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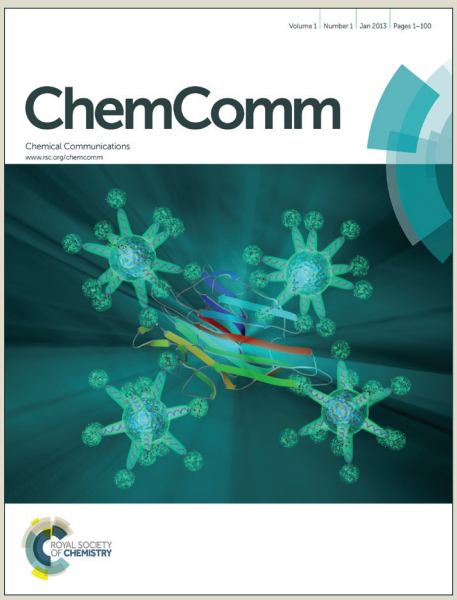

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FEATURE ARTICLE

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, which can provide attractive facilities for investigating physiological and pathological processes of interests with high spatial and temporal resolution, less invasiveness, and rapid response. Due to the versatile roles of hydrogen sulfide (H_2S) in cellular signal transduction and intracellular redox status regulation, the fluorescent probes for the third signalling gasotransmitter detection become bloom in recent years. These probes can offer powerful artifices to investigate physiological actions of H_2S in native environments without disturbing its endogenous distribution. In this feature article, we address the synthesis and design strategies for the development of fluorescent probes based on the reaction-type between H_2S and probes. Moreover, we also highlight the fluorescent probes for other reactive sulfur species, such as sulfane sulfurs and SO_2 derivatives.

Introduction

Fluorescence bioimaging technology has been distinguished readily from biological detection technologies by its several advantages, such as good sensitivity, excellent selectivity, rapid response, and non-invasive detection. In biological systems, the natures of the physiologically active species often involve several features including low concentration, high reactivity and short lifetime. Therefore, there still remains a huge challenge to determine the intracellular concentration of these species accurately. In order to meet these urgent needs, the reaction-based fluorescent probes have been emerging.[1,2]

In general, the design and synthesis strategies for these probes are depending on the physiologically active species' chemical properties. The reaction-based fluorescence probes are mainly composed of two moieties: the fluorescent signal transducer and the fluorescent modulator (**Fig. 1**). The moiety of fluorescent signal transducer takes responsibility for transducing molecular recognition into fluorescence signal that can be detected. It is essential to choose a suitable fluorophore platform as signal transducer. High quantum yield, photostability property and bio-compatibility are critical for bioimaging, because the minimum dosage of probe can avoid disturbing the natural distribution of the physiologically active species. The moiety of fluorescent modulator manipulates the molecular recognition process. A desirable fluorescent modulator can only be triggered by a single reaction switch. The selected reaction is also screened with reasonable reaction kinetic under physiological conditions. After integrating modulator into transducer via conjugated or space bridge, a reaction-based fluorescent probe is available (**Fig. 1**). All the factors that can respond the fluorescent properties will be employed to output the 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. The turn-on signal will provide the ease of measuring low-concentration against to a dark background, which can reduce the false positive signal and increase sensitivity. The appropriate fluorescent probes also possess near-infrared absorption and emission spectra (including two-photon and multi-photon), because the light in this region can maximize tissue penetration while minimizing the absorbance of heme in hemoglobin and myoglobin, water, and lipids. Moreover, the ratiometric probes benefit the ratio of the emission intensity at two different wavelengths, the interference caused by factors such as uneven loading and the inhomogeneous distribution of fluorescent probes in cells can

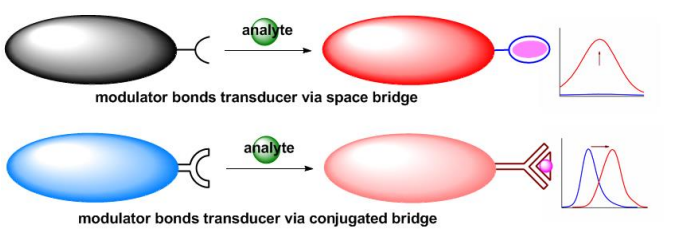


Fig. 1 Schematic illustration for conceiving fluorescent probes.

be cancelled out. Finally, the probes should have low cytotoxicity and suitable water-solubility.

The 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 involved 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.[3] H_2S has been identified as the third gasotransmitter following nitric oxide (NO) and carbon monoxide (CO). Under physiological level, H_2S regulates the intracellular redox status and fundamental signalling processes, including regulation of vascular tone, myocardial contractility, neurotransmission, and insulin secretion. The abnormal level of H_2S in cells will induce many diseases, such as Alzheimer's disease, liver cirrhosis, gastric mucosal injury and arterial and pulmonary hypertension.[4] In recent years, the H_2S-target fluorescence probes receive a booming development which benefits from chemical reactions of H_2S.

The fluorescent probes for GSH, Cys and Hcy have been well reviewed.[5,6] Hitherto, there is few review on H_2S fluorescence probes progress.[7] Now we overview the synthesis and design strategies for the development of fluorescent probes based on the reaction-type between H_2S and probes. We classify these probes according to the reaction types with H_2S. a) H_2S reductive reactions: reducing azides to give amines, reducing nitro/azanol to give amines, and reducing selenoxide to give selenide; b) H_2S nucleophilic reactions: Michael addition reaction, dual nucleophilic reaction, double bond addition reaction, and thiolysis reaction; c) copper-sulfide precipitation reaction. Moreover, we also introduce the fluorescent probes for other reactive sulfur species, such as sulfane sulfurs and SO_2 derivatives. 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.

Fluorescent probes base on reducing azides to amines

Azides and other oxidized nitrogen species can be reduced to amines by H_2S faster than GSH and other thiols, which exhibit a promising method for H_2S detection. After reduced by H_2S, the electron-withdrawing azido group will change into electron-donor amino group. Therefore, exploiting electron-donating ability of different substituent groups will result in versatile fluorescent probes (**Fig. 2**). The fluorescent probes which employ photoinduced electron transfer (PET) mechanism are typically constituted by connecting electron donor/acceptor recognition group to fluorophore via a space bridge. The design principles of such probes are clear, and the resulting phenomena will quench or increase the fluorescence of these probes (**Fig. 2a**). The fluorescent probes that adopt 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 fluorophore. Depending on ICT mechanism, the ratiometric probes can be available really (**Fig. 2b**). The variations of π-conjugated systems triggered by chemical reactions are often followed by an obvious alteration in spectroscopic properties, which is more advantageous to obtain turn-on/ratiometric fluorescent probes (**Fig. 2c**). Moreover, the design strategy that takes advantage of protective group masterly will result in a fantastic probe (**Fig. 2d**). The approaches mentioned above have since been widely adopted for H_2S detection (**Fig. 2**).

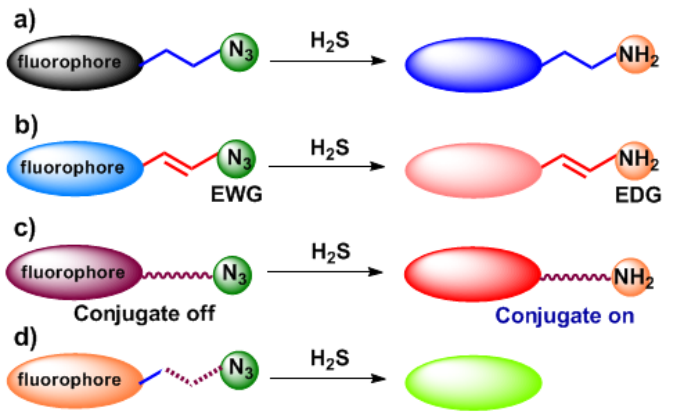
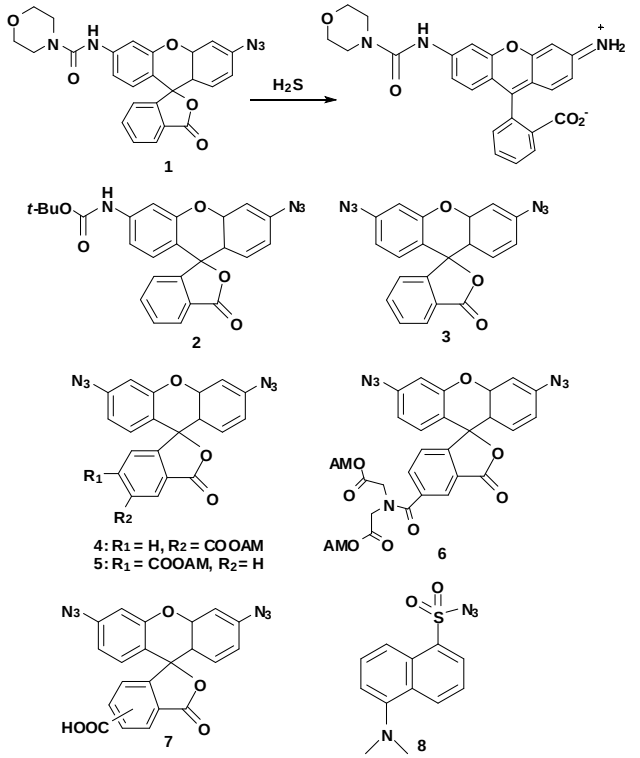


Fig. 2 Summary strategies for fluorescent probes based on reducing azides to amines.

Chang *et al.* exploited the selective H_2S-mediated reduction of azides to develop a series of fluorescent probes for intracellular H_2S detection (**1-6**)[8,9]. The fluorophore of these probes were based on Rhodamine 110. The detection mechanism was illustrated in **Fig. 2c.** After caged Rhodamine 110 by azides at 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 H_2S, the spiro-ring of **1-6** opened, and the π-conjugated structure recovered. Therefore, these H_2S probes would give turn-on response. Probe **1** and **2** could detect H_2S in live HEK293T cells using confocal microscopy, it took about 1h to saturate fluorescence response. Under test conditions, Probe **1** producted Φ = 0.60. Probe **2** producted Φ = 0.51. The detection limit of **1** was 10 μM. However, the concentration of H_2S changed acutely in cells. It was difficult for **1** and **2** to capture H_2S opportunely in cells. After a while, the same group optimized the design strategies and improved the sensitivity and cellular retention for new probes. They reported bis-azido probes (**3-6**) for increasing H_2S sensitivity, and they modified acetoxymethyl ester-protected carboxy group in the new probes to increase cellular trappability (**4-6**). It's worth noting that the probe **6** could direct, real-time detect endogenous H_2S which 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 H_2S production was related to NADPH oxidase–

derived hydrogen peroxide (H_2O_2). This experimental result would establish a link for H_2S/H_2O_2 crosstalk. Under test conditions, Probe **3**, **4**, **5** and **6** products had Φ = 0.92, 0.18, 0.18 and 0.17, respectively. Based on rhodamina 110 as fluorphore, Sun *et al.* also developed a fluorescent probe **7** to trap intracellular H_2S in HeLa cells.[10] Probe **7** displayed 120-fold turn-on response. The detection limit was 1.12×10^{-7} M.

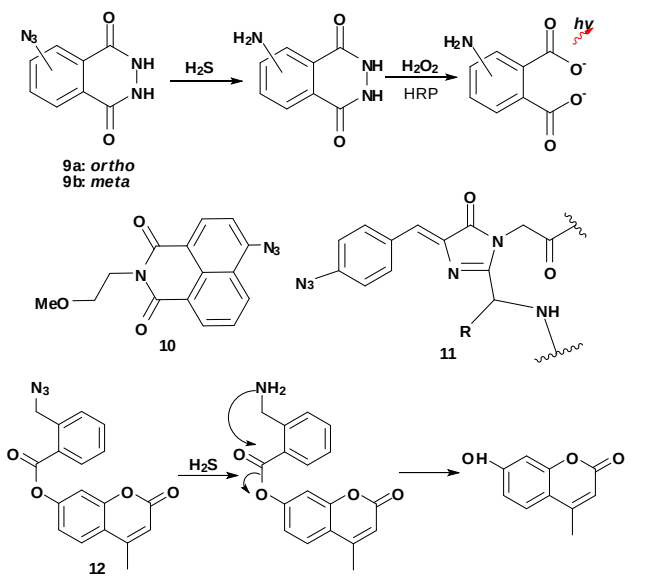
Wang *et al.* developed a turn-on fluorescent probe **8** for H_2S detection in aqueous solutions, blood serum and whole blood.[11] The detection mechanism of the probe was illustrated in **Fig. 2a.** When attached azido group to a strongly electron-withdrawing dansyl fluorophore, the reductive reaction would be accelerated. Probe **8** showed a fast response to H_2S within seconds, which made the quantitative H_2S detection possible regardless of the fast metabolism nature of H_2S in biological systems. The detection limit was 1μM in buffer/Tween and 5 μM in bovine serum. The H_2S concentrations in C57BL6/J mouse model blood was determined to be 31.9±9.4 μM by **8**. The addition of H_2S led to 40-fold fluorescence increase of probe **8**.



Pluth *et al.* reported two reaction-based chemiluminescent probes for H_2S (**9a** and **9b**).[12] Chemiluminescence need not excite by any source, there was little chance for photodegredation of the probe. Furthermore, chemiluminescent probe could avoid biological background interference. Therefore, chemiluminescent methods would provide high signal-to-noise ratios to H_2S detection. **9a** and **9b** combined H_2S-mediated azido group with luminol derived platform. After reduced by H_2S, chemiluminescence resulted from oxidation of the phthalhydrazide moiety which oxidized by H_2O_2 via horseradish peroxidase (HRP) as a catalyst. Probe **9b** was used to detect enzymatically produced H_2S from both isolated CSE enzymes and C6 cell lysates. Probes **9a** and **9b** had strong luminescence responses toward H_2S with 128- and 48-fold, respectively. The detection limits of **9a** and **9b** were 0.7 ± 0.3 and 4.6 ± 2.0 μM, respectively. The group also synthesized fluorescent probe **10** based on azido-naphthalimide.[13] When the probe **10** reacted with H_2S, the azide was reducted to amine following a turn-on fluorescent response. Probe **10** could detect H_2S in HeLa cells. However, GSH would cause interference. Probe **10** producted Φ = 0.096 ± 0.001. The detection limit was 5-10 μM.

Ai *et al.* reported encoded fluorescent proteins (FPs)-based probe **11** to detect H_2S.[14] FPs could self-sufficient in generating intrinsic chromophores from polypeptide sequences. They incorporated *p*-azidophenylalanine (*p*AzF) into peptides of FPs to obtain azide-derived chromophores. This genetically encoded probe could be used to monitor H_2S concentration changes in HeLa cells.

Han *et al.* designed and synthesized a fluorescent probe **12** based on utilization of *o*-(azidomethyl)benzoyl as the hydroxyl protecting group.[15] The detection mechanism of the probe was illustrated in **Fig. 2d**. When H_2S triggered the reduction of azido moiety, the fluorophore 7-hydroxy-4-methylcoumarin would be deprotection, and would release fluorescence. Probe **12** could be used to imaging H_2S in HeLa cells. The detection limit was 10 μM.



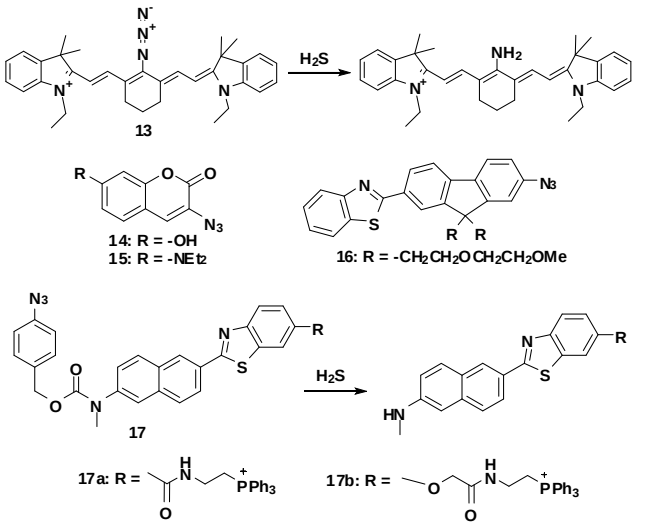
Han *et al.* reported a colorimetric and ratiometric fluorescent probe **13** for detecting H_2S.[16] The detection mechanism of the probe was illustrated in **Fig. 2b**. It was anticipated that the modulation of various electron-donating donors on a cyanine dye can affect its intermolecular electron density distribution. Therefore, controlling electron-donating ability of different substituent groups would result in ICT-induced blue or red

shifts in the emission spectrum of cyanine. When H_2S reduced the azide to amine, the near-infrared heptamethine cyanine platform shifted 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_2S by fluorescent ratio signal in water solution and fetal bovine serum. Probe **13** could monitor H_2S-release by ADT-OH. This probe was able to sense different H_2S 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 fluorenscent probes **14** and **15** for the detection of H_2S.[17] Probes **14** and **15** were both caged by azido grop. When reduced by H_2S, probe **15** showed higher increase of fluorescence intensity, because the electron-donating ability of -NEt_2 was stronger than that of –OH, which was controlled by stronger ICT effects. The products of **14** and **15** were Φ = 0.16 ± 0.013 and Φ = 0.58 ± 0.02. And probe **15** could detect the H_2S in rabbit plasma and PC-3cells.

Cho *et al.* reported a two-photon H_2S probe **16** for deep tissue imaging.[18] The probe employed 7-(benzo[d]thiazol-2-yl)-9,9-(2-methoxyethoxy)ethyl-9H-fluorene as fluorophore. After reduced by H_2S, the TP action cross section of **16** was 302 GM at 750 nm in HEPES buffer. The probe was able to detect endogenously H_2S in HeLa cells and could visualize the overall H_2S distribution at the depths of 90-190 μm in rat hippocampal slice. The products of **16** had Φ = 0.46. The detection limit was 5 - 10 μM. Cho *et al.* next reported another two-photon ratiometric probe **17** for H_2S detection in mitochondria.[19] The detection mechanism of probe **17** was illustrated in **Fig. 2b** and **2d**. 6-(benzo[d]thiazol-2′-yl)-2-(methylamino)naphthalene was selected as the fluorophore, and 4-azidobenzyl carbamate was chosen as the H_2S response-site. The mitochondrial targeting group was triphenylphosphonium salt. After reduced by H_2S, the fluorophore was released. Under test conditions, probe **17a** shifted its emission from 464 nm to 545 nm, the quantum yields of probe **17a** and its product changed from 0.24 to 0.12. And **17b** exhibited emission shift from 420 nm to 500 nm, the quantum yields from 0.23 to 0.50. There was a larger Stoke shift between the probe **17a** and its precursor due to **17a** had a more stable charge-transfer excited state. The two-photon ratiometric probe can be used to detect mitochondrial H_2S levels in living cells and tissues. The probe **17a** demonstrated the relationship between the cystathionine *β*-synthase expression level and the H_2S level in astrocytes. And the experiments showed that genetically mutated Parkinson's disease (PD) -related gene could affect H_2S production in PD patients' brains.

Peng *et al.* and Xu *et al.* reported a two-photo fluorescent probe **18** with near-infrared emission for the detection of H_2S.[20,21] The styrene group was introduced into fluorophore to extend the dicyanomethylenedihydrofuran's conjugation system. After reduced by H_2S, the two-photo action cross section of **18** was 50 GM at 820 nm in DMSO. The probe could give 354-fold fluorescence increase when detected H_2S. And this probe can detect H_2S in commercial fetal bovine serum, MCF-7 cells, HUVEC Cells, rat liver cancer slice and ICR mice. The reported detection limit was 3.05 μM.



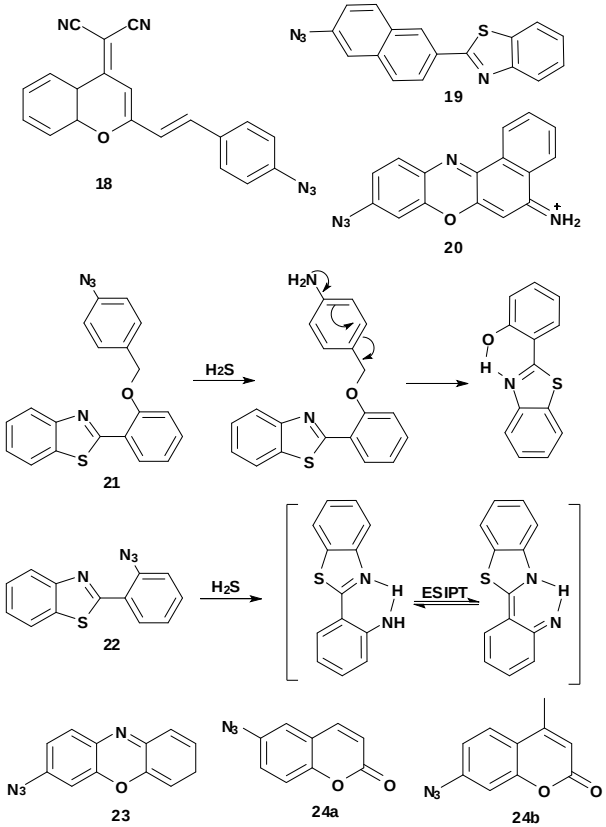
Zhang *et al.* synthesized a two-photo fluorescent probe **19** for the detection of H_2S.[22] This probe chose naphthalene derivative as two-photo fluorophore. The detection mechanism of probe **19** was shown in **Fig. 2b**. Probe **19** had a donor-π-acceptor (D-π-A) structure, the recognition unit azide acted as the electron withdrawing group which could break the D-π-A structure. While H_2S reduced azide to amine, the D-π-A structure recovered, the probe emitted strong fluorescence. The TP action 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_2S in HeLa cells. The detection limit was 20 nM.

Ma *et al.* reported a cresyl violet-based ratiometric fluorescent probe **20**.[23] The probe also had a donor-π-acceptor structure. When the azido group was reduced to amino group, the D-π-A structure changed from electron-withdrawing to electron-donating. The result would lead to a spectroscopic blue or red shift in emission, which could provide a ratiometric method for of H_2S detection with Φ = 0.44 and 0.54, respectively. This probe could be used to detect H_2S in MCF-7 cells and zebrafish by ratiometric imaging. The detection limit of the probe was 0.1 μM.

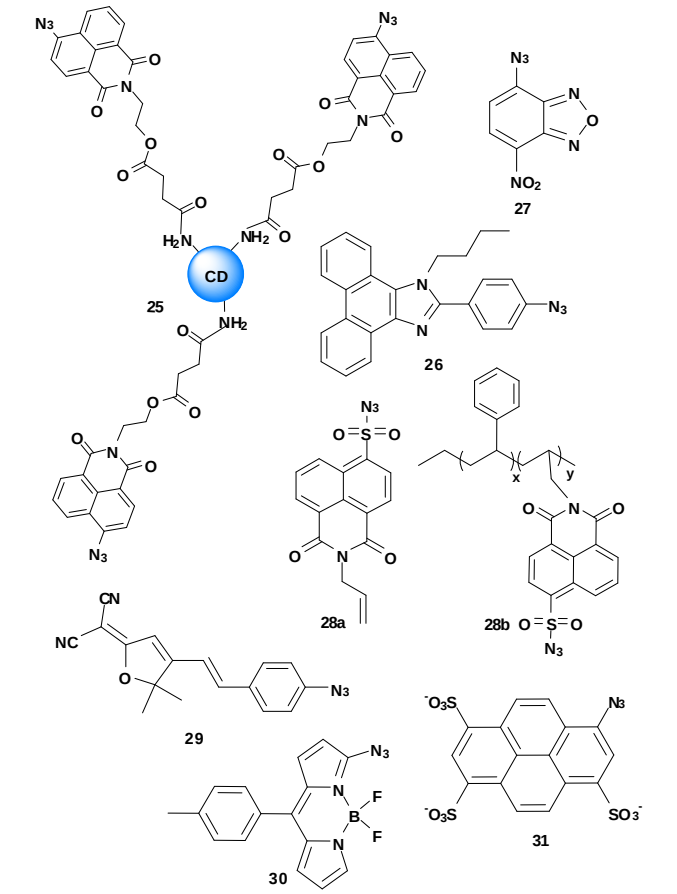
Chang *et al.* reported a ratiometric fluorescent probe **21** based on excited-state intramolecular proton transfer (ESIPT) mechanism for H_2S detection.[24] When the azido group was reduced to amino group, the *p*-aminobenzyl moiety undergone a self-immolate through an intramolecular 1,6-elimination to releasing the ESIPT dye 2-(2'-hydroxyphenyl)-benzothiazole (HBT). The ratio of emission intensity varied 43-fold. The probes that based on ESIPT mechanism often resulted in large Stokes shift. The application of ESIPT could design probes based on their unique sensitive nature to the enviroment. The probe was used to detect H_2S in HeLa cells. The detection limit was 2.4 μM.

Guo *et al.* reported a fluorescent probe 2-(2- azidophenyl) benzothiazole (**22**) based on ESIPT mechanism for H_2S detection.[25] The probe exhibited fluorescence response of 1150-fold with Φ = 0.4138. The probe was used to detect H_2S in B16 cells. The detection limit was 0.78 nM.

Tang *et al.* reported fluorescent probes **23** and **24** for H_2S detection.[26,27] Probe **23** was synthesized based on the fluorophore of phenoxazinon. Probe **23** showed 23-fold increase. The probe can detect H_2S in PBS buffer, fetal bovine serum, and Hela cells. With coumarin as fluorophore, they reported two-photon fluorescent probes **24a** and **24b** for H_2S detection. **24b** showed better selectivity and sensitivity than probe **24a**. The product of **24b** gave Φ = 0.88 ±0.02. Probe **24b** could detect H_2S from both exogenous addition and possible enzymatic production. The imaging of H_2S was achieved in the cardiac tissues of normal rats and atherosclerosis rats.



Zeng *et al.* integrated naphthalimide azide derivative into anchoring site carbon nanodots, and developed a fluorescence resonance energy transfer (FRET) ratiometric fluorescent probe **25**.[28] FRET was the interaction between two excited states fluorophores correlated with distance. The process contained the nonradiative transfer of excitation energy from an excited donor to a proximal ground-state acceptor, and it was convenient to design ratiometric probe which involved the ratio of two emission intensities at different wavelengths. In FRET systems, the emission wavelength of the donor was the excitation wavelength of the acceptor. Therefore, the regulation of precise energy match between carbon nanodots and naphthalimide azide derivative would be beneficial for H_2S detection. Probe **25** could detect H_2S in HeLa and L929 cells. The detection limit was 10 nM.



Lin *et al.* reported probe **26** phenanthroimidazole for H_2S detection.[29] The azido group could withdraw the electrons in phenanthroimidazole, which made the fluorescence weak. Upon treated the probe with H_2S, the fluorescence of the probe could reach saturation within 3 min. The product of **26** was Φ = 0.62. The probe could be used to detect H_2S in HeLa cells with detection limit 8.79 $\times 10^{-7}$ M.

Chen *et al.* reported 7-nitrobenz-2-oxa-1,3-diazole as a colorimetric and fluorescent probe **27** for H_2S detection.[30] When azide was reduced to amine, probe **27** followed by colormetric change from pale-yellow to deep-yellow. The increase of the fluorescence intensity was up to 16-fold. And the probe has been used in imaging the H_2S in living MCF-7 cells. The detection limit was 680 nM.

Wu *et al.* reported polymer-based fluorescent probe **28** for H_2S detection.[31] The monomer **28a** could be further functionalized to polymer **28b**. Na_2S induced 5-fold and 3-fold increase in the fluorescence intensity of **28a** and **28b**, respectively. Probe **28b** could detect H_2S in HeLa cells.

Xu *et al.* reported a fluorescent probe **29** for H_2S detection based on dicyanomethy lenedihydrofuran.[32] The product of **29** gave Φ = 0.018. The probe was able to detect H_2S in living human umbilical vein endothelial cells.

Talukdar *et al.* designed a colorimetric and fluorometric probe **30** for detection of H_2S.[33] The BODIPY-azide could be reduced to BODIPY-amide by H_2S with turn-on fluorescent response. The detection mechanism as was shown in **Fig. 2a**. The fluorescence of **30** was quenched by electron-rich *α*-nitrogen of the azido group. The probe displayed a fast response time in serum albumin (within 30s) with 28-fold fluorescence increase, and the detection limit was 259 nM. The probe was used to detect H_2S in HeLa cells.

Hartman *et al.* reported 8-azidopyrene-1,3,6-trisulfonate probe **31** for H_2S detection with 24-fold fluorescence increase.[34] The probe had a high water solubility at concentrations >100 mM. The probe could measure H_2S in serum.

Fluorescent probes based on reducing nitro groups to amines

There exists a major obstacle to design fluorescent probes by exploiting nitro fluorophores, because the nitro group has always been considered to be a strong quencher for fluorophores. However, the nitro group can be reduced by Na_2S to produce the corresponding amino group under mild conditions, which open a door to design and synthesize new type fluorescent probes containing nitro group for H_2S detection. Taking advantages of this reductive reaction, two mainly types of H_2S fluorescent probes have been developed. One type adopts photoinduced electron transfer (PET) mechanism, which benefit from the strong electron-withdrawing of nitro group (**Fig. 3a**). The other type uses internal charge transfer (ICT) mechanism, which results from a donor-π-acceptor structure caused by strong push-pull electronic effect (**Fig. 3b**).

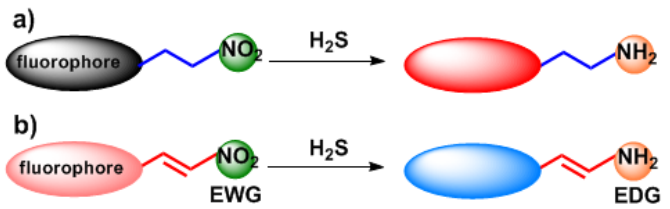


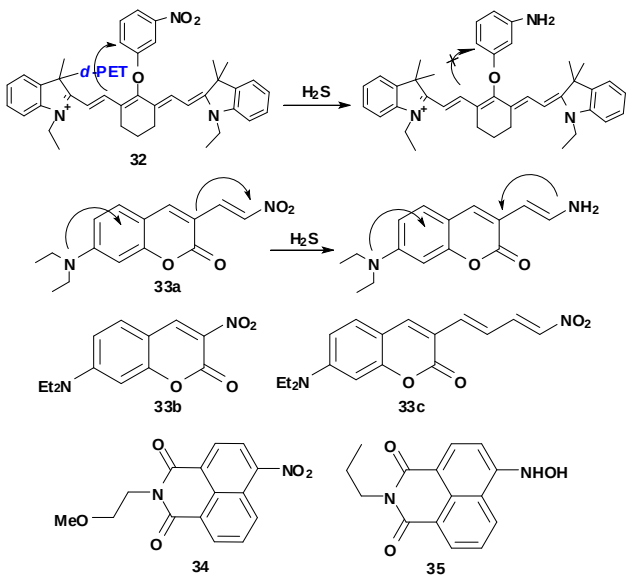
Fig. 3 Summary strategies for fluorescent probes based on reducing nitro groups to amines.

Chen *et al.* designed and synthesized a near-infrared fluorescent probe **32** for H_2S detection in HEPES buffer and in fetal bovine serum.[35] The probe involved PET mechanism while sensing H_2S (**Fig. 3a**). The fluorescence of heptamethine cyanine would be quenched via a photoinduced electron transfer (PET) process from the excited fluorophore to the strong electron-withdrawing of nitro group (donor-excited PET; *d*-PET).[3,36,37] On the other hand, while nitro group was reduced to amino group, there might exist an acceptor-excited PET (*a*-PET) process from amino group to the excited fluorophore since amino group was a strong electron-donating group with lone-pair electrons.[38,39] However, the probe displayed an increasing fluorescence emission, and the quantum yield increased from 0.05 to 0.11. This phenomenon was attributed to substituent effects, that is, the *meta*-position of aromatic ring was non-favorable to the effects of electron donor. The probe had been used to track H_2S in RAW264.7 cells.

Li *et al.* reported colorimetric and ratiometric fluorescent probes **33a-c** based on ICT-strategy for the detection of H_2S.[40] Probe **33a** covered a typical push–pull electronic system crossing coumarin fluorophore. After reacted with H_2S, the push-pull electron system was blocked by push-push electron system, which disturbed ICT mechanism leading to spectral shifts. The D-π-A structures of probes **33a-c** could devote to different sensitivity and selectivity. Reaction of probe **33a** with H_2S resulted in emission shift from 602 nm (Φ = 0.023) to 482 nm (Φ = 0.236). The detection limit of **33a** was 2.5 μM.

Pluth *et al.* reported a 4-nitro naphthalimide fluorescent probe **34** for the detection of H_2S.[13] The functionalization of 4-possition with amino and nitro in the naphthalimide fluorophore platform would result in fluorescence turn-on. Probe **34** was also response to Cys and GSH. Probe **10** producted Φ = 0.096 ± 0.001. The probe was used to detect H_2S in HeLa cells.

Wang *et al.* reported hydroxylamine naphthalimide (**35**) as H_2S fluorescent probe based on ICT mechanism.[41] During the reducing nitro groups to amines process, there would produce hydroxylamine derivatives as intermediate. The hydroxylamine moiety could be reduced more easily than nitro group. Moreover, hydroxylamine moiety was an electron-withdrawing group to naphthalimide, which would quench the fluorescence of naphthalimide. The product of **35** gave Φ = 0.12. The probe could be used to detect H_2S in astrocyte cells.



Fluorescent probes based on reducing selenoxide to selenide

As the active site of the antioxidant enzyme glutathione peroxidase (GPx), organoselenium compounds modulate cellular antioxidant defense systems to defense against reactive oxygen species (ROS) damage via the reduction of reactive oxygen species by bio-thiols. The oxidation-reduction reaction of selenoenzyme depends on a unique ping-pong mechanism between selenoxide and selenide.[42] Taking the advantage of the mimics of the catalytic cycle could develop fluorescent probes for reversible detection of H_2S. Due to selenoxide and selenide are electron-withdrawing group and electron-donating group respectively, the fluorescent probes can be smoothly achieved by photoinduced electron transfer (PET) mechanism (**Fig. 4a**) and internal charge transfer (ICT) mechanism (**Fig. 4b**).

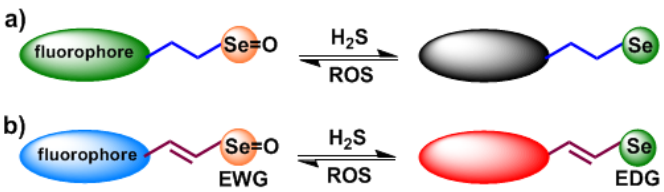
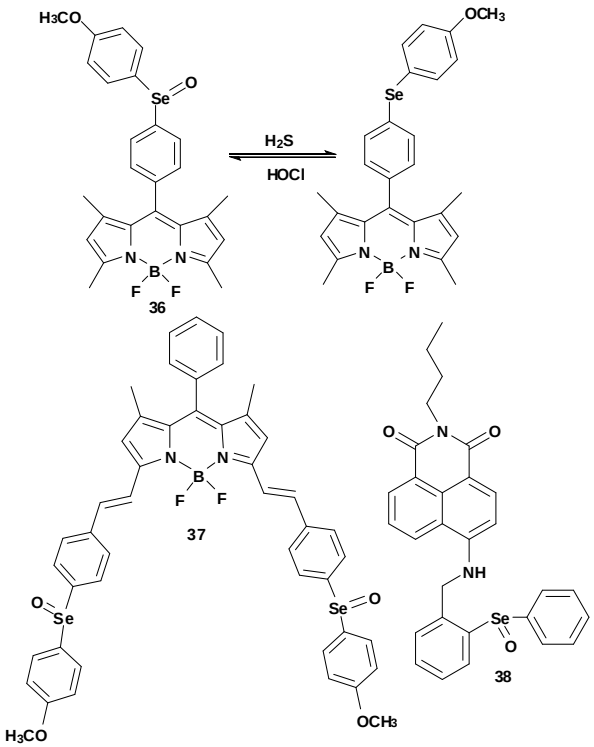


Fig. 4 Summary strategies for fluorescent probes based on reducing selenoxide to selenide.

Han *et al.* developed a series of fluorescence probes (**36, 37, 38**) containing organoselenium moieties that could be used for monitoring the redox cycles between H_2S and ROS. The reversible fluorescence probe **36** could detect the redox cycle between HClO and H_2S. The function mechanism was as shown in **Fig. 4a**. The probe employed BODIPY fluorophore as the signal transducer and 4-methyoxylphenylselanyl benzene (MPhSe) as the modulator.[43] The fluorescence of **36** was quenched as a result of PET between the modulator and the transducer, but Se oxidation 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 H_2S in RAW264.7 cells.

After integrated the modulator (4-methyoxylphenylselenide, MPhSe) into BODIPY platform through a styrene bridge, the probe **37** could function as a near-infrared reversible ratiometric fluorescence probe for the redox cycle between HBrO oxidative stress and H_2S.[44] The function mechanism was as shown in **Fig. 4b**. This approach could facilitate the D-π-A conjugation system and tune the fluorescent emission red-shift efficaciously by 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 had been successfully used to detect the HBrO/H_2S redox cycle in the mouse macrophage cell line RAW264.7.

The probe **38** was based on 1,8-naphthalimide fluorophore.[45] There existed the PET process in **38** (selenide-form) which confirmed by time-dependent density functional theory calculations. However, there also was an excited state configuration twist process in 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 HOCl oxidative stress and H_2S reducing repair in RAW 264.7 cells and in mice.



Fluorescent probes based on the nucleophilic reactions of H_2S

H_2S is a reactive nucleophilic species that can participate in nucleophilic substitution in vivo. The major challenge for H_2S detection is to distinguish H_2S from other biological nucleophiles, such as cysteine and glutathione, which are at micromolar or millimolar concentrations inside most cells. The pKa of H_2S is ~ 7.0 in aqueous solution, whereas other bio-thiols have higher pKa values (Cys: ~ 8.3, GSH: ~ 9.2). Therefore, H_2S is considered to be a stronger nucleophile than other biothiols in physiological media. H_2S can undergo dual nucleophilic reaction as a non-substituted biothiol, however mono-substituted thiols can only take place nucleophilic reaction once. Based on the nucleophilic and dual nucleophilic properties, the fluorescent probes containing bis-electrophilic enters have been devised for H_2S detection. As shown in **Fig. 5a** and **5b**, H_2S can react with the more electrophilic moiety of the fluorescent probe to form an intermediate containing free mercapto (-SH). If another electrophilic site is presented at a suitable position, such as the *ortho*-ester group or α,β-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 **5b** can also react with other biothiols such as Cys and GSH. However, the intermediates cannot continue the next cyclization reaction. Therefore, the fluorescent signal does not suffer from the interference which caused by other biothiols. As a strong nucleophile, H_2S also can interrupt the π-conjugation of the probe, thereby leading to change the probe's emission wavelength (**Fig. 5c**). The removal of strong electron-withdrawing group by H_2S can release the fluorophore (**Fig. 5d**), this strategy will expect to give turn-on fluorescent probes.

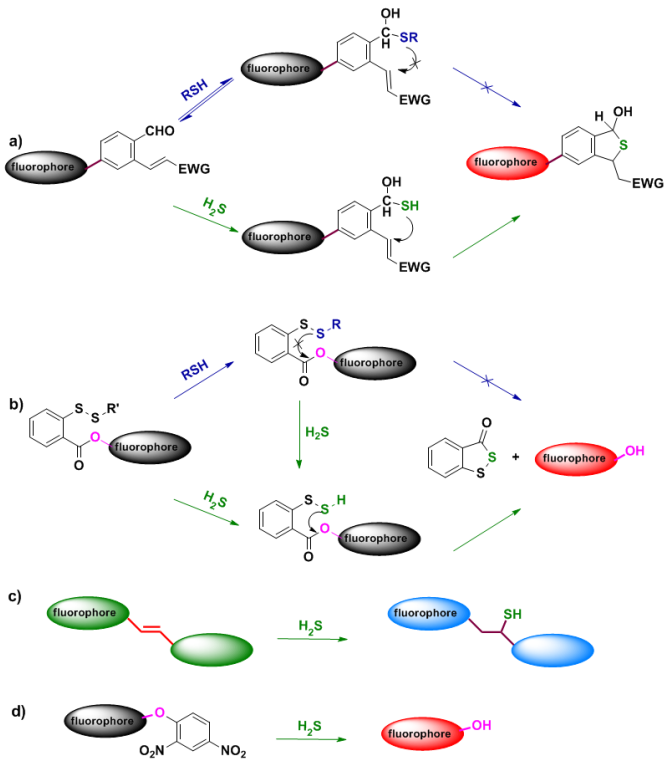


Fig. 5 Summary strategies for fluorescent probes based on the nucleophilic property of H_2S.

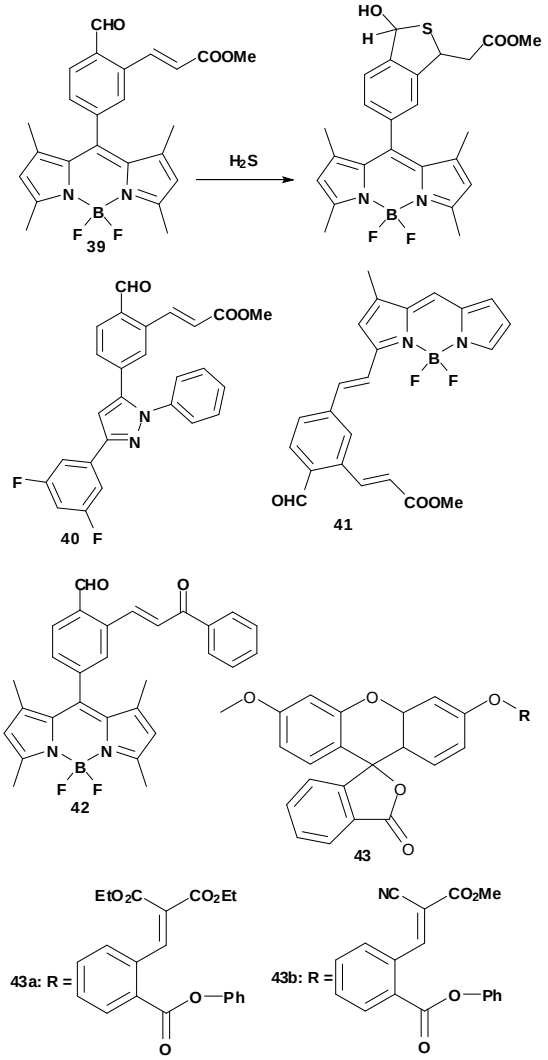
Fluorescent probes based on Michael addition reaction

Qian *et al.* reported two H_2S-selective fluorescent probes **39** and **40**.[46] The probe **39** employed BODIPY as fluorophore, and the probe **40** exploited 1,3,5-triaryl-2-pyrazoline as fluorophore. The H_2S recognition moiety was consisted of an aromatic framework which was substituted by *α,β*-unsaturated acrylate methyl ester and *ortho*-aldehyde (–CHO). The aldehyde group could reversibly react with H_2S to form a hemithioacetal intermediate, which was ready for Michael addition to the proximal acrylate to yield thioacetal (**Fig. 5a**). This tandem reaction could block the PET process, and the probe provided a turn-on response to H_2S. The response of probes **39** and **40** to H_2S with Φ = 0.208 and 0.058. Fluorescent probes **39** and **40** could detect H_2S in HeLa cells.

Li *et al.* reported an ICT-based turn-on fluorescent probe **41** for H_2S detection.[47] The aryl ring which substituted by *ortho*-aldehyde and *α,β*-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_2S in RAW 264.7 macrophage cells. The detection limit was 2.5 μM.

Zhao *et al.* reported a BODIPY-based probe **42** to for H_2S detection.[48] The probe was designed via replacing the *ortho*-acrylate ester with an *α,β*-unsaturated phenyl ketone. The probe was able to response to sulfide the bovine plasma, and the reaction was completed within 120 s at room temperature. The product of **42** gave Φ = 0.10. This fast-response probe offered that the average sulfide concentration in four mice blood plasma was 56.0 ± 2.5 μM. The average sulfide concentration in four C57BL6/J mice brain tissues was estimated to be 7.1 ± 1.4 μmol g^{-1} protein.

Xian *et al.* reported a fluorescent probe **43** for H_2S detection.[49] The design strategy was based on a Michael



addition of H_2S following an intramolecular cyclization to release the fluorophore. Because biothiols could react readily with Michael acceptors at physiological pH at a rapid equilibrium process, monosubstituted biothiols would not consume the probes with the reversible reactions. Probe **43a** and **43b** led to 11-fold and 160-fold turn-on response, respectively. The probe **43b** could detect H_2S in COS7 cells. The detection limit was found to be 1 μM.

Fluorescent probes based on dual nucleophilic reactions

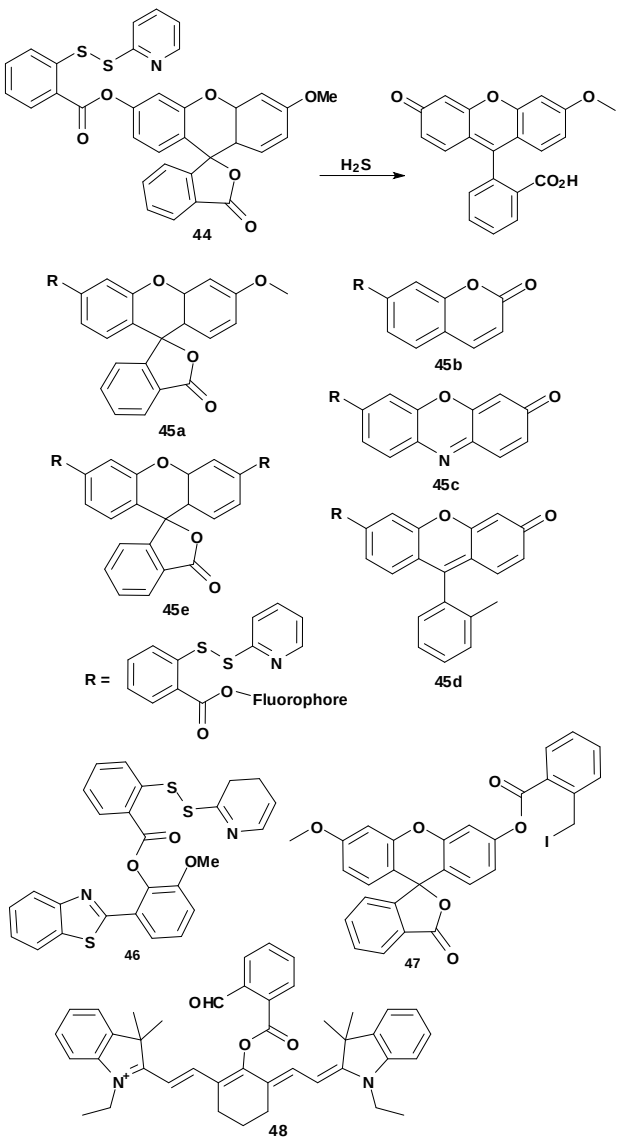
Xian *et al.* designed a series of turn-on probes (**44, 45**) to detect H_2S based on the dual nucleophilic property.[50,51] These fluorescent probes contained reactive disulfide groups. H_2S could react with the disulfide group to give a free -SH containing intermediate, the intermediate next undergone a spontaneous cyclization to release the fluorophore. Other monosubstituted biothiols, such as Cys and GSH, did not lead to interference. The probe **44** could be used to detect H_2S in bovine plasma COS7 cells. The fluorescence quantum increased from 0.003 to 0.392. Xian *et al.* expanded this strategy to prepare and test the probes **45a-e**. The fluorophores, methoxy fluorescein, 7-hydro- xycoumarin, resorufin, and 2-methyl TokyoGreen were chosen as the fluorescent signal transducers. **45d** and **45e** could be used for the production from H_2S donor YZ-4-074 in HeLa cells. The intensities increased 130-, 275-, 68-, 20-, and 60-fold for **45a-e**, respectively. And the detection limits were determined to be 60, 79, 47, 266, and 47 nm for **45a-e**.

Qian *et al.* reported a ratiometric fluorescence probe **46** based on excitedstate intramolecular proton transfer (ESIPT) mechanism for H_2S detection.[52] The fluorophore 2-(2'-hydroxyphenyl) benzothiazole could exhibit a ratiometric detection capability following large Stokes shift. The recognition reaction completed within 2 min. The probe showed a 30-fold fluorescence increase. The probe could be used to detect H_2S in HeLa cells. The detection limit was 0.12 μM.

Guo *et al.* reported a methylfluorescein-based probe **47** for the detection of H_2S.[53] 2-(iodomethyl) benzoate was chosen as H_2S trap group. While exposed to H_2S, the H_2S-induced substitution-cyclization reaction took place smoothly for releasing the fluorophore. The product of **47** gave Φ = 0.379. The probe could detect H_2S in COS-7 cells. The detection limit was 0.10 μM.

Tang *et al.* reported a near-infrared ratiometric fluorescent probe **48** for the detection of H_2S.[54] 2-carboxybenzaldehyde was selected as the H_2S sensing group, with aldehyde and ester as the dual nucleophilic addition position. After reacted with H_2S, the released fluorophore cyanine benefited from tautomerism between enol form and ketone form to give ratiometric detection. There was a 2500-fold increase in ratio response. The probe could target mitochondria and detect the H_2S in HepG2 and A549 cells. The detection limit was 5.0-10 nM.

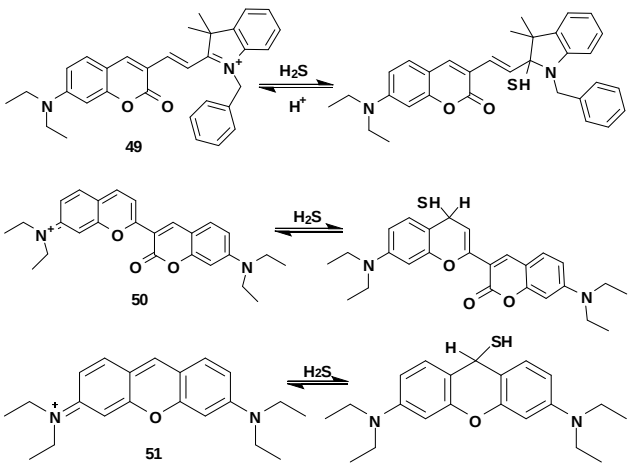
Fluorescent probes based on double bond addition reaction



He *et al.* designed a ratiometric fluorescent probe **49** for H_2S detection.[55] Probe **49** could be considered as a hybrid fluorophore of coumarin and merocyanine through an ethylene group. The probes benefited from the fast HS^- nucleophilic addition to merocyanine moiety in medium of near neutral pH value. After HS^-, the main form of H_2S under the physiological condition, added to the indolenium C-2 atom of **49**, the π-conjugation of the probe was disturbed by eliminating merocyanine emission but retaining coumarin emission, which caused the fluorescent spectrum shifted. The probe had rapid response to the H_2S concentration changes in solution and cells, which were completed within 30 and 80s, respectively. The intensity ratio of **49** increased over 120-fold. This probe could be applied for detecting of H_2S level changes in mitochondria of MCF-7 cells.

Guo *et al.* reported a ratiometric fluorescent probe **50** based on a flavylium derivative and a commercially available

pyronine dye **51** for H_2S detection.[56] Probe **50** gave turn-off response due to the interruption of π-conjugation of pyronine ring. The probe could detect H_2S based on the selective nucleophilic attack of H_2S to the electrically positive benzopyrylium moiety, which would interrupt the π-conjugation, thereby leading to the changes of the emission profile. Probe **50** could provide ratiometric fluorescent response within 10s. There was a 1200-fold increase in ratiometric value. Probe **50** could be used to detect H_2S in HeLa cells and in human serum. The detection limit was 0.14 μM.



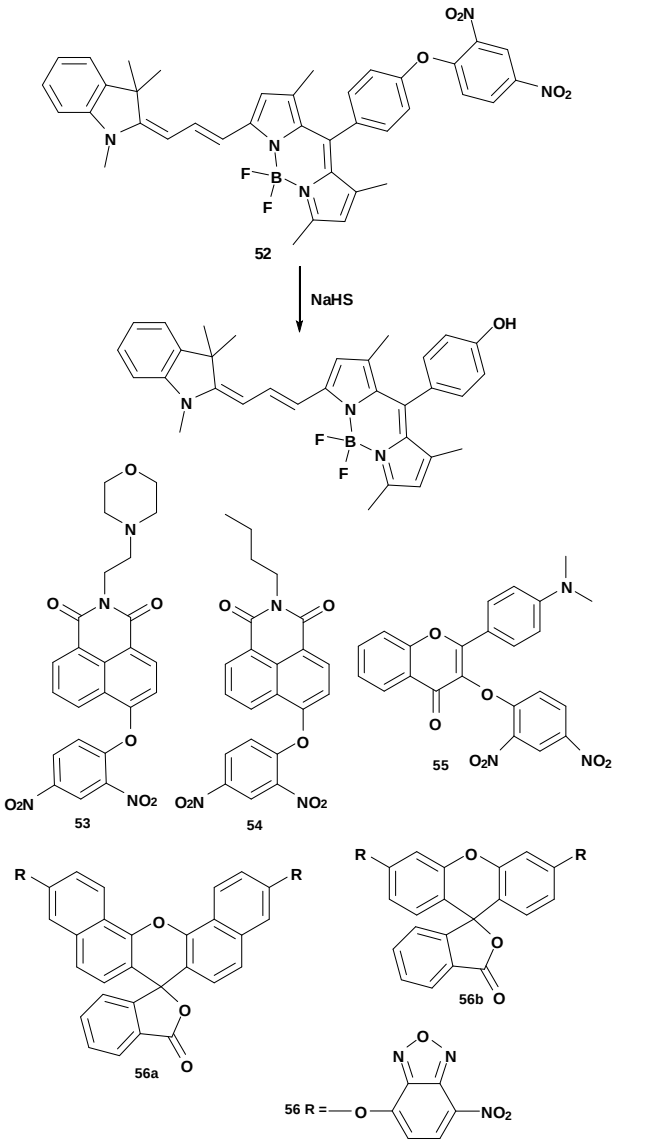
Fluorescent probes based on thiolysis reaction

Lin *et al.* synthesized a near-infrared fluorescent probe **52** based on thiolysis reaction for H_2S detection.[57] The dinitrophenyl group was often used to protect tyrosine in peptide synthesis. Thiols could remove the dinitrophenyl group under basic condition. The probe was prepared through condensation of the BODIPY with Fisher 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 reacted with H_2S, the fluorophore was released with a 18-fold fluorescent increase. This probe had been used to detect H_2S in bovine serum and MCF-7 cells. The detection limit was 5×10^{-8} M.

Xu *et al.* reported a lysosome-targetable fluorescent probe **53** for H_2S detection.[58] The probe was prepared through introducing dinitrophenyl ether group into the 4-position of 1,8-naphthalimide, and 4-(2-aminoethyl)morpholine into the N-imide position as the lysosome-targetable group. The lysosome-targetable probe is of significant meaning for the study of the distribution and function of H_2S in lysosomes of living cells. The fluorescence intensity of **53** increased in 42-fold. The probe could be used to detect H_2S in the lysosomes of MCF-7 cells. The detection limit was 0.48 μM. Xu *et al.* also developed a 1,8-naphthalimide-derived **54** as a two-photo fluorescent probe for H_2S detection based on thiolysis of dinitrophenyl ether.[59] The fluorescence intensity of **54** increased 37-fold. The probe **54** was applicable to detect H_2S in bovine serum and MCF-7 cells. The detection limit was 0.18 μM.

Feng *et al.* reported a 3-hydroxyflavone-based ESIPT probe **55** for H_2S detection.[60] The emission intensity increased 660-fold when detected H_2S. The probe could be used to detect H_2S in biological serum and in simulated wastewater samples. The detection limit was 0.10 μM.

Yi *et al.* reported fluorescent probes **56a** and **56b** for H_2S detection, which employed fluorescein and naphthofluorescein as fluorophores.[61] 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 H_2S. The fluorescence intensity of **56a** and **56b** increased 77-fold and 1000-fold, respectively. The detection limit of **56b** was determined to be 16 μM in solution.



Fluorescent probes based on copper-sulfide precipitation

After formed stable metal complexes with Cu (II), the organic chelators have an efficacious quenching effect on fluorophores due to the paramagnetic Cu (II) center can accept the excited state electronic of fluorophores. It is expected that the removal of Cu^{2+} from the metal center will result in fluorescence recovery. Such fluorescent probes are often ensemble in the fluorophore-chelator-metal ion style (**Fig. 6**). According to hard and soft acids and bases (HSAB) theory, S^{2-} has a strong affinity towards Cu (II). The CuS precipitate is relative stable with $\kappa_{sp} = 1.26 \times 10^{-36}$. After the addition of H_2S in solution, Cu (II) will be eliminated from the metal ligand center, corresponding fluorescent probes again emit fluorescence.

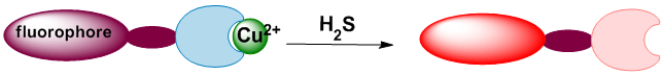


Fig. 6 Summary strategy for fluorescent probes based on copper-sulfide precipitation.

Chang *et al.* developed a fluorescence probe **57** for the selective sensing of S^{2-}.[62] The probe was designed based on a Cu^{2+} complex of fluorescein containing a dipicolylamine chelator. The detection limit was 420 nM in water solution.

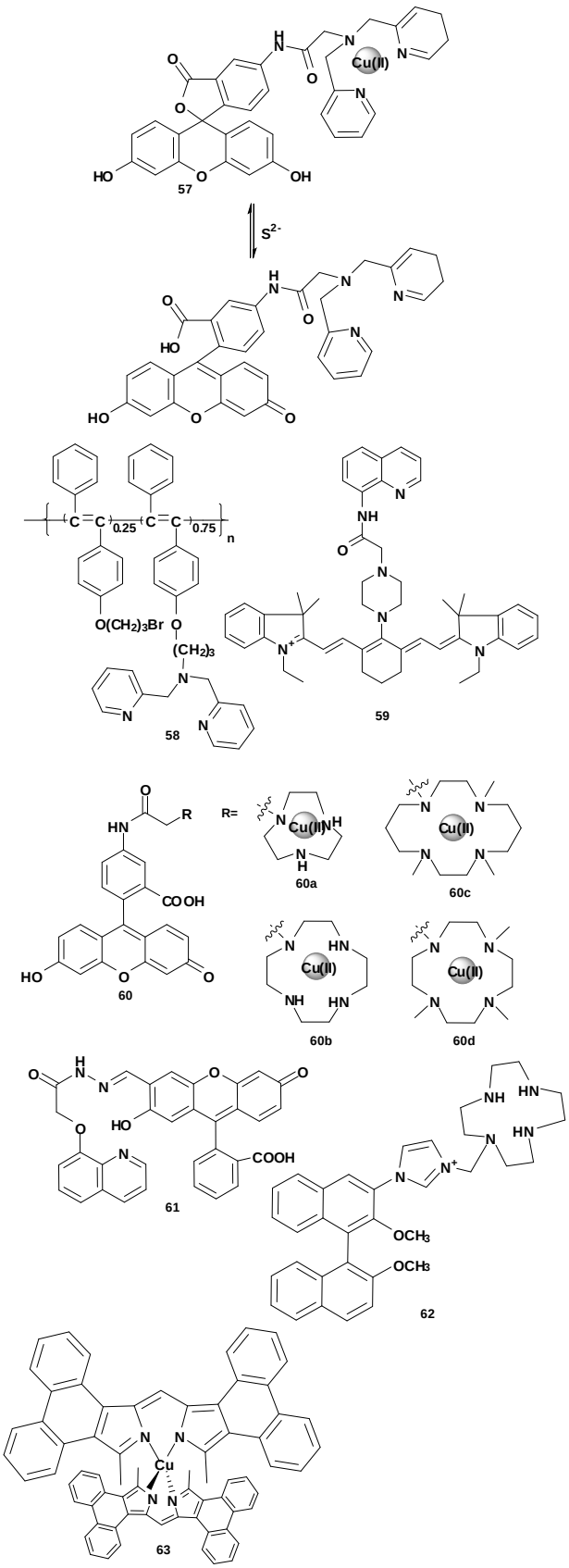
Li *et al.* reported a conjugated polymer fluorescent probe **58** based on disubstituted polyacetylene containing dipicolylamine chelator in the side chains for the detection of Cu^{2+} and S^{2-}.[63] The fluorescence of the probe could be quenched by Cu^{2+}. Based on the displacement strategy that utilizing S^{2-} removed Cu^{2+}, the quenched fluorescence of the probe could recover. The detection limit was 5.0×10^{-7} mol/L.

Lin *et al.* reported a near-infrared fluorescent probe **59** for H_2S detection.[64] The probe was composed of cyanine dye, piperazine linker and 8-aminoquinoline ligand. After deleted Cu^{2+} by H_2S, the probe gave a turn-on response. The probe **59** had $\Phi = 0.11$. The detection limit was 280 nM.

Nagano synthesized a series of azamacrocyclic Cu^{2+}-complex fluorescent probes **60a-d** for H_2S detection.[65] The probe **60b** exhibited high sensitivity and selectivity to detect H_2S, the recognition reaction finished within seconds. It showed fluorescence increase by 50-fold upon addition of H_2S. Probe **60b** could be used to detect H_2S produced by 3-mercaptopyruvate sulfurtransferase (3-MST), pseudoenzymatic H_2S release, and intracellular H_2S in HaLe cells.

Bai *et al.* reported 8-hydroxyquinoline-appended fluorescein derivative **61** for H_2S detection.[66] The fluoresecence of the probe was quenched by Cu^{2+}, which resulted in an off-on type probe for S^{2-} detection with 5-fold increase in the fluorescence intensity. The probe was able to retrievably indicating S^{2-} and Cu^{2+} changes in turn. The probe can detect H_2S in HeLa cells.

Li *et al.* reported a water-soluble fluorescent probe **62** based on 1,1′-bi-2-naphthol derivative for H_2S detection.[67] The metal ligand of the probe was 1,4,7,10-tetraazacyclododecane. This probe could recognize Cu^{2+} and S^{2-} with on-off-on mode. The detection limit for the determination of S^{2-} was 1.6×10^{-5} M.



Shen *et al.* designed a fluorescent probe based on phenanthrene-fused dipyrromethene analogue **63** for H_2S detection.[68] Upon treated with H_2S, the probe had a 14-fold fluorescence increase. The probe could be used as a turn-on fluorescence probe for detecting H_2S in HeLa cells.

Zang *et al.* designed a water-soluble fluorescent probe for H_2S detection based on 3-(1H-benzimidazol-2-yl)-2-hydroxy-benzoate sodium derivatives **64**.[69] The probe was capable for the detection of Cu^{2+} and S^{2-} ions at physiological pH. The displacement mechanism which the probe employed was supported by the fluorescence lifetime data. Free probe **64** exhibited Φ = 0.1063. The detect limition for S^{2-} was determined to be 2.51×10^{-6} M.

Long *et al.* developed a fluorescent probe **65** for the detection of H_2S based on the displacement method.[70] The releasing compound **65** gave a 20-fold fluorescence intensity increase. The probe could be used to detect H_2S in MCF-7 cells. The detection limit was 0.18 μM.

Zhu *et al.* developed a colorimetric probe **66** based on boron-dipyrromethene-Cu^{2+} for detection of H_2S in aqueous media.[71] The probe displayed a 50 nm red-shift of the absorption upon addition of H_2S in solution. The ratio of the absorbance showed a 34-fold ratiometric increase. The detection limit was 1.67×10^{-7} M.

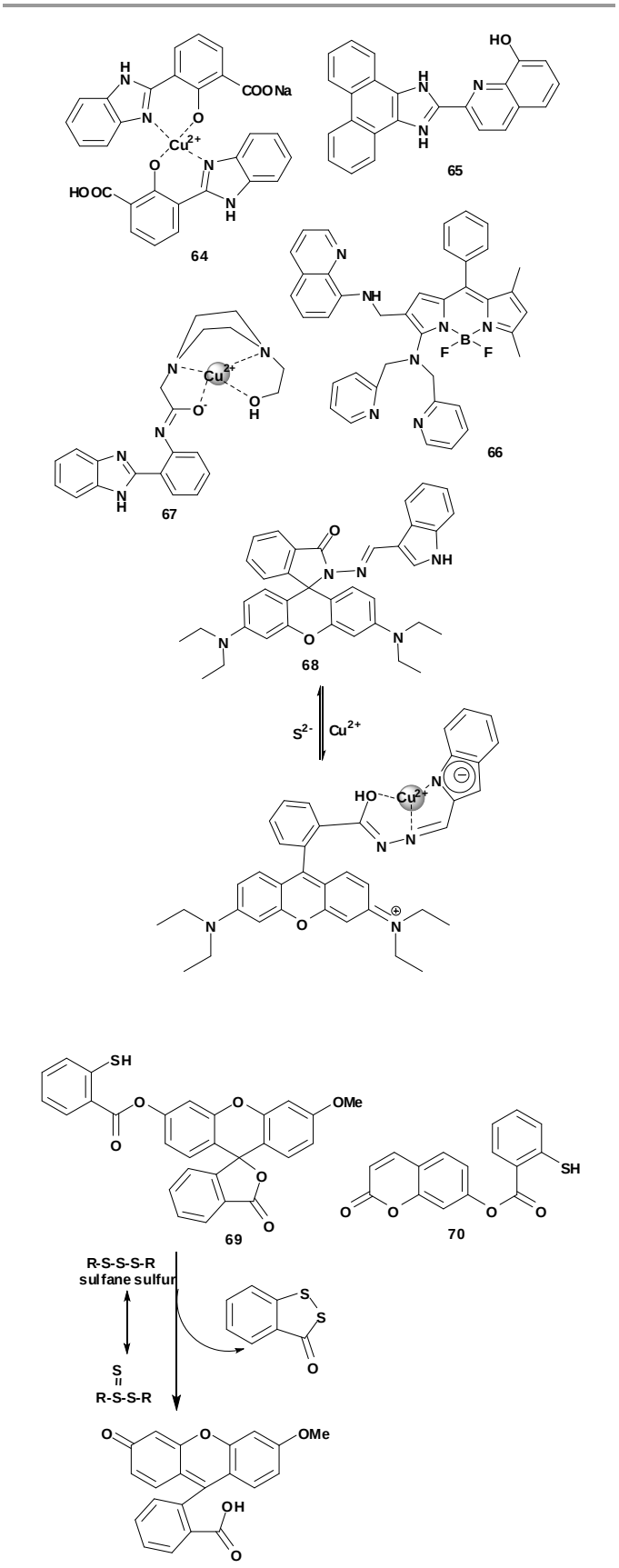
Tang *et al.* reported 2-(2'-aminophenyl)benzimidazole derivatives fluorescent probe **67** for recognition of Cu^{2+} and S^{2-} in water solution.[72] When functioned, the probe displayed excited-state intramolecular proton transfer (ESIPT) feature. The detection limit was 9.12×10^{-7} M.

Ramesh et al. synthesized a near-infrared ratiometric indole functionalized rhodamine derivative probe **68** for the detection of Cu^{2+} and S^{2-}.[73] The probe employed resonance energy transfer (RET) mechanism for detection of Cu^{2+} whose process involved the donor indole and the acceptor Cu^{2+} bound rhodamine moiety. The Cu^{2+} complex would give an on−off response in presence of S^{2-}. The Cu-complex probe **68** had 600-fold decrease of fluorescence intensity upon 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), which is 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_n-S-R), thiosulfate ($S_2O_3^{2-}$), polythionates ($^-SO_3$-S_n-SO_3^-), disulfides and so on.[74-76] As members of reactive sulfur species family, suffane sulfurs exhibit important physiological functions including cellular signal transduction and physiological regulation. The emerging evidences suggest that sulfane sulfurs may be the real signal transduction molecules for cellar events. The rapid production and clearance of H_2S with several biochemical pathways also depend on the metabolic process of suffane sulfurs.

Xian *et al.* reported fluorescent probes **69**, **70** for the sulfane sulfurs detection.[77] the detection mechanism was similar to the probe **44**, which was also illustrated in **Fig.5b**. Sulfane sulfur



compounds were reactive and labile, and there often existed thiosulfoxide tautomers. Therefore, a sulfur atom of sulfane sulfur could react with nucleophilic groups such as mercapto group to produce a reactive intermediate, which undergone intermolecular cyclization reaction immediately to release fluorophore. The fuorescence intensity of both probes increased 50-fold and 25-fold upon detection 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) is considered to be a kind of air pollutant for a long time. Person who is exposure to SO_2 may suffer from respiratory diseases and cancer. SO_2 will be rapidly hydrated to sulphite (SO_3^{2-}) and bisulfite (HSO_3^-) in neutral solution (3:1 M/M). However, endogenous SO_2 can be produced from sulfur-containing amino acids degradation. SO_2 including its derivatives may have physiological roles in the regulation of cardiovascular function in synergy with NO. Sulphite is also used in food as antioxidant to prevent bacterial growth. The design strategies of fluorescence probes for SO_2 detection mainly inspired from the nucleophilic properties of SO_2 (including SO_3^{2-} and HSO_3^-). The reaction mechanisms of these fluorescence probes can be sorted to the nucleophilic addition to aldehydes/ketones (**Fig. 7a**) and the nucleophilic addition to double bond (**Fig. 7b**). SO_2 and its derivatives selectively add to aldehydes/ketones or double bond, leading to the changes in electron-withdrawing effects or π-conjugated system of the probes, which can cause response in fluorescent signal.

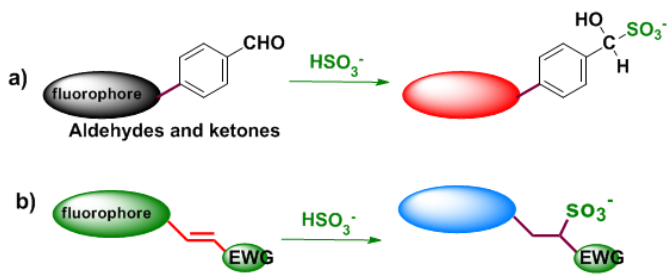


Fig. 7 Summary strategies for fluorescent probes based on the nucleophilic property of SO_2 and its derivatives.

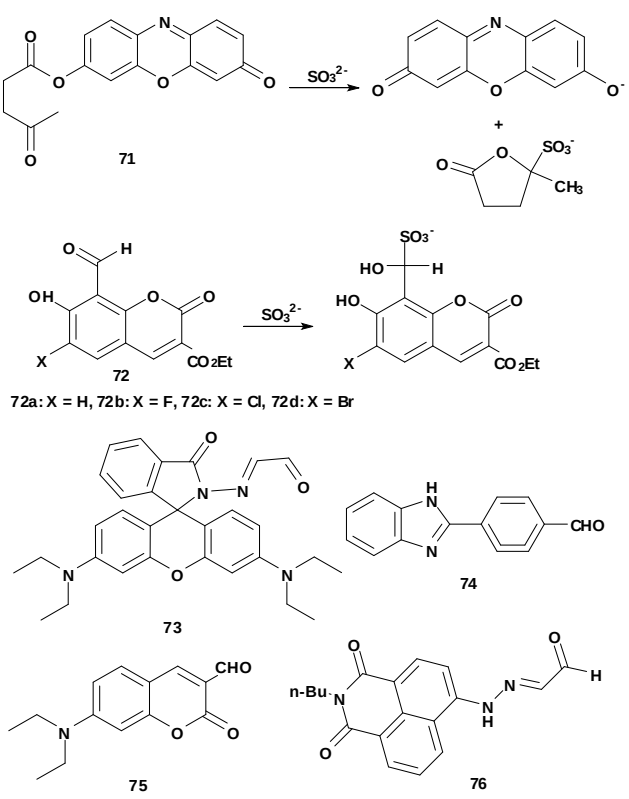
Fluorescent probes based on aldehydes/ketones addition

Chang *et al.* reported a fluorescent probe **71** for sulphite detection.[78] Sulphite could selectively deprotect the resorufin levulinate moiety of the probe. When sulfite reacted with the carbonyl carbon at levulinate, it formed a sulphite-added tetrahedral intermediate. The intermediate next would undergo intermolecular cyclization reaction to release fluorophore. The fluorescence increase was up to 57-fold. The probe has been used to detect sulfite in aqueous solution. The detection limit was 4.9×10^{-5} M.

Guo *et al.* synthesized four coumarin-based fluorescence probes **72a-d** for the detection of bisulfite.[79] Bisulfite could selectively attack 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 1.0×10^{-6} M.

Yang *et al.* developed a rhodamine-based fluorescent probe **73** for the detection of bisulfite.[80] Bisulfite could react with aldehyde to form an aldehyde-bisulfite adduct. Probe **73** also changed from spirolactam (nonfluorescent) to ring-opening spirolactam structure which increased fluorescence emission. The probe could detect bisulfite in aqueous media. The detection limit was 8.9×10^{-7} M. They also presented a ratiometric fluorescent probe 4-(1H-benzimidazol-2-yl)benzaldehyde (**74**) for bisulphite detection.[81] The aldehyde moiety reacted with bisulphite to produce bisulphite 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 μM.

Feng *et al.* developed a coumarin-based fluorescence probe **75** for the detection of bisulfite.[82] The nucleophilic addition reaction with aldehyde would switch-on fluorescent probe for bisulfite flowing the ICT process. The aldehyde–bisulfite adduct produce had a quantum yield of 0.43. The probe could detect bisulfite in solution and in HeLa cells. The detection limit was 3.0 μM.



Guo *et al.* designed a C=N isomerization-based fluorescent probe **76** for bisulfite detection.[83] The C=N isomerization could be inhibited by intramolecular N-H···N=C hydrogen bond. The formation of hydrogen bond would block the C=N rotations,

and fix the molecular structures, resulting in minimizing the nonradiative energy of excited state. The quantum yield was 0.374. The probe could detect bisulfite in locally granulated sugar. The detection limit was 0.1 μM.

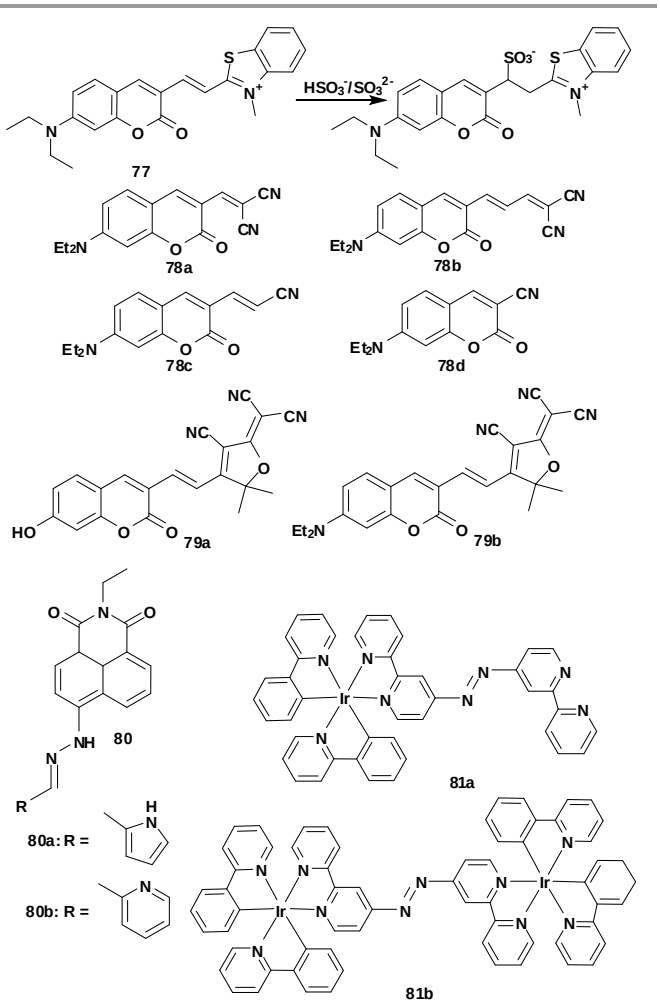
Fluorescent probes based on double bond addition

Guo *et al.* reported a coumarin-hemieyanine fluorescent probe **77** for bisulfite and sulfite detection.[84] The nucleophilic attack of SO_3^{2-}/HSO_3^- toward double bond would interrupt the π-conjugation structure of probe **77**. As a result, the emission profiles before and after adding SO_3^{2-}/HSO_3^- could shift due to the distinct emission between the coumarin-hemieyanine fluorophore and the produced coumarin fluorophore. The probe gave 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 μM.

Li *et al.* reported a colorimetric and ratiometric fluorescent probes **78a-d** for the detection of sulphite.[85] These probes were based on 7-diethylamine coumarin fluorophore which conjugated with cyano group through double bonds. Probes **78a** and **b** provided better response to SO_3^{2-}. SO_3^{2-} undergone Michael addition to *α,ß*-unsaturated double bond, which would interrupt the intramolecular charge transfer (ICT) process of the probe. The probes could detect sulphite in solution. The probe had a 232-fold intensity ratio increase. The detection limit was 58 μM. The same group also presented near-infrared fluorescent probes **79a** and **b** for the colorimetric and ratiometric detection of SO_2 derivatives. [86] The probes were composed of coumarin fluorophores and 2-dicyanomethylene-3cyano-4,5,5-trimethyl-2,5-dihydrofuran which had three cyano groups. Probe **79a** owned better water solubility and electron-withdrawing properties. The ration signal at two different wavelengths could increase 775-fold within 90 seconds. The probe had been used to detect SO_2 derivatives in U-2OS cells. 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.[87] The electron donor property of pyrrole moiety in probe **80a** took the responsibility for fluorescence quenching through photoinduced electron transfer (PET) process. The SO_3^{2-}/HSO_3^- could form hydrogen bonds with pyrrole moiety, and the PET process was blocked. Probe **80a** gave 65-fold fluorescent increase upon detected sulphite. The probe could be used to monitor SO_2 donor real-time released SO_2 and could detect SO_3^{2-}/HSO_3^- in GES-1 cells. The detection limit was 0.56 μM.

Chao *et al.* develop azo group-bridged dinuclear iridium (III) complexs **81a** and **b** as phosphorescent probes for SO_3^{2-}/HSO_3^- detection.[88] When azo group was incorporated into ruthenium(II) complexes, it would become more reactive and could react with SO_3^{2-}/HSO_3^-. Moreover, azo group was 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 undergone nucleophilic addition to azo group, probe **81b** would turn on its luminescent emission. The phosphorescence responses showed a 26-fold and 27-fold increase for sulphite and bisulphite. Probe **81b** was able to detect external and endogenous SO_3^{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 the synthesis and design strategies to the development of reaction-based fluorescent probes for which are classified by the reaction types between analytes (H_2S, sulfane sulfurs and SO_2 derivatives) and probes. According to the reaction types, the probes are illustration through and explained by examples: a) H_2S reductive reactions: reducing azides to give amines, reducing nitro/azanol to give amines, and reducing selenoxide to give selenide; b) H_2S 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 reactive sulfur species fluorescent probes will likely prove to further facilitate analysis for fluorescence bioimaging technology. Compared with traditional methods, the detection of reactive sulfur species via fluorescence spectrometry can lower the external influence to endogenous species distribution, can reduce the time of sample preparation, and can achieve real-time detection, which meet to the high reactivity of these species. Despite the 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 level, yet the fluorescent probes for detection of original reactive sulfur species and distribution under normal physiological conditions are very rare. The distribution of reactive sulfur species in cells can be distinguished by probes which have targeting functions, and the quantitative analysis of reactive sulfur species in cells can be resolved by flow cytometry. For example, endogenous H_2S variation is always restricted to a shape range within a short time, we term it as "H_2S spark" because this is the spontaneous and spatio-temporally localization nature of H_2S biological release. Therefore, to design a probe who can sense "H_2S spark" will be great meaningful. The similar situation also occurs to other reactive small molecules such as NO, H_2O_2, CO, NH_3, SO_2, and sulfane sulfurs.

Organism holds a complex environment, the bioreactive species' generation and decay contain various stages. It is desirable to exploit distinctive design schemes that involve reversible chemoselective reactions and catalytic processes where conceive can simulate enzyme. The reversible strategies suffer from many challenges, such as photostability, without perturbation of target molecules, avoiding cell leakage, and response frequency. Along this line, it is also expected to launch multiresponse probes for bioimaging detection, because the reactive small molecules have signaling cross-talk during the physiological and pathological processes. Compared with single analyte probes, multiresponses probes will provide facilities for improvement of diagnostic and therapy tools, and offer important future directions for biological events.

Above all, the development of perfect probes is a challenging task. It need to master basic concepts and strategies. The development of probes should address key biological problems. Future reaction-based design principles must be pay attention to endogenous reactive small molecules under normal physiological conditions. Whether the probes at which position in the light region (visible region or near-infrared range), the desirable probes should respond remarkably to a minor concentration change, give dependable results, and meanwhile avoid interference from native cellular species, particularly biomoleculars 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 physiological pH range. For real applications of fluorescent probes, especially in clinical diagnostic imaging, is the ultimate goal.

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