Recent progress in H$_2$S activated diagnosis and treatment agents

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Hydrogen sulfide (H$_2$S) is a key biosignal molecule in the human body. Endogenous H$_2$S, as a gas delivery and protective agent in the body, is involved in a variety of physiological processes, including mediating vascular tone and neuromodulation. The production of abnormal H$_2$S levels in the body is related to the occurrence of various diseases, so real-time monitoring of H$_2$S in vivo is very important. However, traditional detection methods face enormous challenges in the in vivo detection of H$_2$S owing to its high volatility and rapid catabolism. Optical probes developed in recent years with the advantages of high sensitivity, short response time, non-invasive nature and capacity for real-time monitoring can overcome the limitations of traditional detection methods and offer the possibility of real-time monitoring of H$_2$S in cells and in vivo. In addition, the production of high concentrations of H$_2$S is closely related to the formation of colon cancer, and H$_2$S-activated treatment agents have been developed for use in this particular tumor microenvironment, which reduce the toxic side effects of traditional therapy on normal tissues and improves the treatment effect. This review summarizes the recent advances in H$_2$S detection probes in vitro and in vivo, as well as H$_2$S-activated tumor treatment agents.

1. Introduction

Hydrogen sulfide (H$_2$S) is an irritating gas with a smell of rotten eggs that has long been considered toxic. Recent studies have shown that H$_2$S is an endogenously unstable gas, which has been identified as a gas carrier, as well as nitric oxide (NO) and carbon monoxide (CO). Endogenous H$_2$S can be enzymatically produced by cystathionine $\gamma$-lyase (CSE), cystathionine $\beta$-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (3MST) in mammalian cells. These enzymes digest cysteine or cysteine derivatives and produce H$_2$S in different organs. It has been shown that H$_2$S is involved in many physiological processes, such as regulating blood pressure, exerting antioxidant and anti-inflammatory effects, and regulating the central nervous system, respiratory and gastrointestinal systems. The physiological concentration of H$_2$S is $0.01-3$ $\mu$M at the cellular level and $30-100$ $\mu$M in the serum. Abnormal levels of H$_2$S in the body can induce several malignant diseases, including Alzheimer’s disease, diabetes, heart disease,
hypothesis and other cardiovascular diseases. Therefore, real-time detection of $\text{H}_2\text{~S}$ levels is important for further study of its physiological and pathological roles in biological systems.

Traditional analytical methods for $\text{H}_2\text{~S}$ mainly include colorimetry, electrochemical analysis, gas chromatography, and sulfide precipitation. These methods need high-standard preparation of samples and collection of $\text{H}_2\text{~S}$ from cells or tissues. However, a fast $\text{H}_2\text{~S}$ catabolism rate leads to fluctuations in its concentration, further resulting in inaccurate measurement. Therefore, the traditional methods have difficulty meeting fast, accurate, and real-time monitoring criteria for $\text{H}_2\text{~S}$ levels in living systems. Optical detection methods are attracting increasing research interest owing to their high sensitivity, short response time, non-invasive nature, capacity for real-time monitoring and easy sample preparation. Based on the good nucleophilic and reducing chemistry of $\text{H}_2\text{~S}$, researchers have been developing optical probes with high sensitivity, selectivity and biocompatibility for the detection of $\text{H}_2\text{~S}$ in biological systems. These probes are based primarily on specific $\text{H}_2\text{~S}$-induced reactions, including azide reduction, nitro reduction, removal of quenchers (such as copper (II)), nucleophilic reactions, to allow fluorescence to be turned on for $\text{H}_2\text{~S}$ detection at different biological levels.

In addition, there have been some reports that CBS is selectively up-regulated and the concentration of $\text{H}_2\text{~S}$ is significantly increased in cancer tissues such as colon, breast and ovarian cancers. $\text{H}_2\text{~S}$ plays an important role in tumor proliferation and metastasis, and has become a new target for cancer treatment. Traditional cancer treatment methods mainly include surgical resection, chemotherapy, radiotherapy and other means. These treatment methods not only have a low cure rate, but also have relatively large side effects. Scientists are working to develop $\text{H}_2\text{~S}$-activated reagents for the treatment of cancer, on account of high concentrations of $\text{H}_2\text{~S}$ in the tumor environment. These mainly include: (i) $\text{H}_2\text{~S}$-activated nanodrug carriers for delivering chemotherapeutic drugs to tumor sites, improving the therapeutic efficiency of cancer while reducing the toxic side effects on normal tissues; (ii) $\text{H}_2\text{~S}$ trapped in normal tissues after intravenous injection, causing damage to normal tissues on light irradiation. The $\text{H}_2\text{~S}$-activated phototherapy agent only produces therapeutic effects at the tumor site, thereby reducing damage to normal tissues.

In this review, we summarize the recent developments of $\text{H}_2\text{~S}$-activated probes in the biomedical field, including fluorescent probes and photoacoustic probes for in vitro and in vivo applications. In addition, the application and advantages of $\text{H}_2\text{~S}$-activated reagents in cancer diagnosis and treatment are also discussed. We also reference the side effects of traditional therapy reagents in the treatment of tumors, and describe the requirements and challenges of $\text{H}_2\text{~S}$-activated reagents. Finally, the possible future application prospects of $\text{H}_2\text{~S}$-activated diagnostic and therapeutic reagents for cancer therapy are also discussed.

2. $\text{H}_2\text{~S}$-activated probes

Abnormal $\text{H}_2\text{~S}$ levels in organisms are associated with the development of many diseases. High-sensitivity probes for $\text{H}_2\text{~S}$ concentrations in animals are very important; they can help us to understand the effects of $\text{H}_2\text{~S}$ on various physiological and pathological processes, and to diagnose related diseases in a timely manner. Probes for $\text{H}_2\text{~S}$ detection in vitro and in vivo are listed in Table 1 and described in detail below.

2.1 $\text{H}_2\text{~S}$ probes in vitro

$\text{H}_2\text{~S}$ intelligent optical probes with high sensitivity, high selectivity, high signal-to-noise ratio and stability are being developed. Fluorescence imaging by fluorescent probe staining is one of the most attractive molecular imaging techniques for $\text{H}_2\text{~S}$ detection in living cells, tissues and living animals. $\text{H}_2\text{~S}$-activated fluorescent probes are mainly based on the difference of emission wavelength before and after response. Although a lot of effort has been expended, fluorescence imaging is limited by problems such as the low concentration of endogenous $\text{H}_2\text{~S}$ and the presence of a large number of interfering molecules, including reduced glutathione, cysteine (Cys) and thiol-containing proteins, in complex living systems. Therefore, it is still a significant challenge to develop highly sensitive and selective fluorescent probes.
Based on the nucleophilic and reductive properties of H₂S, scientists have developed fluorescent probes for H₂S detection founded on the reduction of azides to amines, nucleophilic reactions and copper sulfide precipitation. Liu et al. designed a H₂S fluorescent probe containing bis-electrophile to take advantage of the nucleophilicity of H₂S. The fluorescence intensity of the disulfide-containing probe increased dramatically (55–70-fold) when 50 μM H₂S was presented in solution. In addition, the maximum intensity was reached in 1 h, suggesting that the reaction was fast. The fluorescent probe is selective for H₂S and does not react with other bio-thiols, such as cysteine and glutathione, at the same concentration (100 μM). A fluorophore of dansyl azide (DNS-Az) with high quantum yield was prepared by Peng et al. The azide is reduced to an amine by reduction with H₂S to emit fluorescence for rapid detection of H₂S in vitro. The probe was very sensitive, with a detection limit of 1 μM in buffer/Tween and 5 μM in bovine serum. The reaction was complete in a few seconds, while the fluorescence was enhanced immediately. No obvious response to the probe was observed for most of the tested anions at a concentration of 1 mM, which is a 40-fold higher concentration than that of sulfide. Sasakura et al. designed and synthesized a novel H₂S-detecting fluorescent probe Cyclen-AF + Cu²⁺ (HSip-1) based on the azamacrocyclic ring to form a stable metal complex with Cu²⁺. The paramagnetic Cu²⁺ center could quench the fluorophore’s fluorescence. When H₂S binds to Cu²⁺, Cu²⁺ is released from the azamacrocyclic ring, resulting in enhanced fluorescence. The probe showed a large (50-fold) and immediate increase in the fluorescence intensity upon addition of 10 μM H₂S, whereas almost no fluorescence increment was observed upon the addition of 10 mM GSH. Thus, HSip-1 is more selective for H₂S than previously reported fluorescent probes using 2,4-dinitrosufonyl or azide groups.

For most single-window-response fluorescent probes the experimental results change with the experimental conditions. Ratiometric fluorescent probes are able to overcome the interference due to experimental conditions. Bae et al. reported a H₂S-activated mitochondrially localized two-photon ratiometric fluorescent probe, SHS-M2 (Fig. 1A), which has 6-(benzo[d]thiazol-2-yl)-2-(methylamino) naphthalene as the fluorophore, 4-azidobenzyl carbamate as the H₂S response site, and triphenylphosphonium salt as the mitochondria-targeting moiety. The thiolate-triggered reaction with the azide group would cleave the carbamate linkage and liberate the amino group, accompanied by a decrease in emission intensity at 420 nm and a gradual increase at 500 nm. The color also changes from blue to yellow. Thereby, the emission and the

### Table 1  Summary of recently published reports on H₂S detection probes

<table>
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<tr>
<th>H₂S probe</th>
<th>Reaction mechanism</th>
<th>Wavelength (nm)</th>
<th>Detection limit</th>
<th>Experimental subject</th>
<th>Detection method</th>
<th>Ref.</th>
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<tr>
<td>Cyclen-AF + Cu²⁺ (HSip-1)</td>
<td>Cu²⁺ quenches fluorescence</td>
<td>516</td>
<td>10 μM</td>
<td>HeLa cells</td>
<td>Fluorescence microscopy</td>
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<td>SHS-M2</td>
<td>Azides to amines</td>
<td>464/545</td>
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<td>NanoBODIPY</td>
<td>Nucleophilic reactions</td>
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<td>7 nM</td>
<td>Raw 264.7 macrophage cells</td>
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<td>Coumarin–merocyanine dyad (CPC)</td>
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<td>40 nM</td>
<td>HeLa cells</td>
<td>Confocal microscopy</td>
<td>77</td>
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<tr>
<td>Azide-functionalized O- methylrhodol (MeRho-Az)</td>
<td>Nucleophilic reactions</td>
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<td>86 ± 7 nM</td>
<td>C6 cells and zebrafish</td>
<td>Light sheet fluorescence microscopy</td>
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<td>Ruthenium(ii) complex-based luminescence probe (Ru-MDB)</td>
<td>Nucleophilic reactions</td>
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<td>Zebrafish and mice</td>
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<td>Nucleophilic reactions</td>
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<td>HCT116 tumor mice</td>
<td>Fluorescence imaging</td>
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<tr>
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<td>91 nM</td>
<td>HCT116 tumor cells</td>
<td>Photoacoustic imaging</td>
<td>89</td>
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### Fig. 1

(A) The structure of SHS-M2. (B) Fluorescence response of 1 μM SHS-M2 to 100 μM Na₂S in HEPES buffer from 0 to 60 min. λₑₓ = 373 nm. (C) The relationship between CBS expression and H₂S production in astrocytes of DJ-1 knockout (KO) brain. Brain slices were prepared from wild-type (WT) and DJ-1 KO mice. (1) Hippocampal slices were prepared and stained for GFAP (an astrocyte marker) and CBS-expressed H₂S; (2) H₂S analysis of freshly prepared slices; (3) cortical slices were cultured for 7 days after slicing to stabilize the tissues from slicing stress, and then the H₂S production was measured. Reproduced from ref. 71. Copyright 2013 American Chemical Society.
cross-section of the ratiometric two-photon probe can be increased (Fig. 1B). The probe is more sensitive and the detection limit of \( \text{H}_2\text{~S} \) is 0.4 \( \mu \text{M in vitro} \). The fluorescence intensity of the SHS-M2 after triggering by \( \text{H}_2\text{~S} \) (0.1 \( \text{mM} \)) is 5–8-fold higher than that with 10 \( \text{mM} \) glutathione (GSH) and 1 \( \text{mM} \) cysteine (Cys), which confirms the high selectivity for \( \text{H}_2\text{~S} \) over GSH and Cys. Two-photon microscopy ratiometric imaging of SHS-M2 as a probe can be used to study the relationship between CBS expression and \( \text{H}_2\text{~S} \) levels in cells and brain sections (Fig. 1C).

The main problem of current \( \text{H}_2\text{~S} \) probes is low detection sensitivity. Förster resonance energy transfer (FRET)-based fluorescent probes can eliminate the effect of excitation backscattering on fluorescence detection because of the large offset between donor excitation and acceptor emission.\(^{74,75}\) In addition, two well-separated emission bands with comparable intensities can be used to ensure the accuracy of their strength and ratio. Some fast and accurate ratiometric fluorescent probes for detecting \( \text{H}_2\text{~S} \) have been developed based on FRET.\(^{74,75}\) Zhao \textit{et al.}\(^{76}\) developed a self-assembled micelle aggregate NanoBODIPY fluorescent probe with \( \text{H}_2\text{~S} \)-triggered FRET switch, which consists of a dynamic energy receptor semi-cyanine-BODIPY hybrid dye (BODInD-Cl) and a complementary energy donor (BODIPY1). In the absence of \( \text{H}_2\text{~S} \), a specific FRET from BODIPY1 to BODInD-Cl occurs due to the spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor. In contrast, in the presence of \( \text{H}_2\text{~S} \), the Cl on the aromatic ring in NanoBODIPY is replaced by the \( \text{H}_2\text{~S} \) via nucleophilic substitution and the absorption of the probe is shifted from 540 to 738 nm, resulting in a loss of FRET owing to the lack of overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor (Fig. 2A). This results in a fluorescence signal that simultaneously “turns on” the energy donor BODIPY1 and a fluorescence signal that “closes” the energy acceptor BODInD-Cl. NanoBODIPY can sensitively and quickly detect \( \text{H}_2\text{~S} \) with a detection limit of 7 \( \text{nM} \) by ratiometric fluorescence. The emission intensity gradually increased at 511 nm after adding different concentrations of sodium hydrosulphide (NaHS), accompanied by a loss of emission at 589 nm, and the response was complete within 140 s (Fig. 2B). Through competitive experimental studies, NanoBODIPY showed good selectivity for NaHS with minimal interference from other biologically relevant analytes in PBS buffer (Fig. 2C).

By a similar approach, Feng \textit{et al.}\(^{77}\) reported a FRET-based ratiometric fluorescent probe composed of a coumarin–merocyanine dyad. Before the reaction with \( \text{H}_2\text{~S} \), the emission wavelength of coumarin apparently overlaps with the absorption of merocyanine, and a resonance energy transfer process occurs, so that the probe displays the fluorescence of the cyanine. In the presence of \( \text{H}_2\text{~S} \), the merocyanine moity undergoes a nucleophilic addition reaction with \( \text{H}_2\text{~S} \), and the conjugated system is destroyed; as a result, resonance energy transfer cannot be achieved, and so the fluorescence of coumarin is exhibited. The probe has a detection limit of as low as 40 \( \text{nM} \). It can be used for mitochondrial endogenous and exogenous \( \text{H}_2\text{~S} \) detection; it shows a greater emission shift than other \( \text{H}_2\text{~S} \) probes, and so it exhibits higher selectivity and sensitivity.

### 2.2 \( \text{H}_2\text{~S} \) probes in vivo

Despite rapid progress in the development of \( \text{H}_2\text{~S} \) probes in the past few years, there are still many problems in the transition from solutions, cells and tissues to whole organisms. Tissue penetration, poor spatial resolution in deep biological tissues, fluorophore stability at high excitation wavelengths and other issues have largely limited their application for \textit{in vivo} \( \text{H}_2\text{~S} \) detection.

#### 2.2.1 Fluorescent probes

The light sheet fluorescence microscope (LSFM) is an imaging tool that confines excitation light to a sheet that coincides with the focal plane of a wide field of view imaging system.\(^{78}\) The LSFM can image larger samples than confocal microscopes while enabling rapid imaging. The LSFM combined with a \( \text{H}_2\text{~S} \)-responsive fluorescent probe enables detection of \( \text{H}_2\text{~S} \) levels \textit{in vivo}.\(^{79}\) Hammers \textit{et al.}\(^{80}\) developed an azide-functionalized \( O \)-methylrhodol fluorophore (MeRho-Az) for the detection of \( \text{H}_2\text{~S} \) in live zebrafish (Fig. 3A). The xanthene core modified \( O \)-methylrhodol (MeRhod) is locked in the non-fluorescent spirolactone tautomeric form. The \( \text{H}_2\text{~S} \) reduction of azide regenerates the amine while releasing the fluorescent open tautomer to produce an intense fluorescence, and exhibits a rapid >1000-fold fluorescence response. MeRho-Az can sensitively detect low concentrations of \( \text{H}_2\text{~S} \) with a detection limit of 86 ± 7 \( \text{nM} \). Owing to the pH insensitivity and photostability of MeRho-Az, it can be used for the detection of \( \text{H}_2\text{~S} \) in living organisms. The results showed that the fluorescence signal was rapidly increased after the addition of NaHS to MeRho-Az (Fig. 3B and C). Then MeRho-Az was used to detect endogenous \( \text{H}_2\text{~S} \) in C6 rat glial cells by fluorescence imaging. The fluorescent signal of the C6 rat glial cells in the group treated with AP39 (\( \text{H}_2\text{~S} \) donor) plus MeRho-Az was higher than that for the group treated with MeRho-Az alone. In contrast, the fluorescent signal of the group treated with AOAA

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**Fig. 2** (A) The structure of NanoBODIPY and the FRET process from the complementary energy donor (BODIPY1) to the responsive energy acceptor BODInD-Cl to the responsive energy acceptor BODInD-Cl. (B) Change in ratiometric fluorescence intensity of NanoBODIPY in the presence of NaHS (100 \( \mu\text{M} \)) at different times and fluorescence spectra in various concentrations of NaHS (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 \( \mu\text{M} \), respectively). (C) Ratiometric fluorescence changes of NanoBODIPY in the presence of 100 \( \mu\text{M} \) NaHS and other biologically relevant competing analytes. \( \lambda_ex = 490 \text{ nm} \). Reproduced from ref. 76. Copyright 2015 American Chemical Society.
Phosphorescent transition metal complexes have attracted much attention owing to their strong visible light absorption and emission, large Stokes shift, and stable photochemical properties. A ruthenium(II) complex-based responsive luminescence probe (Ru-MDB) for H$_2$S detection was studied by Du et al. MBDB is a masking moiety for the Ru-MDB complex H$_2$S response. The metal-to-ligand charge transfer (MLCT) excited state of the Ru$^{II}$ complex is destroyed by an intramolecular light-induced electron transfer photo-induced electron transfer (PET) process when the electron acceptor group MDB is linked (Fig. 4A). To utilize the nucleophilic properties of H$_2$S, the new MDB masking group was linked to one of the bipyridine ligands of the Ru$^{II}$ complex through an ester bond that could be cleaved by H$_2$S, resulting in an approximately 86-fold increase in luminescence intensity. The detection limit was measured to be 45 nM, which suggested high sensitivity of Ru-MDB for monitoring H$_2$S in mice. The main characteristics of this probe enabled the monitoring of lysosomal H$_2$S generation in live cells, and the visualization of exogenous/endogenous H$_2$S in live Daphnia magna, zebrafish and mice (Fig. 4B).

Fluorescence imaging in the second near-infrared window (NIR-II, 1000–1700 nm) showed reduced autofluorescence, enhanced tissue penetration, and higher spatial resolution in vivo. Xu et al. designed a H$_2$S-activated NIR-II@Si fluorophore (Fig. 5A) that visualizes colorectal cancer. The probe encapsulates the H$_2$S-responsive fluorophore in the hydrophobic interior of the core–shell silica nanocomposite. The fluorescent nanoprobes comprise two organic chromophores: boron-dipyrromethene (ZX-NIR) dye, which has a maximum emission shift from 600 nm to 900 nm in the presence of H$_2$S to produce NIR-II emission, andaza-BODIPY (aza-BOD), the emission of which remains unchanged at 700 nm, as an internal reference (Fig. 5B and C). The detection limit for H$_2$S was measured to be 37 nM, indicating the high sensitivity of NIR-II@Si for ratiometric detection of H$_2$S. This activatable H$_2$S-specific targeting probe can be used for deep tissue imaging of H$_2$S-rich colon cancer cells. Utilizing the advantages of NIR-II imaging, tumor sites can be selectively targeted in mice (Fig. 5D).
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nmol AOAA, or (5) 300 nmol tumor regions; (2) probe-treated mice at normal sites; (3) probe-using Si@BODPA at di in vivo probe shows an extremely fast response and can detect tran-region. The detection limit was measured to be 53 nM. The probe produces a strong photoacoustic signal output in the NIR absorption around 780 nm (Fig. 6B). Therefore, the Si@BODPA substitution in the presence of H2S results in high NIR

within the nanoparticles (NPs) by aromatic nucleophilic conversion of BODPA to BOD-HS (Fig. 6A); thereby the probe has good water solubility and excellent biocompatibility. Conversion of BODPA to BOD-HS to a ratiometric PA signal in the presence of H2S results in high NIR absorption around 780 nm (Fig. 6B). Therefore, the Si@BODPA probe produces a strong photoacoustic signal output in the NIR region. The detection limit was measured to be 53 nM. The probe shows an extremely fast response and can detect transient changes in H2S. Si@BODPA allows direct photoacoustic tracking of endogenous H2S production in an HCT116 (human colon cancer cell) tumor-bearing mouse model. As shown in Fig. 6C, there was no photoacoustic signal from the normal sites of mice and the tumor site of the mice pre-treated with the CBS inhibitor aminoxyacetic acid (AOAA, 100 nmol) after injection of Si@BODPA, while a photoacoustic signal was observed in the tumors of the mice without and with pretreatment with a CBS activator (S-adenosyl-l-methionine), indicating that Si@BODPA can be used for detection of H2S in vivo.

At present, photoacoustic probes for H2S detection mostly provide single-response photoacoustic signals, and the results will be affected by factors such as instrument, probe concentration and external environment. On the contrary, a ratiometric photoacoustic probe can eliminate the effects of the above factors by using the ratio of two separate wavelength photoacoustic response signals, thereby obtaining reliable experimental results. Ma et al.89 developed a novel ratiometric photoacoustic nanoprobe for in vivo detection of H2S. The nanoprobe AzHD-LP consists of a liposome (LP) with a H2S-responsive near-infrared dye (AzHD) encapsulated inside it (Fig. 7A). After the reduction of azide to amine in the AzHD-LP photoacoustic probe by H2S, the absorption peak appears red-shifted. The absorption of AzHD-LP at 600 nm is reduced, while the absorption at 700 nm is increased, resulting in a ratiometric PA signal in the presence of H2S. The detection limit of AzHD-LP for NaHS in solution was determined to be 91 nM. Furthermore, after AzHD-LP was conjugated to tumor-targeting peptide c(RGDyK), detection of intratumoral H2S production in HCT116 colon tumor mice was achieved under excitation of 532 nm and 700 nm pulsed lasers (Fig. 7B).

In this section, fluorescent probes and photoacoustic (PA) probes for H2S detection are introduced. Although fluorescent probes are widely used in the detection of H2S, their applications in vivo are limited by the autofluorescence and penetration depth. Photoacoustic imaging with high tissue penetration can be used to detect H2S levels in the living body and accurately locate a lesion. However, their sensitivity impedes their further application. As a result, it is necessary to develop better probes. NIR-II fluorescence and NIR-II photoacoustic imaging are emerging technologies that exhibit greater penetration depth and higher sensitivity. Therefore, the design of NIR-II

![Fig. 6](image_url) (A) Schematic illustration of the construction of activatable photoacoustic probes for H2S. (B) The absorption changes of Si@BODPAs (10 μM BODPA1 or BODPA2) in the absence and presence of NaH2S (100 μM). (C) Photoacoustic imaging of tumor-bearing mice using Si@BODPA at different times: (1) saline-treated mice in the tumor regions; (2) probe-treated mice at normal sites; (3) probe-treated mice in the tumor regions; (4,5) mice pre-treated with (4) 100 nmol AOAA, or (5) 300 nmol S-adenosyl-l-methionine for 12 h, were subcutaneously injected with Si@BODPA in the tumor regions. Graph, photoacoustic intensities as a function of time post-injection of Si@BODPA. Reproduced from ref. 88. Copyright 2017 Royal Society of Chemistry.

![Fig. 7](image_url) (A) Illustration of the construction of activatable nanoprobe AzHD-LP and the proposed mechanism for ratiometric photoacoustic (PA) detection of H2S. (B) PA/ultrasound (US) overlaid images of subcutaneous HCT116 tumor in naked mice pretreated with RGD-AzHD-LP, and plot of ratiometric intensity (PA700/PA532) against time. Reproduced from ref. 89. Copyright 2018 Royal Society of Chemistry.
fluorescence probes with weaker autofluorescence and NIR-II PA probes is the way forward.

3. H2S-activated therapeutic reagents

Compared with the traditional treatment of colon cancer, targeted response therapy can reduce side effects and cause more obvious therapeutic effect. Overexpression of cystathionine-β-synthase (CBS) in tumor cells leads to an increase in H2S levels (0.3 to 3.4 mM), especially in colon tumor cells. So, it will be more efficient to use H2S-activated therapy for colon cancer than other tumor microenvironment factors (pH, GSH, etc). Therefore, a series of H2S-activated therapeutic reagents have been designed on account of endogenous hydrogen sulfide, which is highly expressed in colon tumors, including H2S-activated chemotherapy, photodynamic therapy, and photothermal therapy (Table 2).

3.1 Chemotherapy

Chemotherapy is currently the main method used in the clinical treatment of cancer. Current chemical drugs for cancer treatment include doxorubicin (DOX), curcumin and so on. Unfortunately, we have not yet broken through the bottleneck in finding chemical drugs with excellent anti-tumor effects. Since most chemotherapeutic drugs have poor water solubility and low bioavailability, systemic administration is very difficult. The key problem is that normal cells will be damaged when the drugs are administered intravenously, resulting in toxic side effects. Therefore, scientists have long desired to develop a drug carrier from which the release of chemotherapeutic drugs can be stimulated at the tumor site only. In order to increase the targeting effect on tumor tissues and improve the therapeutic effect, a hydrogen sulfide-activated azide-functionalized biocompatible mesoporous silica nanoparticle (MSNPs) was developed by Thirumalaiavan et al. as a specific drug delivery system (Fig. 8A). Further, folic acid (FA) was attached to the surface of the MSNPs to actively target cancer cells. The surface charge of the micelles changes from negative to positive, which promotes the uptake of the materials by the cells and accelerates the release of DOX (Fig. 8D).

Similarly, Zhang et al. used a series of N-(2-hydroxyethyl)-4-azide-1,8-naphthalimide-ended amphiphilic diblock copolymer poly(2-hydroxyethyl methacrylate)-block-poly-methylmethacrylate (N2-Nap-PHEMA-b-PMMA-N3) polymer nano-micelles for loading DOX (Fig. 8C). Under the action of H2S, the charge on the surface of the micelles of these nano-materials is reversed and the azide reduction reaction occurs. The surface charge of the micelles changes from negative to positive, which promotes the uptake of the materials by the cells and accelerates the release of DOX (Fig. 8D).

A pharmaceutical carrier should have excellent biocompatibility. Chen et al. designed a H2S-activated protein cage (CuDOX NP) loaded with chemotherapeutic drugs. They used horse spleen apoferritin (apo-HSF) as a container for copper-complexed doxorubicin to obtain a water-soluble nano-composite. Breaking of the CuDOX coordination interaction by H2S under physiological pH conditions allows the DOX to be released.

Table 2 Summary of recent reports on H2S therapeutic agents

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<td>[Cu2(ZnTepp)2]</td>
<td>Photodynamic therapy</td>
<td>Colon cancer</td>
<td>99</td>
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<td>Electrochromic materials (EMs)</td>
<td>Photodynamic therapy</td>
<td>Colon cancer</td>
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<td>Theranostic prodrug (Nano-TNP-SO)</td>
<td>Photothermal therapy</td>
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<td>103</td>
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<td>Self-assembled H2S response small molecule (SSS)</td>
<td>Photothermal therapy</td>
<td>Colon cancer</td>
<td>104</td>
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slowly released from the protein cage without disrupting the structure of the protein. In vitro cell experiments showed that CuDOX nanoparticles activated by H2S can reduce the premature release of drugs, reduce the toxicity of DOX to normal cells, and enhance the anti-cancer effect.

3.2 Photodynamic therapy

Photodynamic therapy (PDT) is based primarily on the accumulation of non-toxic photosensitizers, oxygen and light to produce reactive oxygen species, particularly singlet oxygen (1O2), which selectively induces apoptosis and necrosis in cancer cells.97–99 PDT serves as a specific method for treatment of cancer because of its multiple merits, including non-invasiveness, obvious therapeutic effect, and lack of inhibition and adverse effects on the host system. However, most PDT agents are extremely hydrophobic, easily aggregate in aqueous solution, and have low accumulation in cancerous tissues, resulting in less generation of 1O2 at the required site. Moreover, they are easily trapped in normal tissues, and damage normal tissues during treatment. Therefore, it is worthwhile to develop intelligent photosensitizer agents (PSs) with good hydrophilicity that selectively accumulate at the tumor site. Effective tumor photodynamic therapy could be achieved by exploiting the high expression of endogenous H2S in colon cancer using a photosensitizer that recovers fluorescence under the activation of H2S. Ma et al.99 reported a nanoscale copper-zinc mixed-metal organic framework photosensitizer, [Cu2(ZnTcpp)-H2O]n (NP-1), activated by H2S for photodynamic therapy of colon cancer (Fig. 9A). 5,10,15,20-Tetrakis(4-methoxycarbonylphenyl)porphyrin (ZnTcpp) is a bridged photosensitive ligand with a mixed metal organic skeleton in which Cu2+ ions serve as a metal node of the skeleton. The paramagnetic Cu2+ ions not only completely quench the ligand fluorescence of the metal–organic framework (MOF) NPs, but also significantly reduce release of reactive oxygen species (ROS). H2S interacts with [Cu2(ZnTcpp)-H2O]n, and Cu2+ ions are taken out from the MOF node to obtain a photosensitizer, and the fluorescence is recovered (Fig. 9B). This open-type fluorescent MOF photosensitizer probe achieves effective cancer treatment through controlled release of photosensitive ligands, and the experimental results showed significant therapeutic effects (Fig. 9C).

In addition, Wu et al.100 reported a class of H2S-activated fluorescent probes and photodynamic smart reagents using electrochromic materials (EMs) with organic π-electron structure (dicationic 1,1,4,4-tetraphenylbutadiene, Z′) as H2S-responsive chromophores. EM12+ is doped into semiconductor polymer nanoparticles (SNPs) to form H2S-activatable fluorescent probes (Z′-SNPs) (Fig. 9D). Within 12+-SNPs, EM12+ can effectively quench the fluorescence of SNP by a fluorescence resonance energy transfer (FRET) process. Subsequent reduction of 12+ to colorless 2 NPs by H2S eliminates the FRET process and restores fluorescence. Further, tumor-targeting ligand folic acid modified fluorescent probes (Z′-SNP830-FA) were used for tumor imaging in H2S-enriched mice. Tumor-targeting and H2S-activatable PSs (Z′-PSs-FA) using EM12+ were further developed by replacing the SNP with organic PS. Z′-PSs-FA accumulates well at the tumor site. After H2S-specific activation, 12+-PSs-FA produces ROS under the action of 808 nm laser irradiation. The reagent exhibits negligible phototoxicity to normal tissues and significant tumor photodynamic therapy effects (Fig. 9E).

In addition, Wang et al.101 have designed and synthesized a theranostic prodrug (TNP-SO) for H2S-activatable near-infrared emission-guided on-demand administration of PDT. The theranostic probe consists of an H2S-activated NIR imaging probe and a sensitizing drug. These two units are connected by a short diglycolamine spacer. The newly obtained small molecule probe is encapsulated into the hydrophobic interior of a silica nanocomposite to produce a nanoprobe with good water solubility and photostability. The absorption of TNP-SO at 509 nm decreased as 677 nm NIR absorption increased after being triggered by H2S. The NIR fluorescence increased linearly with H2S concentration (0–20 μM), and the determined detection limit was 21 nM, indicating that Nano-TNP-SO has high sensitivity for H2S detection. Nanoprobes can also act as good photosensitizers for the efficient production of 1O2. The in vivo results using this probe reveal that cancer imaging accurately guides the location of light exposure to produce the cytotoxic ROS required for on-demand cancer treatment, maximizing treatment efficiency and minimizing side effects.

3.3 Photothermal therapy

Photothermal therapy is a simple, safe, non-invasive treatment method that converts near-infrared laser energy into heat energy to achieve local high-temperature killing of tumor cells.102 Near-infrared photothermal reagents based on photothermal therapy have attracted much attention. Traditional photothermal reagents have limitations such as non-specificity and toxicity. In order to solve these problems, photothermal reagents with intelligent response are required. Shi et al.103 developed a H2S-activated second near-infrared self-assembling

Fig. 9 (A) The simple structural fragment of MOF NP-1 and the proposed strategy for 1O2 generation in cancer therapy. (B) Fluorescence spectra of NP-1 reaction with H2S from 0 to 70 μM. (C) In vivo antitumor efficacy of NP-1 on HCT116 subcutaneous xenograft nude mice. Reproduced from ref. 99. Copyright 2017 WILEY-VCH. (D) Schematic illustration of H2S-activatable 12+-PSs-FA enabling controllable 1O2 generation for PDT. (E) The tumor treatment effect of 12+-PSs-FA. Reproduced from ref. 100. Copyright 2018 American Chemical Society.
fluorescent nanoprobe for guiding photothermal therapy of colon cancer (Fig. 10A). A self-assembled H2S response small molecule (SSS) was designed that contains three triethylene glycol monomethyl ether chain functionalized benzene rings as hydrophilic tails to guide the self-assembly of the SSS. The monochlorinated BODIPY core is the activatable unit based on thiol-halogen nucleophile substitution of H2S. In the absence of H2S, the nanostructured photothermal agent (Nano-PT) produces minimal photothermal effects with absorption and emission at 540 and 589 nm, respectively. However, the H2S response results in high NIR absorption near 790 nm, which not only causes efficient photothermal energy conversion with 785 nm laser irradiation, but also produces bright luminescence in the NIR-II region (Fig. 10B). Using these excellent properties, the Nano-PT enables efficient photothermal ablation of imaging-guided colon cancer tumors (Fig. 10C).

An et al.104 designed an intelligent diagnostic reagent for colon cancer based on the in situ reaction of cuprous oxide (Cu2O) with endogenous H2S at the colon tumor site (Fig. 10D). Highly expressed endogenous H2S in colon tumors reacts with cuprous oxide and produces copper sulfide, which has strong near-infrared absorption, triggering photoacoustic and photothermal effects (Fig. 10E). The design of the in situ reaction at the tumor site reduces the damage to normal tissues during treatment and produces a significant therapeutic effect (Fig. 10F).

4. Summary and outlook

Abnormalities in H2S levels are associated with the development of a variety of diseases, such as colon cancer, breast cancer and ovarian cancer. In order to achieve early prevention and diagnosis of related diseases, research aimed at producing highly sensitive and selective H2S probes has been promoted. Among the available techniques, optical detection methods have higher sensitivity than traditional H2S detection methods. The transition from a single wavelength fluorescent probe to a more sensitive ratiometric fluorescent probe reduces the effects of external environment and other factors. In order to achieve real-time monitoring of H2S in vivo, further development from a short-wavelength fluorescent probe to a second near-infrared fluorescent probe, and photoacoustic probe with high tissue penetration has taken place. More importantly, using the specialized microenvironment with high expression of endogenous H2S at the colon tumor site, H2S-activated intelligent therapeutic agents have been developed. Compared with the traditional reagents, this strategy reduces the damage to normal tissues and shows more obvious therapeutic effects. Although many H2S probes with high sensitivity and high selectivity have been developed so far, as well as H2S smart reagents for cancer treatment, it is still necessary to continue to explore probes with lower side effects before their clinical application.

We believe that the integration of diagnostic and therapeutic agents for H2S detection and related disease treatment has a broad development prospect. In our subsequent research we aim to: (i) develop diagnostic reagents that are easy to prepare, and have good stability and biocompatibility; (ii) combine a variety of methods for tumor diagnosis and treatment, to develop intelligent diagnostic reagents with multi-modal diagnosis and synergistic treatment—for example, combining fluorescent probes with photoacoustic probes;105 (iii) undertake an in-depth study of the side effects of various agents, as well as their potential toxicity. Only once the problems described in this review have been solved, can the reagents be further applied to clinical use.

Conflicts of interest

There are no conflicts of interests to declare.

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Notes and references
