



# A Near-Infrared Ratiometric Fluorescent Probe for Highly Selective Recognition and Bioimaging of Cysteine

Xuan Zhang<sup>1,2\*</sup>, Li Zhang<sup>1†</sup>, Wei-Wei Ma<sup>1†</sup>, Yong Zhou<sup>1</sup>, Zhen-Ni Lu<sup>1</sup> and Suying Xu<sup>3\*</sup>

<sup>1</sup> Key Laboratory of Science and Technology of Eco-Textiles, Ministry of Education, College of Chemistry, Chemical Engineering & Biotechnology, Donghua University, Shanghai, China, <sup>2</sup> State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian, China, <sup>3</sup> Department of Biochemistry, Faculty of Science, Beijing University of Chemical Technology, Beijing, China

## OPEN ACCESS

### Edited by:

Tony D. James,  
University of Bath, United Kingdom

### Reviewed by:

Youjun Yang,  
East China University of Science and  
Technology, China  
Jiangli Fan,  
Dalian University of Technology (DUT),  
China

### \*Correspondence:

Xuan Zhang  
xzhang@dhu.edu.cn  
Suying Xu  
syxu@mail.buct.edu.cn

<sup>†</sup>These authors have contributed  
equally to this work

### Specialty section:

This article was submitted to  
Supramolecular Chemistry,  
a section of the journal  
Frontiers in Chemistry

**Received:** 29 November 2018

**Accepted:** 14 January 2019

**Published:** 01 February 2019

### Citation:

Zhang X, Zhang L, Ma W-W, Zhou Y,  
Lu Z-N and Xu S (2019) A  
Near-Infrared Ratiometric Fluorescent  
Probe for Highly Selective Recognition  
and Bioimaging of Cysteine.  
Front. Chem. 7:32.  
doi: 10.3389/fchem.2019.00032

A benzothiazole-based near-infrared (NIR) ratiometric fluorescent probe (**HBT-Cys**) was developed for discriminating cysteine (Cys) from homocysteine (Hcy) and glutathione (GSH). The probe was designed by masking phenol group in the conjugated benzothiazole derivative with methacrylate group that could be selectively removed by Cys, and therefore an intramolecular charge transfer (ICT) fluorescence was switched on in the NIR region. In the absence of Cys, the probe exhibited a strong blue fluorescence emission at 431 nm, whereas a NIR fluorescence emission at 710 nm was significantly enhanced accompanied by a decrease of emission at 431 nm in the presence of Cys, allowing a ratiometric fluorescence detection of Cys. The fluorescence intensity ratio ( $I_{710\text{nm}}/I_{431\text{nm}}$ ) showed a good linear relationship with Cys concentration of 1–40  $\mu\text{M}$  with the detection limit of 0.5  $\mu\text{M}$ . The sensing mechanism was explored based on MS experimental analysis and DFT theoretical calculation. Moreover, the fluorescent probe was successfully used for fluorescence bioimaging of Cys in living cells.

**Keywords:** NIR ratiometric fluorescent probe, cysteine, benzothiazole derivative, living cells imaging, intramolecular charge transfer

## INTRODUCTION

It has been known that small molecular biothiols, such as L-cystein (Cys), homocysteine (Hcy), and glutathione (GSH) played vital roles in the maintenance of redox homeostasis, intracellular signal transduction, and human metabolism (Shahrokhian, 2001; Giles et al., 2003). Cys is a metabolic product of Hcy and a precursor of the antioxidant GSH, and its normal intracellular level remains to be 30–200  $\mu\text{M}$  (Liu et al., 2014). The deficiency of Cys could cause edema, leucocyte loss, liver damage as well as neurotoxicity, whereas the excess levels of Cys might relate to cardiovascular and Alzheimer's diseases (Lipton et al., 2002; Shao et al., 2012; Dorszewska et al., 2016; Qi et al., 2018). Hence, it has attracted intense interest in the development of novel strategy for detection and imaging of the intracellular Cys, which will further contribute to the better understanding the pathology of associated diseases and their early diagnosis and treatment.

Design and synthesis of small molecule-based fluorescent probe received much attention in molecule recognition and fluorescence signaling in living biosystems due to many advantages of fluorescence technique, such as high sensitivity and selectivity, simplicity, *in vivo* bioimaging (De Silva et al., 1997; Chen et al., 2010, 2016; Chan et al., 2012; Yang et al., 2013; Li et al., 2014; Niu et al., 2015). For bioimaging application, the development of fluorescent probes with near-infrared (NIR, 650–900 nm) emission is more promising due to the merits of deeper tissue penetration and minimum interference from the indigenous fluorescence background of biosystem (Escobedo et al., 2010; Nolting et al., 2011; Yuan et al., 2013; Guo et al., 2014). Additionally, the ratiometric fluorescent probes could provide an inherent reliability originating from its effective self-calibration advantage by monitoring two well-resolved emissions (Kikuchi et al., 2004; Demchenko, 2010; Lee et al., 2015). Although a number of fluorescent probes for Cys detection and imaging have been reported, most of them could not discriminate Cys from Hcy/GSH due to their similar molecular structures and reactivity (Chen et al., 2017, 2018; Li et al., 2017; Liu X. et al., 2017; Nawimanage et al., 2017; Wang F. et al., 2017; Wang Q. et al., 2017, 2018a; Wu et al., 2017; Yin et al., 2017; Yue et al., 2017a,b; Zhang et al., 2017, 2018a,b; Hou et al., 2018; Kim et al., 2018; Ren et al., 2018; Song et al., 2018; Tian et al., 2018; Wang et al., 2018b; Wang J. et al., 2018; Wang L. et al., 2018; Sheng et al., 2019). Several ratiometric fluorescent probes for Cys have been developed (Lv et al., 2014; Feng et al., 2017; Liu G. et al., 2017; Wang F. et al., 2017; Wang L. et al., 2018; Wu et al., 2018; Yue et al., 2018; Zhu et al., 2018), but only a few of them showed the fluorescence emission in NIR region (Feng et al., 2017; Zhu et al., 2018). Therefore, the development of NIR ratiometric fluorescent probe for selective detection of Cys is still a challenging task.

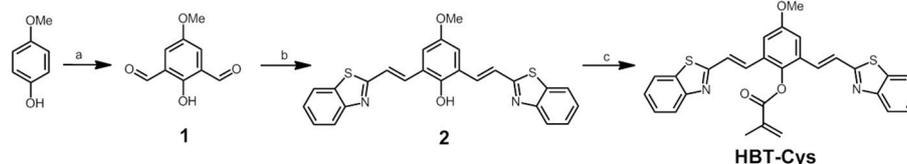
Recently, we have found that the  $\pi$ -conjugation extended benzothiazole derivatives exhibited short-wavelength fluorescence emission in non-polar solvent but the NIR emission in polar solvent, where the NIR fluorescence originated from the deprotonation of phenol group switching on an intramolecular charge transfer (ICT) process (Zhang et al., 2016b). In this work, we envisaged that the masking of phenol group in conjugated benzothiazole derivatives with methacrylate moiety, a Cys-selective recognition site, will result in a short-wavelength emission but a NIR fluorescence will appear in the presence of Cys due to the Cys-selectively induced deprotection, and therefore will allow a NIR ratiometric fluorescence detection of Cys. Accordingly, a new conjugated benzothiazole derivative

(**HBT-Cys**, **Scheme 1**) was synthesized and developed as a novel NIR ratiometric fluorescent probe for Cys detection. The probe distinguished Cys well from GSH/Hcy in a ratiometric manner in aqueous solution and successfully applied in living cells imaging.

## MATERIALS AND METHODS

All the chemicals are analytical grade that was used without purification and purchased from Sinopharm Chemical Reagents Corp. (Shanghai, China). Phosphate buffered saline (PBS, pH = 7.4) was prepared from  $K_2HPO_4$  (0.1 M) and  $KH_2PO_4$  (0.1 M). The stock solution of the probe **3** was prepared in DMF and all of others species solutions were prepared in deionized water.  $^1H$  NMR and  $^{13}C$  NMR spectra were recorded on a Bruker AVANCE III 400 MHz spectrometer. Mass spectra were obtained on AB Sciex MALDI-TOF/TOF<sup>TM</sup> MS. Fluorescence spectra were measured on Edinburgh FS5 spectrofluorometer with Ex/Em slit widths of 5 nm. The absolute fluorescence quantum yields were obtained on Edinburgh FS5 spectrofluorometer equipped with an integrating sphere (EI-FS5-SC-30). Absorption spectra were obtained on a SHIMADZU UV-1800 spectrophotometer. Confocal fluorescence imaging experiments in living Hela cells were carried out with a Carl Zeiss LSM 700 microscope. Theoretical calculations were performed based on the Gaussian 09 package (Frisch et al., 2010). The ground state and the first singlet excited state geometries of the compounds were optimized in the gas phase using density functional theory (DFT) and time-dependent density functional theory (TDDFT) at the B3LYP/6-31+G(d) level, respectively. The fluorescence emission properties were calculated using TDDFT based on the optimized first singlet excited state geometries, respectively.

HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum at 37°C in a 95% humidity atmosphere under 5%  $CO_2$  environment. Then the cells were seeded in confocal microscope culture dishes with a density of  $2 \times 10^5$  cells per well. The cells were then incubated with probe **HBT-Cys** (20  $\mu$  M) for 120 min at 37°C, washed with PBS buffer (10 mM) three times to remove free probe. In the control experiments, the cells were pretreated with NEM (1 mM) for 30 min at 37°C, followed by washing with PBS for three times, and incubated with probe **HBT-Cys** (20  $\mu$  M) for 120 min at 37°C. In another control experiment, the cells were pretreated with NEM (1 mM) for 30 min at 37°C, followed by washing with PBS three times, then incubated with Cys (200  $\mu$  M) for 30 min, and further incubated with probe **HBT-Cys** (20  $\mu$  M)



**SCHEME 1** | Synthesis of **1**, **2**, and **HBT-Cys**. Reagents and conditions: **(a)** hexamethylenetetramine, TFA, 110°C, 72 h; **(b)** 2-methylbenzothiazole,  $AcO_2$ , 145°C, 56 h; pyridine, 115°C, 2 h; water, 100°C, 6 h; and **(c)** methacryloyl chloride, acetone,  $K_2CO_3$ , r.t., 8 h.

for 120 min at 37°C, respectively. All the cells were washed with PBS three times to remove free probe and then imaged at blue and red channels, respectively in a Carl Zeiss LSM 700 microscope.

The synthesis procedures are illustrated in **Scheme 1**. The compound **1** was facily synthesized according to the similar procedure described previously (Zhang and Liu, 2016a). Briefly, under an N<sub>2</sub> atmosphere, 4-methoxyphenol (20 mmol) and hexamethylenetetramine (60 mmol) were dissolved in TFA (15 mL) and refluxed at 110°C for 72 h. The mixture was then cooled down to room temperature and poured into a 3 M HCl solution (120 mL). The crude product was obtained by filtration and further purified by column chromatography (silica gel, CH<sub>2</sub>Cl<sub>2</sub>) to give **1** as a yellow solid (**Figures S3, S4**). Yield: 19%. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>), δ (ppm): 11.09 (s, 1H), 10.24 (s, 2H), 7.60 (s, 2H), 3.82 (s, 3H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>), δ (ppm): 55.89, 121.34, 124.31, 152.12, 156.45, 191.70.

Under an N<sub>2</sub> atmosphere, compound **1** (4 mmol) and 2-methylbenzothiazole (16 mmol) were refluxed in acetic anhydride (3 mL) at 145°C for 56 h. After cooling to room temperature, the solid was obtained by filtration and thoroughly washed by water. The obtained solid was further dissolved in pyridine (19 mL) and refluxed at 115°C for 2 h, then water (10 mL) was added and stirred at 100°C for another 6 h. After cooling down to room temperature, the crude product was collected by filtration and thoroughly washed by CH<sub>2</sub>Cl<sub>2</sub> to give **2** as a yellow solid (**Figures S5, S6**). Yield: 72%. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>), δ (ppm): 9.55 (s, 1H), 8.12 (d, J = 4 Hz, 2H), 8.02-7.98 (m, 4H), 7.68 (d, J = 16 Hz, 2H), 7.53 (t, J = 8 Hz, 2H), 7.45 (t, J = 8 Hz, 4H), 3.86 (s, 3H); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>), δ (ppm): 55.60, 113.19, 122.12, 122.19, 122.42, 125.32, 125.90, 126.44, 132.05, 133.93, 147.90, 153.12, 153.40, 166.72. MALDI-TOF-MS: m/z calcd for C<sub>25</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>S<sub>2</sub>, 442.08; found 442.8298 [M + H]<sup>+</sup> (**Figure S9**).

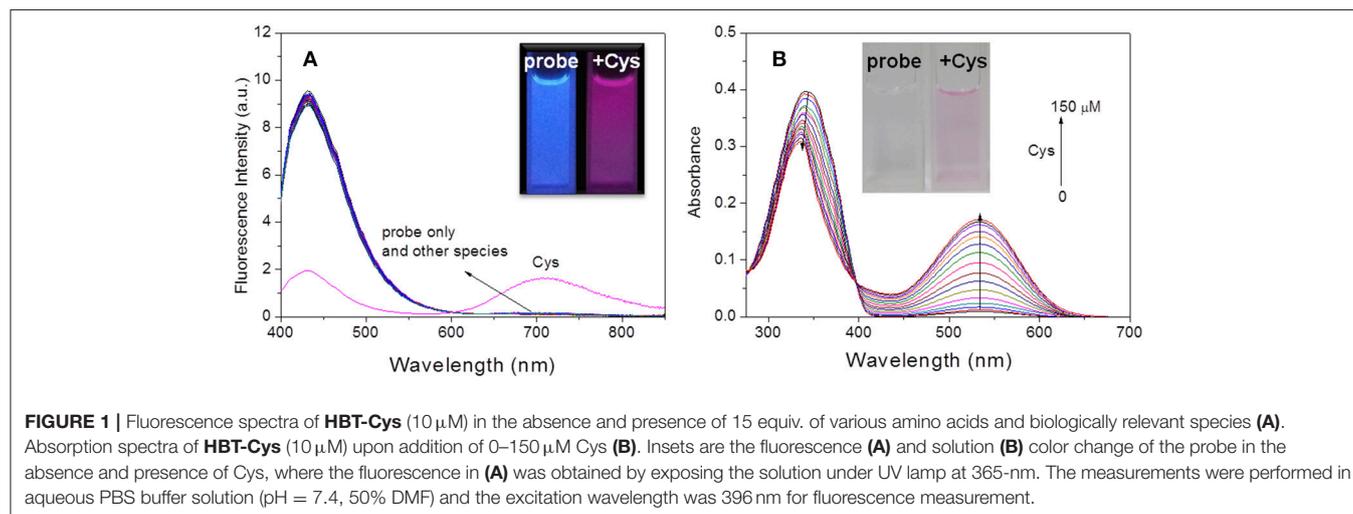
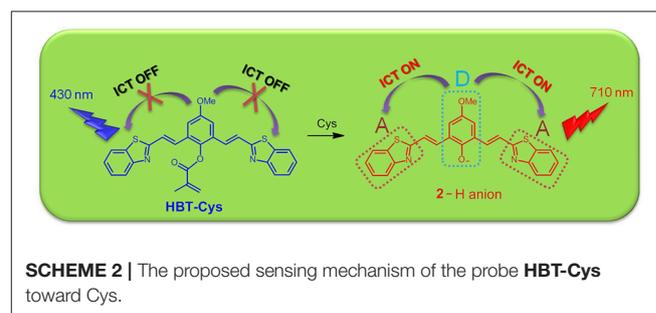
Compound **2** (0.1 mmol) was dissolved in acetone (200 mL) and methacryloyl chloride (0.13 mmol in 5 mL acetone) was slowly added dropwise under stirring at 0°C in the presence of K<sub>2</sub>CO<sub>3</sub> (2.0 mmol). Then the mixture was warmed to room temperature and stirred for another 8 h. The reaction mixture was

filtrated and the filtrate was concentrated in vacuum. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and then washed with water and the organic layer was dried with MgSO<sub>4</sub>. After removing MgSO<sub>4</sub> by filtration, the crude product was obtained by evaporation under reduced pressure and then purified by column chromatography (silica gel, CH<sub>2</sub>Cl<sub>2</sub>/ethyl acetate = 30:1 v/v) to give **HBT-Cys** as yellow solid (**Figures S7, S8**). Yield: 45%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>), δ (ppm): 8.03 (d, J = 8 Hz, 2H), 7.86 (d, J = 8 Hz, 2H), 7.52-7.46 (m, 6H), 7.41 (t, J = 8 Hz, 2H), 7.28 (s, 2H), 6.61 (s, 1H), 5.99 (s, 1H), 3.92 (s, 1H), 2.20 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>), δ (ppm): 18.67, 55.81, 112.86, 121.59, 123.13, 124.90, 125.86, 126.61, 128.52, 130.24, 130.99, 134.14, 135.07, 141.26, 153.31, 157.64, 165.86, 166.61. MALDI-TOF-MS: m/z calcd for C<sub>29</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub>S<sub>2</sub>, 510.11; found 511.2485 [M + H]<sup>+</sup>, 533.2472 [M + Na]<sup>+</sup> (**Figure S10**).

## RESULTS AND DISCUSSIONS

### Design and Synthesis

To design a selective fluorescent probe for Cys, a methacrylate group was chosen as the recognition site which can specifically react with Cys *via* conjugate addition/cyclization reaction (Yang et al., 2011; Ma et al., 2017). The conjugated benzothiazole derivatives displayed the large Stokes' shifted NIR fluorescence emission in deprotonated phenolate anion form due to the strong electron-donating ability of phenolate anion induced

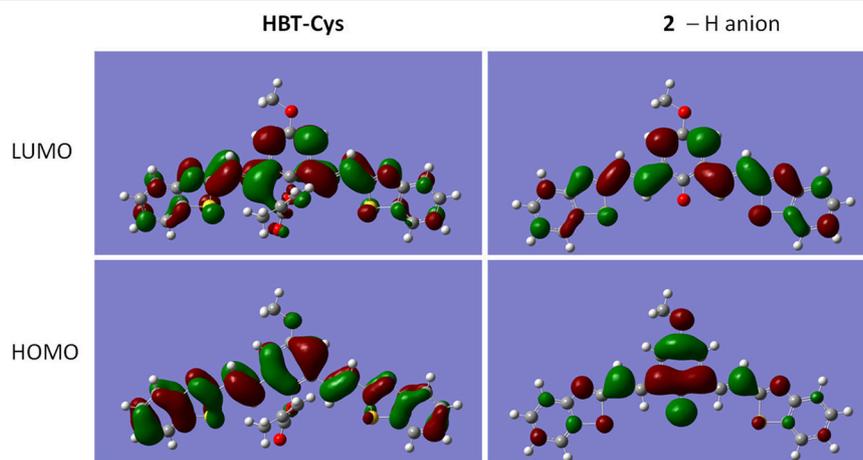


an occurrence of ICT (Karton-Lifshin et al., 2012; Zhang et al., 2016b), but showed a short-wavelength fluorescence in the neutral phenol form, which allowed a ratiometric detection manner based on two emissions. The novel fluorescent probe **HBT-Cys** was thus readily synthesized by combining methacrylate group into conjugated benzothiazole derivative. As shown in **Scheme 1**, the 2,6-diformyl-4-methoxyphenol (**1**) was firstly synthesized from commercially available 4-methoxyphenol *via* Duff reaction, then the conjugated benzothiazole derivative (**2**) was obtained by a direct condensation reaction between **1** and commercially available 2-methylbenzothiazole, and finally the probe **HBT-Cys** was easily afforded by treating **2** with methacryloyl chloride in acetone. The chemical structures of the probe **HBT-Cys** and intermediate compounds were characterized by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and MALDI-TOF-MS.

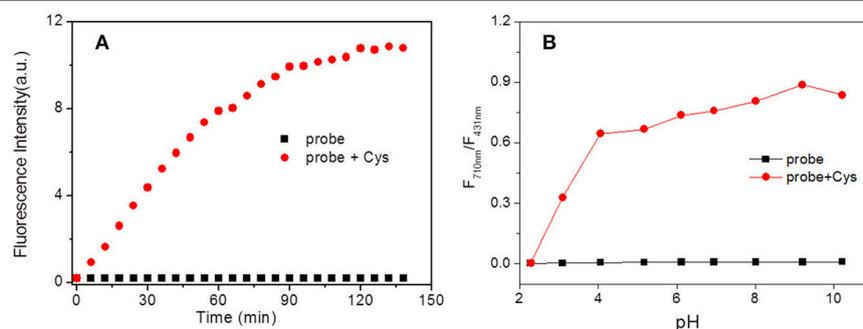
### Spectral Properties

The conjugated benzothiazole derivative **2** showed a short-wavelength emission at 500 nm in  $\text{CHCl}_3$  but a NIR emission at 730 nm in DMF with fluorescence quantum yield of 0.36 and

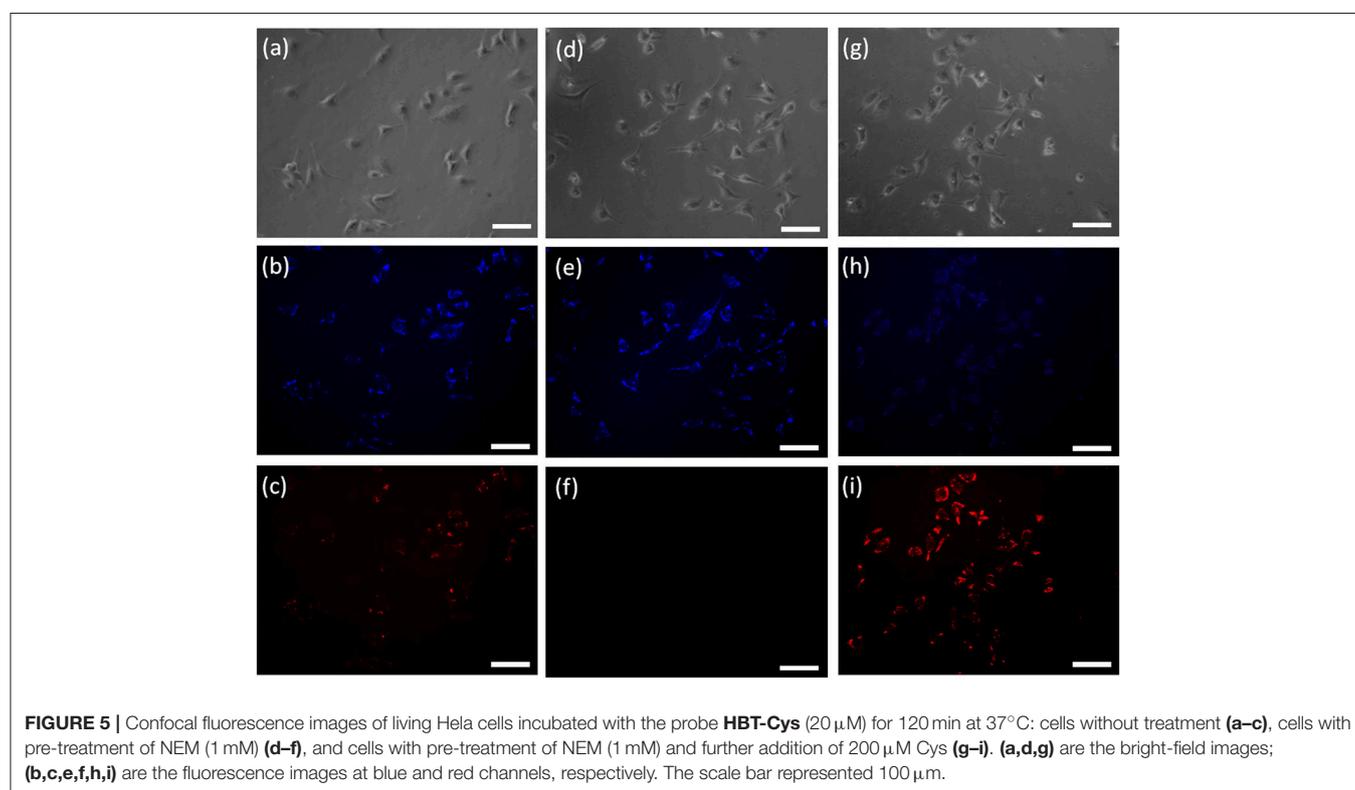
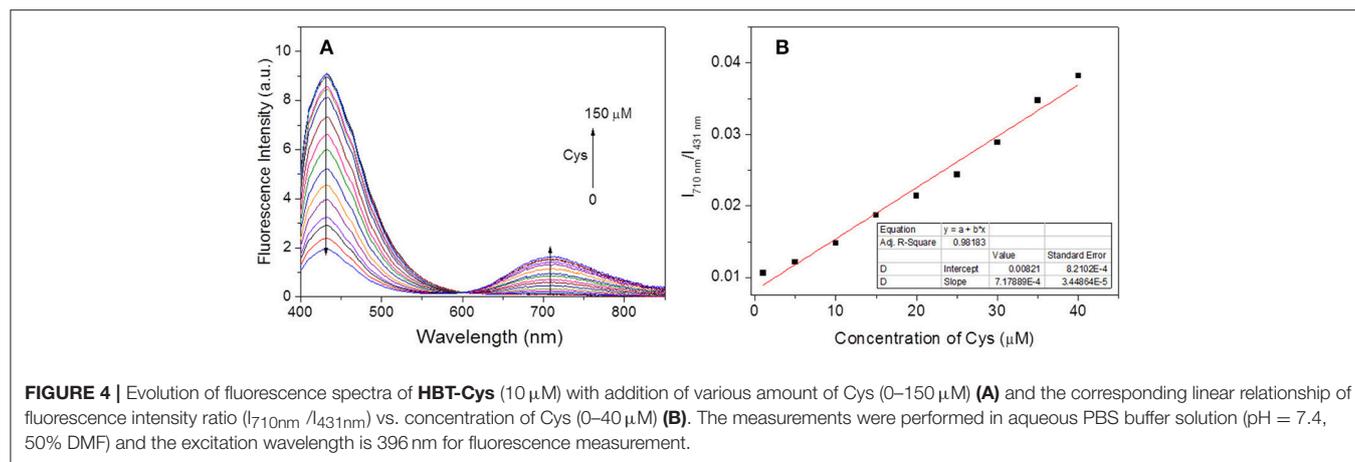
0.24, respectively (**Figure S1**). The NIR fluorescence emission was observed at 710 nm with a fluorescence quantum yield of 0.085 even in the PBS buffer solution (pH = 7.4, containing 50% DMF). These excellent fluorescence properties allowed compound **2** as a suitable platform to construct the fluorescent probe. As expected, the probe **HBT-Cys** showed a short-wavelength emission at 431 nm in the PBS buffer solution (pH = 7.4, containing 50% DMF), but a NIR fluorescence located at 710 nm appeared with decrease of the short-wavelength emission in the presence of Cys (**Figure 1A**). In contrast, the fluorescence spectra of the probe **HBT-Cys** showed no distinct change upon addition of others species such as common amino acids (GSH, Cys, Hcy, Asp, Asn, Ser, Pro, Ala, Gly, Val, Leu, Ile, Thr, Arg, Glu, Gln, Tyr, His, Met, Phe, Trp, Lys, Tau), cations ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ), anions ( $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$ ),  $\text{Na}_2\text{S}$ , mercaptoacetic acid and glucose. An obvious fluorescence color change from blue to red was observed in the presence of Cys (**Figure 1A**). This indicates that the probe **HBT-Cys** exhibited a high selectivity toward Cys in aqueous solution. Meanwhile, the probe **HBT-Cys** only displayed a strong absorption at 343 nm in the PBS buffer solution (pH = 7.4, containing 50% DMF), and the peak at 343 nm gradually



**FIGURE 2** | LUMO and HOMO orbitals of **HBT-Cys** and **2-H** anion in the ground state.



**FIGURE 3** | Time (**A**) and pH (**B**) effect on fluorescence intensity of **HBT-Cys** in the absence and presence of Cys. The fluorescence intensity measured at 710 nm in (**A**). The measurements were performed in aqueous PBS buffer solution (pH = 7.4, 50% DMF) and the excitation wavelength was 396 nm for fluorescence measurement.



decreased with a significant increase of the absorption at 533 nm upon addition of various amounts of Cys, where an isosbestic point was observed at 396 nm and the solution color turned pink from colorless (**Figure 1B**). No significant absorption spectral change was observed in the presence of others species. These spectral properties suggested that the **HBT-Cys** could serve as a NIR ratiometric fluorescent probe for high selective detection of Cys over Hcy/GSH.

### Sensing Mechanism

To confirm that probe **HBT-Cys** has been transformed into **2** in the presence of Cys as shown in **Scheme 2**, ESI-MS mass analysis

of a mixture solution of the probe **HBT-Cys** (10  $\mu\text{M}$ ) with 10 equiv. Cys was conducted. A prominent peak at  $m/z = 441$  corresponding to the  $[2-H]^-$  anion was observed (**Figure S2**), suggesting the fact that Cys took the methacrylate moiety away from probe **HBT-Cys**. The DFT calculation was further performed to gain better insights into the NIR fluorescence and signaling mechanism. **Figure 2** presented the optimized ground state structures of both the probe **HBT-Cys** and **2-H** anion. Obviously, the  $\pi$  electrons of the probe **HBT-Cys** were well-delocalized on the whole molecular skeleton on both the lowest unoccupied molecular orbital (LUMO) and the highest occupied molecular orbital (HOMO). When the  $\pi$  electrons of **2-H** anion

were still delocalized on the whole molecular skeleton on the LUMO, they mainly localized on the phenolate group on the HOMO, implying a potential ICT process from the phenolate donor (D) to two benzothiazole acceptors (A) as shown in . It has been known that the phenol moiety is a latent electron donor but acts as a strong electron donor when it transformed into phenolate, and could therefore switch on an ICT emission (Karton-Lifshin et al., 2012; Zhang et al., 2016b). Based on the TD-DFT calculation on the excited state, the fluorescence emission wavelengths were predicted to be 739 nm for the 2-H anion. The well-reproduction of the experimental result implied that the reliability of present theoretical calculation level. Thus, the signaling mechanism could be rationalized as Cys induced removal of methacrylate group and switch on of ICT emission, shown in **Scheme 2**.

### Response Time and pH Influence

The time-dependent fluorescence response of the probe **HBT-Cys** with and without Cys was performed, respectively. As shown in **Figure 3A**, the NIR fluorescence intensity at 710 nm was dramatically increased over 60 min and leveled off after about 120 min in the presence of Cys. Without Cys, the NIR fluorescence of the probe **HBT-Cys** displayed no significant changes, suggesting that the probe itself is stable enough under experimental condition. Thus, the spectral measurements of **HBT-Cys** were carried out after 120 min upon addition of Cys in the solution.

The pH influence on the fluorescence of the probe both in the absence and presence of Cys were investigated. Without Cys, the fluorescence intensities ratio ( $I_{710\text{nm}}/I_{431\text{nm}}$ ) displayed negligible changes over the range of pH 2.0–10.0 (**Figure 3B**). However, in the presence of Cys, the  $I_{710\text{nm}}/I_{431\text{nm}}$  ratio showed a drastic enhancement after pH > 3 and remained to be almost constant over whole pH region examined. This revealed that the present probe could work in a broad pH region (pH 3.0–10.0) and suitable for imaging under physiological conditions.

### Ratiometric Fluorescence Detection and Imaging of Cys

Under the optimal experimental conditions, the ratiometric fluorescence titrations toward Cys were performed. With excitation at 396 nm (an isosbestic point in absorption spectra, **Figure 1B**), it could be seen that the fluorescence emission gradually decreased at 431 nm and increased at 710 nm with increasing Cys amounts up to 150  $\mu\text{M}$  (**Figure 4A**). The  $I_{710\text{nm}}/I_{431\text{nm}}$  ratios followed a good linear relationship ( $R^2 = 0.9818$ ) with Cys concentration ranging from 1–40  $\mu\text{M}$  (**Figure 4B**). The detection limit was estimated to be 0.5  $\mu\text{M}$  according to  $S/N = 3$ . Hence, the probe **HBT-Cys** could detect Cys quantitatively by ratiometric fluorescence method with excellent sensitivity.

To evaluate the potential practical applications of probe **HBT-Cys**, the fluorescence imaging of Cys in living HeLa cells were also performed with a Carl Zeiss LSM 700 microscope, where dual blue and red channels were monitored, respectively (**Figure 5**). When HeLa cells were incubated with the probe **HBT-Cys** (20  $\mu\text{M}$ ) for 120 min at 37°C, both blue and red fluorescence emissions were observed in two channels

(**Figures 5b,c**), where the NIR emission resulted from the intracellular Cys induced removal of methacrylate group in the probe. In the control experiment, HeLa cells were pretreated with N-ethylmaleimide (NEM, a known scavenger for Cys, 1 mM for 30 min), and thereafter incubated with probe **HBT-Cys** (20  $\mu\text{M}$ ) for another 120 min. While the blue fluorescence remained, there was no fluorescence in the red channel (**Figures 5e,f**). Then the NEM-pretreated HeLa cells were further sequentially incubated with Cys (200  $\mu\text{M}$ ) for 30 min, the probe **HBT-Cys** (20  $\mu\text{M}$ ) for 120 min at 37°C. As a result, a bright fluorescence in the red channel was again observed inside cells accompanied by a weak fluorescence in the blue channel (**Figures 5h,i**). These results suggested that the probe **HBT-Cys** can serve as a promising fluorescent probe for Cys imaging in living cells.

### CONCLUSIONS

In summary, a benzothiazole-based NIR ratiometric fluorescent probe **HBT-Cys** was developed for selective detection of Cys over Hcy and GSH in aqueous solution. The probe was designed by masking the phenol group in the conjugated benzothiazole derivative through methacrylate group that acts both as a trigger of the ICT fluorescence and recognition site for Cys. Upon addition of Cys, the NIR fluorescence emission at 710 nm was significantly increased with decrease of the fluorescence emission at 431 nm. The fluorescence intensity ratio ( $I_{710\text{nm}}/I_{431\text{nm}}$ ) showed a linear relationship with Cys concentration of 1–40  $\mu\text{M}$  with the detection limit of 0.5  $\mu\text{M}$ . Based on mass analysis and DFT calculation, the signaling mechanism of Cys induced removal of methacrylate group and switch-on of the ICT fluorescence was proposed. The fluorescent probe was also successfully used for bioimaging of Cys in living cells, which would provide guidelines for design of novel ratiometric fluorescent probes in future.

### AUTHOR CONTRIBUTIONS

LZ and W-WM were responsible for designing and performing the experiments. YZ and Z-NL were responsible for the characterization of compounds. SX and XZ were responsible for drafting and discussing the manuscript.

### FUNDING

This work was financially supported by the State Key Laboratory of Fine Chemicals, Dalian University of Technology (KF 1715), Shanghai Municipal Natural Science Foundation (16ZR1401700), and the Fundamental Research Funds for the Central Universities (PYBZ1827).

### SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fchem.2019.00032/full#supplementary-material>

## REFERENCES

- Chan, J., Dodani, S. C., and Chang, C. J. (2012). Reaction-based small-molecule fluorescent probes for chemoselective bioimaging. *Nat. Chem.* 4, 973–984. doi: 10.1038/NCHEM.1500
- Chen, C., Zhou, L., Liu, W., and Liu, W. (2018). Coumarinocoumarin-based two-photon fluorescent cysteine biosensor for targeting lysosome. *Anal. Chem.* 90, 6138–6143. doi: 10.1021/acs.analchem.8b00434
- Chen, H., Tang, Y., and Lin, W. (2016). Recent progress in the fluorescent probes for the specific imaging of small molecular weight thiols in living cells. *Trends Anal. Chem.* 76, 166–181. doi: 10.1016/j.trac.2015.11.014
- Chen, X., Zhou, Y., Peng, X., and Yoon, J. (2010). Fluorescent and colorimetric probes for detection of thiols. *Chem. Soc. Rev.* 39, 2120–2135. doi: 10.1039/B925092A
- Chen, Z., Sun, Q., Yao, Y., Fan, X., Zhang, W., and Qian, J. (2017). Highly sensitive detection of cysteine over glutathione and homo-cysteine: new insight into the Michael addition of mercapto group to maleimide. *Biosens. Bioelectron.* 91, 553–559. doi: 10.1016/j.bios.2017.01.013
- De Silva, A. P., Gunaratne, H. Q. N., Gunlaugsson, T., Huxley, A. J. M., McCoy, C. P., Rademacher, J. T., et al. (1997). Signaling recognition events with fluorescent sensors and switches. *Chem. Rev.* 97, 1515–1566. doi: 10.1021/cr960386p
- Demchenko, A. P. (2010). The concept of  $\lambda$ -ratiometry in fluorescence sensing and imaging. *J. Fluoresc.* 20, 1099–1128. doi: 10.1007/s10895-010-0644-y
- Dorszewska, J., Prendecki, M., Oczkowska, A., Dezor, M., and Kozubski, W. (2016). Molecular basis of familial and sporadic Alzheimer's disease. *Curr. Alzheimer Res.* 13, 952–963. doi: 10.2174/1567205013666160314150501
- Escobedo, J. O., Rusin, O., Lim, S., and Strongin, R. M. (2010). NIR dyes for bioimaging applications. *Curr. Opin. Chem. Biol.* 14, 64–70. doi: 10.1016/j.cbpa.2009.10.022
- Feng, S., Fang, Y., Feng, W., Xia, Q., and Feng, G. (2017). A colorimetric and ratiometric fluorescent probe with enhanced near-infrared fluorescence for selective detection of cysteine and its application in living cells. *Dyes Pigm.* 146, 103–111. doi: 10.1016/j.dyepig.2017.07.002
- Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., et al. (2010). *Gaussian 09, Revision C.01*. Wallingford, CT: Gaussian Inc.
- Giles, N. M., Watts, A. B., Giles, G. I., Fry, F. H., Littlechild, J. A., and Jacob, C. (2003). Metal and redox modulation of cysteine protein function. *Chem. Biol.* 10, 677–693. doi: 10.1016/S1074-5521(03)00174-1
- Guo, Z., Park, S., Yoon, J., and Shin, I. (2014). Recent progress in the development of near-infrared fluorescent probes for bioimaging applications. *Chem. Soc. Rev.* 43, 16–29. doi: 10.1039/c3cs60271k
- Hou, X., Li, Z., Li, B., Liu, C., and Xu, Z. (2018). An “off-on” fluorescein-based colorimetric and fluorescent probe for the detection of glutathione and cysteine over homocysteine and its application for cell imaging. *Sens. Actuators B Chem.* 260, 295–302. doi: 10.1016/j.snb.2018.01.013
- Karton-Lifshin, N., Albertazzi, L., Bendikov, M., Baran, P. S., and Shabat, D. (2012). “Donor-two-acceptor” dye design: a distinct gateway to NIR fluorescence. *J. Am. Chem. Soc.* 134, 20412–20420. doi: 10.1021/ja308124q
- Kikuchi, K., Takakusa, H., and Nagano, T. (2004). Recent advances in the design of small molecule-based FRET sensors for cell biology. *Trends Anal. Chem.* 23, 407–415. doi: 10.1016/S0165-9936(04)00608-9
- Kim, Y., Choi, M., Mulay, S. V., Jang, M., Kim, J. Y., Lee, W.-H., et al. (2018). Aqueous red-emissive probe for the selective fluorescent detection of cysteine by deprotection/cyclization cascade resulting in large Stokes' shift. *Chem. Eur. J.* 24, 5623–5629. doi: 10.1002/chem.201706073
- Lee, M. H., Kim, J. S., and Sessler, J. L. (2015). Small molecule-based ratiometric fluorescence probes for cations, anions, and biomolecules. *Chem. Soc. Rev.* 44, 4185–4191. doi: 10.1039/C4CS00280F
- Li, X., Gao, X., Shi, W., and Ma, H. (2014). Design strategies for water-soluble small molecular chromogenic and fluorogenic probes. *Chem. Rev.* 114, 590–659. doi: 10.1021/cr300508p
- Li, Y., Liu, W., Zhang, P., Zhang, H., Wu, J., Ge, J., et al. (2017). A fluorescent probe for the efficient discrimination of Cys, Hcy and GSH based on different cascade reactions. *Biosens. Bioelectron.* 90, 117–124. doi: 10.1016/j.bios.2016.11.021
- Lipton, S. A., Choi, Y. B., Takahashi, H., Zhang, D., Li, W., Godzik, A., et al. (2002). Cysteine regulation of protein function - as exemplified by NMDA-receptor modulation. *Trends Neurosci.* 25, 474–480. doi: 10.1016/S0166-2236(02)02245-2
- Liu, G., Liu, D., Han, X., Sheng, X., Xu, Z., Liu, S., et al. (2017). A hemicyanine-based colorimetric and ratiometric fluorescent probe for selective detection of cysteine and bioimaging in living cell. *Talanta* 170, 406–412. doi: 10.1016/j.talanta.2017.04.038
- Liu, J., Sun, Y.-Q., Huo, Y., Zhang, H., Wang, L., Zhang, P., et al. (2014). Simultaneous fluorescence sensing of cys and GSH from different emission channels. *J. Am. Chem. Soc.* 136, 574–577. doi: 10.1021/ja409578w
- Liu, X., Tian, H., Yang, L., Su, Y., Guo, M., and Song, X. (2017). An ESIPT-based fluorescent probe for sensitive and selective detection of Cys/Hcy over GSH with a red emission and a large Stokes shift. *Tetrahedron Lett.* 58, 3209–3213. doi: 10.1016/j.tetlet.2017.06.082
- Lv, H., Yang, X.-F., Zhong, Y., Guo, Y., Li, Z., and Li, H. (2014). Native chemical ligation combined with spirocyclization of benzopyrylium dyes for the ratiometric and selective fluorescence detection of cysteine and homocysteine. *Anal. Chem.* 86, 1800–1807. doi: 10.1021/ac4038027
- Ma, W.-W., Wang, M.-Y., Yin, D., and Zhang, X. (2017). Facile preparation of naphthol AS-based fluorescent probe for highly selective detection of cysteine in aqueous solution and its imaging application in living cells. *Sens. Actuators B* 248, 332–337. doi: 10.1016/j.snb.2017.03.169
- Navimanager, R. R., Prasai, B., Hettiarachchi, S. U., and McCarley, R. L. (2017). Cascade reaction-based, near-infrared multiphoton fluorescent probe for the selective detection of cysteine. *Anal. Chem.* 89, 6886–6892. doi: 10.1021/acs.analchem.7b01384
- Niu, L.-Y., Chen, Y.-Z., Zheng, H.-R., Wu, L.-Z., Tung, C.-H., and Yang, Q.-Z. (2015). Design strategies of fluorescent probes for selective detection among biothiols. *Chem. Soc. Rev.* 44, 6143–6160. doi: 10.1039/C5CS00152H
- Nolting, D. D., Gore, J. C., and Pham, W. (2011). Near-infrared dyes: probe development and applications in optical molecular imaging. *Curr. Org. Synth.* 8, 521–534. doi: 10.2174/157017911796117223
- Qi, Y., Huang, Y., Li, B., Zeng, F., and Wu, S. (2018). Real-time monitoring of endogenous cysteine levels *in vivo* by near-infrared turn-on fluorescent probe with large Stokes shift. *Anal. Chem.* 90, 1014–1020. doi: 10.1021/acs.analchem.7b04407
- Ren, X., Tian, H., Yang, L., He, L., Geng, Y., Liu, X., et al. (2018). Fluorescent probe for simultaneous discrimination of Cys/Hcy and GSH in pure aqueous media with a fast response under a single-wavelength excitation. *Sens. Actuators B Chem.* 273, 1170–1178. doi: 10.1016/j.snb.2018.04.163
- Shahrokhian, S. (2001). Lead phthalocyanine as a selective carrier for preparation of a cysteine-selective electrode. *Anal. Chem.* 73, 5972–5978. doi: 10.1021/ac010541m
- Shao, J., Sun, H., Guo, H., Ji, S., Zhao, J., Wu, W., et al. (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, 1049–1061. doi: 10.1039/C2SC00762B
- Sheng, H., Hu, Y., Zhou, Y., Fan, S., Cao, Y., Zhao, X., et al. (2019). A highly selective ESIPT-based fluorescent probe with a large Stokes shift for the turn-on detection of cysteine and its application in living cells. *Dyes Pigm.* 160, 48–57. doi: 10.1016/j.dyepig.2018.07.036
- Song, H., Zhang, J., Wang, X., Zhou, Y., Xu, C., Pang, X., and Peng, X. (2018). A novel “turn-on” fluorescent probe with a large Stokes shift for homocysteine and cysteine: performance in living cells and zebrafish. *Sens. Actuators B Chem.* 259, 233–240. doi: 10.1016/j.snb.2017.12.010
- Tian, Y., Zhu, B., Yang, W., Jing, J., and Zhang, X. (2018). A fluorescent probe for differentiating Cys, Hcy and GSH via a stepwise interaction. *Sens. Actuators B Chem.* 262, 345–349. doi: 10.1016/j.snb.2018.01.181
- Wang, F., Feng, C., Lu, L., Xu, Z., and Zhang, W. (2017). A ratiometric fluorescent probe for rapid and sensitive detection of biothiols in fetal bovine serum. *Talanta* 169, 149–155. doi: 10.1016/j.talanta.2017.03.080
- Wang, J., Niu, L., Huang, J., Yan, Z., Zhou, X., and Wang, J. (2018). Thiazolyl substituted NBD as fluorescent probe for the detection of homocysteine and cysteine. *Dyes Pigm.* 158, 151–156. doi: 10.1016/j.dyepig.2018.05.039
- Wang, L., Zhuo, S., Tang, H., and Cao, D. (2018). An efficient fluorescent probe for rapid sensing of different concentration ranges of cysteine with two-stage ratiometric signals. *Dyes Pigm.* 157, 284–289. doi: 10.1016/j.dyepig.2018.05.004
- Wang, Q., Ma, F., Tang, W., Zhao, S., Li, C., and Xie, Y. (2018a). A novel nitroethylene-based porphyrin as a NIR fluorescence turn-on probe for

- biothiols based on the Michael addition reaction. *Dyes Pigm.* 148, 437–443. doi: 10.1016/j.dyepig.2017.09.046
- Wang, Q., Wang, H., Huang, J., Li, N., Gu, Y., and Wang, P. (2017). Novel NIR fluorescent probe with dual models for sensitively and selectively monitoring and imaging Cys in living cells and mice. *Sens. Actuators B Chem.* 253, 400–406. doi: 10.1016/j.snb.2017.06.166
- Wang, Q., Wei, X., Li, C., and Xie, Y. (2018b). A novel p-aminophenylthio- and cyano-substituted BODIPY as a fluorescence turn-on probe for distinguishing cysteine and homocysteine from glutathione. *Dyes Pigm.* 148, 212–218. doi: 10.1016/j.dyepig.2017.09.020
- Wu, Q., Mao, M., Liang, W., and Stadler, F. J. (2018). Quinoline-derived fluorescent probes for the discrimination of Cys from Hcys/GSH and bioimaging in living cells. *Talanta* 186, 110–118. doi: 10.1016/j.talanta.2018.04.044
- Wu, Q., Wu, Y., Yu, C., Wang, Z., Hao, E., and Jiao, L. (2017). A highly selective visible light excitable boron dipyrromethene probe for cysteine over homocysteine and glutathione based on a Michael addition reaction. *Sens. Actuators B Chem.* 253, 1079–1086. doi: 10.1016/j.snb.2017.07.028
- Yang, X.-F., Guo, Y.-X., and Strongin, R. M. (2011). Conjugate addition/cyclization sequence enables selective and simultaneous fluorescence detection of cysteine and homocysteine. *Angew. Chem. Int. Ed.* 50, 10690–10693. doi: 10.1002/anie.201103759
- Yang, Y., Zhao, Q., Feng, W., and Li, F. (2013). Luminescent chemodosimeters for bioimaging. *Chem. Rev.* 113, 192–270. doi: 10.1021/cr2004103
- Yin, C.-X., Xiong, K.-M., Huo, F.-J., Salamanca, J. C., and Strongin, R. M. (2017). Fluorescent probes with multiple binding sites for the discrimination of Cys, Hcy, and GSH. *Angew. Chem. Int. Ed.* 56, 13188–13198. doi: 10.1002/anie.201704084
- Yuan, L., Lin, W., Zheng, K., He, L., and Huang, W. (2013). Far-red to near infrared analyte-responsive fluorescent probes based on organic fluorophore platforms for fluorescence imaging. *Chem. Soc. Rev.* 42, 622–661. doi: 10.1039/c2cs35313j
- Yue, P., Yang, X., Ning, P., Xi, X., Yu, H., Feng, Y., et al. (2018). A mitochondria-targeted ratiometric two-photon fluorescent probe for detecting intracellular cysteine and homocysteine. *Talanta* 178, 24–30. doi: 10.1016/j.talanta.2017.08.085
- Yue, Y., Huo, F., Li, X., Wen, Y., Yi, T., Salamanca, J., et al. (2017a). pH-dependent fluorescent probe that can be tuned for cysteine or homocysteine. *Org. Lett.* 19, 82–85. doi: 10.1021/acs.orglett.6b03357
- Yue, Y., Huo, F., Ning, P., Zhang, Y., Chao, J., Meng, X., et al. (2017b). Dual-site fluorescent probe for visualizing the metabolism of Cys in living cells. *J. Am. Chem. Soc.* 139, 3181–3185. doi: 10.1021/jacs.6b12845
- Zhang, H., Feng, W., and Feng, G. (2017). A simple and readily available fluorescent turn-on probe for cysteine detection and bioimaging in living cells. *Dyes Pigm.* 139, 73–78. doi: 10.1016/j.dyepig.2016.12.011
- Zhang, J., Ji, X., Ren, H., Zhou, J., Chen, Z., Dong, X., et al. (2018a). Meso-heteroaryl BODIPY dyes as dual-responsive fluorescent probes for discrimination of Cys from Hcy and GSH. *Sens. Actuators B Chem.* 260, 861–869. doi: 10.1016/j.snb.2018.01.016
- Zhang, J., Ji, X., Zhou, J., Chen, Z., Dong, X., and Zhao, W. (2018b). Pyridinium substituted BODIPY as NIR fluorescent probe for simultaneous sensing of hydrogen sulphide/glutathione and cysteine/homocysteine. *Sens. Actuators B Chem.* 257, 1076–1082. doi: 10.1016/j.snb.2017.10.133
- Zhang, X., and Liu, J.-Y. (2016a). Solvent dependent photophysical properties and near-infrared solid-state excited state intramolecular proton transfer (ESIPT) fluorescence of 2,4,6-tris(benzothiazolyl)phenol. *Dyes Pigm.* 125, 80–88. doi: 10.1016/j.dyepig.2015.10.002
- Zhang, X., Liu, J.-Y., Ma, W.-W., and Yang, M.-L. (2016b). Near-infrared fluorescence of p-conjugation extended benzothiazole and its application for biothiol imaging in living cells. *J. Mater. Chem. B* 4, 6662–6669. doi: 10.1039/c6tb01465h
- Zhu, X., Yuan, L., Hu, X., Zhang, L., Liang, Y., He, S., et al. (2018). Construction of a fluorine substituted chromenylium-cyanine near-infrared fluorophore for ratiometric sensing. *Sens. Actuators B Chem.* 259, 219–225. doi: 10.1016/j.snb.2017.12.008

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Zhang, Zhang, Ma, Zhou, Lu and Xu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.