Fluorescence chemosensors for hydrogen sulfide detection in biological systems

Zhi Guo,a,b Guiqiu Chen,*a,b Guangming Zeng,*a,b Zhongwu Li,a,b Anwei Chen,c Jiajia Wang,a,b and Longbo Jianga,b

A comprehensive review of the development of H2S fluorescence-sensing strategies, including sensors based on chemical reactions and fluorescence resonance energy transfer (FRET), is presented. The advantages and disadvantages of fluorescence-sensing strategies are compared with those of traditional methods. Fluorescence chemosensors, especially those used in FRET sensing, are highly promising because of their low cost, technical simplicity, and their use in real-time sulfide imaging in living cells. Potential applications based on sulfate reduction to H2S, the relationship between sulfate-reducing bacteria activity and H2S yield, and real-time detection of sulfate-reducing bacteria activity using fluorescence sensors are described. The current challenges, such as low sensitivity and poor stability, are discussed.

1. Introduction

H2S is a colorless gas; it has a distinct smell of rotten eggs, and is mainly produced by the decomposition of organic compounds or as a byproduct of a range of industries, including petroleum refining, farming, waste management, and natural gas production.1,2 The toxic effects of H2S were first documented roughly 300 years ago; since then, many studies have

Zhi Guo received his bachelor’s degree in environmental engineering from Anhui Normal University in 2012. He has received his Master’s degree from Hunan University. Now, he is pursuing his PhD degree in analytical chemistry and biosensors aspects at Hunan University. His research interests include chemical/biological sensors and portable sensing platforms, especially with novel detection systems.

Guiqiu Chen received her PhD degree from Hunan University in 2006 and, at the same time, was appointed as Associate professor of Hunan University. She then worked as a Chemical post-doctoral at Hunan University. She is now a professor and doctoral supervisor in Hunan University. Her research interests were nanosensor synthesis, fluorescence recognition of pollutants and bioimaging, and also water pollution control.
gasotransmitters NO and CO. It has diverse functions in several pathophysiological processes, especially in regulating vascular contractility and neuronal activity.\textsuperscript{16–18} In mammals, H$_2$S production is derived primarily from three enzymes: cystathionine $\gamma$-lyase, CBS, and 3-mercaptoppyruvate sulfur-transferase.\textsuperscript{19,20} The widespread but differential expression of these enzymes in different tissues suggests that H$_2$S has wide importance and significance in the cardiovascular, circulatory, respiratory, urinary, and nervous systems. In addition to the pathophysiological conditions associated with H$_2$S misregulation, H$_2$S can also act on specific cellular targets, including heme proteins,\textsuperscript{21} cysteine (Cys) residues on ATP-sensitive K$^+$ channels,\textsuperscript{22} NO,\textsuperscript{23} and other emerging targets.

Regardless of the dangers or benefits of H$_2$S, it is important to develop efficient methods for the detection of H$_2$S in living systems. The available methods should meet the following four criteria. They should (1) act rapidly (within seconds) under mild conditions; (2) be sensitive for detection under near physiological conditions; (3) show minimal or no interference by other anions in blood serum; and (4) be functional in aqueous solutions and blood plasma.\textsuperscript{24} However, traditional methods of H$_2$S detection, including colorimetry,\textsuperscript{25,26} gas chromatography,\textsuperscript{27,28} electrochemical analysis,\textsuperscript{29,30} and metal-induced sulfide precipitation,\textsuperscript{31} are often limited by poor compatibility with living cells, low temporal resolution, and extensive sample preparation requirements.

Recently, fluorescence imaging has emerged as a hot topic in the field of H$_2$S detection because of its high sensitivity, real-time spatial imaging, and non-damaging detection of targets in living cells or tissues.\textsuperscript{32–43} These strategies are highly desirable compared with traditional methods, and offer high biocompatibility as well as real-time imaging. Fluorescence resonance energy transfer (FRET), a non-radiative energy transfer process, is a widely used sensing mechanism, because it is particularly promising in biological imaging. To date, many FRET processes are based on QDs and covalently linked dye scaffolds. However, there are no FRET processes reported for H$_2$S detection. Although several reviews about H$_2$S sensing in biological systems already exist, the field is advancing at a rapid pace and the current manuscript offers insightful new perspectives on designs and applications.\textsuperscript{44,45} Sulfate reducing bacteria (SRB) are regarded as the main contributor to anaerobic corrosion. It is crucial to determine the activity in the environment instantaneously, but few fluorescence methods have been reported. In this review, we summarize new reaction based fluorescence molecular sensors with different functional groups. Among them, the fluorescence molecular sensors suitable for two-photon microscopy (TPM) are considered as a separate part due to their particular sensing manner. Other fluorescence sensors that employ FRET processes are introduced as a promising method for H$_2$S recognition. We also show the potential use of fluorescence sensors based on QDs and, at the same time, present the challenges faced. Moreover, their potential application in detection of sulfate-reducing bacteria activity is proposed and discussed.

2. Reaction-based molecular sensors for H$_2$S detection derived from functional groups

Recently, a large number of reaction-based sensors for H$_2$S detection have been reported,\textsuperscript{46} these typically offer higher spatiotemporal resolution and greater living-cell compatibility than traditional detection methods. Such reaction-based sensors have been derived from azide or nitro compounds, an azamacrocyclic Cu(II) ion complex, and H$_2$S-specific Michael acceptors. In these sensors, a fluorophore that has a high quantum yield, emits a long wavelength, and responds to hydrosulfide by fluorescence changes is required. When H$_2$S is present, a known unique reaction, followed by an optical change, is triggered. The corresponding optical changes are

Guangming Zeng received his PhD from Wuhan University in 1988. Later, he worked at the College of Environmental Science and Engineering, Hunan University. He is now a professor at Hunan University and the director of Environmental Science and Engineering. His research interests include biosensor and bioimaging, especially fluorescence imaging in biological systems based on QDs. After years of efforts, he has been rewarded with many prizes and has guided hundreds of Master’s degree candidates and doctoral candidates.

Zhongwu Li received his PhD from Institute of Geographical Science and Resources, Chinese Academy of Sciences in 2002. He is an executive director of Chinese Environment, Resource and Ecological Conservation. He is now a professor and doctoral supervisor in Hunan University. His research interests were chemical sensors, and heavy metal detection and control of soil. To date, he has published more than 60 academic papers since joining Hunan University.
usually either linear or non-linear with respect to H₂S concentration, which is crucial for further H₂S quantification.

2.1. Fluorescence molecular sensors derived from azide or nitro compounds

One important approach to designing sulfide-selective optical sensors relies on the unique interactions that occur between sulfides and azides or nitro compounds. One important process in these strategies depends strongly on the reduction of azide and nitro groups on masked fluorophores to generate amines, which resulted in fluorescence emission. As shown in Fig. 1A, H₂S can reduce the nitro group in probe 1 and the azide group in probe 2 to amine groups, thereby creating off-on fluorescence signaling that can be used for H₂S detection. Treatment of a 5 μM solution of probes 1 and 2 with 100 equiv. of H₂S resulted in significantly increased fluorescence. For probe 1, a 15-fold turn-on was observed after 90 min, and a 60-fold turn-on was observed after 45 min for probe 2. The detection limits of probes 1 and 2 for H₂S after 60 min incubation were 5–10 and 1–5 μM, respectively. Human cervical carcinoma cell lines (HeLa cells) were incubated with probes 1 and 2 for 30 min, respectively. As shown in Fig. 1B, the HeLa cell images (a and c) were non-fluorescent (or low-fluorescent) after the incubation. However, the cells became fluorescent or showed fluorescence enhancement (b and d) after 250 μM H₂S was added to the cells of a and c and incubated for another 30 min.

The potential applications of fluorescence detection of H₂S have led to many types of fluorophore being combined with azide or nitro compounds to generate fluorescent probes. Dansyl is a commonly used fluorophore, and is well known for its strong fluorescence and long emission wavelength. A fluorescent dansyl-azide probe (probe 4) was synthesized using dansyl 3 as the precursor (see Fig. 2).24 Probe 4 itself is non-fluorescent. However, on addition of H₂S, a solution of 4 showed strong fluorescence enhancement following reduction of the azido group to an amino group. The addition of 25 μM H₂S led to a 40-fold fluorescence enhancement in 20 mM sodium phosphate buffer (pH 7.5) with 0.5% Tween-20 (buffer/Tween). The detection limit was as low as 1 μM in buffer/Tween and 5 μM in bovine serum, with a signal-to-noise ratio of 3 : 1.

In 2011, Chang et al. developed a pair of new reaction-based fluorescent probes, i.e., sulfidefluor-1 (probe 5) and sulfidefluor-2 (probe 6).
fidefluor-2 (probe 6), for selective imaging of H₂S in living cells; these probes exploit the H₂S-mediated reduction of azides to fluorescent amines (see Fig. 3). Both probes displayed good selectivity for H₂S over abundant biologically relevant thiols, including 5 mM glutathione (GSH) and 5 mM Cys. Probe 5 displayed an approximately three-fold increased response for H₂S than that for most other species tested, and an approximately two-fold increased selectivity versus O₂⁻. For probe 6, better selectivities versus GSH (ca. five-fold), sulfite (ca. four-fold), and O₂⁻ (ca. four-fold) were observed. In addition, a clear increase in intracellular fluorescence intensity was observed for H₂S-treated human embryonic kidney 293 T (HEK293 T) cells using 5 or 6 as the probe. Probe 5 showed a higher turn-on response than probe 6 for the detection of H₂S in cells because of increased lipophilicity and cellular retention.

Similar sensors, CLSS-1 (probe 7) and CLSS-2 (probe 8), were developed with 128- and 48-fold enhanced luminescence responses toward H₂S, with detection limits of 0.7 ± 0.3 and 4.6 ± 2.0 μM, respectively (Fig. 3). They were reaction-based chemiluminescent sulfide sensors. Because chemiluminescence does not require an excitation source, there is little chance of photodegradation of the sensing platform, which may occur in ordinary fluorescence probes. In addition, these chemiluminescent sensors offered high signal-to-noise ratios because biological materials typically did not spontaneously emit light. This special characteristic made the sensors more suitable in biological system application although cysteine derived reductants might interfere in the selectivity of probe 7. Compared with probe 7, probe 8 displays higher selectivity for H₂S over other reactive sulfur, nitrogen, and oxygen species, including GSH, Cys, homocysteine (Hcy), S₂O₃²⁻, NO₂⁻, HNO, ONOO⁻, and NO.

Surfactants such as micelles and other aggregates are widely used in various scientific fields because of their physicochemical properties. Based on the ability of cetyltrimethylammonium bromide (CTAB) micelles to adjust the fluorescence detection sensitivity, two colorimetric and turn-on fluorescent probes (9 and 10) were designed for selective recognition of H₂S (Fig. 4). The probes were constructed by incorporating an azido group into a naphthalimide fluorophore as a specific group for reactions with sulfide based on its reducing properties. In the presence of the cationic surfactant CTAB, the azido groups of the probes were reduced to amino groups by H₂S and the solution changed from colorless to yellow, accompanied by strong yellow-green fluorescence within 10 min. Compared with the reaction in a buffer, the detection limit in CTAB decreased to 20 nM from 0.5 μM. The linear concentration range for H₂S detection was adjusted using differently charged surfactants, and the overall linear range covered five orders of magnitude, from 0.05 μM to 1 mM. The probes were successfully used for rapid and sensitive detection of H₂S levels in fetal bovine serum without any sample pretreatment.

7-Amino-4-methylcoumarin (11) is widely used in fluorescent enzyme assays and can be easily converted to non-fluorescent 7-azido-4-methylcoumarin (probe 12; Fig. 5). In the presence of NaHS, 12 is converted to 11, with a concomitant fluorescence increase that is linear for NaHS concentrations from 100 nM to 100 mM. CBS activity assays showed no responses by the sensors to 10 mM Cys or Hcy, 1 mM pyridoxal 5′-phosphate, or 1 mM S-adenosyl-L-methionine, the allosteric activator of human CBS. These results indicate that the sensors are highly selective.

Fluorescent protein (FP) is widely used in biological imaging, because it does not alter the function or localization of the targeted protein. FP can be engineered to self-assemble into protein particles displaying protein functions.
suitable for various applications in diagnostics or detection. Genetically encoded FPs were modified with sulfide-reactive azide functional groups by expanding the genetic codes of E. coli and mammalian cells (probes 13 and 14, Fig. 6). These structurally modified chromophores were selectively reduced by H2S, resulting in sensitive fluorescence enhancement, which was detectable using fluorescence microscopy techniques (Fig. 7). The fluorescence enhancement showed a linear relationship with H2S in the range of 0–50 μM. Probes 13 and 14 have many advantages such as the addition of cell localization tags to allocate the probe to specific cell subdomains. In addition, the approach described relies on the recognition of target molecules or enzymatic reactions, which is different from the incorporation of unnatural amino acids into FPs based on selective chemical transformations. However, because of the reaction of dithiothreitol with these probes, caution must be exercised to exclude dithiothreitol when measuring H2S in vitro. The reaction with probes may be caused by the strong reductive power of two free thiols of dithiothreitol.

2.2. Fluorescence molecular sensors derived from the azamacrocyclic Cu(n) ion complex

Recently, a new sulfide-selective chemosignaling system was devised based on a dipicolylamine (DPA)–fluorescein complex 16 with Cu2+ (probe 15). The Cu2+ ions in probe 15 form stable species with the targeted sulfide ions. The higher stability of Cu2+-sulfide ion species compared with 15 results in the release of free 16. Concomitantly, the fluorescence of 16 is fully restored by the transformation from probe 15 (quenched, off) to free 16 with a detection limit of 420 nM in 100% aqueous solution in response to sulfide anions (Fig. 8). The probe has selective fluorescence-enhancing behavior exclusively with sulfide ions (the ratio of the final and initial fluorescence intensity (I/I0) at 517 nm = 87). However, a further study confirmed that this probe does not have sufficient selectivity for H2S in the presence of reduced GSH. A clear fluorescence enhancement was observed on the addition of 10 mM GSH. It is well known that azamacrocyclic rings form stable metal complexes with Cu2+, and the paramagnetic Cu2+ center has a pronounced quenching effect on fluorophores. Based on these observations, Nagano et al. designed and synthesized four sensor probes based on a fluorescein (AF) scaffold conjugated with an azama macroyclic Cu2+ complex (Fig. 9). The four macrocyclic fluoresceins, namely 1,4,7-triazacyclononane (17), 1,4,7,10-tetraazaacyclododecane (cyclen, 18), 1,4,8,11-tetraazaacyclotetradecane (19), and N,N9,N9-trimethylcyclen (20), were used as chelators for Cu2+ instead of DPA. Sensitivity and selectivity assays indicated that 18-AF + Cu2+ has excellent selectivity and sensitivity as a fluorescent probe for H2S detection. As shown in Fig. 10, the fluorescence intensity of 18-AF + Cu2+ showed a large and immediate increase with the addition of 10 μM H2S, whereas almost no increase in fluorescence is observed with the addition of 10 mM GSH. In the case of the...
other three probes, 17-AF + Cu$^{2+}$ showed high sensitivity and low selectivity, whereas 19-AF + Cu$^{2+}$ and 20-AF + Cu$^{2+}$ showed low sensitivities and high selectivities. Reaction-based methods derived from azamacrocyclic Cu(II) ion complexes overcome the disadvantages of some other methods, e.g., being a relatively slow reaction and showing poor selectivity for H$_2$S over reactive oxygen species.$^{57,78}$

2.3. Fluorescence molecular sensors derived from H$_2$S-specific Michael acceptors

Recent studies have indicated that many nucleophile-based approaches suffer from irreversible probe deactivation on reaction with other nucleophiles, thereby greatly diminishing the H$_2$S detection capacity.$^{79}$ To detect H$_2$S selectively, it is important to differentiate H$_2$S from other biological nucleophiles, especially thiols such as Cys and GSH. One of the current strategies for addressing this problem is to interdict possible fluorescence changes caused by the reaction of nucleophiles with the probes. The other method is to develop fluorescent probes derived from reversible Michael acceptors (an electrophilic conjugated system acting as an electron acceptor in the Michael addition reaction). These probes allow chemically reversible reactions with thiols prior to reaction with H$_2$S and therefore the probes are not consumed.$^{80}$

As shown in Fig. 11A, H$_2$S reacts with the most electrophilic component of a fluorescent probe such as 21 to form a free-SH-containing intermediate 22. Then the –SH group undergoes spontaneous cyclization to release the fluorophore and form product 24 if another electrophile, such as the ester group shown in 22, is present at a suitable position. The corresponding fluorescence signal correlates well with the H$_2$S concentration. Based on these principles, H$_2$S can be quantified using a convenient and sensitive fluorescence measurement. This strategy can also be used for imaging of H$_2$S in living cells. Although substrate 21 could potentially react with biological thiols such as Cys, product 23 would not undergo cyclization to release the fluorophore. The fluorescence signal should therefore be selective only for H$_2$S.$^{81}$ The selectivity can be further demonstrated in Fig. 11B, from which biological thiols such as cysteine and glutathione would not cause fluorescence enhancement. However, NaHS addition promotes obvious fluorescence intensity enhancement immediately.

In 2012, Xian et al. devised two closely related sensors, probes 25 and 26, which are reversible and are not consumed during the detection process (see Fig. 12).$^{80}$ The two probes were stable toward esterases. H$_2$S can be detected by fluorescence turn-on using these probes, as a result of intramolecular cyclization to release the fluorophore, similar to probe 21. When treated with biological thiols, however, no Michael addition products were isolated. The detection limit for H$_2$S using these probes was found to be $\sim$1 μM, with a linear NaHS concentration range of 1–100 μM. Probe 26 was identified as being better than probe 25, because of its higher sensitivity for, and faster reaction with, H$_2$S. In the treatment of 100 μM NaHS for 30 min with 5 μM probes 25 and 26, probe 25 produced a 11-fold turn-on response. The cyanoacrylate probe 26
proved to be more sensitive to \( \text{H}_2\text{S} \), and gave a 160-fold turn-on response.

Many new but more applicable methods have been proposed based on this strategy. The novel ratiometric fluorescent probe 27 was synthesized based on the selective nucleophilic addition of \( \text{HS}^- \) to a specific merocyanine derivative in a medium of pH 7.4 (Fig. 13).\(^{82} \) The probe responds rapidly to intracellular \( \text{H}_2\text{S} \) and was successfully used for imaging of \( \text{H}_2\text{S} \) in mitochondria of living cells. The detection limit of the ratiometric fluorescence sensor was approximately 1 \( \mu \text{M} \). On titration with \( \text{HS}^- \), the absorption intensity at 588 nm of free 27 decreased gradually, confirming the transformation of 27 to 28 as a result of \( \text{HS}^- \) addition. The solution turned from dark blue to very pale blue, suggesting that \( \text{HS}^- \) can be detected with the naked eye using probe 27.

Probes 29 and 30 were devised based on a chemical strategy for effective in vivo and in vitro detection of \( \text{H}_2\text{S} \) (Fig. 14).\(^{83} \) The reactions of probe 29 (10 \( \mu \text{M} \)) and 30 (5 \( \mu \text{M} \)) with \( \text{Na}_2\text{S} \) (50 \( \mu \text{M} \)) as an aqueous sulfide source, at 37 °C in phosphate-buffered saline, yielded a time-dependent fluorescence increase. A greater than 10-fold increase in the fluorescence intensity, accompanied by a blue shift in the emission maximum from 428 to 391 nm, was observed for probe 29 (\( \varepsilon = 2320 \text{ M}^{-1} \text{ cm}^{-1}, \Phi = 0.058 \)). For 30, a greater than 13-fold increase in the fluorescence intensity of the emission maximum at 510 nm was observed when the probe was excited at 465 nm (\( \varepsilon = 47 \text{ 100 M}^{-1} \text{ cm}^{-1}, \Phi = 0.208 \)). Probes 29 and 30 have excellent selectivities for sulfide, with linear responses in the \( \text{Na}_2\text{S} \) concentration ranges of 10–50 and 5–100 \( \mu \text{M} \), respectively. For 30, the fluorescence intensity increased 2.6–16-fold on addition of 5–100 \( \mu \text{M} \) \( \text{Na}_2\text{S} \). This probe is ~260-fold more selective for \( \text{Na}_2\text{S} \) than for Cys, and ~150-fold more selective for \( \text{Na}_2\text{S} \) than for GSH. Similarly, the responses of probe 29 to all the tested thiols were very low with at least 50–100-fold selectivities for sulfides. These reactions, using \( \text{H}_2\text{S} \) at concentrations as low as 1 \( \mu \text{M} \), produce a color change that is visible to the naked eye. The sensor was also used in a reported study of \( \text{H}_2\text{S} \) imaging in HeLa cells.

3. Fluorescence molecular sensors suitable for TPM

Although some \( \text{H}_2\text{S} \)-triggered specific reactions have been successfully developed for intracellular \( \text{H}_2\text{S} \) imaging,\(^{84–89} \) most of these probes are based on one-photon dyes. The use of such probes for bioimaging with one-photon microscopy (OPM) requires a short excitation wavelength (usually <500 nm), which limits their biological applications because of photobleaching, autofluorescence in cells and tissues, and shallow penetration depths (<100 \( \mu \text{m} \)).\(^{90} \) To address these shortcomings, TPM, which uses two photons of lower energy as the excitation source, has been proposed. TPM has a number of advantages compared with other strategies, including greater penetration depth (>500 \( \mu \text{m} \)), localization of excitation, and extended observation times.\(^{91–93} \) To date, only a few two-photon (TP) probes have been developed for intracellular \( \text{H}_2\text{S} \) imaging.\(^{80} \)

Joe et al. reported a TP probe, 31, which can image intracellular \( \text{H}_2\text{S} \) in mitochondria (Fig. 15).\(^{94} \) According to their study, 6-{[benzo[d]thiazol-2'-yl]-2-(methylamino)naphthalene, 4-azidobenzyl carbamate, and triphenylphosphonium were
used as the fluorophore, and response sites for H2S and mitochondria, respectively. As shown in Fig. 15A, when the reaction with the azide group was triggered by thiolate, the carbamate linkage was cleaved, leading to the release of an amino group. As a result, the emission maximum shifted significantly and the TP cross-section increased, as shown in Fig. 15B. The probe had good selectivity and high sensitivity, with a detection limit of 0.4 μM for Na2S. The probe was also successfully used in astrocyte imaging. The TPM image of 31 merged well with the OPM image of MitoTracker Red, and showed a significant TP cross-section, and a marked blue-to-yellow emission color change in response to H2S (Fig. 16).

Fig. 17 shows a similar probe, 32, which was developed by Joe et al.94 This probe also showed excellent selectivity for H2S, with a detection limit of 0.2 μM. However, the TP action cross-section (Φδmax, where δ is the TP absorption cross-section) values of probe 31 were 1.9–2.3-fold larger than that of 32. The TPM images of cells labeled with 31 were much brighter than those labeled with 32, although both probes showed high photostability. These results indicate that probe 31 is more suitable for H2S detection than probe 32.

At almost the same time, another new TP bioimaging probe, 6-(benzo[d][1hiazol-2’-yl)-2-azidonaphthalene (33), was developed (Fig. 18).90 This probe uses a donor-π-acceptor-structured naphthalene derivative as the TP fluorophore and an azide group as the recognition unit. The probe shows high selectivity and sensitivity to H2S in the linear response concentration range (0–5 μM) with a detection limit of 20 nM. The selective recognition of H2S by 33 is based on the reducing properties of sulfides. Probe 33 alone is non-fluorescent. The addition of NaHS results in conversion of the electron-withdrawing azido group in 33 to an electron-donating amino group with significant enhancement of the characteristic fluorescence emission peak (480 nm). All these features indicate that the probe is suitable for directly monitoring H2S in complex biological samples.

Cho et al. reported a TP probe, 34 (Fig. 19), which showed a 21-fold TP-excited fluorescence enhancement in response to H2S. This probe also selectively detected H2S in rat hippocampal slices at a depth of 90–190 μm using TPM.95 Fan et al. synthesized a TP fluorescent probe, 35, with a near-infrared (NIR) emission, for H2S detection.96 The probe was successfully used for H2S imaging in bovine serum, living cells, tissue, and live mice. The principle of these probes in H2S detection is based on the reduction of the azide group to an amino group, which is similar to the reaction-based methods using azides.

4. Sensors based on FRET for H2S detection

FRET is a non-radiative energy transfer process in which the excitation energy of the donor is transferred to a proximal ground state acceptor via long-range dipole–dipole interactions and/or short-range multipolar interactions.97 The fabrication of fluorescent sensing systems is simple; therefore FRET-based probes are widely used for biological applications.98–108 Unlike one-signal sensors, a FRET-based probe is not dependent on the concentration of a single emissive probe, but uses the ratio of two fluorescence intensities to detect analytes quantitatively. This method can eliminate most ambiguities in the detection by self-calibration of two emission bands.109–111 The efficiency of the FRET-based process is distance dependent, and this provides the basis for the design of FRET probes with analyte-induced donor–acceptor distance changes.112 The distance scale for detection based on FRET is limited by the nature of the dipole–dipole mechanism, which effectively constrains the scale to distances of the order of <100 Å.98 FRET-based probes are used to detect a wide range of species, including DNA, metal ions, and small molecules.
Recently, FRET was successfully used by Strianese et al. in H$_2$S sensing based on fluorescent-labeled cobalt peptide deformylase (Co-PDF; Fig. 20). The absorption spectrum of Co-PDF has a band at 280 nm, and three less intense bands, centered at 320, 560, and 660 nm. H$_2$S quenches the bands of 560 and 660 nm, and two new bands appear, at 625 and 665 nm (Fig. 21A). Based on this, the authors synthesized a probe by covalent attachment to the protein of a fluorescent dye label (Atto620) with an emission spectrum that overlaps with either 625 or 665 nm band of the protein in its H$_2$S-bound state. When the protein is in the H$_2$S-free state, all the energy absorbed by the label is emitted as fluorescence. However, the label fluorescence is (partly) quenched when H$_2$S is present, because of FRET to the 625 and 665 nm bands (Fig. 21B). The detection limit of the proposed system was found to be in the micromolar range. The authors also examined the selectivity of the probe in the presence of biologically relevant and potentially competing thiols such as L-Cys and GSH. The results indicate that the probe was highly selective for H$_2$S, and little interference was observed.

Combining the advantage of the characteristics of an NIR optical response with the sensitivity of a FRET based fluorophoric response for the construction of a chemosensor probe, another FRET-based sensor for S$_2$$^-$ sensing was reported recently. The receptor, L$_1$, was used as a resonance-energy-transfer-based sensor for the detection of Cu$^{2+}$, based on a process involving a donor indole and a Cu$^{2+}$-bound xanthene fragment acceptor. Formation of the corresponding product, an L$_1$–Cu complex, is selectively reversible in the presence of sulfide anions (Fig. 22). HeLa cells were used as a model to check the imaging ability of the probe. The results showed that the L$_1$–Cu complex could readily cross the membrane barrier, permeate into HeLa cells, and rapidly sense intracellular S$_2$$. However, the probe needs a Cu$^{2+}$-bound xanthene fragment as the acceptor to form the L$_1$–Cu complex. This requirement is an obstacle to further applications in S$_2$$^-$ imaging, and Cu$^{2+}$ can cause environmental pollution and cell damage.

Using quantum dots (QDs) as a donor in the sensing process, FRET probes based on QDs have received much attention because of their high fluorescence quantum yields, narrow emission bands, high Stokes shifts, and stability against photobleaching. These QDs based FRET sensing strategies are powerful tools and are promising for applications in biological imaging. Although they have potential merits including high sensitivity, excellent biocompatibility, and in vivo detection, little attention has been paid to endogenous H$_2$S imaging. In order to further understand the pathophysiological effects of endogenous H$_2$S, more work should be performed using fluorescence detection, especially FRET sensing based on QDs.

Fortunately, a FRET ratiometric fluorescence sensor, which employs carbon dots (CDs) as the energy donor and anchoring site, was reported in 2013 (Fig. 23). In the absence of H$_2$S, CD excitation at 340 nm led to emission by the CDs at 425 nm. In the presence of H$_2$S, the CD emission at 425 nm gradually decreased, and a new emission band appeared at 526 nm. This phenomenon was attributed to a reduction of the naphthalimide-azide to naphthalimide-amine by H$_2$S, resulting in FRET by the CDs to naphthalimide-amine. The sensor displayed good selectivity for H$_2$S over a number of biologically relevant thiols such as GSH, Cys, and some other anions. The detection limits were found to be 10 nM in buffer and 19.5 nM in bovine serum, with a wide pH range of 4.0–9.0. More importantly, this system achieved H$_2$S imaging in HeLa and L929 cells.
5. Comparison of fluorescence sensing strategies and traditional methods for H$_2$S detection

Traditional methods for H$_2$S detection include colorimetry, gas chromatography, electrochemical analysis, and metal induced sulfide precipitation. These methods showed various sensitivities to different samples. Although they seem more sensitive in some aspects compared to fluorescence sensing, fluorescence sensing strategies are more appealing and have some other advantages. Table 1 compares traditional methods and new fluorescence-sensing detection methods. In some aspects, the traditional methods, such as gas chromatography, are extremely sensitive to H$_2$S with the reported detection limits below 0.2 pM. However, extreme conditions such as high alkalinity (pH = 8.5) are required to achieve detection. Moreover, traditional methods cannot be used in cell imaging, require large instruments for quantitative H$_2$S detection, and cause cell damage. These requirements mean that many detection methods can be used for imaging in vitro, but not in vivo.

In contrast, fluorescence sensing requires moderate detection conditions such as neutral pH and room temperature. As the data in Table 1 have shown, fluorescent probes have excellent biocompatibilities with HeLa cells, C6 cells, HEK293 cells, and mutant mice. The success of fluorescence imaging in living cells has expanded their application range, especially in clinical and biomedical sciences. Based on these attractive advantages, fluorescence sensing has excellent prospects for application in sulfide imaging. However, it is important to note that challenges such as poor detection limits remain. The intracellular concentration of H$_2$S has been reported to be at nM levels and would rise to low µM levels in response to physiological stimuli under extreme conditions. Without stimulation, the concentration may be lower due to the stimulation increasing H$_2$S release. Moreover, some thiol groups may occupy part of the sensors in the detection process. Therefore, it is important to further improve the sensor’s sensitivity in consideration of the low detection limit. Also, the fluorescence sensors were mainly derived from chemical materials. These materials may degrade or react with other agents in the environment, which limits their stability at long-term usage.

![Fig. 23 Schematic of FRET in S$^{2-}$ sensing.](image)

Table 1  Comparison of detection methods for H$_2$S

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<th>Detection methods</th>
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<td>b</td>
<td>c</td>
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<td>Electrochemical analysis</td>
<td>0.6 nM–10 nM</td>
<td>0.2 nM</td>
<td>c</td>
<td>&gt;7.0</td>
<td>Extracellular</td>
<td>29</td>
</tr>
<tr>
<td>Metal induced sulfide precipitation</td>
<td>10 nM–1 mM</td>
<td>0.31 nM</td>
<td>c</td>
<td></td>
<td>Intra/extracellular</td>
<td>30</td>
</tr>
<tr>
<td>Sensors derived from azide or nitro</td>
<td>100 µM$^a$</td>
<td>1 µM</td>
<td>C57BL6/J mouse model</td>
<td>7.5</td>
<td>Intra/extracellular</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>5 µM and 1 µM</td>
<td></td>
<td>HeLa cells</td>
<td>7.4</td>
<td></td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>5 µM–10 µM</td>
<td></td>
<td>HEK293 T cells</td>
<td>7.4</td>
<td></td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>50 nM$^a$</td>
<td>0.7 nM</td>
<td>C6 cells</td>
<td>7.4</td>
<td></td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>50 nM–1 mM</td>
<td>20 nM</td>
<td>Fetal bovine serum</td>
<td>7.4</td>
<td></td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>10 µM–50 µM</td>
<td>b</td>
<td>HeLa cells</td>
<td>7.4</td>
<td></td>
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<tr>
<td>Sensors derived from an azamacrocyclic Cu(II) ion complex</td>
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<td></td>
<td>HeLa and HEK293 cells</td>
<td>7.4</td>
<td>Intra/extracellular</td>
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<tr>
<td>Sensors derived from H$_2$S specific Michael acceptors</td>
<td>1 µM–100 µM</td>
<td>~1 µM</td>
<td>CO77 cells</td>
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<td>Intra/extracellular</td>
<td>82</td>
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<tr>
<td></td>
<td>50 ng–100 µM</td>
<td>b</td>
<td>MCF-7 cells</td>
<td>7.4</td>
<td></td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>10 µM$^a$</td>
<td>~1 µM</td>
<td>HeLa cells</td>
<td>7.4</td>
<td></td>
<td>83</td>
</tr>
<tr>
<td></td>
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<td>b</td>
<td>HeLa cells</td>
<td>7.4</td>
<td></td>
<td>83</td>
</tr>
<tr>
<td>TPM</td>
<td>20 nM$^a$</td>
<td></td>
<td>HeLa cells</td>
<td>7.4</td>
<td>Intra/extracellular</td>
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<tr>
<td></td>
<td>5 µM–0.2 µM</td>
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<td>94</td>
</tr>
<tr>
<td></td>
<td>100 µM–300 µM</td>
<td>0.2 µM–0.4 µM</td>
<td>HeLa cells</td>
<td>7.4</td>
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<td></td>
<td>25 µM–250 µM</td>
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<td>MCF-7, HeLa cells and mouse tissue</td>
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<td></td>
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<tr>
<td>FRET</td>
<td>10 nM$^a$</td>
<td></td>
<td>HeLa and L929 cells</td>
<td>4.0–9.0</td>
<td>Intra/extracellular</td>
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<td>5 nM$^a$</td>
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<td>HeLa cells</td>
<td>7.3</td>
<td>Intra/extracellular</td>
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</table>

$^a$ No reported results or only uncertain results. $^b$ No reported results. $^c$ No results or results have not been reported yet. LOD: limit of detection.
6. Outlook and further applications

6.1. Fluorescence sensors based on QDs for H₂S detection

As mentioned in the Introduction section, H₂S is a toxic gas that causes biological damage and human diseases. The rotten egg smell of tainted food, water eutrophication, and fecal contamination is always related to H₂S formation. Although recent studies have focused on gaseous signaling, it is important to detect kinetic variations of H₂S to determine its potential effects on the environment, especially in biocorrosion and human health.

The use of fluorescence sensing as a H₂S detection strategy has several advantages such as moderate conditions, in vivo imaging, and high sensitivity. Much work has been carried out on the use of these probes in bioimaging, labeling, separation, disease diagnosis, and therapy. However, there are still several obstacles and limitations such as low sensitivity, low stability, and non-real-time imaging. FRET methods, as a special fluorescence-sensing strategy, are more attractive for specific in vivo and in situ imaging. The use of FRET expands the application range of sulfide fluorescence analysis.

Although numerous examples of fluorescence-sensing strategies in the detection of H₂S are presented in this review, these strategies, especially FRET, remain underused and underappreciated as analytical tools. The primary obstacle is their relatively low sensitivities compared with traditional methods. QDs, because of their unique optical profiles, have been combined with many sensing strategies used in bioanalysis. In the past, many studies have shown that QDs are excellent donors in FRET and have several advantages in metal ions and compound detection compared with molecular fluorophore donors. However, there is no fluorescence detection of sulfide based on QDs reported although some other nanomaterial, such as Au–Ag core–shell nanoparticles, were designed as probes for sulfide mapping in living cells in 2013. The unique optical properties of QDs and the modulation of those properties via FRET would provide researchers with a versatile toolkit for bioanalyses. Multiplex detection is possible using new formats that are simple and flexible and is anticipated to enable the future development of novel diagnostic techniques and intracellular probes. Although significant challenges remain, FRET nanosystems could serve as practical tools for biological studies.

6.2. Possible application in SRB activity detection during anaerobic corrosion

SRB are widespread in marine and terrestrial aquatic environments. They can be found in the sediments of lakes and seas, in flooded soils such as rice paddies, and technical aqueous systems such as sludge digesters and oil tanks. These bacteria have been recognized as a major group of microorganisms linked to anaerobic corrosion. Biocorrosion causes energy and efficiency losses and structural failures, resulting from the corrosion of pipes and equipment. Recently, SRB have been used in the bioprecipitation of metal ions, and their potential use in remediation has received a great deal of attention. Under favorable conditions, an acidic environment and the presence of organic compounds such as acetate as electron donors, SRB can catalyze oxidation reactions and reduce sulfate to H₂S:

\[
2\text{CH}_3\text{CH}_2\text{OH} + 3\text{SO}_4^{2-} + 3\text{H}^+ \\
\rightarrow 3\text{HCO}_3^- + 3\text{H}_2\text{S} + 3\text{H}_2\text{O} + \text{CO}_2 \quad (1)
\]

Then, metal ions present in groundwater form stable, insoluble metal sulfides in the presence of H₂S:

\[
\text{H}_2\text{S} + \text{Metal}^{2+} \rightarrow \text{MetalS}_n^{(s)} + 2\text{H}^+ \quad (2)
\]

Based on this fact, it will be of great significance to indicate sulfate-reducing bacteria activity for anticorrosion and heavy metal removal. However, the currently available characterization methods are inconvenient, because of the need for auxiliary equipment. In 2011, McMahan et al. proposed a method for detecting fecal bacteria via H₂S tests using culturing and molecular methods. However, no specific methods have been reported so far for detecting bacterial activity via H₂S analysis, because many difficulties are encountered. As shown in eqn (1), the SRB activity is directly related to H₂S concentration. A simple, sensitive, and real-time method based on fluorescence-sensing strategies for H₂S detection could be developed for the determination of SRB activity via sulfide detection.

7. Conclusions

H₂S is of particular interest because of the important roles it plays in physiological equilibria. Many methods have therefore been developed to assess the levels of sulfide in drinking water and wastes and for H₂S imaging in living cells. This review provides a comprehensive overview of the development of fluorescence sensors for sulfide detection in recent years, including reaction-based strategies and FRET sensing. The design of FRET-based sulfide-selective chemosensors, especially using QDs as donors, has attracted much interest despite the disadvantage of low sensitivity. Important potential applications in SRB activity determination are discussed and future developments are indicated. Although these new sensors are promising, it is worth noting that much work still remains to be done on improving their sensitivities and stabilities.

Abbreviations

- 3-MST: 3-Mercaptopyruvate sulfurtransferase
- CBS: Cystathionine beta synthase
- CO: Carbon monoxide
- Co-PDF: Cobalt peptide deformylase
- CSE: Cystathionine γ-lyase
- CTAB: Cetyltrimethyl ammonium bromide
- Cys: Cysteine
References
