Supplementary Information

A pH-dependent fluorescent probe that can be tuned for cysteine or homocysteine

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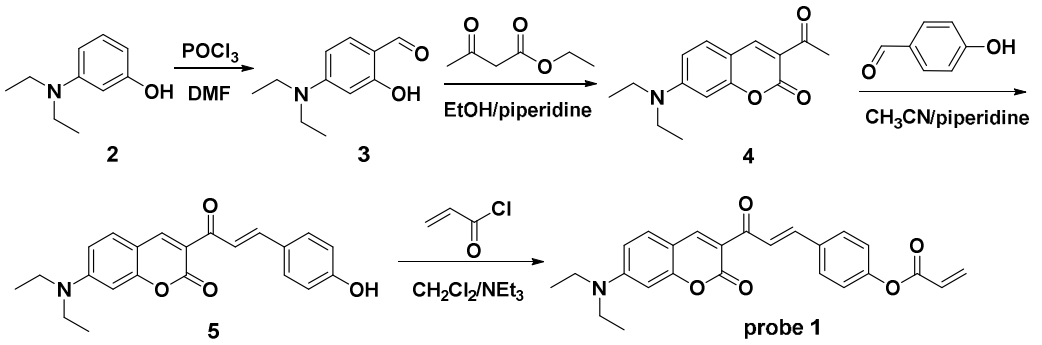
Experimental Section

Experimental Details. All chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified prior to use. Distilled water was used after passing through a water ultra-purification system. TLC analysis was performed using precoated silica plates. Hitachi F−7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. Shanhai Huamei Experiment Instrument Plants, China provided a PO-120 quartz cuvette (10 mm). ^{1}H NMR and 13C NMR experiments were performed with a BRUKER AVANCE III HD 600 MHz and 151 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Coupling constants (*J* values) are reported in hertz. HR MS determinations were carried out on an AB SCIEX TripleTOF 5600 Instruments. ESI-MS was measured with an LTQ-MS (Thermo) Instrument. The cell imaging experiments were measured by a Leica DMi8 fluorescence inversion microscope system and an Olympus FV1000 confocal laser scanning microscope.

Synthesis of probe 1.



Synthesis of compound 5. Compound **4**[S1] (6 mmol, 1.55 g) and *p*-hydroxy benzaldehyde (6 mmol, 0.732 g) was dissolved in 30 mL CH_3CN. 200 μL piperidine was added and the mixture was refluxed for 11 hours. After cooling to room temperature, the mixture was filtrated, washed with 10 mL CH_3CN, and dried under reduce pressure to give 1.23 g (3.4 mmol) yellow powder. ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.08 (s, 1H), 8.58 (s, 1H), 7.80 (d, J = 15.7 Hz, 1H), 7.69 (d, *J* = 9.0 Hz, 1H), 7.63 (d, *J* = 15.7 Hz, 1H), 7.58 (d, *J* = 8.4 Hz, 2H), 6.84 (d, *J* = 8.4 Hz, 2H), 6.81 (d, *J* = 9.2 Hz, 1H), 6.61 (s, 1H), 3.51 (dd, *J* = 13.5, 6.6 Hz, 4H), 1.16 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 185.8, 160.4, 160.4, 158.6, 153.3, 148.6, 143.1, 132.7, 130.9, 126.4, 122.0, 116.4, 116.3, 110.6, 108.4, 96.4, 44.9, 12.8. ESI-MS $[M+Na]^+$: m/z Calcd 386.14, Found 385.83.

Synthesis of Probe 1. To a solution of compound **5** (1 mmol, 0.36 g), triethylamine (3 equiv.) in CH_2Cl_2 (15 mL) at 0 °C, acryloyl chloride (1.3 equiv.) was added gradually and the mixture was stirred for 20 h. After completion of the reaction, the solvent was separated under reduce pressure and the residue was purified by column chromatography using ethyl acetate/ petroleum ether (1:3) to give 0.108 g (0.26 mmol) probe **1** as a yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.62 (s, 1H), 7.96 (d, *J* = 15.8 Hz, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.75 – 7.66 (m, 2H), 7.30 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 9.0 Hz, 1H), 6.63 (s, 1H), 6.57 (d, *J* = 17.4 Hz, 1H), 6.44 (dd, *J* = 17.3, 10.4 Hz, 1H), 6.19 (d, *J* = 9.7 Hz, 1H), 3.52 (dd, *J* = 13.2, 6.3 Hz, 4H), 1.16 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 186.0, 164.4, 160.4, 158.8, 153.5, 152.1, 149.0, 141.3, 134.4, 133.3, 132.9, 130.1, 128.0, 125.8, 122.9, 115.9, 110.8, 108.4, 96.4, 45.0, 12.9. HR MS $[M+H]^+$: m/z Calcd 418.1649, Found 418.1653.

Preparation of Solutions of Probe 1 and Analytes. Stock solution of probe **1** (2 mM) was prepared in DMSO. Stock solutions of Cys (20 mM), Hcy (20 mM), GSH (20 mM) and other amino acids (20 mM) were

prepared by direct dissolution in deionized water. All chemicals used were of analytical grade.

General fluorescence spectra measurements. All the detection experiments were measured in Hepes buffer−DMSO (1:1, v/v). The procedure was as follows: into a Hepes buffer−DMSO (1:1, v/v) solution, containing 30 μM probe **1**, an analyte sample was added. The process was monitored by fluorescence spectrometer (λ_{ex}= 447 nm, slit: 5 nm/5 nm).

Cell Culture and Imaging.

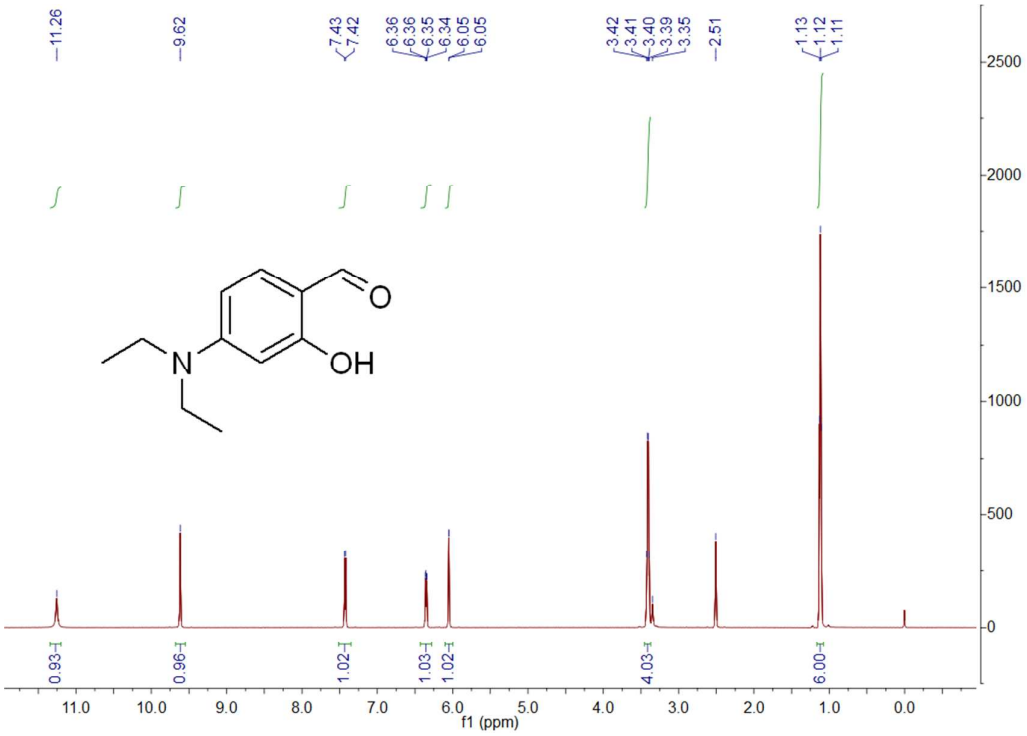
The HepG2 cells were grown in Dulbecco's Modified Eagle's medium supplemented with 12% Fetal Bovine Serum and 1% antibiotics at 37 °C in humidified environment of 5% CO_2. Cells were plated on 6-well plate and allowed to adhere for 24 h. Before the experiments, cells were washed with PBS 3 times. For control, the HepG2 cells were pre-incubated with NEM (1 mM) for 30 min at 37 °C and then incubated with HBSS (Hanks' Balanced Salt Solution (with Ca^{2+}, Mg^{2+})) of pH 7.8 in the presence of 10 μM nigericin and 5 μM probe **1** for 30 min. For Cys, the HepG2 cells were pre-incubated with NEM (1 mM) for 30 min at 37 °C, then incubated with HBSS (Hanks' Balanced Salt Solution (with Ca^{2+}, Mg^{2+})) of pH 7.8 in the presence of 10 μM nigericin and 5 μM probe **1** for 30 min and further incubated with HBSS of pH 7.8 in the presence of 10 μM nigericin and 100 μM Cys for 60 min. For Hcy, the HepG2 cells were pre-incubated with NEM (1 mM) for 30 min at 37 °C, then incubated with HBSS (Hanks' Balanced Salt Solution (with Ca^{2+}, Mg^{2+})) of pH 7.8 in the presence of 10 μM nigericin and 5 μM probe **1** for 30 min and further incubated with HBSS of pH 7.8 in the presence of 10 μM nigericin and 100 μM Hcy for 60 min. Cell imaging was then carried out after washing cells with HBBS buffer.

Cell Viability. Cytotoxicity was assessed by performing MTT assay with the HepG2 cells. Cells were seeded into a 96-well plate at 2 × 103/well and were cultured at 37 °C and 5% CO_2 for 24 h. Different concentrations of probe **1** (0, 2.5, 5, 10, 25, and 50 μM) were then added to the wells. After incubation for 6 or 12 h, MTT (0.5 mg/mL) was added to each well, and the plate was incubated for another 4 h. The optical densities at 490 nm were measured.

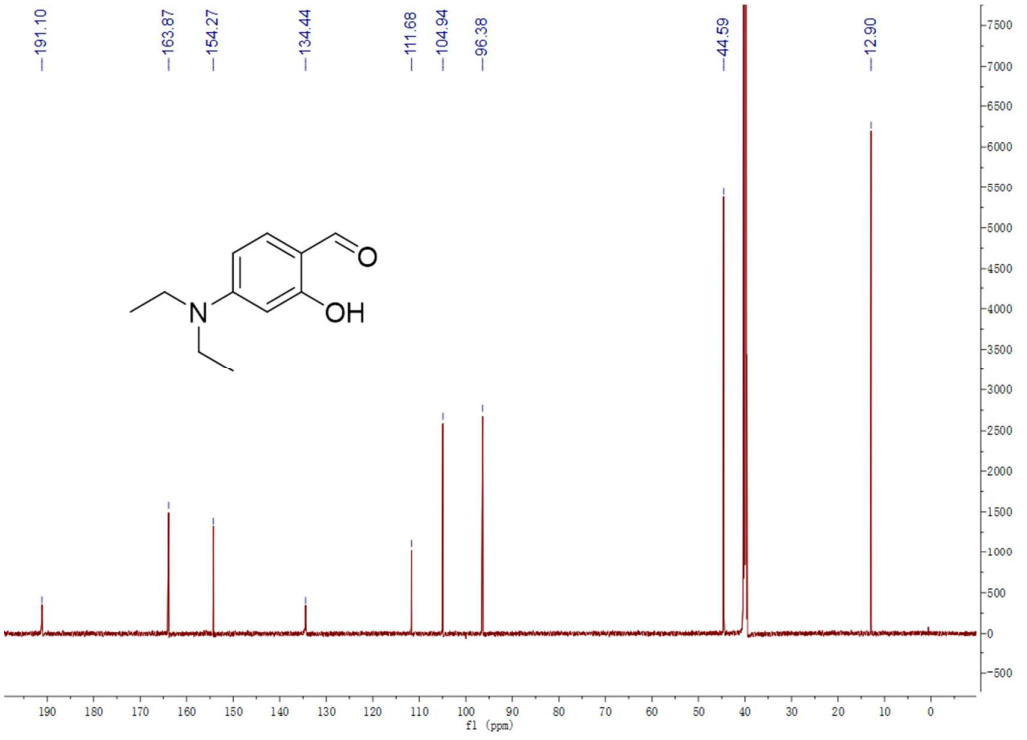
REFERENCES

(S1) Li, X.; Zhao, Y. X.; Wang, T.; Shi, M. Q.; Wu, F. P. *Dyes Pigments*, **2007**, *74*, 108.

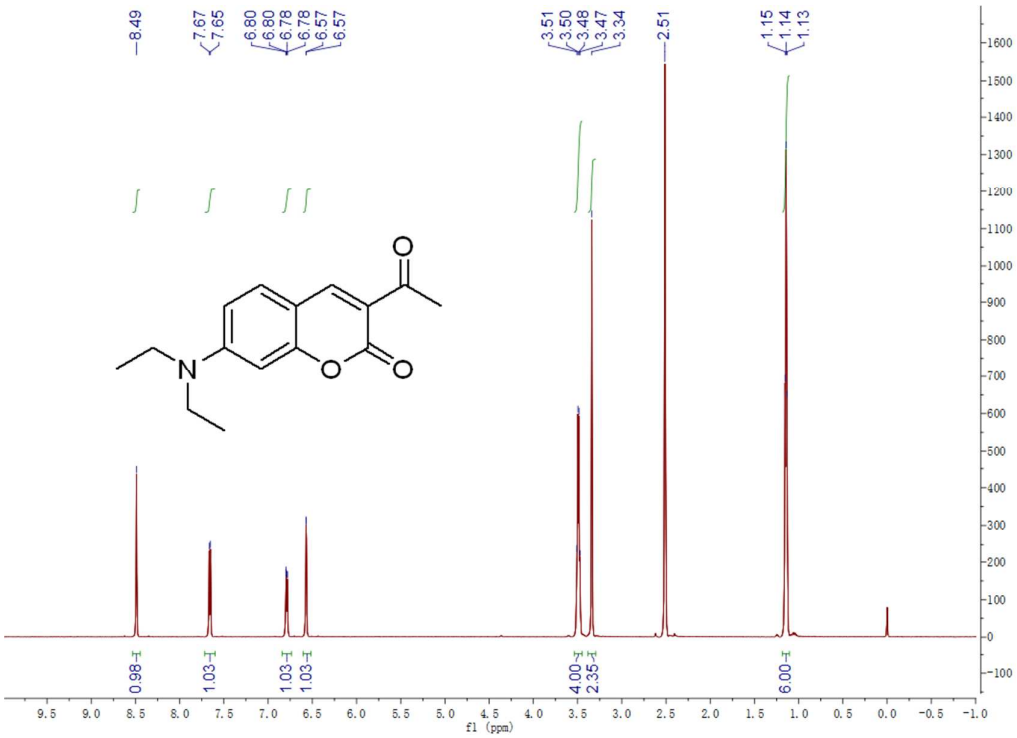
Characterization data for Synthesis:



^{1}H-NMR spectrum of compound **3** in DMSO-d_6



^{13}C-NMR spectrum of compound **3** in DMSO-d_6



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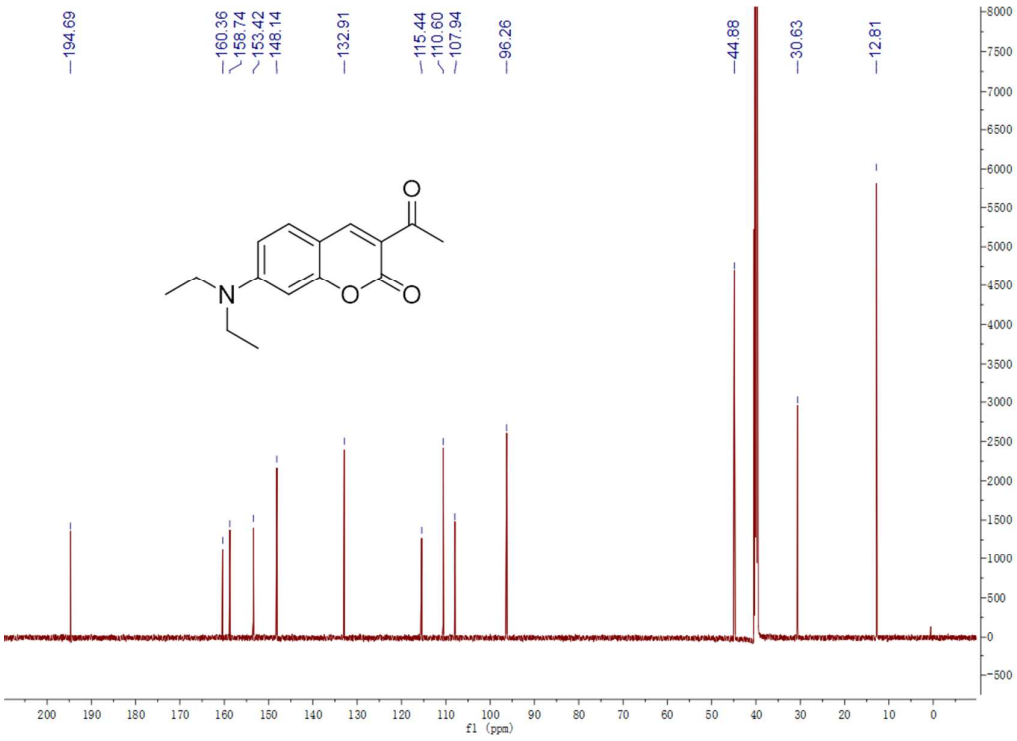
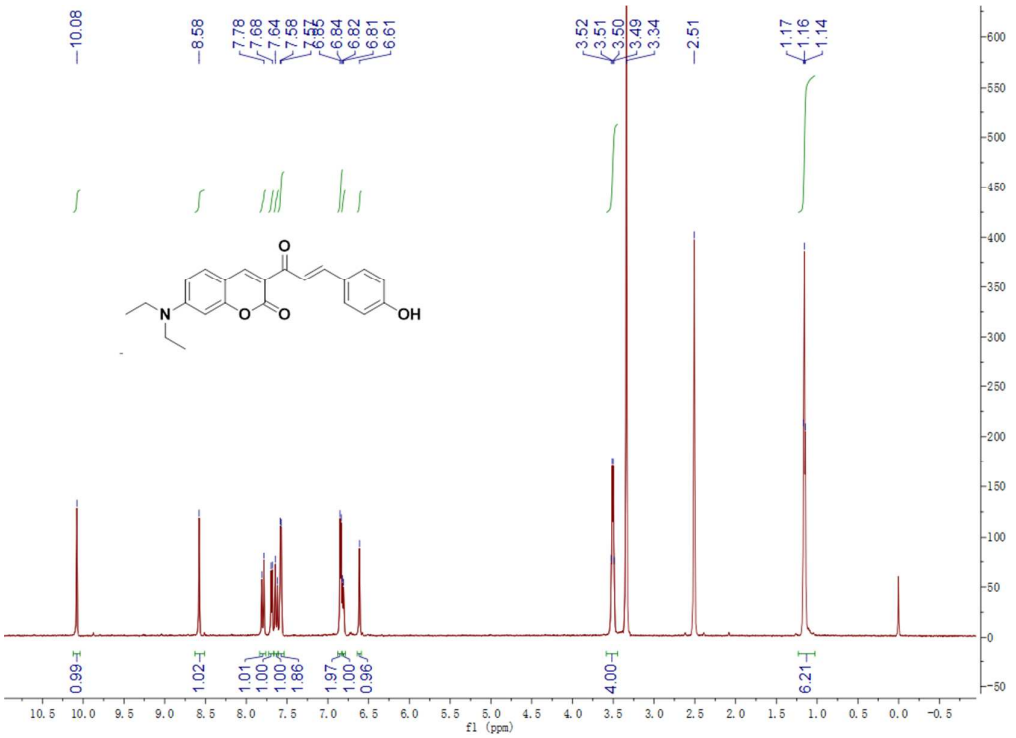
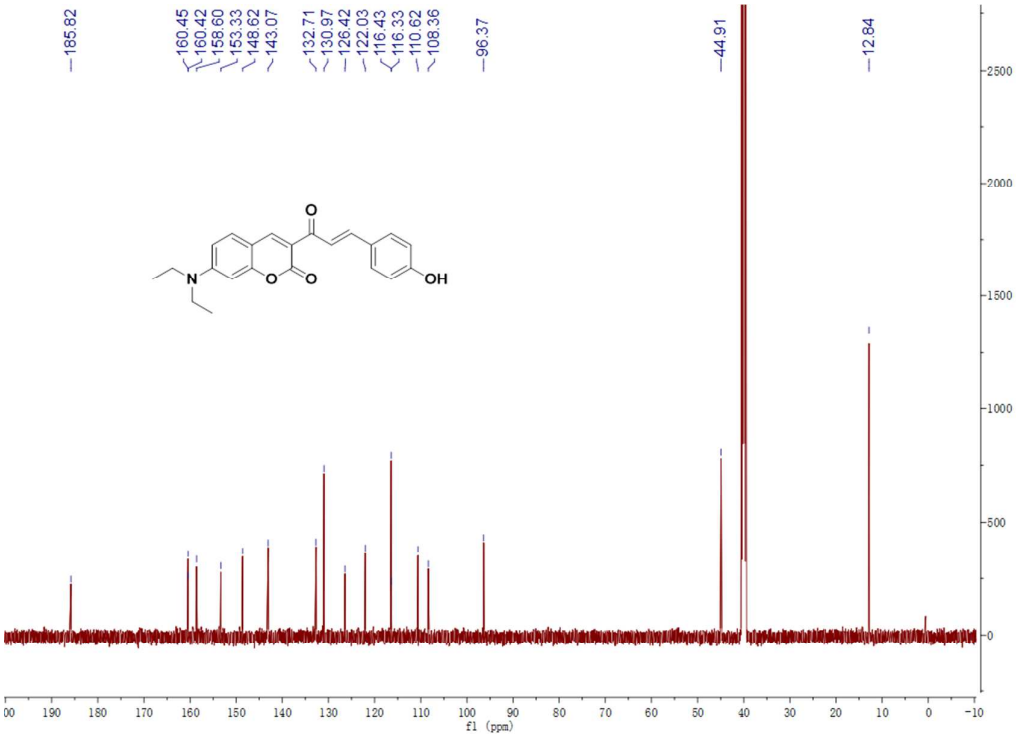


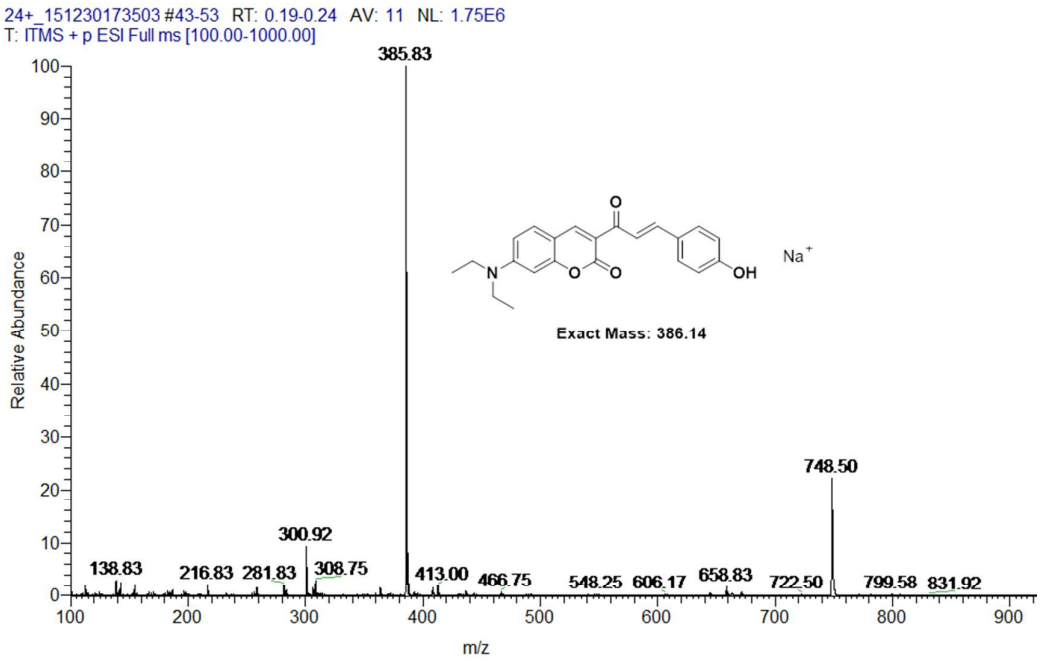
^{13}C-NMR spectrum of compound **4** in DMSO-d_6



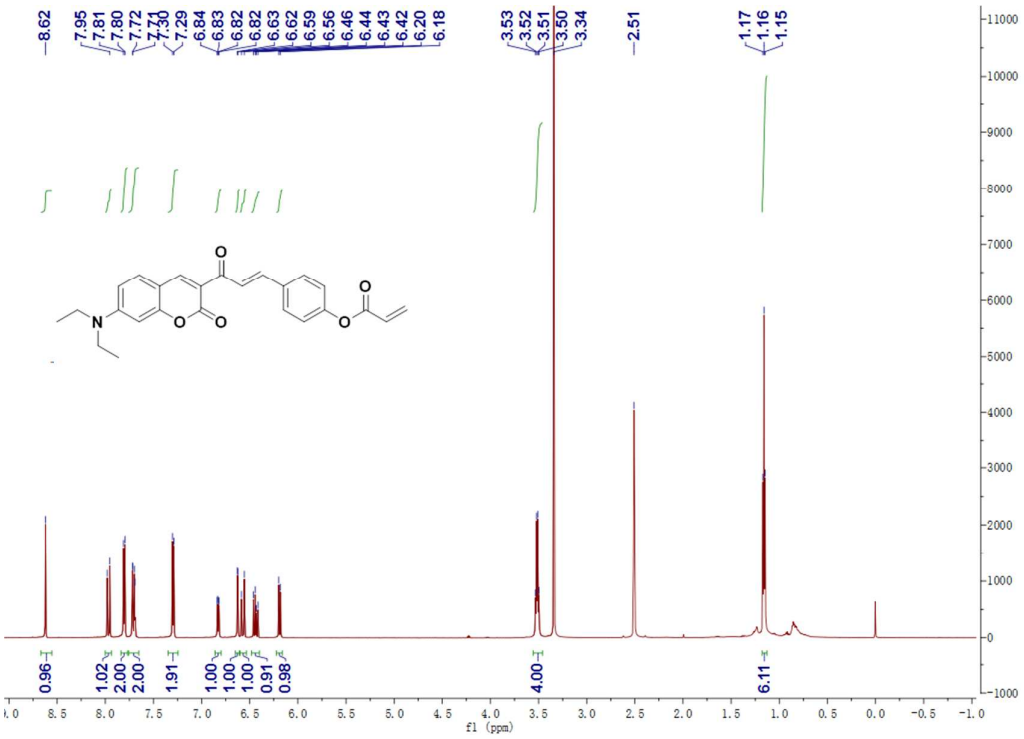
^{1}H-NMR spectrum of compound **5** in DMSO-d_6



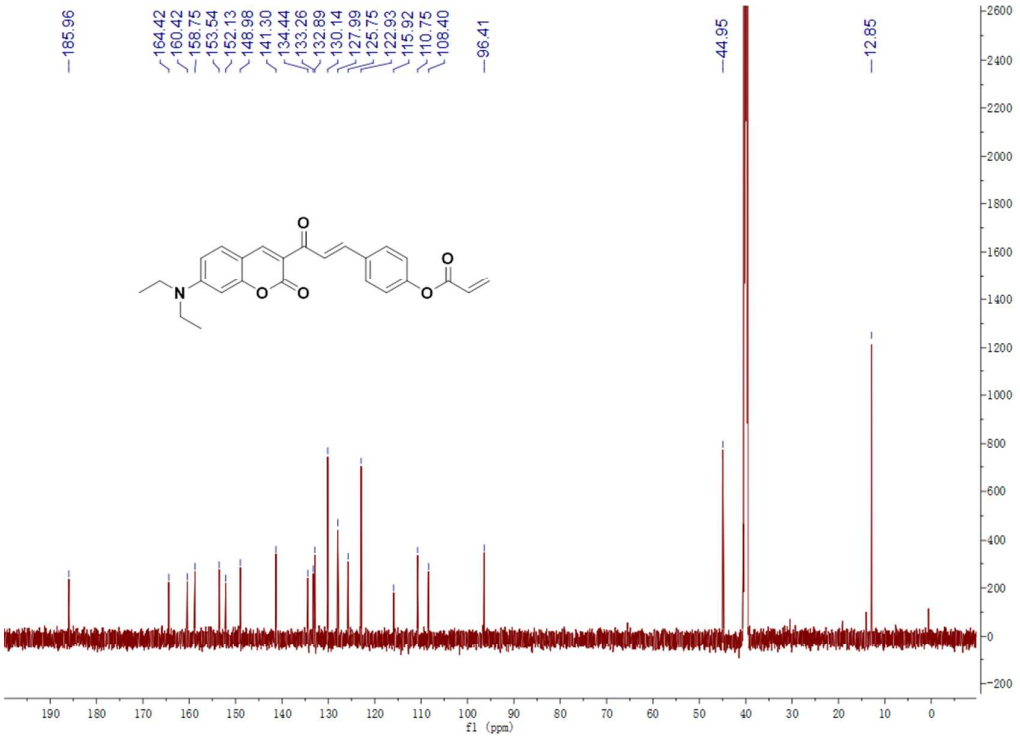
^{13}C-NMR spectrum of compound **5** in DMSO-d_6



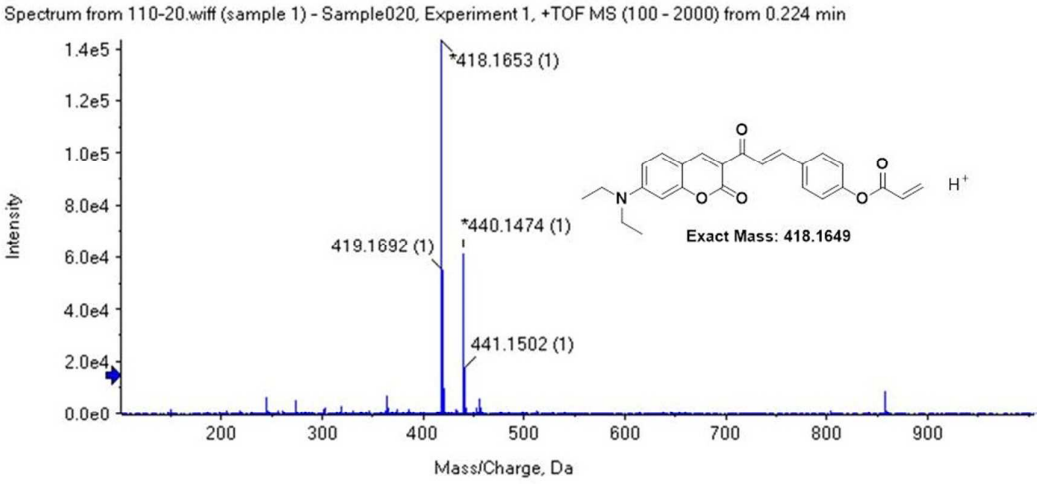
ESI-MS spectrum of compound **5** in MeOH



^{1}H-NMR spectrum of probe **1** in DMSO-d_6

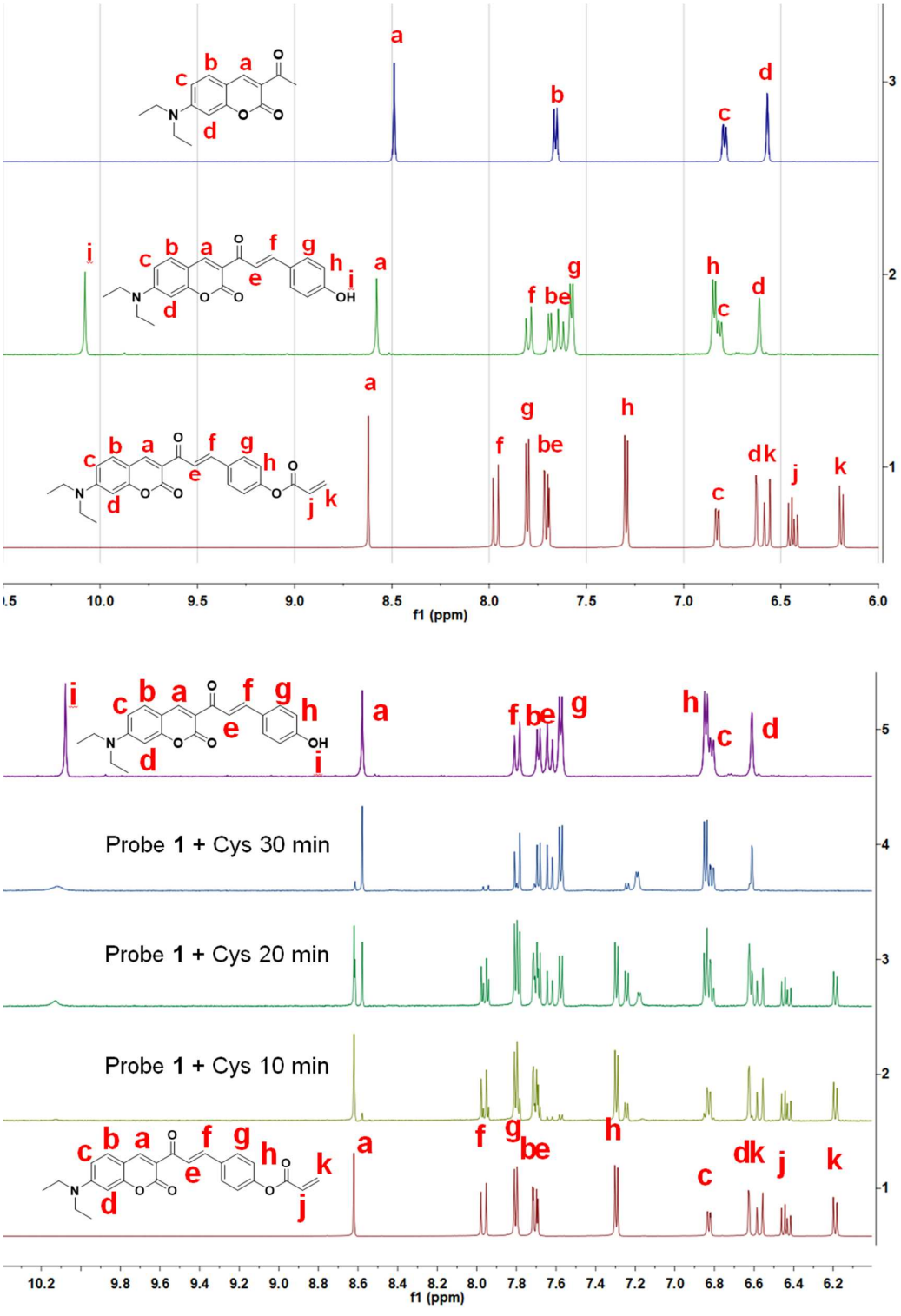


^{13}C-NMR spectrum of probe **1** in DMSO-d_6



HR MS spectrum of probe **1**

Figure S1 ^{1}H NMR affiliation of compound 4, 5, probe 1, probe 1-Cys and probe 1-Hcy.



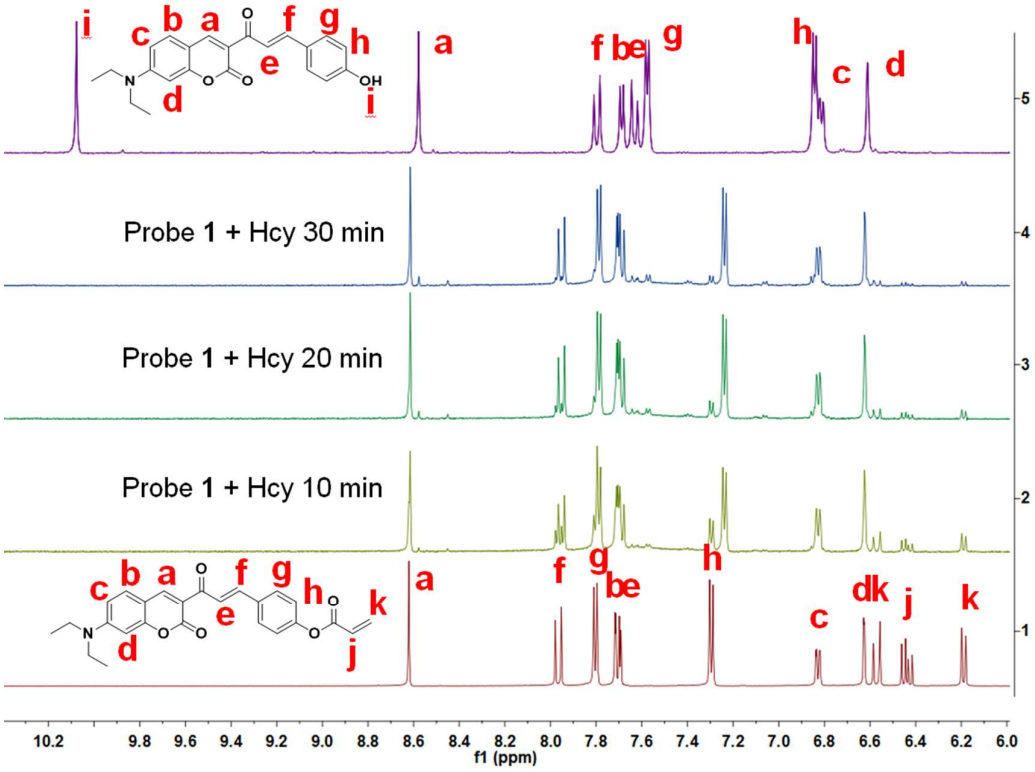

Probe 1 + Hcy 30 min
Probe 1 + Hcy 20 min
Probe 1 + Hcy 10 min
f1 (ppm)

Figure S2 HR MS spectra of the probe 1-Cys system

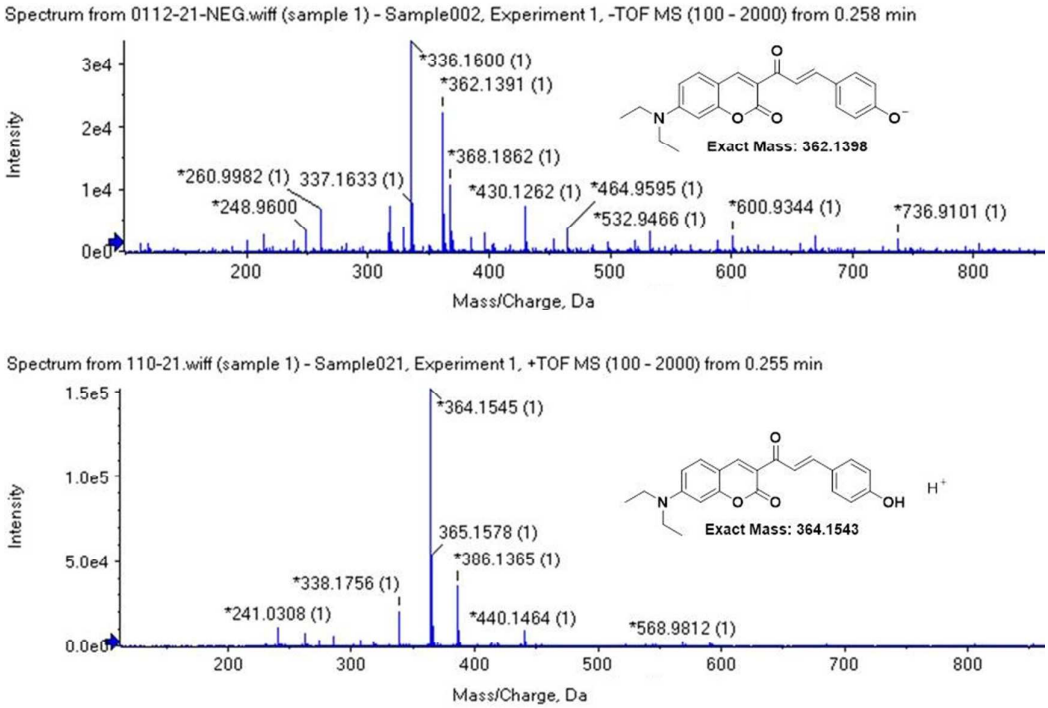


Figure S3 HR MS spectra of the probe 1-Hcy system

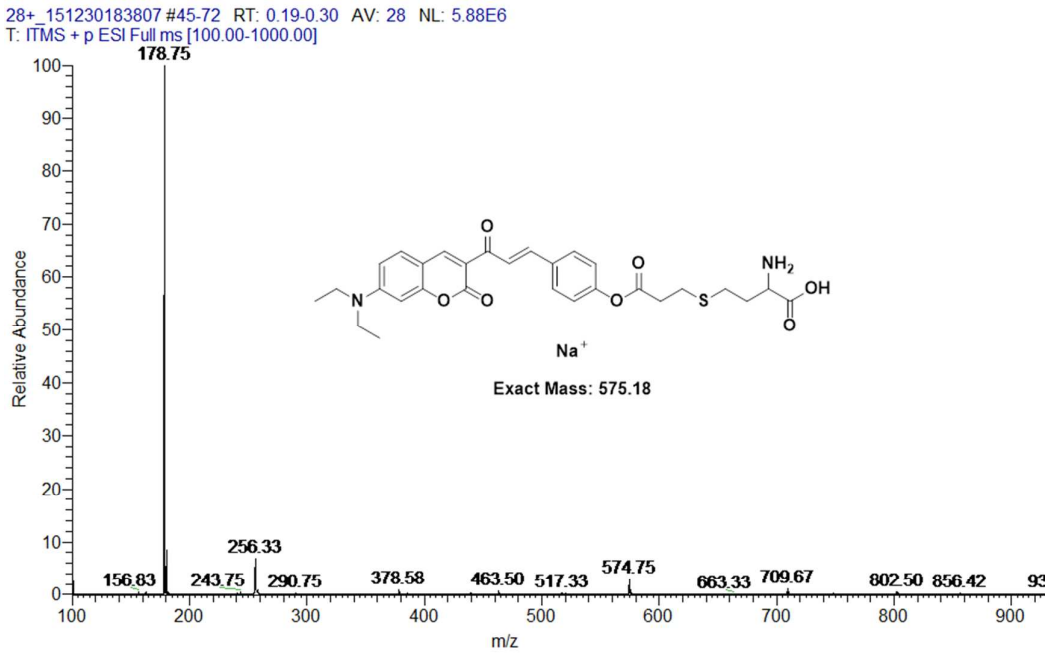
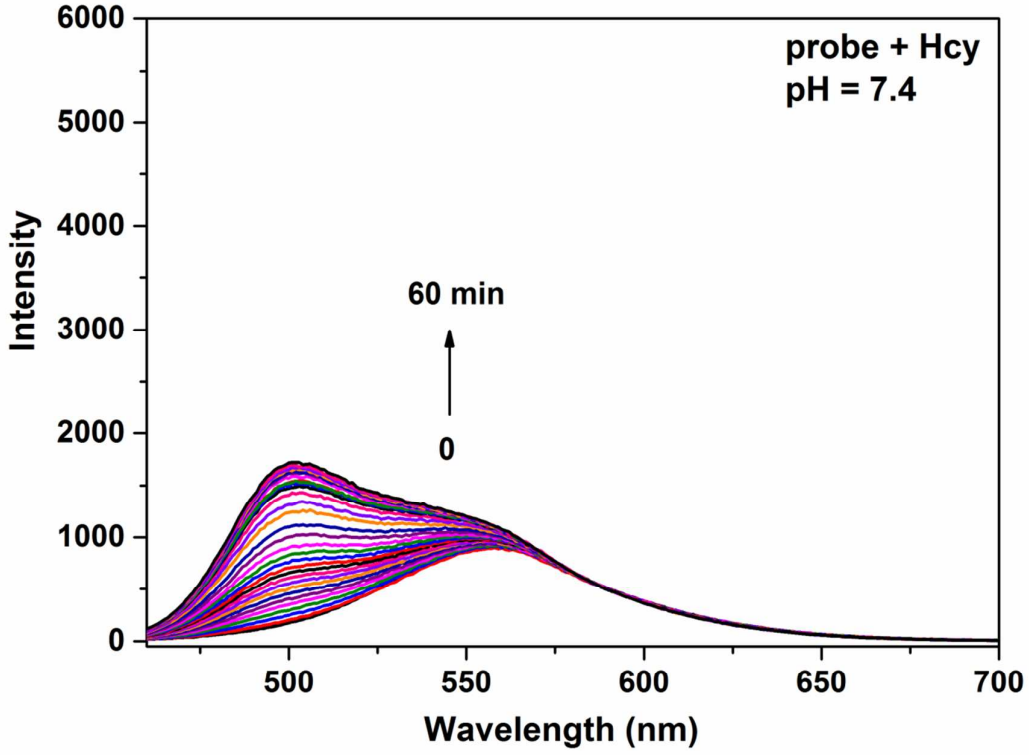
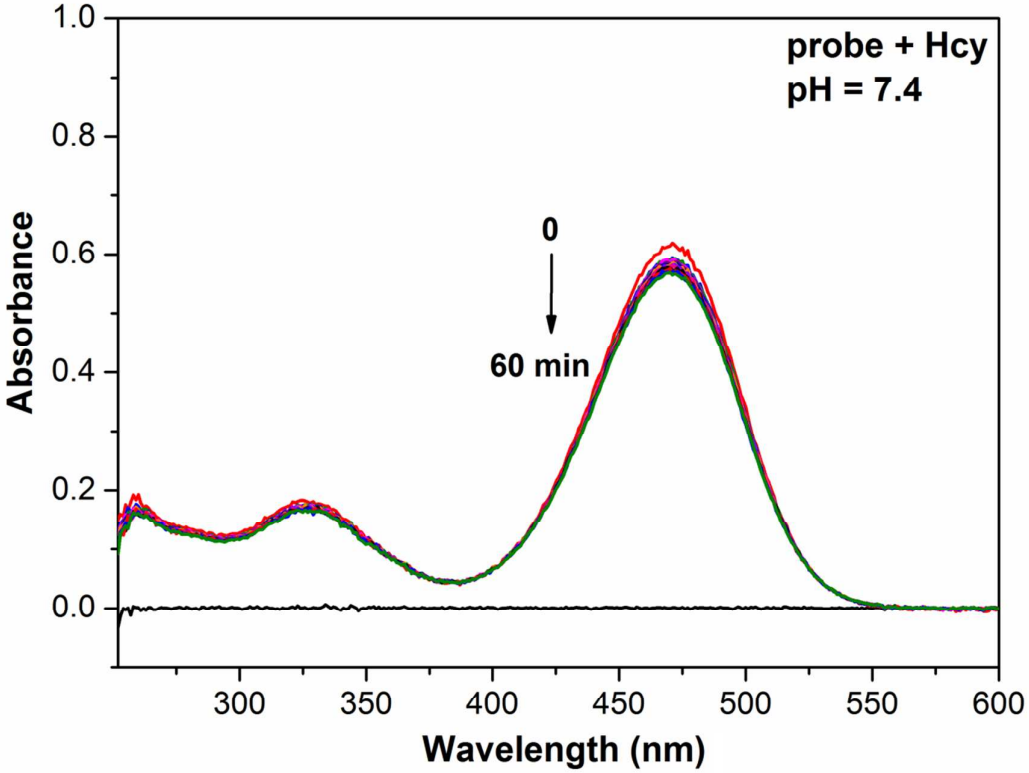
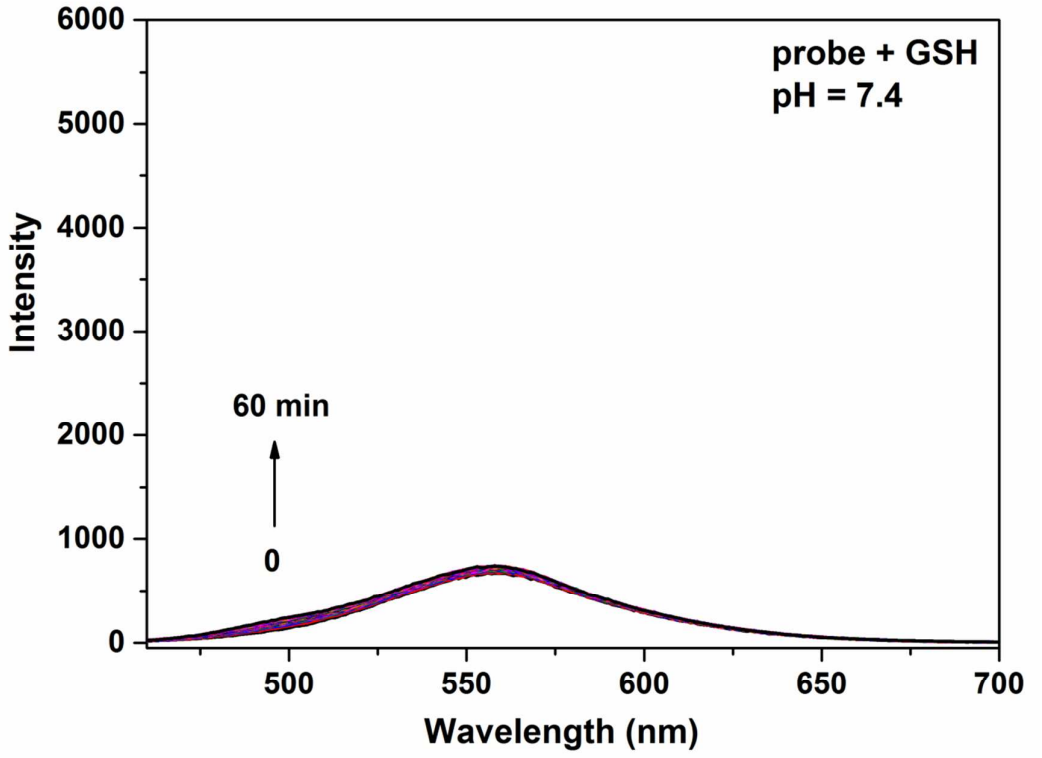


Figure S4 Fluorescent and UV-vis responses of probe 1 towards Hcy and GSH in Hepes buffer/DMSO (1:1, v/v, pH 7.4) solution at 25 °C






probe + GSH
pH = 7.4
Intensity
60 min
0
Wavelength (nm)

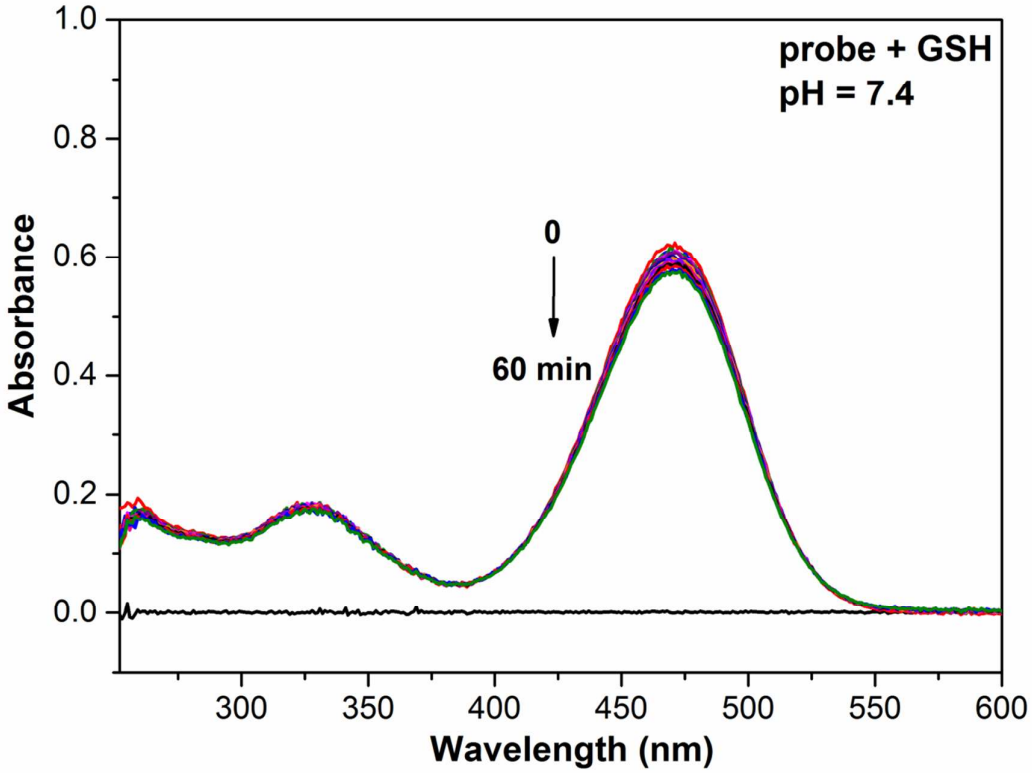
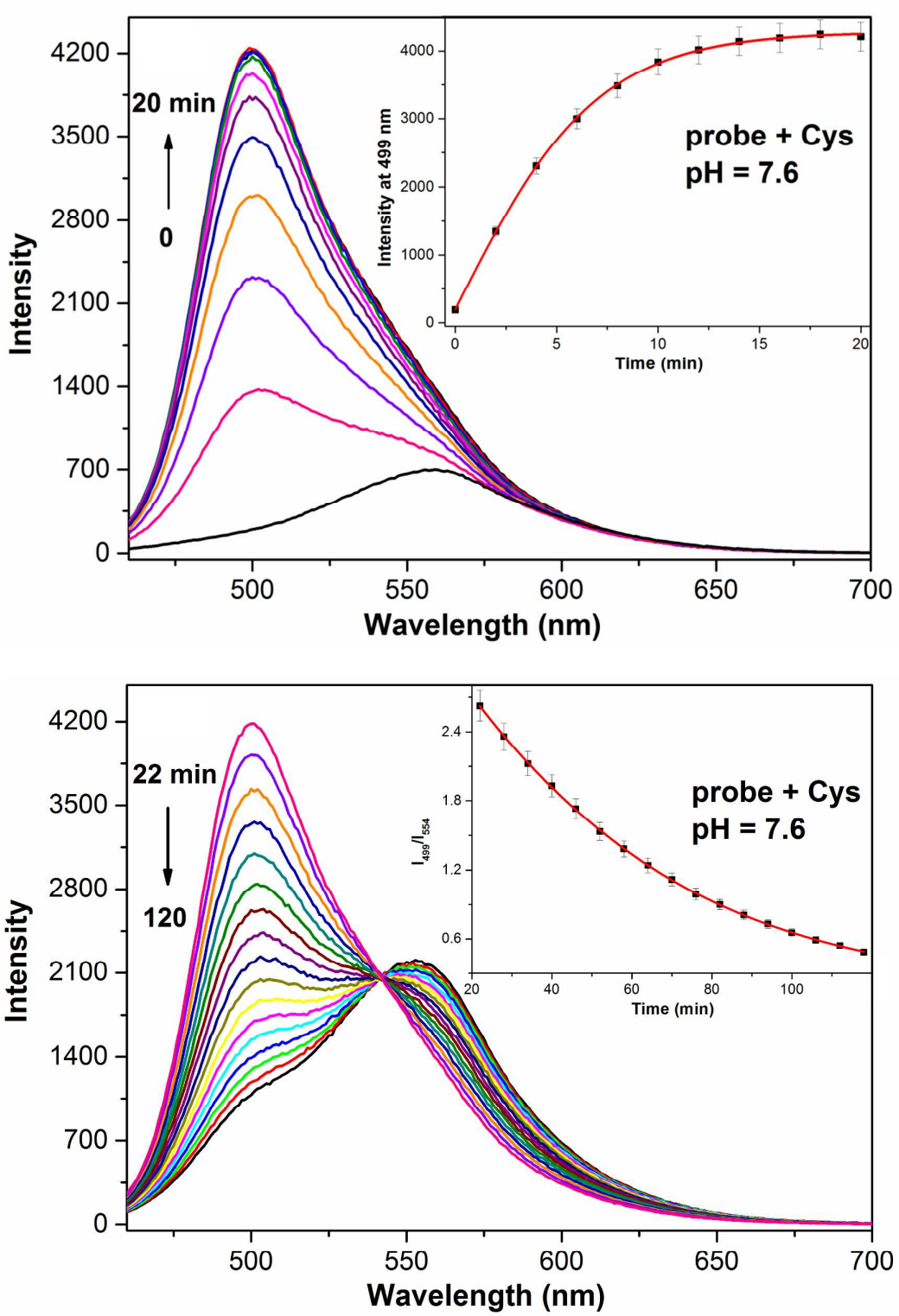
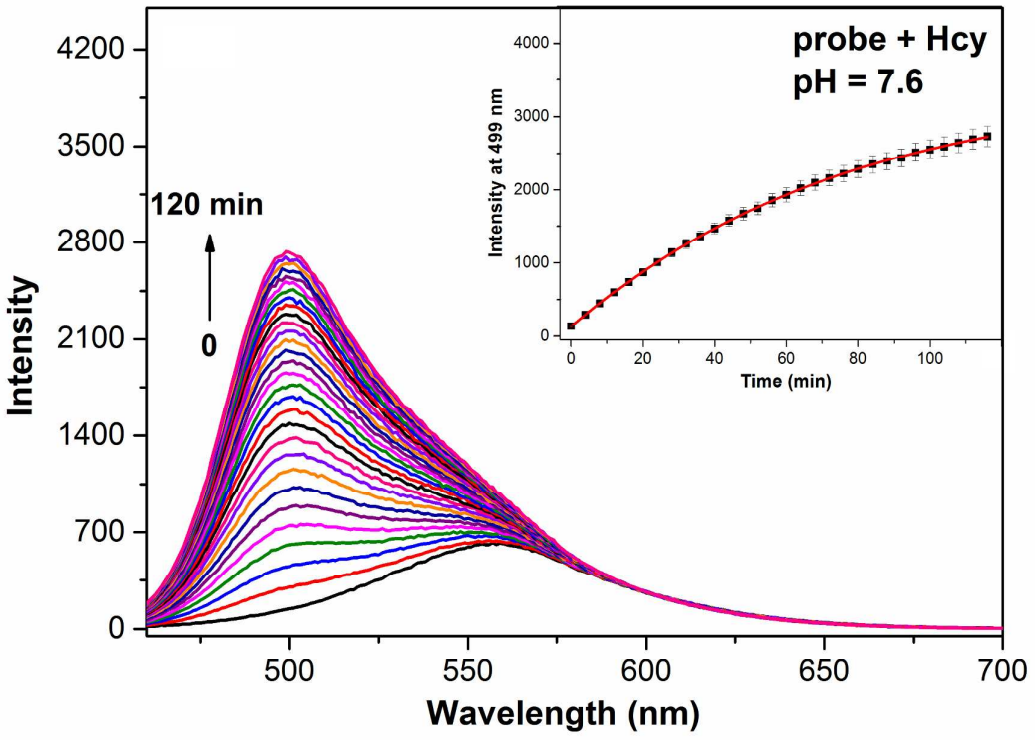
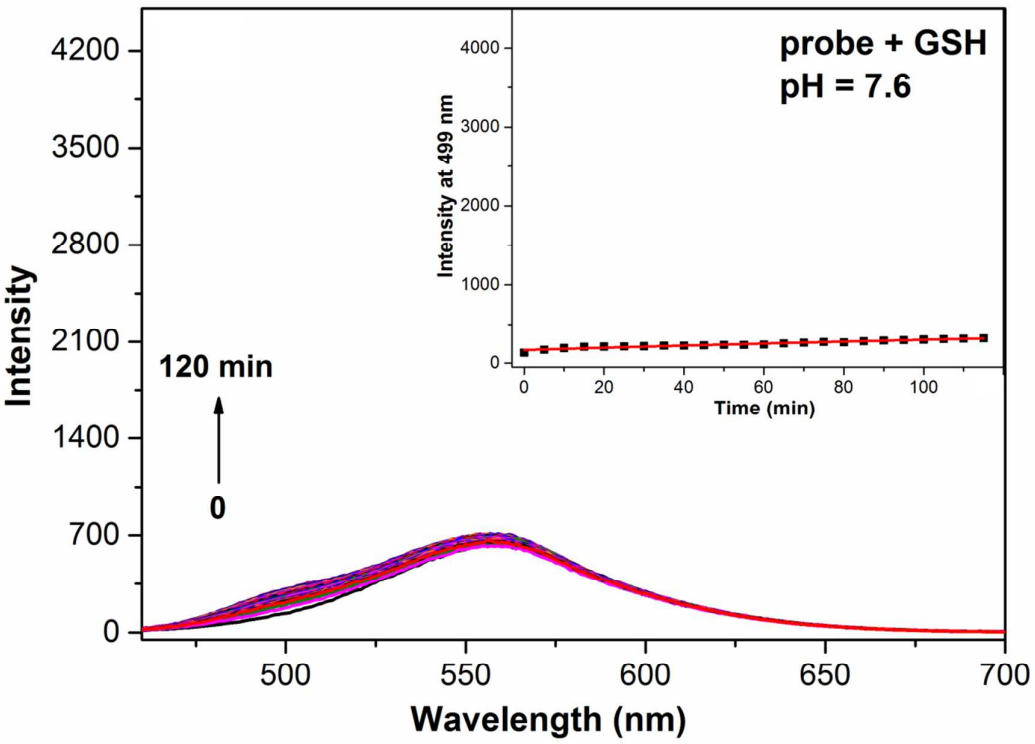
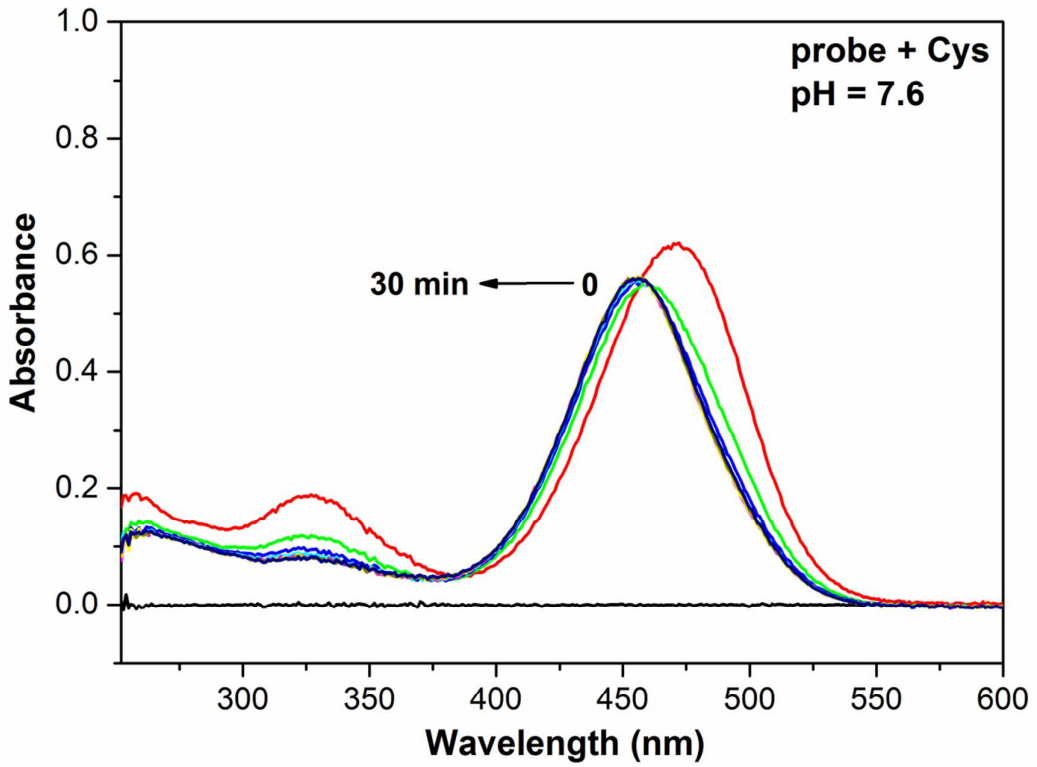

probe + GSH
pH = 7.4
Absorbance
0
60 min
Wavelength (nm)

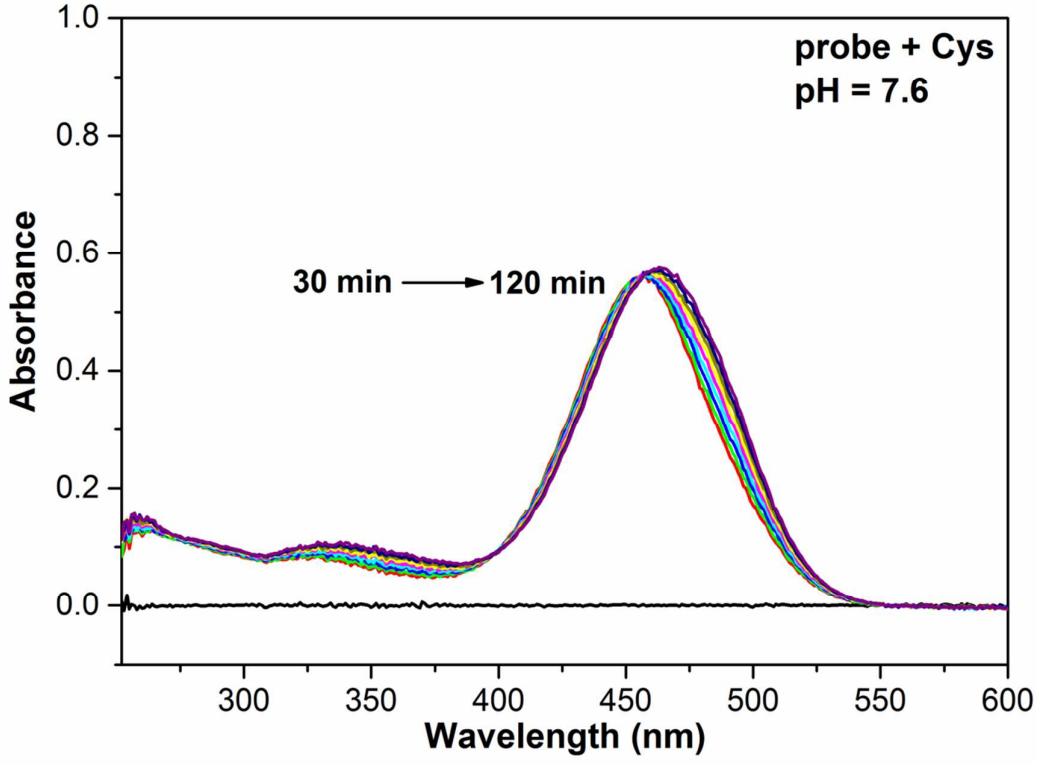
Figure S5 Fluorescent and UV-vis responses of probe 1 towards Cys, Hcy and GSH in Hepes buffer/DMSO (1:1, v/v, pH 7.6) solution at 25 °C

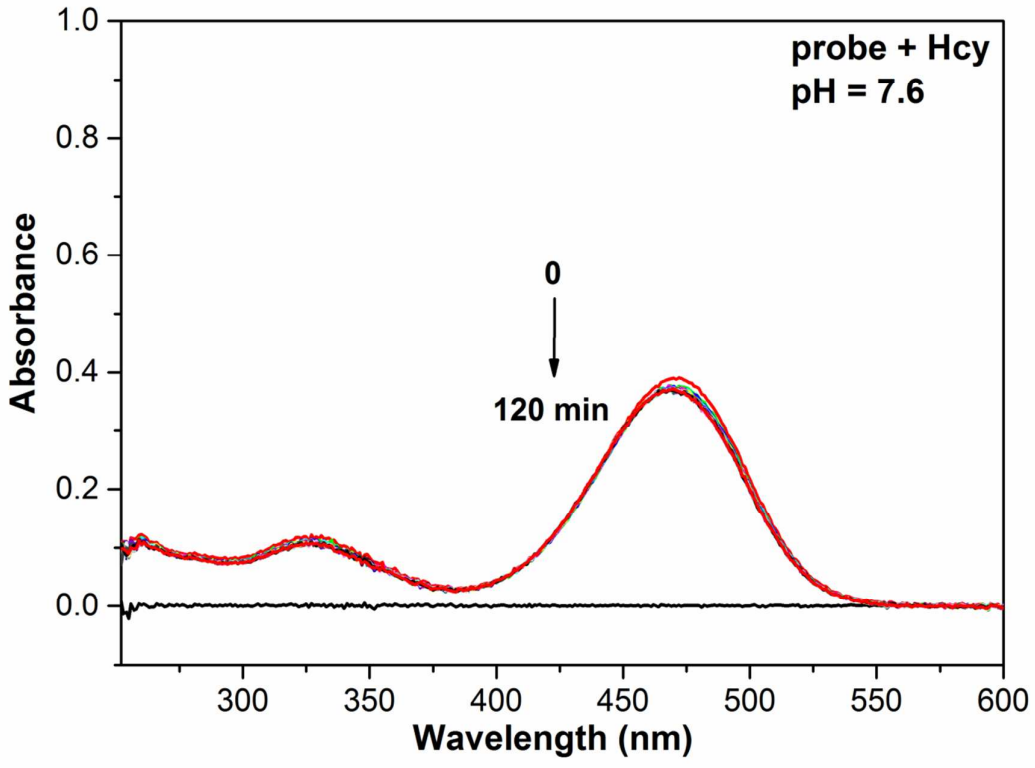



probe + Hcy
pH = 7.6
120 min
0
Intensity
Wavelength (nm)
Intensity at 499 nm
Time (min)


probe + GSH
pH = 7.6
120 min
0
Intensity
Wavelength (nm)
Intensity at 499 nm
Time (min)


probe + Cys
pH = 7.6
30 min
0
Absorbance
Wavelength (nm)
1.0
0.8
0.6
0.4
0.2
0.0
300
350
400
450
500
550
600


probe + Cys
pH = 7.6
30 min
120 min
Absorbance
Wavelength (nm)
1.0
0.8
0.6
0.4
0.2
0.0
300
350
400
450
500
550
600


probe + Hcy
pH = 7.6
0
120 min
Absorbance
Wavelength (nm)
1.0
0.8
0.6
0.4
0.2
0.0
300
350
400
450
500
550
600

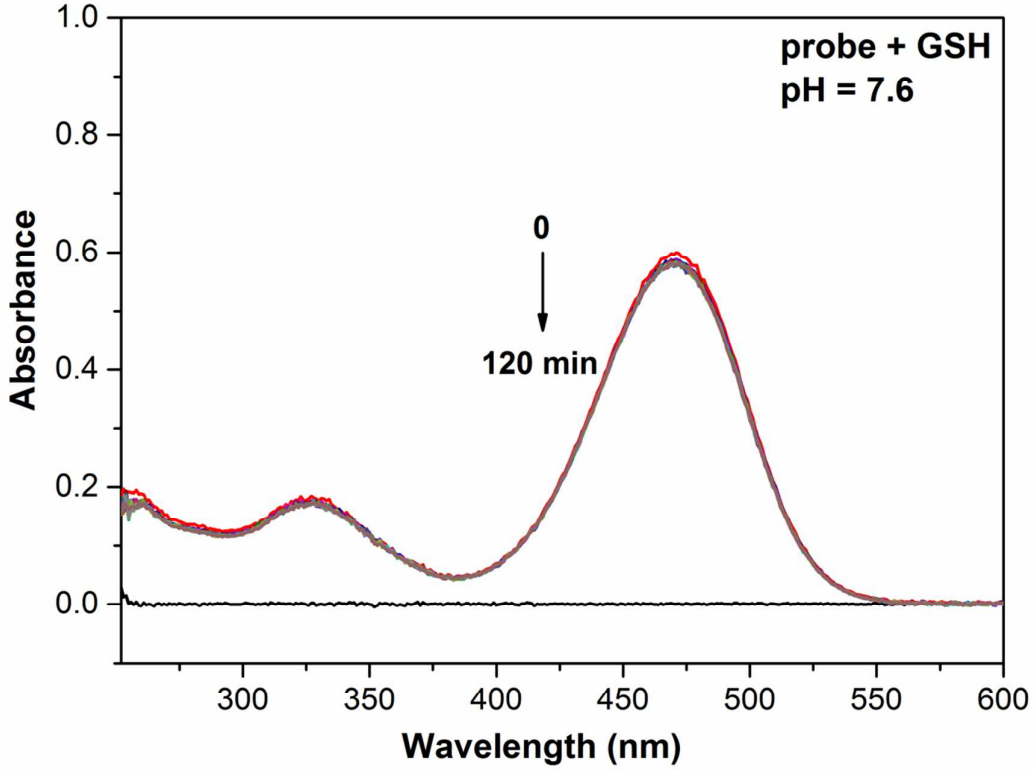
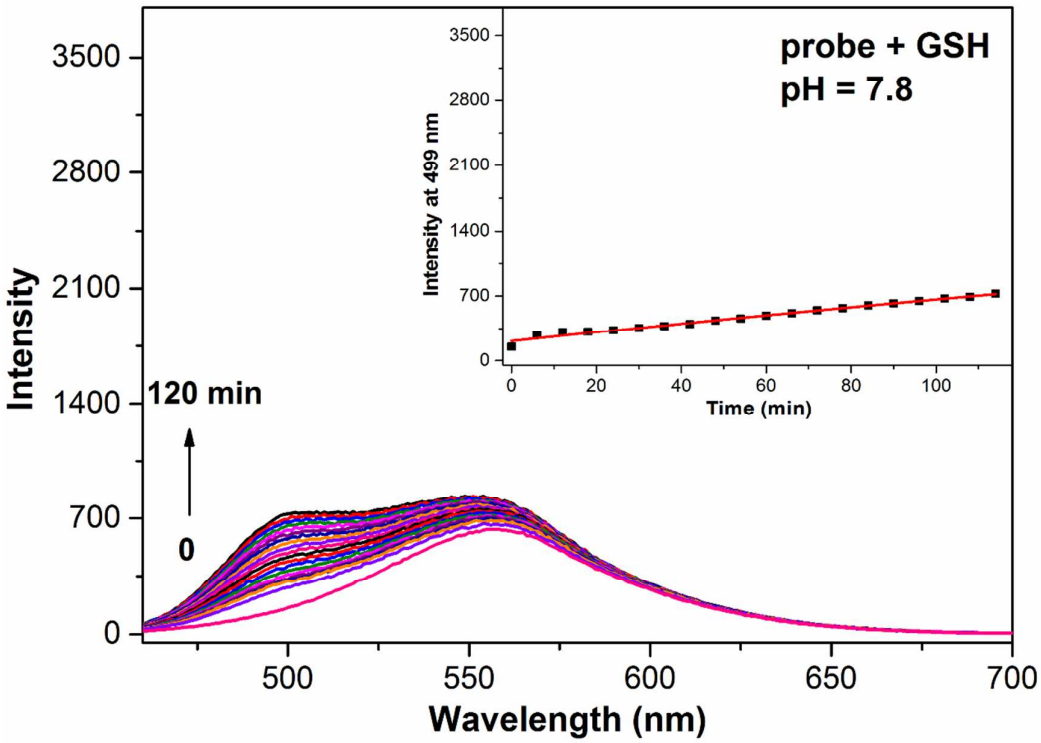
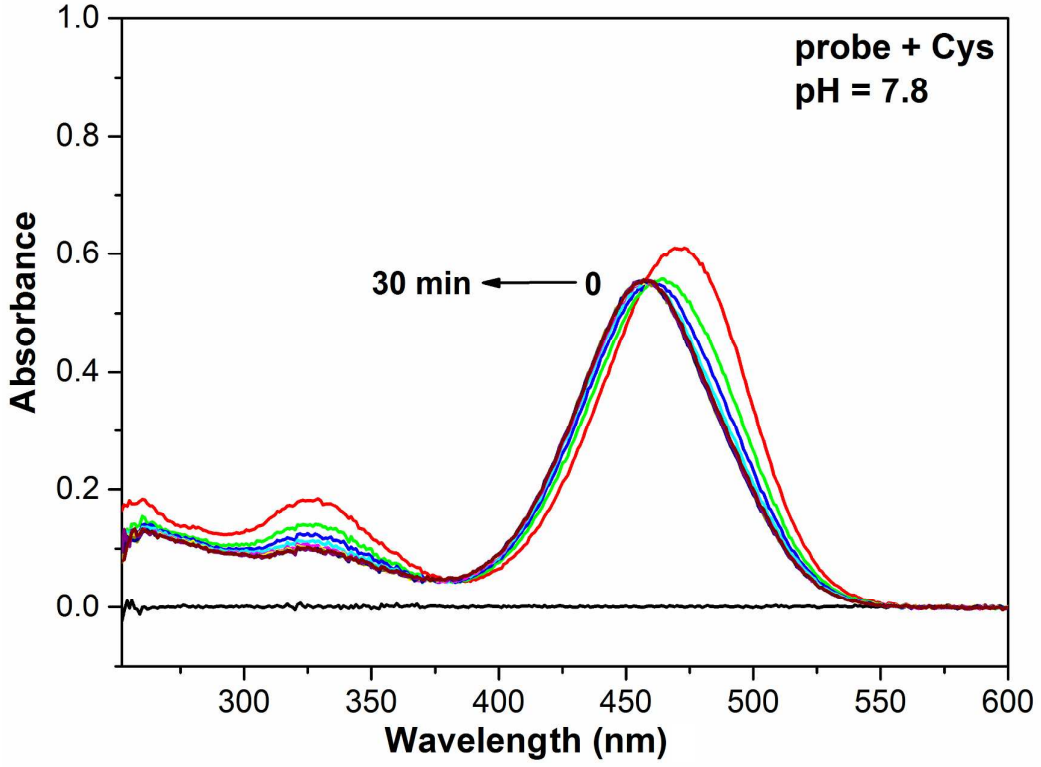
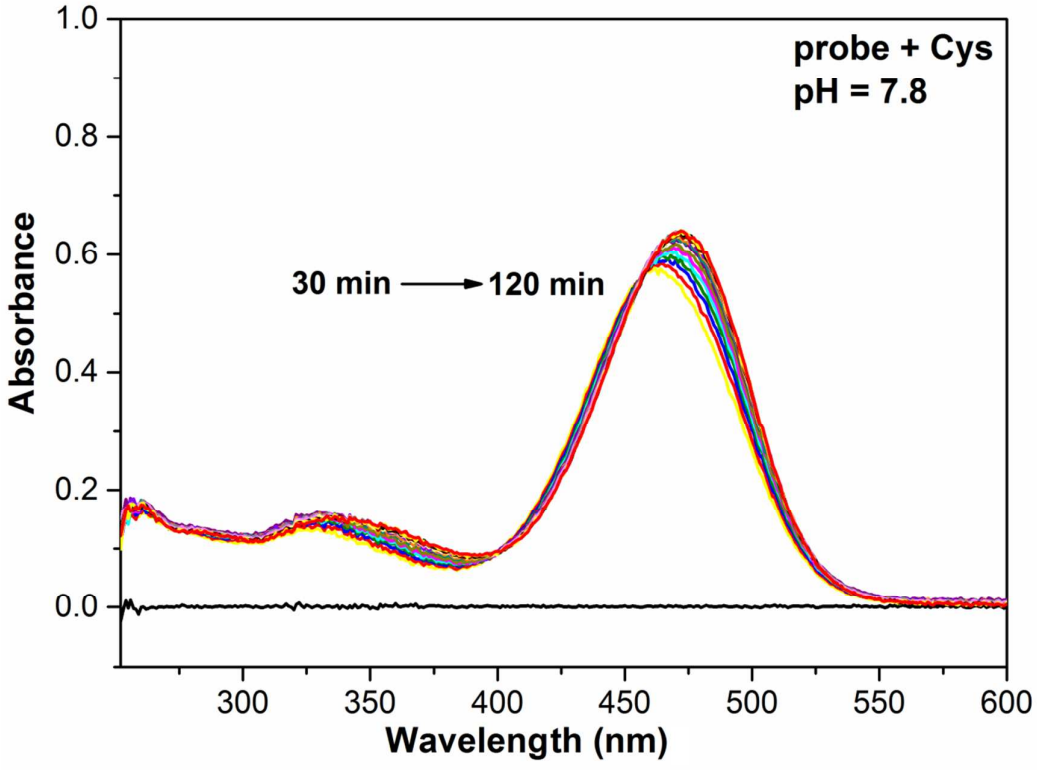
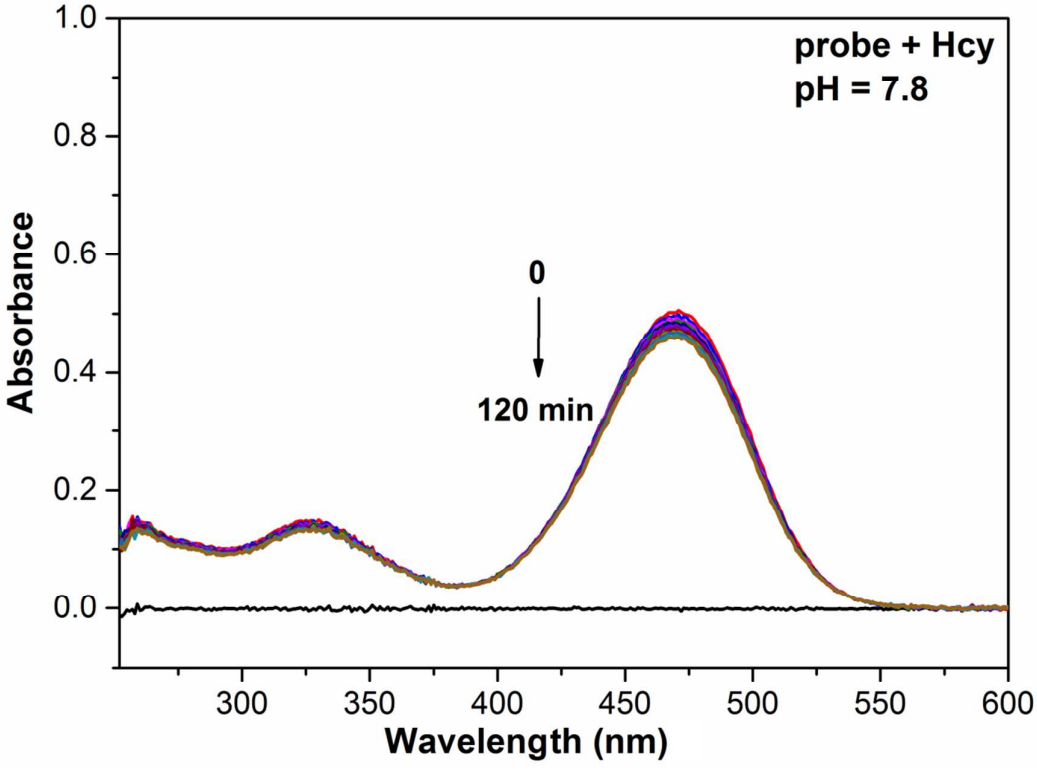

probe + GSH
pH = 7.6
0
120 min
Absorbance
Wavelength (nm)
1.0
0.8
0.6
0.4
0.2
0.0
300
350
400
450
500
550
600

Figure S6 Fluorescent responses of probe 1 towards GSH in Hepes buffer/DMSO (1:1, v/v, pH 7.8) solution at 25 °C and UV-vis responses of probe 1 towards Cys, Hcy and GSH in Hepes buffer/DMSO (1:1, v/v, pH 7.8) solution at 25 °C






probe + Cys
pH = 7.8
30 min
120 min
Absorbance
Wavelength (nm)
1.0
0.8
0.6
0.4
0.2
0.0
300
350
400
450
500
550
600


probe + Hcy
pH = 7.8
0
120 min
Absorbance
Wavelength (nm)
1.0
0.8
0.6
0.4
0.2
0.0
300
350
400
450
500
550
600

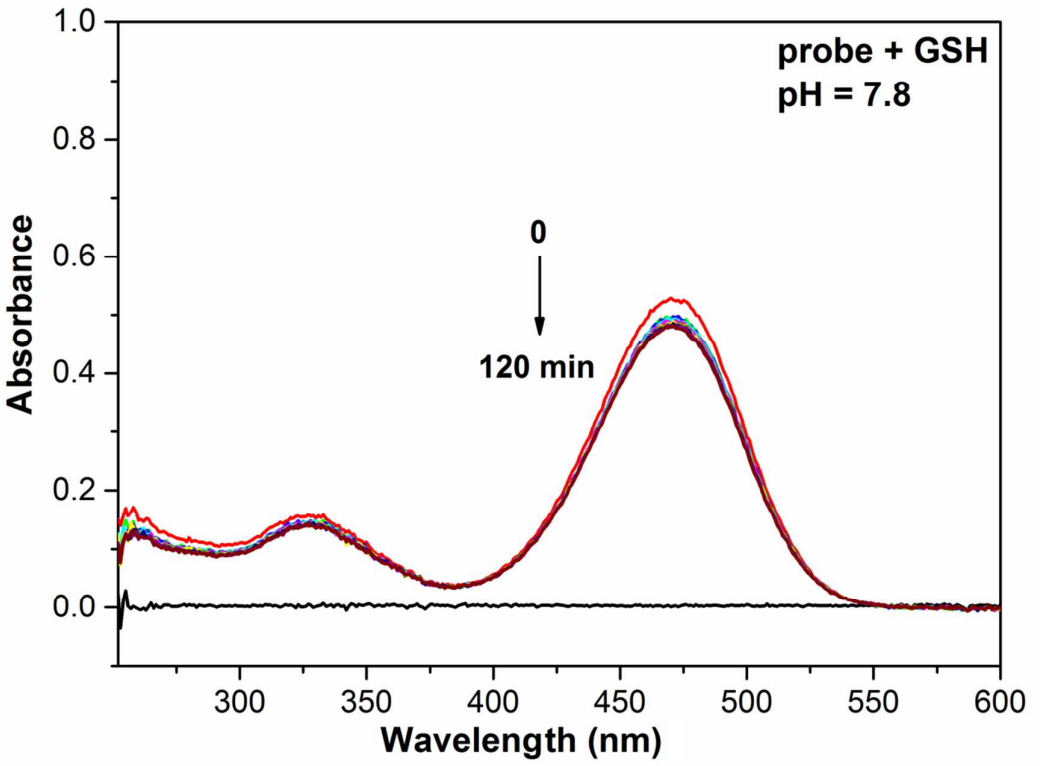
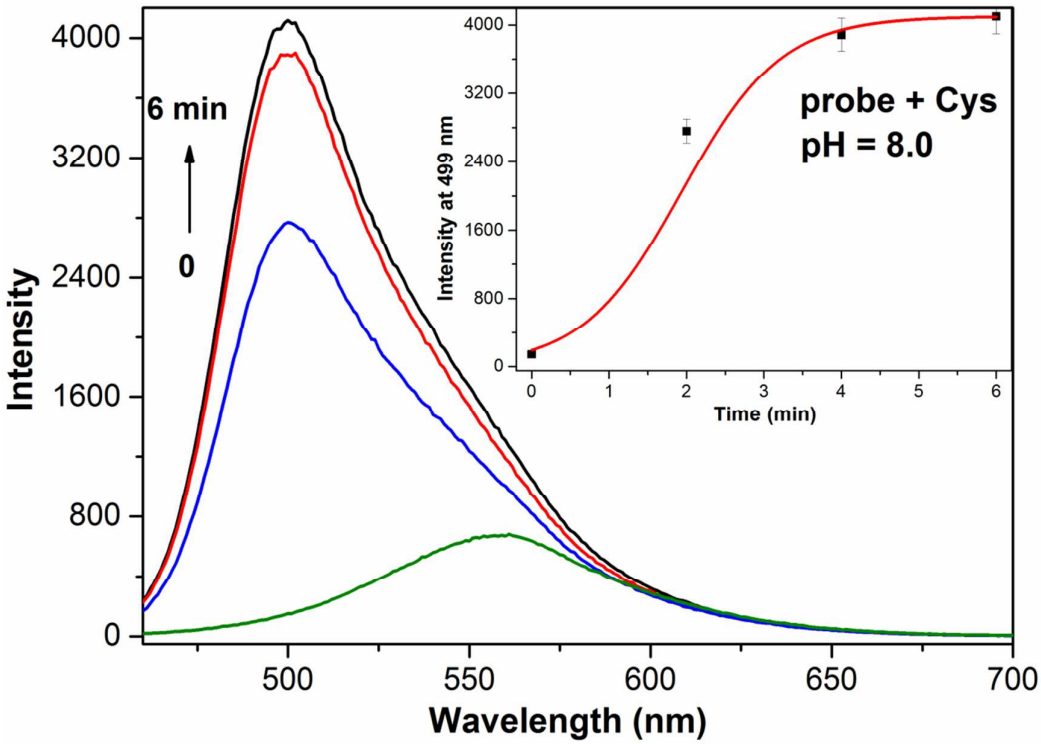
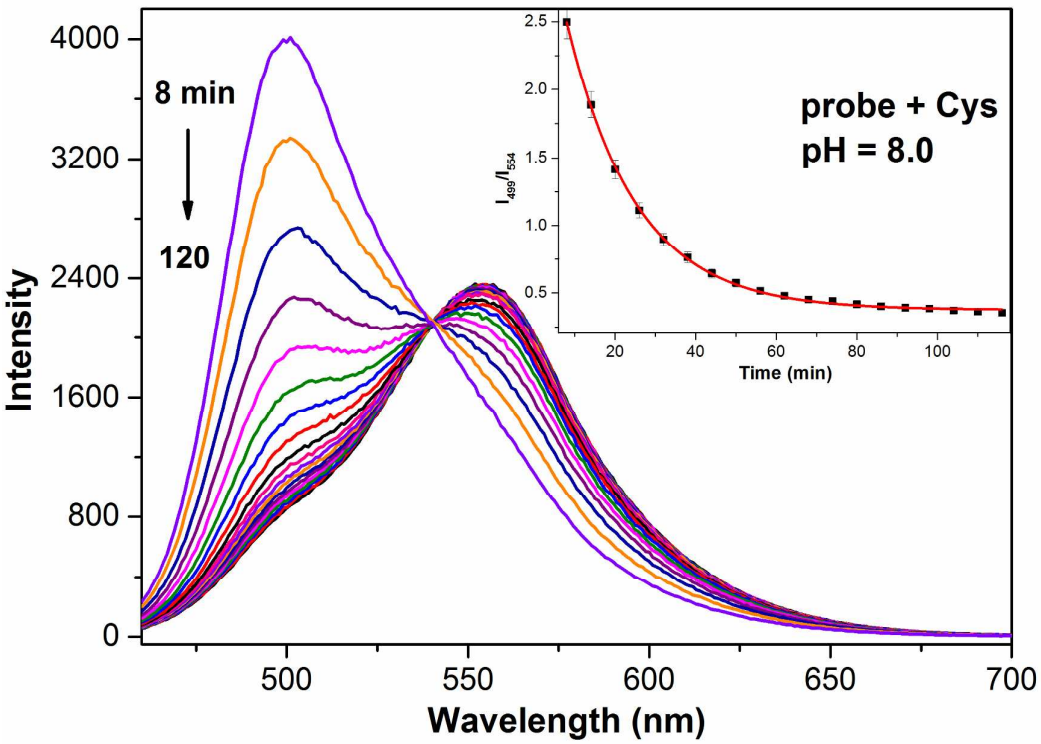
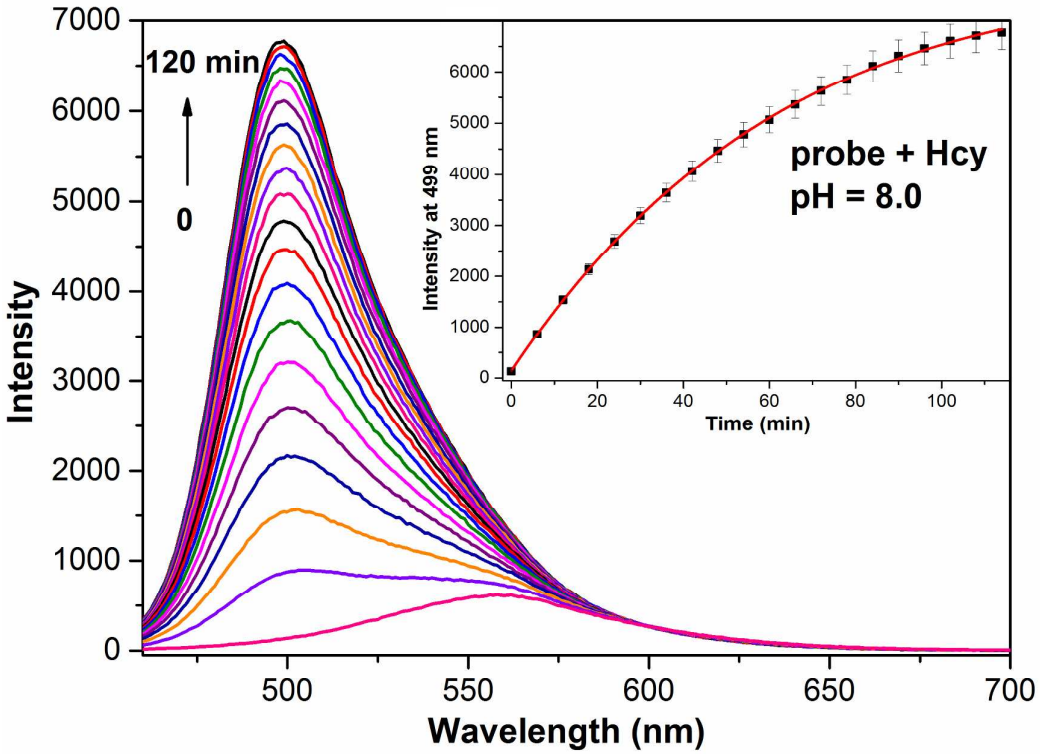

probe + GSH
pH = 7.8
0
120 min
Absorbance
Wavelength (nm)
1.0
0.8
0.6
0.4
0.2
0.0
300
350
400
450
500
550
600

Figure S7 Fluorescent responses of probe 1 towards Cys, Hcy and GSH in Hepes buffer/DMSO (1:1, v/v, pH 8.0) solution at 25 °C






120 min
0
Intensity
Wavelength (nm)
probe + Hcy
pH = 8.0
Intensity at 499 nm
Time (min)

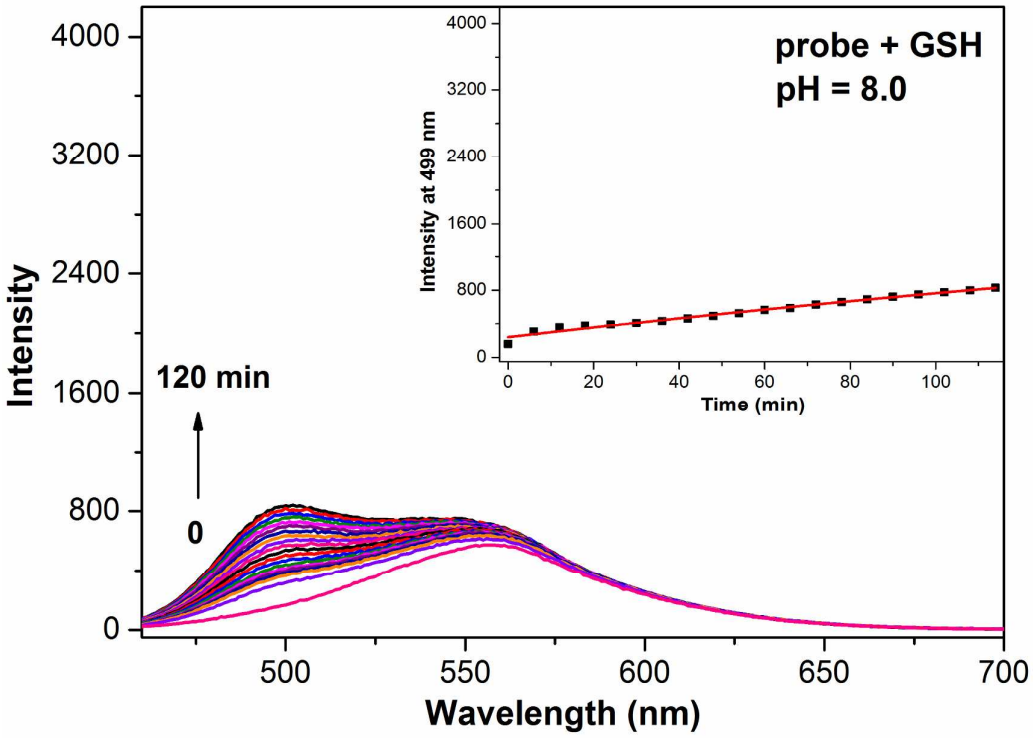

probe + GSH
pH = 8.0
120 min
0
Intensity
Wavelength (nm)
Intensity at 499 nm
Time (min)

Figure S8 Fluorescent responses of probe 1 towards various amino acids in Hepes buffer/DMSO (1:1, v/v) solution at 25 °C

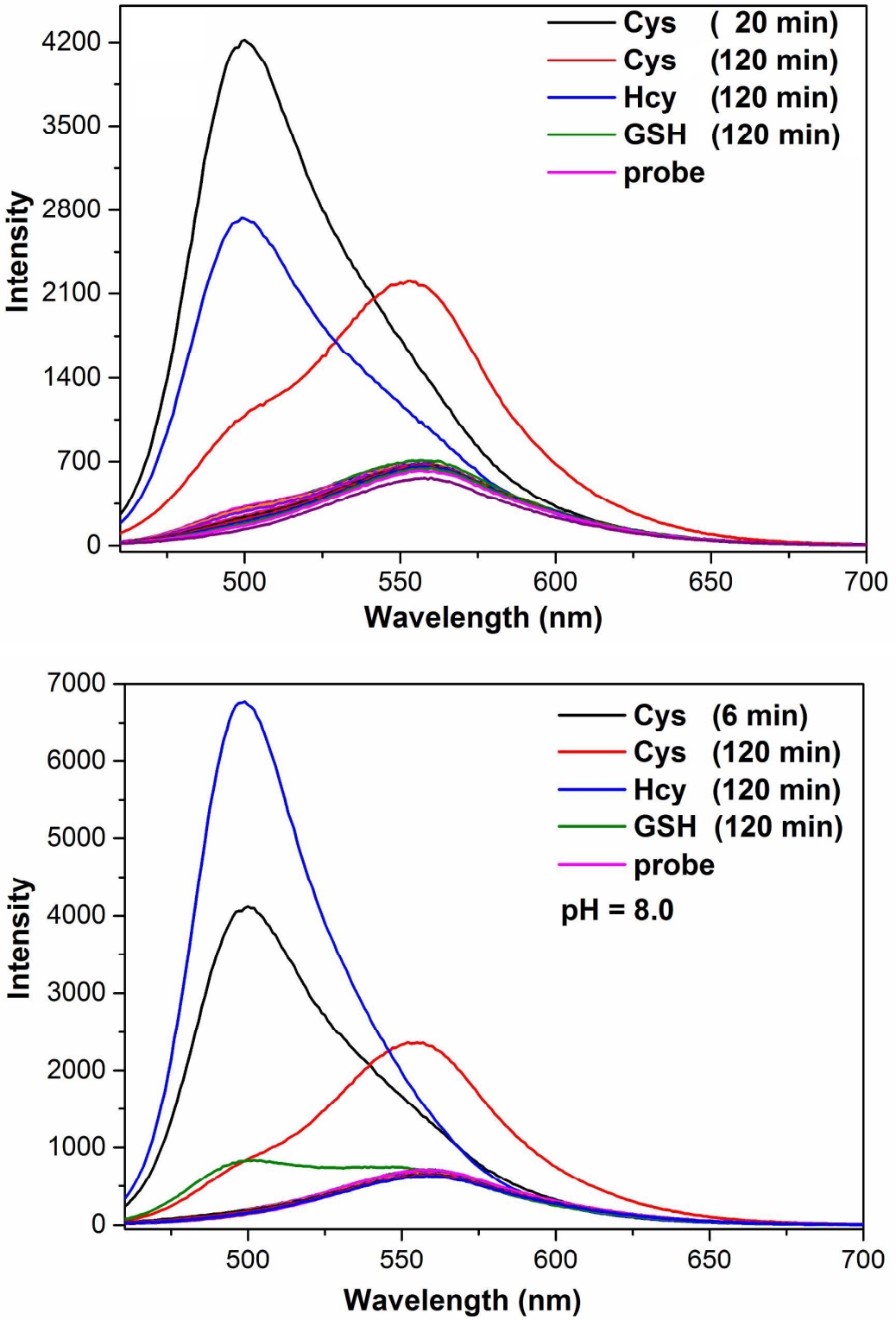


Figure S9 Cell viability values (%) estimated by MTT assay with HepG2 cells, which were cultured in the presence of 0−50 μM probe 1 for 6 and 12 h.

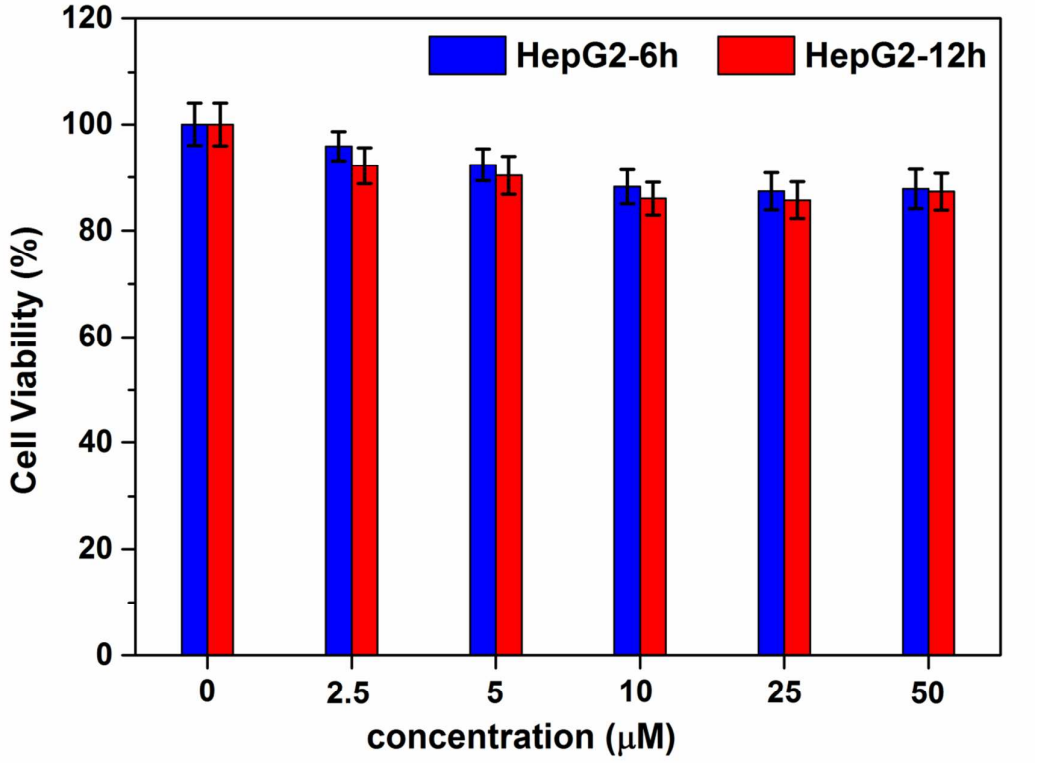


Figure S10 Confocal fluorescent image of HepG2 cells in the pH 7.4 system.

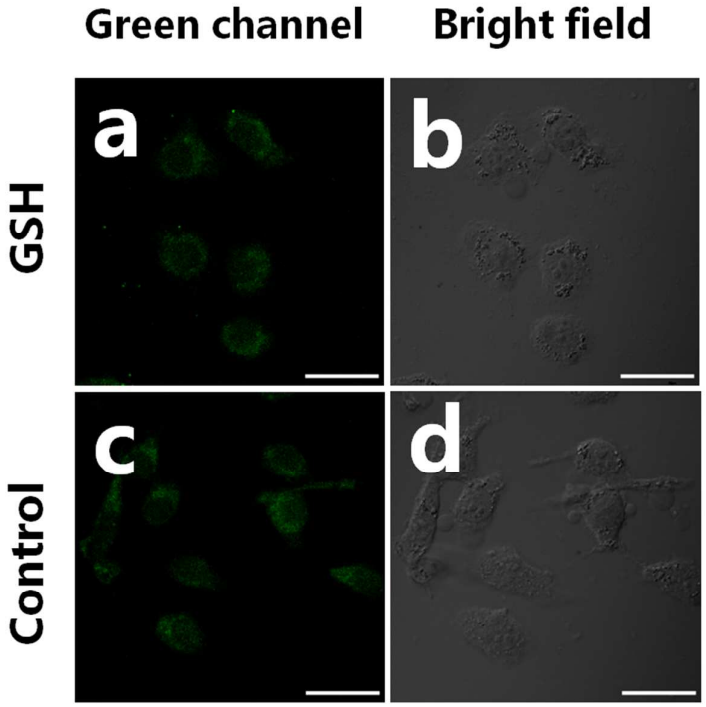


Figure S11 Ratio image of HepG2 cells in the pH 7.8 system (Red channel/Green channel).

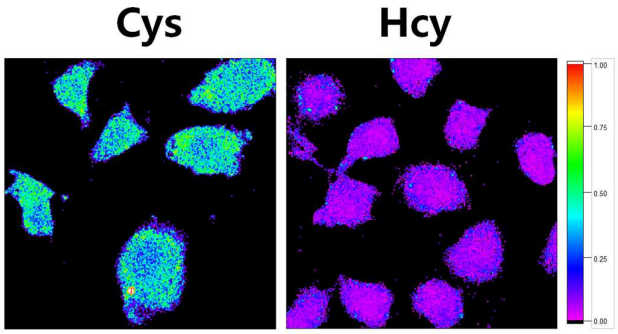


Figure S12 Confocal fluorescent image of HepG2 cells in the pH 7.8 system.