A ratiometric fluorescent probe for simultaneous detection of Cys/Hcy and GSH

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Materials and instruments

Cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), other biologically related species and chemicals were purchased from commercial suppliers and used without any purification. Deionized water was used in all experiments. ^{1}H NMR, ^{13}C NMR and HRMS spectral analysis were recorded on a Bruker 300 MHz spectrometer and Bruker Daltonics MICROTOF-Q II mass spectrometer. UV-vis spectra were obtained with Shimadzu UV-2450 spectrometer and fluorescence spectra were conducted on HITACHI F-4600 spectrometer. HeLa cells were provided by Xiangya Hospital at Central South University (Changsha, China). One-photon microscopy was conducted on a confocal laser microscope (CLSM).

Spectral measurements

Probe **CPR** was dissolved in DMF with a concentration of 10 mM as the stock solution. The solutions of various analytes (100 mM), such as Cys, Hcy, GSH, Lys, Phe, Thr, Met, Val, His, Gly, Ser, Ala, Tyr, Arg, Glu, Ile, KI, KCl, Na_2SO_4, $NaNO_3$, KO_2, $NaNO_2$, H_2O_2 were prepared with deionized water. Typically, the test solution was prepared by adding aqueous solution of analytes into the solution of probe **CPR** (10 mM) in PBS buffer (10 mM, pH = 7.4, containing 30% DMF). The fluorescence spectra were collected with excitation wavelength at 390 nm or 436 nm.

Cell culture and fluorescence imaging experiments

HeLa Cells were grown in Dulbecco's Modified Eagle's medium supplemented with 10% FBS (Fetal Bovine Serum) and 1% antibiotics at 37 °C under an atmosphere of 5% CO_2. MTT assay was used to evaluate the cytotoxic effect. Cells were cultured on the confocal dish for 24 hours and washed with PBS buffer before the imaging experiments. Cells incubated only with **CPR** (5.0 μM) were used as the control groups. For the experimental groups, cells were pretreated with 1.0 mM NEM (N-ethylmaleimide, a trapping reagent for biothiols) for 30 min, and then incubated with 0.3 mM Cys, Hcy and GSH for 15 min, and further incubated with **CPR** for 30 min, respectively. Fluorescence images were collected by a confocal laser microscope:

λ_{ex} = 405 nm, λ_{em} = 450-480 nm and 530-560 nm for the blue and yellow channels, respectively; λ_{ex} = 488 nm, λ_{em} = 580-610 nm for the red channel.

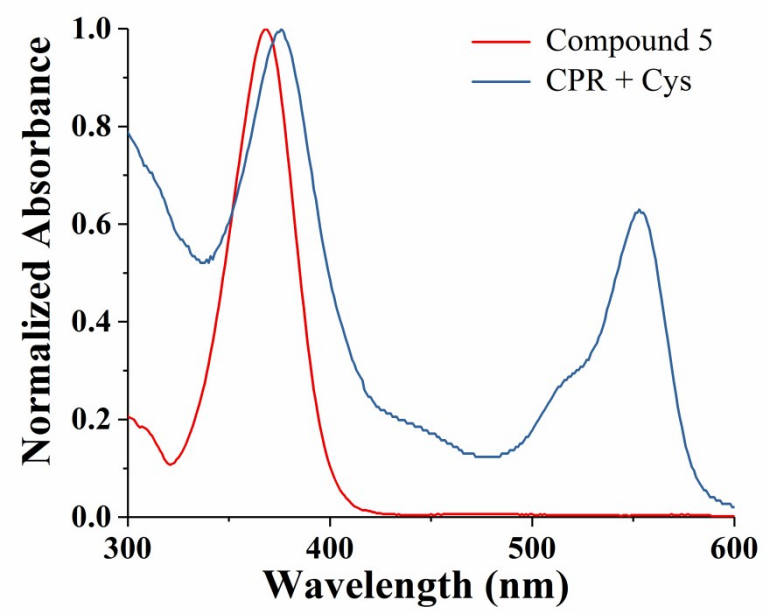

Figure S1. Normalized absorption spectra of compound **5** (red line) and probe **CPR** (5.0 uM) in the presence 60.0 equiv. of Cys (blue line).

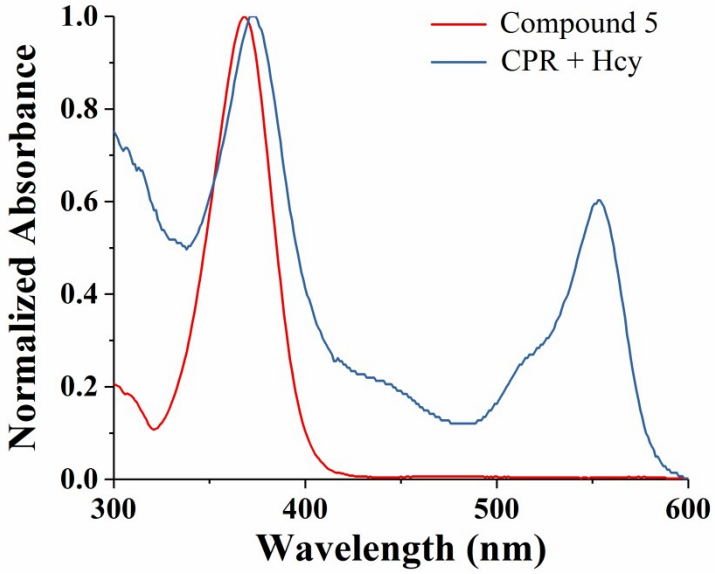

Figure S2. Normalized absorption spectra of compound **5** (red line) and probe **CPR** (5.0 uM) in the presence 60.0 equiv. of Hcy (blue line).

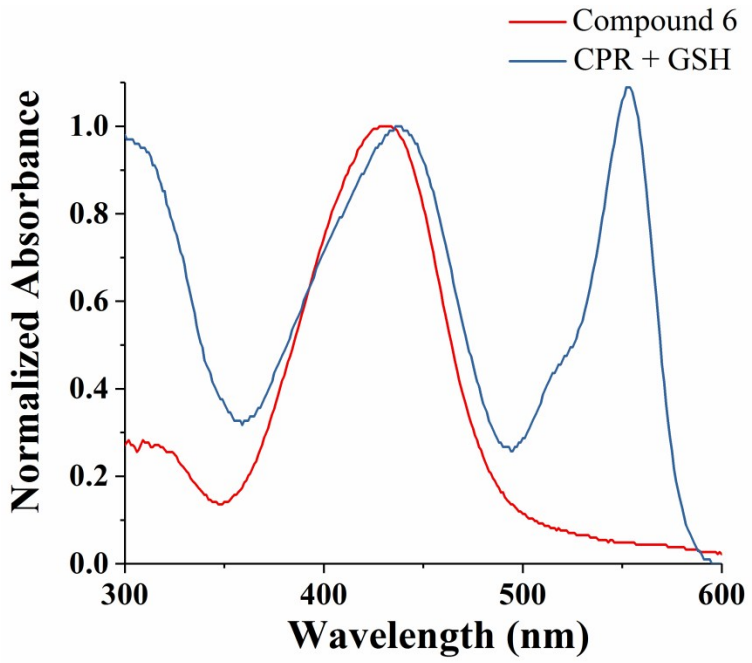

Figure S3. Normalized absorption spectra of compound **6** (red line) and probe **CPR** (5.0 uM) in the presence 20.0 equiv. of GSH (blue line).

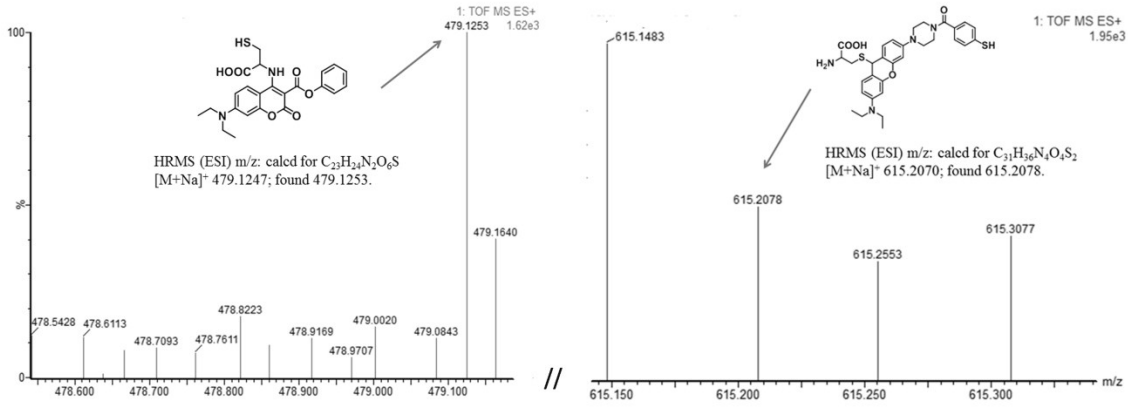

Figure S4. HRMS spectrum of probe **CPR** in the presence of Cys.

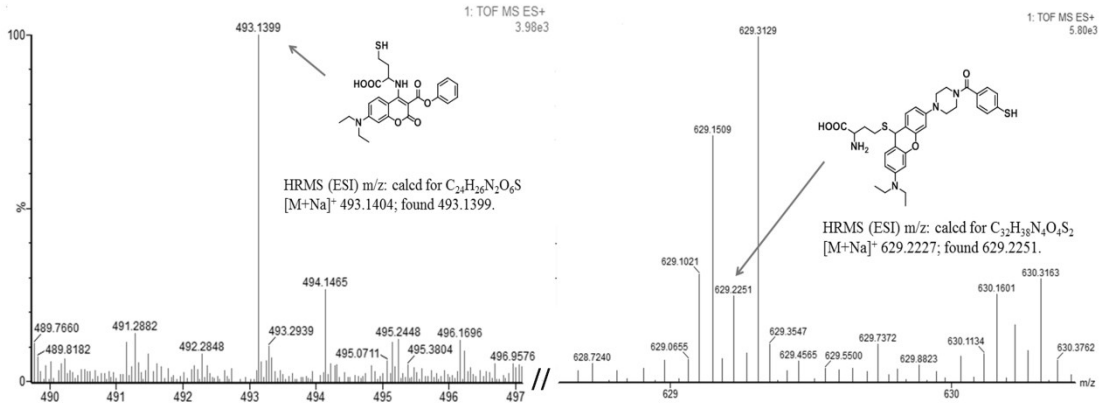

Figure S5. HRMS spectrum of probe **CPR** in the presence of Hcy.

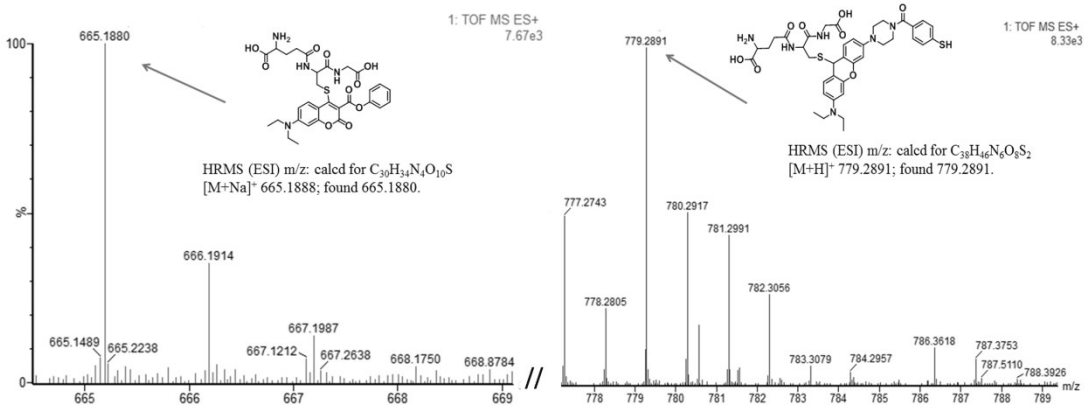

Figure S6. HRMS spectrum of probe **CPR** in the presence of GSH.

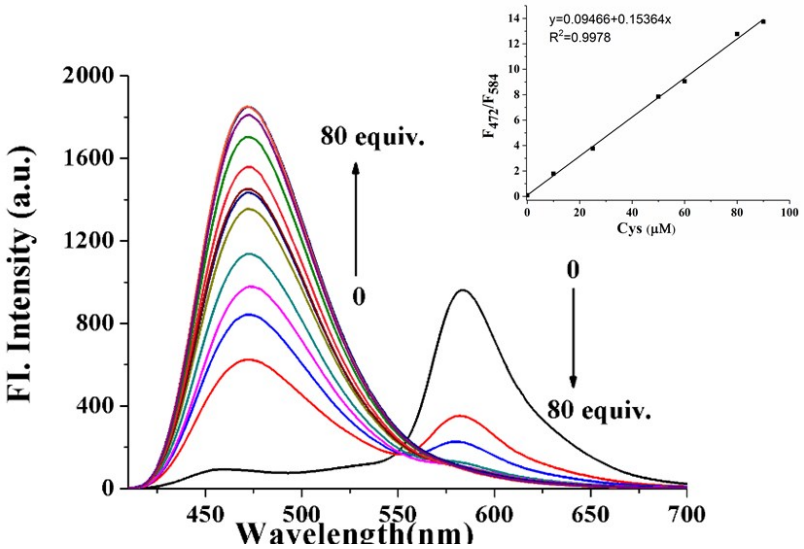

Figure S7. Fluorescence spectra of probe **CPR** (5.0 μM) upon the addition 0.0-80.0 equiv. of Cys. λ_{ex} = 390 nm. Excitation and emission slits: 5/5 nm.

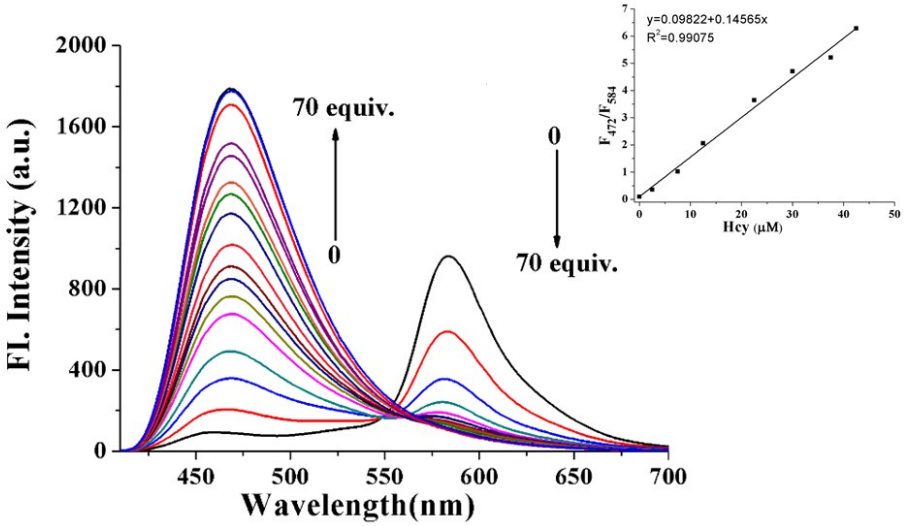

Figure S8. Fluorescence spectra of probe **CPR** (5.0 μM) upon the addition 0.0-70.0 equiv. of Hcy. λ_{ex} = 390 nm. Excitation and emission slits: 5/5 nm.

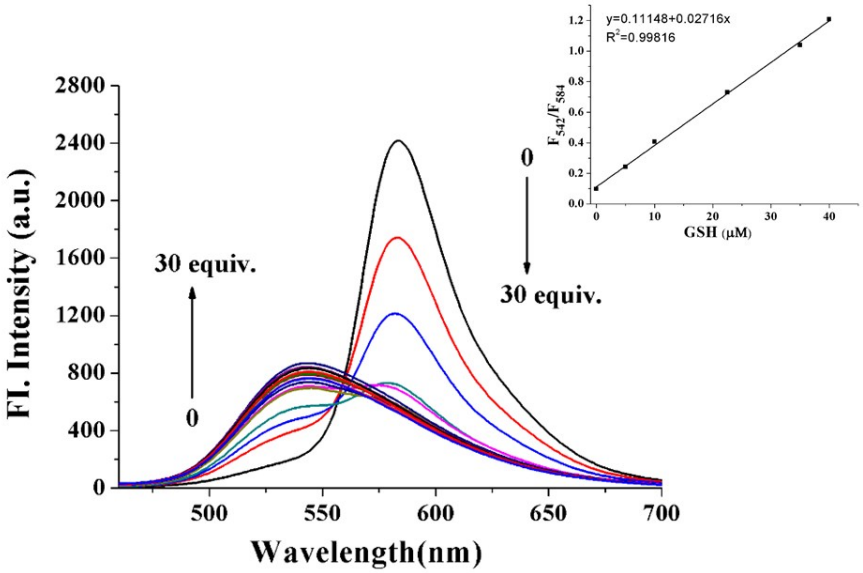

Figure S9. Fluorescence spectra changes of probe **CPR** (5.0 μM) upon the addition 0 to 30 equiv. of GSH. λ_{ex} = 436 nm. Excitation and emission slits: 5/5 nm.

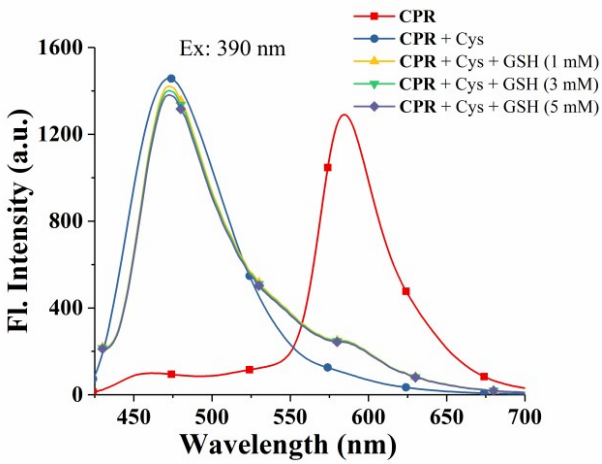

Figure S10. Fluorescence spectra of **CPR** (5.0 μM) upon the addition of 60 equiv. of Cys in the absence/presence of GSH (1mM - 5 mM) after incubation for 40 min.

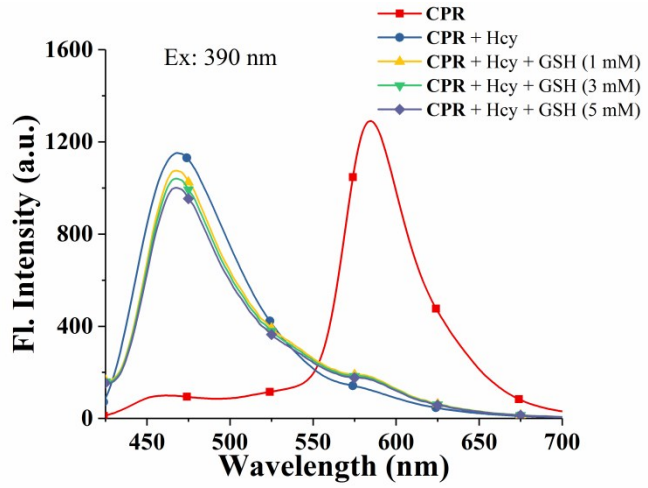

Figure S11. Fluorescence spectra of **CPR** (5.0 μM) upon the addition of 60 equiv. of Hcy in the absence/presence of GSH (1mM - 5 mM) after incubation for 40 min.

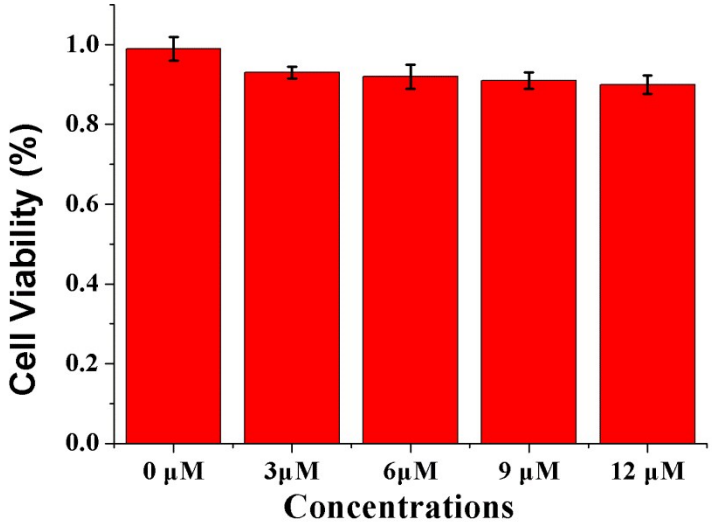

Figure S12 Percentage of viable HeLa cells after treatment with indicated concentrations of probe **CPR** after 24 hours.

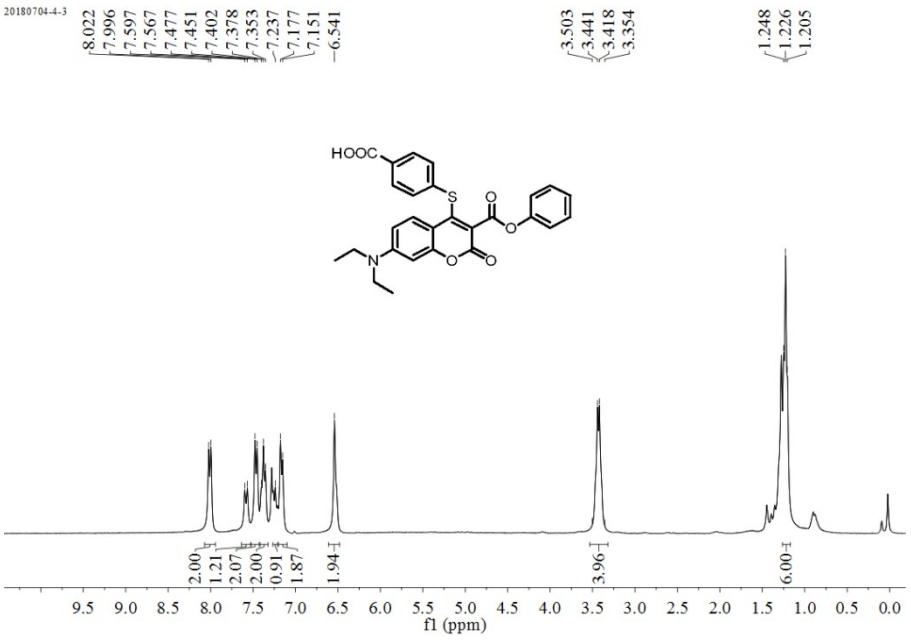

Figure S13. ^{1}H NMR spectrum of compound **3**.

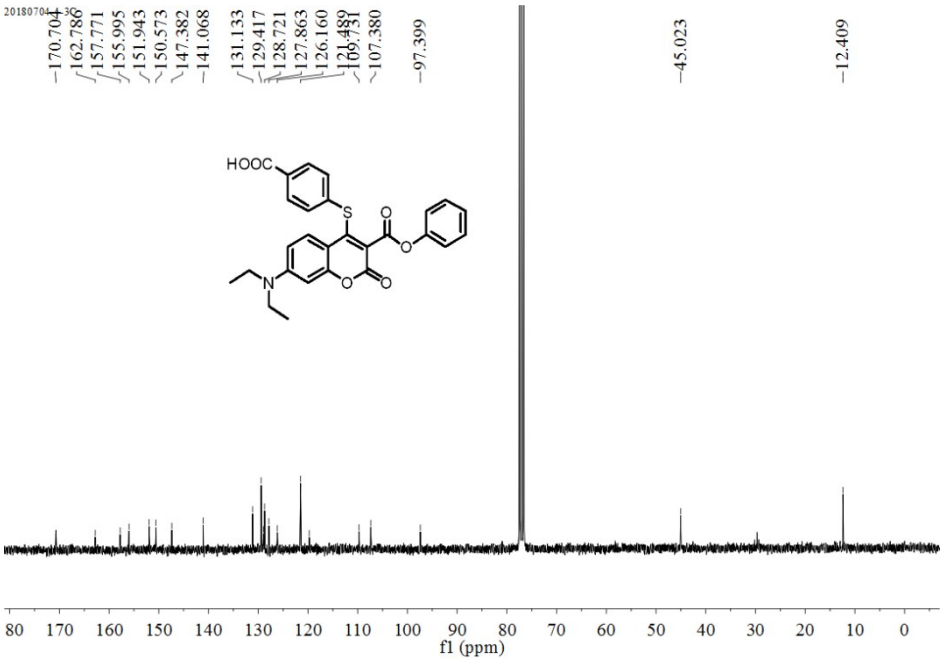

Figure S14. ^{13}C NMR spectrum of compound **3**.

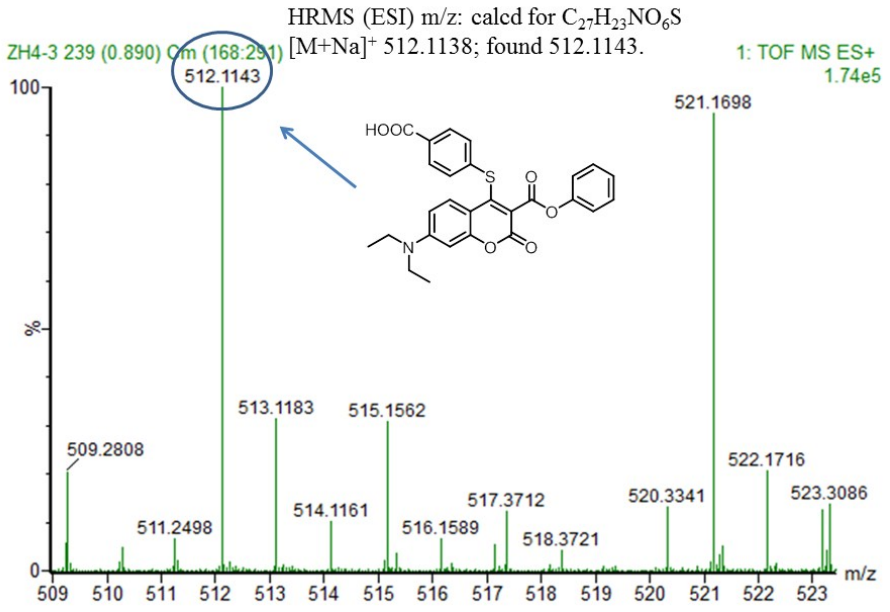

Figure S15. HRMS spectrum of compound **3**.

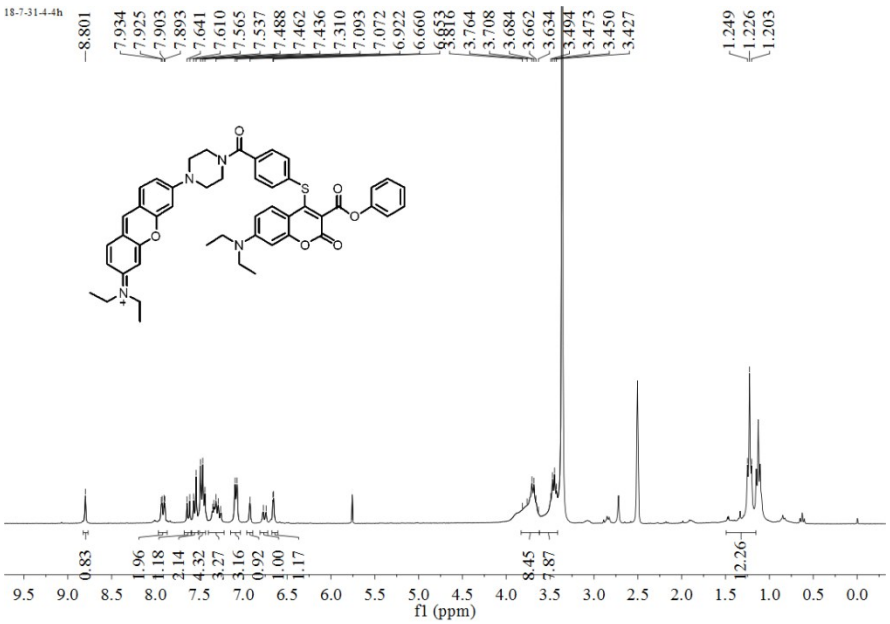

Figure S16. ^{1}H NMR spectrum of probe **CPR**.

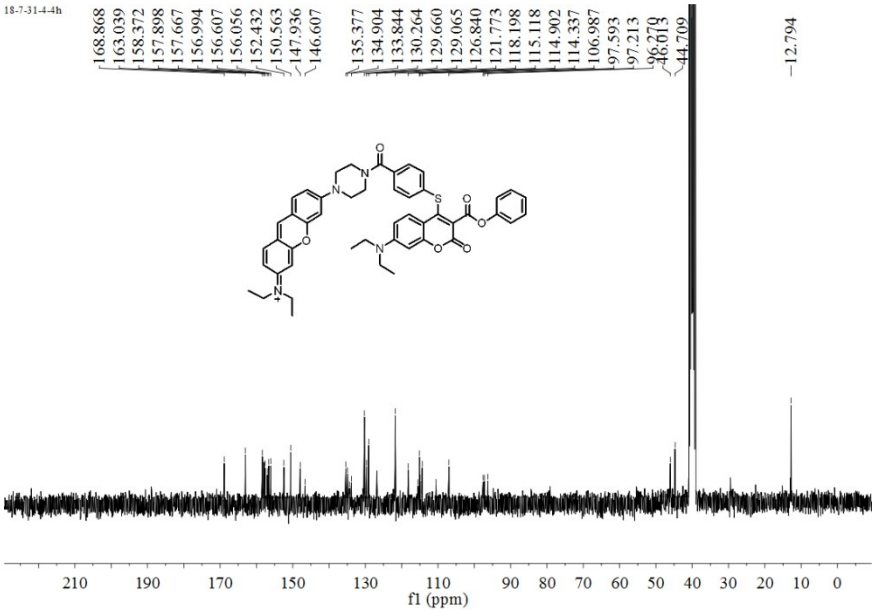

Figure S17. ^{13}C NMR spectrum of probe **CPR**.

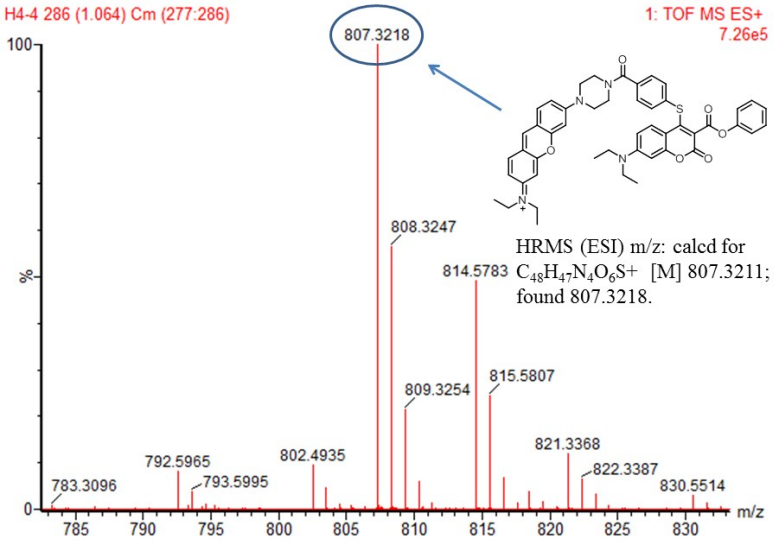

Figure S18. HRMS spectrum of probe CPR.