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ORIGINAL ARTICLE

A Fluorescent Probe Based on Pyrene Ring for Detecting Cys and its Application in Biology

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Abstract

The identification of thiols has become a research hotspot due to its role in biological systems. In this work, we simply constructed a turn-on fluorescent probe named 3-(5-bromopyridin-3-yl)-1-(pyren-1-yl) prop-2-en-1-one, that a combination of pyrene ring and substituted pyridine via the connection of α, β-unsaturated ketone. Cys can destroy the space effect by Michael addition reaction, which makes the fluorescence intensity changes. Furthermore, the probe featured excellent selectivity and high sensitivity (the detection limit was 0.52 μM) by addition of Cys. Moreover, this probe suggested a potential for imaging in vivo owing to the successful imaging of the probe in HepG2 cells, zebrafish, and *Arabidopsis thaliana*.

Keywords Turn-on fluorescent probe · Cys · Space effect · Bioimaging

Introduction

Thiol-based compounds are important signal molecules in the action of biological enzymes and physiological activities, it can regulate the normal redox state of cells, and have significant functions in the physiological activities of organisms [1–4]. Intracellular bio-thiols, such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), play crucial roles in maintaining high-level structures that control redox homeostasis and proteins due to their similar structures [5–7]. For example, Cys, a common amino acid in vivo, helps the metabolism and protein synthesis [8–10]. Abnormal level of aminothiols in biological fluids will cause a variety of diseases, including Alzheimer's disease, cardiovascular disease, and hematopoiesis decrease [11–13]. Biochemical studies suggest that thiols affect the function and structure of lysine, causing the three major structural proteins of the arteries to decay and affecting their growth. Therefore, it is essential to evaluate Cys in vivo.

Fluorescent probes have the advantages of high sensitivity, specificity, simple operation and the ability of real-time detection [14–18]. The discriminative detection of mercaptans has become a hot spot of research because of their obvious roles in biological systems [19, 20]. However, duo to Cys, Hcy and GSH have similar thiol structure, designing and synthesizing fluorescent probes for distinguishing three thiols is a challenge. [21, 22]. So far, several methods have been explored to achieve probes with specific thiol molecular sensing capabilities. For instance, Zhang et al. designed two new BODIPY-based turn-on fluorescent probes for the discrimination of Cys from Hcy and GSH by different fluorescent emission and time response [23]. Wu et al. reported a new quinoline-derived fluorophore with high fluorescence quantum yield based on conjugate addition-cyclization mechanism [24]. Wang' group developed a novel NIR fluorescent probe to differentiate between Cys/Hcy and GSH by dual fluorescence signals [25]. In our group, we always expect to synthesize a specific probe for distinguishing among Cys, Hcy and GSH.

Through our efforts, in this report, we designed and synthesized a turn-on fluorescent probe named 3-(5-

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bromopyridin-3-yl)-1-(pyren-1-yl) prop-2-en-1-one, that the α, β-unsaturated ketone was used as a bridge to connect pyrene and bromopyridine. The structure of the probe was changed by nucleophilic addition reaction of thiols group with the probe so that the fluorescence could be generated from scratch. The probe exhibited excellent sensitivity and selectivity for detecting Cys, and also suggested a potential for imaging in organism (Scheme 1).

Experiment Section

Materials and Measurement

All reagents and solvents were purchased from Tianli Chemical Reagent Tianjin Co., Ltd. They were all analytically pure and used without further purification. Distilled water was used for all experiments. ^{1}H NMR were performed with Bruker instrument with TMS (Tetramethylsilane) as the internal standard for 600 MHz spectrometer (AVANCE III HD). Fluorescence spectra were measured by F-7000 fluorescence spectrophotometer (Hitachi, Japan). Mass spectra were obtained with a Thermo Scientific Q Exactive LC-MS/MS system. The fluorescence images of probe were obtained using a ZEISS LSM 880 confocal laser scanning microscope.

Synthesis of Probe

1-acetyl pyrene (0.35 g, 1.4 mmol), 5-bromopyridine-3-carbaldehyde (1.4 mmol) was added into 1,4-dioxane (15 mL). Then the mixture was poured into 10% aqueous sodium hydroxide (2 mL) and stirred at 20 h. After monitored by TLC, the reaction product was placed in ice water and cooled for about 10 min. Vacuum filtration and repeated washing with ethanol. Then recrystallize with ethanol and dry in vacuum for 12 h. Finally, a pale-yellow solid powder (0.41 g) was obtained (yield 71%). ^{1}H NMR (600 MHz, DMSO-d_6) δ/ppm: 9.00 (d, J = 1.5 Hz, 1H), 8.77–8.70 (m, 3H), 8.59 (d, J = 8.0 Hz, 1H), 8.47–8.40 (m, 3H), 8.37 (d, J = 8.8 Hz, 2H), 8.33–8.29 (m, 1H), 8.18 (t, J = 7.6 Hz, 1H), 8.08 (d, J = 16.0 Hz, 1H), 7.73 (d, J = 16.0 Hz, 1H).(Fig.S1) ^{13}C NMR (151 MHz, DMSO-d_6) δ/ppm: 194.08, 151.91, 149.38, 140.13, 137.75, 133.76, 133.00, 132.89, 131.14, 130.52, 130.40, 130.02, 129.88, 129.42, 127.85, 127.74, 127.33, 127.06, 126.69, 124.90, 124.85, 124.50, 123.97, 121.20. (Fig.S2) HRMS $[M+H]^+$: m/z: calculated 412.02588, found 412.03328.(Fig.S3).

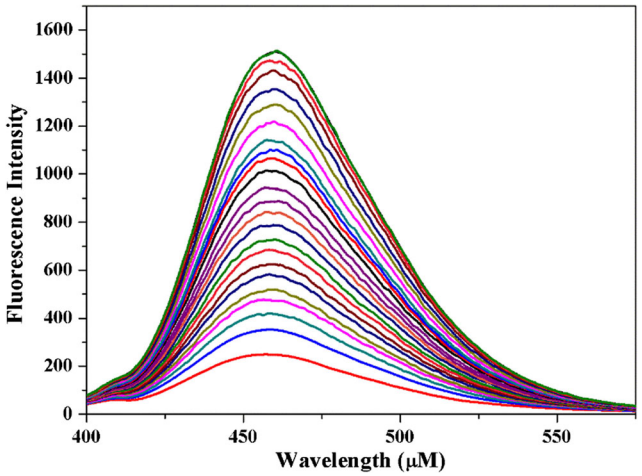
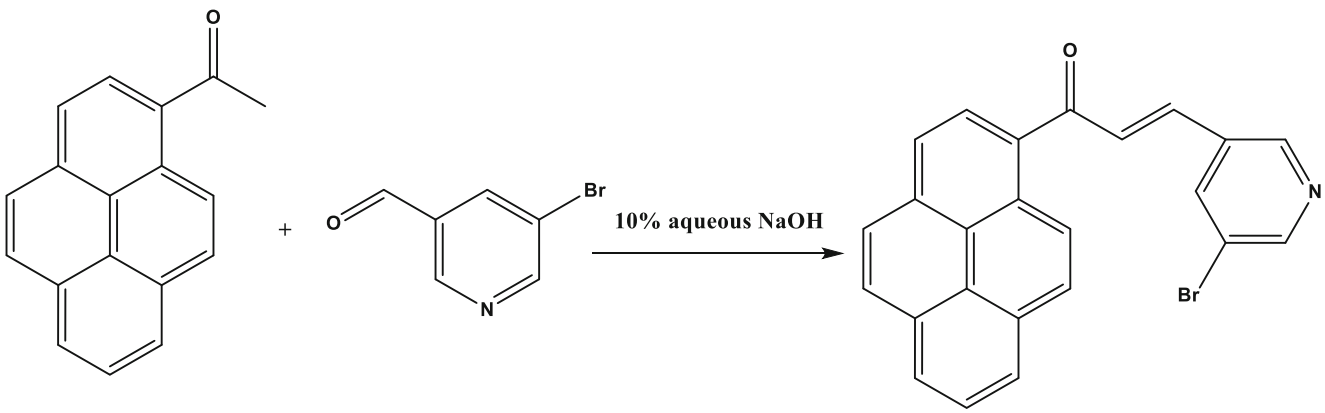


Fig. 1 Fluorescence spectra of probe upon addition of Cys (0–300 μM) in PBS/CH_3CN (v/v, 3/1, pH 7.4) system. (λ_{ex} = 357 nm, slit: 2.5 nm/5 nm)

Optical Studies

The stock solution of probe (1 mM) was prepared in DMSO. The fluorescence spectra experiments were measured in PBS/CH_3CN (v/v, 3/1, pH 7.4) system. Fluorescence intensity was detected separately at excitation of 357 nm with slit 2.5/5 nm. All amino acid solutions Phe, Asp, Tyr, Ala, Val, Gly, Glu, Pro, His, Leu, Ile, GSH, Met, Lys, Ser, Hcy, Cys and ionic solutions Zn^{2+}, Cu^{2+}, Co^{2+}, Mg^{2+}, Ca^{2+}, HSO_3^-, S^{2-}, SO_3^{2-}, $S_2O_3^{2-}$ were prepared in redistilled water.



Scheme 1 The synthetic route of probe by one-step reaction

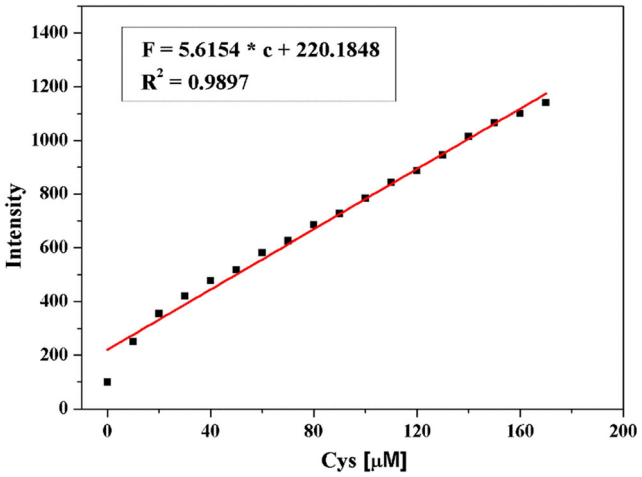


Fig. 2 The linear correlation between fluorescence intensity and Cys concentrations at 450 nm (0–170 μM). (λ_{ex} = 357 nm, slit: 2.5 nm/5 nm)

Zebrafish Culture and Imaging

The zebrafish were kept at 28 °C and cultured in E3 embryo media (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 5–10% methylene blue; pH 7.5). For imaging experiment, 5-day-old zebrafish were placed in E3 embryo media containing 10 μM of probe and fed for 30 min. In the control experiment, 1 mM NEM (N-ethyl maleimide, sulfhydryl scavenger) was treated with zebrafish for 30 min, then incubated with Cys (100 μM) for 30 min.

Results and Discussion

Characteristic Spectrum

The spectroscopic properties of probe 1 (10 μM) towards Cys were determined in PBS/CH_3CN (*v*/v, 3/1, pH 7.4) system. Under this condition, the probe itself had small fluorescence intensity at 450 nm when the excitation was at 357 nm by according to Fig. 1. With the addition of Cys (0–300 μM), the fluorescence intensity gradually increased by nearly 15-fold fluorescence. Furthermore, a good linear correlation (R^2 = 0.9897) was obtained between the fluorescence intensity (450 nm) and concentration (0–170 μM). The regression equation was F = 5.6154 × [Cys] + 220.1848 (Fig. 2). The

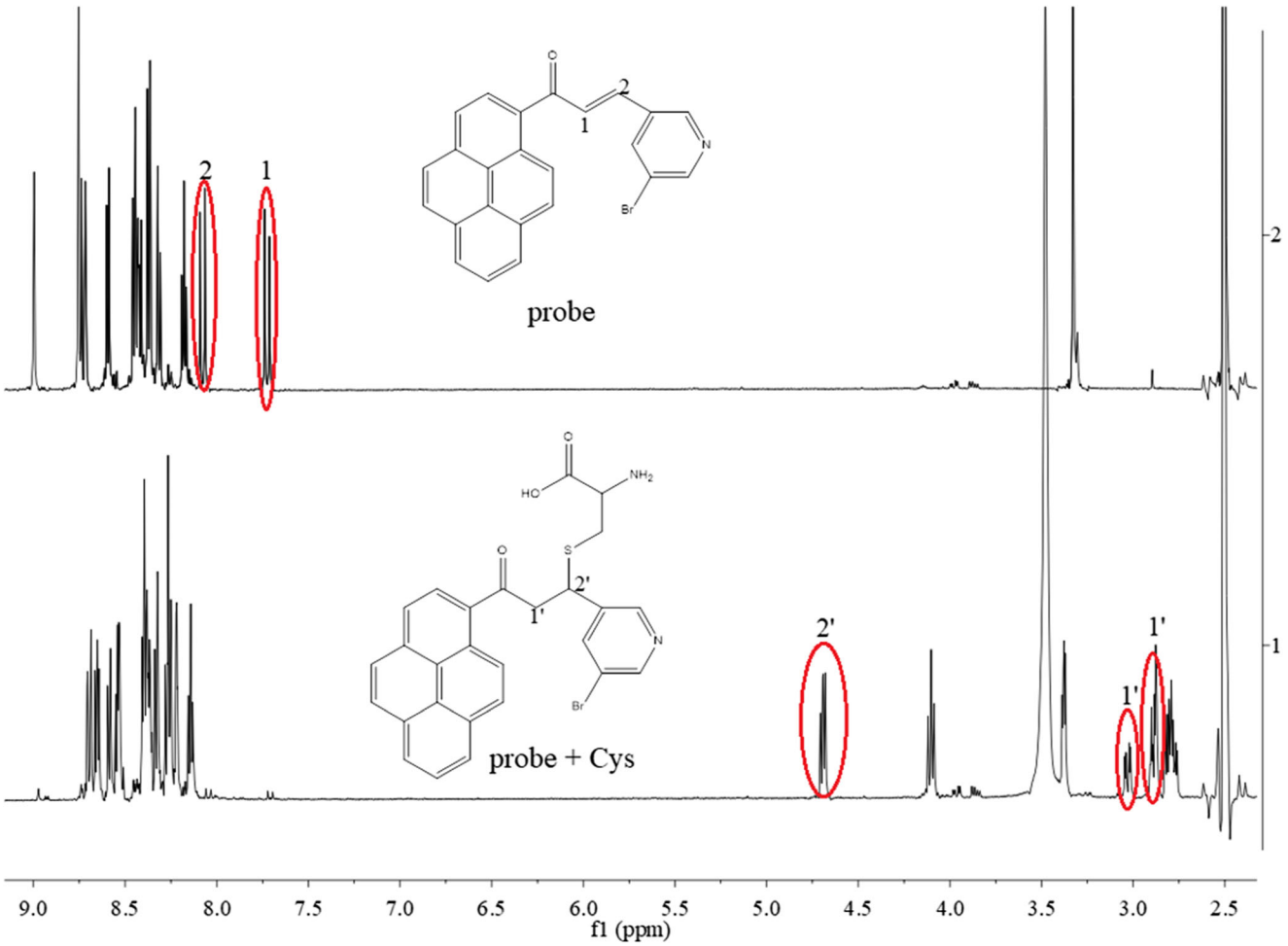
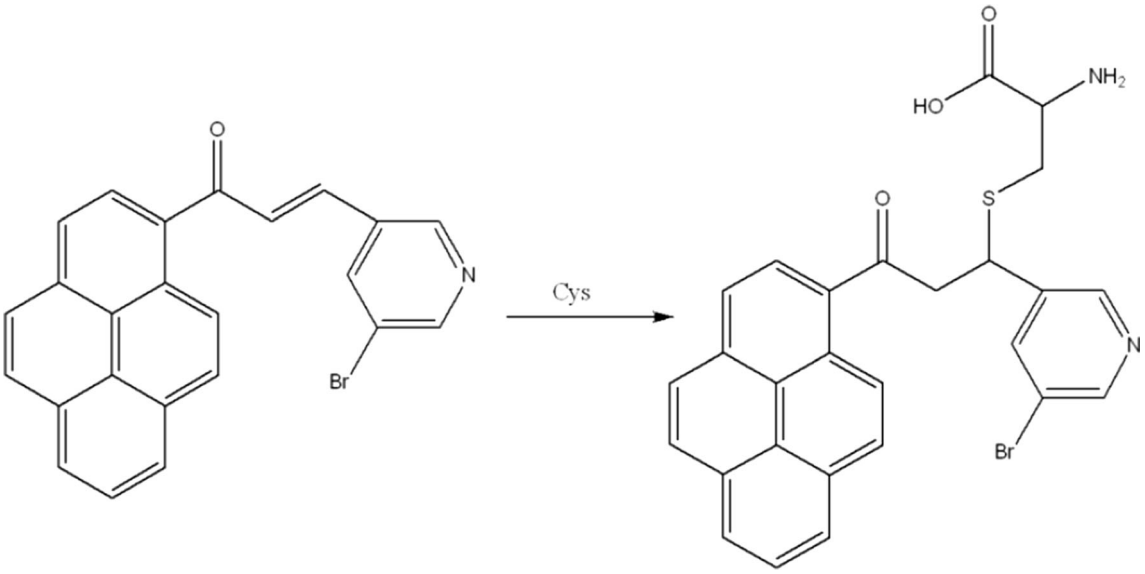


Fig. 3 The ^{1}H NMR of probe in DMSO-d_6 before and after Cys addition



Scheme 2 Reaction process of probe for Cys

detection limit was calculated to be 0.52 μM given the 3σ/k (by the IUPAC).

Proposed Response Mechanism

We hypothesized that the reaction occurred by the fact that the double bond of the probe was attacked after the addition of Cys, resulting in a change in fluorescence intensity. Due to the attack of the thiol on the carbon-carbon double bond of α, β-unsaturated ketone, the π-conjugation between pyrene ring and the pyridine system of the probe was destroyed. To further verify the proposed reaction mechanism, ^{1}H NMR spectroscopy of probe in the presence of Cys was investigated (Fig. 3). The double bond peak of probe disappeared at δ 8.08 ppm (H_1) and 7.73 (H_2) ppm, and a series of new peaks appeared near the δ 4.09 ppm ($H_{2'}$), 3.04 ppm ($H_{1'}$), 2.88 ppm ($H_{1'}$) which is attributed to the paraffin peaks of Cys. All the above results further support the proposed sensing mechanism what we proposed in Scheme 2.

Selective Response of Probe

To value the effect of disturbing analytes including amino acids and some related ions towards probe, the selectivity and interference experiments were investigated. As shown in Fig. 4, 500 μM Asp, Ile, Ala, Phe, Leu, Met, Ser, Lys, Val, Gly, Try, Glu, Pro, His, GSH, Hcy, S^{2-}, SO_3^{2-}, HSO_3^-, $S_2O_3^{2-}$, Zn^{2+}, Cu^{2+}, Co^{2+}, Mg^{2+}, Ca^{2+} and 300 μM Cys were poured

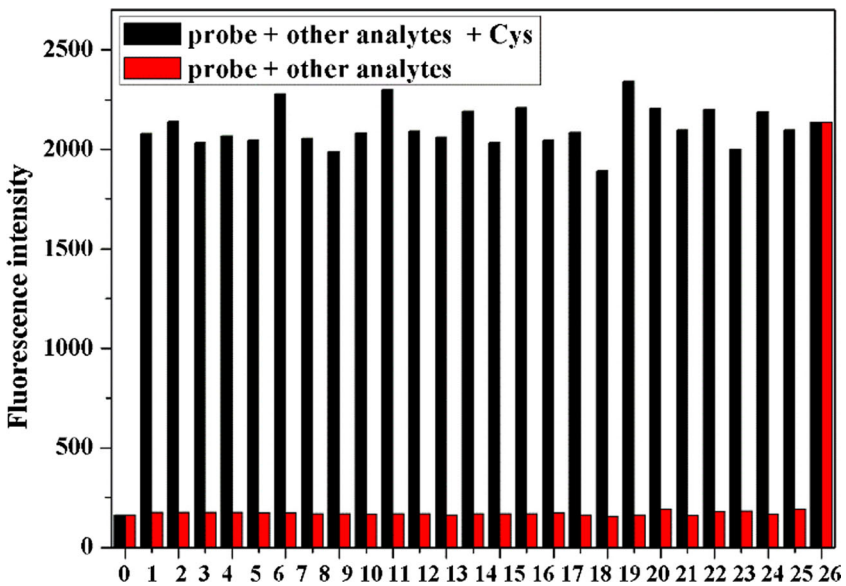


Fig. 4 The selectivity (red) and anti-interference ability (black) of the probe (10 μM) after addition of other analytes at 450 nm in PBS/CH_3CN (*v*/v, 3/1, pH 7.4) system. (0) probe, (1) Asp, (2) Ile, (3) Ala, (4) Phe, (5) Leu, (6) Met, (7) Ser, (8) Lys, (9) Val, (10) Gly, (11) Try, (12) Glu, (13) Pro, (14) His, (15) GSH, (16) Hcy, (17) S^{2-}, (18) SO_3^{2-}, (19) HSO_3^-, (20) $S_2O_3^{2-}$, (21) Zn^{2+}, (22) Cu^{2+}, (23) Co^{2+}, (24) Mg^{2+}, (25) Ca^{2+}, (26) Cys. (λ_{ex} = 357 nm, slit: 2.5 nm/5 nm)

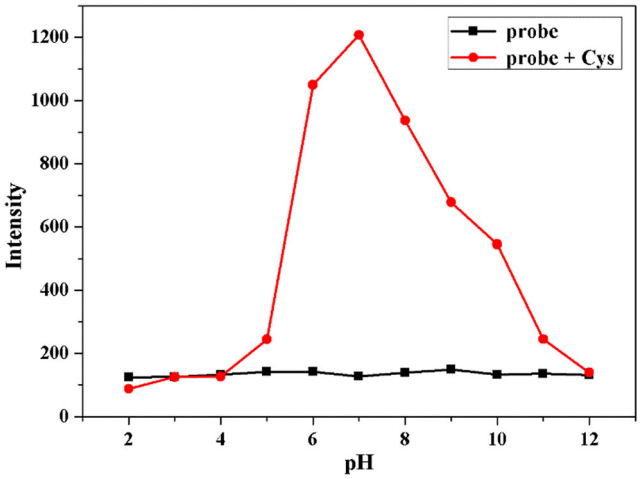


Fig. 5 The fluorescence intensities of probe 1 (10 μM) in the presence of 100 μM Cys in PBS/CH_3CN (v/v = 3/1) at 450 nm. (λ_{ex} = 357 nm, slit: 2.5 nm/5 nm)

into the solution of probe, respectively. We can observe that probe has good selectivity for Cys in various analytes. At the same time, we also tested the anti-interference ability of other analytes to detect Cys in which other analytes had almost no change in fluorescence intensity.

The Effect of pH

To ensure the optical stability of probe in the absence and presence Cys at 450 nm in PBS/CH_3CN (*v*/v, 3/1) system, the pH effects were measured at different pH values (pH 2–12). Figure 5 displayed that the probe itself exhibited almost non-fluorescent in the range of pH. When Cys (100 μM) was added, the fluorescence intensity of the probe increases sharply between 5 and 7 and decreases between 7 and 11 as the pH increased. Therefore, this probe had good performance for detecting Cys at physiological pH environment.

Cellular Imaging

To demonstrate the potential biological relevance, we had done some experiments with HepG2 cells. As shown in Fig. 6a2, the cells exhibited weak fluorescence when it was incubated with the probe. In the control group, NEM pre-incubated cells further cultured with the probe showed non-fluorescence signals (Fig. 6b2). When cells were incubated with Cys (100 μM) after cells co-incubated with NEM and the probe, it could be observed obvious blue fluorescence signals (Fig. 6c2). These results

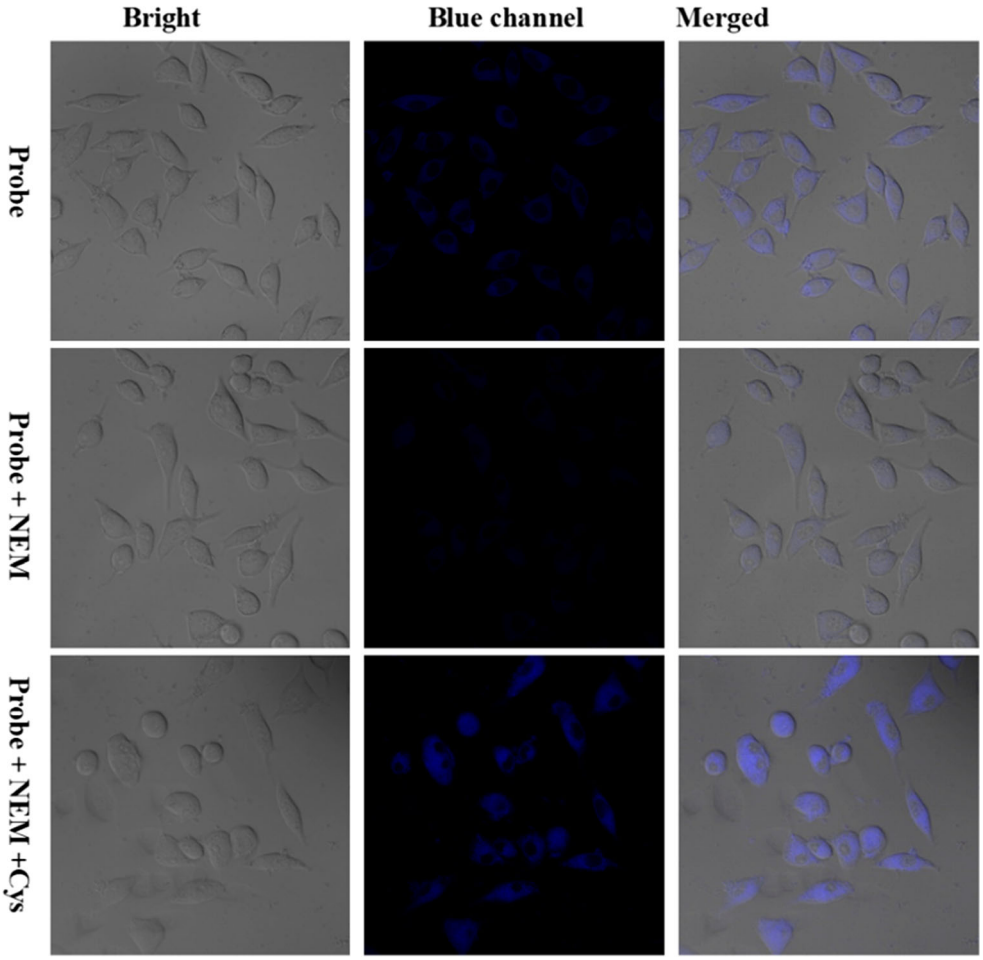


Fig. 6 Fluorescence images of living HepG2 cells. (A) cells incubated with probe (10 μM) for 15 min; (B) cells incubated with NEM (1 mM) for 40 min, and treated with probe (10 μM) for 15 min; (C) cells sequentially treated with NEM (1 mM, 40 min), probe (10 μM, 15 min), Cys (100 μM, 15 min). (Blue channel: λ_{em} = 410–550 nm, λ_{ex} = 405 nm)

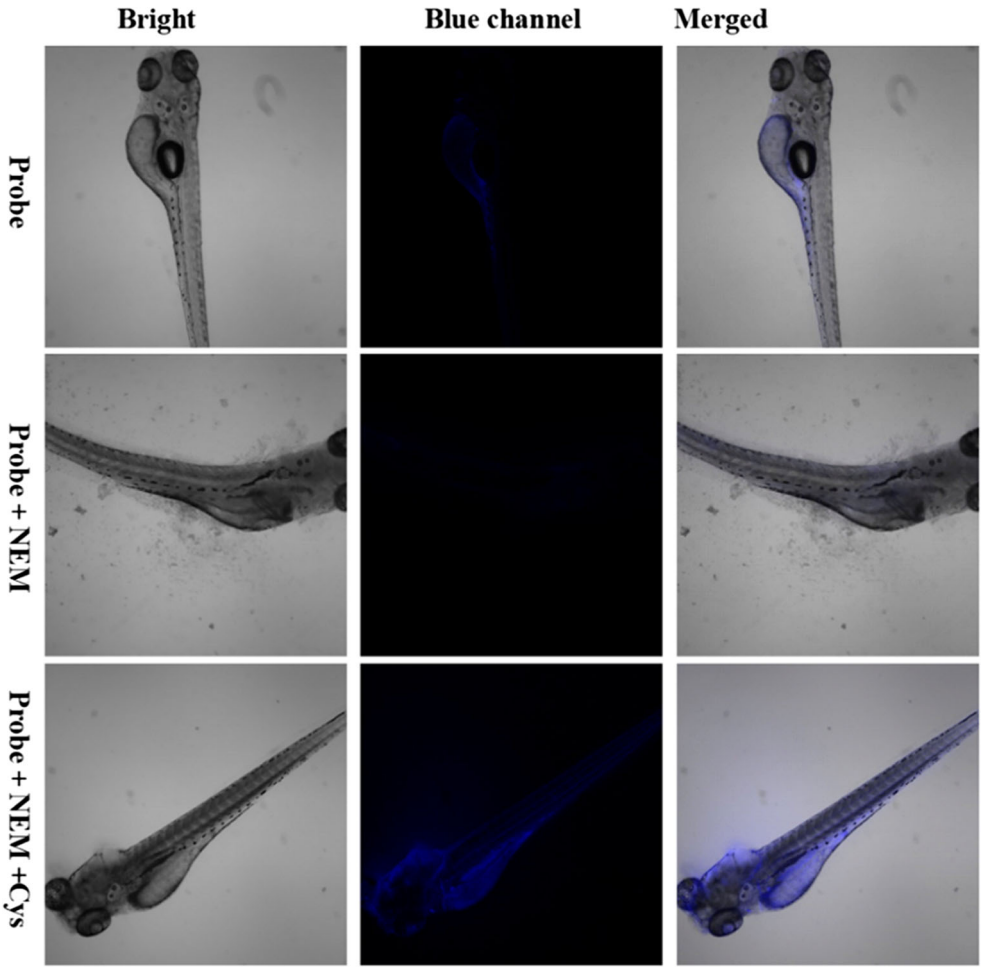


Fig. 7 Fluorescence images of zebrafish. **a** zebrafish fed with probe (10 μM) for 30 min; **b** zebrafish fed with with NEM (1 mM) for 40 min, and treated probe (10 μM) for 30 min; **c** zebrafish sequentially treated with NEM (1 mM, 40 min), probe (10 μM, 30 min), Cys (100 μM, 30 min). (Blue channel: λ_{em} = 410–550 nm, λ_{ex} = 405 nm)

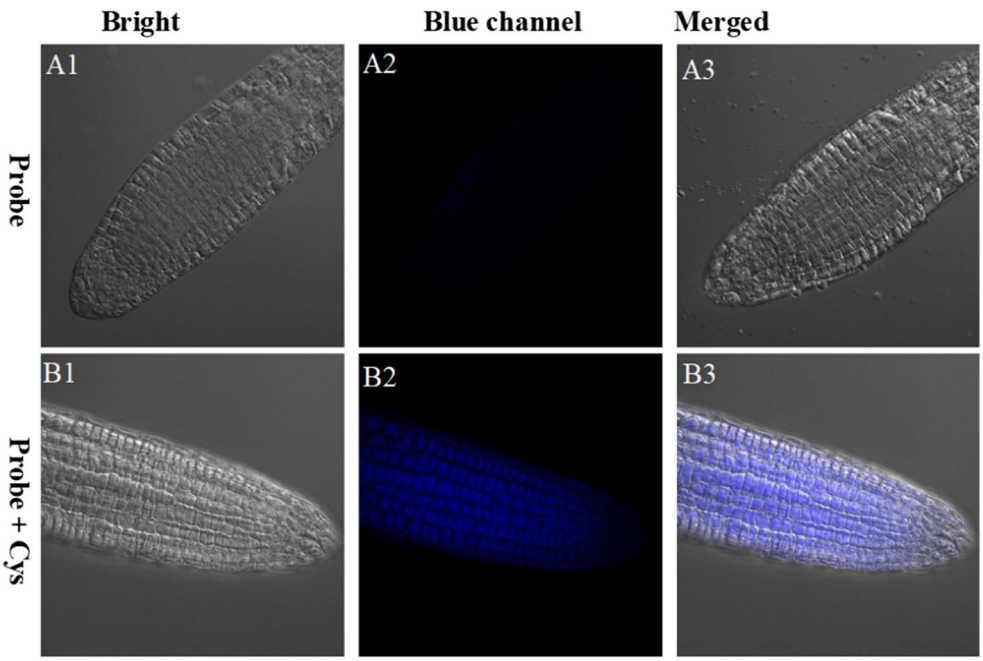


Fig. 8 Fluorescence images of Arabidopsis root tip. **a** Arabidopsis root tip treated with probe (10 μM) for 30 min; **b** Arabidopsis root tip treated with probe (10 μM) for 30 min, the treated with Cys (100 μM) for 30 min. (Blue channel: λ_{em} = 410–550 nm, λ_{ex} = 405 nm)

implied that the probe has the potential to image endogenous and exogenous Cys in living cells.

Imaging of Zebrafish and Arabidopsis Root Tip

To further verify cell permeability of probe, we further studied probe in living zebrafish imaging. As exhibited in Fig. 7a2, zebrafish showed weak fluorescence after incubation with the probe. As a comparison, the NEM-pretreated zebrafish further incubated with probe, the fluorescence signals disappeared (Fig. 7b2). When Cys was added, the fluorescence intensity changes drastically (Fig. 7c2). Miraculously, Arabidopsis root tip was co-incubated with probe and Cys emitted a violent fluorescence emission (Fig. 8b2). Those results demonstrated that the probe has excellent cell membrane permeability and presents a good prospect for the detection of Cys in organisms.

In Conclusions

In summary, a turn-on fluorescent probe for detecting Cys was designed and synthesized. Due to Michael addition reaction, the π-conjugated system of the probe was destroyed by the sulfhydryl attack of the carbon-carbon double bond of α, β-unsaturated, which results in the change of fluorescence emission. Simultaneously, the probe has high selectivity, sensitivity and lower detection limit. Fluorescence imaging experiment has illustrated that the probe presents a good prospect for the detection of Cys in organisms.

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References

1. Gutscher M, Pauleau A, Marty L, Brach T, Gh SY, Meyer A et al (2008) Real-time imaging of the intracellular glutathione redox potential. Nat Methods 5:553–559
2. Go YM, Jones DP (2013) Thiol/disulfide redox states in signaling and sensing. Crit Rev Biochem Mol 48:173–191
3. Niu W, Guo L, Li Y, Shuang S, Dong C, Wong MS (2015) Highly selective two-photon fluorescent probe for ratiometric sensing and imaging cysteine in mitochondria. Anal Chem 88:1908–1914
4. Zhang W, Liu J, Yu Y, Han Q, Chen T, Shen J et al (2018) A novel near-infrared fluorescent probe for highly selective detection of cysteine and its application in living cells. Talanta 185:477–482
5. Ke W, Hanjing P, Binghe W (2014) Recent advances in thiol and sulfide reactive probes. J Cell Biochem 115(6):1007–1022
6. Zhang H, Liu R, Liu J, Li L, Wang P, Yao SQ, Xu Z, Sun H (2015) A minimalist fluorescent probe for differentiating Cys, Hcy and GSH in live cells. Chem Sci 7(1):256–260
7. Sudha S, Alexa B, Jacob S, Jacques PF, Rosenberg IH, D'Agostino RB et al (2002) Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. New Engl J Med 346(7):476–483
8. Schulz JB, Lindenau J, Seyfried J, Dichgans J (2010) Glutathione, oxidative stress and neurodegeneration. FEBS J 267(16):4904–4911
9. Tang LJ, Xu D, Tian MY, Yan XM (2019) A mitochondria-targetable far-red emissive fluorescence probe for highly selective detection of cysteine with a large stokes shift. J Lumin 208:502–508
10. Xie XX, Yin CX, Yue YK, Huo FJ (2018) Rational design of fluorescent probe for Cys dynamics imaging in living cell. Sensors Actuators B Chem 267:76–82
11. Wang Q, Hong W, Huang J, Nan L, Gu Y, Peng W (2017) Novel NIR fluorescent probe with dual models for sensitively and selectively monitoring and imaging Cys in living cells and mice. Sensors Actuators B Chem 253:400–406
12. Wang XF, Cynader MS (2001) Pyruvate released by astrocytes protects neurons from copper-catalyzed cysteine neurotoxicity. J Neurosci 21(10):3322–3331
13. Shahrokhian S (2001) Lead phthalocyanine as a selective carrier for preparation of a cysteine-selective electrode. Anal Chem 73(24):5972–5978
14. Tang Y, Jin L, Yin B (2017) A dual-selective fluorescent probe for GSH and Cys detection: emission and pH dependent selectivity. Anal Chim Acta 993:87–95
15. Jung HS, Chen XQ, Kim JS, Yoon JY (2013) Recent progress in luminescent and colorimetric chemosensors for detection of thiols. Chem Soc Rev 42(14):6019–6031
16. Chen H, Tang Y, Lin W (2016) Recent progress in the fluorescent probes for the specific imaging of small molecular weight thiols in living cells. TrAC Trends Anal Chem 76:166–181
17. Yin CX, Xiong KM, Huo FJ, Salamanca JC, Strongin RM (2017) Fluorescent probes with multiple binding sites for the discrimination of Cys, Hcy, and GSH. Angew Chem Int Ed 56(43):13188–13198
18. Shao J, Sun H, Guo H, Ji S, Zhao J, Wu W, Yuan X, Zhang C, James TD (2012) A highly selective red-emitting FRET fluorescent molecular probe derived from BODIPY for the detection of cysteine and homocysteine: an experimental and theoretical study. Chem Sci 3(4):1049–1061
19. Tang LJ, Tian MY, Chen HB, Yan XM, Zhong KL, Bian YJ (2018) An ESIPT-based mitochondria-targeted ratiometric and NIR-emitting fluorescent probe for hydrogen peroxide and its bioimaging in living cells. Dyes Pigments 158:482–489
20. Yue YK, Yin CX, Huo FJ, Chao JB, Zhang YB (2016) Thiol-chromene click chemistry: a turn-on fluorescent probe for specific detection of cysteine and its application in bioimaging. Sensors Actuators B Chem 223:496–500
21. Niu LY, Chen YZ, Zheng HR, Wu LZ, Tung CH, Yang QZ (2015) Design strategies of fluorescent probes for selective detection among biothiols. Chem Soc Rev 44(17):6143–6160
22. Zhou Y, Yoon JY (2012) Recent progress in fluorescent and colorimetric chemosensors for detection of amino acids. Chem Soc Rev 41(1):52–67
23. Jian Z, Xin J, Hang R, Zhou J, Chen Z, Dong X et al (2018) Meso-heteroaryl BODIPY dyes as dual-responsive fluorescent probes for discrimination of Cys from Hcy and GSH. Sensors Actuators B Chem 260:861–869

24. Wu QQ, Mao M, Liang WL, Stadler FJ (2018) Quinoline-derived fluorescent probes for the discrimination of Cys from Hcys/GSH and bioimaging in living cells. Talanta 186:110–118
25. Peng W, Yan W, Nan L, Huang J, Wang Q, Gu Y (2017) A novel DCM-NBD conjugate fluorescent probe for discrimination of Cys/Hcy from GSH and its bioimaging applications in living cells and animals. Sensors Actuators B Chem 245:297–304