Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for

A cysteine probe with high selectivity and sensitivity promoted by response-assisted electrostatic attraction

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Experimental

1.1. General Methods and materials

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-300 (300 MHz) spectrometer with TMS as the reference. UV-vis spectra were recorded on Perkin Elmer Lambda 3500 UV-vis spectra with a 1.0 cm quartz cell. PL spectra were conducted on Fluorescence Spectrophotometer (RF-540). MALDI-TOF mass spectra were recorded on a Shimadzu MALDI AXIMA-CFR+ Spectrometer. Confocal microscopy experiments were conducted with Leica TCS MP5 Ⅱ. All the biological analytes including BSA, Insulin, ATP, GSH, and other amino acids were purchased from Aldrich and used without further purification. Other commercially available chemicals were purchased from J&K Scientific Ltd., Beijing, China. Twice distilled water was used in the whole research.

1.2. Synthesis

1.2.1. Synthesis of 3-acetyl-7- (diethylamino)-2H-1-benzopyran-2-one (3)

Ethyl acetoacetate (1.3 g, 10 mmol) and 4-(diethylamino) salicylaldehyde (2 g, 10 mmol) was dissolved in absolute methanol (20 mL), and then three drops of piperidine were added as catalyst. The mixture was stirred at room temperature for 10 h, and then a bright yellow precipitate was formed, it was collected by filter and washed with cold absolute ethanol (5 mL). Recrystallization from absolute ethanol gave compound 2 as a yellow solid (2 g, 7.8 mmol) in 78 % yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (s, 1H), 7.40 (d, J = 9.0 Hz, 1H), 6.61 (dd, J = 9.0, 2.4 Hz, 1H), 6.47 (d, J = 2.2 Hz, 1H), 3.46 (q, J = 7.1 Hz, 4H), 2.68 (s, 3H), 1.24 (t, J = 7.1 Hz, 6H).

*1.2.2. Synthesis of **2***

3-Acetyl-7-diethylaminocoumarin (3; 200.0 mg, 0.77 mmol) and 3-pyridinecarboxaldehyde (85.8 mg, 0.80 mmol) were dissolved in ethanol (20 mL), and then three drops of piperidine were added as a catalyst. The mixture was heated to reflux for 12 h, and the solvent was removed under reduced pressure. The resulting residue was then purified by chromatography on silica gel to give 2 as a brick-red solid (92 mg, 0.26 mmol) in 34 % yield: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.83 (s, 1H), 8.59 (d, J = 4.7 Hz, 1H), 8.56 (s, 1H), 8.22 (d, J = 15.8 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 15.8 Hz, 1H), 7.43 (d, J = 9.0 Hz, 1H), 7.34 (dd, J = 7.9, 4.9 Hz, 1H), 6.63 (dd, J = 9.0, 2.3 Hz, 1H), 6.49 (d, J = 2.1 Hz, 1H), 3.47 (q, J = 7.1 Hz, 4H), 1.25 (t, J = 7.1 Hz, 6H).

*1.2.3. Preparation of probe **1***

Compound **2** (100.0 mg, 0.28 mmol) and excess iodomethane were mixed with acetonitrile (15 mL), and then the mixture was as stirred in darkness at room

temperature for 10 h, and then a bright a brick-red precipitate was gained by addition of cold absolute ethanol (10 mL), the precipitate was collected by filter and recrystallization from absolute ethanol gave **1** (80 mg, 0.22 mmol, 82 %). ^{1}H NMR (300 MHz, DMSO): δ 9.39 (s, 1H), 8.96 (d, *J* = 6.0 Hz, 1H), 8.86 (d, *J* = 8.2 Hz, 1H), 8.65 (s, 1H), 8.17 (dd, *J* = 15.0, 5.4 Hz, 2H), 7.71 (dd, *J* = 12.6, 3.2 Hz, 2H), 6.84 (dd, *J* = 9.0, 2.0 Hz, 1H), 6.63 (s, 1H), 4.36 (s, 3H), 3.50 (q, *J* = 7.1 Hz, 4H), 1.48 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, DMSO): δ 185.16, 159.99, 158.58, 153.53, 149.09, 145.48, 145.23, 142.94, 134.88, 133.99, 132.80, 131.07, 127.81, 114.31, 110.64, 108.10, 96.16, 48.14, 44.47, 12.61. MS: [M^{+}] at 363.0.

1.2.4. Spectroscopic data

Stock solutions (1 mM) of the biological analytes (BSA, Cys, Hcy, GSH, Ala, Arg, Asp, Glu, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val, Fe^{3+}, Zn^{2+}, H_2O_2, ATP, and glucose) were prepared in PBS buffer solution (pH 7.4). Probe **1** (10 μM) was also prepared in this PBS buffer solution. All UV/Vis and fluorescence titration experiments were performed using 10 μM of **1** in PBS buffer solution with varying concentrations of analytes at room temperature. The time dependences of the response of **1** (10 μM) to thiols (10 μM-1 mM, respectively) were determined by mixing the two reactants in PBS buffer solution (pH 7.4). Kinetic measurements of the reactions were carried out in a single-mixing mode.

1.2.5. DFT/TDDFT Calculations

The UV-vis absorption and the emission properties of **1** were studied with DFT/TDDFT calculations at the B3LYP/6-31G(d)/level using Gaussian 09. Water was used as the solvent in the calculations (PCM model). First, the optimized ground-state geometry of **1** was obtained for several possible conformers. Using the lowest energy conformer of **1**, the structure of **1**-Cys was obtained. The UV-vis absorption was calculated by the TDDFT method based on the ground-state geometry (vertical excitation, Franck-Condon principle). The geometry of excited state was optimized and the emission was calculated with the TDDFT method (usually excited state is responsible for the fluorescence, Kasha's role). The vertical excitation and the emission related calculations were based on the optimized excited state.

1.2.6. Fluorescence quantum yields

The fluorescence quantum yields of the probe **1** were measured with quinine sulfate as standard (ϕ = 0.546 in 0.05 M H_2SO_4), according to Eq. (1), where ϕ is the quantum yield, *F* is the measured integrated emission intensity, and *A* is the optical density (absorbance). The *R* refers to the fluorophore of unknown quantum yield, and s refers to the reference fluorophore (quinine sulfate) of known quantum yield:

$$\phi_R = \phi_S \left(\frac{F_R}{F_S}\right)\left(\frac{A_S}{A_R}\right)\left(\frac{\eta_R^2}{\eta_S^2}\right) \quad (1)$$

1.2.7. Detection limit[1]

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of 1 without Cys was measured by 10 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and the Cys concentration could be obtained in the 0-10 μM (R = 0.9934). 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 25 nM at S/N = 3.

1.2.8. Fluorescence detection of Cys34 within Bovine serum albumin (BSA)

Native BSA (66 mg, 0.1 mM) was dissolved in 10 mL of PBS buffer solution (pH 7.4), and then stored at 4 ℃. BSA were treated with 0.1 mM of probe **1** for 2 hours, and then fluorescent experiments were conducted. Denatured BSA was prepared in the following ways. (1) Native BSA (66 mg, 0.1 mM) was dissolved in 10 mL of PBS buffer solution (pH 7.4), to which guanidininum hydrochloride (GdnHCl) (5 mM) was added, the mixed solution was slowly stirring for 30 min at room temperature, and then stored at 4 ℃; (2) A solution of 10 mL native BSA (0.1 mM) in PBS buffer solution (pH 7.4) was continuously vibrated by ultrasonic oscillator for 30 min at room temperature, and then stored at 4 ℃. Both native and denatured BSA was treated with 0.1 mM of probe 1 for 2 hours, and then fluorescent experiments were conducted.

1.2.9. Reduction of disulfide bonds in protein by dithiothreitol (DTT)

Native BSA (66 mg, 0.1 mM) in PBS buffer solution was treated with DTT (10 mM) for 3 h, and then the excess DTT was removed by a dialysis membrane (1000 Da). The reduction procedure of insulin is the same to BSA.

1.2.10. Cell culture and confocal microscopy experiments

The HeLa cells were cultured on the surface of glass slide in SPP medium (1% proteose peptone, 0.2% glucose, 0.1% yeast extract, 0.003% EDTA ferric sodium salt) at 37˚C. For confocal microscopy experiments, HeLa cells were stained with compound **2** (5 μM), co-stained with probe **1** (5 μM) and Moti-tracker (2 μM) for 1 h at 37 ˚C and washed 3 times with PBS, and then used individual exciting light (λ_{450} nm for **1** and λ_{540} nm for Mito-tracker, respectively) to gain fluorescent pictures.

Synthetic route of probe 1:

Reagents and conditions: (a) $CH_3COCH_2COOC_2H_5$, methanol, piperidine, r.t, 10 h; (b) 3-pyridinecarboxaldehyde, piperidine, ethanol, reflux, 12 h; (c) iodomethane, acetonitrile, darkness, r.t, 10 h.

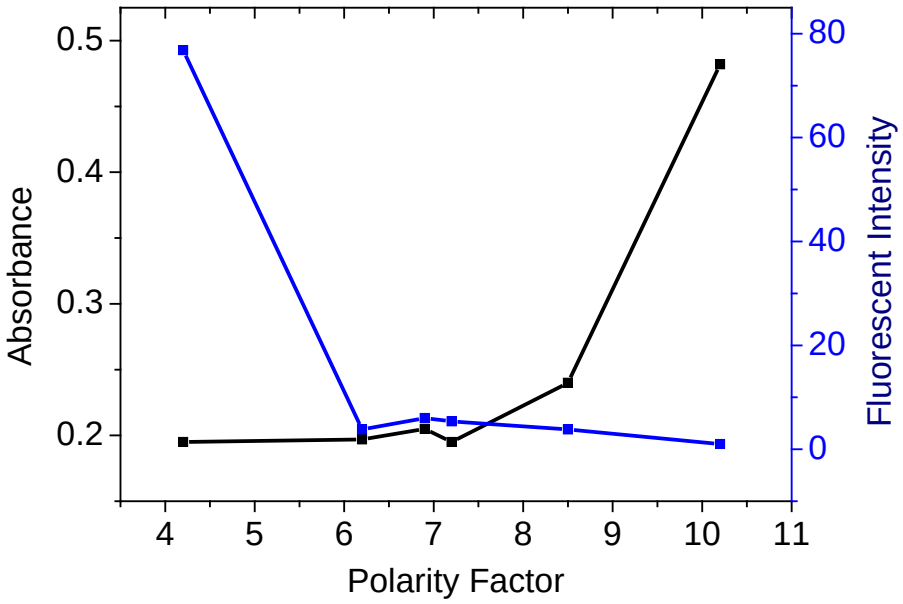

Figure S1. Absorbance and fluorescence changes of probe **1** (10 μM) in various solutions with different polarities.

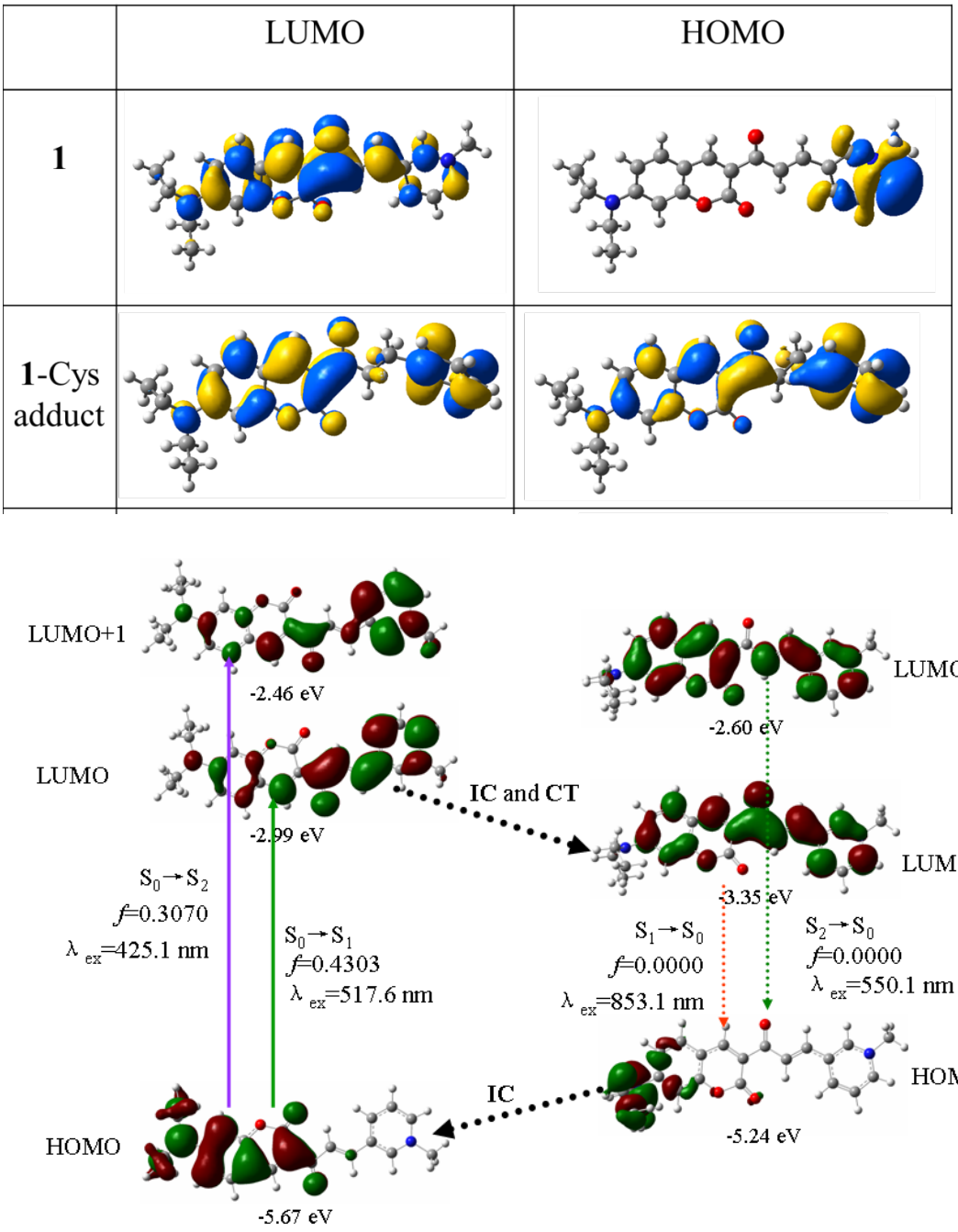

Figure S2. Rationalization of the UV−vis absorption and the weak emission of 1: the frontier molecular orbitals (MOs) involved in the vertical excitation (i.e., UV−vis absorption, the left columns) and emission (the right column). IC stands for internal conversion and CT stand for conformation transformation. Excitation and radiative decay processes are marked as solid lines and the nonradiative processes are marked by dotted lines.

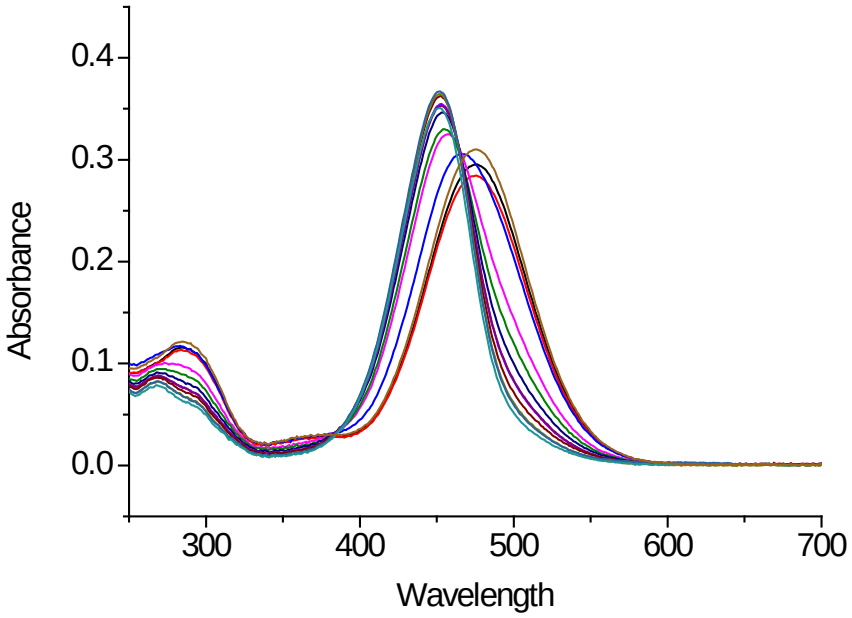

Figure S3. Absorbance spectra of probe **1** (10 μM) in PBS buffer solution (pH 7.4), upon addition of Cys (0-10 μM).

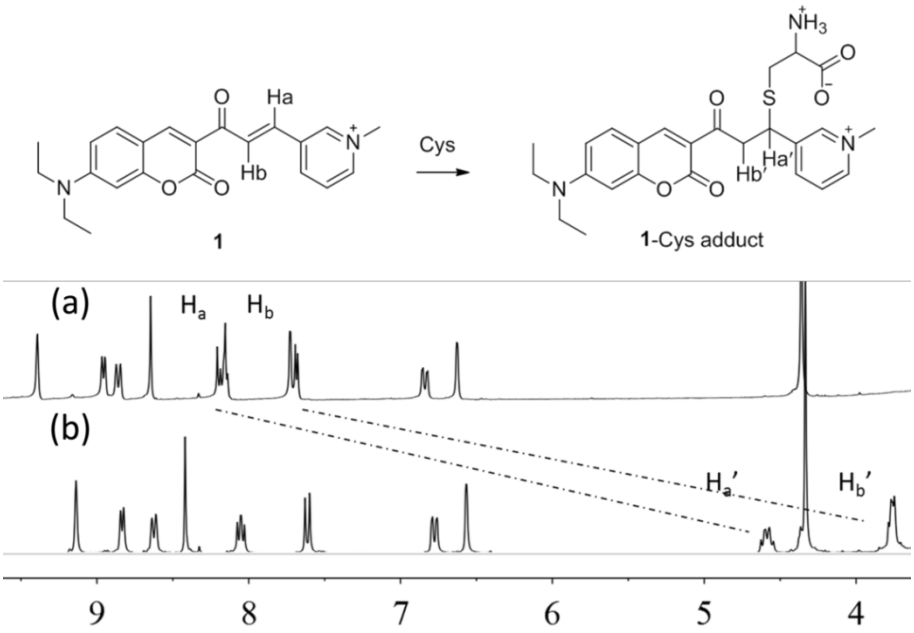

Figure S4. ^{1}H NMR spectral changes of probe **1** (10 mg) upon addition of Cys (1 equiv.) in DMSO-d_6 at room temperature. a) Probe **1** only, b) probe **1**-Cys adduct.

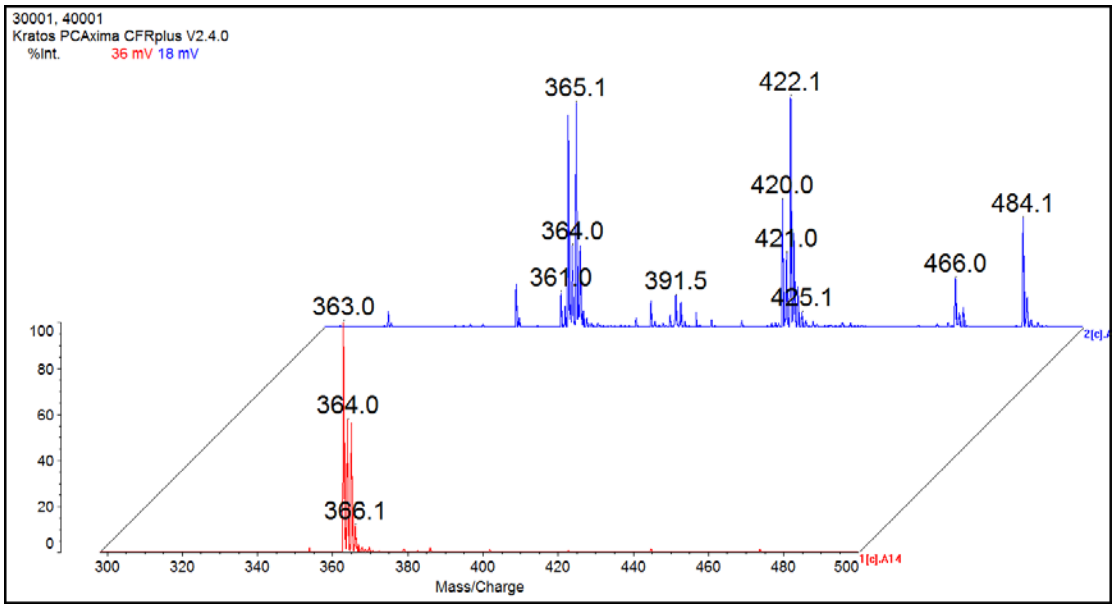

Figure S5. TOF-MS spectral changes of probe **1** upon addition of Cys (1 equiv.) in PBS buffer solution (pH 7.4) at room temperature. Probe **1** only (bottom), probe **1**-Cys adduct (top).

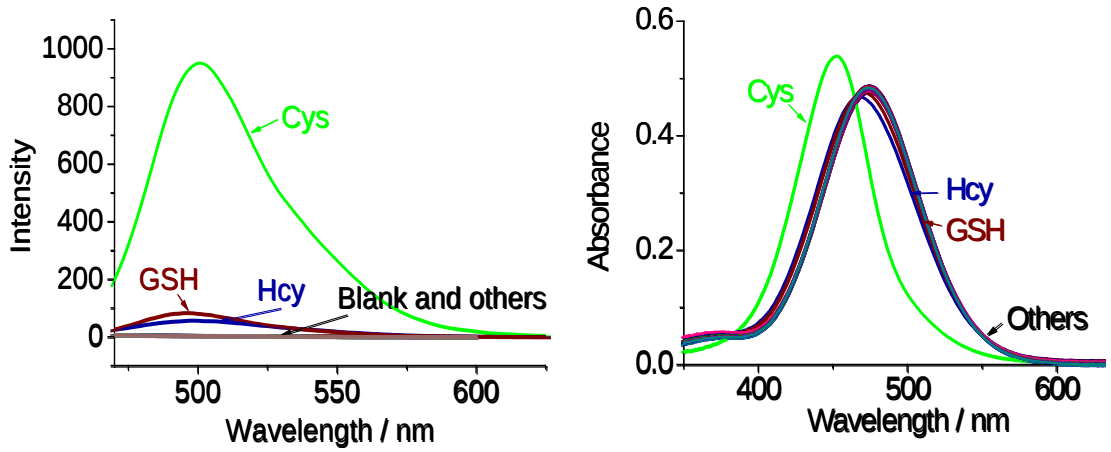

Figure S6. Fluorescence spectra (a) and absorbance spectra (b) of probe **1** (10 μM) towards various analytes (1 mM) in PBS buffer solution (pH 7.4).

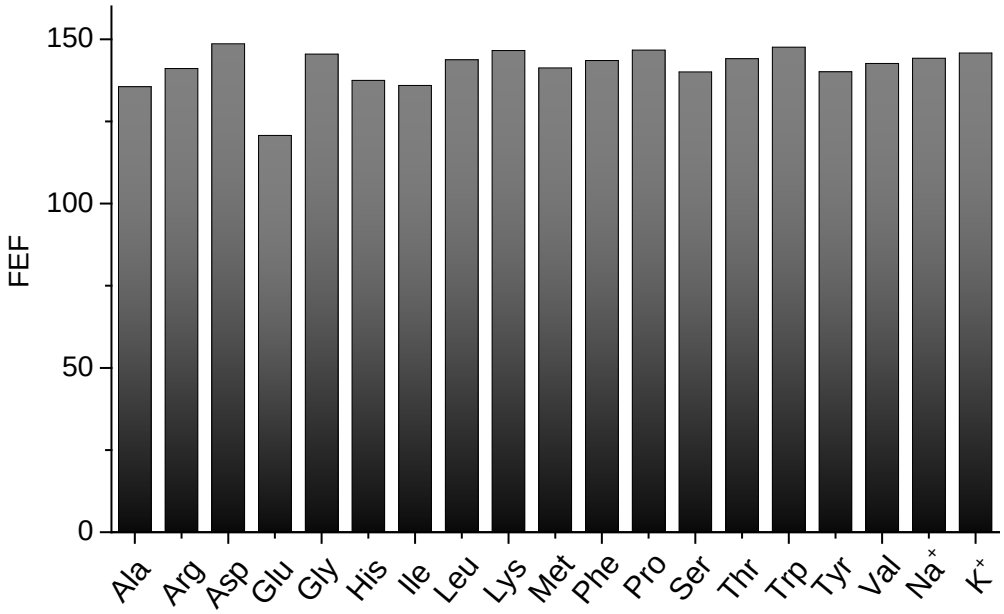

Figure S7. Fluorescence-enhancement factor (FEF) of probe **1** (10 μM) toward Cys (0.1 mM) in the presence of different competing analytes (1 mM) in the PBS buffer solution (pH 7.4).

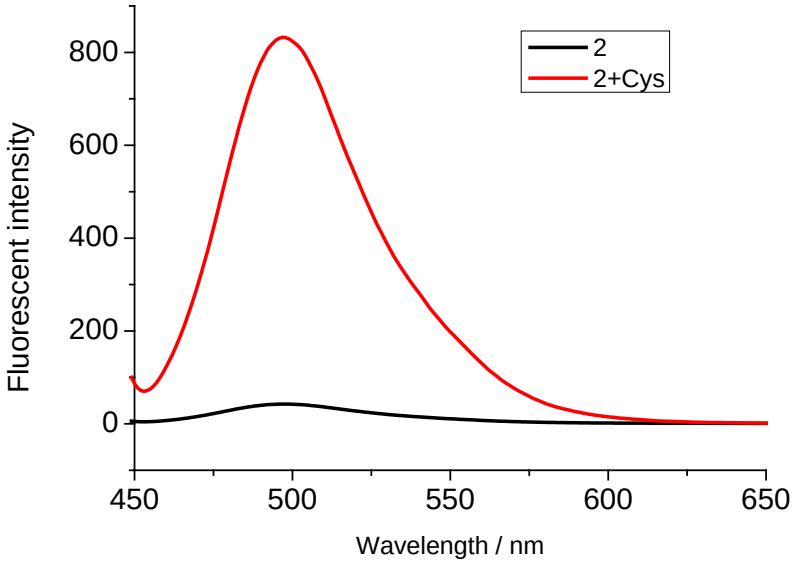

Figure S8. Fluorescent response of compound **2** (10 μM) towards Cys (1 mM) in PBS buffer solution containing 1% methanol (pH 7.4).

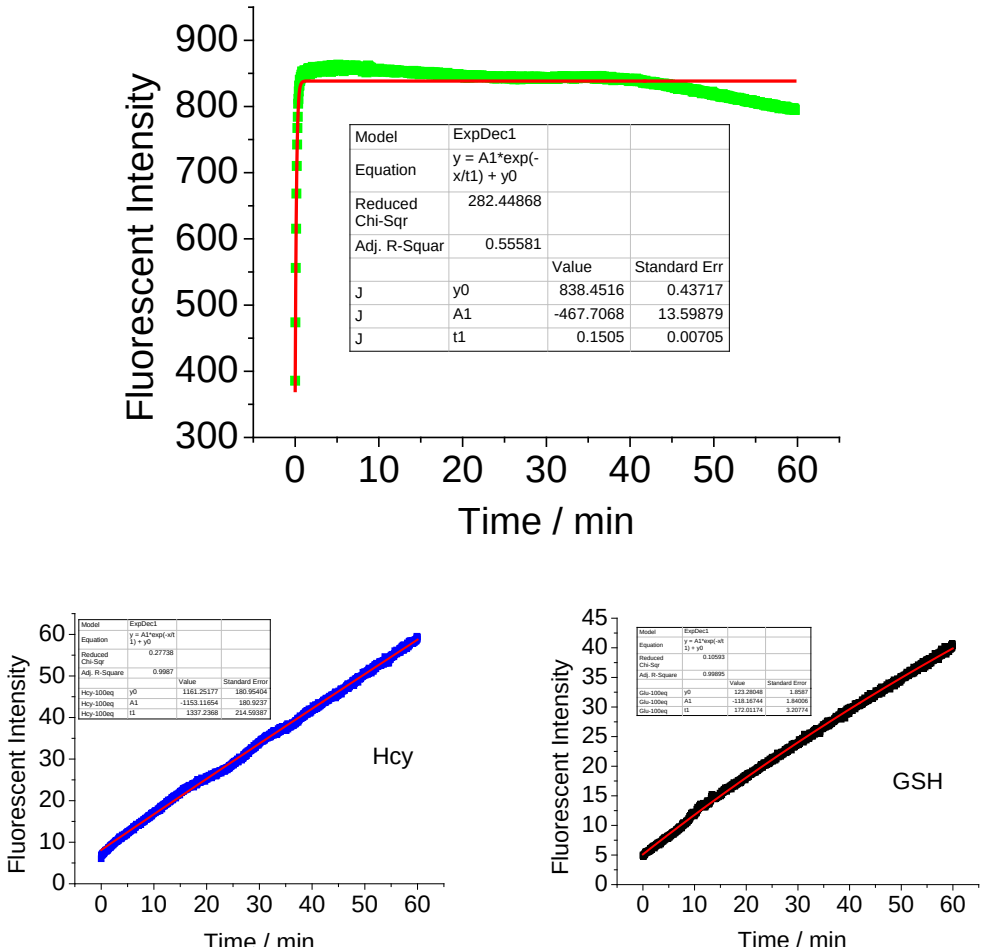

Figure S9. Time course of the fluorescence response of probe 1 (10 μM) in PBS buffer solution (pH 7.4), regarding the presence of 1 mM of Cys, Hcy, or GSH. The fluorescence intensity was recorded near 508 nm at room temperature. The boxes indicate kinetic analysis results based on first order decay model.

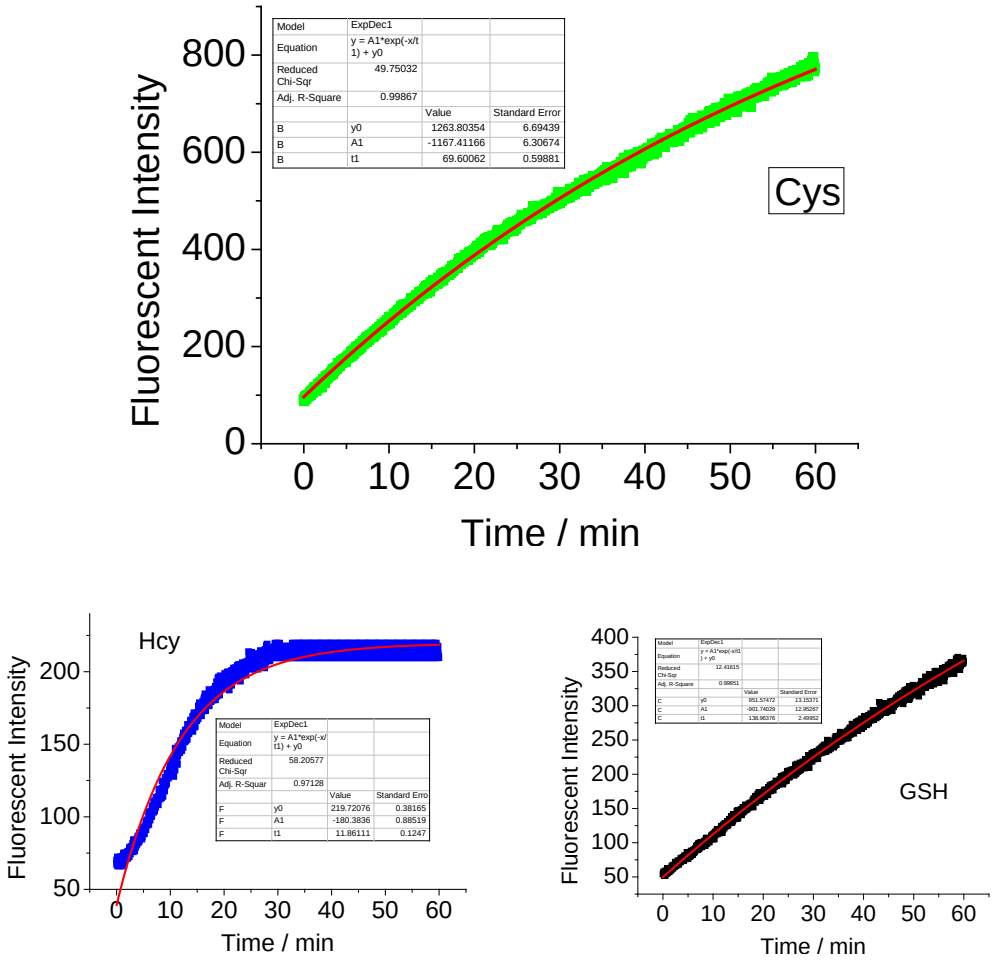

Figure S10. Time course of the fluorescence response of compound **2** (10 μM) in PBS buffer solution containing 1% methanol (pH 7.4), regarding the presence of 1 mM of Cys, Hcy, or GSH. The fluorescence intensity was recorded near 500 nm at room temperature. The boxes indicate kinetic analysis results based on first order decay model.

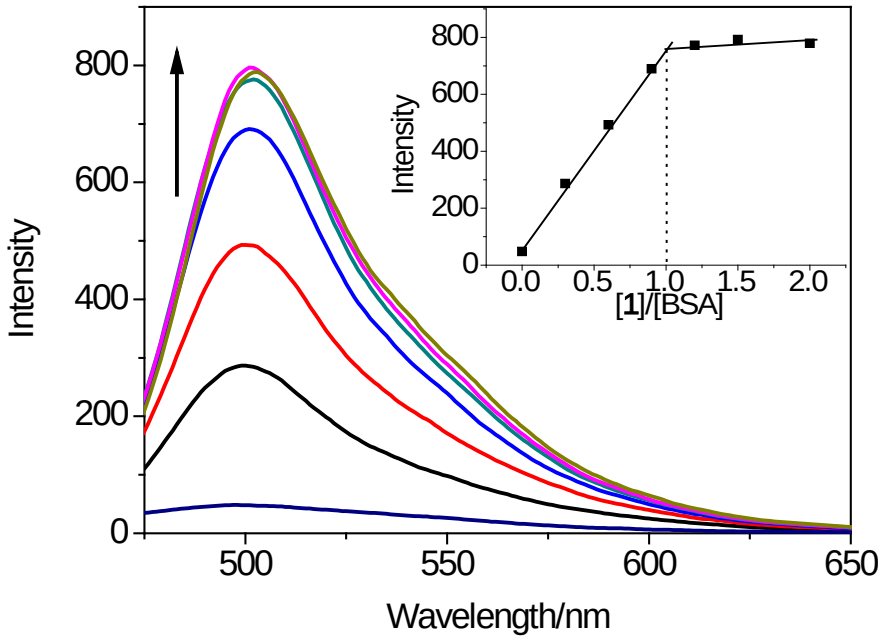

Figure S11. Fluorescent titrations of BSA (10 μM) towards probe **1** (0-2 equiv.) in PBS buffer solution (pH 7.4). Inset: the stoichiometric ratio between BSA and Probe **1**.

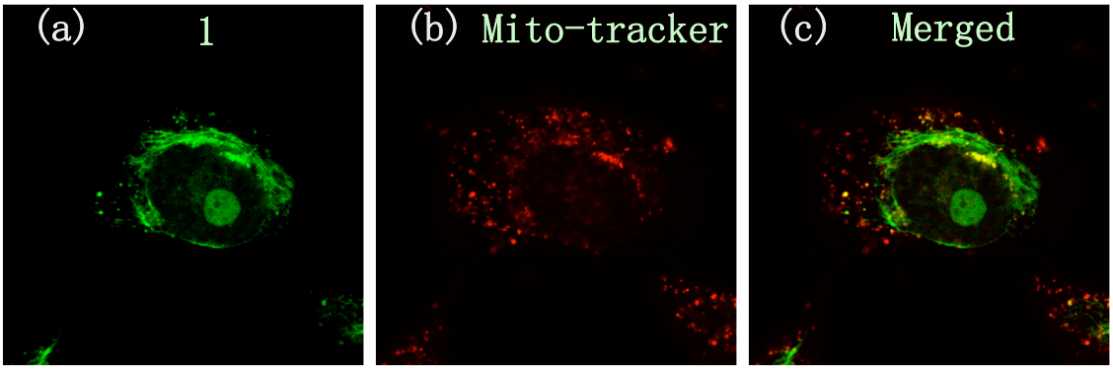

Figure S12. Confocal fluorescence images of single HeLa cell co-stained with probe **1** (5 μM) and Mito-tracker (2 μM).

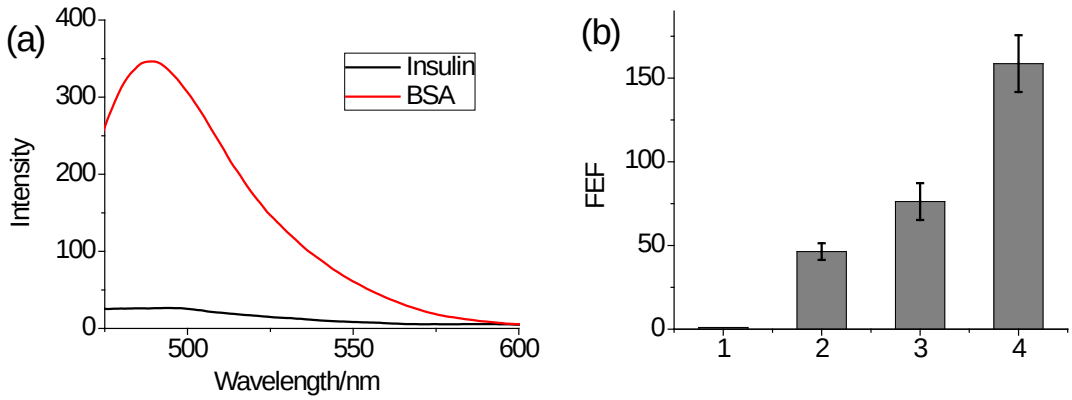

Figure S13. (a) The fluorescent response of probe **1** towards native insulin (without free Cys), and native BSA (with only one free cys34). All the concentrations of proteins are maintained at 0.1 mM in PBS buffer solution (pH 7.4). The date was recorded after 2 h. (b) Fluorescent responses of probe **1** (0.1 mM) in PBS buffer solution (pH 7.4) at room temperature towards different BSA (0.1 mM): (1) without BSA, (2) native BSA, (3) denatured BSA treated with GdnHCl (5 mM), (4) denatured BSA by ultrasound.

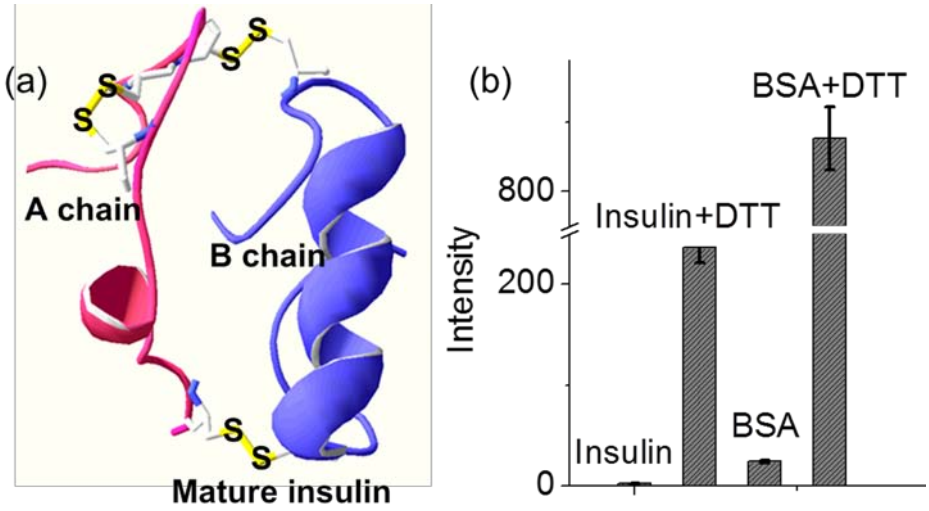

Figure S14. (a) The structure of mature insulin containing three disulfide bonds. (b) The fluorescent response of probe **1** (1 mM) towards native insulin (without free Cys), DTT treated insulin, native BSA (with only one free cys34), and DTT treated BSA. All the concentrations of proteins are maintained at 0.1 mM in PBS buffer solution (pH 7.4). The date was recorded after 30 min.

Figure S15. The modeling structure of BSA obtained, where Cys34 is covalently conjugated with probe **1**.

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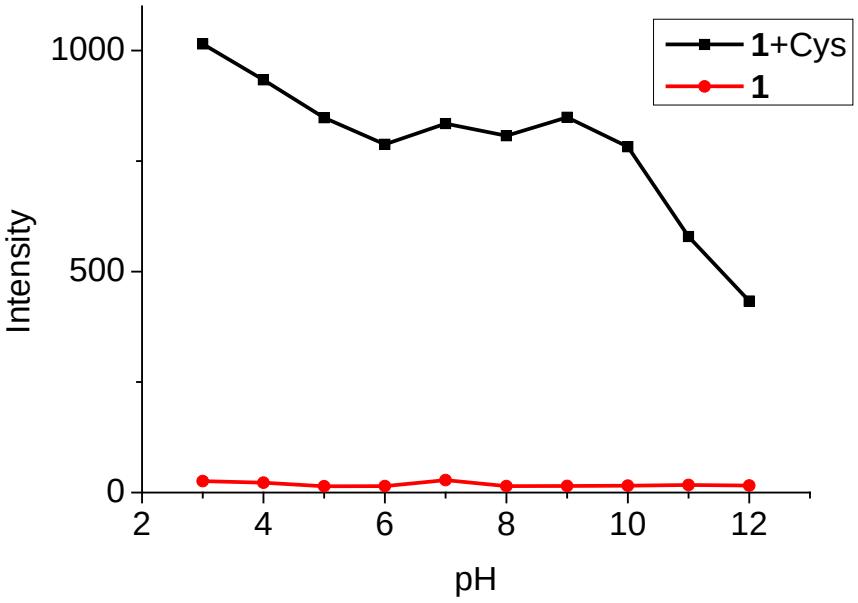


Figure S16. The fluorescence intensities for probe **1** in the absence or presence of Cys (10 equiv.) at various pH values.

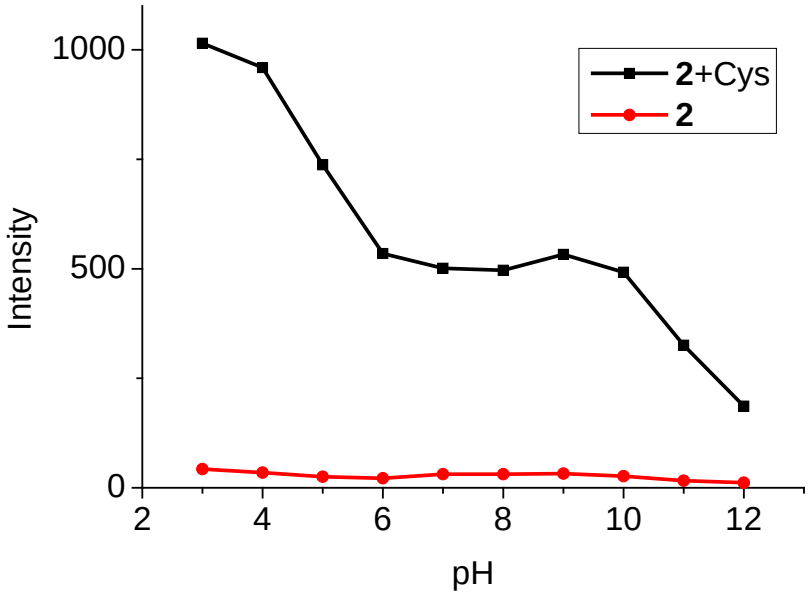


Figure S17. The fluorescence intensities for compound **2** in the absence or presence of Cys (10 equiv.) at various pH values.

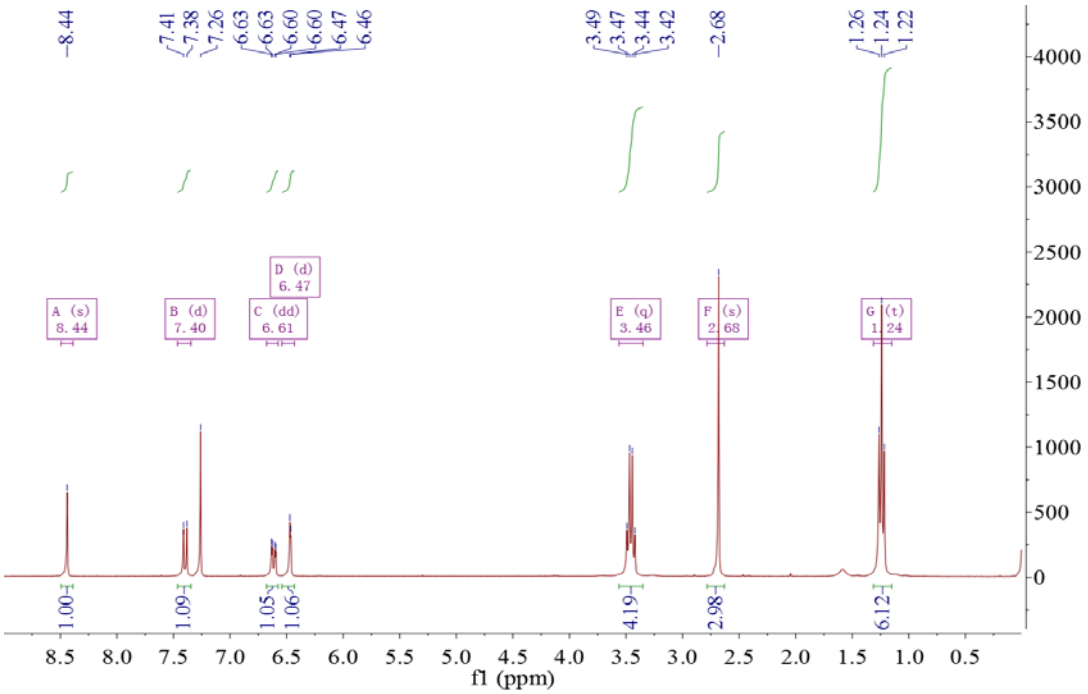


Figure S18. ^{1}H NMR spectra of **3**.

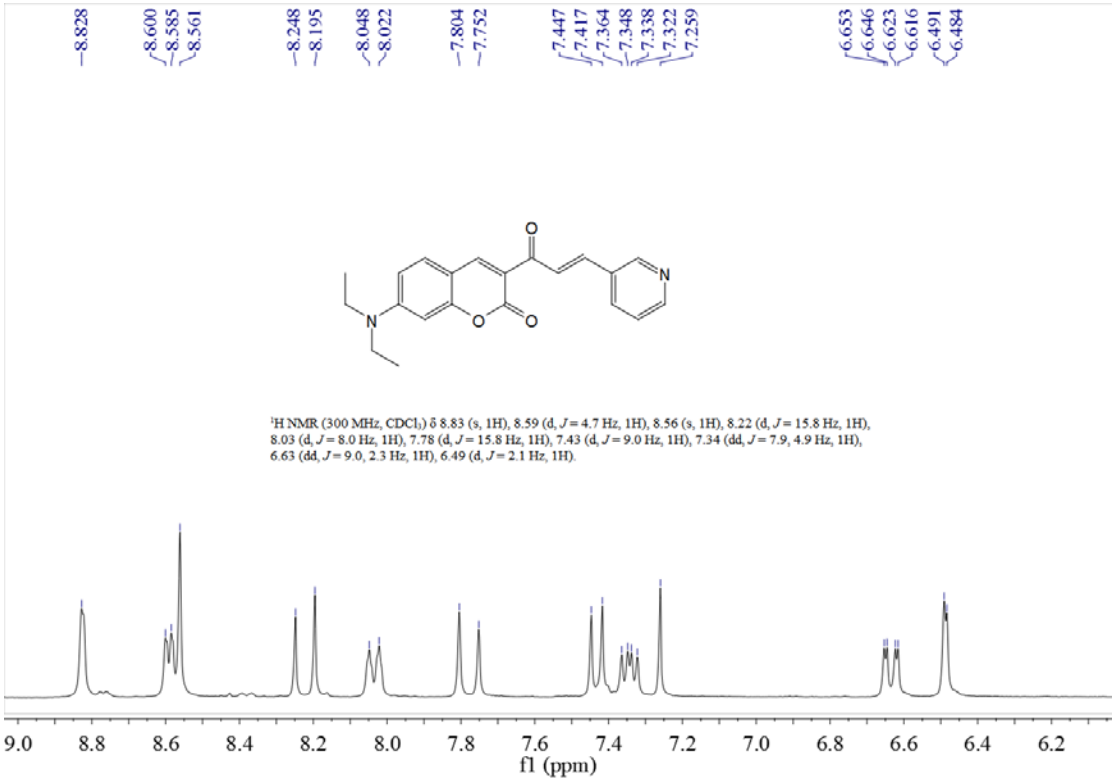
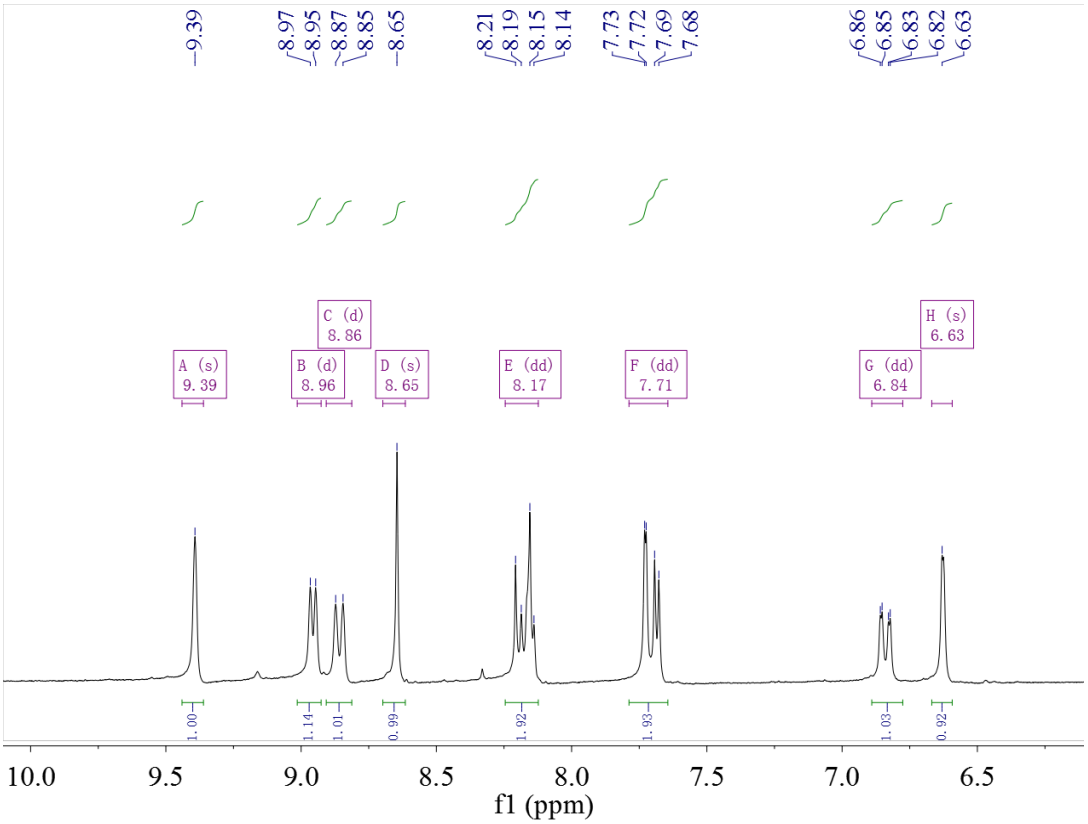


Figure S19. ^{1}H NMR spectra of compound **2**



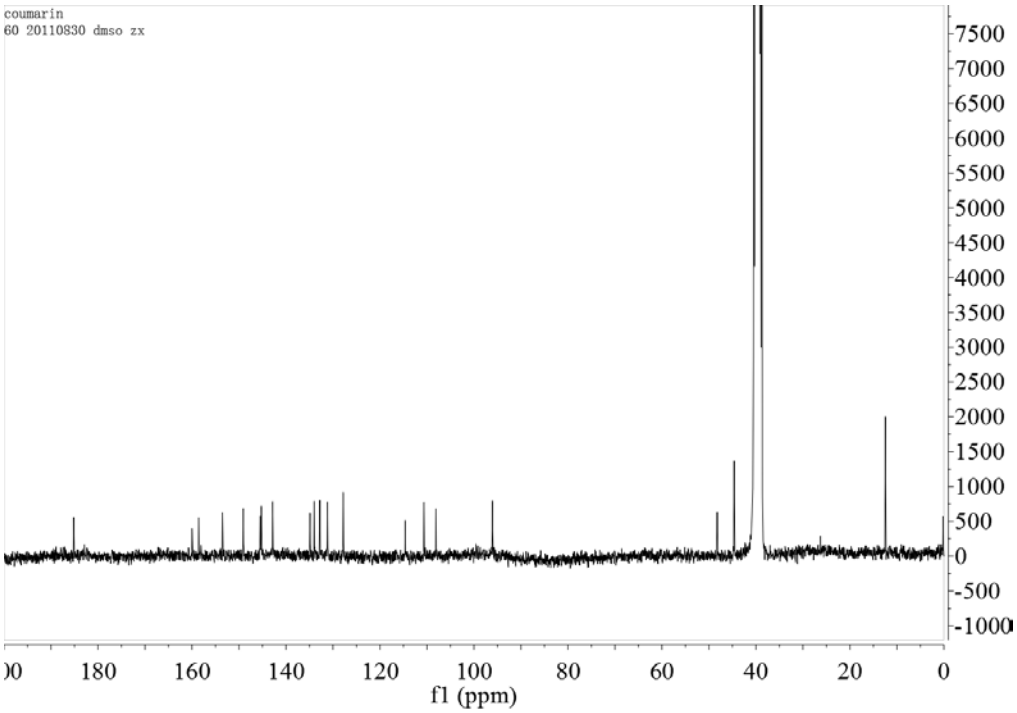


Figure S20. ^{1}H NMR and ^{13}C NMR spectra of probe **1**.

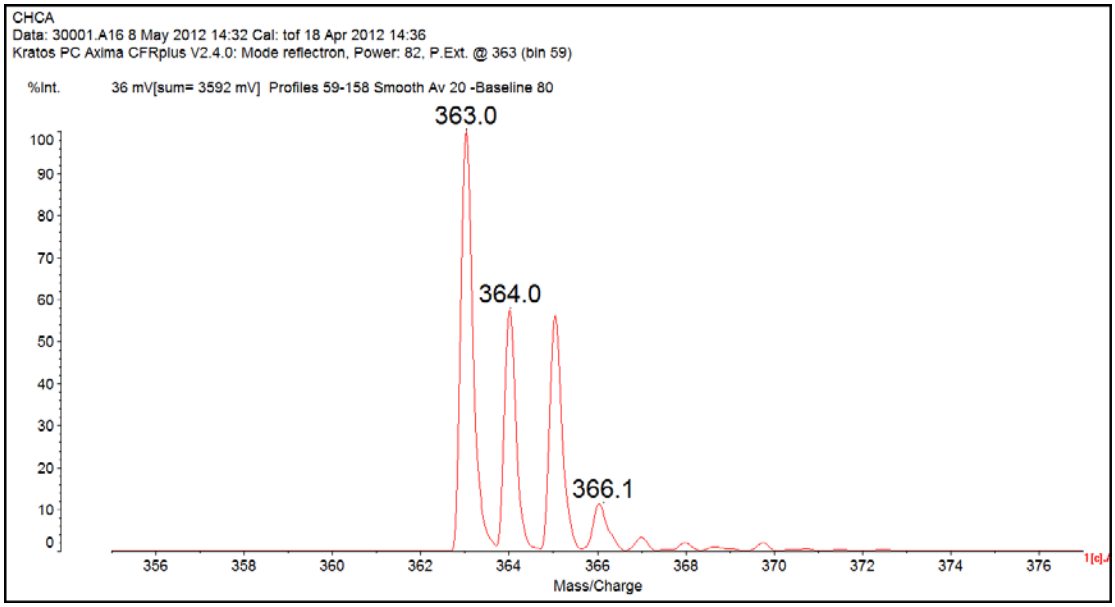


Figure S21. TOF-MS spectra of probe **1**.

References

1. H. A. Clark, R. Kopelman, R. Tjalkens and M. A. Philbert, *Analytical Chemistry*, 1999, **71**, 4837-4843.