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A red-emitting fluorescent probe for biothiols detection with a large Stokes shift

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ABSTRACT

A highly selective red-emitting fluorescent probe for biothiols detection was developed. The addition of biothiols to the solution of this probe, **1**, resulted in a strong red fluorescence with an obvious color change from red to claybank. This colorimetric and fluorescent dual probe exhibited a low detection limit (15 nM) and a large Stokes shift (147 nm) in detecting biothiols. Importantly, the utility of this probe for sensing intracellular thiols was successfully demonstrated in living HNE-2 cells.

1. Introduction

Biothiols, such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) (Fig. 1), are of great importance in many physiological processes.[1,2] Cys is a precursor for the production of protein and its deficiency can cause many health issues such as growth retardation, lethargy, hair depigmentation, liver damage, muscle and fat loss, and skin lesions.[3,4] An elevated level of Hcy in human blood is a risk factor for cardiovascular and neurodegenerative diseases, inflammatory bowel disease, osteoporotic fractures and Alzheimer's disease.[5–8] GSH, the most abundant intracellular non-protein thiol, also plays crucial roles in maintaining the reducing environment in cells and acts as a redox regulator.[9–13] As a consequence, it's very meaningful to develop efficient methods for the detection of biothiols in biological samples.

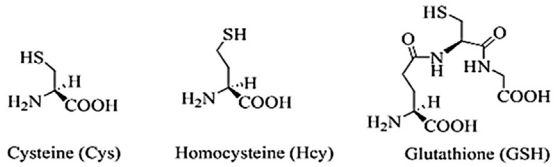

Fig. 1. The structures of biothiols.

Due to many advantages including sensitivity, easy operation, non-destructive detection and high spatiotemporal resolution, fluorescent probes are the ideal tools for the detection of biothiols in vitro/vivo, and therefore have drawn considerable attention in the past decade.[14–19] It's well known that fluorescent probes with long excitation and emission wavelength are favorable in fluorescent detection because the long wavelength photons have deeper tissue penetration, less photo-damage to cells, and smaller affection from background. In addition, the large Stokes shift is also desirable in the design of fluorescent probes because self-absorption and the interference from auto-fluorescence can be greatly minimized when the incident light and fluorescence signal are well separated.[17,20–24]

Our previous study showed that iminocoumarin dye **4** had an emission in red spectral region, a reasonable quantum yield and a large Stokes shift (147 nm).[25] These above-mentioned merits make dye **4** a good candidate for the design of fluorescent probes.

In this work, we employed dye **4** as the scaffold to develop a red-emitting fluorescent probe, **1**, for the detection of biothiols.

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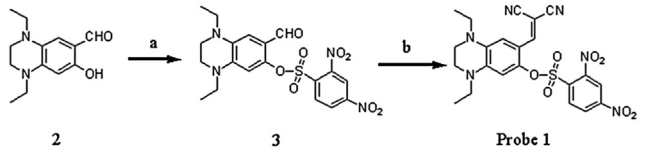
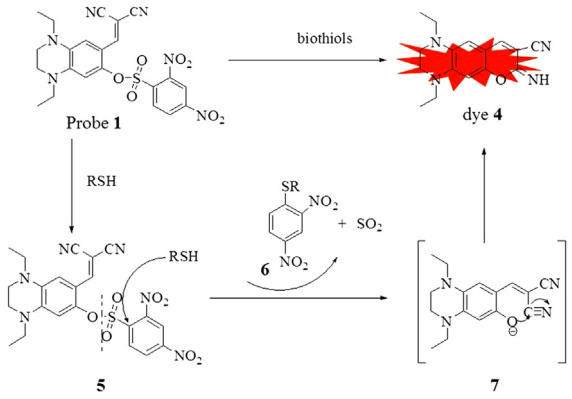
The synthetic route for Probe **1** is outlined in Scheme 1. Probe **1** was essentially none-fluorescent due to the photo-induced electron transfer (PET) process caused by the 2,4-dinitrobezensulfonate group (**DNBS**). We speculated that the biothiols would cleave the **DNBS** moiety in Probe **1** to generate the intermediate **7**, which is subjected to a subsequent cyclization reaction to form the red-emitting fluorescent dye, **4** (shown in Scheme 2).[26–28]



Scheme 1. Synthetic route of Probe **1**. (a) 2,4-dinitrobenzenesulfonyl chloride, DCM, triethylamine, 0 °C, 3 h, yield 50.8%; (b) malononitrile, triethylamine, DCM, 25.0 °C, 4 h, yield 33.5%.



Scheme 2. The proposed sensing mechanism of Probe **1** with biothiols.

2. Results and discussion

2.1. The sensing properties of Probe 1 toward biothiols

The optical response of Probe **1** for biothiols was investigated in HEPES buffer (10.0 mM, pH 7.4) with 30% CH_3CN. As shown in Fig. 2, Probe **1** featured an absorption band with a maximum at 498 nm and was almost non-fluorescent. The addition of excessive Cys to the solution of Probe **1** resulted in a 26 nm blue-shift from 498 nm to 472 nm in the absorption spectrum accompanying with an obvious color change from red to claybank.

Next, fluorescence behaviour of Probe **1** in response to different amount of Cys was investigated. Upon the treatment of Cys, the solution of Probe **1** exhibited a significant fluorescence enhancement with λ_{max}=619 nm. The absorption and emission spectra of Probe **1** with Cys were in accordance with the spectral characteristic of dye **4**, suggesting that Cys cleaved **DNBS** moiety in Probe **1**, and dye **4** was subsequently formed. The titration experiment on the solution of Probe **1** (10.0 μM) with different concentrations of Cys showed that the fluorescence intensity at 619 nm increased with increasing the concentration of Cys and leveled off when 7.0 equiv of Cys was added (shown in Fig. 3). The enhancement was up to 30-fold. A plot of the fluorescence intensity at 619 nm versus the concentration of Cys in the range of 0.0–10.0 μM displayed a good linearity (R=0.9977). The detection limit was calculated to be 1.5×10^{-8} M based on S/N=3, which was sufficiently low for the detection of Cys in biological samples (Fig. 4). In addition, Hcy and GSH were subjected to the same experiments and similar results were obtained (shown in Figs. S1

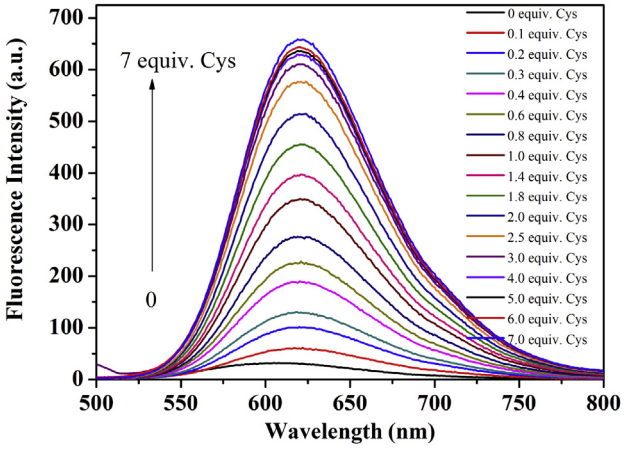


Fig. 3. Fluorescence spectra of Probe **1** (10.0 μM) in response to different concentrations of Cys in HEPES buffer (10.0 mM, pH 7.4) with 30% CH_3CN. Excitation wavelength: 485 nm. Excitation and emission slits: 5.0 nm/5.0 nm.

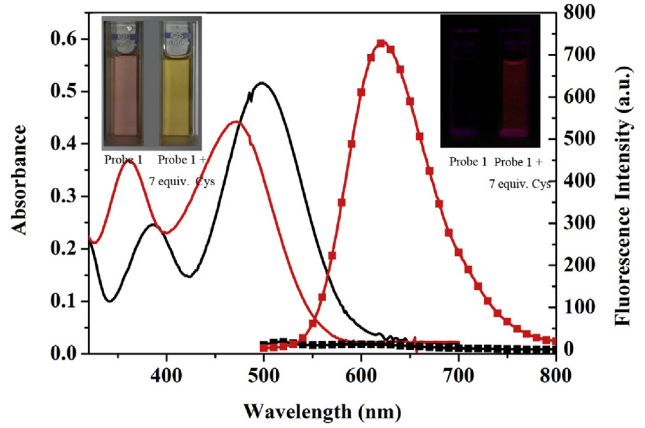


Fig. 2. Absorption (solid lines) and emission (dotted lines) spectra of Probe **1** (black lines) and Probe **1** with 7.0 equiv Cys (red lines) in HEPES buffer (pH=7.4, containing 30% CH_3CN, v/v). Inset: the color photos of the solution of Probe **1** (10.0 μM) in the absence/presence of Cys (7.0 equiv) (left); the fluorescence photos of the solution of Probe **1** (10.0 μM) in the absence/presence of Cys (7.0 equiv) under a 365 nm UV lamp (right). Spectra were recorded after incubation Probe **1** with Cys for 60 min. Excitation wavelength: 485 nm. Excitation and emission slits: 5.0 nm/5.0 nm.

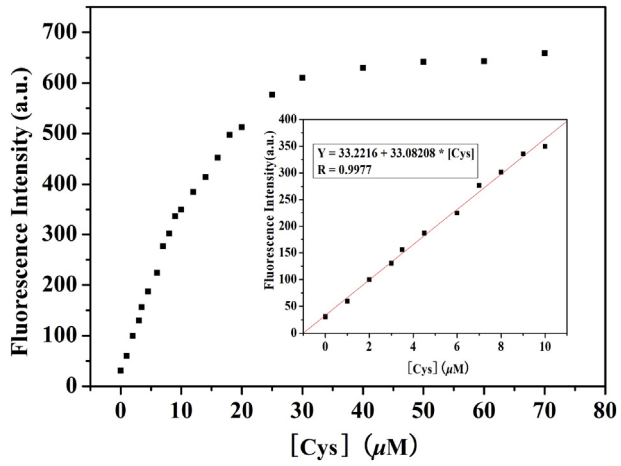


Fig. 4. Plot of fluorescence intensity at 619 nm of Probe **1** (10.0 μM) against the concentration of Cys in HEPES buffer (10.0 mM, pH 7.4) with 30% CH_3CN. Inset: the linear relationship between the fluorescence intensity at 619 nm and Cys concentration (0.0–10.0 μM).