Supporting Information for "Selective Turn-on Fluorescent Probes for Homocysteine and Their Bioimaging Applications"

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Experimental Section

General Methods. Unless otherwise noted, materials were obtained from Aldrich and were used without further purification. Melting points were measured using a Büchi 530 melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker 30 0 MHz or Varian 500 MHz. Chemical shifts are given in ppm and coupling constants (J) in Hz. UV absorption spectra were obtained on UVIKON 933 Double Beam UV/VIS S pectrometer. Fluorescence emission spectra were obtained using RF-5301/PC Spectrofl uorophotometer (Shimadzu)

Synthesis of P-Hcy-1. A solution of 1-Hydroxypyrene-2-carbaldehyde (50 mg, 0.2 mm ol) in 10 mL of dichloroethane containing anhydrous K_2CO_3 (0.11 g, 0.8 mmol) was stir red at room temperature for 10 min. Propinoyl chloride (0.028 g, 0.3 mmol) was then dr opwise using a syringe. The mixture was stirred at reflux 1h to complete the reaction. T he mixture was filtered and the filtrate was concentrated in vauo giving a residue that w as subjected to column chromatography (silica) using CH_2Cl_2 as eluent to give pure **P-H cy-1** (0.058 g, 94%); mp 142˚C (dec.); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.31 (t, J = 7.5 Hz, 3H), 3.05 (q, J = 7.5Hz, 2H), 8.19-8.44 (m, 7H), 8.83 (s, 1H), 10.47 (s, 1H); ^{13}C N MR (125 MHz, DMSO-d_6) δ 191.53, 174.14, 144.84, 132.09, 131.97, 130.02, 129.30, 1 28.97, 128.89, 128.22, 128.12, 127.56, 127.08, 126.83, 125.86, 124.49, 123.88, 121.36, 27.48, 9.51; FAB-MS m/z =303.1021 $[M+H]^{+}$, calcd for $C_{20}H_{14}O_3$ =302.0943.

Synthesis of P-Hcy-2. To a solution of 1-hydroxypyrene-2-carbaldehyde (0.2 g, 0.82 m mol) and dry triethylamine (1.2 mL, 8.2 mmol) in dry CH_2Cl_2 (20 mL) under nitrogen at 0˚C was added acryloyl chloride (0.66 mL, 8.2 mmol) dropwise. The mixture was stirre d for 4 h at room temperature andconcentrated in vacuo. The residue was subjected to si lica gel column chromatography using hexane/CH_2Cl_2 (v/v 8:2) as eluent to obtain yello w powder of **P-Hcy-2** (0.054g, 22%); mp 138˚C (dec.); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.35 (dd, J = 9.0 and 6.0 Hz, 1H), 6.71-6.75 (m, 2H), 8.14-8.24 (m, 2H), 8.28-8.44 (m, 5H), 8.84 (s, 1H), 10.40 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 190.96, 165.29, 14 3.87, 135.27, 131.80, 131.63, 129.99, 129.17, 128.76, 128.72, 127.90, 127.78, 127.64, 1

27.52, 126.95, 126.65, 125.49, 124.06, 123.56, 120.69; FAB-MS m/z =300.0786 $[M+H]^+$, calcd for $C_{20}H_{12}O_3$ =300.0786.

Fluorescence Studies. Stock solutions (0.01 M) of Hcy, Cys and GSH were prepared in distilled water. Stock solutions of the probes (1 mM) were also prepared in DMSO. Test solutions were prepared by placing 30 μL of the probe stock solution into a test tube, dil uting the solution to 3 mL with HEPES buffer (0.01M, pH 7.4), and adding an appropria te aliquot of each substrate containing stock solution. For all measurements, the excitati on wavelength was 350 nm for **P-Hcy-1** and 376 nm for **P-Hcy-2**. Fluorescence Spectra were measured 10 min after addition of **P-Hcy-1** and **P-Hcy-2**.

Fluorescence detection of intracellular Hcy using Hcy probe. HeLa cells were cultur ed in Dulbecco's modified Eagle's medium (DMEM, Invitrogen), supplemented with 1 0% (v/v) fetal bovine serum (Gibco), 100 units/mL penicillin (Gibco) and 100 μg/mL st reptomycin (Gibco) in a humidified incubator with 5% CO_2 at 37 ^oC. The cells were stai ned with 60 μM Hcy probe (**P-Hcy-1** or **P-Hcy-2**) for 20 min to detect intracellular Hcy . On the other hand, HeLa cells were incubated with 20 μM Hcy for 30 min, washed wit h PBS to remove excess Hcy, and then stained with 60 μM Hcy probe for 20 min. In ad dition, Hcy-treated cells were incubated with 500 μM NEM for 20 min and stained with 60 μM Hcy probe for 20 min after washing with PBS. The stained cells were imaged usi ng confocal microscopy (LSM 510 META, Carl Zeiss) (Hcy probes; λ_{ex} = 405 nm, λ_{em} = 420 – 480 nm). To test whether Hcy probes detect intracellular Cys, HeLa cells were pre-treated with 500 μM NEM for 20 min to remove the endogenous thiol containing m olecules. After washing with PBS, the cells were further incubated with 20 μM Cys for 20 min and stained with either 60 μM Hcy probe or 20 μM Cys probe for 20 min. The tr eated cells were analyzed by confocal microscopy (Cys probe; λ_{ex} = 633 nm, λ_{em} = 650 nm). To test whether Hcy probe detects intracellular GSH, HeLa cells were incubated w ith 250 μM α-lipoic acid for 48 h to induce generation of GSH in cells. After washing w ith PBS, the cells were treated with either 60 μM Hcy probe or 20 μM thiol probe. The t reated cells were analyzed by confocal microscopy (thiol probe; λ_{ex} = 488 nm, λ_{em} = 50 5 – 530 nm).

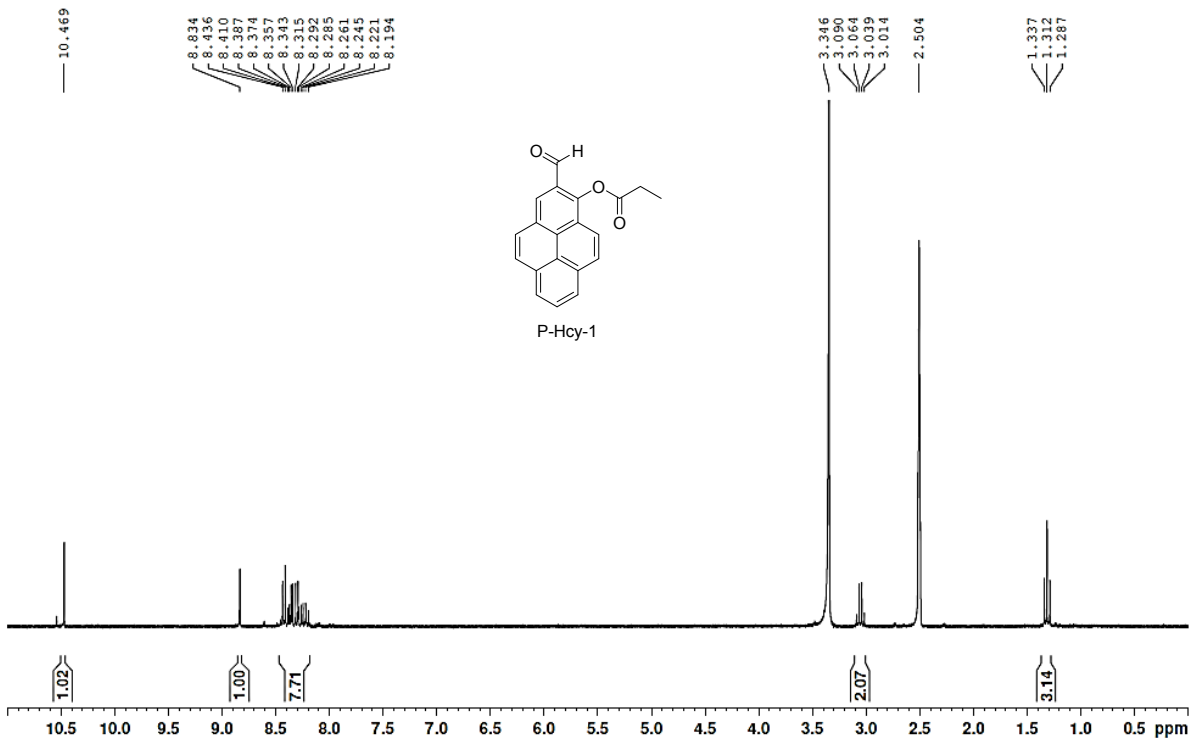


Fig S1. ^{1}H NMR (DMSO-d_6, 300 MHz) spectrum of **P-Hcy-1**.

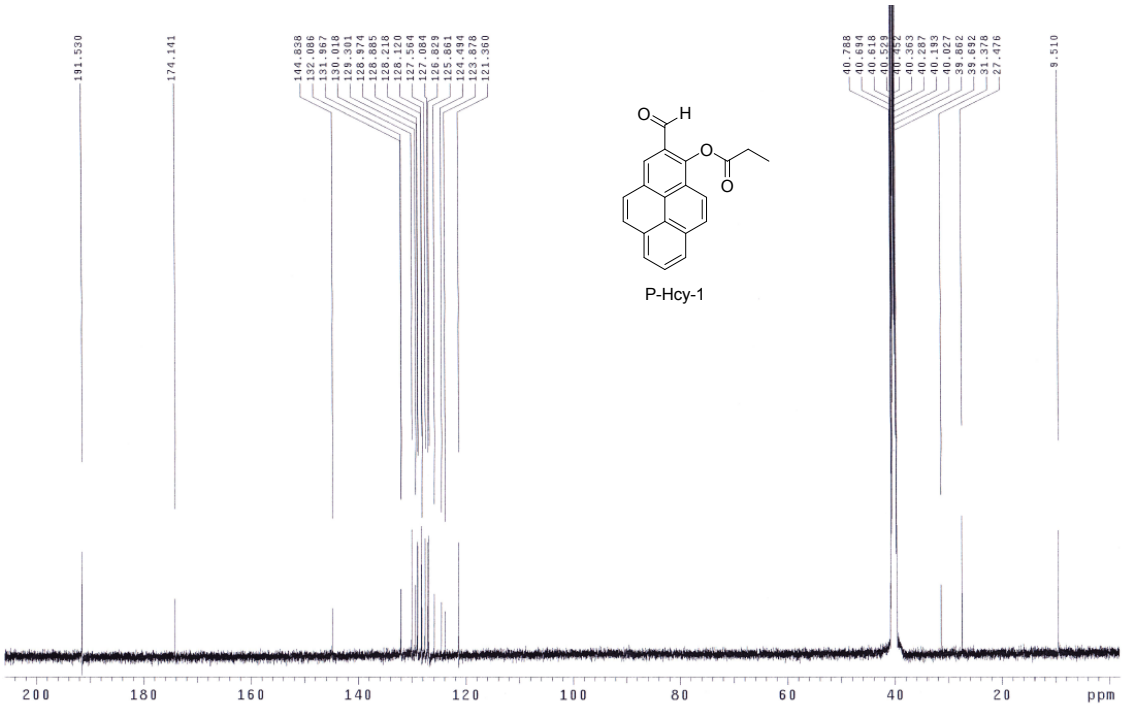


Fig S2. ^{13}C NMR (DMSO-d_6, 125 MHz) spectrum of **P-Hcy-1**.

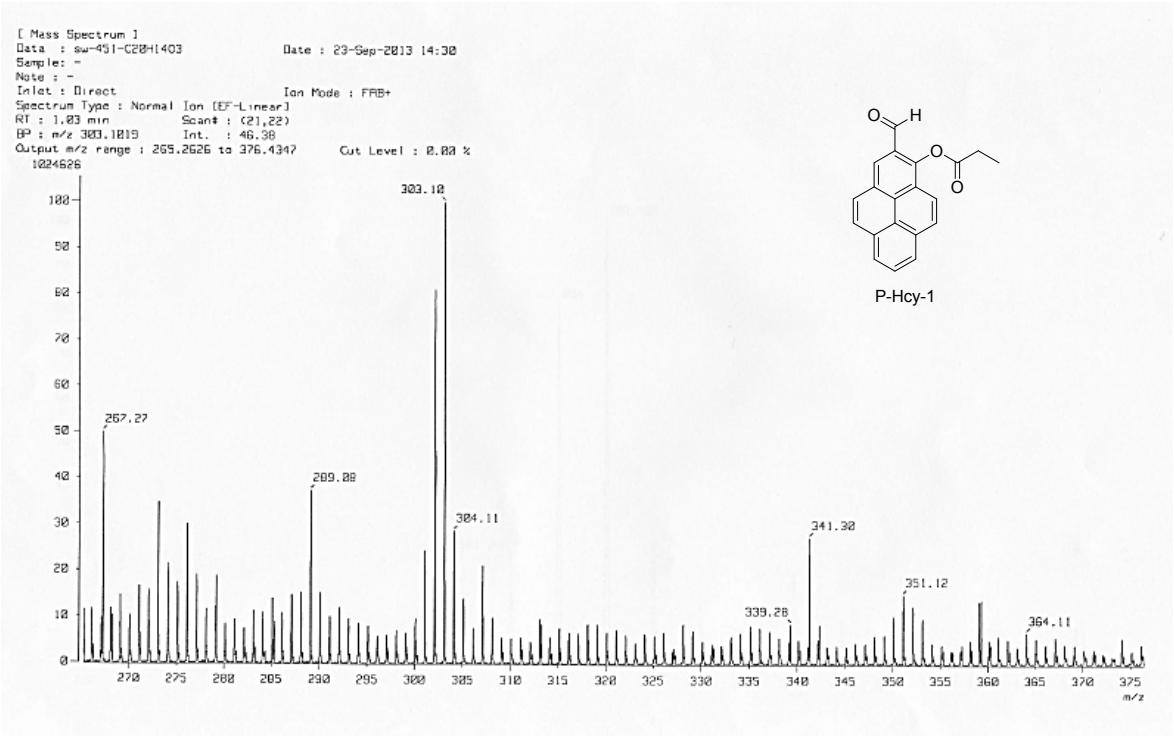


Fig S3. The FAB mass spectrum of **P-Hcy-1**.

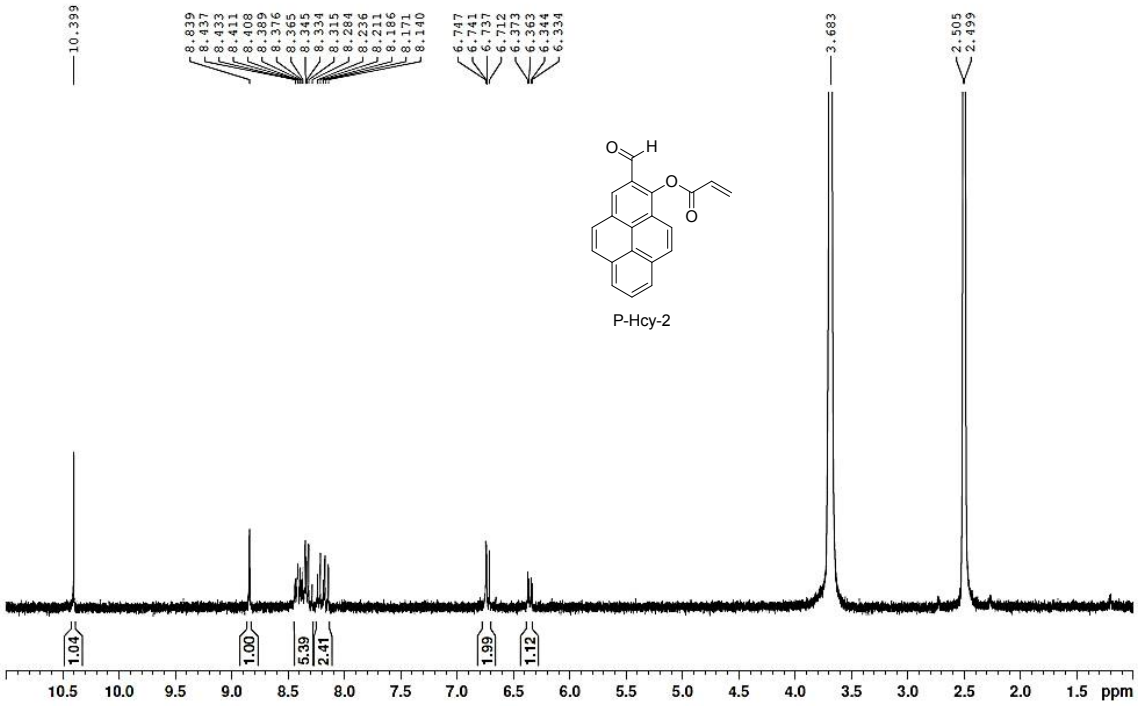


Fig S4. ^{1}H NMR (DMSO-d_6, 300 MHz) spectrum of **P-Hcy-2**.

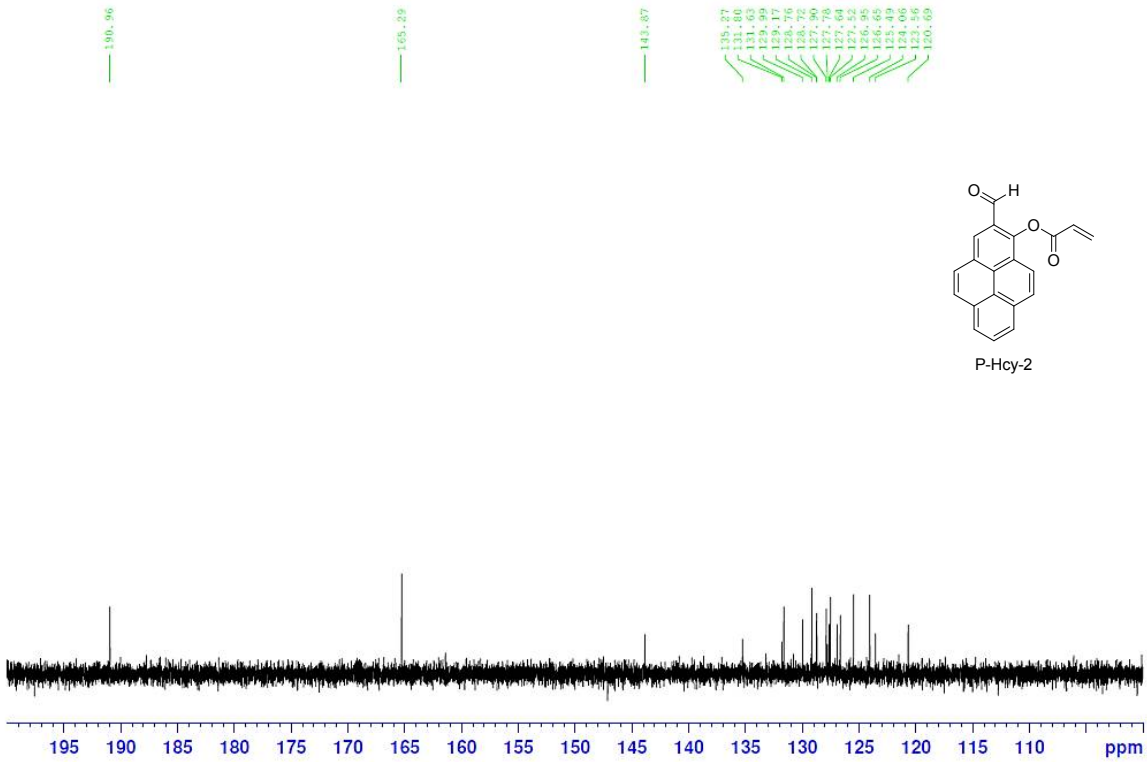


Fig S5. ^{13}C NMR (DMSO-d_6, 125 MHz) spectrum of **P-Hcy-2**.

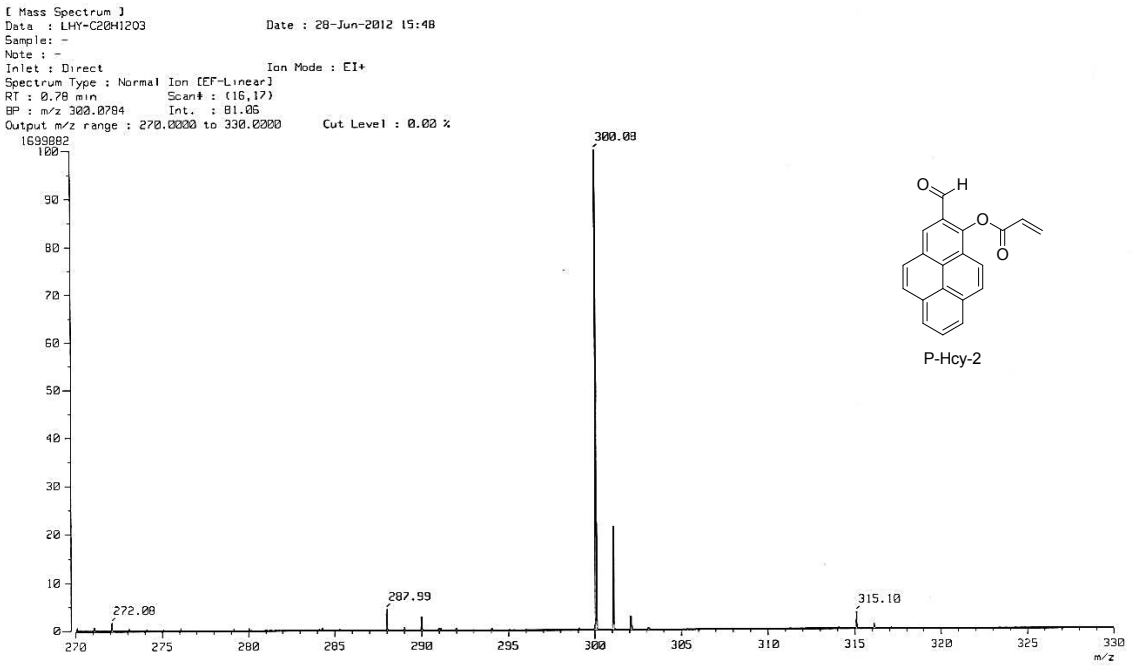


Fig S6. The FAB mass spectrum of **P-Hcy-2**.

Table S1. Detection limits, dissociation constants and response times of reported Hcy p robes, **P-Hcy-1** and **P-Hcy-2**.

	Detection limit	Dissociation constant (Kd)	Response time
P-Hcy-1	1.94×10^{-6} M		10min
P-Hcy-2	1.44×10^{-7} M		5min
Ref. 8(a)			4min
Ref. 8(b)		$3.23 (\pm 0.20) \times 10^{-4}$ M	
Ref. 8(c)	4.5×10^{-4} M		
Ref. 8(d)			20min
Ref. 8(e)		$6.05 (\pm 1.16) \times 10^{-4}$ M	
Ref. 8(f)	42 nM		10min

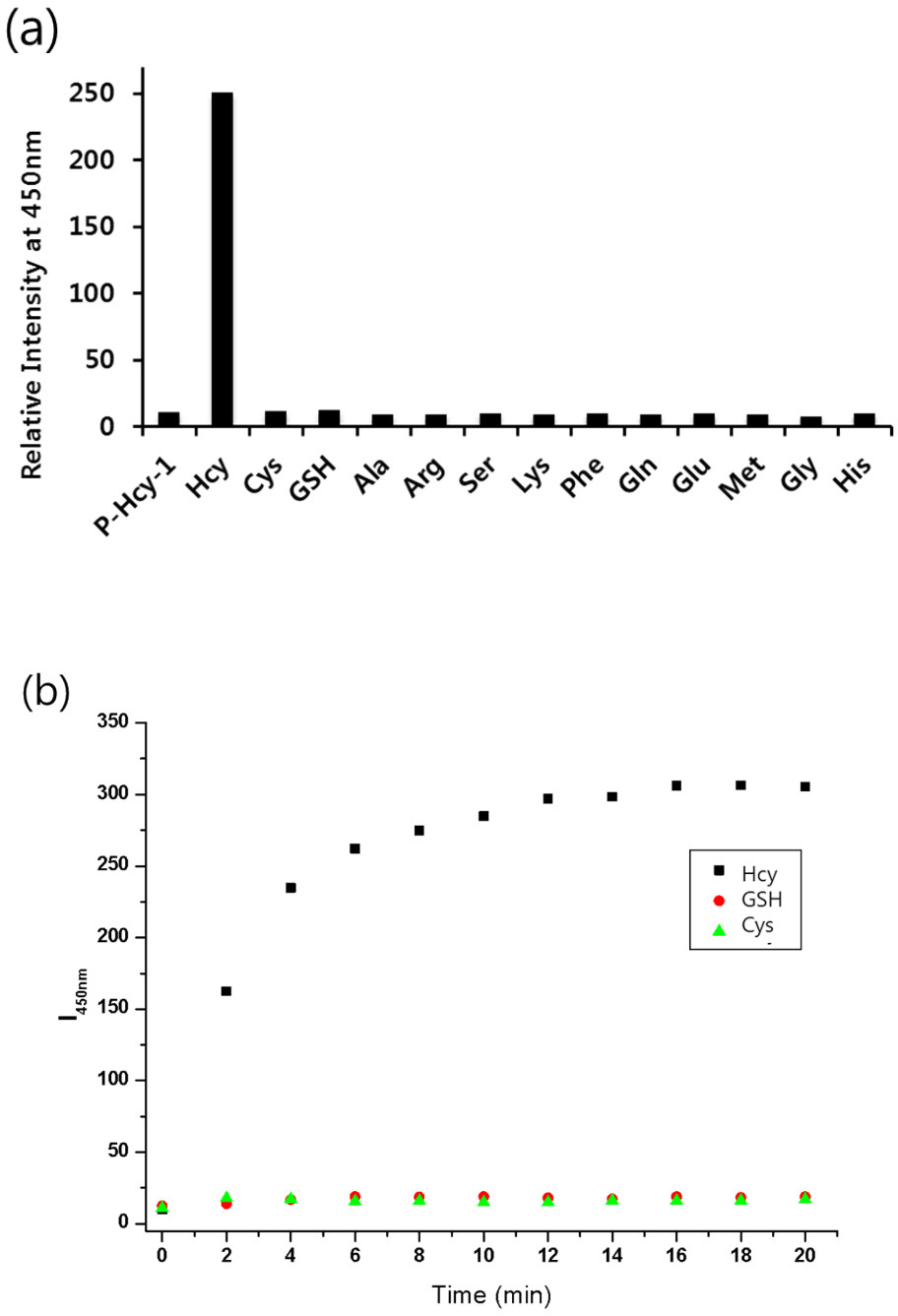


Fig S7. Selective response of **P-Hcy-1** to Hcy. (a) Relative fluorescence intensities of **P-Hcy-1** (10 μM) upon addition of 10 equiv. of various amino acids or GSH in HEPES buffer at 450nm. (b) Time-dependent change of **P-Hcy-1** (10 μM) with the addition of 10 equiv. of Hcy, Cys and GSH in HEPES (0.01M, pH 7.4) containing 10% DMSO (excitation wavelength: 350 nm).

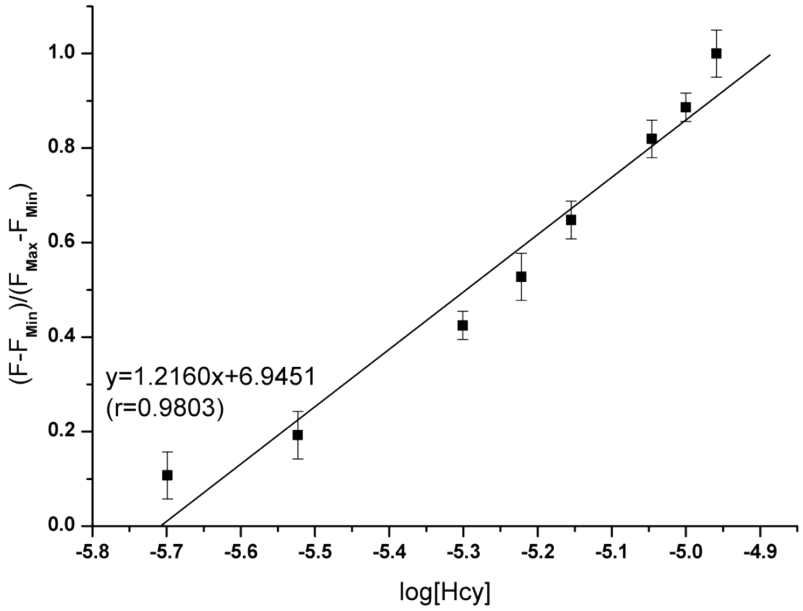


Fig S8. Normalized fluorescence responses of **P-Hcy-1** (1 μM) to changing Hcy concentrations in DMSO-HEPES (0.01M, pH 7.4) (1:9, v/v). (Detection limit = 1.94×10^{-6} M).

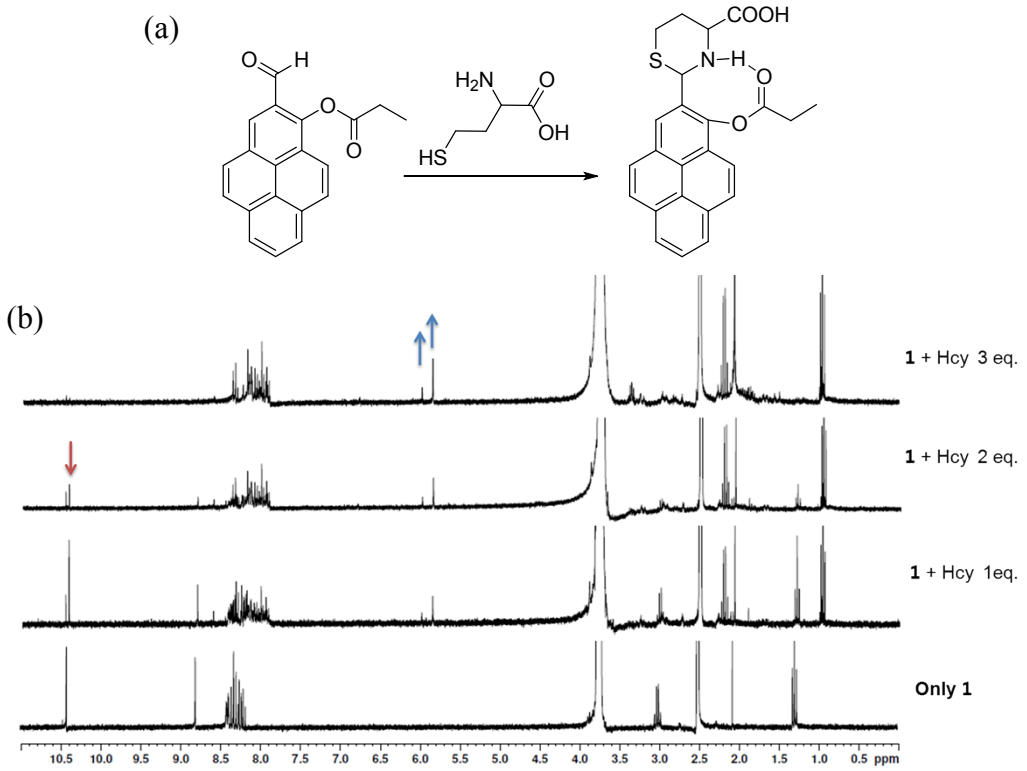


Fig S9. (a) Possible mechanism for the reaction between **P-Hcy-1** and Hcy. (b) ^{1}H NMR spectral change of **P-Hcy-1** upon addition of Hcy in DMSO-d_6:D_2O (9:1, v/v).

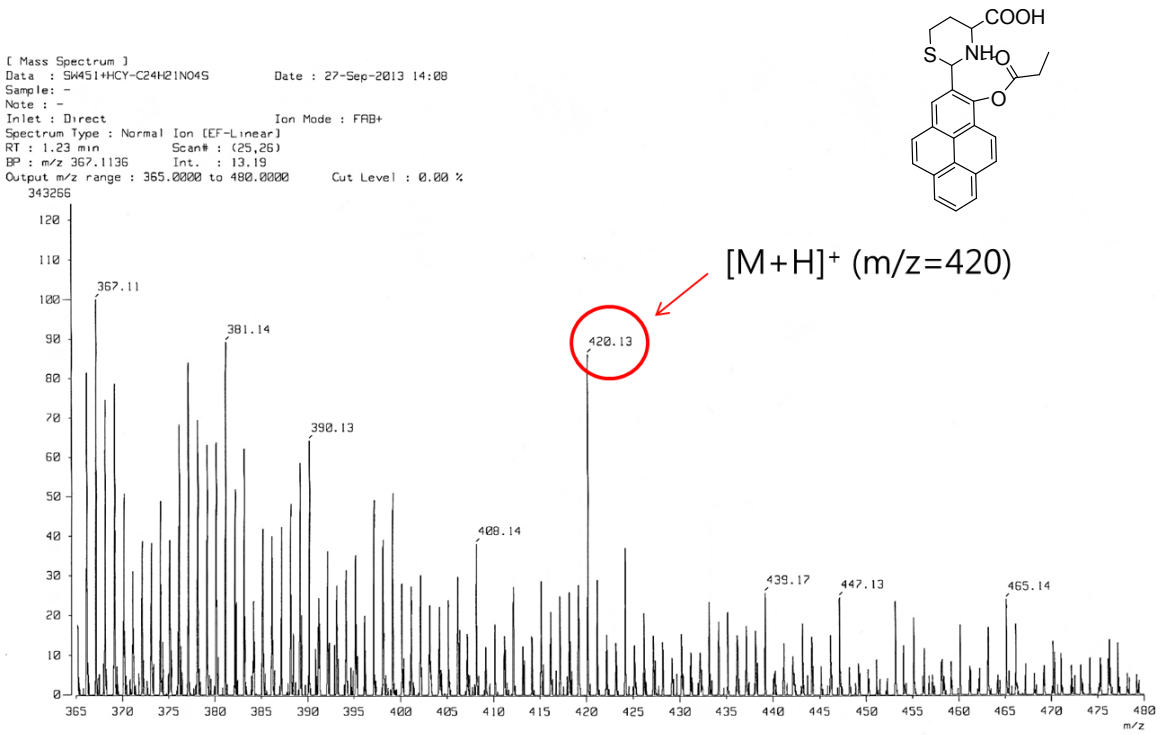


Fig S10. The FAB mass spectrum of **P-Hcy-1**+Hcy.

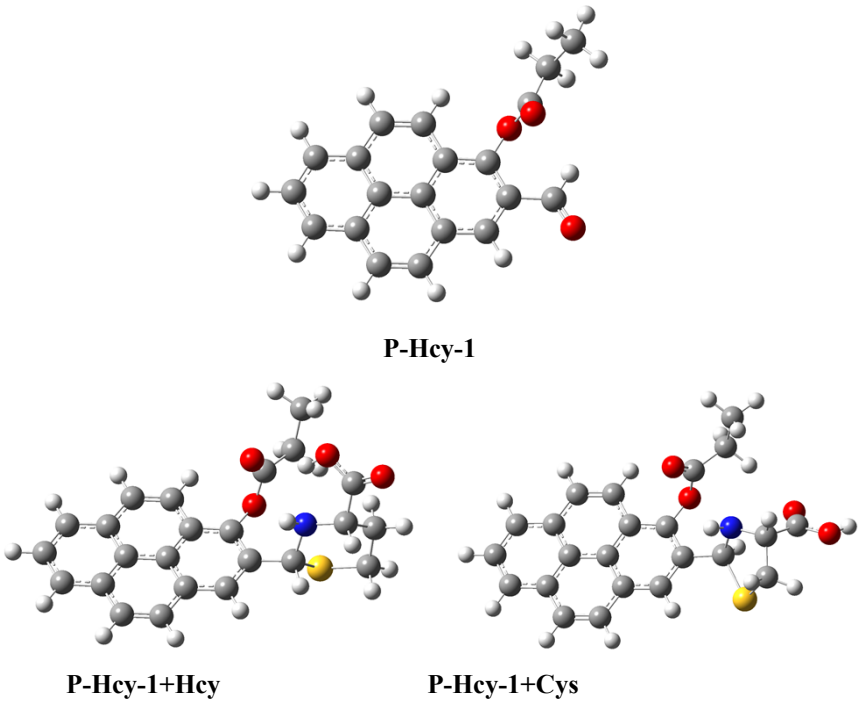
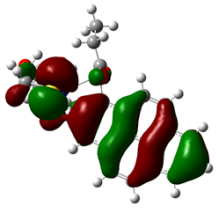
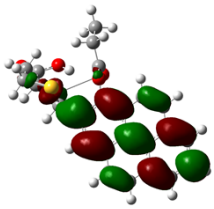
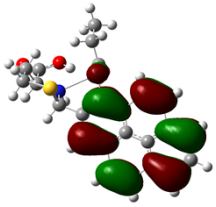
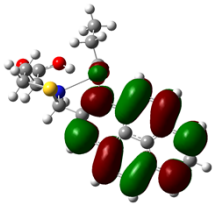


Fig S11. Optimized structures of **P-Hcy-1**, **P-Hcy-1**+Hcy and **P-Hcy-1**+Cys.

(a) P-Hcy-1+Hcy

Excitation contribution	Molecular orbital	
HOMO-1 → LUMO+1 4.0 %	 HOMO-1	 LUMO+1
HOMO → LUMO 96.0 %	 HOMO	 LUMO

(b) P-Hcy-1+Cys

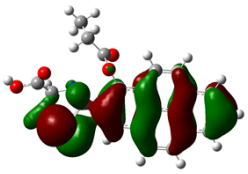
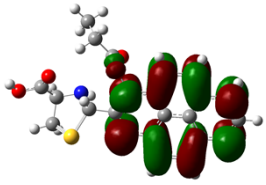
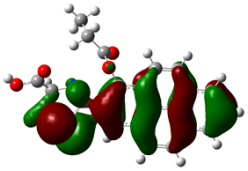
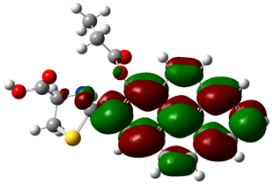
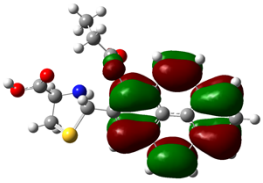
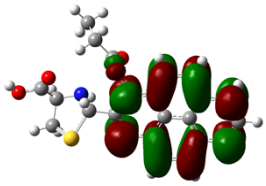
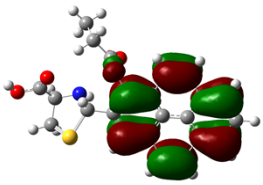
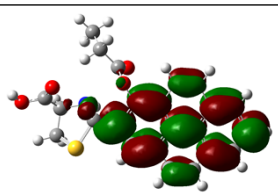
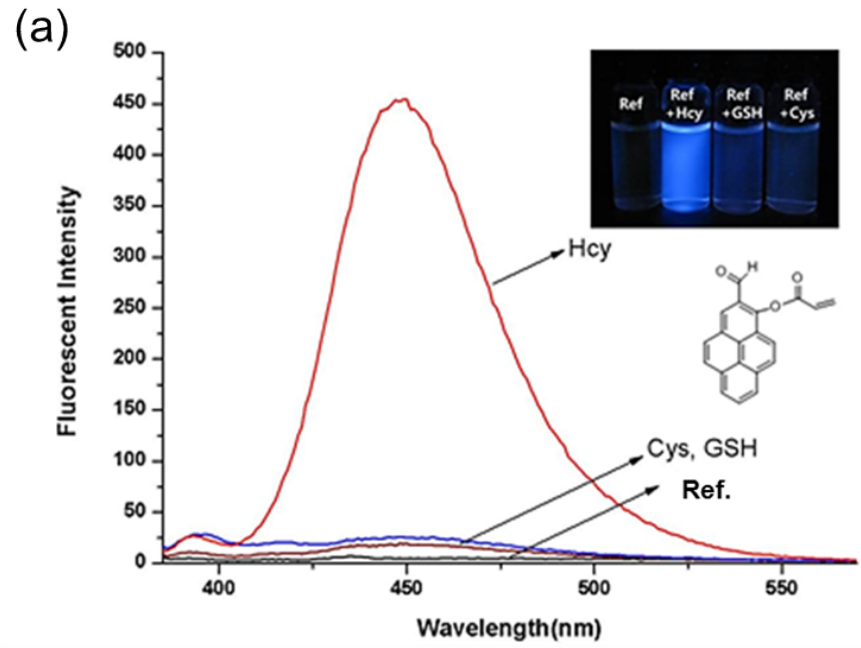
Excitation contribution	Molecular orbital	
HOMO-1 → LUMO 3.8 %	 HOMO-1	 LUMO
HOMO-1 → LUMO +1 3.0 %	 HOMO-1	 LUMO+1
HOMO → LUMO 83.1 %	 HOMO	 LUMO
HOMO → LUMO+1 10.1 %	 HOMO	 LUMO+1

Fig S12. Molecular orbitals and excitation contributions of the excitation for (a)**P-Hcy-1** +Hcy and (b) **P-Hcy-1**+Cys.



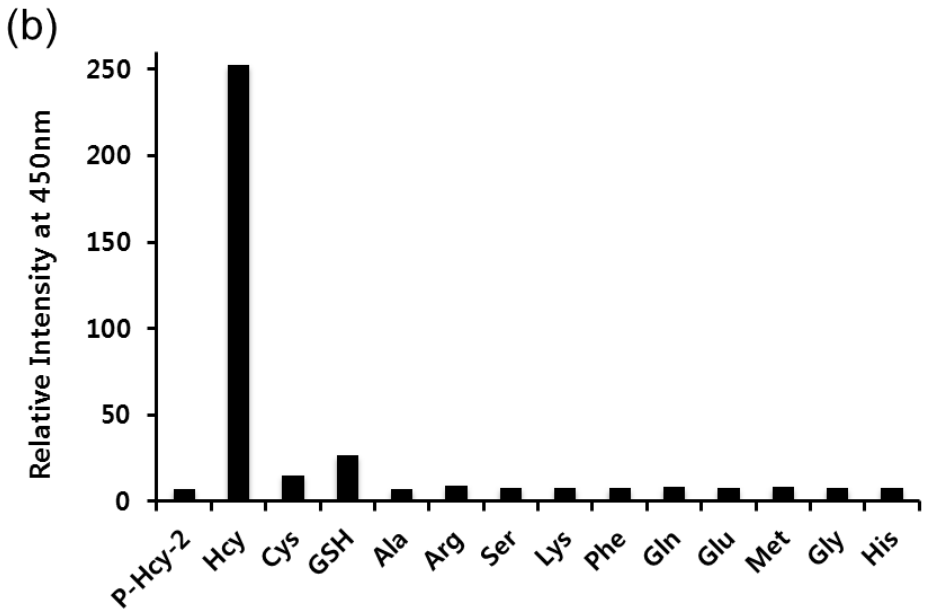


Fig S13. Selective response of **P-Hcy-2** to Hcy. (a) Fluorescence spectra of **P-Hcy-2** (1 0 μM) in HEPES (0.01 M, pH 7.4) containing 10% DMSO upon addition of 10 equiv. o f Hcy, Cys and GSH (excitation wavelength: 376 nm, slit: 3 × 5 nm). Inset: an image of **P-Hcy-2** in the absence and presence of Hcy, Cys and GSH under a handheld UV lamp at 365 nm. (b) Relative fluorescence intensities of **P-Hcy-2** (10 μM) upon addition of 10 equiv. of various amino acids or GSH in HEPES buffer at 450 nm.

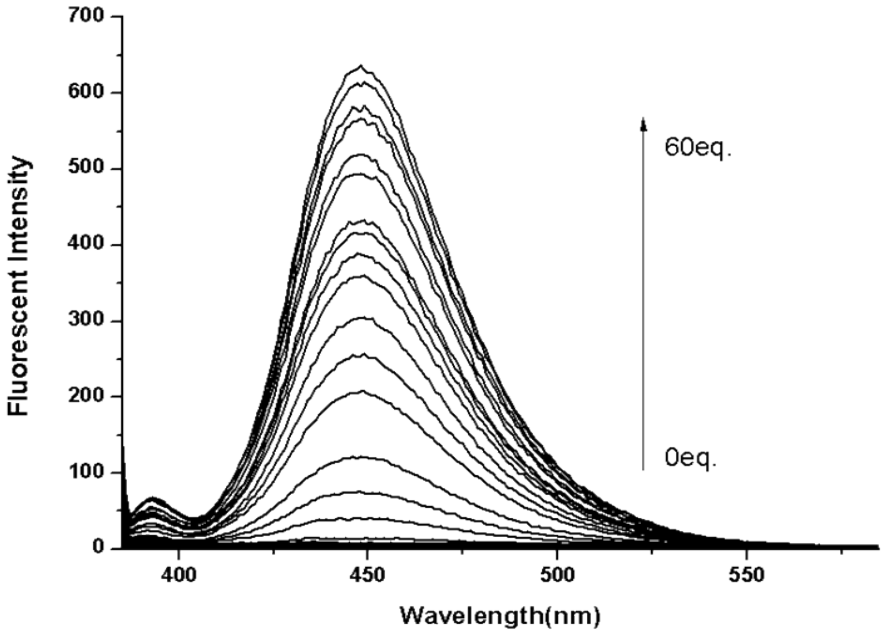
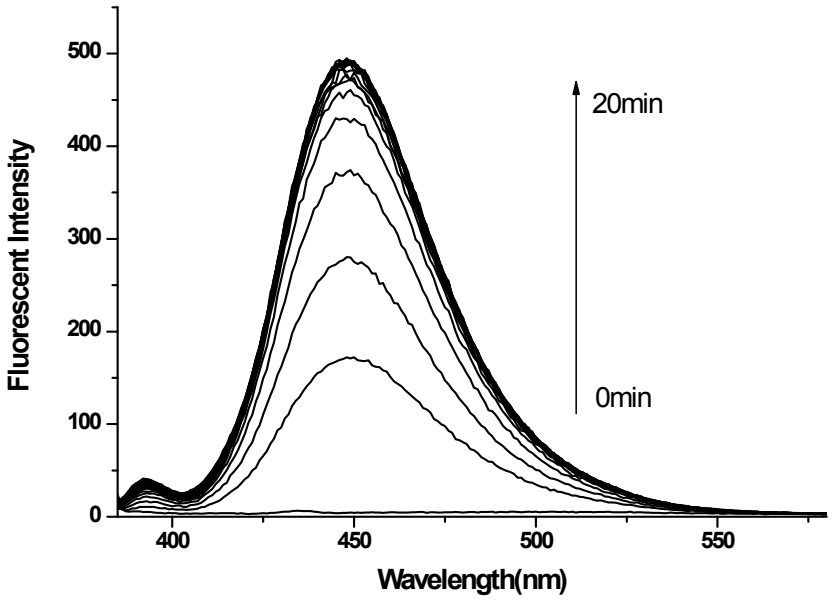


Fig S14. Fluorescence changes of **P-Hcy-2** (10 μM) with Hcy in DMSO-HEPES (0.01M, pH 7.4) (1:9, v/v). (Excitation wavelength: 376 nm) (Slit: 3 × 5 nm).



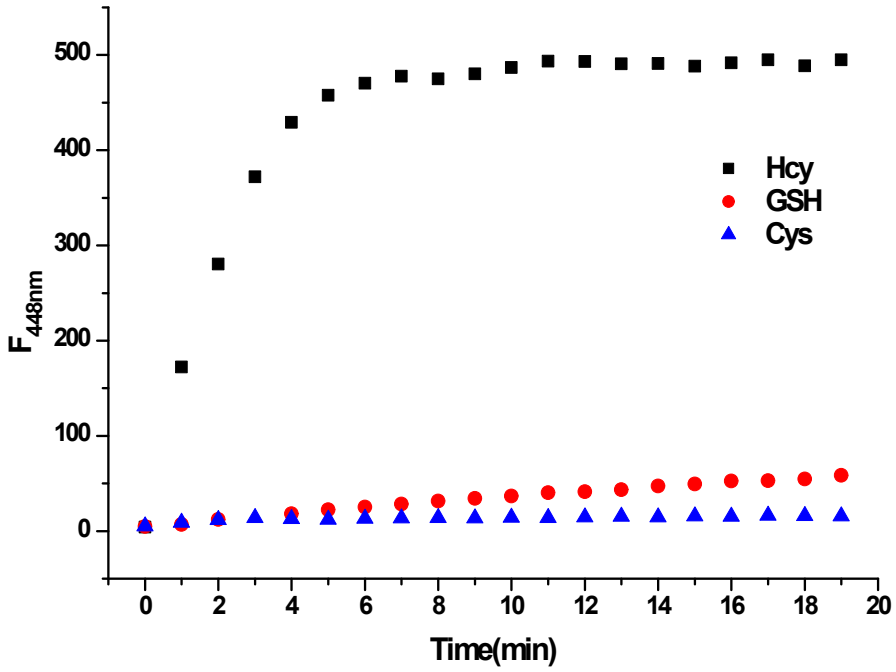


Fig S15. (a) Time-dependent change of **P-Hcy-2** (10 μM) with the addition of 10 equiv. of Hcy in DMSO-HEPES (0.01M, pH 7.4) (1:9, v/v). (Excitation wavelength: 376 nm) (Slit: 3 × 5 nm). (b) Time-dependent change of **P-Hcy-2** (10 μM) with the addition of 10 equiv. of Hcy, Cys and GSH in DMSO-HEPES (0.01M, pH 7.4) (1:9, v/v). (Excitation wavelength: 376 nm) (Slit: 3 × 5 nm).

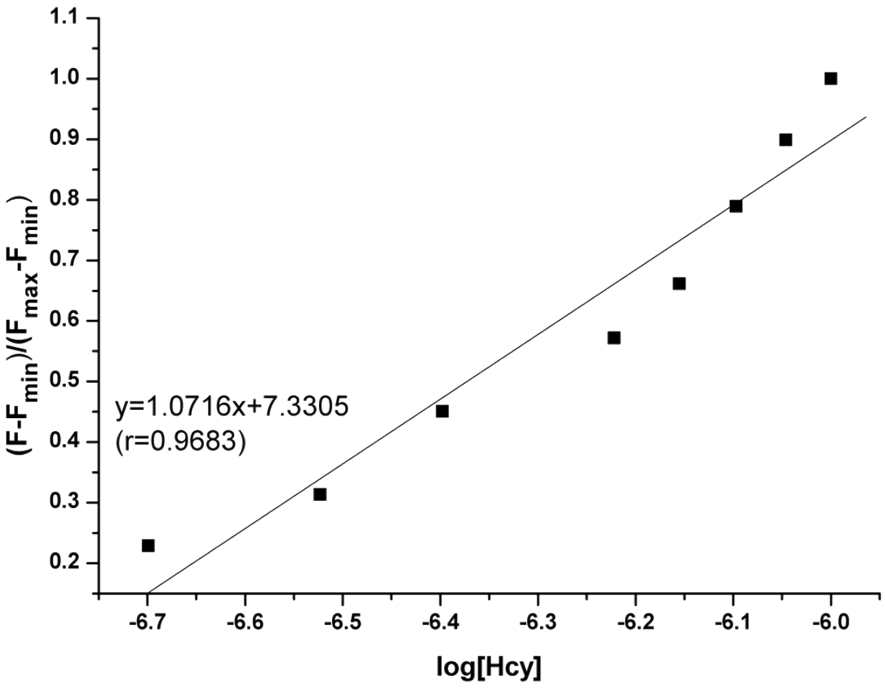


Fig S16. Normalized fluorescence responses of **P-Hcy-2** (0.1 μM) to changing Hcy con centrations in DMSO-HEPES (0.01M, pH 7.4) (1:9, v/v). (Detection limit = 1.44×10^{-7} M)

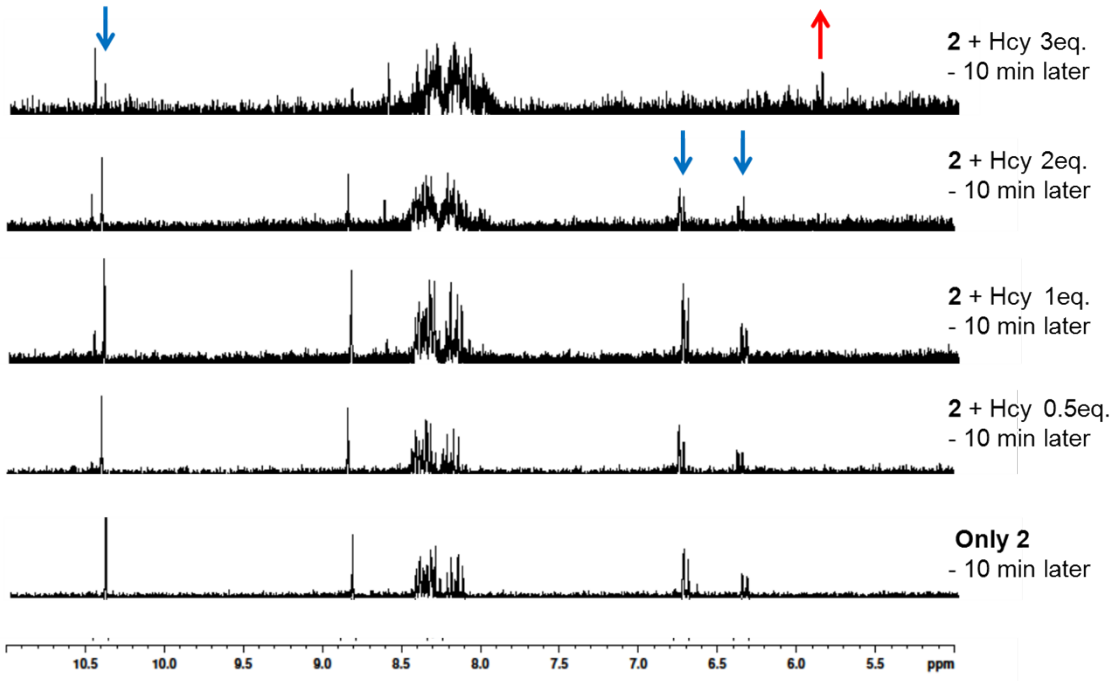


Fig S17. ^{1}H NMR spectral change of **P-Hcy-2** upon addition of Hcy in DMSO-d_6:D_2O (9:1, v/v).

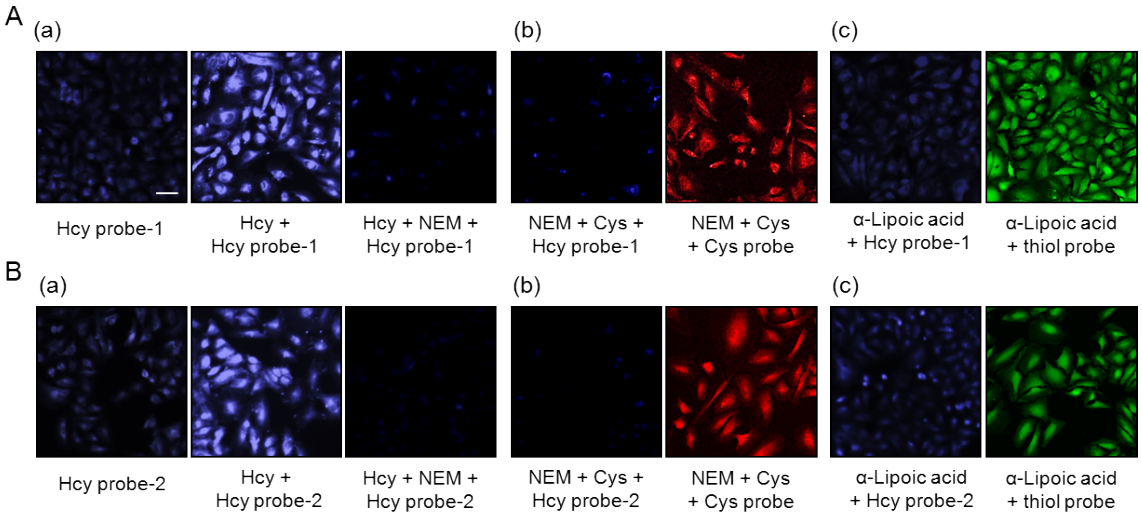
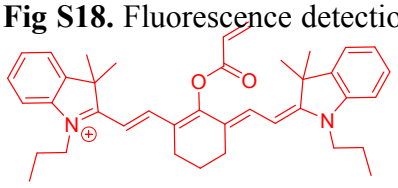
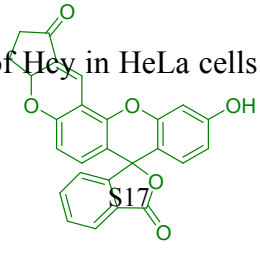


Fig S18. Fluorescence detection of Hcy in HeLa cells using (A) **P-Hcy-1** and (B) **P-Hc**



Cys probe



Thiol probe

y-2. (a) Left; fluorescence image of cells incubated with 60 μM Hcy probe for 20 min. Middle; fluorescence image of cells treated with 20 μM Hcy for 30 min, followed by tre atment with 60 μM Hcy probe for 20 min. Right; fluorescence image of cells treated wit h 20 μM Hcy for 30 min, incubated with 500 μM NEM for 20 min to remove intracellul ar biothiols and then stained with 60 μM Hcy probe for 20 min. (b) Fluorescence image of cells incubated with 500 μM NEM for 20 min, supplemented with 20 μM Cys for 20 min and then stained with either (left) 60 μM Hcy probe or (right) 20 μM Cys probe for 20 min. (c) Fluorescence image of cells incubated with 250 μM α-lipoic acid for 48 h to enhance production of GSH in cells, followed by treatment with either (left) 60 μM Hcy probe or (right) 20 μM thiol probe for 20 min. Scale bar represents 50 μm. (C) Structure of Cys and thiol probes used for this study.