Supplementary Information

A novel and effective benzo[d]thiazole-based fluorescent probe with dual recognition factors for highly sensitive and selective imaging of cysteine in vitro and in vivo

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Supplementary Figures

1. Characterization of BT-OH and BT-AC

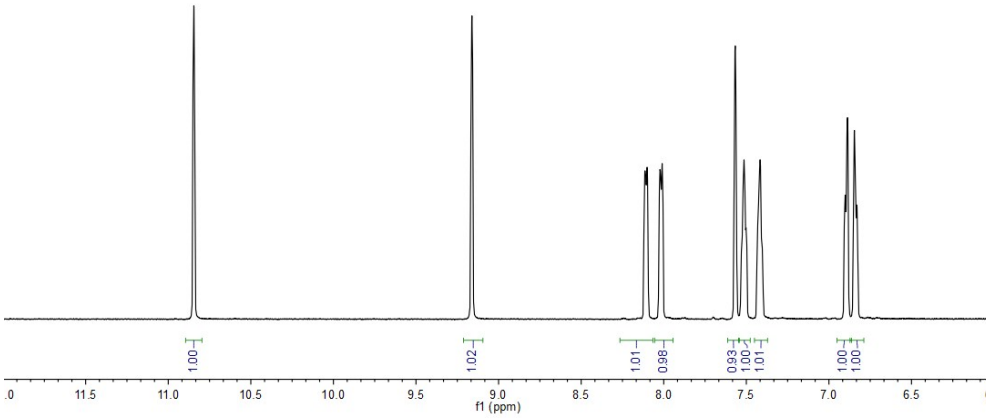

Figure S1. ^{1}H-NMR spectra of **BT-OH**

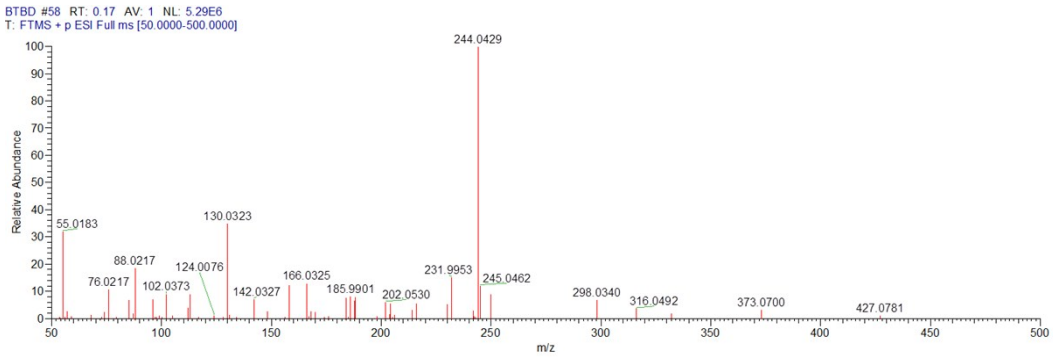

Figure S2. LC-HRMS spectra of **BT-OH**

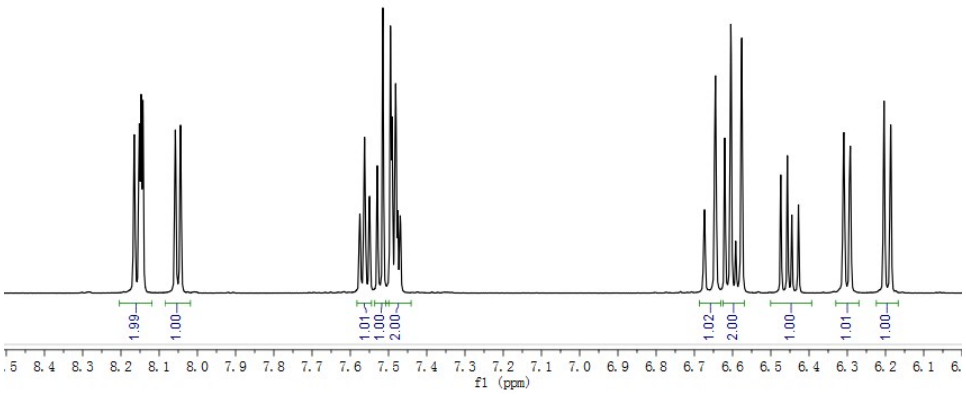

Figure S3. ^{1}H -NMR spectra of **BT-AC**

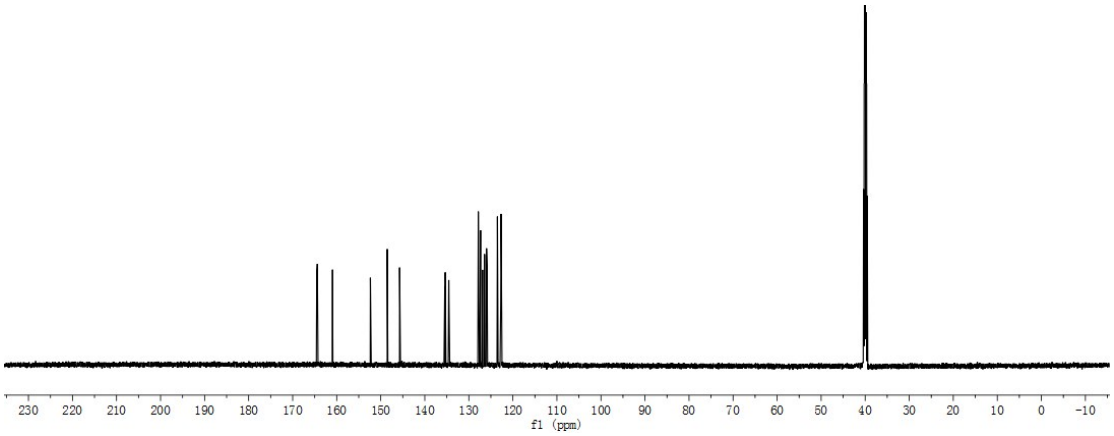

Figure S4. ^{13}C-NMR spectra of **BT-AC**

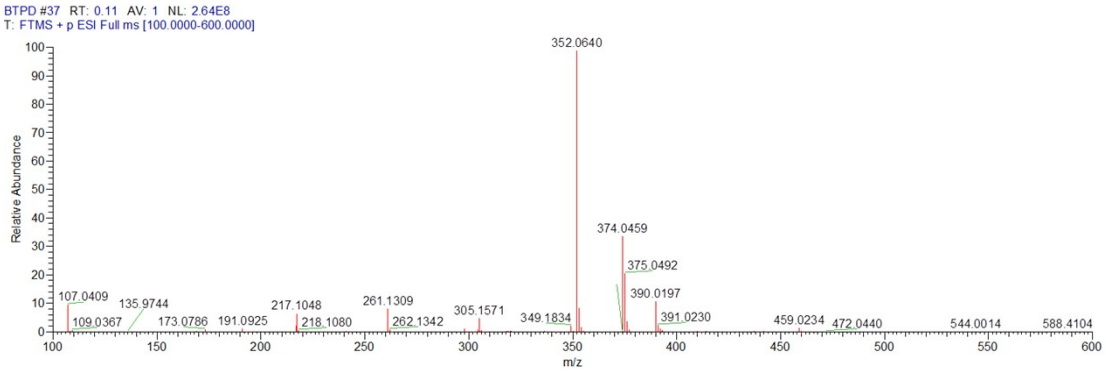

Figure S5. LC-HRMS spectra of **BT-AC**

2. Optimization of experimental conditio

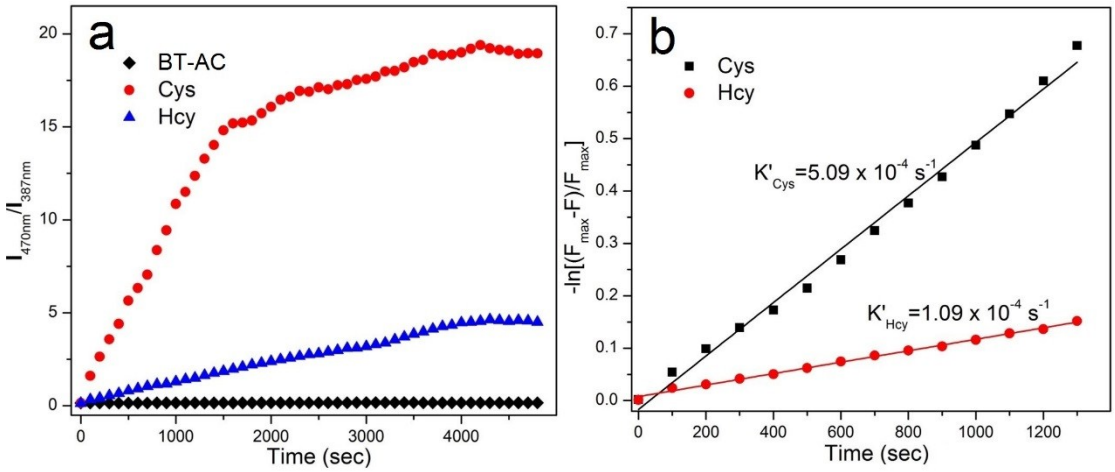

Fig S6. Time-dependent fluorescence ratiometric response (I_{470nm}/I_{387nm}) and pseudo-first-order kinetic plots of **BT-AC** (10 μM) at 470 nm with and without biothiols (200 μM) in DMSO-HEPES buffer solution (7:3, v/v, pH 7.4, λ_{ex} = 335 nm).

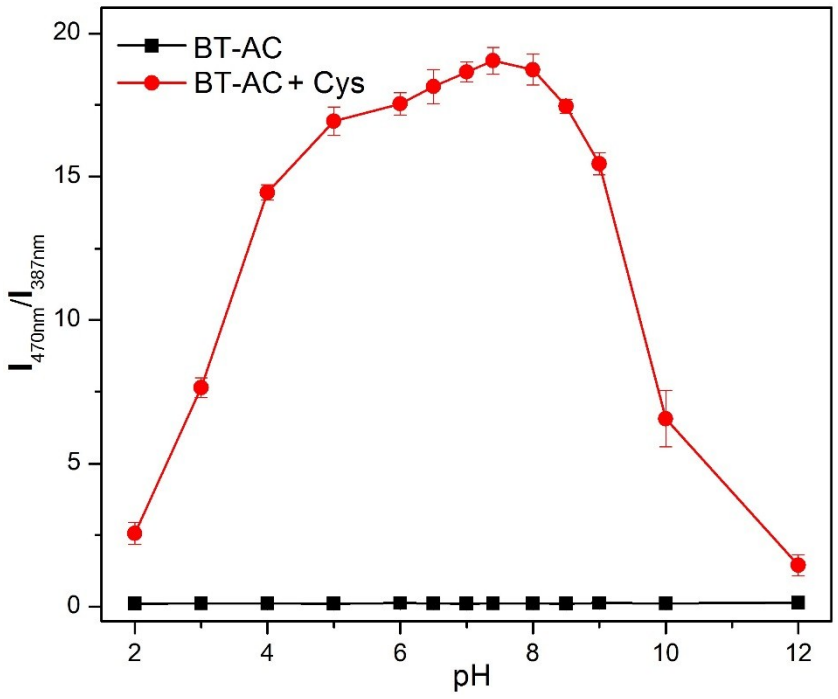

Figure S7. Ratiometric response (I_{470nm}/I_{387nm}) of **BT-AC** (10 μM) with or without biothiols (200 μM) in DMSO-HEPES buffer solution (**BT-AC** 10 mM, pH = 2, 3, 4, 5, 6, 6.5, 7, 7.4, 8, 8, 8.5, 9, 9.5, 10 and 12, λ_{ex} = 335 nm, slit: 10.0 nm). Data are mean±SE (bars) (n = 3).

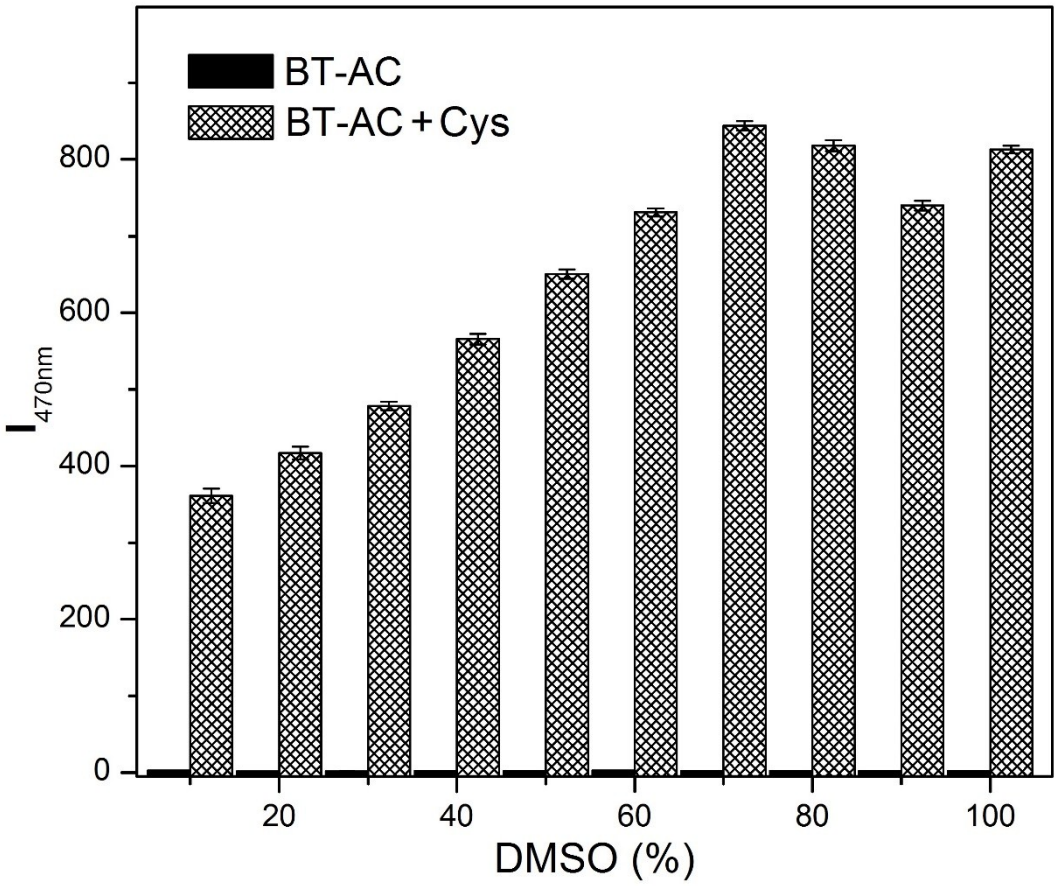

Figure S8. Fluorescence response of **BT-AC** (10 μM) without and with biothiols (200 μM) in HEPES buffer solution with different ratios of DMSO and HEPES. (pH 7.4, λ_{ex} = 335 nm, slit: 10.0 nm). Data are mean ± SE (bars) (n = 3).

3. Study on reaction mechanism of BT-AC with Cys/Hcy

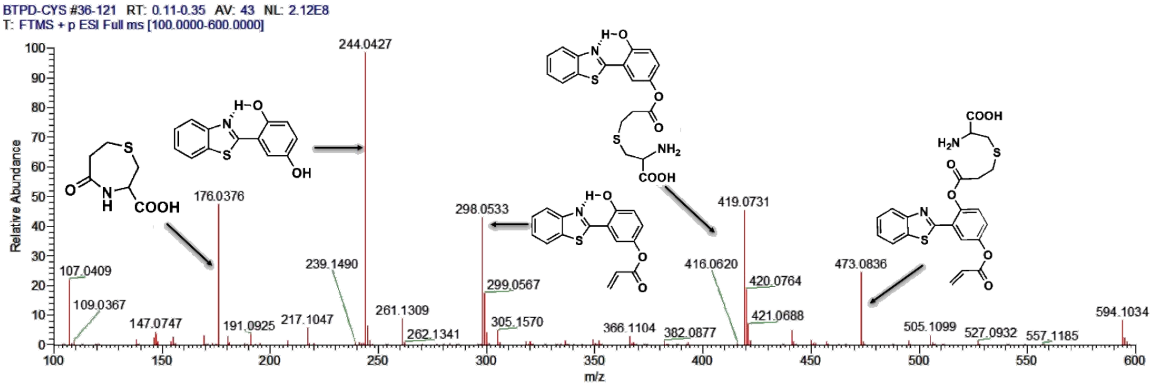

Figure S9. LC- HRMS spectrum of the products of the reaction between **BT-AC** and Cys

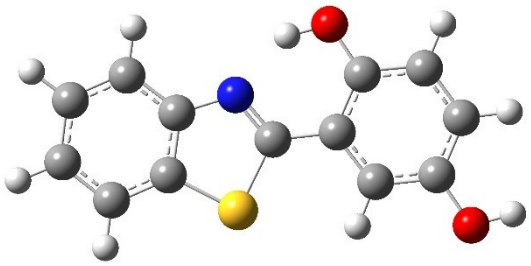

Figure S10. Optimized structure of probe **BT-OH**.

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%mem=8gb

%nproc=4

b3lyp/6-31g** cube=orbitals

Title Card Required

C	-4.00162	-0.62551	-0.30079
C	-2.60668	-0.86214	-0.30112
C	-1.71769	0.22216	-0.30121
C	-2.23378	1.51251	-0.30098
C	-3.62116	1.73592	-0.30066
C	-4.51917	0.67069	-0.30056
C	-3.28135	-2.99823	-0.30116
H	-0.64804	0.03745	-0.30147
H	-1.55547	2.36084	-0.30105
H	-4.00200	2.75316	-0.30048
H	-5.59048	0.84698	-0.30031
N	-2.24602	-2.19633	-0.30133
S	-4.85456	-2.15829	-0.30074
C	-3.17065	-4.45046	-0.30132
C	-4.31818	-5.2672	-0.30104
C	-1.8853	-5.06101	-0.30178
C	-4.21537	-6.65048	-0.30118
H	-5.31072	-4.82723	-0.3007
C	-1.80146	-6.46072	-0.30191

C	-2.94522	-7.24668	-0.30161
H	-0.81427	-6.91105	-0.30224
H	-2.85407	-8.33152	-0.30171
O	-0.73061	-4.36629	-0.30203
O	-5.37928	-7.3778	-0.30088
H	-0.95597	-3.40077	-0.30183
H	-5.15948	-8.32176	-0.30108

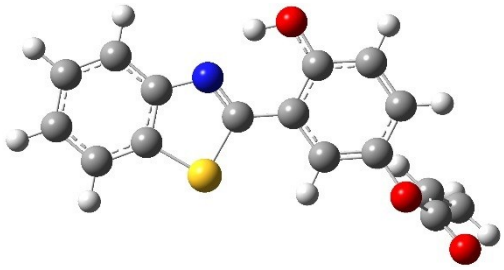

Figure S11. Optimized structure of probe **BT-OH-AC**.

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%nproc=4

b3lyp/6-31g** cube=orbitals

Title Card Required

C	-3.34053	0.05144	0.38569
C	-2.23037	-0.43764	-0.34177
C	-1.25198	0.45481	-0.80272
C	-1.39999	1.80921	-0.53038
C	-2.50743	2.28428	0.19317
C	-3.48877	1.41222	0.65888
C	-3.26074	-2.39546	0.00743
H	-0.40126	0.07583	-1.36022
H	-0.64962	2.51162	-0.88116

H	-2.6018	3.34747	0.39409
H	-4.34214	1.78309	1.21808
N	-2.223	-1.80693	-0.53222
S	-4.386	-1.28557	0.82847
C	-3.482	-3.83443	-0.05415
C	-4.6151	-4.42822	0.53573
C	-2.5399	-4.66858	-0.72134
C	-4.8128	-5.79629	0.47374
H	-5.3495	-3.82253	1.05794
C	-2.75985	-6.05606	-0.76328
C	-3.87748	-6.61955	-0.16808
H	-2.02207	-6.67077	-1.26816
H	-4.03308	-7.69394	-0.18611
O	-1.43347	-4.19882	-1.31872
O	-5.89959	-6.34972	1.14371
C	-6.97966	-6.89822	0.48311
O	-7.78014	-7.52584	1.13222
C	-7.09536	-6.6424	-0.97919
H	-6.38284	-5.97717	-1.45378
C	-8.07627	-7.22227	-1.67623
H	-8.19746	-7.04545	-2.74081
H	-8.78148	-7.88836	-1.1875
H	-1.41361	-3.21257	-1.19898

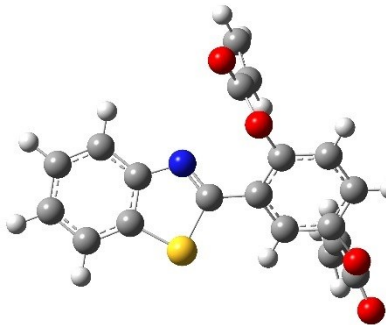

Figure S12. Optimized structure of probe **BT-AC**.

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%mem=8gb

%nproc=4

b3lyp/6-31g** cube=orbitals

Title Card Required

C	-3.04472	-0.58653	0.42656
C	-1.90272	-1.23284	-0.10802
C	-0.85719	-0.46723	-0.64624
C	-0.96955	0.91661	-0.64147
C	-2.10881	1.54716	-0.10906
C	-3.15677	0.80598	0.4302
C	-3.01172	-3.05614	0.50112
H	0.01619	-0.97101	-1.04746
H	-0.16691	1.5225	-1.05169
H	-2.17369	2.63151	-0.11586
H	-4.03222	1.29867	0.8417
N	-1.9247	-2.61331	-0.05027
S	-4.15998	-1.79129	1.03401
C	-3.32725	-4.47856	0.69597
C	-4.66348	-4.91273	0.69912
C	-2.31408	-5.44315	0.89418
C	-4.97735	-6.25717	0.87816
H	-5.46645	-4.19738	0.55169
C	-2.64369	-6.78479	1.09363
C	-3.97157	-7.20214	1.081
H	-1.83977	-7.49085	1.2745
H	-4.23781	-8.24215	1.23777
O	-0.99226	-5.0773	1.04344
O	1.02904	-4.62188	0.25454

C	-0.101	-4.9422	-0.00967
C	-0.59673	-5.26378	-1.37366
C	0.26229	-5.32949	-2.39328
H	-0.06833	-5.56379	-3.40074
H	-1.65606	-5.44602	-1.51476
H	1.3208	-5.14426	-2.23585
O	-6.29722	-6.67222	0.94987
C	-7.17603	-6.5746	-0.11463
O	-8.35393	-6.68939	0.11297
C	-6.58766	-6.39064	-1.46865
H	-5.51072	-6.44561	-1.5832
C	-7.38888	-6.18152	-2.51688
H	-6.99178	-6.06387	-3.52063
H	-8.46571	-6.12602	-2.38642

4. Practical application potential of BT-AC

Figure S13. Fluorescence changes in test paper (under a 365 nm UV lamp) with probe **BT-AC** upon addition of some representative analytes (1. **BT-AC**; 2. buffer; 3. Pro; 4. Asp; 5. Try; 6. Arg; 7. Tyr; 8. His; 9. Glu; 10. Lys; 11. Thr, 12.glucose; 13. K^+; 14. Ca^{2+}; 15. Na^+; 16. Mg^{2+}; 17. Zn^{2+}; 18. Fe^{3+}; 19. Cu^{2+}, 20. H_2O_2; 21. NaHS; 22. Cys; 23. Hcy; 24. GSH).

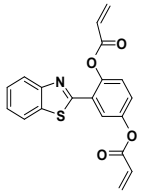
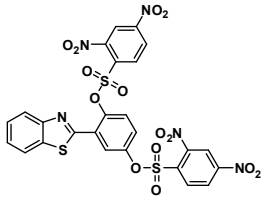
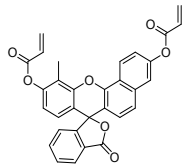
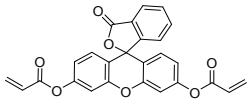
5. The program of gradient elution in LC-HRMS

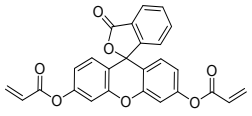
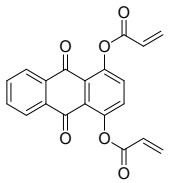
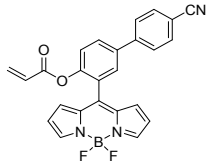
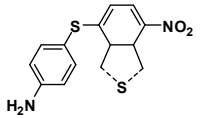
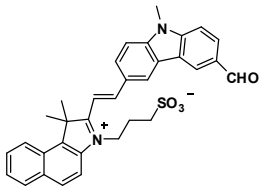
Table S1. The program of gradient elution in LC-HRMS.

Time (min)	Flow rate (mL/min)	%A	%B	curve
0.25	0.3	95%	5%	6
3	0.3	60%	40%	6
5	0.3	5%	95%	6
7	0.3	5%	95%	6
8	0.3	95%	5%	11
10	0.3	95%	5%	11

6. Comparison of BT-AC with other fluorescent probes for Cys

Table S2. Comparison of several recent reported probes for the detection of Cys.

Probe	Molecular weight	Stoke shift (nm)	Solvent (pH=7.4)	Excitation and Emission wavelength	Reaction sites	Time (min)	Detection Limit (μM)	Reference
	351.4	135	DMSO-HEPES (7/3，v/v)	E_x = 335 nm, E_m = 470 nm	acrylate group	20	0.032	This work
	703.6	160	DMSO-HEPES (4/6，v/v)	E_x=350nm, E_m=430nm/510nm,	2,4-dinitrobenzenesulfonyl group	20	0.17-0.40	1
	504.5	71	CTAB buffer	E_x = 562 nm, E_m = 621 nm	acrylate group	20	0.20	2
	440.4	70	EtOH-water (2/3, v/v)	E_x = 450 nm, E_m = 520 nm	acrylate group	60	0.50	3

	440.4	37	EtOH-PBS (2/8, v/v)	E_x = 478 nm, E_m = 515 nm	acrylate group	15	0.077	4
	348.4	35	CH_3CN–HEPES (2/8, v/v)	E_x = 470 nm, E_m = 565 nm	acrylate group	90	0.158	5
	440.2	30	CH_3CN-H_2O (1:1, v/v)	E_x = 503 nm, E_m = 525 nm	acrylate group	150	0.037	6
	306.4	60	HEPES (0.01 M, pH 7.4) containing 1% DMSO	E_x = 475 nm E_m = 535 nm	p-aminophenylthioether	60	0.1	7
	550.7	95	PBS / DMSO （2/1， v / v）	E_x = 497 nm E_m = 590 nm	Carbazole and benzoindole	10	0.06	8

7. References

1. M.Q. Zhu, X.N Liu, Y.N Yang, L.J. Wang, X.Q. Wu, S.S. Fan, Z. Wang, R.M. Hua, Y. Wang and Q. X. Li, *J Mol Liq*, 2019, **287**, 111016.
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