Installation of efficient quenching groups of a fluorescent probe for the specific detection of cysteine and homocysteine over glutathione in solution and imaging of living cells

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ABSTRACT
Herein, we report the synthesis and characterisation of a new fluorescent probe 4-(7-nitro-benzo[1,2,5]oxadiazol-4-yl)-benzaldehyde (NBOB) installed with quenching groups for highly selective and sensitive sensing of biothiols. The probe itself is non-fluorescent due to the presence of quenching groups and photoinduced electron transfer (PET) process. Thus, sensitivity of the probe towards thiols was significantly improved by quenching effects. NBOB has been shown to exhibit selective reactivity towards cysteine (Cys) and homocysteine (Hcy) over glutathione (GSH) under stoichiometric conditions. The response mechanism was proved by 1H NMR, LCMS and theoretical calculation. The probe NBOB has been shown to react with Cys present in Vero cells by fluorescence microscopy.

1. Introduction
Biological thiols, such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), play crucial roles in many biological systems, especially biological redox status and abnormal levels of these biothiols are related to a number of diseases (1–3). For example, Cys deficiency is associated with many syndromes such as slow growth in children, hair depigmentation, oedema, lethargy, liver damage, loss of muscle and fat, skin lesions and weakness (4, 5). High concentrations of Hcy are linked with cardiovascular disease and Alzheimer’s disease (6, 7). GSH deficiency is also involved in many diseases such as liver damage, leucocyte loss, cancer, AIDS and neurodegenerative diseases (8, 9). Therefore, the rapid, convenient, selective and sensitive
detection of trace amounts of these biothiols is of great importance.

Among various detection methods, fluorescent sensors-based detection has proven to be one of the most convenient methods due to its simplicity, low cost, high sensitivity and great effective molecular tools for intracellular bioimaging. Accordingly, great efforts have been devoted in the past decade to develop reaction-based fluorescent sensors for the detection of thiols in living systems (10–13).

Most of the existing probes utilise the strong nucleophilicity of the thiol group, for which different reaction-based mechanisms have been operated such as Michael addition (14, 15), cyclisation reaction with aldehyde (16, 17), cleavage reaction such as cleavage of sulfonamide and sulfonate esters by thiols (18–20), nucleophilic substitution (12, 21), disulfide exchange reaction (18, 22) and others (23–25) are explored. These sensors distinguish biothiols from other amino acids. However, it is still a challenge to discriminate among thiol-containing molecules with their similar structures and reactivities. By means of the cyclisation of Cys/Hcy with aldehydes or acrylates, pioneered by the Strongin group, selective detection of Cys/Hcy over GSH was achieved (14, 15). However, many of them suffer from a long response time (18, 24, 26–31), low sensitivity (18, 23, 24), a complicated synthesis process (12, 28, 31, 32) or need short UV light excitation (21, 24, 32, 33). On the other hand, the development of reaction-based fluorescent sensors for quick discrimination of Cys/Hcy without interference from GSH remains a tough task. Thus, new thiol probes with improved properties are still expected to be developed.

To develop highly reactive and sensitive fluorescent probes for biothiols, we herein introduce a novel design strategy through installation of quenching groups along with one group is highly sensitive to nucleophilic addition within the same framework of a fluorophore. A fluorophore is attached to a reactive aldehyde group and has been reported as a useful reactive unit for thiol detection in the previous studies (34–36). However, the presence of quenching groups such as an aromatic aldehyde and nitro groups can quench the fluorescence due to the existence of a low-lying n–π* transition explains the efficient intersystem crossing process and enhance photoinduced electron transfer (PET) effect (37). In general, the fluorescence of aromatic hydrocarbons bearing an –NO_2 substituent cannot be observed. That being said, many nitroaromatics do exhibit fluorescence. Our choice of fluorophore, NBD, is remarkably found to exhibit weak fluorescence despite the presence of –NO_2 substituent (38). We envisage that fluorescence will be heavily quenched through the combined quenching groups and hence the probe 4-(7-nitrobenzo[1,2,5]oxadiazol-4-yl)-benzaldehyde (NBOB) is non-fluorescent (Φ = 0.021). The background fluorescence will be minimised, and as a result, the fluorescence turn-on fold can be significantly increased upon reaction with thiols. However, when treated with biological thiols, probe NBOB exhibits a very rapidly, time-dependent enhancement of its fluorescence signal (Φ = 0.392). A single-reactive substance can only react with one of the reactive (quenching) groups, and the presence of another quenching group can still partially quench the fluorescence. Such a strategy should greatly improve the selectivity of the fluorescent probe towards specific analyte detection. Based on our design strategy, we hypothesise that the quenching probe has exhibits several meritorious features for sensing biothiols such as high selectivity and sensitivity, a rapid detection process with colorimetric and fluorescence dual signal changes, and shows excellent performance in living cell imaging.

2. Experimental

2.1. General information and materials

The 1H and 13C NMR spectra were recorded on a Bruker AM-400 spectrometer using Me_4Si as the internal standard. The 1H NMR chemical shift values are expressed in ppm (δ) relative to CHCl_3 (δ = 7.26 ppm). Mass spectra were carried out using Water’s QTOF Micro YA 263 mass spectrometer. UV-visible and fluorescence spectra measurements were performed on a SHIMADZU UV-1800 and a PerkinElmer LS-55 spectrofluorimeter, respectively. DMSO of analytical grade were purchased from Spectrochem. All other materials for synthesis were purchased from Aldrich Chemical Co. and used without further purification. The solutions of different anions were prepared from their perchlorate, chloride or nitrate salts of analytical grade, and then subsequently diluted to prepare working solutions. The following abbreviations are used to describe spin multiplicities in 1H NMR spectra: s = singlet; d = doublet; t = triplet; m = multiplet.

2.2. Preparation of NBOB

A mixture compound 4-Chloro-7-nitro-benzo[1,2,5]oxadiazole (1) (250 mg, 1.25 mmol) and 4-formyl phenylboronic acid (264 mg, 1.75 mmol) in toluene (10 mL) was degassed with nitrogen for 20 min and then to it added potassium fluoride (218 mg, 3.75 mmol), 2-(Dicyclohexylphosphino)-2’-methylbiphenyl (9.1 mg, 0.025 mmol) followed by the addition of palladium acetate (2.8 mg, 0.0125 mmol) at rt. The reaction mixture was then heated at 70 °C for 12 h. TLC showed SM was consumed. The reaction mixture was filtered through Celite bed and washed with ethyl acetate. The combined organic solution was washed with 1 N NaOH followed by the brine wash, dried over sodium sulfate and
concentrated. The crude mass was purified by column chromatography on silica, product eluted with 20% ethyl acetate/hexane afforded compound NBOB (100 mg, 30%) as yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) δ (ppm): 10.14 (s, 1H), 8.61 (d, J = 7.7 Hz, 1H), 8.22 (d, J = 8.0 Hz, 2H), 8.1 (d, J = 8.2, 2H), 7.84 (d, J = 7.6 Hz, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$): 191.36, 149.50, 143.22, 138.84, 137.64, 137.08, 136.02, 130.64, 130.38, 129.78, 127.46. MS (ESI): m/z calc. for C$_{13}$H$_7$N$_3$O$_4$+: 269.22; found: 270.1 [M+H]$^+$. 

2.3. Preparation of test solution for UV–vis and fluorescence study

Bulk solution of NBOB, anion and cationic salts were made up in DMSO-water (4:1, v/v). For UV–vis and fluorescence titrations, stock solution of NBOB was prepared (c = 1.0 × 10$^{-5}$ M) in DMSO-H$_2$O (4:1, v/v). The solution of the guest anions salts in the order of (2.0 × 10$^{-4}$ M) was prepared in Millipore water.

2.4. Computational studies

All geometries NBOB, NBOB–Cys and NBOB–Hcy were optimised by density functional theory (DFT) calculations using Gaussian 09 (B3LYP/6-31G(d,p)) software package.

2.5. Cell culture

Vero cell (very thin endothelial cell) (Vero 76, ATCC No CRL-1587) lines were prepared from continuous culture in Dulbecco’s modified Eagle’s medium (DMEM, Sigma Chemical Co., St. Louis, MO) supplemented with 10% foetal bovine serum (Invitrogen), penicillin (100 μg/mL) and streptomycin (100 μg/mL). The Vero 76 were obtained from the American Type Culture Collection (Rockville, MD) and maintained in DMEM containing 10% (v/v) foetal bovine serum and antibiotics in a CO$_2$ incubator. Cells were initially propagated in 75 cm$^2$ polystyrene, filter-capped tissue culture flask in an atmosphere of 5% CO$_2$ and 95% air at 37 °C. The cells were then seeded onto 96-well plates (approximately 10$^4$ cells per well) for 24 h. Next day, media was removed and various concentrations of probe NBOB, NBOB–Cys and NBOB–Hcy complex (0, 15, 25, 50, 75 and 100 μM) made in DMEM were added to the cells and incubated for 24 h. Solvent control samples (cells treated with DMSO in DMEM), no cells and cells in DMEM without any treatment were also included in the study. Following incubation, the growth media was removed, and fresh DMEM containing MTT solution was added for 24 h. Solvent control samples (cells treated with DMSO in DMEM), no cells and cells in DMEM without any treatment were also included in the study. Following incubation, the growth media was removed, and fresh DMEM containing MTT solution was added. The plate was incubated for 3–4 h at 37 °C. Subsequently, the supernatant was removed, the insoluble coloured formazan product was solubilised in DMSO, and its absorbance was measured in a microtiter plate reader (Perkin–Elmer) at 570 nm. The assay was performed in triplicate for each concentration of probe NBOB, NBOB–Cys and NBOB–Hcy complex. The OD value of wells containing only DMEM medium was subtracted from all readings to get rid of the background influence. Data analysis and calculation of standard deviation was performed with Microsoft Excel 2007 (Microsoft Corporation).
3. Results and discussion

3.1. Synthesis

In this work, we constructed a novel fluorescent probe, \textbf{NBOB}, through Suzuki-Miyaura cross-coupling of 4-Chloro-7-nitrobenzo[1,2,5]oxadiazole with 4-formylphenylboronic acid (39) as a starting material (Scheme 1).

Reagents and Conditions: (a) 4-formyl phenylboronic acid, KF, 2-(Dicyclohexylphosphino)-2'-methylbiphenyl, palladium acetate, toluene, 70 °C, 12 h.

The probe can react with biothiols Cys/Hcys very fast through a cyclisation reaction with aldehyde, thereby giving remarkable spectral changes under physiological conditions. All the new compounds were characterised by $^1$HNMR, $^{13}$C NMR and HRMS (Figure S1–S7 of the Supplementary Information, available online).

3.2. Sensing mechanism and characterisation of \textbf{NBOB} with Cysteine

A remarkable fluorescence colour change of aqueous \textbf{NBOB} solution (DMSO/HEPES buffer, v/v 1:4, pH 7.4) from colourless to intense greenish-yellow upon incubation with Cys can be observed by the naked eye under a handheld UV lamp (Figure 2(a) inset) This shows the potential utility of \textbf{NBOB} as a reactive fluorescent probe for biothiols.

To understand the sensing mechanism of \textbf{NBOB} to thiols, $^1$H NMR and mass spectroscopic analysis were performed. To confirm the formation of thiazolidine \textbf{NBOB}–Cys and thiazinane \textbf{NBOB}–Hcy, probe \textbf{NBOB} was treated with Cys/Hcy, and the reaction products were isolated by column chromatography over silica gel column. The partial $^1$H NMR spectra of \textbf{NBOB} and the isolated \textbf{NBOB}–Cys are shown in Figure 1 (Figure S2 and S5 of the Supplementary Information, available online). The resonance signal corresponding to the aldehyde proton at 10.14 ppm disappeared along with the appearance of two new peaks at 5.82 and 5.63 ppm assigned to the methine protons of

![Scheme 1. Scheme for synthesis of \textbf{NBOB}. Reagents & Conditions: (a) 4-formyl phenylboronic acid, KF, 2-(Dicyclohexylphosphino)-2'-methylbiphenyl, palladium acetate, toluene, 70 °C, 12 h.](image1)

![Figure 1. (Colour online) The $^1$H NMR spectra of \textbf{NBOB} and \textbf{NBOB}–Cys in DMSO-d6.](image2)

![Figure 2. (Colour online) (a) Fluorescence (Excitation = 382 nm) titration of probe \textbf{NBOB} (1 x 10^{-5} M) in DMSO/PBS buffer, v/v, 1:4, pH 7.4 after addition of 0–100 equiv. of Cys. Inset shows the fluorescent colour change after the addition of Cys in \textbf{NBOB} solution. (b) Change in fluorescence intensity (at 530 nm) of \textbf{NBOB} vs. concentration of Cys.](image3)
the thiazolidine. These results suggested the formation of thiazolidine, consistent with a previous report (40–42).

LCMS spectrum analysis also confirmed the cyclisation reaction between Cys and NBOB. For pure probe NBOB, a characteristic peak at \( m/z = 270.10 \) was obtained which corresponds to the species \([\text{NBOB}+\text{H}]^+\). Upon the addition of Cys, a new peak at \( m/z = 373.00 \) (assigned to the thiazolidine) appeared along with the disappearance of the peak at \( m/z = 270.10 \) (Figure S1 and S6 of the Supplementary Information, available online).

### 3.3. Fluorescence and UV–vis study

The reactions of NBOB with Cys/Hcys and GSH were monitored at varying analyte concentrations by fluorescence spectroscopy in HEPES buffer (10 mM, pH 7.4) by exciting the solutions at 382 nm and measuring the emission intensity.

Probe NBOB showed almost no fluorescence (\( \Phi = 0.021 \)) (nearly the same as that of buffer only) in HEPES buffer without Cys (Figure 2). These results are consistent with our design that the 7-nitro-benzo[1,2,5]oxadiazole (NBD) fluorescence in NBOB is heavily quenched through the PET from benzoazadiazole group to benzaldehyde unit and the combined usage of quenching groups (Scheme 2).

However, a significant turn-on dual channel emissive fluorescence response with the maximum emission at 530 and 585 nm was observed upon the addition of Cys (Figure 2), which was ascribed to the prohibition of PET upon sensing.

After reacting with Cys, the increase in maximum fluorescence intensity is more than 600-fold. Such type of dual channel emissive fluorescent probe for Cys/Hcys is still rare in the literature. From these results, we can conclude that the fluorescence turn-on fold upon reacting with biothiols (Cys/Hcys) for NBOB-probe is indeed greatly increased, which agrees well with our initial hypothesis.

To gain detailed information about the sensitivity of NBOB, the fluorescence intensity change was closely monitored by addition of various concentrations of Cys into the probe (Figure 2(a) and (b)). The fluorescence emission at 530 or 585 nm revealed an excellent linear relationship with Cys concentrations ranging between 0 and 2.5 equiv., implying that Cys can be quantitatively detected in a wider concentration range.

The detection limit was calculated to be 4.5 μM (Figure S8 of the Supplementary Information, available online) based on a signal-to-noise value of 3 viz. 3σ/slope method (43), thus indicating that NBOB could afford the quantitative determination of thiol. Additionally, almost no further change in fluorescence occurred when the amount of Cys was increased to 5.0 equiv. These results suggest that NBOB molecules could be converted completely into the adduct NBOB–Cys with a large fluorescence quantum yield (\( \Phi = 0.392 \)).

In good agreement with the results of the fluorescence spectral studies, then, we examined the UV/Vis spectral properties of the probe NBOB (1 mM) in the absence or presence of Cys/Hcy.

As designed, in aqueous HEPES buffer, the free sensor only displayed the two characteristic absorption peaks of the NBD and benzaldehyde at 382 and 275 nm, respectively, but no featured absorption band arises due to conjugation between the two moieties, as the NBOB contains two electron withdrawing groups at the two ends. The UV/Vis spectra displayed the characteristic increase in absorption bands at 275 and 382 nm on gradual addition of Cys (0–10 equiv.) to the solution of probe 1 (1 × 10⁻⁵ M) within 5 min, and a colour change from colourless to light brown was observed by the naked eye (Figure 3).

To examine the selectivity, probe NBOB (5 μM) in DMSO-HEPES buffer (20 mM, pH 7.4) was incubated with various natural amino acids (30 equiv.) including Hcy, GSH, His, Glu, Asp, Val, Phe, Tyr, Ala, Ser, Arg, Pro, Thr, Gly, Trp, Ile and Lys and monitored by fluorescence spectroscopy. As shown in Figure 4, probe NBOB displays significant fluorescence enhancement not only towards Cys, but also towards Hcy and minimum response to GSH (Figure S16 of the Supplementary Information, available online), which indicates that probe NBOB can be used to detect two biothiols rapidly and simultaneously.

We believe that the aldehyde group, as the specific reaction point for Cys/Hcy, was linked directly to the substituted NBD skeleton. The aldehyde group in NBOB can form thiazolidine and thiazine with Cys/Hcy during cyclisation reaction through more feasible cyclic five- and
In the absence of Cys, the free probe is stable over a wide range of pH values from 2 to 12, and displayed the obvious response for Cys in the region of 5–9. At pH 7.4, probe NBOB exhibited a drastic change in the emission from 0.01 in the absence of Cys to ~600 in the presence of Cys (30 equiv.), a very large (~600-fold) enhancement. This indicates that the probe may be suitable for bio-applications at the physiological pH.

3.5. Kinetic study

Furthermore, we moved forward to examine the kinetic profiles of the reaction at different concentrations of Cys at room temperature. Through monitoring the fluorescence emission at 585 nm, the Cys-mediated cyclisation reaction was found to obey a pseudo-first-order law of rate constant $K' = 0.003 \text{ s}^{-1}$ (Figure 6) under Cys excess conditions. Probe NBOB (1.0 $\times$ 10$^{-5}$ M) was incubated with 50 equiv. Cys in HEPES buffer at 25 ºC and it shows fast fluorescence increase at the beginning. Subsequently, the fluorescence intensity underwent a gradual increase and finally reached a plateau. Based on the results at different concentrations of Cys, the second-order rate constant was also determined to be 1.3 M$^{-1}$ s$^{-1}$ (Figure S19 of the Supplementary Information, available online).

The time-dependent fluorescence response of probe NBOB in the presence of Cys or Hcy was measured at 37 ºC in aqueous HEPES buffer (20 mM, pH 7.4) containing DMSO and indicates that there were significant spectral changes within minutes of addition of Cys/Hcy (Figure 7).

The fluorescence intensity was linearly proportional to the Cys from 0 to 40 $\mu$M range ($R^2 = 0.944$), indicating the suitability of NBOB for quantitative detection of Cys (Figure 2(b) and Figure S8 of the Supplementary Information, available online). The reaction with Cys essentially reached completion after 5 min (and 8 min for Hcy)
ring fragment has not attended planarity to the adjacent benzaldehyde with dihedral angle 35°, and hence, the ICT character of the probe is only very weak as a result of no absorption and emission shift happen. In addition, we also performed TD-DFT calculations for the NBOB and the NBOB+Cys thiazolidine product also. The vertical transitions i.e. the calculated λmax, main orbital transition and oscillator strength (f) are listed in Table S2 in the Supporting Information. In the case of the NBOB probe, TDDFT calculations provided absorption band at ~396 and ~278 nm belonging to the S0→S2 (f = 0.4592) and S0→S9 (f = 0.1132) energy states, respectively (Table S2 of the Supplementary Information, available online). The energy minimisation structure of NBOB, NBOB–Cys and NBOB–Hcy were shown in Figure 8. These are the main contributing transitions, which correspond to energy states arises from HOMO→LUMO (~88.50%) and HOMO-5→LUMO (~36.90%) HOMO→LUMO+2 (~29.40%), respectively. These values are consistent with the absorbance bands at 382 and 275 nm obtained experimentally. However, the probe

(Figure 6) and, comparable to previous reports (44, 45). An assay time of 5 min was chosen in the evaluation of the selectivity and sensitivity of probe NBOB towards Cys/Hcy.

3.6. Theoretical study

To further understand the change of the photophysical properties of probe NBOB and the corresponding products obtained from the reaction between probe NBOB and Cys i.e. NBOB+Cys thiazolidine were examined by DFT and time-dependent density function theory (TDDFT) calculations at the B3LYP/6-31+G(d,p) level of the Gaussian 09 program. The optimised geometries and calculated electron distributions in the frontier molecular orbitals HOMO (the highest occupied molecular orbitals) and LUMO (the lowest unoccupied molecular orbitals) of NBOB and the NBOB+Cys thiazolidine are shown in (Figure S11 of the Supplementary Information, available online). In particular, for the probe NBOB, electron clouds in both the HOMO and LUMO are widely distributed at the NBD and benzaldehyde moieties, respectively. As well as planar NBD

![Figure 6](image)

**Figure 6.** (Colour online) (a) Change in fluorescence intensity (at 530 nm) of NBOB, with the addition of 10 equiv. of Cys with respect to time. (b) Pseudo-first-order kinetic plot of NBOB with Cys in DMSO:H2O [NBOB (1.0 × 10−5 M) with Cys (5.0 × 10−4 M)].

![Figure 7](image)

**Figure 7.** (Colour online) Time-dependent fluorescence spectra of NBOB in DMSO/PBS buffer, v/v, 1:4, pH 7.4 (1 × 10−5 M) in the presence of Cys (a) and Hcy (b).
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from the higher states $S_2$ or $S_9$ state to the $S_0$ would proceed via the intermediate $S_1$ state characteristic of a non-radiative pathway from the NBD-moiety to the benzaldehyde moiety, which could be the main reason for the non-fluorescent nature of the probe. The energy gaps between HOMO and LUMO in the probe $\text{NBOB}$ and $\text{NBOB}+\text{Cys}$ thiazolidine product were 79.21 and 62.03 kcal mol$^{-1}$, respectively [Figure S11 and S12 (for $\text{NBOB}$, the $S_0 \rightarrow S_1$ transition (i.e. a HOMO-1 → LUMO or a HOMO-1 → LUMO+1 electronic excitation) provided an oscillator strength, $f = 0.0001$ indicating a forbidden transition process and $S_1$ as the dark state (Table S1 and S2 of the Supplementary Information, available online). Notably, the allowed transitions obtained from this calculation were $S_0 \rightarrow S_2$ and $S_0 \rightarrow S_9$, confirming $S_2$ and $S_9$ act as the emissive states. These data indicate that an emission of electron from the higher states $S_2$ or $S_9$ state to the $S_0$ would proceed via the intermediate $S_1$ state characteristic of a non-radiative pathway from the NBD-moiety to the benzaldehyde moiety, which could be the main reason for the non-fluorescent nature of the probe. The energy gaps between HOMO and LUMO in the probe $\text{NBOB}$ and $\text{NBOB}+\text{Cys}$ thiazolidine product were 79.21 and 62.03 kcal mol$^{-1}$, respectively [Figure S11 and S12 (for

Figure 8. (Colour online) Energy minimisation structure of (a) $\text{NBOB}$ (b) $\text{NBOB}–\text{Cys}$ and (c) $\text{NBOB}–\text{Hcy}$.

Figure 9. (Colour online) Confocal fluorescence images of probe in Vero 76 cells. (a) Bright field image of the cells. (b) Only Cys at 2.0 $\times$ 10$^{-5}$ M concentration. (c) Bright field image of the cells treated with Cys at 2.0 $\times$ 10$^{-5}$ M, and probe $\text{NBOB}$ at concentrations 2.0 $\times$ 10$^{-6}$ M. (d) Stained with probe $\text{NBOB}$ at concentration 2.0 $\times$ 10$^{-6}$ M, all images were acquired with a 60× objective lens.
NBOB and NBOB-Hcy of the Supplementary Information, available online). The HOMO–LUMO energy gap of thiazolinedine becomes smaller relative to that of probe NBOB.

3.7. Live cell imaging

Finally, the probe was used for imaging live Vero cells (very thin endothelial cell) (Vero 76, ATCC No CRL-1587). However, to materialise this objective, it is a prerequisite to assess the cytotoxic effect of probe NBOB on live cells. The well-established MTT assay, which is based on mitochondrial dehydrogenase activity of viable cells were adopted to study cytotoxicity of above-mentioned compounds at varying concentrations mentioned in method section. Cytotoxicity measurements for each experiment show that probe NBOB does not exert any adverse effect on cell viability; the same are the cases when cells were treated with varying concentrations of Cys. The cells were seeded on a 24-well plate at a density of 40,000 cells per well in culture medium overnight (see the Experimental Section for details of the Supplementary Information, available online).

In one well, cells were pretreated with N-ethylmaleimide (a thiol-blocking agent) for 30 min to remove free thiols and then incubated with NBOB (20 μM) for another 30 min, followed by washing with HEPES (pH 7.4) for three times and it showed almost no fluorescence (Figure 9(a) and (b)). By contrast, when Vero cells were pretreated with 0.5 mM Cys and then incubated with NBOB, a marked increase in yellowish-green emission was observed under excitation at 405 nm (Figure 9(c) and (d)). The results show that NBOB is cell-permeable and senses thiols, which can be detected by cellular imaging.

4. Conclusion

In summary, we developed a novel strategy by installing quenching groups for the rational design of a highly selective and sensitive fluorescent probe (NBOB) for Cys/Hcy based on the cyclisation reaction between Cys/Hcy and aldehyde. This probe can be easily prepared and shows several appealing sensing properties for biothiols based on the quenching effects, the fluorescence of the probe can be maximally inhibited, and the turn-on response upon thiol activation is much larger which leads to the high sensitivity and fast response of the probe towards thiols. The probe was also applied to the biological imaging of Cys or Hcy inside living cells. We hope the results presented here may contribute to the development of novel reaction-based probes for the detection of biological small molecules. Furthermore, we expect that the novel design strategy could be employed as a general method for preparation of other kinds of fluorescent probes.

Supplemental material

Supplemental data for this article can be accessed online here: http://dx.doi.org/10.1080/10610278.2016.1170127

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Disclosure statement

No potential conflict of interest was reported by the authors.

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