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An ESIPT-based fluorescent probe for sensitive and selective detection of Cys/Hcy over GSH with a red emission and a large Stokes shift

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ABSTRACT

An ESIPT-based fluorescent probe (Probe **1**) using acrylate as recognition group for the selective and sensitive detection of cysteine/homocysteine (Cys/Hcy) has been developed. In the presence of Cys/Hcy, this probe was transformed into 1,3-bis(bispyridin-2ylimino)isoindolin-4-ol (dye **4**) which displayed red fluorescence with a large Stokes shift (217 nm) when excited. The detection limits are as low as 5.4 nM and 7.0 nM for Cys and Hcy respectively (based on S/N = 3). Importantly, this probe has been successfully demonstrated for the detection of intracellular Cys/Hcy in living cells.

Introduction

Biological thiols such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) are of great importance in various physiological and pathological processes.[1–4] Numerous studies have shown that abnormal levels of biothiols *in vivo* can cause many diseases.[5] For instance, elevated level of Cys can lead to neurotoxicity,[6,7] while its deficiency can cause slow growth, hair depigmentation, dropsy, somnolence, liver damage, muscle and fat loss, skin lesions, and asthenia.[8] Moreover, the level of Hcy is considered to be closely associated with cardiovascular diseases and neural tube defects.[9] GSH has important functions in maintaining redox activities, xenobiotic metabolism, signal transmission, and gene regulation in cells.[10–12] As a consequence, the valid approaches, especially those that can differentiate these three species, for the detection of biothiols with high sensitivity and selectivity are highly demanded.

Fluorescent technique has been a dominant methodology which is extensively used for the detection of various species by virtue of its easy operation, high sensitivity, and high spatial and temporal resolution.[13–17] In recent years, although many fluorescent probes has been reported for the detection of biothiols,[18–25] only a handful of them are able to discriminate these three biothiols because of their similar structure and reactivity. The assembly of an acrylate moiety into fluorescent dyes was proved to be a valid method for discriminating Cys/Hcy over GSH and other amino acids.[26–29] So far, acrylate-based fluorescent probes with both red/NIR emission and large Stokes shift for specific detection of Cys/Hcy are rare.[30] Fluorescent probes with long wavelength emission is preferred in fluorescent sensing by virtue of deep tissue penetration, minimal interference from background, and less photo-damage to biological samples.[31] Also, large Stokes shift is a favorable property for fluorescent probes, which can reduce self-absorption and minimize auto-fluorescence, and therefore improve the detection sensitivity.[32–34]

1,3-Bis(bispyridin-2ylimino)isoindolin-4-ol (shown in Scheme 1), dye **4**, was a desired scaffold to construct fluorescent probes due to the long emission wavelength (λ_{em} = 585 nm) and large Stokes shift (217 nm) resulting from the excited-state intramolecular photon transfer (ESIPT) process.[32] In this study, we developed Probe **1** by assembling an acrylate moiety into dye **4** as a sensing group for sensitive detection of Cys/Hcy over GSH and other amino acids. The synthetic route for Probe **1** was presented in Scheme 1. We expected that the acrylate moiety in Probe **1** would induce a photo-induced electron transfer (PET) process and inhibit ESIPT process in its excited state and therefore would quench its fluorescence effectively.[30] In the presence of Cys/Hcy, Probe **1** underwent a conjugate addition reaction with Cys/Hcy to generate a thioether,[35,36] which was further transformed into dye **4** through an intramolecular cyclization reaction (shown in Scheme 2). As for GSH, the corresponding intermediate thioether could not undergo intramolecular cyclization reaction due to the kinetically unfavourable ten-membered ring formation (shown in Scheme S1).[37–39] As a

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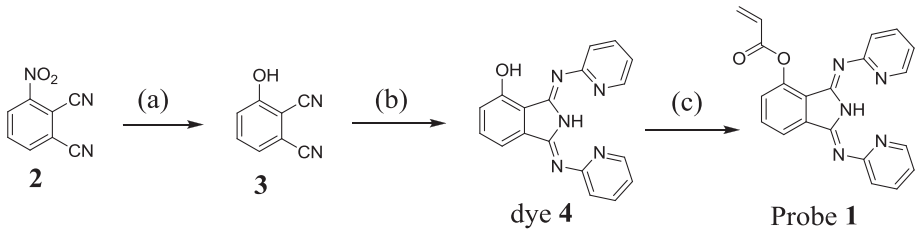
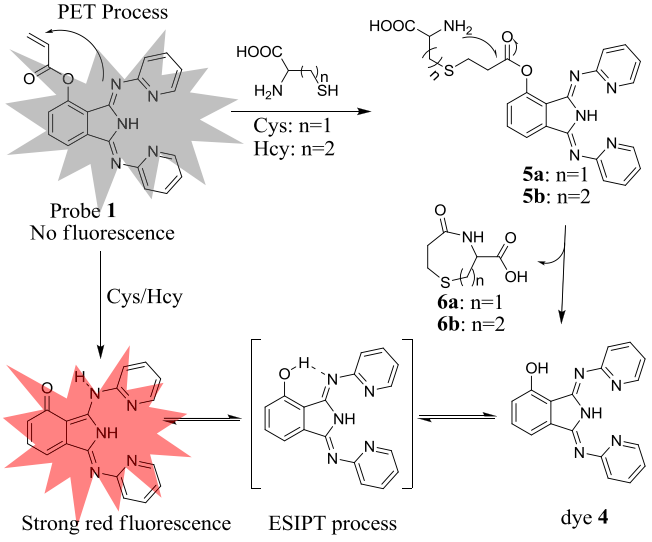


Scheme 1. Synthetic route of Probe **1**. (a) K_2CO_3, $NaNO_2$, DMSO, 130 °C, 30 min, yield 54%; (b) 2-aminopyridine, $CaCl_2$, *n*-BuOH, 110 °C, 5 days, yield 25%; (c) acryloyl chloride, DCM, triethylamine, r.t., 10 min, yield 81.2%.



Scheme 2. Proposed reaction mechanism of Probe **1** with Cys/Hcy.

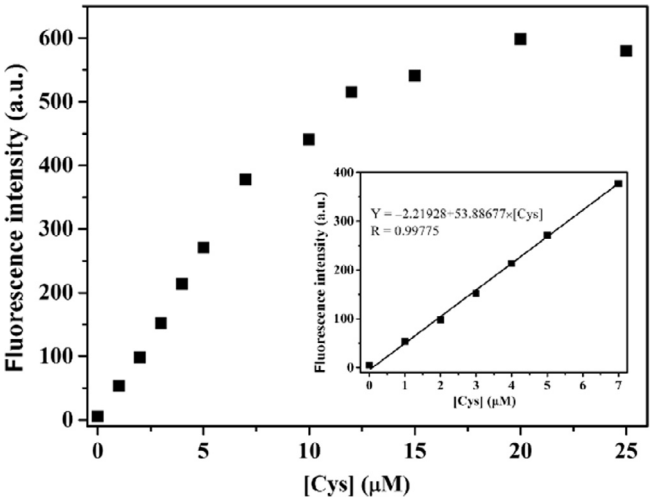


Fig. 2. Plot of fluorescence intensity at 585 nm of Probe **1** (5.0 μM) *vs* Cys concentration. Inset: a linear correlation between the fluorescence intensity at 585 nm and concentration of Cys. Data were obtained after incubation for 20 min and excitation wavelength was 368 nm.

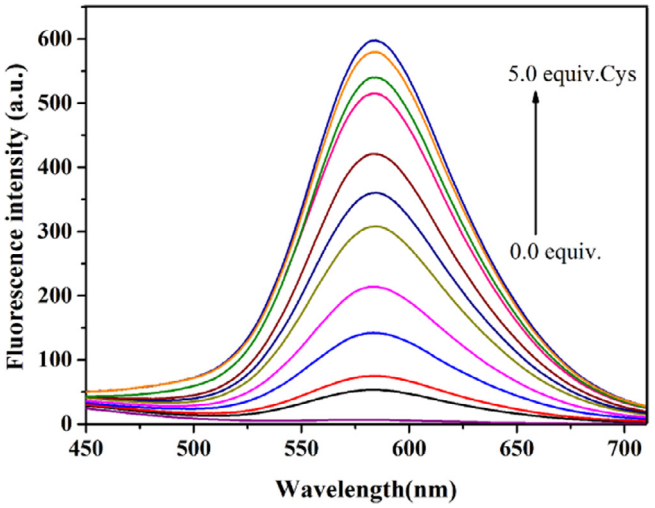


Fig. 1. Fluorescent spectra of Probe **1** (5.0 μM) treated with different concentrations of Cys (0.0–5.0 equiv.) in PBS buffer (10.0 mM, pH 7.4, 1.0 mM CTAB). Spectra were obtained after incubation for 20 min and the excitation wavelength was 368 nm.

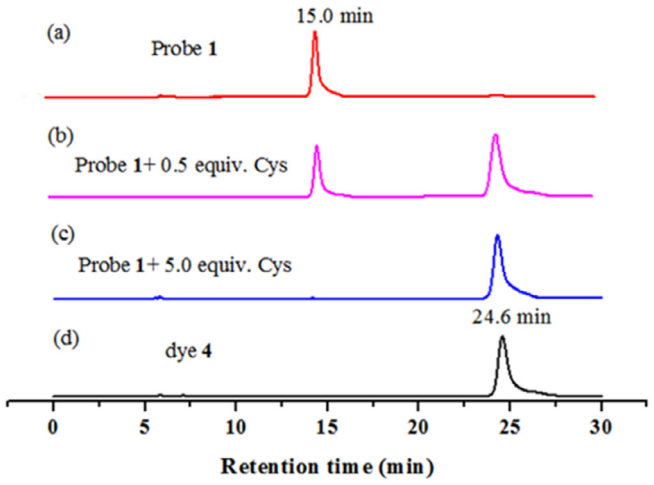


Fig. 3. HPLC chromatograms: (a) Probe **1** (50.0 μM); (b) Probe **1** (50.0 μM) treated with 0.5 equiv. of Cys; (c) Probe **1** (50.0 μM) treated with 5.0 equiv. of Cys; (d) dye **4** (50.0 μM). The data was recorded after incubation for 20 min in PBS buffer (10.0 mM, pH 7.4, 1.0 mM CTAB). Condition: eluent, H_2O/CH_3CN (v/v, 3/7); flow rate, 1.0 mL/min; temperature, 25 °C; detection wavelength, 370 nm; injection volume, 20.0 μL.

consequence, Probe **1** would be able to specifically detect Cys/Hcy against GSH and other amino acids with a red emission and a large Stokes shift.

Results and discussion

The sensitivity of Probe **1** was investigated by incubating this probe with different concentrations of Cys/Hcy in PBS buffer (10.0 mM, pH 7.4, 1.0 mM CTAB). The results were shown in Fig. 1. As we expected, the solution of Probe **1** had no fluorescence. However, the addition of Cys to the solution of Probe **1** resulted in a remarkable fluorescence enhancement with a maximum at 585 nm. Moreover, the fluorescence intensity was gradually increased along with the added amount of Cys. When 5.0 equiv.