Research progress in endogenous H₂S-activatable nanoplatforms for cancer theranostics

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Abstract

Hydrogen sulfide (H₂S) is a critical signaling molecule that exists in a wide variety of organizational categories, which has significant influences on several physiological functions containing vasodilatory modulation and neurotransmitter regulation. Dysregulated production and abnormal contents of H₂S are considered to be the characteristics of different types of disease occurrence; the detection and real-time monitoring of endogenous H₂S in tissues and living cells is of great significance. However, most conventional H₂S detection methods still suffer from some inevitable drawbacks including low bioavailability, accuracy, and sensitivity, making them difficult to apply in visualizing endogenous H₂S in vivo. Optical probes constructed with the features of fast responsive time, excellent selectivity and sensitivity, as well as noninvasive performance, providing the possibility of detecting and monitoring H₂S in real-time at the cellular and mouse levels. Such methods show the application prospect, which could avoid the defects of conventional detection approaches. Furthermore, a certain concentration of H₂S can influence the therapeutic efficacy, for example, anti-inflammatory and protection against oxidative stress, during the treatment of cancer and neurodegenerative diseases. Because the generation of overexpressed exogenous H₂S is closely tied to the tumor formation, the development of H₂S-responsive theranostic nanoplatfroms is highly needed for H₂S-related tumor diagnosis and treatment. The theranostic nanoplatfroms are expected to maximize therapeutic effectiveness and minimize side effects to normal tissues. In this review article, the current research progress, challenges, and future possibilities of H₂S-activatable nanoplatfroms for H₂S detection and malignant tumor theranostics are summarized.

Abbreviations: CBS, cystathionine β-synthase; CEW, chicken egg white; COF, covalent organic framework; CPT-11, camptothecin-11; CT, computerized tomography; DOX, doxorubicin; EM, electrochromic materials; ESIPT, excited state intramolecular proton transfer; FRET, Förster resonance energy transfer; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; ICT, intramolecular charge transfer; LP, liposome; LRET, luminous resonance energy transfer; MOF, metal-organic framework; NIR, near-infrared; NIR-II, second near-infrared; PA, photoacoustic; PDT, photodynamic therapy; PET, positron emission tomography; PTT, photothermal therapy; ROS, reactive oxygen species; SAM, S-adenosyl-L-methionine; SPNs, semiconducting polymer nanoparticles; UCL, upconversion luminescence; UCNPs, upconversion nanoparticles.
1 | INTRODUCTION

Hydrogen sulfide (H$_2$S) has long been considered as a colorless, flammable, and toxic gas with the characteristic smell of rotten eggs, and also viewed as the third gasotransmitter in the following of nitric oxide (NO) and carbon monoxide (CO).$^{1-4}$ Accumulating results indicate that the generation of H$_2$S occurs mainly through enzymatic pathways. Enzymatic generation of endogenous H$_2$S by cystathionine $\gamma$-lyase, cystathionine $\beta$-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase in mammalian cells has been confirmed to be organ specific in accordance with the category of enzymes.$^{5-9}$ Moreover, the distribution and modulation of H$_2$S generating enzymes are intricate, and the knowledge on the biological impact of endogenous H$_2$S is amplifying. Recent studies have shown that endogenously generated H$_2$S has special influences on pathological and physiological functions, containing the modulation of vasorelaxation, stimulation of cellular mitochondrial bioenergetics, anti-inflammation, reducing oxidative stress, neuromodulation, protection from reperfusion injury after myocardial infarction, inhibition of insulin resistance, and promotion of angiogenesis.$^{10-13}$

The physiological concentration of H$_2$S ranges 0.01-3 µM at the cellular level and 30-100 µM in the serum.$^{14,15}$ An abnormal concentration of endogenous H$_2$S may trigger many diseases such as Down syndrome, Alzheimer’s disease, cirrhosis, diabetes, and cancer, which has been demonstrated based on previous reports.$^{16-20}$ Therefore, investigations on endogenous H$_2$S, such as the real-time monitoring of H$_2$S and utilizing H$_2$S to explore particular theranostic nanoagents, have aroused a considerable concern in the critical fields of biomedicine, nanomaterials, and chemical science.

The evaluation of H$_2$S contents in vitro and in vivo is of great significance to comprehension of the relevant physiological processes and diagnosis of some diseases. Several methods have been proposed for the visualization of H$_2$S generation and distribution, containing electrochemical assay, gas chromatography, and the most widespread sulfide precipitation.$^{21-24}$ Nevertheless, such conventional detection methods are usually restricted by complex sample fabrication, low temporal resolution, and possible destruction of tissues or cells. For these reasons, a fluorescence-based method has appeared and received wide attention as a feasible approach for in situ and real-time detection of H$_2$S in biological systems, based on its special advantages of high specificity and sensitivity, shorter response time, excellent biocompatibility, and negligible cell damage simultaneously.$^{25,26}$ On account of the unique nucleophilic and reducing capacity of H$_2$S, scientists have innovatively explored fluorescent probes with excellent sensitivity, chemical selectivity, and water solubility for H$_2$S inspection.$^{27}$ So far, the recognizable process of these probes is mainly conducted through H$_2$S-triggered Michael reaction, reduction reaction of azide and nitroso with H$_2$S to amine, precipitation with copper (II) ion, and thiolysis of dinitrophenyl ether or other groups by H$_2$S.$^{28}$

Accordingly, emerging evidence has revealed that the H$_2$S-generating enzyme CBS is optionally up-modulated, bringing out an increased rate of H$_2$S formation in cancer cells (such as colon cancer cells, breast cancer cells, prostate cancer cells, and ovarian cancer cells) as compared with that in noncancerous tissues or nontransformed cells.$^{29}$ Such H$_2$S could promote cancer cell bioenergetics, proliferation, migration, and invasion, as well as serve as an emerging target for oncotherapy.$^{30,31}$ According to the present condition, major diagnostic and therapeutic approaches for malignant tumor in clinical are imaging diagnosis (including computerized tomography (CT) and magnetic resonance imaging), surgical excision, chemotherapy, and radiotherapy. However, many problems are still existent, for instance low selectivity, apparent toxic side effect, and recurrence, which are seriously hindering its further development. Utilizing overexpressed endogenous H$_2$S as an initiator to activate the ingenious nanotheranostic reagents with high selectivity has been considered a promising strategy for cancer therapy with reduced side effects and enhanced therapeutic effect. Up to now, some smart nanomaterials have been designed and exploited as H$_2$S-activated theranostic agents.

In this review, we take a deep insight on the development of H$_2$S-activatable smart theranostic agents, and these nanoplatforms demonstrated favorable prospects for diagnosis prevention and anticancer treatment applications (Scheme 1). These nanomaterials can be primarily divided into three categories according to their functions: (a) ingenious nanoprobe for the detection of H$_2$S levels, (b) smart nanoagents for H$_2$S-activatable bioimaging, and (c) nanotheranostic agents with H$_2$S-responsive performance for cancer diagnostics and treatment. Thus, H$_2$S-activatable nanotheranostic agents as promising systems for the diagnosis and therapeutics of cancer are summarized in this review. In addition, current challenges and further requirements of H$_2$S-activatable nanotheranostic agents are also discussed.
SCHEME 1  Schematic illustration of in situ chemical reaction by endogenous H₂S-activatable nanoplatforms and their applications in fluorescence probes, bioimaging, and cancer therapy

2 | FLUORESCENT PROBE FOR H₂S DETECTION

As an endogenous signaling molecule, H₂S involves a great deal of physiological functions to maintain cellular health. How to accurately locate molecular targets of H₂S remains an ongoing exploration and a major challenge. Thereby, the quantitative analysis of cellular H₂S level is crucial for biological research and clinical diagnosis. There are some significant problems that should be taken into consideration when constructing a H₂S-responsive nanoplatform. The heart of the problem is the discrimination of H₂S over other biological sulfur species such as cysteine and glutathione. Low detection sensitivity and extended response time are other principal obstacles that need to be considered. In comparison with the conventional detection methods, fluorescent probes possess the specific advantages of monitoring H₂S in real time in biological systems, because they are highly sensitive to biological targets, no significant damage to tissue, and easy to detect. Therefore, it is important to fabricate highly sensitive and selective fluorescent probes, which could be applied for monitoring and tracking H₂S in biosystems (as shown in Table 1).

2.1 | Turn-on near-infrared fluorescent probes

On account of the nucleophilic and reduction performance of H₂S, researchers have constructed multiple fluorescent probes for detecting H₂S by the reduction of azide to amine, nucleophilic reaction, and so on. Erman et al developed an excited state intramolecular proton transfer (ESIPT)-based turn-on fluorescent probe with fast responsiveness, high sensitivity, and high selectivity. The probe was made by conjugating 3-hydroxyflavone with an electrophilic cyanate motif (Figure 1A). A cyanate (RO-CN) can be served as a reaction precursor to interact with sulfur species to generate thiocarbamates that frequently hydrolyze to hydroxyl derivatives in aqueous solution. With the stimulation of H₂S, the probe

| H₂S probe                          | Reaction mechanism         | Wavelength (nm) | Detection limit | Experimental subject | Detection method            | Ref. |
|-----------------------------------|-----------------------------|-----------------|-----------------|----------------------|-----------------------------|------|
| ESIPT-based fluorescent dye       | Nucleophilic reaction       | 525             | 0.25 µM         | A549 cells           | Fluorescent microscopy      | 43   |
| NBD-based probe                   | Azide to amine              | 589             | 0.057 µM/0.58 µM| HEK293 cells         | Fluorescent microscopy      | 44   |
| 1-NH₂                             | Azide to amine              | 670             | 3.05 µM         | MCF-7 cells          | Two-photon microscopy       | 46   |
| TpASH-NPHS                        | Azide to amine              | 535             | 0.11 µM         | HepG2 cells          | Two-photon microscopy       | 47   |
| TAB-2-Cu²⁺                        | Copper sulfide precipitation| 500-550         | 47 nM/123 nM    | NIH/3T3 cells        | Two-photon microscopy       | 48   |
| p-Nitrobenzyl-based probe (RHP-2) | Nitro to amino              | 467/532         | 270 nM/280 nM   | MCF-7 cells          | Confocal microscopy         | 54   |
| SHS-M2                            | Azide to amine              | 420/500         | 0.4 µM          | DJ-1-deficient astrocytes and brain slices | Two-photon microscopy | 21   |
| NanoBODIPY                        | Nucleophilic reaction       | 511/589         | 7 nM            | Raw 264.7 macrophage cells | Confocal microscopy | 59   |
| Coumarin-merocyanine dyad (CPC)   | Nucleophilic reaction       | 474/587         | 40 nM           | HeLa cells           | Confocal microscopy         | 60   |
triggered the fracture of the external group and produced free hydroxyl group, achieving the turn-on responsive fluorescence. A characteristic emission peak at 525 nm could be detected on account of the ESIPT-regulated fluorescence response, as well as a new absorption peak at 425 nm appears with the decline in absorption band at 297 nm (Figure 1B). Such probe exhibits the exceptional selectivity, prominent fluorescence improvement (more than 200-folds), low detection limit, and fast response time to H$_2$S in compared with other reactive species based on the fluorescence emission spectra (Figure 1C). The probe indicated the potential application prospect for detecting H$_2$S in living cells (Figure 1D). In addition, Xi et al constructed two fluorescent probes by integrating fluorescein (probe 1) or rhodamine B (probe 2) with 7-nitro-1,2,3-benzoxadiazole (NBD) moiety for the fast and selective detection of H$_2$S. The fluorescence emission intensity of probe 1 was obviously increased with the addition of H$_2$S, with the detection limit of 0.057 µM. Similarly, with the introduction of H$_2$S, the probe 2 displays a significant absorption increase at 567 nm, and meanwhile a new characteristic peak appears at 530 nm. After reacted with H$_2$S, the fluorescence intensity increase at 589 nm was much smaller than that for probe 1, due to the quenching of rhodamine B fluorescence by NBD group based on the photo-triggered electron transfer effect. The detection limit of probe 2 was determined to be 0.58 µM, indicating that probe 2 could selectively detect H$_2$S. The smart NBD-based turn-on fluorescent probes can serve as fluorescent agents for H$_2$S detection and endogenous H$_2$S-responsive fluorescent imaging.

2.2 | Two-photon fluorescent probes

In comparison with the conventional one-photon microscopy imaging, two-photon microscopy imaging by using two photons of low energy to achieve the excited state of a fluorophore possesses more unique advantages including deep tissue penetration, low phototoxicity, and self-absorption. Nevertheless, almost all currently reported two-photon probes have short emission wavelengths in the range of 380-550 nm, which severely limited their biological application. Thus, the development of novel two-photon fluorescent probes with near-infrared (NIR) fluorescence emission is of great importance. In order to meet the above requirements and clarify the practical application of fluorescent probes, a smart two-photon fluorescent probe based on benzopyran derivative (1-NH$_2$) was synthesized by linking a styrene group to extend the emission wavelength to the NIR region by Sun et al. Then, they prepared the probe by substituting the amino group with azide group, which can be reacted with H$_2$S to generate 1-NH$_2$ with apparent fluorescence changes for detection of H$_2$S in vitro and in vivo. After H$_2$S treatment, the intrinsic absorption at 400 and 425 nm significantly declined and a new characteristic absorption peak emerged at around 505 nm along with the redshift of the peak, causing the color change of the solution from yellow to orange red. The probe can be used for H$_2$S evaluation with the detection limit of 3.05 µM. The probe was utilized to understand endogenous H$_2$S-triggered imaging in living cells, tissues, and in vivo mice, which exhibited the desired two-photon absorption performance. In addition, Zhang et al reported two-photon covalent-organic framework (COF)-based hybrid nanoprobe with low biological toxicity and excellent photostability and imaging performance. The probe, namely, TpASH-NPHS, integrates the unique advantages of COFs and small molecule probes. In the presence of H$_2$S, the probe exhibited an apparent increase in fluorescence signal intensity at the wavelength of 535 nm by reducing the azide group to amine group, and the detection limit for H$_2$S was determined to be 0.11 µM. The probe could serve as a model analyte for targeting detection of...
endogenous H₂S without interference from other intracellular biomacromolecules. The two-photon fluorescent COF-based probe can also be utilized for detection and imaging of H₂S in living cells and deep tissues irradiated with NIR laser, hence greatly minimizing the tissue autofluorescence, reducing tissue damage, and improving tissue penetration depth.

**π-Conjugated triarylboron compounds** constructed by electron donor groups could display intramolecular charge transfer (ICT) with a large dipole moment due to the strong electron deficiency of boron, enabling them to possess large two-photon absorption cross section. Thus, the π-conjugated triarylboron compounds could be used as fluorescent probes with high fluorescence quantum yield and photostability in different research fields. Combined with the remarkable characteristics of π-conjugated triarylboron derivatives along with quick response ability between sulfide anion and copper (II) ion, turn-on two-photon fluorescent probes based on triarylboron compounds conjugated with cyclen and diphenylamine (namely, TAB-1, TAB-2, and TAB-3) were fabricated by Liu and co-workers (Figure 2A). The interaction between copper ion and cyclen impedes the ICT process, which leads to the fluorescence quenching. Cu²⁺ can be detached from the fluorescent probe to restore the fluorescence in the presence of H₂S with high selectivity and sensitivity (Figure 2B). The detection limit of TAB-2-Cu²⁺ was determined to be 47 and 123 nM through monitoring changes in fluorescence and lifetime, respectively. The living cell imaging results revealed that TAB-2-Cu²⁺ with good cellular membrane permeability and low toxicity was uniformly distributed in mitochondria, which endows it as a specific fluorescent probe for detecting endogenous H₂S (Figure 2C).

### 2.3 Ratiometric fluorescent probes

For most of reported single-signal emission fluorescent probes, the experimental results are easily influenced by the experimental conditions, which may result in an inaccurate detection of H₂S. In comparison with turn-on fluorescent probes, ratiometric fluorescent probes are more accurate for detecting H₂S, independent of variables in quantitative analysis such as excitation intensity variations, environmental factors, light scattering, and probe concentrations. Therefore, the development of ratiometric fluorescent probes is of great importance, which is able to eliminate the interference caused by external experimental conditions. Zhang et al constructed p-nitrobenzyl-based ratiometric fluorescent probe (RHP-2) with the advantages of low detection limit, excellent photostability, and high selectivity. Such probe was synthesized by connecting a p-nitrobenzyl group to 1,8-naphtha-limide via a carbamate linkage. RHP-2 exhibited a characteristic single fluorescence emission peak at 467 nm without introduction of H₂S. In the presence of H₂S, the fluorescence intensity of the characteristic peak at 467 nm decreased gradually, together with the emergence of a new characteristic peak at 532 nm due to the nitro-reduction mechanism. Simultaneously, the fluorescence emission color of the solution transformed from blue into green, suggesting that RHP-2 could serve as a ratiometric fluorescent probe for H₂S detection. The detection limit of RHP-2 for H₂S was determined to be 270 and 280 nM in phosphate buffered solution and fetal bovine serum, separately. RHP-2 is biocompatible and capable of ratiometrically imaging endogenous H₂S in living cells, mouse, and hippocampus.

As discussed above, the current reported turn-on fluorescent probes can be affected by the experimental conditions, containing laser power and fluorescent probe distribution,
making them impossible to quantitatively determine H$_2$S. Lately, mitochondrial-targeted ratiometric fluorescent probes for determination of H$_2$S were also constructed, where the excitation wavelength is located in the short wavelength region with lower tissue penetration depth. Therefore, it is significant to design and fabricate two-photon ratiometric fluorescent probes for endogenous H$_2$S detection, because two near-infrared photons as the irradiation source have the unique advantages, such as deep tissue penetration depth and accurate excitation position. Bae et al. designed a mitochondrial-targeted two-photon ratiometric fluorescent probe (SHS-M2), which was constructed by 6-(benzo[d]thiazol-2'-yl)-2-(methylamino)naphthalene as the fluorophore, triphenylphosphonium salt as the mitochondrial targeted factor, and 4-azidobenzyl carbamate as the H$_2$S activating site, as revealed in Figure 3A. After treatment with H$_2$S, the thiolate-induced interaction with the azide group would cleave the carbamate linkage and generate the amino group, together with a significant decrease in emission intensity at 420 nm and a concomitant increase at 500 nm (Figure 3B). Such probe demonstrated two-photon-activated fluorescence, and a significant change in emission color from blue to yellow can be detected. Moreover, the detection limit of SHS-M2 for H$_2$S was measured to be 0.4 µM in vitro, indicating its high sensitivity. The probe exhibited significantly higher response to H$_2$S than to glutathione and cysteine, thereby affirming the probe with high sensitivity for quantitative analysis of intracellular H$_2$S with the minimum interference.
More significantly, SHS-M2 could serve as a two-photon ratiometric fluorescent probe to judge different H$_2$S levels generated by various CBS expression levels (Figure 3C-E).

### 2.4 | FRET fluorescent probes

Recently, a large number of ratiometric fluorescent probes based on Förster resonance energy transfer (FRET) process have appeared.\(^5^7\) FRET refers to the interaction between two different fluorescence groups by a nonconjugated link in the same molecule. To a certain extent, the emission wavelength of the donor and the absorption wavelength of the acceptor can effectively overlap, and the donor may absorb radiation energy and transfer to the acceptor. A FRET-based process can effectively avoid the influence of excitation background scattering on fluorescence detection thanks to the remarkable shift between donor excitation and acceptor emission.\(^5^8\) Moreover, two well-disconnected emission bands with analogous intensity can be more precise in determining their intensities and ratios. Therefore, the development of a new ratiometric fluorescent probe with extraordinarily fast, sensitive, and selective detection of H$_2$S is extremely desired. Zhao et al. presented a FRET switchable self-assembled micellar nanoprobe (NanoBODIPY; Figure 4A) by encapsulating a semi-cyanine-BODIPY hybrid dye (BODInD-Cl) and its complementary energy donor (BODIPY1) into the hydrophobic interior of an amphiphilic copolymer (mPEG-DSPE).\(^5^9\) An apparent redshift in the absorption band of BODInD-Cl was detected upon H$_2$S activation, where the Cl group on the aromatic ring is replaced by H$_2$S based on nucleophilic substitution, resulting in the loss of FRET and the restoration of donor fluorescence. NanoBODIPY is highly sensitive to H$_2$S with a detection limit of 7 nM, proving its great prospects for H$_2$S detection. Also, a significant ratiometric fluorescence change was detected, and the fluorescence intensity of NanoBODIPY1 at 511 nm exhibited an apparent increase along with the decrease of emission intensity at 589 nm simultaneously (Figures 4B and 4C). Such research demonstrated a H$_2$S-triggered ratiometric fluorescent probe with fast responsiveness and selectivity based on FRET process for the detection of endogenous H$_2$S in living cells (Figure 4D).

Similarly, Feng et al developed a FRET-based ratiometric fluorescent probe (namely CPC), which is composed of coumarin-merocyanine dyad.\(^6^0\) In the absence of H$_2$S, the probe demonstrated merocyanine fluorescence emission, as the emission spectrum of coumarin overlaps obviously with the absorption spectrum of merocyanine for generating FRET process. In contrast, the reaction between merocyanine moiety and H$_2$S provides a nucleophilic substitution product (CPC-S), which is not a fluorescent receptor on account of the damage of the conjugated system and the disappearance of the FRET process. The probe exhibits only the characteristic emission signal of the coumarin unit. The detection limit of the probe was determined to be 40 nM. In addition, the probe presented an apparent variation of dual-emission peaks at 474 and 587 nm upon H$_2$S addition under a single wavelength irradiation, and the fluorescence intensity ratio of the emission peaks ($I_{474}/I_{587}$) raised. The probe can be used as a tool for ratiometric detection of endogenous H$_2$S in mitochondria, which has high selectivity and sensitivity to H$_2$S.

### 3 | H$_2$S-ACTIVATABLE NANOPROBES FOR BIOIMAGING

Although fluorescence-based detection approaches and a variety of fluorescent probes have been developed and applied for real-time monitoring of H$_2$S,\(^6^1-6^3\) many issues still exist, such as low tissue penetration depth, poor photostability, and low spatial resolution, which seriously hinder their practical applications for in vivo endogenous H$_2$S detection.\(^6^4\) Currently, endogenous H$_2$S-responsive imaging probes for in vivo bioimaging are still rare, particularly for the imaging diagnosis of H$_2$S-correlated diseases, such as cancer. Suitable fluorescent probes with fast response, high selectivity, and high sensitivity for the imaging of endogenous H$_2$S in vivo are desired to satisfy the current application requirements (Table 2).

#### 3.1 | Second near-infrared fluorescence imaging probes

To date, H$_2$S-activatable fluorescent and chemiluminescent probes have been presented and utilized for imaging diagnosis and real-time detection of H$_2$S levels.\(^6^5-6^7\) However, the fluorescence emission centers of the current reported fluorescent probes are mainly located in the visible or first near-infrared region (650-900 nm), and inevitably there are still many problems that need to be solved, such as low tissue penetration depth and serious background interference from living organisms.\(^6^8-7^1\) Fluorescence imaging in the second near-infrared window (NIR-II; 1000-1700 nm) has aroused wide attention, as it possesses the advantages of reduced autofluorescence, deeper tissue penetration, and higher spatial resolution.\(^7^2\) Therefore, the development of H$_2$S-activatable NIR-II emission fluorescent probes with high sensitivity, excellent spatial resolution, and tumor-targeted properties holds great prospects for improving the diagnostic accuracy. Recently, Zhao et al. developed a H$_2$S-activatable NIR-II@Si fluorescent nanoprobe to image veracious location of colorectal cancer.\(^7^3\) The visualized nanoprobe was synthesized by the encapsulation of the H$_2$S-responsive fluorescent probe borondipyromethene (ZX-NIR) and aza-BODIPY (inert to H$_2$S) in the hydrophobic interiors of micellar aggregates based on
FIGURE 4 (A) Chemical structures of BODInD-Cl, BODIPY1, and mPEG-DSPE, as well as formation of micellar aggregate NanoBODIPY for modulating FRET from the complementary energy donor (BODIPY1) to the responsive energy acceptor (BODInD-Cl). (B) Time-dependent ratiometric fluorescence changes of NanoBODIPY in the presence of NaHS (100 µM) in PBS (pH 7.4) at 37°C. Inset shows changes of fluorescence spectra in the absence and presence of NaHS. (C) Fluorescence spectra of NanoBODIPY in the presence of various concentrations of NaHS (0-10.0 µM) in PBS buffer (pH 7.4) at 37°C. (D) Ratiometric fluorescence changes of NanoBODIPY in the presence of 100 µM NaHS and other biologically relevant competing analytes in PBS buffer (pH 7.4) at 37°C. λex = 490 nm. Reproduced with permission. Copyright 2015, American Chemical Society

TABLE 2 Summary of recently reported H2S imaging probes

| H2S probe     | Reaction mechanism | Wavelength (nm) | Detection limit | Experimental subject | Detection method | Ref. |
|---------------|--------------------|-----------------|-----------------|----------------------|-----------------|-----|
| NIR-II@Si     | Nucleophilic reaction | 700/900          | 37 nM           | HCT116 tumor mice    | Fluorescence imaging | 73  |
| Ag-CEW        | Chemical reaction   | 1090             | 35 nM           | HCT116 tumor mice    | Fluorescence imaging | 74  |
| TPE-BODIPY-C1 | Nucleophilic reaction | 920             | 0.65 µM         | HCT116 tumor mice    | Fluorescence imaging | 75  |
| Si@BODPA      | Nucleophilic reaction | 780             | 53 nM           | HCT116 tumor mice    | PA imaging       | 83  |
| AzHD-LP       | Azide to amine      | 600/700          | 91 nM           | HCT116 tumor mice    | PA imaging       | 81  |
| HS-CyBZ       | Nucleophilic reaction | 630/805          | 0.5 µM          | HCT116 tumor mice    | PA imaging       | 84  |
| UCNPs@mSiO2-MC| Nucleophilic reaction | 540/800          | 0.58 µM         | HeLa cells           | UCL imaging      | 104 |
| UCNPs@CD-CHC1 | Nucleophilic reaction | 541/800          | 0.13 µM         | Lipopolysaccharide-induced inflammation | UCL imaging | 105 |
| 64Cu-cyclen   | Chemical reaction   | _               | 0.15 µM         | Myocardial infarction mode | PET imaging | 109 |

the self-assembly strategy, followed by covalent cross-linking with N-trimethoxysilylpropyl-N,N,N-tri-n-butylammonium bromide (TBNBr) to form the silica shell (Figure 5A). When the nanoprobe coexists with H2S, the NIR-II fluorescence emission could be detected at the wavelength of 900 nm (Figures 5B and 5C). As a comparison, aza-BODIPY was utilized as a reference, and its absorption band and fluorescence emission intensity exhibited no apparent changes in the absence
of H$_2$S. The detection limit of NIR-II@Si was evaluated to be 37 nM, revealing its high sensitivity for ratiometric detection of H$_2$S. This H$_2$S-responsive specific nanoprobe can also be used as a visualization tool for in vivo bioimaging of H$_2$S-overexpressed cancer cells (Figure 5D). Taking advantages of the NIR-II fluorescence imaging and the high levels of H$_2$S in cancer cells, the probe may be able to accurately locate the tumor and visualize the diagnosis and treatment process.

In addition, Hao et al designed an endogenous H$_2$S-activated NIR-II emitting optical probe (Ag-CEW) for specific diagnosis of colorectal cancer (Figure 6A). The Ag-CEW complex was prepared by utilizing chicken egg white (CEW) as the surface ligands for chelating Ag$^+$ at room temperature. Ag-CEW revealed an obvious NIR-II fluorescence emission based on endogenous H$_2$S-triggered in situ chemical reaction to generate Ag$_2$S quantum dots. In the presence of H$_2$S, Ag-CEW exhibited an apparent NIR-II fluorescence emission peak at around 1090 nm and ultrasensitive detection capability of H$_2$S with the detection limit of 35 nM, confirming its accurate detection of H$_2$S (Figure 6B). Moreover, the Ag-CEW complex was successfully used for in vivo NIR-II fluorescence imaging-induced specific diagnosis of colon cancer with high sensitivity, and the interference signal was thoroughly shielded by reticuloendothelial system through in situ H$_2$S activation (Figure 6C). Wang et al.

3.2 | Photoacoustic imaging nanoprobes

Photoacoustic (PA) imaging is a state-of-the-art imaging modality, which depends on the translation of excitation light into ultrasonic waves according to the PA effect, and allows deeper tissue imaging penetration and higher in vivo spatial...
resolution when comparison with traditional optical imaging techniques. As a result of these significant advantages, PA imaging as a noninvasive imaging tool has been extensively applied in biology and medicine. Recently, a H$_2$S-triggered PA nanoprobe was presented through the encapsulation of semi-cyanine-BODIPY hybrid dye (BODPA) in the hydrophobic interior of a core-shell silica nanosystem (abbreviated as Si@BODPA), which exhibited excellent water solubility and biocompatibility, as demonstrated in Figure 7A. When introducing H$_2$S into Si@BODPA aqueous solution, the BODPA dye was converted into its thiol adduct based on nucleophilic substitution reaction, which demonstrated a significant absorption in NIR region at the wavelength of around 780 nm with a strong PA signal (Figure 7B). Furthermore, such nanoprobe revealed a quick response to capture transient H$_2$S with the detection limit of 53 nM. What’s more, this nanoprobe could act as a potential system for PA imaging of overexpressed endogenous H$_2$S in a colorectal tumor-bearing mouse model. As shown in Figures 7C and 7D, no prominent PA signal was found in the normal site and the tumor site of the mice pre-injected with CBS inhibitor aminooxyacetic acid (100 nmol) after the administration of Si@BODPA, whereas there was an apparent signal enhancement detected with or without pre-injection of a CBS activator S-adenosyl-L-methionine (SAM), suggesting that Si@BODPA could effectively detect H$_2$S in vivo. Such nanoprobe shows high selectivity to endogenous H$_2$S and could be used to perform real-time monitoring of H$_2$S-relevant biological functions.

At present, the PA probes for H$_2$S detection were designed under a single PA signal mode, which might lead to inaccurate detection results due to some uncertain factors such as equipment performance, ambient conditions, and photo-bleaching. As an obvious contrast, the development of ratiometric PA probes is of great significance, which could obliterate the defects by the self-calibration utilizing two noninterfering PA signals at disconnected wavelength, ultimately realizing reliable analysis results. Lately, a ratiometric PA nanoprobe (AzHD-LP) consisting of H$_2$S-responsive NIR dye (AzHD) and a liposome (LP) was designed by Ma and co-workers (Figure 8A). The H$_2$S-triggered ratiometric PA nanoprobe showed redshifts of two obvious characteristic absorption peaks when the azide group was reduced to the amine group. The absorption intensity of AzHD-LP centered at the wavelength of 600 and 700 nm decreased and increased, separately. Moreover, AzHD-LP could effectively respond to NaHS aqueous solution with the detection limit of 91 nM. AzHD-LP was further modified with the peptide c (RGDyK) to achieve a tumor-targeting nanoprobe. The inspection of intratumoral overexpressed H$_2$S with high sensitivity was acquired on colon tumor-bearing mouse modal, where the PA signals could be detected at 532 and 700 nm simultaneously (Figure 8B). The turn-on ratiometric PA probe activated by...
FIGURE 7  (A) Schematic illustration for the construction of activatable PA probes to H2S. (B) Absorption changes of Si@BODPA (10 µM BODPA1 or BODPA2) in the absence and presence of NaHS (100 µM). (C) In vivo PA imaging of tumor-bearing mice using Si@BODPA180(7) at different time points after the injection: (1) saline-treated mice into the tumor regions; (2) probe-treated mice at normal sites; (3) probe treated mice in the tumor regions; (4 and 5) mice pretreated with (4) 100 nmol aminooxyacetic acid or (5) 300 nmol SAM for 12 h, and then subcutaneously injected with Si@BODPA180(7) in the tumor regions. (D) PA intensity as a function of time upon post-injection of Si@BODPA180(7). Reproduced with permission.© Copyright 2017, Royal Society of Chemistry
H$_2$S with a remarkable dual-absorbance response as well as high sensitivity and selectivity was achieved.

Up to now, single mode fluorescent probes or PA probes have been widely investigated for H$_2$S detection. Single-channel optical or PA imaging has its inevitable disadvantages including limited tissue penetration depth as well as low sensitivity and resolution, which is not reliable for detecting applications. Therefore, the NIR ratiometric dual-modality imaging probes are urgently needed. Recently, Chen et al developed an optical/PA dual-modality H$_2$S-activatable ratiometric probe (HS-CyBz) for in/ex vivo ratiometric optical and PA imaging based on the up-modulation of endogenous H$_2$S.$^{84}$ Two well-separated characteristic emission bands centered at 805 and 630 nm could be detected in the emission spectrum of HS-CyBz. In the presence of H$_2$S, the emission intensity of the former decreases and that of the latter increases dramatically. The H$_2$S detection limit of this probe was measured to be 0.5 µM, revealing its excellent ratiometric response performance to H$_2$S. Then, the in vivo optical/PA imaging on HCT116 tumor-bearing mice with high levels of endogenous H$_2$S was performed. As shown in Figures 8C and 8D, the signal ratios of both F$_{620}$/F$_{790}$ and PA$_{825}$/PA$_{775}$ at
the tumor sites exhibited a significant improvement after the administration of HS-CyBz, in contrast with that at the normal sites. This research confirms that ratiometric optical/PA dual-modality imaging probes are more reliable, showing high efficacy and sensitivity for the detection of H₂S.

### 3.3 UCL imaging probes

Upconversion nanoparticles (UCNPs) can convert longer wavelength irradiation (NIR) to shorter wavelength visible light emission with a considerable anti-Stokes shift. In comparison with inorganic semiconductor nanoparticles or organic dyes, UCNPs demonstrate outstanding characteristics of negligible autofluorescence from biological tissues, deep tissue penetration depth, no photobleaching, and less damage to biological tissues. As a consequence, UCNPs are considered as desirable nanoprobes for monitoring living cells and animal imaging. To acquire specific performance, UCNPs need to interact with other chromophores with identifiable sites, based on the FRET process. Several UCNP/chromophore-based FRET nanoplatforms have been presented for monitoring biological species and toxins. In these applications, the UCNPs (donor) transfer energy to the organic chromophores (acceptor) to cause changes in the upconversion luminescence (UCL) emission. Therefore, UCNP/chromophore nanoplatforms show promising prospects for being as H₂S detection probes.

Because the multicolor luminescence of UCNPs can be modulated by doping various ions, several chromophores with different absorption bands could be conjugated and fabricated for specific H₂S activation. As such, Peng et al developed three H₂S-activatable chromophores interacted with UCNPs having varied doping ions. Such H₂S sensors showed responsive luminescence emission signals within the visible-NIR region. Although these UCNP/chromophore systems displayed different FRET efficiency, they all demonstrated excellent selectivity and rapid responsiveness in living cells. Dop- ing Tm³⁺ into UCNPs produces UCL emission signal at 800 nm, which can be used as a potential candidate for ratiometric detection of H₂S with enhanced sensitivity. Moreover, Liu et al developed porous silica-coated UCNPs followed by conjugation with mercocyanine (UCNPs@mSiO₂-MC) for determination of H₂S by monitoring the ratio changes of the UCL intensity at various wavelengths (Figure 9A). As shown in Figure 9B, UCNPs@mSiO₂-MC displayed an ascended ratiometric fluorescence signal (I₅₄₀/I₈₀₀) for higher sensitivity with the detection limit at approximately 0.58 µM, which was lower than that of another mercocyanine-based H₂S nanosystem (about 1.0 µM). Analogously, Zhou et al designed coumarin-hemicyanine (CHC1)-conjugated α-cyclodextrin-modified upconversion nanophosphors (UCNPs@CD-CHC1) as a ratiometric UCL nanoprobe for effective H₂S detection (Figure 9C). By determining the ratio of UCL signal intensity at the wavelengths of 541 and 800 nm, in comparison with single UCL signals (1.85 µM), such nanoprobe exhibited a high sensitivity to H₂S with the detection limit of 0.13 µM (Figure 9D). This UCNPs@CD-CHC1 could be utilized for ratiometric UCL detection of pseudoenzymatic H₂S generation in living cells, demonstrating its capability to monitor lipopolysaccharide-triggered inflammation in the liver tissues of mouse models (Figure 9E).

### 3.4 PET imaging probes

Even though fluorescence-based imaging has been proposed as the principal method for inspection of H₂S, its applications in live-animal imaging are restricted due to the limited quantitative analysis. Positron emission tomography (PET) imaging technique is highly sensitive and noninvasive for molecular imaging of metabolism, signal transduction, and gene expression from assay to clinic. As expected, targeted and sensitive PET nanoprobes have also been presented for H₂S-activatable imaging. For instance, Yoo et al adopted the radioisotope-based immobilization technique for the detection, quantification, and in vivo imaging based on endogenous H₂S via PET imaging. The macrocyclic ⁶⁴Cu complexes could react with endogenous H₂S to product insoluble ⁶⁴CuS nanoparticles (Figure 10A). ⁶⁴Cu-cyclen demonstrated high sensitivity and selectivity for H₂S as compared with other potential nanodetectors containing polysulfides. Because of the difference of physical properties, intravenously administrated ⁶⁴Cu-cyclen and ⁶⁴Cu-cyclam were rapidly cleared from the body, whereas the formed ⁶⁴CuS nanoparticles were fossilized for more than 4 h after interacting with H₂S (Figure 10B). Once ⁶⁴Cu-cyclen was intravenously injected into tumor-bearing mice, an ascended H₂S content within the inflamed parts of the paw was distinctly observed and quantified through Cerenkov luminescence and PET imaging simultaneously (Figures 10C and 10D). Furthermore, the ⁶⁴Cu-cyclen could be also utilized to study the defect site in the myocardium from a severe myocardial infarction model, as exhibited in Figure 10E-H. As far as it goes, such probe exhibited a good prospect as a powerful nanosystem, ending efficient detection, precise quantification, and distinct imaging of living animals with abnormal H₂S.

In this section, the research progress on NIR-II fluorescent, PA, UCL, and PET imaging probes for H₂S detection and bioimaging is summarized. Different imaging modes have inherent advantages, for instance, NIR-II fluorescence imaging possesses low autofluorescence, PA imaging presents deep tissue penetration depth, high temporal-spatial resolution, and sensitive noninvasiveness for molecular imaging assays. Some drawbacks could be avoided by combining multiple imaging modes into a single probe.
Taking the advantages of ratiometric imaging and theranostic requirements into consideration, the development of endogenous H2S-responsive ratiometric multimodality imaging nanoprobes with therapeutic functions is highly desired.

### 4 | H2S-ACTIVATABLE NANOAGENTS FOR CANCER THERAPY

H2S, as a crucial signaling molecule in human body, has a crucial impact on health and diseases. Current studies have shown that H2S is overproduced (0.3-3.4 mM) in some tumor cells, such as colon cancer, due to the selective up-modulation of CBS. The generated H2S could promote tumor growth, proliferation, and angiogenesis, and it also provides nutrients to the tumor. The endogenous H2S could be used as an emerging anticancer therapeutic target for specific tumor therapy, which might solve the current existing problems, for example, misdiagnosis, recurrence, and metastasis.

Therefore, the development of endogenous H2S-responsive theranostic nanoagents have been regarded as an effective strategy to enhance the therapeutic effect according to the fact that H2S is overexpressed in colon cancer cells, as shown in Table 3. Thus, H2S-activatable nanoagents with different treatment modalities containing chemotherapy, photodynamic therapy (PDT), and photothermal therapy (PTT) are summarized as follows.

#### 4.1 | Chemotherapy

Chemotherapy is the main mean of clinical cancer treatment. Major FDA-approved chemotherapy drugs include doxorubicin (DOX), paclitaxel, and so on. It is unfortunate that we have not broken through the bottleneck of discovering new anticancer drugs with absolute therapeutic efficacy. Currently, the chemotherapy drug developed have some
FIGURE 10  (A) Schematic illustration for the production of $^{64}$CuS via $^{64}$Cu-cyclen reaction with H₂S. (B) Detection of H₂S in vivo: (i) Cerenkov luminescence images of the SD rats injected with Matrigel alone (top left), Matrigel + NaCl (top right), H₂S gas solution (bottom left) and NaHS (bottom right), respectively, on the back at 0-h postinjection of $^{64}$Cu-cyclen; (ii) PET image of the injection sites at 4-h postinjection (“L” stands for liver); (iii and iv) Cerenkov luminescence images of SD rats injected with $^{64}$CuCl₂ (top left), $^{64}$CuS (top right), $^{64}$Cu-cyclen (bottom left), and $^{64}$Cu-cyclam (bottom right) on the back at (iii) 0-h postinjection and (iv) 4-h postinjection; (v) Quantitative analysis of clearance from the injection sites. (C) Cerenkov luminescence images of BALB/c mice with paw inflammation (developed by complete Freund’s adjuvant) at 1-h postinjection of various probes. (D) PET (maximum intensity projection)/CT images obtained from the BALB/c mice with paw inflammation at 1-h postadministration of different probes. (E) Transverse PET/CT images of the rats with acute myocardial infarction mode after 4-h injection of $^{64}$Cu-cyclen. (F) Transverse PET/CT images of the same mode after 1-h postinjection of $[^{18}$F]FDG. (G) Co-registered fusion image of $^{64}$Cu-cyclen and $^{18}$F-FDG. (H) Quantitative analysis of PET imaging of the myocardial infarction model ($n = 4$). Reproduced with permission.109 Copyright 2016, Wiley-VCH

TABLE 3  Summary of recently reported H₂S therapeutic agents

| Therapeutic agent type          | Therapeutic strategy | Tumor species | Ref. |
|--------------------------------|----------------------|---------------|------|
| NPs@BOD/CPT                    | Chemotherapy         | Colon cancer  | 120  |
| N₃-Nap-PHEMA-b-P MMA-N3        | Chemotherapy         | Cervical cancer | 121  |
| CuDOX NP                      | Chemotherapy         | Cervical cancer | 122  |
| [Cu₂(ZnTepp)₂H₂O]₃            | PDT                  | Colon cancer  | 129  |
| Theranostic prodrug (Nano-TNP-SO) | PDT                  | Colon cancer  | 130  |
| Electrochromic materials (EMs) | PDT                  | Colon cancer  | 131  |
| Self-assembled H₂S responsive small molecule (SSS) | PTT                  | Colon cancer  | 141  |
| Cu₂O                          | PTT                  | Colon cancer  | 142  |
| Au@Cu₂O                       | PTT                  | Colon cancer  | 143  |

Disadvantages of poor water solubility, low biological availability, and systemic toxicity, which limited their practical applications.116-118 The main problem is that the administration of drugs systematically can result in undefined damage to normal tissues.119 Therefore, researchers are committed to developing ingenious anticancer nanoplatforms, from which the on-demand precision drug release could be controlled by endogenous small molecules within tumor regions. In order to improve the specific targeting of tumor cells and anticancer treatment performance, a H₂S-responsive theranostic nanosystem with the generation of NIR-triggered hyperthermia capacity for imaging-directed and photo-controlled drug release in H₂S-overexpressed cancer cells was reported by Shi and co-workers (Figure 11A).120 Such nanoplatform (NPs@BOD/CPT) consists of borondipyrrmethene (InTBOC-Cl) as a H₂S-triggered NIR photothermal agent and a drug camptothecin-11 (CPT-11), encapsulated by thermosensitive nanoparticles. In the absence of H₂S, InTBOC-Cl exhibits no significant hyperthermia effect when exposed to NIR laser and the loaded drugs are encapsulated inside NPs@BOD/CPT nanoparticles without leakage. On the contrary, the introduction of H₂S induces significant NIR
absorption at 756 nm, which could generate hyperthermia effect to exceed the melting point of nanoparticles and release CPT-11 simultaneously. In addition, H$_2$S-induced NIR-II fluorescence emission could be used for disease diagnostics and imaging-guided real-time monitoring of cancer treatment. NPs@BOD/CPT displayed a significant sensitivity for H$_2$S detection with a detection limit of 79 nM. NPs@BOD/CPT was confirmed to be a H$_2$S-triggered and photo-controlled drug delivery nanosystem for chemotherapy, showing an apparent tumor growth inhibition effect in vivo (Figure 11B).

Zhang et al constructed a H$_2$S-responsive charge reversal polymer micelle nanocarrier for H$_2$S detection and targeted drug release to achieve tumor theranostics. They fabricated a polymer micelle (N$_3$-Nap-PHEMA$_{45}$-b-PMMA$_{42}$-N$_3$) consisting of azide-based H$_2$S nanoprobe (N$_3$-Nap) and chemotherapy drug DOX ended with amphiphilic block copolymers (Figure 11C). With the introduction of H$_2$S, the surface charge of the micelles transformed from negative to positive, as the azide group in the H$_2$S probe is reduced to the amine group. The micelles showed more effective uptake through electrostatic attraction, and then released the encapsulated drug to obtain enhanced therapeutic effect based on the H$_2$S-triggered targeting. In vivo fluorescence imaging and therapeutic experimental results indicate that such DOX-loaded micelles possess excellent imaging effect and demonstrate selective release of anticancer drug at the tumor sites (Figure 11D). Similarly, Chen et al reported a H$_2$S-responsive protein cage (CuDOX) for controlled

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**FIGURE 11** (A) Schematic illustration of NPs@BOD/CPT for cancer imaging and photo-controlled on-demand drug release. (B) Localization of free DOX by the costaining experiments with Hoechst in HCT116 cells. Emergence of red fluorescence in nuclei upon continuous illumination of HCT116 cells containing NPs@BOD/DOX, illumination of HCT116 cells containing NPs@DOX, and HepG2 cells containing NPs@BOD/DOX. Scale bar = 10 µm. Reproduced with permission. Copyright 2019, Wiley-VCH. (C) Possible mechanism of monitoring H$_2$S, H$_2$S-triggered charge reversal, and cell uptake process for N$_3$-Nap-PHEMA$_{45}$-b-PMMA$_{42}$-N$_3$ micelles. (D) Biodistribution of DOX in 4T1 tumor-bearing mice at 4-h postinjection. Data are presented as percentage injected dose per gram (%ID/g). Reproduced with permission. Copyright 2016, American Chemical Society. (E) Schematic representation of reaction between H$_2$S and CuDOX NP, showing front-view geometry and method for drug incorporation into apo-HSF cavity and H$_2$S-activated drug release. (F) Cumulative time-dependent DOX release from CuDOX NP solution with or without Na$_2$S at pH 7.4 and 6.4 at 37°C following the same procedure. Inset: photographs of CuDOX NP solution after Na$_2$S-treatment with increasing Na$_2$S/Cu$^{2+}$ ratios from left to right. Reproduced with permission. Copyright 2017, American Chemical Society.
release of DOX, where commercial horse spleen apoferritin (apo-HSF) was used as a nanocarrier for loading copper-pre-coordinated DOX (Figure 11E). Upon $\text{H}_2\text{~S}$ activation under the condition of physiological pH, the coordination interaction between copper ion and DOX is disconnected to allow the release of chemotherapeutic drug from protein cage (Figure 11F). In vitro cell experiments demonstrated that CuDOX had good selectivity to cancer cells with excessive expression of $\text{H}_2\text{~S}$ that can induce drug release in tumor cells and reduce toxic side effects to normal cells. $\text{H}_2\text{~S}$ plays an important role in theranostics, motivating researchers to synthesize smart nanoplatforms for $\text{H}_2\text{~S}$-responsive advanced diagnosis and tumor treatment.

### 4.2 Photodynamic therapy

PDT involves a nontoxic photosensitizer that acts as a light-responsive drug to produce cytotoxic reactive oxygen species (ROS) by exposing to external laser in the presence of tissue oxygen, resulting in cellular function disorder and cell apoptosis. PDT is a potential method for cancer treatment with the advantages of noninvasiveness, negligible adverse effect, and significant therapeutic effect in comparison with traditional treatment methods. However, most reported photosensitizers have a low accumulation effect at the tumor sites, leading to lower production of ROS in the tumor sits, and thus the therapeutic effect of PDT is often not satisfactory. In addition, it is possible to damage normal tissues in the process of treatment. Therefore, designing and fabricating antitumor theranostic nanoprobes that combine diagnosis capacity to discriminate tumors and guide the on-demand drug administration to cancer are of great significance, which could maximize therapeutic effectiveness and prevent damage to normal tissues. Ma et al developed a smart $\text{H}_2\text{~S}$-activatable single-component metal-organic framework (MOF) photosensitizer with controllable singlet-oxygen ($^{1}\text{O}_2$) generation for PDT (Figure 12A). They employed zinc-metalated molecule (ZnTcpp) as a photosensitizer to coordinate with Cu$^{2+}$ ion as building blocks to achieve copper-zinc-mixed MOF. Cu$^{2+}$ ion not only acts as a quenching agent to attenuate the fluorescence of the MOF nanoparticles but also minimizes the generation efficiency of ROS. When $\text{H}_2\text{~S}$ is introduced, the Cu$^{2+}$ ion would react with $\text{H}_2\text{~S}$ to be released from the framework, and then fluorescent MOF nanoparticles and controllable $^{1}\text{O}_2$ production were achieved (Figure 12B). In vitro and in vivo experimental results confirmed that the nanoparticles could specifically respond to endogenous $\text{H}_2\text{~S}$ and exhibit an excellent treatment efficacy with controllable release of photoactive species (Figure 12C).

Wang et al reported a $\text{H}_2\text{~S}$-triggered theranostic prodrug (TNP-SO) for selective detection of overexpressed $\text{H}_2\text{~S}$ in cancer cells, and NIR fluorescence imaging-directed on-demand administration for PDT (Figure 12D). The theranostic prodrug was synthesized by the encapsulation of a $\text{H}_2\text{~S}$-responsive NIR imaging probe and a photosensitizer into diethylene glycol-amine species. By interacting with $\text{H}_2\text{~S}$, the absorption intensity of TNP-SO at 500 nm decreased evidently along with a new absorption band appeared at 655 nm. The NIR fluorescence intensity increased gradually upon increasing the $\text{H}_2\text{~S}$ concentration, and the detection limit of the probe was measured to be 21 nM, suggesting that the TNP-SO possesses high sensitivity for $\text{H}_2\text{~S}$ detection (Figures 12E and 12F). The TNP-SO nanoprobe could also be used as a photosensitizer for the generation of cytotoxic ROS. Such probe was also applied for in vivo PDT, which displayed the selective detection of $\text{H}_2\text{~S}$-overexpressed cancer cells and accurately imaging-guided ROS generation when exposed to laser irradiation for on-demand cancer treatment with low side effects (Figure 12G). Furthermore, Ye et al put forward a type of $\text{H}_2\text{~S}$-activatable fluorescence nanoprobe and photodynamic treatment agent based on electrochromic materials (EM) with a specific organic $\pi$-electron structure (dicaticonic 1,1,4,4-tetraarylbutadiene, 1$^{2+}$). The $\text{H}_2\text{~S}$-responsive chromophore (1$^{2+}$-SNPs) was prepared by doping EM 1$^{2+}$ into semiconducting polymer nanoparticles (SPNs), as shown in Figure 12H. Within 1$^{2+}$-SNPs, the EM 1$^{2+}$ with apparent absorption in the range of 500-800 nm can effectively quench the fluorescence produced by SPNs based on the FRET process. As activated by $\text{H}_2\text{~S}$, the 1$^{2+}$ could be converted into colorless species 2, serving as a strong reductant in the process of electrochemistry-triggered electron transfer. Furthermore, $\text{H}_2\text{~S}$-activatable selective tumor PDT together with fluorescence tumor imaging was achieved in tumor-bearing mice after intravenous administration of folic acid-modified EM 1$^{2+}$ photosensitizer upon NIR light excitation, enabling improved specificity and efficacy for cancer treatment. This research displays a method of designing smart $\text{H}_2\text{~S}$-responsive EM as both fluorescent probes and photosensitizers for in vivo bioimaging and PDT of $\text{H}_2\text{~S}$-overexpressed tumors (Figure 12I).

### 4.3 Photothermal therapy

PTT has aroused widespread attention in the field of cancer therapy, based on the distinct advantages of simplicity, noninvasiveness, and controllable therapeutics. For PTT, photothermal agents are utilized to capture light and transform it into heat energy for thermal ablation of tumor cells and tissues. Until now, a variety of NIR-responsive photothermal agents have been developed for PTT and demonstrated significant therapeutic effects, whereas some photothermal nanomaterials did not show significant specificity to tumor-related components, which might cause
FIGURE 12 (A) Simple structural fragment of MOF NP-1 and the proposed strategy for \( ^1 \)O\(_2\) generation in cancer therapy. (B) Fluorescence spectra of NP-1 obtained upon titration with NaHS from 0 to 70 \( \mu \)m, \( \lambda_{ex} = 420 \) nm. (C) In vitro tumor growth inhibition curves after different treatments. Reproduced with permission. Copyright 2017, Wiley-VCH. (D) Schematic illustration for the generation of Nano-TNP-SO. (E) Time-dependent emission spectral changes of Nano-TNP-SO (TNP-SO 5 \( \mu \)M) in the presence of 100 \( \mu \)M NaHS in PBS solution (pH 7.4, room temperature). \( \lambda_{ex} = 640 \) nm. (F) Synthesis of the target compound TNP-SO and possible mechanism of interaction with H\(_2\)S. (G) In vivo tumor growth curves of mice by administration of various treatments. Reproduced with permission. Copyright 2019, Royal Society of Chemistry. (H) \( ^{125} \)I-SNPs showing low fluorescence (OFF) owing to the fluorescence quenching effect of EM \( ^{125} \)I via a FRET process. The H\(_2\)S-triggered reduction of \( ^{125} \)I-SNPs can eliminate the FRET process and recover fluorescence (ON), allowing for noninvasive imaging of hepatic or tumor H\(_2\)S in living mice. (I) Plots of tumor volumes after various treatments. Reproduced with permission. Copyright 2018, American Chemical Society.

toxic side effects and damage to normal tissues. To address current existing challenges, PTT agents specifically activated by tumor-related components are of great significance. Shi et al reported the in situ-generated, NIR light-activatable self-assembled photothermal agent for imaging-directed PTT based on the overexpressed endogenous H\(_2\)S in colon cancer. The H\(_2\)S-triggered photothermal nanoagent (SSS) with excellent water solubility was developed, which consisted of triethylene glycol monomethyl ether chain-modified phenyl ring as a hydrophilic phase to trigger the self-assembly (Figure 13A). H\(_2\)S as an activator could interact with the monochlorinated BODIPY core based on the thiol-halogen nucleophilic substitution reaction. No significant hyperthermia could be detected in the absence of H\(_2\)S, and meanwhile the characteristic absorption and emission peaks of the BODIPY unit can be observed at the wavelength of 540 and 589 nm, respectively. Surprisingly, the addition of H\(_2\)S to the photothermal agent resulted in
the appearance of distinct characteristic absorption band at the wavelength of 790 nm, which not only triggered high photothermal conversion efficiency for PTT under 785 nm laser irradiation, but also achieved excellent fluorescence imaging (Figure 13B). Utilizing these unique features, the nanotheranostic agent could trigger effective inhibition of H₂S-excessive tumor cells directed by NIR fluorescence imaging (Figure 13C).

An et al fabricated an on-demand theranostic nanoplatform by using Cu₂O reacted with overexpressed H₂S in tumor
for colon cancer treatment with diagnosis and therapeutic functions simultaneously.\textsuperscript{142} Once the nanoparticles reach the tumor site, they react with overexpressed endogenous \( \text{H}_2\text{S} \) in colon cancer cells to produce copper sulfide (CuS) nanoparticles with significant absorption in NIR biological transmission window. Compared with the generated CuS nanoparticles, no apparent absorption band of Cu\(_2\)O nanoparticles was found in the NIR region, indicating that Cu\(_2\)O nanoparticles could serve as a potential system for \( \text{H}_2\text{S} \)-activatable PA imaging and PTT, as presented in Figure 13D. This research confirmed that the metal oxide nanoparticles could be activated by endogenous \( \text{H}_2\text{S} \) in tumor cells, demonstrating obviously enhanced PA imaging and photothermal therapeutic effect on \( \text{H}_2\text{S} \)-overexpressed colon cancer cells (Figure 13E). The proposed \( \text{H}_2\text{S} \) responsive mechanism opens up a way for the treatment of colon cancer. Furthermore, Yang et al developed a theranostic agent (Au@Cu\(_2\)O) responsive to endogenous \( \text{H}_2\text{S} \) with triggered PA imaging for highly effective antitumor therapy (Figure 13F).\textsuperscript{143} The imaging and hyperthermia effect of endogenous \( \text{H}_2\text{S} \)-triggered Au@Cu\(_2\)O are originated from the localized surface plasmon resonance between a noble metal (Au) and a plasmonic semiconductor (Cu\(_2\)O). In comparison with Cu\(_2\)O alone, the fabricated Au@Cu\(_2\)O nanoparticles exhibited prominently increased NIR absorption on account of the improved local field. Au@Cu\(_2\)O also possesses unique advantages of fast response and anti-interference performance. The photothermal conversion efficiency of Au@Cu\(_2\)O was enhanced by approximately 50\% with the introduction of \( \text{H}_2\text{S} \) (Figure 13G). In addition, the Au@Cu\(_2\)O nanoparticles demonstrated PA imaging-guided in vivo tumor growth inhibition upon the stimulation of endogenous \( \text{H}_2\text{S} \).

5 | CONCLUSION AND OUTLOOK

\( \text{H}_2\text{S} \) is a major chemical mediator that plays an important role in various physiological functions and diseases. To acquire early prevention and accurate diagnosis of related diseases, scientists have devoted to constructing smart \( \text{H}_2\text{S} \)-responsive nanoprobes with excellent sensitivity and selectivity. Compared with traditional detection techniques, fluorescence-based methods have appeared as useful approaches for real-time monitoring of endogenous \( \text{H}_2\text{S} \). The emergence of ratiometric fluorescent probes with excellent sensitivity could eliminate distractions from external environment, which are better than single emission wavelength fluorescent probes. The transition from one-photon to two-photon fluorescent probes could achieve deeper tissue penetration, lower phototoxicity, and better self-absorption performance. To achieve more efficient and accurate detection of \( \text{H}_2\text{S} \) in vivo, NIR-II fluorescent probes and PA probes have been proposed to replace short-wavelength probes, which endow fast responsiveness, high sensitivity, and deep tissue penetration depth. Because the overexpressed CBS promotes the generation of endogenous \( \text{H}_2\text{S} \) in cancer cells, \( \text{H}_2\text{S} \)-responsive therapeutic nanoagents have been presented, where \( \text{H}_2\text{S} \) could act as a potential pharmacological imaging target for the identification of malignant cancer. In comparison with the reported theranostic agents, in situ endogenous \( \text{H}_2\text{S} \)-activatable theranostics agents for cancer treatment. Nevertheless, developing novel \( \text{H}_2\text{S} \)-responsive nanoplatforms with “all-in-one” performance for translating into clinical applications is still immensely challenging, but greatly significant.

It is confident that the integration of smart diagnostic and therapeutic nanomaterials for the detection and real-time monitoring of \( \text{H}_2\text{S} \) and relevant disease treatment has capacious development foreground. In subsequent investigations, it is expected to construct therapeutic agents that are activated by endogenous substances in tumor with high selectivity and excellent biocompatibility. The development of degradable \( \text{H}_2\text{S} \)-activatable nanomaterials with multimodality imaging-based diagnosis and synergistic treatment performance is also urgently desired. In addition, it is crucial to systematically evaluate the biosafety of \( \text{H}_2\text{S} \)-responsive nanomaterials at the cellular and animal levels by analyzing their blood circulation, metabolism, and pharmacological toxicity in vitro and in vivo. Once these major issues are solved, we can afford fundamental basis for future research and practical applications of these nanosystems in disease theranostics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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