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**COMMUNICATION**

# A selective fluorescent turn-on NIR probe for cysteine†

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**A selective and sensitive turn-on fluorescent NIR probe for cysteine has been developed. Cleavage of 2,4-dinitrobenzenesulfonyl (DNBS) with thiols switches the weakly fluorescent aza-BODIPY dye ($\lambda_{em}$ = 734 nm, $\Phi_f$ = 0.03) to a strongly fluorescent species in the NIR region ($\lambda_{em}$ = 755 nm, $\Phi_f$ = 0.14).**

Low molecular weight thiols (LMWTs) play vital roles in living organisms.[1] For example, cysteine (Cys) is an essential biological molecule required for its detoxifying function, immunological competence, and growth and delay of senility of cells and tissues in living systems.[1,2] Lack of cysteine causes health problems such as retarded growth, hair depigmentation, and liver damage.[3] Therefore, selective and sensitive detection of intracellular cysteine has attracted increasing interest.[4]

Among the available techniques to detect and quantify these thiols, luminescent methods have been extensively pursued due to their simplicity and high sensitivity.[5] This process of detecting thiols involves a specific reaction between probes and thiols, such as cleavage reaction by thiol,[6] cyclization with aldehyde,[7] metal complex-displacement coordination,[8] and others.[9] The cleavage reaction by deprotection of the 2,4-dinitrobenzenesulfonyl (DNBS) group was found to primarily switch a PET[10] or ICT[11] process to achieve a turn-on fluorescent probe (Fig. 1). For example, Maeda *et al.* reported a probe for thiols based on rhodamine in 2005;[12] Hu and Zhao subsequently reported PET probes based on BODIPY;[13] changes to the fluorophore, such as benzo[*d*]oxazol-4-ylmethanamine, 1,10-phenanthrolin-5-amine and others led to fluorescent probes for thiols too.[14] Therefore, these probes have great potential for applications in the detection of thiols *in vitro* and in environmental studies. However, the emission range (400–600 nm) of these probes for detecting thiols is in the visible region. NIR fluorescent dyes can greatly reduce background absorption, fluorescence, light scattering, and improve the detectable sensitivity and selectivity.[15] More importantly, NIR fluorescent dyes have deep penetration to achieve a distinct image *in vivo*. BODIPY dyes are well-known to be highly fluorescent, very stable, and exceptionally insensitive to the polarity of solvents as well as to pH.[16] We had reported novel NIR aza-BODIPY dyes ($\lambda_{abs}$ = 740 nm) in previous work.[17] Herein we designed a NIR probe for detecting cysteine **1** (Scheme 1, see ESI†). DNBS is a good potent electron accepter and can disturb the electronic structure of the BODIPY fluorophore,[13b] thus probe **1** is hypothesized to be weakly fluorescent due to photoinduced electron transfer, while thiol-specific cleavage reaction will release the hydroxyl group, and in turn makes the BODIPY fluorophore **2** strongly fluorescent (Scheme 1).

The spectroscopic properties of **1** in the absence and presence of cysteine were examined. The absorption and emission maxima of probe **1** were 717 ($\varepsilon$ = 48 000 $M^{-1}$ $cm^{-1}$) and 734 nm respectively, and probe **1** was weakly fluorescent ($\Phi_f$ = 0.03) (the solid curve in Fig. 2). Upon addition of cysteine, the absorption and emission maxima were red-shifted to 735 ($\varepsilon$ = 63 000 $M^{-1}$ $cm^{-1}$) and 755 nm respectively, and a significant increase in fluorescence quantum yield ($\Phi_f$ = 0.14) was observed (the dashed curve in Fig. 2, Scheme 1), which is in good agreement with a typical PET process.[13,18] To the best of our knowledge, **1** is the first example of a NIR probe for detecting thiol

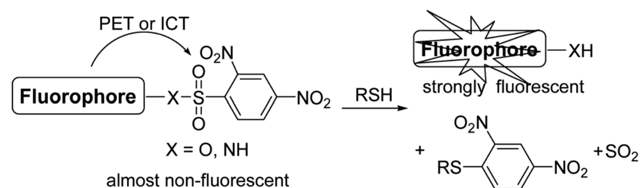
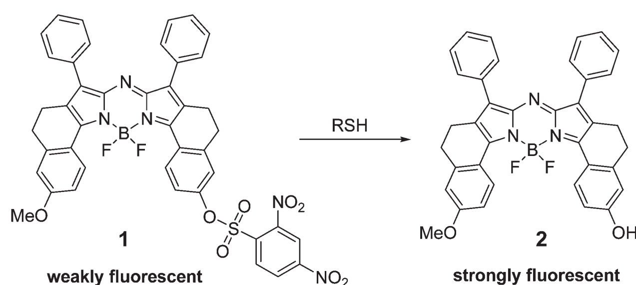



**Fig. 1** Concept of a turn-on probe based on deprotection of the 2,4-dinitrobenzenesulfonyl group.




**Scheme 1** Design of a fluorescent turn-on NIR probe **1** and cleavage of probe **1** with thiol(s) to generate **2**.

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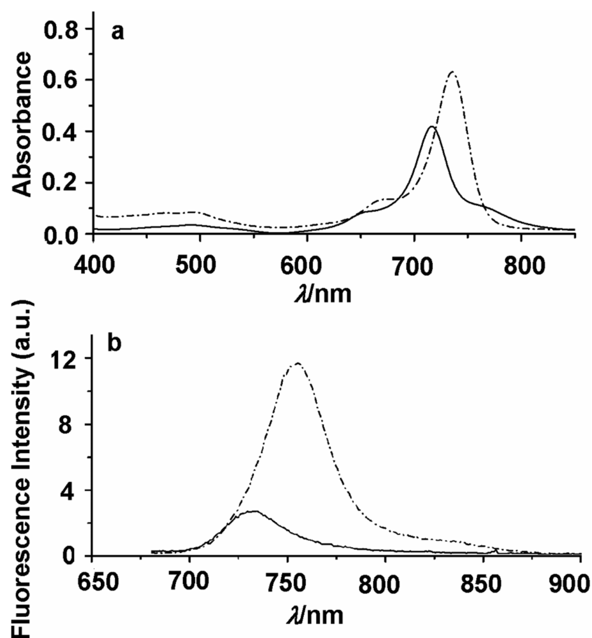


**Fig. 2** (a) Absorption and (b) emission spectra ($\lambda_{ex}$ = 670 nm) of probe **1** before (solid curve) and after (dashed curve) the addition of cysteine. Final concentrations of **1** and cysteine are 2.0 × $10^{-5}$ M and 2.0 × $10^{-3}$ M, respectively, in MeCN–$H_2O$–DMSO (79 : 20 : 1, v/v/v; pH = 7.5). Data were collected before or 1 h after the addition of cysteine at 20 °C.

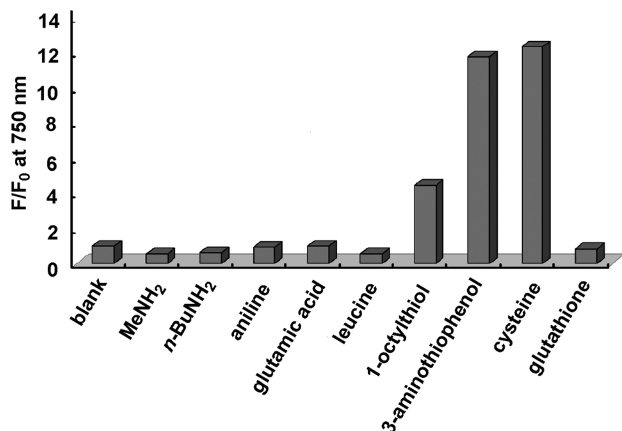


**Fig. 3** Selectivity of probe **1** toward various analytes. Relative fluorescence intensity of 20 μM probe **1** (MeCN–$H_2O$–DMSO = 79 : 20 : 1, v/v/v; pH = 7.5) was measured at 755 nm ($\lambda_{ex}$ = 670 nm) after incubation at 20 °C for 1 h in the presence of 2 × $10^{-3}$ M (final concentrations) of analytes.

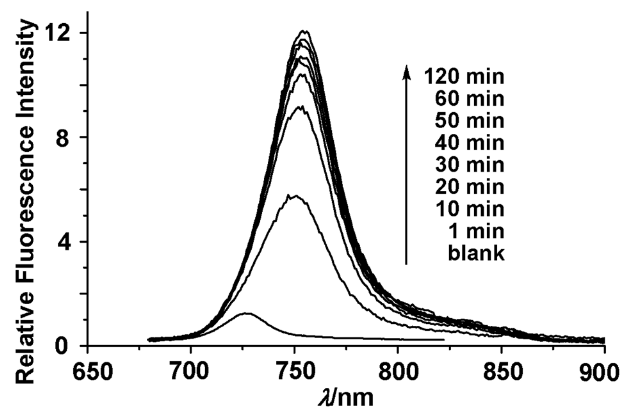


**Fig. 4** Fluorescence emission–time profile of probe **1** towards cysteine. The fluorescence intensity of 20 μM probe **1** (MeCN–$H_2O$–DMSO = 79 : 20 : 1, v/v/v; pH = 7.5) was studied at 20 °C in the absence and presence of cysteine (100 equiv.) after the specified time periods (1, 10, 20, 30, 40, 50, 60, 120 min) with $\lambda_{ex}$ = 670 nm.

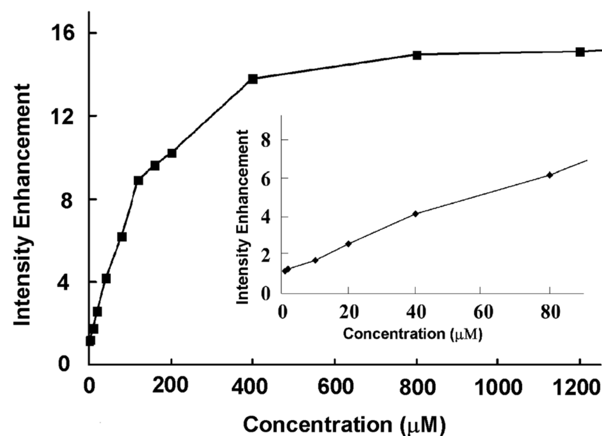


**Fig. 5** Fluorescence response of 20 μM probe **1** (MeCN–$H_2O$–DMSO = 79 : 20 : 1, v/v/v) at 755 nm upon reacting with cysteine in 1, 2, 10, 20, 40, 80, 120, 160, 200, 400, 800, 1200 μM concentrations after 1 h of incubation at 20 °C. The excitation wavelength was 670 nm. The inner panel displays the fluorescence enhancement of probe **1** toward cysteine at 1, 2, 10, 20, 40, 80 μM.

based on aza-BODIPY. These observations suggest that the direct attachment of a DNBS group to the aza-BODIPY core induced PET and quenched effectively the fluorophore's fluorescence. A remarkable shift of the absorption (18 nm) and emission (21 nm) maxima between the probe **1** and the corresponding cleavage product **2** was observed.

The selectivity of probe **1** towards various amino compounds and biological thiols was investigated. As shown in Fig. 3, probe **1** was highly selective to thiols with remarkable fluorescence intensity enhancement for cysteine, 3-aminothiophenol, and 1-octylthiol, while slight enhancement for $MeNH_2$, *n*-$BuNH_2$, aniline, glutamic acid, leucine and glutathione was observed. Thus probe **1** exhibited the high selectivity toward thiols, especially cysteine.

The response of probe **1** toward cysteine was carried out (Fig. 4). After the addition of cysteine, a dramatic fluorescence intensity increase was observed in a short time frame (<50 min). No further remarkable enhancement was observed in an extended reaction period up to 120 min.

The sensitivity of probe **1** was then studied by fluorescence response towards various concentrations of cysteine (Fig. 5). A distinct response of the probe **1** (20 μM) to cysteine in the concentration range of 1–120 μM was observed. When 20 equiv. of cysteine (400 μM) was added, the enhancement of fluorescence intensity reached the maximum in 1 h. Further increase of cysteine concentration did not provide further enhancement of

fluorescence intensity. Notably, the detection limit to cysteine was found to be $5 \times 10^{-7}$ M.

The effect of the pH on the fluorescence intensity and the reactivity of probe **1** were examined. Probe **1** was very stable in a range of pH 5–9, and no pH-dependent fluorescence change was observed (see Fig. S1, ESI†). However, in the presence of cysteine, the fluorescence intensity of probe **1** slightly varied with pH value (pH = 5–9),[13a] and probe **1** exhibited the most sensitive response under weakly basic conditions (pH = 8) as a result of the ionization of cysteine.[19]

In conclusion, we have developed a new fluorescent turn-on NIR probe based on the aza-BODIPY dye. Probe **1** is weakly fluorescent. Cleavage of DNBS with thiols releases the aza-BODIPY fluorophore **2** with concurrent fluorescence in the NIR region ($\lambda_{em}$ = 755 nm, $\Phi_f$ = 0.14). It is highly selective towards cysteine and the detection is rapid. An increase in the of concentration of cysteine affords a quicker and more dramatic response, and the detection limit to cysteine reaches $5 \times 10^{-7}$ M. Probe **1** can be applied to neutral conditions with a pH range (7–8) that is compatible with most biological applications. Importantly, the remarkable shift of the absorption (18 nm) and emission (21 nm) maxima between probe **1** and the corresponding cleavage product **2** is observed to distinctly raise the detection sensitivity. Efforts to create a new fluorescent turn-on NIR-emitting thiol probe based on a NIR dye with high fluorescence contrast are now ongoing in our lab.

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