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Graphic Abstract

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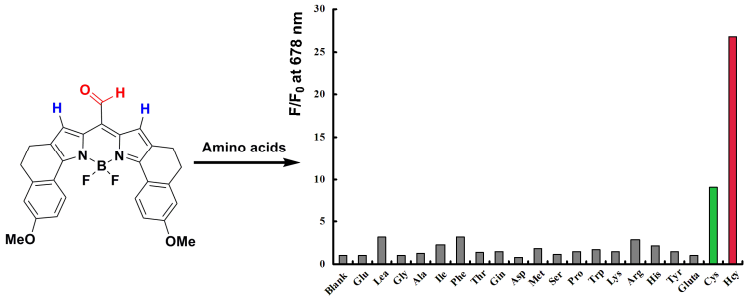

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ARTICLE TYPE

A turn-on NIR fluorescent probe for the detection of homocysteine over cysteine†

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The BODIPY-based turn-on NIR fluorescent probe containing a *partially exposed* aldehyde group at the *meso* position for the detection of homocysteine over cysteine was developed. Such probe is potentially useful for the discrimination of Hcy from Cys.

Low molecular weight thiols such as cysteine (Cys), homocysteine (Hcy) play vital roles in maintaining biological systems.[1] Cys and Hcy are essential biological molecules required for detoxifying function, immunological competence, as well as growth and delay of senility of cells and tissues in living systems.[2] The deficiency of Cys is associated with retarded growth, hair depigmentation, lethargy, liver damage, skin lesions, and weakness.[3] Hcy has been implicated in various types of vascular and renal diseases. An elevated level of Hcy in human blood (=> 15 μm) is a risk factor for Alzheimer's disease, cardiovascular disease, neural tube defect, inflammatory bowel disease, and osteoporosis.[4] Since abnormal levels of Cys/Hcy closely interrelats to different diseases, the detection and discrimination between Cys and Hcy become significant to disease prediction and early diagnosis.

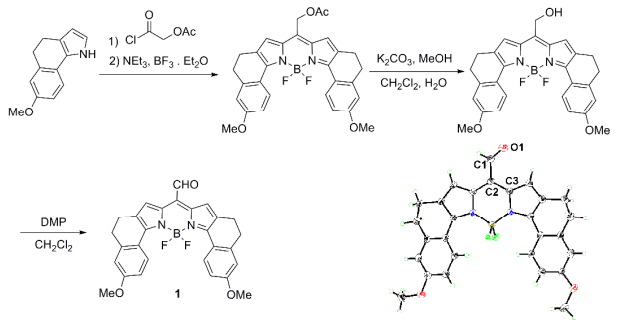
In comparison with other technologies to detect and quantify thiols, luminescent methods have been extensively pursued due to their simplicity and high sensitivity.[5] The processes of detecting thiols generally involve some specific reactions between probes and thiols, such as cyclization with aldehyde, Michael addition, cleavage reaction by thiols, metal complexes-oxidation–reduction, metal complexes-displace coordination and others.[6] Although many fluorescent probes for thiols have been developed,[6] due to the close resemblance of Cys and Hcy, selective probes for the detection and discrimination between Cys and Hcy are limited. Recent publications were disclosed as visible-region fluorescent probes for the discrimination of Cys from Hcy.[7] A Rhodamine-based probe for discrimination of Cys from Hcy based on cyalization with aldehyde was developed by Peng *et. al*.[7a] A cysteine probe based on response-assisted electrostatic attraction was prepared by Wu *et. al*.[7b] A benzothiazole-based fluorescent probe by Strongin *et. al* using Michael addition-cyclization was reported to discriminate Cys from Hcy.[7c] Same cascade strategy was also used by Yoon, Zhu, and Chen *et. al*. for the development of a cysteine probe.[7d-f] In comparison, the pioneer works to specifically detect Hcy were so scarce and only provided by Strongin group and Li group: Strongin *et. al*. developed a colorimetric method for the selective detection of Hcy at neutral pH; Li *et. al*. first reported a highly selective luminescent chemosensor for Hcy over Cys.[8] Herein, we report a BODIPY-based turn-on NIR fluorescent probe **1** containing a *partially exposed* aldehyde group for the detection of Hcy over Cys, as well as for potential discrimination of Hcy from Cys.

Our long-going interest exists in the design and synthesis of BODIPY-based dyes as NIR fluorescent probes.[9] BODIPY dyes are known to be highly fluorescent, very stable, and exceptionally insensitive to the polarity of solvents as well as to pH, and widespreadly applied as fluorophores in probes or sensors.[10] NIR fluorescent dyes can greatly reduce background absorption, fluorescence, light scattering, and improve the detectable sensitivity and selectivity.[11] An example of a BODIPY-based NIR probe for thiols was recently documented by us.[12] Herein, NIR probe **1** (Scheme 1) we discovered takes advantages of conformationally restricted BODIPY for the long absorption maximum,[9] an aldyhyde group for the cyclization to allow detection of thiols,[13] as well as the weak fluorescence of *meso*-aldehyde containing BODIPY dye.[14]

It is well-known that the selective reaction of aldehydes with N-terminal cysteines to form thiazolidines has been used to label and immobilize peptides and proteins.[15] Recently, this chemistry was applied to sense Hcy and Cys since the sensors with aldehyde functionality can form a rapid 6- or 5-membered ring with 1,3- or 1,2-aminothiols.[6a] To simply synthesize a target compound, we first synthesized 1,3,5,7-tetramethyl-4,4-difluorobora-8-aldehyde-3*a*,4*a*-diaza-*s*-indacene.[14] The studies on this *meso*-aldehyde containing BODIPY dye demonstrated that no response to Cys or Hcy was observed, which are in consistent with the reported literature.[16] This suggested that the steric hindrance of the methyl groups at 1- and 7-positions in BODIPY prevented the cyclization of aldehyde with Hcy or Cys.[6a] The 1- and 7-protons in probe **1** may provide a reasonable steric

interation to allow detection of homocysteine and cysteine (Scheme 1). Therefore, compound **1** with the hydrongen atom at 1- and 7-positions in BODIPY was developed.

Utilizing 7-methoxy-4,5-dihydro-1H-benzo[*g*]indole,[17] probe **1** was successfully synthesized by the literature method (Scheme 1).[14] Solidstate structure of **1** was confirmed by X-ray crystallographic analysis. The dihedral angle of the O1-C1-C2-C3 was 13.6° .



Scheme 1 A turn-on NIR fluorescent probe **1** for detection of Hcy over Cys and its ORTEP diagram

The fluorescence sensing behavior of **1** toward Hcy or Cys was examined using a 20 μM solution of **1** in a MeCN/H_2O (8 : 2, v/v) solution at pH 7.2[18] in the absence and presence of Hcy or Cys (Fig. 1). The absorption maximum of probe **1** was 760 nm, and no fluorescence of **1** was observed in organic solvent (e.g. MeCN, $CHCl_3$). Similar to the reported BODIPY dye of 1,3,5,7-tetramethyl-4,4-difluorobora-8-aldehyde-3*a*,4*a*-diaza-*s*-indacene,[14] the n–π* transition resulted in the nonemissive behavior for probe **1**. The observed weak fluorescence (Φ_f = 0.06) of probe **1** in MeCN/H_2O (8 : 2, v/v) was attributed to the partial hydrolysis of the aldehyde group.[14] Upon addition of Hcy, the absorption maximum was blue-shifted to 661 nm, and the emission maximum was 678 nm in NIR region, respectively. A significant fluorescence enhancement by 30 folds was observed, and the fluorescent quantum yield was measured to be 0.92. In the case of Cys, upon addition of Cys under the same conditions, the absorption and emission maxima were blue-shifted to 672 and 682 nm respectively; the fluorescence intensity was increased by 9 folds (Φ_f = 0.39). It was noteworthy that the clear difference of probe **1** responded to Hcy/Cys from the emission spectra within the same time interval was noticed as shown in Fig. 1.

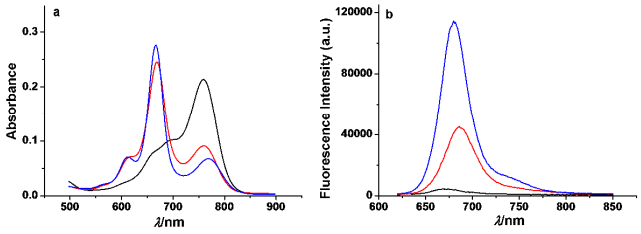


Fig. 1 (a) Absorption and (b) emission spectra (λ_{ex} = 620 nm) of probe **1** prior to (black curve) and after the addition of Hcy (blue curve) or Cys (red curve). Final concentrations of **1** and Hcy or Cys are 2.0 × 10^{-5} M and 2.0 × 10^{-3} M, respectively, in MeCN/H_2O (8 : 2, v/v; pH = 7.2). Data were collected before or 4 h after the addition of Hcy or Cys at 20 °C.

The selectivity profile of probe **1** towards various amino acids was provided. As shown in Fig. 2, among various amino acids, only Cys and Hcy lead to obvious fluorescence enhancement effect. It should be noted that probe **1** was more selective to Hcy over Cys, which suggests that **1** could be a Hcy-selective fluorescent probe.

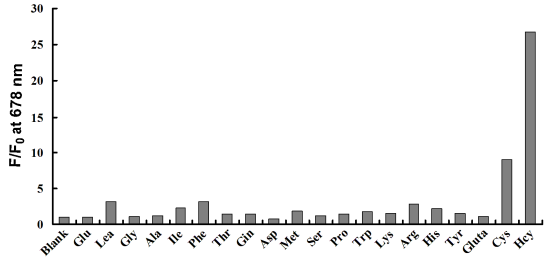


Fig. 2 Relative responses of probe **1** toward various analytes. Relative fluorescence intensity of 20 μM probe **1** in MeCN/H_2O (8/2, v/v; pH = 7.2) was measured at 678 nm (λ_{ex} = 620 nm) after incubation at 20 °C for 4 h in the presence of 2 × 10^{-3} M (final concentrations) of analytes.

The responses of probe **1** to various concentrations of Hcy were then studied by absorption and fluorescence spectroscopies (Fig. 3). The absorption spectra varied in a ratiometric response with an isosbetic point at 687 nm, which could be adopted for the quantitative estimation of concentration of Hcy (Fig. 3a). The fluorescence changed with increasing amounts of Hcy displayed a linear response to the amount of Hcy up to 200 μM (Fig. 3b). Probe **1** exhibited less intensified response to Cys as shown in both absorption and fluorescence spectra (Fig. S1†).

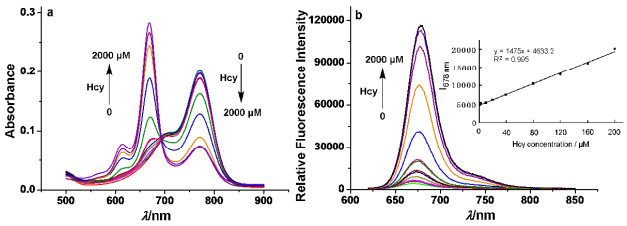
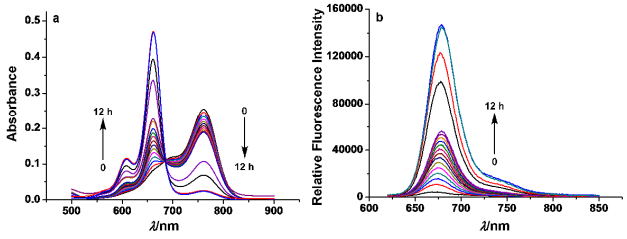


Fig. 3 Absorption and Fluorescence responses of 20 μM probe **1** (MeCN/ H_2O = 8 : 2, v/v) upon reacting with Hcy in 1, 2, 10, 20, 40, 80, 120, 160, 200, 400, 800, 1200, 1600, 2000 μM concentration after 4 h of incubation at 20 °C. The excitation wavelength was 620 nm. The inner panel displays the fluorescence enhancement of probe **1** toward Hcy of 1, 2, 10, 20, 40, 80 120, 160, 200 μM.



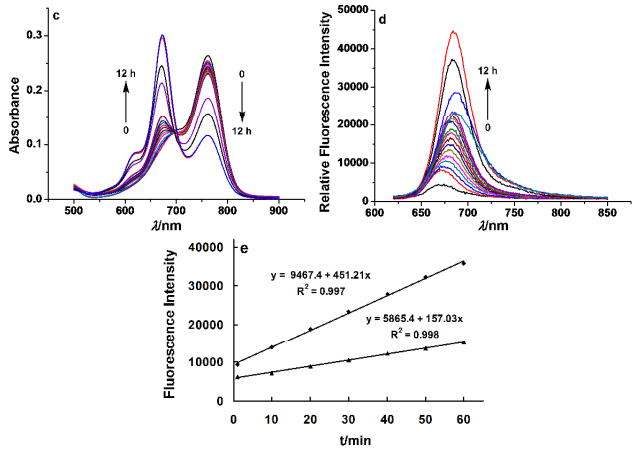


Fig. 4 Time-dependent changes (1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 240, 360, 540, 720 min.) of absorption (a, c) and fluorescence (b, d) spectra (λ_{ex} = 620 nm) of probe **1** (20 μM, MeCN/H_2O (8/2, v/v; pH = 7.2)) with Hcy or Cys (1.0×10^{-3} M) at 20 °C. Changes of time-dependent fluorescence intensity (e) of **1** in the presence of Hcy(◆) and Cys(▲). λ_{ex} = 620 nm.

For better understanding the difference between Hcy and Cys for the response to probe **1**, the time-dependent spectra of **1** were monitored in the presence of Hcy or Cys. As shown in Fig. 4a and 4c, upon addition of Hcy/Cys to probe **1**, both absorption spectra changed ratiometrically, and the formation of isosbestic point was noticed at 687 nm for Hcy, and 696 nm for Cys, respectively. Moreover, the fluorescence enhancement exhibits in a time dependent manner with a linear response (coefficient of 0.997 for Hcy, and 0.998 for Cys, respectively) in a time frame from 0 to 60 min (Fig. 4b, 4d and 4e). From the time dependent fluorescence intensity changes of probe **1**, the selectivity for Hcy over Cys is obvious. The difference on responsive rate (451.21/157.03 ≈ 3) provides a potential application to discriminate Hcy from Cys.

To gain further insight into the different effect of Hcy and Cys to probe **1**, the interference experiments were investigated (Fig. 5). Upon addition of Cys (0.1–50 equiv.) to probe **1** in the presence of Hcy (10 equiv.), it was found that the enhanced fluorescent intensity was 25% while 10 equiv. of Cys presents, and 50% with 50 equiv. of Cys. Therefore, the fluorescence intensity of probe **1** to Cys was not sensitive. In a stark contrast, upon addition of Hcy (0.1–50 equiv.) to probe **1** in the presence of Cys (10 equiv.), the effect of enhancement on fluorescent intensity was dramatic, with 2-fold enhancement for 10 equiv. of Hcy, and 5-fold for 50 equiv. of Hcy.

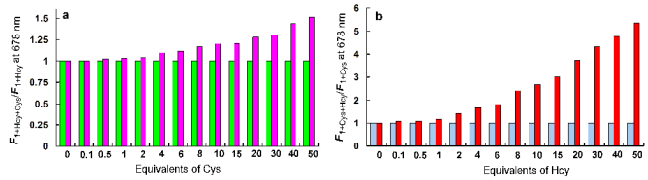


Fig. 5 Ratio of relative fluorescence intensity of probe **1** (20 μM, MeCN/H_2O (8/2, v/v; pH = 7.2)) in presence of various equivalents of Cys or Hcy at 678 nm (λ_{ex} = 620 nm) after 4 h of incubation at 20 °C. (a) The green bar represents the normalized fluorescence intensity after addition of Hcy (10 equiv.), and the pink bar represents the normalized fluorescence intensity after the subsequent addition of Cys (0 to 50 equiv.). (b) The blue bar represents the normalized fluorescence intensity after addition of Cys (10 equiv.), and the red bar represents the normalized fluorescence intensity after the subsequent addition of Hcy (0 to 50 equiv.).

In conclusion, the fluorescence turn-on NIR probe **1** containing a *partially exposed* aldehyde group at the *meso* position for the detection of homocysteine over cysteine was developed. Probe **1** is weakly fluorescent (Φ_f = 0.06 in MeCN/H_2O (8 : 2, v/v)). Upon addition of Hcy or Cys to probe **1**, the fluorescence (Φ_f = 0.92 for Hcy; Φ_f = 0.39 for Cys) was released, and ratiometric responses were observed, with isosbestic point noticed at 687 nm for Hcy and 696 nm for Cys, respectively. Probe **1** was demonstrated to be a Hcy-selective fluorescent probe with linear response to Hcy from 2 to 200 μM. From the time dependent fluorescence intensity changes of probe **1**, the selectivity for Hcy over Cys is obvious. The kinetic modes provide a potential application to discriminate Hcy from Cys. Further efforts for the water-soluble version of modifications are ongoing in our lab.

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Notes and references

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†Electronic Supplementary Information (ESI) available: Experimental details and ^1H, ^{13}C NMR spectra. See DOI: 10.1039/b000000x/
‡*These authors contributed equally to this work*

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