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A highly sensitive two-photon fluorescent probe for glutathione with near-infrared emission at 719 nm and intracellular glutathione imaging

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Abstract

A near-infrared turn-on two-photon fluorescent probe ST-BODIPY for glutathione-specific detection was designed and synthesized by attaching triphenylamine to BODIPY skeleton through the Knoevenagel condensation to prolong the maximum emission wavelength to the NIR region. And 2,4-dinitrobenzenesulfonyl group (DNBS), as the fluorescence quencher and thiol recognition moiety, was modified in 8 position of BODIPY. In the presence of GSH, the probe afforded an "off-on" signal response with a significant NIR fluorescence enhancement centered at 719 nm accompanying by quantum yield increased to 0.44, which was ascribed to the glutathione-induced S_NAr (aromatic substitution) reaction. Surprisingly, we found that the probe could discriminate GSH from other biothiols including Cys and Hcy upon the addition of intracellular concentrations of them. Time-dependence also demonstrated that the probe could distinguish GSH from Cys and Hcy under physiological environment. The limit of detection (LOD) for GSH was calculated as 25.46 nM from the titration experiments, which is lower than most previously reported GSH-selective probes. Under the Ti:sapphire pulsed laser's 800 nm irradiation, ST-BODIPY toward GSH generated an "off-on" signal response with a significant enhancement of fluorescence emission at 719 nm after treatment with

GSH. Besides, the 2PA cross section value (σ_2) was calculated to be 410 GM, suggesting that it could not only function well as an excellent two-photon fluorescent probe for the detection of intracellular GSH, but also be applied for two-photon imaging with high sensitivity in living cells. Moreover, ST-BODIPY probe has been successfully employed for monitoring exogenous and endogenous GSH in MCF-7 cells with satisfying results, perhaps it was feasible for detecting abnormal contents of GSH in a biological system and accomplishing the goal of maintaining normal human activities.

Key words : two-photon; near-infrared; Glutathione sensor; bistriphenylamine-BODIPY; endogeous cell imaging.

1. Introduction

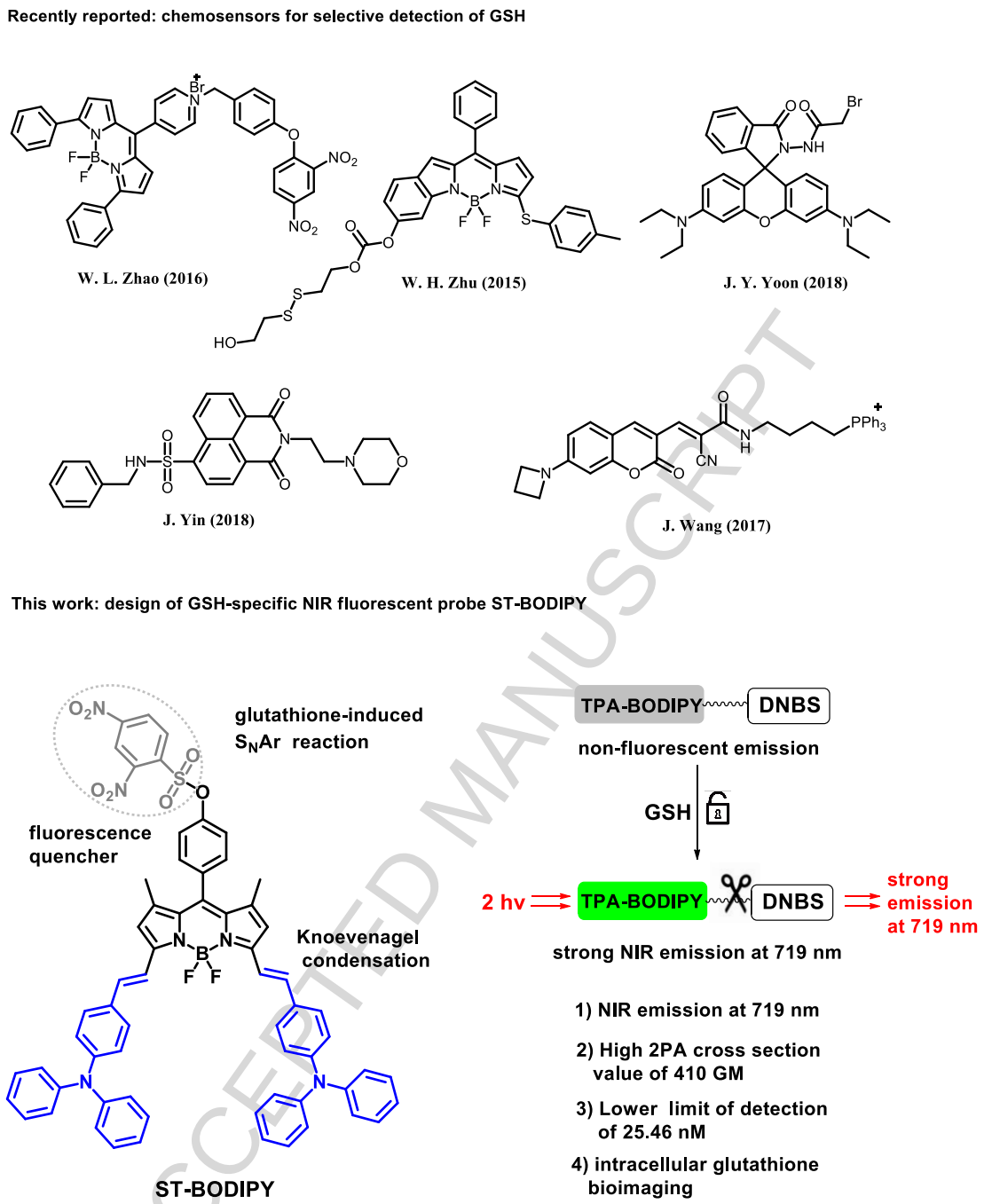
Intracellular biothiol, including cysteine (Cys), glutathione (GSH), and homocysteine (Hcy), in biological systems have drawed considerable interest in recent years due to the essential influence on involving in various physiological and pathological processes[1-3]. Among these intracellular biothiol, GSH, the most abundant intracellular non-proteinogenic biothiol, whose normal intracellular level is in the range of 1.0 to 15.0 mM[4-6], is regarded as an important biomolecule in participating in the process of the intracellular redox activities to regulate the metabolism and cellular homeostasis[7-11]. However, variations in the levels of GSH directly implicated many diseases, such as liver damage, leucocyte loss, psoriasis, cancer, and HIV infection[12-18]. Accordingly, selective and sensitive detection of GSH

is of great benefit to monitoring these biothiols under complicated biological conditions[19-22].

In the past few years, thiol-based fluorescent probes have attracted great attention for the purpose of detecting biothiols. However, on account of the fact that Cys/Hcy have similar reactivity of the thiol groups in the thiol-containing amino acids, there were scarcely sensors that could detect GSH from Cys and Hcy[23-30]. Though a few probes provided selective fluorescent detection for GSH (Scheme 1), the absorption and emission of them were in the visible range (300-650 nm)[31-33]. Accordingly, Zhao et al[34] developed BODIPY-based fluorescent probe used to detect mitochondrial GSH in living cells with detection limit of 109 nM and its fluorescence emission was located at 599 nm. Kim et al[35] has explored GSH-selective one-photon fluorescent probe based on rhodamine using for HeLa cells and zebrafish imaging, whose fluorescence emission wavelength was 579 nm and its detection limit was 0.17 μM. Superior to one-photon, two-photon probes, especially taking with a large two-photon excitation cross-section, provided lots of advantages in cell imaging[36,37]. Considering the fact that near-infrared (NIR, 650-900 nm) two-photon fluorescent dyes would display extraordinary advantages by virtue of the less damage to living cells and better tissue penetration in the living systems, the exploring of two-photon near-infrared (650-900 nm) GSH-selective probes were in urgent need, especially NIR fluorescence emission wavelength reached 700 nm [38, 39].

Herein, A near-infrared two-photon fluorescent probe ST-BODIPY was designed for the selective detection of GSH by introducing triphenylamine into 3 and 5 position

of BODIPY. As a result of extended conjugation, the ST-BODIPY revealed emission well-into the near-infrared region. 2,4-dinitrobenzenesulfonyl group(DNBS) was incorporated at the 8-position of BODIPY as the fluorescence quencher and thiol recognition moiety (Scheme 1). In the presence of GSH, the probe displayed an "off-on" signal response accompanying with an obvious NIR fluorescence enhancement centered at 719 nm with lower limit of detection, which attributed to the glutathione-induced S_NAr reaction resulting in the cleavage of the DNBS group from the probe molecule. Importantly, experimental results demonstrated the probe could discriminate GSH from Cys and Hcy under intracellular concentrations. What's more, ST-BODIPY could be served as a two-photon fluorescent turn-on probe for detection of GSH and its 2PA cross section value (σ_2) was 410 GM at 719 nm excited by the Ti:sapphire pulsed laser's 800 nm irradiation, indicating that it was suitable for the two-photon fluorescence bioimaging. At last, the probe was also used to detect endogenous and exogenous GSH in living cells, which further displayed its valuable applications in biological system.



Scheme. 1. Recently reported GSH-selective chemosensors and design of specific GSH-selective NIR fluorescent probe ST-BODIPY in this work

2. Experimental section

2.1 Materials and general methods

Reagents

2,4-dinitrobenzenesulfonyl group was purchased from Aladdin. Triphenylamine, piperidine and toluene were purchased from Macklin Biochemical Co.,Ltd (Shanghai, China). Triethylamine was purchased from Adamas. acetic acid and ethyl acetoacetate were purchased from Maya reagent company. Indocyanine green (ICG) was purchased from Sigma-Aldrich. All solvents used in spectroscopic measurements were of analytical grade.

The used amino acides were obtained by commercial purchase. Glutathione (GSH), cysteine (Cys), and homocysteine (Hcy) were purchased from Macklin Biochemical Co., Ltd (Shanghai, China), Shanghai HuiXing Biochemical Reagent Co., Ltd, Tokyo Chemical industry Co., Ltd (Shanghai, China) respectively. Other amino acids were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

Apparatus

Chemical structures were verified by NMR analysis and mass spectrometry. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker ADVANCE 500 NMR spectrometer and Bruker AVANCE III HD 600 NMR spectrometer in DMSO-d_6 with tetramethylsilane (TMS) as internal standard. Chemical shifts were given in parts per million (ppm). The mass spectra data were obtained on a Broker Autoflex TOF/TOF mass spectrometry. UV-visible absorption spectra were determined on a Shimadzu UV-3600 Spectrophotometer. Fluorescence spectra were measured on a HORIBA FL-4 Max spectrometer. 1×1×3 cm quartz cuvettes were used for absorption and emission spectral titration. The two-photon fluorescence emission spectra were acquired by recording with Exemplar Plus modular spectrophotometer (MODEL NO.

Mira 900) in the following standard configuration: wavelength range of 200-1080 nm, 25 μm slit, a LVF filter, a ruled grating (800 mm/250 nm), and a spectral resolution of 1.5 nm, using a mode-locked Ti:sapphire pulsed laser with the two-photon excitation condition(λ_{ex} = 800 nm, 76 MHz, 100 fs).

Calculation of Detection limit

The detection limit, indicating the sensitivity of the probe, was found from 10 blank solutions based on the definition by IUPAC (CDL=3 Sb/m). Detection limit for GSH was calculated by Eq.

$$\text{Detection limit} = 3\delta/k$$

Where δ standard deviation of 10 blank measurements and k=slope obtained from the graph of fluorescence intensity ratio vs concentration of GSH[40].

Determination of the Fluorescence Quantum

The quantum yield was calculated according to the equation:

$$\varphi_s = \varphi_r \times \frac{A_r}{A_s} \times \frac{F_s}{F_r} \times \left(\frac{n_s}{n_r}\right)^2$$

Where A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts s and r refer to the unknown and to the standard, respectively, indocyanine green (ICG, Φ_f = 0.13 in DMSO)[41] was used as reference[42].

MTT cytotoxicity assay

Cytotoxicity of the probe ST-BODIPY was examined by vitro MTT assay. The logarithmic phase of MCF-7 cells were inoculated in a 96-well cell culture plate (1×10^4 cells per well), and incubated at 37 ℃ under an atmosphere of 5% CO_2 for 24 hours. Next, the cells were incubated with different concentrations (0-20 μM) of ST-BODIPY for another 24 h. Then, the cells were incubated with 5 mg/mLMTT. After that the supernatant was removed following by the addition of 100 mL DMSO to each well and oscillation for 5 minutes. The absorbance of each well of the 96-well plate was then obtained at 570 nm on a Benchmark Plus plate reader. Cell viability was calculated according to the formula:

$$\text{Cell viability (\%)} = A/A_0 \times 100\%$$

where A is average value of sample OD570, A_0 is average value of blank OD570 and OD570 is the absorbance measured at 570 nm[43].

Cell culture

MCF-7 cells, human breast cancer cells, were firstly cultured in 1640 medium supplemented with 10 % fetal bovine serum (FBS) in a humidified incubator at 37 ℃ under an atmosphere of 5% CO_2, Then were seeding on confocal dishes and allowed to cultivate for 24 h. Before fluorescence imaging, all the cells were washed with PBS thrice and then incubated with PBS free fresh media for subsequent cell imaging. When starting the imaging of endogenous GSH detection, the cells were pretreated with 100 μM N-Ethylmaleimide (NEM), a well-known thiol-blocking agent, for 90 min before incubation with ST-BODIPY (10.0 μM), then incubated with GSH (160

μM) for 30 min. Confocal fluorescence microscopy (FLUOVIEW FV3000. OLYMPUS) was used for all the imaging.[44]

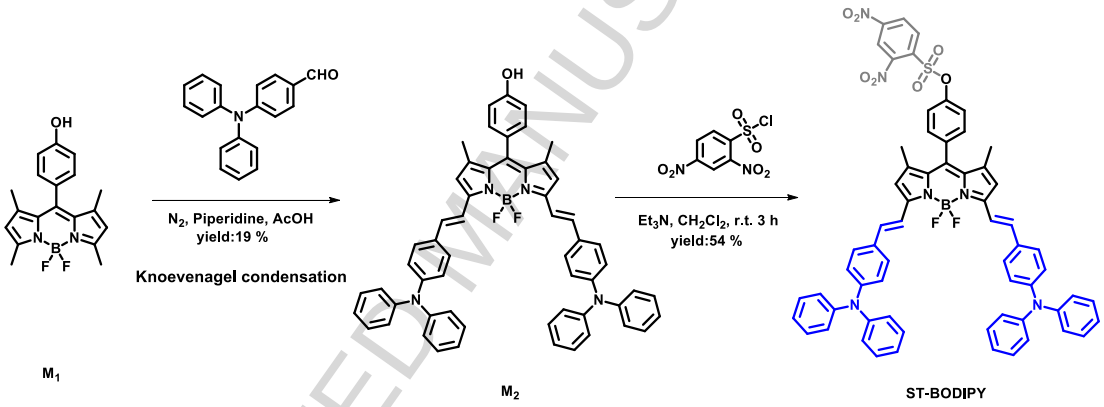
2.2 Synthesis

The synthetic route of ST-BODIPY is presented in scheme 2 and compound M_1[45,46] and 4-(diphenylamino)benzaldehyde[47] was obtained according to literature procedures.

The synthesis of M_2: Compound M_1 (340 mg, 1.0 mmol), 4-(diphenylamino)benzaldehyde (109 mg, 4.0 mmol) and piperidine (250 μL) were dissolved in dry toluene (20.0 mL) in a Dean-Stark apparatus, followed by the addition of three drops of AcOH. And then stirred at 100 ℃ for 12 h under a nitrogen atmosphere. The solvent was concentrated in vacuo and flash column chromatography (petroleum ether/dichloromethane, 1:2) afforded the product (162 mg, 19 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.83 (s, 1 H), 7.48-7.45 (m, 6 H), 7.39-7.34 (m, 10 H), 7.17 (d, J = 8.2 Hz, 2 H), 7.14 (t, J_1 = 7.1 Hz, J_2 =7.1 Hz, 4 H), 7.09 (d, J = 8.2 Hz, 8 H), 6.96-6.93 (m, 6 H), 6.92 (s, 2 H), 1.49 (s, 6 H).

The synthesis of ST-BODIPY: Compound M_2 (190 mg, 0.2 mmol), excess Et_3N (156 μL) and 2,4-dinitrobenzenesulfonyl chloride (125 mg, 0.4 mmol) were dissolved in CH_2Cl_2. After being stirred at room temperature for 3 h, The reaction mixture was washed with water, and the organic layers were combined and dried over anhydrous Na_2SO_4. The solvent was concentrated at reduced pressure, and the residue was purified by column chromatography (petroleum ether/dichloromethane, 1:1) afforded the product (130 mg, 54 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.14 (s, 1 H), 8.60 (d,

J = 8.7 Hz,1 H), 8.19 (d, *J* = 8.6 Hz, 1 H), 7.53-7.47 (m, 8 H), 7.41-7.38 (m, 4 H), 7.37 (t, J_1 = 7.9 Hz, J_2 = 7.7 Hz, 8 H), 7.14 (t, J_1 = 7.4 Hz, J_2 = 7.4 Hz, 4 H), 7.10 (d, *J* = 7.7 Hz, 8 H), 6.96-6.94 (m, 6 H), 1.34 (s, 6 H). ^{13}C NMR (300 MHz, DMSO-d_6) δ: 152.20, 151.51, 148.93, 148.36, 148.13, 146.35, 145.32, 140.39, 137.59, 137.41, 136.55, 135.37, 134.40, 133.79, 132.29, 130.99, 130.11, 129.64, 129.44, 129.30, 128.44, 127.18, 126.24, 124.89, 123.99, 122.77, 121.57, 121.07, 119.45, 118.54, 118.34, 115.97, 14.32. HRMS m/z calcd for $C_{63}H_{47}BF_2N_6O_7S$: $[M]^-$ = 1080.3288, found: 1080.3253.



Scheme 2 Synthetic route of ST-BODIPY

3. Results and discussion

3.1 Design and synthesis of the conjugated bistriphenylamine-BODIPY NIR fluorescent probe

In the design of NIR fluorescent probe ST-BODIPY depicted in Scheme 2, to afford the NIR dye Compound M_2, the 3 and 5 position of Compound M_1 were conjugated with 4-(diphenylamino)benzaldehyde through Knoevenagel condensation in toluene under a nitrogen atmosphere with catalysis of piperidine and AcOH. The 2,4-dinitrobenzenesulfonyl (DNBS) unit, as the fluorescence quencher and thiol

recognition moiety, linked to the 8 position of BODIPY. In the presence of GSH, glutathione-induced S_NAr reaction led to cleavage of the DNBS to generate free fluorophore in concert with turn-on fluorescence. The data of the structure characterization are described in the experimental section and supporting information (Fig. S1-S4, Supporting information). The target product ST-BODIPY were confirmed by ^1H NMR, ^{13}C NMR and mass pectrometry.

3.2 Two-photon turn-on NIR fluorescent probe ST-BODIPY for glutathione based on GSH-induced S_NAr reaction

Under the two-photon excitation condition, the probe alone displayed non-fluorescent emission on account of DNBS as fluorescence quencher moiety that switched off the TPEF(two-photon excited fluorescence) signal. However, in the presence of GSH, an apparent emission band centered at 719 nm appeared which attributed to GSH-induced S_NAr reaction (Fig. 1). The remarkable variation was accompanied by a dramatic color change from colorless to brightly pink under Ti:sapphire pulsed laser's irradiation, which was easily observed by naked eyes (Scheme 3). What's more, the 2PA cross section value (σ_2) of ST-BODIPY for GSH was examined to be 410 GM with rhodamine B[48] used as calibration standard (both at the concentration of 10^{-4} M) according to the equation[49]:

$$\varphi_s = \varphi_r \times \frac{A_r}{A_s} \times \frac{F_s}{F_r} \times (\frac{n_s}{n_r})^2$$

Here, Subscripts s and r stand for the unknown and to the standard, σ is the TPA cross-section value, c is the concentration of solution, n is the refractive index of the solution, F is the TPEF integral intensities of the solution emitted at the exciting

wavelength, and Φ is the fluorescence quantum yield. All the Two-photon experimental results demonstrated that ST-BODIPY was favorable for the detection of GSH as a Two-photon fluorescent turn-on NIR fluorescent probe. Maybe it was expected to have great potential applications in two-photon fluorescence bioimaging.

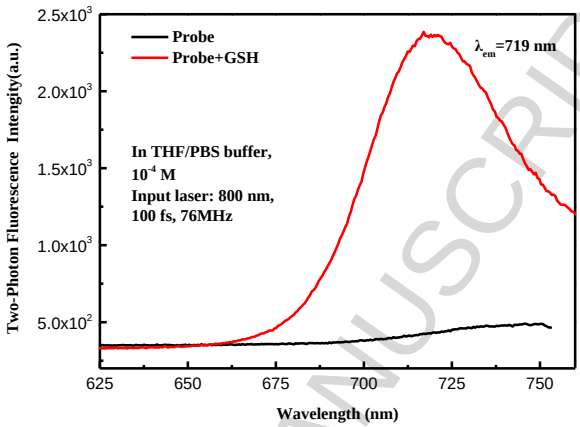
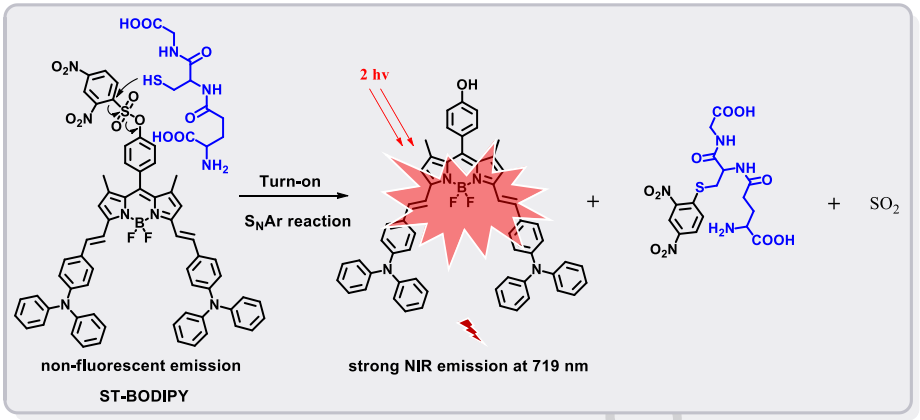


Fig. 1 Two-photon excitation fluorescence spectra of ST-BODIPY (100 μM) in the absence or presence of 1600 μM of GSH. Conditions: PBS buffer (pH= 7.4) and THF (1/1, v/v). λ_{ex} = 800 nm.

the proposed mechanism of NIR fluorescent probe ST-BODIPY for GSH was that the fluorescence enhancement response of ST-BODIPY was via thiol group of GSH induced S_NAr reaction which led to the cleavage of the DNBS group from the probe molecule (Scheme 3). To verify the mechanism, ST-BODIPY was treated with GSH in DMSO-d_6 at room temperature and then identified by ^1H NMR. As depicted in Fig. 2, the three protons on 2,4-dinitrobenzenesulfonyl group upfield shifted from 9.14, 8.60, 8.19 ppm (H_a, H_b, H_c) to 8.91, 8.52, 8.02 ppm ($H_{a'}$, $H_{b'}$, $H_{c'}$) respectively, which could be ascribed to the fact that the electron withdrawing O=S=O on

2,4-dinitrobenzenesulfonyl group was replaced by electron donating −S group after the nucleophilic aromatic substitution reaction.



Scheme 3. Proposed reaction mechanism for GSH-selective detection and its turn-on fluorescence response toward GSH under 800 nm two-photon excitation light

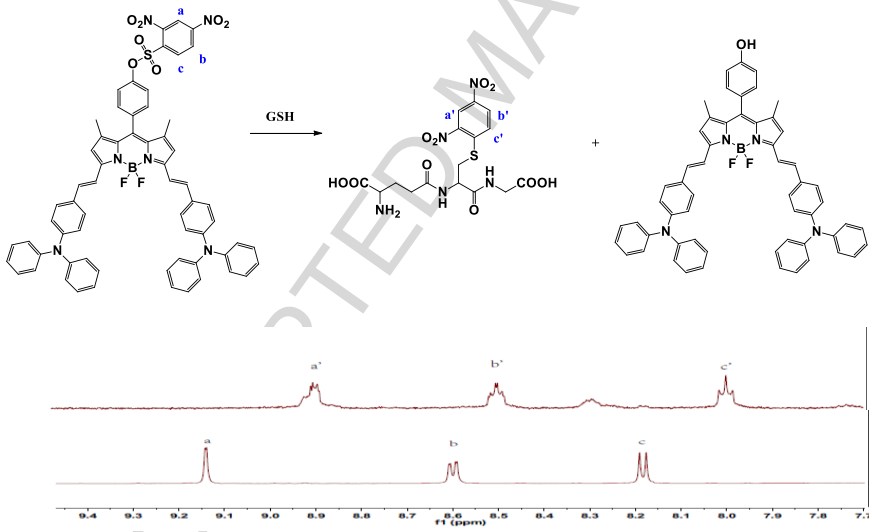


Fig. 2 Partial ^{1}H NMR spectra of ST-BODIPY, ST-BODIPY + GSH in DMSO-d_6

3.3 Glutathione-specific detection of NIR fluorescent probe

As displayed in Fig. 3a, ST-BODIPY alone exhibited non-fluorescence. However, the addition of GSH to the solution of ST-BODIPY generated a significant fluorescence enhancement at 719 nm indicating an “off-on” signal response. Besides,

the probe ST-BODIPY had no influence on the thiol-lacking amino acid, such as Gly, Pro, Tyr, Ala, Arg, His, Thr, Asp, Glu, Ser, leu, Val, Trp. As is known to us, GSH is the most abundant intracellular non-proteinogenic biothiol with intracellular concentration of 1-15 mM compared with Cys (30-200 μM) and Hcy (12.4 μM). Intracellular concentrations of Cys, Hcy, and GSH were respectively added to the solution of ST-BODIPY, the probe showed remarkably selective response toward GSH with a high quantum yield of 0.44. In contrast, the probe exhibited weak fluorescence towards Cys (Φ_F = 0.23) and Hcy (Φ_F = 0.12) (Table 1). These results indicated that ST-BODIPY can discriminate GSH from Cys and Hcy. Furthermore, due to the co-existence of various amino acids in the physiological environment, the interference from other amino acids should be taken into account. As shown in Fig. 3b, competitive experiment indicated that the probe can be utilized for the selective detection of GSH and not be interfered by other amino acids.

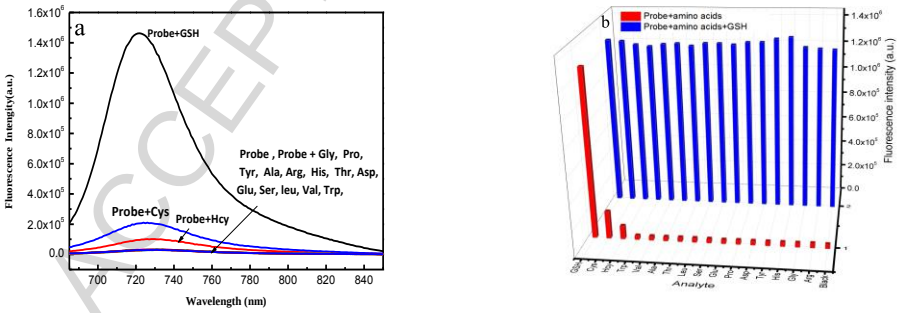


Fig. 3 (a) The fluorescence intensity of probe ST-BODIPY (10 μM, λ_{ex} = 670 nm) in the absence and presence of thiol amino acids including Cys (30 μM), Hcy (12.5 μM) and GSH (1 mM) in intracellular concentrations and other thiol-lacking amino acids (200 μM). (b) Fluorescence responses of ST-BODIPY in the presence of different

amino acids. The blue bars showed the intensities of ST-BODIPY + GSH (160 μM) + 200 μM amino acids. The red bars displayed the intensities probe ST-BODIPY in the absence and presence of thiol amino acids including Cys (30 μM), Hcy (12.5 μM) and GSH (1 mM) in intracellular concentrations and other thiol-lacking amino acids (200 μM). Conditions: PBS buffer (pH= 7.4) and THF (1/1, v/v).

The absorption spectral properties of NIR fluorescent probe ST-BODIPY were examined in a PBS buffer (pH 7.4) and THF (1/1, v/v) mixed solvent system. According to Fig. S5, the probe ST-BODIPY showed two absorption peaks at 435 nm ($\varepsilon = 3.14 \times 10^4\ cm^{-1} \cdot M^{-1}$) and 691 nm ($\varepsilon = 6.91 \times 10^4\ cm^{-1} \cdot M^{-1}$).

Table.1 The detailed photophysical properties of ST-BODIPY in the absence and presence of GSH and other amino acids

compounds	λ_{abs} (nm)	ε_{max} ($10^4\ cm^{-1}M^{-1}$)	λ_{em} (nm)	Fluorescence quantum yield (Φ_F)
probe	691	6.91	----	----
Probe+GSH	679	7.86	719	0.44
Probe+Hcy	679	7.89	726	0.12
Probe+Cys	679	7.70	720	0.23
Probe+His	693	6.88	----	----

λ_{abs}: UV absorption maximum; ε_{max}: molar extinction coefficient; λ_{em}: fluorescence maximum; Φ_F: fluorescence quantum yield. Conditions: PBS buffer (pH= 7.4) and THF (1/1, v/v).

3.4 Glutathione-sensitive NIR fluorescent probe ST-BODIPY with low detection limit

The fluorescence titration experiment was carried out to evaluate the ability of NIR fluorescent probe ST-BODIPY to detect GSH (Fig. 4a). In the free probe, there was almost no emission peak, while the emission band centered at 719 nm was observed and enhanced up to a saturation gradually after adding GSH with the increase of the concentrations. The emission spectra of ST-BODIPY to GSH had a good liner relationship and the detection limit for GSH was obtained to be 25.46 nM (3σ/k) (Fig. 4b), which was lower than most probes' in previous work.

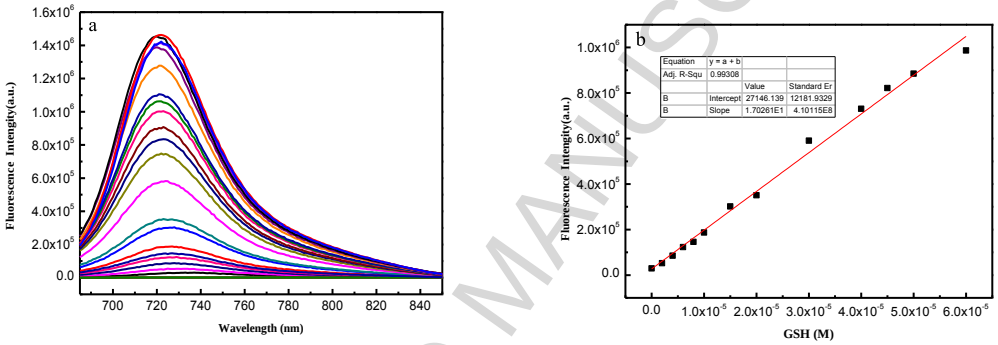


Fig.4 (a) Fluorescence spectra of probe ST-BODIPY (10 μM, λ_{ex} = 670 nm) with increasing amount of GSH. (b) Calibration curve of the emission response as a function of GSH concentration. Conditions: PBS buffer (pH= 7.4) and THF (1/1, v/v).

To further details of the applicability of the probe to detecting GSH in biological environment, the influence of the pH on GSH before and after adding GSH has been examined shown in Fig. 5. Emission intensity of the probe toward GSH between pH 6.0-9.0 maintained almost constant values, demonstrating that the NIR probe ST-BODIPY could work under physiological pH conditions, such as in living cells and tissues. Moreover, Time dependence on the recognition behavior of probe ST-BODIPY toward GSH, Hcy, and Cys testified that reaction rate to GSH was much

faster than Cys and Hcy upon the addition of intracellular concentrations of them (Fig. 6), which reconfirmed that probe ST-BODIPY showed remarkable selective detection towards GSH under physiological condition.

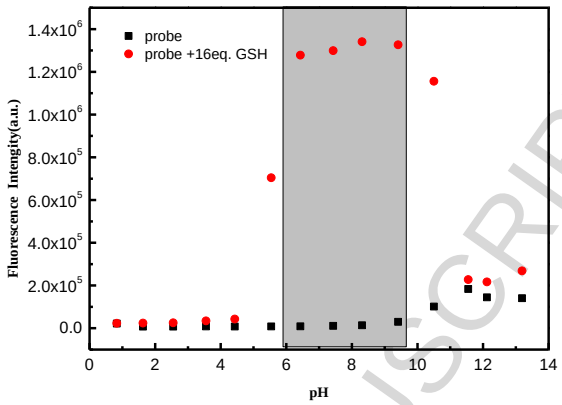


Fig. 5 Fluorescence intensity of ST-BODIPY (10 μM, λ_{ex} = 670 nm) in the absence (●) or presence (■) of GSH (160 μM) as a function of pH. Conditions: PBS buffer (pH= 7.4) and THF (1/1, v/v).

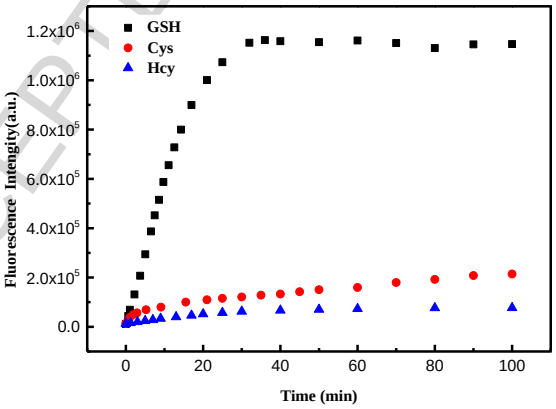


Fig. 6 Time-dependent changes of fluorescence spectra of probe ST-BODIPY (10 μM) with GSH (1 mM), Cys (30 μM) and Hcy (12 μM) in THF/PBS buffer (pH=7.4, 1: 1, v/v) (λ_{ex}=670 nm)

3.5 Endogenous and exogenous cell imaging of NIR fluorescent probe ST-BODIPY

Conventional MTT assay was investigated to certify cytotoxicity of the probe. As displayed in Fig. 7, it showed no apparent loss of cell viability when the probe was exposed in concentrations of 0-20 μM for 24 h under the experimental conditions,. This result demonstrated that the probe has low cytotoxicity to cultured cells.

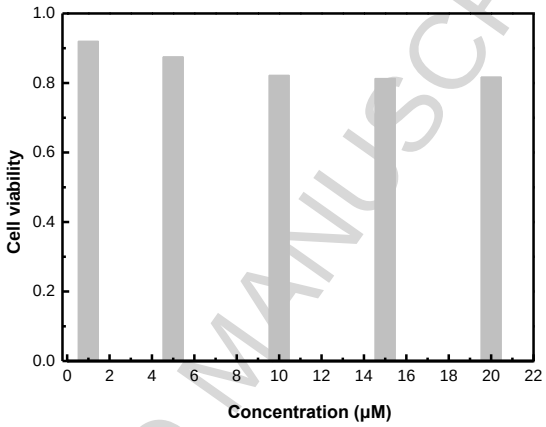


Fig. 7 Cytotoxicity of probe in MCF-7 cells treated with different concentrations of ST-BODIPY for 24 h, and cell viabilities were determined by the MTT assay. Datas were expressed as mean ± SD.

Based on above results, the practical utility of probe ST-BODIPY for selective fluorescent imaging of GSH in living cells was studied (Fig. 8). A significant bright NIR fluorescence was found when MCF-7 cells were incubated with probe ST-BODIPY (Fig. 8 (a - c)), indicating that probe ST-BODIPY was capable of permeating into cells and reacting with endogenous GSH to generate NIR fluorescence. When the MCF-7 cells were preincubated with 0.5 mM N-ethylmaleimide (NEM), a known thiol-blocking reagent, before incubating with

ST-BODIPY(10 uM), no emission was detected (Fig. 8 (d - f)), which demonstrated that thiol species were completely reacted by NEM. Upon the addition of GSH (1 mM) to the NEM pretreated MCF-7 cells followed by incubating with ST-BODIPY (10 μM), an outstanding NIR red fluorescence emerged (Fig. 8 (g - i)). All these results showed that probe ST-BODIPY not only could be used to detect the endogenous GSH, but also could be applied for cell imaging upon addition of GSH in living cells. Perhaps it could be further applied for detecting abnormal contents of GSH in a biological system and maintaining normal human activities.

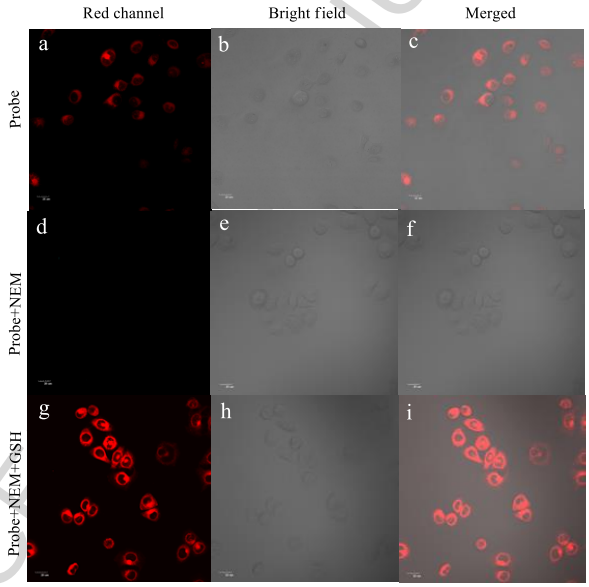


Fig. 8 The upside (a-c): confocal microscopy images of MCF-7 cells incubated with probe ST-BODIPY (10 μM) for 90 min; the middle (d-f): confocal microscopy images of MCF-7 cells preincubated with NEM (0.5 mM) for 30 min and then treated with probe ST-BODIPY (10 μM) for 90 min; the downside (g-i): confocal microscopy images of MCF-7 cells incubated with NEM (0.5 mM) for 30 min, then incubated

with ST-BODIPY (10 μM) for 90 min, and finally added GSH (1 mM). Excitation at 600 nm.

4. Conclusion

To sum up, we have developed a turn-on near-infrared two-photon fluorescent probe ST-BODIPY for the selectively and sensitively detection of GSH from other amino acids. Briefly, 4-(diphenylamino)benzaldehyde was conjugated at 3-position and 5-position of BODIPY with longer wavelength as NIR-fluorophore by Knoevenagel condensation. 2,4-dinitrobenzenesulfonyl (DNBS) was introduced at 8-position of BODIPY playing a role of fluorescence quencher and thiol recognition moiety. Under the Ti:sapphire pulsed laser's excitation at 800 nm, ST-BODIPY could be regarded as a two-photon fluorescent turn-on probe for GSH with the appearance and remarked enhancement of fluorescence emission peak at 719 nm accompanied by a dramatic color change from colorless to brightly pink. Due to its effective TPA action cross section value (σ_2=410 GM), it could be used for two-photon imaging for GSH with high sensitivity in living cells. In THF/PBS mixture solution, ST-BODIPY had a special ability to recognize GSH over Cys and Hcy when intracellular concentrations of them were added which was also testified from time-dependence. In the addition of GSH, the enhancement of fluorescence emission was observed at 719 nm on account of the GSH-induced S_NAr reaction that led to the cleavage of the DNBS group and the release of the fluorophore. Furthermore, this probe displayed high sensitivity for GSH and its detection limit was as low as 25.46 nM. The quantum yield was worked out to be 0.44. Furthermore, ST-BODIPY has been successfully

used for detection of endogenous and exogenous GSH. cell imaging experiment certified that the probe ST-BODIPY could infiltrate into MCF-7 cells and react with endogenous GSH to generate NIR fluorescence, which further displays its valuable applications in biological system.

Conflicts of interest

There are no conflicts to declare.

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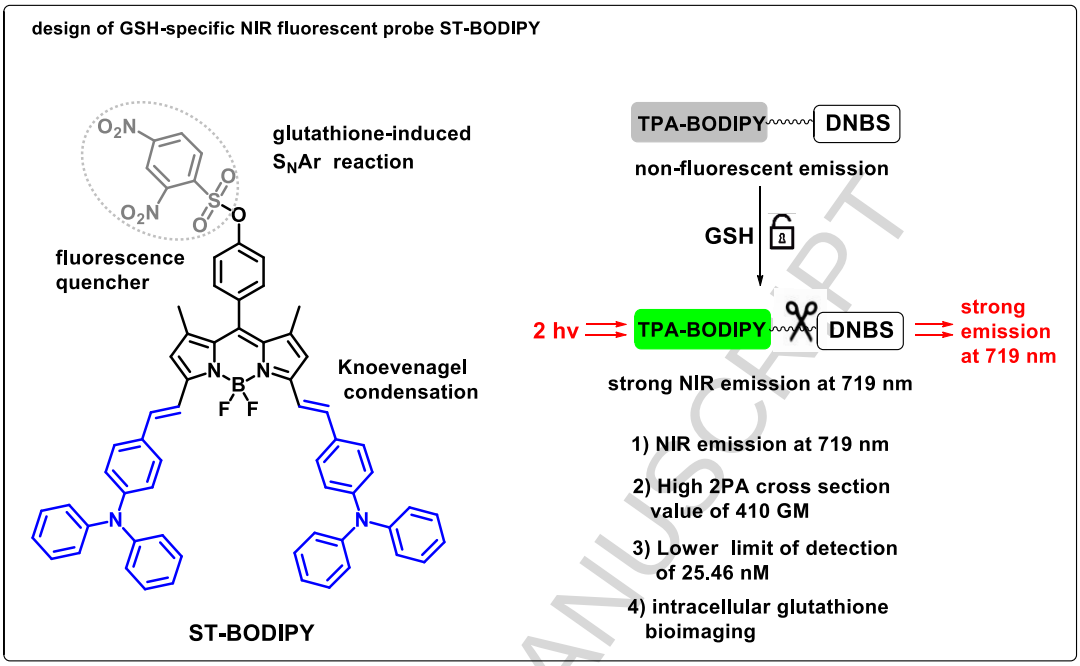
Graphical abstracts:



Highlights:

(1) ST-BODIPY was designed by attaching triphenylamine to BODIPY skeleton through Knoevenagel condensation to afford the NIR dye.

(2) Emission wavelength of ST-BODIPY reached NIR region which was at 719 nm.

(3) ST-BODIPY was served as two-photon fluorescent probe with large 2PA cross section value (σ_2) of 410 GM.

(4) ST-BODIPY was applied for endogenous and exogenous cell imaging.