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A fluorescent dyad with large emission shift for discrimination of cysteine/homocysteine from glutathione and hydrogen sulfide and the application of bioimaging

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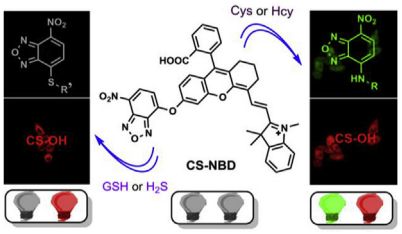
HIGHLIGHTS

- A fluorescent dyad (**CS-NBD**) was developed for efficiently discriminating Cys/Hcy from GSH and H_2S.
- Probe **CS-NBD** generates two different sets of fluorescence signal in two emission bands responding to Cys/Hcy and GSH/H_2S.
- Probe **CS-NBD** was employed to distinguish Cys and Hcy in living cells by dual-color fluorescence imaging.

GRAPHICAL ABSTRACT



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ABSTRACT

Biothiols, as reactive sulfur species (RSS), play important roles in human physiology, and they have a close connection of generation and metabolism pathways among of them. It is challenging to discriminate biothiols from each other due to the similar chemical structures and properties of them. Herein, we develop a fluorescent hybrid dyad (**CS-NBD**) for efficiently discriminating cysteine (Cys)/homocysteine (Hcy) from glutathione (GSH) and hydrogen sulfide (H_2S) by a dual-channel detection method. **CS-NBD** performs inherently no fluorescence in ranging from visible to near infrared region. However, upon addition of Cys (2–150 μM)/Hcy (2–200 μM), **CS-NBD** generates significant fluorescence enhancement in two distinct emission bands (Green-Red), while encounter of GSH (2–100 μM) or H_2S (2–70 μM) induces the fluorescence increase only in the red channel. The detection limit was determined to be 0.021 μM for Cys, 0.037 μM for Hcy, 0.028 μM for GSH, and 0.015 μM for H_2S, respectively (S/N = 3). The interval distance between two emission bands is up to 163 nm, which is favourable to acquire the accurate data in measurement due to the reducing of crosstalk signals. **CS-NBD** is also successfully applied to distinguish Cys/Hcy in cellular context by dual-color fluorescence imaging.

1. Introduction

Reactive sulfur species (RSS), containing hydrogen sulfide (H_2S), hydrogen polysulfides (H_2S_n, n > 1), mercaptoamino-acids, and sulfur dioxide (SO_2), is an essential biological group of substances to maintain the health of cellular. RSS are active as antioxidants and signaling agents in various organ tissue and play vital roles in human physiology physiological processes, such as neuromodulation, synaptic transmission, cardioprotection, vasodilation, inflammation, lipid metabolism, insulin secretion, and colonic motility [1–7]. Biothiols including H_2S, cysteine (Cys), homocysteine (Hcy) and

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glutathione (GSH) have a close relationship in generation and metabolism processes and their concentration level have a significant effect on each other. Endogenous H_2S, the simplest biothiol, can be produced by the catalysis reactions of Cys and Hcy with cystathionine β-synthase (CBS) or cystathionine γ-lyase (CSE) [8]. GSH, the most abundant intracellular non-protein biothiol, is a species of mercaptoamino-acids like Cys and Hcy. GSH can keep the reduced state of Cys in proteins, and also prevent the damage from oxidative stress by trapping free radicals in cells [9,10]. In addition, Cys is a precursor of GSH, meanwhile, Hcy is also the precursor of Cys [11,12]. Their abnormal levels are associated with many diseases, such as liver damage, skin lesions, slow growth, Alzheimer's, cancer, and cardiovascular disease [10,13–15]. Therefore, the selective detection of biothiols in biological samples is significant for better understanding the relationships among of them in their generation and metabolism mechanisms and their physiological roles in biological systems.

Due to the high sensitivity, good selectivity and simple operation, fluorescent probes have attracted great attention. In addition, the inherence of fluorescence imaging method is very suitable for real-time and in-situ detection of target analytes with nondestructive feature in living samples [16–18]. Hence, a great of efforts have been made to develope fluorescent biothiols probes over the past decade years [19–26]. However, owing to biothiols all contain a highly reactive sulfydryl group (-SH), they possess the similar chemical properties, such as nucleophilicity and reducibility. It results in great challenge for developing selective fluorescent biothiol probes, especially discriminating Cys/Hcy or GSH from H_2S, because H_2S possesses much stronger nucleophilic and reducing property than other biothiols. Furthermore, the fluorescent probes possessing two or more emission bands are more favorable than single emission band-based probes, because the former is more conducive to eliminate the disadvantage of accurateness induced by false positive signal in measurement [27–29]. Thereby, we developed a hybrid fluorescent dyad (**CS-NBD**) with two distinct emission bands for selective detection of Cys/Hcy form GSH and H_2S (Scheme 1). We comprehensively investigated the optical response of the probe **CS-NBD** to biothiols and its application for fluorescence imaging in living cells. When **CS-NBD** responding to Cys/Hcy or GSH/H_2S in aqueous solution, its mixture displays different response fluorescence signals distinguishing from free probe in two distinct emission bands (green-red), which is propitious to eliminate the disadvantage of detection induced by the crosstalk signals. The probe was also successfully applied to distinguish Cys/Hcy from GSH and H_2S in living cells. Thus, probe **CS-NBD** has a potential to investigate the relationships among of them in their generation and metabolism processes and their physiological roles in biological systems.

2. Material and methods

2.1. Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Twice distilled water was used throughout all experiments. The instruments used in this work were listed in supporting information.

2.2. Cell culture

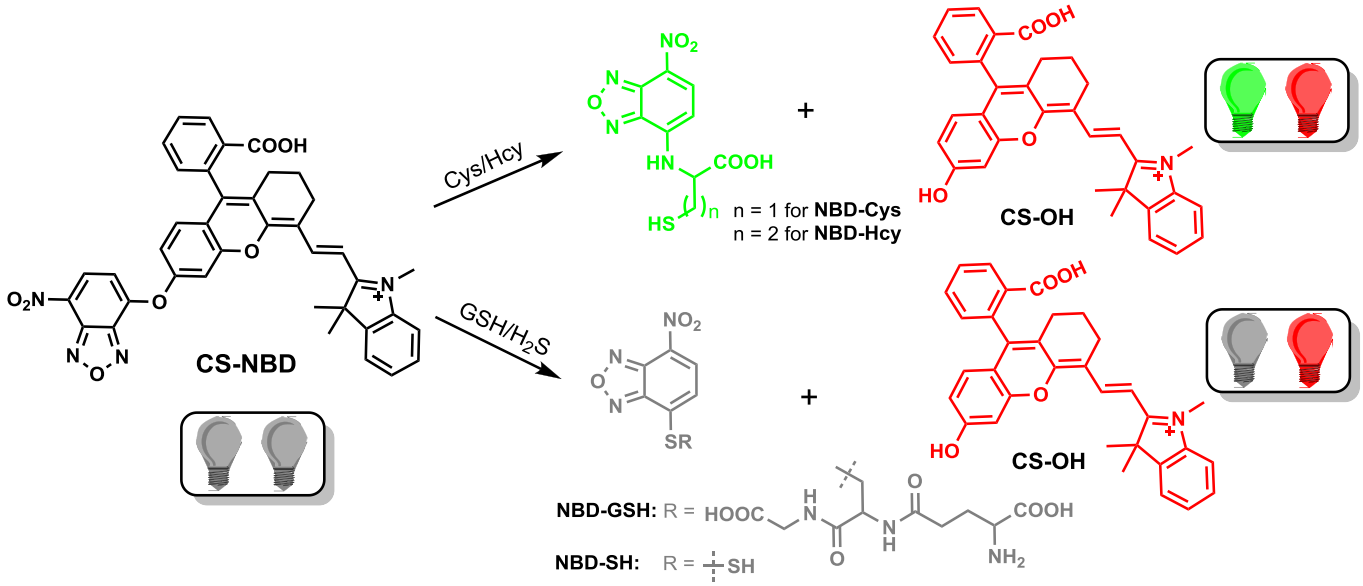
HeLa hepatoma cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) and grown in the constant-temperature incubator with an atmosphere of 5% CO_2 and 95% air at 37 °C.

2.3. Cytotoxicity assay

The cytotoxicity of probe **CS-NBD** at different concentration (5, 10, and 25 μM) was evaluate by a standard MTT assay according to previous procedure [30].

2.4. Bioimaging for discriminating Cys/Hcy in living cells

The cell experiment was divided into five groups. As the control experiment, the first group is that HeLa cells were pretreated with NEM (0.5 mM) for 30 min, subsequently co-incubated with **CS-NBD** (5 μM) and Hoechst (1 μM) for 30 min, then imaged after washing by PBS buffer. In experimental groups, the cells were pretreated with NEM (0.5 mM) for 30 min, subsequently incubated with Cys (250 μM), Hcy (250 μM), or Na_2S (100 μM) for 15 min, washed by



Scheme 1. The proposed response process of probe **CS-NBD** with respective biothiols and the changes of fluorescence signal in two distinct emission bands.