Electronic Supplementary Material (ESI) for Analyst.

Supplemental Information for

Mitochondria-Targeted Ratiometric Fluorescent Imaging of Cysteine

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Experimental Section:

Synthesis of Compound 1

2-Methylbenzothiazole (3.18 mL, 25 mmol) and Iodoethane (10.5 mL, 125 mmol) ware added in thick glass tube, and the mixture was stirred at 140℃ for 12 h. After cooled to room temperature, the reaction extracted with water and ethyl acetate, and the water phase was dried by rotary evaporator to obtain Compound **1** (5.49 g, 72% yield). HRMS: Calculated for $C_{10}H_{12}NS^+$ 178.0685; Found: 178.0732 (Figure S4).

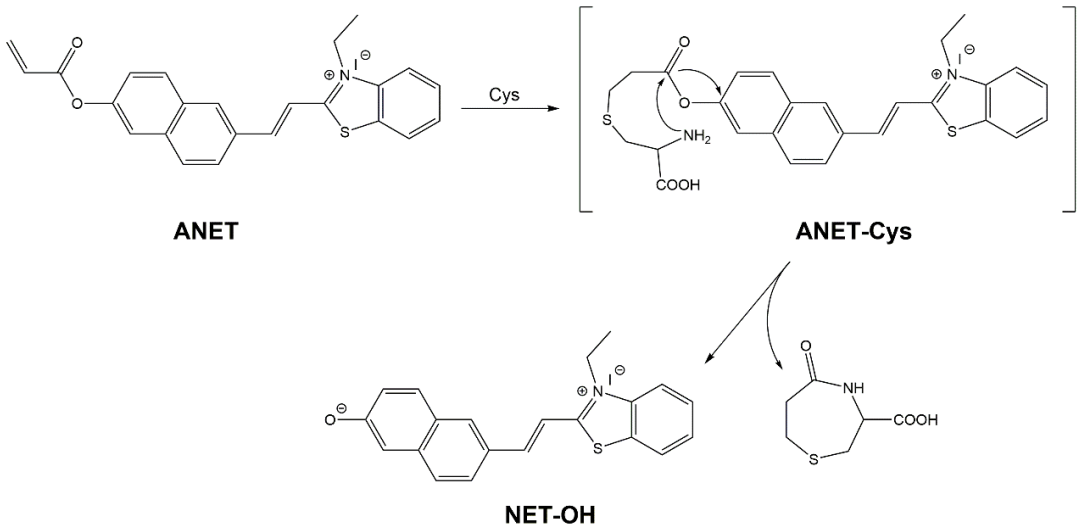
Synthesis of Compound NET-OH

Compound **1** (304.9 mg, 1.0 mmol), 6-Hydroxy-2-naphthaldehyde (258.3 mg, 1.5 mmol) and 400 μL Piperidine ware dissolved in 100 mL EtOH, and the mixture was stirred at 105℃ for 24 h. After the reaction was completed, the mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was extracted with water and ethyl acetate, and the product was in the water layer. The water was removed by rotary evaporator to obtain **NET-OH** (280.4 mg, 63% yield). HRMS: Calculated for $C_{21}H_{18}NOS^+$ 332.1104; Found: 332.1285 (Figure S5).

Synthesis of Compound ANET

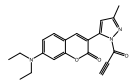
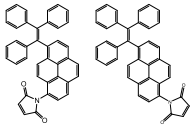
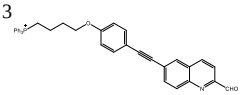
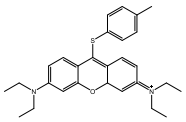
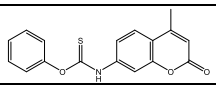
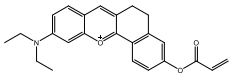
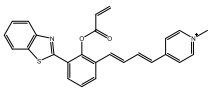
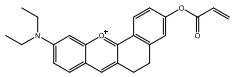
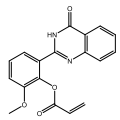
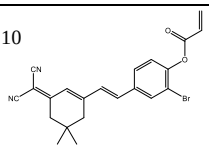
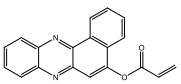
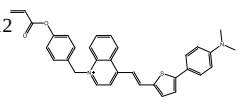
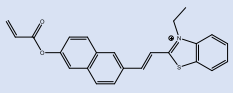
NET-OH (91.8 mg, 0.2 mmol), Acryloyl chloride (200 μL, 2.45 mmol) and Na_2CO_3 150.0 mg, 1.42 mmol) ware added in 20 mL ACN, and the mixture was stirred at room temperature for 48 h. After filtered the mixture, the obtained solution was dried by rotary evaporator, purified by column chromatography on silica gel by using (CH_2Cl_2: CH_3OH=20: 1) to obtain **ANET** (52.3 mg, 51% yield).[1-3] HRMS: Calculated for $C_{24}H_{20}NO_2S^+$ 386.1209; Found: 386.1443 (Figure S6).

Supplementary Figures:



Scheme S1. The reaction mechanism of **ANET** with Cys.

Table S1. Comparison of **ANET** with other representative Cys fluorescent probes.

Structures	Responsive group	Targeting ability	Selectivity	DL (μM)	Ratio	Stokes shift	Reference
1 		No	Cys/Hcy	Cys: 0.049 Hcy: 0.051	No	65	43
2 		No	Cys/Hcy	Not mentioned	No	125/127	44
3 		Mito	Cys/Hcy	Not mentioned	Yes	157→140	45
4 		Mito	Cys/Hcy	Cys: 0.022 Hcy: 0.023	Yes	85→40	46
5 		No	Cys	0.16	No	103	47
6 		No	Cys	0.12	No	45	28
7 		No	Cys	0.102	No	302	38
8 		No	Cys	0.243	No	50	48
9 		No	Cys	16.7	No	145	49
10 		No	Cys	0.087	No	161	50
11 		No	Cys	0.0018	Yes	95→70	51
12 		Yes	Cys	0.021	No	260	52
13. This work 		Yes	Cys	0.074	Yes	119→163	

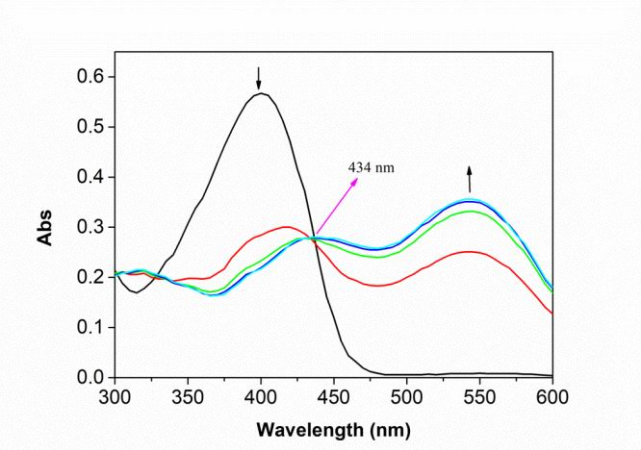


Figure S1. The time-dependent UV spectrum of **ANET** (50 μM)+Cys (50 μM) in DMSO: PBS buffer (1/1 v/v) at pH 7.4.

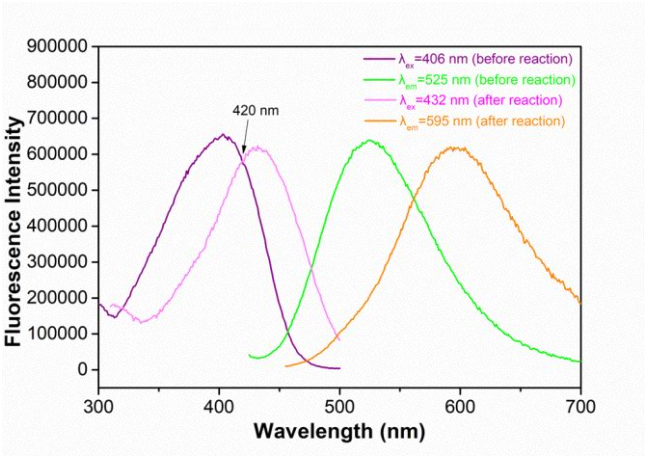


Figure S2. The excitation and emission spectra of **ANET** (10 μM) before and after reaction with Cys (10 μM) in DMSO: PBS buffer (1/1 v/v) at pH 7.4.

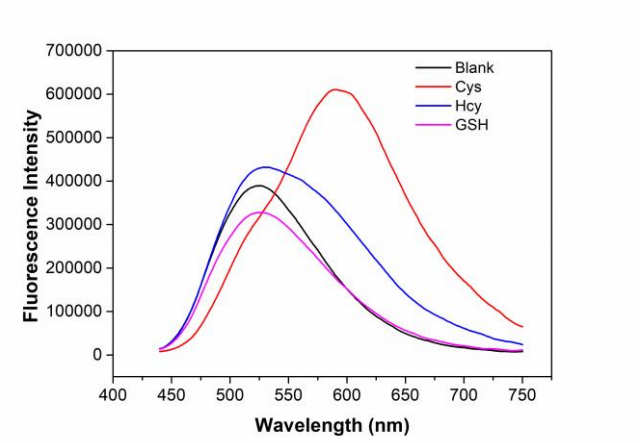


Figure S3. Fluorescence spectra of **ANET** (10 μM) upon addition of 10 μM Cys, 10 μM Hcy, 1mM GSH after 10 min respectively.

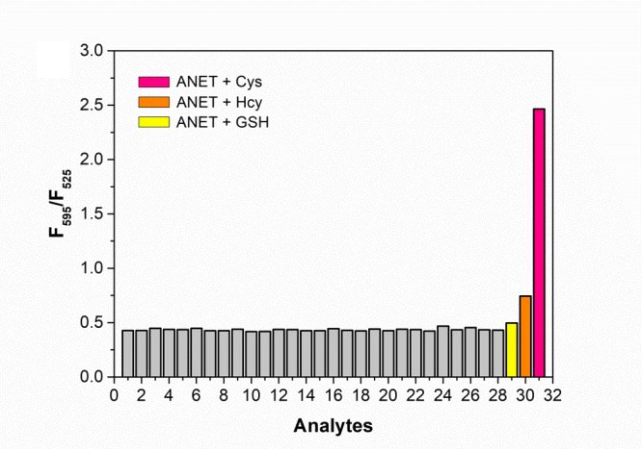


Figure S4. Fluorescence intensity at F_{595}/F_{525} of **ANET** (10 μM) in the presence of various analytes for 10 min: (1) Blank, (2) Cl^-, (3) Br^-, (4) CO_3^{2-}, (5) SO_4^{2-}, (6) S^{2-}, (7) NO^{3-}, (8) NO^{2-}, (9) K^+ (140 mM), (10) Mg^{2+}, (11) Zn^{2+}, (12) Fe^{2+}, (13) Ala, (14) Arg, (15) Asp, (16) Glu, (17) Gly, (18) His, (19) Leu, (20) Lys, (21) Ile, (22) Met, (23) Phe, (24) Pro, (25) Ser, (26) Thr, (27) Try, (28) Val, (29) GSH (1 mM), (30) Hcy (10 μM), (31) Cys (10 μM), the concentration of other analytes is 100 μM

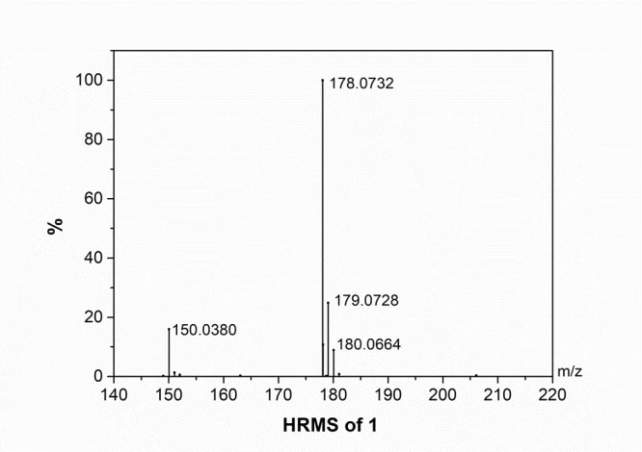


Figure S5. ESI-MS spectrum of **1** in positive ion mode.

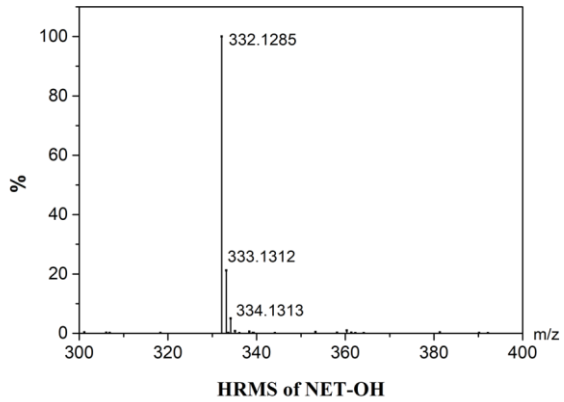


Figure S6. ESI-MS spectrum of **NET-OH** in positive ion mode.

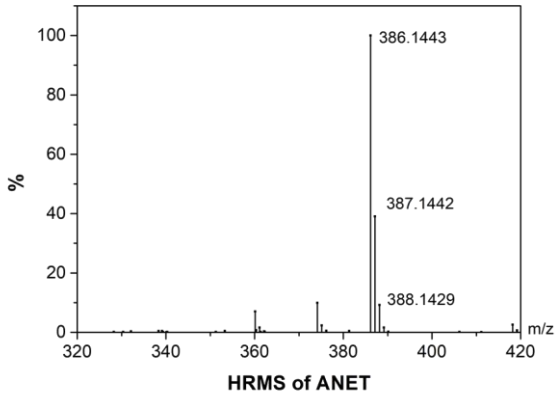


Figure S7. ESI-MS spectrum of **ANET** in positive ion mode.

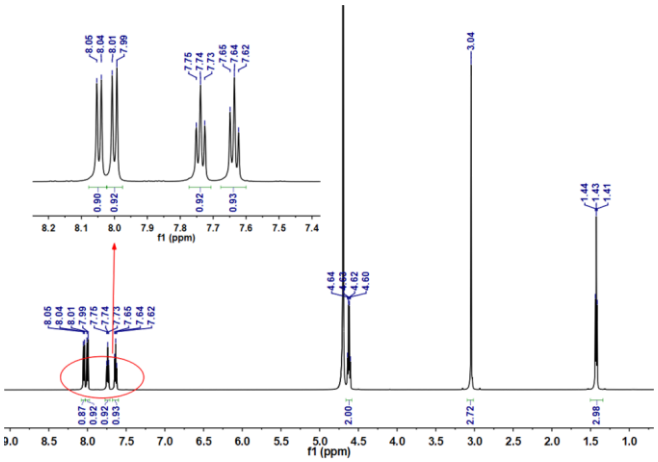


Figure S8. ^{1}H NMR spectrum of **Compound 1** in D_2O.

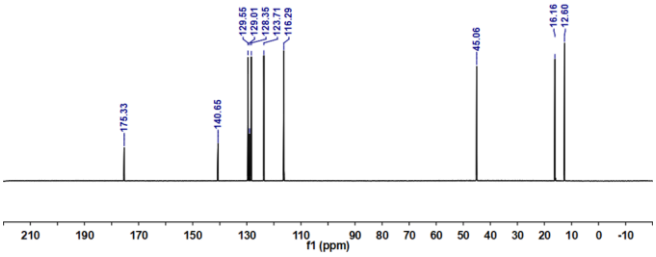


Figure S9. ^{13}C NMR spectrum of **Compound 1** in D_2O

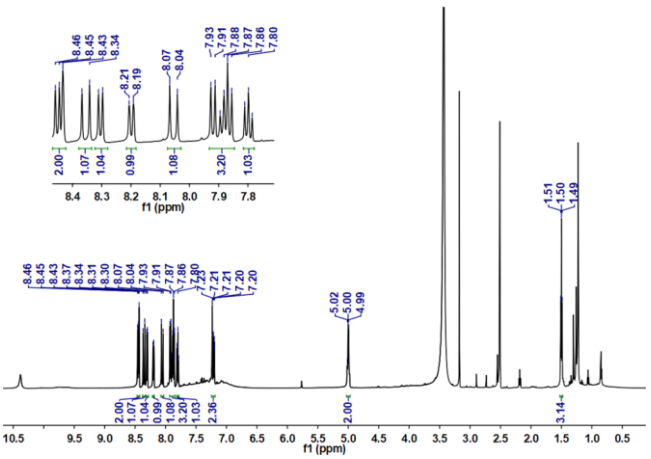


Figure S10. ^{1}H NMR spectrum of **NET-OH** in DMSO-*d6*.

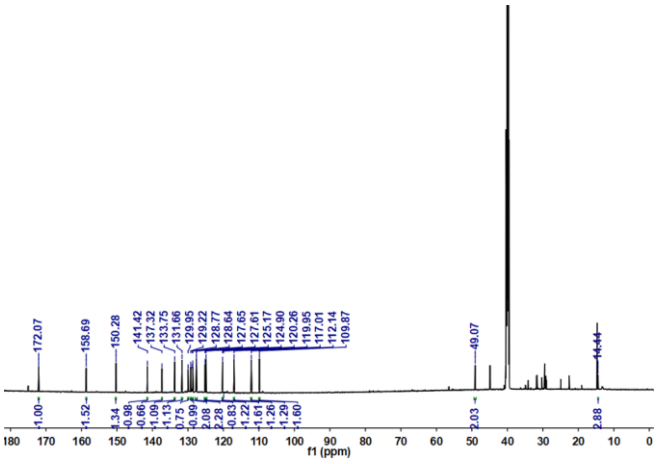


Figure S11. ^{13}C NMR spectrum of **NET-OH** in DMSO-*d6*.

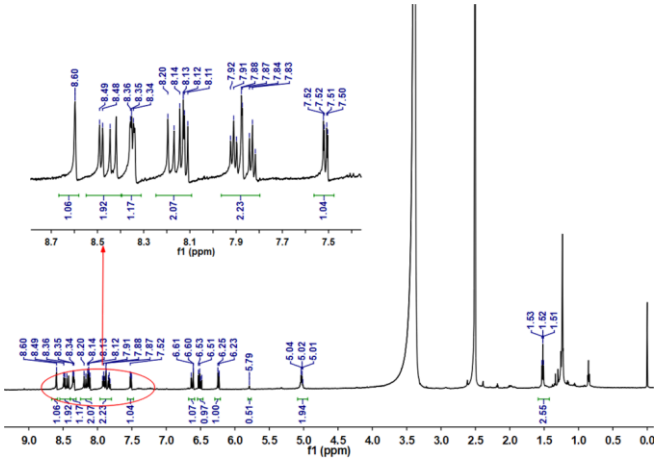


Figure S12. ^{1}H NMR spectrum of **ANET** in DMSO-*d6*.

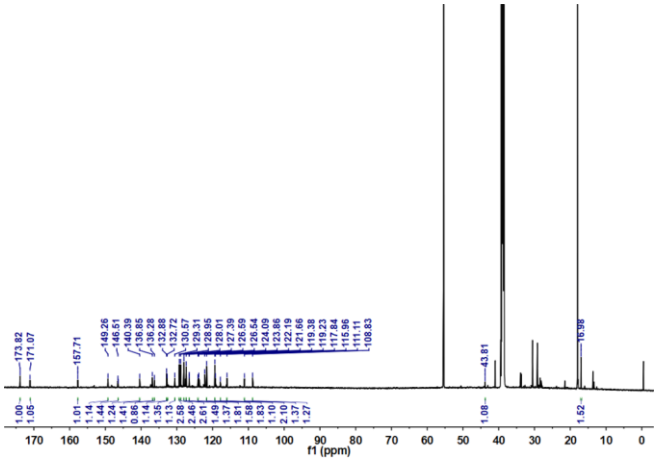


Figure S13. ^{13}C NMR spectrum of **ANET** in DMSO-*d6*.

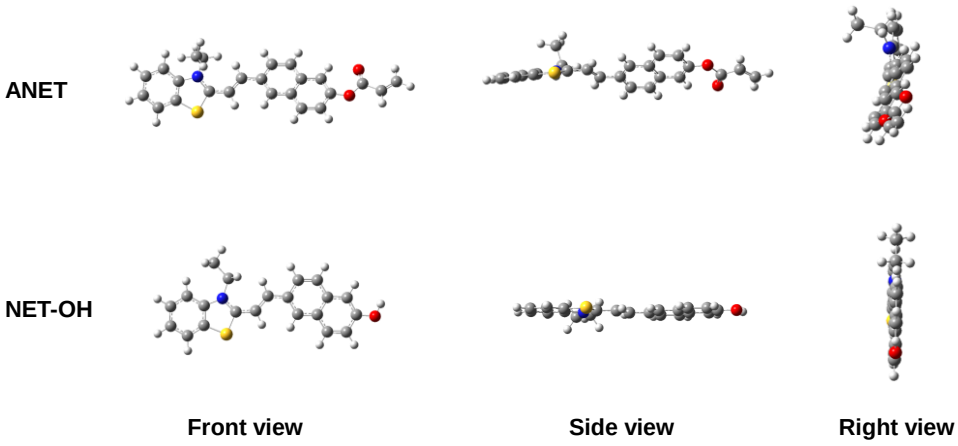


Figure S14. Energy-optimized geometries of **ANET** and **NET-OH**.

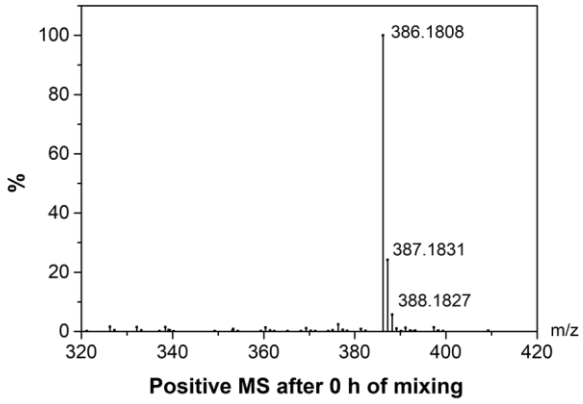


Figure S15. ESI-MS spectrum of **ANET**+Cys in positive ion mode for 0 h.

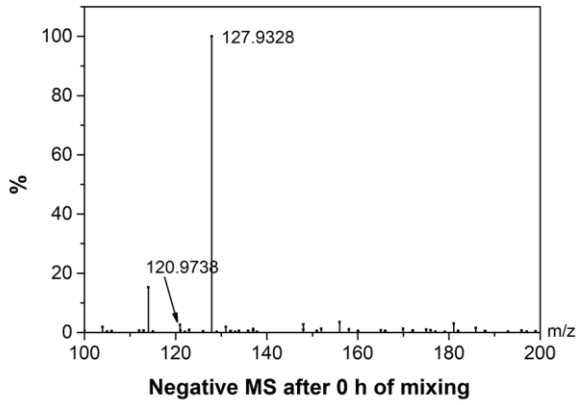


Figure S16. ESI-MS spectrum of **ANET**+Cys in negative ion mode for 0 h.

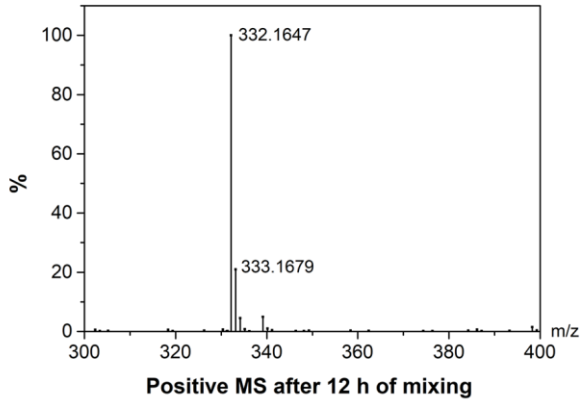


Figure S17. Positive ion mode ESI-MS spectrum of **ANET** reacted with 2 equiv Cys for 12 h in CH_3CN/H_2O (1/1 v/v).

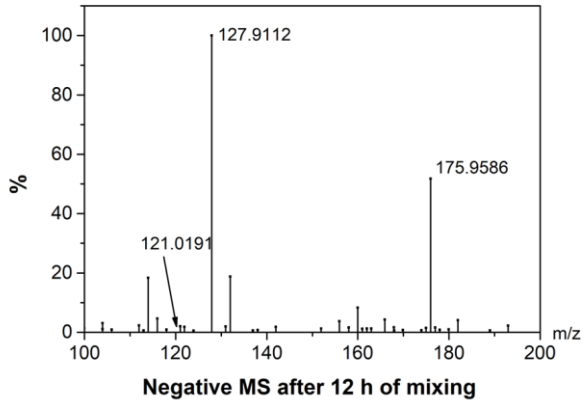


Figure S18. Negative ion mode ESI-MS spectrum of **ANET** reacted with 2 equiv Cys for 12 h in CH_3CN/H_2O (1/1 v/v).

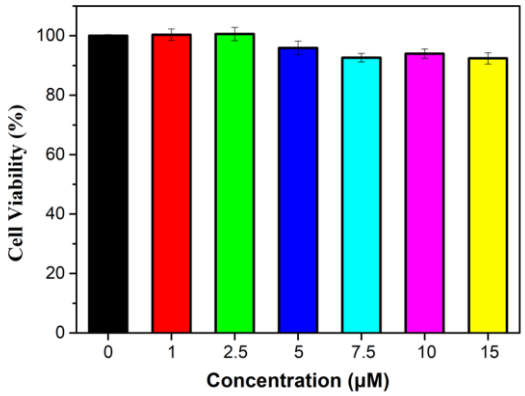


Figure S19. The cell cytotoxicity of various concentration of **ANET** in HepG2 cells for 12 h.

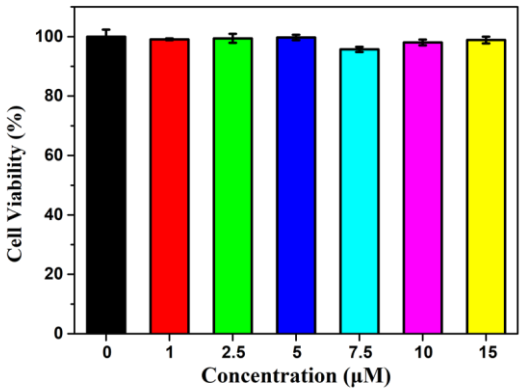


Figure S20. The cell cytotoxicity of various concentration of **ANET** in Hela cells for 12 h.

Reference:

1. S. Li, D. Song, W. Huang, Z. Li and Z. Liu, Analytical Chemistry, 2020, 92, 2802-2808.

2. B. Lin, L. Fan, Y. Zhou, J. Ge, X. Wang, C. Dong, S. Shuang and M. S. Wong, Journal of Materials Chemistry B, 2020, 8, 10586-10592.

3. W. Niu, L. Guo, Y. Li, S. Shuang, C. Dong and M. S. Wong, Analytical Chemistry, 2016, 88, 1908-1914.