corp., USA). UV-vis spectra were recorded on a Shimadzu UV-1700 spectrometer. PL spectra were measured on a Perkin-Elmer LS 55 spectrofluorometer. The particle size and size distribution were determined by laser light scattering (LLS) with a particle size analyzer (90 Plus, Brookhaven Instruments Co., USA) at a fixed angle of 90° at room temperature. $^1$H NMR and $^{13}$C NMR spectra were measured on a Bruker ARX 400 NMR spectrometer. The HPLC profiles and mass spectra were acquired using a Shimadzu IT-TOF. High resolution mass spectra (HRMS) were recorded on a Finnigan MAT TSQ 7000 Mass Spectrometer. The preparative HPLC experiments used 0.1% TFA/$H_2O$ and 0.1% TFA/acetonitrile as eluents and the flow rate was 2 mL/min.

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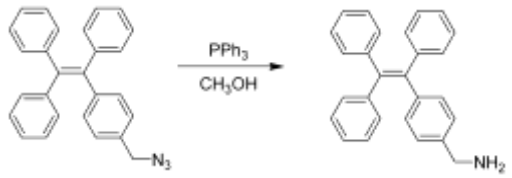
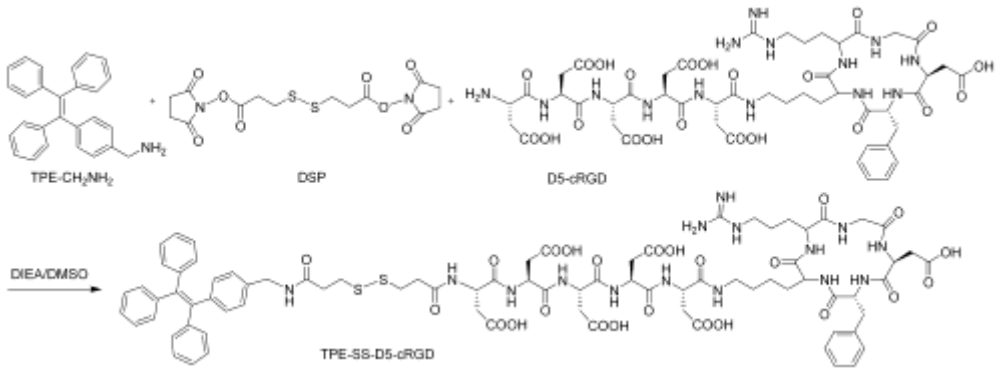
**Synthesis of 1-[(4-aminomethyl)phenyl]-1,2,2-triphenylethene (TPE-$CH_2NH_2$):** Into a nitrogen-filled 50 mL two-necked round bottle flask, a solution of 1-[(4-azidomethyl)phenyl]-1,2,2-triphenylethene[1] (0.194 g, 0.5 mmol), triphenylphosphine (0.197 g, 0.75 mmol) in 10 mL of anhydrous methanol was added. The mixture was refluxed overnight. After cooling down to room temperature, the solvent was evaporated under reduced pressure. The crude product was purified by silica-gel chromatography to obtain a white product in 70% yield (0.13 g).




**Scheme S1.** Synthetic route to compound TPE-$CH_2NH_2$.

**Synthesis, Purification and Characterization of Probe TPE-SS-D5-cRGD.** In a typical reaction, TPE-$CH_2NH_2$ (5.0 mg, 13.9 μmol) and D5-cRGD (16.4 mg, 13.9 μmol) were dissolved in DMSO (0.5 mL) with a catalytic amount of DIEA (1.0 μL) and the mixture was stirred at room temperature for 10 min. Then DSP (5.6 mg, 13.9 μmol) in DMSO (0.5 mL) was added quickly to the above solution. The reaction was continued with stirring at room temperature for another 24 h. The final product was purified by prep-HPLC (solvent A: water with 0.1% TFA, solvent B: $CH_3CN$ with 0.1% TFA) and lyophilized under vacuum to yield the probe as white powders in 45% yield (10.7 mg).




**Scheme S2**. Synthetic route to probe TPE-SS-D5-cRGD.

**Synthesis of Non-targetable Probe TPE-SS-D5.** Non-targetable probe TPE-SS-D5 was prepared by a similar procedure but D5 was used instead of D5-cRGD. TPE-$CH_2NH_2$ (5.0 mg, 13.9 μmol) and D5 (8.2 mg, 13.9 μmol)

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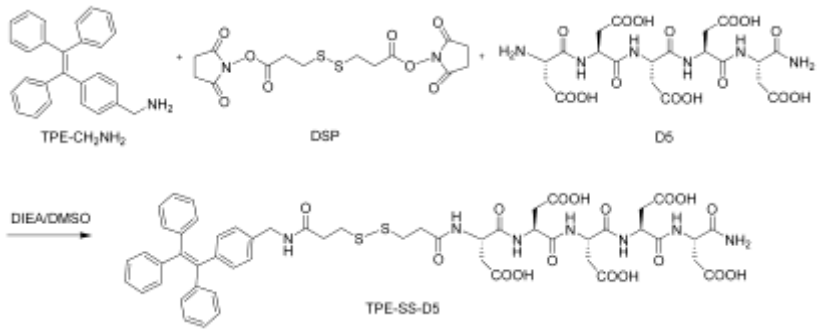
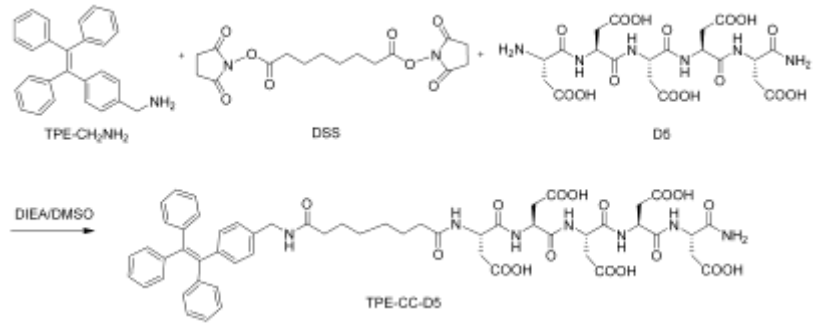
were dissolved in DMSO (0.5 mL) with a catalytic amount of DIEA (1.0 μL) and the mixture was stirred at room temperature for 10 min. Then DSP (5.6 mg, 13.9 μmol) in DMSO (0.5 mL) was added quickly to the above solution. The reaction was continued with stirring at room temperature for another 24 h. The final product was purified by prep-HPLC and lyophilized under vacuum to yield the probe as white powders in 49% yield (7.7 mg).




**Scheme S3**. Synthetic route to probe TPE-SS-D5.

**Synthesis of the Control Probe TPE-CC-D5.** The control probe TPE-CC-D5 was prepared by a similar procedure as TPE-SS-D5 using DSS as a coupling reagent in place of DSP. TPE-$CH_2NH_2$ (5.0 mg, 13.9 μmol) and D5 (8.2 mg, 13.9 μmol) were dissolved in DMSO (0.5 mL) with a catalytic amount of DIEA (1.0 μL) and the mixture was stirred at room temperature for 10 min. Then DSS (5.1 mg, 13.9 μmol) in DMSO (0.5 mL) was added quickly to the above solution. The reaction was continued with stirring at room temperature for another 24 h. The final product was purified by prep-HPLC and lyophilized under vacuum to yield the probe as white powders in 44% yield (6.7 mg).




**Scheme S4**. Synthetic route to probe TPE-CC-D5.

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**General procedure for probe activation by GSH.** DMSO stock solutions of TPE-SS-D5-cRGD, TPE-SS-D5 and TPE-CC-D5 were diluted with a mixture of DMSO and PBS (v/v = 1/199). Next, each probe was incubated with GSH at room temperature and the change of fluorescence intensity was measured. The solution was excited at 312 nm, and the emission was collected from 385 to 585 nm.

**Cell Culture.** U87-MG human glioblastoma and MCF-7 breast cancer cell lines were provided by American Type Culture Collection (ATCC). The cells were cultured in DMEM (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated FBS (Invitrogen), 100 U/mL penicillin, and 100 μg/mL streptomycin (Thermo Scientific) and maintained in a humidified incubator at 37 °C with 5% $CO_2$. Before experiment, the cells were precultured until confluence was reached.

**Confocal Imaging.** U87-MG and MCF-7 cells were cultured in the chambers (LAB-TEK, Chambered Coverglass System) at 37 °C. After 80% confluence, the culture medium was removed and washed twice with 1× PBS buffer. The probe in DMSO stock solution was then added to the chamber to reach a final concentration of 50 μM (1.0% DMSO). In some experiments, the cells were pre-incubated with media containing cRGD (50 μM) or BSO prior to probe incubation. After incubation at 37 °C for 2 h, the medium was replaced by fresh DMEM and incubated for further 4 h. After that, the cells were washed twice with ice-cold PBS and fixed with fresh 4% paraformaldehyde for 10 min at room temperature and permeabilized with 0.1% Triton X-100 in 1 × PBS for 5 min. The cell nucleus was stained with propidium iodide (Invitrogen, Carlsbad, CA) following the standard protocol of the manufacturer. The cells were then imaged immediately by confocal laser scanning microscope (CLSM, Zeiss LSM 410, Jena, Germany) equipped with DAPI and Texas Red filters with imaging software (Fluoview FV500). The images were analyzed by Image J 1.43× program (developed by NIH, http://rsbweb.nih.gov/ij/).

**Cytotoxicity of the Probes.** 3-(4,5-Dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were used to assess the metabolic activity of U87-MG cancer cells. U87-MG cells were seeded in 96-well plates (Costar, IL, USA) at an intensity of $4 \times 10^4$ cells $mL^{-1}$. After 24 h incubation, the medium was replaced by the probe suspension at a concentration of 50 μM (1 % DMSO). After incubation at 37 °C for 2 h, the medium was

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replaced with fresh DMEM and incubated at 37 °C. After the designated time intervals, the wells were washed twice with 1 × PBS buffer, and 100 μL of freshly prepared MTT (0.5 mg $mL^{-1}$) solution in culture medium was added into each well. The MTT medium solution was carefully removed after 3 h incubation in the incubator at 37 °C. DMSO (100 μL) was then added into each well and the plate was gently shaken to dissolve all the precipitates formed. The absorbance of MTT at 570 nm was monitored by the microplate reader (Genios Tecan). Cell viability was expressed by the ratio of absorbance of the cells incubated with probe suspension to that of the cells incubated with culture medium only.

**References**

(S1) Shi, H. B.; Kwok, R. T. K.; Liu, J. Z.; Xing, B. G.; Tang, B. Z.; Liu, B. *J. Am. Chem. Soc.* **2012**, *134*, 17972.

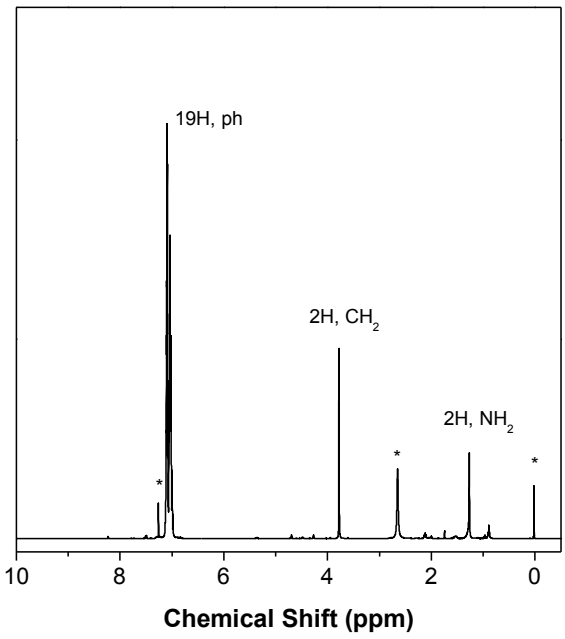


**Fig. S1.** $^{1}H$ spectrum of TPE-$CH_2NH_2$ in $CDCl_3$.

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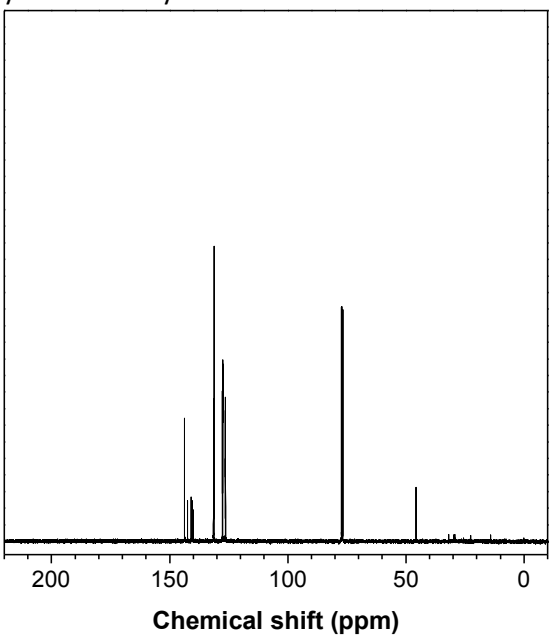


**Fig. S2.** $^{13}C$ spectrum of TPE-$CH_2NH_2$ in $CDCl_3$.

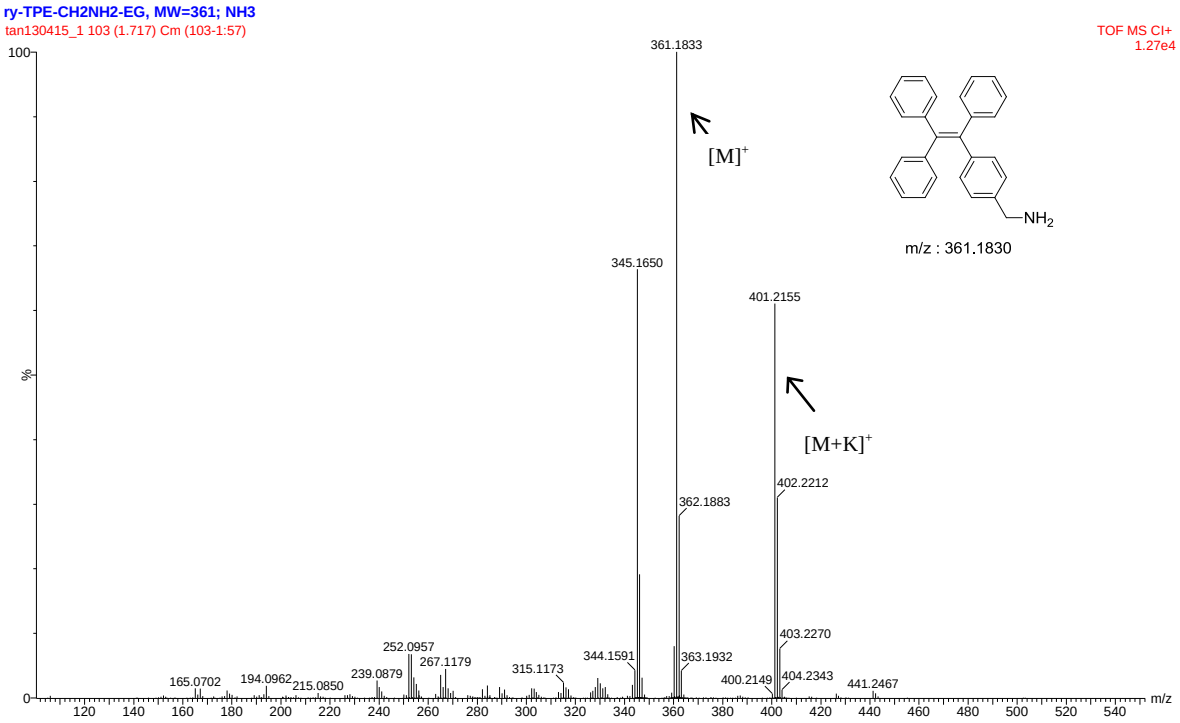


**Fig. S3**. High resolution mass spectrum (MALDI-TOF) of TPE-$CH_2NH_2$.

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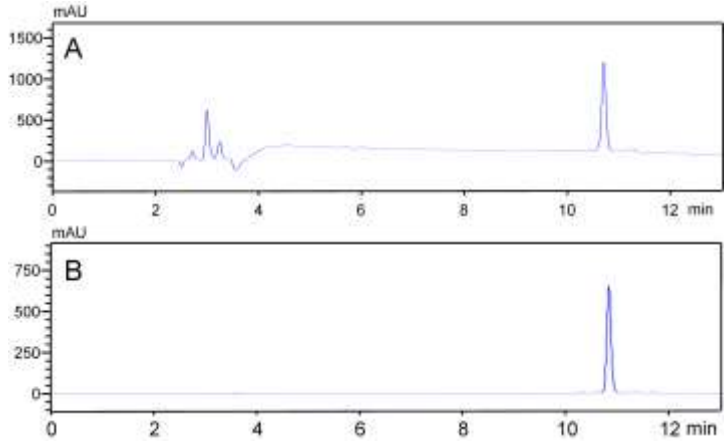


**Fig. S4**. HPLC spectra of TPE-SS-D5-cRGD monitored with absorbance at 214 nm (A) and 312 nm (B).

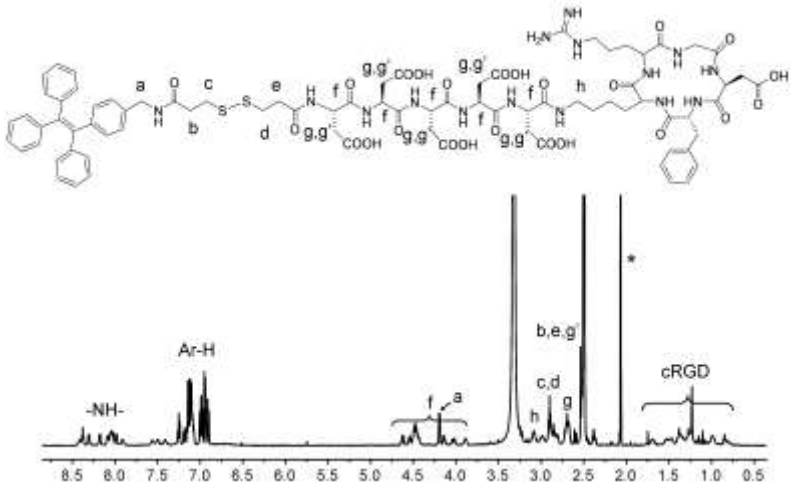


**Fig. S5**. $^{1}$H NMR spectrum of TPE-SS-D5-cRGD in DMSO-$d_6$. (* = Acetone)

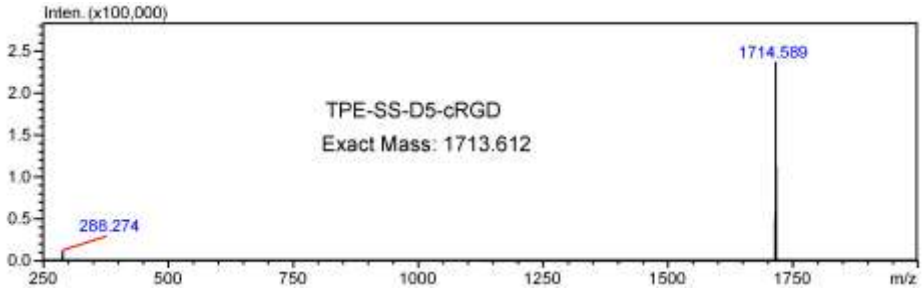


**Fig. S6**. Mass spectrum of TPE-SS-D5-cRGD.

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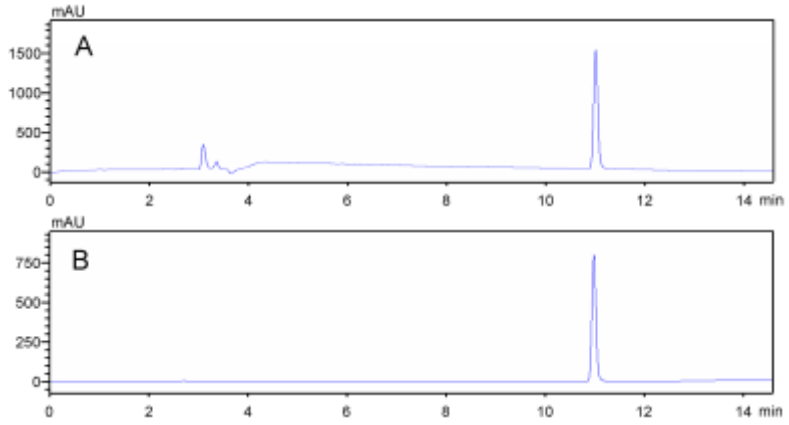


**Fig. S7**. HPLC spectra of TPE-SS-D5 monitored with absorbance at 214 nm (A) and 312 nm (B).

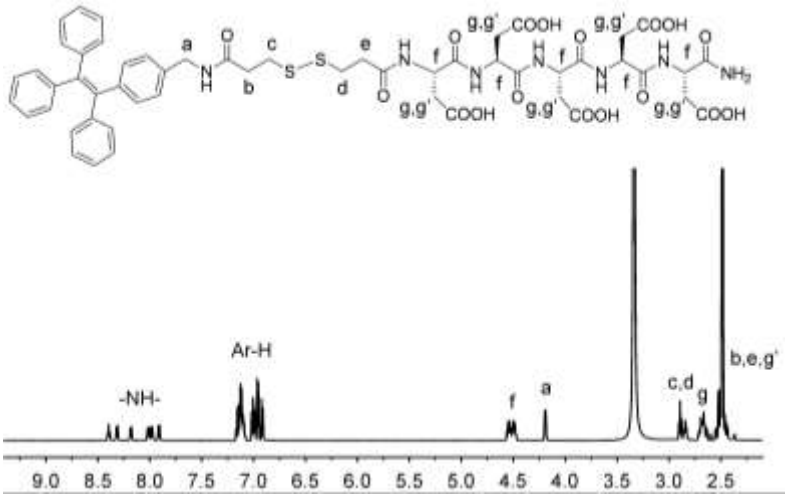


**Fig. S8**. $^{1}$H NMR spectrum of TPE-SS-D5 in DMSO-$d_6$.

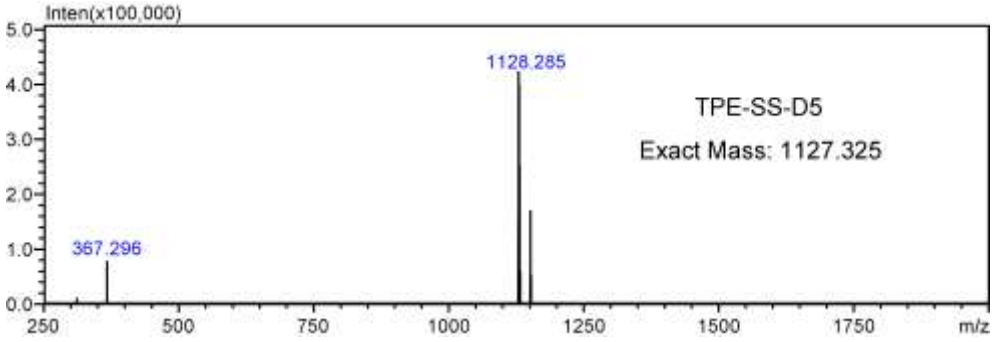


**Fig. S9**. Mass spectrum of TPE-SS-D5.

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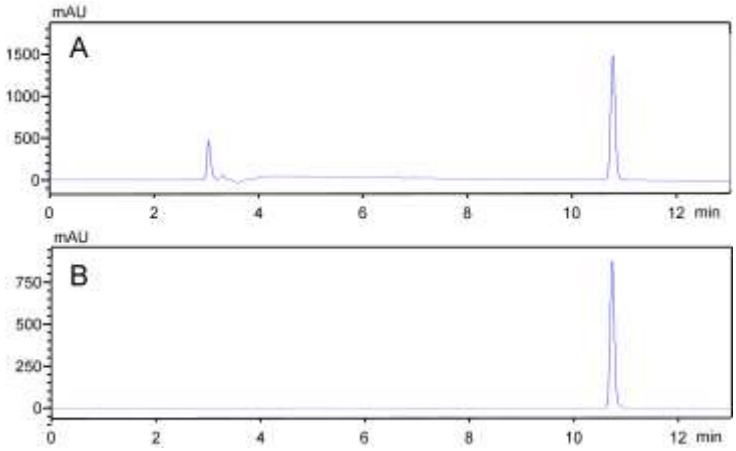


**Fig. S10**. HPLC spectra of TPE-CC-D5 monitored with absorbance at 214 nm (A) and 312 nm (B).

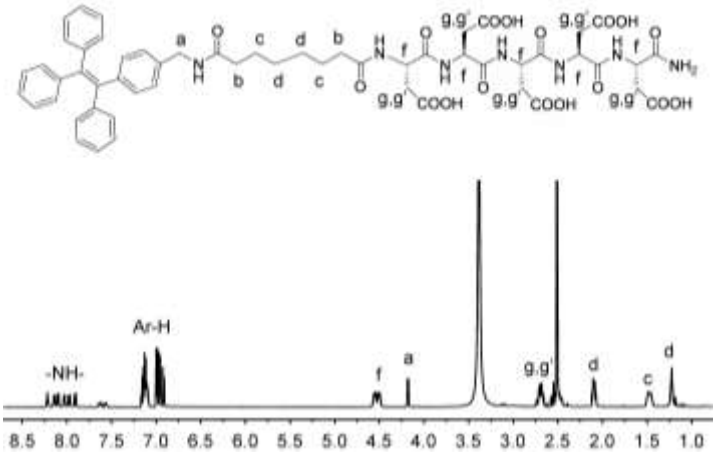


**Fig. S11**. $^{1}$H NMR spectrum of TPE-CC-D5 in DMSO-$d_6$.

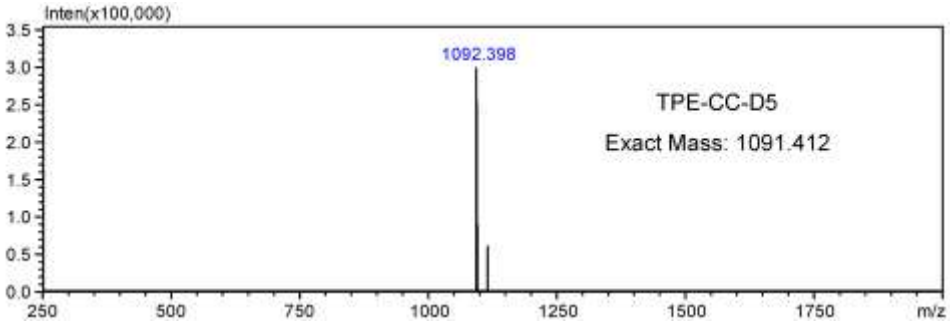


**Fig. S12**. Mass spectrum of TPE-SS-D5.

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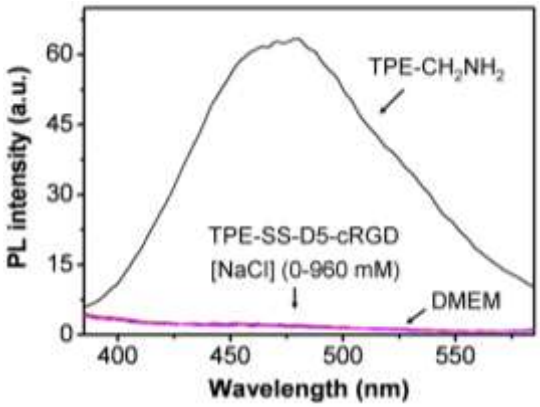


**Fig. S13.** PL spectra of TPE-$CH_2NH_2$ and TPE-SS-D5-cRGD in DMSO/water (v/v = 1/199) with NaCl concentrations varying from 0, 60, 240, 480 to 960 mM and in cell culture medium (DMEM). TPE-$CH_2NH_2$] = [TPE-SS-D5-cRGD] = 10 μM; $\lambda_{ex}$ = 312 nm.

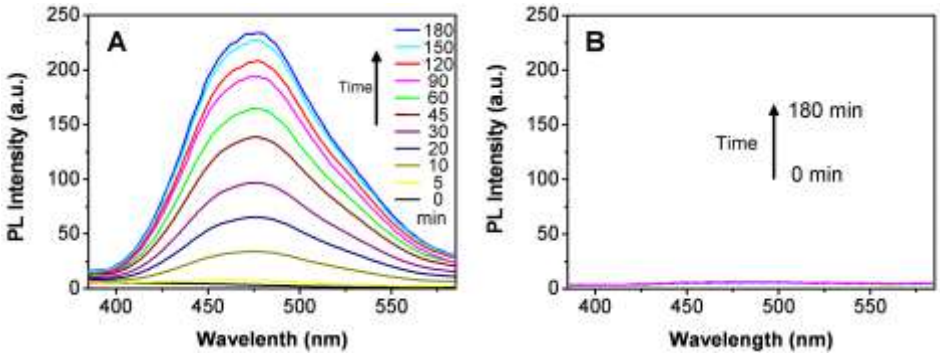


**Fig. S14**. Time-dependent fluorescence spectra of TPE-SS-D5 (A) and TPE-CC-D5 (B) (10 μM) upon treatment with GSH (1.0 mM) in DMSO/PBS (v/v = 1/199).

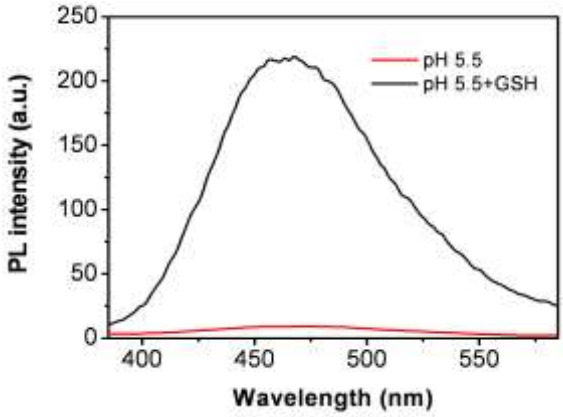


**Fig. S15.** Fluorescence spectra of TPE-SS-D5-cRGD (10 μM) in the absence or presence of 1 mM GSH in acetate buffer (pH 5.5).

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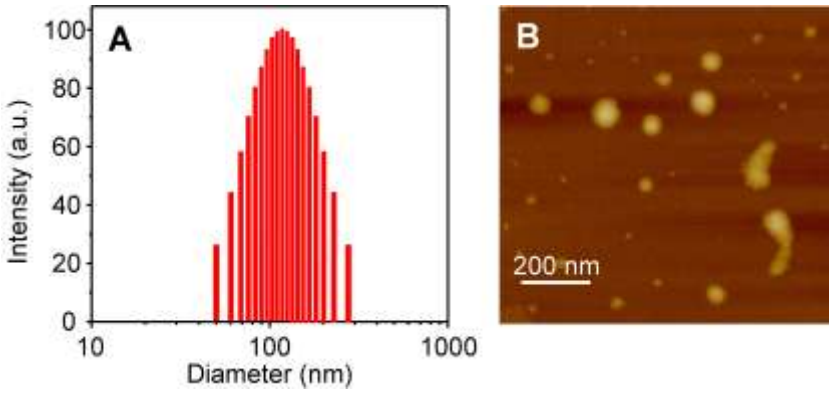


**Fig. S16**. Hydrodynamic diameters of TPE-SS-D5-cRGD (10 μM) upon incubation with 1 mM GSH for 3 h in DMSO/water (v/v = 1/199) measured from LLS (A) and AFM (B).

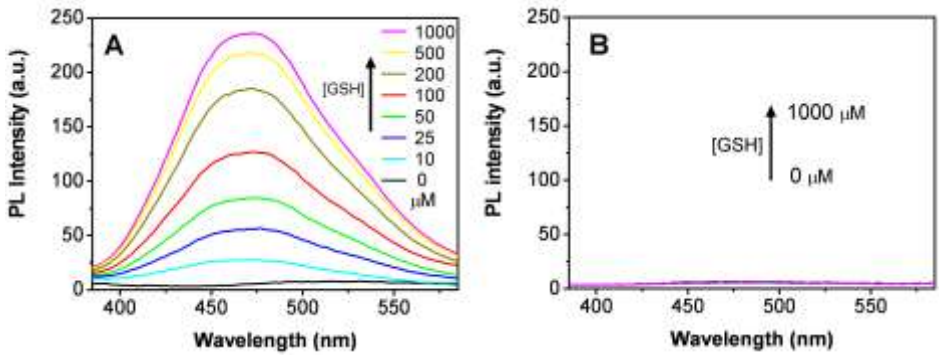


**Fig. S17**. PL spectra of TPE-SS-D5 (A) and TPE-CC-D5 (B) in the presence of GSH with concentrations varying from 0, 10, 25, 50, 100, 200 to 1000 μM in DMSO/PBS (v/v = 1/199).

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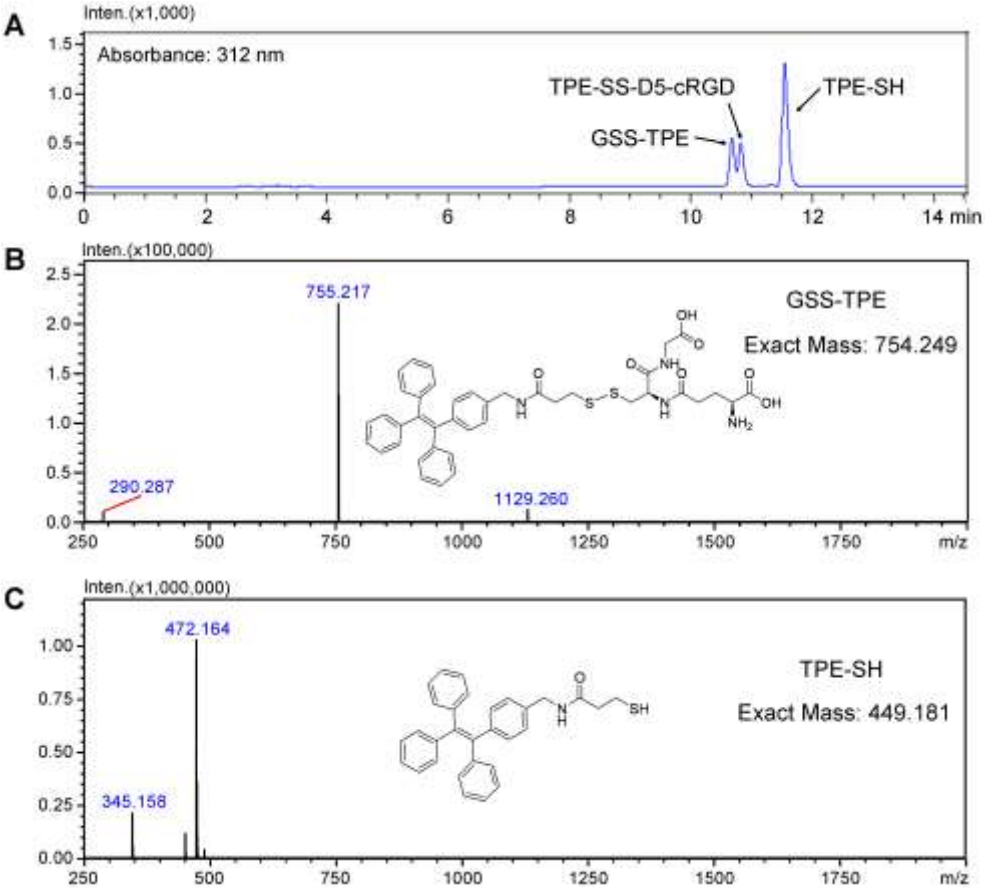


**Fig. S18.** Hydrolysis of TPE-SS-D5-cRGD (10 μM) by 1 mM GSH at t = 3 h monitored by LC-MS (A). Peaks in the chromatograms were detected by monitoring the UV/Vis absorbance at 312 nm. The corresponding mass spectra of newly formed peaks for GSS-TPE (B) and TPE-SH (C) are determined by IT TOF-MS spectrometry.

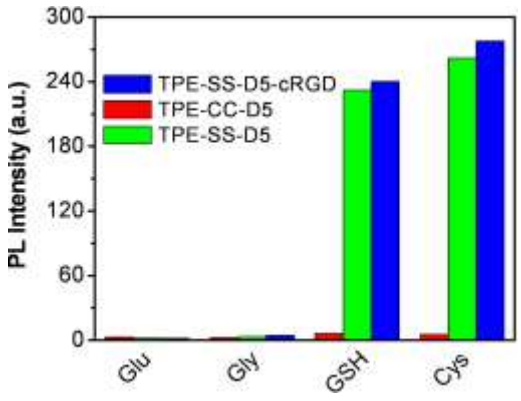


**Fig. S19**. Fluorescence response of TPE-SS-D5-cRGD toward 1.0 mM of GSH, Cys, Glu and Gly in DMSO/PBS (v/v = 1/199).

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**Table S1.** Optical properties of quinoline sulfate, TPE-$CH_2NH_2$, TPE-SS-D5-cRGD, TPE-SS-D5, TPE-CC-D5, GSS-TPE and TPE-SH in DMSO/PBS (v/v = 1/199).

| Samples | $\lambda_{ex}$ [a] | $\lambda_{em}$ [b] | $\Phi$ [c] |
|---|---|---|---|
| Quinoline sulfate | 346 | 450 | 0.54 |
| TPE-$CH_2NH_2$ | 312 | 476 | 0.23 |
| TPE-SS-D5-cRGD | 312 | 472 | 0.001 |
| TPE-SS-D5 | 312 | 474 | 0.001 |
| TPE-CC-D5 | 312 | 474 | 0.001 |
| TPE-SH | 312 | 470 | 0.19 |
| GSS-TPE | 312 | 470 | 0.12 |

[a] $\lambda_{ex}$ is the excitation maximum; [b] $\lambda_{em}$ is the emission maximum; [c] $\Phi$ is quantum yield which is determined using quinoline sulfate as the standard in aqueous solution.

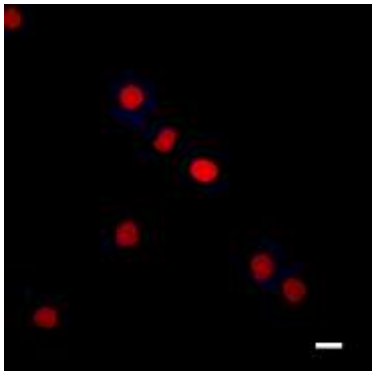

**Fig. S20.** CLSM images of U87-MG cells pre-incubated with 25 μM free cRGD before incubation with TPE-SS-D5-cRGD. Nucleus stained with propidium iodide (red). The scale bar is 20 μm.

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**Fig. S21.** CLSM images of living U87-MG cells incubation with TPE-SS-D5-cRGD. All images share the same scale bar (20 μm).

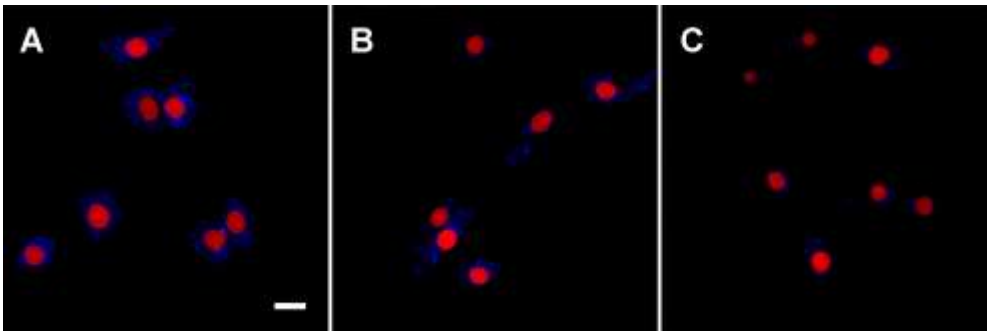


**Fig. S22**. Confocal microscopy images of U87-MG cells pretreated with BSO at concentrations of 25 μM (A), 50 μM (B) and 100 μM (C) for 2 h followed by incubation with TPE-SS-D5-cRGD. All images share the same scale bar (20 μm).

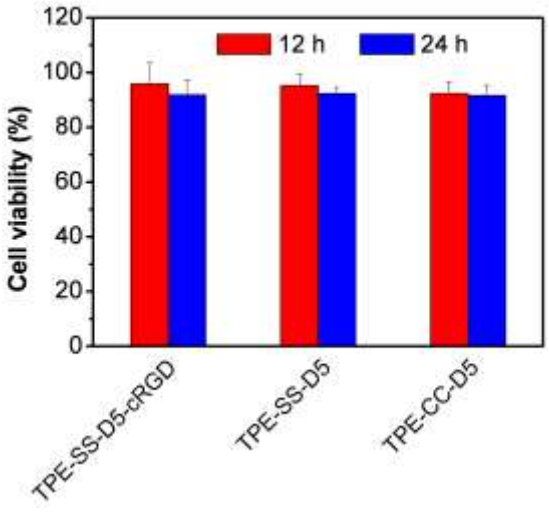


**Fig. S23.** Metabolic viabilities of U-87MG cancer cells after incubation with TPE-SS-D5-cRGD, TPE-SS-D5, TPE-CC-D5 at a concentration of 50 μM for 12 and 24 h.