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ARTICLE

A highly selective ratiometric fluorescent probe for biothiol and imaging in live cells

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Baozhen Gao[a], Lixia Cui[a], Yong Pan[b], Guomei Zhang[a], Ying Zhou[a], Caihong Zhang[a,*], Shaomin Shuang[a], Chuan Dong[a, *]

A new N-butyl-4-amino-1,8-naphthalimide-based colorimetric and ratiometric fluorescent probe for the detection of biothiols (cysteine, homocysteine, and glutathione) were designed and synthesized. The probe exhibited good selectivity and sensitivity toward biothiols over other non-thiol-bearing amino acids and common anions with significant changes in both color (from colorless to yellow) and fluorescence (from blue to yellow-green). The mechanism is based on cleavage of disulfide by thiols followed by an intramolecular cyclization to give the product, N-butyl-4-amino-1,8-naphthalimide, which showed outstanding intramolecular charge transfer (ICT). Therefore, ratiometric fluorescence signal was observed. Furthermore, the probes were successfully applied for visualizing endogenous thiols in living cells.

1 Introduction

Biological thiols such as glutathione (GSH), cysteine (Cys), and homocysteine (Hcy) have similar structure and play essential roles in many physiological processes. The abnormal levels of intracellular biothiols will initiate many diseases [1-3]. Among these agents, GSH is the most abundant intracellular nonprotein thiol (about 0.1~10 mM) [4], and play a critical role in maintaining redox homeostasis of the cell and is directly linked with many diseases including cancer, Parkinson's diease, Alzheimer disease, etc [5-8]. Thus, it is important to develop efficient methods for the detection and quantification of biothiols in physiological media for academic research and for the diagnosis of the related diseases.

Currently, among the major methods for biothiols detection, fluorescent probes have been recognized as the efficient molecular tools due to their high selectivity, low cost and ability to detect and image the intracellular biothiols [9], in particular, ratiometric fluorescent probes have attracted increasing attention which based on the ratio of emission intensity from two different wavelengths can eliminate the influence of probe concentration, environment and excitation intensity [10-13]. To date, a variety of ratiometric fluorescent biothiols probes have been reported based on different mechanisms: 1) thiol cleavage reaction [14-17], 2) thiol nucleophilic reaction [18-21], 3) metal complexes-displace coordination and others [22-26]. Among the various strategies, the probes based on cleavage of disulfide by thiols have been most actively developed. A number of excellent disulfide probes for biothiols have been exploited such as Pullela et al. tethered fluorescein and rhodamine by disulfid containing linker [27]; Karuso et al. jioned two molecules of fluorescein by a short disulfide-containing linker [28]; Chmielewski et al. synthesized a new rhodamine derivative bearing disulfide unit [29]. Some reports use GSH-triggered disulfide-based cleavable reactions for targeted drug delivery [30–35]. Although many promising probes have been reported for thiols detection in recent years, it still remains a challenge to improve methodology for this purpose.

In this work, we report a new ratiometric fluorescent probe for the detection of biothiols based on the nucleophilic cleavage-cyclization of disulfides. N-butyl-4-amino-1,8-naphthalimide was chosen as the fluorophore due to its desirable optical properties, such as high photostability, large Stokes' shift, insensitivity to pH [36–39]. 2-(pyridin-2-yldisulfanyl)ethanol owning to its sensitivity and rapid cleavage by biothiols and an appropriate linker was employed for reaction acceptor (Scheme 1). The probe showed high selectivity for biothiols over other amino acids and anions. It was successfully applied for the GSH bioimaging in live cells.

2 Experimental

2.1 Chemicals

4-Bromo-1,8-naphthalic anhydride, EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride), DMAP (4-dimethylaminopyridine) and other chemicals were purchased from Sigma-Aldrich or Aldrich and were used as received. Solvents were dried according to standard procedures. All biothiols (including cysteine (Cys), homocysteine (Hcy), glutathione (GSH)), amino acids and other anions were analytical grade and were purchased from Beijing Experiment Reagent Co., Ltd (Beijing, China).

2.2 Apparatus

[a] Department of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, China. E-mail: chzhang@sxu.edu.cn; Fax: +86-351-7011688; Tel: +86-351-7010588
[b] State Key Laboratory of NBC Protection for Civilian, Research Institute of Chemical Defense, Yangfang, Changping District, Beijing, 102205, China.

† Electronic supplementary information (ESI) available: ^{1}H NMR, ^{13}CNMR and MS spectra of probe, ratiometric responses towards Cys and Hcy, time-dependent fluorescence spectral changes of probe with biothinols, the effects of pH, Determination of quantum yield.

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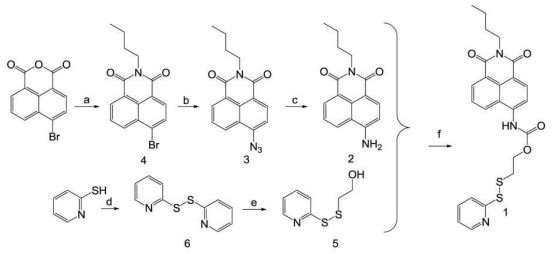
^{1}H-NMR and ^{13}C-NMR spectra were collected on a 300 MHz spectrometer. Fluorescence excitation and emission spectra were measured on a Cary Eclipse fluorescence spectrophotometer. High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer. Fluorescence imaging was performed with a fluorescence microscope with a GFP light cube for fluorescence channel and 40×objectives.

2.3 Preparation and characterization of probe

Synthesis of probe (1) is summarized in Scheme 1. N-butyl-4-amino-1,8-naphthalimide (2) [40, 41] and 2-(pyridine-2-yldisulfanyl)ethanol (5) were synthesized by the literature methods. Synthesis of probe 1 is described below [42].

To a mixture of N-butyl-4-amino-1,8-naphthalimide 2 (60mg, 0.14 mmol) and DIPEA (67 mg, 0.52 mmol) in dry toluene (5 mL) was added a solution of triphosgene (52 mg, 0.17 mmol) in toluene dropwise in ice bath for 2h. Then the resulting solution was heated to reflux for 3h. After cooling to room temperature, the reaction mixture was flushed with nitrogen gas and removal of unreacted phosgene gas. A solution was diluted with CH_2Cl_2 (6 mL) and added the 2-(pyridine-2-yldisulfanyl)ethanol (31.32 mg, 0.18 mmol) and the solution was stirred at room temperature for overnight. The reaction was then concentrated and purified by flash column chromatography (silica gel, 5:95 MeOH/CH_2Cl_2) to give probe as a yellow solid (45 mg, 0.09 mmol, 40.5% yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57-8.64 (m, 2H), 8.45-8.48 (d, 1H, J = 8.47), 8.24-8.33 (m, 2H,) 7.61-7.78 (m, 3H), 7.08-7.12 (m, 1H), 4.52-4.56 (t, 2H, J = 4.54 Hz), 4.15-4.20 (t, 2H J = 4.17), 3.13-3.17 (t, 2H, J = 3.15), 1.66-1.77 (m, 2H), 1.38-1.51 (m, 2H), 0.95-1.00 (t, 3H, J = 0.97); ^{13}C NMR (100 MHz, CDCl3) δ 164.4, 163.9, 159.8, 153.0, 150.0, 139.0, 137.4, 132.6, 131.5, 129.1, 126.9, 126.3, 123.6, 123.2, 121.4, 120.6, 118.2, 117.0, 64.2, 40.5, 37.7, 30.4, 20.6, 14.1; HRESI-MS: calcd for $C_{24}H_{23}N_3O_4S_2$ $[M+H]^+$ 482.1203, found 482.11966.(Fig.S1)



a) n-BuNH_2, EtOH, reflux, 89%; b) NaN_3, DMF, 90 °C, reflux, 82%; c) NH_4Cl, Zn, reflux, 65%; d) $CHCl_3$, SO_2Cl_2; $NaHCO_3$, 31%; e) 2-mercaptoethanol, MeOH,89.5%; f) DIPEA, triphosgene, 40.5%.

Scheme 1 The synthesis route of probe.

2.4 General UV–vis and fluorescence spectra measurements

Stock solutions of probe 1 (0.25mM) were prepared in DMF (N, N-Dimethylformamide). Biothiols solutions, amino acids solutions and anion solution were also prepared using the deionized water. UV–vis and fluorescence spectra were obtained in DMF: water (1:9 v/v, PES buffer, pH 7.4) solutions. Fluorescence measurements were carried out with a slit width of 5 nm (λex = 420nm).

2.5 Cell imaging experiments with GSH

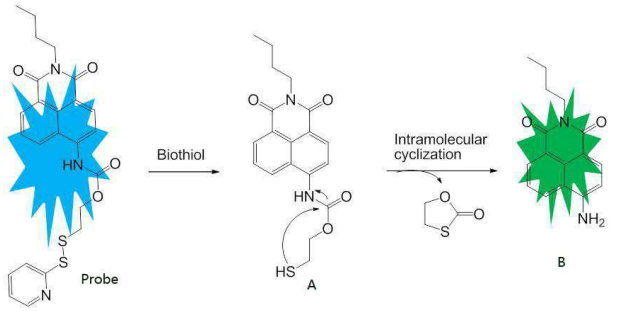
HeLa cells were cultured in Dulbecco's modified Eagle's Medium (DMEM, Cellgro company) supplemented with 10% fetal bovine serum, 4mM glutamine, 100 IU/mL penicillin, and 100ug/mL streptomycin at 37 °C and with 5% CO_2 for two days. Cells were transferred to 24-well plates one day before imaging. Before use, the adherent cells were washed once with FBS-free DMEM. Then the cell was treated with GSH (200 μM) in culture media for 30 min at 37°C and washed with phosphate-buffered saline (PBS). After washing with PBS to remove the remaining GSH, the cells were further incubated with the probe (5 μM) for 120min in PBS buffer (pH 7.4, containing 100μM CTAB). Cell imaging was carried out after washing the cells with PBS (pH 7.4). All of the microscopy images were taken on a fluorescence microscope with blue and green channels.

3 Results and discussion

3.1 Design principle of the probe

The disulfide bond can undergo cleavage via reaction with the thiols, many effective fluorescent probes were developed based on it and proved to have high selectivity and sensitivity for biothiols. In order to further improve the fluorescence properties, and to use them for the quantitative detection of biothiols in complex samples, development of ratiometric fluorescent probes based on this strategy was required.

Herein, we used N-butyl-4-amino-1,8-naphthalimide linked with pyridine by disulfide-containing linker to prepare a ratiometric fluorescent probe for biothiols (Scheme 2). Because carbamate bond was formed, the electron-withdrawing group weakened the ICT effect and resulted in a fluorescent emission wavelength blue shift. Upon reacting with biothiols, the disulfide bond cleavage generated the intermediate A, having a free -SH group. Then the intramolecular cyclization of A should proceed to release the byproduct, 1,3-oxathiolan-2-one and the fluorophore, N-butyl-4-amino-1,8-naphthalimide B, which showed outstanding intramolecular charge transfer (ICT). Therefore, ratiometric signal was observed. This mechanism has been proved by reaction between probe and GSH, and reaction product B was confirmed by ^{1}H NMR (Fig. S2).



Scheme 2. Proposed detection mechanism of probe against biothiols.

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3.2 Ratiometric and colorimetric responses towards biothiols

With the probe in hand, we first investigated its fluorescence spectroscopic properties in a mixture of DMF/buffer (1:9, 50 mM PBS buffer, pH 7.4). The probe showed a wide fluorescence emission band (420-650nm) with a peak at 482 nm. The fluorescence quantum yield was 0.11.

The probe (1μM) was treated with GSH, Cys, and Hcy separately (all at 1 mM), the fluorescence emission intensity of 482 nm obviously decreases, accompanying appearance of a new emission band (470−670 nm) with a peak at 540 nm, exhibiting a ratiometric change with a large red-shift over 60 nm for the probe as shown in Fig. 1. Meanwhile, the color of the solution changed gradually from colorless to yellow, which was clearly recognizable by naked eyes. We also noted that the solution's fluorescence color changed from blue to yellow-green under illumination with a 365 nm UV lamp. These results demonstrated that the probe was sensitive to these biothiols.

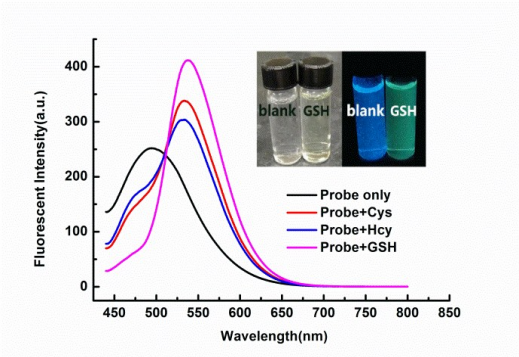

Fig.1 The emission spectra of probe (1 μM) in 50 mM PBS buffer (pH 7.4, 10% DMF) with Cys, Hcy and GSH (1 mM) for 120 min at 25 °C, λex=420 nm. Inset: the color changes of probe without and with addition of GSH. left: under ambient lighting, right: under UV irradiation.

The fluorescence titrations were also investigated under the same condition as shown in Fig. 2. Upon the addition of GSH, the emission band at 482 nm decreased and a new band at 540 nm gradually enhanced. The changes occur with an obvious isosbestic point at 505 nm. It was found that with increased concentrations of GSH (20 μM -200 μM), 0~6-fold enhancements of the fluorescence intensity ratios could be observed. A good linearity (R = 0.9961) between the fluorescence intensity ratio (F_{540}/F_{482}) and the GSH concentration in the range of 20 μM -200 μM was obtained, as depicted in insert Fig. 2. The detection limit for GSH, based on the definition by IUPAC (CDL=3Sb/m), was calculated to be 3.7 μM [43]. These results showed that the probe could be applied for quantitative measurement of GSH concentrations. Similar results were also observed upon the addition of Cys and Hcy into the solution of probe (Fig. S3, S4).

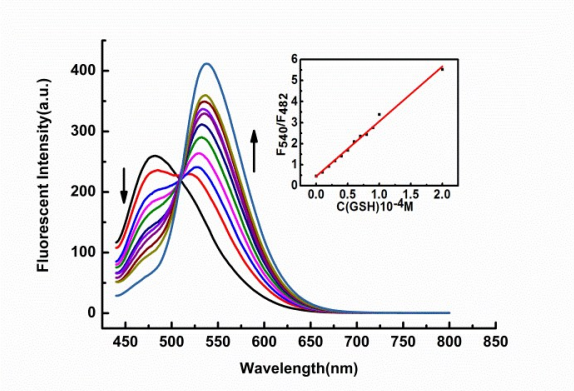

Fig.2. The emission spectra of probe (1 μM) in 50 mM PBS buffer (pH 7.4, 10% DMF) with different concentrations of GSH (0, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200 μM) for 120 min at 25 °C, λex=420 nm. Insert is the concentration of GSH dependence of the ratiometric fluorescence signal (F_{540}/F_{482}).

3.3 Time-dependence in the detection process of biothiols

We examined the time-dependent fluorescence changes of the probe (1μM) towards GSH. It was found that in the presence of 1 mM GSH, the emission intensity at 482 nm was increased at first ('1-4' in the Fig.3), the changes took place in 8min under these experimental conditions, then decreased gradually, while the emission at 540 nm increases (in the Fig.3). Within 120 min, the fluorescence intensity reaches a plateau (insert fig.3).

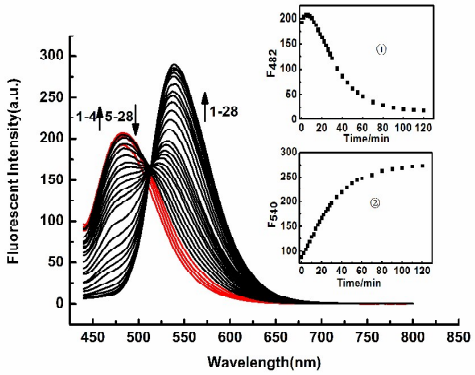

Fig.3. Time-dependent fluorescence response of the probe (1 uM) to GSH (1 mM) in 50 mM PBS buffer (pH 7.4, 10% DMF). Insert: ① Fluorescence intensity of probe at 482 nm upon reaction with GSH. ② Fluorescence intensity of probe at 540 nm upon reaction with GSH.

The above changes are consistent with the mechanism of probe to GSH. The increased emission intensity at 482 nm was assigned the formation of intermediate A, which can formed readily as the result of disulfide cleavage. Then A followed by slow intramolecular cyclization and cleavage of a neighboring carbamate bond to give B. Corresponding, the emission intensity at 482 nm was decreased and the emission at 540 nm was increased. Similar results were achieved for Cys and Hcy (Fig.S5).

3.4 pH effects

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The pH dependence of the thiol-mediated disulfide bond cleavage step was also investigated and as seen in Figure S6, The probe appeared to be stable in the pH range 4-10 as almost no fluorescence changes were observed. In the presence of biothiols, probe gave strong ratiometric fluorescence responses at pH (7-10). In contrast, the probe did not show significant signals change at acidic pH (4-6). Such findings showed that probe could be used to detect cellular thiols in biological conditions.

3.5 Selectivity over biothiols

To assess the selectivity of the probe toward thiols, the probe (1μM) was treated with various thiol-bearing amino acids including GSH, Cys, Hcy and other non-thiol-bearing amino acids (L-histidine (His), L-glutamic acid (Glu), L-asparagine (Asp), L-valine (Val), L-phenylalanine (Phe), L-tyrosine (Tyr), L-alanine (Ala), L-serine (Ser), L-leucine (Leu), L-arginine (Arg), L-proline (Pro), L-threonine (Thr), L-glutamine (Glu), L-tryptophan (Trp), L-isoleucine (Ile), L-lysine (Lys)) (all at 1 mM). Fluorescence signals were recorded after mixing for 120 min. As shown in Fig. 4 (a), the fluorescence ratio (F_{540}/F_{482}) of probe exhibited a significant increase upon reaction with GSH, Cys, Hcy, whereas little fluorescence intensity changes were observed in the presence of other non-thiol-bearing amino acid. Moreover, when biothiols (including Cys, Hcy and GSH) co-existed with non-thiol-bearing amino acid, the fluorescence ratio signal showed an obvious increase. These results clearly demonstrated the excellent selectivity of probe even when other non-thiol-bearing amino acid coexisted with biothiols.

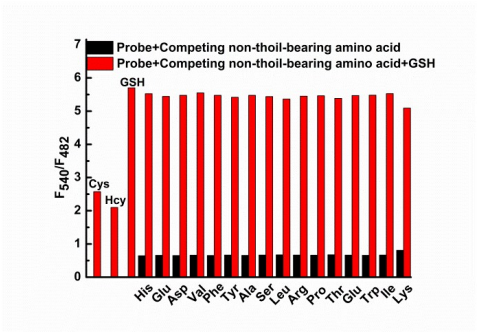
In order to check the influence of anions to the probe toward GSH, the probe (1μM) was treated with some common anions such as Cl^-, Br^-, I^-, SO_3^{2-}, $C_2O_4^{2-}$, CO_3^{2-}, SO_4^{2-}, NO_3^-, $S_2O_3^{2-}$, Ac^- and PO_4^{3-}. As shown in Fig. 4 (b), these anions exhibited no noticeable fluorescence and had no interference to the detection of biothinols.

3.6 Cellular imaging

The cytotoxicity of the probe is an important consideration for intracellular imaging applications. So the cytotoxicity of the probe was evaluated using the HeLa cell line by the MTT assay (Fig.5). From the results we can see that the probe exhibited little cytotoxicity for cells, even at the concentration of 20 μM, the cell viability is still more than 93%. The results demonstrated that the probe has good biocompatibility and is appropriate for the detection of biothiols in live cells.

It is well accepted that living cells maintain high concentrations of biothiols, especially GSH. In order to evaluate the capability of probe for selective fluorescence detection of intracellular GSH, fluorescence imaging in live HeLa cells was carried out. The cells were treated with 5μM probe for 120 min, after removal of excess probe and washed. As shown in Fig.6 (a, b and c), we could observe not only bule fluorescence ,but weakly green fluorescence, it showed that the probe was able to penetrate the cell membrane and react with intracellular thiols, resulting in green fluorescence signals. In one control experiment, HeLa cells were pre-treated with GSH (200 μM) for 30 min, leading to an increased cellular thiol concentration. When probe was incubated with these cells, we could observe obvious blue fluorescence decrease and green fluorescence increase (Fig. 5 d, e and f). In another control experiment, HeLa cells were pretreated with 500 μM N-methylmaleimide (NMM) for 20 min to deplete intracellular thiols, then the probe (5 μM) was applied to cells. The results were shown in Fig.5 (g, h and i), the blue fluorescence were obvious and green fluorescence were not observed, These results clearly demonstrated that the probe is suitable for fluorescence imaging of GSH in living cells.

a



b

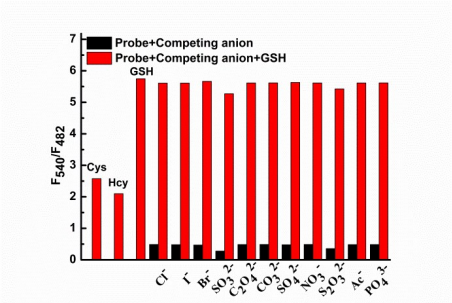


Fig.4(a) The ratiometric fluorescence intensity of probe (1 μM) in 50 mM PBS buffer (pH 7.4, 10% DMF) upon the addition of 1 mM GSH in the presence of 1mM non-thiol-bearing amino acids (His, Glu, Asp, Val, Phe, Tyr, Ala, Ser, Leu, Arg, Pro, Thr, Glu, Trp, Ile, Lys). λex=420 nm. Bars represent fluorescence intensity ratio F_{540}/F_{482}. Black bars: probe + various non-thiol-bearing amino acid. Red bar: probe + various non-thiol-bearing amino acid + GSH. λ ex = 420 nm. Each spectrum was acquired 120 min after various analytes addition at 25 °C.

Fig.4(b) The ratiometric fluorescence intensity of probe (1 μM) in 50 mM PBS buffer (pH 7.4, 10% DMF) upon the addition of 1 mM GSH in the presence of 1mM common anions (Cl^-, I^-, Br^-, SO_3^{2-}, $C_2O_4^{2-}$, CO_3^{2-}, SO_4^{2-}, NO_3^-, $S_2O_3^{2-}$,Ac^-,PO_4^{3-}). λex=420 nm. Bars represent fluorescence intensity ratio F_{540}/F_{482}. Black bars: probe + various anions. Red bar: probe + anion + GSH. λ ex = 420 nm. Each spectrum was acquired 120 min after various analytes addition at 25 °C.

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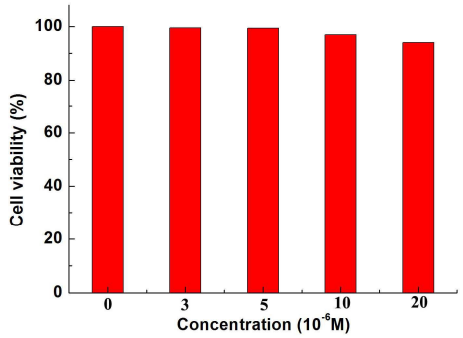

Fig.5 Vibility of HeLa cells after incubation with probe at difference concentration (0, 3, 5,10 and 20 μM) for 2h based on the results from three separate MTT assays

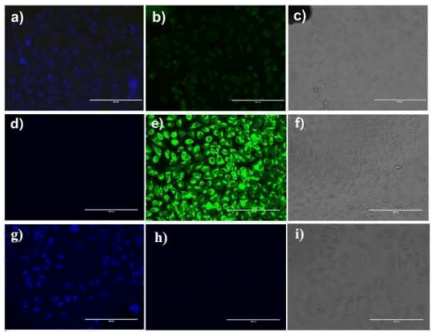

Fig.6 Fluorescence images of living Hela cells: Hela cells on 24-well plate were incubated with probe (5 μM) for 120 min: a) blue channel, b) green channel, and c) bright-field transmission image; HeLa cells was treated with 200μM GSH for 30min and further incubated probe (5μM) for 120 min: d) blue channel, e) green channel, and f) bright-field transmission image. Cells were pre-incubated with 500μM NMM for 20 min, washed, and then treated with probe (5μM) for 120 min: g) blue channel, h) green channel, and i) bright-field transmission image.

4 Conclusions

In conclusion, we have reported the design, synthesis, and properties of a ratiometric fluorescent probe for biothinols detection. The probe exhibited high selectivity towards biothinols than other non-thiol-bearing amino acids and common anions. It was successfully applied for the GSH bioimaging in living cells.

Acknowledgements

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Notes and references

1 T.P. Dalton, H.G. Shertzer, A. Puga, *Annual Review of Pharmacology and Toxicology*, 1999, **39**, 67-101.
2 Z.A. Wood, E. Schroder, J. Robin Harris, L.B. Poole, *Trends. Biochem. Sci.*, 2003, **28**, 32-40.
3 J. Liu, Y.Q. Sun, Y.Y. Huo, H.X. Zhang, L.F. Wang, P. Zhang, D. Song, Y.W. Shi, W. Guo, *J. Am. Chem. Soc.,* 2014, **136**, 574-577.
4 M.H. Lee, J.H. Han, P. Kwon, S. Bhuniya, J.Y. Kim, J.L. Sessler, C. Kang, J.S. Kim, *J. Am. Chem. Soc.,* 2012, **134**, 1316-1322.
5 H.J. Forman, H.Q. Zhang, A. Rinna, *Molecular Aspects of Medicine,* 2009, **30**, 1-12.
6 D. Han, N. Hanawa, B. Saberi, N. Kaplowitz, *Am. J. Physiol.*, 2006, **291**(1,Pt. 1), G1-G7.
7 D. Ścibior, M. Skrzycki, M. Podsiad, H. Czeczot, *Clin. Biochem.,* 2008, **41**, 852-858.
8 S.C. Lu, *Curr. Top. Cell. Regul.,* 2000, **36**, 95-116.
9 K. Wang, H.J. Peng, B.H. Wang, *J. Cell. Biochem.,* 2014, **115**, 1007-1022.
10 W. Lin, L. Yuan, Z. Cao, Y. Feng, J. Song, *Angew. Chem. Int. Ed.,* 2010, **49**, 375-379.
11 D. Srikun, E.W. Miller, D.W. Domaille, C.J. Chang, *J. Am. Chem. Soc.,* 2008, **130**, 4596-4597.
12 J. Liu, Y.Q. Sun, J.Y. Zhang, T. Yang, J.B. Cao, L.S. Zhang, W. Guo, *Chem. Eur. J.,* 2013, **19**, 4717-4722.
13 C.H. Zhang, B. Peng, W. Chen, S.M. Shuang, M. Xian, C. Dong, *Dyes and Pigments,* 2015, **121**, 299-304.
14 M.H. Lee, J.H. Han, P.S. Kwon, S. Bhuniya, J.Y. Kim, J.L. Sessler, C. Kang, J.S. Kim, *J. Am. Chem. Soc.,* 2012, **134**, 1316-1322.
15 S.Q. Wang, Q.H. Wu, H.Y. Wang, X.X. Zheng, S.L. Shen, Y.R. Zhang, J.Y. Miao, B.X. Zhao, *Biosens. Bioelectron,* 2014, **55**, 386-390.
16 L.L. Long, W.Y. Lin, B.B. Chen, W.S. Gao, L. Yuan, *Chem. Commun.,* 2011, **47**, 893-895.
17 C.H. Zhang, G.M. Zhang, L.H. Feng, J.F. Li, *Sensors and Actuators B*, 2015, **216**, 412-417.
18 W.Y. Lin, L.L. Long, L. Yuan, Z.M. Cao, B.B. Chen, W. Tan, *Org. Lett.,* 2008, **10**, 5577-5580.
19 X.M. Liu, N. Xi, S.J. Liu, Y. Ma, H.R. Yang, H.R. Li, J.H. He, Q. Zhao, F.Y. Li, W. Huang, *J. Mater. Chem.,* 2012, **22**, 7894-7901.
20 X.Q. Chen, S.K. Ko, M.J. Kim, I. Shin, J. Yoon, *Chem. Commun.,* 2010, **46**, 2751-2753.
21 V. Hong, A.A. Kislukhin, M.G. Finn, *J. Am. Chem. Soc.,* 2009, **131**, 9986-9994.
22 Y.T. Yang, F.J. Huo, C.X. Yin, A.M. Zheng, J.B. Chao, Y.Q. Li, Z.X. Nie, R. Martínez-Máñez, D.S. Liu, *Biosensors and Bioelectronics,* 2013, **47**, 300-306.
23 X.Q. Chen, Y. Zhou, X.J. Peng, J. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 2120-2135.
24 K.H. Hong, S.Y. Lim, M.Y. Yun, J.W. Lim, J.H. Woo, H. Kwon, H.J. Kim, *Tetrahedron Lett.,* 2013, **54**, 3003-3006.
25 X.H. Cao, Y.Q. Wu, K.Y. Liu, X.D. Yu, B. Wu, H.Z. Wu, Z.G. Gong, T. Yi, *J. Mater. Chem.*, 2012, **22**, 2650-2657.
26 L. Song, T. Jia, W.J. Lu, N.Q. Jia, W.B. Zhang, J.H. Qian, *Org. Biomol. Chem.,* 2014, **12**, 8422-8427.
27 P.K. Pullela, T. Chiku, M.J. Carvan, D.S. Sem, *Anal. Biochem.,* 2006, **352**, 265-273.
28 A.M. Piggott, P. Karuso, *Anal. Chem.,* 2007, **79**, 8769-8773.
29 M.M. Pires, J. Chmielewski, *Org. Lett.,* 2008, **10**, 837-840.
30 M.H. Lee, J.Y. Kim, J.H. Han, S. Bhuniya, J.L. Sessler, C. Kang, J.S. Kim, *J. Am. Chem. Soc.,* 2012, **134**, 12668-12674.
31 M. Lelle, S. Kaloyanova, C. Freidel, M. Theodoropoulou, M. Musheev, C. Niehrs, G. Stalla, K. Peneva, *Mol. Pharmaceutics,* 2015, **12**, 4290-4300.
32 P. Liu, X. Wang, K. Hiltunen, Z. Chen, *ACS Appl. Mater. Interfaces*, 2015, **7**, 26811-26818.

View Article Online
DOI: 10.1039/C6RA04564B

33 A. Wierzba, M. Wojciechowska, J. Trylska, D. Gryko, *Bioconjugate Chem.,* 2016, **27**, 189-197.
34 H. Yang, Q. Wang, S. Huang, A. Xiao, F. Li, L. Gan, X. Yang, *ACS Appl. Mater. Interfaces,* 2016, **8**, 7729-7738.
35 M.H. Lee, J.L. Sessler, J.S. Kim, *Acc. Chem. Res.,* 2015, **48**, 2935-2946.
36 R.M. Duke, E.B. Veale, F.M. Pfeffer, P.E. Kruger, T. Gunnlaugsson, *Chem. Soc. Rev.,* 2010, **39**, 3936-3953.
37 Z.C. Xu, K.H. Baek, H.N. Kim, J.N. Cui, X.H. Qian, D.R. Spring, I. Shin, J. Yoon, *J. Am. Chem. Soc.,* 2009, **132**, 601-610.
38 Z.C. Xu, J. Yoon, D.R. Spring, *Chem. Commun.,* 2010, **46**, 2563-2565.
39 B.C. Zhu, X.L. Zhang, Y.M. Li, P.F. Wang, H.Y. Zhang, X.Q. Zhuang, *Chem. Commun.,* 2010, **46**, 5710-5712.
40 L.A. Montoya, M.D. Pluth, *Chem. Commun.,* 2012, **48**, 4767-4769.
41 J. Jiang, H. Jiang, W. Liu, X.L. Tang, X. Zhou, W.S. Liu, R.T. Liu, *Org. Lett.,* 2011, **13**, 4922-1925.
42 X.L. Liu, X.J. Du, C.G. Dai, Q.H. Song, *J. Org. Chem.,* 2014, **79**, 9481-9489.
43 Y.S. Xie, P.C. Wei, X. Li, T. Hong, K. Zhang, H. Furuta, *J. Am. Chem. Soc.,* 2013, **135**, 19119-19122.

View Article Online
DOI: 10.1039/C6RA04564B