Journal of Materials Chemistry B

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

rsc.li/materials-b

View Article Online
DOI: 10.1039/C7TB00098G

Journal Name

ARTICLE

A NIR fluorescent chemodosimeter for imaging endogenous hydrogen polysulfides *via* the CSE enzymatic pathway

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Sodium polysulfides (Na_2S_n, n > 1) as an important raw material in chemical industry can give hydrogen polysulfides (H_2S_n, n > 1) in physiological conditions. Hydrogen polysulfides are important reactive sulfide species that play crucial roles in biological systems. The simple detection of H_2S_n in solution and biosystems becomes very important as a consequence of the foregoing applications. Herein, we reported a NIR fluorescent chemodosimeter, **Cy-S_n**, displaying turn-on fluorescence after reaction with H_2S_n, which exhibits good selectivity and sensitively in solution and biosystems. Moreover, it was applied sucessfully to monitor endogenous hydrogen polysulfides *via* the cystathionine γ-lyase (CSE) enzymatic pathway during inflammation and anti-inflammation in living RAW 264.7 cells. The mice experiments indicated that the chemodosimeter has a good potential to be employed in the imaging of living biosystems.

Introduction

Sodium polysulfides (Na_2S_n, n > 1), as an important raw material in chemical industry, has been widely used in hydrometallurgy, rubber, leather industry, agriculture and etc. It is also an important source of sulfur dye. Sodium polysulfides could be produced from sodium sulfide and sulfur in industry and quantification of the concentration by titrimetry which takes much time.[1,2] Excess of sodium polysulfides could result sulphur-bearing effluent and destruction of the environmental system.[3] It has been reported that the effluent could cause damage to organism.[4,5] Simple detection of polysulfides may be request for ecological protection and industrial application. In physiological conditions, sodium polysulfides can give the equivalent of H_2S_n (n > 1).[6] As a derivative of sodium polysulfides, hydrogen polysulfide (H_2S_n, n > 1) received less attention having been considered as oxidized forms of H_2S. In present of ROS, H_2S can form disulphide species (1). However, a 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).[7]

$$2H_2S + 1/2O_2 \rightarrow H_2S_2 + H_2O \qquad (1)$$

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

Recent reports have suggested that H_2S_n might be a new group of signaling molecules. Besides, more and more studies imply that biological activities associated with H_2S may actually be executed by H_2S_n.[8,9] For instance, recent research demonstrated that H_2S_n features hundreds times more potent than that of H_2S in inducing Ca^{2+} influx in astrocytes *via* S-sulfhydration on TRAP1 channels. In addition, many results revealed H_2S_n are more effective than H_2S in S-sulfhydration.[10] This posttranslational modification was previously thought to be mediated by the reaction of of H_2S with oxidised cysteine residues such as S-sulfenic acid (S–OH) and S-nitrosothiol (SNO).[11-13] However, recent results have demonstrated that H_2S_n plays an important role in many protein S-sulfhydration.[14-16] It was also found that H_2S_n can activate tumor suppressors, ion channels, and transcription factors with higher potency than H_2S.[17] So it is of great significance to recognize H_2S_n in biosystems.

In recent years, a few analytical technologies have been developed to determination of polysulfides in the chemical system, such as titrimetry,[18,19] spectrophotometry and polarography typically involve indirect assays or reaction of the sample with reagents such as sulfite or indine.[20,21] However, most of these methods are effective for extracellular polysulfides. Fluorescent probes have become powerful tools for monitoring the extracellular and intracellular levels of biologically relevant species and to understand their functions because of the rapid, nondestructive, selective, and sensitive advantages of emission signals.[22-31] Up to date, some fluorescent probes have been developed for imaging H_2S_n in living cells.[6,32-35] However, only small amount of them could image H_2S_n with a NIR fluorescent emission.[36,37] Therefore, it is significant to develop a NIR fluorescent probe for imaging H_2S_n *in vitro* and *in vivo*.

The semiheptamethine derivative, a classic NIR dye scaffold, has been wildly exploited as a fluorophore in the detection of various species,[38] such as palladium,[39] hydrazine,[40] ROS,[41-45] pH,[46,47] thiols,[48,49] and nitroreductase.[50] Herein, we developed a novel fluorescent probe, **Cy-S_n**, based on a semiheptamethine derivative for polysulfides with NIR fluorescent emission. The proposed probe

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†Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

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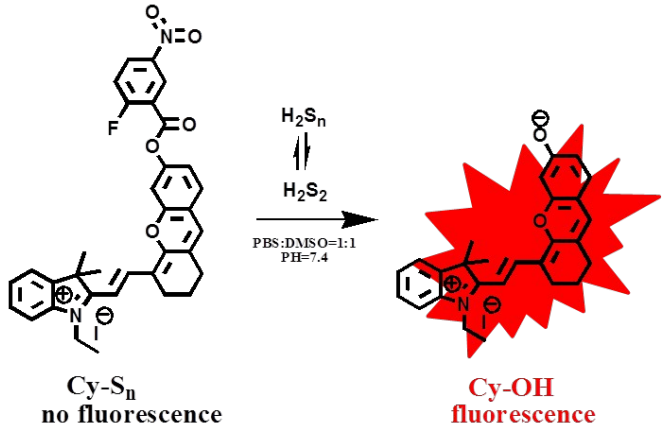
DOI: 10.1039/C7TB00028C

features a semiheptamethine cation unit as the fluorophore and an electrophilic polysulfides capture group *p*-nitrofluorobenzoate as the polysulfides recognition unit as well as a fluorescence quencher. Upon reaction with H_2S_n, the nucleophilic aromatic substitution to fluorobenzoiates affords the persulfide intermediates, which subsequently undergoes a cyclization to form benzodithiolone products and release **Cy-OH** with a NIR fluorescent emission (Scheme 1).



Scheme 1. The structure and reaction mechanism of the probe **Cy-S$_n$**.

Results and discussion

Systhesis of probe Cy-Sn

Cy-S$_n$ was synthesized according to the route shown in Scheme S1 as a blue solid in several steps and has been characterized by ^{1}H NMR, ^{13}C NMR and HRMS.

UV-vis and fluorescence responses

In these experiments, fresh prepared solutions of Na_2S_2 and Na_2S_4 were used as the equivalent of H_2S_2 and H_2S_4. The absorption and fluorescence properties of **Cy-S$_n$** were determined in PBS (pH = 7.4) solution containing 50% of DMSO (Fig. 1a). **Cy-S$_n$** has a maximum absorption peak at around 590 nm. After treatment of **Cy-S$_n$** with Na_2S_4, the maximum absorption peak shifted from 590 nm to 700 nm. The photo-stability of both **Cy-S$_n$** and **Cy-S$_n$** treated with Na_2S_4 is much better than **Cy7-Cl** (structure in Fig. S1b) after exposure to high density light (500W, tungsten lamp) for 100 min, which is a critical factor for bioimaging *in vivo* (Fig. S1a).

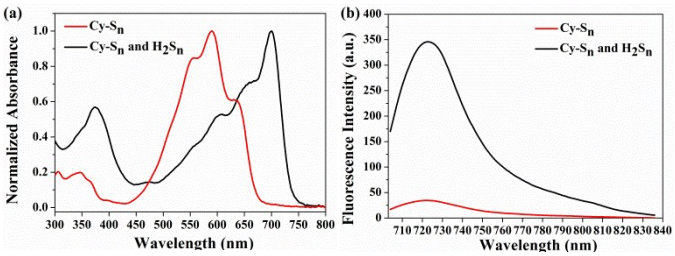


Fig. 1 (a) UV-vis absorption, (b) fluorescence emission spectrum of 5 μM **Cy-S$_n$** before and after addition of 300 μM H_2S_n.

Probe **Cy-S$_n$** showed almost no fluorescence emission upon excitation at 720 nm. Addition of Na_2S_4 resulted in an obvious increase at 720 nm (Fig. 1b). Mass spectrometry data along with absorption and emission spectra showed that the *p*-nitrofluorobenzoate group in **Cy-S$_n$** can capture H_2S_n sensitively and generate **Cy-OH** as the fluorescence product (Fig. S2). The fluorescence intensity of **Cy-S$_n$** increased obviously at 720 nm after treatment of H_2S_n, indicating that the probe is suited for the recognition of H_2S_n both *in vivo* and *in vitro*.

To further identify the performance of **Cy-S$_n$**, the fluorescence of the probe was measured by fluorescence titration experiment. In a certain range, the fluorescence intensity increased gradually following the increased concentration of Na_2S_4 (Fig. 2a and Fig. 2b). With increasing concentrations of Na_2S_4, the fluorescence titration spectrum exhibited two different enhancement profiles between the fluorescence intensity at 720 nm and the concentration of Na_2S_4 (Fig. 2c). The linear correlations are $y_1 = 11.20799\ x_1 + 32.41159$ (Na_2S_4 0—10 μM) and $y_2 = 1.18938\ x_2 + 137.979$ (Na_2S_4 10—100 μM). The detection limit of H_2S_n was calculated according to the equation DL= 3σ/S, where σ is the standard deviation of a blank measurement and S is the slope between intensity versus sample concentration. The detection limit of **Cy-S$_n$** was calculated to be 2.2 × 10^{-8} M. The time-dependent fluorescent change measurements showed the response time of **Cy-S$_n$** toward H_2S_n was 5 min (Fig. 2d). The pH experiments indicated that **Cy-S$_n$** could detect H_2S_n in physiological pH range (Fig. S3).

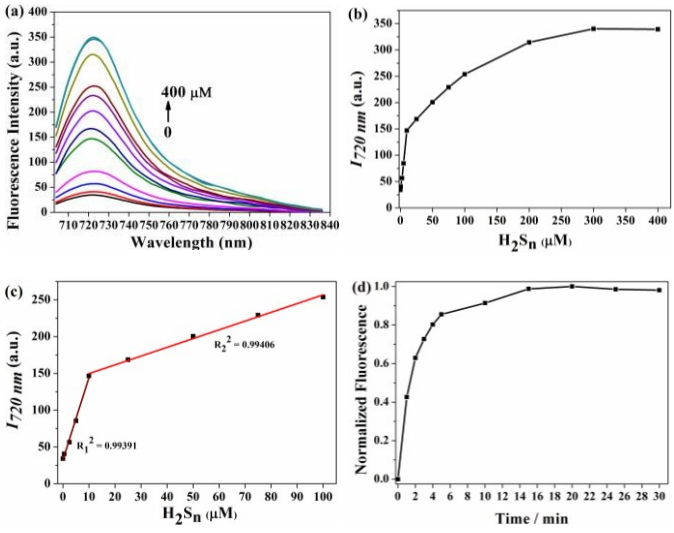


Fig. 2 (a) and (b) Emission spectra of probe **Cy-S$_n$** (5 μM) in the presence of different concentration of Na_2S_4 (0, 0.5, 2.5, 5, 10, 25, 50, 75, 100, 200, 300 and 400 μM, 5 min) ; (c) two different linear correlations between emission intensity and concentration of Na_2S_4 at 720 nm; (d) Time-dependent fluorescence changes of **Cy-S$_n$** (5 μM) upon addition of Na_2S_4 (300 μM) at 720 nm. Emission spectra obtained in solution (DMSO/PBS = 5/5, pH = 7.4) upon excitation at 680 nm.

H_2S_n selective and competitive experiment

To test the selectivity of the probe for H_2S_n, **Cy-S$_n$** was treated with a series of RSS and ROS. As shown, no significant fluorescence increase was observed for any of these RSS compounds (Fig. 3a).

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Only Na_2S_2 and Na_2S_4 could induce obvious fluorescence intensity (720 nm). Moreover, obvious fluorescence enhancements were still observed when Na_2S_4 and other coexisted in solution. Compared to Na_2S_2 and Na_2S_4, almost the same levels of fluorescence turn-on responses were observed for most of these compounds. These results clearly demonstrated that **Cy-S_n** shows high activity and selectivity to recognize H_2S_n in a complex environment (Fig. S4 and Fig. S5). On the other hand, the probe did not give response to the common ROS including hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), superoxide (O_2^-), singlet oxygen ($^1O_2$) and hypochlorite (ClO^-). However fluorescence enhancements were observed when **Cy-S_n** was treated with the mixture of ROS and H_2S especially for ClO^-/H_2S system (Fig. 3b, Fig. S6 and Fig. S7). Among them, hypochlorite (ClO^-) is the most effective ROS coverting H_2S to H_2S_n in the solution. This result is consistent with reports by Nagy et al. that H_2S_n can be generated rapidly from hypochlorous acid and H_2S.[51]

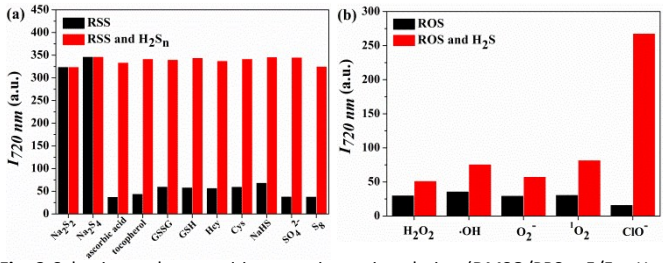

Fig. 3 Selective and competitive experiment in solution (DMSO/PBS = 5/5, pH = 7.4) with the emission intensity at 720 nm. (a) Fluorescence response of **Cy-S_n** (5 μM) to various RSS (300 μM) with H_2S_n (red) and without (black). Each spectrum was recorded at 5 min after addition of the analytes (from left to right represent Na_2S_2, Na_2S_4, ascorbic acid, tocopherol, GSSG, GSH, HCy, Cys, NaHS, SO_4^{2-}, S_8,). (b) Fluorescence response of **Cy-S_n** (5 μM) to various ROS (300 μM) with (red) and without (black) H_2S (300 μM). Each spectrum was recorded at 5 min after addition of the analytes (from left to right represent H_2O_2, ·OH, O_2^-, $^1O_2$ and ClO^-).

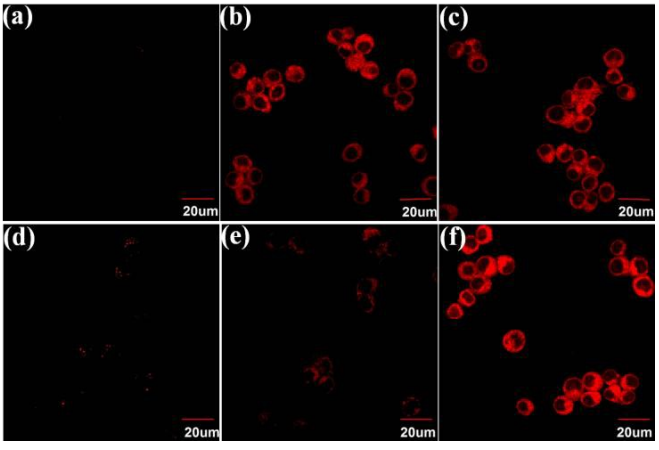

Fig. 4 Confocal microscope images of RAW264.7 cells. Cells were treated with **Cy-S_n** for 15 min, then PBS (a), Na_2S_2 (b) and Na_2S_4 (c) for 20 min. From (d) to (f), cells were treated with **Cy-S_n** for 15 min, then NaClO, NaHS and mixture of NaClO and NaHS for 20 min. Confocal microscope image of cells upon excitation at 635 nm, emission window 650—750 nm.

Fluorescence bio-imaging and cytotoxicity assay

The ability of **Cy-S_n** to recognize H_2S_n in living cells was investigated. As a control, little fluorescence was observed when RAW264.7 cells were treated with **Cy-S_n** (5 μM) for 15 min at 37 °C (Fig. 4a). After RAW264.7 cells incubated with **Cy-S_n** (5 μM) for 15 min and subsequently Na_2S_2 and Na_2S_4 (50 μM) for 20 min at 37 °C, red fluorescence was obtained in the cells by a confocal fluorescence microscope imaging upon excitation at 635 nm (Fig. 4b and Fig. 4c). These results showed that **Cy-S_n** could selectively recognize exogenous H_2S_n in living cells.

H_2S_n derivatives are short-lived species and can readily decompose in buffer. It may be a reliable and sustainable system from H_2S and NaClO for *in situ* generation of H_2S_n. Herein, **Cy-S_n** was used to monitor the *in situ* generation of H_2S_n in living cells. RAW264.7 cells were incubated with **Cy-S_n** (5 μM) for 15 min. Subsequently the cells were incubated with NaHS (50 μM), NaClO (50 μM) or the mixture of NaHS (50 μM) and NaClO (50 μM) for 20 min at 37 °C. Neither H_2S nor ClO^- gave noticeable fluorescence responses in cells. H_2S_n from the reaction of H_2S and NaClO led to fluorescence enhancement in cells, respectively. (Fig. 4d—4f). This result is consistent with the reports by Xian et al. that H_2S can react with NaClO and generate H_2S_n *in situ*[52] which could be selectively recognized by **Cy-S_n** in living cells.

Intracellular H_2S_n is close to cystathionine γ-lyase (CSE)[53] which could be regulated by inflammation and anti-inflammation. CSE can be overexpressed when induced by lipopolysaccharide (LPS) which cause inflammation in RAW264.7 cells. On the other hand, dexamethasone can down-regulate CSE expression in LPS-treated cells during anti-inflammation.[54,55] **Cy-S_n** was applied to monitor endogenous hydrogen polysulfides *via* the CSE enzymatic pathway during inflammation and anti-inflammation. RAW264.7 cells were incubated with **Cy-S_n** (5 μM) for 15 min at 37 °C. There existed noticeable fluorescence intensity enhancement after the cells being stimulated with LPS for 24 h. However, weakened fluorescence was obtained when the cells co-incubated with dexamethasone and LPS (Fig. 5). As control, almost no fluorescence was observed when the cells were co-incubated with PPG (DL-propargylglycine, an inhibitor of CSE) and LPS. All these results demonstrated that **Cy-S_n** could recognize exogenous and endogenous H_2S_n in living cells.

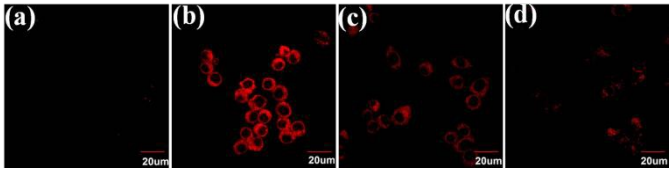

Fig. 5 Confocal microscope images of RAW264.7 cells. Cells were treated with **Cy-S_n** for 15 min, then PBS (a), 1 μg/mL LPS for 24 h (b), 1 μg/mL LPS and 1 nM dexamethasone for 24 h (c), pretreated with 1mM PPG 8 h then 1 μg/mL LPS for 24 h (d). Confocal microscope image of cells upon excitation at 635 nm, emission window 650—750 nm

We further tested the cytotoxicity of **Cy-S_n** by using a cell viability assay. RAW264.7 cells were treated with **Cy-S_n** (2.5 and 5 μM) for 24 h, and then cell viability was evaluated using a MTT assay. The results showed that cell viability was over 90%, indicating that **Cy-S_n** had low cytotoxicity (Fig. 6).

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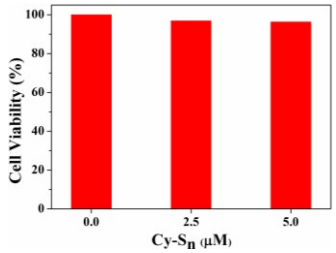

Fig. 6 Cytotoxicity of **Cy-S$_n$** probe. Cell viability of RAW264.7 cells incubated with **Cy-S$_n$** (2.5 and 5 μM) for 24 h.

Being encouraged by successful utilization of **Cy-S$_n$** in imaging H_2S_n in living cells, we evaluated the suitability of **Cy-S$_n$** for monitoring H_2S_n in living mice. **Cy-S$_n$** (50 μM, 100 μL PBS) and Na_2S_4 (100 μM, 100 μL PBS) were subcutaneous injection into a shaved living mouse in order. As shown in Fig.7, after being incubated for a certain time, fluorescence intensity increased over time and be saturated at about 60 min. With the above results, it was revealed that **Cy-S$_n$** could recognize H_2S_n in living mice.

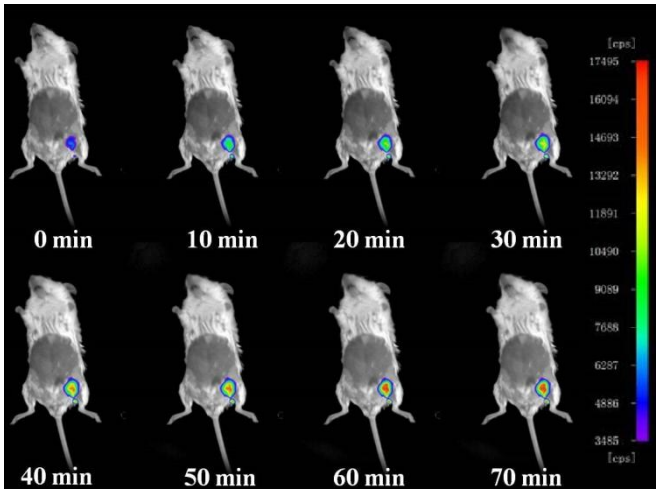

Fig. 7 Fluorescence images of a shaved living mouse given a subcutaneous injection of **Cy-S$_n$** (50 μM, 100 μL PBS) and a subsequent subcutaneous injection of Na_2S_4 (100 μM, 100 μL PBS). Images were taken after incubation for 0, 10, 20, 30, 40, 50, 60 and 70 min, respectively. The images were taken with an excitation at 630 nm and emission at 700 ± 10 nm.

The application in the detection of sodium polysulfide in solution

It is an important process to quantification of sodium polysulfide in the raw solution after synthesis. **Cy-S$_n$** was used to quantification of sodium polysulfide in the raw solution. The raw sodium polysulfide solution was prepared according to the literature and the concentration of sodium polysulfide was determined by titrimetry and fluorimetry based on **Cy-S$_n$**.[1] Compared to the conventional titrimetry, the concentration determined by **Cy-S$_n$** showing ingored difference indicated that **Cy-S$_n$** may have a potential application in industry.(Table 1)

Table 1. Concentration of sodium polysulfides in raw solution

sodium polysulfide	titrimetry[a]	fluorimetry
c (mol/L)	0.393	0.368

[a] data from literature [1]

Experimental

General

All solvents and reagents used were reagent grade and were used without further purification unless specified. The stock solution of **Cy-S$_n$** was dissolved in DMSO at a concentration of 1 mM and stored in a refrigerator for use. Na_2S_2 and Na_2S_4 solution were fresh prepared at the concentration of 100 mM before use. ^{1}H NMR and ^{13}C NMR spectra were recorded on a VARIAN INOVA-400 (or a Bruker Avance II 400 MHz or 500 MHz) spectrometer. Chemical shifts (δ) in ppm (in $CDCl_3$ or Acetone-d^6, with TMS as the internal standard). A VARIAN CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812-M018) was used for fluorescence spectroscopy. A Perkin Elmer Lambda 35 UV/VIS spectrophotometer (Perkin Elmer) was used for measuring absorption spectroscopy. HRMS spectrometric data were reported on HP1100LC/MSD MS and an LC/Q-TOF-MS instruments. A Model PHS-3C meter was used for all pH measurements. The HCl or NaOH stock solution was prepared at a concentration of 1 M for slight pH changes. Silica gel (100 - 200 mesh) and aluminum oxide (neutral. 100-200 mesh) bought from Qingdao Ocean Chemicals were used for flash column chromatography. Doubly purified water used in all experiments was from Milli-Q systems. All measurements were recorded at room temperature.

Synthesis of Compounds

Synthesis of compound **Cy-OH**

Cy-OH was synthesized according to the procedure published in literature.[56] Resorcinol (275.6 mg, 2.48 mmol) and triethylamine (0.3 mL) were placed in a flask containing DMF (5 mL) at room temperature under a nitrogen atmosphere. After stirring for 10 min, compound 2 (529.70 mg, 0.83 mmol) in DMF (2 mL) was introduced to the mixture via a syringe, subsequently the mixture was heated to 50 °C for 5 h. The solvent was removed under vacuum. The crude product was purified by silica gel flash chromatography to give compound **Cy-OH** as a blue-green solid (262.38 mg, yield 60.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 13.1 Hz, 1H), 7.32 (dd, J = 11.1, 6.4 Hz, 3H), 7.20 – 7.14 (m, 1H), 7.13 – 6.90 (m, 3H), 5.81 (d, J = 13.5 Hz, 1H), 4.00 (d, J = 7.0 Hz, 2H), 2.68 (s, 2H), 2.59 (s, 2H), 1.90 – 1.86 (m, 2H), 1.67 (s, 6H), 1.40 (t, J = 6.9 Hz, 3H). HRMS (ESI) calcd for $C_{27}H_{28}NO_2^+$ $[M]^+$ 398.2115, found 398.2118. **Cy-OH** was used next step without other characterization.

Synthesis of 2-fluoro-5-nitrobenzoyl chloride

2-fluoro-5-nitrobenzoicacid (185.11 mg, 1 mmol) was placed in a flask containing anhydrous CH_2Cl_2 (5 mL), and then the solution was stirred 0 °C under a nitrogen atmosphere for 30 min. Oxalyl chloride (750 mg, 5.91 mmol) and anhydrous DMF (2 drops) were added slowly. The mixture was stirred 0 °C for 1 h and overnight at room

DOI: 10.1039/C7TB00098G

temperature. After removing the solvent under vacuum, the crude product was used next step directly.

Synthesis of compound **Cy-S_n**

To solution of **Cy-OH** (84.02 mg, 0.16 mmol) in anhydrous CH_2Cl_2 (5 mL) was added 4-dimethylaminopyridine (39.09mg, 0.32 mmol) and 2-fluoro-5-nitrobenzoyl chloride (64.95mg, 0.32 mmol) at room temperature. After 30 min, the mixture was concentrated under vacuum to give the crude product, which was purified by silica gel flash chromatography to afford the desired product (85.38 mg, 77.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (dd, *J* = 6.0, 2.9 Hz, 1H), 8.64 (d, *J* = 15.2 Hz, 1H), 8.59 – 8.49 (m, 1H), 7.50 (t, *J* = 4.6 Hz, 4H), 7.47 (d, *J* = 3.1 Hz, 1H), 7.45 – 7.40 (m, 1H), 7.20 (d, *J* = 1.9 Hz, 1H), 7.18 – 7.11 (m, 2H), 6.77 (d, *J* = 15.1 Hz, 1H), 4.68 (d, *J* = 7.3 Hz, 2H), 2.82 (s, 2H), 2.75 (s, 2H), 2.00 – 1.90 (m, 2H), 1.78 (s, 6H), 1.54 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.42, 166.59, 164.42, 160.16, 159.35, 153.09, 151.81, 146.54,142.43, 140.98, 130.90, 130.81, 130.72, 130.27, 129.48, 128.59, 128.40, 128.13, 122.56, 120.41, 118.95, 118.69, 115.92, 113.45, 109.35, 106.94, 51.15, 42.05, 29.61, 28.39, 24.44, 20.27, 13.33. HRMS (ESI) calcd for $C_{34}H_{30}FN_2O_5^+$ $[M]^+$ 565.2133, found 565.2136.

Cell culture and confocal fluorescence imaging

RAW264.7 cells were cultured in DMEM supplemented with 10% fetal bovine serum at 37 °C in an atmosphere containing 5% CO_2. For live cell imaging, cells grown in a glass bottom dish were incubated with compound for 30 min and wash with DMEM three times. Then cells were imaged using a confocal fluorescence microscope (OLYMPUS, FV1000) for fluorescence imaging. The images of **Cy-OH** were obtained with the emission in the range of 650—750 nm for fluorescence imaging.

Cytotoxic assay

RAW264.7 cells were used for estimating the cytotoxicity of **Cy-S_n**. Briefly, cells were seeded in a 96-well plate. On the following day, cells were incubated with 5 μM **Cy-S_n** for 12 h and 24 h at 37 °C. Subsequently, 100 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical Co. USA) was added into each well, followed by further incubation for 24 h at 37 °C. The DMEM was remove and DMSO (200 μL/well) added. Optical density (OD) was measured by a microplate reader (Spectra Max M5, Molecular Devices) at 570 nm with subtraction of the absorbance of the cell-free blank volume at 630 nm. The relative cell viability (100%) was calculated using the following formula: cell viability (%) = $(OD_{dye} - OD_{K\ dye}) / (OD_{control} - OD_{K\ control}) \times 100$, where dye stands for the **Cy-S_n** sample.

Fluorescence imaging in mice

All procedures were carried out in compliance with the Guide for the Care and Use of Laboratory Animal Resources and the National Research Council, and were approved by the Institutional Animal Care and Use Committee of the NIH. The bioimaging experiments on living mice utilized the NightOWL II LB983 small animal in vivo imaging system equipped with a sensitive Charge Coupled Device (CCD) camera, with the excitation at 630 nm and the 700 ± 10 nm emission filter. Healthy mice (seven weeks old, 20-25 g) were used, and animals had free access to food and water. The mice were anesthetized, and the abdominal fur was removed.

Application of Cy-S_n for raw sodium polysulfide solution.

Synthesis of raw sodium polusufides solution

Sodium polysulfides solution was prepared from sodium sulfide ($Na_2S{\cdot}9H_2O$, 0.042 mol) and sulfur (0.094 mol) in deionized water (100 mL) according to the literature.[1]

Quantification of sodium polysulfides in the raw solution

The sodium polysulfides solution was diluted and added to the PBS solution (pH = 7.4) containing 50% of DMSO. The concentration of sodium polysulfide in the PBS/DMSO solution was determined and then calculated the concentration of raw solution.

Conclusions

In summary, we have developed the near-infrared probe **Cy-S_n**, which selectively responds to H_2S_n with a good detection limit as low as 2.2×10^{-8} M and low cytotoxicity to biological systems. **Cy-S_n** has been applied to image endogenous generated H_2S_n *via* the CSE enzymatic pathway during inflammation and anti-inflammation *in situ*. The experiments of imaging H_2S_n in mouse demonstrate that **Cy-S_n** could recognize H_2S_n in living animals. Moreover, **Cy-S_n** could be used to quantification of sodium polysulfides in the raw solution. Therefore, this new probe shows great potential for recognition of H_2S_n both *in vitro* and *in vivo*.

Acknowledgements

This work was financially supported by the National Science Foundation of China (21421005, 21422601, 21576037 and U1608222).

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