Supporting Information

Fluorescein propiolate: A propiolate-decorated fluorescent probe with remarkable selectivity towards cysteine

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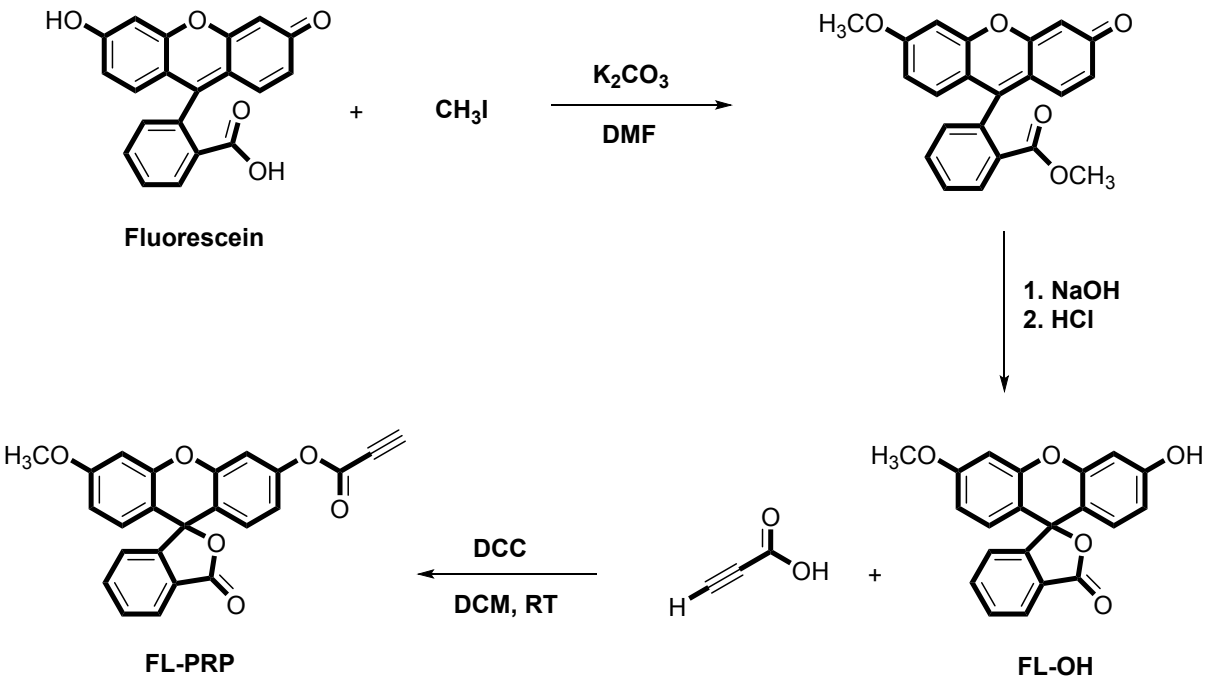
1.General Methods

All reagents were purchased from commercial suppliers (Aldrich and Merck) and used without further purification. ^{1}H NMR and ^{13}C NMR were measured on a Varian VNMRJ 600 Nuclear Magnetic Resonance Spectrometer. Mass analysis were conducted with Tandem Gold Triple Quadrupole LC-MS/MS device. UV absorption spectra was obtained on Shimadzu UV-2550 Spectrophotometer. Fluorescence emission spectra was obtained using Varian Cary Eclipse Fluorescence spectrophotometer. Cell imaging was performed with Zeiss Axio fluorescence microscope. Samples were contained in 10.0 mm path length quartz cuvettes (2.0 mL volume). Upon excitation at 460 nm, the emission spectra were integrated over the range 480 nm to 700 nm (Both excitation and emission slit width 5 nm / 5 nm).

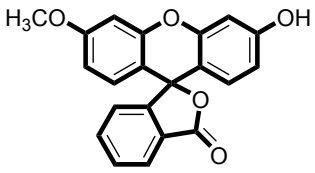
2. Synthesis of Probe Molecule

The synthesis pathway for **FL-PRP** was shown in Scheme 1. **FL-OH** was synthesized by using literature procedure.[1]



Scheme 1: Synthesis pathway of **FL-PRP**

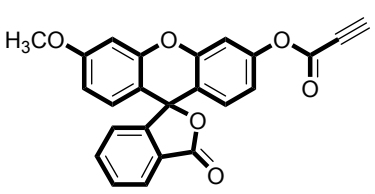
Synthesis of FL-OH



FL-OH

CH_3I (2.84 g, 20 mmol) was added to the mixture of fluorescein (3.32 g, 10 mmol) and K_2CO_3 (2.77 g, 20 mmol) in DMF (10 mL) at room temperature. After stirring for 24 hours, the reaction mixture was diluted with H_2O (150 mL), the resulting precipitate was filtered, washed with water, the filtrate was extracted with ethyl acetate, the organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The combined residue was subjected to silica gel chromatography with CH_2Cl_2/CH_3OH (50:1) to give a yellow solid (91 %). 10% aqueous solution of NaOH (10 mL, 25 mmol) was added to the solution of resulting compound (3.60 g, 10 mmol) in CH_3OH (36 mL) at 30 °C. After stirring for 5 hours, CH_3OH was evaporated and the reaction mixture was diluted with H_2O (100 mL). The solution was acidified to pH 5 with 1 M HCl, the resulting precipitate was filtered, washed with water and dried under vacuum to give a pale-yellow solid **FL-OH** (69 %) which was used for next synthesis without further purification.

Synthesis of FL-PRP



FL-PRP

To a solution of **FL-OH** (346 mg, 1 mmol) and propiolic acid (190 µL, 3 mmol) in dry DCM (10 ml) was added DCC (206 mg. 1 mmol). The reaction mixture stirred for 1h at room temperature under Argon atmosphere. After completion of reaction, solvent was removed under vacuum and the resulting residue extracted 3 times with DCM. The organic layer was dried over $MgSO_4$ filtered and concentrated. The resultant residue was purified by column chromatography (eluent: DCM) to effort **FL-PRP** as a white solid. (179 mg, 45 % yield). ^{1}H NMR (400 MHz , $CDCl_3$) δ = 8.03 (d, J = 7.8 Hz, 1 H), 7.68 (t, J =7.2 Hz, 1 H), 7.63 (t, J =7.2 Hz, 1 H), 7.17 (d, J = 7.8 Hz, 1 H), 7.14 (s, 1H), 6,86-6.83 (m, 2H), 6.78 (s, 1H), 6.71-6.69 (m, 1H), 6.64-6.63 (m, 1H), 3.84 (s, 3 H), 3.13 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.9, 164.2, 155.6, 154.8, 154.5, 153.5, 152.9, 137.9, 132.6, 132.0, 131.7, 129.1, 127.8, 126.6, 120.3, 119.6, 114.8, 113.4, 112.8, 103.5, 84.9, 80.1, 76.6, 58.3. MS (LC-MS): m/z Calcd. For $C_{24}H_{14}O_6$: 398.1 Found: 399.1 $[M+H]^+$

Reference

1. J. Zhang, Y.-Q. Sun, J. Liu and W. Guo, *Chem Commun*., 2013, **49**, 11305.

3.Determination of Detection Limit

The detection limit was calculated based on the fluorescence titration. To determine the detection limit, the emission intensity of **FL-PRP (**10.0 μM) without Cysteine was measured by 10 times and standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and Cysteine concentration could be obtained in the 0,1 – 0,9 μM (R = 0.976). The detection limit is then calculated with the equation: detection limit = 3σbi/m, where σbi is the Standard deviation of blank measurements; m is the slope between intensity versus sample concentration. The detection limit was measured to be 182 nM.

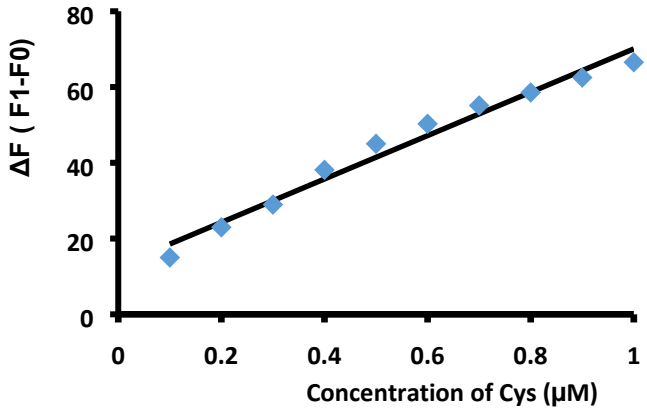


Figure S1. Fluorescence changes of **FL-PRP** (10.0 μM) upon addition of Cys (0.1 to 0.9 μM) in 6:4 CH_3CN: PBS buffer at pH 7.0 (λ_{ex}:460 nm, at 25 °C).

4. Kinetic Studies

The reactions of **FL-PRP** (20 μM) with Cysteine, Homocysteine and Glutathione (5 equiv.) in 6:4 CH_3CN: PBS buffer were monitored by the fluorescence intensity at 460 nm at 25 °C. The pseudo-first-order rate constant for the reaction was determined by the following equation:

$$\ln [(Fmax-Ft)/Fmax] = -k' t$$

where Ft and Fmax are the fluorescence intensities at 460 nm at time t and the maximum value obtained after the reaction was complete, respectively, and k′ is the pseudo-first-order rate constant. The plot for the reactions between the **FL-PRP** and Cysteine, Homocysteine and Glutathione (5 equiv.) are shown in Figure S4. The negative slope of the line represents the pseudo-first-order rate constant.

5. Cell Studies and Cell viability Assay

A549 Human Lung Adeno carcinoma cell lines were grown in DMEM supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5 % CO_2 at 37 ^oC. The cells were plated on 12 mm cover glasses in 6-well plate and allowed to grow for 24h. Before the experiments, cells were treated with 2 mM N-methylmaleimide (NMM). After 30 min of NMM-treatment at 37 ^oC, cells were washed with PBS three times and then the cells were incubated **FL-PRP** (5 μM) for 20 min at 37 ^oC then washed with PBS three times. After incubating with Cysteine (10 μM) for 20 min at 37 ^oC, cells were rinsed with PBS three times, and DAPI for 10 min at 37^oC then washed with PBS three times. Then, the fluorescence images were acquired through a Zeiss Axio fluorescence microscope.

Cell viability assay (MTT assay): Cells were incubated with **FL-PRP** for 0.5, 1, 2, 12 and 24 hours and cytotoxic effects were determined by tetrazolium (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) based colorimetric assay. At the end of incubation periods, medium of the cells were removed and cells were washed by pre-warmed phosphate buffered saline (PBS) to remove any trace of compounds and to prevent colour interferance during optical density (OD) determination. MTT solution (0.5 mg/mL in PBS) was added into each well and incubated for 3.5 hours. After the incubation time plates were centrifuged at 1800 rpm for 10 minute at room temperatures to avoid accidental removal of formazan crystals. Crystals were dissolved with 100 μL DMSO. The absorbance was determined at 540 nm. Results were represented as percentage viability and calculated by the following formula:

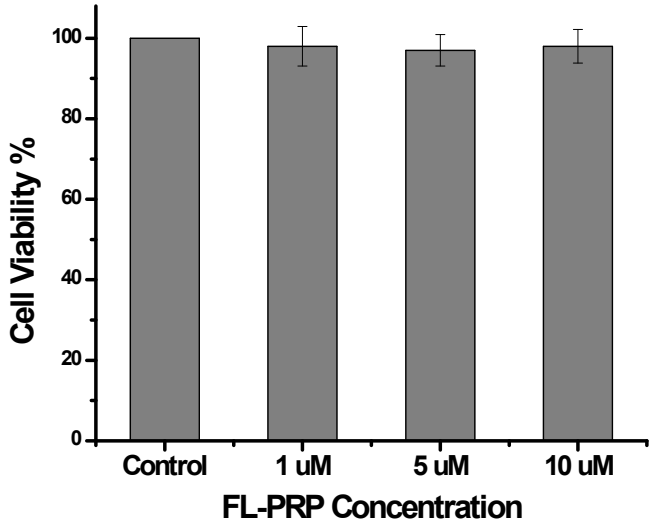


Figure S2: % of cell viability of A-549 cells incubated with (1-10 μM) **FL-PRP** for 24 h.

6. Emission and Absorption Spectra of FL-PRP

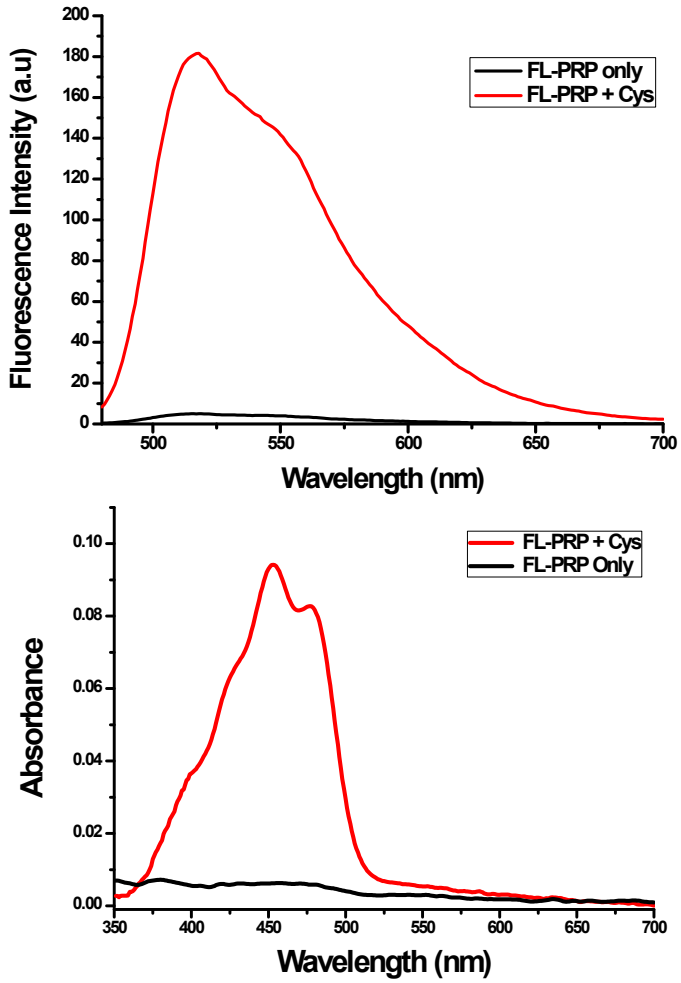


Figure. S3. Emission **and** Absorption spectra of **FL-PRP** (10 μM) and Cys (10 equiv.) in 6:4 CH_3CN: PBS buffer at pH 7.4 (λ_{ex}:460 nm, at 25 °C).

7. Time-dependent Fluorescence Change of FL-PRP

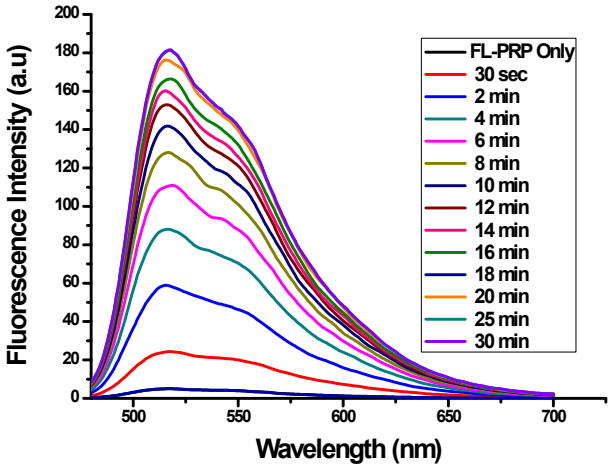
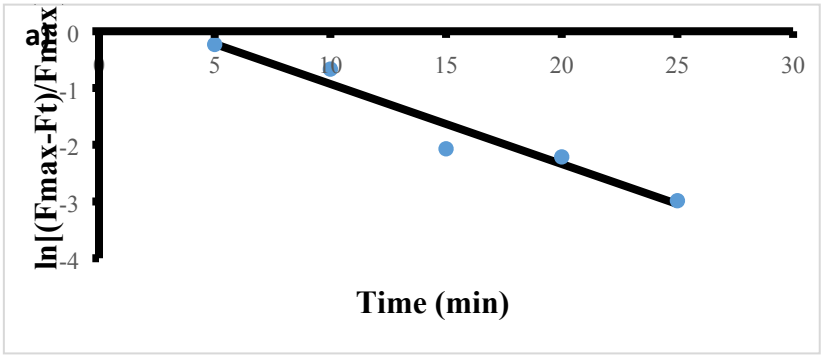
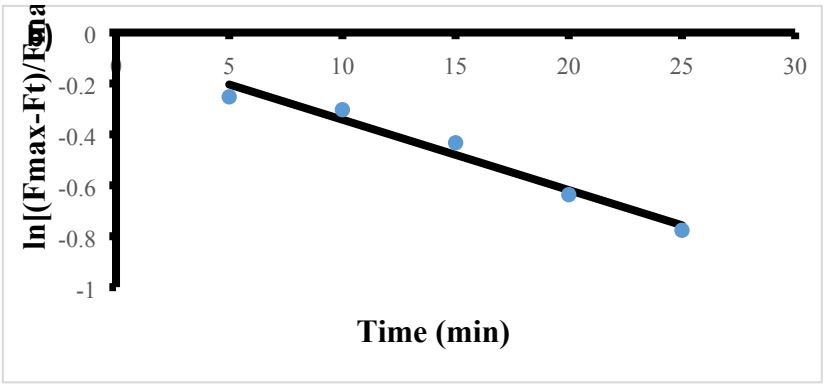


Figure S4. Time-dependent fluorescence change of **FL-PRP** (10 μM) and Cys (10 equiv.) in 6:4 CH_3CN: PBS buffer at pH 7.4 (λ_{ex}:460 nm, at 25 °C).





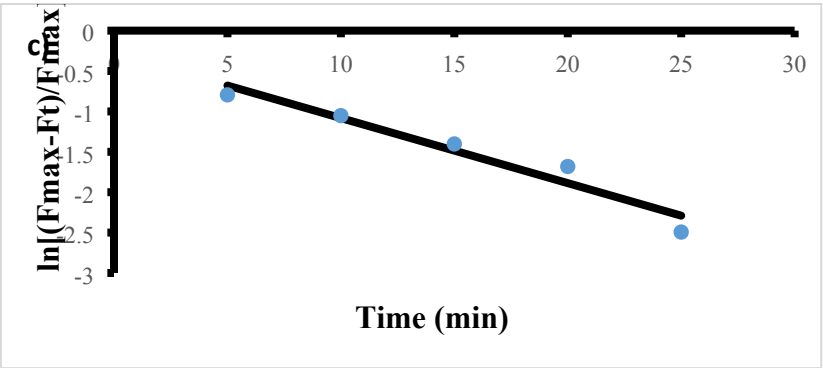


Figure S5. A Pseudo-first-order kinetic plots of the reaction between **FL-PRP** (10 μM) in the presence of 5.0 equivalent of **a)** Cysteine, **b)** Homocysteine and **c)** Glutathione measured in 6:4 CH_3CN: PBS buffer at pH 7.4 (λ_{ex}: 460 nm, at 25 °C).

8. Fluorescence Titration of FL-PRP

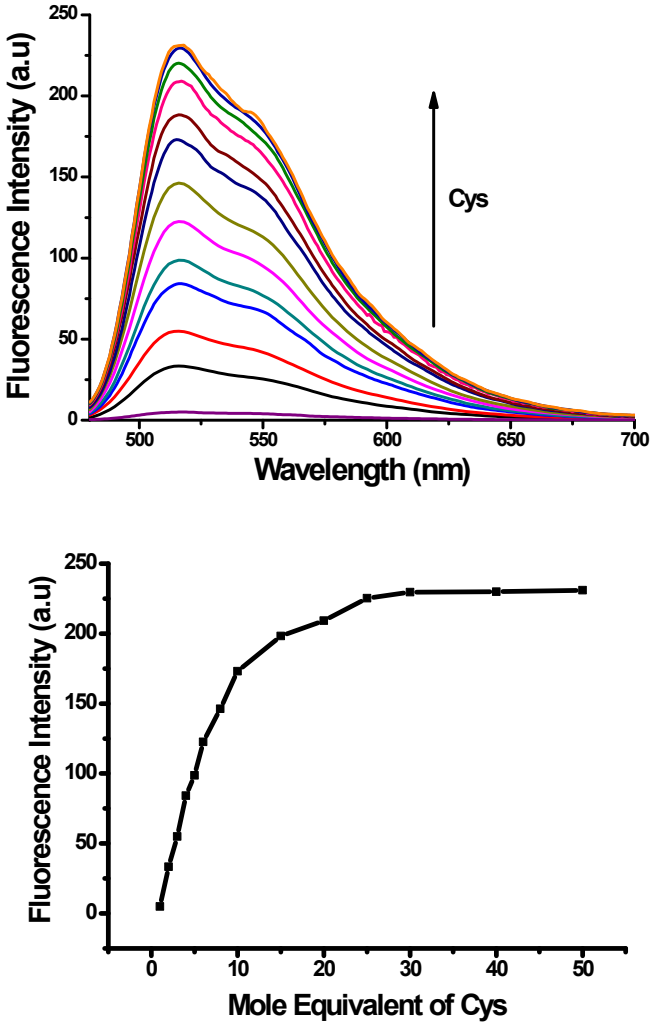


Figure S6. Fluorescence spectra of **FL-PRP** (10μM) in 6:4 CH_3CN: PBS buffer at pH 7.4 (λ_{ex}: 460 nm, at 25 °C) in the presence of Cys (mole equivalents 1-50)

9. Fluorescence Response of FL-PRP with Cysteine and other Biologically Active Compounds

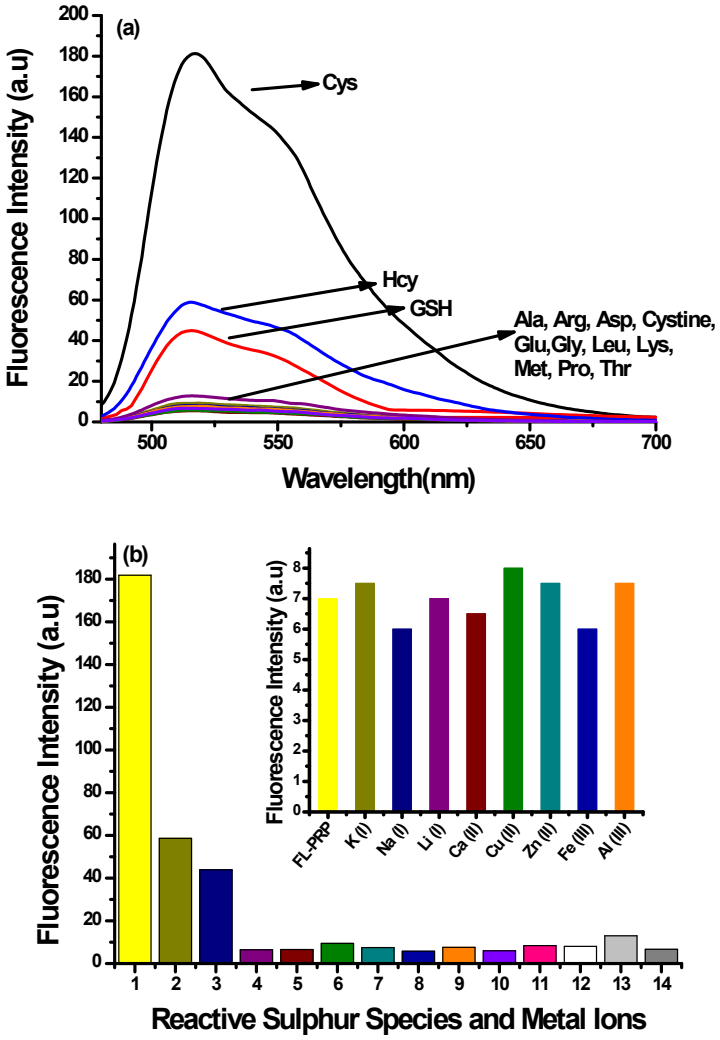


Figure S7. Fluorescence intensities of **FL-PRP** (10 μM) in CH_3CN: PBS buffer (10 mM, pH 7.4, 6:4 v/v) in the presence of reactive sulphur species and other amino acids (10 equiv.), (λ_{ex}:460 nm). 1, Cys; 2, Hcy; 3, GSH; 4, Ala; 5, Arg; 6, Asp; 7, Cystine; 8, Glu; 9, Gly; 10, Leu; 11, Lys; 12, Met; 13, Pro; 14, H_2S (source: Na_2S). Inset: Fluorescence intensities of **FL-PRP** (10 μM) in the presence of metal ions (10 equiv.).

10. The Fluorescence Response of FL-PRP in the presence of Cysteine and Other Amino Acids

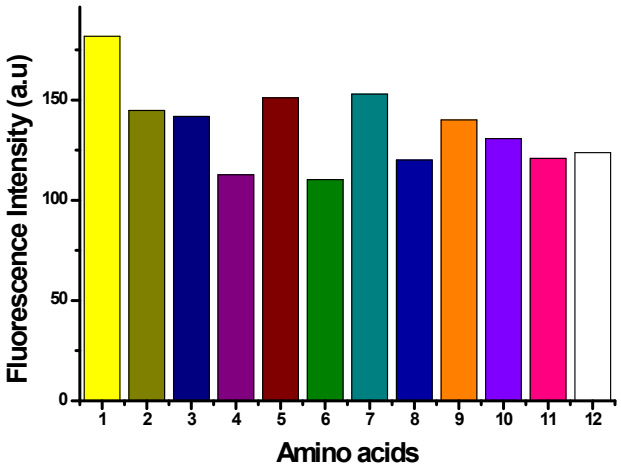


Figure S8. Fluorescence intensities of **FL-PRP** (10μM) in 6:4 CH_3CN: PBS buffer in the presence of 50 equiv. amino acids interests and 10 equiv. Cys (λ_{ex}: 460 nm). 1, Cys; 2, Ala; 3, Arg; 4, Asp; 5, Cystine; 6, Glu; 7, Gly; 8, Leu; 9, Lys; 10, Met; 11, Pro; 12, H_2S.

11. Effect of pH

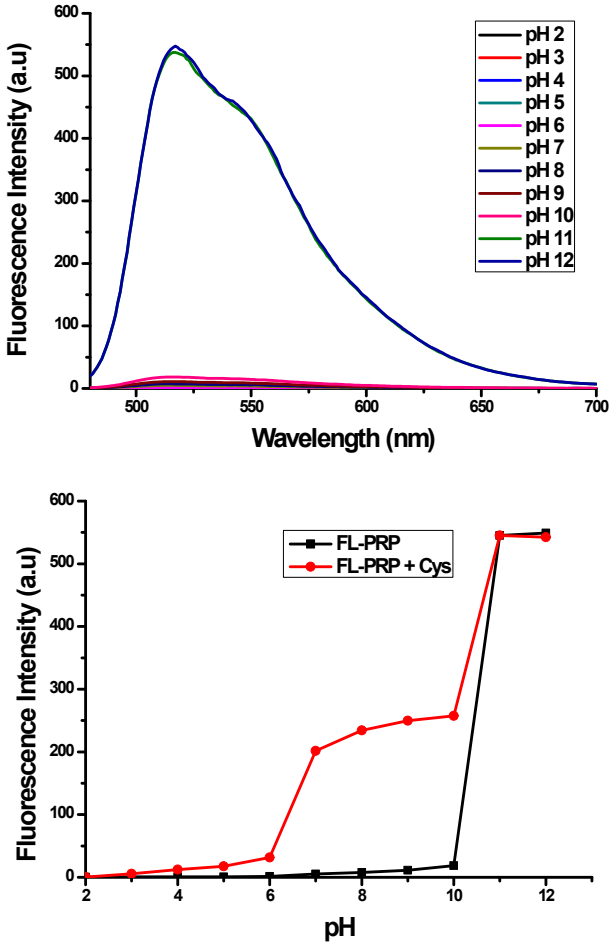
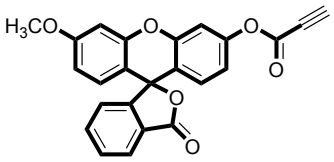


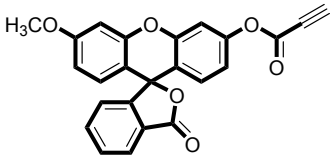
Figure S9. Effect of pH on the fluorescence intensity of **FL-PRP** (10μM) in 6:4 CH_3CN: PBS buffer in the absence and presence of Cys (20 equiv.) (λ_{ex}:460 nm).

^{1}H NMR of FL-PRP



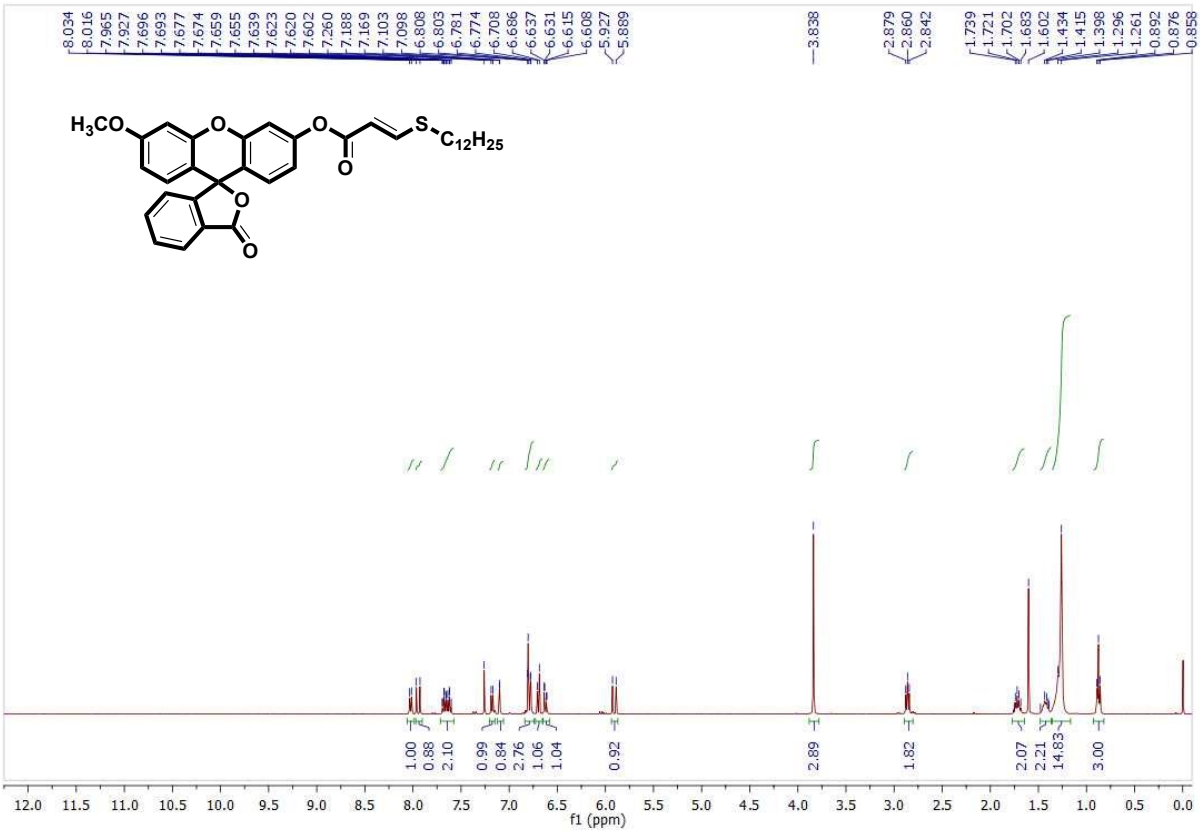
FL-PRP

^{13}C NMR of FL-PRP

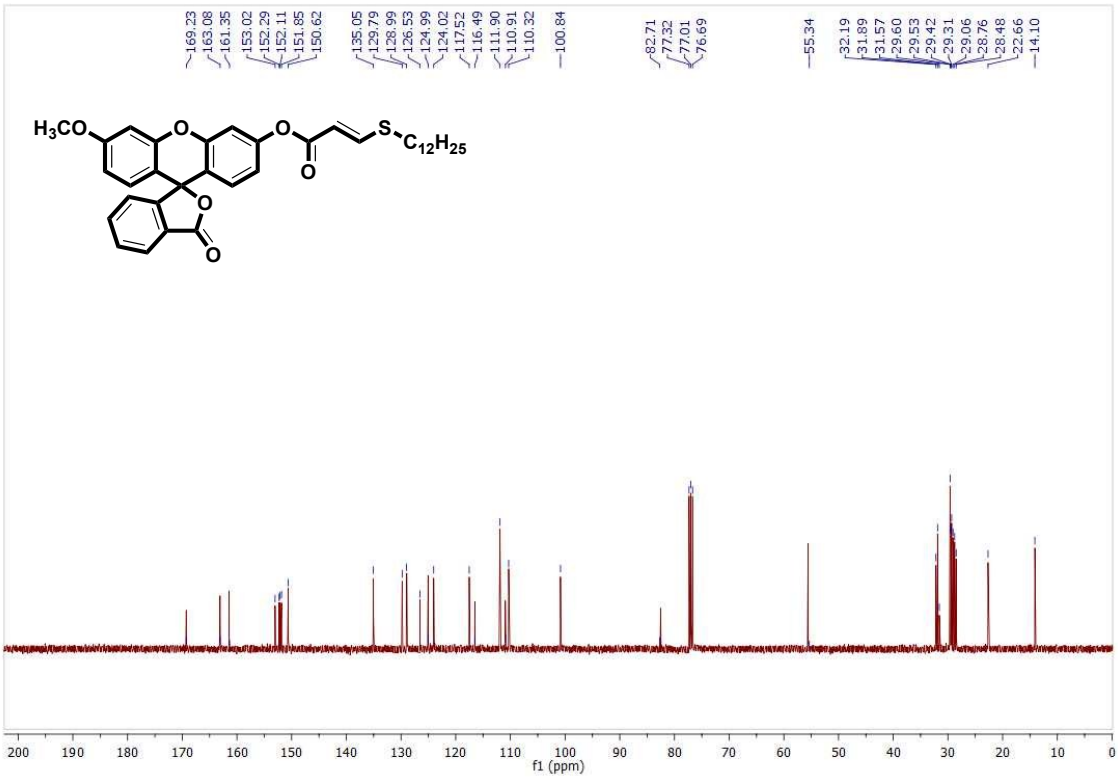


FL-PRP

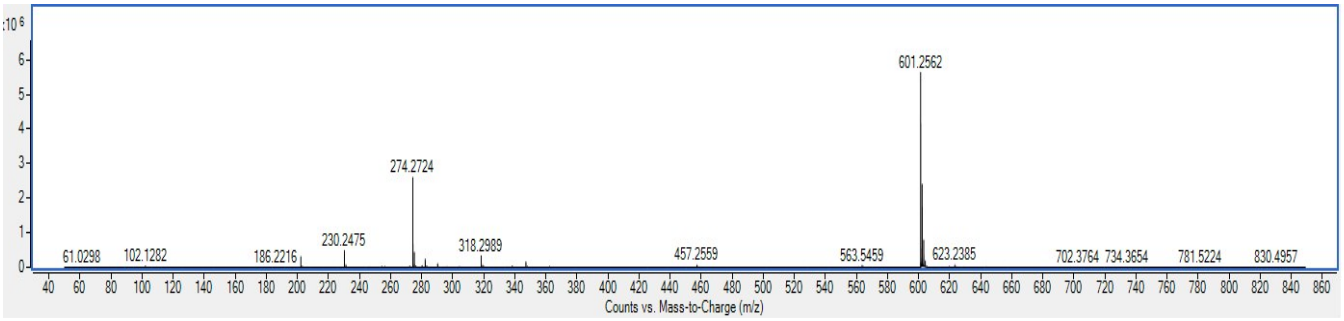
^{1}H NMR of FL-Dodec



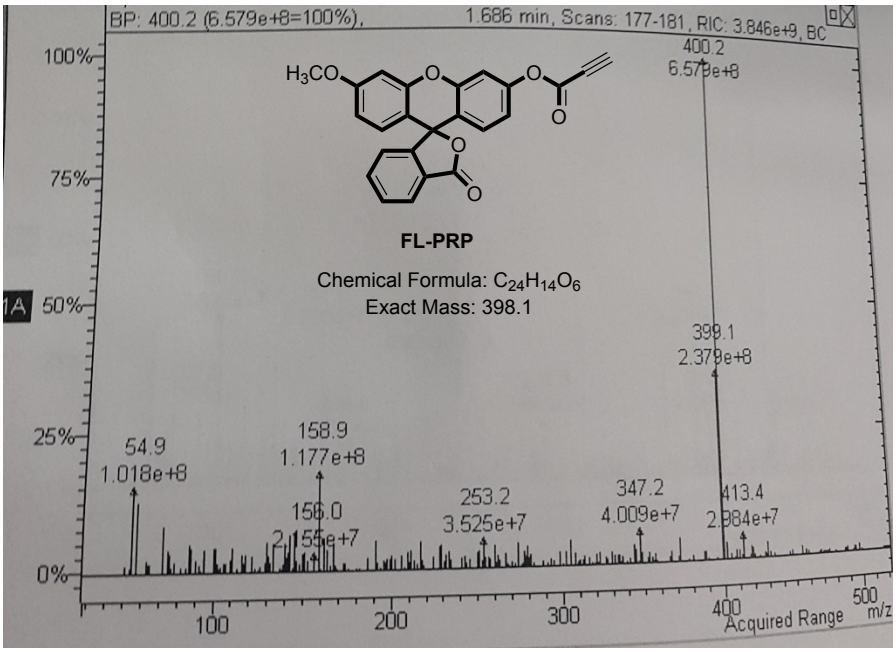
^{13}C NMR of Dodec



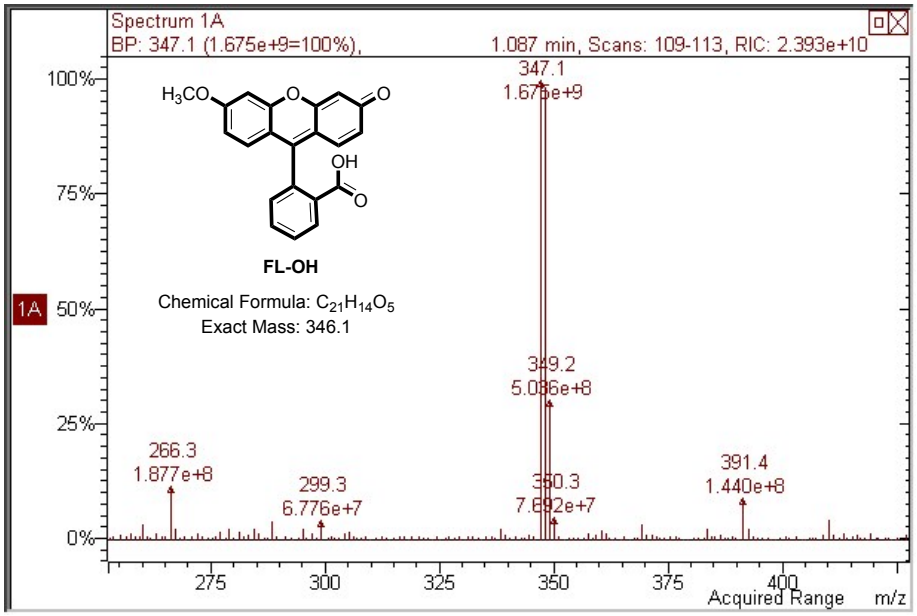
Synthesis of FL-Dodec: To a solution of **FL-PRP** in CH_3CN (2 ml) was added and dodecanethiol (1,2 eq.). The reaction mixture was stirred for 30 min. at room temperature under argon atmosphere. After completion of reaction, solvent was removed under vacuum and the resulting residue was extracted with DCM. The organic layer was dried over $MgSO_4$ filtered and concentrated. The resultant residue was purified by column chromatography (eluent: DCM) to effort **FL-Dodec**. ^{1}H NMR (400 MHz , $CDCl_3$) δ = 8.03 (d, *J* = 7.4 Hz, 1 H), 7.95 (d, *J* =15.1 Hz, 1H), 7.65 (ddt, *J* =14.7, 8.3, 4.2 Hz, 2H), 7.18 (d, *J* = 7.4 Hz, 1H), 7.09 (m, 1H), 6,81-6.77 (m, 3H), 6.71-6.69 (m, 1H), 6.62 (dd, *J* = 8.8, 2.5 Hz, 1H), 5.91 (d, *J* =15.1 Hz, 1 H), 3.84 (s, 3H), 2.86 (t, *J* =7.4 Hz, 2H), 1.75-1.68 (m, 2H) 1.48- 1.39 (m, 2H), 1.30-1.26 (m, 16H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 169.2, 163.1, 161.4, 153.0, 152.3, 152.1, 151.9, 150.6, 135.1, 129.8, 129.0, 126.5, 125.0, 124.0, 117.5, 116.5, 111.9, 110.9, 110.3, 100.8, 82.7, 55.3, 32.2, 31.9, 31.6, 29.6, 29.5, 29.4, 29.3, 29.1, 28.8, 28.5, 22.7, 14.1. HRMS: m/z: Calcd. for ($C_{36}H_{40}O_6S$) [M+H$^+$]: 601.2579; found, 601.2562.



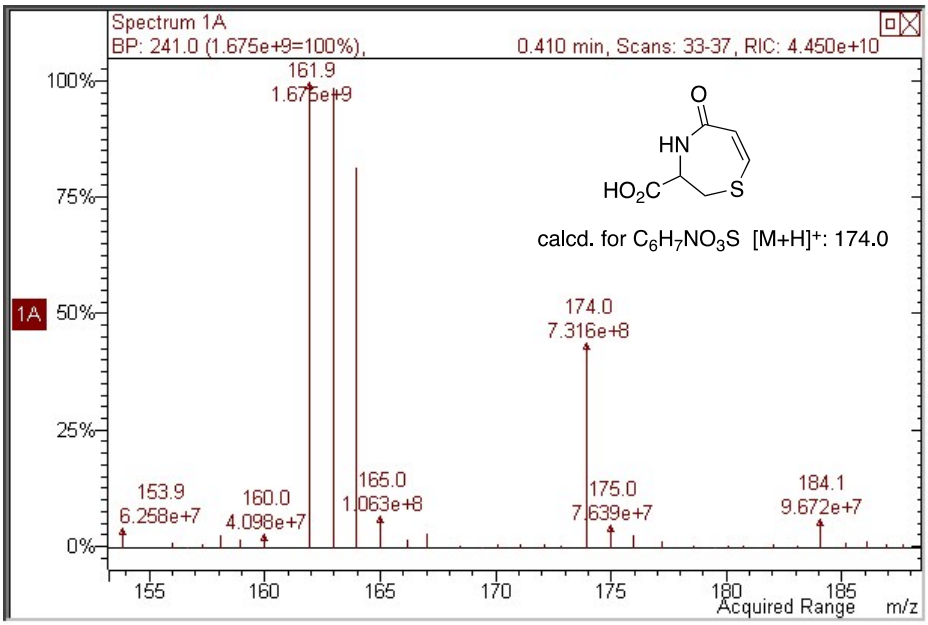
Mass spectra of **FL-Dodec**



Mass spectra of **FL-PRP**

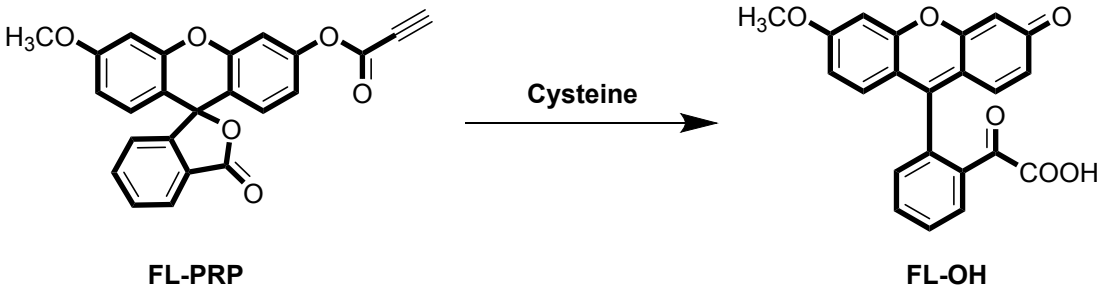


Mass spectra of **FL-OH** in the crude reaction mixture (**FL-PRP** + Cysteine (5 eq.))



Mass spectra of the cyclization product in the crude reaction mixture (**FL-PRP** + Cysteine (5 eq.))

TLC Image of the Hydrolysis Reaction of FL-PRP Mediated with Cysteine



FL-PRP **FL-OH**

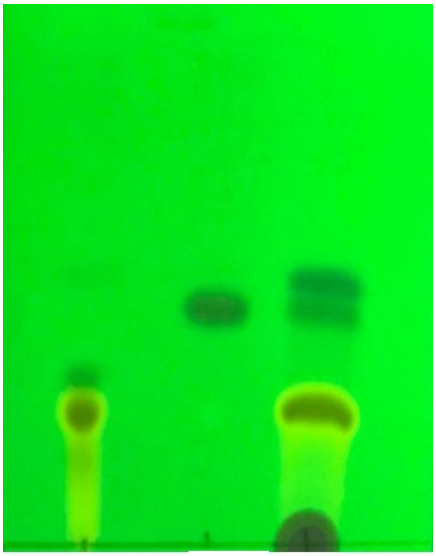


Figure S10. TLC image of the hydrolysis reaction of **FL-PRP** mediated with cysteine