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ARTICLE TYPE

A colorimetric and near-infrared fluorescent probe for biothiols and its application in living cells†

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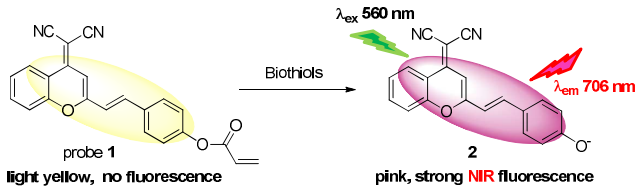
A new near-infrared (NIR) fluorescent probe, which contains a conjugated dicyanomethylene-benzopyran structure as the NIR fluorophore and an acrylate moiety as the reaction site, was found to be a promising NIR probe for biothiols (Cys, Hcy and GSH). This probe shows rapid response, high selectivity and sensitivity for biothiols (particularly for Cys and Hcy), accompanied by distinct color changes and significant NIR fluorescence turn on responses. The detection limit for Cys was found to be 81 nM. In addition, the imaging of biothiols by this probe was also successfully applied in living cells, indicating that this probe hold promise for applications in biological samples.

Introduction

As important biomolecules, biothiols such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) play vital roles in a wide range of physiological and pathological processes arising from their biological redox chemistry.[1] Abnormal levels of these biothiols have been reported to be closely related to many diseases.[2] For example, Cys deficiency is involved in many syndromes such as slow growth in children, hair depigmentation, edema, lethargy, liver damage, loss of muscle and fat, skin lesions, and weakness, while an elevated level of Hcy in the blood is a well-known risk factor for a number of diseases, such as Alzheimer's, cardiovascular diseases and osteoporosis.[3] Therefore, sensitive and convenient methods for selective determination of biothiols are of great importance for various investigations in biochemistry as well as assistance in diagnosis of the related diseases in clinic.

Fluorescent detection has shown ever-increasing interest in recent years due to its simplicity, convenient, low cost, high sensitivity and capability of real-time detection and spatial imaging. Accordingly, development of fluorescent probes for fluorescent detection of biothiols have drawn considerable attention in the past decade.[4] Utilizing the strong nucleophilicity and the high binding affinity for metal ions of thiols, a number of fluorescent probes for biothiols have been developed based on various fluorophores and different approaches such as nucleophilic addition (mainly via Michael addition),[5] cyclization reaction with aldehyde,[6] conjugate addition–cyclization reaction with an acrylate,[7] cleavage of sulfonamide and sulfonate esters,[8] nucleophilic substitution,[9] disulfide exchange reaction,[10] binding to metal complex[11] and others[12]. However, most of these probes have emissions in the visible region, in which the fluorescence imaging can be easily interfered by cell auto-fluorescence. For this reason, near-infrared (NIR, 650-900 nm) fluorescent probes are more suitable for biological imaging applications, because they produce lower background fluorescence with less scattering, penetrate much deeper into tissues than UV and visible light and cause less damage to biological samples.[13] However, only a few NIR fluorescent probes for biothiols (simultaneous detection of Cys, Hcy and GSH) have been reported to date.[5l,8e,9g,11g] Therefore, the development of new NIR fluorescent probes for selective, sensitive and convenient detection of biothiols is still highly demanded.



Scheme 1. Structure of probe **1** and sensing of biothiols.

Herein, we report a new NIR fluorescent probe, which can be used to detect biothiols conveniently with high selectivity and sensitivity. This probe is based on a dicyanomethylene-benzopyran (DCMB) structure and acrylate-functionalized (probe **1** in Scheme 1). Recently, acrylate-functionalized probes for biothiols have attracted much attention due to the reaction between thiol-containing amino acids (particularly Cys) and an acrylate moiety can be conducted efficiently in aqueous solution under mild conditions.[7] On the other hand, DCMB-based probes have received much attention in recent years due to their emissions are located at the NIR region.[8e,14] Besides, compared to the most frequently used NIR fluorophores such as squaraine and cyanine, the DCMB fluorophore has better photostability and larger Stokes shift,[15] which makes DCMB-based probes highly valuable for NIR fluorescent sensing of biologically important species. Most importantly, probe **1** developed in this work not only possesses several essential sensing properties including high selectivity, high sensitivity and fast response for biothiols, but also shows distinct colorimetric and significant enhanced NIR fluorescence signal outputs. This enables a convenient visible detection by the ''naked-eye'' and a highly sensitive NIR fluorescent detection for

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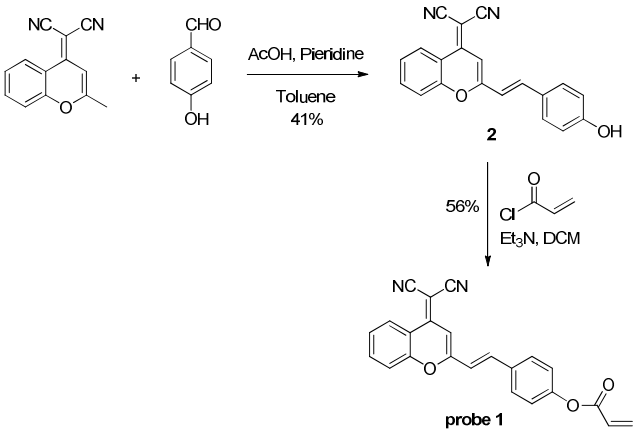
biothiols. In addition, this probe can be readily prepared and can be applied to image biothiols in living cells, thus making it a promising NIR probe for applications involving detection of biothiols both *in vivo* and *in vitro*.

Results and discussion

1. Probe synthesis

The synthesis of probe **1** is outlined in Scheme 2. Briefly, probe **1** can be readily prepared by the reaction of **2** with acryloyl chloride in the presence of triethylamine at room temperature, and its structure was confirmed by ^{1}H NMR, ^{13}C NMR, IR and HRMS spectroscopy. Compound **2** is a known molecule and was prepared by the literature method.[8e] Detailed synthetic procedures and structure characterizations are given in the experimental section and in the ESI†.



Scheme 2. Synthesis of probe **1**.

2. Sensing properties of probe 1

With probe **1** in hand, Cys as one of the typical biothiols was first used to examine its sensing properties. The sensing ability of probe **1** for Cys was investigated in DMSO-PBS buffer (10 mM, pH 7.4, 1:1, v/v) by absorption and fluorescence spectroscopy. Considering aggregation of probe **1** and fluorophore **2** (the expected product) may occur in aqueous solution due to their low water solubility, their spectra changes at different concentrations were studied. Since the absorption spectra of both **1** and **2** were found proportional to their concentrations from 0-50 μM (Fig. S1a-d, ESI†), aggregations are not likely to occur for both of them if their concentrations are used below 50 μM under the test conditions. However, the fluorescence of **2** was only found proportional to its concentration from 0-20 μM, and above that, it can be partially quenched (Fig. S1e-f, ESI†). This indicates that the concentration of probe **1** can be used up to 20 μM for an accurate analysis. Thus, 20 μM of probe **1** was used for sensing of Cys. As shown in Fig. 1, probe **1** (20 μM) appears a light yellow solution with maximum absorption at 395 nm and shows almost no fluorescent. Upon addition of Cys (100 μM) and after 30 min, the maximum absorption of the solution red-shifted to 555 nm ($\Delta\lambda$ = 160 nm), accompanied by a distinct color change from light yellow to pink (Fig. 1a) and a significant enhancement of NIR fluorescence around 706 nm (Fig. 1b). To obtain more information on the reaction kinetics, time-dependent analyses of probe **1** in the absence and presence of Cys were carried out by monitoring the fluorescence intensity change at 706 nm as a function of time in buffer solution at 37 °C. As shown in Fig. 2, without Cys, probe **1** displays no noticeable changes in the fluorescence intensity at 706 nm, indicating that probe **1** is stable under this condition. However, when being treated with Cys (1 equiv. or 5 equiv.), the solution shows a large increase in the fluorescent intensity at 706 nm until it reaches a plateau after about 15 min. The observed pseudo-first-order rate constant k_{obs} for the reaction between probe **1** and Cys under stoichiometric conditions (20 μM of each) was determined to be about 0.167 min^{-1} ($t_{1/2}$ = 4.15 min, Fig. S2, ESI†), indicating that the reaction between probe **1** and Cys is very fast. Since some reported acrylate-functionalised biothiol probes need a long response time (more than 40 min) for Cys,[7a,d,g] the fast response of probe **1** to Cys is an advantage. Clearly, the above results show that probe **1** could serve as a dual colorimetric and NIR fluorescent turn-on sensor for rapid detection of Cys in aqueous solution under mild conditions.

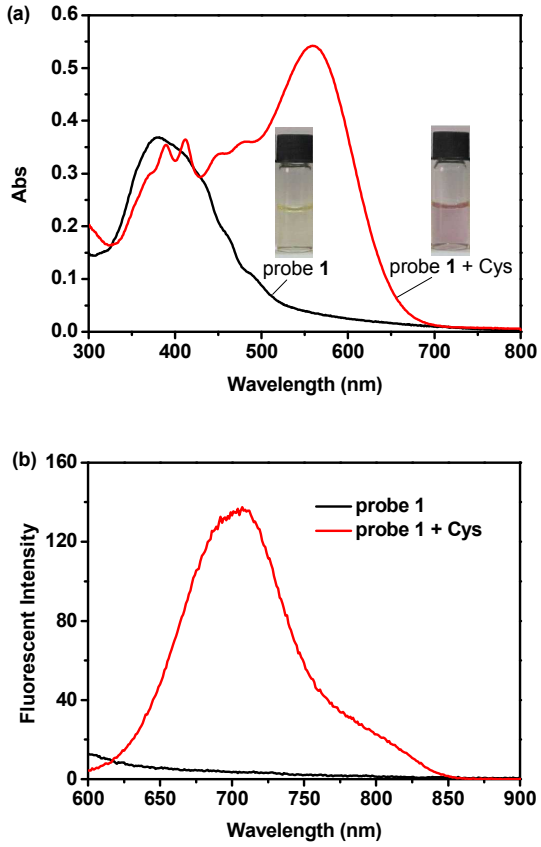


Fig. 1 (a) UV-Vis spectral changes of probe **1** (20 μM) upon treatment with Cys (5 eq) after 30 min in PBS buffer. (b) Fluorescence spectral changes of probe **1** (20 μM) upon treatment with Cys (5eq) after 30 min in PBS buffer. All spectra were measured in DMSO-PBS buffer (10 mM, pH 7.4, 1:1, v/v) at 37 °C. For fluorescent measurement, λ_{ex} = 560 nm; slit width (10, 10).

The absorbance and fluorescence changes of the probe **1** solution upon addition of different concentrations of Cys were then measured, respectively. As shown in Fig. S3-S4 (ESI†), progressive increases in absorption at 555 nm and fluorescence

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intensity at 706 nm were observed with increasing concentrations of Cys, and saturation occurred when Cys was added more than 5 equiv. to the concentration of probe **1**. To get insight into its sensitivity, the detection limit of probe **1** for Cys was determined based on the fluorescence titration. As shown in Fig. 3, a linear calibration curve can be found between the fluorescent intensity changes at 706 nm and the concentration of Cys in the range of 0 to 10 μM (R^2 = 0.99982). Thus, the detection limit of probe **1** for Cys was measured to be about 81 nM based on the signal to noise ratio (S/N) = 3 under the test conditions, which indicates that probe **1** can be used as a highly sensitive sensor for the quantitative detection of Cys.

The effect of pH on the absorption and fluorescence signal response of probe **1** towards Cys was also investigated. As shown in Fig. S5 (ESI†), probe **1** itself is not pH sensitive unless the pH > 9. However, in the presence of Cys, a large enhancement in the absorbance intensity at 560 nm and the fluorescence intensity at 706 nm can be observed over a wide pH range from 6 to 10 with maximum changes at pH over 8. This indicates that probe **1** can be used to detect Cys over a relatively wide pH range.

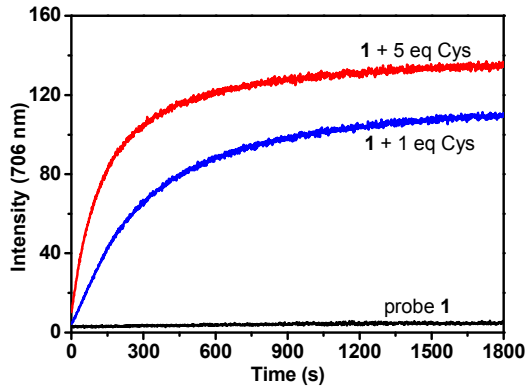
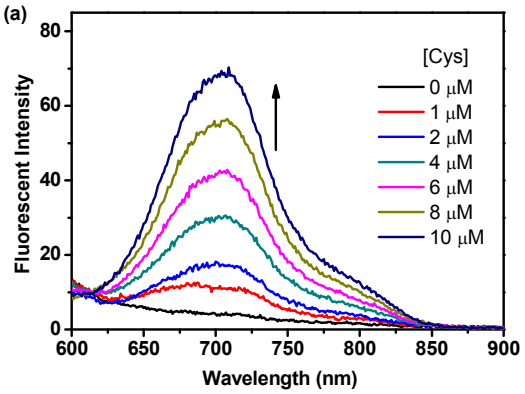


Fig. 2 Time-dependent of fluorescence kinetics spectra of probe **1** (20 μM) upon addition of Cys (0, 20 and 100 μM) in DMSO-PBS buffer (10 mM, pH 7.4, 1:1, v/v) at 37 °C. All the reactions are monitored every 1 s at 706 nm. λ_{ex} = 560 nm; slit width: (10, 10).



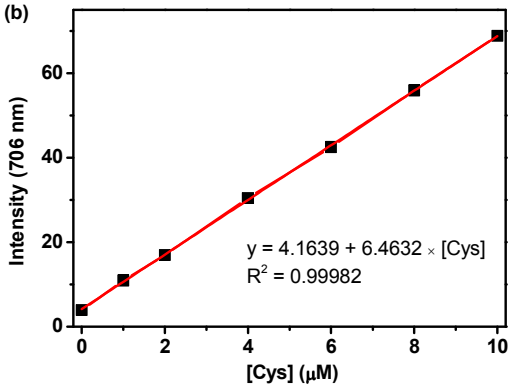
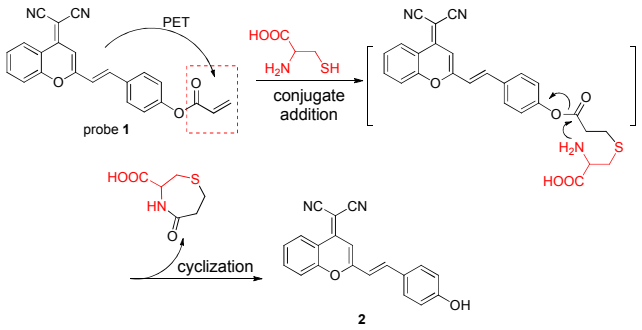


Fig. 3 (a) Fluorescence spectra changes of probe **1** (20 μM) upon addition of different concentrations of Cys in DMSO-PBS buffer (10 mM, pH 7.4, 1:1, v/v) at 37 °C. Final concentration of Cys: 0, 1, 2, 4, 6, 8 and 10 μM, respectively. (b) Fluorescence intensity changes at 706 nm of probe **1** (20 μM) against concentration of Cys. Each spectrum was obtained 30 min after Cys addition. λ_{ex} = 560 nm; slit width: (10, 10).

Probe **1** itself shows almost no fluorescence, which can be ascribed to the quenching effect of an alkene-induced PET (photoinduced electron-transfer) process.[7a] The significant NIR fluorescence enhancement of probe **1** for Cys suggests that Cys triggered the cleavage of acryloyl group in probe **1** and simultaneously released the NIR fluorophore **2**. To understand the sensing process, a feasible sensing mechanism was proposed according to the literature[7] for probe **1** towards sensing of Cys (Scheme 3). The reaction of Cys with probe **1** involves two steps. A Michael addition reaction of the thiol of Cys to the acryloyl group of probe **1** was first taken place, and then followed by a spontaneous intramolecular cyclization to release the NIR fluorescent compound **2**. To test this, the fluorescent product from the reaction of probe **1** with Cys was isolated, and the TLC, fluorescence, ^{1}H NMR and MS analysis showed that the product is **2** (Fig. S6-S8, ESI†). Therefore, the sensing process of probe **1** for Cys is most likely happened as shown in Scheme 3.



Scheme 3. A plausible mechanism of response of probe **1** to thiols.

The above results show that probe **1** can be used as a dual colorimetric and NIR fluorescent sensor for rapid and sensitive detection of Cys in aqueous solution under mild conditions. In order to evaluate its selectivity, its responses toward addition of biothiols GSH and Hcy, and other natural amino acids such as phenylalanine (Phe), glutamine (Gln), tryptophan (Trp), threonine (Thr), methionine (Met), glutamic acid (Glu), isoleucine (Ile),

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glycine (Gly), tyrosine (Tyr), arginine (Arg), serine (Ser), alanine (Ala), aspartic acid (Asp), leucine (Leu), histidine (His), and lysine (Lys) were then investigated. A colorimetric assay is firstly explored as it is simple. As shown in Fig. 4, the color of the probe **1** solution was obviously changed only upon addition of biothiols (especially Cys and Hcy). To get a better understanding, the UV-vis and fluorescent spectra changes of probe **1** towards these amino acids were then investigated. As shown in Fig. 5, noticeable UV-vis spectral changes and significant fluorescence enhancement of the probe **1** solution were only observed upon addition of biothiols. In contrast, besides the above mentioned amino acids, other amino-containing compounds such as $H_2NCH_2CH_2NH_2$, $OHCH_2CH_2NH_2$, $C_6H_5CH_2NH_2$, and $C_6H_5NH_2$ showed almost no changes to the probe **1** solution. This indicates that probe **1** showed high selectivity for biothiols over these amino-containing species. Moreover, as shown in Fig. 6, fluorescent detection of biothiols (Cys was used as a representative for biothiols) in the presence of these amino-containing species was found also effective, indicating that these non-thiol-containing amino species showed no interference to the detection of biothiols by probe **1**. In addition, we also tested the response of probe **1** to other potential interfering anion species such as F^-, Cl^-, Br^-, I^-, NO_3^-, NO_2^-, AcO^-, SCN^-, CO_3^{2-}, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, HS^-, S^{2-}, CN^-, and some reactive oxygen species such as hypochlorite, hydrogen peroxide, hydroxyl radical, and superoxide, in which superoxide has been found very reactive to a recently reported biothiol probe.[16] As shown in Fig. S9 and S10 (ESI†), these anion and reactive oxygen species also showed almost no effect to the probe **1** solution (compared with Cys), indicating that probe **1** is inert to these anion and reactive oxygen species. Therefore, all these investigations indicate that probe **1** is highly selective for biothiols.

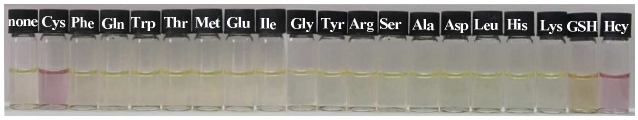
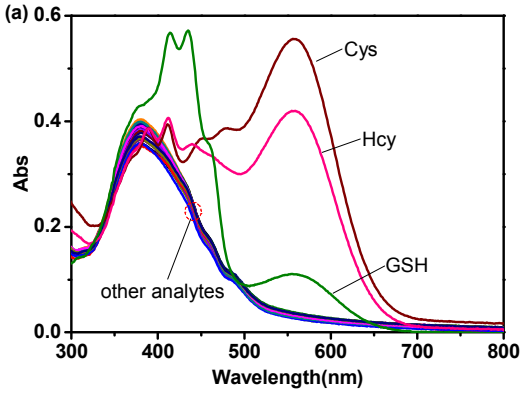


Fig. 4 Color changes of probe **1** (20 μM) upon addition of 100 μM of different analytes (from left to right: none, Cys, Phe, Gln, Trp, Thr, Met, Glu, Ile, Gly, Tyr, Arg, Ser, Ala, Asp, Leu, His, Lys, GSH and Hcy) in DMSO-PBS buffer (10 mM, pH 7.4, 1:1, v/v) after 30 min at room temperature.



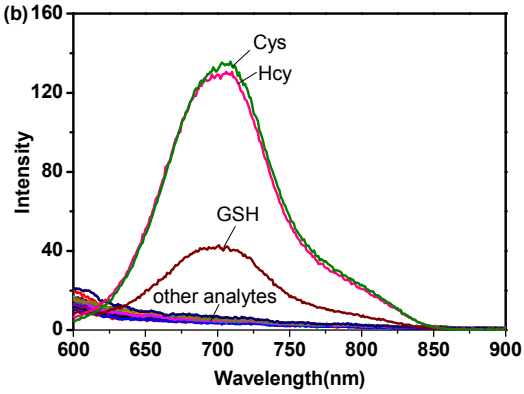


Fig. 5 Absorption responses (a) and fluorescence responses (b) of probe **1** (20 μM) upon addition of various analytes (Phe, Gln, Trp, Thr, Met, Glu, Ile, Gly, Tyr, Arg, Ser, Ala, Asp, Leu, His, $H_2NCH_2CH_2NH_2$, $OHCH_2CH_2NH_2$, $C_6H_5CH_2NH_2$, $C_6H_5NH_2$, Cys, Hcy, and GSH, 100 μM of each) in DMSO-PBS buffer (10 mM, pH 7.4, 1:1, v/v) at 37 °C and each spectrum was obtained 30 min after addition of the analyte.

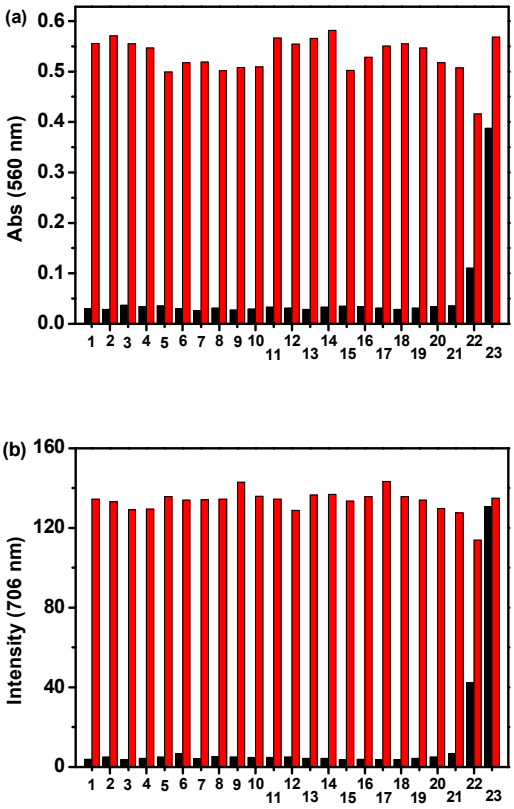


Fig. 6 (a) Absorbance intensity changes (at 560 nm) and (b) fluorescence intensity changes (at 706 nm) of probe **1** (20 μM) for Cys in the presence of various amino acids (100 μM). Black bars represent the addition of a single analyte including: 1. none, 2. Phe, 3. Gln, 4. Trp, 5. Thr, 6. Met, 7. Glu, 8. Ile, 9. Gly, 10. Tyr, 11. Arg, 12. Ser, 13. Ala, 14. Asp, 15. Leu, 16. His, 17. Lys, 18. $C_6H_5NH_2$, 19. $C_6H_5CH_2NH_2$, 20. $OHCH_2CH_2NH_2$, 21. $H_2NCH_2CH_2NH_2$, 22. GSH, and 23. Hcy. Red bars represent the subsequent addition of Cys (100 μM) to the mixture. All experiments were performed in DMSO-PBS buffer (10 mM, pH 7.4, 1:1, v/v) at 37 °C and data was obtained 30 min after addition of each analyte.

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The response differences of probe **1** for Cys, Hcy and GSH were also investigated by time dependent absorption spectra and fluorescent intensity changes. As shown in Fig. S11 and S12 (ESI†), the absorption (560 nm) or fluorescence intensity (706 nm) change speed is arranged in the order of Cys > Hcy > GSH, which indicates that probe **1** is most reactive for Cys between these three biothiols. This is consistent with that of the recently reported acrylate-functionalized probes for biothiols.[7e-f]

3. Practical applications

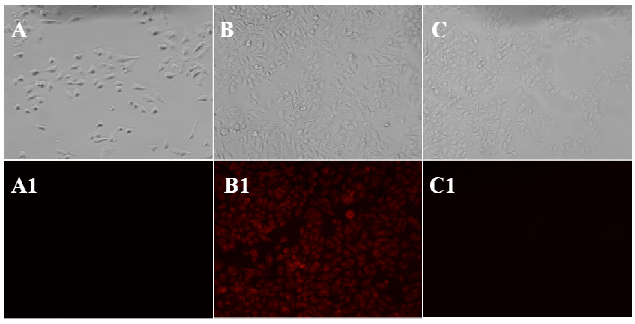

Fig. 7. Imaging of biothiols by probe **1** in living cells. Top: bright field images. A: HeLa cells; B: HeLa cells were incubated with probe **1** (50 μM) for 60 min. C: HeLa cells were pre-incubated with 500 μM NEM and then treated with probe **1** (50 μM) for 60 min. Bottom: A1, B1 and C1 are fluorescence images of A, B and C, respectively.

Based on the above results, the practical utilities of probes **1** for cell imaging of biothiols were then explored in living HeLa cells. As shown in Fig. 7, HeLa cells alone showed no fluorescence (A1) and when they were incubated with probe **1** for 60 min, the cells started to show red fluorescence (B1). As a control experiment, N-ethylmaleimide (NEM), a known thiol trapping reagent was added to the cell culture prior to the addition of probe **1**, and in this case, no fluorescence was observed (C1). These results indicate that probe **1** is able to detect biothiols in living cells.

Experimentals

General

Starting materials were purchased from commercial suppliers and used without further purification. All aqueous solutions and buffers were prepared with using distilled water that had been passed through a Millipore-Q ultrapurification system. TLC analysis was performed using precoated silica plates. Melting points were determined using an X-4 apparatus and are not corrected. IR spectra were recorded on a Perkin-Elmer Spectrum BX FT-IR spectrophotometer as KBr pellets and were reported in cm^{-1}. NMR spectra were measured on Varian Mercury 400 and 600 instruments. Electrospray mass spectra (ESI-MS) were acquired on Agilent 1100 Series LC/MS ion trap mass spectrometers and 6530 Accurate-Mass QTOF spectrometer coupled to an Agilent HPLC 1200 series (Agilent Technologies). UV-vis spectra were recorded on an Agilent Cary 100 UV-vis spectrophotometer. Fluorescent spectra were recorded on an Agilent Cary Eclipse fluorescence spectrophotometer. Both UV-vis and fluorescence spectrophotometer are equipped with a temperature controller. Standard quartz cuvettes with a 10 mm lightpath were used for all UV-vis spectra and fluorescent spectra measurements. Cell imaging was performed in an inverted fluorescence microscopy with a 20× objective lens.

Solutions preparation and optical mesurements: Stock solutions analytes including biothiols Cys, Hcy, GSH, other amino acids such as Phe, Gln, Trp, Thr, Met, Glu, Ile, Gly, Tyr, Arg, Ser, Ala, Asp, Leu and His (5 mM of each) as well as anions F^-, Cl^-, Br^-, I^-, NO_3^-, NO_2^-, AcO^-, SCN^-, CO_3^{2-}, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, HS^-, S^{2-} and CN^- (50 mM of each, sodium salts) were prepared in ultrapure water. Stock solution of probe **1** (1 mM) was prepared in HPLC grade DMSO. For optical measurements, a solution of probe **1** (20 μM) was prepared in DMSO-H_2O solution (1:1, v/v, 10 mM PBS buffer). Then 3.0 mL of the probe **1** solution was placed in a quartz cell until the temperature reached at 37 °C over a few minutes. The UV-vis or fluorescent spectra were recorded upon addition of various analytes. An excitation and emission slit of 10 nm was used for fluorescence measurements.

Cell imaging experiments: HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (Fetal Bovine Serum), 100mg/mL penicillin and 100 μg/mL streptomycin in a 5% CO_2, water saturated incubator at 37 °C, and then were seeded in a 12-well culture plate for one night before cell imaging experiments. For living cells imaging experiment of probe **1**, cells were incubated with 50 μM probe **1** (with 1% DMSO, v/v) for 60 min at 37 °C and washed three times with prewar med PBS, and then imaged immediately. For N-ethylmaleimide (NEM) treated experiments, HeLa cells were pretreated with 0.5 mM NEM for 60 min at 37 °C, washed three times with prewarmed PBS, and then incubated with 50 μM probe **1** for 60 min at 37 °C. Cell imaging was then carried out after washing cells with prewarmed PBS buffer.

Synthesis of compound 2. 2-(2-methyl-4H-chromen-4-ylidene) malononitrile (208 mg, 1.0 mmol) and 4-hydroxybenzaldehyde (388 mg, 1.1 mmol) were dissolved in 30 mL toluene. And then piperidine (0.5 mL) and acetic acid (0.5 ml) were added. A dean-stark head was fitted and the reaction mixture was heated under reflux for 12 h. After the completion of the reaction, the mixture was allowed to cool to room temperature and then condensed under reduced pressure. A pure enough red solid (205 mg, 41%) was obtained after filtration and washing with cold CH_2Cl_2. Mp: 256-258˚C. TLC (silica plate): R_f 0.15 (hexane:ethyl acetate 3:1, v/v). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.73 (d, J = 8.3 Hz, 1H), 7.91 (t, J = 7.8 Hz, 1H), 7.79 (d, J = 8.3 Hz, 1H), 7.70 (d, J = 16.0 Hz, 1H), 7.63 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 7.8 Hz, 1H), 7.27 (d, J = 16.0 Hz, 1H), 6.95 (s, 1H), 6.85 (d, J = 8.5 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.1, 158.8, 152.7, 152.0, 139.2, 135.2, 130.3, 126.0, 124.6, 118.9, 117.4, 117.1 116.0, 115.8, 105.6, 59.0. IR (KBr, cm^{-1}): 3395, 2270, 2204, 1625, 1589, 1550, 1481, 1452, 1409, 1334, 1306, 1263, 1198, 1164, 1142, 975, 838, 811, 765, 742, 641; MS (EI): m/z 312.24 (M^+, 100%); HR-MS (ESI): m/z, calcd for $C_{20}H_{13}N_2O_2^+$ ($M + H^+$) 313.0972; Found 313.0956.

Synthesis of probe 1. To a stirred solution of compound **2** (100 mg, 0.32 mmol) in dry $CH_2 Cl_2$ (10 ml) was added 0.5 ml of triethylamine. The mixture was then cooled to 0˚C and a solution of acryloyl chloride (0.5 mL) in dry CH_2Cl_2 (5 ml) was added dropwise. After being stirred at 0˚C for 30 minutes, the mixture was then stirred at room temperature for 3h. After the completion

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of the reaction, the mixture was condensed under reduced pressure. The residue was recrystallized with acetone. A yellow solid was obtained (66 mg, 56%). Mp: 230-232˚C. TLC (silica plate): R_f 0.33 (hexane:ethyl acetate 3:1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.90 (d, J = 8.3 Hz, 1H), 7.75 (t, J = 7.8 Hz, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 16.0 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.45 (t, J = 7.8 Hz, 1H), 7.23 (d, J = 8.5 Hz, 1H), 6.85 (s, 1H), 6.77 (d, J = 16.0 Hz, 1H), 6.64 (d, J = 17.4 Hz, 1H), 6.34 (dd, J = 17.4, 10.5 Hz, 1H), 6.06 (d, J = 10.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.2, 157.2, 152.8, 152.2, 137.7, 134.7, 133.2, 132.4, 129.0, 127.6, 126.0, 125.8, 122.4, 118.9, 118.6, 117.8, 116.7, 115.6, 107.0, 63.1; IR (KBr, cm^{-1}):3446, 2274, 2207, 1630, 1596, 1555, 1500, 1456, 1405, 1326, 1297, 1261, 1199, 1169, 1137, 979, 839, 748; MS (EI): m/z 366.24 (M^+, 36%), 312.24 (M^+ - CH_2=CHCO, 100%); HR-MS (ESI): m/z, calcd for $C_{23}H_{15}N_2O_3^+$ ($M + H^+$) 367.1077; Found 367.1075.

Conclusions

In summary, we developed a new NIR fluorescent turn-on probe **1** for biothiols. This probe shows fast response, high selectivity and sensitivity for biothiols with colorimetric and NIR fluorescent dual signal changes. Moreover, it can be used for imaging of biothiols in living cells. Considering its easy preparation and large Stokes shift together, this new probe showed great potential for applications involving detection of biothiols both *in vivo* and *in vitro*.

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Notes and references

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1 L. Packer, *Biothiols in Health and Disease*, CRC press, 1995.

2 (*a*) S. Seshadri, A. Beiser, J. Selhub, P. F. Jacques, I. H. Rosenberg, R. B. D'Agostino, P. W. F. Wilson and P. A. Wolf, *N. Engl. J. Med*., 2002, **346**, 476–483; (*b*) S. Y. Zhang, C.-N. Ong and H.-M. Shen, *Cancer Lett*., 2004, **208**, 143–153; (*c*) O. Nygard, J. E. Nordrehaug, H. Refsum, P. M. Ueland, M. Farstad and S. E. Vollset, *N. Engl. J. Med*., 1997, **337**, 230–237.

3 (*a*) C. E. Paulsen and K. S. Carroll, *Chem. Rev*., 2013, **113**, 4633–4679; (b) S. Shahrokhian, *Anal. Chem*., 2001, **73**, 5972–5978.

4 For useful reviews, see (*a*) X. Chen, Y. Zhou, X. Peng and J. Yoon, *Chem. Soc. Rev*., 2010, **39**, 2120–2135; (*b*) Y. Zhou and J. Yoon, *Chem. Soc. Rev*., 2012, **41**, 52–67; (*c*) H, Peng, W, Chen, Y, Cheng, L, Hakuna, R, Strongin and B. Wang, *Sensors*, 2012, **12**, 15907–15946; (*d*) H. S. Jung, X. Chen, J. S. Kim and J. Yoon, *Chem. Soc. Rev*., 2013, **42**, 6019–6031; (*e*) C. Yin, F. Huo, J. Zhang, R. Martínez-Máñez, Y. Yang, H. Lv and S. Li, *Chem. Soc. Rev*., 2013, **42**, 6032–6059.

5 (*a*) X. Chen, S.-K. Ko, M. J. Kim, I. Shin and J. Yoon, *Chem. Commun*., 2010, **46**, 2751–2753; (*b*) H. Kwon, K. Lee and H.-J. Kim, *Chem. Commun*., 2011, **47**, 1773–1775; (*c*) G.-J. Kim, K. Lee, H. Kwon and H.-J. Kim, *Org. Lett*., 2011, **13**, 2799–2801; (*d*) H. S. Jung, K. C. Ko, G.-H. Kim, A.-R. Lee, Y.-C. Na, C. Kang, J. Y. Lee and J. S. Kim, *Org. Lett*., 2011, **13**, 1498–1501; (*e*) Y.-Q. Sun, M. Chen, J. Liu, X. Lv, J. Li and W. Guo, *Chem. Commun*., 2011, **47**, 11029–11031; (*f*) H.-Y. Shiu, M.-K. Wong and C.-M. Che, *Chem. Commun*., 2011, **47**, 4367–4369; (*g*) D. Kand, A. M. Kalle, S. J. Varma and P. Talukdar, *Chem. Commun*., 2012, **48**, 2722–2724; (*h*) H. S. Jung, J. H. Han, T. Pradhan, S. Kim, S. W. Lee, J. L. Sessler, T. W. Kim, C. Kang and J. S. Kim, *Biomaterials*, 2012, **33**, 945–953; (*i*) H. S. Jung, T. Pradhan, J. H. Han, K. J. Heo, J. H. Lee, C. Kang and J. S. Kim, *Biomaterials*, 2012, **33**, 8495–8502; (*j*) B. K. McMahon and T. Gunnlaugsson, *J. Am. Chem. Soc*. 2012, **134**, 10725–10728; (*k*) H. Wang, G. Zhou, C. Mao and X. Chen, *Dyes Pigm*., 2013, **96**, 232–236; (*l*) X.-D. Liu, R. Sun, Y. Xu, Y.-J. Xu, J.-F. Ge and J.-M. Lu, *Sens. Actuators, B*, 2013, **178**, 525–531; (*m*) P. Das, A. K. Mandal, U. Reddy G., M. Baidya, S. K. Ghosh and A. Das, *Org. Biomol. Chem*., 2013, **11**, 6604–6614.

6 (*a*) M. Hu, J. Fan, H. Li, K. Song, S. Wang, G. Cheng and X. Peng, *Org. Biomol. Chem*., 2011, **9**, 980–983; (*b*) X. Liu, N. Xi, S. Liu, Y. Ma, H. Yang, H. Li, J. He, Q. Zhao, F. Li and W. Huang, *J. Mater. Chem*., 2012, **22**, 7894–7901; (*c*) P. Wang, J. Liu, X. Lv, Y. Liu, Y. Zhao and W. Guo, *Org. Lett*., 2012, **14**, 520–523; (*d*) F. Kong, R. Liu, R. Chu, X. Wang, K. Xu and B. Tang, *Chem. Commun*., 2013, **49**, 9176–9178; (*e*) P. Das, A. K. Mandal, N. B. Chandar, M. Baidya, H. B. Bhatt, B. Ganguly, S. K. Ghosh, and A. Das, *Chem.–Eur. J*. 2012, **18**, 15382–15393.

7 (*a*) X. Yang, Y. Guo and R. Strongin, *Angew. Chem., Int. Ed*., 2011, **50**, 10690–10693; (*b*) H. Wang, G. Zhou, H. Gai and X. Chen, *Chem. Commun*., 2012, **48**, 8341–8343; (*c*) X. Yang, Y. Guo and R. M. Strongin, *Org. Biomol. Chem*., 2012, **10**, 2739–2741; (*d*) Z. Guo, S. Nam, S. Park and J. Yoon, *Chem. Sci*., 2012, **3**, 2760–2765; (*e*) X. Xiong, F. Song, G. Chen, W. Sun, J. Wang, P. Gao, Y. Zhang, B. Qiao, W. Li, S. Sun, J. Fan and X. Peng, *Chem.–Eur. J*., 2013, **19**, 6538–6545; (*f*) J. Shi, Y. Wang, X. Tang, W. Liu, H. Jiang, W. Dou and W. Liu, *Dyes. Pigm*., 2014, **100**, 255–260; (*g*) B. Liu, J. Wang, G. Zhang, R. Bai and Y. Pang, *ACS Appl. Mater. Interfaces*, 2014, **6**, 4402−4407.

8 (*a*) S. Ji, H. Guo, X. Yuan, X. Li, H. Ding, P. Gao, C. Zhao, W. Wu, W. Wu and J. Zhao, *Org. Lett*., 2011, **12**, 2876–2879; (*b*) L. Yuan, W. Lin, S. Zhao, W. Gao, B. Chen, L. He and S. Zhu, *J. Am. Chem. Soc*., 2012, **134**, 13510–13523; (*c*) X.-D. Jiang, J. Zhang, X. Shaoa and W. Zhao, *Org. Biomol. Chem*., 2012, **10**, 1966–1968; (*d*) M. Wei, P. Yin, Y. Shen, L. Zhang, J. Deng, S. Xue, H. Li, B. Guo, Y. Zhang and S. Yao, *Chem. Commun*., 2013, **49**, 4640–4642; (*e*) M. Li, X. Wu, Y. Wang, Y. Li, W. Zhu, and T. D. James, *Chem. Commun*., 2014, **50**, 1751–1753; (*f*) J. Yin, Y. Kwon, D. Kim, D. Lee, G. Kim, Y. Hu, J.-H. Ryu and J. Yoon, *J. Am. Chem. Soc*., 2014, **136**, 5351–5358.

9 (*a*) M. Zhang, M. Yu, F. Li, M. Zhu, M. Li, Y. Gao, L. Li, Z. Liu, J. Zhang, D. Zhang, T. Yi and C. Huang, *J. Am. Chem. Soc*. 2007, **129**, 10322–10323; (*b*) L.-Y. Niu, Y.-S. Guan, Y.-Z. Chen, L.-Z. Wu, C.-H. Tung and Q.-Z. Yang, *J. Am. Chem. Soc.*, 2012, 134, 18928–18931; (*c*) X.-F. Yang, Q. Huang, Y. Zhong, Z. Li, H. Li, M. Lowry, J. O. Escobedo and R. M. Strongin, *Chem. Sci*., 2014, **5**, 2177–2183; (*d*) S.-Y. Lim, K.-H. Hong, D. I. Kim, H. Kwon and H.-J. Kim, *J. Am. Chem. Soc*., 2014, **136**, 7018–7025; (*e*) Y.-S. Guan, L.-Y. Niu, Y.-Z. Chen, L.-Z. Wu, C.-H. Tung and Q.-Z. Yang, *RSC Adv*., 2014, **4**, 8360–8364; (*f*) H. Lv, X.-F. Yang, Y. Zhong, Y. Guo, Z. Li and Hua Li, *Anal. Chem*. 2014, **86**, 1800–1807; (*g*) H. Zhang, Y. Huo, Y. Shi and W. Guo, *Chem. Sci.*, 2014, **5**, 3183–3188.

10 (*a*) J. H. Lee, C. S. Lim, Y. S. Tian, J. H. Han and B. R. Cho, *J. Am. Chem. Soc*., 2010, **132**, 1216–1217; (*b*) C. S. Lim, G. Masanta, H. J. Kim, J. H. Han, H. M. Kim and B. R. Cho, *J. Am. Chem. Soc*., 2011, **133**, 11132–11135; (*c*) M. H. Lee, J. H. Han, P.-S. Kwon, S. Bhuniya, J. Y. Kim, J. L. Sessler, C. Kang and J. S. Kim, *J. Am. Chem. Soc*., 2012, **134**, 1316–1322; (*d*) B. C. Zhu, X. L. Zhang, Y. M. Li, P. F. Wang, H. Y. Zhang and X. Q. Zhuang, *Chem. Commun*., 2010, **46**, 5710–5712; (*e*) C. Ding, H. Li, X. Li and S. Zhang, *Chem. Commun*., 2010, **46**, 7990–7992.

11 (*a*) H. S. Jung, J. H. Han, Y. Habata, C. Kang and J. S. Kim, *Chem. Commun*., 2011, **47**, 5142–5144; (*b*) H. Wang, G. Zhou and X. Chen, *Sens. Actuators, B*, 2013, **176**, 698–703; (*c*) Y.-B. Ruan,

RSC Advances Accepted Manuscript

A.-F. Li, J.-S. Zhao, J.-S. Shen and Y.-B. Jiang, *Chem. Commun.*, 2010, **46**, 4938–4940; (*d*) Y.-K. Yang, S. Shim and J. Tae, *Chem. Commun*., 2010, **46**, 7766–7768; (*e*) N. Y. Kwon, D. Kim, G. Jang, J. H. Lee, J.-H. So, C.-H. Kim, T. H. Kim and T. S. Lee, *ACS Appl. Mater. Interfaces*, 2012, **4**, 1429–1433; (*f*) Y. Bao, Q. Li, B. Liu, F. Du, J. Tian, H. Wang, Y. Wang and R. Bai, *Chem. Commun*., 2012, **48**, 118–120; (*g*) C. Luo, Q. Zhou, B. Zhang and X. Wang, *New J. Chem*., 2011, **35**, 45–48.

12 (*a*) B. Zhu, X. Zhang, H. Jia, Y. Li, S. Chen and S. Zhang, *Dyes Pigm*., 2010, **86**, 87–92; (*b*) R. Wang, L. Chen, P. Liu, Q. Zhang and Y. Wang, *Chem.–Eur. J*., 2012, **18**, 11343–11349.

13 (*a*) Z. Guo, S. Park, J. Yoon and I. Shin, *Chem. Soc. Rev*., 2014, **43**, 16–29; (*b*) L. Yuan, W. Lin, K. Zheng, L. He and W. Huang, *Chem. Soc. Rev*., 2013, **42**, 622–661.

14 (*a*) X. Huang, Z. Guo, W. Zhu, Y. Xie and He Tian, *Chem. Commun*., 2008, 5143–5145; (*b*) W. H. Zhu, X. M. Huang, Z. Q. Guo, X. Wu, H. Yu and H. Tian, *Chem. Commun*., 2012, **48**, 1784-1786; (*c*) J. Cao, C. Zhao, W. Zhu, *Tetrahedron Lett.*, 2012, **53**, 2107–2110; (*d*) W. Sun, J. Fan, C. Hu, J. Cao, H. Zhang, X. Xiong, J. Wang, C. Shuang, S. Sun and X. Peng, *Chem. Commun*., 2013, **49**, 3890–3892. (e) D. Yu, F. Huang, S. Ding and G. Feng, *Anal. Chem*., 2014, **86**, 8835– 8841.

15 Z. Guo, W. Zhu and H. Tian, *Chem. Commun*., 2012, **48**, 6073–6084.

16 Kim, W. S. Choi, and D. G. Churchill, *Org. Lett*., 2013, **15**, 3630–3633.

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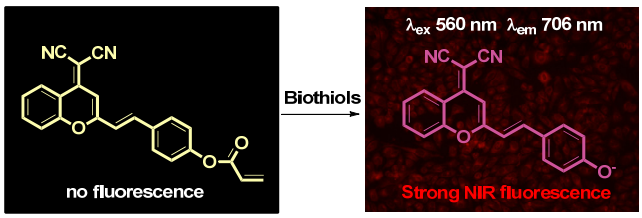
A new highly selective and sensitive colorimetric and NIR fluorescent probe for detection and bioimaging of biothiols was reported.



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