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Highly Selective Colorimetric and Fluorescent BODIPY Dyes for Sensing of Cysteine and/or Homocysteine

Qinghua Wu, Jinyuan Zhou, Yangchun Wu, Changjiang Yu, Erhong Hao, * *and Lijuan Jiao* *

Laboratory of Functionalized Molecular Solids, Ministry of Education, and Anhui Key Laboratory of Molecular Based Materials, College of Chemistry and Material Science, Anhui Normal University, Wuhu, 241000, China.

*To whom correspondence should be addressed. E-mail: haoehong@ahnu.edu.cn, jiao421@mail.ahnu.edu.cn

Abstract

By taking the advantage of "turn off" or "turn on" fluorescence of the visible light excitable BODIPYs, a selective fluorescent and colorimetric "naked eye" sensing of cysteine (Cys) and/or homocysteine (Hcy) in methanol-HEPES buffer (45 mM, pH = 7.2, v/v = 1/1) solution over various Communon amino acids and related thio-containing compounds has been achieved based on the reaction of the formyl groups on α- and β-formyl BODIPYs **1** and **3** with Cys/Hcy. β–FormylBODIPY **1** showed a selective response for both Cys and Hcy over the other analytes, with an apparent color change from green to light brown due to a dramatic quenching of the fluorescence intensity. In contrast, α–formylBODIPY **3** gave "naked eye" discrimination between Cys and Hcy, with a dramatic color change from light orange to yellowish green because of a significant increase of the green fluorescence intensity with the addition of Cys. Possible mechanism for sensing of Cys and/or Hcy by BODIPYs **1** and **3** was studied by NMR, HRMS and DFT calculation. α–formylBODIPY **3** has been applied to the biological imaging of Cys in living cells.

Keywords: BODIPY, formylation, dyes, cysteine, homocysteine.

1. Introduction

Cysteine (Cys) and homocysteine (Hcy) are essential biological thiols relevant to the growth of cells and tissues in living systems. Alternations of the levels of these specific biological thiols have been linked to a number of diseases, such as liver damage, neutral tube defects, Alzheimer's, cardiovascular, and various types of vascular and renal diseases.[1-2] Thus, their detection has attracted wide research interests,[3-6] with significant effort being devoted to the development of fluorescent, ratiometric, and colorimetrical sensors for Cys/Hcy.[7-14] Among those, colorimetric sensors are of particular interest and more favorable since they can be used for the so-called "naked eye" detection of analyte without resorting to any spectroscopic instruments. Usually, upon changes in their chemical environment, colorimetric sensors change color based on the large absorption shifts. On the other hand, most of these sensors, including several boron dipyrrin (BODIPY)-based sensors show high sensitivity toward thiol-containing compounds, and many show high selectivity for Cys and Hcy over other Communon thiols.[7-9] However, only a few of them could discriminate between Cys and Hcy, because of the structural similarity of these two species that differ only by a single methylene unit in their side chains.[9,14]

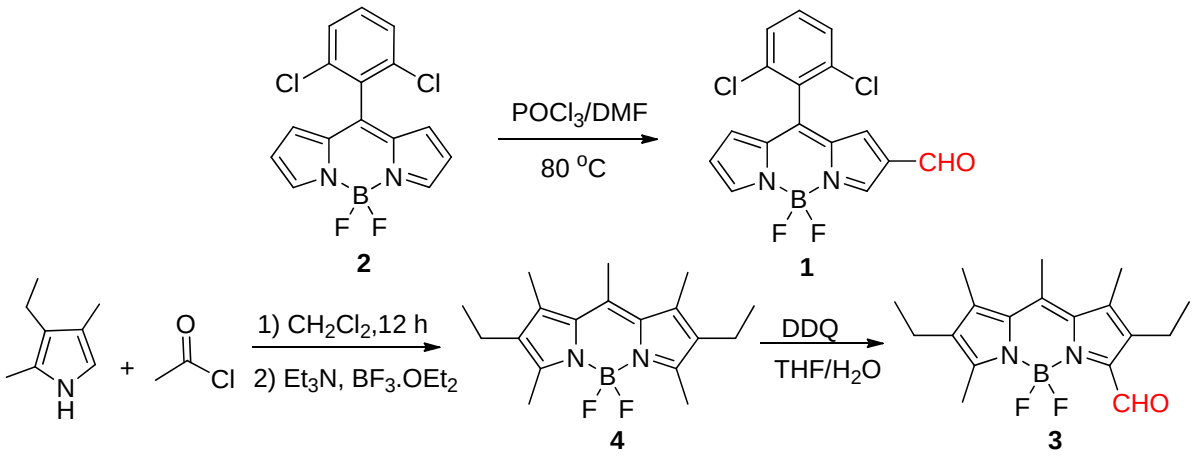
The mechanism for sensing of Cys/Hcy is mainly using the strong nucleophilicity of the thiol group via various types of reactions, including the cyclization and Michael addition. The installation of aldehyde groups to chromophores has successfully generated a number of probes suitable for the Cys/Hcy detection at different wavelengths, based on the specific reaction between aldehyde and Cys/Hcy to afford the corresponding thiazolidine and thiazine groups.[7e,12,15]

BODIPY dyes have excellent photophysical properties, and have attracted wide research interests in highly diverse fields.[16-17] With various types of formylBODIPYs readily available,[18-19] herein, we report β–formylBODIPY **1** and α–formylBODIPY **3** (Scheme 1) for the highly selective fluorescent and

colorimetric detection of Cys and/or Hcy in methanol-HEPES buffer (45 mM, pH = 7.2, v/v = 1/1) solution by taking the advantage of "turn off or "turn on" mechanism of the visible light excitable fluorescent molecules. The location of the formyl group greatly affected the selectivity of these formylBODIPYs: β–formylBODIPY **1** gave a selective response for both Cys and Hcy, while the α–formylBODIPY **3** showed "naked eye" discrimination between Cys and Hcy with a dramatic color change from light orange to yellowish green upon the addition of Cys.

2. Results and Discussion:

2.1 Synthesis of FormylBODIPYs



Scheme 1. Syntheses of BODIPYs **1** and **3**.

Synthetic routes for BODIPYs **1** and **3** were shown in Scheme 1. β–FormylBODIPY **1** was synthesized in 81% yield from the formylation of BODIPY **2**,[18] while α–FormylBODIPY **3** was prepared in 90% yeild from the oxidation of the α–methyl group on BODIPY **4** using DDQ in THF/water mixture solvent according to literature (Scheme 1).[19] The precursor BODIPY **4** was obtained from the acid-catalyzed condensation between 4–ethyl–3,5–dimethylpyrrole and acetyl chloride in dichloromethane, and the subsequent complexation with BF_3 by following a literature precedure.[19]

2.2 Characteristic Spectrum and Design of FormylBODIPY Probes

Photophysical properties of BODIPYs **1**-**4** in methanol were summarized in Table S1. The installation of a formyl group at the β-position of the BODIPY **2** gave a slight blue-shift in both the absorption (4 nm) and emission (1 nm) maxima for BODIPY **1**. High fluorescence quantum yield (0.75) was still observed for BODIPY **1** in methanol, giving a greenish solution under daylight due to the existence of strong visible light excited fluorescence. In contrast, nearly 90% of the fluorescence intensity in BODIPY **4** (as a well known laser dye PM 567, Φ = 0.91 in methanol[20]), disappeared in its oxidation to BODIPY **3** (Φ = 0.07 in methanol).

Since the installation of these aldehyde groups to the BODIPY chromophore has lead to either a red- or blue-shift of the spectra for BODIPY core, respectively. The conversion of these aldehyde groups to thiazolidines/thiazinanes by Cys and Hcy may generate a dual absorption bands for the BODIPY chromophore. As a result, α– and β–formylBODIPYs **1** and **3** would be suitable for the ratiometrical detection of Cys/Hcy. Recently, Shao and coworkers[7e] have used a *meso*–(4–formylphenyl)BODIPY for the Cys/Hcy detection. In their report, α–(4–formylstyrene)BODIPY, in which the aldehyde group is conjugated to the BODIPY core via a styrene bridge failed to react with Cys and Hcy under various temperature and pH conditions. The author attributed this failure of the reaction to the conjugative effect of the BODIPY core on the aldehyde group, which decreased the electropositivity of the carbon in the aldehyde group. In contrast to their report, our α–formylBODIPY **3**, in which the aldehyde group is direct connecting at the α–position of the BODIPY core gave a highly selective response for Cys.

2.3 Sensing of Biothiols by β–FormylBODIPY Probe 1

Initially, time dependent absorption spectral changes demonstrated that BODIPY **1** reacted with Cys in MeOH/HEPES (45 mM, pH 7.2, 1:1, v/v) to form a new species as expected (Figure 1a). The equilibrium was reached at around 60 min (Figure 1b), which is comparable to previous results

involving the reaction between aldehyde group and Cys.[21] All the tests were taken 1h later after addition of the Cys/Hcy to ensure the reaction reaching equilibrium.

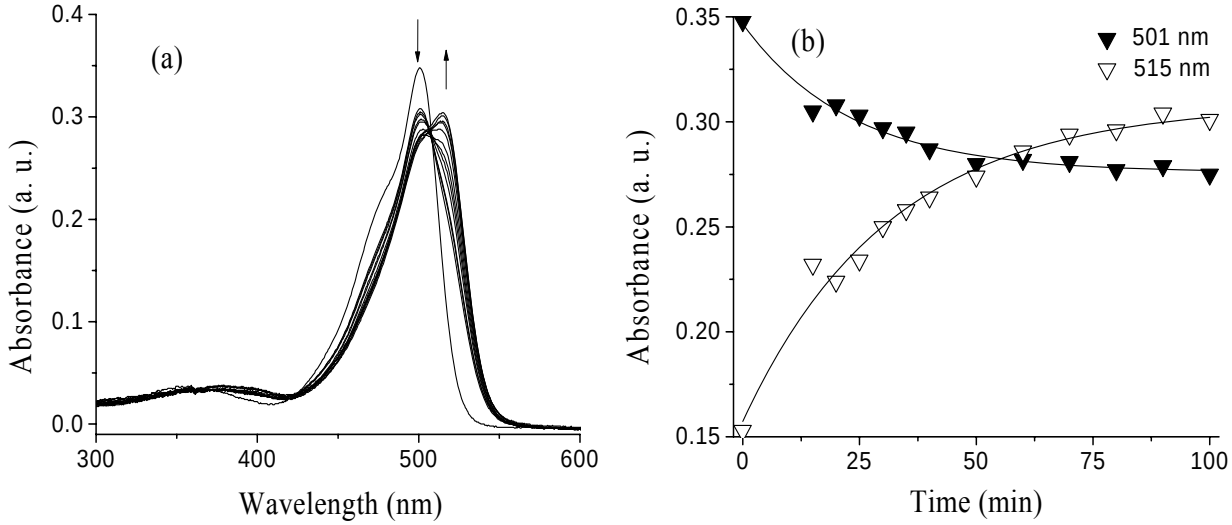


Figure 1. Time-dependent absorption spectral changes (a) and the absorption intensity changes (b) of **1** (1×10^{-5} M) in MeOH/HEPES (45 mM, pH 7.2, 1:1, v/v) with 200 equiv of Cys.

The sensing behavior of BODIPY **1** to Cys and Hcy was investigated by UV-vis and fluorescence spectroscopy. As shown in Figure 2a, BODIPY **1** (1×10^{-5} M) showed a maximum absorbance at 501 nm in MeOH/HEPES buffer solution (45 mM, pH = 7.2, v/v = 1/1). With increasing the concentration of Cys, it was gradually shifted to 516 nm with an isosbestic point at 507 nm. Similar bathochromic shift (from 501 to 516 nm, with an isosbestic point at 503 nm) was also observed for Hcy (Figure 2b). Associated with these red-shifts of the spectra, an apparent color change from green to light brown was observed upon addition of Cys and Hcy to the solution of BODIPY **1** as demonstrated in the inset picture in Figure 2a. Since both BODIPY **1** and its resultant product after reaction with Cys and Hcy have strong absorption at visible wavelength (Figure 2), this dramatic color change should be attributed to the quenching of its green daylight fluorescence upon addition of Cys and Hcy.

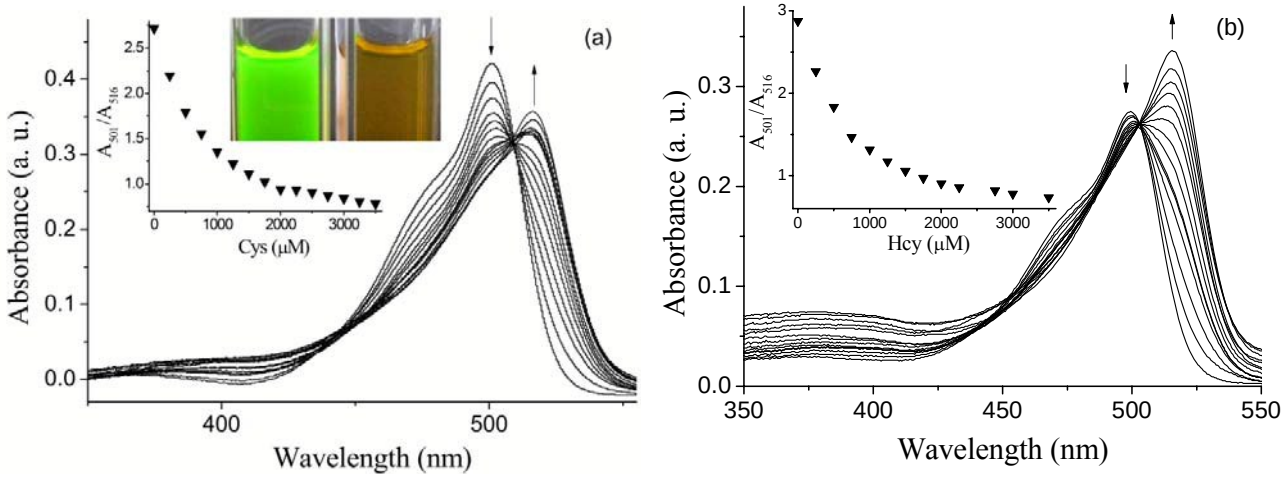


Figure 2. Absorption spectral changes of BODIPY **1** (10 μM) in MeOH/HEPES (45 mM, pH = 7.2, v/v = 1/1) solution upon addition of 0–350 equiv of Cys (a) or Hcy (b). Each spectrum was recorded after 60 min. Inset plots: ratiometric calibration curve A_{501}/A_{516} as a function of Cys (a) or Hcy (b) concentration. Inset picture: color changes of BODIPY **1** (1×10^{-4} M) with the addition of 200 equiv of Cys under daylight irradiation condition.

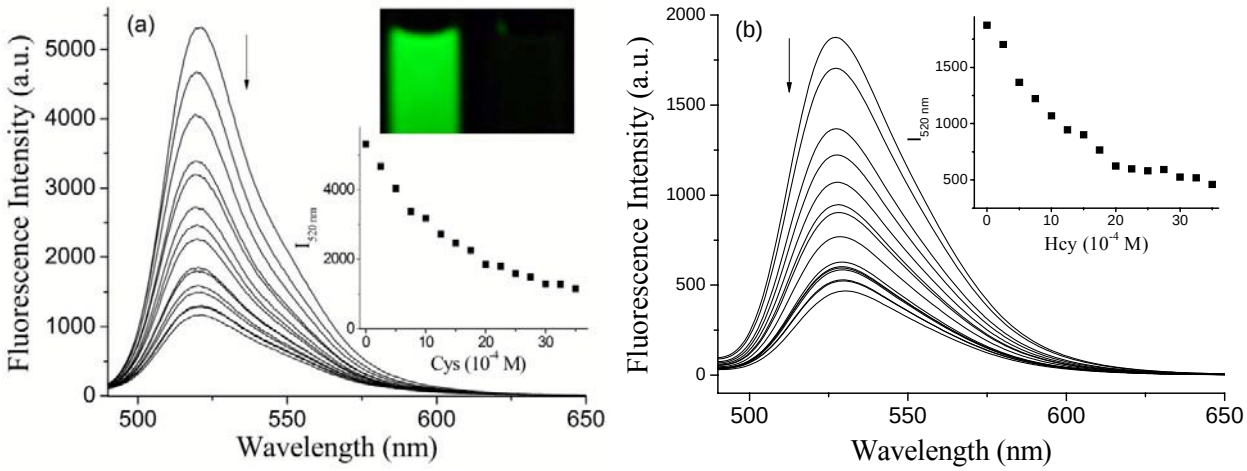


Figure 3. Fluorescence spectral changes of BODIPY **1** (1 μM) in MeOH/HEPES (45 mM, pH = 7.2, v/v = 1/1) solution upon addition of 0–350 equiv of Cys (a) and Hcy (b). Inset plot: titration curve of BODIPY **1** with Cys (a) or Hcy (b) (0-350 μM). Inset picture: color change of BODIPY **1** (1×10^{-4} M) with the addition of 200 equiv of Cys under UV-irradiation condition (365 nm).

Indeed, besides the visible color change, the addition of Cys and Hcy also caused a significant

quenching of the strong green fluorescence of BODIPY **1** in MeOH/HEPES buffer solution (45 mM, pH = 7.2, v/v = 1/1) as shown in Figure 3. The nearly shut down of the fluorescence of BODIPY **1** was observed with the addition of 350 equiv of Cys as shown in the inset picture in Figure 3a. A similar quenching of the fluorescence intensity was observed with the addition of Hcy (Figure 3b).

To investigate the selectivity of BODIPY **1** to Cys and Hcy, the optical response of BODIPY **1** to various amino acids and related thiol-containing compounds in a MeOH/HEPES (45 mM, pH = 7.2, v/v = 1/1) solution was investigated by UV-vis and fluorescence emission. BODIPY **1** (1×10^{-5} M) was treated with 200 equiv of Cys (or Hcy) or 400 equiv of a series of Communon amino acids and related thio-containing compounds, separately. As shown in Figure 4a, only Cys and Hcy caused an apparent red-shift of the absorption maximum (from 501 to 516 nm). By contrast, other amino acids caused no discernible changes in absorption for BODIPY **1**, and do not interfere with its Hcy/Cys response.

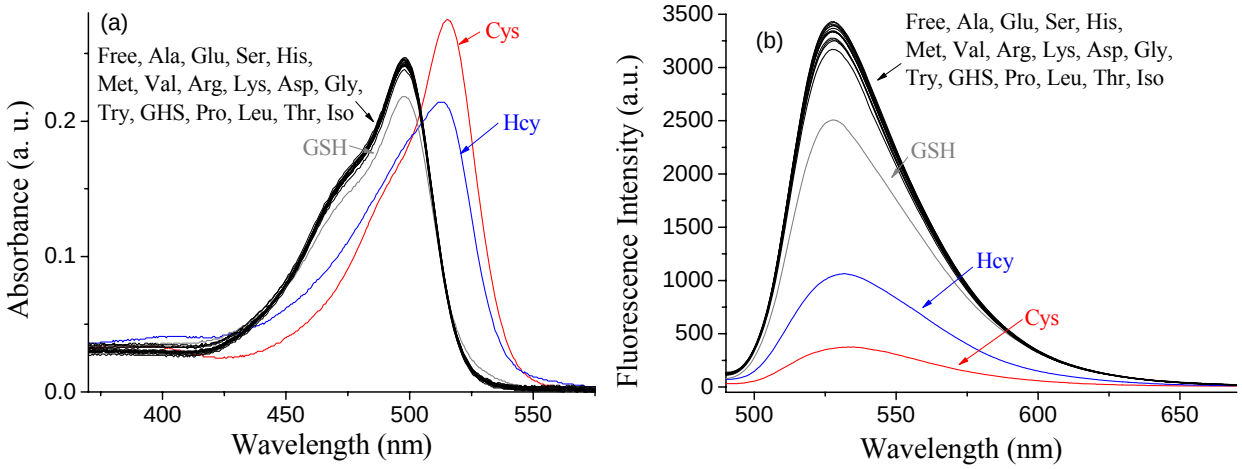


Figure 4. Absorption (a, 1×10^{-5} M) and emission (b, 1×10^{-6} M, λ_{ex} = 480 nm) spectra of BODIPY **1** with or without 200 equiv of Cys/Hcy or 400 equiv of various other amino acids and thio-containing compounds in a MeOH/HEPES (45 mM, pH = 7.2, v/v = 1/1) solution. Slit was set at 5.0 nm.

In addition, a selective fluorescence response for Cys and Hcy was also observed for BODIPY **1**, respectively (Figure 4b). The addition of 200 equiv of Cys or Hcy caused a significant quenching of the

fluorescence intensity of BODIPY **1**. The fluorescence quantum yield of **1** in this buffer solution was 0.61. Upon addition 200 equiv of Cys, nearly 87% fluorescence was quenched, giving fluorescence quantum yield of 0.08 for **1**–Cys. In contrast, no obvious changes of the fluorescence intensity were observed with the addition of 400 equiv of other amino acids and related thiol-containing compounds, separately. BODIPY **1** exhibits higher selectivity for Cys and Hcy over other Communon amino acids and related thio-containing compounds (Figure S1 in Supporting information): the further addition of 400 equiv of other competing species into the solution of **1**–Cys or **1**–Hcy caused little variations in fluorescence intensity, in comparison with **1**–Cys or **1**–Hcy alone.

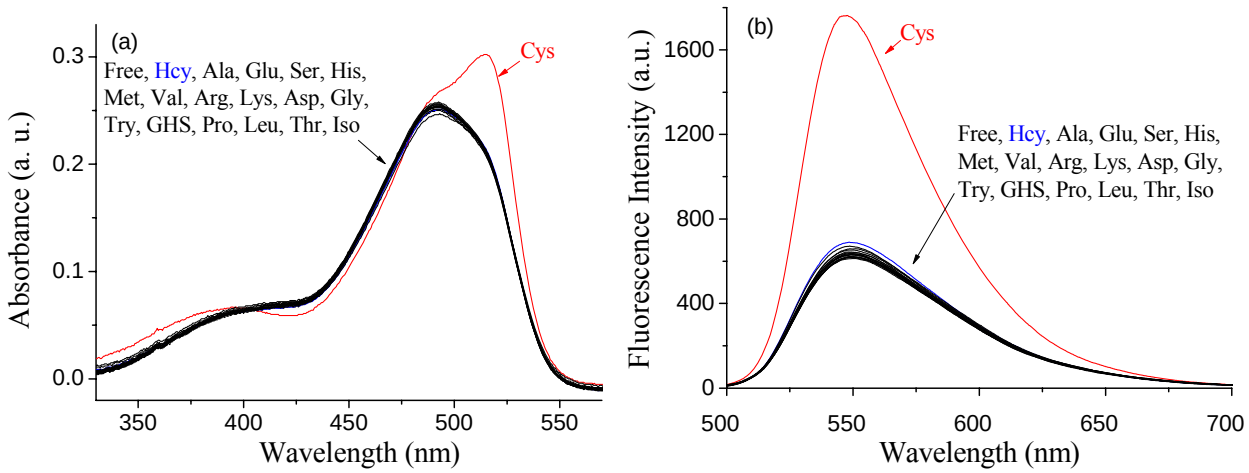


Figure 5. Absorption (a, 1×10^{-5} M) and emission (b, 1×10^{-6} M, $\lambda_{ex} = 480$ nm) spectra of BODIPY **3** with or without 200 equiv of Cys/Hcy or 400 equiv of various other amino acids and thio-containing compounds in a MeOH/HEPES (45 mM, pH = 7.2, v/v = 1/1) solution.

2.4 Sensing of Biothiols by α–FormylBODIPY Probe 3

Unlike β–formylBODIPY **1**, the α–formylBODIPY **3** gave a selective response to Cys over other amino acids, including Hcy (Figure S2). BODIPY **3** (1×10^{-5} M) in a MeOH/HEPES (45 mM, pH = 7.2, v/v = 1/1) solution was treated with 200 equiv of Cys (or Hcy) or 400 equiv of a series of Communon amino acids and related thio-containing compounds, separately. The addition of Cys caused a 25 nm

red-shift of maximum absorption (from 491 nm to 516 nm) of BODIPY **3** as shown in Figure 5a. In contrast, no obvious absorption change was observed with the addition of increasing concentration of Hcy and other amino acids, separately.

The addition of Cys also caused a significant increase in the fluorescence intensity of BODIPY **3**. No obvious changes of the fluorescence intensity were observed for other competing species, including Hcy (Figure 5b). Unlike β–formylBODIPY **1** which shows selectivity for both Cys and Hcy, the α–formylBODIPY **3** is only highly selective for Cys. The high selectivity of BODIPY **3** for Cys was further confirmed by the competition experiment. As shown in Figure S3, the further addition of 400 equiv of other competition species including Hcy into the solution of **3**–Cys caused no further enhancement of the fluorescence intensity in comparison with **3**–Cys alone.

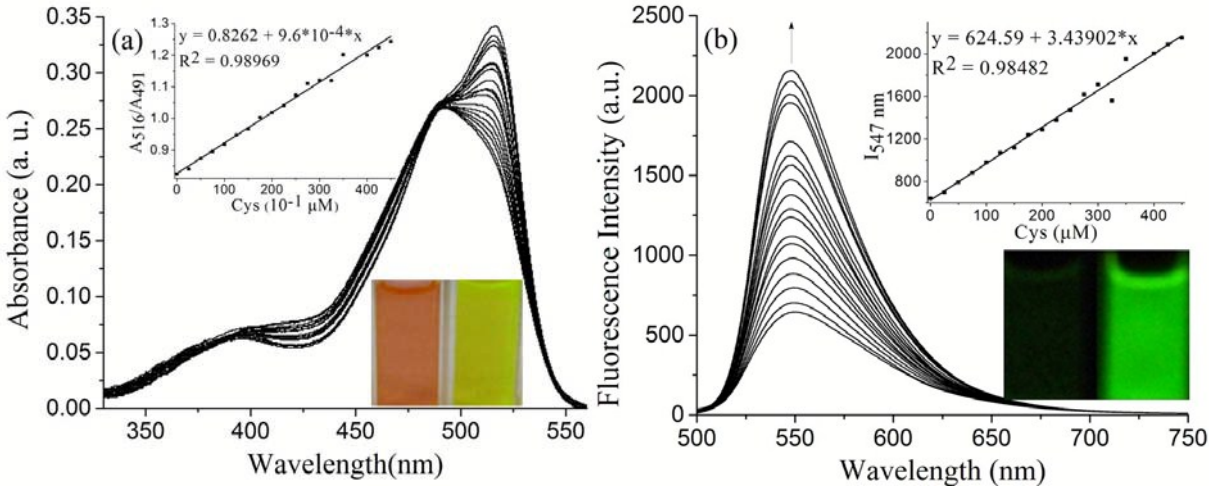


Figure 6. Absorption (a, 1×10^{-5} M) and fluorescence (b, 1×10^{-6} M) spectral changes of BODIPY **3** in MeOH/HEPES (45 mM, pH = 7.2, v/v = 1/1) solution upon addition of Cys. Each spectrum was recorded after 60 min. Inset plots: (a) ratiometric calibration curve A_{516}/A_{491} as a function of Cys concentration (0–4.5×10^{-3} M); (b) fluorescence titration curve of BODIPY **3** with Cys (0–4.5×10^{-4} M). Inset pictures: color changes of BODIPY **3** (1×10^{-4} M) with the addition of 200 equiv of Cys at (a) daylight or (b) UV irradiation (365 nm) conditions.

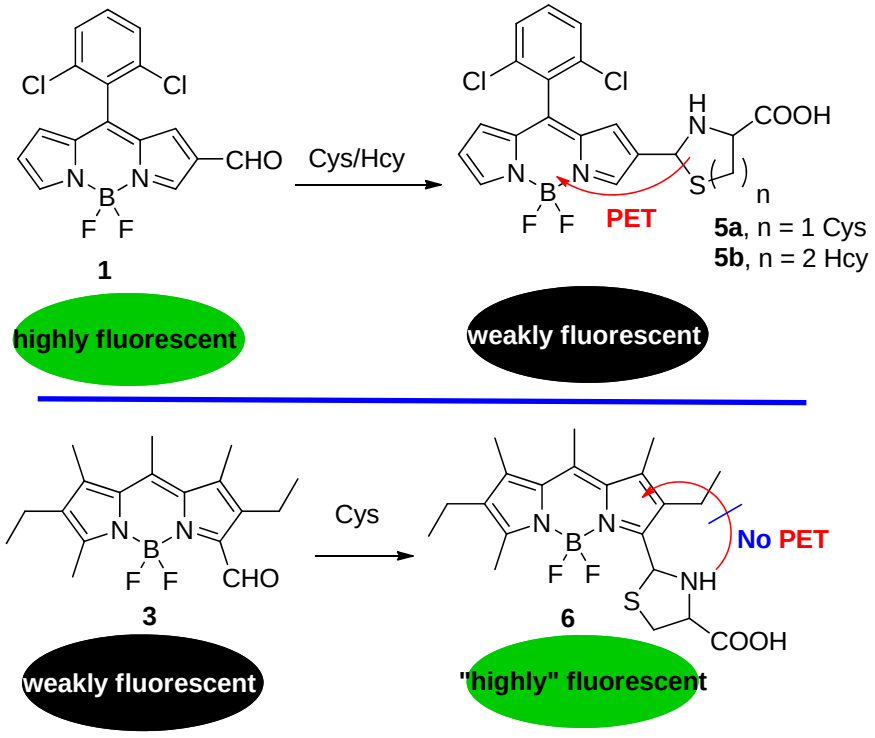
Subsequently, the UV-vis and fluorescence titrations of BODIPY **3** toward increasing amount of Cys were performed as shown in Figure 6. With the addition of increasing concentration (0–450 equiv.) of Cys, the absorption peak of BODIPY **3** was red shifted from 491 to 516 nm. When the changes at 491 and 516 nm were monitored, the absorbance ratios at these two wavelengths are linearly related to the concentration of Cys (0–4500 μM), which means it can detect the normal human physiological Cys concentration. In consistent with this red-shift, a distinct color change from light orange to yellowish green was observed under ambient light condition that could be observed by naked eyes. In contrast to the ordinary color change, this dramatic color change observed here may be due to the generation of the strong green daylight fluorescence for the initially weak daylight fluorescent solution BODIPY **3** (light brown) upon addition of Cys.

The addition of increasing concentration (0–450 equiv.) of Cys also caused a significant increase of the fluorescence intensity of BODIPY **3** as shown in Figure 6b. BODIPY **3** has very low fluorescence quantum yield 0.09 in the buffer solution. Upon addition of Cys, nearly 3 fold enhanced fluorescence was observed, giving fluorescence quantum yield of 0.26 for **3**–Cys. As a result, this dramatic increasing of the fluorescence intensity was visualized as a dramatic color change from dark to bright green under hand-hold UV-irradiation lamp (365 nm). Competition experiments for BODIPY **3** (Figure S3 in Supporting information) also show little variations in fluorescence intensity, in comparison with **3**–Cys alone indicating higher selectivity for Cys over other Communon amino acids including Hcy. Moreover, there was a good linearity between the fluorescence intensity and the concentrations of Cys in the range of 0–400 uM with a detection limit of 3.3 uM (Fig. 6b). Similarly, the observed absorption change A_{516}/A_{491} is proportion to the concentrations of Cys in the range of 0–4 mM with a good

linearity (Fig. 3a). These results implied that chemodosimeter **3** might detect Cys qualitatively and quantitatively by the fluorescence spectrometry method.

2.5 Mechanism of 1 and 3 in sensing Cys and Hcy

Aldehyde group has been known to specifically react with Cys and Hcy to afford thiazolidine and thiazinane,[7e, 12, 15, 21] respectively. The selective "turn off" sensing of Cys/Hcy (BODIPY **1**) and "turn on" sensing of Cys over HCy (BODIPY **3**) are remarkable, which promoted us to study the possible sensing mechanism. In case of our β–formylBODIPY **1**, this cyclization reaction will give BODIPYs **5a** and **5b** as shown in Scheme 2. The formation of **5a** and **5b** has been confirmed by exact ESI-TOF MS and ^{1}H-NMR titration (Figure S5 in Supporting information). Intramolecular photoinduced electron transfer (PET) from thiazolidine and thiazinane to BODIPY core was proposed to quench the fluorescence in **5a** and **5b** as shown in Scheme 2.



Scheme 2. Proposed mechanism for sensing of Cys and/or Hcy by BODIPYs **1** and **3**

While for BODIPY **3** which can selectively discriminate Cys over Hcy, Cys may form the generally more favored 5-membered ring heterocycle **6** upon reaction with aldehyde group, as compared to Hcy, which gives 6-membered ring product, since the thiol group of Cys is sterically less hindered than those of Hcy and GSH as reported.[21c-d, 22] Following these previous results, we attributed the different sensing behavior of these two formylBODIPYs to the sterical hindrance effect aroused from the adjacent pyrrolic alkyl substituent in BODIPY **3**, which makes it more difficult to react with Hcy to form six-member-ring thiazinane product. Another possible origin of this preference could be rationalized on the basis of the different nucleophilicities of the thio groups, since the pKa of Cys (8.30) is lower than those of Hcy (8.87) and GSH (9.20).[22d, 23]

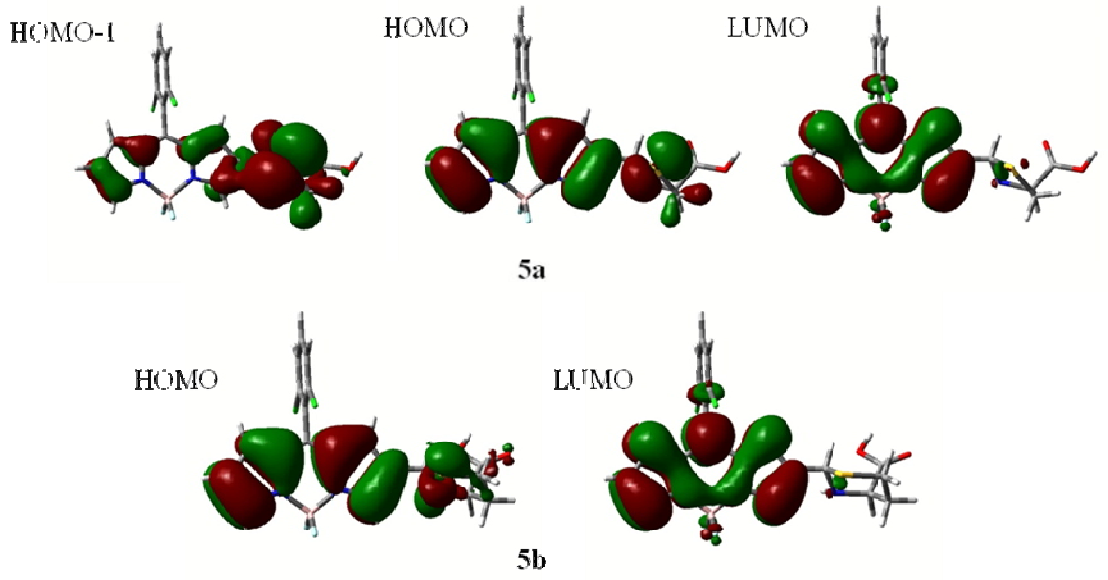


Figure 7. Mechanism of the PET effect: main frontier MOs of BODIPY **5a** and **5b**, calculated with DFT/TDDFT using Gaussian 09.

2.6 Quantum Chemical Calculations

BODIPY **3,** which shows a very weak fluorescence, gave strong green fluorescent BODIPY **6** after reaction with Cys (Scheme 2). The formation of **6** has been detected by TLC and confirmed by exact

ESI-TOF MS and ^{1}H-NMR titration (Figure S6 in Supporting information). In contrast to BODIPY **5**, BODIPY **6** shows strong green fluorescence which indicates no PET from thiazolidine to BODIPY core. To help confirm the PET mechanism, theoretical calculations based on density functional theory (DFT) and time-dependent DFT (TDDFT) were performed for BODIPYs **1**, **3**, **5a**, **5b** and **6** (see Supporting Information for more details). The TDDFT calculated absorption spectrum (Figure S12 in Supporting information) is in good agreement with the UV-vis absorption spectral (Figures 4 and 5). The strategy we used in the rationalization of the fluorescence properties of the molecular sensors is to study the properties of the lowest-lying singlet excited state (S1), which is responsible for the fluorescence emission (Kasha's rule).[24] The S1 state will be a dark state if the oscillator strength of the S0→S1 transition is close to zero (forbidden transition).

For the BODIPY **1**, a fully allowed S0 to S1 transition was found (Figure S7 in Supporting information). The HOMO→LUMO transition is mainly consisted of the S1 (S0→S1, excitation energy of 421.2 nm, oscillator strength f = 0.5492). This result indicates that S0→S1 transition is quantum mechanically allowed, as well as the S1→S0 transition. Thus, BODIPY **1** is probably fluorescent. By examining the molecular orbitals we found the HOMO and LUMO of BODIPY **1** show significant overlap, so there is no charge transfer and the fluorescence is intensified when compared with the BODIPYs **5a** and **5b**. The TDDFT calculated parameter of BODIPY **5a** shows S0→S1 with excitation energy of 464.70 nm, oscillator strength f = 0.2787; S0→S2 with excitation energy of 423.7 nm, oscillator strength f = 0.2783. This two excited states may all contribute to the maximum absorption. The S0→S1 is mainly contributed by HOMO→LUMO and S0→S2 is contributed by HOMO-1→LUMO. The singlet excited states of BODIPY **5b** shows the S1 is a dark state, supported by the oscillator strength excitation of f = 0.0461. The main component of the allowed S0→S2 transition

(excitation energy of 441.18 nm, oscillator strength f = 0.4114) of BODIPY **5b** is localized at HOMO→LUMO. S2 can be populated by direct photo-excitation, then *via* internal conversion to the S1 state. However, S1→S0 is non-radiative. The TDDFT calculations also predict, to some extent, electron transfer (ET) from the thiazolidines/thiazinanes unit to BODIPY unit for BODIPY **5a** and **5b**, which is in agreement with the normal PET effect, or a-PET (fluorophore as the electron acceptor in PET), [25] which leads to fluorescence quenching.

The oscillator strength of BODIPY **6** is f = 0.4993 which it's mainly localized at HOMO→LUMO. This result indicates that S0→S1 transition is quantum mechanically allowed. For BODIPY **6** the frontier MOs of transition state are significant overlap (Figure S10 in Supporting information) as those of BODIPY **1**, so there is less chance for possible electron transfer (ET) from the thiazolidines unit to BODIPY unit. These results may explain why the fluorescence of BODIPY **6** is not quenching.

2.7 Live-cell Imaging

To examine the possibility of probe **3** for the image of Cys in living cells, we further applied **3** which is dissolved in DMSO/HEPES (v/v = 1/99) to human gastric cancer SGC7901 cells. After incubated with 5 mM Cys in culture medium for 30 min, washed three times with PBS, and then incubated with 10 μM **3** for 2h, the cells showed strong green fluorescence (Figure 8a) when imaged with a fluorescence microscope using FITC (green channel) filter set. In contrast, when cells were pretreated with 20 mM NEM (N-ethylmaleimide, as a thio-reactive compound) for 30 min, washed three times with PBS, and then incubated with 10 μM **3** for 2h, only very weak green fluorescence (Figure 8c) was recorded using green channel. Bright-field transmission images of cells confirmed that the cells were viable throughout the imaging experiments (Figures 8b and 8d). These results suggest **3** can enter cells and make fluorescent turn-on detection of Cys in cells.

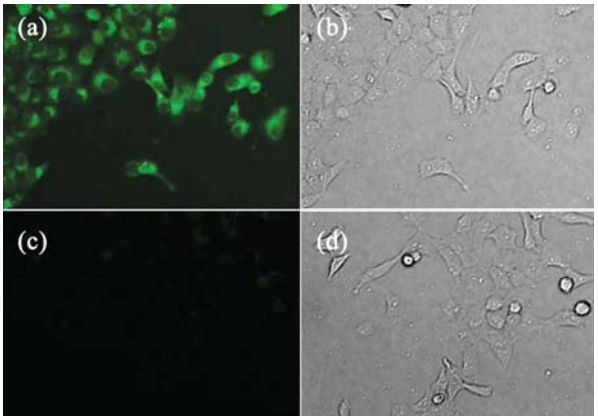


Figure 8. Fluorescence (a) and brightfield (b) image of living SGC7901 cells pretreated with 5 mM Cys for 30 min and then incubated with 10 μM **3** for 2h. Fluorescence (c) and brightfield (d) image of living SGC7901 cells pretreated with 20 mM NEM for 30 min, washed with PBS and then incubated with 10 μM **3** for 2h.

3. Conclusions

In summary, we have developed highly selective probes for cysteine and/or homocysteine using α– and β–formylBODIPYs: β–formylBODIPY **1** showed a selective response for cysteine and homocysteine over various amino acids and related thio-containing compounds with a dramatic color change from green to light brown, while α–formylBODIPY **3** showed a highly selective "naked-eye" detection of cysteine over homocysteine and other competing species with color changes from light orange to yellowish green. Our highly selective "naked-eyes" sensing of cysteine and/or Hcy is achieved by using visible light excitable formylBODIPY chromophores, based on the turn-off and turn-on daylight fluorescence of the chromophore caused by the addition of cysteine and/or homocysteine.

4. Experimental Section

General. Reagents were purchased as reagent-grade and used without further purification unless otherwise stated. Solvents were used as received from Communercial suppliers unless noted otherwise.

Double distilled water was used for spectra detection. Methanol was HPLC grade without fluorescent impurities. All reactions were performed in oven-dried or flame-dried glassware unless otherwise stated, and were monitored by TLC using 0.25 mm silica gel plates with UV indicator (60F-254). ^{1}H and ^{13}C NMR are obtained on a 300 MHz NMR spectrometer at room temperature. Chemical shifts (δ) are given in ppm relative to $CDCl_3$ (7.26 ppm for ^{1}H and 77 ppm for ^{13}C) or to internal TMS (0 ppm for ^{1}H). UV-visible absorption spectra were recorded on a Shimadzu UV2450 spectrophotometer (190-900 nm scan range). Fluorescence emission spectra were recorded on a Hitachi F-4600 FL Spectrophotometer. Stock solutions of amino acids were prepared in distilled water. The stock solutions for BODIPYs **1** and **3** (1 mM) were prepared in anhydrous methanol, and were used to prepare the methanol-HEPES buffer (45 mM, pH = 7.2, v/v = 1/1) solution.

Synthesis of β-formylBODIPY 1: A mixture of DMF (3 mL, 39 mmol) and $POCl_3$ (3 mL, 32 mmol) was stirred in the ice-cold condition for 5 min under argon. After warming up to room temperature, the reaction mixture was further stirred for 30 min. Then BODIPY **4** (60 mg, 0.18 mmol) in $ClCH_2CH_2Cl$ (10 mL) was added into the reaction mixture. After raising temperature to 80 ^{o}C, the reaction mixture was further stirred for 10 h, cooled to room temperature, and slowly poured into a saturated aqueous solution of K_2CO_3 (200 mL) under the ice-cold condition. After warming up to room temperature, the reaction mixture was further stirred for 1 h, extracted with CH_2Cl_2 (3 × 100 mL). The organic layer was combined, washed with water (2 × 100 mL), dried over anhydrous $NaSO_4$ and removed solvent under reduced pressure. The crude product was further purified using silica gel column chromatography (CH_2Cl_2/hexane = 3/2, v/v) to give the β-formylBODIPY **1** as reddish-brown powder in 72% yield (47 mg). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.84 (s, 1H), 8.29 (s, 1H), 8.23(s, 1H), 7.53-7.49 (m, 3H), 7.06 (s, 1H), 6.95 (d, J = 4.5 Hz, 1H), 6.72 (d, J = 4.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 184.7, 151.4,

143.6, 142.9, 137.5, 134.9, 133.9, 133.4, 131.8, 130.2, 128.4, 126.8, 122.3. HRMS (EI) calcd for $C_{16}H_9BCl_2F_2N_2O$ $[M]^+$ 364.0153, found 364.0156.

Synthesis of α-FormylBODIPY 3: To a degassed solution of **4** (64 mg, 0.2 mmol) in 8 mL THF/H_2O (v/v = 100/1) was dropwisely added a solution of DDQ (180 mg, 0.8 mmol) in 2 mL THF at 0 °C. The reaction mixture was slowly raised to room temperature and left stirred overnight. The reaction was quenched by adding 20 mL of water. The resulting mixture was extracted by 50 mL of dichloromethane and washed with water. Organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum to get the crude product. The crude product was further purified using column chromatography (silica gel, hexane/EtOAc = 6/1, v/v) to give the desired BODIPY **3** in 90% yield (60 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.35 (s, 1H), 2.80 (brs, 2H), 2.69 (s, 3H), 2.60 (s, 3H), 2.45-2.47 (m, 2H), 2.44 (s, 3H), 2.39 (s, 3H), 1.08 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): 186.2, 164.2, 142.3, 142.1, 138.9, 137.6, 137.0, 136.6, 132.5, 132.1, 17.9, 17.8, 17.3, 15.1, 14.8, 14.5, 13.6, 13.3. ESI-MS calcd for $C_{18}H_{23}N_2OBF_2$ $[M^+]$: 332.2, found: 332.2.

Amino Acids Titration of BODIPYs 1 and 3. UV-vis and fluorescence titrations were carried out in a methanol-HEPES buffer (45 mM, pH = 7.2, v/v = 1/1) solution of BODIPYs **1** and **3**. Typically, a few microliters of stock solution of the analytes (amino acids and related thio-containing compounds) were added into 3 mL of buffer solutions of BODIPYs **1** and **3**, respectively. The addition was limited to 10 μL so that the volume change was insignificant. The samples containing various analytes or different concentrations of Cys/Hcy were kept at 37 ^{o}C for 1 hour before recording the UV-vis absorption and fluorescence emission spectra of the samples. For the fluorescence emission measurement, the samples were excited at 480 nm, and emission was collected from 490 to 700 nm.

The Detection Limit. The detection limit of BODIPY **3** toward Cys was calculated based on the fluorescence titration. BODIPY **3** was employed at 1 μM and the slit was adjusted to 5.0 nm. To determine the S/N ratio (signal-to-noise ratio), the emission intensity of **3** without Cys was measured by 10 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and the Cys concentration could be obtained in the 0 -400 μM (R^2 = 0.9848), as shown in Fig. 6b. The detection limit is then calculated with the equation: detection limit = $3\sigma_{bi}/m$, where σ_{bi} is the standard deviation of blank measurements, m is the slope between intensity versus sample concentration.

Fluorescence Imaging: Human gastric cancer SGC7901 cells were cultured in DMEM supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin sulfate (100 μg/ml), and maintained at 37°C with 5% CO_2 in a humidified incubator. One day before imaging, cells were seeded in 6-well flat-bottomed plates in an atmosphere of 5% CO_2, 95% air at 37°C. Fluorescence imaging of intracellular cysteine was observed under OLYMPUS-IX71 inverted fluorescence microscope and imaged using FITC (greeen channel) filter set or TRITC (red channel) filter set. The microscope settings (brightness, contrast, and exposure time) were held constant before and after pretreatment of cells with N-ethylmaleimide to compare the relative intensity of intracellular cysteine fluorescence.

The cells were treated with 5 mM Cys in culture media for 30 min at 37°C with 5% CO_2 in a humidified incubator. After washing with phosphate buffered saline (PBS) to remove the remaining NEM, the cells were further incubated with 10 μM of **3** in culture media (containing less than 1% DMSO from the 1 mM stock solution of **3** in DMSO) for 30 min at 37°C with 5% CO_2 in a humidified incubator. For the control experiment, the cells were treated with 20 mM N-ethylmaleimide (NEM) in culture media for 30 min at 37°C with 5% CO_2 in a humidified incubator. After washing with PBS to

remove the remaining NEM, the cells were further incubated with 10 μM of **3** in culture media for 30 min at 37°C with 5% CO_2 in a humidified incubator. Fluorescence imaging was then carried out after washing the cells with the PBS.

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Electronic supplementary information (ESI) available. Experimental section (instrumentation, relative determination of fluorescence quantum yield Φ), calculated frontier orbitals, absorption and fluorescence emission spectra, spectroscopic/photophysical data of BODIPYs **1**-**4**, and copies of NMR spectra and Mass spectra. For ESI or other electronic format see:

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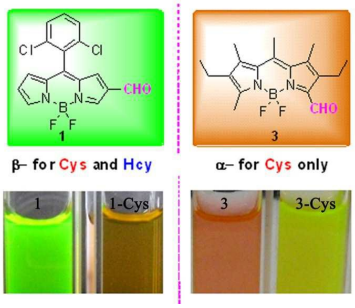
Graphical Abstract



Selective “naked eye” and fluorescent sensing of biothiols by visible-light excitable α- and β-formyl BODIPYs **1** and **3** was developed.