Fluorescent probes for hydrogen sulfide detection and bioimaging

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In comparison with other biological detection technologies, fluorescence bioimaging technology has become a powerful supporting tool for intracellular detection, and can provide attractive facilities for investigating physiological and pathological processes of interest with high spatial and temporal resolution, less invasiveness, and a rapid response. Due to the versatile roles of hydrogen sulfide (H₂S) in cellular signal transduction and intracellular redox status regulation, fluorescent probes for the detection of this third signaling gasotransmitter have rapidly increased in number in recent years. These probes can offer powerful means to investigate the physiological actions of H₂S in its native environments without disturbing its endogenous distribution. In this feature article, we address the synthesis and design strategies for the development of fluorescent probes for H₂S based on the reaction type between H₂S and the probes. Moreover, we also highlight fluorescent probes for other reactive sulfur species, such as sulfane sulfurs and SO₂ derivatives.

Introduction

Fluorescence bioimaging technology can be distinguished readily amongst biological detection technologies by its many advantages, such as good sensitivity, excellent selectivity, rapid response, and non-invasive detection. In biological systems, features of physiologically active species include low concentration, high reactivity and short lifetimes. Therefore, it still remains a huge challenge to determine the intracellular concentration of these species accurately. In order to meet these urgent needs, reaction-based fluorescent probes have emerged.1,2

In general, the design and synthetic strategies of these probes depend on the chemical properties of the physiologically active species. The reaction-based fluorescent probes are mainly composed of two moieties: the fluorescence signal transducer and the fluorescence modulator (Fig. 1). The fluorescence signal transducer moiety transduces molecular recognition into a fluorescence signal that can be detected. It is essential to choose a suitable fluorophore platform as the signal transducer. High quantum yield, photostability and bio-compatibility are critical for bioimaging, such that the minimum dosage of the probe can avoid disturbing the natural

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distribution of the physiologically active species. The fluorescence modulator moiety manipulates the molecular recognition process. A desirable fluorescence modulator can only be triggered by a single reaction switch. The selected reaction is also screened to ensure it possesses reasonable reaction kinetics under physiological conditions. After integrating the modulator into the transducer via a conjugated or a space bridge, a reaction-based fluorescent probe is generated (Fig. 1). All the features of the fluorescence response can be employed as outputs based on their signal changes, such as absorption, emission spectra, and fluorescence lifetime. It is generally recognized that turn-on fluorescent probes are more efficient compared with turn-off probes. Turn-on signals provide ease of measuring low concentrations against a dark background, which can reduce false positive signals and increase sensitivity. Appropriate fluorescent probes also possess near-infrared absorption and emission spectra (including two-photon and multi-photon), because the light in this region shows maximized tissue penetration while minimizing the absorbance of heme in hemoglobin and myoglobin, water, and lipids. Moreover, ratiometric probes benefit from measuring the ratio of the emission intensity at two different wavelengths, since the interference caused by factors such as uneven loading and the inhomogeneous distribution of fluorescent probes in cells can be cancelled out. Finally, the probes should have low cytotoxicity and appropriate water solubility.

Intracellular reactive sulfur species (RSS) is a general term for sulfur-containing biomolecules. These molecules play critical roles in physiological and pathological processes. Glutathione (GSH), the most abundant intracellular nonprotein thiol, can control intracellular redox activity, intracellular signal transduction, and gene regulation. Cysteine (Cys) is implicated in slow growth in children, liver damage, skin lesions, and loss of muscle and fat. Homocysteine (Hcy) is a risk factor for Alzheimer’s disease and cobalamin (vitamin B12) deficiency. H2S has been identified as the third gasotransmitter following nitric oxide (NO) and carbon monoxide (CO). At physiological levels, H2S regulates the intracellular redox status and fundamental signalling processes, including regulation of vascular tone, myocardial contractility, neurotransmission, and insulin secretion. Abnormal levels of H2S in cells can induce many diseases, such as Alzheimer’s disease, liver cirrhosis, gastric mucosal injury and arterial and pulmonary hypertension. In recent years, the development of fluorescent probes for H2S has rapidly increased, benefiting from the chemical reactions of H2S.

The fluorescent probes for GSH, Cys and Hcy have been well reviewed. Hitherto, there have been few reviews on the progress of fluorescent probes for H2S. Now, we overview the synthesis and design strategies for the development of fluorescent probes based on the reaction type between H2S and the probes. We classify these probes according to their reaction type with H2S: (a) H2S reductive reactions: reducing azides to amines, reducing nitro/azanol to give amines, and reducing selenoxide to give selenide; (b) H2S nucleophilic reactions: Michael addition reactions, dual nucleophilic reactions, double bond addition reactions, and thiolysis reactions; (c) copper sulfide precipitation reaction. Moreover, we also introduce fluorescent probes for other reactive sulfur species, such as sulfane sulfurs and SO2 derivatives. The detection of sulfane sulfurs is mainly based on the nucleophilic addition reaction, and the detection of SO2 derivatives is based on their nucleophilic and reductive properties.
Fluorescent probes based on reducing azides to amines

Azides and other oxidized nitrogen species can be reduced to amines by H2S faster than by GSH and other thiols, signifying a promising method for H2S detection. Upon reduction by H2S, the electron-withdrawing azido group will be converted into an electron-donating amino group. Therefore, exploiting the electron-donating ability of different substituent groups will result in versatile fluorescent probes (Fig. 2). The fluorescent probes employing the photoinduced electron transfer (PET) mechanism are typically constructed by connecting an electron donor/acceptor recognition group to a fluorophore via a spacer bridge. The design principles of such probes are clear, and the resulting processes will quench or increase the fluorescence of these probes (Fig. 2a). The fluorescent probes that adopt an internal charge transfer (ICT) mechanism typically contain a strong push-pull electronic system, wherein the electron donating group (EDG) and the electron withdrawing group (EWG) are conjugated to the fluorophore. Depending on the ICT mechanism, ratiometric probes can be readily available (Fig. 2b). The changes in the π-conjugated systems triggered by chemical reactions are often followed by a notable alteration in spectroscopic properties, which is advantageous to obtain turn-on/ratiometric fluorescent probes (Fig. 2c). Moreover, design strategies that take advantage of protecting group chemistry will result in excellent probes (Fig. 2d). The approaches mentioned above have since been widely adopted for H2S detection (Fig. 2).

Chang et al. have exploited the selective H2S-mediated reduction of azides to amines to develop a series of fluorescent probes for intracellular H2S detection (1–6). The fluorophores of these probes were based on Rhodamine 110. The detection mechanism was illustrated in Fig. 2c. After caging Rhodamine 110 by azides at the 3 or 6 positions, the probes 1–6 adopted a closed lactone conformation, and exhibited no absorption features. When azides were reduced to amines by H2S, the spiro-rings of 1–6 opened, and the π-conjugated structure was recovered. Therefore, these H2S probes gave a turn-on response. Probes 1 and 2 could detect H2S in live HEK293T cells using confocal microscopy, and it took about 1 h to saturate the fluorescence response. Under test conditions, Probe 1 gave a fluorescence quantum yield, ϕ, of 0.60. For Probe 2, ϕ = 0.51. The detection limit of 1 was 10 μM. However, the concentration of H2S changed acutely in cells, and it was difficult for 1 and 2 to capture H2S opportunely in cells. Later, the same group optimized these design strategies and improved the sensitivity and cellular retention in their new probes. They reported bis-azido probes (3–6) for increasing H2S sensitivity, and they incorporated an acetoxyethyl ester-protected carboxy group into the new probes to increase cellular trappability (4–6). It is worth noting that probe 6 could, directly and in real-time, detect endogenous H2S which was produced in live human umbilical vein endothelial cells upon stimulation with vascular endothelial growth factor (VEGF). The detection limit of 6 was 500 nM. They also revealed that endogenous H2S production was related to NADPH oxidase-derived hydrogen peroxide (H2O2). This experimental result would establish a link for H2S/H2O2 crosstalk. Under test conditions, probes 3, 4, 5 and 6 displayed fluorescence quantum yields of 0.92, 0.18, 0.18 and 0.17, respectively. Based on Rhodamine 110 as the fluorophore, Sun et al. also developed a fluorescent probe 7 to trap intracellular H2S in HeLa cells. Probe 7 displayed a 120-fold fluorescence enhancement as a turn-on response, and the detection limit was 1.12 × 10−7 M.

Wang et al. developed a turn-on fluorescent probe 8 for H2S detection in aqueous solutions, blood serum and whole blood. The detection mechanism of the probe was illustrated in Fig. 2a. When the azido group was attached to a strongly electron-withdrawing dansyl fluorophore, the reductive reaction was accelerated. Probe 8 showed a fast response to H2S, within seconds, which made quantitative H2S detection possible regardless of the fact that it is rapidly metabolized in biological systems. The detection limit was 1 μM in buffer/Tween and 5 μM in bovine serum. The H2S concentrations in C57BL6/J mouse model blood were determined to be 31.9 ± 9.4 μM using 8. The addition of H2S led to a 40-fold increase in the fluorescence of probe 8.

Pluth et al. reported two reaction-based chemiluminescent probes for H2S (9a and 9b). Since chemiluminescence does not require excitation by any source, there is little chance of photo-degradation of the probe. Furthermore, chemiluminescent probes can avoid biological background interference. Therefore, chemiluminescent methods can provide high signal-to-noise ratios in H2S detection. 9a and 9b combined an H2S-mediated azido group with a luminol derived platform. After reduction by H2S, chemiluminescence resulted from the oxidation of the...
The azide to an amine, the near-infrared heptamethine cyanine platform showed a shift in its emission spectra from 710 nm to 750 nm. The quantum yields of probe 13 and its product changed from 0.11 to 0.12. The probe could evaluate H$_2$S by fluorescence ratio signals in aqueous solution and fetal bovine serum. Probe 13 could monitor H$_2$S release by ADT-OH. This probe was able to sense different H$_2$S levels in RAW 264.7 cells using confocal microscopy ratiometric imaging. The detection limit was 0.08 μM.

Li et al. reported two coumarin-based fluorescent probes 14 and 15 for the detection of H$_2$S. Probes 14 and 15 were both caged by an azido group. When reduced by H$_2$S, probe 15 showed a larger increase in fluorescence intensity, because the electron-donating ability of –NEt$_2$ was stronger than that of –OH, and was controlled by stronger ICT effects. 14 and 15 gave quantum yields of 0.16 ± 0.013 and 0.58 ± 0.02, respectively, and probe 15 could detect H$_2$S in rabbit plasma and PC-3 cells.

Cho et al. reported a two-photon (TP) H$_2$S probe 16 for deep tissue imaging. The probe employed 7-[benzo[d][1h]azol-2-yl]-9,9-(2-methoxyethoxy)ethyl-9H-fluorene as the fluorophore. After reduction by H$_2$S, the TP absorption cross section of 16 was 302 GM at 750 nm in HEPES buffer. The probe was able to detect endogenous H$_2$S in HeLa cells and could visualize the overall H$_2$S distribution at depths of 90–190 μm in a rat hippocampal slice. The products of 16 had $\Phi = 0.46$. The detection limit was 5–10 μM. Cho et al. next reported another two-photon ratiometric probe 17 for H$_2$S detection in mitochondria. The detection mechanism of probe 17 was illustrated in Fig. 2b and d. 6-(Benzo[d][1h]azol-2-yl)-2-(methylamino)naphthalene was selected as the fluorophore, and 4-azidobenzyl carbamate was chosen as the H$_2$S response site. The mitochondrial targeting group was a triphenylphosphonium salt. After reduction by H$_2$S, the fluorophore was released. Under test conditions, probe 17a showed a shift in emission from 464 nm to 545 nm, and the quantum yields of probe 17a and its product changed from 0.24 to 0.12. 17b exhibited a shift in emission from 420 nm to 500 nm, and in quantum yield from 0.23 to 0.50. There was a larger Stokes shift between the probe 17a and its precursor due to the fact that 17a had a more stable charge-transfer excited state. The two-photon ratiometric probe can be used to detect mitochondrial H$_2$S levels in living cells and tissues. The probe 17a demonstrated the relationship between the cystathionine β-synthase expression level and the H$_2$S level in astrocytes. Furthermore, the experiments showed that a genetically mutated Parkinson's disease (PD)-related gene could affect H$_2$S production in the brains of PD patients.

Peng et al. and Xu et al. reported a two-photon fluorescent probe 18 with near-infrared emission for the detection of H$_2$S. A styrene group was introduced into the fluorophore to extend the conjugation system of the dicyanomethylene-dihydrofuran. After reduction by H$_2$S, the two-photon absorption cross section of 18 was 50 GM at 820 nm in DMSO. The probe could give a 354-fold fluorescence increase upon detection of H$_2$S. Furthermore, this probe can detect H$_2$S in commercial fetal bovine serum, MCF-7 cells, HUVEC cells, rat liver cancer slices and ICR mice. The reported detection limit was 3.05 μM.
Zhang et al. synthesized a two-photon fluorescent probe 19 for the detection of H$_2$S. This probe employed a naphthalene derivative as the two-photon fluorophore. The detection mechanism of probe 19 was shown in Fig. 2b. Probe 19 had a donor–π-acceptor (D–π–A) structure, and the recognition unit azide acted as the electron withdrawing group which could break the D–π–A structure. When H$_2$S reduced the azide to an amine, the D–π–A structure recovered and the probe emitted strong fluorescence. The TP absorption cross section of product 19 was estimated to be 110.98 GM at 760 nm, and probe 19 showed a 21-fold TP excited fluorescence increase. The probe could be used to detect endogenous H$_2$S in HeLa cells, and the detection limit was 20 nM.

Ma et al. reported a cresyl violet-based ratiometric fluorescent probe 20. The probe also had a donor–π-acceptor structure. When the azido group was reduced to an amino group, the D–π–A structure changed from electron-withdrawing to electron-donating. The result led to a spectroscopic blue or red shift in emission, which could provide a ratiometric method of H$_2$S detection with $F = 0.44$ and 0.54, respectively. This probe could be used to detect H$_2$S in MCF-7 cells and zebrafish by ratiometric imaging. The detection limit of the probe was 0.1 μM.

Chang et al. reported a ratiometric fluorescent probe 21 based on an excited-state intramolecular proton transfer (ESIPT) mechanism for H$_2$S detection. When the azido group was reduced to an amino group, the p-aminobenzyl moiety underwent an intramolecular 1,6-elimination to release the ESIPT dye 2-(2'-hydroxyphenyl)-benzothiazole (HBT). The ratio of emission intensity varied 43-fold. The probes based on the ESIPT mechanism often resulted in large Stokes shifts. The ESIPT mechanism could be exploited to design probes based on their unique, environment-sensitive nature. The probe was used to detect H$_2$S in HeLa cells, and the detection limit was 2.4 μM.

Guo et al. reported a fluorescent probe 2-(2-azidophenyl) benzothiazole (22) based on the ESIPT mechanism for H$_2$S detection. The probe exhibited a 1150-fold increase in fluorescence with $\Phi = 0.4138$. The probe was used to detect H$_2$S in B16 cells, and the detection limit was 0.78 nM.

Tang et al. reported fluorescent probes 23 and 24 for H$_2$S detection. Probe 23 was synthesized based on the fluorophore phenoxazinon, and showed a 23-fold increase in fluorescence. The probe can detect H$_2$S in PBS buffer, fetal bovine serum, and HeLa cells. With coumarin as a fluorophore, these authors reported two-photon fluorescent probes 24a and 24b for H$_2$S detection. 24b showed better selectivity and sensitivity than probe 24a. The product of 24b gave $\Phi = 0.88 \pm 0.02$. Probe 24b could detect H$_2$S from both exogenous addition and possible enzymatic production, and imaging of H$_2$S was achieved in the cardiac tissues of normal rats and atherosclerotic rats.

Zeng et al. integrated a naphthalimide azide derivative into the anchoring site of carbon nanodots, and developed a fluorescence resonance energy transfer (FRET) ratiometric fluorescent probe 25. FRET is the interaction between two excited state fluorophores correlated with distance. The process involves the nonradiative transfer of excitation energy from an excited donor to a proximal ground-state acceptor, and it is convenient in the design of ratiometric probes involving the ratio of two emission intensities at different wavelengths. In FRET systems, the emission wavelength of the donor is the excitation wavelength of the acceptor. Therefore, regulation of the precise energy match between carbon nanodots and the naphthalimide azide derivative would be beneficial for H$_2$S detection. Probe 25 could detect H$_2$S in HeLa and L929 cells, and the detection limit was 10 nM.
Lin et al. reported probe 26, phenanthroimidazole, for H₂S detection. The azido group could withdraw the electrons from phenanthroimidazole, which made the fluorescence weak. Upon treatment of the probe with H₂S, the fluorescence of the probe could reach saturation within 3 min. The product of 26 gave \( F = 0.62 \). The probe could be used to detect H₂S in HeLa cells with a detection limit of 8.79 \( \mu \)M.

Chen et al. reported 7-nitrobenz-2-oxa-1,3-diazole as a colorimetric and fluorescent probe 27 for H₂S detection. When the azide was reduced to an amine, probe 27 showed a change in colour from pale-yellow to deep-yellow. The increase of the fluorescence intensity was up to 16-fold. The probe was used to image H₂S in living MCF-7 cells, and the detection limit was 680 nM.

Wu et al. reported the polymer-based fluorescent probe 28 for H₂S detection. The monomer 28a could be further functionalized to polymer 28b. Na₂S induced a 5-fold and a 3-fold increase in the fluorescence intensity of 28a and 28b, respectively. Probe 28b could detect H₂S in HeLa cells.

Xu et al. reported a fluorescent probe 29 for H₂S detection based on dicyanomethylenehydrofuran. 29 gave \( F = 0.018 \), and the probe was able to detect H₂S in living human umbilical vein endothelial cells.

Talukdar et al. designed a colorimetric and fluorometric probe 30 for the detection of H₂S. The BODIPY-azide could be reduced to a BODIPY-amide by H₂S with a turn-on fluorescent response. The detection mechanism was as shown in Fig. 2a.

The fluorescence of 30 was quenched by the electron-rich \( \alpha \)-nitrogen of the azido group. The probe displayed a fast response time in serum albumin (within 30 s) with a 28-fold fluorescence increase, and the detection limit was 259 nM. The probe was used to detect H₂S in HeLa cells.

Hartman et al. reported the 8-azidopyrene-1,3,6-trisulfonate probe 31 for H₂S detection with a 24-fold fluorescence increase. The probe had a high water solubility at concentrations >100 mM, and could measure H₂S in serum.

**Fluorescent probes based on reducing nitro groups to amines**

There exists a major obstacle in the design of fluorescent probes by exploiting nitro fluorophores, because the nitro group has always been considered to be a strong quencher of fluorophores. However, the nitro group can be reduced by Na₂S to produce the corresponding amino group under mild conditions, which opens a door to the design and synthesis of new types of fluorescent probe containing a nitro group for H₂S detection. Taking advantage of this reductive reaction, two main types of H₂S fluorescent probes have been developed. One type adopts the photoinduced electron transfer (PET) mechanism, which benefits from the strongly electron-withdrawing nature of the nitro group (Fig. 3a). The other type uses the internal charge transfer (ICT) mechanism, which results from a donor–π–acceptor structure caused by a strong push–pull electronic effect (Fig. 3b).

Chen et al. designed and synthesized a near-infrared fluorescent probe 32 for H₂S detection in HEPES buffer and in fetal bovine serum. The probe involved the PET mechanism in sensing H₂S (Fig. 3a). The fluorescence of heptamethine cyanine would be quenched via a photoinduced electron transfer (PET) process from the excited fluorophore to the strongly electron-withdrawing nitro group (donor-excited PET; d-PET). On the other hand, while the nitro group was reduced to an amino group, there might exist an acceptor-excited PET (a-PET) process from the amino group to the excited fluorophore, since the amino group is a strongly electron-donating group with lone-pair electrons. However, the probe displayed an increase in fluorescence emission, and the quantum yield increased from 0.05 to 0.11. This phenomenon was attributed to substituent effects; that is, it was not favorable for the substituent in the \( \alpha \)-position of the aromatic ring to act as an electron donor. The probe has been used to track H₂S in RAW264.7 cells.

Li et al. reported colorimetric and ratiometric fluorescent probes 33a–c based on an ICT strategy for the detection of
H₂S.⁴⁰ Probe 33a demonstrated a typical push–pull electronic system, crossing the coumarin fluorophore. After reaction with H₂S, the push–pull electron system was blocked by a push–push electron system, which disturbed the ICT mechanism, leading to spectral shifts. The D–π–A structures of probes 33a–c could result in different sensitivities and selectivities. The reaction of probe 33a with H₂S resulted in an emission shift from 602 nm (Φ = 0.023) to 482 nm (Φ = 0.236). The detection limit using 33a was 2.5 μM.

Pluth et al. reported a 4-nitronaphthalimide fluorescent probe 34 for the detection of H₂S.¹³ The functionalization of the 4-position with amino and nitro in the naphthalimide fluorophore platform resulted in fluorescence turn-on. Probe 34 was also responsive to Cys and GSH. Probe 34 gave Φ = 0.096 ± 0.001, and the probe was used to detect H₂S in HeLa cells.

Wang et al. reported hydroxylamine naphthalimide (35) as a fluorescent probe for H₂S based on the ICT mechanism.⁴¹ During the reduction of nitro groups to amines, hydroxylamine derivatives are produced as intermediates. The hydroxylamine moiety can be reduced more easily than the nitro group. Moreover, the hydroxylamine moiety is an electron-withdrawing group, and can quench the fluorescence of naphthalimide. 35 gave Φ = 0.12, and the probe could be used to detect H₂S in astrocyte cells.

Fluorescent probes based on reducing selenoxide to selenide

As the active site of the antioxidant enzyme glutathione peroxidase (GPx), organoselenium compoundsmodulate cellular antioxidant defense systems in defense against damage by reactive oxygen species (ROS) via the reduction of reactive oxygen species by biothiols. The oxidation–reduction reaction of the selenoenzyme depends on a unique ping-pong mechanism between selenoxide and selenide.⁴² Taking advantage of mimics of the catalytic cycle could aid in the development of fluorescent probes for the reversible detection of H₂S. Since selenoxide and selenide are electron-withdrawing and electron-donating groups, respectively, fluorescent probes can be smoothly achieved by a photoinduced electron transfer (PET) mechanism (Fig. 4a) and an internal charge transfer (ICT) mechanism (Fig. 4b).

Han et al. developed a series of fluorescent probes (36, 37, 38) containing organoselenium moieties that could be used for monitoring the redox cycles between H₂S and ROS. The reversible fluorescent probe 36 could detect the redox cycle between HClO and H₂S. The mechanism was as shown in Fig. 4a. The probe employed a BODIPY fluorophore as the signal transducer and 4-methoxyphenylselanyl benzene (MPhSe) as the modulator.⁴³ The fluorescence of 36 was quenched as a result of PET between the modulator and the transducer, but oxidation of Se prevented the PET, causing the fluorescence emission to be “switched on”. The quantum yield increased from 0.13 to 0.96. The probe could be used to detect the redox cycle induced by HClO and H₂S in RAW264.7 cells.

After integration of the modulator (4-methoxyphenylselane, MPhSe) into the BODIPY platform through a styrene bridge, probe 37 could function as a near-infrared reversible ratiometric fluorescent probe for the redox cycle between HBrO oxidative stress and H₂S.⁴⁴ The mechanism was as shown in Fig. 4b. This approach could facilitate the D–π–A conjugation system and tune the red-shift of the emission efficaciously due to the strong electron-donating properties of the selenide group. After selenide was oxidized to selenoxide by HBrO, the fluorescence of the probe would blue-shift because of the electron-withdrawing effect of selenoxide. The quantum yield increased from 0.00083 to 0.206 (at 635 nm). The probe has been successfully used to detect the HBrO/H₂S redox cycle in the mouse macrophage cell line RAW264.7.

The probe 38 was based on a 1,8-naphthalimide fluorophore.⁴⁵ There exists a PET process in 38 (selenide-form), which was confirmed by time-dependent density functional theory calculations. However, there also was an excited state configuration twist process in the selenide-form, but not in its selenoxide form (38). This excited state configuration twist would cause fluorescence quenching. The quantum yield of 38 increased from 0.04 to 0.45. The probe was capable of detecting HClO oxidative stress and H₂S reducing repair in RAW 264.7 cells and in mice.

Fluorescent probes based on the nucleophilic reactions of H₂S

H₂S is a reactive nucleophilic species that can participate in nucleophilic substitution in vivo. The major challenge in H₂S detection is to distinguish H₂S from other biological nucleophiles,
such as cysteine and glutathione, which are present at micromolar or millimolar concentrations inside most cells. The $pK_a$ of H$_2$S is $\sim 7.0$ in aqueous solution, whereas other bio-thiols have higher $pK_a$ values (Cys: $\sim 8.3$, GSH: $\sim 9.2$). Therefore, H$_2$S is considered to be a stronger nucleophile than other biothiols in physiological media. H$_2$S can undergo dual nucleophilic reaction as a non-substituted biothiol, however mono-substituted thiols can only undergo one nucleophilic reaction. Based on the nucleophilic and dual nucleophilic properties, fluorescent probes containing bis-electrophilic entities have been devised for H$_2$S detection. As shown in Fig. 5a and b, H$_2$S can react with the more electrophilic moiety of the fluorescent probe to form an intermediate containing free mercapto (–SH). If another electrophilic site is present at a suitable position, such as an ortho-ester group or an $\alpha,\beta$-unsaturated acrylate group, the –SH group can undergo Michael addition (Fig. 5a) or a spontaneous cyclization (Fig. 5b) to trigger the fluorescent switch turn-on. The fluorescent probes based on these strategies, as shown in Fig. 5a and b, can also react with other biothiols such as Cys and GSH. However, the intermediates cannot continue to the next cyclization reaction. Therefore, the fluorescent signal does not suffer from the interference caused by other biothiols. As a strong nucleophile, H$_2$S also can interrupt the π-conjugation of the probe, thereby leading to a change in the emission wavelength of the probe (Fig. 5c). The removal of the strong electron-withdrawing group by H$_2$S can release the fluorophore (Fig. 5d); this strategy is expected to give turn-on fluorescent probes.

**Fluorescent probes based on Michael addition reaction**

Qian et al. reported two H$_2$S-selective fluorescent probes 39 and 40. Probes 39 and 40 exploited 1,3,5-triaryl-2-pyrazoline as the fluorophore. The H$_2$S recognition moiety consisted of an aromatic framework which was substituted by an $\alpha,\beta$-unsaturated acrylate methyl ester and ortho-aldehyde (–CHO). The aldehyde group could reversibly react with H$_2$S to form a hemithioacetal intermediate, which was suitable for Michael addition to the proximal acrylate to yield thioacetal (Fig. 5a). This tandem reaction could block the PET process, and the probe showed a turn-on response to H$_2$S. Probes 39 and 40 to H$_2$S gave $\Phi = 0.208$ and 0.058, and these probes could detect H$_2$S in HeLa cells.

Li et al. reported an ICT-based turn-on fluorescent probe 41 for H$_2$S detection. The aryl ring substituted by an ortho-aldehyde and an $\alpha,\beta$-unsaturated acrylate methyl ester was conjugated to the BODIPY fluorophore via styrene. The quantum yield increased from 0.006 to 0.13. The probe could detect H$_2$S in RAW 264.7 macrophage cells, and the detection limit was 2.5 μM.

Zhao et al. reported a BODIPY-based probe 42 for H$_2$S detection. The probe was designed by replacing the ortho-acrylate ester with an $\alpha,\beta$-unsaturated phenyl ketone. The probe was able to respond to sulfide in bovine plasma, and the reaction was completed within 120 s at room temperature. 42 gave $\Phi = 0.10$. This fast-response probe suggested that the average sulfide concentration in the blood plasma of four mice was 56.0 ± 2.3 μM. The average sulfide concentration in four C57BL6/J mouse brain tissues was estimated to be 7.1 ± 1.4 μmol g$^{-1}$ protein.

Xian et al. reported a fluorescent probe 43 for H$_2$S detection. The design strategy was based on a Michael addition of...
H₂S followed by an intramolecular cyclization to release the fluorophore. Because biothiols could react readily with Michael acceptors at physiological pH in a rapid equilibrium process, monosubstituted biothiols would not consume the probes in reversible reactions. Probe 43a and 43b led to 11-fold and 160-fold turn-on responses, respectively. The probe 43b could detect H₂S in COS7 cells, and the detection limit was found to be 1 μM.

Fluorescent probes based on dual nucleophilic reactions
Xian et al. designed a series of turn-on probes (44, 45) to detect H₂S based on the dual nucleophilic property.50,51 These fluorescent probes contained reactive disulfide groups. H₂S could react with the disulfide group to give a free –SH containing intermediate; the intermediate next underwent a spontaneous cyclization to release the fluorophore. Other monosubstituted biothiols would not consume the probes in reversible reactions. Probe 43a and 43b led to 11-fold and 160-fold turn-on responses, respectively. The probe 43b could detect H₂S in COS7 cells, and the detection limit was found to be 1 μM.

Qian et al. reported a ratiometric fluorescent probe 46 based on the excited state intramolecular proton transfer (ESIPT) mechanism for H₂S detection.52 The fluorophore 2-(2'-hydroxyphenyl) benzothiazole exhibited ratiometric detection capability, following a large Stokes shift. The recognition reaction completed within 2 min. The probe showed a 30-fold fluorescence increase, and could be used to detect H₂S in HeLa cells. The detection limit was 0.12 μM.

Guo et al. reported a methylfluorescein-based probe 47 for the detection of H₂S.53 2-(iodomethyl) benzoate was chosen as the H₂S trapping group. Upon exposure to H₂S, the H₂S-induced substitution–cyclization reaction took place smoothly to release the fluorophore. 47 showed Φ = 0.379, and the probe could detect H₂S in COS7 cells. The detection limit was 0.10 μM.

Tang et al. reported a near-infrared ratiometric fluorescent probe 48 for the detection of H₂S.54 2-Carboxybenzaldehyde was selected as the H₂S sensing group, with the aldehyde and the ester at the dual nucleophilic addition positions. After reaction with H₂S, the released fluorophore cyanine benefited from tautomerism between the enol and the ketone forms to allow ratiometric detection. There was a 2500-fold increase in ratio response. The probe could target mitochondria and detect the H₂S in HepG2 and A549 cells; the detection limit was 5.0–10 nM.

Fluorescent probes based on a double bond addition reaction
He et al. designed a ratiometric fluorescent probe 49 for H₂S detection.55 Probe 49 could be considered as a hybrid fluorophore of coumarin and merocyanine through an ethylene linkage. The probes benefited from the fast HS⁻ nucleophilic addition to the merocyanine moiety in a medium of nearly neutral pH. After HS⁻, the main form of H₂S under physiological conditions, added to the indolenium C-2 atom of 49, the π-conjugation of the probe was disturbed, eliminating the emission of merocyanine but retaining that of coumarin, causing a shift in the fluorescence spectrum. The probe showed rapid responses to the changes of H₂S concentration in solution and cells, which were completed within 30 and 80 s, respectively. The intensity ratio of 49 increased over 120-fold. This probe was applied for the detection of changes in the level of H₂S in the mitochondria of MCF-7 cells.

Guo et al. reported a ratiometric fluorescent probe 50 based on a flavylum derivative and a commercially available pyronine dye 51 for H₂S detection.56 Probe 50 gave a turn-off response due to the interruption of the π-conjugation of the pyronine ring. The probe could detect H₂S based on the selective nucleophilic attack of H₂S on the electrically positive benzopyrpylium moiety, which would interrupt the π-conjugation, thereby leading to changes in the emission profile. Probe 50 could provide a ratiometric fluorescent response within 10 s.
was a 1200-fold increase in the ratiometric value. Probe 50 could be used to detect $\text{H}_2\text{~S}$ in HeLa cells and in human serum; the detection limit was 0.14 $\mu$M.

**Fluorescent probes based on a thiolysis reaction**

Lin *et al.* synthesized a near-infrared fluorescent probe 52 based on a thiolysis reaction for $\text{H}_2\text{~S}$ detection. The dinitrophenyl group has often been used to protect tyrosine in peptide synthesis. Thiols could remove the dinitrophenyl group under basic conditions. The probe was prepared through condensation of the BODIPY with a Fischer aldehyde, and then caged by 1-fluoro-2,4-dinitrobenzene. The probe is non-fluorescent due to the d-PET process from the excited dye to the strong electron-withdrawing group. After reaction with $\text{H}_2\text{~S}$, the fluorophore was released with an 18-fold increase in fluorescence. This probe was used to detect $\text{H}_2\text{~S}$ in bovine serum and MCF-7 cells, and the detection limit was $5 \times 10^{-8}$ M.

Xu *et al.* reported a lysosome-targeting fluorescent probe 53 for $\text{H}_2\text{~S}$ detection. The probe was prepared by introducing a dinitrophenyl ether group into the 4-position of 1,8-naphthalimide, and 4-(2-aminoethyl)morpholine into the N-imide position as the lysosome-targeting group. The lysosome-targeting probe is of significance for the study of the distribution and function of $\text{H}_2\text{~S}$ in lysosomes of living cells. The fluorescence intensity of 53 increased 42-fold. The probe could be used to detect $\text{H}_2\text{~S}$ in the lysosomes of MCF-7 cells. The detection limit was 0.48 $\mu$M. Xu *et al.* also developed a 1,8-naphthalimide-derived probe 54 as a two-photon fluorescent probe for $\text{H}_2\text{~S}$ detection based on the thiolysis of dinitrophenyl ether. The fluorescence intensity of 54 increased 37-fold. The probe 54 was applied to detect $\text{H}_2\text{~S}$ in bovine serum and MCF-7 cells, and the detection limit was 0.18 $\mu$M.

Feng *et al.* reported a 3-hydroxyflavone-based ESIPT probe 55 for $\text{H}_2\text{~S}$ detection. The emission intensity increased 660-fold when detecting $\text{H}_2\text{~S}$. The probe could be used to detect $\text{H}_2\text{~S}$ in biological serum and in simulated wastewater samples. The detection limit was 0.10 $\mu$M.

Yi *et al.* reported fluorescent probes 56a and 56b for $\text{H}_2\text{~S}$ detection, which employed fluorescein and naphthofluorescein as fluorophores. Based on the thiolysis of (7-nitro-1,2,3-benzoxadiazole) ether, the probes could release the fluorophores and give a turn-on response to $\text{H}_2\text{~S}$. The fluorescence intensity of 56a and 56b increased 77-fold and 1000-fold, respectively. The detection limit of 56b was determined to be 16 $\mu$M in solution.

**Fluorescent probes based on copper sulfide precipitation**

After forming stable metal complexes with Cu(II), organic chelators have an efficacious quenching effect on fluorophores due to the fact that the paramagnetic Cu(II) center can accept the excited state electrons of the fluorophores. It is expected that the removal of Cu$^{2+}$ from the complex will result in fluorescence recovery. Such fluorescent probes are often assembled in a fluorophore–chelator–metal ion manner (Fig. 6). According to the hard and soft acids and bases (HSAB) theory, $\text{S}^{2-}$ has a strong affinity for Cu(II). The CuS precipitate is relatively stable, with $K_{\text{sp}} = 1.26 \times 10^{-36}$. After the addition of $\text{H}_2\text{~S}$ in solution,
Cu(II) will be eliminated from the complex, and the corresponding fluorescent probes will again become emissive.

Chang et al. developed a fluorescent probe 57 for the selective sensing of S\(2^–\). The probe was designed based on a Cu\(^{2+}\) complex of fluorescein containing a dipicolylamine chelator. The detection limit was 420 nM in aqueous solution.

Li et al. reported a conjugated polymer fluorescent probe 58 based on a disubstituted polyacetylene containing a dipicolylamine chelator in the side chains for the detection of Cu\(^{2+}\) and S\(^{2–}\). The fluorescence of the probe could be quenched by Cu\(^{2+}\). Based on the displacement strategy using S\(^{2–}\) to remove Cu\(^{2+}\), the quenched fluorescence of the probe could recover. The detection limit was 5.0 \(\times\) 10\(^{-7}\) mol L\(^{-1}\).

Lin et al. reported a near-infrared fluorescent probe 59 for H\(_2\)S detection. The probe was composed of a cyanine dye, a piperazine linker and an 8-aminooquinoline ligand. After removal of Cu\(^{2+}\) by H\(_2\)S, the probe gave a turn-on response. The probe 59 showed \(\Phi = 0.11\), and the detection limit was 280 nM.

Nagano synthesized a series of azamacroyclic Cu\(^{2+}\)-complex fluorescent probes 60a–d for H\(_2\)S detection. The probe 60b exhibited high sensitivity and selectivity in the detection of H\(_2\)S, and the recognition reaction finished within seconds. It showed a 50-fold fluorescence increase upon the addition of H\(_2\)S. Probe 60b could be used to detect H\(_2\)S produced by 3-mercaptopyruvate sulfurtransferase (3-MST), pseudoenzymatic H\(_2\)S release, and intracellular H\(_2\)S in HeLa cells.

Bai et al. reported an 8-hydroxyquinoline-appended fluorescein derivative 61 for H\(_2\)S detection. The fluorescence of the probe was quenched by Cu\(^{2+}\), which resulted in an off–on type probe for S\(^{2–}\) detection with a 5-fold increase in the fluorescence intensity. The probe was able to reversibly indicate S\(^{2–}\) and Cu\(^{2+}\) changes in turn, and could detect H\(_2\)S in HeLa cells.

Li et al. reported a water-soluble fluorescent probe 62 based on a 1,1′-bi-2-naphthol derivative for H\(_2\)S detection. The metal ligand of the probe was 1,4,7,10-tetraazacyclododecane. This probe could recognize Cu\(^{2+}\) and S\(^{2–}\) in an on–off–on mode. The detection limit for the determination of S\(^{2–}\) was 1.6 \(\times\) 10\(^{-5}\) M.

Shen et al. designed a fluorescent probe based on phenanthrene-fused dipyrromethene analogue 63 for H\(_2\)S detection. Upon treatment with H\(_2\)S, the probe showed a 14-fold fluorescence increase. The probe could be used as a turn-on fluorescent probe for detecting H\(_2\)S in HeLa cells.

Zang et al. designed a water-soluble fluorescent probe for H\(_2\)S detection based on a 3-(1H-benzimidazol-2-yl)-2-hydroxybenzoate sodium derivative, 64. The probe was capable of the detection of Cu\(^{2+}\) and S\(^{2–}\) ions at physiological pH. The displacement mechanism the probe employed was supported by the fluorescence lifetime data. Free probe 64 exhibited \(\Phi = 0.1063\). The detection limitation of S\(^{2–}\) was determined to be 2.51 \(\times\) 10\(^{-6}\) M.

Long et al. developed a fluorescent probe 65 for the detection of H\(_2\)S based on the displacement method. The released compound 65 gave a 20-fold fluorescence intensity increase. The probe could be used to detect H\(_2\)S in MCF-7 cells. The detection limit was 0.18 \(\mu\)M.

Zhu et al. developed a colorimetric probe 66 based on borondipyrromethene-Cu\(^{2+}\) for the detection of H\(_2\)S in aqueous media. The probe displayed a 50 nm red-shift in absorption upon the addition of H\(_2\)S in solution. The ratio of the absorbance showed a 34-fold ratiometric increase. The detection limit was 1.67 \(\times\) 10\(^{-7}\) M.

Tang et al. reported the 2-[2′-aminophenyl]benzimidazole derivative fluorescent probe 67 for recognition of Cu\(^{2+}\) and S\(^{2–}\) in aqueous solution. The probe displayed an excited-state intramolecular proton transfer (ESIPT) mechanism, and the detection limit was 9.12 \(\times\) 10\(^{-7}\) M.

Ramesh et al. synthesized a near-infrared ratiometric indole functionalized rhodamine derivative probe 68 for the detection...
of Cu$^{2+}$ and S$^{2-}$. The probe employed the resonance energy transfer (RET) mechanism for the detection of Cu$^{2+}$, the process of which involved a donor indole and an acceptor Cu$^{2+}$ bound rhodamine moiety. The Cu$^{2+}$ complex gave an on-off response in the presence of S$^{2-}$. The Cu-complex probe 68 showed a 600-fold decrease of fluorescence intensity upon the addition of S$^{2-}$. The probe could detect Cu$^{2+}$ and S$^{2-}$ in HeLa cells.

**Fluorescent probes for sulfane sulfurs**

Sulfane sulfurs are the uncharged form of sulfur (S$^0$), and are attached to proteins through a covalent bond between the S$^0$ atom and other sulfur atoms, such as elemental sulfur (S$_8$), persulfides (R–S–SH), polysulfides (R–S–S–S–R), thiosulfate (S$_2$O$_3$$^2-$), polythionates (SO$_4$$^2-$), disulfides and so on. As members of the family of reactive sulfur species, sulfane sulfurs exhibit important physiological functions including cellular signal transduction and physiological regulation. The emerging evidence suggests that sulfane sulfurs may be the real signal transduction molecules for cellular events. The rapid production and clearance of H$_2$S in several biochemical pathways also depends on the metabolic process of sulfane sulfurs.

Xian et al. reported fluorescent probes 69, 70 for the detection of sulfane sulfurs. The detection mechanism was similar to that of probe 44, which is also illustrated in Fig. 5b. Sulfane sulfur compounds are reactive and labile, and there often exist thiosulfoxide tautomers. Therefore, a sulfur atom of a sulfane sulfur could react with nucleophilic groups such as the mercapto group to produce a reactive intermediate, which immediately underwent an intermolecular cyclization reaction to release the fluorophore. The fluorescence intensity of both probes increased 50-fold and 25-fold upon the detection of sulfane sulfurs. Probe 69 was used to detect sulfane sulfide both in H9c2 and HeLa cells. The detection limits were 32 nM (for 69) and 73 nM (for 70).

**Fluorescent probes for SO$_2$ and its derivatives**

Sulfur dioxide (SO$_2$) has long been considered as an air pollutant. People who are exposed to SO$_2$ may suffer from respiratory diseases and cancer. SO$_2$ is rapidly hydrated to sulfite (SO$_3$$^2-$) and bisulfite (HSO$_3^-$) in neutral solutions (3:1 M/M). However, endogenous SO$_2$ can be produced from the degradation of sulfur-containing amino acids. SO$_2$, and its derivatives, may have physiological roles in the regulation of cardiovascular function in synergy with NO. Sulfite is also used in food as an antioxidant to prevent bacterial growth. The design strategies of fluorescent probes for SO$_2$ detection are mainly inspired by the nucleophilic properties of SO$_2$ (including SO$_3$$^2-$ and HSO$_3^-$). The reaction mechanisms of these fluorescent probes can be sorted according to the nucleophilic addition to aldehydes/ketones (Fig. 7a) and the nucleophilic addition to double bonds (Fig. 7b). SO$_2$ and its derivatives selectively add to aldehydes/ketones or to double bonds, leading to changes in the electron-withdrawing effects.
or the π-conjugated systems of the probes, which can cause a response in terms of the fluorescence signal.

**Fluorescent probes based on aldehyde/ketone addition**

Chang et al. reported fluorescent probe 71 for sulfite detection.\(^{78}\) Sulfite could selectively deprotect the resorufin levulinate moiety of the probe. When sulfite reacted with the carbonyl carbon of the levulinate, a sulfite-added tetrahedral intermediate was formed. The intermediate would next undergo an intramolecular cyclization reaction to release the fluorophore. The fluorescence increase was up to 57-fold. The probe was used to detect sulfite in aqueous solution. The detection limit was 4.9 \(\times 10^{-5}\) M.

Guo et al. synthesized four coumarin-based fluorescent probes 72a–d for the detection of bisulfite.\(^{79}\) Bisulfite could selectively attack the aldehyde moieties of the probes. The quantum yields of products of 72a–d were 0.33, 0.62, 0.52 and 0.26, respectively. These probes were used to detect sulfite in granulated sugar. The detection limit was 1.0 \(\times 10^{-6}\) M.

Yang et al. developed a rhodamine-based fluorescent probe 73 for the detection of bisulfite.\(^{80}\) Bisulfite could react with the aldehyde to form an aldehyde–bisulfite adduct. Probe 73 also changed from a spirolactam (nonfluorescent) to a ring-opened spirolactam structure, increasing the fluorescence emission. The probe could detect bisulfite in aqueous media. The detection limit was 8.9 \(\times 10^{-7}\) M. These authors also presented a ratiometric fluorescent probe 4-(1H-benzimidazol-2-yl)benzaldehyde (74) for bisulfite detection.\(^{81}\) The aldehyde moiety reacted with bisulfite to produce a bisulfite adduct, which resulted in different electron-withdrawing effects and triggered the ICT process. The probe could detect bisulfite in aqueous media. The detection limit was 0.4 \(\mu\)M.

Feng et al. developed a coumarin-based fluorescent probe 75 for the detection of bisulfite.\(^{82}\) The nucleophilic addition reaction with aldehyde would switch-on the fluorescent probe for bisulfite, initiating the ICT process. The aldehyde–bisulfite adduct produced had a quantum yield of 0.43. The probe could detect bisulfite in solution and in HeLa cells. The detection limit was 3.0 \(\mu\)M.

Guo et al. designed a C═N isomerization-based fluorescent probe 76 for bisulfite detection.\(^{83}\) The C═N isomerization could be inhibited by the intramolecular N⋯H⋯N═C hydrogen bond. The formation of the hydrogen bond would block the C═N rotations, and fix the molecular structures in place,
resulting in minimizing the nonradiative energy of the excited state. The quantum yield was 0.374. The probe could detect bisulfite in locally granulated sugar; the detection limit was 0.1 mM.

**Fluorescent probes based on double bond addition**

Guo et al. reported a coumarin–hemicyanine fluorescent probe 77 for bisulfite and sulfite detection. The nucleophilic attack of SO$_3^{2-}$/HSO$_3^-$ on the double bond interrupted the $\pi$-conjugated structure of probe 77. As a result, the emission profiles before and after adding SO$_3^{2-}$/HSO$_3^-$ shifted due to the distinct emissions of the coumarin–hemicyanine fluorophore and the produced coumarin fluorophore. The probe gave a 1110-fold increase in the ratiometric signal. The probe could be used to detect SO$_3^{2-}$/HSO$_3^-$ in HeLa cells. The detection limit was 0.38 mM.

Li et al. reported colorimetric and ratiometric fluorescent probes 78a–d for the detection of sulfite. These probes were based on the fluorophore 7-diethylamine coumarin, which conjugated with the cyano group through double bonds. Probes 78a and b provided the best responses to SO$_3^{2-}$. SO$_3^{2-}$ underwent a Michael addition to the $\alpha,\beta$-unsaturated double bond, which interrupted the intramolecular charge transfer (ICT) process of the probe. The probes could detect sulfite in solution. Probe 78a had a 232-fold intensity ratio increase. The detection limit was 58 mM. The same group also presented near-infrared fluorescent probes 79a and b for the colorimetric and ratiometric detection of SO$_3$ derivatives. The probes were composed of coumarin fluorophores and 2-dicyanomethylene-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran, which had three cyano groups. Probe 79a possessed better water solubility and electron-withdrawing properties. The signal ratio at two different wavelengths could increase 775-fold within 90 seconds. The probe was used to detect SO$_2$ derivatives in U-2OS cells, and the detection limit was 0.27 nM.

Weng et al. reported fluorescent probes (80a and b) based on 4-hydrazinyl-1,8-naphthalimide for SO$_3^{2-}$/HSO$_3^-$ detection. The electron donating pyrrole moiety in probe 80a was responsible for fluorescence quenching through the photoinduced electron transfer (PET) process. The SO$_3^{2-}$/HSO$_3^-$ could form hydrogen bonds with the pyrrole moiety, and the PET process was blocked. Probe 80a gave a 65-fold fluorescent increase upon the detection of sulfite. The probe could be used to monitor the SO$_2$ released from an SO$_3$ donor in real time and could detect SO$_3^{2-}$/HSO$_3^-$ in GES-1 cells. The detection limit was 0.56 mM.

Chao et al. developed azo group-bridged dinuclear iridium(III) complexes 81a and b as phosphorescent probes for the detection of SO$_3^{2-}$/HSO$_3^-$. When the azo group was incorporated into ruthenium(n) complexes, it became more reactive and could react with SO$_3^{2-}$/HSO$_3^-$. Moreover, the azo group is an electron-withdrawing group which could quench the luminescence of luminescent metal complexes employing the metal-to-ligand charge-transfer (MLCT) mechanism. After the SO$_2$ derivatives underwent nucleophilic addition to the azo group, probe 81b became luminescent. The phosphorescence responses
showed a 26-fold and 27-fold increase for sulfite and bisulfite. Probe 81b was able to detect external and endogenous SO$_2^{2-}$/HSO$_3^-$ in HepG2 cells. The detection limits were 0.24 μM for SO$_3^{2-}$ and 0.14 μM for HSO$_3^-$.

Conclusions and prospects

In this feature article, we have summarized the synthetic and design strategies in the development of reaction-based fluorescent probes which are classified by the reaction types between analytes (H$_2$S, sulfane sulfurs and SO$_2$ derivatives) and probes. According to the reaction types, the probes are illustrated through and explained by examples: (a) H$_2$S reductive reactions: reducing azides to give amines, reducing nitro/ azanol to give amines, and reducing selenoxide to give selenide; (b) H$_2$S nucleophilic reactions: Michael addition reaction, dual nucleophilic reaction, double bond addition reaction, and thiolysis reaction; (c) copper sulfide precipitation reaction. The detection of sulfane sulfurs is mainly based on the nucleophilic addition reaction, and the detection of SO$_2$ derivatives is based on the nucleophilic and reductive properties of SO$_2$ derivatives.

Rapid recent developments in fluorescent probes for reactive sulfur species will likely prove to further facilitate analysis by fluorescence bioimaging technology. Compared with traditional methods, the detection of reactive sulfur species via fluorescence spectrometry can lower the external influence on the endogenous species distribution, can reduce the time of sample preparation, and can achieve real-time detection, in line with the high reactivity of these species. Despite the fact that reaction-based probes deliver us unique and versatile approaches for examining a wide range of reactive species in chemical and biological systems, there also exist many obstacles in terms of new reaction types, selectivity, sensitivity, response time, and fundamental applications. Although the reported detection limits are down to micromolar or even nanomolar levels, fluorescent probes for the detection of original reactive sulfur species and their distribution under normal physiological conditions are very rare. The distribution of reactive sulfur species in cells can be elucidated by probes which have targeting functions, and the quantitative analysis of reactive sulfur species will likely prove to further facilitate analysis.

Future reaction-based design principles must pay attention to the endogenous reactive small molecules under normal physiological conditions. Regardless of the position of the probes in the spectrum (visible region or near-infrared range), desirable probes should respond well to a minor concentration change, give dependable results, and meanwhile avoid interference from native cellular species, particularly biomolecular species such as glutathione and cysteine, and so on. All these will require the probes to exhibit good selectivity, high sensitivity, good photostability, low cytotoxicity, suitable water solubility, and the ability to work within the physiological pH range. Real applications of fluorescent probes, especially in clinical diagnostic imaging, is the ultimate goal.

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Notes and references
