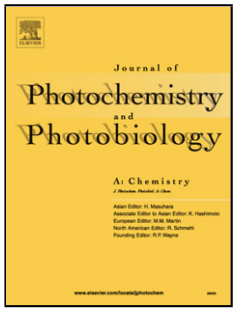
Journal Pre-proof



A highly selective “Turn On” fluorescent probe based on FRET mechanism for hydrogen sulfide detection in living cells

Pakornsiri Sontisiri, Peerada Yingyuad, Panumart Thongyoo

PII: S1010-6030(19)31828-3

DOI: https://doi.org/10.1016/j.jphotochem.2020.112401

Reference: JPC 112401

To appear in: *Journal of Photochemistry & Photobiology, A: Chemistry*

Received Date: 25 October 2019

Revised Date: 16 January 2020

Accepted Date: 19 January 2020

Please cite this article as: Sontisiri P, Yingyuad P, Thongyoo P, A highly selective “Turn On” fluorescent probe based on FRET mechanism for hydrogen sulfide detection in living cells, *Journal of Photochemistry and amp; Photobiology, A: Chemistry* (2020), doi: https://doi.org/10.1016/j.jphotochem.2020.112401

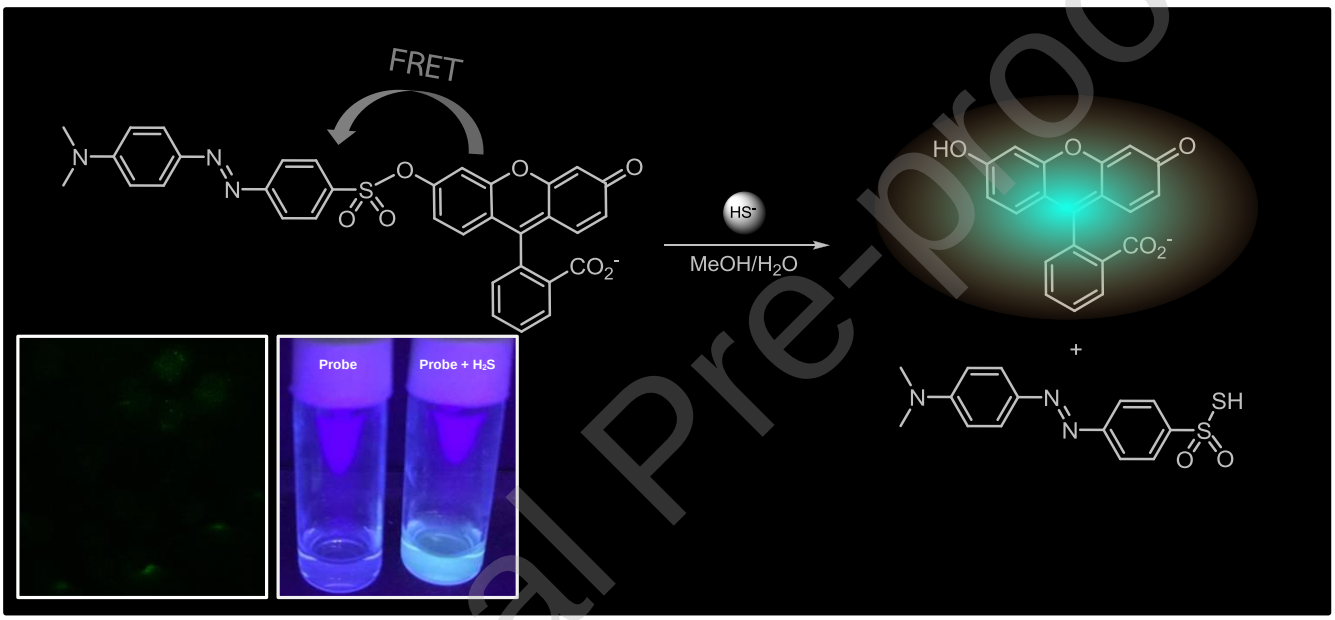
A highly selective "Turn On" fluorescent probe based on FRET mechanism for hydrogen sulfide detection in living cells

Pakornsiri Sontisiri,[1] Peerada Yingyuad[2] and Panumart Thongyoo[1,*]

[1]Medicinal Chemistry Research Unit, Chemistry Department, Thammasat University, Pathumthani, Thailand 10210; Tel: +66(2)5644440
[2]Chemistry Department, Kasetsart University, Bangkok, Thailand 10900; Tel: +66(2)5625555

Corresponding Authors: +66-2-5644440 ext 2408
Email address: tpanumas@tu.ac.th

Graphical Abstract



Highlights

- A novel fluorescent "OFF-ON" probe based on a FRET approach was successfully developed.
- A dabsylfluorescein probe demonstrated a superb selectivity and great specificity towards H_2S.
- A dabsylfluorescein probe can potentially be applied for the visualization of H_2S in living cells.

Abstract

A novel class of fluorescent "turn on" probe based on the fluorescence resonance energy transfer (FRET) approach was designed, and successfully synthesized for the detection of hydrogen sulfide (H_2S) both *in vitro* and *in vivo*. This new H_2S

responsive fluorescent probe was developed based on the basis of the dabsyl and fluorescein FRET system. In the presence of H_2S, a fluorescence enhancement was markedly observed mainly due to the inhibition of the FRET process. This probe could quantitatively measure the level of H_2S with the detection limit of 0.02 μM. Cell imaging results demonstrated the potential applicability of a dabsylfluorescein probe for H_2S detection in living cells.

Keywords: Hydrogen Sulfide, Dabsyl, FRET, Fluorescent Probe

1. Introduction

In recent years, much effort has been made to the design and development of novel highly selective and efficient fluorescent sensors for the detection of important biomarkers mainly due to their important roles in the biological processes particularly sulfur containing biomarkers [1-4]. Hydrogen sulfide (H_2S) has been recognized as an important gas signaling molecule with pathological and biological effects causing a detrimental impact to human health and environmental issues [5]. In addition, it has particularly played a great role in a number of physiological and biological processes, namely the blood pressure regulation, cardiac response to ischemia injury, inflammation, immune response and digestive system [6, 7]. Importantly, the over production of H_2S has been an effective indicator in some ailments, particularly diabetes, Alzheimer's disease, liver cirrhosis and Down's syndrome [8-10]. Accordingly, the development of highly sensitive and selective analytical processes for detecting H_2S level in environment and biological systems is of considerably importance. Today, a number techniques have been successfully applied to quantify the level of H_2S content in environmental and physiological systems, namely colorimetric analyses, electrochemical methods and chromatography [11-15]. Unfortunately, these analyses have experienced with specific limitations, particularly a sophisticated instrumentation and very high cost. Presently, the deveopment of novel fluorescent sensors has attracted of great interest, and become one of suitable approaches for quantifying the content of H_2S with the major advantages in terms of a distinct specificity with great selectivity, convenience and the applicability for real-time and on-site detection. Typically, there are two main strategies applicable for the development of H_2S-responsive fluorescent probes either as nucleophile or reductant [16-24], both of which are varied in terms of mechanistic pathways (namely photoinduced electron transfer (PET), fluorescence resonance energy transfer (FRET) or chelation enhanced fluorescence (CHEF) and H_2S-recognition warheads.

To date, a variety of fluorescent probes with great sensitivity and selectivity towards H_2S have successfully been developed, and successfully utilized for the fabrication of a thiol responsive fluorescent “OFF-ON” probe. Maleimide-type probe, monobromobimane and 2, 4-dinitrobenzenesulfonyl (DBS)[25] moieties are general examples of H_2S sensitive units, demonstrating the potential applicability to the detection of thiol containing species *via* the Michael addition reaction, alkylation of thiols and nucleophilic aromatic substitution (*via* S_NAr mechanism) respectively, the latter of which is highly activated by the presence of electron withdrawing groups (two nitro groups) at an aromatic ring, and is significantly required for the S_NAr mechanism. Unfortunately, those previously reported probes were considerably suffered from a limited solubility, high fluorescent background and particularly some probes were relatively less reactive for thiol containing species. Notably, nearly all H_2S recognition based probes were designed, and successfully synthesized according to the conjugation of fluorescent reporting moieties and masking units, which were once deprotected by the interection with H_2S, enabling to restore the fluorescence emission. This approach was extensively studied for the dectection of H_2S in either *in vitro* or *in vivo* imaging together with the animal bioimaging study. Additionally, those

masking moieties were required to specifically response towards the presence of H_2S over other sulfur species. Examples of selected sulfur-sensitive masking moieties applied in the development of fluorescent probes are shown in **Figure 1**. Interestingly, only a few publications related to fluorescent probes based on the FRET mechanism for H_2S detection have been investigated [26-38]. Recently, Zhao and co-workers have published a novel fluorescent sensor for H_2S detection based on a FRET mechanism by conjugating between a fluorescein and an azidocoumarin scaffold *via* a piparazine linker. In the presence of H_2S, an azidocoumarin-fluorescein conjugate was subsequently converted to an aminocoumarin-fluorescein conjugate, which effectively restored the FRET mechanism.[26]

(Figure 1)

Nowadays, a significant number of FRET based fluorescent sensors have been documented. However, there are some drawbacks in terms of a delayed response, a short excitation wavelength and high background fluorescence which significantly diminished the efficacy of FRET based fluorescent sensors. This has led us to design and to synthesize a new FRET pair, which showed the potential applicability for the development of a thiol responsive fluorescent "OFF-ON" probe. Therefore, the development of highly sensitive and greatly selective platform for H_2S detection is of our interest.

2. Experimental section

2.1 Materials and methods

All general laboratory chemicals and reagents obtained from chemical suppliers (Aldrich Chemical Co.) were used without further purification. Acetonitrile, dichloromethane, methanol, toluene, ethyl acetate, acetone and concentrate H_2SO_4 were purchased from RCI Labscan, Thailand. All other chemicals used in this investigation were of analytical grade and used directly without further purification. Double distilled water was used to prepare all the solutions.

2.2 Synthesis of dabsyl chloride

A dabsyl acid (10.0 g, 0.03 mol) was dissolved in dichloromethane (150 mL). To this solution was slowly added by thionyl chloride (22 mL, 0.30 mol) and three drops of dimethyl formamide (DMF) as a catalyst. The reaction mixture was allowed to reflux for 6 hrs. The reaction was evaporated to dryness, and purified by column chromatography with silica gel (20% ethyl acetate: hexane) to afford a deep red solid (7.80 g, 79%). m.p. = 262 °C, ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.22 (s, 6H), 6.87 (d, 2H, J = 7.9 Hz), 8.08 (d, 2H, J = 8.5 Hz), 8.14 (d, 4H, J = 8.7 Hz); ^{13}C-NMR (100MHz, $CDCl_3$): δ 45, 111.5, 121.0, 124.7, 126.5, 142.8, 149.0, 152.4, 152.6; IR (KBr) cm^{-1}: 2895, 1603, 1430, 1364, 1180, 1134, 822, 683, 585; HRMS: m/z calculated for $C_{14}H_{14}ClN_3O_2S$: 323.7979$[M]^+$, found: 405.2534$[M+ACN+K]^+$.

2.3 Synthesis of dabsylfluorescein

A fluorescein (50 mg, 0.15 mmol) was dissolved in acetonitrile (2.0 mL). To this solution was subsequently added by dabsyl chloride (122 mg, 0.375 mmol) dissolved in acetonitrile (2.5 mL). The reaction mixture was added by trimethylamine (37.8 mg, 52 μL). This reaction mixture was allowed to stir for overnight. The reaction was evaporated to dryness, and purified by column chromatography with silica gel (40% ethyl acetate: hexane) to afford a bright orange solid (70 mg, 75%). m.p. = 257 °C, ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.14 (s, 6H), 6.80 (d, 2H, J = 9.1 Hz), 6.93 (d, 2H, J = 8.7 Hz), 7.01 (dd, 2H, J = 8.6, 2.3 Hz), 7.28 (s, 2H), 7.30 (d, 1H, J = 2.3 Hz), 7.67 (dd, 1H, J = 7.5, 1.2 Hz), 7.73 (dd, 1H, J =

7.5, 1.2 Hz), 7.96 (dd, 4H, J = 8.7, 2.9 Hz), 8.08 (d, 1H, J = 7.4 Hz), 8.32 (d, 2H, J = 8.6 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 40.2, 110.7, 111.5, 116.6, 117.9, 122.2, 124.2, 125.3, 125.7, 126.2, 128.8, 129.0, 130.0, 131.3, 135.4, 143.8, 151.7, 152.5, 153.1, 156.7, 164.4, 169.2; IR (KBr) cm^{-1}: 3425, 2927, 1768, 1755, 1604, 1600, 1421, 1365, 1191, 1134, 1087, 992, 834, 605; HRMS: m/z calculated for $C_{34}H_{25}N_3O_7S$: 619.1413$[M]^+$, found: 701.4941$[M+2ACN+H]^+$.

2.4 Preparation of ligand solutions for fluorescence study

Stock solutions (10 mM) of HS^-, cysteine, glutathione, alanine, N_3^-, Br^-, NO_3^-, CO_3^{2-}, Ca^{2+}, I^- and F^- in 30 % methanol/phosphate buffer (10.0 mM, pH 7.4) were prepared. Test solutions were prepared by adding 10 µL of the probe stock solution into test tubes, added an appropriate aliquot of each metal stock, and diluted the solution to 2 mL with 30 % methanol/phosphate buffer (10.0 mM, pH 7.4). For all measurements, excitation wavelength was set at 495 nm, excitation and emission slit widths was 1 nm.

2.5 Preparation of dabsylfluorescein solutions for fluorescence study

A stock solution (250 µM) of dabsylfluorescein in DMSO was prepared. Test solutions were prepared by adding 400 µL of the probe stock solution into test tubes, added an appropriate aliquot of each ligand stock solution, and diluted the solution to 2 mL with 30 % methanol/phosphate buffer (10.0 mM, pH 7.4). For all measurements, excitation wavelength was set at 495 nm, excitation and emission slit widths were 1 nm.

3. Results and discussion

3.1 The synthesis of a novel FRET based fluorescent sensor (dabsylfluorescein)

In this research, we designed the synthetic strategy of a novel H_2S responsive FRET based fluorescent probe based on the basis of using H_2S as nucleophile *via* the H_2S induced thiolysis approach. A new candidate for a highly selective and specific fluorescent "OFF-ON" probe for H_2S detection *via* a FRET mechanism was developed and subsequently synthesized based on the basis of the dabsyl and fluorescein FRET approach to afford an "OFF-ON" fluorescent probe which fluorescently emitted in the presence of H_2S. A fluorescein was employed as a fluorescent signaling reporter mainly due to their high quantum yields and the great photo-stability. In order to explore a suitable FRET system, an important pre-requisite for the discovery of an efficient FRET system has been the significant overlap between the acceptor absorption and the donor emission. To this approach, a highly specific and selective fluorescent "OFF-ON" probe *via* a FRET mechanism was designed by using a fluorescein as a fluorescein signaling reporter, which was completely quenched by the presence of a dabsyl unit, consisting of two benzene rings connected by an azo bridge. A dabsyl chloride was selected as a quencher moiety covalently conjugated with a fluorescein *via* O-sulfonylation to afford "dabsylfluorescein". According to their spectroscopic properties, the emission of the fluorescein fluorophore as the donor emission is shown at 520 nm (green region) upon the excitation at 485 nm, and the acceptor absorption of a dabsyl chloride is demonstrated at 485 nm as shown in **Figure 2**. Notably, an acceptor absorption of a dabsyl chloride and the donor emission of a fluorescein have shown the significant overlap, making these two candidates the most appropriate FRET pairs for the design of a fluorescent "OFF-ON" probe based on FRET approach as illustrated in **Figure 2**. Furthermore, the close proximity between donor and acceptor units has been the major pre-requisite for the development of fluorescent probe based on a FRET approach. In order to reduce the distance between fluorescein and dabsyl moieties and to minimize the π-π stacking between two moieties, fluorescein and dabsyl moieties were directly conjugated without the uses of linkers to provide the

great advantage for a FRET mechanism, which effectively quenched the fluorescent intensity of a fluorescein mainly due to the generation of FRET mechanism. Interestingly, a collision quenching (*via* the π-π interaction) between a donor and an acceptor moieties could also be the quenching mechanism, which potentially quenched a fluorescent intensity.[39, 40]

(Figure 2)

To the synthetic approach, a dabsyl chloride (known as a dark quencher) was firstly synthesized using a dabsyl acid (widely known as a methyl orange) in the presence of a thionyl chloride, and DMF as a catalyst to afford a dabsyl chloride in a great yield (95%). Notably, a dabsyl chloride was utilized in this strategy mainly due to its absorption property, showing an absorption peak centered at 485 nm (green region) which considerably overlapped with an emission region of fluorescein, implying that a dabsyl moiety could absorb an emitted photon generated by a fluorescein moiety once it was excited. Then, a dabsyl chloride was subsequently coupled to fluorescein *via* an O-sulfonylation in the presence of trimethylamine (Et_3N) to afford a dabsylfluorescein in a great yield (70%) as shown in **Figure 3**. Typically, fluorescein is widely recognized as a well-known fluorescent dye with a great fluorescence quantum yield ($\Phi = 0.79$), excellent solubility, great photo-stability and very high molar extinction coefficient. According to this synthetic point of view, the fabrication of this dabsylfluorescein was quite facile and simple in which the synthetic strategy was readily accomplished *via* only two simple reactions with an excellent yield (75%) relative to other H_2S responsive fluorescent probes in which multistep complicated organic syntheses were required.[27, 28, 31-35, 37] The resulting dabsyl chloride and dabsylfluorescein were further characterized according to spectroscopic analyses using ^{1}H-NMR, ^{13}C-NMR, UV-Vis spectroscopy, fluorescence spectroscopy and ESI-MS, respectively (**Figure S1-S6**).

(Figure 3)

3.2 The optical (UV-Vis and fluorescence) properties of dabsylfluorescein towards hydrogen sulfide (H_2S)

The spectroscopic analyses of a dabsylfluorescein were further characterized using UV-Vis spectroscopy and fluorescence spectroscopy. The absorption behavior of a dabsyl fluorescein was measured in 30% methanol-phosphate buffer (10 mM, pH 7.4) at a final concentration of 8.4 μM, showing a strong absorption signal with a maximum absorption wavelength at 480 nm, corresponding to the π-π* transition of both dabsyl and fluorescein parts. Additionally, a weak broad absorption peak at 290 nm was also noticed, corresponding to the π-π* transition of dabsyl and fluorescein parts as shown in **Figure 4**. Upon the addition of HS^-, a strong absorption signal at 480 nm was slightly red-shifted to 493 nm (bathochromic shift), corresponding to the absorption wavelength of a free fluorescein. This indicated that a thiolysis of dabsylfluorescein was clearly generated to afford a free fluorescein. The mechanistic study of the H_2S induced thiolysis was further investigated.

(Figure 4)

The fluorescence response of a dabsylfluorescein towards H_2S was further investigated in 30% methanol-phosphate buffer (10 mM, pH 7.4) at a final concentration of 8.4 μM. Upon the excitation at 480 nm, a dabsylfluorescein presented a broad

weak emission signal at 520 nm mainly owing to the formation of the FRET process. Briefly, a fluorescein was covalently conjugated with a dabsyl unit *via* O-sulfonylation, causing the FRET process which effectively quenched the fluorescent intensity of a dabsylfluorescein as confirmed by the decrease of a quantum yield (Φ = 0.005) relative to that of standard fluorescein (Φ = 0.79). The detail of quantum yield measurement was shown in SI. Interestingly, upon the addition of HS^- (17.0 μM) a dabsylfluorescein showed a strong emission signal centered at 520 nm, which implied the generation of a fluorescein as shown in **Figure 5**. This was ascribed as the result of the termination of a FRET process *via* the H_2S induced thiolysis approach of a dabsylfluorescein.

3.3 The selectivity and specificity of a dabsylfluorescein towards various biological markers and ions

The selectivity of a dabsylfluorescein (8.4 μM) over various biological markers and ions (17.0 μM) was next investigated using UV-Vis spectrometry and fluorescence spectrometry in 30% methanol-phosphate buffer (10.0 mM, pH 7.4) with an excitation wavelength at 480 nm. As shown in **Figure 4**, only an absorption spectrum of a dabsylfluorescein in the presence of HS^- showed an absorption response. While, the absorption spectra of other biological markers and ions; cations (Ca^{2+}), anions (I^-, F^-, CO_3^{2-}, Br^-, NO_3^-, N_3^-), non-thiol amino acid (alanine) and reactive thiol species (GSH and cysteine) did not response as shown in **Figure S7**. Experimental results obviously revealed the specificity of a dabsylfluorescein towards HS^- over other reactive thiol species (GSH and Cys).

(Figure 5)

The selectivity of a dabsylfluorescein (8.4 μM) over various biological markers and ions (17.0 μM) was next investigated using fluorescence spectrometry in 30% methanol-phosphate buffer (10.0 mM, pH 7.4). As shown in **Figure 6**, a dabsylfluorescein fluorescently emitted at 520 nm upon an excitation wavelength at 480 nm. While, the fluorescent intensity of the corresponding dabsylfluorescein was significantly decreased as confirmed by a calculated quantum yield (Φ = 0.005) relative to that of fluorescein (Φ = 0.79) alone. Typically, a fluorescein demonstrated an emission wavelength at 520 nm with a great quantum yield (Φ = 0.79). Once covalently conjugated to a dabsyl unit *via* an O-sulfonylation, the resulting dabsylfluorescein demonstrated very low fluorescent emission. This was attributed to the presence of electron from nitrogen atom at an azo (–N=N–) moiety playing a particular role to generate the FRET process, which significantly reduced the fluorescent intensity of a dabsylfluorescein. The proposed mechanism of the sensing of H_2S was demonstrated in **Figure 7**.

The selectivity of a dabsylfluorescein towards H_2S, other biological markers and ions was further investigated by monitoring the fluorescent intensity response overtime after the addition of various biological markers and ions; cation (Ca^{2+}), non-thiol amino acid (alanine), bio-thiol species (glutathione (GSH) and cysteine) and anions (HS^-, N_3^-, Br^-, NO_3^-, CO_3^{2-}, I^-, F^-). The fluorescent intensity of a dabsylfluorescein (5 μM, 1 mL) upon the addition of various biological markers and ions (50 μM, 1 mL) was further investigated in 30% methanol-phosphate buffer (10.0 mM, pH 7.4). Interestingly, it was found that only HS^- demonstrated a significant fluorescence enhancement at 520 nm, while the addition of other biological markers and ions did not give any fluorescence response. Additionally, the sensitivity of a dabsylfluorescein towards biological thiols (GSH and cysteine) was successfully examined. According to results in **Figure 6**, biological thiols did not give any fluorescence response possibly due to their higher pKa values of biological thiols (pKa = 8.2 for Cys and

pKa = 9.3 for GSH) relative to that of H_2S (pKa = 7). This undoubtedly affected to its reactivity to launch the nucleophilic attack at a sulfonate ester. Therefore, the presence of biological thiols did not interfere with the H_2S initiated a fluorescence response. This clearly confirmed the selectivity of a dabsylfluorescein towards HS^- as shown in **Figure 6**.

(Figure 6)

(Figure 7)

3.4 The sensing mechanism of dabsylfluorescein towards hydrogen sulfide (H_2S)

The mechanistic study of sensing a dabsylfluorescein *via* H_2S was further investigated using ESI-MS spectroscopy. The H_2S induced thiolysis approach of a dabsylfluorescein was generated by the nucleophilic attack of HS^- to the sulfonate ester moiety to afford a fluorescently emitted fluorescein and dabsyl-SH as shown in **Figure 6**. As expected, the molecular masses of two species (both a fluorescein and a dabsyl-SH) were found, and subsequently confirmed by using LC-ESI-MS spectroscopy. According to the LC-ESI-MS results, the molecular masses of dabsyl-SH at 384.9148$[M+ACN+Na]^+$ and fluorescein at 333.0757$[M+H]^+$ were clearly observed as depicted in **Figure S8-S9**, respectively. This clearly indicated that the sensing mechanism of a dabsylfluorescein *via* HS^- was achieved *via* a thiolysis of HS^- at the sulfonate ester moiety. This was significantly different from other sulfonate ester based fluorescent probes whereas the sensing mechanism took place *via* the nucleophilic aromatic substitution (S_NAr) at an aromatic ring activated by the presence of two nitro groups [25]. Notably, under this condition, the hydrolysis of a dabsylfluorescein did not take place as the molecular mass of a dabsyl acid did not observe. Next, we turned our attention to the sensing properties of a dabsylfluorescein towards HS^- using a fluorometric titration experiment as depicted in **Figure 8**. The fluorescent intensity steadily increased at 520 nm upon the gradual addition of HS^- (from 10 equivalents to 50 equivalents) attributed to the termination of the FRET process as previously mentioned. A linear relationship between the fluorescent intensity of a dabsylfluorescein and HS^- concentration ranging from 135 nM to 5 μM was observed. The detection limit was calculated to be 0.02 μM. These results confirmed that a dabsylfluorescein could quantitatively measure the level of H_2S with the great sensitivity.

(Figure 8)

3.5 The competitive experiment of dabsylfluorescein towards various biological markers and ions

To further investigate the selectivity of a dabsylfluorescein toward H_2S, the competition experiment was studied by the addition of HS^- (25 μM, 250 μL) to a dabsylfluorescein (5 μM, 500 μL) in the presence of various biological markers and ions (25 μM, 250 μL), such as N_3^-, NO_3^-, Ala, Br^-, Ca^{2+}, cysteine, CO_3^{2-}, F^-, glutathione (GSH) and I^-. Notably, a dabsylfluorescein nicely responded to HS^- in the presence of other biological thiols; namely glutathione (GSH), and cysteine. This clearly confirmed that a dabsylfluorescein was highly selective over HS^- ion as shown in **Figure 9**.

(Figure 9)

3.6 The bio-imaging application

This dabsylfluorescein demonstrated the great lipophilic property mainly due to the presence of a dabsyl moiety, providing the great cell membrane permeability as confirmed by *in vivo* bio-imaging experiment. The cell internalization ability of a dabsylfluorescein for sensing the level of the intramolecular hydrogen sulfide (H_2S) in living cell (HeLa cells) was evaluated using a confocal microscope. Firstly, HeLa cells were incubated with a solution of a dabsylfluorescein (50 μM, 10% DMSO-PBS, pH 7.4) for 30 min at 37 °C, and then washed with PBS to remove an excess dabsylfluorescein. Then, HeLa cells were co-incubated with NaSH (100 μM) for 15 min at 37 °C to initiate a fluorescence response. According to the results from a confocal fluorescence microscope, it clearly demonstrated that a dabsylfluorescein enabled to permeate and to interact with intracellular thiols, resulting in an increased fluorescence response in HeLa cells. This was ascribed as the result of the alteration of the intracellular thiol level in HeLa cells as depicted in **Figure 10.** According to the bright-field image, HeLa cells were viable throughout the experiment. Finally, we could summarize that a dabsylfluorescein was a cytomembrane-permeable probe for the detection of thiols in living cells.

(Figure 10)

4. Conclusion

In summary, we have reported a new synthetic strategy of a FRET based fluorescent probe based on the basis of the dabsyl and fluorescein system. To this approach, a fluorescein was completely quenched by the presence of a dabsyl unit, consisting of an azo bridge linked between two benzene rings, and firstly reported here for the first time. In the presence of H_2S, a fluorescence enhancement was markedly observed mainly due to the inhibition of the FRET process *via* the H_2S induced a thiolysis approach, clearly showing a fluorometric response for H_2S detection with the great sensitivity and selectivity toward H_2S over other biological markers and ions. Under a UV lamp, the detection strategy gives rise to a fluorescent response, which could readily be visualized with the naked-eye. Importantly, the utilization of a dabsyl unit as an effective quencher could potentially be a promising platform for the development of fluorescent probes for H_2S detection in which a dabsylfluorescein displayed broad absorption peaks at 250 nm, and 380-550 nm, which potentially quenched a wide range of fluorescent reporting dyes, possessing an absorption range approximately 380-550 nm, ranging from a coumarin, fluorescein, rhodamine, rhodol to fludol. Interestingly, a dabsylfluorescein has shown the ability for the cell internalization into cancerous cells (HeLa cell lines). Therefore, this novel dabsylfluorescein could be potentially applicable for the H_2S detection in living cells and biological systems.

Novelty Statement

This research aimed to the design, and syntheses of a novel fluorescent “OFF-ON” probe based on a FRET mechanism. To the best of our knowledge, this work reported the discovery of a novel efficient FRET pair approach on the basis of the dabsyl and fluorescein system, which was reported here for the first time, and it was potentially applied for the syntheses of a dabsylfluorescein applicable for the

detection of H_2S in living cells

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://xxxxxxxxxxxxxxx

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This research is supported by Thammasat University Research Fund under the TU Research Scholar, Contract number 2/53/2561. PS thanks to the Royal Golden Jubilee scholarship Ph.D. program (RGJ-PHD). The authors acknowledge the Central Scientific Instrument Center (CSIC), Thammasat University Center of Scientific Equipment for Advanced Research (TUCSEAR), Department of Chemistry, Faculty of Science and Technology, Thammasat University.

References

[1] K. Singh, A.M. Rotaru, A.A. Beharry, Fluorescent Chemosensors as Future Tools for Cancer Biology, ACS Chemical Biology 13(7) (2018) 1785-1798.

[2] X. Chen, Y. Zhou, X. Peng, J. Yoon, Fluorescent and colorimetric probes for detection of thiols, Chemical Society Reviews 39(6) (2010) 2120-2135.

[3] M.E. Langmuir, J.-R. Yang, A.M. Moussa, R. Laura, K.A. LeCompte, New naphthopyranone based fluorescent thiol probes, Tetrahedron Letters 36(23) (1995) 3989-3992.

[4] D.P. Li, J.F. Zhang, J. Cui, X.F. Ma, J.T. Liu, J.Y. Miao, B.X. Zhao, A ratiometric fluorescent probe for fast detection of hydrogen sulfide and recognition of biological thiols, Sensors and Actuators, B: Chemical 234 (2016) 231-238.

[5] C. Szabõ, Hydrogen sulphide and its therapeutic potential, Nature Reviews Drug Discovery 6(11) (2007) 917-935.

[6] R. D'Emmanuele Di Villa Bianca, R. Sorrentino, V. Mirone, G. Cirino, Hydrogen sulfide and erectile function: A novel therapeutic target, Nature Reviews Urology 8(5) (2011) 286-289.

[7] C. Szabo, Gaseotransmitters: New frontiers for translational science, Science Translational Medicine 2(59) (2010).

[8] D. Giuliani, A. Ottani, D. Zaffe, M. Galantucci, F. Strinati, R. Lodi, S. Guarini, Hydrogen sulfide slows down progression of experimental Alzheimer's disease by targeting multiple pathophysiological mechanisms, Neurobiology of Learning and Memory 104 (2013) 82-91.

[9] B.D. Paul, J.I. Sbodio, R. Xu, M.S. Vandiver, J.Y. Cha, A.M. Snowman, S.H. Snyder, Cystathionine γ-lyase deficiency mediates neurodegeneration in Huntington's disease, Nature 508(7498) (2014) 96-100.

[10] L.F. Hu, M. Lu, C.X. Tiong, G.S. Dawe, G. Hu, J.S. Bian, Neuroprotective effects of hydrogen sulfide on Parkinson's disease rat models, Aging Cell 9(2) (2010) 135-146.

[11] Y. Zhao, X. Zhu, H. Kan, W. Wang, B. Zhu, B. Du, X. Zhang, A highly selective colorimetric chemodosimeter for fast and quantitative detection of hydrogen sulfide, Analyst 137(23) (2012) 5576-5580.

[12] L.A. Montoya, T.F. Pearce, R.J. Hansen, L.N. Zakharov, M.D. Pluth, Development of selective colorimetric probes for hydrogen sulfide based on nucleophilic aromatic substitution, Journal of Organic Chemistry 78(13) (2013) 6550-6557.

[13] V.K. Gupta, H. Karimi-Maleh, R. Sadegh, Simultaneous determination of hydroxylamine, phenol and sulfite in water and waste water samples using a voltammetric nanosensor, International Journal of Electrochemical Science 10(1) (2015) 303-316.

[14] J. Furne, A. Saeed, M.D. Levitt, Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values, American Journal of Physiology - Regulatory Integrative and Comparative Physiology 295(5) (2008) R1479-R1485.

[15] U. Hannestad, S. Margheri, B. Sörbo, A sensitive gas chromatographic method for determination of protein-associated sulfur, Analytical Biochemistry 178(2) (1989) 394-398.

[16] A.R. Lippert, E.J. New, C.J. Chang, Reaction-based fluorescent probes for selective imaging of hydrogen sulfide in living cells, Journal of the American Chemical Society 133(26) (2011) 10078-10080.

[17] 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, Angewandte Chemie - International Edition 50(41) (2011) 9672-9675.

[18] W. Sun, J. Fan, C. Hu, J. Cao, H. Zhang, X. Xiong, J. Wang, S. Cui, S. Sun, X. Peng, A two-photon fluorescent probe with near-infrared emission for hydrogen sulfide imaging in biosystems, Chemical Communications 49(37) (2013) 3890-3892.

[19] 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, Analytica Chimica Acta 859 (2015) 59-65.

[20] L.A. Montoya, M.D. Pluth, Selective turn-on fluorescent probes for imaging hydrogen sulfide in living cells, Chemical Communications 48(39) (2012) 4767-4769.

[21] M.Y. Wu, K. Li, J.T. Hou, Z. Huang, X.Q. Yu, A selective colorimetric and ratiometric fluorescent probe for hydrogen sulfide, Organic and Biomolecular Chemistry 10(41) (2012) 8342-8347.

[22] X. Li, J. Cheng, Y. Gong, B. Yang, Y. Hu, Mapping hydrogen sulfide in rats with a novel azo-based fluorescent probe, Biosensors and Bioelectronics 65 (2015) 302-306.

[23] X. Cao, W. Lin, K. Zheng, L. He, A near-infrared fluorescent turn-on probe for fluorescence imaging of hydrogen sulfide in living cells based on thiolysis of dinitrophenyl ether, Chemical Communications 48(85) (2012) 10529-10531.

[24] D. Maity, A. Raj, P.K. Samanta, D. Karthigeyan, T.K. Kundu, S.K. Pati, T. Govindaraju, A probe for ratiometric near-infrared fluorescence and colorimetric hydrogen sulfide detection and imaging in live cells, RSC Advances 4(22) (2014) 11147-11151.

[25] H. Maeda, H. Matsuno, M. Ushida, K. Katayama, K. Saeki, N. Itoh, 2,4-Dinitrobenzenesulfonyl fluoresceins as fluorescent alternatives to Ellman's reagent in thiol-quantification enzyme assays, Angewandte Chemie - International Edition 44(19) (2005) 2922-2925.

[26] Q. Zhao, F. Huo, J. Kang, Y. Zhang, C. Yin, A novel FRET-based fluorescent probe for the selective detection of hydrogen sulfide (H2S) and its application for bioimaging, Journal of Materials Chemistry B 6(30) (2018) 4903-4908.

[27] K. Huang, M. Liu, Z. Liu, D. Cao, J. Hou, W. Zeng, Ratiometric and colorimetric detection of hydrogen sulfide with high selectivity and sensitivity using a novel FRET-based fluorescence probe, Dyes and Pigments 118 (2015) 88-94.

[28] Y. Tang, G.F. Jiang, A novel two-photon fluorescent probe for hydrogen sulfide in living cells using an acedan-NBD amine dyad based on FRET process with high selectivity and sensitivity, New Journal of Chemistry 41(14) (2017) 6769-6774.

[29] L. He, X. Yang, Y. Liu, W. Lin, Colorimetric and ratiometric fluorescent probe for hydrogen sulfide using a coumarin-pyronine FRET dyad with a large emission shift, Analytical Methods 8(45) (2016) 8022-8027.

[30] C. Wei, L. Wei, Z. Xi, L. Yi, A FRET-based fluorescent probe for imaging H 2 S in living cells, Tetrahedron Letters (2013) 6937-6939.

[31] C. Wei, R. Wang, L. Wei, L. Cheng, Z. Li, Z. Xi, L. Yi, O-fluorination of aromatic azides yields improved azido-based fluorescent probes for hydrogen sulfide: Synthesis, spectra, and bioimaging, Chemistry - An Asian Journal 9(12) (2014) 3586-3592.

[32] L. Yuan, Q.P. Zuo, FRET-based mitochondria-targetable dual-excitation ratiometric fluorescent probe for monitoring hydrogen sulfide in living cells, Chemistry - An Asian Journal 9(6) (2014) 1544-1549.

[33] B. Chen, P. Wang, Q. Jin, X. Tang, Chemoselective reduction and self-immolation based FRET probes for detecting hydrogen sulfide in solution and in cells, Organic and Biomolecular Chemistry 12(30) (2014) 5629-5633.

[34] J. Zhang, R. Wang, Z. Zhu, L. Yi, Z. Xi, A FRET-based ratiometric fluorescent probe for visualizing H 2 S in lysosomes, Tetrahedron 71(45) (2015) 8572-8576.

[35] K. Huang, L. Yu, P. Xu, X. Zhang, W. Zeng, A novel FRET-based ratiometric fluorescent probe for highly sensitive detection of hydrogen sulfide, RSC Advances 5(23) (2015) 17797-17801.

[36] C. Zhang, L. Wei, C. Wei, J. Zhang, R. Wang, Z. Xi, L. Yi, A FRET-ICT dual-quenching fluorescent probe with large off-on response for H 2 S: Synthesis, spectra and bioimaging, Chemical Communications 51(35) (2015) 7505-7508.
[37] K. Huang, M. Liu, X. Wang, D. Cao, F. Gao, K. Zhou, W. Wang, W. Zeng, Cascade reaction and FRET-based fluorescent probe for the colorimetric and ratiometric signaling of hydrogen sulfide, Tetrahedron Letters 56(24) (2015) 3769-3773.
[38] X. Feng, T. Zhang, J.T. Liu, J.Y. Miao, B.X. Zhao, A new ratiometric fluorescent probe for rapid, sensitive and selective detection of endogenous hydrogen sulfide in mitochondria, Chemical Communications 52(15) (2016) 3131-3134.
[39] H. Kashida, K. Morimoto, H. Asanuma, A stem-less probe using spontaneous pairing between Cy3 and quencher for RNA detection, Science and Technology of Advanced Materials 17(1) (2016) 267-273.
[40] M.K. Johansson, H. Fidder, D. Dick, R.M. Cook, Intramolecular dimers: A new strategy to flourescence quenching in dual-labeled oligonucleotide probes, Journal of the American Chemical Society 124(24) (2002) 6950-6956.
[41] Q. Zhao, J. Kang, Y. Wen, F. Huo, Y. Zhang, C. Yin, "Turn-on" fluorescent probe for detection of H2S and its applications in bioimaging, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 189 (2018) 8-12.
[42] Z. Wu, Z. Li, L. Yang, J. Han, S. Han, Fluorogenic detection of hydrogen sulfide via reductive unmasking of o-azidomethylbenzoyl-coumarin conjugate, Chemical Communications 48(81) (2012) 10120-10122.
[43] X. Wang, J. Sun, W. Zhang, X. Ma, J. Lv, B. Tang, A near-infrared ratiometric fluorescent probe for rapid and highly sensitive imaging of endogenous hydrogen sulfide in living cells, Chemical Science 4(6) (2013) 2551-2556.
[44] Y. Yang, Y.U. Liu, L. Yang, J.U.N. Liu, K.U.N. Li, S. Luo, A coumarin-based colorimetric fluorescent probe for hydrogen sulfide, Journal of Chemical Sciences 127(3) (2015) 359-363.
[45] E. Karakuş, M. Üçüncü, M. Emrullahoğlu, Electrophilic Cyanate As a Recognition Motif for Reactive Sulfur Species: Selective Fluorescence Detection of H2S, Analytical Chemistry 88(1) (2016) 1039-1043.
[46] X. Jin, S. Wu, M. She, Y. Jia, L. Hao, B. Yin, L. Wang, M. Obst, Y. Shen, Y. Zhang, J. Li, Novel Fluorescein-Based Fluorescent Probe for Detecting H2S and Its Real Applications in Blood Plasma and Biological Imaging, Analytical Chemistry 88(22) (2016) 11253-11260.
[47] X. Lin, X. Lu, J. Zhou, H. Ren, X. Dong, W. Zhao, Z. Chen, Instantaneous fluorescent probe for the specific detection of H2S, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 213 (2019) 416-422.
[48] C. Liu, B. Peng, S. Li, C.-M. Park, A.R. Whorton, M. Xian, Reaction Based Fluorescent Probes for Hydrogen Sulfide, Organic Letters 14(8) (2012) 2184-2187.

Figures and Legends

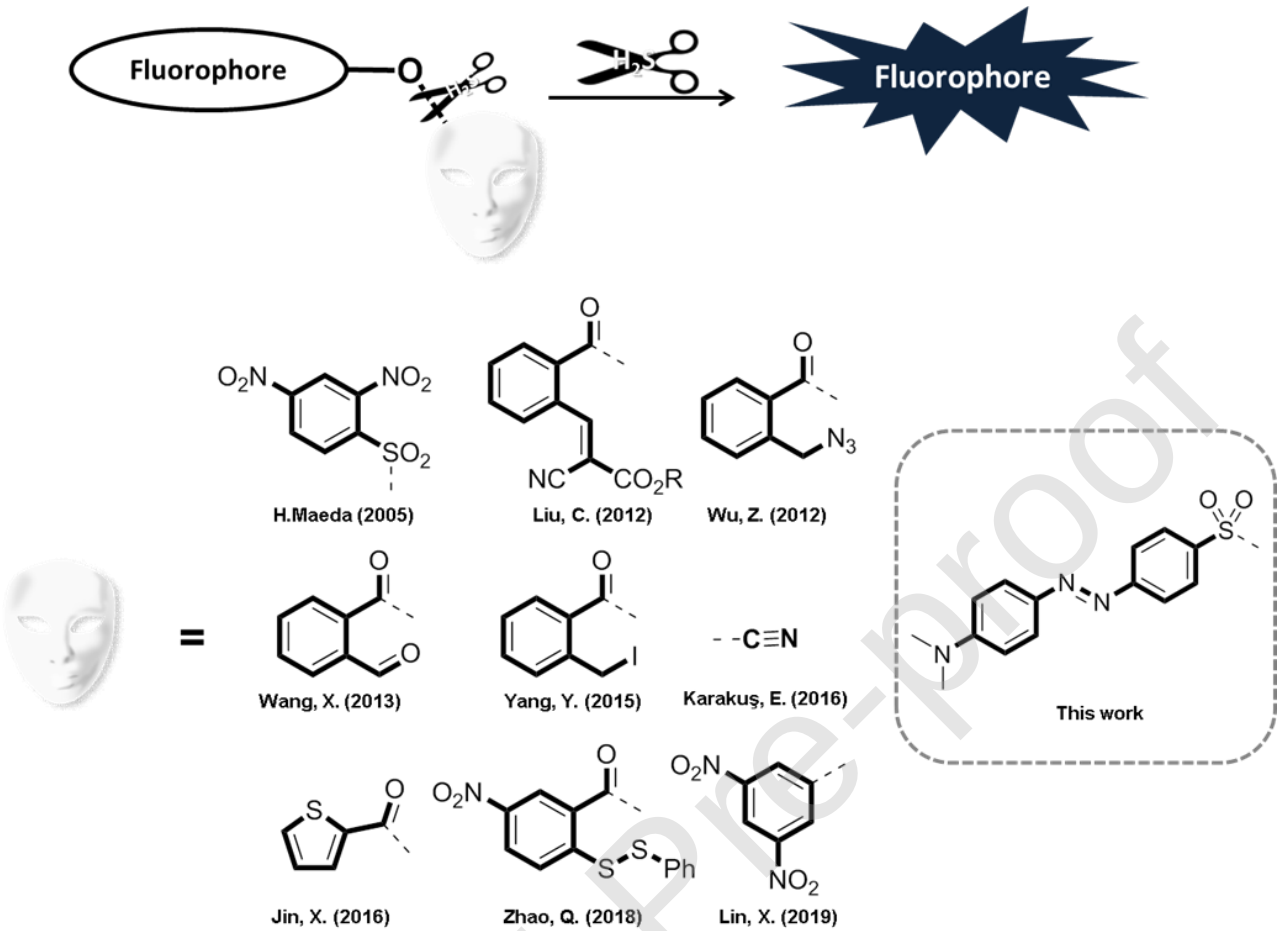


Figure 1 Selected sulfur-sensitive masking moieties applied in the development of fluorescent probes [25, 41-48]. Chemically sensitive bonds towards sulfur attack are illustrated as a dashed line.

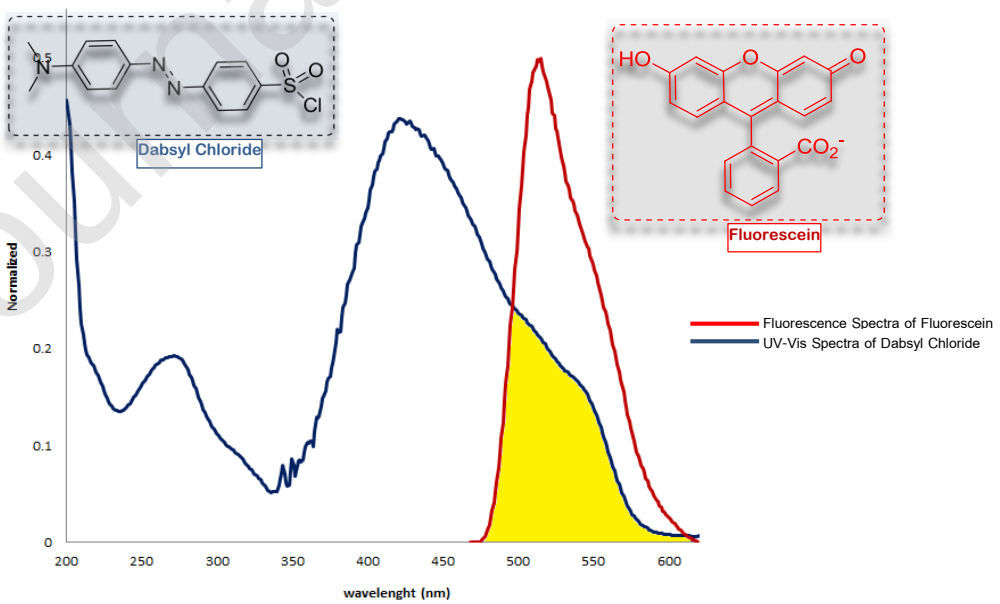


Figure 2 Normalized UV–Vis spectra of a dabsyl chloride (Navy) and fluorescence spectra of a fluorescein (Red). The spectral overlapping between absorption spectrum (an acceptor) and emission spectrum (a donor) is shown in yellow area.

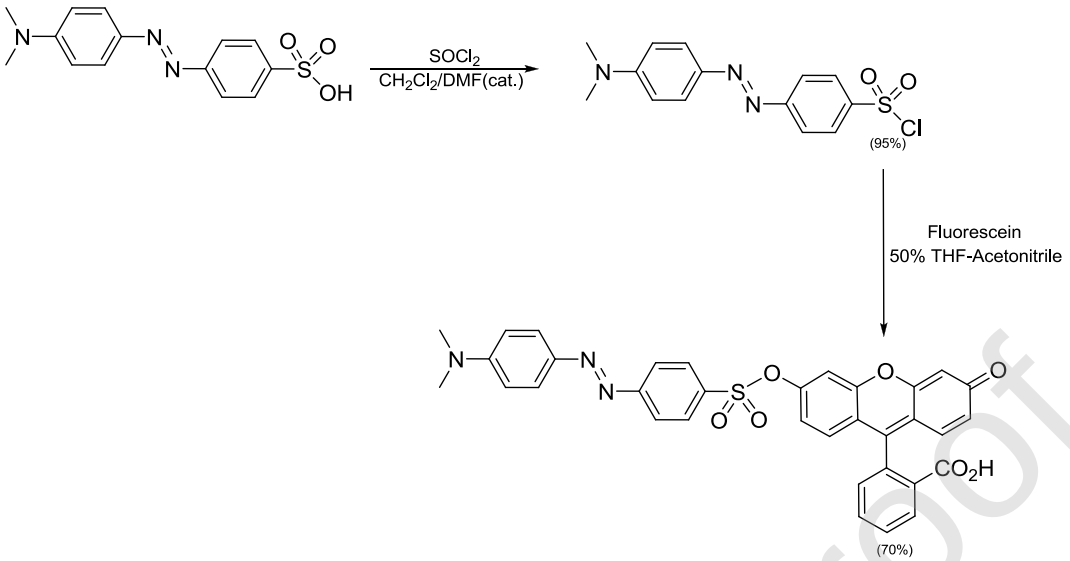


Figure 3 The synthesis of a dabsylfluorescein

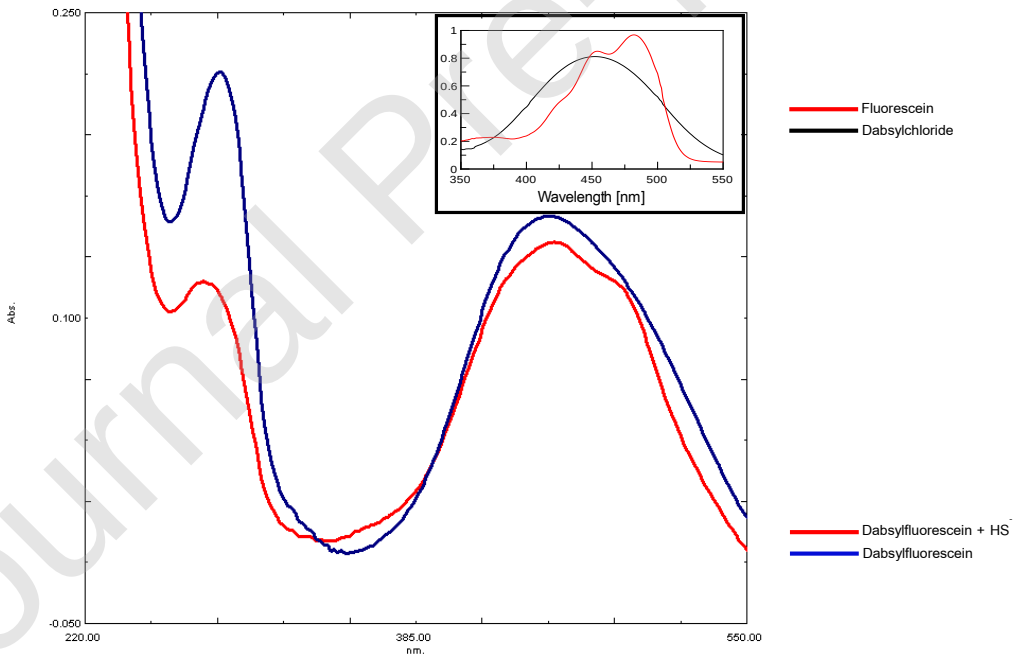


Figure 4 UV–Vis spectra of a dabsylfluorescein (8.4 μM) in the presence of 5.0 equiv. of HS^- (17.0 μM, Red) and a dabsylfluorescein without HS^- in 30% CH_3OH-phosphate buffer (10 mM, pH 7.4, Navy). (Inset) UV-Vis spectra of dabsylchloride and fluorescein (8.4 uM) in 30% methanol-phosphate buffer (10.0 mM, pH 7.4).

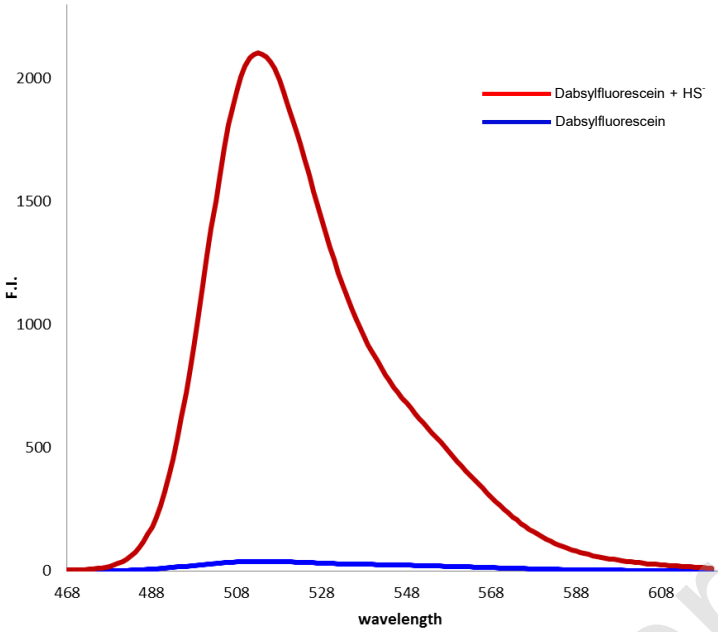
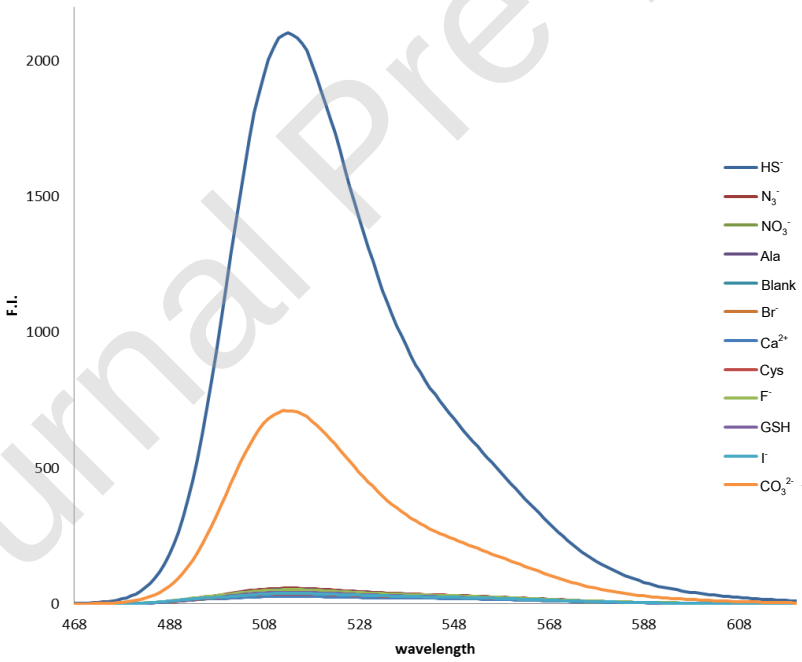


Figure 5 (Red) Emission spectra of a dabsylfluorescein (8.4 uM) in the presence of 2.0 equiv. of HS^- (17.0 uM) in 30% methanol-phosphate buffer (10.0 mM, pH 7.4) and (Blue) a dabsylfluorescein (5.0 μM) without any ions in 30% methanol-phosphate buffer (10.0 mM, pH 7.4).

(a)



(b)

Figure 6 The selectivity of a dabsylfluorescein in the presence of various ions in 30% methanol-phosphate buffer (10 mM, pH 7.4). (a) Fluorescence spectra of a dabsylfluorescein (5.0 uM, 1 mL) in the presence of various ions (10 equiv.) at 50 uM in 30% methanol-phosphate buffer (10.0 mM, pH 7.4). (b) Color changes of dabsylfluorescein upon the addition of HS^- (50 uM) and other ions (50 uM) measured in 10% methanol-phosphate buffer (10 mM, pH 7.4) under UV light. λ_{ex} = 480 nm, λ_{em} = 520 nm.

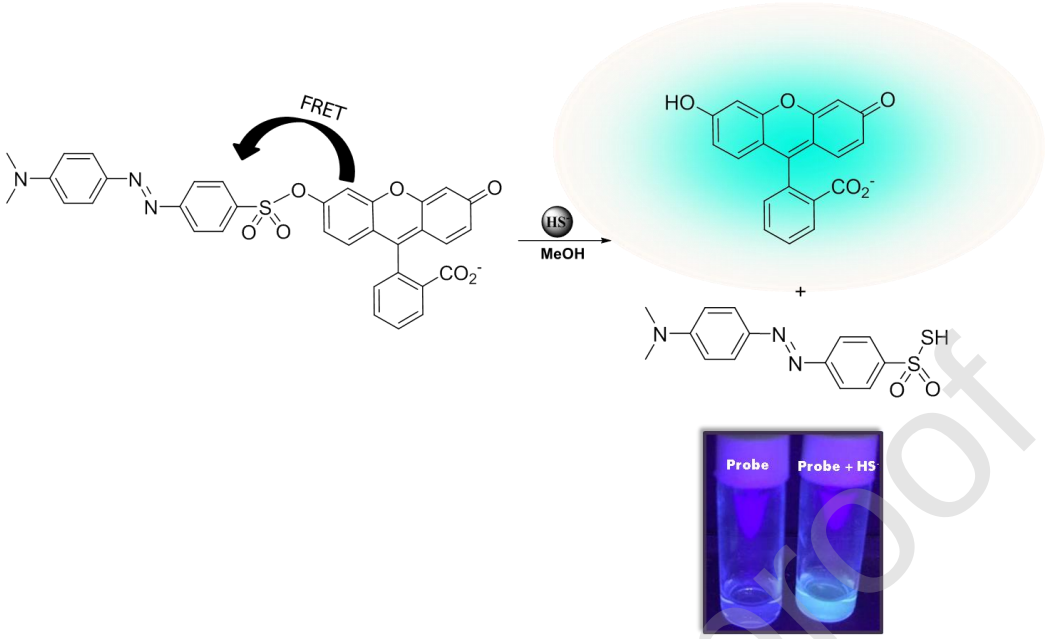


Figure 7 Graphic of the proposed mechanism of the sensing of HS^-

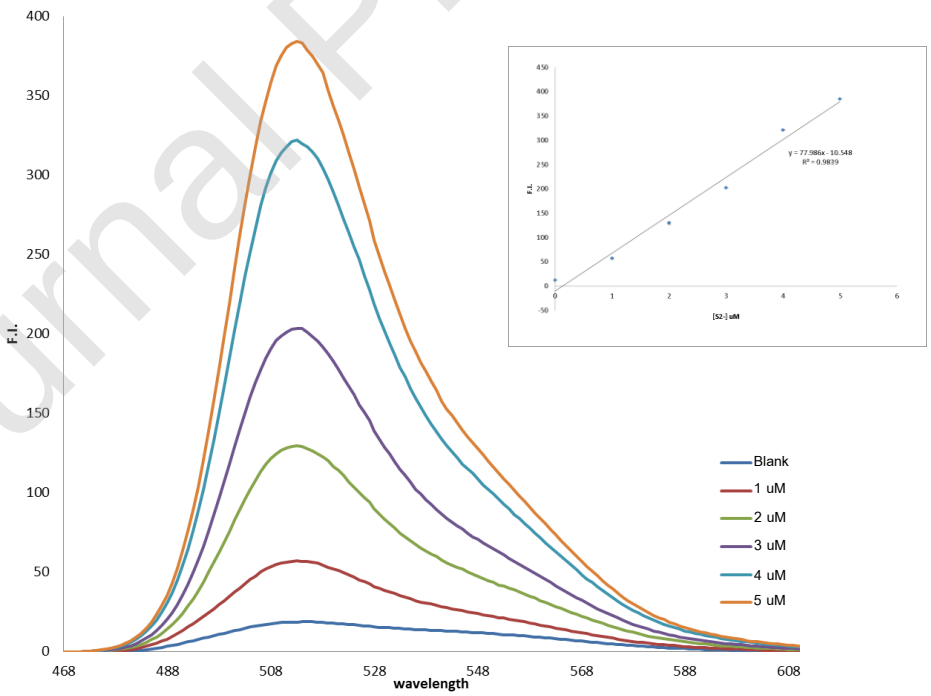


Figure 8 The variation of fluorescence emission spectra of a dabsylfluorescein with a gradual addition of HS^- (10-50 equiv.) in 30% methanol-phosphate buffer (10 mM, pH 7.4). Excitation wavelength was set at 480 nm. LOD = 0.02 uM

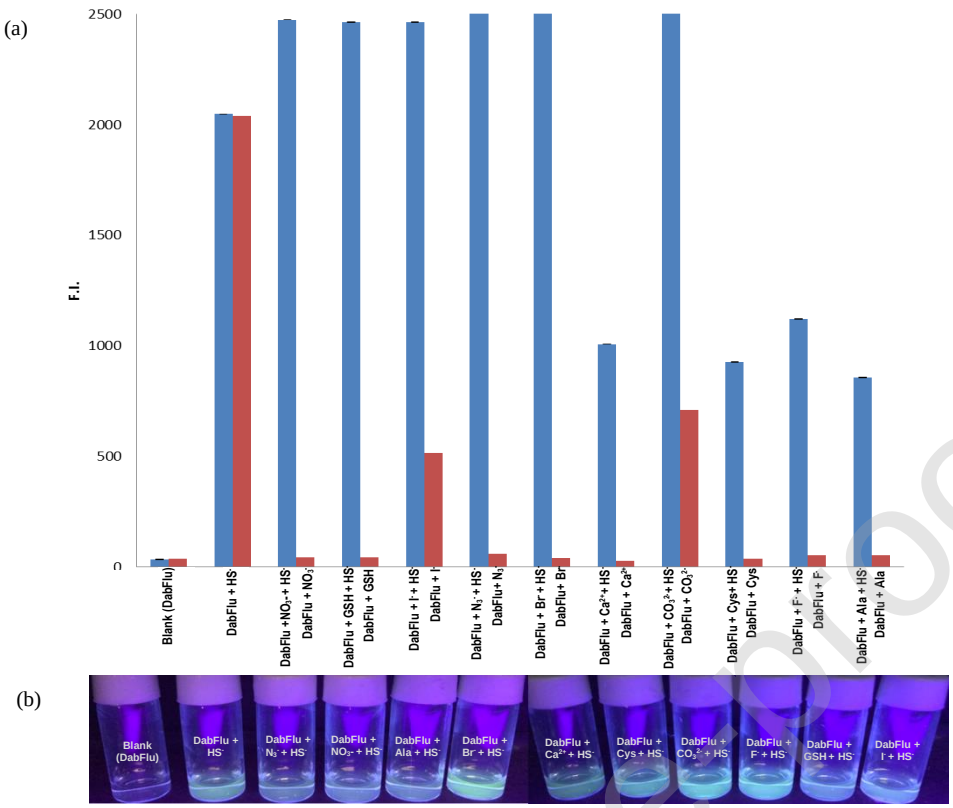


Figure 9 (a). The competitive selectivity of a dabsylfluorescein towards HS^- (5.0 equiv.) in the presence of other ions (5.0 equiv.) in 30% methanol-phosphate buffer (10 mM, pH 7.4)(blue bar), and the selectivity of a dabsylfluorescein towards other ions (5.0 equiv.)(red bar). (b). Color changes of dabsylfluorescein mixed with HS^- in the presence of other ions or other biomarkers measured in 10% methanol-phosphate buffer (10 mM, pH 7.4) under UV light. Excitation wavelength was set at 480 nm and blank = dabsylfluorescein (DabFlu).

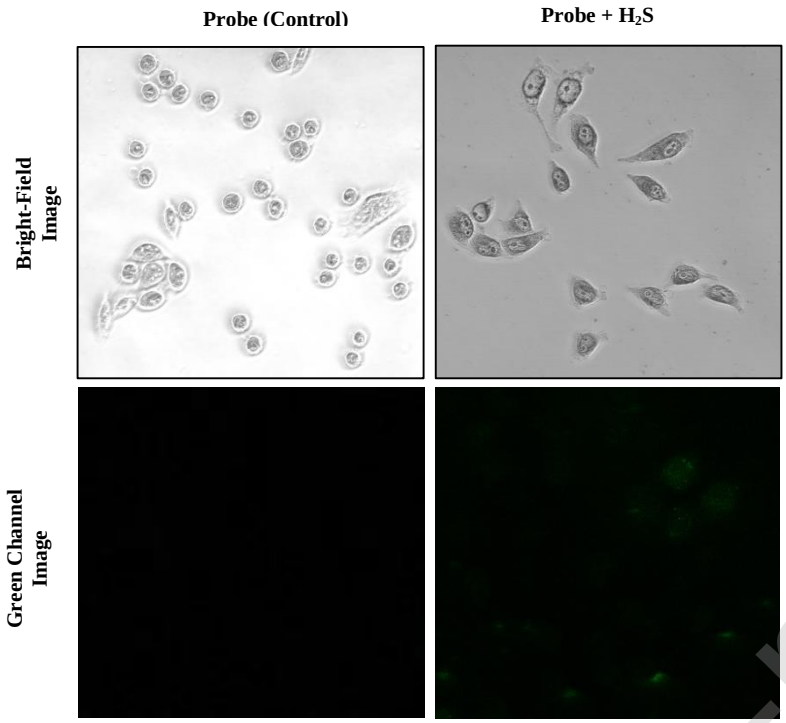


Figure 10 Confocal fluorescence images of HeLa cells. Cells were incubated with dabsylfluorescein (25 μM) for 30 min, and subsequently treated with NaSH (50 μM) for 20 min in 10% DMSO-Phosphate buffer (10 mM, pH 7.4); (a) Bright-field images, (b) Fluorescence images and (c) Merged bright-field and fluorescence images. Excitation wavelength was set at 480 nm. The images were acquired using confocal fluorescence microscope at 40 × magnifications.