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Fluorescence chemosensors for hydrogen sulfide detection in biological systems

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A comprehensive review of the development of H_2S 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 H_2S, the relationship between sulfate-reducing bacteria activity and H_2S 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

H_2S 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 H_2S were first documented roughly 300 years ago; since then, many studies have focused on its potential damage to biological systems.[3–8] H_2S is extremely dangerous to the human body because of its reducibility and high lipid solubility even at low levels.[9] Previous reports have shown that the cystathionine β-synthase (CBS) gene, which is one of the three enzymes able to produce H_2S, is overexpressed in Down's syndrome.[10] The secretion level of insulin, a crucial factor in avoiding the development of diabetes, is reduced using high concentrations of H_2S.[11] It has also been reported that deregulation of H_2S increases the possibility of liver cirrhosis.[12] In addition to biological damage, this odorous and toxic gas is corrosive and damages pipelines and catalysts and the ceramic membranes used in syngas separations.[13–15]

Recent evidence has shown that H_2S acts as a third endogenously generated gaseous signaling compound with cytoprotective properties; the other two are the well-known

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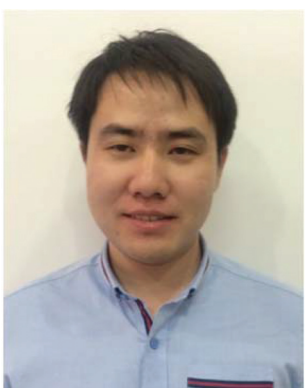

Zhi Guo

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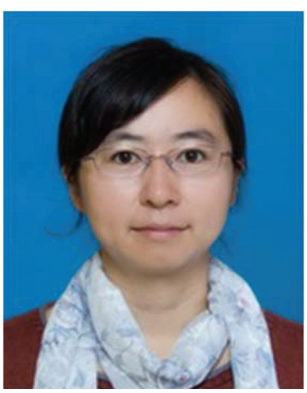

Guiqiu Chen

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gasotransmitters NO and CO. It has diverse functions in several pathophysiological processes, especially in regulating vascular contractility and neuronal activity.[16–18] In mammals, H_2S production is derived primarily from three enzymes: cystathionine γ-lyase, CBS, and 3-mercaptopyruvate sulfurtransferase.[19,20] The widespread but differential expression of these enzymes in different tissues suggests that H_2S has wide importance and significance in the cardiovascular, circulatory, respiratory, urinary, and nervous systems. In addition to the pathophysiological conditions associated with H_2S misregulation, H_2S can also act on specific cellular targets, including heme proteins,[21] cysteine (Cys) residues on ATP-sensitive K^+ channels,[22] NO,[23] and other emerging targets.

Regardless of the dangers or benefits of H_2S, it is important to develop efficient methods for the detection of H_2S 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.[24] However, traditional methods of H_2S detection, including colorimetry,[25,26] gas chromatography,[27,28] electrochemical analysis,[29,30] and metal-induced sulfide precipitation,[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_2S detection because of its high sensitivity, real-time spatial imaging, and non-damaging detection of targets in living cells or tissues.[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_2S detection. Although several reviews about H_2S 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.[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_2S 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_2S detection derived from functional groups

Recently, a large number of reaction-based sensors for H_2S detection have been reported;[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_2S-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_2S is present, a known unique reaction, followed by an optical change, is triggered. The corresponding optical changes are

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usually either linear or non-linear with respect to H_2S concentration, which is crucial for further H_2S 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, H_2S 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 H_2S detection.[47] Treatment of a 5 μM solution of probes **1** and **2** with 100 equiv. of H_2S 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 H_2S 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 H_2S was added to the cells of a and c and incubated for another 30 min.

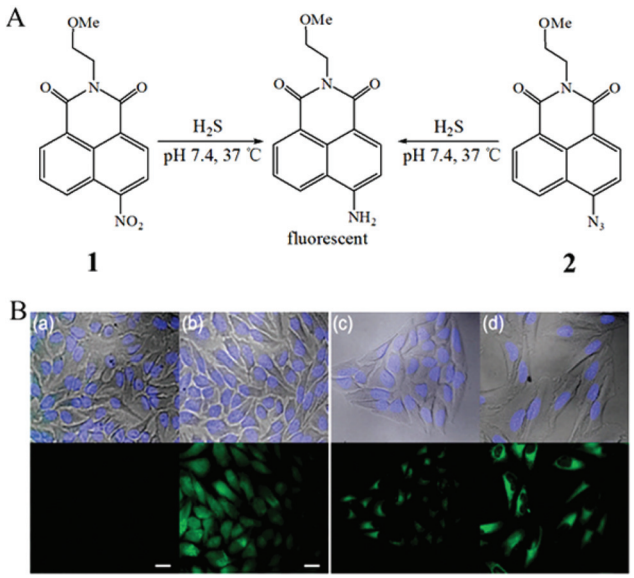

Fig. 1 (A) Proposed reaction of probes **1** and **2** with H_2S. (B) Fluorescence microscopic imaging of living HeLa cells incubated with probes (a) **1**; (b) **1** with H_2S; (c) **2**; (d) **2** with H_2S.[47]

The potential applications of fluorescence detection of H_2S 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.[48–56] 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 H_2S, a solution of **4** showed strong fluorescence enhancement following reduction of the azido group to an amino group. The addition of 25 μM H_2S 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 sul-

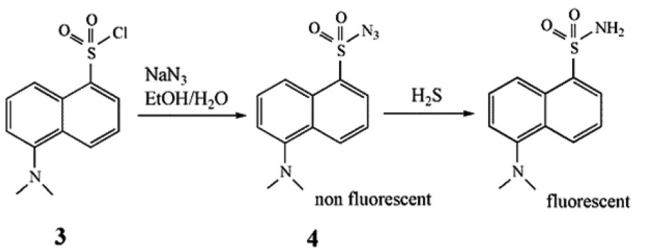

Fig. 2 Proposed reaction of probe **4** with H_2S.[24]

Anwei Chen

Anwei Chen completed his PhD recently under the supervision of Prof. Zeng and Prof. Chen in Hunan University. He has published nine papers and in 2012 he received the Scholarship Award for Excellent Doctoral Student granted by the Ministry of Education, which is awarded annually to the doctoral student showing the best research potential and academic merit.

Jiajia Wang

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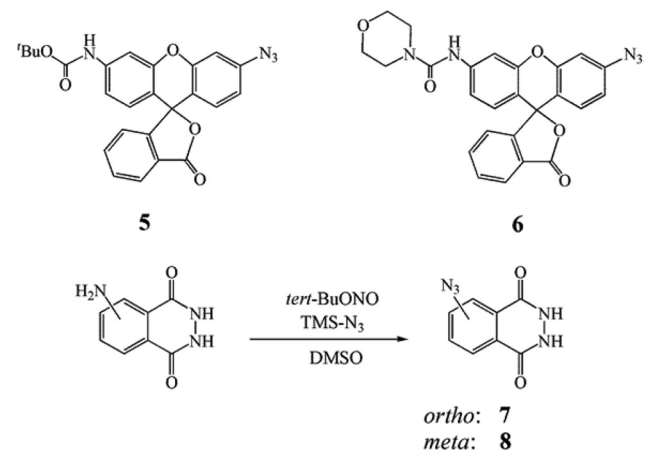
fidefluor-2 (probe **6**), for selective imaging of H_2S in living cells; these probes exploit the H_2S-mediated reduction of azides to fluorescent amines (see Fig. 3).[57] Both probes displayed good selectivity for H_2S over abundant biologically relevant thiols, including 5 mM glutathione (GSH) and 5 mM Cys. Probe **5** displayed an approximately three-fold increased response for H_2S than that for most other species tested, and an approximately two-fold increased selectivity *versus* O_2^-. For probe **6**, better selectivities *versus* GSH (*ca*. five-fold), sulfite (*ca*. four-fold), and O_2^- (*ca*. four-fold) were observed. In addition, a clear increase in intracellular fluorescence intensity was observed for H_2S-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 H_2S 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 H_2S, with detection limits of 0.7 ± 0.3 and 4.6 ± 2.0 μM, respectively (Fig. 3).[58] 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 H_2S over other reactive sulfur, nitrogen, and oxygen species, including GSH, Cys, homocysteine (Hcy), $S_2O_3^{2-}$, NO_2^-, 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



5 **6**

Fig. 3 Structures of **5**–**8**.[57,58]

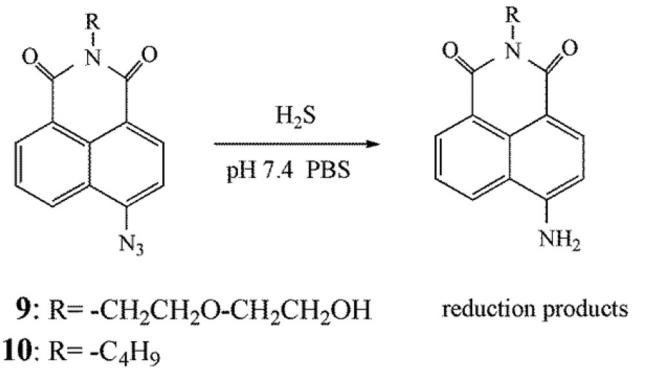


Fig. 4 Proposed reaction of probes **9** and **10** with H_2S.[59]

fluorescent probes (**9** and **10**) were designed for selective recognition of H_2S (Fig. 4).[59] 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 H_2S 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 H_2S 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 H_2S levels in fetal bovine serum without any sample pretreatment.[59]

7-Amino-4-methylcoumarin (**11**) is widely used in fluorogenic enzyme assays and can be easily converted to non-fluorescent 7-azido-4-methylcoumarin (probe **12**; Fig. 5).[60] 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.[61–70] FP can be engineered to self-assemble into protein particles displaying protein functions

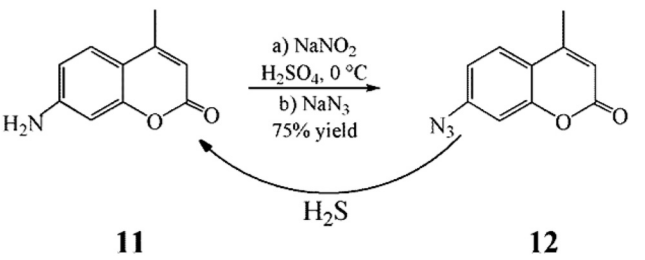
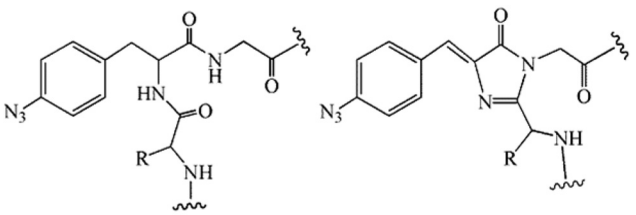


Fig. 5 Proposed reaction of **12** with H_2S.[60]



13 14

Fig. 6 Structures of **13** and **14**.[72]

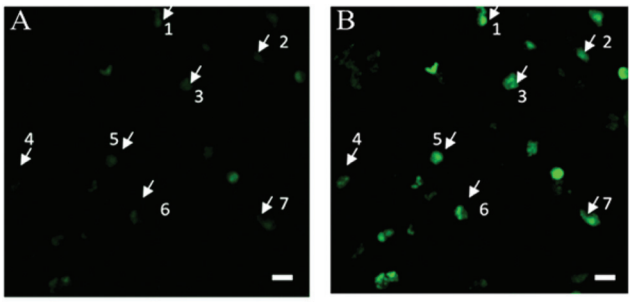


Fig. 7 Fluorescence microscopic imaging of living HeLa cells before (A) and after (B) the addition of buffered NaHS.[72]

suitable for various applications in diagnostics or detection.[71] 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).[72] These structurally modified chromophores were selectively reduced by H_2S, resulting in sensitive fluorescence enhancement, which was detectable using fluorescence microscope techniques (Fig. 7). The fluorescence enhancement showed a linear relationship with H_2S 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.[73] In addition, the approach described relies on the recognition of target molecules or enzymatic reactions,[74] 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 H_2S *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(II) ion complex

Recently, a new sulfide-selective chemosignaling system was devised based on a dipicolylamine (DPA)–fluorescein complex **16** with Cu^{2+} (probe **15**).[75] The Cu^{2+} ions in probe **15** form stable species with the targeted sulfide ions. The higher stability of Cu^{2+}–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/I_o) at 517 nm = 87). However, a further study confirmed that this probe does not have sufficient selectivity for H_2S in the presence of reduced GSH. A clear fluorescence enhancement was observed on the addition of 10 mM GSH.[76]

It is well known that azamacrocyclic rings form stable metal complexes with Cu^{2+}, and the paramagnetic Cu^{2+} center has a pronounced quenching effect on fluorophores.[77] Based on these observations, Nagano *et al.* designed and synthesized four sensor probes based on a fluorescein (AF) scaffold conjugated with an azamacrocyclic Cu^{2+} complex (Fig. 9).[76] The four macrocyclic fluoresceins, namely 1,4,7-triazacyclononane (**17**), 1,4,7,10-tetraazacyclododecane (cyclen, **18**), 1,4,8,11-tetraazacyclotetradecane (**19**), and *N*,*N*′9,*N*″9-trimethylcyclen (**20**), were used as chelators for Cu^{2+} instead of DPA. Sensitivity and selectivity assays indicated that **18**-AF + Cu^{2+} has excellent selectivity and sensitivity as a fluorescent probe for H_2S detection. As shown in Fig. 10, the fluorescence intensity of **18**-AF + Cu^{2+} showed a large and immediate increase with the addition of 10 μM H_2S, whereas almost no increase in fluorescence is observed with the addition of 10 mM GSH. In the case of the

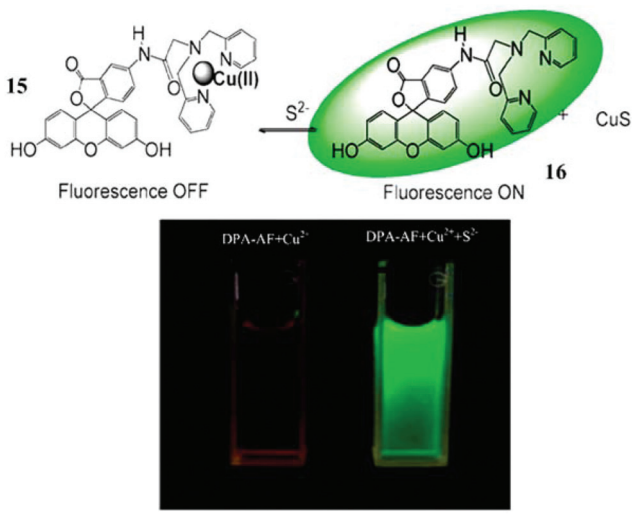


Fig. 8 Signaling of sulfide ions by **15**.[75]

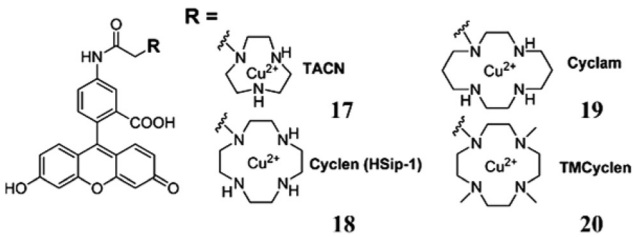


Fig. 9 Structures of four macrocyclic fluorescein–Cu^{2+} conjugate.[76]

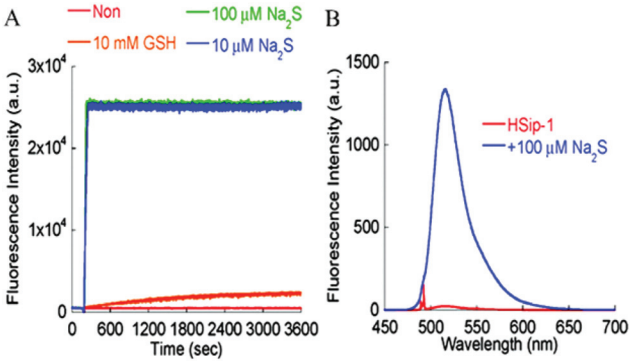


Fig. 10 (A) Time course of reactions of **18** without addition (red) or with addition of 10 mM GSH (orange), 100 μM Na_2S (green), or 10 μM Na_2S (blue). (B) Fluorescence spectra of 1 μM **18** before (red) and after (blue) reaction with 100 μM Na_2S.[76]

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_2S over reactive oxygen species.[57,78]

2.3. Fluorescence molecular sensors derived from H_2S-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_2S detection capacity.[79] To detect H_2S selectively, it is important to differentiate H_2S 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_2S and therefore the probes are not consumed.[80]

As shown in Fig. 11A, H_2S 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_2S concentration. Based on these principles, H_2S can be quantified using a convenient and sensitive fluorescence measurement. This strategy can also be used for imaging of H_2S 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_2S.[81] The selectivity can be further demonstrated in Fig. 11B, from which biological

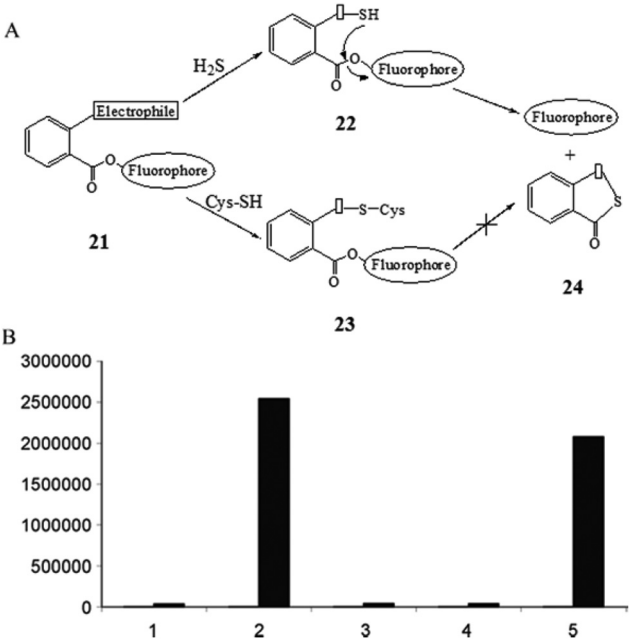


Fig. 11 (A) Proposed reaction mechanism of **21** with H_2S. (B) Fluorescence response of probe **21** toward H_2S and other thiols: (1) **21** only (100 μM), (2) **21** (100 μM) + NaHS (50 μM); (3) **21** (100 μM) + cysteine (50 μM); (4) **21** (100 μM) + glutathione (50 μM), and (5) **21** (100 μM) + glutathione (50 μM) + NaHS (50 μM).[81]

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_2S 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_2S using these probes was found to be ~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_2S. 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**

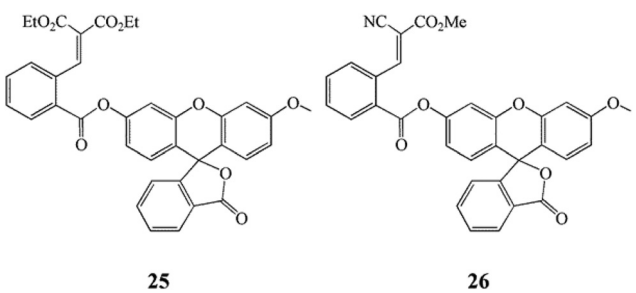


Fig. 12 Structures of **25** and **26**.[80]

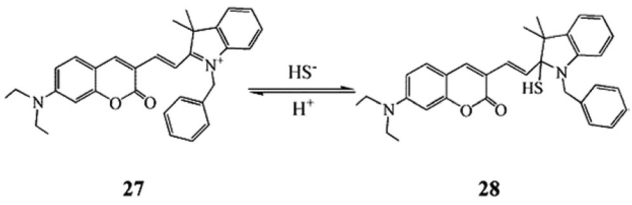


Fig. 13 Proposed reaction of **27** with sulfide.[82]

proved to be more sensitive to H_2S, 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 HS^- to a specific merocyanine derivative in a medium of pH 7.4 (Fig. 13).[82] The probe responds rapidly to intracellular H_2S and was successfully used for imaging of H_2S in mitochondria of living cells. The detection limit of the ratiometric fluorescence sensor was approximately 1 μM. On titration with HS^-, the absorption intensity at 588 nm of free **27** decreased gradually, confirming the transformation of **27** to **28** as a result of HS^- addition. The solution turned from dark blue to very pale blue, suggesting that 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 H_2S (Fig. 14).[83] The reactions of probe **29** (10 μM) and **30** (5 μM), with Na_2S (50 μM) 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** (ε = 2320 M^{-1} cm^{-1}, Φ = 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 (ε = 47 100 M^{-1} cm^{-1}, Φ = 0.208). Probes **29** and **30** have excellent selectivities for sulfide, with linear responses in the Na_2S concentration ranges of 10–50 and 5–100 μM, respectively. For **30**, the fluorescence intensity increased 2.6–16-fold on addition of 5–100 μM Na_2S. This probe is ~260-fold more selective for Na_2S than for Cys, and ~150-fold more selective for Na_2S 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 H_2S at concentrations as low as 1 μM, produce a color change that is visible to the naked eye. The sensor was also used in a reported study of H_2S imaging in HeLa cells.

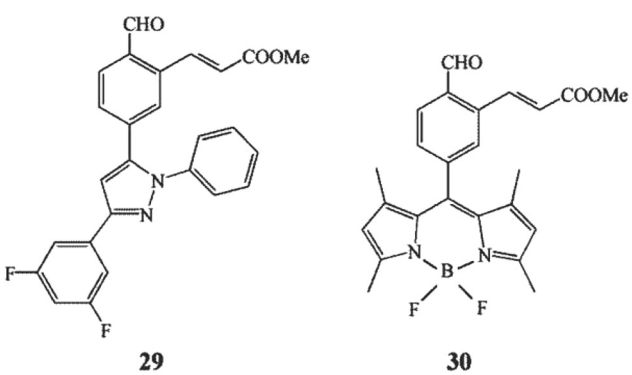


Fig. 14 Structures of **29** and **30**.[83]

3. Fluorescence molecular sensors suitable for TPM

Although some H_2S-triggered specific reactions have been successfully developed for intracellular H_2S 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 μm).[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 μm), localization of excitation, and extended observation times.[91–93] To date, only a few two-photon (TP) probes have been developed for intracellular H_2S imaging.[80]

Joe *et al.* reported a TP probe, **31**, which can image intracellular H_2S in mitochondria (Fig. 15).[94] According to their study, 6-(benzo[*d*]thiazol-2′-yl)-2-(methylamino)naphthalene, 4-azidobenzyl carbamate, and triphenylphosphonium were

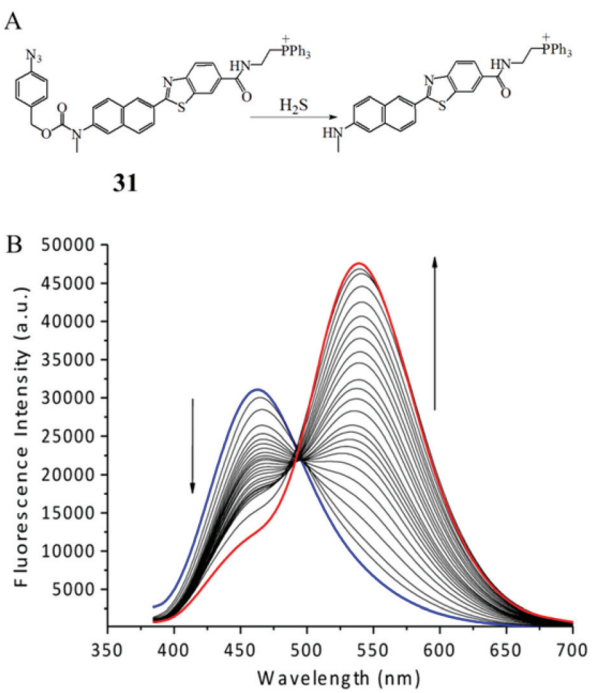


Fig. 15 (A) Proposed reaction of probe **31** with H_2S. (B) Fluorescence response with time for the reactions of **31** with Na_2S in HEPES buffer.[94]

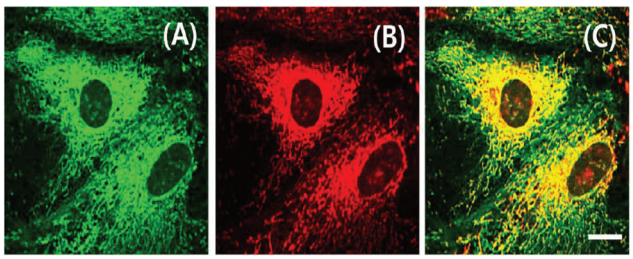


Fig. 16 (A) TPM and (B) OPM images of astrocytes colabeled with (A) **31** and (B) MitoTracker Red FM. (C) Merged image.[94]

used as the fluorophore, and response sites for H_2S 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 Na_2S. 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 H_2S (Fig. 16).

Fig. 17 shows a similar probe, **32**, which was developed by Joe *et al.*[94] This probe also showed excellent selectivity for H_2S, with a detection limit of 0.2 μM. However, the TP action cross-section ($\Phi\delta_{max}$, 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 H_2S detection than probe **32**.

At almost the same time, another new TP bioimaging probe, 6-(benzo[*d*]thiazol-2′-yl)-2-azidonaphthalene (**33**), was developed (Fig. 18).[90] This probe uses a donor–π-acceptor-

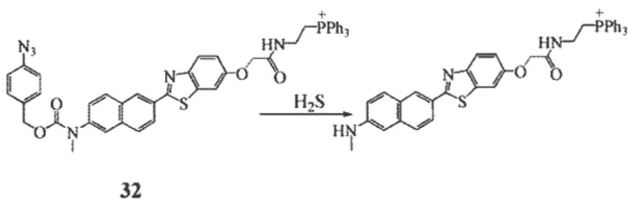


Fig. 17 Proposed reaction of probe **32** with H_2S.[94]

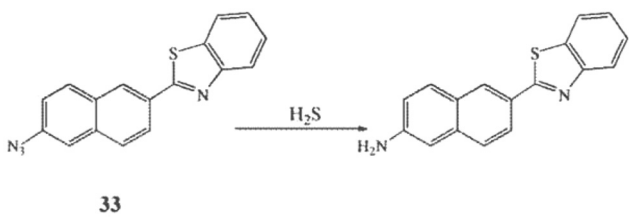


Fig. 18 Proposed reaction of probe **33** with H_2S.[90]

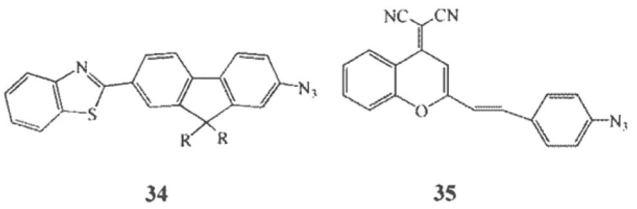


Fig. 19 Structures of **34** and **35**.[95,96]

structured naphthalene derivative as the TP fluorophore and an azide group as the recognition unit. The probe shows high selectivity and sensitivity to H_2S in the linear response concentration range (0–5 μM) with a detection limit of 20 nM. The selective recognition of H_2S 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 H_2S 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 H_2S. This probe also selectively detected H_2S 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 H_2S detection.[96] The probe was successfully used for H_2S imaging in bovine serum, living cells, tissue, and live mice. The principle of these probes in H_2S 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 H_2S 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.

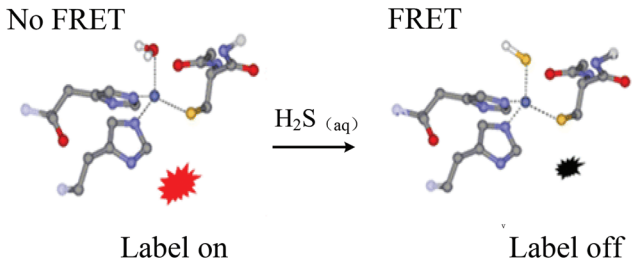


Fig. 20 Schematic of FRET based on a fluorescent label of Co-PDF.[113]

Recently, FRET was successfully used by Strianese *et al.* in H_2S sensing based on fluorescent-labeled cobalt peptide deformylase (Co-PDF; Fig. 20).[113] The absorption spectrum of Co-PDF has a band at 280 nm, and three less intense bands, centered at 320, 560, and 660 nm.[114] H_2S 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_2S-bound state. When the protein is in the H_2S-free state, all the energy absorbed by the label is emitted as fluorescence. However, the label fluorescence is (partly) quenched when H_2S 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_2S, 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.[115] The receptor, L_1, was used as a resonance-energy-transfer-based sensor for the detection of Cu^{2+}, based on a

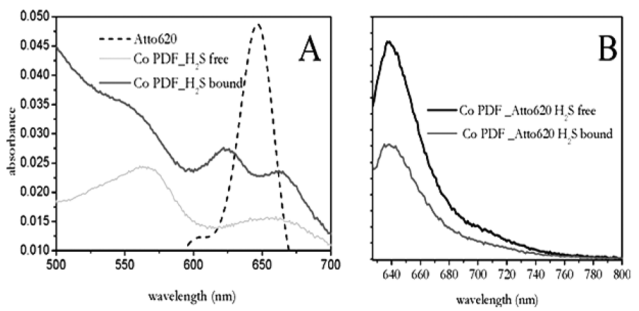


Fig. 21 (A) Absorption spectrum of H_2S-free Co-PDF (gray) and the H_2S-bound form (dark gray) and emission spectrum (dotted trace) of Atto620 (λ_{max} = 645 nm). (B) Fluorescence emission spectrum (λ_{ex} = 620 nm) of Atto620-labeled H_2S-free (black trace) and H_2S-bound (gray trace) Co-PDF.[113]

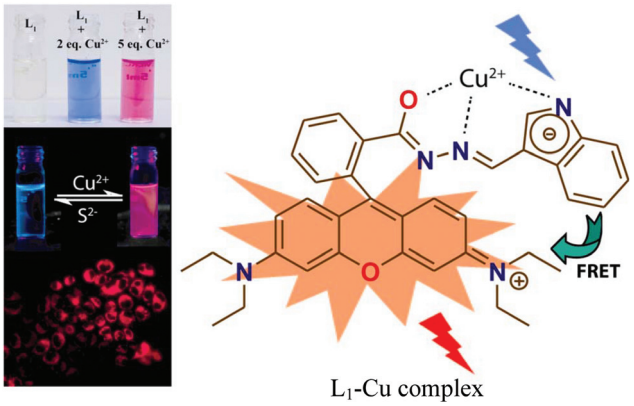


Fig. 22 Schematic of FRET in S^{2-} sensing.[115]

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.[116–121] 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_2S imaging. In order to further understand the pathophysiological effects of endogenous H_2S, 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).[122] In the absence of H_2S, CD excitation at 340 nm led to emission by the CDs at 425 nm. In the presence of H_2S, 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_2S, resulting in FRET by the CDs to naphthalimide-amine. The sensor displayed good selectivity for H_2S 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_2S imaging in HeLa and L929 cells.

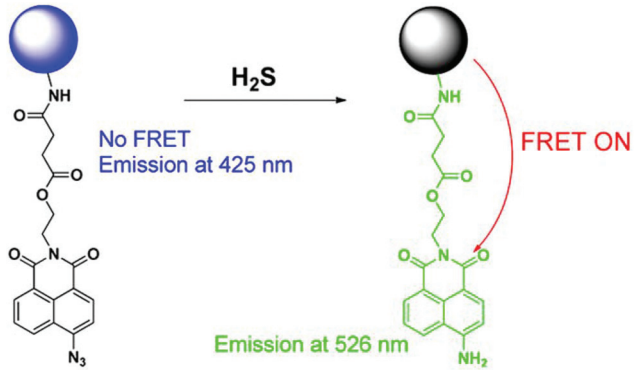


Fig. 23 Schematic of FRET in S^{2-} sensing.[122]

5. Comparison of fluorescence sensing strategies and traditional methods for H_2S detection

Traditional methods for H_2S 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_2S with the reported detection limits below 0.2 pM. However, extreme conditions such as high alkalinity (pH = 8.5) are required to achieve detection.[27] Moreover, traditional methods cannot be used in cell imaging, require large instruments for quantitative H_2S 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.[125–129] 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_2S has been reported to be at nM levels and would rise to low μM levels in response to physiological stimuli under extreme conditions.[11,76,130] Without stimulation, the concentration may be lower due to the stimulation increasing H_2S release.[131,132] 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.

Table 1 Comparison of detection methods for H_2S

Detection methods	Linear range	LOD	Biological application	pH	Intracellular/ extracellular	Reference
Colorimetry	1.112 mM[a]	[b]	[c]	9.0	Intracellular/ extracellular	123
Gas chromatography	[a]	0.2 pM	[c]	8.5	Extracellular	28
Electrochemical analysis	0.6 nM–10 nM	0.2 nM	[c]	>7.0	Intracellular/	29
	[a]	10 nM	[c]	<6.8	extracellular	30
Metal induced sulfide precipitation	10 nM–1 mM	0.31 nM	[c]	6	Extracellular	31
Sensors derived from azide or nitro	100 μM[a]	1 μM	C57BL6/J mouse model	7.5	Intracellular/	24
	[a]	5 μM and 1 μM	HeLa cells	7.4	extracellular	47
	[a]	5 μM–10 μM	HEK293 T cells	7.4		57
	50 nM[a]	0.7 nM	C6 cells	7.4		58
	50 nM–1 mM	20 nM	Fetal bovine serum	7.4		59
	10 μM–50 μM	[b]	HeLa cells	7.4		72
Sensors derived from an azamacrocyclic Cu(II) ion complex	[a]	420 nM	[c]	7.0	Intracellular/	75
	[a]	<10 μM	HeLa and HEK293 cells	7.4	extracellular	76
Sensors derived from H_2S specific Michael acceptors	1 μM–100 μM	~1 μM	COS7 cells	7.4	Intracellular/	80
	10 μM[a]	[a]	COS7 cells	7.4	extracellular	81
	[a]	~1 μM	MCF-7 cells	7.4		82
	10 μM–50 μM	[b]	HeLa cells	7.4/7.0		83
TPM	5 μM[a]	20 nM	HeLa cells	7.4	Intracellular/	90
	[a]	0.2 μM and 0.4 μM	HeLa cells	7.4	extracellular	94
	100 μM–300 μM	5 μM–10 μM	HeLa cells	7.2		95
	25 μM–250 μM	3.05 μM	MCF-7, HeLa cells and mice tissue	7.4		96
FRET	[a]	10 nM	HeLa and L929 cells	4.0–9.0	Intracellular/	122
	[a]	[b]	HeLa cells	7.3	extracellular	124

[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 H_2S detection

As mentioned in the Introduction section, H_2S 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 H_2S formation. Although recent studies have focused on gaseous signaling, it is important to detect kinetic variations of H_2S to determine its potential effects on the environment, especially in biocorrosion and human health.

The use of fluorescence sensing as a H_2S 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 H_2S 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.[133–142] 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.[143] 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.[144–146] These bacteria have been recognized as a major group of microorganisms linked to anaerobic corrosion.[147–152] Biocorrosion causes energy and efficiency losses and structural failures, resulting from the corrosion of pipes and equipment.[153] Recently, SRB have been used in the bioprecipitation of metal ions, and their potential use in remediation has received a great deal of attention.[154–161] Under favorable conditions, *i.e.*, an acidic environment and the presence of organic compounds such as acetate as electron donors, SRB can catalyze oxidation reactions and reduce sulfate to H_2S:[162–165]

$$2CH_3CH_2OH + 3SO_4^{2-} + 3H^+ \rightarrow 3HCO_3^- + 3H_2S + 3H_2O + CO_2 \quad (1)$$

Then, metal ions present in groundwater form stable, insoluble metal sulfides in the presence of H_2S:

$$H_2S + Metal^{2+} \rightarrow MetalS_{(s)} + 2H^+ \quad (2)$$

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.[164–167] In 2011, McMahan *et al.* proposed a method for detecting fecal bacteria *via* H_2S tests using culturing and molecular methods.[168] However, no specific methods have been reported so far for detecting bacterial activity *via* H_2S analysis, because many difficulties are encountered. As shown in eqn (1), the SRB activity is directly related to H_2S concentration. A simple, sensitive, and real-time method based on fluorescence-sensing strategies for H_2S detection could be developed for the determination of SRB activity *via* sulfide detection.

7. Conclusions

H_2S 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 H_2S 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

DPA	Dipicolylamine
FP	Fluorescent protein
FRET	Fluorescence resonance energy transfer
GSH	Glutathione
HeLa cells	Human cervical carcinoma cell line
HEK293 T cells	Human embryonic kidney 293 T cells
Hcy	Homocysteine
KATP channels	ATP-sensitive K^+ channels
NBD-SH	Nitrobenzofurazan thiol
NBD-SR	Nitrobenzofurazan thioethers
NO	Nitric oxide
OPM	One-photon microscopy
QDs	Quantum dots
SRB	Sulfate reducing bacteria
TP	Two-photon
TPM	Two-photon microscopy

Notes

The authors declare no competing financial interest.

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References

1 E. T. Kato Jr., C. M. P. Yoshida, A. B. Reis, I. S. Melo and T. T. Franco, *Polym. Int.*, 2011, **60**, 951–956.
2 K. J. Iversen and M. J. S. Spencer, *J. Phys. Chem. C*, 2013, **117**, 26106–26118.
3 M. Hoffman, A. Rajapakse, X. L. Hen and K. S. Gates, *Chem. Res. Toxicol.*, 2012, **25**, 1609–1615.
4 S. H. Libardi, H. Pindstrup, D. R. Cardoso and L. H. Skibsted, *J. Agric. Food Chem.*, 2013, **61**, 2883–2888.
5 C. Ramos-Alvarez, B. K. Yoo, R. Pietri, I. Lamarre, J. L. Martin, J. Lopez-Garriga and M. Negrerie, *Biochemistry*, 2013, **52**, 7007–7021.
6 L. Y. Hu, S. L. Hu, J. Wu, Y. H. Li, J. L. Zheng, Z. J. Wei, J. Liu, H. L. Wang, Y. Liu and H. Zhang, *J. Agric. Food Chem.*, 2012, **60**, 8684–8693.
7 S. P. Li, K. D. Hu, L. Y. Hu, Y. H. Li, A. M. Jiang, F. Xiao, Y. Han, Y. S. Liu and H. Zhang, *J. Agric. Food Chem.*, 2014, **62**, 1119–1129.
8 M. R. Jackson, S. L. Melideo and M. S. Jorns, *Biochemistry*, 2012, **51**, 6804–6815.
9 R. J. Reiffenstein, W. C. Hulbert and S. H. Roth, *Annu. Rev. Pharmacol. Toxicol.*, 1992, **32**, 109–134.
10 P. Kamoun, M. C. Belardinelli, A. Chabli, K. Lallouchi and B. Chadefaux-Vekemans, *Am. J. Med. Genet. A.*, 2003, **116A**, 310–311.
11 W. Yang, G. Yang, X. Jia, L. Wu and R. Wang, *J. Physiol.*, 2005, **569**, 519–531.
12 S. Fiorucci, E. Antonelli, A. Mencarelli, S. Orlandi, B. Renga, G. Rizzo, E. Distrutti, V. Shah and A. Morelli, *Hepatology*, 2005, **42**, 539–548.
13 S. Rezaei, A. Tavana, J. A. Sawada, L. Wu, A. S. M. Junaid and S. M. Kuznicki, *Ind. Eng. Chem. Res.*, 2012, **51**, 12430–12434.
14 Z. A. Premji, J. M. H. Lo and P. D. Clark, *J. Phys. Chem. A*, 2014, **118**, 1541–1556.
15 C. P. O'Brien, J. B. Miller, B. D. Morreale and A. J. Gellman, *J. Phys. Chem. C*, 2012, **116**, 17657–17667.
16 L. Li, A. Hsu and P. K. Moore, *Pharmacol. Ther.*, 2009, **123**, 386–400.
17 L. Li, P. Rose and P. K. Moore, *Annu. Rev. Pharmacol. Toxicol.*, 2011, **51**, 169–187.
18 M. R. Filipovic, M. Eberhardt, V. Prokopovic, A. Mijuskovic, Z. Orescanin-Dusic, P. Reeh and I. Ivanovic-Burmazovic, *J. Med. Chem.*, 2013, **56**, 1499–1508.
19 H. Kimura, *Amino Acids*, 2011, **41**, 113–121.
20 R. Wang, *Physiol. Rev.*, 2012, **92**, 791–896.
21 R. Pietri, E. Roman-Morales and J. Lopez-Garriga, *Antioxid. Redox Signaling*, 2011, **15**, 393–404.
22 B. Jiang, G. H. Tang, K. Cao, L. Y. Wu and R. Wang, *Antioxid. Redox Signaling*, 2010, **12**, 1167–1178.
23 M. R. Filipovic, J. L. Miljkovic, T. Nauser, M. Royzen, K. Klos, T. Shubina, W. H. Koppenol, S. J. Lippard and I. Ivanovic-Burmazovic, *J. Am. Chem. Soc.*, 2012, **134**, 12016–12027.
24 H. J. Peng, Y. F. Cheng, C. F. Dai, A. L. Adrienne, L. King, B. L. Predmore, D. J. Lefer and B. H. Wang, *Angew. Chem., Int. Ed.*, 2011, **50**, 9672–9675.
25 Y. J. Peng, J. Nanduri, G. Raghuraman, D. Souvannakitti, M. M. Gadalla, G. K. Kumar, S. H. Snyder and N. R. Prabhakar, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 10719–10724.
26 M. N. Hughes, M. N. Centelles and K. P. Moore, *Free Radical Biol. Med.*, 2009, **47**, 1346–1353.
27 J. Furne, A. Saeed and M. D. Levitt, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 2008, **295**, R1479–R1485.
28 J. Radford-Knaery and G. A. Cutter, *Anal. Chem.*, 1993, **65**, 976–982.
29 R. F. Huang, X. W. Zheng and Y. J. Qu, *Anal. Chim. Acta*, 2007, **582**, 267–274.
30 J. E. Doeller, T. S. Isbell, G. Benavides, J. Koenitzer, H. Patel, R. P. Patel, J. R. Lancaster Jr., V. M. Darley-Usmar and D. W. Kraus, *Anal. Biochem.*, 2005, **341**, 40–51.
31 H. Li, Y. Tian, Z. F. Deng and Y. Liang, *Analyst*, 2012, **137**, 4605–4609.
32 K. Awasthi, T. Nakabayashi and N. Ohta, *J. Phys. Chem. B*, 2012, **116**, 11159–11165.
33 A. J. Lee, X. Y. Wang, L. J. Carlson, J. A. Smyder, B. Loesch, X. M. Tu, M. Zheng and T. D. Krauss, *Nano Lett.*, 2011, **11**, 1636–1640.
34 T. Kaji, T. Yamada, R. Ueda and A. Otomo, *J. Phys. Chem. Lett.*, 2011, **2**, 1651–1656.

35 X. Li, Y. He and L. Qu, *Langmuir*, 2013, **29**, 2439–2445.
36 Y. Q. Tu, W. Li, P. Wu, H. Zhang and C. X. Cai, *Anal. Chem.*, 2013, **85**, 2536–2542.
37 J. C. Kraft and R. J. Y. Ho, *Biochemistry*, 2014, **53**, 1275–1283.
38 L. Wei, C. Liu, B. Chen, P. Zhou, H. C. Li, L. H. Xiao and E. S. Yeung, *Anal. Chem.*, 2013, **85**, 3789–3793.
39 M. Sigdel, M. L. Pusey and R. S. Aygun, *Cryst. Growth Des.*, 2013, **13**, 2728–2736.
40 Y. Zhang, J. M. Liu and X. P. Yan, *Anal. Chem.*, 2013, **85**, 228–234.
41 Y. K. Xu, S. Hwang, S. Kim and J. Y. Chen, *ACS Appl. Mater. Interfaces*, 2014, **6**, 5619–5628.
42 N. Kumar, V. Bhalla and M. Kumar, *Coord. Chem. Rev.*, 2013, **257**, 2335–2347.
43 G. K. Kolluru, X. Shen, S. C. Bir and C. G. Kevil, *Nitric Oxide*, 2013, 5–20.
44 W. Xuan, C. Sheng, Y. Cao, W. He and W. Wang, *Angew. Chem., Int. Ed.*, 2012, **51**, 2282–2284.
45 V. S. Lin and C. Chang, *J. Curr. Opin. Chem. Biol.*, 2012, **16**, 595–601.
46 A. R. Lippert, *J. Inorg. Biochem.*, 2014, **133**, 136–142.
47 L. A. Montoya and M. D. Pluth, *Chem. Commun.*, 2012, **48**, 4767–4769.
48 K. K. Lin, S. C. Wu, K. M. Hsu, C. H. Hung, W. F. Liaw and Y. M. Wang, *Org. Lett.*, 2013, **15**, 4242–4245.
49 M. Gomez-Mendoza, M. L. Marin and M. A. Miranda, *J. Phys. Chem. Lett.*, 2011, **2**, 782–785.
50 J. Rohacova, G. Sastre, M. L. Marin and M. A. Miranda, *J. Phys. Chem. B*, 2011, **115**, 10518–10524.
51 M. Gomez-Mendoza, M. L. Marin and M. A. Miranda, *J. Phys. Chem. B*, 2012, **116**, 14776–14780.
52 B. H. Shankar and D. Ramaiah, *J. Phys. Chem. B*, 2011, **115**, 13292–13299.
53 Y. Cao, L. P. Ding, S. H. Wang, Y. Liu, J. M. Fan, W. T. Hu, P. Liu and Y. Fang, *ACS Appl. Mater. Interfaces*, 2014, **6**, 49–56.
54 S. D. Tito, F. Morvan, A. Meyer, J. J. Vasseur, A. Cummaro, L. Petraccone, B. Pagano, E. Novellino, A. Randazzo, C. Giancola and D. Montesarchio, *Bioconjugate Chem.*, 2013, **24**, 1917–1927.
55 K. Matsuoka, H. Arai, H. Oka, T. Koyama and K. Hatano, *ACS Macro Lett.*, 2012, **1**, 266–269.
56 Y. X. Cheng, J. Hao, L. A. Lee, M. C. Biewer, Q. Wang and M. C. Stefan, *Biomacromolecules*, 2012, **13**, 2163–2173.
57 A. R. Lippert, E. J. New and C. J. Chang, *J. Am. Chem. Soc.*, 2011, **133**, 10078–10080.
58 T. S. Bailey and M. D. Pluth, *J. Am. Chem. Soc.*, 2013, **135**, 16697–16704.
59 H. Y. Tian, J. H. Qian, H. Y. Bai, Q. Sun, L. Y. Zhang and W. B. Zhang, *Anal. Chim. Acta*, 2013, **768**, 136–142.
60 M. K. Thorson, T. Majtan, J. P. Kraus and A. M. Barrios, *Angew. Chem., Int. Ed.*, 2013, **52**, 4641–4644.
61 D. M. Shcherbakova, M. A. Hink, L. Joosen, T. W. J. Gadella and V. V. Verkhusha, *J. Am. Chem. Soc.*, 2012, **134**, 7913–7923.
62 F. V. Subach and V. V. Verkhusha, *Chem. Rev.*, 2012, **112**, 4308–4327.
63 S. C. Alford, Y. D. Ding, T. Simmen and R. E. Campbell, *ACS Synth. Biol.*, 2012, **1**, 569–575.
64 P. Dedecker, F. C. D. Schryver and J. Hofkens, *J. Am. Chem. Soc.*, 2013, **135**, 2387–2402.
65 H. Hoi, T. Matsuda, T. Nagai and R. E. Campbell, *J. Am. Chem. Soc.*, 2013, **135**, 46–49.
66 J. G. Park, Y. Qin, D. F. Galati and A. E. Palmer, *ACS Chem. Biol.*, 2012, **7**, 1636–1640.
67 A. W. Smith, A. A. Smoligovets and J. T. Groves, *J. Phys. Chem. A*, 2011, **115**, 3867–3875.
68 Z. Y. Tian and A. D. Q. Li, *Acc. Chem. Res.*, 2013, **46**, 269–279.
69 A. E. Jablonski, J. C. Hsiang, P. Bagchi, N. Hull, C. I. Richards, C. J. Fahrni and R. M. Dickson, *J. Phys. Chem. Lett.*, 2012, **3**, 3585–3591.
70 T. Yamada, H. Yoshimura, A. Inaguma and T. Ozawa, *Anal. Chem.*, 2011, **83**, 5708–5714.
71 A. C. Jahns, Y. Maspolim, S. X. Chen, J. M. Guthrie, L. F. Blackwell and B. H. A. Rehm, *Bioconjugate Chem.*, 2013, **24**, 1314–1323.
72 S. Chen, Z. J. Chen, W. Ren and H. W. Ai, *J. Am. Chem. Soc.*, 2012, **134**, 9589–9592.
73 P. J. Dittmer, J. G. Miranda, J. A. Gorski and A. E. Palmer, *J. Biol. Chem.*, 2009, **284**, 16289–16297.
74 A. Ibraheem and R. E. Campbell, *Curr. Opin. Chem. Biol.*, 2010, **14**, 30–36.
75 M. G. Choi, S. Cha, H. Lee, H. L. Jeon and S. K. Chang, *Chem. Commun.*, 2009, 7390–7392.
76 K. Sasakura, K. Hanaoka, N. Shibuya, Y. Mikami, Y. Kimura, T. Komatsu, T. Ueno, T. Terai, H. Kimura and T. Nagano, *J. Am. Chem. Soc.*, 2011, **133**, 18003–18005.
77 T. Koike, T. Watanabe, S. Aoki, E. Kimura and M. Shiro, *J. Am. Chem. Soc.*, 1996, **118**, 12696–12703.
78 M. Hatsuo, Y. Kayoko, K. Iho, H. Leila, I. Norio, N. Shinsaku, K. Naoko, S. Keiichiro and U. Tadayuki, *Chem. – Eur. J.*, 2007, **13**, 1946–1954.
79 L. A. Montoya, T. F. Pearce, R. J. Hansen, L. N. Zakharov and M. D. Pluth, *J. Org. Chem.*, 2013, **78**, 6550–6557.
80 C. R. Liu, B. Peng, S. Li, C. M. Park, A. R. Whorton and M. Xian, *Org. Lett.*, 2012, **14**, 2184–2187.
81 C. R. Liu, J. Pan, S. Li, Y. Zhao, L. Y. Wu, C. E. Berkman, A. R. Whorton and M. Xian, *Angew. Chem., Int. Ed.*, 2011, **50**, 10327–10329.
82 Y. C. Chen, C. C. Zhu, Z. H. Yang, J. J. Chen, Y. F. He, Y. Jiao, W. J. He, L. Qiu, J. J. Cen and Z. J. Guo, *Angew. Chem., Int. Ed.*, 2013, **52**, 1688–1691.
83 Y. Qian, J. Karpus, O. Kabil, S. Y. Zhang, H. L. Zhu, R. Banerjee, J. Zhao and C. He, *Nat. Commun.*, 2011, **2**, 495.
84 F. Hou, L. Huang, P. Xi, J. Cheng, X. Zhao, G. Xie, Y. Shi, F. Cheng, X. Yao, D. Bai and Z. Zeng, *Inorg. Chem.*, 2012, **51**, 2454–2460.
85 F. B. Yu, P. Li, P. Song, B. S. Wang, J. Z. Zhao and K. L. Han, *Chem. Commun.*, 2012, **48**, 2852–2854.

86 Q. Q. Wan, Y. C. Song, Z. Li, X. H. Gao and H. M. Ma, *Chem. Commun.*, 2013, **49**, 502–504.
87 W. M. Xuan, R. Pan, Y. T. Cao, K. J. Liu and W. Wang, *Chem. Commun.*, 2012, **48**, 10669–10671.
88 J. Liu, Y. Q. Sun, J. Y. Zhang, T. Yang, J. B. Cao, L. S. Zhang and W. Guo, *Chem. – Eur. J.*, 2013, **19**, 4717–4722.
89 T. Y. Liu, Z. C. Xu, D. R. Spring and J. N. Cui, *Org. Lett.*, 2013, **15**, 2310–2313.
90 G. J. Mao, T. T. Wei, X. X. Wang, S. Y. Huan, D. Q. Lu, J. Zhang, X. B. Zhang, W. H. Tan, G. L. Shen and R. Q. Yu, *Anal. Chem.*, 2013, **85**, 7875–7881.
91 F. Helmchen and W. Denk, *Nat. Methods*, 2005, **2**, 932–940.
92 S. Yao and K. D. Belfield, *Eur. J. Org. Chem.*, 2012, 3199–3127.
93 L. Li, J. Ge, H. Wu, Q. H. Xu and S. Q. Yao, *J. Am. Chem. Soc.*, 2012, **134**, 12157–12167.
94 S. K. Bae, C. H. Heo, D. J. Choi, D. Sen, E. H. Joe, B. R. Cho and H. M. Kim, *J. Am. Chem. Soc.*, 2013, **135**, 9915–9923.
95 S. K. Das, C. S. Lim, S. Y. Yang, J. H. Han and B. R. Cho, *Chem. Commun.*, 2012, **48**, 8395–8397.
96 W. Sun, J. L. Fan, C. Hu, J. F. Cao, H. Zhang, X. Q. Xiong, J. Y. Wang, C. Shuang, S. G. Sun and X. J. Peng, *Chem. Commun.*, 2013, **49**, 3890–3892.
97 K. E. Sapsford, L. Berti and I. L. Medintz, *Angew. Chem., Int. Ed.*, 2006, **45**, 4562–4589.
98 G. W. Chen, F. L. Song, X. Q. Xiong and X. J. Peng, *Ind. Eng. Chem. Res.*, 2013, **52**, 11228–11245.
99 W. Lin, Y. F. Du, Y. T. Zhu and X. Chen, *J. Am. Chem. Soc.*, 2014, **136**, 679–687.
100 M. Schifferer and O. Griesbeck, *J. Am. Chem. Soc.*, 2012, **134**, 15185–15188.
101 B. G. Abraham, N. V. Tkachenko, V. Santala, H. Lemmetyinen and M. Karp, *Bioconjugate Chem.*, 2011, **22**, 227–234.
102 B. G. Keller, A. Kobitski, A. Jäschke, G. U. Nienhaus and F. Noé, *J. Am. Chem. Soc.*, 2014, **136**, 4534–4543.
103 T. Kato, H. Kashida, H. Kishida, H. Yada, H. Okamoto and H. Asanuma, *J. Am. Chem. Soc.*, 2013, **135**, 741–750.
104 V. Biju, A. Anas, H. Akita, E. S. Shibu, T. Itoh, H. Harashima and M. Ishikawa, *ACS Nano*, 2012, **6**, 3776–3788.
105 C. A. Alabi, K. T. Love, G. Sahay, T. Stutzman, W. T. Young, R. Langer and D. G. Anderson, *ACS Nano*, 2012, **6**, 6133–6141.
106 K. D. Wegner, P. T. Lanh, T. Jennings, E. Oh, V. Jain, S. M. Fairclough, J. M. Smith, E. Giovanelli, N. Lequeux, T. Pons and N. Hildebrandt, *ACS Appl. Mater. Interfaces*, 2013, **5**, 2881–2892.
107 A. M. Dennis, W. J. Rhee, D. Sotto, S. N. Dublin and G. Bao, *ACS Nano*, 2012, **6**, 2917–2924.
108 A. Y. Kobitski, S. Schäfer, A. Nierth, M. Singer, A. Jäschke and G. U. Nienhaus, *J. Phys. Chem. B*, 2013, **117**, 12800–12806.
109 M. Royzen, Z. Dai and J. W. Canary, *J. Am. Chem. Soc.*, 2005, **127**, 1612–1613.
110 K. Kiyose, H. Kojima, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2006, **128**, 6548–6549.
111 A. Ajayaghosh, P. Carol and S. Sreejith, *J. Am. Chem. Soc.*, 2005, **127**, 14962–14963.
112 L. Yuan, W. Y. Lin, K. B. Zheng and S. S. Zhu, *Acc. Chem. Res.*, 2013, **46**, 1462–1473.
113 M. Strianese, G. J. Palm, S. Milione, O. Kühl, W. Hinrichs and C. Pellecchia, *Inorg. Chem.*, 2012, **51**, 11220–11222.
114 P. T. Rajagopalan, S. Grimme and D. Pei, *Biochemistry*, 2000, **39**, 779–790.
115 C. Kar, M. D. Adhikari, A. Ramesh and G. Das, *Inorg. Chem.*, 2013, **52**, 743–752.
116 A. P. Litvin, E. V. Ushakova, P. S. Parfenov, A. V. Fedorov and A. V. Baranov, *Phys. Chem. C*, 2014, **118**, 6531–6535.
117 R. Freeman, J. Girsh and I. Willner, *ACS Appl. Mater. Interfaces*, 2013, **5**, 2815–2834.
118 A. Wagh, S. Y. Qian and B. Law, *Bioconjugate Chem.*, 2012, **23**, 981–992.
119 A. M. Dennis, W. J. Rhee, D. Sotto, S. N. Dublin and G. Bao, *ACS Nano*, 2012, **6**, 2917–2924.
120 P. T. Burks, A. D. Ostrowski, A. A. Mikhailovsky, E. M. Chan, P. S. Wagenknecht and P. C. Ford, *J. Am. Chem. Soc.*, 2012, **134**, 13266–13275.
121 H. Peng, L. J. Zhang, T. H. M. Kjällman, C. Soeller and J. Travas-Sejdic, *J. Am. Chem. Soc.*, 2007, **129**, 3048–3049.
122 C. M. Yu, X. Z. Li, F. Zeng, F. Y. Zheng and S. Z. Wu, *Chem. Commun.*, 2013, **49**, 403–405.
123 D. Jiménez, R. Martínez-Máñez, F. Sancenón, J. V. Ros-Lis, A. Benito and J. Soto, *J. Am. Chem. Soc.*, 2003, **125**, 9000–9001.
124 C. Kar, M. D. Adhikari, A. Ramesh and G. Das, *Inorg. Chem.*, 2013, **52**, 743–752.
125 M. Hoffman, A. Rajapakse, X. L. Shen and K. S. Gates, *Chem. Res. Toxicol.*, 2012, **25**, 1609–1615.
126 Y. J. Chen, X. M. Gao, X. P. Di, Q. Y. Ouyang, P. Gao, L. H. Qi, C. Y. Li and C. L. Zhu, *ACS Appl. Mater. Interfaces*, 2013, **5**, 3267–3274.
127 M. D. Pluth, T. Spencer Bailey, M. D. Hammers and L. A. Montoya, *Biochalcogen Chem.: Biol. Chem. Sulfur Selenium Tellurium, ACS Symp. Ser.*, 2013, **1152**, 15–32.
128 C. X. Duan and Y. G. Liu, *Curr. Med. Chem.*, 2013, **20**, 2929–2937.
129 B. Peng and M. Xian, *Asian J. Org. Chem.*, 2014, **3**, 914–924.
130 L. Li, M. Bhatia, Y. Z. Zhu, Y. C. Zhu, R. D. Ramnath, Z. J. Wang, F. B. M. Anuar, M. Whiteman, M. Salto-Tellez and P. K. Moore, *FASEB J.*, 2005, **19**, 1196–1198.
131 G. D. Yang, L. Y. Wu, B. Jiang, W. Yang, J. S. Qi, K. Cao, Q. H. Meng, A. K. Mustafa, W. T. Mu, S. M. Zhang, S. H. Snyder and R. Wang, *Science*, 2008, **322**, 587–590.
132 A. Papapetropoulos, A. Pyriochou, Z. Altaany, G. Yang, A. Marazioti, Z. Zhou, M. G. Jeschke, L. K. Branski, D. N. Herndon, R. Wang and C. Szabo, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 21972–21977.

133 G. Aragay, F. Pino and A. Merkoçi, *Chem. Rev.*, 2012, **112**, 5317–5338.

134 Y. Q. Wang, B. Yan and L. X. Chen, *Chem. Rev.*, 2013, **113**, 1391–1428.

135 S. F. Liu, X. Zhang, Y. M. Yu and G. Z. Zou, *Anal. Chem.*, 2014, **86**, 2784–2788.

136 S. Su, J. W. Fan, B. Xue, L. H. Yuwen, X. F. Liu, D. Pan, C. H. Fan and L. H. Wang, *ACS Appl. Mater. Interfaces*, 2014, **6**, 1152–1157.

137 J. Yao, M. Yang and Y. X. Duan, *Chem. Rev.*, 2014, **114**, 6130–6178.

138 J. B. Liu, Y. Liu, X. H. Yang, K. M. Wang, Q. Wang, H. Shi and L. Li, *Anal. Chem.*, 2013, **85**, 11121–11128.

139 M. Li, H. L. Gou, I. Al-Ogaidi and N. Q. Wu, *ACS Sustainable Chem. Eng.*, 2013, **1**, 713–723.

140 B. H. Dong, C. Y. Li, G. C. Chen, Y. J. Zhang, Y. Zhang, M. J. Deng and Q. B. Wang, *Chem. Mater.*, 2013, **25**, 2503–2509.

141 Y. C. Wang, R. Hu, G. M. Lin, I. Roy and K. T. Yong, *ACS Appl. Mater. Interfaces*, 2013, **5**, 2786–2799.

142 H. Zhang, L. Zhang, R. P. Liang, J. Huang and J. D. Qiu, *Anal. Chem.*, 2013, **85**, 10969–10976.

143 S. Chatterjee and T. K. Mukherjee, *J. Phys. Chem. C*, 2013, **117**, 10799–10808.

144 D. Goevert and R. Conrad, *Environ. Sci. Technol.*, 2008, **42**, 7813–7817.

145 B. D. Gibson, R. T. Amos and D. W. Blowes, *Environ. Sci. Technol.*, 2011, **45**, 2863–2870.

146 M. Natter, J. Keevan, Y. Wang, A. R. Keimowitz, B. C. Okeke, A. Son and M. K. Lee, *Environ. Sci. Technol.*, 2012, **46**, 5744–5755.

147 F. Sarioğlu, R. Javaherdashti and N. Aksöz, *Int. J. Pressure Vessels Piping*, 1997, **73**, 127–131.

148 B. Zheng, Y. Zhao, W. Xue and H. Liu, *Surf. Coat. Technol.*, 2012, **216**, 100–105.

149 H. A. Videla and L. K. Herrera, *Int. Biodeterior. Biodegrad.*, 2009, **63**, 896–900.

150 T. S. Rao, T. N. Sairam, B. Viswanathan and K. V. K. Nair, *Corros. Sci.*, 2000, **42**, 1417–1431.

151 C. Sun, J. Xu and F. H. Wang, *Ind. Eng. Chem. Res.*, 2011, **50**, 12797–12806.

152 J. Liu, J. S. Zheng and L. M. Xu, *Mater. Corros.*, 2001, **52**, 833–837.

153 Q. Xin, X. W. Zhang and L. C. Lei, *Ind. Eng. Chem. Res.*, 2008, **47**, 9644–9650.

154 M. A. Harris and S. Ragusa, *Environ. Geol.*, 2000, **40**, 195–215.

155 K. R. Waybrant, D. W. Blowes and C. J. Ptacek, *Environ. Sci. Technol.*, 1998, **32**, 1972–1979.

156 V. P. Utgikar, H. H. Tabak, J. R. Haines and R. Govind, *Biotechnol. Bioeng.*, 2003, **82**, 306–312.

157 T. K. Tsukamoto, H. A. Killion and G. C. Miller, *Water Res.*, 2004, **38**, 1405–1418.

158 T. Luo, H. X. Tian, Z. Guo, G. Q. Zhuang and C. Y. Jing, *Environ. Sci. Technol.*, 2013, **47**, 10939–10946.

159 A. F. Hofacker, A. Voegelin, R. Kaegi and R. Kretzschmar, *Environ. Sci. Technol.*, 2013, **47**, 7739–7746.

160 H. Bai, Y. Kang, H. E. Quan, Y. Han, J. Sun and Y. Feng, *J. Environ. Manage.*, 2013, **129**, 350–356.

161 H. Bai, Y. Kang, H. E. Quan, Y. Han and Y. Feng, *Int. J. Miner. Process.*, 2012, **112–113**, 71–76.

162 A. C. Davis, B. M. Patterson, M. E. Grassi, B. S. Robertson, H. Prommer and A. J. Mckinley, *Environ. Sci. Technol.*, 2007, **41**, 7131–7137.

163 J. Y. Cao, Y. Y. Li, G. J. Zhang, C. Yang and X. Y. Cao, *Int. J. Miner. Process.*, 2013, **125**, 27–33.

164 J. Y. Cao, G. J. Zhang, Z. S. Mao, Y. Y. Li, Z. H. Fang and C. Yang, *Int. J. Miner. Process.*, 2012, **106–109**, 58–64.

165 G. Gonzalez-Gil, S. I. C. Lopes, P. E. Saikaly and P. N. L. Lens, *Bioresour. Technol.*, 2012, **126**, 238–246.

166 K. U. Kjeldsen, C. Joulian and K. Ingvorsen, *Environ. Sci. Technol.*, 2004, **38**, 2038–2043.

167 E. B. Ekstrom and F. M. M. Morel, *Environ. Sci. Technol.*, 2008, **42**, 93–99.

168 L. McMahan, A. A. Devine, A. M. Grunden and M. D. Sobsey, *Appl. Microbiol. Biotechnol.*, 2011, **92**, 1287–1295.