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A remarkable ratiometric fluorescent chemodosimeter for very rapid detection of hydrogen sulfide in vapour phase and living cells

Cite this: DOI: 10.1039/c3njoooooox

Received ooth XXXXX 2013,
Accepted ooth XXXXX 2013

DOI: 10.1039/c3njoooooox

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A ratiometric fluorescent and colorimetric probe for hydrogen sulfide has been developed by combining benzthiazole and cyanine moiety. Due to its fast response and a large Stokes shift, it was used to develop a fluorescent probe for sensitive and selective detection of hydrogen sulfide. Moreover, this probe detects SH^- both in solid and vapor phase. Its potential for biological applications was confirmed by employing it for fluorescence imaging of SH^- in living cells.

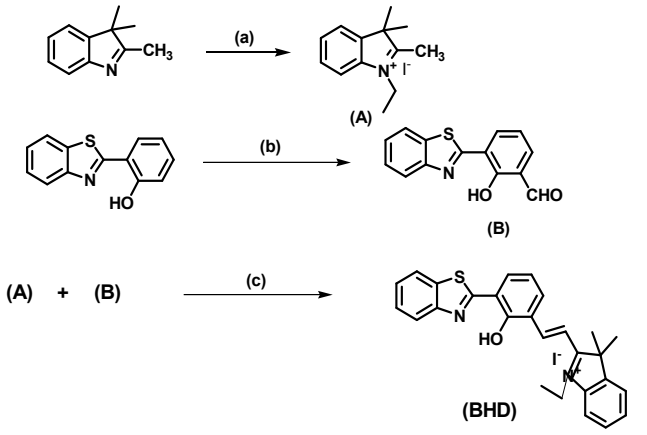
Introduction:

Hydrogen sulfide (H_2S) is an interesting target for the development of new sensors. H_2S is widely used in industrial activities, such as production of organ sulfur compounds, as a precursor to metal sulfides, modification of catalysts, and the delignification of pulp.[1] H_2S is involved in a wide range of physiological functions, such as modulation of blood pressure, reduction of ischemia reperfusion injury[2], regulation of inflammation[3], and suppression of oxidative stress.[4] Notably, the level of endogenous biological H_2S has been reported as a wide range from undetectable to approximately 300 μM, with its actual concentrations remaining controversial.[5] The endogenous levels of H_2S are believed to be related with some diseases like Alzheimer's disease, Down's syndrome, diabetes and liver cirrhosis.[6] Moreover, it has indeed been known as a toxic gas for hundreds of years,[7] and as such the detection of sulfides (in water, hydrogen sulfide is essentially present under the two forms H_2S and SH-, with pK_{a1} = 6.76 at 37°C,[8] when pK_{a2} is well above 14),[9] has gained significant importance within the analytical community.[10] H_2S is recognized as the third most important gasotransmitter for regulating cardiovascular, neuronal, immune, endocrine, and gastrointestinal systems after nitric oxide and carbon monoxide.[11] Therefore, easy and convenient signaling of toxic H_2S is very important for the rapid assessment of this widely used and environmentally important species.

To date, a variety of analytical techniques, including colorimetry, electrochemical assay, gas chromatography and sulfide precipitation[12] have been exploited for the purpose of hydrogen sulfide analysis. Although these methods provide sensitive analysis, they require complicated sample preparation, sophisticated instrumentation, or destruction of tissues or cells. On the other hands, fluorescent probes play an important role in this respect, due to their great temporal and spatial resolution capability, as well as high sensitivity, simplicity of implementation, fast response times and offering application methods for not only in vitro assays but also in vivo imaging studies.[13] More recently, several types of fluorescent probes for the detection of H_2S have been reported, including: (1) reducibility,[14] (2) two steps nucleophilic reactions,[15] (3) sulfide-selective chemosignal with Cu^{2+} complex,[16] (4) sulfide-selective cleavage benzenesulfonate or benzenesulfonamide.[17] Among these probes, ratiometric fluorescent probes allow the measurement of emission intensities at two different wavelengths. This should provide a built-in correction for various environmental effects and gives greater precision to the data analysis relative to single-channel detection. Currently, a few ratiometric fluorescent probes for H_2S have been reported.[18] But, most of them still display a delayed response time (more than 20 min) except for the probe (30 s) reported by Guo and He[19]. However, the selective detection of H_2S is still challenging due to the interference of other biothiols (GSH, Cys, Hcy, and thiol-containing proteins). Therefore, there is a need to develop new probes for H_2S with improved

properties.Recently Mahapatra et al[20] reported very similar type compound in which CN^- attacked the double bond.There are other more many example of addition of cyanide to double bond. But addition of SH^- to double bond is really a great challenge.

The probe BHD was synthesized on the basis of the route shown in Scheme 1. Compound (A)[21] and Compound (B)[22] are synthesized according to reported procedure. Compounds (A) and (B) were refluxed in ethanol having a catalytic amount of piperidine to afford the target probe BHD. The structure of the receptor was confirmed by ^1H NMR, ^{13}C NMR and HRMS mass spectra. Moreover, detection of SH^- in vivo in live RAW 264.7 cells under confocal microscopy has also been demonstrated in this study.



Scheme 1: Synthetic route to BHD. Reagents and conditions: (a) ethyl iodide, toluene, reflux, 8 h; (b) Hexamine, AcOH, Toluene, reflux, 21 hr (c) ethanol, piperidine (cat), reflux, 12 h

Result and Discussion:

For the preparation of probe BHD frist the salt of compound (A) was prepared. Then compound (B) was prepared by Duff reaction. Now, compound (A) and (B) coupled together in presence of catalytic amount of piperidine to get the probe BHD. Now the probe was used for UV and fluorescence study.

Photo physical sensing property of the probe :

The sensing ability of probe BHD for SH^- was investigated in a $CH_3CN–H_2O$ solution (4 : 6, v/v, 10 mM HEPES, pH 7.4). Under this condition, the probe solution is pink due to internal charge transfer (ICT) effect of the whole molecule. With increasing NaSH (a commonly employed H_2S donor) concentration (1 equiv), the absorption peaks at 570 nm gradually decreased and the absorption around 428 nm increased simultaneously with a clear isosbestic point at 484 nm, along with an obvious color change from pink to yellow (Figure 1a). Thus, this detection behavior could be easily seen with the naked eye under a normal UV lamp. The hypsochromic shift was apparently attributed to the ICT.[23] This phenomenon was likely due to the the formation of the BHD–SH adduct as well as the clean chemical transformation. In addition, the BHD–SH adduct could be easily obtained by treating BHD with 1 equiv NaSH in CH_3CN with good yield (ESI†). It was found that the adduct is stable enough in solid state, but gradually decomposed to its starting material in organic solution, suggesting that the chemical reaction is reversible (Scheme 2). In fact, in the presence of 1 equiv. of SH^-, a 21 fold enhancement in the ratiometric value of A_{428}/A_{570} (0.12 to 2.5) was achieved with respect to the SH^- free solution (Figure 1b). Other common anions and thiols (F^-, Cl^-, Br^-, CN^-, HSO_3^-, OCl^-, $S_2O_3^{2-}$, $S_2O_4^{2-}$, SO_3^{2-}, NO_3^-, H_2O_2, Cys, HCy), however, had no effect on the absorption of probe BHD indicating that our probe showed a selective response towards SH^- over other anions and thiols (Figure 2).

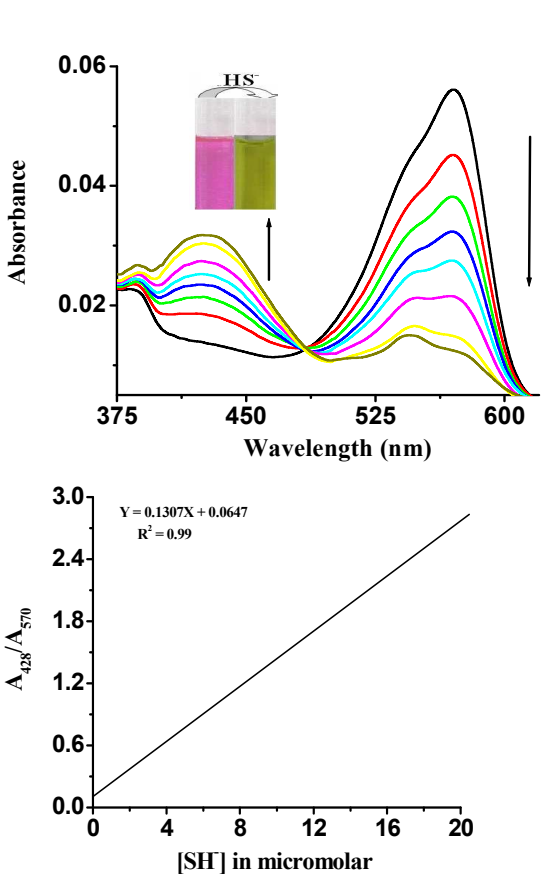


Figure 1: (a) UV absorption spectra of sensor BHD in the presence of an increased concentration of hydrogen sulfide (0–200 μM) in $CH_3CN–H_2O$ solution (4 : 6, v/v, 10 mM HEPES, pH 7.4); the inset shows the color change detectable by the naked eye of sensor BHD with the addition of hydrogen sulfide (b) Absorbance ratio changes (A_{428}/A_{570}) of BHD upon gradual addition of SH^- (1 equiv.)

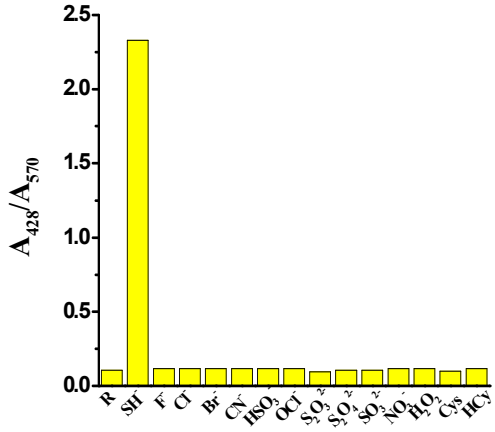
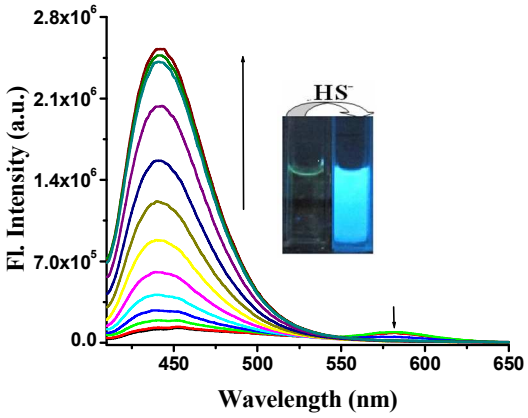


Figure 2: Relative absorbance of the BHD in presence of anions and thiols at pH 7.4 in CH_3CN:H_2O (4:6, V/V)

The emission spectra and fluorescence titration experiments of BHD with H_2S were recorded in a CH_3CN–H_2O solution (4 : 6, v/v, 10 mM HEPES, pH 7.4). Free probe (Φ = 0.002) shows two well-resolved emission bands centered at 440 and 585 nm (λex = 430 nm). The distinct gap between the two bands is over 145 nm, which makes this probe favorable for the dual emission ratiometric imaging owing to the minimum overlap between the two bands. When NaSH was added to the solution of probe (Φ = 0.13), the intensity of the emission peak at 585 nm gradually decreased with the simultaneous increased intensity at 440 nm (Figure 3a), with a clear isosbestic point at 544 nm. The slight red-fluorescent solution exhibited blue fluorescence, when SH^- was gradually added to the solution of the BHD (Figure 3a, inset). The large hypsochromic shift of the ICT band from 585 to 440 nm suggests that the π-conjugation and the ICT progress of BHD were both inhibited by the nucleophilic addition of SH^- to BHD. The fluorescence was stabilized after the amount of added SH^- reached 1.2 equiv. In fact in the presence of 1 equiv. of hydrogen sulfide, a 104-fold enhancement in the ratiometric value of F_{440}/F_{585} (1.38 to 144.18) is achieved with respect to the SH^- free solution (Figure 3b).

(a)



(b)

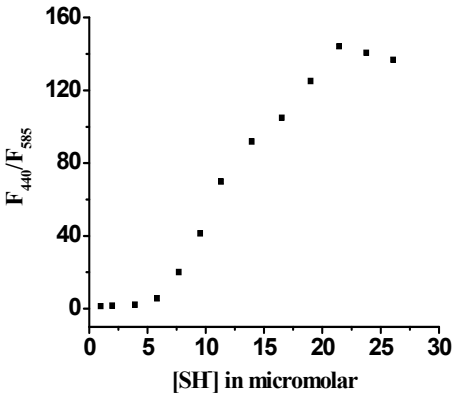


Figure 3: (a) Fluorescence titration spectra of BHD (c = 2.0 × 10^{-5} M) in the presence of 1 equiv. of SH^- (c = 2.0 × 10^{-4} M) at pH 7.4 in CH_3CN–H_2O (4 : 6, v/v). The naked eye color and fluorescence change of the BHD on addition of SH^- (inset) (b) Fluorescence ratio changes (F_{440}/F_{585}) of BHD upon gradual addition of SH^- (1 equiv.)

For assessing the specific nature of BHD towards SH^-, we investigated the effects of relevant physiological anions and thiols (F^-, Cl^-, Br^-, CN^-, HSO_3^-, OCl^-, $S_2O_3^{2-}$, $S_2O_4^{2-}$, SO_3^{2-}, NO_3^-, H_2O_2, Cys, HCy). Only nucleophile CN^- induces slight change of the ratio (Figure 4a) .We also examined several other nucleophiles, such as ethanol, ethyl amine, GSH, ammonia, NH_2OH, ethylenediamine, urea, thiourea, CH_3NH_2 (Figure S1). Similarly, these species induced no obvious change of emission of BHD. After adding SH^- into the testing solution, the fluorescence ratio of our probe increased and other anions and thiols remain inert toward the probe. These selectivity profiles showed that BHD had good selectivity and sensitivity to SH^- under simulated physiological conditions. Practical applicability of the BHD as a SH^- signaling device was verified by competitive signaling experiments with different anions and thiols (Figure 4b). The BHD probe exhibited unaltered signaling for SH^- in the presence of commonly encountered anions and thiols. The calculated detection limit is 2.054 μM based on K X Sb1/S, [24] where Sb1 is the standard deviation of blank measurements and S is the slope of the calibration curve.

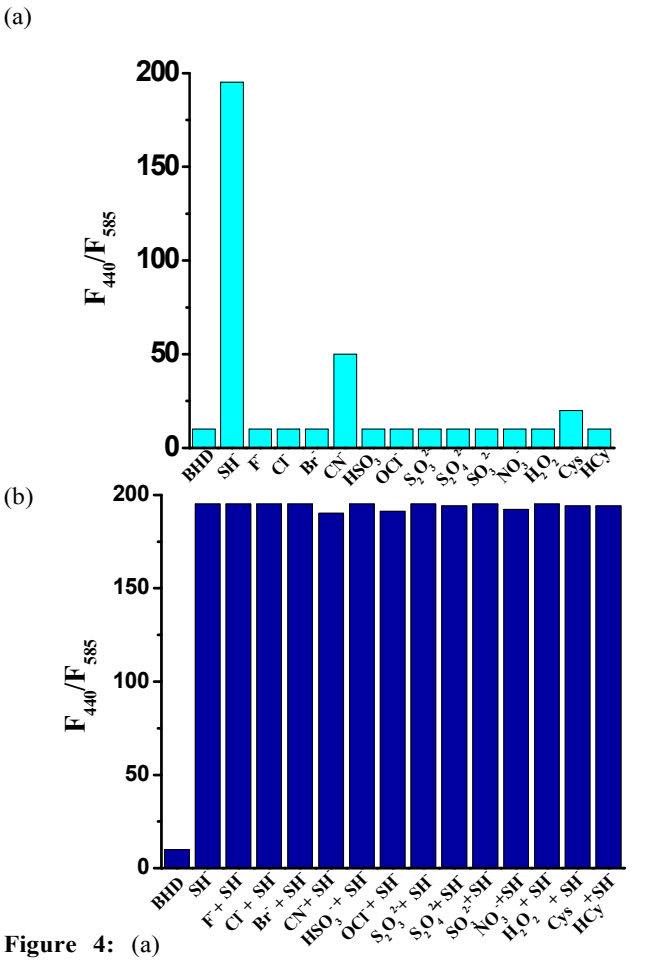
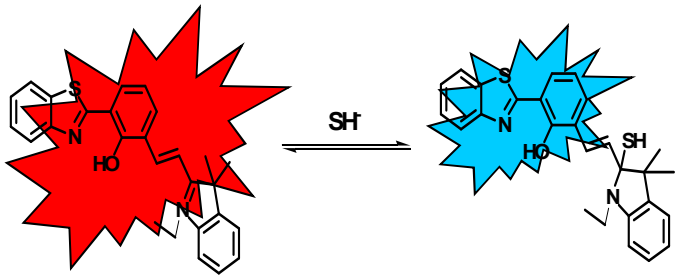


Figure 4: (a) Comparison of emission intensity ratio (F_{440}/F_{585}) of probe BHD at pH 7.4 in $CH_3CN:H_2O$ (4:6, V/V) in the presence of various anions and thiols (b) Competitive fluorescent signaling of SH^- (1 equiv.) by BHD in presence of various anions and thiols (10 equiv.), λ_{ex} = 430 nm.

Moreover, to test the selectivity of BHD in a 100% aqueous solution, fluorescence titration was further carried out with different cations such as F^-, Cl^-, Br^-, CN^-, HSO_3^-, OCl^-, $S_2O_3^{2-}$, $S_2O_3^{2-}$, $S_2O_4^{2-}$, SO_3^{2-}, NO_3^-, Cys and Hcy in water. In this case, the intensity of the emission peak at 585 nm gradually decreased with the simultaneous increased intensity at 440 nm only when SH^- was added to the solution of BHD (Figure S3). Therefore, in water, BHD showed specific selectivity towards SH^-.

Sensing Mechanism of receptor towards hydrogen sulfide:



Scheme 2: The proposed H_2S sensing mechanism towards the probe BHD

Based on these experimental observations we outlined the plausible signaling mechanism (Scheme 2). As a good nucleophile, SH^- can undergo addition to electrophilic centers in fluorescent molecules. The subsequent nucleophile attacked on the cyanine moiety in our probe, which successfully showed a unique colorimetric and ratiometric response.

^{1}HNMR and HR MS study of receptor and adduct:

The ability of BHD to detect hydrogen sulfide was studied by ^{1}HNMR, ^{13}C NMR and HRMS mass spectra. Almost all proton signals from the benzthiazole-cyanine conjugated system underwent an obvious up-field shift after SH- addition; this shift is consistent with the proposed product where the electron-withdrawing ability of indolenium N^+ has disappeared. The BHD sensor displayed a characteristic m/z peak at 425.1688. However, after the addition of an excess amount of SH- this peak totally disappeared. At the same time, a new m/z peak at 458.6201 assigned to the corresponding BHD-SH adduct appeared. All these results strongly demonstrated that the probe is highly effective for hydrogen sulfide.

Bio image of SH^-:

Cell viability assay:

Considering the thermodynamically favourable binding properties of BHD with SH^-, a practical application leading to the further examination of the ability of the probe (BHD) to sense SH^- in living cells was needed. In order to fulfil this objective, it is important to determine the cytotoxic effect of BHD and SH^- and the complex on live cells. The well-established MTT assay, which is based on the mitochondrial dehydrogenase activity of viable cells, was adopted to study cytotoxicity of the aforementioned compounds at varying concentrations mentioned in the Materials and Methods section. Fig. S3 shows that the probe compound did not exert any adverse effect on cell viability; the same is the case when cells were treated with varying concentrations of SH^-.

However, exposure of HCT cells to the probe-SH complex resulted in a decline in cell viability above the 20 μM concentration. The effect was more pronounced in higher

concentrations and showed an adverse cytotoxic effect in a dose-dependent manner. The viability of HCT cells was not influenced by the solvent (DMSO) as evidenced in Fig. S3, leading to the conclusion that the observed cytotoxic effect could be attributed to the probe-SH complex. The results obtained in the in vitro cytotoxic assay suggested that in order to pursue fluorescence imaging studies of the probe-SH complex in live cells, it would be prudent to choose a working concentration of 20 μM for the probe compound. Hence, to assess the effectiveness of compound BHD as a probe for the intracellular detection of SH^- by fluorescence microscopy, RAW cells were treated with 20 μM SH^- for 1 h followed by a 10 μM probe solution to promote the formation of probe-SH complex. On the basis of the established 1 : 1 stoichiometry of binding between BHD and SH^-, it can be reasonably assumed that the concentration of the complex formed in the HCT cells would be much lower than the concentration (20 μM) at which a marginal cytotoxic effect of the complex was observed (Fig. S3).

Fluorescent imaging of live-cells:

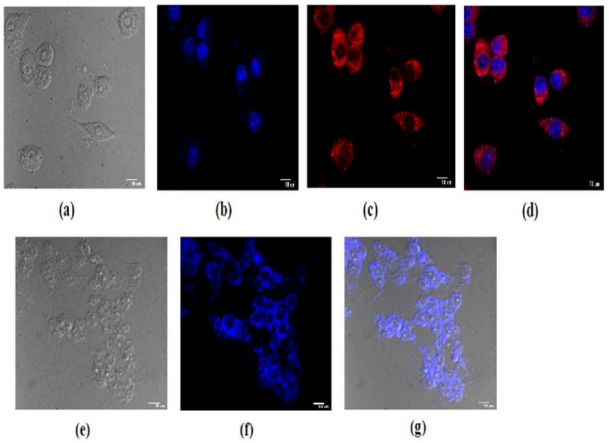


Figure 5: Confocal microscopic images of RAW 264.7 control cells (a–d) and SH- treated (e–g) cells pretreated with Probe BHD. All the images were acquired at 60X objective lens. SH^- treated cells do not emit fluorescence as control. (a) Bright field image of the cells (b) Cells treated with nuclei counterstained with DAPI (1 μg/ml) (c) only probe BHD at c = 1.0 X 10^{-5} M concentration (excitation wave length 430) (d) overlay image of (b) and (c) in dark field (e) bright field image of RAW cells treated with probe BHD and SH^- (f) cells treated with Probe (c = 1.0 X 10^{-5} M) and SH^- (c = 2.0 X 10^{-5} M) (g) Overlapping image of (e) and (f).

Fluorescence microscopic studies revealed a lack of fluorescence for RAW cells when treated with either the probe compound or SH^- alone. Upon incubation with SH- followed by the probe compound, a striking switch-on fluorescence was observed inside the RAW cells, which indicated the formation of the probe-SH complex, as observed earlier in solution studies. Further, an intense blue fluorescence was conspicuous in the perinuclear region of the RAW cells (Fig. 5, panel f and g), which indicates that the probe can easily penetrate the cell membrane and can be used to probe SH^- in the cells. The fluorescence microscopic analysis strongly suggested that probe compound could readily cross the membrane barrier, permeate into the RAW cells, and rapidly sense intracellular SH^-. The bright field images of treated cells did not reveal any gross morphometric change before and after the treatment suggesting that the RAW cells were viable. These findings open up the way for potential in vivo biomedical applications of the sensor.

Applications:

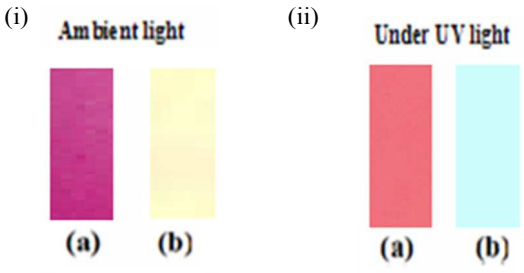


Figure 6: Color changes of BHD on test paper in the (a) absence and (b) presence of hydrogen sulfide under ambient and UV light.

Many sensors for SH^- detection can only be performed in solution, which limits their application under special circumstances, such as on-site detection in situ. To demonstrate the practical application of our sensor, we prepared TLC plates of the BHD to determine the suitability of a "dip-stick" method for the detection of SH^- (Figure 6). Finally, to confirm the practical ability of the probe, hydrogen sulfide was also detected in gas phase. The fluorescence response of BHD solutions before and after exposure to saturated hydrogen sulfide vapor is illustrated in Figure 7a. We also analyzed SH^- in distilled water, tap water, and simulated wastewater. The changes in the signaling of BHD for distilled water, tap water, and simulated wastewater[25] were measured for a series of H_2S concentrations in aqueous CH_3CN solution. As shown in Figure 7b, the responses of BHD for H_2S in tap water and simulated wastewater were in good agreement with the signaling in distilled water. This observation suggests that the probe BHD could be useful for the convenient optical signaling of hydrogen sulfide in chemical and environmental applications.

(a)

(b)

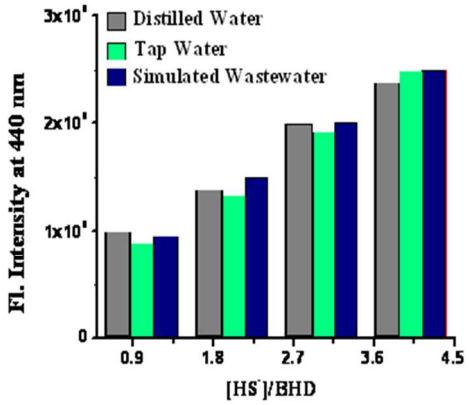


Figure 7: (a) Change in the TLC plate (coated with BHD) in the presence of hydrogen sulfide vapor (b) Fluorescence detection of hydrogen sulfide in distilled water, tap water and simulated waste water by BHD. [BHD] = 2.0 X 10^{-6} M, at pH 7.4 in CH_3CN–H_2O (4 : 6, v/v). λex = 430 nm.

Conclusions

We have here designed and constructed a new ratiometric fluorescent H_2S sensor utilizing ICT. This probe can detect SH^- selectively with a high level of sensitivity under physiological conditions based on the novel nucleophilic addition reaction of SH- towards electrically positive cyanine moiety. Moreover, the strategy enables the determination of H_2S with the attractive ratiometric fluorescence method, through which a huge H_2S-induced change in the intensity ratio and a big emission shift could be obtained before and after its addition. The probe also detects H_2S by naked eye. The probe shows an excellent performance in the ‘‘dip stick’’ method. This probe was successfully used in real water samples. In addition, biological imaging studies followed by cytotoxicity assay strongly supports its potential in practical applications to detect intracellular SH- in live cells.

Experimental Section:

General:
The chemicals and solvents were purchased from Sigma-Aldrich Chemicals Private Limited and were used without further purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Brucker 500 MHz instruments respectively. For NMR spectra, $CDCl_3$ was used as solvent with TMS as an internal standard. Chemical shifts are expressed in δ □□units and ^{1}H–^{1}H Hz. UV-vis titration experiments were performed on a JASCO UV-V530 spectrophotometer and fluorescence experiment was done using PTI fluorescence spectrophotometer with a fluorescence cell of 10 mm path. IR spectra were recorded on a JASCO FT/IR-460 plus spectrometer, using KBr discs.

Methods for the preparation of receptor:

Synthesis of receptor (BHD): Compound (A) (500mg, 2.65 mmol) and compound (B) (675 mg, 2.64 mmol) were refluxed for 12 hours in EtOH in presence of catalytic amount of Piperidine (2-3 drops) to afford a deep red color solution. Then the solvent was evaporated and the crude material is mixed with silica gel for column chromatography using 0 to 20% MeOH in $CHCl_3$ as a solvent to afford receptor BHD (870mg, Yield: 77 %). **^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm):** 10.139 (s, 2H), 8.710 (d, 1H, J = 7.5 Hz), 8.392 (m, 1H), 7.987 (q, 2H, J = 8 Hz), 7.685 (m, 2H), 7.639 (m, 4H), 7.434 (m,1H), 5.000 (dd, 1H, J = 7 Hz), 4.774 (dd, 1H, J = 7.5 Hz), 1.649 (m, 6H), 1.308 (m, 2H), 0.872 (s, 3H). **^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm):** 177.47, 167.17, 157.11, 153.77, 151.41, 141.48, 139.41, 135.28, 132.44, 131.54, 128.01, 126.25, 123.75, 122.92, 121.15, 119.44, 114.05, 47.42, 29.02, 25.82, 12.53. **MS (HR MS): (m/z, %):** 425.1688 [(M^+, 100 %]

Synthesis of BHD-SH adduct: BHD is mixed with one equivalent NaSH in acetonitrile at room temperature to give a yellow colored solution. On removing the solvent, got a yellow solid product which was used for MASS and IR spectroscopy.
^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 10.059 (s, 2H), 8.708 (d, 1H, J = 7.5 Hz), 8.389 (m, 1H), 7.986 (q, 2H, J = 8 Hz), 7.683 (m, 2H), 7.638 (m, 4H), 7.432 (m,1H), 5.000 (dd, 1H, J = 7 Hz), 4.773 (dd, 1H, J = 7.5 Hz), 1.647 (m, 6H), 1.305 (m, 2H), 0.872 (s, 3H). **MS (HR MS):** (m/z, %): 458.6201 [(M^+, 100 %]

Determination of fluorescence quantum yield:

Here, the quantum yield φ was measured by using the following equation,

$$\varphi_x = \varphi_s (F_x / F_s)(A_s / A_x)(n_x^2/ n_s^2)$$

Where,
X & S indicate the unknown and standard solution respectively,
φ = quantum yield,
F = area under the emission curve, A = absorbance at the excitation wave length,

n = index of refraction of the solvent. Here φ measurements were performed using anthracene in ethanol as standard [φ = 0.27] (error ~ 10%).
The quantum yield of **BHD** itself is 0.002 is remarkably change into 0.13 an enhancement around 65 fold is observed.

Method for the preparation of TLC plate sticks:

Thin layer chromatography (TLC) plate sticks were easily prepared by immersing a TLC plate into a solution of BHD (2 X 10^{-4} M) in CH_3CN (1 mM) and then exposing it to air to evaporate the solvent. The detection of SH^- was carried out by inserting the TLC plate into SH^- solution and evaporating the solvent to dryness.

Details of live-cell imaging

Materials Methods

Human colorectal carcinoma cell line, HCT 116 and RAW 264.7 macrophages were obtained from NCCS, Pune and maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma Chemical) supplemented with 10% fetal bovine serum (Invitrogen), penicillin (100 µg/ml), and streptomycin (100 µg/ml) in a CO_2 incubator. Cells were initially propagated in 25 cm^2 tissue culture flask in an atmosphere of 5% CO_2 and 95% air at 37°C humidified air till 70- 80% confluency.

Fluorescent imaging studies:
For fluorescent imaging studies, 7.5×10^3 RAW cells in 150 µl media were seeded on sterile 12 mm diameter Poly L lysine coated cover-slip and kept in a sterile 35 mm covered Petri dish and incubated at 37°C in a CO_2 incubator for 24-30 h. Next day cells were washed three times with phosphate buffered saline (pH 7.4) and fixed using 4% paraformaldehyde in PBS (pH 7.4) for 10 minutes at room temperature washed with PBS followed by permeabilization using 0.1% saponin for 10 minutes. Then the cells were incubated with 2.0×10^{-5} M SH^- dissolved in 100 µl DMEM at 37 °C for 1 h in a CO_2 incubator and observed under 60 X magnification of Andor spinning disc confocal microscope. The cells were again washed thrice with PBS (pH 7.4) to get rid of excess HS and incubated in DMEM containing probe (BHD) to a final concentration of 1.0×10^{-5} M followed by washing with PBS (pH 7.4) three times to remove excess probe outside the cells. Again, images were acquired. Before fluorescent imaging all the solutions were aspirated out and cover slips containing cells were mounted onto slides in a mounting medium containing DAPI, (4',6-Diamidino-2-Phenylindole) in 1µg/ml concentration. DAPI is a popular nuclear counterstain used in multicolor fluorescent imaging of cells. It preferentially stains dsDNA and its blue fluorescence can be easily detected in any other background.. Finally the slides were stored in dark before microscopic images are acquired.

Cytotoxicity assay:

The cytotoxic effects of probe, SH^- and probe−SH complex were determined by an MTT assay following the manufacturer's instruction (MTT 2003, Sigma-Aldrich, MO). HCT cells were cultured into 96-well plates (approximately 10^4 cells per well) for 24 h. Next day media was removed and various concentrations of probe, SH- and probe−SH complex (10, 20, 30, 50, 75, and 100 µM) made in DMEM were added to the cells and incubated for 24 h. Solvent control samples (cells treated with DMSO in DMEM), no cells and cells in DMEM without any treatment were also included in the study. Following incubation, the growth media were removed, and fresh DMEM containing MTT solution was added. The plate was incubated for 3−4 h at 37°C. Subsequently, the supernatant was removed, the insoluble colored formazan product was solubilized in DMSO, and its absorbance was measured in a microtiter plate reader (Perkin-Elmer) at 570 nm. The assay was performed in triplicate for each concentration of probe, SH^- and probe−SH complex. The OD value of wells containing only DMEM medium was subtracted from all readings to get rid of the background influence. Data analysis and calculation of standard deviation were performed with Microsoft Excel 2007 (Microsoft Corporation).

General method of UV-vis and fluorescence titrations:

For UV-vis and fluorescence titrations, stock solution of the sensor was prepared ($c = 2 \times 10^{-5}$ ML^{-1}) in CH_3CN: H_2O (4:6, v/v). The solution of the guest cation was prepared (2×10^{-4} ML^{-1}) in CH_3CN: H_2O (4:6, v/v) at pH 7.4 by using 10 mM HEPES buffer. The original volume of the receptor solution is 2 ml. Solutions of the sensor of various concentrations and increasing concentrations of cations, anions and amine containing compounds were prepared separately. The spectra of these solutions were recorded by means of UV-vis and fluorescence methods.

Acknowledgements

Authors thank the DST (Govt. of India) for financial supports. S.P. acknowledges the UGC for providing fellowships.

Notes and references

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† Electronic Supplementary Information (ESI) available: [details of synthetic procedure with charectarisation and spectral data are avialable]. See DOI: 10.1039/b000000x/

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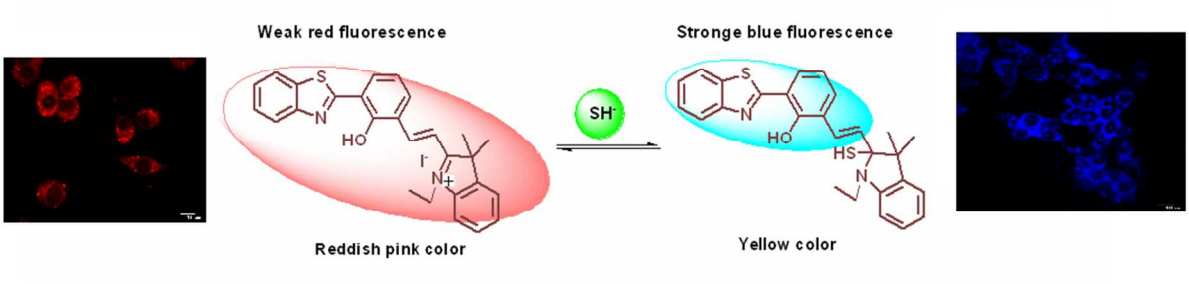
TOC graphic



TOC Synopsis

A ratiometric fluorescent probe having a fast response and a large Stokes shift detects SH^- both in solid and vapour phases and this probe is used for fluorescence imaging of SH^- in living cells.