



RAPID COMMUNICATION

A simple and sensitive fluorescent probe for specific detection of cysteine

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Abstract. A fluorescent probe, with simplicity of structure and convenience of synthesis, is capable of detecting cysteine over other biothiols, such as homocysteine and glutathione. The detection limit of 60 nM and 190-fold increase in fluorescence intensity offer advantage for detection of Cys in biological systems. Furthermore, the probe **1** could be applied in bioimaging.

Keywords. Fluorescence probe; cysteine; selectivity; bioimaging.

1. Introduction

Bio-thiols such as cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) play pivotal roles in various biological processes including protein synthesis, detoxification, metabolism, signal transduction and gene regulation.^{1,2} In addition, these bio-thiols are essential in regulating of the redox state of proteins and in protein structure. More importantly, these species are involved in many crucial functions, for example, cysteine (Cys) and homocysteine (Hcy) are related to variety of human diseases like neurotoxicity, skin lesions, edema, muscle weakness, lethargy, slow growth, hair depigmentation,³ cardiovascular⁴ and Alzheimer's diseases.⁵ Similarly, glutathione (GSH) is the most abundant intracellular non-protein thiol and is considered to be an indicator of oxidative stress.^{6,7} As a consequence, detection of these low molecular weight thiols is important and timely. Therefore, a great many of detection methods were developed, but the fluorescence probe detection obtained considerable attention on the basis of its simplicity, efficiency, low detection limits and ease of handling.^{8–10} Up to now, the fluorescence probes were designed and synthesized mainly according to the different chemical reaction mechanisms, such as Michael addition, cyclization, conjugate

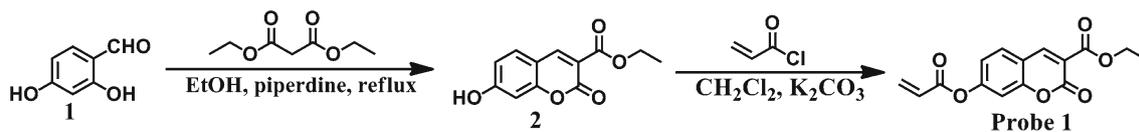
addition-cyclization, cleavage reaction by thiols, thiol-halogen nucleophilic substitution, disulfide exchange, and others.^{4,11–16} However, selective detection of Cys is difficult in view of structural similarity of Cys, Hcy and GSH. Thus, development of probes to selectively detect Cys also remains challenging for the scientific community.

Among these approaches, the selective detection of Cys based on the conjugate addition-cyclization of Cys with acrylates has proven to be specifically effective, this strategy has attracted broad attention.^{17–24} Moreover, coumarin, a well-known fluorophore, exhibits low cytotoxicity and possesses favorable photophysical properties such as a large Stokes shift, visible excitation and emission wavelengths, good photostability, reasonable fluorescent quantum yield and easy synthesis.^{25–27} Thus, in this work, we designed and synthesized fluorescent probe **1** (ethyl 7-(acryloyloxy)-2-oxo-2*H*-chromene-3-carboxylate) with a latent coumarin fluorophore and a receptor of acrylate. We envision that the probe **1** is capable of detecting selectively Cys over Hcy and GSH.

2. Experimental

Unless otherwise noted, materials were purchased from commercial suppliers and used without further purification. All the solvents were purified and dried according to

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Scheme 1. Synthesis of probe 1.

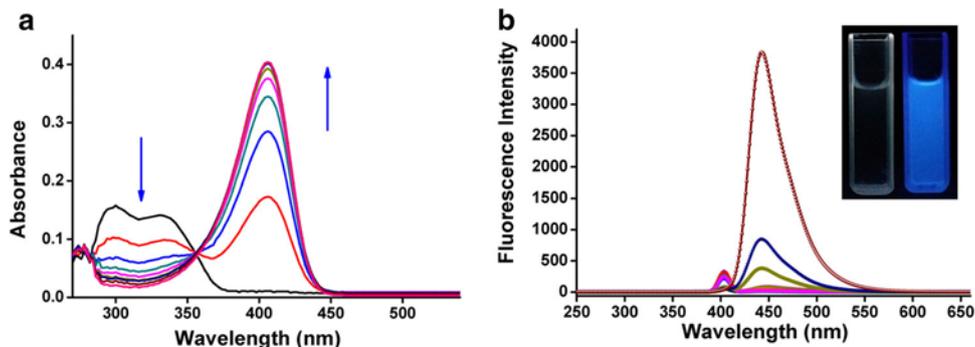


Figure 1. (a) With addition of Cys (100 μM), UV-Vis absorption spectra of probe **1** (10 μM) with increase of incubation time from 0 to 15 min. (b) Fluorescence spectra of probe **1** (10 μM) in the presence of various analytes (100 μM) in an aqueous PBS buffer (pH 7.4, containing 20% acetonitrile); $\lambda_{\text{ex}} = 406$ nm. Inset shows a picture of probe **1** without and with Cys under ultraviolet radiation.

general methods. Synthesis and Characterization of probe **1**, and other experimental details are given in Supporting Information.

3. Results and Discussion

Keeping the following considerations in mind, namely, acrylates can recognize Cys based on conjugate addition-cyclization and coumarin is a well-known fluorophore, probe **1** was synthesized (Scheme 1, for details and characterization see the Supporting Information) and structure confirmed by NMR and HRMS (ESI). Initially, we explored the UV-Vis absorption spectrum of probe **1** (10 μM) in phosphate buffer (PBS pH 7.4, containing 20% acetonitrile). Probe **1** had obvious absorption peaks at 300 and 335 nm. However, with addition of Cys (100 μM), the absorption bands at 300 and 335 nm gradually decreased with concomitant growth of a new band at 406 nm (Figure 1a). Thus, 406 nm was identified as excitation spectrum wavelength. Subsequently, the fluorescence intensity of probe **1** was screened with the different amino acids (L-Cys, Hcy, GSH, Arg, Asp, Tyr, Pro, Lys, Gly, Ala, Val, Phe, Thr, DL-Met, His, Ser, Leu, Trp, Ile, L-Met, Glu, at 100 μM) in phosphate buffer (PBS pH 7.4, containing 20% acetonitrile) (Figure 1b). Interestingly, a remarkable 190-fold enhancement in fluorescence intensity was observed with the addition of Cys and the solution, after 5 min incubation time, showed strong blue fluorescence with

an excitation at 406 nm. However, other amino acids did not cause distinct change of fluorescence intensity; Hcy and GSH showed slight interference, however. Therefore, the probe **1** could selectively detect Cys level.

In order to explore the effects of other analytes for detection of Cys, we carried out the competition experiment (Figure S1 in Supplementary Information). The results indicated that fluorescence intensity of probe **1** for Cys did not change clearly in the presence of other amino acids or metal ions, even Hcy and GSH. Moreover, there is only 20% decrease in fluorescence intensity of probe **1** toward Cys in the presence of GSH/Hcy (200 μM). Thus, these experimental results further showed that the probe **1** could distinguish Cys from other amino acids. In addition, the fluorescence intensity was enhanced gradually with the increase of Cys content from 1 equiv. to 10 equiv. (Figure 2a). Subsequently, we further investigated the properties of the probe **1**. To investigate the fluorescence spectral response of probe **1** for Cys, we extended the action time of Cys to 20 min. The result displayed that the increase of fluorescence intensity reached the plateau stage after 10 min (Figure 2b). Thus, this response is faster than those reported previously.²⁴ Furthermore, the fluorescence intensity of probe **1** toward Hcy/GSH increased with extension of response time. However, this enhancement is slower and weaker than of probe **1** toward Cys. Meanwhile, the probe **1** did not induce any obvious change by itself, which meant the probe **1** was stable under this reaction condition. On the same basis,

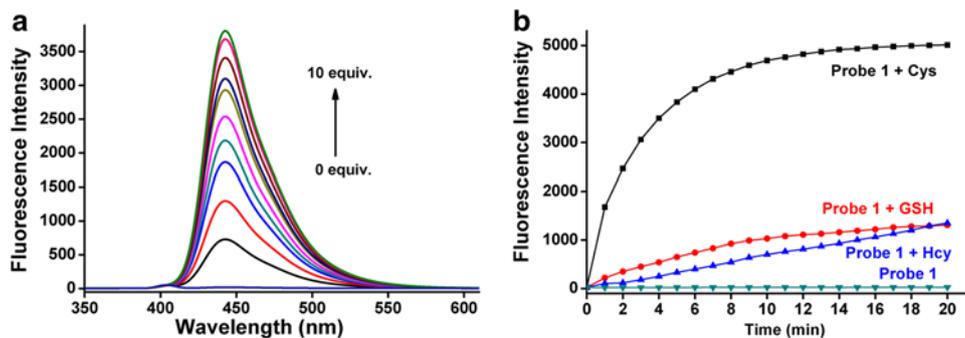
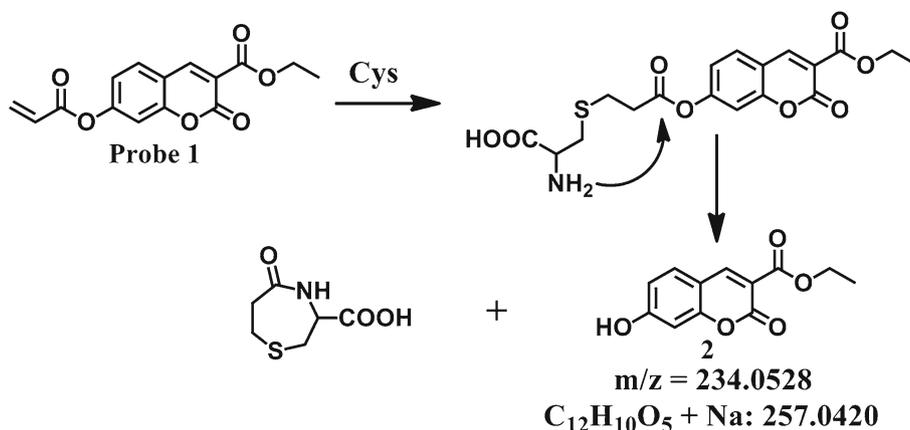


Figure 2. (a) Emission spectra of probe **1** (10 μM) with increase of Cys concentration (from 0 to 10 equiv.) after incubation of 5 min at room temperature. (b) Time-dependent fluorescence intensity of probe **1** (10 μM) in the presence and absence of Cys (10 equiv.), $\lambda_{\text{ex}} = 406 \text{ nm}$.



Scheme 2. Proposed reaction mechanism of probe **1** with Cys.

the detection limit (3 s/m, $n = 20$) of Cys was evaluated to be 60 nM (Figure S2 in Supplementary Information).^{28,29} More importantly, the fluorescence intensity of probe **1** was weak in pH 3.0–9.0; however, with addition of Cys, the fluorescence intensity was enhanced obviously in pH 6.0–9.0. Thus, probe **1** is capable of detecting selectively Cys level under the conditions of physiological pH (Figure S3 in Supplementary Information).

We further investigated the reaction mechanism between probe **1** and Cys. Cys (100 μM) was added to the solution of probe **1** (10 μM), and we completed high resolution mass spectrometry analysis of the mixture. The peak at $m/z = 257.0421$, corresponding to product **2** (M+Na) was clearly discovered (Figure S4 in Supplementary Information). Therefore, we propose a probable reaction mechanism (Scheme 2). The initial conjugate addition of Cys to acrylate moiety and subsequent intramolecular cyclization afforded the final fluorescent compound, which caused increase of fluorescence intensity. Meanwhile, we measured the *pseudo*-

first-order rate constant k' at room temperature. The *pseudo*-first-order rate constants for Cys, Hcy, and GSH were measured as $k' = 1.2 \times 10^{-2} \text{ s}^{-1}$, $1.0 \times 10^{-3} \text{ s}^{-1}$, $1.5 \times 10^{-3} \text{ s}^{-1}$, respectively (Figure S5 in Supplementary Information). Thus, Cys induced a rapid cyclization reaction of a stable seven-membered ring, which is kinetically favorable. This difference of reaction rate made that the probe **1** could selectively and quickly detect Cys over Hcy/GSH.

Lastly, we studied the application of probe **1** in live-cell imaging. Before the cell imaging was investigated, we first measured the cytotoxic activity of probe **1** by MTT method.³⁰ Interestingly, the results displayed that the probe **1** (20 μM) did not induce apparent cell death within 24 h of incubation time (Figure 6 in Supplementary Information). Subsequently, we recorded the bioimaging of probe **1** (Figure 3). When the HeLa cells were incubated with probe **1** (10 μM) for 1 h, they became blue fluorescent. More importantly, when the cells were pretreated with thiol scavenger (N-ethyl maleimide, NEM, 1 mM) for 30 min, the blue fluores-

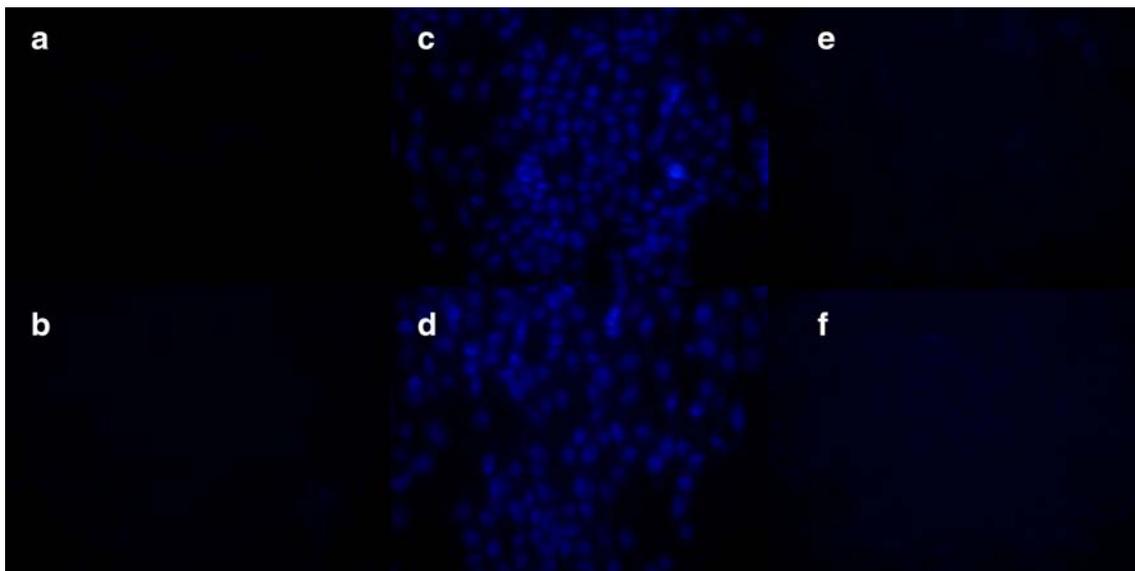


Figure 3. Bioimaging of HeLa cells. (A) Blank; (B) Cells after incubation with DMSO for 1 h; (C, D) Cells after incubation with probe **1** (10 μ M) for 1h; (E, F) cells which were pre-incubated with NEM (1 mM) for 30 min. $\lambda_{\text{ex}} = 406$ nm.

cence did not appear with cells incubated by probe **1**. Therefore, probe **1** can be applied for sensing Cys in living cells.

4. Conclusions

We designed and synthesized the fluorescent probe **1**, which displayed a highly selective and sensitive response to Cys over Hcy/GSH in the tests. Moreover, the 190-fold increase in fluorescence intensity, and detection limit of 60 nM offered a powerful method for practical detection of Cys in biological systems. Also, the probe **1** could be applied in bioimaging. Thus, we believe that this new probe will be of great benefit for gerater understanding of the roles of Cys in the biological systems.

Supplementary Information (SI)

All additional information pertaining to the characterization of compounds using ^1H NMR, ^{13}C MNR spectra, and HRMS, and experimental methods are given in the Supporting Information, which is available at www.ias.ac.in/chemsci.

Acknowledgements

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