Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 265 (2022) 120390

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

An ESIPT-based fluorescent probe with fast-response for detection of hydrogen sulfide in mitochondria

Yuting Du *, Hongliang Wang, Ting Zhang, Wei Wen, Zhiying Li, Minjie Bi, Juan Liu

Department of Chemistry, Xinzhou Teachers University, Xinzhou, Shanxi 034000, China

HIGHLIGHTS

- **HBTP-H_2S** reacts with H_2S *via* nucleophilic addition reaction.
- **HBTP-H_2S** is an excited-state intramolecular proton transfer (ESIPT) fluorescent probe for rapidly distinguish H_2S.
- **HBTP-H_2S** exhibits ultrafast-response (within 30 s), good selectivity and large Stokes shift (188 nm) for detection of H_2S.
- **HBTP-H_2S** has been successfully applied for imaging of mitochondrial H_2S in live cells.

ARTICLE INFO

Article history:
Received 15 April 2021
Received in revised form 23 August 2021
Accepted 6 September 2021
Available online 10 September 2021

Keywords:
Fluorescent probe
Hydrogen sulfide
Excited-state intramolecular proton transfer
Mitochondria-targeting

ABSTRACT

Excited-state intramolecular proton transfer (ESIPT) has recently received considerable attention due to its dual fluorescent changes and large Stokes shift. Hydrogen sulfide (H_2S) is a gas signal molecule that plays important roles in modulating the functions of different systems. Herein, by modifying 2-(2́-hydroxyphenyl) benzothiazole (HBT) scaffold, a novel near-infrared mitochondria-targeted fluorescent probe **HBTP-H_2S** has been rationally designed based on excited-state intramolecular proton transfer (ESIPT) effect. The nucleophilic addition reaction of the H_2S with probe **HBTP-H_2S** caused the break of the conjugated skeleton, resulting the shifting of maximum emission peak from 658 nm to 470 nm. **HBTP-H_2S** showed fast-response response time, good selectivity and a large Stokes shift (188 nm) toward H_2S. Most importantly, inspired by the inherent advantages of the probe, **HBTP-H_2S** was successfully employed to monitor mitochondrial H_2S in HepG2 cells.

1. Introduction

Hydrogen sulfide (H_2S) is a colorless gas with foulodor of rotten egg and has been confirmed as gas signal molecule[1-3]. In mammals, mostly H_2S is generated by using sulfur-containing amino acids as a substrate that is catalyzed by enzymes[4]. Moreover, H_2S has been identified as a gasotransmitter, which regulats numerous important physiological functions such as nervous, cardiovascular and immune systems[5-7]. However, the abnormal levels of H_2S lead to many human diseases including diabetes[8], Alzheimer's disease[9], liver cirrhosis[10] and the symptoms of Down's syndrome [11-13]. In addition, higher levels of H_2S promote tumor proliferation and growth in cancer cells[14-17].

Mitochondria is the most capital organelle in eukaryotic cells and a major source of intracellular reactive sulfur species[18,19]. Mitochondrial H_2S plays a critical role in the physiological and pathological processes[20-23]. Hence, developing efficient tools to monitor mitochondrial H_2S levels will be promising for further exploring its pathophysiological roles.

The traditional methods of H_2S mainly contain sulfide selective electrodes[24,25], gas chromatography[26,27], colori-metric [28,29] and methylene blue assay[30]. However, the processes of treating samples are complicated and these technology are not capable of real-time monitor the H_2S. Therefore, various organic fluorescent probes have been explored in recent years owing to the advantages such as handy staining processes, flexible molecular design strategies and high sensitivity[31-33]. Accordingly, a lot of fluorescent probes for H_2S have been developed[34-36], most of which are based on the chemical properties of H_2S, such as its reducing properties[37-47], high binding affinity towards copper ions[48,49], specific nucleophilicity[50-58], thiolysis of 7-nitro-1,2,3-benzoxadiazole (NBD) moiety[59-62] and azide reduction[63-66]. Most probes cover either intramolecular charge transfer (ICT) [67] or photoinduced electron transfer (PET) processes, which have displayed only small stokes shifts or relatively weak

* Corresponding author.
E-mail address: yutingdu123@163.com (Y. Du).

https://doi.org/10.1016/j.saa.2021.120390
1386-1425/

fluorescence. Compare with ICT and PET processes, excited state intramolecular proton transfer (ESIPT) process recently received considerable attention due to its large stokes shift[68,69], improved sensitivity[70,71], high fluorescence quantum yield and dual fluorescence intensity changes[72,73]. Therefore, the development of ESIPT-based fluorescent probes for H_2S is valuable. Moreover, because the ESIPT process is often inhibited in polar and hydrogen bond donating solvents. Thus, mosts of fluorescent probes which based on ESIPT usually works with the assistant of some surfactants such as cetyltrimethylammonium bromide (CTAB) that creates a sufficiently hydrophobic environment[68]. However, due to the toxicity of surfactants at high concentrations, thus, the use of surfactants may limit the biological application of ESIPT-based probes.

A few fluorescent probes that based on ESIPT for detection of H_2S have been reported[74-77] (see the Supplementary Table S1). However, it remains a challenge to develop fluorescent probes with excellent sensitivity and high selectivity for detection of mitochondrial H_2S. Additionally, since H_2S has a strong nucleophilic addition reaction property, it can change the fluorescence after reacting with the probe **HBTP-H_2S**((E)-4-(3-(benzo[d]thiazol-2-yl)-2-hydroxy-5-methylstyryl)-1-(2-hydroxyethyl)pyridin-1-ium) (Scheme 1). However, there are hardly probes to detect H_2S by the reaction mechanism of nucleophilic addition[78].

Accordingly, inspired by previous works[74-77], we designed and synthesized **HBTP-H_2S** to detect H_2S which used HBT (2-(2′-Hydroxyphenyl) benzothiazole) as an ESIPT fluorophore[79] and pyridinium scaffold as mitochondrial targetable group without the need of introducing CTAB (Scheme 1). Due to the strong electron-pulling ability of the pyridine salt, the fluorescence of **HBTP-H_2S** showed red emission (658 nm). Meanwhile, pyridinium scaffold has strong electrophilic ability. While **HBTP-H_2S** undergoes a nucleophilic addition reaction with H_2S (a stronger nucleophile), which resulting the conjugate structure of **HBTP-H_2S** destroyed and leads to the shifting of maximum emission peak from 658 nm to 470 nm. Furthermore, the probe was with fast-response response time (within 30 s) in monitoring mitochondrial H_2S and excellent selectivity. Most importantly, **HBTP-H_2S** exhibites near-infrared fluorescence characteristics, which is advantageous in biological applications.

2. Experiment section

2.1. Instruments

Deionized water was used during all experiments. ^{1}H NMR and ^{13}C NMR spectra were carried out on a 600 MHz Bruker ADVANCE III spectrometer. High-resolution mass spectra were collected on a High-Resolution Mass Spectrometer (HRMS, Bruker APEX Ⅱ 47e mass spectrometer). The UV–vis absorption spectra were measured by using an Evolution 220 spectrometer (Thermo Fisher Scientific). Fluorescent spectra and the absolute quantum yield (Φ_{fl}(%)) of **HBTP-H_2S** were captured on FS5 spectrofluorometer. Unless otherwise stated, all excitation and emission bandwidths were both set at 2 nm, respectively. Images were acquired using Leica Fluorescent Microscope (DM4000B) with × 20 objective lens.

2.2. Synthesis of HBTP-H_2S

The probe **HBTP-H_2S** was readily synthesized by using simple and cheap raw materials. The details of synthesis procedure and the characterization of compounds were included in the Supporting Information.

HBTP-H_2S, Greenyellow solid; ^{1}H NMR (600 MHz, DMSO d_6, ppm) δ 13.05 (s, 1H), 8.85 (d, J = 6.9 Hz, 2H), 8.23 (d, J = 7.0 Hz, 2H), 8.21–8.17 (m, 1H), 8.12 (d, J = 16.4 Hz, 1H), 8.08 (dt, J = 8.1, 0.8 Hz, 1H), 7.79–7.74 (m, 2H), 7.66 (d, J = 16.4 Hz, 1H), 7.58 (ddd, J = 8.3, 7.1, 1.3 Hz, 1H), 7.50 (ddd, J = 8.2, 7.2, 1.2 Hz, 1H), 5.24 (t, J = 5.3 Hz, 1H), 4.56 (t, J = 5.0 Hz, 2H), 3.84 (q, J = 5.1 Hz, 2H), 2.36 (s, 3H); ^{13}C NMR (150 MHz, DMSO d_6, ppm): δ 168.66, 154.46, 153.46, 151.26, 145.17, 135.42, 132.99, 132.88, 131.16, 129.55, 127.60, 126.51, 124.97, 124.08, 123.94, 122.87, 122.40, 117.60, 62.54, 60.50, 20.40; HRMS (ESI) Calcd. for $C_{23}H_{21}O_2N_2S$ $(M + H)^+$: 389.1314, Found 389.1318.

2.3. Spectral properties of HBTP-H_2S

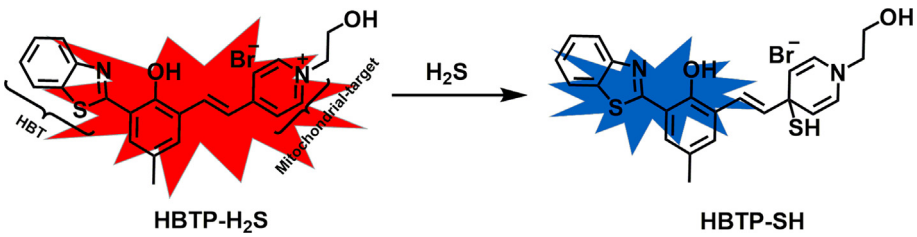
The stock solutions of **HBTP-H_2S** (10 mM) were prepared in DMSO. Sodium sulfide ($Na_2S{\cdot}9H_2O$) was diluted to 100 mM by double distilled water as the source of H_2S. The fluorescence and UV/vis spectra were obtained after a certain amount of Na_2S or another analyte was added to a solution of probe. The test solutions of **HBTP-H_2S** (5 μM) were prepared by diluting the stock solution and all the spectra experiments were measured in CH_3CN / PBS buffer (v/v = 1:1, 10 mM, pH = 7.4). The selectivity of **HBTP-H_2S** for biothiols (Cys, GSH and Hcy), Thioacetamide (TAA) and different anions (Br^-, Cl^-, F^-, I^-, SO_4^{2-}, SO_3^{2-}, NO_3^-, NO_2^-, $S_2O_3^{2-}$, CN^-) were evaluated by the fluorescent response with addition of excess biological species (3 mM). All solutions of the anions were produced in deionized water.

2.4. Cytotoxicity of HBTP-H_2S

HepG2 (4×10^4 cells/well) cells were seeded in 96-well plates for 24 h followed by replacement of the culture medium with fresh medium and then incubation of the cells with **HBTP-H_2S** for another 24 h at 37 °C. Subsequently, 0.5 mg/mL of MTT solution was added, and the cells were incubated for another 4 h at 37 °C. The OD values were measured by microplate reader (Bio-Rad M680, USA) at 570 nm.

2.5. Cell culture and fluorescent imaging

HepG2 cells were obtained from the Shanghai Biochemistry and Cell Biology Cell Bank of the Chinese Academy of Sciences. HepG2 cells were cultured in RPMI medium 1640 supplemented with 10% FBS (fetal bovine serum), 1% penicillin and 1% streptomycin in an atmosphere of 5% CO_2 at 37 °C. For cells imaging studies, HepG2



Scheme 1. Design of fluorescent probe **HBTP-H_2S** and its sensing mechanism for H_2S.

cells were seeded in 6-well plates (4×10^5 cells/well) for 24 h and replaced the old medium with fresh medium. The cells were preincubated with Na_2S (250, 500 or 1000 μM, respectively) for 30 min and treated with **HBTP-H_2S** (10 μM) for another 30 min. For imaging of H_2S in mitochondria, the cells were incubated with 500 nM Mito-Tracker Green for 30 min, then incubated cells with 5 μM **HBTP-H_2S** for another 30 min. The cells were washed with PBS three times to wash away the excess probe and imaged with fluorescent microscope. Fluorescent imaging was acquired by fluorescent microscope (Leica DM 4000B, United States).

3. Result and discussion

3.1. Synthesis of HBTP-H_2S

HBTP-H_2S was prepared by following the synthesis procedure shown in Scheme 2[80-82]. In brief, Compound 1 was synthesized through cyclization of 5-methylsalicylaldehyde and 2-aminobenzenethiol. Then compound 1 was used to react with hexamethylenetetramine to obtain compound 2. Finally, compound 2 was reacted with 1-(2-hydroxyethyl)-4-methylpyridin-1-ium to get the probe **HBTP-H_2S**.

3.2. Fluorescent response of HBTP-H_2S to H_2S and the detection mechanism

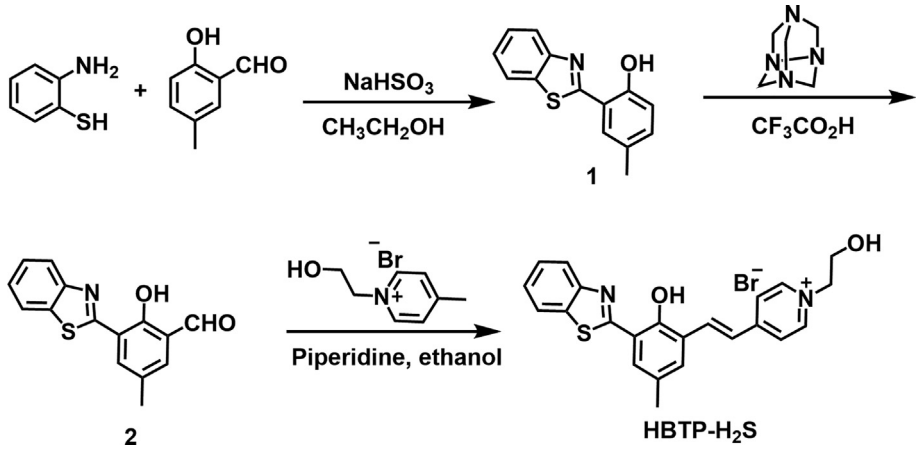
Firstly, we examined the photostability of the probe **HBTP-H_2S**. As shown in Fig. S1, the probe was stable for 30 min. Simultaneously, under the visible light or UV-light (254 nm) for 30 min, **HBTP-H_2S** was always stable, suggesting that **HBTP-H_2S** is photostability extracellularly. Then, the response of the **HBTP-H_2S** to H_2S were studied in PBS/CH_3CN buffer (10 mM, pH = 7.4, 1/1, v/v). As seen in the Fig. 1A, the probe **HBTP-H_2S** exhibited two peaks at about 314 and 400 nm. When the probe reacts with Na_2S, it causes the two peaks red shifted at about 380 and 584 nm respectively. For the macroscopic performance, the color changed from faint yellow to peach, indicating that a new product might have been generated. At the same time, an sharply decreased was observed at 658 nm while a new emission band at 470 nm was appeared in the case of **HBTP-H_2S** add Na_2S (Fig. 1B). Notably, Na_2S trigged a large Stokes shift (188 nm), which enabled the ratiometric detection of by measuring the fluorescence intensity ratio F_{658}/F_{470}. As indicated above, **HBTP-H_2S** could serve as an efficient fluorescent probe for sensing of H_2S .

Considering the solvent effect of ESIPT mechanism, the fluorescent spectra and the absolute quantum yield (Φ_{fl}(%)) of **HBTP-H_2S** were also examined in different solvent such as acetonitrile, dimethyl sulfoxide, methanol and tetrahydrofuran. These results illustrated that **HBTP-H_2S** exhibited a strong keto-form emission in acetonitrile based on an ESIPT process, but a weakened enol-form emission in ethanol and methanol (Fig. S2). Because methanol can form intermolecular hydrogen bonds with **HBTP-H_2S**. Meanwhile, the absolute quantum yield (Φ_{fl}(%)) of **HBTP-H_2S** in polar solvent is high nonpolar solvent except dimethyl sulfoxide (Table S2), suggesting that nonpolar solvent and dimethyl sulfoxide showed that disadvantage of ESIPT process because they were no hydrogen protons. Additionally, due to the strong electron-pulling ability of the methyl pyridine salt, the fluorescence of **HBTP-H_2S** showed only keto tautomer emission (658 nm) in PBS/CH_3CN buffer (10 mM, pH = 7.4, 1/1, v/v).

To further verify our putative reaction mechanism, 5 μM probes were incubated with 1 mM of Na_2S in PBS/CH_3CN buffer (10 mM, pH = 7.4, 1/1, v/v) for 10 min and detected by using HRMS mass and ^1H NMR spectrometry. We discovered that a new peak appeared at M/Z 423.11 $[M]^+$ (Fig. S3), proving that the mass peak was owing to the molecular of the compound **HBTP-SH**. Moreover, the main product of the structure was characterized *via* NMR spectra (Fig. S4). When we add excessive Na_2S, almost all proton signals from the pyridinium scaffold conjugated system underwent an obvious up-field shift, this shift is consistent with the proposed product where the electron-withdrawing ability of indolenium N^+ has disappeared. Meanwhile, we assumed that HS^- addition to the *para*-position of C = N^+ and the vinyl protons at 8.099 (Hb) and 7.537 (Ha) were upfield shifted to 8.082 (Hb) and 7.441 (Ha) upon Na_2S addition at room temperature. This mechanism is similar to the previously reported detection of H_2S[55,78,83] (Scheme S2). The above results support the original intention of our design (Scheme 1).

3.3. Sensitivity and response time of the probe HBTP-H_2S towards H_2S

The capability of **HBTP-H_2S** to sense H_2S were measured in PBS/CH_3CN buffer (10 mM, pH = 7.4, 1/1, v/v). Studies on titration experiments showed that with the rise concentration of Na_2S, the ratio F_{658}/F_{470} showed an excellent linear relation to H_2S (Fig. 2A) and the linear equation was concluded as y = -0.1392x + 36.1807 (R^2 = 0.99977). The detection limit for H_2S was determined to 8.5 μM according to the 3σ/k method. Additionally, pyridinium scaffold of **HBTP-H_2S** has strong electrophilic ability. Simultaneously, H_2S has a lower pKa value than other biothiols (H_2S: ~7.0 [84]; Cys: ~8.3; GSH: ~9.2) , therefore, it is a stronger nucleophile and small steric hindrance. Hence, the detection limit for H_2S was



Scheme 2. Synthetic route of the probe **HBTP-H_2S**.

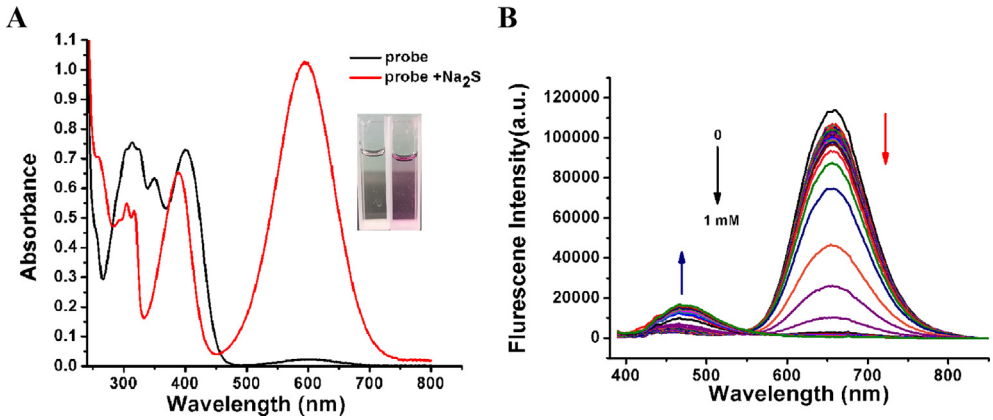


Fig. 1. Optical response of **HBTP-H_2S** to H_2S. (A) UV–vis absorption spectra of **HBTP-H_2S** (5 μM) before and after reaction with Na_2S (1 mM) for 10 min in PBS/CH_3CN buffer (10 mM, pH = 7.4, 1/1, v/v). Insert images were taken under sunlight. (B) Fluorescent spectra of **HBTP-H_2S** (5 μM) before and after reaction with Na_2S (1 mM) for 1 min in PBS/CH_3CN buffer (10 mM, pH = 7.4, 1/1, v/v). λex = 380 nm, slit width (ex/em) = 2/2 nm.

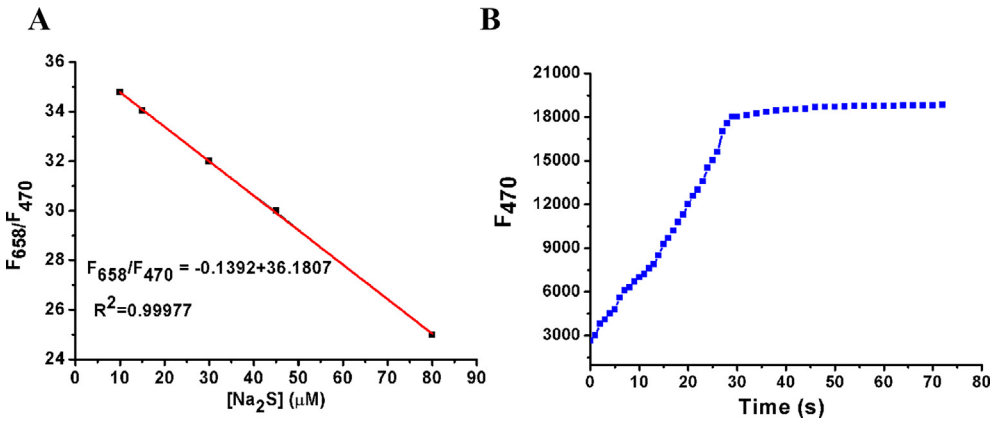


Fig. 2. (A) The linear relationship between the ratio F_{658}/F_{470} of **HBTP-H_2S** (5 μM) and Na_2S (0–80 μM). (B) Time-dependent fluorescent spectra of **HBTP-H_2S** (5 μM) in the presence of 1 mM Na_2S. All spectra were measured in PBS/CH_3CN buffer (10 mM, pH = 7.4, 1/1, v/v). λex = 380 nm, slit width (ex/em) = 2/2 nm.

as low as 8.5 μM. It was reported that probes can detect H_2S with fast response time[85,86]. Therefore, the time-dependent fluorescent spectrum were performed to determine the reaction time of **HBTP-H_2S** with Na_2S in PBS/CH_3CN buffer (10 mM, pH = 7.4, 1/1, v/v). As the reaction progressed, **HBTP-H_2S** displayed emission at maximum of 470 nm with fast response which reached to the saturated intensities within 30 s and finished within 1 min, suggesting the feasibility for rapid detection of H_2S (Fig. 2B).

3.4. Selectivities and pH response of the probe HBTP-H_2S towards H_2S

Subsequently, the selectivity of **HBTP-H_2S** towards H_2S were investigated by the fluorescent changes in the presence of various interfering species including thiol compounds (Cys, GSH, and Hcy), TAA and different anions (Br^-, Cl^-, F^-, I^-, SO_4^{2-}, SO_3^{2-}, NO_3^-, NO_2^-, $S_2O_3^{2-}$, CN^-) at 3 mM. According to the results Fig. 3A, except SO_3^{2-} caused negligible spectral changes, none of the species causes an obvious fluorescent change, suggesting that the probe can detect H_2S with high selectivity. Notably, the classical nucleophile CN^- induces almost no change of the ratio; the protonation of CN^- in neutral medium might be the origin, since HCN possess a pKa about 9.2 [87]. Moreover, endogenous H_2S has been found in high concentrations (10 to 600 μM) in the brain of bovine, rat, and human[88]. Next, we attempted to examine the effect of pH on the fluorescent properties of **HBTP-H_2S**. It was shown that Fig. 3B, the probe itself was not significantly varied the ratio F_{658}/F_{470} in the pH range of 5–10, illustrating that **HBTP-H_2S** is stable in this pH range. Meanwhile, because the mitochondrial pKa is approximately 8.8, therefore OH^- would not react with the probe at the same condition. Whereas the pH ranges from 5 to 10 in the presence of Na_2S, the ratio F_{658}/F_{470} showed decreasing. This is because that ESIPT mechanism is sensitive to the changes of pH. In brief, the probe under physiological conditions can ensure the ratio F_{658}/F_{470} when detecting of H_2S, suggesting the potential of the probe in biological research.

3.5. Biological applications

Inspired by the in vitro results described above, **HBTP-H_2S** was applied for the detection of H_2S in HepG2 cells. Firstly, the cytotoxicity of **HBTP-H_2S** was examined by the MTT assay, we discovered that 10 μM probe was non-cytotoxic and thus used in the following experiments (Fig. S5). As shown in the Fig. 4A, HepG2 cells were directly incubated with **HBTP-H_2S** for 30 min, we observed that the light red fluorescence of **HBTP-H_2S**, suggesting that the content of H_2S is very little in cells. Next, the exogenous H_2S donor sodium sulfide (250, 500 and 1000 μM) was added, we found that with the increase in the concentration of Na_2S, the fluorescent intensity decreased in a certain concentration dependent.

Because **HBTP-H_2S** contains a positively charged pyridinium moiety and the mitochondrial membrane potential is negative, we further tested its ability to localize in mitochondria by means

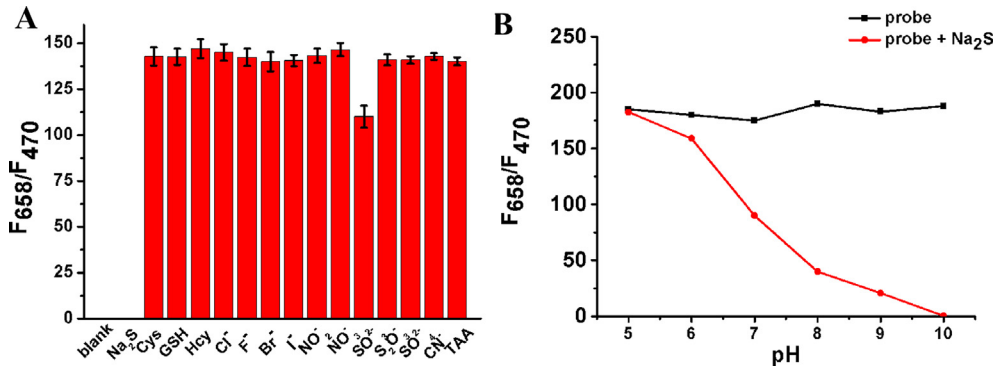


Fig. 3. (A) Changes in the ratioratio F_{658}/F_{470} of **HBTP-H_2S** (5 μM) in the presence of various relevant anions (3 mM) or biothiols (Cys, Hcy, GSH, 3 mM) after incubating for 10 min in PBS/CH_3CN buffer (10 mM, pH = 7.4, 1/1, v/v) at room temperature. (1) blank (the ratioratio F_{658}/F_{470} without the probe), (2) Na_2S, (2) Cys, (3) GSH, (4) Hcy, (5) Cl^-, (6) F^-, (7) Br^-, (8) I^-, (9) NO_2^-, (10) NO_3^-, (11) SO_3^{2-}, (12) $S_2O_3^-$, (16) SO_4^{2-}, (17) CN^-, (18) TAA. (B) pH effect on the ratio of F_{658}/F_{470} of **HBTP-H_2S** (5 μM) in the absence and presence of Na_2S (1 mM). All experiments were performed in PBS/CH_3CN buffer (10 mM, pH = 7.4, 1/1, v/v) at room temperature.

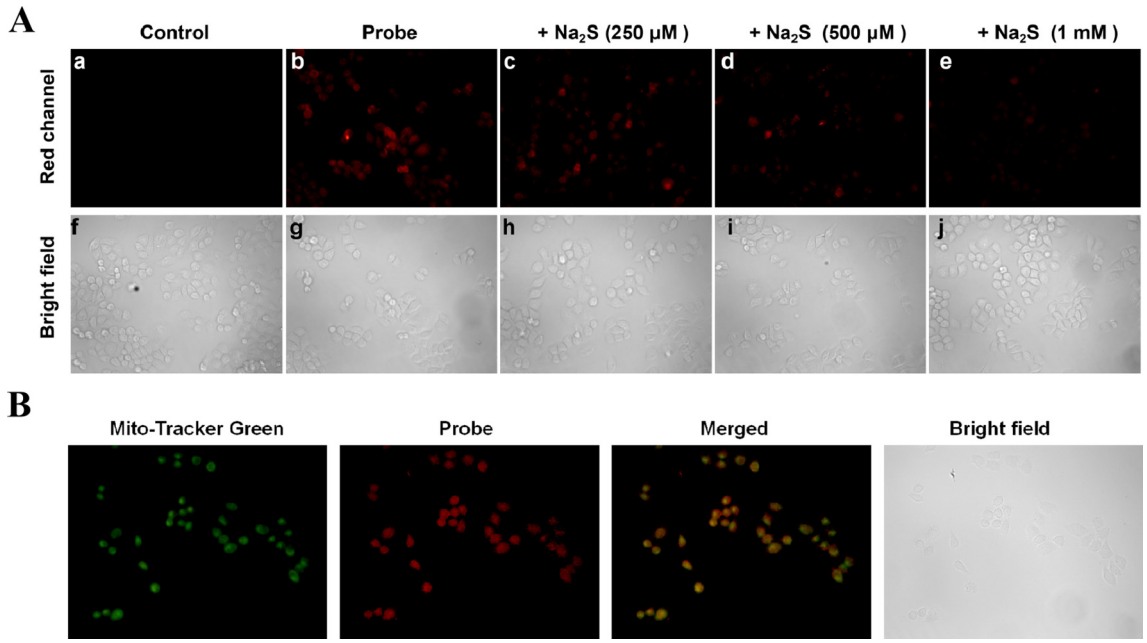


Fig. 4. Fluorescent images of H_2S in HepG2 cells. (A) Cells were stained with 10 μM **HBTP-H_2S**, and then incubated with 250, 500 and 1000 μM Na_2S for another 30 min before imaging. (B) The cells were incubated with 500 nM Mito-Tracker Green for 30 min after incubated cells with 5 μM **HBTP-H_2S** for another 30 min. Scale bar: 50 μm.

of the co-localization experiments. The cells were preincubated with 500 nM Mito-Tracker Green (a commercially available mitochondrial dye) for 30 min and then incubated with 5 μM **HBTP-H_2S** for another 30 min. Fig. 4B showed that the strong yellow fluorescence of **HBTP-H_2S** overlapped very well with the Mito-Tracker Green. The overlap coefficient is being 0.88. These results strongly support that the probe **HBTP-H_2S** can selectively and efficiently localize in to mitochondria.

4. Conclusion

To sum up, a new NIR fluorescent probe **HBTP-H_2S** was developed, which is capable of imaging H_2S of mitochondrial in HepG2 cells. The probe was composed of HBT (2-(2′-Hydroxyphenyl) benzothiazole) and pyridinium scaffold and detected mitochondrial H_2S in HepG2 cells. Under the physiological conditions, **HBTP-H_2S** exhibits fast-response (within 30 s), large Stokes shift (188 nm), higher sensitivity and higher selectivity towards H_2S than other levels of thiol compounds. Furthermore, **HBTP-H_2S** is also used to bioimage mitochondrial H_2S in living cells.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by Scientific and Technological Innovation Programs of Higher Education Institutions in Shanxi (No. 2020L0545), Xinzhou Teachers University Fund (No. 2019KY09), Xinzhou Teachers University PhD startup fund (No. 00000460), the Fund for Shanxi "1331 Project" Key Subjects Construction and the Top Science and Technology Innovation Teams of Xinzhou Teachers University.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2021.120390.

References

[1] H. Kimura, Y. Nagai, K. Umemura, Y. Kimura, Physiological roles of hydrogen sulfide: synaptic modulation, neuroprotection, and smooth muscle relaxation, Antioxid. Redox. Sign. 7 (2005) 795–803.

[2] C. Szabó, Hydrogen sulphide and its therapeutic potential, Nat. Rev. Drug Discov. 6 (11) (2007) 917–935.

[3] E. Culotta, D. Koshland, No news is dood news, Science 18 (1992) 1863–1865.

[4] W. Xuan, C. Sheng, Y. Cao, W. He, W. Wang, Fluorescent probes for the detection of hydrogen sulfide in biological systems, Angew. Chem. Int. Ed. Engl. 51 (10) (2012) 2282–2284.

[5] R. Wang, Physiological Implications of Hydrogen Sulfide: A Whiff Exploration That Blossomed, Physiol. Rev. 92 (2012) 791–896.

[6] C. Szabo, Gasotransmitters in cancer: From pathophysiology to experimental therapy, Nat. Rev. Drug Discov. 15 (3) (2016) 185–203.

[7] L. Li, P. Rose, P.K. Moore, Hydrogen sulfide and cell signaling, Annu. Rev. Pharmacol. Toxicol. 51 (1) (2011) 169–187.

[8] L. Wu, W. Yang, X. Jia, G. Yang, D. Duridanova, K. Cao, R. Wang, Pancreatic islet overproduction of H_2S and suppressed insulin release in Zucker diabetic rats, Lab. Investig. 89 (1) (2009) 59–67.

[9] K.o. Eto, T. Asada, K. Arima, T. Makifuchi, H. Kimura, Brain hydrogen sulfide is severely decreased in Alzheimer's disease, Biochem. Biophs. Res. Commun. 293 (5) (2002) 1485–1488.

[10] S. Fiorucci, E. Antonelli, A. Mencarelli, S. Orlandi, B. Renga, G. Rizzo, E. Distrutti, V. Shah, A. Morelli, The third gas: H_2S regulates perfusion pressure in both the isolated and perfused normal rat liver and in cirrhosis, Hepatology 42 (2005) 539–548.

[11] X.u. Wang, J. Sun, W. Zhang, X. Ma, J. Lv, B.o. Tang, A near-infrared ratiometric fluorescent probe for rapid and highly sensitive imaging of endogenous hydrogen sulfide in living cells, Chem. Sci. 4 (6) (2013) 2551, https://doi.org/10.1039/c3sc50369k.

[12] H. Zhang, Y. Xie, P. Wang, G. Chen, R. Liu, Y.-W. Lam, Y.i. Hu, Q. Zhu, H. Sun, An iminocoumarin benzothiazole-based fluorescent probe for imaging hydrogen sulfide in living cells, Talanta 135 (2015) 149–154.

[13] C. Coletta, K. Modis, B. Szczesny, A. Brunyanszki, G. Olah, E.C. Rios, K. Yanagi, A. Ahmad, A. Papapetropoulos, C. Szabo, Regulation of Vascular Tone, Angiogenesis and Cellular Bioenergetics by the 3-Mercaptopyruvate Sulfurtransferase/H_2S Pathway: Functional Impairment by Hyperglycemia and Restoration by DL-alpha-Lipoic, Acid. Mol. Med. 21 (2015) 1–14.

[14] C. Szabo, M.R. Hellmich, Endogenously produced hydrogen sulfide supports tumor cell growth and proliferation, Cell Cycle 12 (18) (2013) 2915–2916.

[15] C. Szabo, C. Coletta, C. Chao, K. Modis, B. Szczesny, A. Papapetropoulos, M.R. Hellmich, Tumor-derived hydrogen sulfide, produced by cystathionine-β-synthase, stimulates bioenergetics, cell proliferation, and angiogenesis in colon cancer, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 12474–12479.

[16] C. Coletta, A. Papapetropoulos, K. Erdelyi, G. Olah, K. Modis, P. Panopoulos, A. Asimakopoulou, D. Gero, I. Sharina, E. Martin, C. Szabo, Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation, Proc. Natl. Acad. Sci. U. S. A. 109 (23) (2012) 9161–9166.

[17] D.D. Wu, M.L. Li, W.K. Tian, S.W. Wang, L.Z. Cui, H. Li, H.J. Wang, A.L. Ji, Y.Z. Li, Hydrogen sulfide acts as a double-edged sword in human hepatocellular carcinoma cells through EGFR/ERK/MMP-2 and PTEN/AKT signaling pathways, Sci. Rep. 7 (2017) 5134–5148.

[18] R.G. Kenny, C.J. Marmion, Toward Multi-Targeted Platinum and Ruthenium Drugs-A New Paradigm in Cancer Drug Treatment Regimens?, Chem Rev. 119 (2019) 1058–1137.

[19] E.F. Fang, Y. Hou, K. Palikaras, B.A. Adriaanse, J.S. Kerr, B. Yang, S. Lautrup, M.M. Hasan-Olive, D. Caponio, X. Dan, P. Rocktaschel, D.L. Croteau, M. Akbari, N.H. Greig, T. Fladby, H. Nilsen, M.Z. Cader, M.P. Mattson, N. Tavernarakis, V.A. Bohr, Mitophagy inhibits amyloid-beta and tau pathology and reverses cognitive deficits in models of Alzheimer's disease, Nat. Neurosci. 22 (2019) 401–412.

[20] S. Herzig, R.J. Shaw, AMPK: guardian of metabolism and mitochondrial homeostasis, Nat. Rev. Mol. Cell Biol. 19 (2) (2018) 121–135.

[21] L. Castro, V. Tortora, S. Mansilla, R. Radi, Aconitases: nonredox iron-sulfur proteins sensitive to reactive species, Acc Chem Res 52 (2019) 2609–2619.

[22] J. Zhang, Q. Liu, Y. Ba, J. Cheng, H. Yang, Y. Cui, J. Kong, X. Zhang, F-containing initiator for ultrasensitive fluorescent detection of lung cancer DNA via atom transfer radical polymerization, Anal. Chim Acta 1094 (2020) 99–105.

[23] K. Ronaldson-Bouchard, S.P. Ma, K. Yeager, T. Chen, LouJin Song, D. Sirabella, K. Morikawa, D. Teles, M. Yazawa, G. Vunjak-Novakovic, Advanced maturation of human cardiac tissue grown from pluripotent stem cells, Nature 556 (7700) (2018) 239–243.

[24] A. V, Kroll, V, Smorchkov, A. Y. Nazarenko, Electrochemical sensors for hydrogen and hydrogen sulfide determination, Sens. Actuators B Chem. 21 (1994) 97–100.

[25] M. García-Calzada, G. Marbán, A.B. Fuertes, Potentiometric determination of sulphur in solid samples with a sulphide selective electrode, Anal. Chim. Acta 380 (1) (1999) 39–45.

[26] W. Wardencki, Problems with the determination of environmental sulphur compounds by gas chromatography, J. Chromatogr A. 793 (1) (1998) 1–19.

[27] T.W. Mitchell, J.C. Savage, D.H. Gould, High-performance liquid chromatography detection of sulfide in tissues from sulfide-treated mice, J. Appl. Toxicol. 13 (6) (1993) 389–394.

[28] M.G. Choi, S. Cha, H. Lee, H.L. Jeon, S.-K. Chang, Sulfide-selective chemosignaling by a Cu^{2+} complex of dipicolylamine appended fluorescein, Chem. Commun. (47) (2009) 7390, https://doi.org/10.1039/b916476f.

[29] M.M.F. Choi, Fluorimetric optode membrane for sulfide detection, Analyst 123 (7) (1998) 1631–1634.

[30] J.K. Fogo, M. Popowsky, Spectrophotometric determination of hydrogen sulfide, Anal. Chem. 21 (6) (1949) 732–734.

[31] N. Kumar, V. Bhalla, M. Kumar, Recent developments of fluorescent probes for the detection of gasotransmitters (NO, CO and H_2S), Coordin. Chem. Rev. 257 (15-16) (2013) 2335–2347.

[32] X. Tian, L.C. Murfin, L. Wu, S.E. Lewis, T.D. James, Fluorescent small organic probes for biosensing, Chem. Sci. 12 (10) (2021) 3406–3426.

[33] B. Ma, D.-H. Tian, S. Yan, X.-C. Li, F. Dai, B. Zhou, Developing a styrylpyridinium-based fluorescent probe with excellent sensitivity for visualizing basal H_2S levels in mitochondria, Sens. Actuators B Chem. 327 (2021) 128937.

[34] L. Li, M. Bhatia, Y.Z. Zhu, Y.C. Zhu, R.D. Ramnath, Z.J. Wang, F.B.M. Anuar, M. Whiteman, M. Salto-Tellez, P.K. Moore, Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse, FASEB J. 19 (9) (2005) 1196–1198.

[35] B. Wang, P. Li, F. Yu, P. Song, X. Sun, S. Yang, Z. Lou, K. Han, A reversible fluorescence probe based on Se-BODIPY for the redox cycle between HClO oxidative stress and H_2S repair in living cells, Chem. Commun. 49 (10) (2013) 1014–1016.

[36] Z. Lou, P. Li, K. Han, Redox-Responsive Fluorescent Probes with Different Design Strategies, Acc. Chem. Res. 48 (5) (2015) 1358–1368.

[37] A.R. Lippert, E.J. New, C.J. Chang, Reaction-Based Fluorescent Probes for Selective Imaging of Hydrogen Sulfide in Living Cellsang, J. Am. Chem. Soc. 133 (26) (2011) 10078–10080.

[38] H. Peng, Y. Cheng, C. Dai, A.L. King, B.L. Predmore, D.J. Lefer, B. Wang, A Fluorescent Probe for Fast and Quantitative Detection of Hydrogen Sulfide in Blood, Angew. Chem. Int. Ed. 50 (41) (2011) 9672–9675.

[39] S. Elsayed, C. de la Torre, L.E. Santos-Figueroa, C. MarinHernandez, R. Martinez-Manez, F. Sancenon, A.M. Costero, S. Gil, M. Parra, Azide and sulfonylazide functionalized fluorophores for the selective and sensitive detection of hydrogen sulfide, Sensors and Actuators, B: Chemical 207 (2015) 987–994.

[40] Y.-W. Duan, X.-F. Yang, Y. Zhong, Y. Guo, Z. Li, H. Li, A ratiometric fluorescent probe for gasotransmitter hydrogen sulfide based on a coumarin-benzopyrylium platform, Anal. Chim. Acta 859 (2015) 59–65.

[41] F. Yu, P. Li, P. Song, B. Wang, J. Zhao, K. Han, An ICT-based strategy to a colorimetric and ratiometric fluorescence probe for hydrogen sulfide in living cells, Chem. Commun. 48 (23) (2012) 2852, https://doi.org/10.1039/c2cc17658k.

[42] Y. Jiang, Q. Wu, X. Chang, A ratiometric fluorescent probe for hydrogen sulfide imaging in living cells, Talanta 121 (2014) 122–126.

[43] Q. Wan, Y. Song, Z. Li, X. Gao, H. Ma, In vivo monitoring of hydrogen sulfide using a cresyl violet-based ratiometric fluorescence probe, Chem. Commun. 49 (5) (2013) 502–504.

[44] Z. Wu, Z. Li, L. Yang, J. Han, S. Han, Fluorogenic detection of hydrogen sulfide via reductive unmasking of o-azidomethylbenzoyl-coumarin conjugate, Chem. Commun. 48 (81) (2012) 10120, https://doi.org/10.1039/c2cc34682f.

[45] R. Wang, F. Yu, L. Chen, H. Chen, L. Wang, W. Zhang, A highly selective turn-on near-infrared fluorescent probe for hydrogen sulfide detection and imaging in living cells, Chem. Commun. 48 (96) (2012) 11757, https://doi.org/10.1039/c2cc36088h.

[46] Y. Wen, F. Huo, J. Wang, C. Yin, Molecular isomerization triggered by H_2S to an NIR accessible first direct visualization of Ca2+-dependent production in living HeLa cells, J. Mater. Chem. B. 7 (43) (2019) 6855–6860.

[47] W. Zhang, F. Huo, C. Yin, Photocontrolled single-/dual-site alternative fluorescence probes distinguishing detection of H_2S/SO_2 in vivo, Org. Lett. 21 (13) (2019) 5277–5280.

[48] X. Qu, C. Li, H. Chen, J. Mack, Z. Guo, Z. Shen, A red fluorescent turn-on probe for hydrogen sulfide and its application in living cells, Chem. Commun. 49 (68) (2013) 7510, https://doi.org/10.1039/c3cc44128h.

[49] F. Hou, L. Huang, P. Xi, J.u. Cheng, X. Zhao, G. Xie, Y. Shi, F. Cheng, X. Yao, D. Bai, Z. Zeng, A Retrievable and Highly Selective Fluorescent Probe for Monitoring Sulfide and Imaging in Living Cells, Inorganic Chemistry 51 (4) (2012) 2454–2460.

[50] C. Wei, Q. Zhu, W. Liu, W. Chen, Z. Xi, L. Yi, NBD-based colorimetric and fluorescent turn-on probes for hydrogen sulfide, Org. Biomol. Chem. 12 (3) (2014) 479–485.

[51] E. Karakus, M. Ucuncu, M. Emrullahoglu, Electrophilic Cyanate As a Recognition Motif for Reactive Sulfur Species: Selective Fluorescence Detection of H_2S, Anal. Chem. 88 (2016) 1039–1043.

[52] W. Niu, L.i. Fan, M. Nan, Z. Li, D. Lu, M.S. Wong, S. Shuang, C. Dong, Ratiometric Emission Fluorescent pH Probe for Imaging of Living Cells in Extreme Acidity, Anal. Chem. 87 (5) (2015) 2788–2793.

[53] L.A. Montoya, M.D. Pluth, Hydrogen sulfide deactivates common nitrobenzofurazan-based fluorescent thiol labeling reagents, Anal. Chem. 86 (12) (2014) 6032–6039.

[54] Y. Chen, C. Zhu, Z. Yang, J. Chen, Y. He, Y. Jiao, W. He, L. Qiu, J. Cen, Z. Guo, A ratiometric fluorescent probe for rapid detection of hydrogen sulfide in mitochondria, Angew. Chem. Int. Ed. 52 (6) (2013) 1688–1691.
[55] M. Ren, B. Deng, X. Kong, K. Zhou, K. Liu, G. Xu, W. Lin, A TICT-based fluorescent probe for rapid and specific detection of hydrogen sulfide and its bioimaging applications, Chem. Commun. 52 (38) (2016) 6415–6418.
[56] Y. Yang, C. Yin, F. Huo, Y. Zhang, J. Chao, A ratiometric colorimetric and fluorescent chemosensor for rapid detection hydrogen sulfide and its bioimaging, Sens. Actuators B Chem. 203 (2014) 596–601.
[57] J. Kang, F. Huo, Y. Yao, C. Yin, A high signal-to-background ratio H_2S-specific fluorescent probe based on nucleophilic substitution and its bioimaging for generation H_2S induced by Ca^{2+} in vivo, Dyes Pigm. 171 (2019) 107755.
[58] J. Wang, Y. Wen, F. Huo, C. Yin, Based 'successive' nucleophilic substitution mitochondrial-targeted H_2S red light emissive fluorescent probe and its imaging in mice, Sens. Actuators B Chem. 297 (2019) 126773.
[59] Y. Zhang, H. Chen, D. Chen, D.i. Wu, X. Chen, S.H. Liu, J. Yin, A fluorescent turn-on H_2S-responsive probe: design, synthesis and application, Org. Biomol. Chem. 13 (38) (2015) 9760–9766.
[60] Y. Wang, X. Lv, W. Guo, A reaction-based and highly selective fluorescent probe for hydrogen sulfide, Dyes Pigm. 139 (2017) 482–486.
[61] Y.L. Pak, J. Li, K.C. Ko, G. Kim, J.Y. Lee, J. Yoon, Mitochondria-targeted reactionbased fluorescent probe for hydrogen sulfide, Anal. Chem. 88 (10) (2016) 5476–5481.
[62] K. Zhang, J. Zhang, Z. Xi, L.-Y. Li, X. Gu, Q.-Z. Zhang, L. Yi, A new H_2S-specific near-infrared fluorescence-enhanced probe that can visualize the H_2S level in colorectal cancer cells in mice, Chem. Sci. 8 (4) (2017) 2776–2781.
[63] H. Peng, Y. Cheng, C. Dai, A.L. King, B.L. Predmore, D.J. Lefer, B. Wang, pH-Tunable calcium phosphate covered mesoporous silica nanocontainers for intracellular controlled release of guest drugs, Angew. Chem. Int. Ed. 123 (2011) 9015–9019.
[64] L.A. Montoya, M.D. Pluth, Selective turn-on fluorescent probes for imaging hydrogen sulfide in living cells, Chem. Commun. 48 (39) (2012) 4767, https://doi.org/10.1039/c2cc30730h.
[65] S.i. Chen, Z.-J. Chen, W. Ren, H.-W. Ai, Reaction-Based Genetically Encoded Fluorescent Hydrogen Sulfide Sensors, J. Am. Chem. Soc. 134 (23) (2012) 9589–9592.
[66] Y.Q. Zhang, L. Zhang, A novel "turn-on" fluorescent probe based on hydroxy functionalized naphthalimide as a logic platform for visual recognition of H_2S in environment and living cells, Spectrochim. Acta Part A-Mol. Biomol. Spectrosc. 235 (2020) 118331.
[67] L. Wang, W.G. Yang, Y.Y. Song, Y.H. Hu, Novel turn-on fluorescence sensor for detection and imaging of endogenous H2S induced by sodium nitroprusside, Spectrochim. Acta Part A-Mol. Biomol. Spectrosc. 243 (2020) 118775.
[68] A.C. Sedgwick, L. Wu, H.-H. Han, S.D. Bull, X.-P. He, T.D. James, J.L. Sessler, B.Z. Tang, H.e. Tian, J. Yoon, Excited-state intramolecular proton-transfer (ESIPT) based fluorescence sensors and imaging agents, Chem. Soc. Rev. 47 (23) (2018) 8842–8880.
[69] A. Bhattacharyya, S.K. Mandal, N. Guchhait, Imine-amine tautomerism vs keto-enol tautomerism: acceptor basicity dominates over acceptor electronegativity in the ESIPT process through a six-membered intramolecular H-bonded network, J. Phys. Chem. A 123 (2019) 10246–10253.
[70] T. Liu, Q.L. Yan, L. Feng, X.C. Ma, X.G. Tian, Z.L. Yu, Isolation of gammaglutamyl-transferase rich-bacteria from mouse gut by a near-infrared fluorescent probe with large Stokes shift, Anal. Chem. 90 (2018) 9921–9928.
[71] P. Majumdar, J. Zhao, 2-(2-hydroxyphenyl)-benzothiazole (HBT)-rhodamine dyad: acid-switchable absorption and fluorescence of excited-state intramolecular proton transfer (ESIPT), J. Phys. Chem. B 119 (2015) 2384–2394.
[72] Y. Wang, H. Xiang, R. Zhao, C. Huang, A renewable test strip combined with solidstate ratiometric fluorescence emission spectra for the highly selective and fast determination of hydrazine gas, Analyst 143 (16) (2018) 3900–3906.
[73] L. Wu, Y. Wang, M. Weber, L. Liu, A.C. Sedgwick, S.D. Bull, C. Huang, T.D. James, ESIPT-based ratiometric fluorescence probe for the intracellular imaging of peroxynitrite, Chem. Commun. 54 (71) (2018) 9953–9956.
[74] H. Guan, A. Zhang, P. Li, L. Xia, F. Guo, Feng Guo[*,†], ESIPT Fluorescence Probe Based on Double-Switch Recognition Mechanism for Selective and Rapid Detection of Hydrogen Sulfide in Living Cells, ACS, Omega 4 (5) (2019) 9113–9119.
[75] C.Y. Wu, X.J. Hua, B. Gua, P. Yina, W. Sua, Y.Q. Lia, Q.J. Lua, Y.Y. Zhanga, H.T. Lia, A lysosome-targeting colorimetric and fluorescent dual signal probe for sensitive detection and bioimaging of hydrogen sulfide, Anal. Methods 10 (2018) 604–610.
[76] Y. Lu, B.L. Dong, W.H. Song, X.Q. Kong, A.H. Mehmood, W.Y. Lin, A PET and ESIPT based fluorescent probe for the imaging of hydrogen sulfide (H_2S) in live cells and zebrafishes, Anal. Methods 11 (2019) 3301–3306.
[77] J.J. Liu, X.Z. Chen, Y.Y. Zhang, G. Gao, X.Y. Zhang, S.C. Hou, Y.X. Hou, A novel 3-hydroxychromone fluorescence probe for hydrogen sulfide based on an excited-state intramolecular proton transfer mechanism, New J. Chem. 42 (2018) 12918–12923.
[78] H.B. Fang, Y.C. Chen, X.C. Shi, Y. Bai, Z.Y. Chen, Z. Han, Y.M. Zhang, W.J. He, Z.J. Guo, Tuning lipophilicity for optimizing the H_2S sensing performance of coumarin-merocyanine derivatives, New J. Chem. 43 (2019) 14800–14805.
[79] P.F. Barbara, L.E. Brus, P.M. Rentzepis, Intramolecular proton transfer and excitedstate relaxation in 2-(2-hydroxyphenyl)benzothiazole, J. Am. Chem. Soc. 102 (1980) 5631–5635.
[80] H. Wen, Q. Huang, X.F. Yang, H. Li, Spirolactamized benzothiazole-substituted N, Ndiethylrhodol: a new platform to construct ratiometric fluorescent probes, Chem. Commun. 49 (2013) 4956–4958.
[81] Dipendra Dahal, Lucas McDonald, Xiaoman Bi, Chathura Abeywickrama, Farai Gombedza, Michael Konopka, Sailaja Paruchuri, Yi Pang, An NIR-emitting lysosome-targeting probe with large Stokes shift via coupling cyanine and excited-state intramolecular proton transfer, Chem. Commun. 53 (26) (2017) 3697–3700.
[82] Lingyu Zeng, Shiyu Chen, Tian Xia, Cheng Zhong, Zhihong Liu, Designing two-photon fluorescent probes based on the target-induced enhancement of the absorption cross-section, Chem. Commun. 50 (76) (2014) 11139–11142.
[83] Yuncong Chen, Chengcheng Zhu, Zhenghao Yang, Junjie Chen, Yafeng He, Yang Jiao, Weijiang He, Lin Qiu, Jiajie Cen, Zijian Guo, A Ratiometric Fluorescent Probe for Rapid Detection of Hydrogen Sulfide in Mitochondria, Angew. Chem. Int. Ed. 125 (6) (2013) 1732–1735.
[84] Nathan L. Whitfield, Edward L. Kreimier, Francys C. Verdial, Nini Skovgaard, Kenneth R. Olson, Reappraisal of H_2S/sulfide concentration in vertebrate blood and its potential significance in ischemic preconditioning and vascular signaling, Am. J. Physiol. Regul. Integr. Comp. Physiol. 294 (6) (2008) R1930–R1937.
[85] Xianfeng Lin, Xiuhong Lu, Junliang Zhou, Hang Ren, Xiaochun Dong, Weili Zhao, Zhongjian Chen, Instantaneous fluorescent probe for the specific detection of H_2S, Spectrochim. Acta Part A-Mol. Biomol. Spectrosc. 213 (2019) 416–422.
[86] Keli Zhong, Xiaoling Hu, Shiyi Zhou, Xiuying Liu, Xue Gao, Lijun Tang, Xiaomei Yan, Mitochondria-Targeted Red-Emission Fluorescent Probe for Ultrafast Detection of H_2S in Food and Its Bioimaging Application, J. Agric. Food Chem. 69 (16) (2021) 4628–4634.
[87] X. Lv, J. Liu, Y.L. Liu, Y. Zhao, Y.Q. Sun, P. Wang, W. Guo, Ratiometric fluorescence detection of cyanide based on a hybrid coumarin–hemicyanine dye: the large emission shift and the high selectivity, Chem. Commun. 47 (2011) 12843–12845.
[88] A. Papapetropoulos, A. Pyriochou, Z. Altaany, G. Yang, A. Marazioti, Z. Zhou, M. G. Jeschke, L.K. Branski, D.N. Herndon, R. Wang, C. Szab, Hydrogen sulfide is an endogenous stimulator of angiogenesis, Proc. Natl. Acad. Sci. USA 106 (2009) 21972.