**A fluorescence enhancement probe based on BODIPY for
the discrimination of cysteine from homocysteine and
glutathione**

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ABSTRACT:

Herein, a fluorescent probe BODIPY-based glyoxal hydrazone (BODIPY-GH) (**1**) for cysteine based on inhibiting of intramolecular charge transfer (ICT) quenching process upon reaction with the unsaturated aldehyde has been synthesized, which exhibits longer excitation wavelength, selective and sensitive colorimetric and fluorimetric response toward cysteine in natural media. The probe shows highly selectivity towards cysteine over homocysteine and glutathione as well as other amino acids with a significant fluorescence enhancement response within 15 min. In the presence of 50 equiv. of homocysteine, the emission increased slightly within 15 min and completed in 2.5h to reach its maximum intensity. Therefore, the discrimination of cysteine from homocysteine and glutathione can be achieved through detection of probe **1**. It shows low cytotoxicity and excellent membrane permeability toward living cells, which was successfully applied to detect and image intracellular cysteine effectively by confocal fluorescence imaging.

Keywords: BODIPY; fluorescent probes; living cells; cysteine; bioimaging

1. Introduction

Biothiols, such as cysteine (Cys), homocysteine (Hcy), and glutathione (GSH), play the significant roles in maintaining redox balance of various physiological processes. Among them, Cys involved in protein synthesis, detoxification, metal binding, post-translational modification, and metabolism (Reddie and Carroll 2008; Weerapana et al. 2010). Cys deficiency could lead to slow growth, skin lesions, liver damage, etc (Shahrokhian 2001). The elevated levels of Cys could induce severe

neurotoxicity and cardiovascular diseases (Wang and Cynader 2001). Therefore, it is of great importance to detect Cys in biological systems for understanding its biological functions and accelerates the advancement of diagnosis and therapy means.

A variety of ingenious fluorescent probes have been developed for biothiols so far, and their design strategies are mainly based on the strong nucleophilicity of thiol group combined with various reaction mechanisms such as Michael addition, cyclization, displacement of coordination, and cleavage reactions (He et al. 2015; Yin et al. 2013; Zhang et al. 2015; Zhou and Yoon 2012) and Cys-induced aggregation with nanosensors (Lu and Zu 2007; Xiao et al. 2011). Because of the considerably challenging due to the similar structures and reactivity of Cys/Hcy/GSH, the early-stage strategies are generally aimed at distinguishing Cys/Hcy/GSH from thiol-free amino acids. New strategy for discriminating Cys from its competitive species has been developed greatly. Many excellent works provide the important tools for studying Cys-relevant biological functions (Gao et al. 2015; Jia et al. 2015; Niu et al. 2013). Among these various sensing mechanisms, the cyclization of N-terminal Cys residues (containing both SH and NH₂ groups) reacting with unsaturated aldehydes to form thiazolidines has been used to design chemodosimeters for the detection of Cys over Hcy successfully (Li et al. 2009; Wang et al. 2005).

4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) (Loudet and Burgess 2007) derivatives may be favored fluorophores as fluorescent sensors. The worthy qualities of BODIPY (Treibs and Kreuzer 1968), contain relatively high fluorescence

quantum yields and molar absorption coefficients, stabilization towards light and chemicals. The BODIPY core can be postfunctionalized easily, leading to dyes with custom-made, fine-tuned spectroscopic properties. A series of BODIPY-based fluorescent chemosensors have been designed to detect various kinds of analytes, such as cations (Yang et al. 2016), anions (Ekmekci et al. 2008; Hudnall and Gabbai 2008), molecules (Niu et al. 2012, 2013), polarity (Sunahara et al. 2007), pH (Han et al. 2009). Following the strategy in work of Pi Wang et al. (Wang et al. 2012), a BODIPY-based glyoxal hydrazone (**BODIPY-GH**) (**1**) for the fluorescence turn-on detection of Cys has designed. The design and plausible working mechanism of this probe is suggested in Scheme 1. It is based on inhibiting ICT **quenching process upon reaction with the unsaturated aldehyde to form 5-membered ring heterocycle**, enabling the highly selective and sensitive detection of Cys over Hcy and GSH as well as other amino acids. The potential of the probe for detecting and imaging Cys in cells have been demonstrated successfully.

Scheme 1.

2. Experimental

2.1 Instrument and reagent

All of the chemicals of reagent grade quality were purchased from commercial way and used as received. ¹H and ¹³C NMR spectra were received with a Bruker DRX-400 and DRX-400/4 spectrometer. Mass spectra were detected in E.I. Mode. High-resolution mass spectra (HRMS) were obtained on a Bruker microTOF-Q II

mass spectrometer. Melting points were obtained with an X-4 precise micro melting point cryoscope (Beijing Fukai Instrument Co.) and are uncorrected. All pH measurements were made with a pH-10C digital pH meter.

2.2 The synthesis of probe BODIPY-GH

Scheme 2.

3,5-dichloro-8-phenyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (**3**) was synthesized as depicted previously (Xu et al. 2005).

3-chloro-3a, 4a-diaza-4,4-difluoro-5-hydrazinyl-8-phenyl boron dipyrromethene (**2**) was synthesized according to the procedures reported in the literature (Dilek and Bane 2008). ¹H NMR (CDCl₃, 400 MHz): δ 6.16 (d, 1H, J=4.0 Hz, H-a), 6.32(d, 1H, J=4.0 Hz, H-b), 6.59(d, 1H, J=4.0 Hz, H-c), 6.86(d, 1H, J=4.0 Hz, H-d), 7.44 (m, 5H (ph)), 7.57(s, 1H, H-e). MS (ESI) m/z calcd. for C₁₅H₁₂BClF₂N₄ 332.5; found 331.1 (M-1); 311.0 (M-1-HF); 316.0 (M-NH₂, 100%).

Synthesis of BODIPY-GH (**1**): 2 mL of glyoxal was added to (71 mg, 0.215 mmol) of compound **2**. Stirring in ethanol was performed for 2 h until the reaction appeared complete by TLC. The excess glyoxal was removed under reduced pressure. The crude residue was purified through column chromatography over silica with ethyl acetate and petroleum ether (1:10) to obtain 68 mg of **1** in 85% of yield; Red solid, mp 110-112 °C. R_f 0.49 (1:5 ethyl acetate: petroleum ether). ¹H NMR (CDCl₃, 400 MHz): δ 6.288(d, 1H, J=3.6 Hz, H-a), 6.586(d, 1H, J=3.6 Hz, H-b), 6.747(d, 1H, J=4.8 Hz, H-c), 6.972(d, 1H, J=4.4 Hz, H-d), 7.429(d, 1H, J=7.6 Hz, H-f), 7.480 (m, 5H (ph)), 9.632(d, 1H, J=7.6 Hz, H-g), 9.793(s, 1H, NH, H-e); ¹³C NMR (CDCl₃): δ

110.96, 115.19, 125.69, 128.44, 128.52, 128.59, 130.28, 130.41, 132.35, 132.69,
133.11, 135.09, 135.46, 138.86, 143.08, 157.14, 189.83. HRMS: calcd. for [**1**+H⁺]
(C₁₇H₁₃BClF₂N₄O) 373.0839, found 373.0837.

1-Cys: The mixture of **1** (0.073 mmol) and Cys (0.58 mmol) was stirred in 40
mL of EtOH-H₂O (3:1, v/v) at room temperature. TLC shows that the reaction could
be completed within 1 hr, and provided **1-Cys** product mainly. The mixture was
poured in water extracted with dichloromethane and then evaporated to dryness under
reduced pressure. The solid residue was chromatographed on silica gel using
dichloromethane and MeOH (4:1) as an eluent to give **1-Cys** to obtain 17 mg of **1** in
50% of yield as diastereomers;. ¹H NMR (CDCl₃, 400 MHz): 2.85-2.91 (4H),
6.10-6.82 (4H), 6.85-7.5 (6H), 7.99 (1H). HRMS: calcd. for [**1-Cys**+H⁺]
(C₂₀H₁₈BClF₂N₅O₂S) 476.0931, found 476.0910.

2.3 General method for measurements of photophysical properties

UV-vis absorption spectra were obtained on a Varian UV-Cary5000
spectrophotometer. In order to get the corrected steady-state excitation and emission
spectra, an Edinburgh instrument FLS920 spectrofluorometer and a F7000
spectrofluorometer was used. Rhodamine 6G in H₂O ($\lambda_{\text{ex}} = 483 \text{ nm}$, $\phi_{\text{f}} = 0.76$) was
used as a fluorescence standard (Olmsted 1979).

2.4 Time-resolved fluorescence spectroscopy

Fluorescence lifetimes were detected by a FLS920. The monitored wavelength
was 545 nm. Fluorescence decay histograms were recorded using the time-correlated

single photon counting technique in 2048 channels through a FLS920 spectrometer equipped with a supercontinuum white laser (400-700 nm). Histograms of the instrument response functions (using LUDOX scatterer) and sample decays were obtained until it typically reached 1.0×10^4 counts. The fitting parameters (decay times and pre-exponential factors) were decided by minimizing the reduced chi-square χ^2 .

2.5 Cell culture, fluorescence imaging and cytotoxicity Tests

To obtain the cell permeability of **1**, porcine kidney 15 (PK15) cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) subjoined with 10% FBS (fetal bovine serum). The cell lines were kept in a moist atmosphere containing 5% CO₂ at 37 °C. Cells were incubated with **1** (15 µM) in 1.0 mL of fresh culture medium for 2 h after removal of the culture medium. Subsequently, the cells were treated with 500 µM Cys. Cells were incubated for 30 min and rinsed with phosphate-buffered saline (PBS) three times to remove free compound before imaging. PK15 cells only incubated with 15 µM **1** for 30 min acted as a control. Confocal fluorescence images of PK15 cells were carried out on an Olympus FV1000-IX81 laser confocal microscope.

The cytotoxic activity experiment of complex against PK15 cells was tested according to standard 3-(4,5-dimethylthiazole)-2,5-diphenyltetrazolium bromide (MTT) assay procedures. PK15 cells were seeded in 96-well assay plates at a density of 10^4 cells per well (100 µL total volume/well) for 24 h. The as-prepared **1**, (5 µM, 10 µM, 25 µM, 50 µM and 100 µM) were added in the serum-free medium and

incubated with the cells for 24 h after that. The optical absorbance of the cells was detected at 450 nm through a microplate reader (German Berthold Mithras2LB943). The control experiment was finished by detecting the growth culture medium without **1**.

3. Results and discussion

3.1 Fluorescent response of **1 toward Cys.**

The absorption and emission spectra of **1** were recorded in different solvents was shown in Fig. S1. The absorption spectra of **1** are of similar shape as those of previously described boron dipyrromethene dyes: i.e., a narrow absorption band with a maximum around 524 nm (MeCN) and 541 nm (cyclohexane) (is attributed to the 0–0 vibrational band of S_0 - S_1 transition). Additionally, a weaker, broad absorption band is found in the UV at around 350 nm, this broader and weaker absorption band is attributed to the S_0 - S_2 transition. **1** in different solvents shows an emission spectrum with a maximum at ~558 nm, shaped like the mirror image of the absorption spectrum, displaying the typical emission features of BODIPY. The ϕ_f values are low in MeCN (0.02). The low ϕ_f value for **1** in polar solvents can be attributed to an efficient quenching via an excited-state ICT process from the oxygen atom of the aldehyde group to the strongly electron deficient BODIPY acceptor. The quantum yield of fluorescence ϕ_f increases with decreasing polarity of the solvent from MeCN (0.02) to cyclohexane (0.2). Table S1 summarizes the photophysical data of **1** in several solvents.

Upon addition of 50 equiv of biothiols GSH to a 20 μ M solution of **1** in acetonitrile/PBS buffer (2 : 8, v/v, 10 mM, pH 7.4) after 15 min and 150 min, no change in the UV/vis absorption and fluorescence spectra could be found. Among the biothiols, only the addition of Cys to a solution of **1** in acetonitrile/PBS buffer after 15 min results in a significant (approximately 50 nm) hypsochromic shift of the absorption spectrum and is accompanied by a increase in fluorescence emission intensity (Fig. S2 and S3, Table S2). The addition of Hcy to a solution of **1** in acetonitrile/PBS buffer after 15 min almost no affects the absorption and emission spectra. But after 150 min, the absorption and emission spectral changes of **1** in the presence of Hcy are similar to that of Cys. The results of **1** in the presence of biothiols after 15 min and 150 min are summarized in Table S2.

The absorption and fluorescence emission spectra of **1** as a function of [Cys] (in addition of Cys after 15 min) are shown in Fig. 1. Upon increasing the Cys concentration, the lowest energy absorption band (with maximum at 528 nm in the absence of Cys) decreases in intensity while a new peak appears at approximately 478 nm whose intensity increases with higher Cys concentration. The relative contributions of the 528/478 nm signals change with varying [Cys] and the vis absorption spectra show isosbestic points at 489 and 393 nm. The process showed visible color change of **1** from purple to yellow under daylight (Fig. 1a, inset). At ambient temperature, probe **1** exhibited a weak and broad emission band centered at 545 nm. Additions of Cys to a solution of **1** in acetonitrile/PBS buffer enhance the

fluorescence emission intensity without spectra shift. The quantum yield ϕ_f of **1** increases from 0.015 in the absence of Cys to 0.254 with addition of 500 μ M Cys. The low ϕ_f value for free **1** (0.015) in acetonitrile/PBS buffer can be attributed to the ICT character of the excited state. The emission increased gradually as a result of the formation of 5-membered ring of thiazolidine. Upon binding Cys, the electron-donating properties of the oxygen atom of the aldehyde group are reduced, decreasing the ICT-character of the excited state and causing a 17-fold amplification of the fluorophore's quantum yield.

The time-dependent fluorescence response of **1** (20 μ M) in the presence of biothiols (Cys and Hcy) was investigated in acetonitrile/PBS buffer (2 : 8, v/v, 10 mM, pH 7.4) at room temperature. Upon addition 50 equiv. of Cys, the emission band centered at 545 nm quickly increased in the initial 1 min and reached relative maximum within 5 min and maintained its strong fluorescence in 2.5 hours (Fig. S4a). The fluorescence response of **1** with Hcy under identical conditions were also studied (Fig. S4b). In the presence of Hcy, the emission increased slightly within 15 minutes and completed in 150 min to reach its maximum intensity. The formation rate of heterocycle is further slower than Cys. Therefore, the discrimination of Cys from Hcy and GSH was achieved through detection of probe **1** within 15 min.

The fluorescence intensity of **1** at maximum emission increased linearly with the amount of Cys in the range of 0–250 μ M. The calibration curve of fluorescent intensity (F) vs the concentration of Cys at 547 nm is shown in inset of Fig. 1b.

215 $F = 91.91 + 10.15 \times [\text{Cys}]$ ($r = 0.9979$, $[\text{Cys}] = 0\text{--}50\ \mu\text{M}$)

216 $F = 345.87 + 5.22 \times [\text{Cys}]$ ($r = 0.9974$, $[\text{Cys}] = 50\text{--}250\ \mu\text{M}$)

217 The detection limit (DL) was estimated based on the following equation:

218 $\text{DL} = 3.3\sigma/k$, where σ is the standard deviation of the blank sample and k is the slope of

219 the calibration curve. From the linear equation of spectra titration at 547 nm, the

220 detection limit of **1** for Cys was calculated to be 54.6 nM (10.92 ppb, 0–50 μM) and

221 106 nM (21.2 ppb, 50–250 μM) (the intracellular concentration for Cys: 30–200 μM ;

222 (Park and Imlay 2003)).

223 As shown in Table S3, the detection limit obtained from this method was found

224 to be higher or comparable than those of others. The result indicates that the

225 BODIPY-based glyoxal hydrazone could be potentially utilized as an excellent optical

226 sensor for the quantitative analysis of Cys in biological samples. Fluorescence

227 emission titration spectral (Fig. S5) of **1** with Hcy was recorded 150 min after Hcy

228 addition to confirm the response behaviour of **1**, which shows good linear

229 enhancement with the increasing concentration of Hcy.

230 According to the literatures reported, upon reaction with the unsaturated

231 aldehyde, potentially heightened selectivity for Cys over Hcy is predicted and Cys

232 may form the generally more favored 5-membered ring thiazolidine, as compared to

233 Hcy (6-membered ring thiazinane) (Li et al. 2009; Mei et al. 2012; Starkenmann 2003;

234 Wang et al. 2005). Because **1** possesses similar unsaturated aldehyde group, the

235 plausible mechanism for its specific selectivity for Cys over Hcy is illustrated in

Scheme 1. To confirm the formation of 1-Cys and 1-Hcy, the ^1H NMR spectra of **1** in a solution of D_2O –DMSO (1: 20, v/v) with Cys (1 equiv.) or Hcy (1 equiv.) at different reaction time was monitored at room temperature (Fig. S6). In the presence of 1 equiv. of Cys after 5 min, the resonance signal corresponding to the aldehyde proton at 9.51 ppm (asterisk) disappeared; However, concomitantly, two new peaks at 5.12 and 5.25 ppm (triangular) assigned to the methine proton of the thiazolidine diastereomer emerged, consistent with the previous reports (Hu et al. 2011; Lin et al. 2008; Wang et al. 2012). In the presence of 1 equiv. of Hcy, the resonance signal corresponding to the aldehyde proton (asterisk) decreased slightly within 5 min and disappeared after 60 min, two new peaks at 5.07 and 5.12 ppm (triangular) assigned to the methine proton of the thiazinane diastereomer emerged more and more apparent within 60 min. It is verified that the formation rate of heterocycle in the reaction of **1** with Hcy is further slower than Cys. The reaction product of **1** with Cys was isolated and characterized by standard method. The ^1H NMR spectra and HRMS spectra (SI) of sensing reaction product of **1** with Cys were measured, which was also confirmed by absorption spectra and fluorescence emission spectra (Fig. S7). The absorption and fluorescence spectra of **1**-Cys (the isolated product) are almost the same as **1**+Cys before isolated. This formation was further characterized by mass spectrometry analysis, in which the peaks at m/z 476.0910 (calcd = 476.0931) corresponding to [**1**-Cys+ H^+] was clearly observed (SI). After treatment with Cys, the aldehyde group in **1** disappeared and the ICT effect from aldehyde group removed, therefore a turn-on

fluorescent response of BODIPY fluorochrome showed. **1** showed a preference for Cys over Hcy because the Cys reaction product was formed at a faster rate.

Figure 1.

3.2 Selective fluorescence response of **1** turn-on fluorescence toward Cys

In order to check the responsive properties of **1** the interference of the intracellular species, the fluorescence responses of **1** to various amino acids and NaS₂ were measured under physiological conditions. As shown in Fig. 2a, only Cys induced a significant enhancement of fluorescence intensity under the given conditions. The addition of other amino acids could not cause any obvious fluorescent change. Moreover, the competitive experiments were done to deeply examine the selectivity and anti-interference of **1**. As shown in Fig. 2b, it clearly showed that the fluorescence emission of **1** in the presence of Cys was still outstanding, and the selective sensing behavior for Cys was hardly interfered by commonly coexistent amino acids.

In addition, the fluorescence intensity changes of **1** as a function of pH in the presence and absence of Cys system were also investigated (Fig. S8). For **1**, it showed a failing response at higher pH due to the formation of intramolecular hydrogen bond of aldehyde group. It was stable within the pH range of 6–9. In the presence of Cys, **1** exhibited no dramatic spectral change in wide pH range of 5–10, and it was sensitive and stable for Cys within the pH range of 5.25–8.93, in which most biological samples can be tested. As a result, **1** could be applied in living cells without strong interference from pH effects.

Figure 2.

3.3 The fluorescence decay of **1**

Fluorescence decay traces of **1** at different concentrations of Cys were tracked through the single-photon timing method. The analyses of **1** in terms of τ_i and α_i were demonstrated incorporating in single decay surface curves recorded at the same excitation wavelength (483 nm). The fluorescence lifetime measurements of **1** demonstrate that the fluorescence decay curves consisted of two exponential components. Detailed photophysical properties of **1** about decay times τ_1 , τ_2 and average lifetime τ_A are listed in Table S2 and Table S4. The fluorescence decay is fitted to the bi-exponential profile with the lifetime of ~1.21 ns and ~0.43 ns in MeCN (Fig. S9). The fast component (~0.43 ns) is present with large amplitude (96%). In THF and cyclohexane, the fluorescence decay also shows two main components. But the two lifetime τ increase both when the solvent changes from MeCN (1.21 and 0.43 ns) to THF (1.32 and 0.81 ns) and cyclohexane (2.37 and 0.86 ns). The relative amplitudes α_1 (%) increases (from 4% to 84%) with decreasing solvent polarity. The slower decay time can be attributed to the lifetime of the LE (local excited) state and the short decay time can be considered as the lifetime of the ICT state. In more polar solvents, this LE emission is strongly quenched.

Upon the addition of different concentration of Cys to **1** in acetonitrile/PBS buffer, the two lifetimes are almost constant (~1.1 and ~0.8 ns), but the longer lifetime increases in the amplitude (18% to 45%). The ICT can be efficiently blocked by

reaction with Cys. The reaction of Cys with aldehyde suppresses the ICT. Fig. 3 presents the fluorescence decay of **1** before and after addition of Cys in acetonitrile/PBS buffer, the fluorescence decay became slower in the presence of Cys. The average life time τ_A also become longer after in addition of Cys (Table S4).

Figure 3.

3.4 Application of **1** for cell imaging

Capability of **1** for intracellular sensing was first assessed in PK15 cells. The experiment results show that **1** can be efficiently internalized by the endocytosis of PK15 cells. After co-incubation with **1** in serum-free cell culture medium at 37 °C for 2 h, appreciable amounts of **1** was uptaken into the PK15 cells, which were corroborated by confocal microscopy. As shown in Fig. 4, the PK15 cells kept good shape and appeared viable, which exhibited very weak and green emission from the cytoplasm through comparing with bright-field images of cells. However, the cells treated with **1** were further incubated with Cys (500 μ M) for another 30 min at 37 °C; All cells displayed remarkable green fluorescence, much stronger than fluorescence of **1**-stained cells. The data demonstrate that the fluorescent intensity changed apparently with the presence of Cys and the concentration variation of Cys could be assessed by serving the novel **1** as a chemosensor. The biocompatibility of **1** was assessed by the cellular cytotoxicity experiment. As shown in Fig. S10, five different amounts of **1** were incubated with PK15 cells for 24 h and the cell viability was not changed obviously, which exhibited the low cytotoxicity and good biocompatibility of **1**.

Figure 4.

4. Conclusion

In summary, we have demonstrated a novel fluorescent turn-on sensor **1** for Cys over Hcy/GSH as well as other amino acids based on inhibiting of ICT quenching process upon reaction with the unsaturated aldehyde to form 5-membered ring thiazolidine. Sensor **1** displays a highly selective and fast fluorescent enhancement towards Cys with an obvious fluorescent enhancement, which affords a high sensitivity to Cys with a detection limit of 54.6 nM observed. The probe was applied to the biological imaging of Cys inside living cells successfully. Further work aimed at applications to optimize the sensing method by modifying the reaction group and fluorophore.

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Notes

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401 Figure captions

402 **Scheme 1.** A plausible mechanism of response of **1** to Cys/Hcy.

403 **Scheme 2.** Synthesis of **1**.

404 **Fig. 1.** (a) UV-vis absorption changes and (b) fluorescence spectral changes of **1** (20
 405 μM) upon addition of Cys. Each spectrum was recorded 15 min after Cys addition in
 406 acetonitrile/PBS buffer (2 : 8, v/v, 10 mM, pH 7.4) at room temperature. (a, inset):

407 Visible color change of **1** under daylight as a function of the concentrations of Cys at
408 0 μM and 700 μM , respectively. (b, inset): the fluorescence intensity at 545 nm as a
409 function of the concentrations of Cys. $\lambda_{\text{ex}}=483$ nm. **Relative standard deviation (RSD)**
410 **is about 6.7%, n=4.**

411 **Fig. 2.** (a) Emission response at 545 nm of **1** (20 μM) upon addition of 50 equiv. of
412 various amino acids and NaS_2 . Each data point was acquired 15 min after addition of
413 different amino acids in acetonitrile/PBS buffer (2 : 8, v/v, 10 mM, pH 7.4) at room
414 temperature. (a, inset): fluorescent color change of **1** under UV light as a function of
415 Cys, Hcy and GSH, respectively. (b) Fluorescence ratio of **1** at 545 nm in
416 acetonitrile/PBS buffer solutions to various species, and its competition graph with
417 Cys. Black bars represent addition of various amino acids to the solution of **1**. Red
418 bars represent addition of Cys and various amino acids to the solution of **1**. $\lambda_{\text{ex}} = 483$
419 nm. **RSD is about 8.1%, n=4.**

420 **Fig. 3.** Representative fluorescence decay curves (collected at 545 nm) of **1** in
421 acetonitrile/PBS buffer (2 : 8, v/v, 10 mM, pH 7.4) excited at 483 nm and **1** (20 μM)
422 in addition of Cys. Each data point was acquired 15 min after addition of Cys.

423 **Fig. 4.** Confocal fluorescence and bright-field images of PK15 cells: The images of
424 the first row is blank and nothing incubated with cells; The second row is
425 fluorescence images of PK15 cells incubated with **1** (15 μM); The third row is
426 fluorescence images of PK15 cells pretreated with 500 μM Cys and incubated with **1**
427 (15 μM). **Scale bar = 25 μm .**

Scheme 1

Scheme 2

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Figure 1

443
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Figure 2

Figure 3

Figure 4