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A near-infrared fluorescent probe for the detection of hydrogen polysulfides biosynthetic pathways in living cells and in vivo

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Abstract: Hydrogen polysulfides (H_2S_n, n > 1), derived from hydrogen sulfide (H_2S), have been considered to be involved in cytoprotective processes and redox signaling. The emerging evidences imply that the actual signaling molecule is H_2S_n rather than H_2S. In this work, we present a near-infrared fluorescent probe BD-ss for the selective detection of H_2S_n biosynthetic pathways in living cells and in vivo. The probe is constructed by equipping a bis-electrophilic H_2S_n capture group *p*-nitrofluorobenzoiate to a near-infrared fluorophore azo-BODIPY. BD-ss can provide a remarkable turn-on fluorescence response for assessing endogenous H_2S_n formation ways in serum, in living cells and in vivo.

Introduction

The performance of reactive sulfur species (RSS) has involved in every aspect of cell biology, from protein function to redox signal transduction. More and more evidences indicate that a variety of diseases arise due to RSS dysregulation which modified with reactive oxygen and reactive nitrogen species (ROS and RNS).[1] RSS mainly include glutathione (GSH), cysteine (Cys), hydrogen sulphides (H_2S), hydrogen polysulfides (H_2S_n, n>1), persulfides, and S-modified cysteine adducts such as S-nitrosothiols and sulfenic acids. Among them, H_2S is now recognized as the third gasotransmitter that plays important roles in biological systems.[2] However, H_2S_n, the direct redox forms of H_2S which are considered to be involved in cytoprotective processes and redox signaling, have always drawn less attention due to lacking of selective chemical tools. From the redox chemistry perspective, the redox couple of H_2S and H_2S_n are very likely to coexist in biological systems. In the presence of ROS, H_2S can form disulphide species. The disulphide species can also be reduced to H_2S (**1**). However, disulphide species will rapidly undergo further redox equilibrium reactions to produce other hydrogen polysulfides which is controlled by pH and the relative amount of the oxidized versus reduced forms (**2**).[3] At high (millimolar) concentrations, hydropolysulfides can also be formed by autoxidation of H_2S.[4] It is worth noting that the biosynthetic pathways and biofunctions of H_2S_n are still under investigation. H_2S_n may have their own biosynthetic pathways from H_2S in presence of ROS.[3] H_2S_n can also behave as the precursors of H_2S through their reducibility.[5] Therefore, some biological mechanisms that are previously attributed to H_2S may actually be mediated by H_2S_n.[4] For example, polysulfides can activate transient receptor potential channels in astrocytes, which previously has been contributed to the activating of H_2S.

$$2H_2S \xrightleftharpoons{|O|} H_2S_2 + H_2O \qquad (1)$$

$$nHS_2^- \rightleftharpoons HS_{n+1}^- + (n-1)\,HS^- \qquad (2)$$

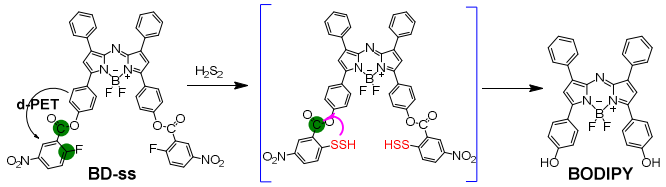
In order to better understand the function and biological properties of H_2S_n, it is urgent to develop highly selective and accurate methods for detection H_2S_n in biosystems. The traditional method for detecting polysulfides is to measure UV absorption peaks at 290-300 nm and 370 nm.[6] However, this traditional detection method requires the reduction of polysulfides to H_2S. Therefore, the traditional method cannot meet the demands of biological in situ detection by sensitivity and selectivity. Additionally, H_2S_n is species in biosystems. For this reason, fluorescent probe will be the desired chemical tool for the detection of intracellular H_2S_n because of its high sensitivity, selectivity and real-time detection.[7] Unfortunately, there is very few report on fluorescent probes for H_2S_n detection so far.[8] Xian's group has developed fluorescent probes with emission located in visible region for selective detection of exogenous H_2S_n in living cells. Compared with short wavelength emission, near-infrared (NIR) light has been drawn immense attention, because the NIR light can improve tissue depth penetration and minimize the effect from the background autofluorescence.[7,9] With this in mind, we attempt

to present a new NIR fluorescent probe for the detection of H_2S_n in living cells and in vivo.

Results and discussion

Probe design and detection mechanism

We conceive that H_2S_n hold two electrophilic mercapto groups (–SH), that is, such compounds can perform bis-nucleophilic reactions in biological systems. This chemical property may provide an opportunity for the selective capture of H_2S_n. We select a bis-electrophilic nitro-activated fluorobenzoiate[8a] as modulator for the probe BD-ss (Scheme 1). The electrophilic groups are often considered to be a strong quencher for the fluorophore. Therefore, we anticipate that fluorescence properties of a fluorophore can be manipulated through a photoinduced electron transfer (PET) process from the excited fluorophore to the strong electron-withdrawing group (donor-excited PET, d-PET).[10] To achieve our design strategy, we next particularly choose azo-BODIPY as the fluorophore, because of its high molar absorption coefficient, good photostability, and NIR emission. After integrated nitro-activated fluorobenzoiate into azo-BODIPY platform, the fluorescence of the fluorophore is effectively quenched by the d-PET process between the modulator and the fluorophore.



Scheme 1. Structure of BD-ss and proposed mechanism of BD-ss against H_2S_2

H_2S_n include a large number of active species, however, there exist rapid dynamic equilibrium between them (Scheme 1).[11] Therefore, in this work, hydrogen disulfide (H_2S_2) is always used as the primary model compound of H_2S_n. In our experiments, freshly prepared solutions of Na_2S_2 were used as the source of H_2S_2. The proposed detection mechanism of BD-ss is illustrated in Scheme 1. H_2S_2 begins nucleophilic aromatic substitution (S_NAr) via replacing F-atom to form an intermediate containing free -SH group. Subsequently, the free –SH group undergoes a spontaneous intramolecular cyclization with the ester group to release the azo-BODIPY fluorophore. This tandem reaction will eliminate the d-PET process, and trigger the fluorescence switch turn-on significantly. In order to affirm the detection mechanism of our probe, we performed a reaction of BD-ss with a biothiol model N-Acetyl-L-cysteine methyl ester. Fluorescent intensity had no changes when N-Acetyl-L-cysteine methyl ester was added. However, after Na_2S_2 was added to the reaction mixture, as expected, a remarkable increase in fluorescence intensity was observed. The results indicate that the relevant biothiols cannot interfere the detection of H_2S_n (Scheme S1, Electronic Supplementary Information, ESI).

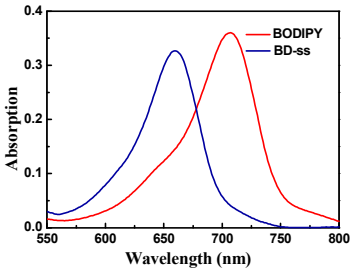


Fig. 1 UV–vis absorption spectral of probe BD-ss (10 μM) before and after addition of Na_2S_2 (20 μM). The blue curve is the absorption spectrum of BD-ss. The red curve was recorded after treatment with Na_2S_2.

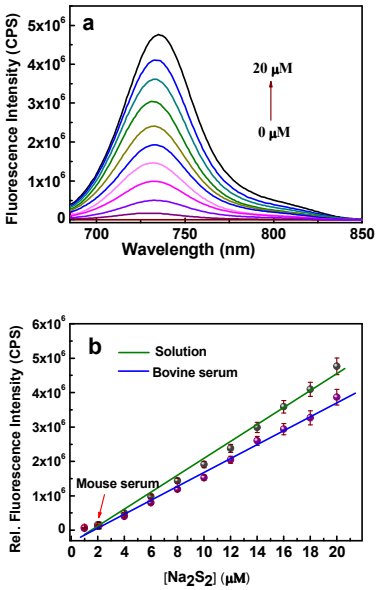


Fig. 2 a) Fluorescence spectra of BD-ss (10 μM) upon addition of Na_2S_2 (0 – 20 μM). Spectra were acquired in 10 mM HEPES buffer (pH 7.4, 0.4% Tween 80) after incubation with various concentrations of Na_2S_2 for 5 min at 37 °C. b) The corresponding linear relationship between the fluorescent intensity and Na_2S_2 concentrations (0-20 μM) in buffer solution and in serum. λ_{ex} = 707 nm, λ_{em} = 737 nm. The red point is the mean fluorescence intensity in mice serum.

Spectroscopic properties

The absorption and fluorescence spectra of BD-ss (10 μM) were examined under simulated physiological conditions (10 mM HEPES buffer, pH 7.4). As is well recognized, the environment of the cell is liposoluble, so we employ 0.4% Tween 80 to simulate the hydrophobicity of the cells, since Tween 80 is a nonionic surfactant and has been widely used in foods, pharmaceutical preparations, and cosmetics due to its effectiveness at low concentrations and relative low toxicity. BD-ss exhibited an absorption peak centred at 660 nm ($\varepsilon_{660\ nm}$ = 3.2 × 10^5 cm^{-1} M^{-1}). After treated BD-ss with Na_2S_2, a new absorption peak appeared at 707 nm ($\varepsilon_{707\ nm}$ = 3.6 × 10^5 cm^{-1} M^{-1}) indicating that BD-ss had reacted with H_2S_2 and induced the cleavage of ester group to release the fluorophore (Fig. 1). Upon addition of different concentration of Na_2S_2 (0 - 20 μM), the fluorescent profile gradually increased in the NIR region

(Fig. 2a). The fluorescence intensities at 737 nm were linearly related to the concentrations of Na_2S_2 under the given range (Fig. 2b). The regression equation was $F_{737\ nm} = 3.56\times10^5$ $[Na_2S_2]$ μM + 1.48×10^5 with r = 0.9970. The detection limit was determined to be 50 nM (3σ/κ) under the experimental conditions. The results demonstrate that BD-ss can potentially detect H_2S_n both qualitatively and quantitatively under simulated physiological conditions. We also explored the ability of BD-ss to quantify H_2S_n in serum sample. We prepared a simulated solution containing 20% fetal bovine serum to test the probe. Different concentrations of Na_2S_2 (0 - 20 μM) were added to the samples containing BD-ss (10 μM). As shown in Fig. 2b, the fluorescence intensities at 737 nm were linearly related to the concentration of Na_2S_2. The regression equation was $F_{737\ nm} = 2.08 \times 10^5$ $[Na_2S_2]$ μM - 3.30×10^3, with r = 0.9945. The different profiles of the two calibration curve could attribute to the reaction between H_2S_n and biomolecules in serum. The result indicates that our probe can determine H_2S_n both qualitatively and quantitatively in serum.

The physiologically relevant H_2S concentration is estimated ranging from nano- to millimolar levels.[2] However, the endogenous H_2S metabolism can be reversed through oxygen-dependent sulfane sulfur production. Sulfane sulfur are uncharged form of sulfur (S^0) with six valence electrons, which can be reversibly attached to proteins via covalent bond between S^0 and other sulfur atoms. They are mainly present in dihydropolysulfides (H-S_n-SH, n ≥ 1), hydropolysulfides (R-S_n-SH, n ≥ 1), polysulfides (R-S-S_n-S-R, n ≥ 1), and elemental sulfur (S_8).[2c] Sulfane sulfur play important roles in vivo. To completely comprehend the biofunctions of sulfane sulfur in vivo, it is necessary to develop methods that are sensitive enough to evaluate sulfane sulfur levels in biological samples. The traditional methods for the total sulfane sulfur assessment offer the concentrations ranging 1.3 to 85 μM.[12] As far as we known, to date, the concentration of H_2S_n is unavailable. Therefore, we next applied our probe to directly detect H_2S_n concentration in the BALB/c mouse serum (n = 5). The concentration of H_2S_n in the serum of mice was 2.01 μM (the red point in Fig. 2b). Our calibration curve covers the range of endogenous levels of H_2S_n, which indicates that our probe is very suitable for the detection of H_2S_n in biological samples.

Selectivity to H_2S_n

To test the selectivity for H_2S_n, BD-ss was treated with a series of biorelated RSS. As shown in Fig. 3, only H_2S_n could trigger strong fluorescence response. Other RSS, such as Cys, Hcy, GSH, CysSSCys, GSSG, Cys-poly-sulfide, S_8, $S_2O_3^{2-}$ and HSO_3^-, could not induce any fluorescence increase. However, NaHS (a common source of H_2S) would cause a little enhancement in fluorescence intensity due to the autoxidation of H_2S to H_2S_n at high concentration.[5] We also tested the response of BD-ss to other reductive species, such as ascorbic acid and tocopherol. There was also no fluorescence response to be found. Additionally, we also tested whether the effect of common physiological metal ions and anions could induce interference or not. As shown in Fig. S5 (see ESI), the probe did not give any fluorescence response to these metal ions and anions. Since the probe showed high selectivity towards H_2S_n, the competition experiments were performed in the presence of Na_2S_2. When Na_2S_2 (10 μM) and other RSS coexisted, we still observed satisfactory fluorescence response. These results demonstrate that our probe can be used for the selective detection of H_2S_n in the presence of biothiols and other physiological species.

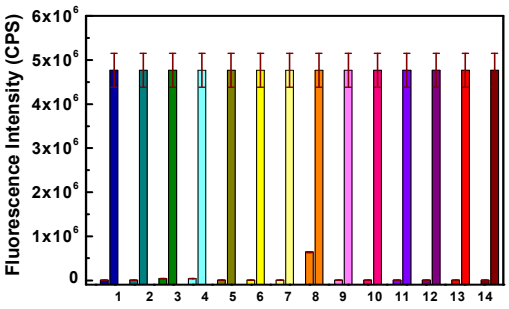


Fig. 3 Fluorescence responses of BD-ss (10 μM) to biologically relevant RSS. In each group, the bars represent relative responses at 737 nm of BD-ss to RSS and the mixture of RSS with 10 μM Na_2S_2, respectively. Legend: (1) blank + 20 μM Na_2S_2; (2) blank +20 μM Na_2S_4; (3) 1 mM Cys + Na_2S_2; (4) 1 mM Hcy + Na_2S_2; (5) 10 mM GSH + Na_2S_2; (6) 1 mM CysSSCys + Na_2S_2; (7) 1 mM GSSG + Na_2S_2; (8) 0.5 mM NaHS + Na_2S_2; (9)1 mM Cys-poly-sulfide + Na_2S_2; (10) 0.5 mM S_8 + Na_2S_2; (11) 0.5 mM $Na_2S_2O_3$ + Na_2S_2; (12) 0.5 mM $NaHSO_3$ + Na_2S_2; (13) 1 mM ascorbic acid + Na_2S_2; (14) 1 mM tocopherol + Na_2S_2. Data were recorded in 10 mM HEPES buffer (pH 7.4 and 0.4% Tween 80) at 37 °C for 35 min. λ_{ex} = 707 nm, λ_{em} = 737 nm.

Biosynthetic pathways of H_2S_n

The biosynthetic pathways of H_2S_n are under investigation until now, the recent studies suggest that H_2S_n may derive from H_2S in the presence of ROS.[3,13] We next employed BD-ss to detect in situ H_2S_n generation from H_2S and ROS. In this work, H_2O_2 was chosen on behalf of ROS. As shown in Fig. 4, the probe BD-ss displayed no response to H_2O_2. When NaHS was premixed with H_2O_2, a little fluorescence enhancement was obtained, which indicated the formation of H_2S_n in the systems. However, the formation process of H_2S_n was very slow. It was reported that glutathione peroxidase (GPx) could scavenge ROS through converting reduced biothiols (RSH) to oxidized biothiols (RSSR),[14] which inspired us that GPx might be involved in the H_2S_n biosynthesis. We added GPx into the system as the catalyst. Excitingly, a remarkable increase in fluorescence intensity was observed within 20 min (Fig. 4), which demonstrated GPx was participated in the reaction between H_2O_2 and H_2S. Moreover, some reports suggest that persulfides can be synthesized from cystine through cystathionine γ-lyase (CSE).[15] Therefore, we applied the probe to examine if H_2S_n could generate via enzymatic activity, including enzyme CSE and the related enzyme cystathioneine β-synthase (CBS). As indicated in Fig. 4, both of the enzymes CSE and CBS could induce the fluorescence intensity increase when cystine was used as substrate, while the fluorescence response to CBS was much weaker than that of CSE. We

attributed this phenomenon to the different physiological functions of the enzyme CSE and CBS in biosystems. The reaction of enzyme CSE with cystine mainly focused on the deriving hydropolysulfide species, while the enzyme CBS firstly generated polysulfides and then converted to hydropolysulfides in the presence of cystine and glutathione.[5,15] All of these results illustrate that our probe can be suitable for detection of H_2S_n not only generated from ROS and H_2S, but also enzymatic produced in biochemical systems.

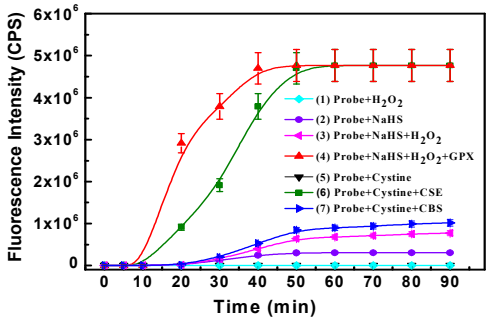


Fig. 4 Fluorescence responses of BD-ss (10 μM) towards (1)-(4) H_2O_2 (50 μM) in the presence of NaHS (50 μM) or GPx (50 U/L), and (5)-(7) hydrogen polysulfides catalysed by CSE (50 μg/mL) and CBS (50 μg/mL) using cystine (1.25 mM) as a substrate. Data were acquired in 10 mM HEPES buffer (pH 7.4, 0.4% Tween 80) at 37 °C for 35 min. λ_{ex} = 707 nm, λ_{em} = 737 nm.

Imaging H_2S_n in Cells

Having demonstrated the selectivity and sensitivity of BD-ss for H_2S_n, we next assessed the ability of BD-ss to respond to H_2S_n in the mouse macrophage cell line RAW264.7 cells. The first group (Fig. 5a-e): RAW264.7 cells were incubated with BD-ss (1 μM) for 15 min at 37 °C as control (Fig. 5a). The fluorescence intensity would increase rapidly after the cells were incubated with Na_2S_2 (5 μM) for 6 min, 9 min, 15 min, and 20 min at 37 °C (Fig. 5b-e). The fluorescence intensity would saturate after incubated Na_2S_2 for 15 min (Fig. 6a). The results illustrate that BD-ss can be used for detecting exogenously added H_2S_n in living cells. It has been reported that H_2S can be stored as H_2S_n in sulfane sulfur pool when H_2S at high concentration.[16] Next, we applied our probe to monitor the conversion process of H_2S to H_2S_n over time in RAW264.7 cells. The cells in Fig. 5f were incubated with BD-ss for 15 min at 37 °C, and then washed with RPMI-1640. The cells were further incubated with NaHS (50 μM) for 0.5h, 1h, 2h, and 3h at 37 °C, respectively. As shown in Fig. 5g-j, the confocal fluorescence images grew brighter as the incubation time from 0.5 to 3 h (Fig. 6b). The results indicate that the cells can convert excess H_2S to H_2S_n.

Hitherto, the biosynthetic pathways of H_2S_n are far from indubitability. Some investigations propose that H_2S_n can be formed when H_2S react with ROS, such as HClO, and H_2O_2.[3,11,13] In vitro test, we also confirmed that H_2S_n could be derived from H_2S in presence of H_2O_2, which catalysed by GPx. Therefore, we turned our attention to verify H_2S_n production from ROS and H_2S in RAW264.7 cells. The cells in Fig. 5k were pretreated with phorbol 12-myristate 13-acetate (PMA, 10 nM) for 30 min to stimulate the overproduction of ROS.[17] Then the cells incubated

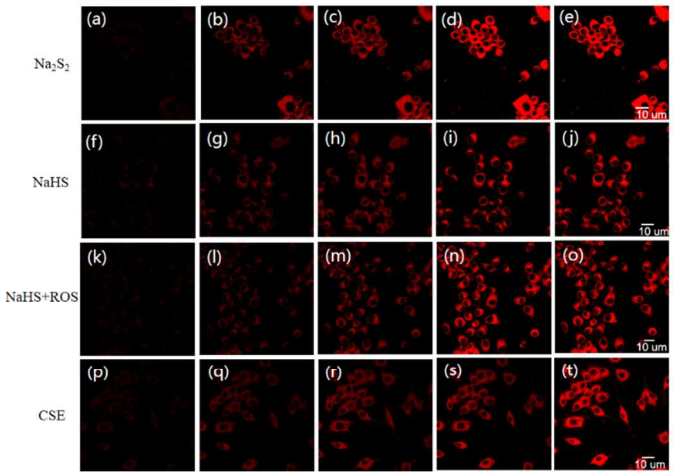


Fig. 5 Confocal microscopy images of living RAW264.7 cells for visualizing H_2S_n level changes using BD-ss (1μM). Images displayed represent emission intensity collected in optical window between 700 and 800 nm upon excitation at 635 nm. (a), (f), and (p) RAW264.7 cells incubated with BD-ss for 15 min at 37 °C, and washed with RPMI-1640. (b)-(e) The cells incubated with 5 μM Na_2S_2 for 6 min, 9 min, 15 min, and 20 min at 37 °C. (g)-(j) RAW264.7 cells incubated with NaHS (50 μM) for 0.5h, 1h, 2h, and 3h at 37 °C. (k) The cells pretreated with PMA (10 nM) for 30 min to overproduce ROS. (l)-(o) the cells were incubated with NaHS (50 μM) for 3, 6, 8, and 15 min. (q)-(t) RAW264.7 cells were stimulated by LPS (1 μg/ml) to monitor H_2S_n produced endogenously for 8h, 9h, 10h, and 16h.

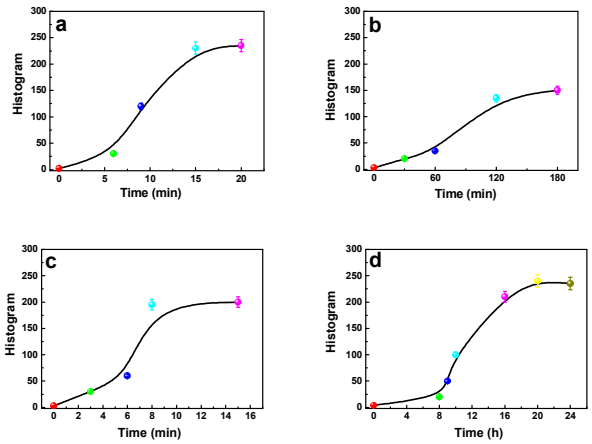


Fig. 6 Histogram of time dependent intensities of the images in Fig. 5. a) The histogram of Fig. 5a - 5e; b) The histogram of Fig. 5f - 5j; c) The histogram of Fig. 5k - 5o; d) The histogram of Fig. 5p - 5t.

with BD-ss for 15 min. After washed with RPMI-1640 to remove excess BD-ss, the cells were incubated with NaHS (50 μM) for 3, 6, 8, and 15 min (Fig. 5l-o). As expected, the fluorescence intensity increased (Fig. 6c), which provided clear demonstration that ROS could react with H_2S to form H_2S_n using GPx as a catalyst in living cells due to RAW264.7 cells contain GPx to regulate antioxidants and anti-inflammatory activities.

After having been confirmed the detection of H_2S_n supplemented exogenously, we next assessed the capability of BD-ss to detect endogenous H_2S_n. The formation of intracellular H_2S_n may be closely related with cystine and cystathionine γ-lyase (CSE).[5,15] We sought to determine whether our probe could detect endogenous H_2S_n that derived from cystine and CSE in RAW264.7 cells. CSE mRNA can overexpress when induced by lipopolysaccharide (LPS) in RAW264.7 cells.[18] RAW264.7 cells were incubated with BD-ss for 15 min. After washed with RPMI-1640, the cells were stimulated with LPS (1μg/mL). We selected the time points at 8h, 9h, 10h, and 16h to monitor the production of endogenous H_2S_n (Fig. 5q-t). As expected, there existed obvious fluorescence intensity increase over time (Fig. 6d). As control experiment, the cells were pretreated with a CSE inhibitor, DL-propargylglycine (PAG, 1 mM),[19] and were incubated with BD-ss (1 μM) for 15 min. Subsequently, the cells were stimulated with LPS for 24h. As shown in Fig. 7, the cells gave attenuated fluorescence response, confirming that CSE contributed to the H_2S_n generation. All these results indicate that our probe can directly detect endogenous and exogenous H_2S_n level changes in living cells.

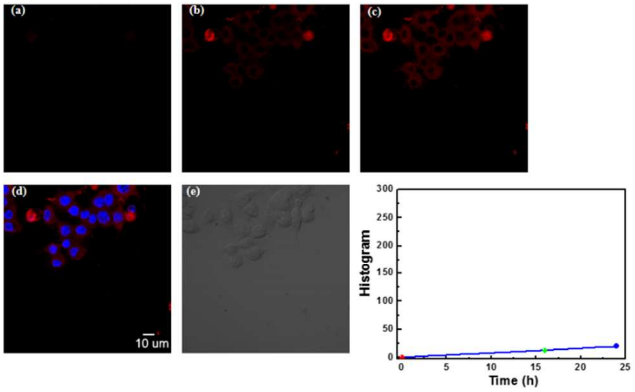


Fig. 7 Fluorescence confocal microscopic images of RAW264.7 cells pretreated by the CSE inhibitor. The cells were treated DL-propargylglycine (1 mmol/L) for 8 h, and then washed with RPMI -1640 and loaded with 1 μM BD-ss under 37 °C for 15 min (a). The cells were next incubated with LPS (1 μg/ml). The cells showed much weaker fluorescence response at (b) 16h and (c) 24h.

Imaging H_2S_n in Vivo

In order to highlight the advantages of our NIR probe, we finally assessed the capability of BD-ss for visualizing H_2S_n formation in living animals. We utilized BALB/c mice as the biological model to examine the potentiality. The mice in Fig. 8a (group b) were injected intraperitoneal (i.p.) cavity with LPS (100 μL, 10 μg/mL) for 24 h to induce CSE mRNA overexpress.[18] And then these mice were loaded with BD-ss (50 μL, 10 μM) for the next 30 min. There was a dramatic fluorescence increase in group b. The control mice which were given an i.p. injection of BD-ss (50 μL, 10 μM) displayed faint fluorescence (group a). The results indicated that our probe can detect the endogenous H_2S_n formation in vivo. Moreover, the quantification of mean fluorescence intensities for each group were shown in Fig. 8b The mean fluorescence intensity of group b is ~180 times higher than that of the control group. All these results convince that our probe can be used to image H_2S_n in living animals, which revealed the potential application of the new near-infrared fluorescent probe in vivo.

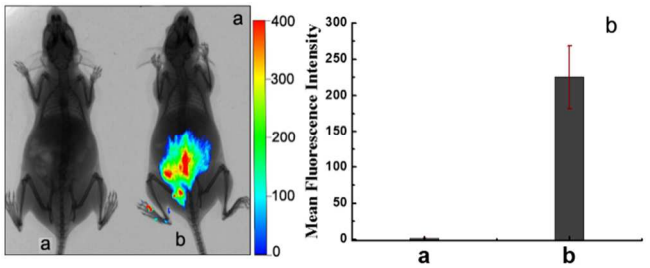


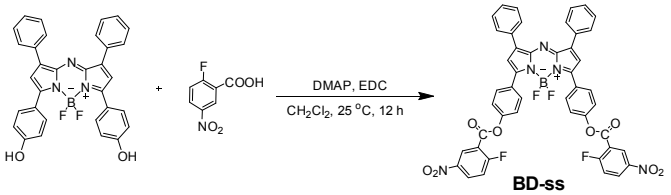
Fig. 8 Representative fluorescence images of mice visualizing H_2S_n level changes using BD-ss. Images constructed from 720 nm fluorescence collection window, λ_{ex} = 710 nm. (a) Group a was injected i.p. with BD-ss (10 μM, 50 μL in 1:9 DMSO/saline v/v) for 30 min. Group b was injected i.p. with LPS (10 μg/mL, 100 μL in 1:9 DMSO/saline v/v) for 24 h, and then loaded with BD-ss (10 μM, 50 μL in 1:9 DMSO/saline v/v) for 30 min. (b) Quantification of total photon flux from each group. The total number of photons from the entire peritoneal cavity of the mice was integrated. n = 5, Error bars are ± SEM.

Conclusions

In summary, we have developed a new NIR fluorescent probe BD-ss which exhibits high selectivity and sensitivity for H_2S_n both in serum, in living cells and in vivo. The probe is constructed by equipping a bis-electrophilic H_2S_n capture group *p*-nitrofluorobenzoiate to a near-infrared azo-BODIPY fluorophore. When exposed to H_2S_n, BD-ss releases a remarkable turn-on fluorescence response. In addition, BD-ss can also be used for monitoring H_2S_n in living cells not only exogenously added but also produced via enzymatic stimulation. This successful example will open up a new avenue to develop promising probes for the advancement of bioimaging of H_2S_n.

Experimental Section

Synthesis and characterization of probe BD-ss



Scheme 1. Synthetic route of BD-ss

BODIPY was synthesized in our laboratory according to the reported protocol.[20] A mixture of BODIPY (53.0 mg, 0.1 mmol), 2-fluoro-5-nitrobenzoic acid (37.0 mg, 0.2 mmol), 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC, 38.4 mg, 0.2 mmol) and 4-dimethylaminopyridine (DMAP, 2.44 mg, 0.02 mmol) in CH_2Cl_2 (50 mL) was stirred for 12 hours at 25 °C.[20] Then the mixture was neutralized with dilute HBr, and partitioned

between CH_2Cl_2 (50 mL) and H_2O (50 mL). Then the organic phase was evaporated under reduced pressure and resulted residue was subjected to column chromatography for purification (CH_2Cl_2). Probe BD-ss was obtained as a green solid. Yield: 37.4 mg, 43.3 %. ^{1}H NMR (500 MHz, $CDCl_3$-D_1) δ(ppm): 8.11 (m, 2H), 8.02 (m, 2H), 7.65-7.36 (m, 15H), 7.24-7.20 (m, 5H), 7.19 (s, 1H), 4.25 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$-D_1) δ(ppm): 166.80, 165.01, 163.19, 157.22, 156.03, 153.97, 153.45, 152.05, 142.67, 136.15, 134.32, 132.15, 131.01, 130.59, 130.08, 129.45, 129.04, 128.95, 128.54, 128.20, 127.95, 127.15, 121.75, 121.03, 117.75, 117.36, 92.30. LC-MS (ESI$^+$): m/z $C_{46}H_{26}BF_4N_5O_8$ calcd. 863.1811, found [M$^+$] 863.1813.

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Notes and references

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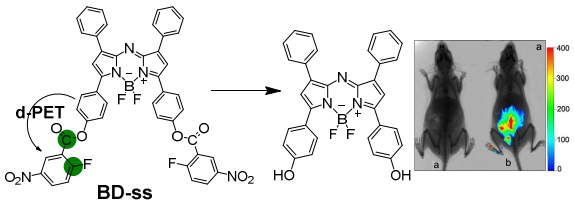
Graphic abstract



We present a new NIR fluorescent probe for the biosynthetic pathways of H_2S_n in living cells and in vivo.