Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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Electronic Supporting Information

Lysosomal-targeted fluorescent probe based pH regulating reactivity for tracking of Cysteine dynamics in oxidative stress

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1. Experimental Section

Materials. The chemicals used in the experiments were purchased from Shanghai Aladdin Biochemical Technology Co, Ltd. and did not require further purification for use. Roswell Park Memorial Institute 1640 (RPMI 1640) was purchased from Beijing Solarbio Science&Technoiogy Co., Ltd. The distilled water used was processed by a water ultrapure purification system, and the PBS buffer solution was made from commercially available PBS phosphate buffer dry powder (2 L/bag) dissolved in distilled water and fixed to 2 L. All sample solutions were prepared from solid samples dissolved in DMSO or water.

Instruments. Hitachi U-3900 UV-Vis spectrophotometer was employed to measure UV-Vis spectra. Hitachi F-7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. ^{1}H and ^{13}C NMR experiments were performed with a Bruker Avance III HD 600 and 151 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Coupling constants (J values) are reported in hertz. ESI determinations were carried out on AB Triple TOF 5600plus System (AB SCIEX, Framingham, U.S.A.). The cell imaging experiments were measured by a Zeiss LSM880 confocal laser scanning microscope.

Colocalization. Hela cells were cultured overnight on 35 mm glass-bottom culture dishes and washed with PBS (3 times × 2 mL). After incubation with 10 μM **ECMA** for 10 min, the cells were washed with PBS three times. Then, Lyso-Tracker Red (LTR) was added and co-incubated for another 30 min at 37 ℃. And then washed with PBS (10 mM, pH 7.4) for three times. Fluorescence images were acquired on a fluorescence confocal microscope with an orange channel for **ECMA** and a red channel for Lyso-Tracker Red, respectively.

Experimental process of real-time fluorescence imaging of cells: Hela cells were first pretreated with **ECMA** for 10 min, the excess **ECMA** was then washed out by PBS (3 times × 2 mL). The pretreated Hela cells were subsequently co-incubated with Cys or H_2O_2, respectively. Finally, real-time imaging experiments were carried out on a confocal microscope (10 min). (Orange channel: λ_{em} = 542-602 nm, λ_{ex} = 488 nm; Blue channel: λ_{em} = 460-520 nm, λ_{ex} = 405 nm)

2. Synthesis and Characterization

Synthesis of compound 1. $POCl_3$ (0.6 mL) was slowly added to a round bottom flask containing 0.8 mL *N, N*-Dimethylformamide (DMF). The reaction mixture was stirred at 0 ℃ for 2 h. Then, 7-(diethylamino)coumarin (480 mg, 2.2 mmol) dissolved in 4 mL DMF was added dropwise to the mixed solution of the above followed by stirring at 60 ℃ for 12 h. Subsequently, the reaction solution was poured into 200 mL of ice water, the pH was adjusted to 5-6, and the precipitate was filtered and washed with water three times. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.39 (s, 1H), 7.66 (d, J = 9.0 Hz, 1H), 6.81 (dd, J = 9.1, 2.5 Hz, 1H), 6.59 (d, J = 2.4 Hz, 1H), 3.50 (q, J = 7.1 Hz, 4H), 1.15 (t, J = 7.0 Hz, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 187.57, 161.18, 158.92, 153.91, 146.58, 133.53, 113.64, 110.94, 108.15, 96.85, 45.02, 12.83. HR-MS: [**Compound 1** + H]$^+$ calculated for $C_{14}H_{16}NO_3^+$ 246.1125, found 246.1127.

Synthesis of probe ECMA. Compound **1** (490 mg, 2 mmol) and 3-morpholino-3-oxopropanenitrile (460 mg, 3 mmol) with piperidine (30 μL) was dissolved in anhydrous ethanol (20 mL). The mixture was

heated at 80 ℃ for 5 h. Then, the solvent was evaporated and the crude solid was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH, v/v = 50/1) on silica gel to obtain the desired product (320 mg, yield 41.9%) as an orange-red powder. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.65 (s, 1H), 7.97 (s, 1H), 7.68 (s, 1H), 7.59 (d, J = 9.0 Hz, 1H), 7.56 (d, J = 9.0 Hz, 1H), 7.44 (s, 1H), 6.82 (dd, J = 9.1, 2.5 Hz, 1H), 6.79 (dd, J = 9.0, 2.5 Hz, 1H), 6.64 (d, J = 2.6 Hz, 1H), 6.59 (d, J = 2.4 Hz, 1H), 3.64 – 3.59 (m, 12H), 3.53 – 3.49 (m, 12H), 1.16 – 1.13 (m, 12H). ^{13}C NMR (151 MHz, DMSO): δ 162.99, 161.59, 160.55, 159.50, 157.56, 157.33, 153.22, 153.00, 145.77, 144.67, 143.56, 142.60, 132.10, 131.86, 118.14, 116.93, 111.74, 111.07, 110.83, 110.64, 108.12, 108.08, 105.50, 103.16, 97.02, 96.76, 66.36, 66.05, 47.19, 44.95, 42.31, 12.83. HR-MS: $[M + H]^+$ Calcd for $C_{21}H_{24}N_3O_4^+$ 382.1761, found 382.1756.

Synthesis route

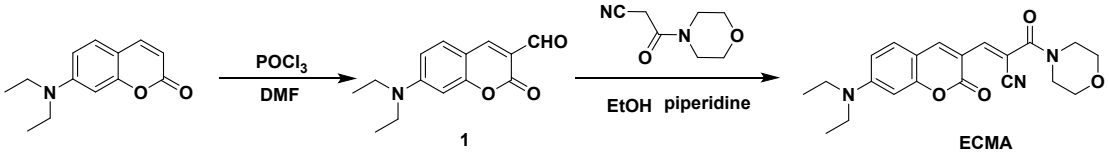

Figure S1. Synthesis route of **ECMA**

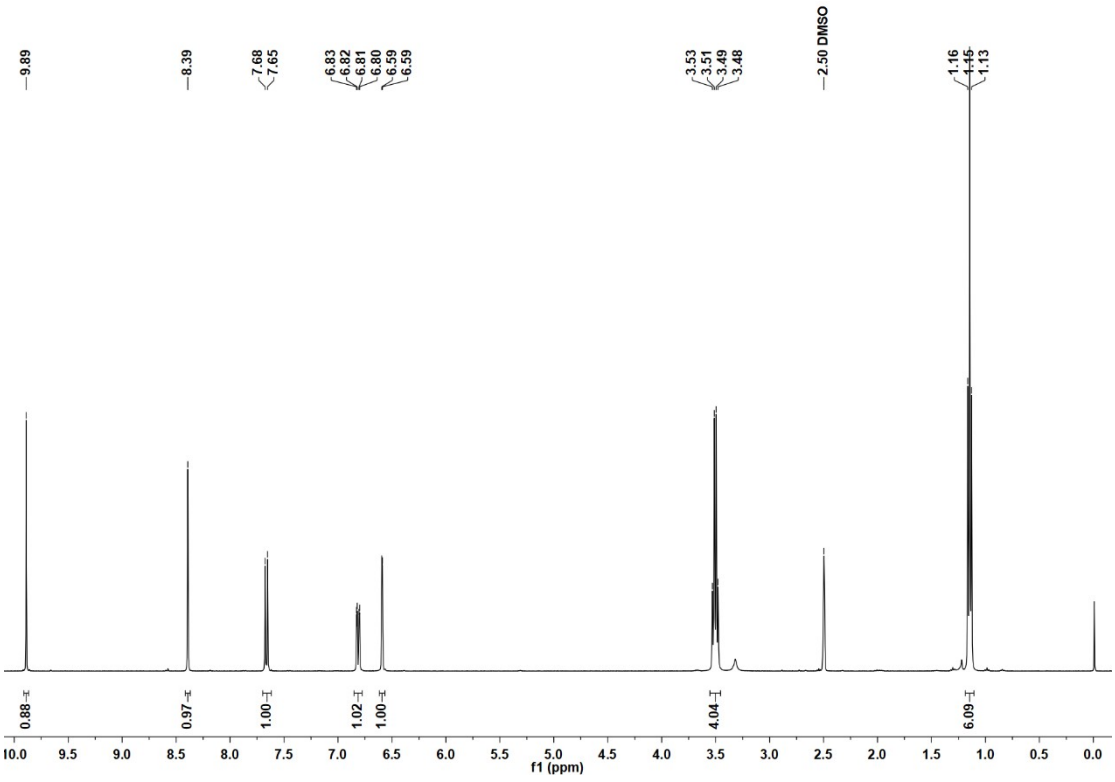

Figure S2. The ^{1}H NMR (600 MHz) spectra of Compound **1** in DMSO.

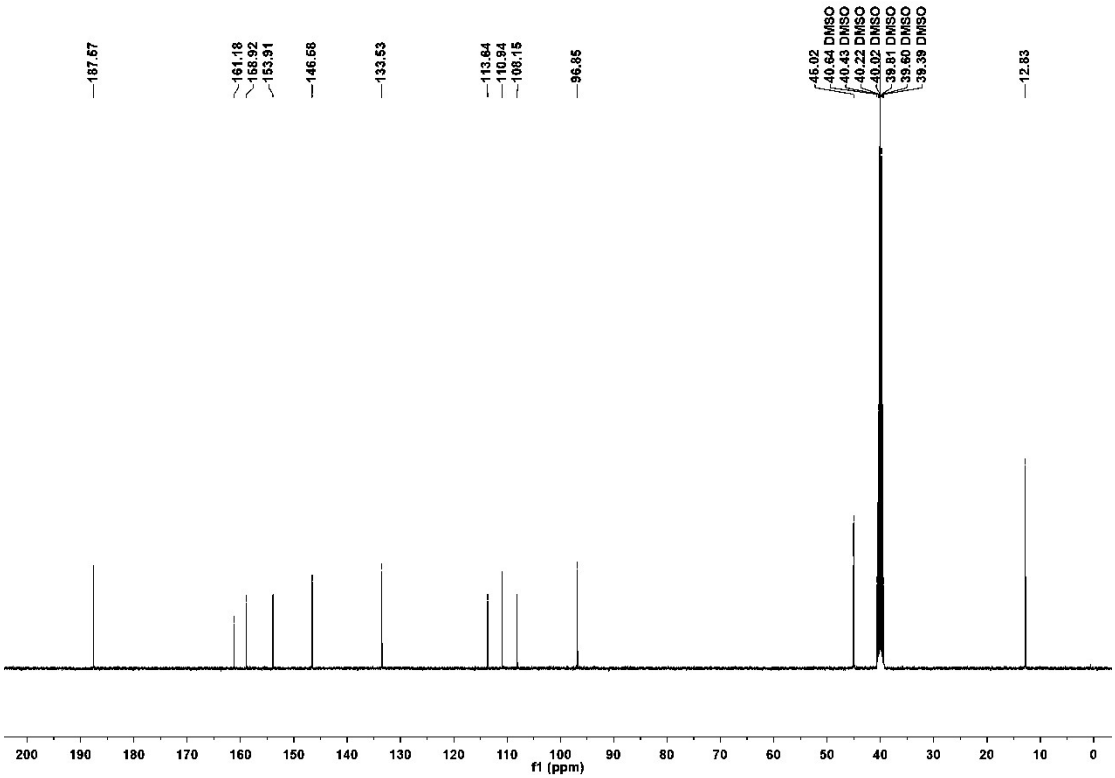

Figure S3. The ^{13}C NMR (151 MHz) spectra of Compound **1** in DMSO.

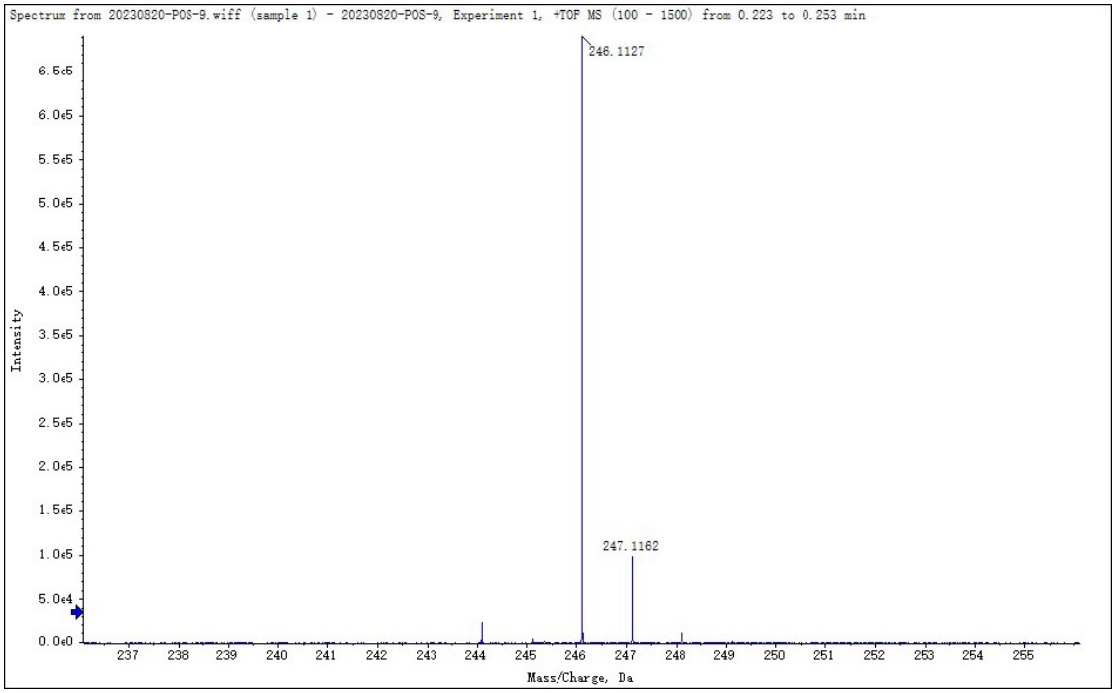

Figure S4. HR-MS spectra of Compound **1**

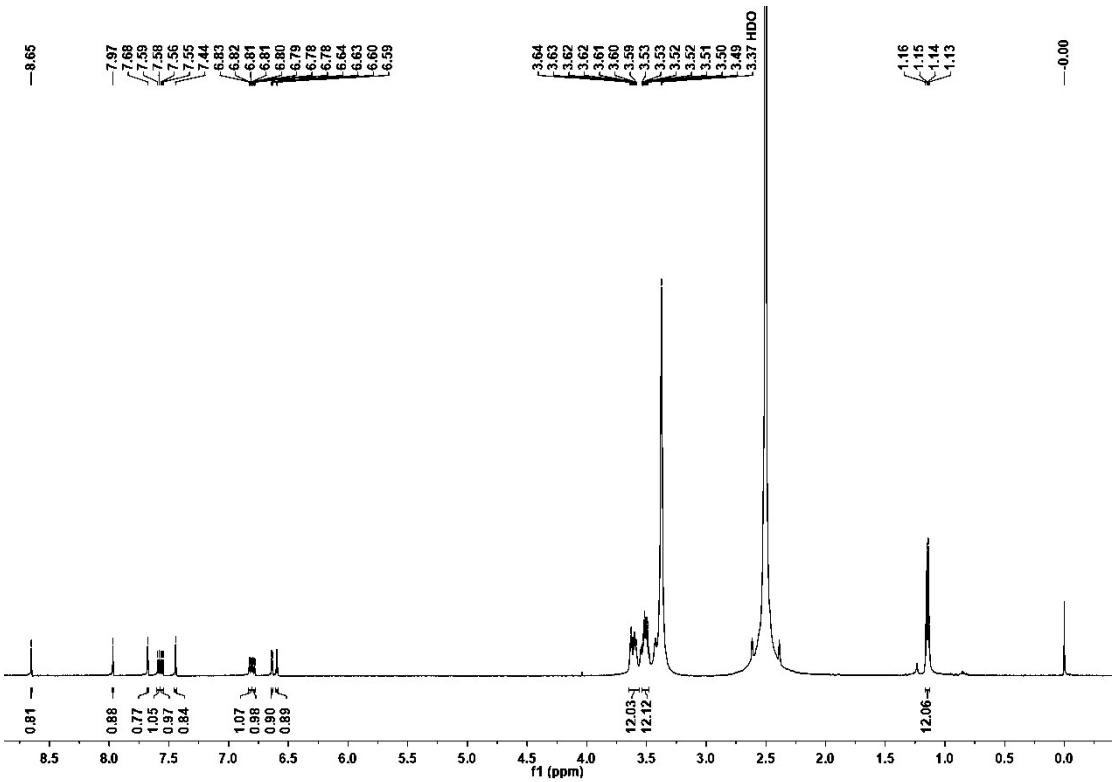

Figure S5. The ^{1}H NMR (600 MHz) spectra of Compound **ECMA** in DMSO-d_6.

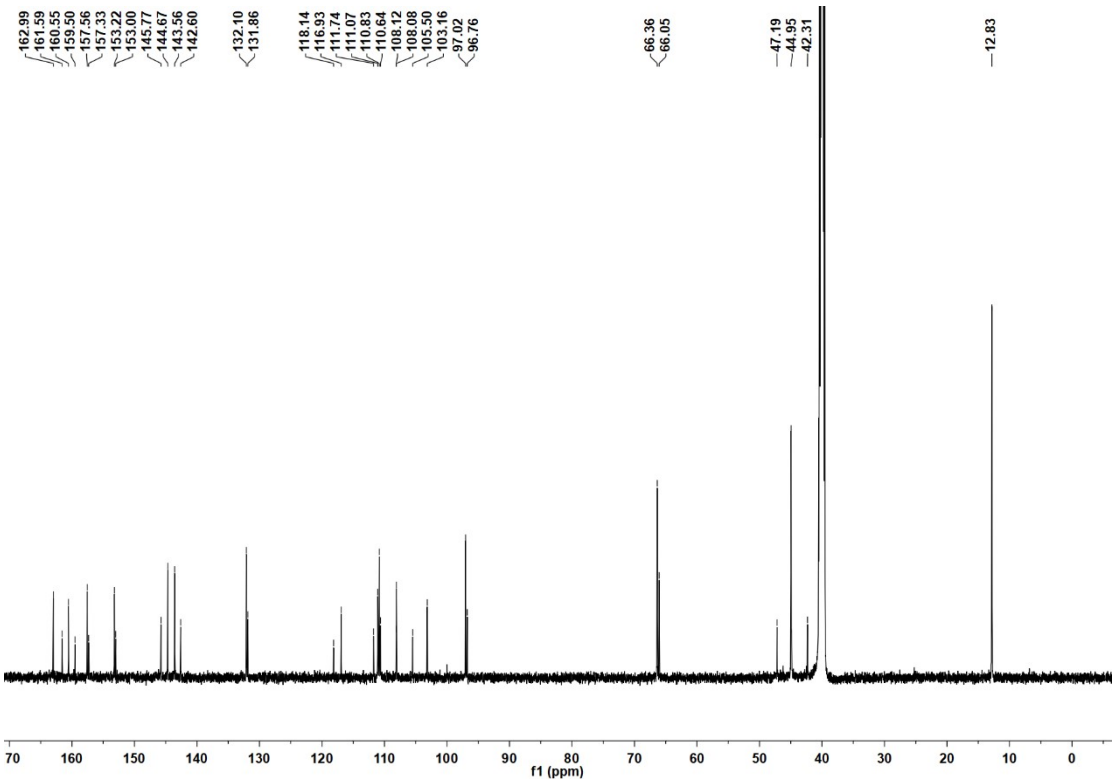

Figure S6. The ^{13}C NMR (151 MHz) spectra of Compound **ECMA** in DMSO-d_6.

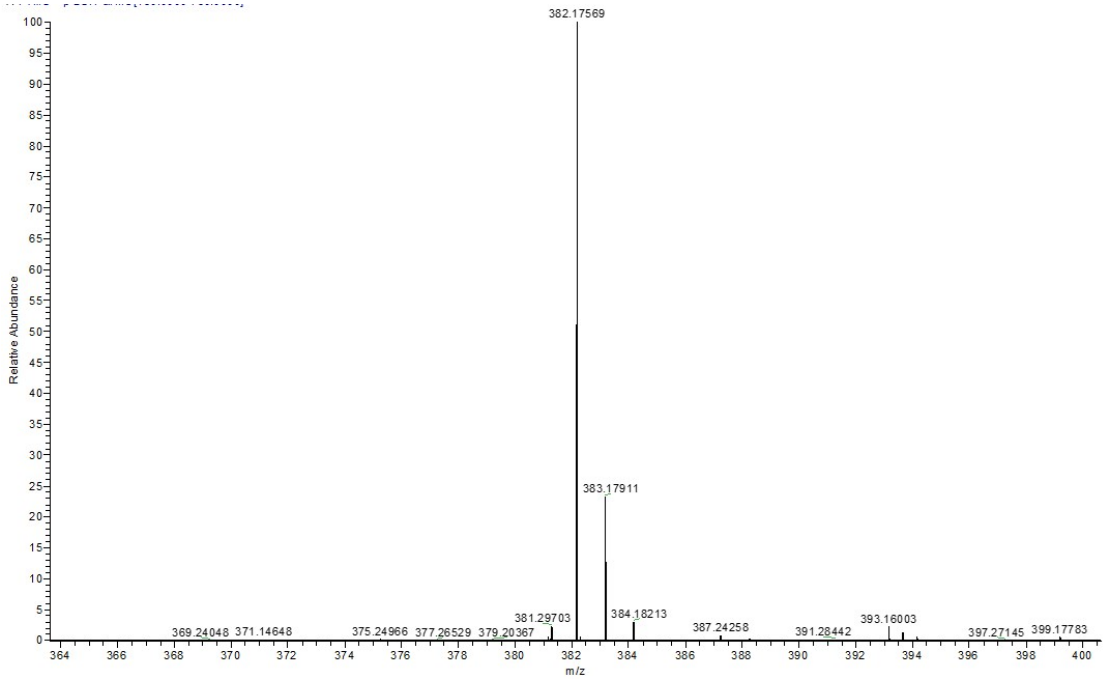

Figure S7. HR-MS spectra of Compound **ECMA**

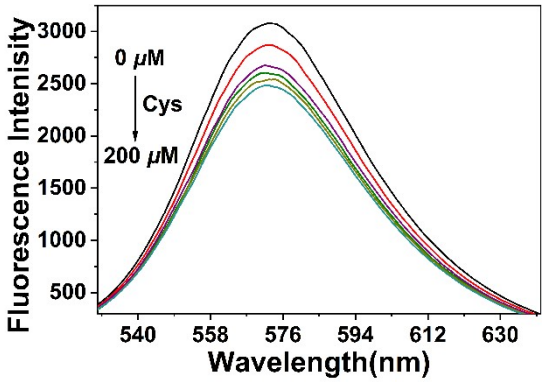

Figure S8: Fluorescence emission titration of **ECMA** in the presence of different concentration of Cys (0-200 μM) at pH = 5, λ_{ex} = 475 nm, λ_{em} = 572 nm. Slit = 5 nm/5 nm.

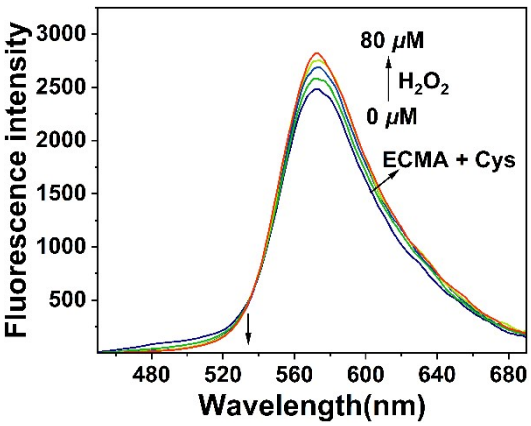

Figure S9: Spectral response of **ECMA** with Cys upon addition of H_2O_2 (0-80 μM) at pH = 5, λ_{ex} = 475

nm, λ_{em} = 572 nm. Slit = 5 nm/5 nm.

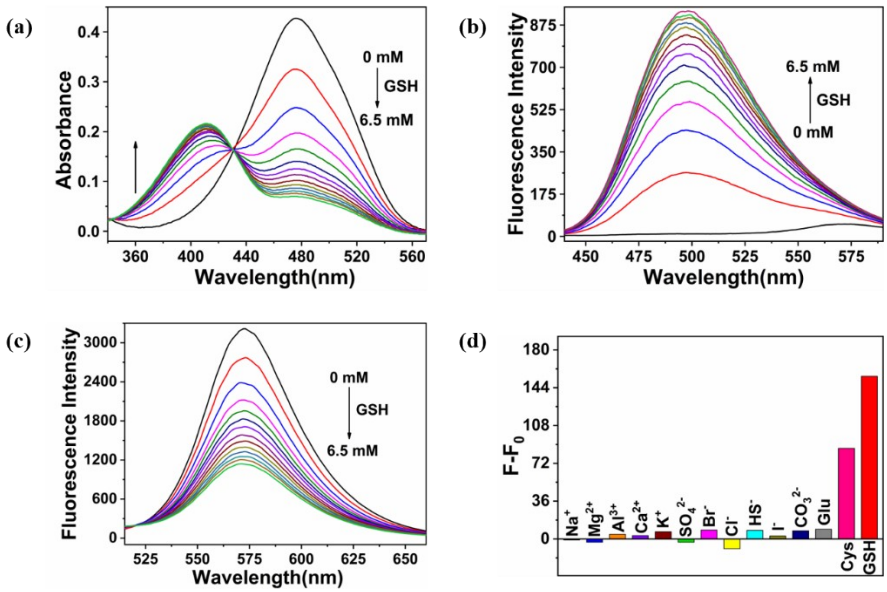

Figure S10: Spectral properties of **ECMA** toward GSH were investigated in PBS systems. (a) UV-vis absorption spectra of **ECMA** in the presence of different concentration of GSH (0-6.5 mM) (b) and (c) Fluorescence titration spectra of **ECMA** upon addition of GSH (0-6.5 mM), λ_{ex} = 475 nm, λ_{em} = 575 nm λ_{ex} = 400 nm, λ_{em} = 500 nm. (d) The competitive selectivity of **ECMA** (10 μM) towards various biologically relevant species (100 μM) at 490 nm.

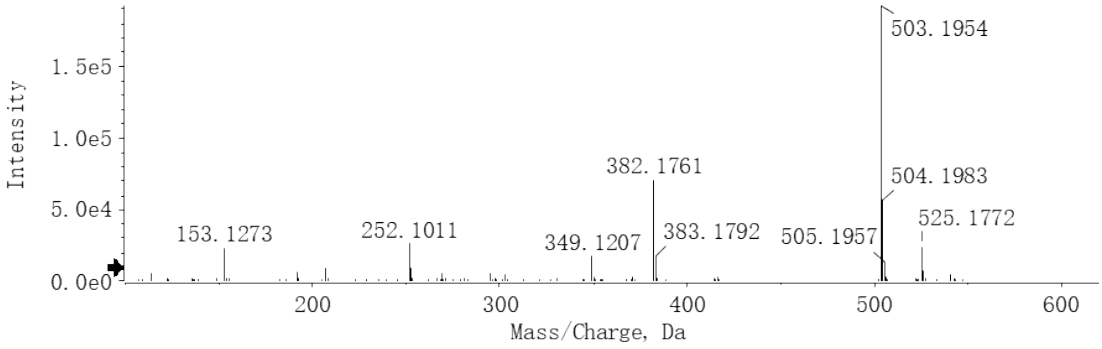

Figure S11: The ESI-MS of product obtained by reaction of probe **ECMA** and Cys.

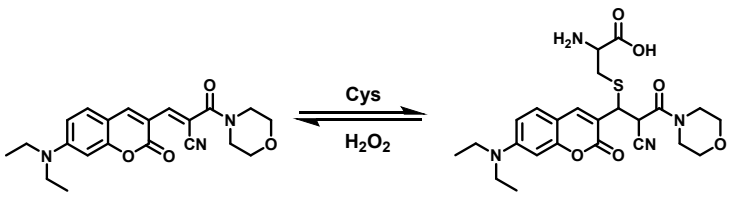

Figure S12: The reaction mechanism of **ECMA**.

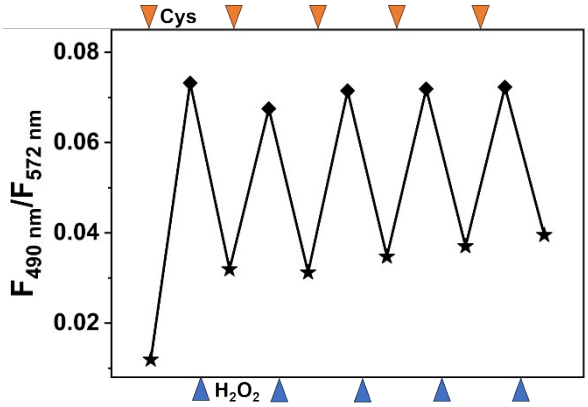

Figure S13: Reversible cyclic response test of **ECMA**

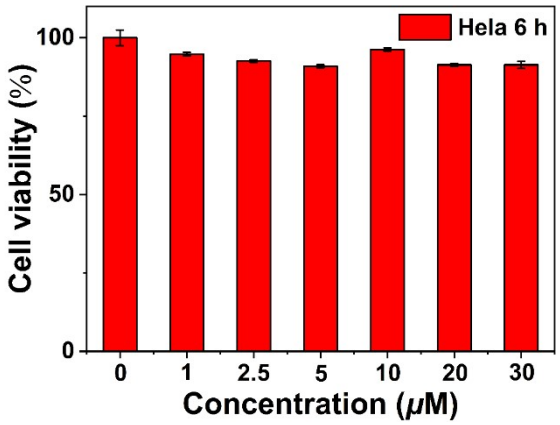

Figure S14: The cytotoxicity test of probe **ECMA**

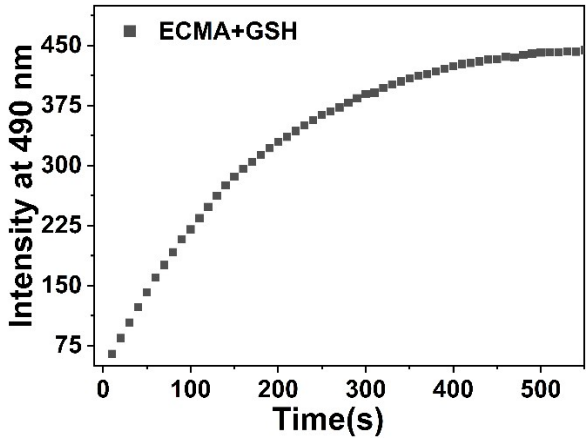

Figure S15: Kinetic test of **ECMA** (10 μM) reaction to GSH (1 mM) at PH=7.4.

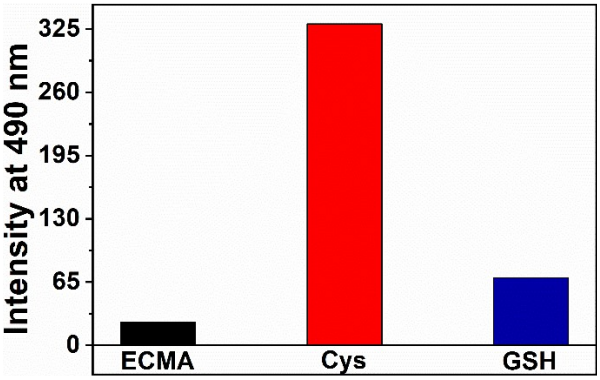

Figure S16: The reactivity of **ECMA** to Cys (200 μM) and GSH (6.5 mM) was observed at PH=5.