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A Water-Soluble Dual-site Fluorescent Probe for Rapid Detection of Cysteine with High Sensitivity and Specificity

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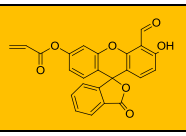
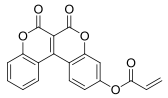
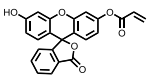
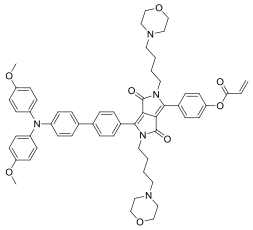
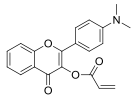
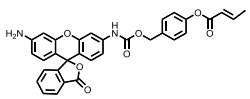
1. Comparisons about some work for the detection of Cys.

Table S1. A comparison table about some representative reported work for the detection of Cys.

Probe		Solvent (pH=7.4)	Time for Cell experiment (Min)	Time for naked-eye recognition (s)	Detection Limit (μM)	Reference
J-L1		HEPES (pH=6.8)	2	5	0.0617	***Our work***
A		PBS	5	600	-	1
B		EtOH-PBS (2/8, v/v)	30	600	0.121	2
C		PBS-DMSO (6/4, v/v)	20	6000	-	3
D		DMSO-PBS (1/4, v/v)	15	300	0.2	4
E		HEPES	10	2400	7.62	5

Reference:

1. C. Chen, L. Zhou, X. Huang and W. Liu, *J. Mater. Chem. B*, 2017, **5**, 5892-5897.

2. H. Wang, G. Zhou, H. Gai and X. Chen. *Chem*. *Commun*., 2012, **10**, 2739-2741.

3. X. Zhang, Y. Hang, W. Qu, Y. Yan, P. and J. Hua, *RSC. Adv.*, 2016, **6**, 20014-20020.

4. Y. Liu, D. Yu, S. Ding, Q. Xiao, J. Guo and G. Feng, *ACS Appl. Mater. Inter.*, 2014, **6**, 17543-17550.

5. Lim, S.-Y.; Yoon, D.-H.; Ha, D.Y.; Ahn, J.M.; Kim, D.I.; Kown, H.; Ha, H.-J.; Kim, H.-J. *Sens. Actuators B*, 2013, **188**, 111–116.

2. General information.

The commercially available chemicals were used without further purification. All of solvents used in experiments were analytical-reagent grade. ^{1}H and ^{13}C NMR spectra were measured on the Bruker ADVANCE III 400 MHz, JEOL JNM-ECS M Hz using TMS as an internal standard. Mass spectra were determined on a Bruker esquire 6000 spectrometer. UV-vis spectra were performed on an Agilent Carry 5000 UV-Vis-NIR spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-7000 spectrophotometer equipped with quartz cuvettes of 1 cm path length. All pH measurements were made with a pH-10C digital pH meter.

Various stock solutions (10.0 mM) of the perchlorate salts of Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, PO_4^{3-}, $H_2PO_4^{-}$, CO_3^{2-}, SO_4^{2-}, Cl^{-} and Br^{-}; Cys, Hcy, GSH, Glu, Gly, Cys-Cys, Cit, Asp, The, Phe, Pro, Asn, Leu, Gln, Ile, Thr, Lys, Orn, Arg, Val, Met, Ala and His in deionized water were prepared. Stock solutions of **J-L1** (10.0 mM) were also prepared in DMSO. Test solutions were prepared by placing 2.0 μL of the probe stock solution into a test tube, and then diluting to 2.0 mL with HEPES buffer solution, followed by the addition of an appropriate aliquot of each metal ion or amino acid's stock solution. For all measurements, fluorescence spectra were obtained by excitation at 365 nm. Fluorescence quantum yields were determined in solution, using quinine sulfate (Φ = 0.546 in 0.05 M H_2SO_4) as a standard.

The HeLa cells were grown in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS (fetal bovine serum), 2 mM of glutamine, penicillin (100 units/mL), and streptomycin (100 units/mL) under an atmosphere of 5% CO_2 and 95% air at 37 ℃. These cells were then seeded in a 12-well plate at a density of 1.0×10^4 cells per well in culture media. Before the experiments, they were immediately rinsed with HEPES buffer (pH = 6.8), and then were treated with **J-L1** (10.0 μM) in culture media for 2 min at 37 ℃ in a humidified incubator. For the control experiment, the HeLa cells were treated with 50.0 μM of *N*-ethylmaleimide (NEM) in culture media for 30 min at 37 ℃ in a humidified incubator. After washing with HEPES

buffer (pH = 6.8), the cells were further incubated with **J-L1** (10.0 μM) in culture media for 2 min. The bright field and fluorescence images were acquired with Floid cell imaging station (Life Technology).

3. The synthetic route of probe J-L1.

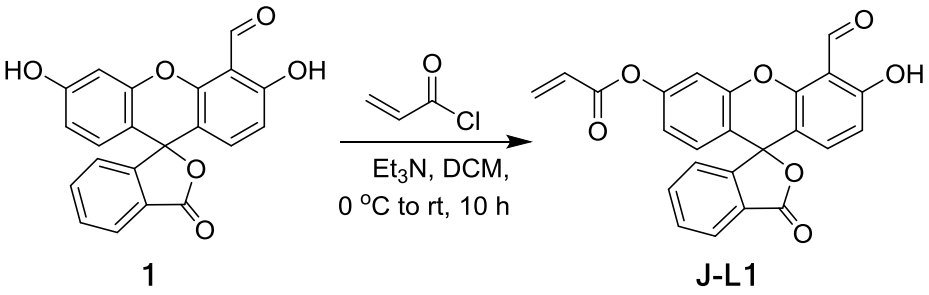


Figure S1. The synthesis of probe **J-L1.**

The compound **1** was synthesized according to a known procedure.[6] Firstly compound **1** (0.33 mmol, 118.8 mg) was dissolved in CH_2Cl_2 (10 mL) under argon, followed by the addition of dry triethylamine (0.5 mmol, 69 μL) dropwise. The reaction mixture was then stirred for 5 min at room temperature and cooled to 0℃ by ice-bath. Acryloyl chloride (2.0 mmol, 160 μL) was added dropwise, and the resulting mixture turned to be turbid. When the reaction mixture turned back to transparent, stirring was continued for 10 h at room temperature. The mixture was quenched by water (10 mL), then extracted by CH_2Cl_2 (15×3 mL). The combined organic phase was collected. The white solid powder of **J-L1** (31.4 mg, 23.1%) was obtained via column chromatography and vacuum drying. The probe **J-L1** had almost no fluorescence emission, and its fluorescence quantum yield was determined in solution, using quinine sulphate (Φ = 0.001 in 0.05 M H_2SO_4) as a standard.[7] m.p.: 80.4-82.0 ℃. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 11.88 (s, 1H), 10.62 (s, 1H), 8.05 (d, J = 6.4 Hz, 1H), 7.78 (dd, J = 20.0, 6.8 Hz, 2H), 7.47 (s, 1H), 7.38 (d, J = 6.8 Hz, 1H), 7.00 (d, J = 8.4 Hz, 2H), 6.90 (d, J = 8.4 Hz, 1H), 6.74 (d, J = 9.2 Hz, 1H), 6.56 (d, J = 17.2 Hz, 1H), 6.45 6.38 (m, 1H), 6.18 (d, J = 9.6 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 192.1, 167.9, 163.5, 162.8, 151.9, 151.2, 150.9, 149.5, 135.9, 134.5, 132.6, 129.3, 128.2, 126.3, 125.3, 124.3, 122.9, 117.5, 115.6, 113.2, 109.4, 108.3, 107.8, 80.2 ppm. ESI-MS: *m/z* 415.1 $[M + H]^+$. HRMS (ESI): calcd. for $C_{24}H_{15}O_7$ $[M + H]^+$ 415.0812; found: 415.0808.

Reference:

6. W. Wang, O. Rusin, X. Xu, K. K. Kim, J. O. Escobedo, S. O. Fakayode, K. A. Fletcher, M. Lowry, C. M. Schowalter, C. M. Lawrence, F. R. Fronczek, I. M. Warner and R. M. Strongin, *J. Am. Chem. Soc*., 2005, **127**, 15949-15958.
7. W. H. Melhuish, *J. Phys. Chem. B*, 1961, **65**, 229-235.

4. ^{1}H, ^{13}C NMR and ESI-MS copies of probe J-L1.

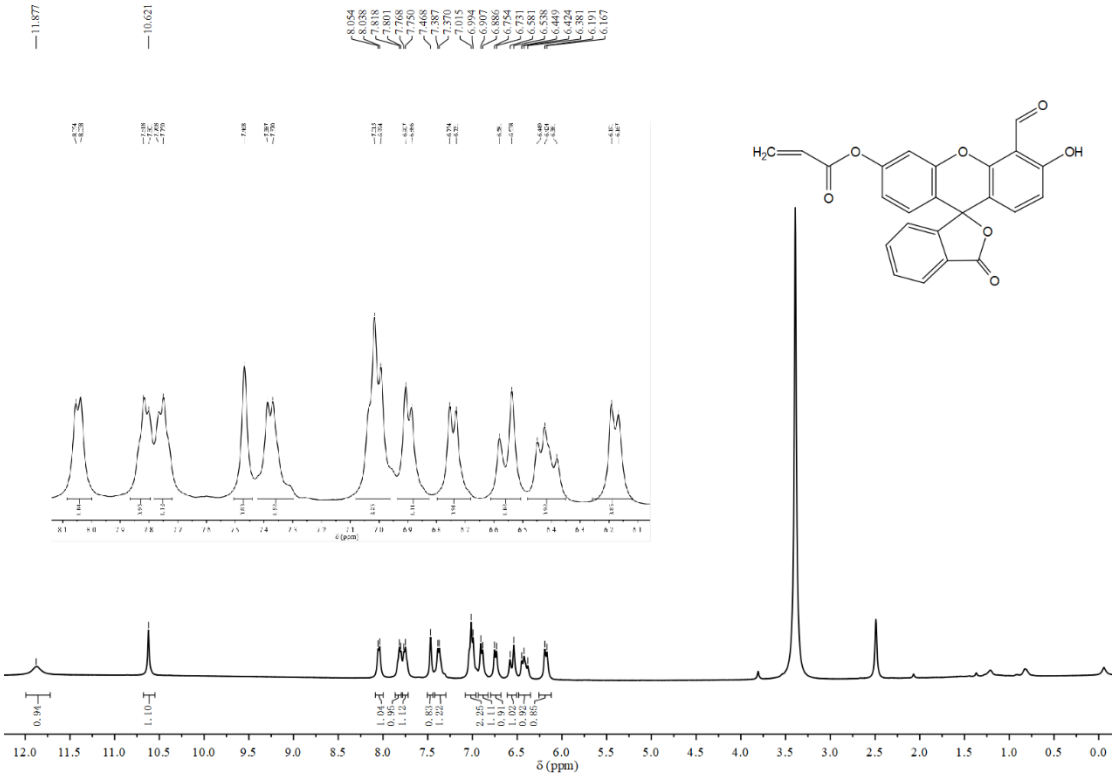


Figure S2. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of probe **J-L1**.

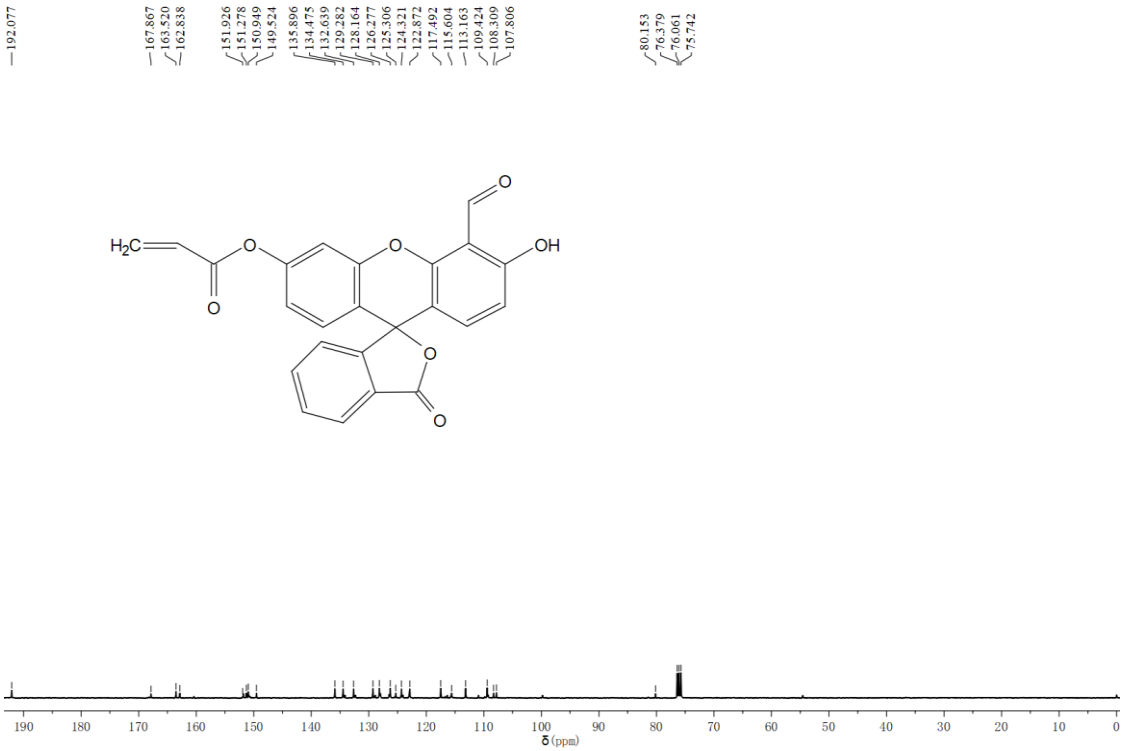


Figure S3. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of probe **J-L1**.

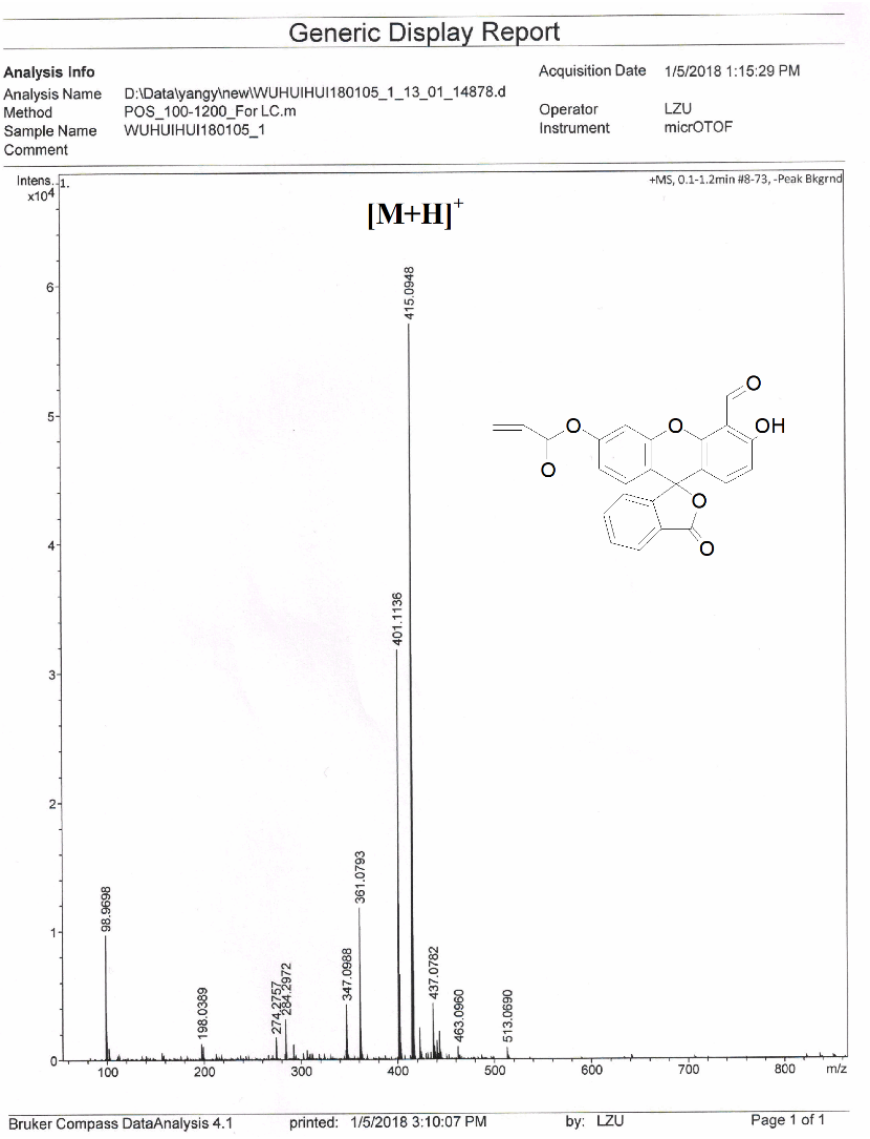


Figure S4. ESI-MS spectrum of probe **J-L1**.

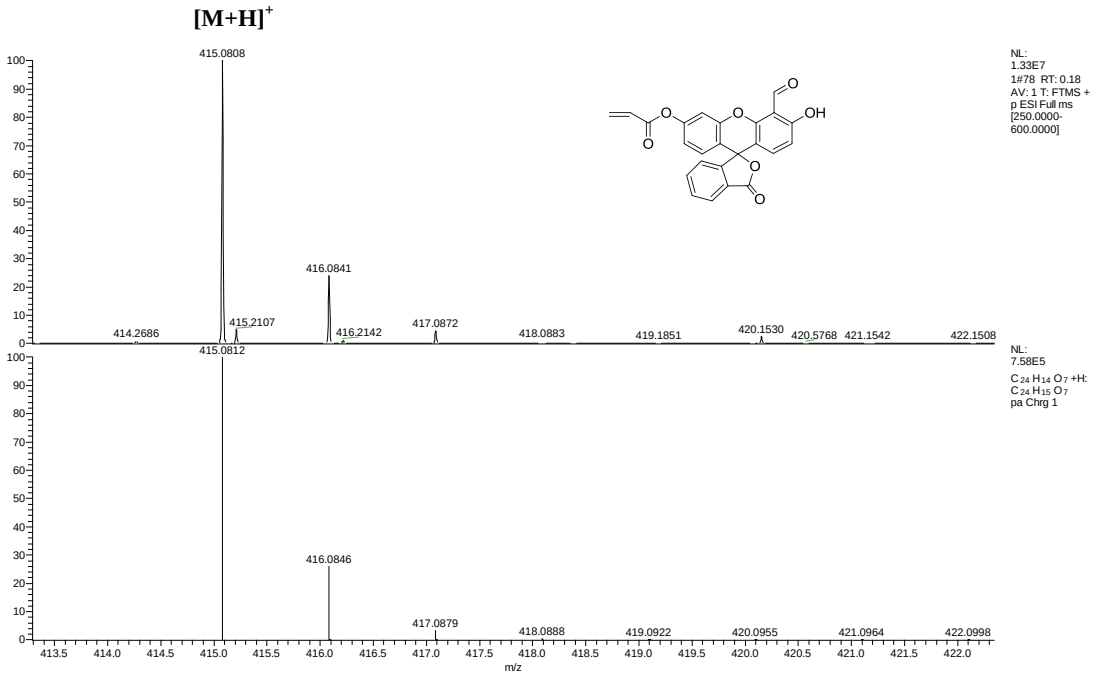


Figure S5. HRMS spectrum of probe **J-L1**.

5. The selection of solvents for probe J-L1 detecting Cys.

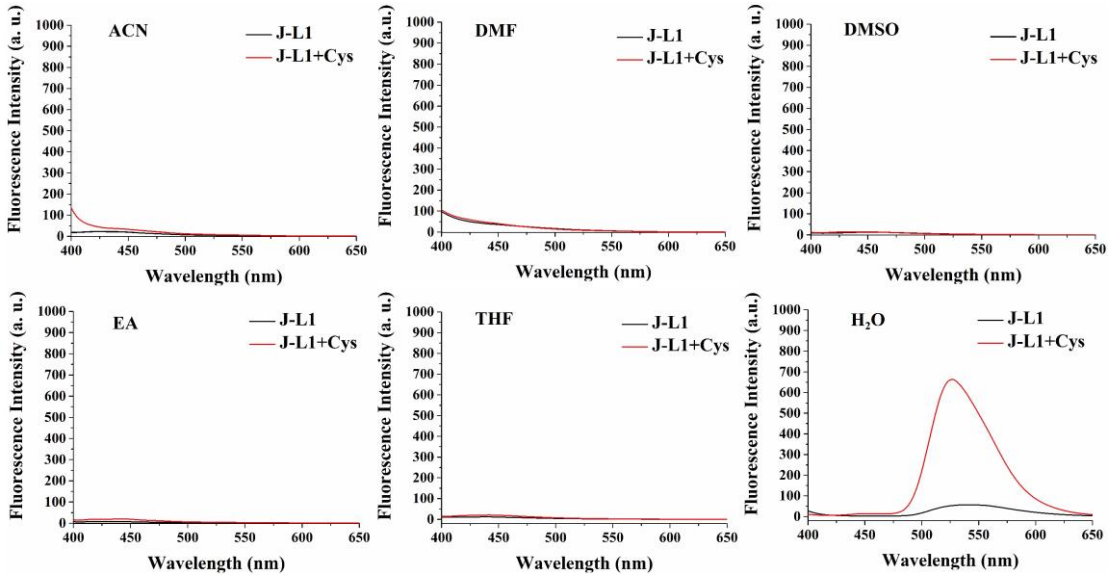


Figure S6. The fluorescence emission of probe **J-L1** (10.0 μM) and its response to Cys (10.0 μM) in different solvents. λ_{ex} = 365 nm.

6. Stabilities of the probe J-L1 and mixture J-L1 + Cys.

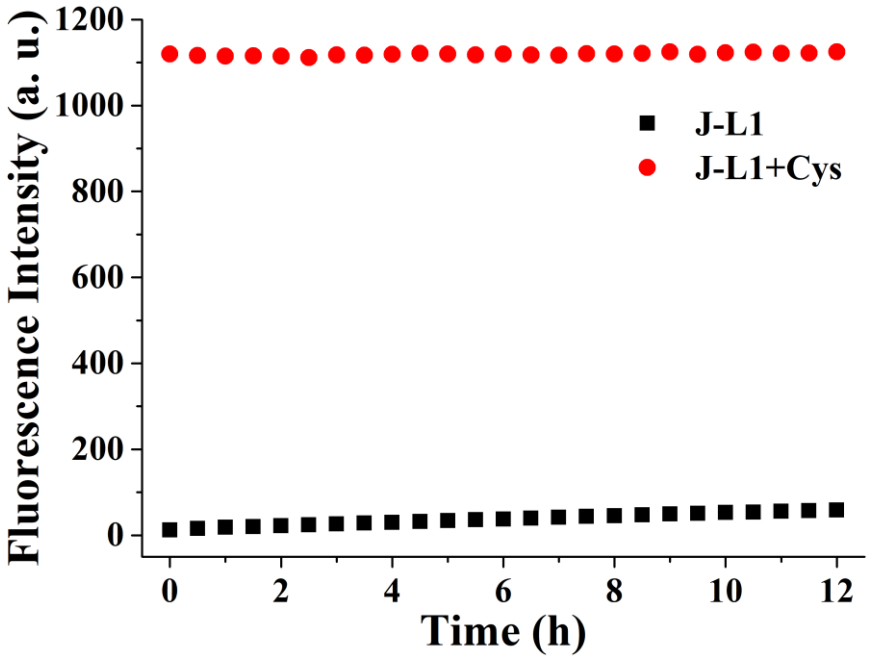


Figure S7. Spot chart of fluorescent intensity changes at 543 nm (**J-L1**) and 525 nm (**J-L1** + Cys) over time (pH = 6.8).

7. The influence of different pH values on the recognition of biological thiols.

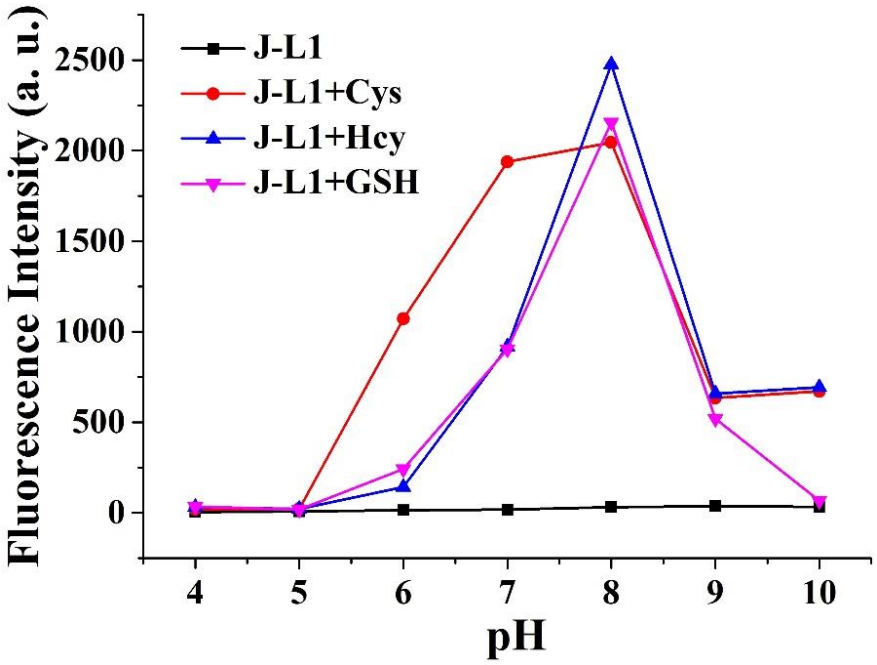


Figure S8. The fluorescence emission intensity at 543 nm of probe **J-L1** (10.0 μM) and the fluorescence emission intensity at 525 nm of **J-L1**+Cys, **J-L1**+Hcy and **J-L1**+GSH in different pH conditions. λ_{ex} = 365 nm.

8. The fluorescent changes of J-L1 responses to biothiols.

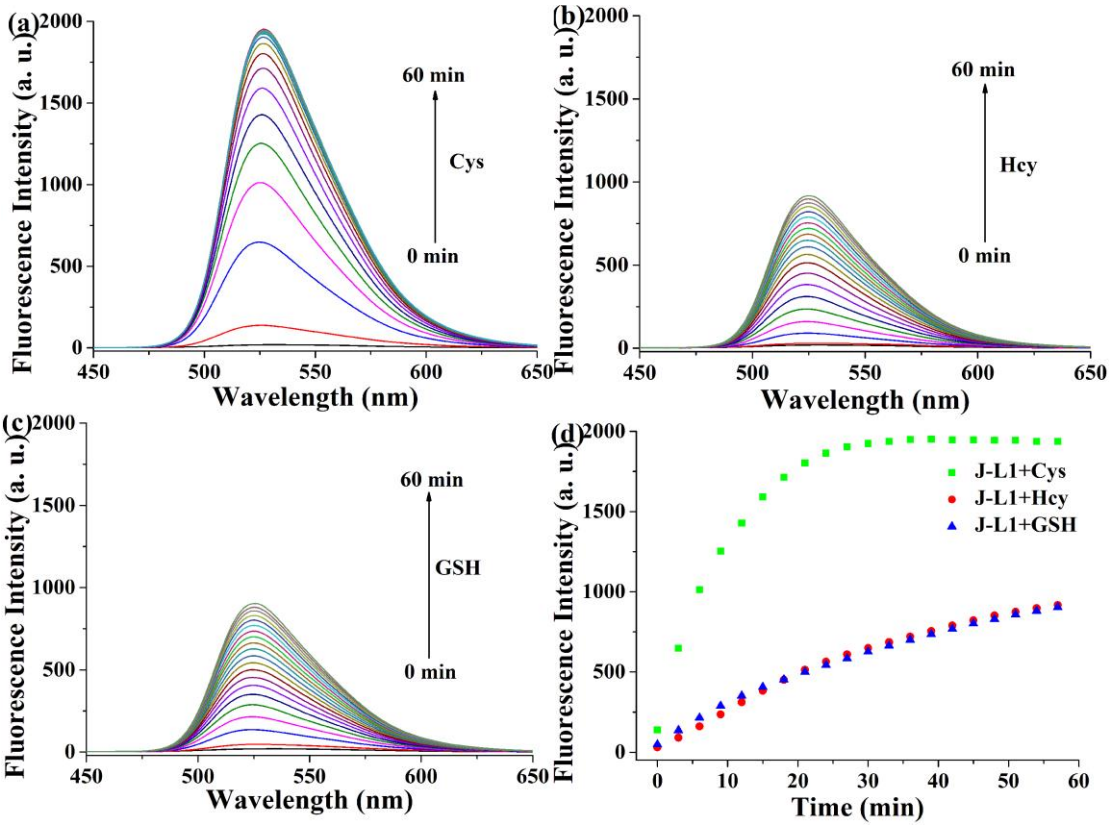


Figure S9. The fluorescent changes of **J-L1** (10.0 μM) responses to mercaptoamino acids (120.0 μM) within 60 min (pH = 7.4). (a) Cys; (b) Hcy; (c) GSH; (d) Spot chart of fluorescent intensity changes at 525 nm (λ_{ex} = 365 nm).

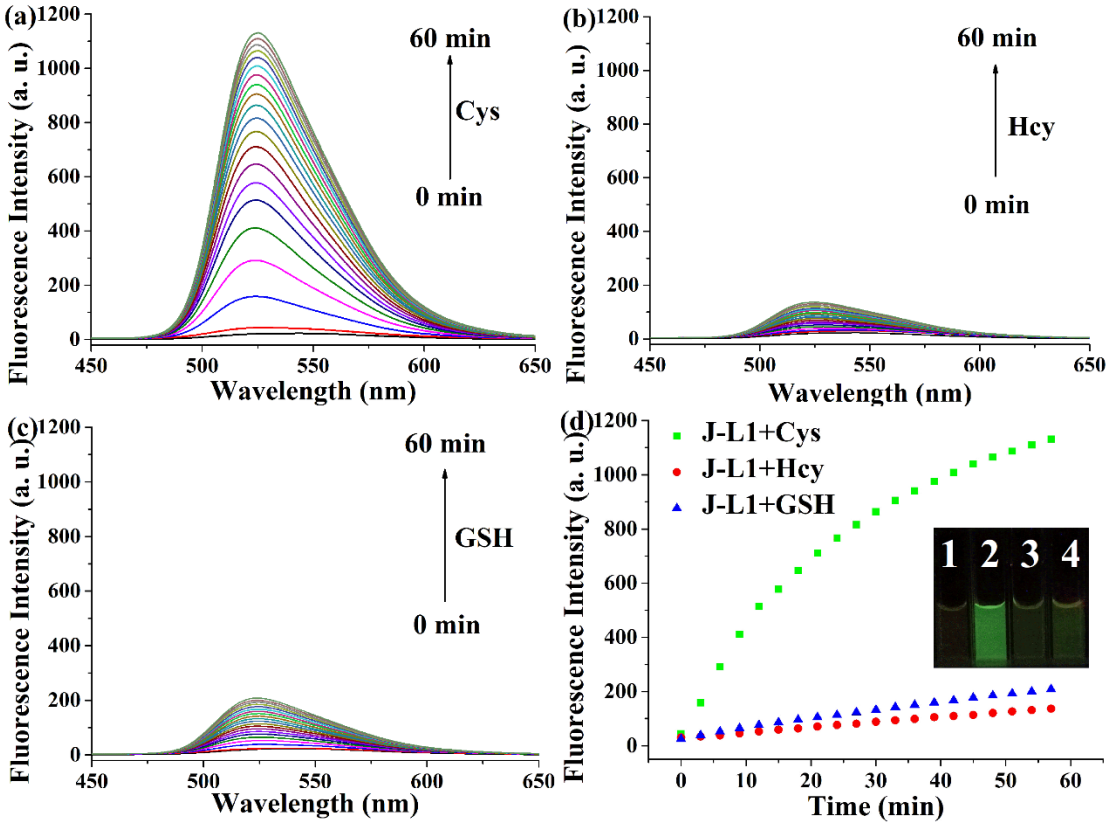


Figure S10. The fluorescent changes of **J-L1** (10.0 μM) responses to mercaptoamino acids (120.0 μM) within 60 min. (a) Cys; (b) Hcy; (c) GSH; (d) Spot chart of fluorescent intensity changes at 525 nm (λ_{ex} = 365 nm). Inset: the fluorescence images of various mixture (1: **J-L1**; 2: **J-L1** + Cys; 3: **J-L1** + Hcy; 4: **J-L1** + GSH).

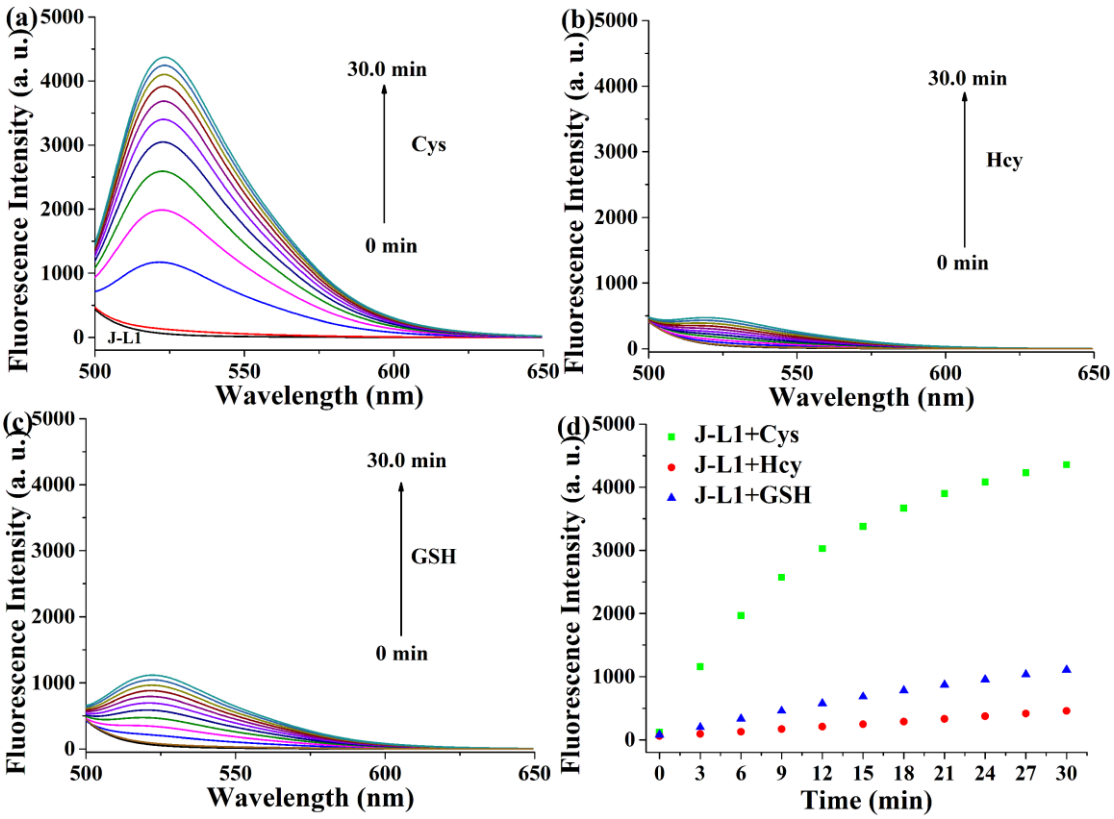


Figure S11. The fluorescent changes of **J-L1** (10.0 μM) responses to mercaptoamino acids (120.0 μM) within 30 min (pH = 6.8). (a) Cys; (b) Hcy; (c) GSH; (d) Spot chart of fluorescent intensity changes at 525 nm (λ_{ex} = 485 nm).

9. The changes of UV-vis spectrum.

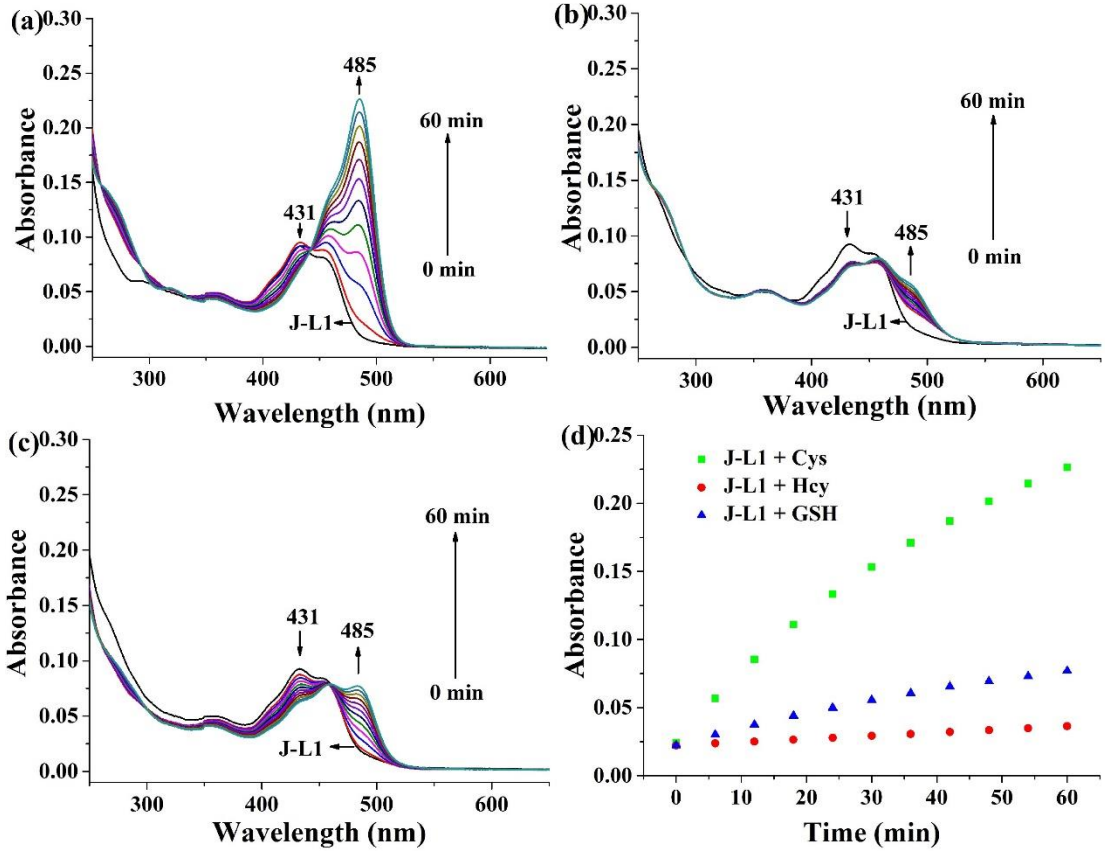


Figure S12. The changes of UV-vis spectra of (a) **J-L1** + Cys; (b) **J-L1** + Hcy (c) **J-L1** + GSH over time and (d) dot plot at 485nm.

10. The naked-eye rapid detection of Cys under visible light and UV light.

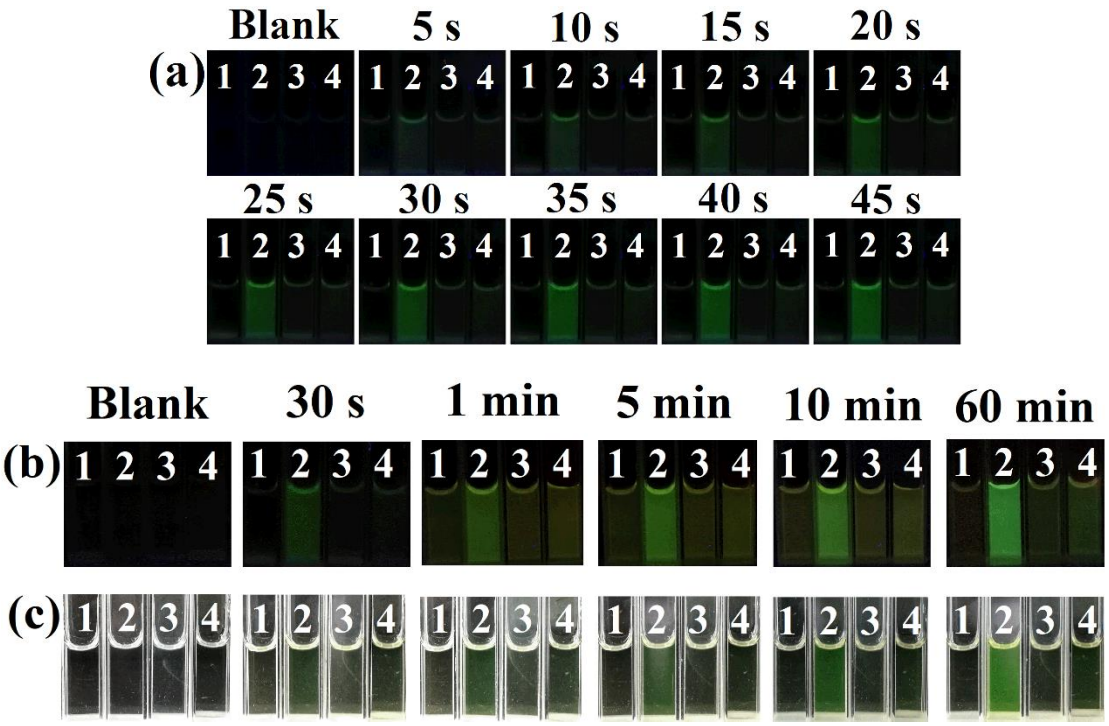


Figure S13. Naked-eye photos of **J-L1** (10.0 μM) responses to 120.0 μM various biomercaptan molecules (1: blank; 2: Cys; 3: Hcy; 4: GSH**)**: (a)(b) Under UV light at 365 nm; (c) Under visible light.

11. The kinetic study for the reaction of probe J-L1 and Cys.

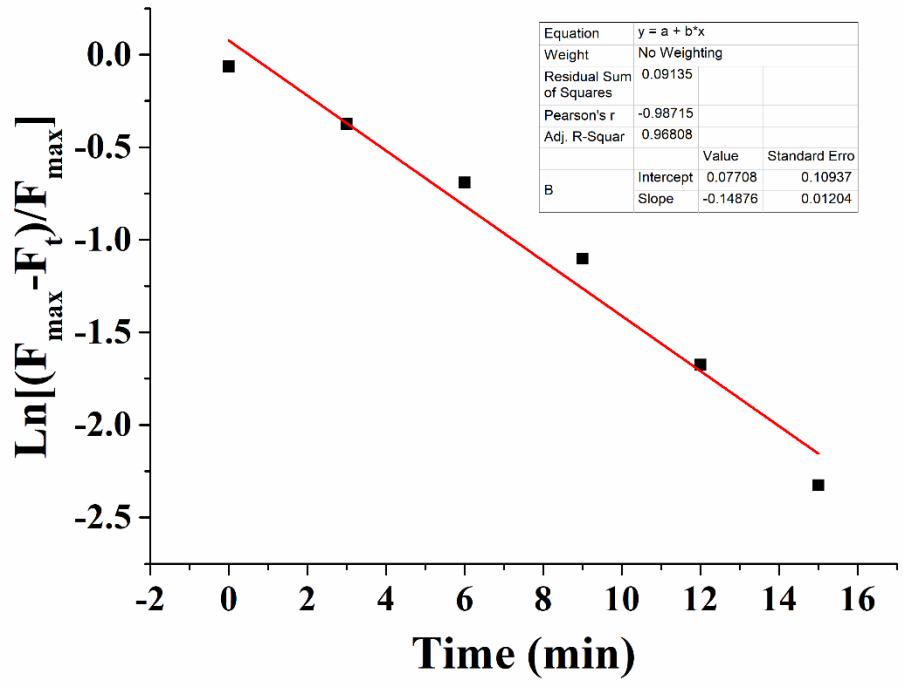


Figure S14. Pseudo first-order kinetic plots of the reaction of probe **J-L1** (10.0 μM) with Cys (120.0 μM) in HEPES buffer (10.0 mM, pH=6.8), λ_{ex} = 365 nm, λ_{em} = 525 nm.

The result of the analysis as follows：

$Ln[(F_{max}-F_t)/(F_{max})] = -k't$

Where F_{max} and F_t are the maximum value obtained after the reaction was completed and the fluorescence intensity at 540 nm at time t. k' is the pseudo-first-order rate constant.

Therefore, the apparent pseudo-first-order rate constant k' was 0.0248 s^{-1}.

12. Determination of the detection limit of J-L1 to Cys.

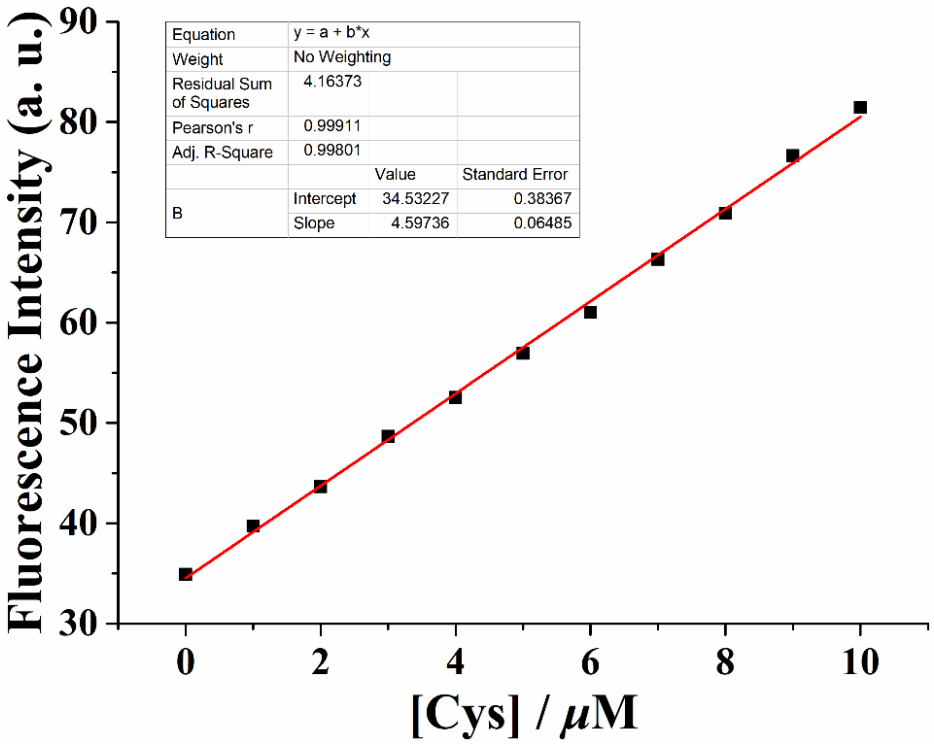


Figure S15. Plot of the intensity at 525 nm for a mixture of probe **J-L1** (10.0 μM) and Cys in HEPES (10.0 mM, pH=6.8) solution in the range 0 — 10.0 μM, λ_{ex} = 365 nm, λ_{em} = 525 nm.

The fluorescence detection limit of probe **J-L1** toward Cys was examined from the titration spectra of probe **J-L1** with Cys. The below equation was used for the plot that determined the LOD.

The result of the analysis as follows：

Linear Equation： Y= 34.53227 + 4.59736 × X

$\delta = \sqrt{\frac{\sum(F_0 - \overline{F_0})^2}{N-1}}$ = 0.0946 (N=20); S = 4.597 × 10^6; K=3;

LOD = $K \times \delta / S$ = 6.17 × 10^{-8} M = 0.0617 μM

Where N, S, F_0 and $\overline{F_0}$ are the number of replicates for the measurements, slope, fluorescence intensity and mean of fluorescence intensity of the blank solution of probe **J-L1**, respectively.

13. Competition experiments of J-L1 responses to Cys.

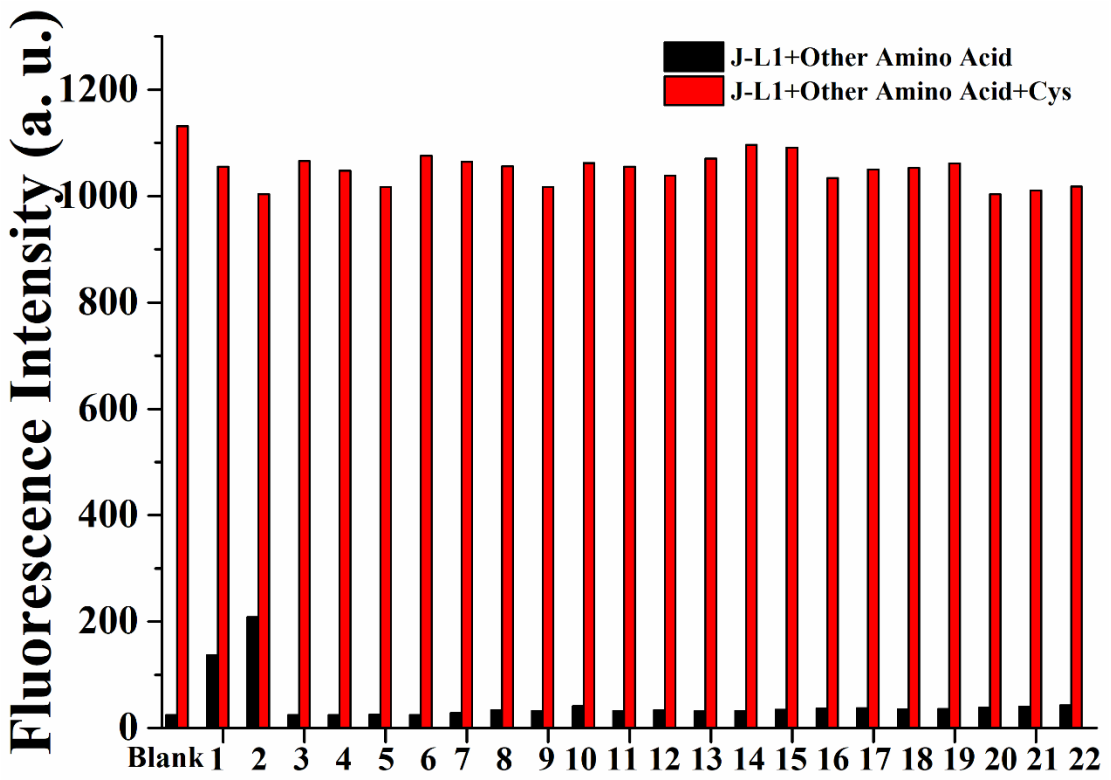


Figure S16. Fluorescence intensity of probe **J-L1** responses to various amino acids (120.0 μM) and further with 120.0 μM Cys. From 1 to 22: Hcy, GSH, Glu, Gly, Cys-Cys, Cit, Asp, The, Phe, Pro, Asn, Leu, Gln, Ile, Thr, Lys, Orn, Arg, Val, Met, Ala, His. All spectra were recorded after 60 min.

14. ESI-MS spectrum of J-L1 responses to Cys.

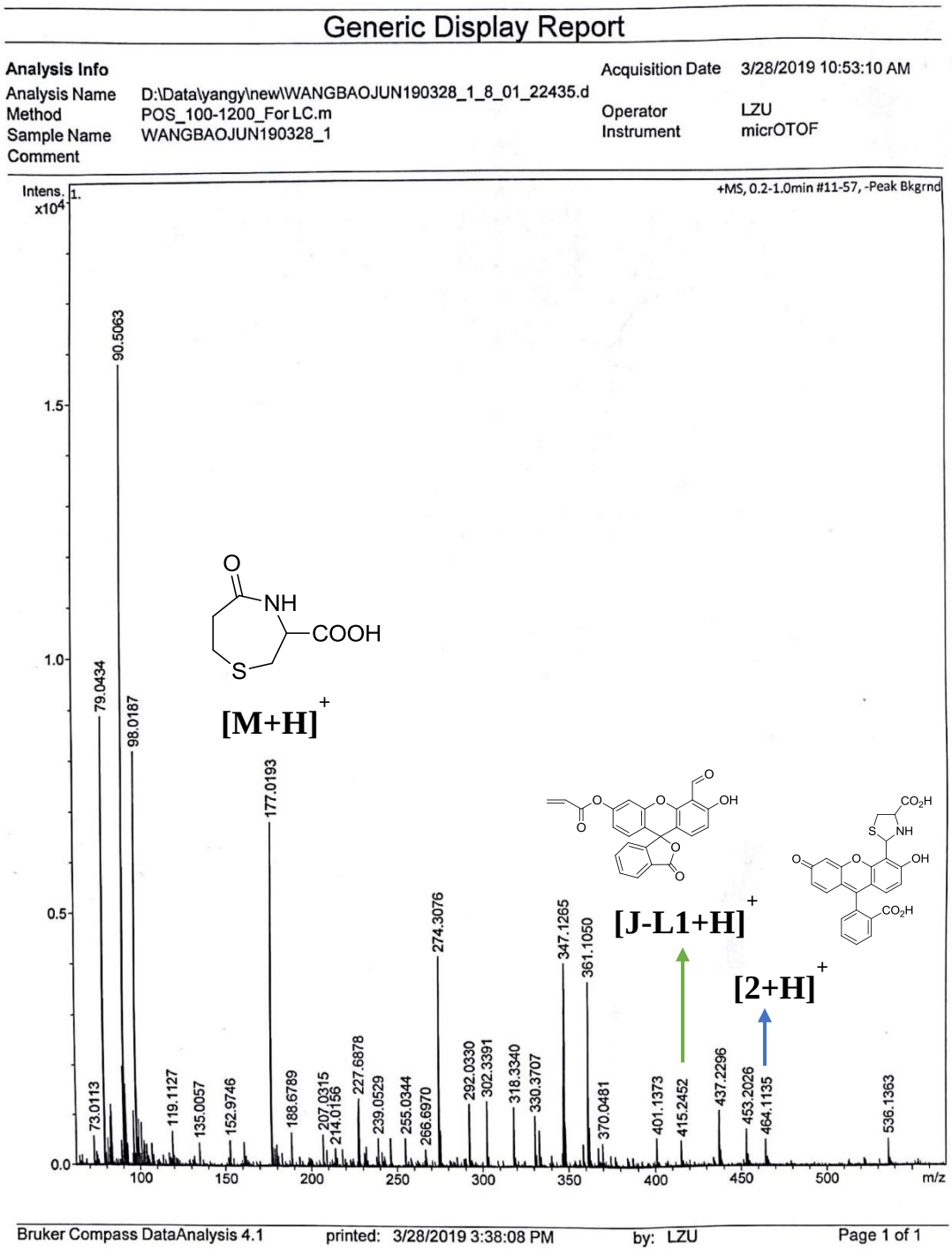


Figure S17. ESI-MS spectrum of **J-L1** responses to Cys.

15. Optimized structures and computed total energies of probe J-L1 and compound 2.

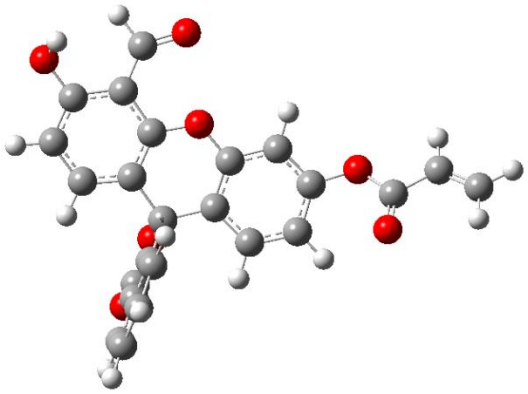


Figure S18. Optimized structure of probe **J-L1**.

B3LYP/6-31g(d,p) geom=connectivity, E= –1449.5434545 a.u.

Charge = 0 Multiplicity = 1

C	-1.498546373	1.233258864	1.339854841
C	-1.366271838	-0.160716843	1.303958683
C	-0.224275685	-0.702854914	0.731684818
C	0.796354206	0.097165839	0.195870767
C	0.627352248	1.481974972	0.261464455
C	-0.511873845	2.060673401	0.824692871
C	2.05317185	-0.518101777	-0.384992572
C	2.988657234	0.539373029	-0.938525718
C	2.69006382	1.886164842	-0.817714405
O	1.553980224	2.36243945	-0.23154291
C	4.20452328	0.169096497	-1.551593888
C	5.076939966	1.132742334	-2.069226445
C	4.776811192	2.476212611	-1.963211373
C	3.583195499	2.911489501	-1.288460169
C	1.764915657	-1.635405667	-1.380469162
C	2.263197743	-2.831448038	-0.881460089
C	2.891532502	-2.57200198	0.434660879

O	2.757832178	-1.232142193	0.70171327
C	1.116393446	-1.595157629	-2.611552366
C	0.987525778	-2.792097168	-3.320338358
C	1.493458081	-3.999397888	-2.812656857
C	2.141308776	-4.032311301	-1.579100337
O	3.436148425	-3.338317335	1.19002349
O	5.640196159	3.406771471	-2.488949338
C	-4.762910878	2.321621781	2.581088209
C	-6.079355772	2.102043342	2.580593909
O	-2.562633361	1.882879633	1.955666829
C	-3.856559766	1.446592045	1.800271033
O	-4.184183213	0.499285953	1.123986247
C	3.354232134	4.264508157	-1.059932787
O	2.331044227	4.847114536	-0.526163927
H	-2.142284267	-0.798075908	1.703282439
H	-0.114109908	-1.781963289	0.701890048
H	-0.611046748	3.138681988	0.861841011
H	4.454334441	-0.881419396	-1.640540797
H	5.989569201	0.845669437	-2.579443119
H	0.721282481	-0.666208069	-3.010992011
H	0.485284872	-2.789696106	-4.283270128
H	1.376171991	-4.912153938	-3.388575154
H	2.540969217	-4.951374921	-1.162698756
H	5.130103632	4.046571798	-3.009312493
H	-4.306138897	3.132297034	3.139059908
H	-6.761125499	2.730479485	3.143647842
H	-6.497938196	1.279746377	2.008450839
H	4.141303786	4.989589984	-1.317579228

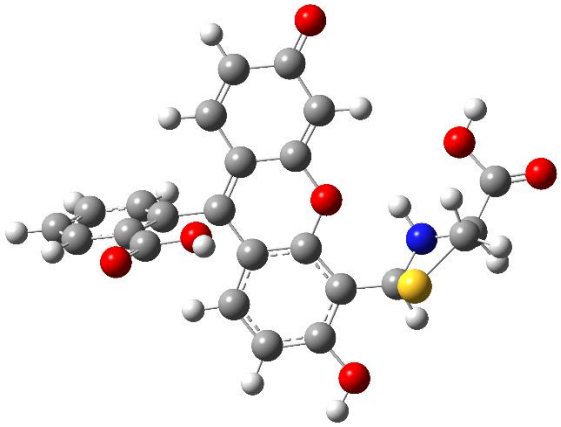


Figure S19. Optimized structure of compound **2**.

B3LYP/6-31g(d,p) geom=connectivity, E = –1903.83796862 a.u.

Charge = 0 Multiplicity = 1

C	-1.40623311	0.35309254	-0.61459509
C	-0.67004759	1.51452012	-1.09438314
C	-1.47746383	2.69733286	-1.43914833
C	-2.83065306	2.70629038	-1.33604271
C	-3.56672318	1.54204134	-0.87931139
C	-2.75968652	0.38972278	-0.52007514
O	-3.40632380	-0.76204951	-0.04943905
C	-4.94100318	1.48676036	-0.76093937
C	-4.78483920	-0.84001426	0.06230816
C	-5.58414971	0.26831831	-0.30341067
C	-6.98291895	0.12575610	-0.18770172
C	-7.54204050	-1.05567983	0.27460838
C	-6.71255757	-2.12753127	0.64110719
C	-5.31031923	-2.05191816	0.53929286
O	-7.23471677	-3.31499494	1.12308220
C	-4.48947091	-3.20953994	1.00589454
O	0.58722889	1.51863797	-1.21063678

C	-5.79519992	2.64716063	-1.17336931
C	-6.07454367	2.77821771	-2.54642402
C	-6.87623111	3.81488861	-3.03221963
C	-7.42173239	4.74926508	-2.14545151
C	-7.15565574	4.63718364	-0.78260800
C	-6.34773476	3.59947424	-0.27982089
N	-3.28312712	-3.49293772	0.31354078
C	-2.39997824	-4.33182096	1.11521782
C	-2.22443868	-3.67192977	2.53370402
S	-3.89952102	-2.91525705	2.97244209
C	-6.13915283	3.61462535	1.19100111
O	-6.66614006	4.42844877	1.96255376
O	-5.29225065	2.63787125	1.65511205
C	-1.05956692	-4.52354558	0.44798961
O	-0.39282319	-5.55704352	0.45777285
O	-0.61327078	-3.35548549	-0.14234103
H	-0.85654805	-0.53725239	-0.33710520
H	-0.92937545	3.56790358	-1.78179469
H	-3.39641854	3.59354363	-1.59614433
H	-7.62588558	0.95166418	-0.46525449
H	-8.62007696	-1.15302549	0.35900161
H	-8.20706162	-3.28913070	1.20045946

16. The cytotoxicity of probe J-L1 on living HeLa cells.

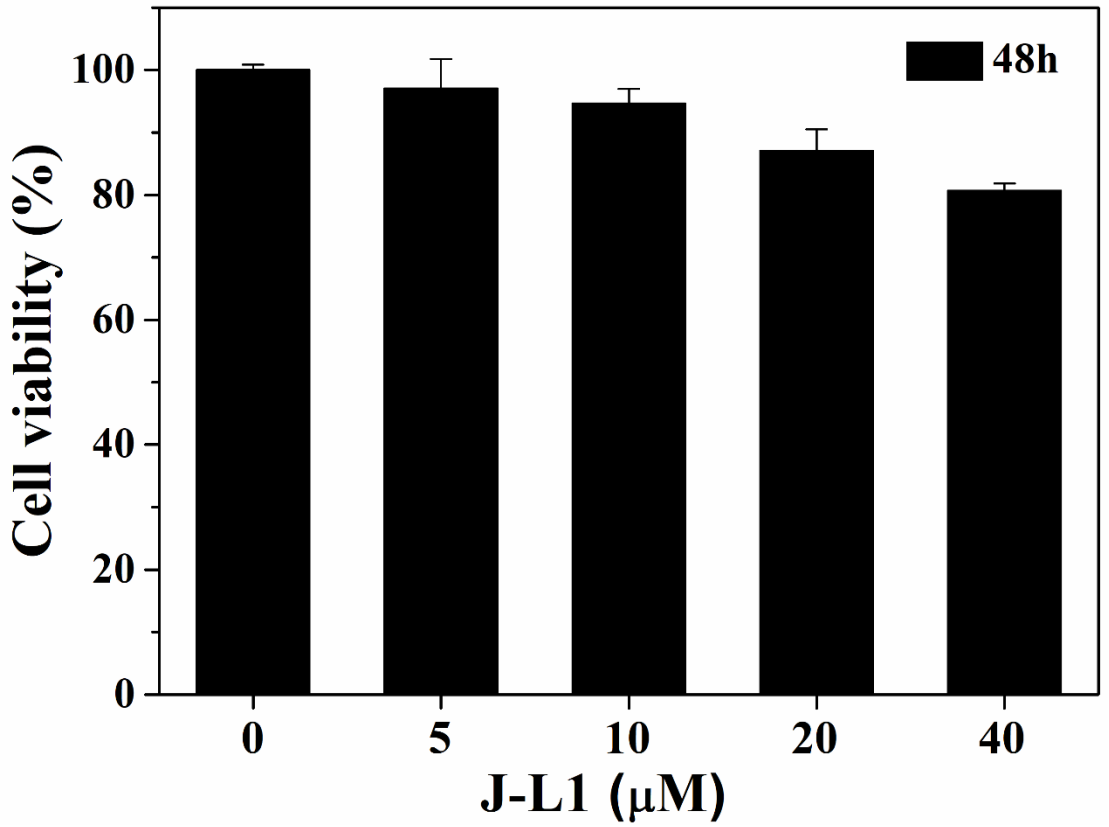


Figure S20. The cytotoxicity of probe **J-L1** in living HeLa cells for 12 h: 0 μM, 5.0 μM, 20.0 μM and 40.0 μM.

17. The fluorescent response of J-L1 (10.0 μM) to several common ions (10.0 μM) in cell.

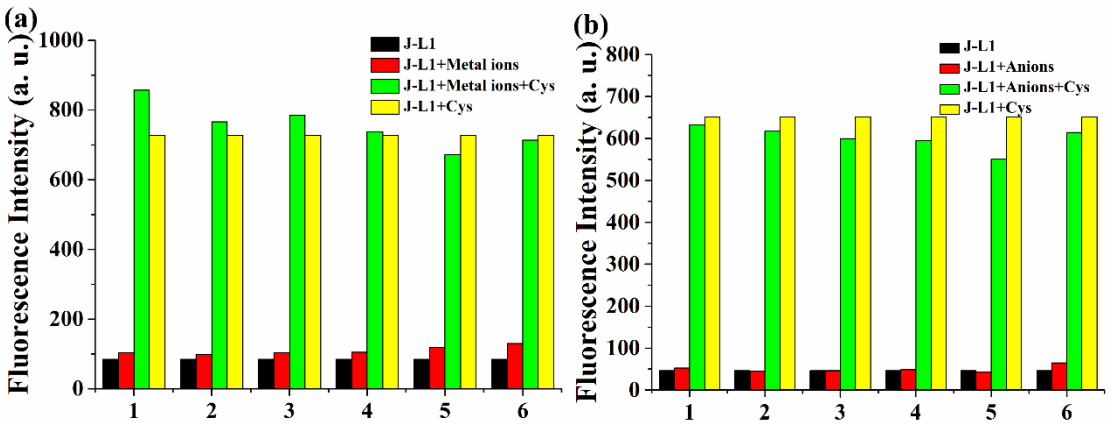


Figure S21. The fluorescent response of **J-L1** (10.0 μM) to several common ions in cell. (a) From 1 to 6: Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, Cu^{2+} and Zn^{2+}; (b) From 1 to 6: PO_4^{3-}, $H_2PO_4^{-}$, CO_3^{2-}, SO_4^{2-}, Cl^{-} and Br^{-} (λ_{ex} = 365 nm).