

Supporting Information

Light-Up Probe based on AIEgens: Dual Signal Turn-On for Cascade Caspase Activation Monitoring

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Supplementary Methods

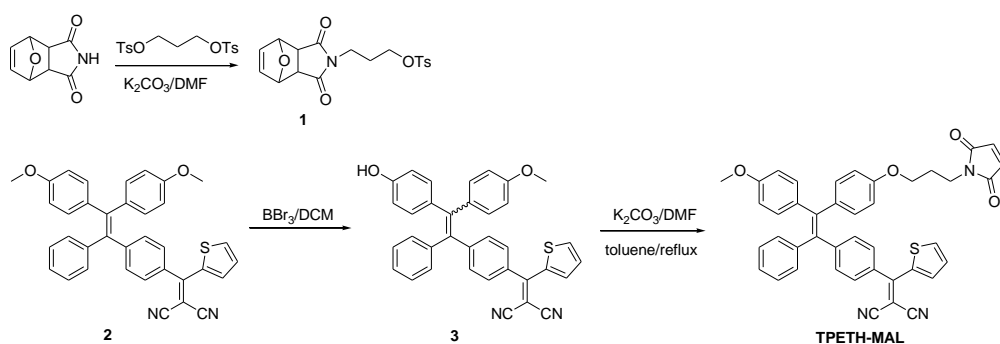
Materials and Methods

Materials and characterization: Trifluoroacetic acid (TFA), copper (II) sulfate (CuSO_4), sodium ascorbate, anhydrous dimethyl sulfoxide (DMSO), anhydrous *N,N*-dimethylformamide (DMF), diosgenin, hydrogen peroxide (H_2O_2), doxorubicin (DOX), 5-fluorouracil (5-Fu), *N,N*-diisopropylethylamine (DIEA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and other chemicals were all purchased from Sigma-Aldrich or Alfa Aesar and used as received without further purification. Tetrahydrofuran (THF) and dichloromethane were dried by distillation using sodium as drying agent and benzophenone as indicator. All non-aqueous reactions were carried out under nitrogen atmosphere in oven-dried glassware. Deuterated solvents with tetramethylsilane (TMS) as internal reference were purchased from Cambridge Isotope Laboratories Inc.. Alkyne- and cysteine-dual functionalized peptides CDVEDIETDPra (Cys-Asp-Val-Glu-Asp-Ile-Glu-Thr-Asp-Pra) and CDVEDLEHDPra (Cys-Asp-Val-Glu-Asp-Leu-Glu-His-Asp-Pra) were purchased from GL Biochem Ltd (Shanghai). 1,1-Dimethyl-2-[4-(azidomethyl)phenyl]-3,4,5-triphenylsilole (TPS- N_3) was prepared according to the previous report.¹

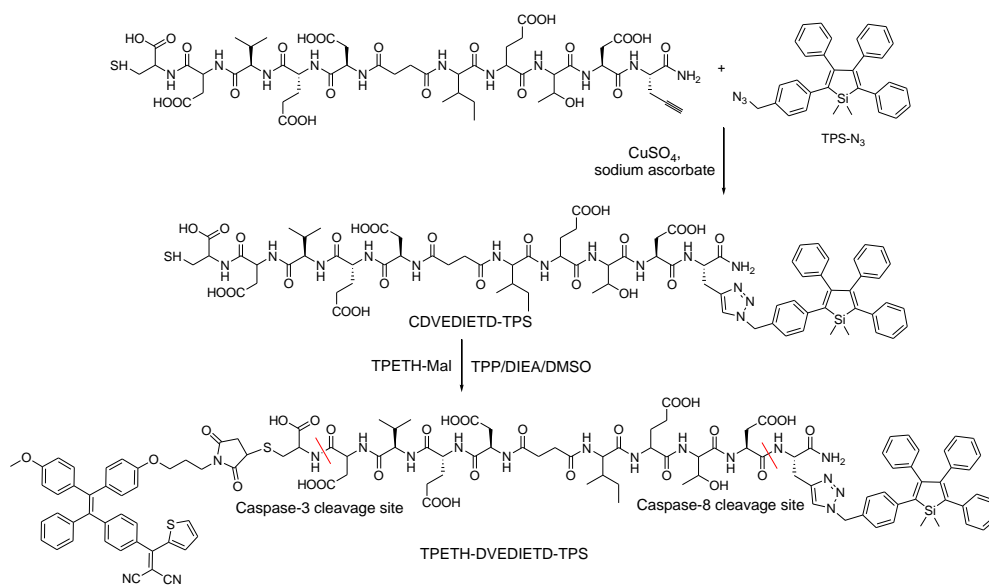
Dulbecco's Modified Essential Medium (DMEM) is a commercial product of Invitrogen. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, United States). Phosphate-buffer saline (PBS, 10 \times) buffer with pH = 7.4 is a commercial product of 1st BASE (Singapore). Milli-Q water (18.2 M Ω) was used to prepare the buffer solutions from the 10 \times PBS stock buffer. 1 \times PBS contains NaCl (137 mM), KCl (2.7 mM), Na_2HPO_4 (10 mM), and KH_2PO_4 (1.8 mM). Recombinant human caspase-8 and caspase-3 were purchased from R&D Systems. Caspase-8, caspase-9 and caspase-3 inhibitors were purchased from Biovision. Cleaved caspase-3 (Asp175) (5A1E) rabbit mAb (#9664); Cleaved Caspase-9 (Asp330) (D2D4) Rabbit mAb (#7237) and Cleaved Caspase-8 (Asp391) (18C8) Rabbit mAb (#9496) were purchased from Cell Signaling. Mouse anti-rabbit IgG-Texas Red (TR) was

purchased from Santa Cruz. Hoechst 33342, fetal bovine serum (FBS) and trypsin-EDTA solution were purchased from Life Technologies.

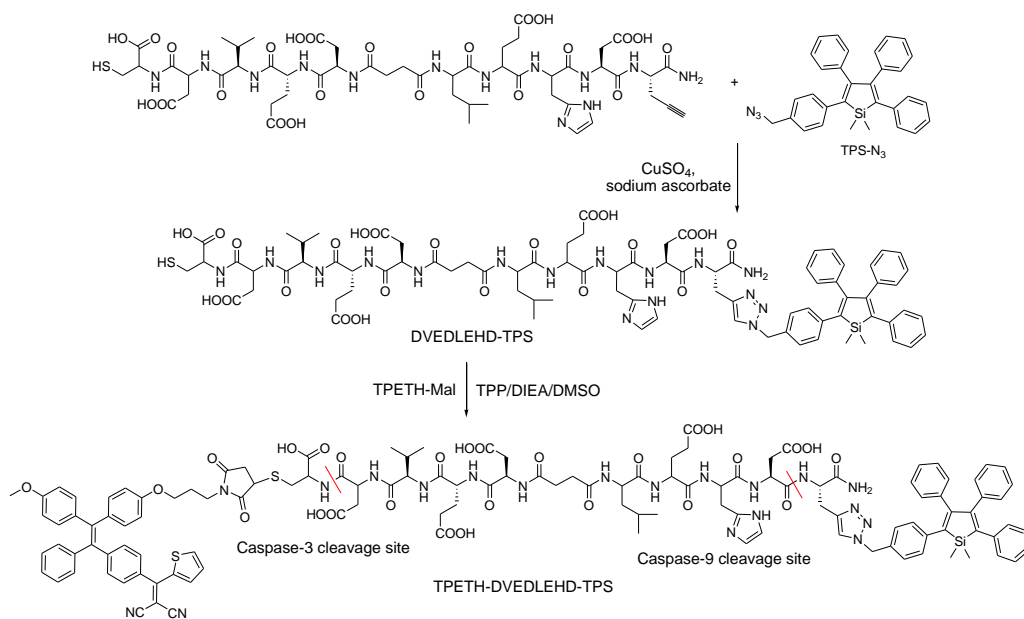
NMR spectra were measured on a Bruker ARX 300/400/500 NMR spectrometer. Chemical shifts are reported in parts per million referenced with respect to residual solvent ($\text{CDCl}_3 = 7.26$ ppm and $(\text{CD}_3)_2\text{SO} = 2.50$ ppm) for ^1H NMR and ($\text{CDCl}_3 = 77.0$ ppm and $(\text{CD}_3)_2\text{SO} = 40.0$ ppm) for ^{13}C NMR. The extent of reaction was monitored by thin layer chromatography (TLC) using Merck 60 F254 pre-coated silica gel plates with fluorescent indicator UV254. After the plates were subjected to elution in the TLC chamber, the spots were visualized under UV light or using the appropriate stain (I_2 , KMnO_4 , ninhydrin or ceric ammonium molybdate (CAM)). Flash column chromatography was carried out using Merck silica gel (0.040-0.063). A 0.1% trifluoroacetic acid solution in H_2O and acetonitrile was used as the eluent for high-performance liquid chromatography (HPLC) experiments (Agilent). Mass spectra were recorded on Agilent 5975 DIP-MS for electron impact (EI) and the AmaZon X LC-MS for electrospray ionization (ESI). Particle size and size distribution were determined by laser light scattering (LLS) with a particle size analyzer (90 Plus, Brookhaven Instruments Co., United States) at a fixed angle of 90° at room temperature. TEM images were obtained from a JEOL JEM-2010 transmission electron microscope with an accelerating voltage of 200 kV. UV-vis absorption spectra were taken on a Shimadzu Model UV-1700 spectrometer. Photoluminescence (PL) spectra were measured on a Perkin-Elmer LS 55 spectrofluorometer. All UV and PL spectra were collected at 24 ± 1 °C.



Scheme S1. Synthetic route to TPETH-Mal.

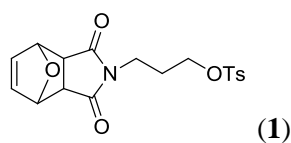


Scheme S2. Synthetic route to TPETH-DVEDIETD-TPS (Probe 1), and its cleavage sites by caspase-8 and caspase-3.



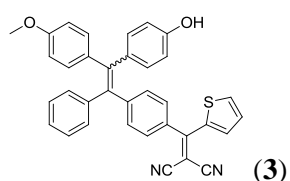
Scheme S3. Synthetic route to TPETH-DVEDLEHD-TPS (Probe 2), and its cleavage sites by caspase-9 and caspase-3.

Synthesis of compound 1.



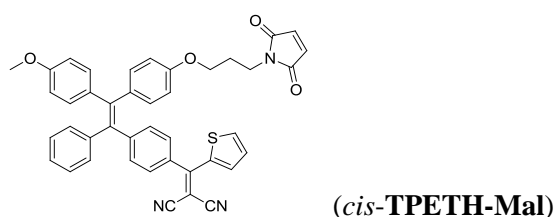
To the solution of 3a,4,7,7a-tetrahydro-1*H*-4,7-epoxyisoindole-1,3(2*H*)-dione (500 mg, 3.0 mM) in DMF (20 mL) was added propane-1,3-diyl bis(4-methylbenzenesulfonate) (760 mg, 2.0 mM) and potassium carbonate (700 mg, 5.1 mM). The resulting mixture was stirred at 50 °C for 8 h. Then the mixture was cooled down to room temperature and the solvent was removed under reduced pressure. To the residue was added water (50 mL) and ethyl acetate (100 mL). The organic phase was separated and washed further with brine (50 mL×3), dried over sodium sulfite. The mixture was then filtered and the filtrate was concentrated and purified with chromatography (Hexane/EA (v/v) = 10/1 to 1/1) to give compound **1** as a white solid (290 mg, 38.4% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.77 (m, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 6.48 (t, *J* = 0.8 Hz, 2H), 5.19 (t, *J* = 0.8 Hz, 2H), 4.00 (t, *J* = 6.4 Hz, 2H), 3.52 (t, *J* = 6.8 Hz, 2H), 2.81 (s, 2H), 2.43 (s, 3H), 1.89-1.95 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 175.9, 144.7, 136.4, 132.7, 129.7, 127.9, 80.8, 67.6, 47.3, 35.3, 26.8, 21.5; HRMS (ESI), calcd for [M+Na]⁺: 400.0825, found: 400.0835.

Synthesis of compound 3.



The compound **2** was prepared according to the similar procedure reported previously.^[2] Boron tribromide (1.0 M in dichloromethane, 0.50 mL) was added to the solution of compound **2** (160 mg, 0.29 mM) in dichloromethane (10 mL) at 0 °C. The reaction mixture was then stirred at room temperature for 2 h. The reaction was cooled under ice-water bath and was quenched by addition of water (5 mL). The organic layer was collected, washed with brine (15 mL), dried over Na₂SO₄. The mixture was filtered and the filtrate was concentrated under reduced pressure. The desired red residue was purified by column chromatography (hexane/ethyl acetate (v/v) = 20/1 to 3/1) to give compound **3** as a red solid (80 mg, 51.6% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.78-7.81 (m, 1H), 7.71-7.73 (m, 1H), 7.11-7.22 (m, 7H), 7.05-7.07 (m, 2H), 6.86-6.90 (m, 4H), 6.64-6.67 (m, 2H), 6.57-6.60 (m, 2H), 3.75 (s, 3H).

Synthesis of TPETH-Mal.



To the solution of compound **3** (54 mg, 0.1 mM) in acetonitrile (5 mL) was added compound **2** (50 mg, 0.13 mM) and potassium carbonate (28 mg, 0.2 mM). The resulting mixture was stirred at 50 °C for 12 h. Then the reaction mixture was cooled down to room temperature and the solid was removed by filtration. The filtrate was subsequently concentrated and purified with chromatography (hexane/EA (v/v) = 10/1 to 3/1) to give the key intermediate (48 mg), which was then dissolved in toluene (5 mL) and refluxed for 20 h. The solvent was removed and the red residue was further purified with chromatography (hexane/EA (v/v) = 10/1 to 3/1) to give TPETH-Mal as red solid (36 mg, 53.4% yield). The *cis* isomer was further obtained by HPLC purification.^[3] ¹H NMR (400 MHz, CDCl₃) δ 7.82 (dd, $J_1 = 5.0$ Hz, $J_2 = 1.0$ Hz, 1H), 7.73 (dd, $J_1 = 4.0$ Hz, $J_2 = 1.0$ Hz, 1H), 7.23 (dd, $J_1 = 5.0$ Hz, $J_2 = 4.0$ Hz, 1H), 7.11-7.23 (m, 7H), 7.07 (m, 2H), 6.96 (m, 2H), 6.90 (m, 2H), 6.69 (s, 2H), 6.60-6.65 (m, 4H), 3.92 (t, $J = 6.0$ Hz, 2H), 3.75 (s, 3H), 3.72 (t, $J = 7.0$ Hz, 2H), 2.06 (m, 2H); HRMS (ESI), calcd for [M+Na]⁺: 696.1927, found: 696.1932.

Synthesis of the probe TPETH-DVEDIETD-TPS (Probe 1). TPS-N₃ (5 mg, 10.7 μ M) and CDVEDIETDPra (13.1 mg, 10.7 μ M) were first dissolved in DMSO/H₂O (v/v = 10:1) and mixed thoroughly. Into the mixture, sodium ascorbate (1.8 mg, 9.0 μ M) and CuSO₄ (2.4 mg, 4.5 μ M) were added to initiate the click reaction and the mixture was shaken at room temperature for another 24 h. The mixture was separated with HPLC using reverse column with acetonitrile, water and 0.1% TFA as gradient elution buffer to give CDVEDIETD-TPS as a white solid (6.3 mg, 35.0% yield). To the solution of TPETH-Mal (1.0 mg, 1.5 μ M) in DMSO (0.4 mL) was added CDVEDIETD-TPS (1.7 mg, 1.0 μ M), triphenylphosphine (0.5 mg, 1.9 μ M) and *N,N*-diisopropylethylamine (DIEA, 1 μ L). The resulting mixture was stirred at room temperature for 4 h. The mixture was separated with HPLC using reverse column with acetonitrile, water and 0.1% TFA as gradient elution buffer to give Probe 1 as a red powder (1.1 mg, 46.0% yield). MS (ESI), calcd for [M+2H]²⁺: 1187.9, found: 1187.9.

Synthesis of the probe TPETH-DVEDLEHD-TPS (Probe 2). Probe 2 was prepared using a similar procedure as Probe 1 with CDVEDLEHDPra instead of CDVEDIETDPra. Typically, TPS-N₃ (5 mg, 10.7 μ M) and

CDVEDLEHDPra (13.5 mg, 10.7 μM) were first dissolved in DMSO/H₂O (v/v = 10:1) and mixed thoroughly. Into the mixture, sodium ascorbate (1.8 mg, 9.0 μM) and CuSO₄ (2.4 mg, 4.5 μM) were added to initiate the click reaction and the mixture was shaken at room temperature for another 24 h. The mixture was separated with HPLC using reverse column with acetonitrile, water and 0.1% TFA as gradient elution buffer to give DVEDLEHD-TPS as a white solid (7.2 mg, 39.0% yield). Then DVEDLEHD-TPS (1.8 mg, 1.0 μM) and TPETH-Mal (1.0 mg, 1.5 μM) were dissolved in DMSO (0.4 mL) before triphenylphosphine (0.5 mg, 1.9 μM) and DIEA (1 μL) were added. The resulting mixture was stirred at room temperature for 4 h. The mixture was separated with HPLC using reverse column with acetonitrile, water and 0.1% TFA as gradient elution buffer to give Probe 2 as a red powder (1.2 mg, 41.0% yield). MS (ESI), calcd for [M+2H]²⁺: 1205.9, found: 1205.8.

General procedure for enzymatic assay. DMSO stock solutions of probes were diluted with a mixture of DMSO and PBS (v/v = 1/99) to 10 μM . Next, the probes were incubated with caspase-8 or caspase-3 at 37 $^{\circ}\text{C}$ and the changes of fluorescence intensity of TPS and TPETH were measured. The PL spectra of TPS were collected from 400 to 650 nm under excitation at 365 nm; the PL spectra of TPETH were collected from 525 to 825 nm under excitation at 430 nm.

Cell Culture. Human cervix carcinoma HeLa cells were provided by American Type Culture Collection (ATCC). The cells were cultured in DMEM medium containing 100 $\mu\text{g mL}^{-1}$ streptomycin, 10% heat-inactivated FBS, 100 U mL^{-1} penicillin, and maintained in a humidified incubator with 5% CO₂ at 37 $^{\circ}\text{C}$.

Confocal Imaging. HeLa cells were cultured in 8-well chambers (Thermo Scientific) at 37 $^{\circ}\text{C}$. After 80% confluence, the culture medium was removed and washed twice with 1 \times PBS. After incubation with Probe 1 for 2 h at 37 $^{\circ}\text{C}$, the cells were washed twice with 1 \times PBS buffer and treated with apoptosis inducers (H₂O₂ or diosgenin) for different time. The cells were then washed twice with 1 \times PBS and the cell nuclei were live stained with Hoechst 33342, following the standard protocols of the manufacturer (Life Technologies) and imaged immediately by confocal microscope (CLSM, Zeiss LSM 410, Jena, Germany). For colocalization with active caspase-8 or caspase-3 antibody, the cells were fixed with 3.7% formaldehyde in 1 \times PBS for 15 min at room temperature, washed twice with cold 1 \times PBS again, and permeabilized with 0.1% Triton X-100 in 1 \times PBS for 10 min. The cells were then blocked with 2% Bovine Serum Albumin (BSA) in 1 \times PBS for 30 min and washed twice with 1 \times PBS. The cells were subsequently incubated with a mixture of anti-caspase-8/3 antibody in 1 \times PBS (v/v = 1/99) for 1 h at room temperature, washed once with 1 \times PBS buffer, and then incubated with mouse anti-rabbit IgG-TR (0.8 $\mu\text{g mL}^{-1}$) in 1 \times PBS for 1 h, followed by washing with 1 \times PBS. For Hoechst 33342 imaging, the excitation wavelength was 405

nm and the emission filter was 430–470 nm, laser power: 5%, high voltage (HV): 480 V and offset: -4; for TPS residue detection, the excitation wavelength was 405 nm, and the emission filter was 505–525 nm, laser power: 5%, HV: 450 V and offset: -2; for TPETH residue detection, the excitation wavelength was 405 nm and the emission wavelength was collected above 650 nm, laser power: 5%, HV: 550 V and offset: -2.

Flow Cytometry Study. HeLa cells in 24-well plate (Costar, IL, USA) were pre-cultured overnight and incubated with Probe 1 for the designated time and treated with H₂O₂ (1.0 mM) for different time. After incubation, the cells were washed with 1 × PBS and treated with trypsin, washed with the medium twice and subjected to flow cytometry analysis using Cyan-LX (DakoCytomation). The cells without any treatment were used as control. The mean fluorescence was determined by counting 10,000 events.

Cytotoxicity Studies. MTT assays were used to assess the cell viability of HeLa cells after incubation with the probes. The cells in 96-well plates (Costar, IL, USA) were incubated with the probes for designated time in the dark. The cells were further incubated in fresh medium for 48 h and washed with 1 × PBS. Then MTT in 1 × PBS solution (100 μL, 0.5 mg mL⁻¹) was added into each well. After incubation for 3 h, the supernatant was discarded and the precipitate was dissolved in DMSO (100 μL) with gentle shaking. The absorbance of MTT at 570 nm was monitored by the microplate reader (Genios Tecan). The cells without any treatment were used as control.

References:

- [1] Y. Y. Yuan, R. T. K. Kwok, B. Z. Tang, B. Liu, *J. Am. Chem. Soc.* **2014**, *136*, 2546-2554
- [2] Y. Y. Yuan, C.-J. Zhang, S. D. Xu, B. Liu, *Chem. Sci.* **2016**, *7*, 1862-1866.
- [3] Y. Y. Yuan, C.-J. Zhang, R. T. Kwok, S. D. Xu, R. Y. Zhang, J. E. Wu, B. Z. Tang, B. Liu, *Adv. Funct. Mater.* **2015**, *25*, 6586-6595.

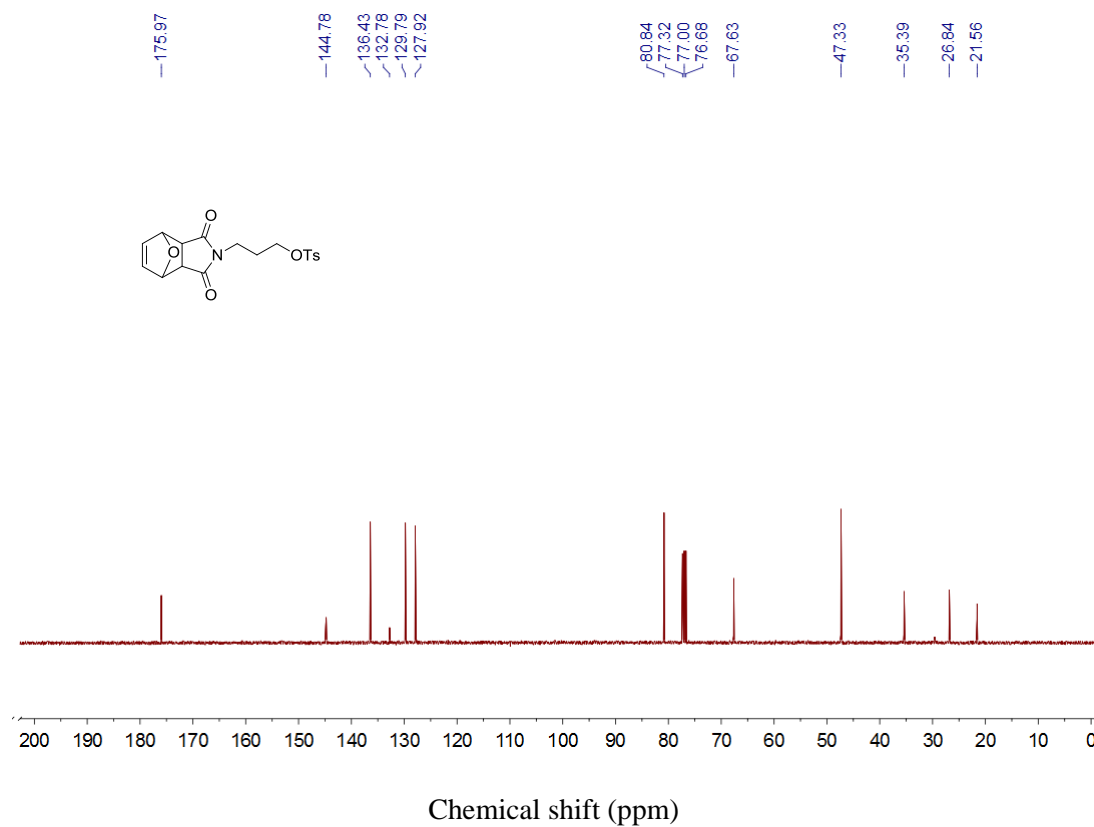
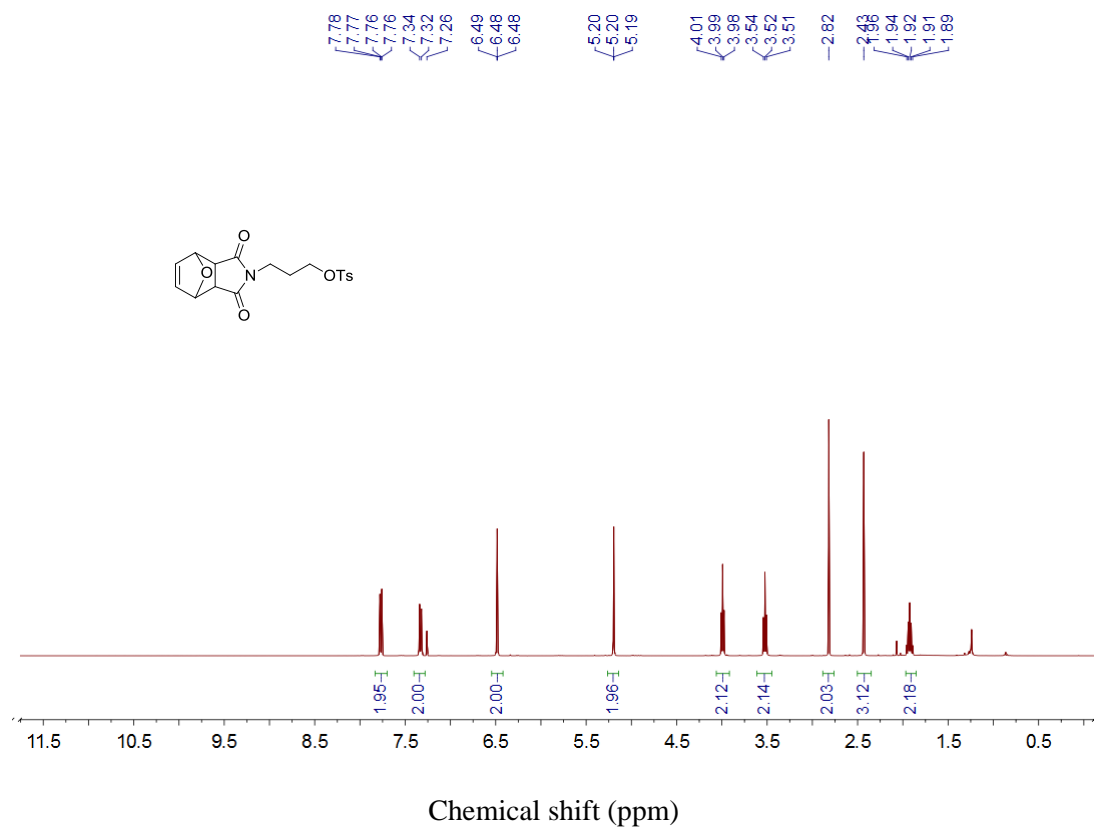


Figure S1. ^1H and ^{13}C -NMR spectra of compound 1.

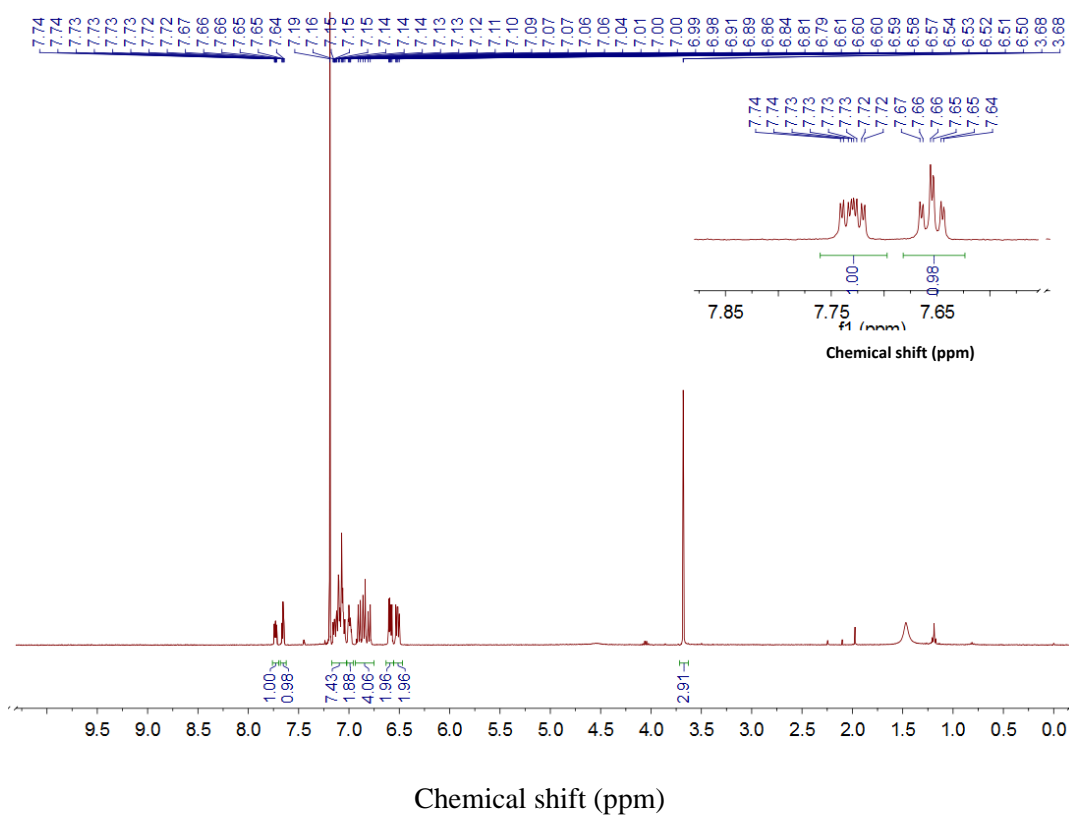


Figure S2. ^1H NMR spectrum of compound **3**.

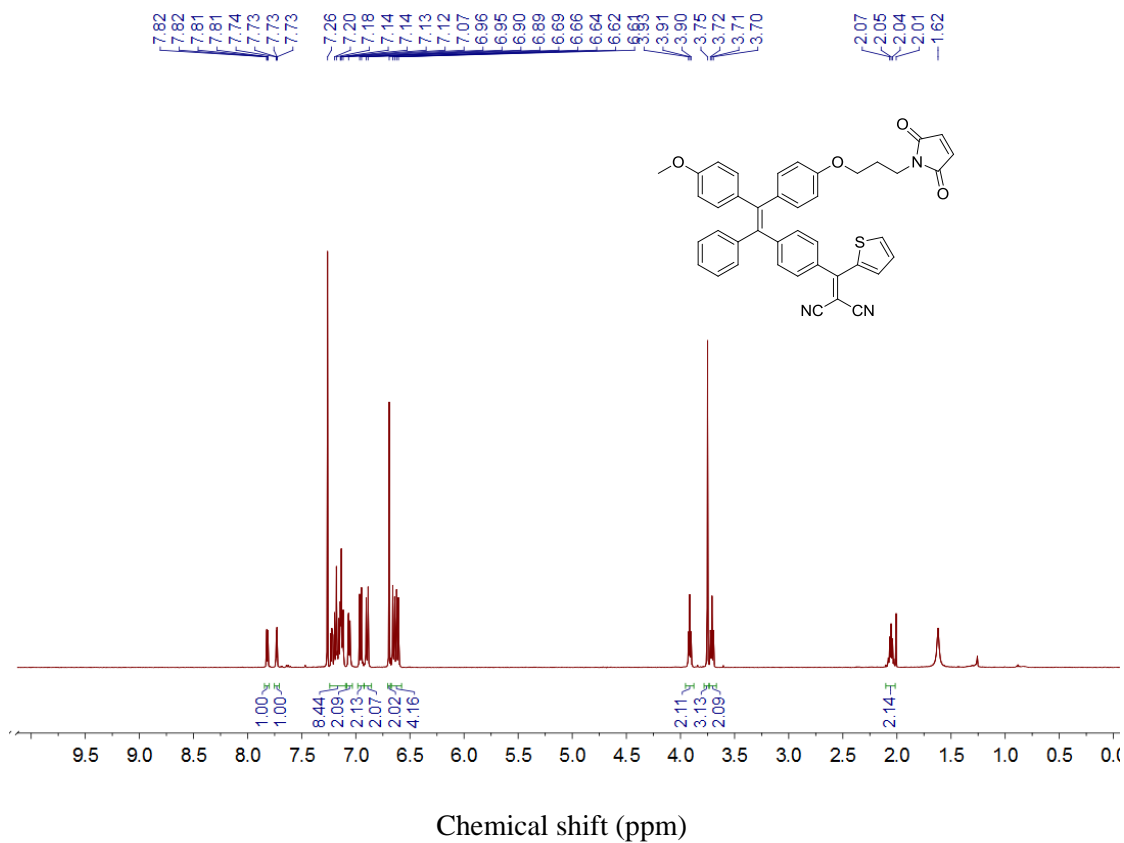


Figure S3. ^1H NMR spectrum of *cis*-TPETH-Mal (isomer-1).

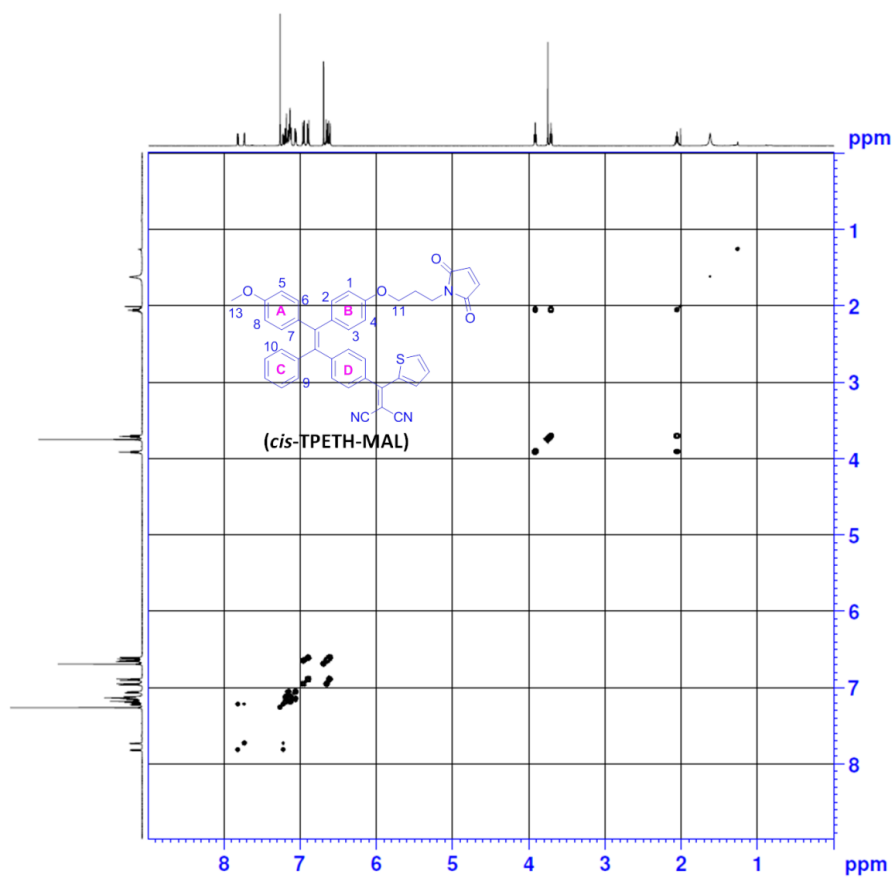


Figure S4. COSY-NMR of *cis*-TPETH-Mal.

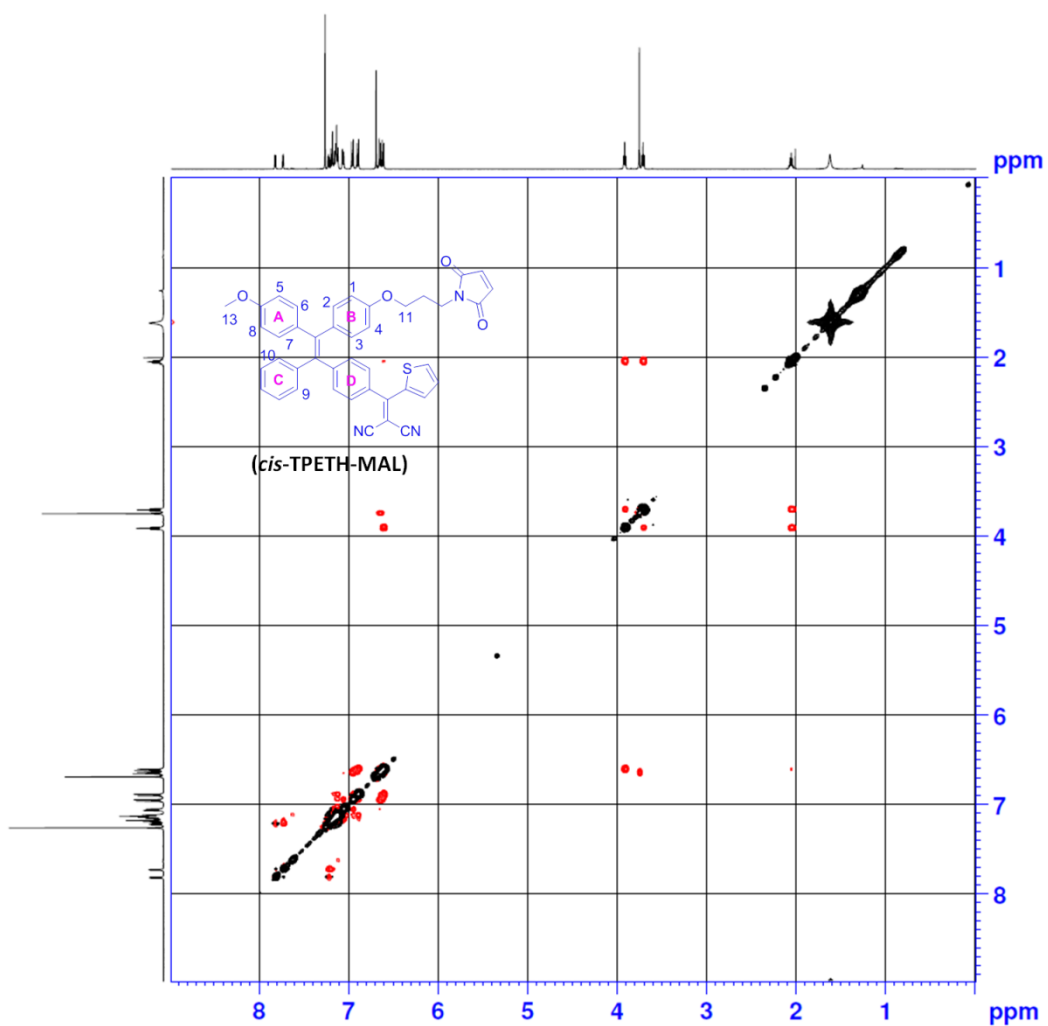


Figure S5. NOESY-NMR of *cis*-TPETH-Mal.

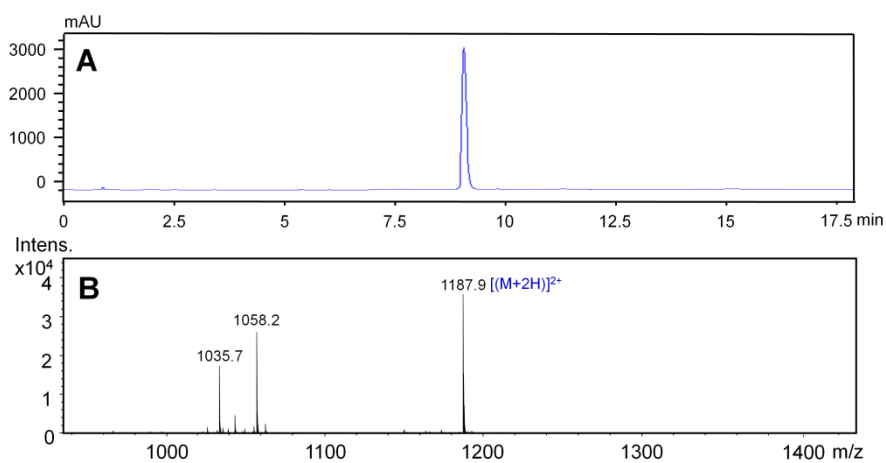


Figure S6. HPLC (A) and ESI mass spectra (B) of Probe 1. The HPLC spectrum was recorded at absorbance of 405 nm.

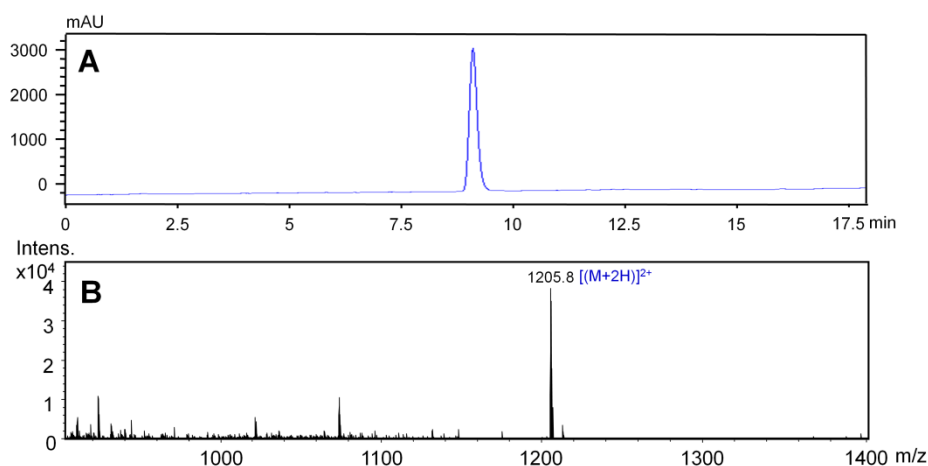


Figure S7. HPLC (A) and ESI mass spectra (B) of Probe 2. The HPLC spectrum was recorded at absorbance of 405 nm.

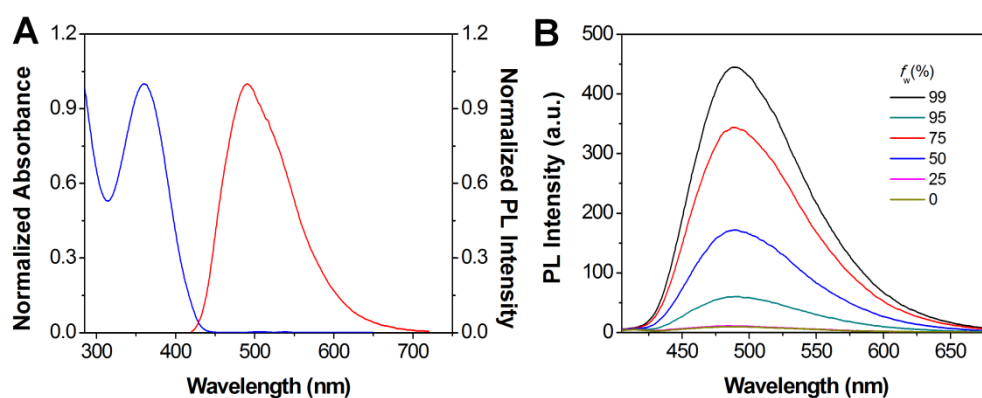


Figure S8. (A) Normalized absorption and PL spectra of TPS-N₃ in DMSO/water mixture ($v/v = 1/99$). (B) PL spectra of TPS-N₃ in DMSO/water mixtures with different water fractions (f_w).

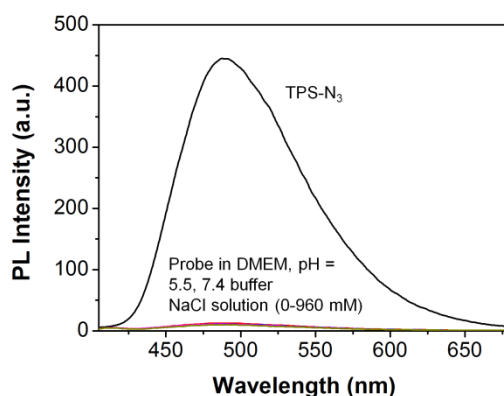


Figure S9. PL spectra of Probe 1 (10 μM) referenced against free TPS-N₃ in water containing [NaCl] ranging from 0, 250, 500, 960 mM, in cell culture medium (DMEM) or in buffer solutions with pH of 7.4 and 5.5 (DMSO/PBS buffer and DMSO/acetate buffer, $v/v = 1/99$), respectively.

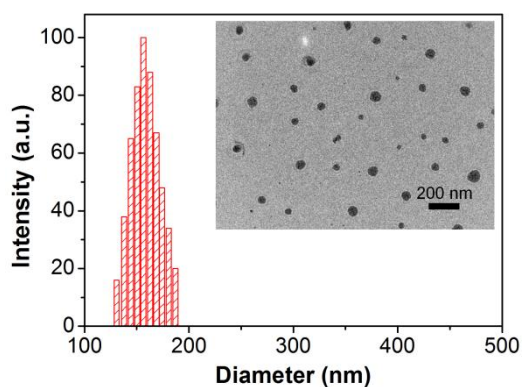


Figure S10. Hydrodynamic diameters obtained from LLS and TEM images for the TPS residue of Probe 1 in DMSO/PBS (v/v = 1/99) after treatment with caspase-8.

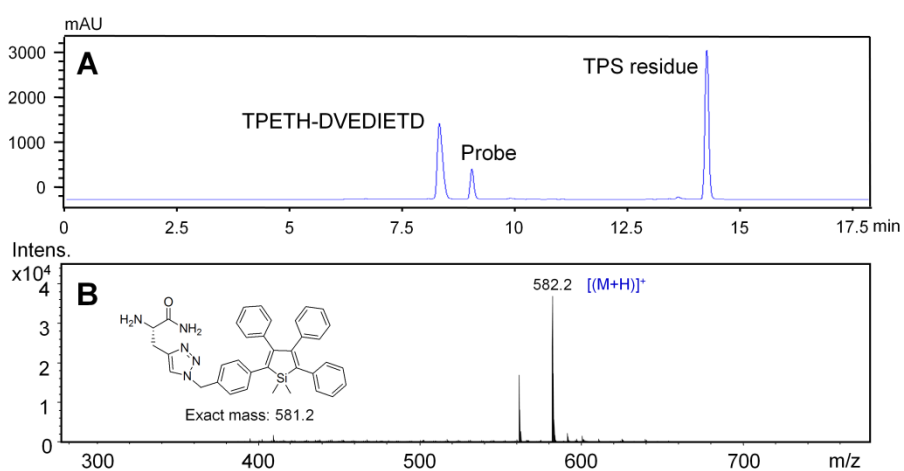


Figure S11. The caspase-8 catalyzed hydrolysis of Probe 1 (10 μM) monitored by reverse phase HPLC (A) and mass studies of the TPS residue (B).

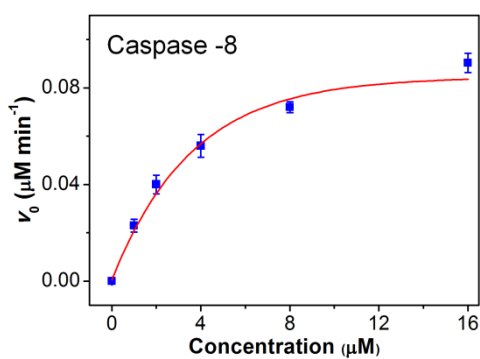


Figure S12. Michaelis-Menton plot for the hydrolysis of Probe 1 at different concentrations when incubated with caspase-8 (100 pM).

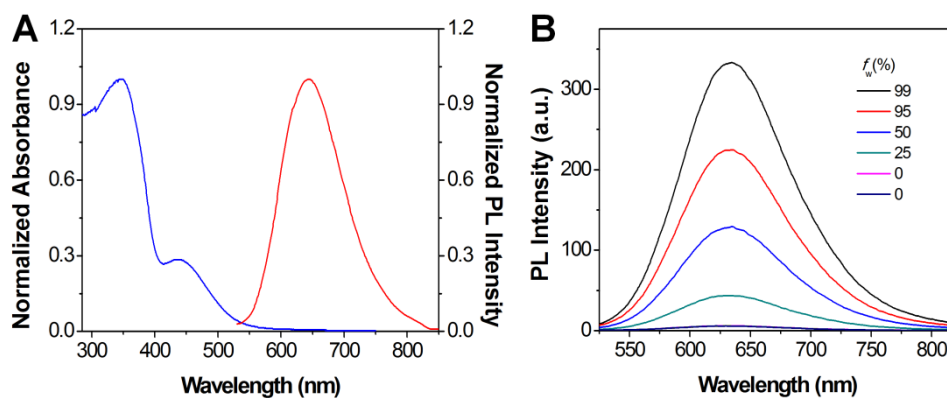


Figure S13. (A) Normalized absorption and PL spectra of TPETH-Mal in DMSO/water mixtures ($v/v = 1/99$); (B) PL spectra of TPETH-Mal in DMSO/water mixtures at different water fractions (f_w).

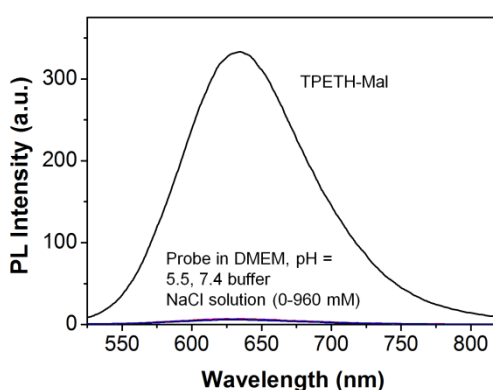


Figure S14. PL spectra of Probe 1 ($10 \mu\text{M}$) referenced against free TPETH-Mal in water containing $[\text{NaCl}]$ ranging from 0, 250, 500, 960 mM, in cell culture medium (DMEM) or in buffer solutions with pH of 7.4 and 5.5 (DMSO/PBS buffer and DMSO/acetate buffer, $v/v = 1/99$), respectively.

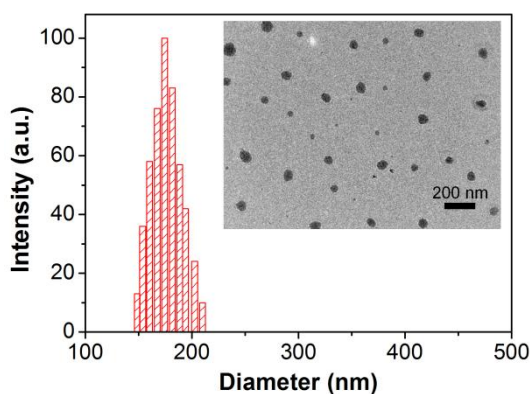


Figure S15. Hydrodynamic diameters obtained from LLS and TEM images for the TPETH residues of Probe 1 in DMSO/PBS ($v/v = 1/99$) after treatment with caspase-3.

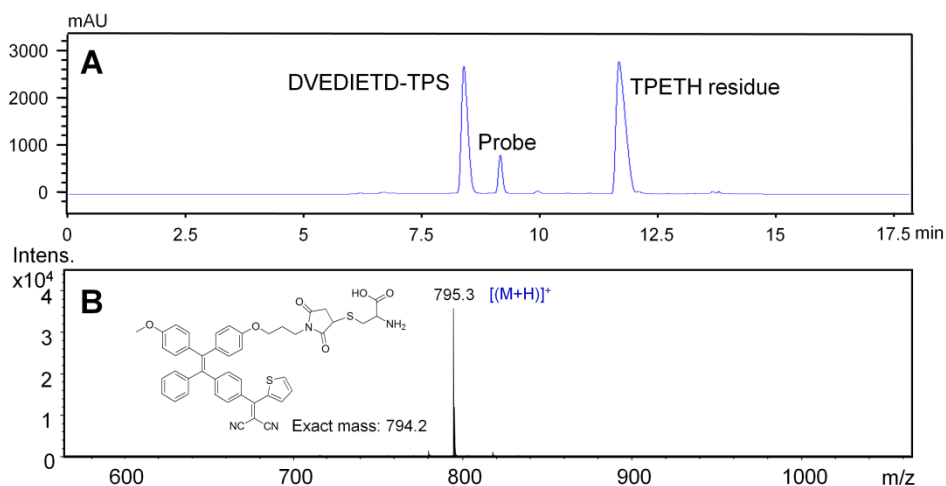


Figure S16. The caspase-3 catalyzed hydrolysis of Probe 1 (10 μM) monitored by reverse phase HPLC (A) and mass studies of the TPETH residue (B).

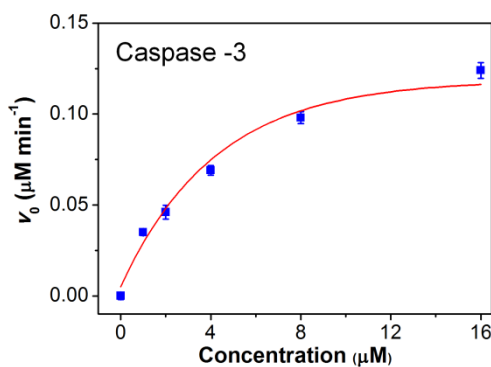


Figure S17. Michaelis-Menton plot for the hydrolysis of Probe 1 at different concentrations when incubated with caspase-3 (100 pM).

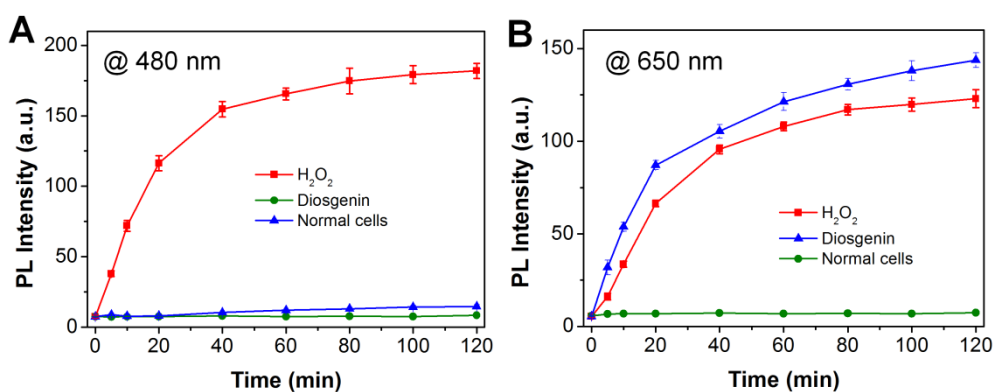


Figure S18. Time-dependent PL intensity changes of Probe 1 after incubation with the lysate of normal and apoptotic HeLa cells (induced by treating the cells with H_2O_2 (1.0 mM) or diosgenin (10 μM) for 1 h monitored at 480 nm (A) or 650 nm (B).

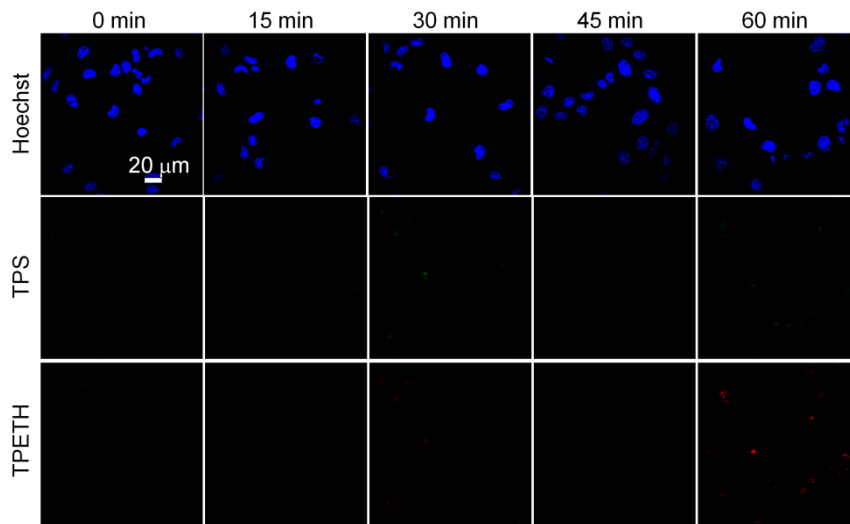


Figure S19. Confocal images of HeLa cells incubated with Probe 1 (10 μ M) for 2 h and further incubated in fresh medium for different time. Blue fluorescence (nucleus dyed with Hoechst 33342, E_x : 405 nm, E_m : 430-470 nm); Green fluorescence (TPS residue, E_x : 405 nm, E_m : 505-525 nm); Red fluorescence (TPETH residue, E_x : 405 nm, E_m : > 650 nm).

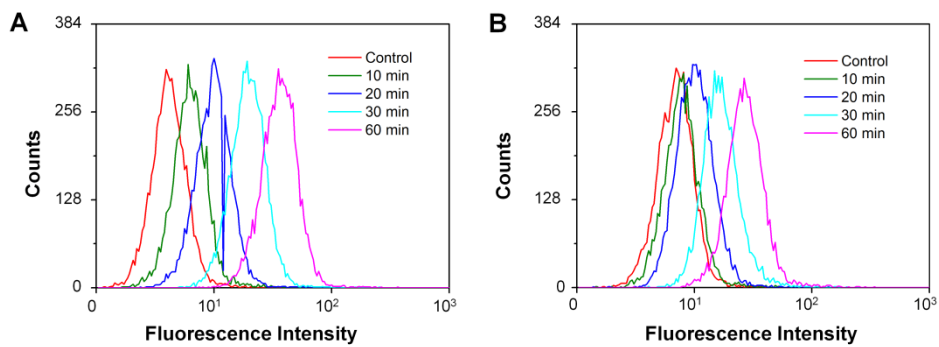


Figure S20. Flow cytometric analyses of TPS (A) and TPETH residue (B) fluorescence in Probe 1 pretreated HeLa cells for 2 h after further treatment with H_2O_2 (1.0 mM) for different time.

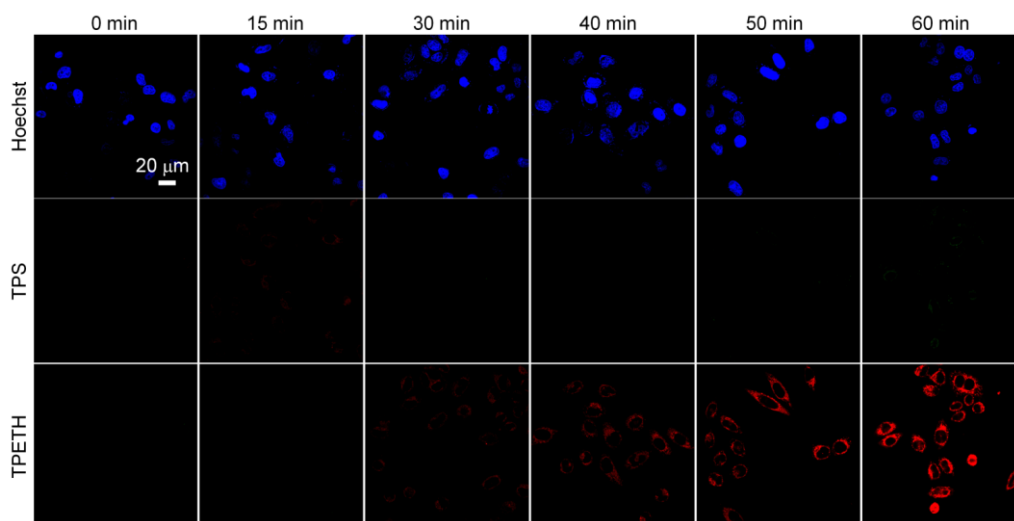


Figure S21. Confocal images of HeLa cells after treatment with Probe 1 (10 μM) for 2 h and further incubated with diosgenin for different durations. Blue fluorescence (nucleus dyed with Hoechst 33342, E_x : 405 nm, E_m : 430–470 nm); Green fluorescence (TPS residue, E_x : 405 nm; E_m : 505–525 nm); Red fluorescence (TPETH residue, E_x : 405 nm, E_m : > 650 nm nm).

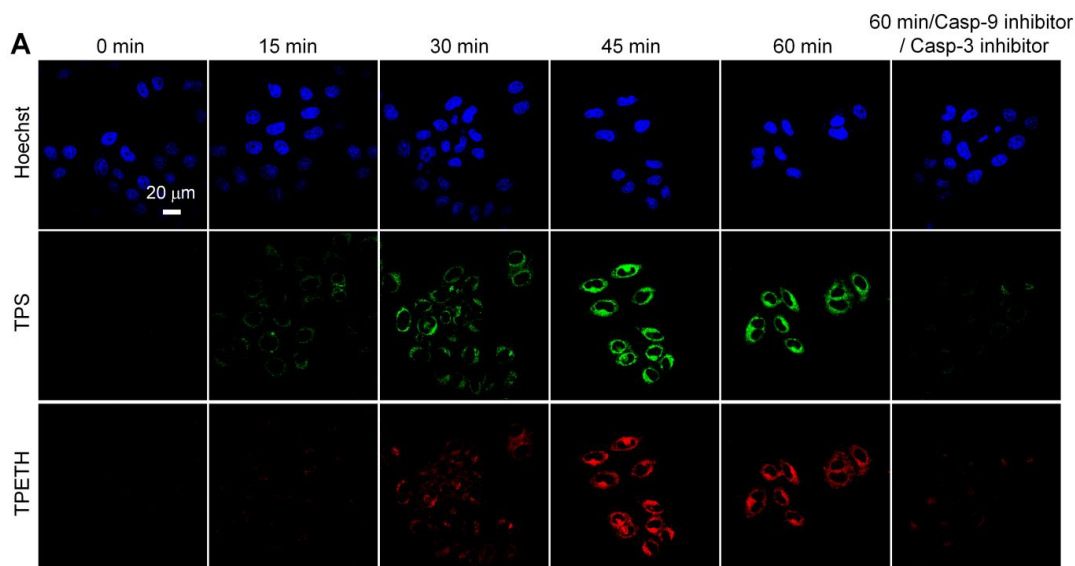


Figure S22. Confocal images of HeLa cells after treatment with Probe 2 (10 μM) for 2 h and further incubated with diosgenin (10 μM) for different durations or in the presence of caspase-9 and caspase-3 inhibitors (50 μM). Blue fluorescence (nucleus dyed with Hoechst 33342, E_x : 405 nm, E_m : 430–470 nm); Green fluorescence (TPS residue, E_x : 405 nm; E_m : 505–525 nm); Red fluorescence (TPETH residue, E_x : 405 nm, E_m : > 650 nm nm).

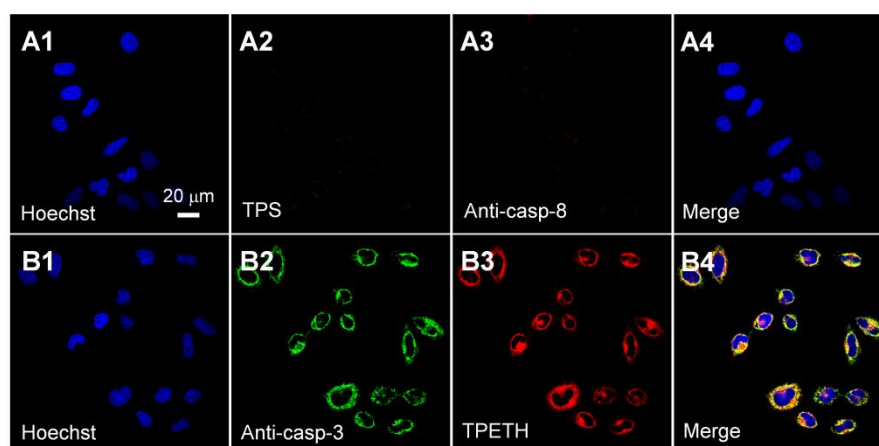


Figure S23. (A) Confocal images of diosgenin (10 μM) induced apoptotic HeLa cells pretreated with Probe 1 (10 μM) for 2 h and further stained with anti-casp-8 antibody and Texas Red-labelled secondary antibody. Blue fluorescence (nucleus dyed with Hoechst 33342, A1, E_x : 405 nm, E_m : 430–470 nm); Green fluorescence (TPS residue, A2, E_x : 405 nm; E_m : 505–525 nm); Red fluorescence (Texas Red, A3, E_x : 543 nm, E_m : 610–640 nm); A4 is the overlay of images A1–A3. (B) Confocal images of diosgenin induced apoptotic HeLa cells pretreated with Probe 1 (10 μM) for 2 h and further stained with anti-casp-3 antibody and Texas Red-labelled secondary antibody. Blue fluorescence (nucleus dyed with Hoechst 33342, B1, E_x : 405 nm, E_m : 430–470 nm); Green fluorescence

(Texas Red, B2, E_x : 543 nm, E_m : 610–640 nm); red fluorescence (TPETH residue, B3, E_x : 405 nm, E_m : > 650 nm); B4 is the overlay image of B1-B3. The fluorescence of Texas Red in B2 was artificially labeled with green color. Due to the low absorbance of TPETH residue at 543 nm, its spectral overlap with Texas Red is negligible. All images share the same scale bar (20 μ m).

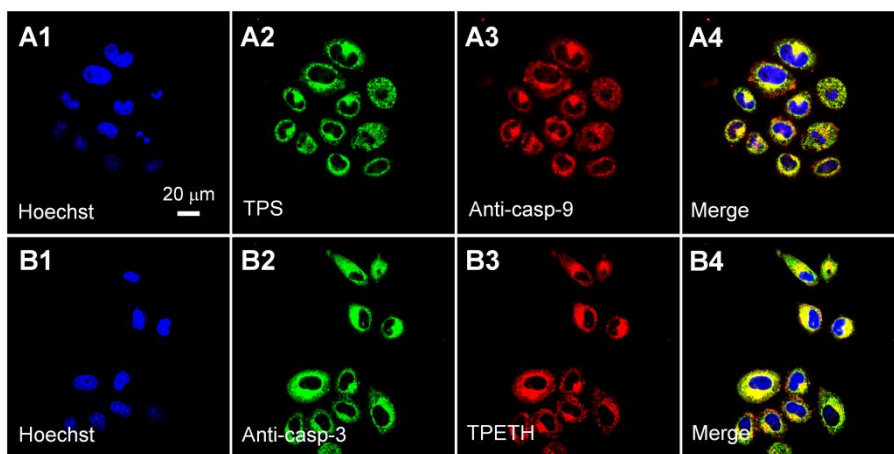


Figure S24. (A) Confocal images of diosgenin (10 μ M) induced apoptotic HeLa cells treated with Probe 2 (10 μ M) for 2 h and further stained with anti-casp-9 antibody and Texas Red-labelled secondary antibody. Blue fluorescence (nucleus dyed with Hoechst 33342, A1, E_x : 405 nm, E_m : 430-470 nm); Green fluorescence (TPS residue, A2, E_x : 405 nm; E_m : 505-525 nm); Red fluorescence (Texas Red, A3, E_x : 543 nm, E_m : 610–640 nm); A4 is the overlay of images A1-A3. (B) Confocal images of diosgenin (10 μ M) induced apoptotic HeLa cells treated with Probe 2 (10 μ M) for 2 h and further stained with anti-casp-3 antibody and Texas Red-labelled secondary antibody. Blue fluorescence (nucleus dyed with Hoechst 33342, B1, E_x : 405 nm, E_m : 430-470 nm); Green fluorescence (Texas Red, B2, E_x : 543 nm, E_m : 610–640 nm); red fluorescence (TPETH residue, B3, E_x : 405 nm, E_m : > 650 nm); B4 is the overlay image of B1-B3. The fluorescence of Texas Red in B2 was artificially labeled with green color. Due to the low absorbance of TPETH residue at 543 nm, its spectral overlap with Texas Red is negligible. All images share the same scale bar (20 μ m).

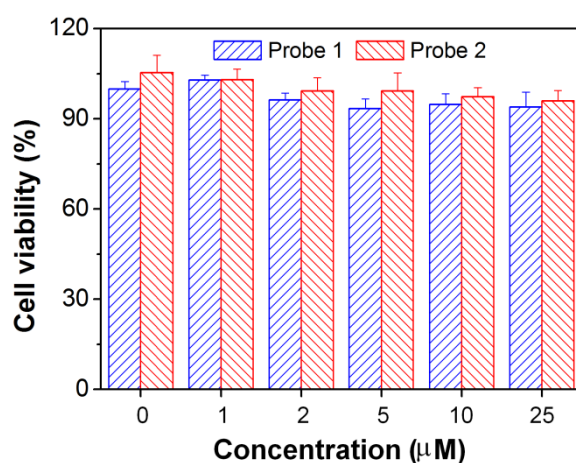


Figure S25. The viability of HeLa cells upon incubation with Probe 1 or Probe 2 at different concentrations for 48 h. Data represent mean values \pm standard deviation, n = 3.

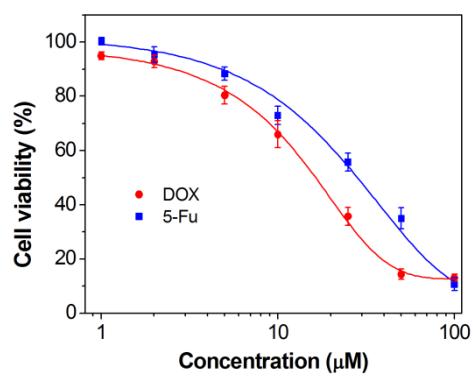


Figure S26. The viability of HeLa cells upon treatment with DOX or 5-Fu at different concentrations for 24 h. Data represent mean values \pm standard deviation, n = 3.

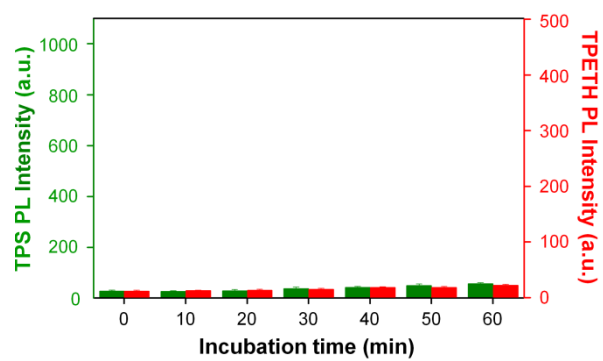


Figure S27. Time-dependent PL intensity changes of TPS (green) and TPETH (red) residues in Probe 1 (10 μ M) pre-treated HeLa cells for 2 h and further incubated in fresh medium for different time. Data represent mean values \pm standard deviation, n = 3.