

### **Contributor's Details:**

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Contributor 1:

Joshua J. Woods  
Robert F. Smith School for Chemical and Biomolecular Engineering  
Department of Chemistry and Chemical Biology  
Cornell University  
Ithaca, NY, USA 14853-1301

Contributor 2:

Justin J. Wilson\*  
Department of Chemistry and Chemical Biology  
G50A Baker Laboratory  
Cornell University  
Ithaca, NY, USA 14853-1301  
Phone: 607-255-4344  
E-mail: jjw275@cornell.edu  
\*Corresponding author

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### **Title:**

Bioinorganic Chemistry of Hydrogen Sulfide: Detection, Delivery, and Interactions with Metalloproteins

### **Name and Affiliation of Contributors:**

Joshua J. Woods  
*Robert F. Smith School for Chemical and Biomolecular Engineering*  
*Department of Chemistry and Chemical Biology*  
*Cornell University*  
*Ithaca, NY, USA*

Justin J. Wilson\*  
*Department of Chemistry and Chemical Biology*  
*Cornell University*  
*Ithaca, NY, USA*

\*corresponding author  
e-mail: jjw275@cornell.edu

### **Abstract:**

Hydrogen sulfide (H<sub>2</sub>S) has received significant attention in the past two decades for its role in biological processes. Despite numerous studies on the biological activity of this gas, the specific nature of the interaction between H<sub>2</sub>S and metalloproteins and biologically relevant metal ions remains largely unknown. The current understanding of the chemical biology of H<sub>2</sub>S with metalloproteins and enzymes is discussed herein. The utility of inorganic complexes as tools for the

detection and delivery of H<sub>2</sub>S under biologically relevant conditions is also discussed.

**Keywords:**

Hydrogen sulfide, gasotransmitters, metalloproteins, bioinorganic chemistry

## 1. INTRODUCTION

For the majority of the 20<sup>th</sup> century, the gaseous small molecule hydrogen sulfide (H<sub>2</sub>S) has been known for its toxicity and characteristic rotten egg smell. Only after a series of investigations on the metabolism of sulfur-containing amino acids was it discovered that H<sub>2</sub>S is produced endogenously in mammals.<sup>1-8</sup> Following this revelation, H<sub>2</sub>S is now recognized to be the third gasotransmitter as a small gaseous molecule that mediates cellular signaling processes, joining carbon monoxide (CO) and nitric oxide (NO; see eibc2724) in this role.<sup>9</sup> The biological activity of H<sub>2</sub>S and related polysulfides (H<sub>2</sub>S<sub>n</sub>) is highly diverse, and within the last two decades they have received significant attention for their ability to regulate cell death signaling,<sup>10,11</sup> cancer biology,<sup>12</sup> the cardiovascular system,<sup>13,14</sup> the nervous system,<sup>15,16</sup> and metabolism<sup>17,18</sup>. It has been hypothesized that a number of the observed biological actions of H<sub>2</sub>S stem from its interaction with biologically relevant metals and metalloproteins.<sup>19-21</sup> Thus, in depth investigations of the coordination chemistry of H<sub>2</sub>S and HS<sup>-</sup> in simple transition metal complexes have shed light on these interactions and highlighted the diverse reactivity of this gas and related sulfur species with metal ions (see eibc0213).<sup>22,23,32-35,24-31</sup> In addition to its roles in maintaining normal biological function, H<sub>2</sub>S has received significant attention for its therapeutic potential for the treatment of pathological conditions such as ischemic-reperfusion injury, stroke, and Alzheimer's disease.<sup>36-39</sup> As such, there has been a significant amount of research devoted to developing molecules to detect and deliver H<sub>2</sub>S in biological systems.<sup>40</sup> These compounds have been invaluable for studying the biological activity of this gas. This article will focus on the role of inorganic chemistry in the biology of H<sub>2</sub>S. Specifically, a description of the nature of chemical interactions of H<sub>2</sub>S with metalloproteins and the use of metal complexes for both the detection and delivery of this gasotransmitter will be provided. The body of work presented in this review highlights the significance of bioinorganic H<sub>2</sub>S chemistry and will motivate future work in this area.

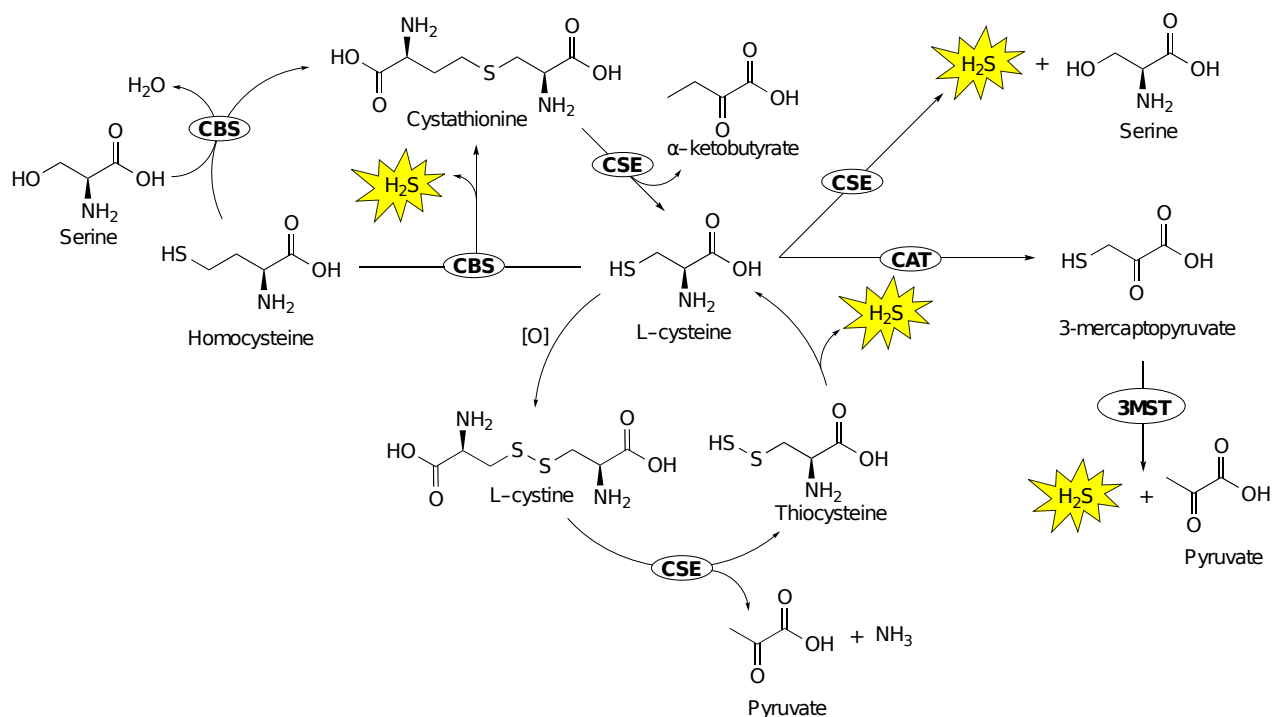
## 2. PROPERTIES AND ENDOGENOUS H<sub>2</sub>S PRODUCTION

H<sub>2</sub>S is weakly acidic with pK<sub>a</sub> values of 7.02 and >17 at 25 °C.<sup>41,42</sup> In pH 7.4 aqueous solution, approximately 72% of the total hydrogen sulfide exists in the form of HS<sup>-</sup><sub>(aq)</sub> and 28% as H<sub>2</sub>S<sub>(aq)</sub>. Despite early speculation to the contrary,<sup>43,44</sup> the dianionic S<sup>2-</sup> species does not exist in aqueous solution.<sup>45</sup> H<sub>2</sub>S is slightly hydrophobic with a water-octanol partition coefficient of 0.64 at pH 7.4, thereby enabling it to efficiently permeate cell membranes.<sup>46</sup> The anion HS<sup>-</sup> is a potent nucleophile that readily reacts with reactive oxygen, nitrogen, and sulfur species (ROS, RNS, and RSS, respectively) in addition to biologically relevant metals and metalloproteins.<sup>20,47</sup> This high nucleophilicity has been implicated as a key feature of the biological chemistry of H<sub>2</sub>S and has also been leveraged in molecular strategies to detect this gasotransmitter in solution (vide infra). In complex biological systems, sulfur can

attain oxidation states ranging from -2 in H<sub>2</sub>S to +6 in SO<sub>4</sub><sup>2-</sup>. With the sulfur atom of H<sub>2</sub>S in its lowest oxidation state, this molecule can be easily oxidized under biological conditions, giving rise to other biologically relevant RSS (see eibc0215.pub2).<sup>48</sup> Its high nucleophilicity and ability to undergo a wide range of redox reactions make studying the biological chemistry of H<sub>2</sub>S particularly challenging.

Enzymatic production of H<sub>2</sub>S relies primarily on the activity of cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and the combined action of cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfurtransferase (3MST).<sup>49,50</sup> H<sub>2</sub>S can also be produced nonenzymatically through metabolism of naturally occurring polysulfides, bacterial reduction of sulfur containing species in the intestinal tract, and catalytic reduction of sulfur-containing amino acids in blood.<sup>51-53</sup> A summary of the major production pathways for enzymatic production of H<sub>2</sub>S is given in Figure 1. CBS is expressed primarily in the central nervous system and liver,<sup>54</sup> whereas CSE is more broadly distributed throughout the rest of the body. For example, CSE knockout (CSE<sup>-/-</sup>) mice have decreased circulating H<sub>2</sub>S levels compared to the wild-type mice.<sup>55</sup> Canonically, CBS catalyzes the pyridoxal 5'-phosphate (PLP) dependent condensation of L-homocysteine and L-serine to generate L-cystathionine,<sup>56</sup> and CSE promotes cleavage of L-cystathionine to yield L-cysteine, ammonia, and α-ketobutyrate. Both enzymes are able to produce H<sub>2</sub>S through a number of different pathways starting from cysteine or homocysteine.<sup>10,57-59</sup> CBS is allosterically activated by S-adenosyl-L-methionine (AdoMet), which binds to the regulatory C-terminal domain.<sup>60</sup> It has been suggested that CBS is further regulated by NO and CO, which can bind to the heme center located in the N-terminal domain.<sup>61-66</sup> In contrast to CBS, the biological regulation of CSE is poorly understood. It has been suggested that H<sub>2</sub>S production by CSE increases in the presence of calcium and/or calmodulin.<sup>55</sup> Other studies have found that elevated Ca<sup>2+</sup> levels decrease H<sub>2</sub>S production.<sup>67</sup> Unlike CBS, which is constitutively expressed within cells, the expression of CSE is highly inducible; its expression in tissues is modulated by a range of stimuli including endoplasmic reticulum (ER) stress, oxidative stress, nitric oxide (NO), and nutrient deprivation.<sup>68</sup>

CAT and 3MST generate H<sub>2</sub>S through a sequential two-step reaction process. L-cysteine is converted to 3-mercaptopyruvate by CAT, which in turn is converted to pyruvate by 3MST with the release of H<sub>2</sub>S.<sup>69</sup> This pathway was first discovered in CBS<sup>-/-</sup> mice, where the brain levels of H<sub>2</sub>S were relatively unchanged compared to those in wild-type mice.<sup>70</sup> Rather little is understood of the activity and regulation of H<sub>2</sub>S production via this pathway, although it has been shown that CAT is highly regulated by cellular Ca<sup>2+</sup> levels.<sup>71</sup> In the structure of 3MST, a central cysteine residue (Cys248) is required for enzyme function.<sup>69</sup>

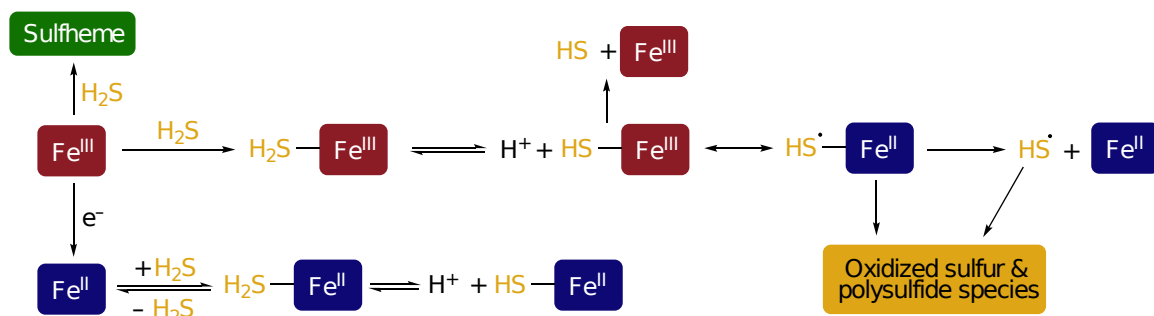


**Figure 1.** Summary of the major  $\text{H}_2\text{S}$  production pathways by cystathionine- $\beta$ -synthase (CBS), cystathionine- $\gamma$ -lyase (CSE), and the combined action of cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfurtransferase (3MST).

### 3. INTERACTIONS WITH METALLOPROTEINS

#### 3.1 Hemeproteins and Synthetic Heme Systems.

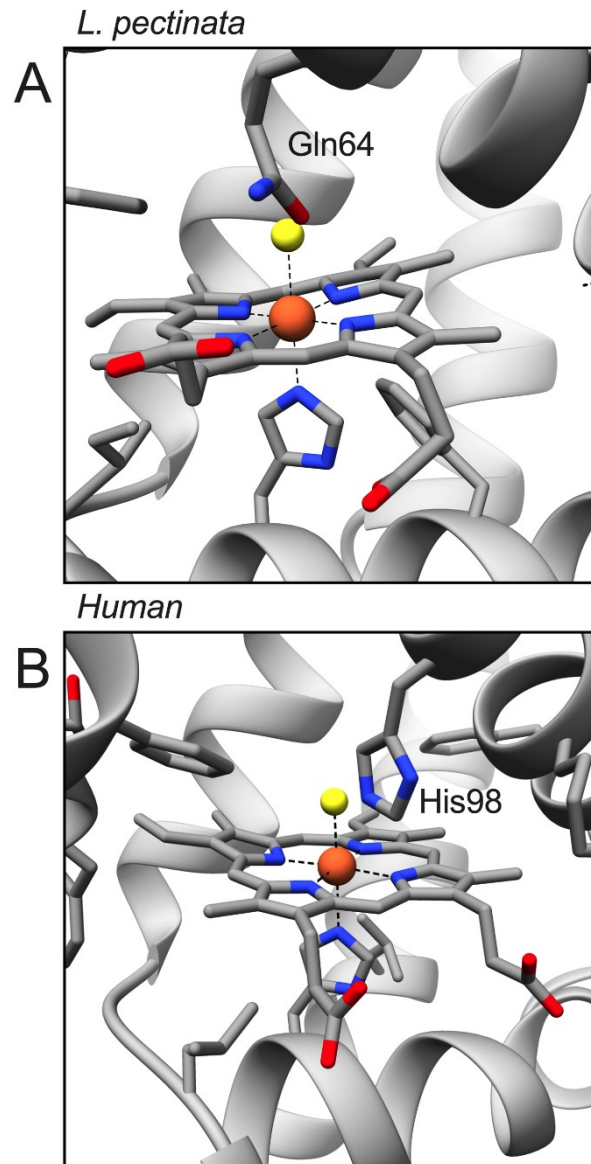
Heme-containing systems are interesting platforms that can bind to and activate a number of small molecules including  $\text{O}_2$ ,  $\text{CO}$ , and  $\text{NO}$ . The interactions of these and related small molecules with both natural and synthetic heme systems have been studied in great detail (see eibc0056, eibc0097, eibc0099, eibc0100).<sup>72-74</sup> Consequently, the chemistry of  $\text{H}_2\text{S}$  with hemeproteins, which has been recently reviewed,<sup>10,20,26,75</sup> has received significant attention compared to other metalloproteins. The nature of these reactions, summarized in Figure 2, is highly dependent on the surface accessibility of the heme center, the polarity of the local protein environment, and the identity and position of distal residues within the active site.<sup>76,77</sup>



**Figure 2.** Possible pathways for  $\text{H}_2\text{S}$  interactions with heme proteins.

A multitude of studies involving neuroglobins,<sup>78,79</sup> hemoglobins,<sup>80-83</sup> myoglobins,<sup>84-86</sup> metalloenzymes,<sup>87-90</sup> and synthetic heme systems<sup>91,92,101,93-100</sup> have highlighted the diverse reactivity of H<sub>2</sub>S with heme centers. H<sub>2</sub>S or HS<sup>-</sup> can bind ferric heme (Fe<sup>III</sup>) and, in some cases, reduce the ferric center to ferrous (Fe<sup>II</sup>) iron to generate hydrothiyl (HS<sup>•</sup>)-Fe<sup>II</sup> type complexes.<sup>93,96</sup> These compounds undergo further reactions to generate oxidized sulfur and polysulfide species (Figure 2).<sup>83,84,86,88,99,102,103</sup> Such polysulfides have been implicated in the S-sulfhydration of various proteins, which mediates many of the biological roles of H<sub>2</sub>S.<sup>103-106</sup> Ferric heme can reversibly bind H<sub>2</sub>S/HS<sup>-</sup> for transport or storage to prevent sulfide toxicity.<sup>84,107,108</sup> For example, it has been speculated that neuroglobin plays a protective role by binding sulfide under conditions of elevated brain sulfide levels.<sup>79</sup> Additionally, H<sub>2</sub>S has been shown to attenuate lipid peroxidation in atherosclerotic lesions to prevent a proinflammatory response. It was hypothesized that this result was due to the ability of H<sub>2</sub>S to prevent formation of oxidized hemoglobin,<sup>109</sup> illustrating how the cardioprotective effects of this gas may arise from its interaction with metalloproteins. Ferrous heme may also bind sulfide, but with significantly lower affinity than the corresponding Fe<sup>III</sup> species (Figure 2).<sup>75,95,108</sup>

One of the best characterized examples of H<sub>2</sub>S/HS<sup>-</sup> binding to naturally occurring heme proteins can be found in studies of the hemoglobin I (HbI), II (HbII), and III (HbIII) proteins isolated from the mollusk *L. pectinata*.<sup>110</sup> These mollusks exist in a symbiotic relationship with autotrophic bacteria, which oxidize H<sub>2</sub>S. HbI is a sulfide-reactive hemoglobin that is functionally devoted to sulfide transport. Nucleophilic attack of H<sub>2</sub>S on oxygen-bound HbI displaces the bound superoxide anion. The resulting ferric hemoglobin sulfide transports the bound sulfide to the autotrophic bacteria. By contrast, HbII and HbIII only bind H<sub>2</sub>S in the absence of O<sub>2</sub>. On the basis of these results, it was hypothesized that HbI is selectively used to transport H<sub>2</sub>S, whereas HbII and HbIII facilitate delivery of O<sub>2</sub>.<sup>108</sup> X-ray crystallographic analysis of sulfide-bound HbI revealed the importance of a glutamine residue (Gln64) at the distal ligand binding site (Figure 3A). It is hypothesized that this residue is important in facilitating the relatively fast sulfide association rate, in addition to stabilizing the bound sulfide through hydrogen-bonding interactions. Four distal phenylalanine residues, which are unique to HbI, create a hydrophobic pocket that stabilizes the iron-bound sulfide and protects it against oxidation or solvation.<sup>107,111</sup> In human hemoglobin-sulfide, the distal histidine residue (His98; Figure 3B) participates in hydrogen-bonding interactions similar to Gln64 in HbI.<sup>103</sup> It was hypothesized that ferric human hemoglobin acts similar to *L. pectinata* HbI to transport H<sub>2</sub>S in the blood.<sup>112</sup> Given that human hemoglobin binds H<sub>2</sub>S with considerably lower affinity and is subsequently reduced to generate ferrous hemoglobin and RSS,<sup>84</sup> this transport mechanism is unlikely.

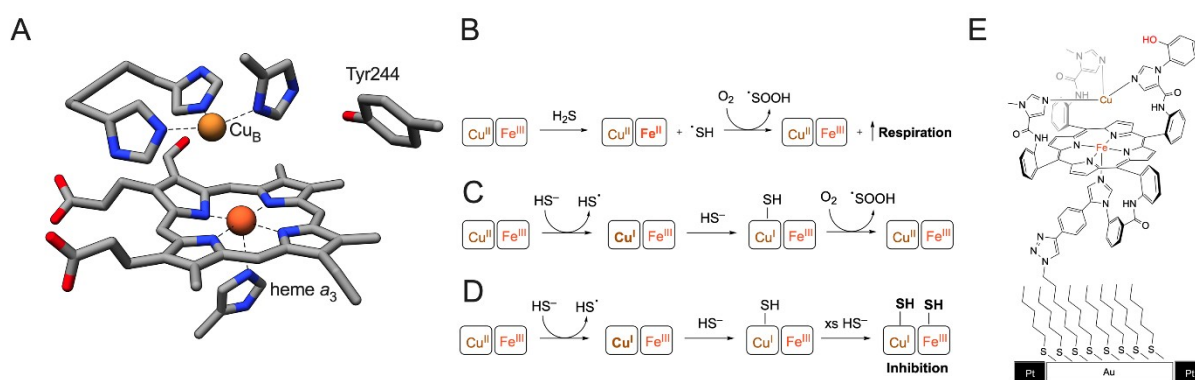


**Figure 3.** X-ray crystal structures of sulfide-bound (A) Hbl from *L. pectinata*<sup>107</sup> and (B) human hemoglobin<sup>103</sup> (PDB: 1MOH, 5UCU).

In addition to hemoglobin and myoglobin, the heme proteins cytochrome *c* (Cc)<sup>102</sup> and cytochrome *c* oxidase (CcO)<sup>113</sup> have received significant attention for their reactivity with H<sub>2</sub>S, as well as the other two gasotransmitters, CO and NO.<sup>102,114-116</sup> CcO is the final enzyme of the mitochondrial electron transport chain that uses electrons supplied by Cc to reduce O<sub>2</sub> to water. The protons that are produced from this reaction are translocated across the mitochondrial membrane to generate an electrochemical potential that is used for downstream production of ATP. The active site of this transmembrane metalloenzyme contains a heme center (a<sub>3</sub>) and a Cu<sup>II</sup> center (Cu<sub>B</sub>; Figure 4A).

The ability of H<sub>2</sub>S to attenuate the activity of CcO has been known since 1929.<sup>117</sup> The nature of the interaction between CcO and H<sub>2</sub>S is complex and the

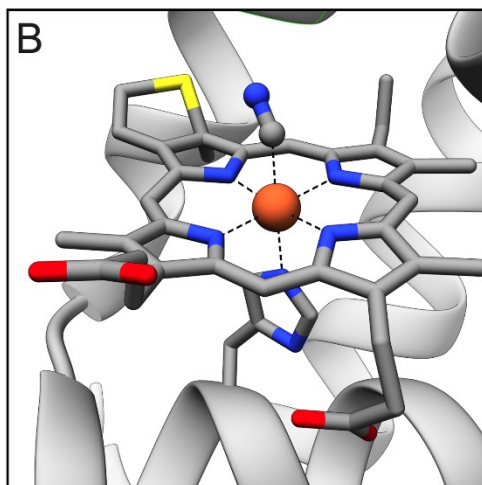
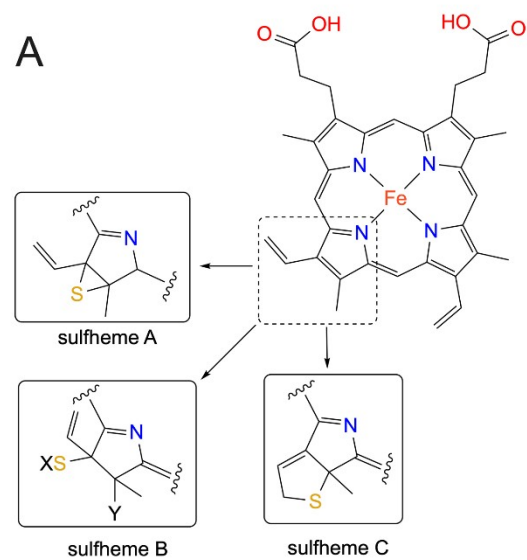
biological consequences of this interaction are highly concentration dependent.<sup>113,118,119</sup> At low concentrations (~1 equiv.), H<sub>2</sub>S reduces heme a<sub>3</sub>, but the low binding affinity of H<sub>2</sub>S for the ferrous heme (~12.5 μM) prevents sulfide binding to the Fe<sup>II</sup> center (Figure 4B). This reduction event, which leads to production of RSS and increased O<sub>2</sub> consumption, may suggest that H<sub>2</sub>S and related RSS act as substrates for mitochondrial energy production in conditions of low energy supply, such as hibernation.<sup>113,120-123</sup> At slightly higher H<sub>2</sub>S concentrations (2-3 equiv.), the Cu<sup>II</sup> site is reduced and reversibly binds H<sub>2</sub>S. This interaction is reversed by O<sub>2</sub>, which oxidizes the Cu<sup>I</sup> center, releasing the bound H<sub>2</sub>S (Figure 4C).<sup>119</sup> When a large excess of H<sub>2</sub>S (>4 equiv.) is supplied, reduction of the Cu<sup>II</sup> center and subsequent binding of H<sub>2</sub>S induces a conformational change that allows for another equivalent of H<sub>2</sub>S to bind heme a<sub>3</sub>, which results in irreversible inhibition of CcO (Figure 4D).<sup>118,120</sup> A functional small-molecule model of the CcO active site (Figure 4E) was employed to gain further insight on the interactions of this enzyme with H<sub>2</sub>S.<sup>119</sup> This model complex, which faithfully captures the coordination environments and proximities of the copper Cu<sub>B</sub> center, heme a<sub>3</sub>, and the putative redox-active Tyr244, was found to be well suited as a mimic for the CcO active site, which exhibited reactivity with H<sub>2</sub>S that strongly supported hypotheses formulated based on previous studies using the intact enzyme.



**Figure 4.** (A) Structure of the active site of human CcO (PDB 5Z62).<sup>124</sup> (B-D) Proposed interactions between CcO and (B) low ( $\leq 1$  equiv.), (C) moderate (2 - 3 equiv.), and (D) high (>4 equiv.) H<sub>2</sub>S concentrations. (E) Structure of the CcO model complex, attached to a rotating gold electrode, that was used to probe the reaction between CcO and H<sub>2</sub>S.

In addition to metal reduction and coordination, an alternative mode of interaction of H<sub>2</sub>S with hemes arises from its direct incorporation into one of the heme pyrrole rings to furnish sulfheme derivatives. This activity was observed in early investigations of the interaction between H<sub>2</sub>S and hemoglobin or myoglobin.<sup>125-128</sup> The three general classes of sulfheme derivatives, sulfheme A (an episulfide), sulfheme B (a ring-opened episulfide), and sulfheme C (a thiochlorin) are shown in Figure 5.<sup>129-134</sup> In comparison to natural heme proteins, sulfheme proteins have significant differences in their ligand-binding properties, which arise predominately from electronic rather than steric effects.<sup>128,134</sup> The exact mechanisms of sulfheme formation are unclear, but have been suggested to involve oxoferryl (Fe<sup>IV</sup>=O) intermediates.<sup>10,89,135</sup> The secondary coordination sphere,

specifically a His residue in the distal position of the enzyme active site, has been shown to be important for the production sulfheme species in some cases.<sup>77,135</sup> For example, human hemoglobin, which contains this distal residue, forms sulfheme derivatives whereas Hbl from *L. pectinata*, which contains distal Gln instead, does not. Site-directed mutagenesis of this Gln residue to a His residue allowed this protein to convert to the sulfheme form upon exposure to H<sub>2</sub>S.<sup>77</sup> By contrast, CcO, which contains His residues near the active site, does not form sulfheme in the presence of H<sub>2</sub>S. In this particular case, it has been hypothesized that these His residues do not have the proper orientation and therefore cannot interact with the heme center to promote sulfheme formation.<sup>76</sup> Sulfheme derivatives of the metalloenzymes catalase and lactoperoxidase have also been observed.<sup>89,136</sup> Given their poor affinity for O<sub>2</sub> and inability to be converted back to normal hemoproteins,<sup>125,137,138</sup> it is unlikely that these species play a role in cellular signaling, but instead mediate the toxic effects of this gas. For example, elevated sulfheme levels induce a toxic condition known as sulfhemoglobinemia due to their inability to adequately bind O<sub>2</sub>.<sup>139</sup>





**Figure 5.** (A) Diagram of three sulfheme products formed through covalent addition of sulfide to the porphyrin ring. (B) X-ray crystal structure of cyanide-bound myoglobin containing sulfheme C (PDB: 1YMC).<sup>134</sup>

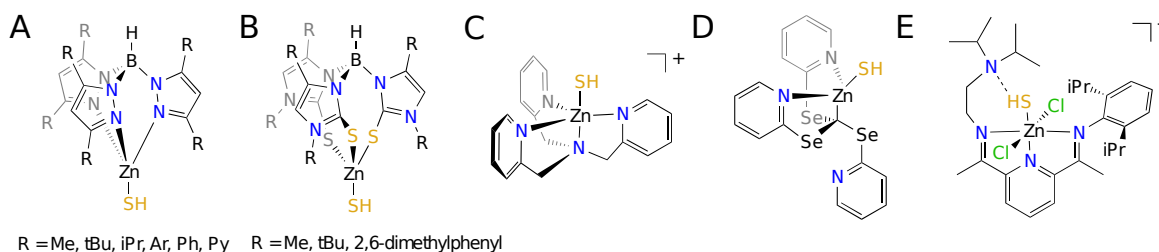
### 3.2 Zinc-Containing Proteins

The interactions between spectroscopically silent Zn-containing proteins and H<sub>2</sub>S have received relatively little attention compared to those for other metalloproteins.<sup>10</sup> Proteins containing the zinc finger (ZF) motif are particularly important, as evidenced by their high abundance in eukaryotic cells. ZFs are small, folded domains that are stabilized by coordination to a structural Zn<sup>II</sup> ion with a combination His and Cys residues. This class of proteins plays an important role in eukaryotic cells as transcription factors that regulate gene transcription.<sup>140</sup> Although Zn<sup>II</sup> is not redox active, Cys-bound ZF domains undergo S-sulfhydration in response to H<sub>2</sub>S exposure.<sup>141-143</sup> The mechanism of S-sulfhydration of the specific ZF protein tristetraprolin was investigated. A key discovery from this study was that this process occurs only in the presence of O<sub>2</sub>.<sup>142</sup> The Zn<sup>II</sup> center is thought to facilitate the S-sulfhydration by shifting the redox potential of the Cys and enabling its oxidation by O<sub>2</sub> and subsequent sulfhydration by H<sub>2</sub>S.

In addition to being a key structural requisite for ZFs, Zn<sup>II</sup> plays a catalytic role in several enzymes, which may also react with H<sub>2</sub>S. For example, the Zn-containing enzyme phosphodiesterase 5 is inhibited by nanomolar concentrations of H<sub>2</sub>S.<sup>144</sup> The mechanism of this inhibition has not been fully elucidated, but the direct coordination of H<sub>2</sub>S to the catalytic Zn<sup>II</sup> center could not be ruled out. Similarly, the inhibition of carbonic anhydrase by H<sub>2</sub>S arises from the direct coordination of this gasotransmitter to the His<sub>3</sub>Zn active site found in most isoforms of this enzyme.<sup>145-147</sup> A model complex of the dinuclear Zn<sup>II</sup> enzyme CS<sub>2</sub> hydrolase, which is found in achaeobacteria, was shown to produce H<sub>2</sub>S and CO<sub>2</sub> via hydrolysis of CS<sub>2</sub>,<sup>148</sup> suggesting that CS<sub>2</sub> is a biologically relevant source of H<sub>2</sub>S.<sup>149</sup>

Lastly, H<sub>2</sub>S regulates proteins with alternative functions. For example, H<sub>2</sub>S is also known to interact with metallothioneins, Cys-rich proteins that bind Zn<sup>II</sup> and detoxify heavy metal ions. In this capacity, H<sub>2</sub>S prevents Cd<sup>II</sup>-induced toxicity by stabilizing the Zn<sup>II</sup>-containing isoform of metallothionein-1.<sup>150</sup> Zn proteins also have a potential role for transport of H<sub>2</sub>S. In the giant tubeworm *R. pachyptila*, which inhabits sulfide-rich environments and lives in a symbiotic relationship with sulfide-oxidizing bacteria,<sup>151</sup> uses a unique form of hemoglobin that contains 12 Zn<sup>II</sup> centers in a hollow cavity, which bind and capture H<sub>2</sub>S for transport through the bloodstream.<sup>152</sup>

The reactivity of the ZF and catalytic Zn<sup>II</sup> proteins with H<sub>2</sub>S has prompted the evaluation of small-molecule complexes to model this chemistry.<sup>147</sup> The tris-pyrazolylborate ligand system has been used extensively to mimic the His<sub>3</sub> coordination environment of Zn-containing proteins (Figure 6A). Reactions of these model complexes with H<sub>2</sub>S have confirmed that SH<sup>-</sup> can coordinate directly to the Zn<sup>II</sup> center. These studies have demonstrated the importance of bulky, nonpolar ligands to stabilize the resulting Zn-SH complex.<sup>153-157</sup> Other Zn-SH complexes supported by the tris(thioimidazole)hydroborate (Figure 6B),<sup>158,159</sup> tris(2-pyridylmethyl)amine (Figure 6C),<sup>160,161</sup> tris(2-pyridylseleno)methyl (Figure 6D),<sup>162</sup> and a substituted pyridinediimine ligand (Figure 6E)<sup>163</sup> have also been reported.

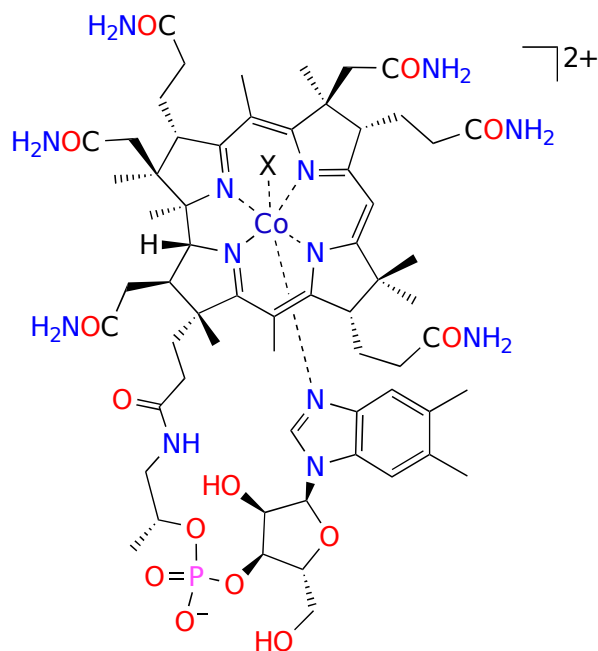


**Figure 6.** General structures of reported Zn-SH coordination compounds.

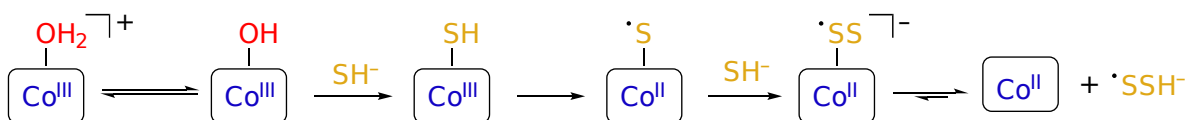
### 3.3 Cobalamin

In addition to the metalloprotein examples discussed above,  $\text{H}_2\text{S}$  also reacts with other redox-active biomolecules. Of particular relevance to the field of bioinorganic chemistry, one of the more interesting reaction partners of  $\text{H}_2\text{S}$  is vitamin  $\text{B}_{12}$ , or cobalamin, a Co-containing molecule that is essential for DNA synthesis and metabolism (Figure 7A; see eibc2489).<sup>164</sup> Only a handful of reports have explored the reactivity of  $\text{H}_2\text{S}$  with cobalamin derivatives and as such, relatively little is known about the biological implications of the reactivity between these molecules.  $\text{H}_2\text{S}$  can displace strongly nucleophilic ligands, such as  $\text{OH}^-$  or  $\text{CN}^-$ , from the  $\text{Co}^{\text{III}}$  center of cobalamin to furnish stable  $\text{Co}^{\text{II}}$  derivatives and  $\text{RSS}^-$ .<sup>165-167</sup> Under anaerobic conditions the generated  $\text{Co}^{\text{II}}$  species readily react with methyl iodide to generate methylcobalamin, which can be cleanly converted back to  $\text{Co}^{\text{III}}$  aquocobalamin in the presence of light, demonstrating that the reaction between cobalamin and  $\text{H}_2\text{S}$  is reversible.<sup>167,168</sup> Kinetic studies have suggested that the reaction between  $\text{H}_2\text{S}$  and cobalamin under anaerobic conditions proceeds via initial binding of  $\text{H}_2\text{S}$  to the  $\text{Co}^{\text{III}}$  center followed by reduction to  $\text{Co}^{\text{II}}$  through an inner-sphere electron transfer reaction. Addition of a second equivalent of  $\text{H}_2\text{S}$  to the  $\text{Co}^{\text{II}}$  sulfide complex produces polysulfide derivatives (Figure 7B). In the presence of  $\text{O}_2$ , the reaction products are much more diverse and include  $[\text{Co}^{\text{II}}\text{-S-S-Co}^{\text{II}}]$  type species and sulfur-modified corrinoids.<sup>166,167</sup> Given the ability of cobalamin and its derivatives to bind  $\text{H}_2\text{S}$ , it has been demonstrated that administration of the vitamin  $\text{B}_{12}$  analogue cobinamide in rabbits prevents sulfide poisoning in vivo by decreasing plasma  $\text{H}_2\text{S}$  levels.<sup>169</sup>

A

X = H<sub>2</sub>O

B



**Figure 7.** (A) Chemical structure of aquocobalamin. (B) Proposed reaction pathway between cobalamin and H<sub>2</sub>S under anaerobic conditions. For more details, see Ref. 167.

## 4. METAL COMPLEXES FOR H<sub>2</sub>S DETECTION

### 4.1 Common H<sub>2</sub>S Detection Methods

Given the growing importance of H<sub>2</sub>S in biology and bioinorganic chemistry, methods for quantification and detection of H<sub>2</sub>S in biological systems have become the focus of intensive study. The spectrophotometric methylene blue (MB) assay is one of the oldest, albeit very reliable, method for sulfide detection and quantification.<sup>170-172</sup> In this assay, MB is generated through the FeCl<sub>3</sub>-catalyzed reaction between *p*-dimethylamino aniline and H<sub>2</sub>S. The characteristic absorbance of MB at 670 nm allows for direct quantification of H<sub>2</sub>S. The MB assay requires the use of acidic aqueous solutions. These conditions, however, can extract acid-labile sulfur from other biological sources, thereby complicating analysis of H<sub>2</sub>S in biological or pH-sensitive systems. Thus, the use of this assay is limited in measuring H<sub>2</sub>S production from donors that are activated under acidic conditions, such as phosphinodithioates.<sup>173-175</sup> An alternative procedure involving precipitation of H<sub>2</sub>S as zinc sulfide prior to performing the MB reaction can be used to circumvent these challenges.<sup>176</sup> Despite its widespread use, the MB method is relatively insensitive with a limit of detection (LOD) of ~2 μM, thus making it insufficient for detecting low concentrations of H<sub>2</sub>S that may be physiologically relevant in biological systems.<sup>177</sup> Another H<sub>2</sub>S detection method, called the monobromobiane

(mBB) method, is generally more accurate and is significantly more sensitive (LOD ~ 2 nM) than MB. This technique relies on the reaction of mBB with H<sub>2</sub>S to furnish sulfide dibimane, which can be detected either spectrophotometrically or by fluorescence spectroscopy.<sup>178,179</sup> The mBB method requires basic conditions and low oxygen concentrations in order to obtain the highest sensitivity for H<sub>2</sub>S. Additionally, coupling this technique to HPLC analysis provides information on the speciation of H<sub>2</sub>S and H<sub>2</sub>S<sub>n</sub> because mBB also reacts with biological thiols and poly- and persulfide species.<sup>180</sup> The resulting products of these reactions are different and can be resolved by HPLC. However, this additional reactivity can be problematic when trying to use the mBB method to measure H<sub>2</sub>S under normoxic, physiological conditions.

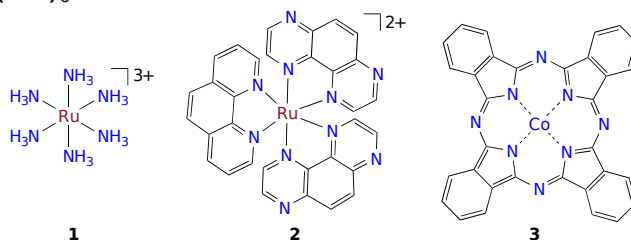
Amperometric electrodes have also been developed to complement spectrophotometric techniques for H<sub>2</sub>S detection.<sup>181,182</sup> In general, these sensors consist of an ion-selective membrane and a polarizing voltage, which allows H<sub>2</sub>S permeation into the electrode. The interior of the electrode contains a strongly basic solution of Fe(CN)<sub>6</sub><sup>3-</sup>, which is reduced selectively by H<sub>2</sub>S to Fe(CN)<sub>6</sub><sup>4-</sup>. The generated ferrocyanide is re-oxidized by a platinum electrode, which generates a current relative to the amount of H<sub>2</sub>S present.<sup>183,184</sup> This technique is advantageous over other methods in that it can be used in unadulterated biological samples, such as tissue homogenates, cultured cells, and even circulating blood of living animals,<sup>185</sup> allowing for time-resolved measurement of H<sub>2</sub>S dynamics. Sulfide electrodes are limited in that they cannot provide information at sub-cellular resolution and are known to be highly pressure and temperature sensitive, requiring frequent recalibration.<sup>21</sup>

The past decade has seen rapid development and implementation of fluorescent and colorimetric reaction-based H<sub>2</sub>S probes for use in biological applications. These systems generally consist of a chromophore appended with a sulfide-sensitive protecting group. Attack by H<sub>2</sub>S releases the active species, resulting in a colorimetric or fluorescence response. Several recent reviews provide a comprehensive background on recent advances in the development of such sensors.<sup>186-191</sup> Compared to the methods discussed above, these probes allow for spatiotemporal monitoring of H<sub>2</sub>S levels in cells and tissues that is minimally invasive. This section will focus on the use of inorganic complexes for H<sub>2</sub>S detection. Compared to sensors based on organic dyes, metal-based lumiphores generally have the advantage of high photostability, large Stokes shifts, which reduces self-quenching, and long-lived luminescence lifetimes (see eibc0253), which permits time-gated luminescence imaging and minimizes interference from biological autofluorescence.<sup>192-194</sup>

## 4.2 Metal-Based H<sub>2</sub>S Sensors

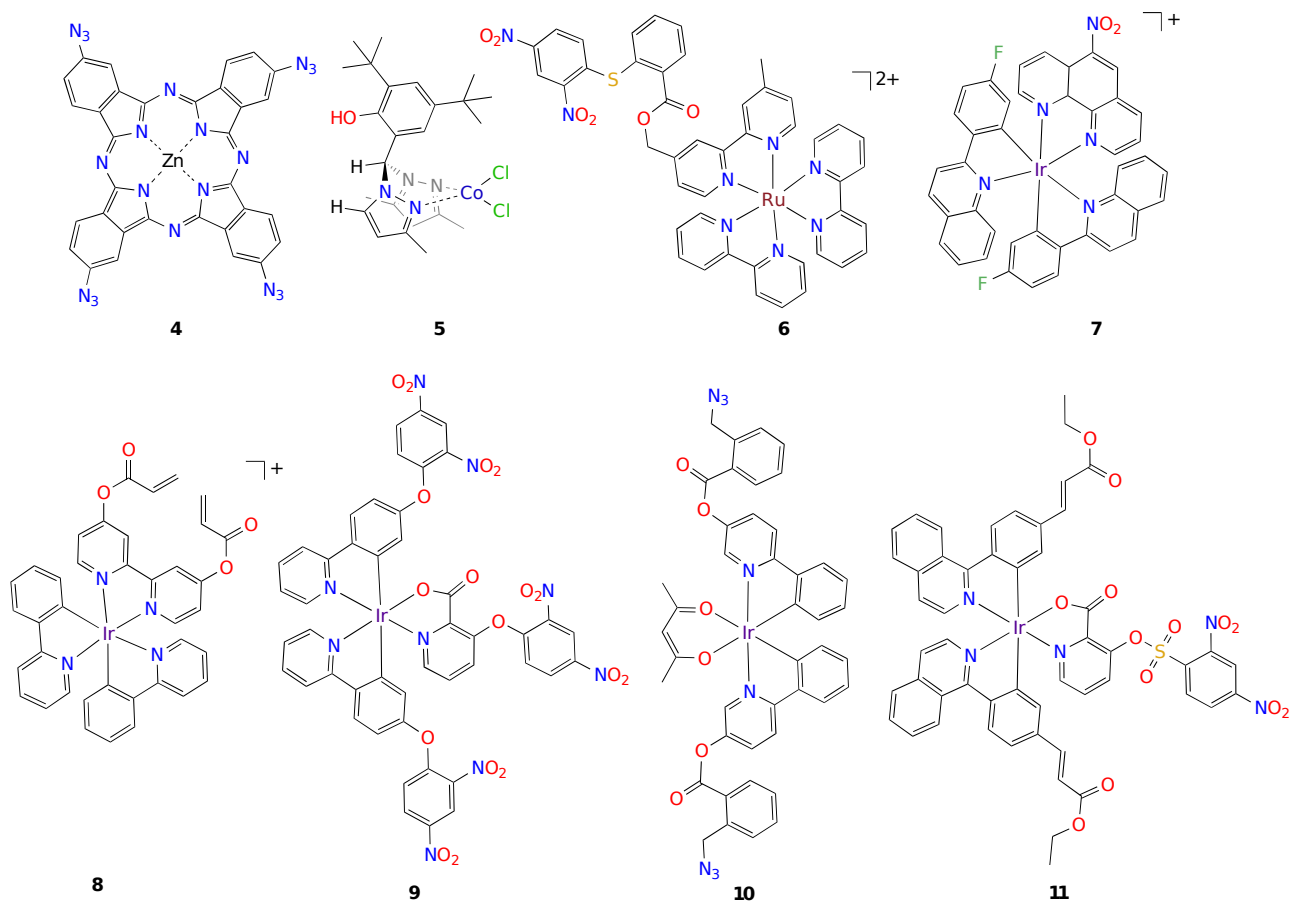
Although we note that metal-organic framework (MOF)-based sensing platforms have recently been explored for the detection of H<sub>2</sub>S,<sup>195,196</sup> this section will focus solely on small-molecule probes. In a general sense, metal-based sensors take advantage of the strong reducing power and nucleophilicity of H<sub>2</sub>S and HS<sup>-</sup>.<sup>186</sup> For example, the reduction of the [Ru(NH<sub>3</sub>)<sub>6</sub>]<sup>3+</sup> (**1**, Figure 8) by sulfide in pH 7.4 aqueous solution was employed to develop a microchip-based system for continuous monitoring of H<sub>2</sub>S levels in the central nervous system of guinea pigs.<sup>197</sup> Similarly, electron transfer between H<sub>2</sub>S and a Ru<sup>II</sup> polypyridyl complex (**2**, Figure 8) induces a phase shift in the luminescence of the complex, which was leveraged to develop reversible sensors capable of monitoring H<sub>2</sub>S dynamics over extended periods of

time.<sup>198</sup> In complex **3** (Figure 8), the Co<sup>II</sup> center is reduced by H<sub>2</sub>S to Co<sup>I</sup>, resulting in a shift in the electronic absorbance spectrum. The metal center can be cleanly oxidized back to Co<sup>II</sup> upon exposure to air, thereby conferring a degree of reversibility to this system.<sup>199</sup> In a different study which highlights the use of metalloproteins for this application, reduction of the Cu<sup>II</sup> center of a fluorescently-tagged azurin protein isolated from *P. aeruginosa* by H<sub>2</sub>S resulted in an increase in fluorescence.<sup>200</sup> This response could be reversed back to the quenched state upon treatment with K<sub>3</sub>Fe(CN)<sub>6</sub>.



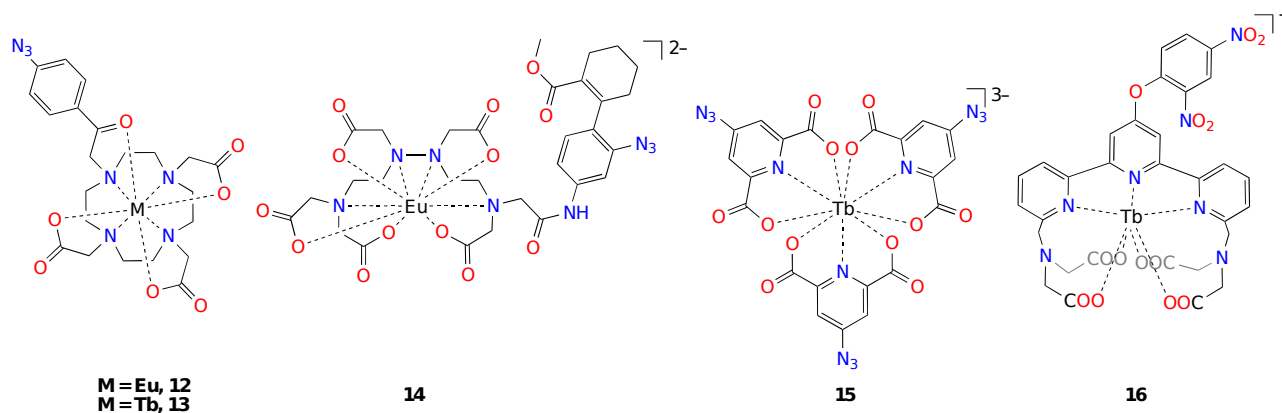
**Figure 8.** Metal complexes that detect H<sub>2</sub>S through reduction.

Luminescent Zn<sup>II</sup> (**4**),<sup>201</sup> Co<sup>II</sup> (**5**),<sup>202</sup> Ru<sup>II</sup> (**6**)<sup>198,203</sup> and Ir<sup>III</sup> (**7-11**)<sup>204-208</sup> complexes have been reported as H<sub>2</sub>S sensors (Figure 9). With the exception of **5**, which appears to sense H<sub>2</sub>S through non-covalent interactions,<sup>202</sup> these scaffolds include H<sub>2</sub>S-reactive masking groups, such as an azides, nitros, dinitrophenyl sulfonyl, or dinitrophenyl ethers. These functional groups can quench the photoexcited metal-to-ligand charge transfer state (MLCT) via photoinduced electron transfer (PET), thereby preventing photoluminescent emissive decay pathways. Nucleophilic attack of H<sub>2</sub>S on these groups modifies them such that PET to the excited state is no longer energetically viable, resulting in an increase in metal-based photoluminescence intensity. Of these compounds, only **6** and **7** have been used in biological contexts. The high sensitivity of **6** towards H<sub>2</sub>S (LOD = 45 nM) enabled the monitoring of lysosomal H<sub>2</sub>S levels in live cells, as well as endogenous H<sub>2</sub>S levels in living zebrafish and mice.<sup>203</sup> Despite the significantly lower sensitivity of **7** (LOD = 4.35 μM) compared to **6**, this compound was similarly able to detect H<sub>2</sub>S in vitro and in vivo.<sup>208</sup> Complex **6** was also activated by hypoxic environments, suggesting that its H<sub>2</sub>S-detection capabilities under these conditions is limited. Although compounds **8-11** have not been studied in biological settings, they offer interesting opportunities for the development of electrochemiluminescent (ECL) sensors, which rely on the generation of luminescent excited states by redox chemistry rather than photon absorption.<sup>209</sup>



**Figure 9.** Luminescent metal complexes for H<sub>2</sub>S detection.

The narrow emission energies, long-lived luminescence lifetimes, and large Stokes shifts of the photoexcited LaPorte forbidden f-f states of the lanthanides Eu<sup>III</sup> and Tb<sup>III</sup> (see eibc2067, eibc2068)<sup>210,211</sup> have also been leveraged in the development of luminescent H<sub>2</sub>S-responsive probes (**12-16**; Figure 10).<sup>212-217</sup> These complexes contain an H<sub>2</sub>S-reactive functional group such as an azide or dinitrophenyl ether, which quenches the complex photoexcited state in a similar manner to the transition metal complexes discussed above. The reaction of these groups with H<sub>2</sub>S restores luminescence by converting these groups to forms that cannot engage in PET. Compound **12** was the first example of a kinetically stable Eu<sup>III</sup> complex that could be used for detection of H<sub>2</sub>S. The biological compatibility of **12** was demonstrated by using it to detect this gasotransmitter in human serum.<sup>212</sup> More recently, a Eu<sup>III</sup> probe bearing a functionalized aminopolycarboxylate ligand (**14**) was reported. This probe can detect H<sub>2</sub>S produced by CSE in aqueous solution, and was used in a high-throughput screening assay to identify potential inhibitors of this enzyme.<sup>215</sup> Another lanthanide-based H<sub>2</sub>S sensor, a Tb<sup>III</sup> complex bearing azide-substituted pyridine carboxylate ligands (**15**), was able to detect H<sub>2</sub>S at concentrations as low as 10 nM in aqueous solution. This compound was used in a paper-based assay to detect trace H<sub>2</sub>S exhaled by mice.<sup>213</sup> Lastly, a Tb<sup>III</sup> complex containing a 2,6-dinitrophenol functionalized terpyridine type ligand (**16**) was used to detect H<sub>2</sub>S in living cells.<sup>216</sup>

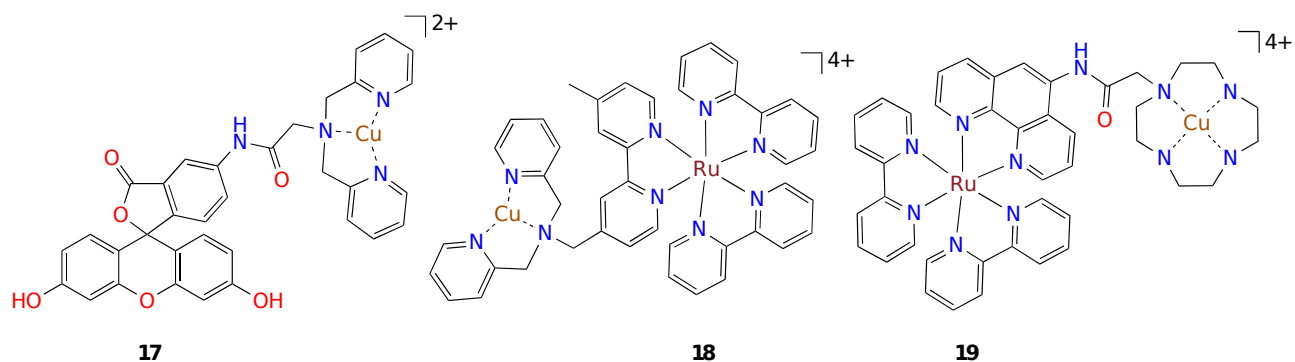


**Figure 10.** Luminescent lanthanide-based H<sub>2</sub>S-sensing complexes.

### 4.3 Metal Displacement Sensors

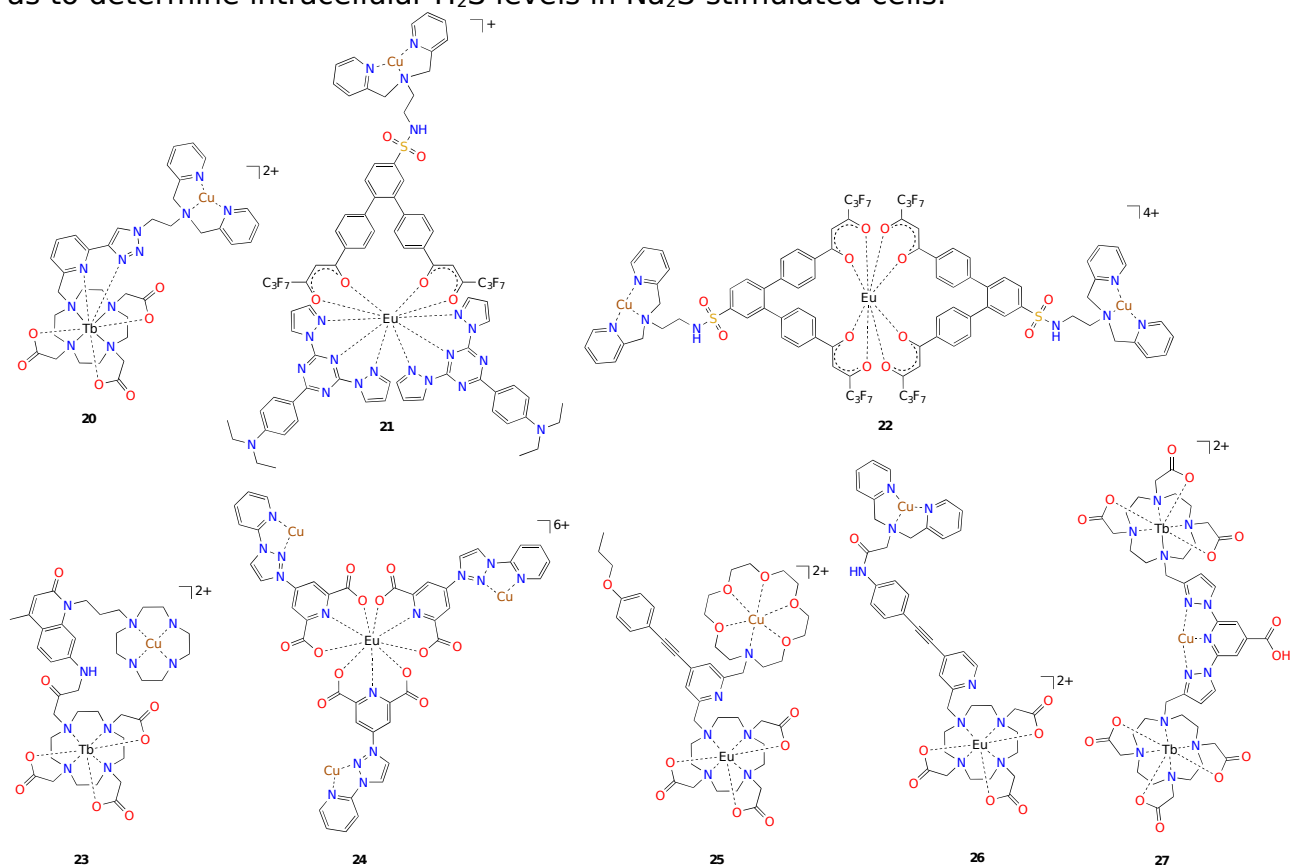
The characteristic low solubility of metal sulfide salts offers a possible strategy for the detection of H<sub>2</sub>S through metal displacement reactions. Since 1685,<sup>218,219</sup> H<sub>2</sub>S has been used to precipitate small concentrations of Cu<sup>II</sup>, Sn<sup>II</sup>, Pd<sup>II</sup>, and Hg<sup>II</sup> as a means of diagnosing their presence. As such, compounds containing these metal ions, in addition to Zn<sup>II</sup>, Cd<sup>II</sup>, Pb<sup>II</sup>, and Ag<sup>I</sup>, have all been utilized for detection of this gas.<sup>220–227</sup> Generally, this strategy relies on compounds that contain both a photoluminescent dye and a metal ion bound by a chelator, such as 1,4,7,10-tetraazacyclododecane (cyclen) or di-(2-picoyl) amine (DPA).<sup>188,194</sup> One of the first sensors employing this approach combined the fluorescent dye fluorescein with the DPA ligand bound to Cu<sup>II</sup> (**17**, Figure 11).<sup>228</sup> The emission of fluorescein is quenched by the paramagnetic Cu<sup>II</sup> center,<sup>229</sup> but is restored upon exposure to H<sub>2</sub>S, which removes the Cu<sup>II</sup> in the form of insoluble CuS.<sup>220</sup> Following this example, sensors containing organic fluorescent dyes such as rhodamine, anthracene, and 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) that act through metal ion displacement have been reported.<sup>187,230,231</sup> The following discussion focuses specifically on inorganic lumiphores that detect H<sub>2</sub>S through metal displacement reactions.

The heterobimetallic Ru<sup>II</sup>-Cu<sup>II</sup> complex [Ru(bpy)<sub>2</sub>(bpy-DPA)]<sup>4+</sup> (**18**; bpy-DPA = 4-methyl-4'-[N,N-bis(2-picoyl)amino-methylene]-2,2'-bipyridine; Figure 11) was reported to be a sensitive (LOD = 20 nM) turn-on luminescent H<sub>2</sub>S sensor that is activated by precipitation of CuS.<sup>232</sup> This complex was also used as an ECL sensor to measure sulfide concentration in the cortex of adult male rats.<sup>233</sup> A similar ruthenium complex containing phenanthroline appended with a cyclen chelator was also shown to sense H<sub>2</sub>S in the presence of Cu<sup>II</sup> (**19**, Figure 11). This complex is capable of detecting H<sub>2</sub>S over a broad pH range and can detect H<sub>2</sub>S in vitro with a rapid turn-on response time.<sup>234</sup>



**Figure 11.** Luminescent H<sub>2</sub>S sensors that detect H<sub>2</sub>S through metal displacement.

A number of Cu<sup>II</sup> displacement sensors have been reported that capitalize on the long-lived luminescence of the lanthanides Eu<sup>III</sup> and Tb<sup>III</sup> (**20-27**, Figure 12).<sup>235-242</sup> Although some of these compounds, such as **20**, were used to monitor H<sub>2</sub>S in petroleum plant waste streams,<sup>237</sup> a few of these probes have found applications in biological settings. Heterobimetallic Eu<sup>III</sup>-Cu<sup>II</sup> compounds **21** and **22** bearing β-diketonate ligands and DPA Cu<sup>II</sup>-chelating moieties were found to be relatively nontoxic and capable of selectively detecting intracellular H<sub>2</sub>S in vitro.<sup>241,242</sup> More recently, **23** was reported as a luminescent probe with a rapid turn-on response, high sensitivity, and good selectivity for H<sub>2</sub>S. Furthermore, this compound could be used to measure H<sub>2</sub>S production from both the slow-releasing H<sub>2</sub>S donor morpholin-4-ium 4-methoxyphenyl(morpholino)phosphinodithioate (GY4137) and CSE, as well as to determine intracellular H<sub>2</sub>S levels in Na<sub>2</sub>S-stimulated cells.<sup>235</sup>

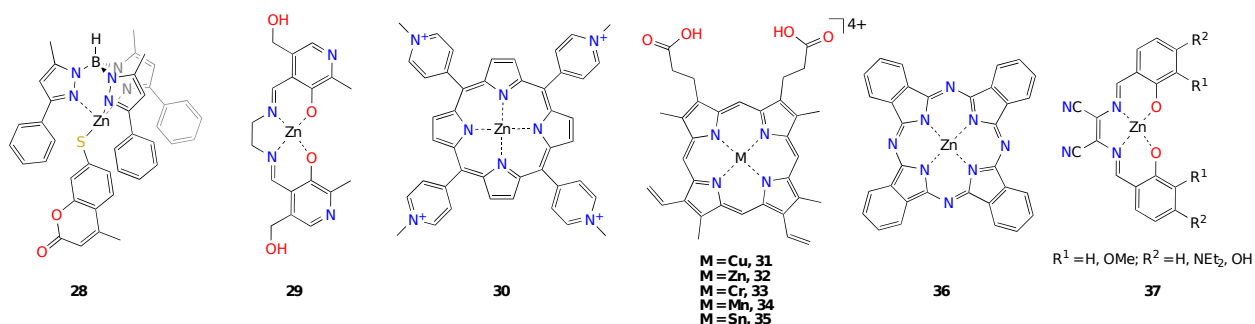




**Figure 12.** Luminescent lanthanide-based H<sub>2</sub>S probes that rely on metal displacement for detection.

#### 4.4 H<sub>2</sub>S Detection via Metal Coordination

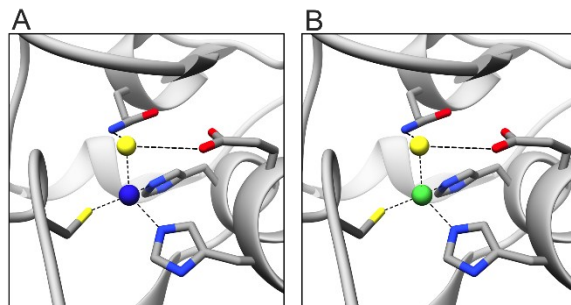
Researchers have developed probes that can detect H<sub>2</sub>S via changes in fluorescence or absorbance upon direct coordination of this gas transmitter to the metal center. The Zn<sup>II</sup> tris(pyrazoyl)borate complex **28** containing 7-mercapto-4-methylcoumarin was the earliest coordination-based H<sub>2</sub>S sensor with a LOD of 1 μM.<sup>243</sup> Binding of H<sub>2</sub>S to the Zn center results in the release of the coumarin ligand and a concomitant color change, making **28** a colorimetric sensor. Pyridoxal (**29**)<sup>244,245</sup>, porphyrin (**30-35**),<sup>246-248</sup> phthalocyanine (**36**),<sup>199</sup> and salen (**37**)<sup>249</sup> complexes have also been shown to sense H<sub>2</sub>S via direct metal coordination (Figure 13). Compound **31** displays decent selectivity for H<sub>2</sub>S over a range of biologically relevant anions, thiols and oxidants.<sup>247</sup> A second study found that **31** failed to bind sulfide in organic solution, a result that reveals the importance of solvent effects and proton availability for this class of sensors.<sup>248</sup> The absorption spectra of porphyrin compounds **34** and **35** undergo significant changes in the presence of excess HS<sup>-</sup>. The metal center, however, undergoes subsequent reduction to give unresolved reaction products,<sup>248</sup> which might hinder their use as colorimetric H<sub>2</sub>S sensors.



**Figure 13.** Inorganic complexes that detect H<sub>2</sub>S upon metal coordination.

Researchers have also leveraged the ability of metalloproteins and enzymes to bind H<sub>2</sub>S for the development of coordination-based sensors. The general approach combines an H<sub>2</sub>S-binding metalloprotein with a fluorescent tag. The metalloprotein chromophores of these systems exhibit significant shifts in their absorbance wavelengths upon binding to H<sub>2</sub>S. The change in absorbance of the metalloprotein chromophore can modulate the emission intensity of fluorescent tag by attenuating or enhancing the availability of photons from the excitation source. This strategy relies on the use of fluorescent tags with excitation wavelengths near absorbance maxima of the protein or enzyme in the absence of H<sub>2</sub>S, which prevents fluorescence of the tag. Coordination of H<sub>2</sub>S/HS<sup>-</sup> to the metal center causes the protein absorbance to shift, unmasking the fluorophore, which results in an increase in fluorescence intensity. Fluorophore-tagged analogues of Horse skeletal muscle myoglobin isolated from horse skeletal muscle,<sup>250</sup> cobalt peptide deformylase (PDF) from *E. coli*,<sup>251</sup> and Hbl from *L. pectinata*<sup>252</sup> have all been used as H<sub>2</sub>S sensors of this type with good selectivity over other biological thiols and sensitivity in the nanomolar range. The metal-sulfide interaction in PDF was supported by X-ray

crystallography, which demonstrated that both the Co and Ni-containing forms of this enzyme coordinate H<sub>2</sub>S (Figure 14).<sup>251</sup>



**Figure 14.** X-ray structures of (A) Co-PDF and (B) Ni-PDF (PDB: 4AZ4, 4AL2)

The coordination-based approach for H<sub>2</sub>S detection is intriguing given the low stability and high reactivity of many metal hydrogen sulfide and hydrosulfido complexes.<sup>26,27,253-256</sup> In contrast to the reaction-based and metal displacement sensors, these systems can potentially give rise to reversible H<sub>2</sub>S probes, an important property that could be used to monitor H<sub>2</sub>S dynamics in biological or industrial settings. For example, treatment of the hydrosulfide adduct of **33** or **36** with acetic acid or oxygen regenerates the parent complexes.<sup>199</sup> Similarly, purging solutions of H<sub>2</sub>S-treated Hbl with argon produces the free protein, which can then detect additional sulfide.<sup>252</sup> Together these studies highlight the utility of the coordination-based approach for development of reversible sensors.

## 5. METAL COMPLEXES AS AGENTS FOR H<sub>2</sub>S DELIVERY

### 5.1 Simple Inorganic Salts

Despite the numerous advances in understanding the biological activity of H<sub>2</sub>S, it remains a challenge to deliver this gas to biological systems. The use of H<sub>2</sub>S in its gaseous state is challenged by its toxicity, flammability, and volatility.<sup>257,258</sup> Simple metal sulfide salts such as Na<sub>2</sub>S and NaSH are the most commonly employed sources of H<sub>2</sub>S in biological studies.<sup>258</sup> Although these salts are generally easier to handle than gaseous H<sub>2</sub>S, they are typically obtained commercially with very low purity levels,<sup>259</sup> a limitation that makes it challenging to discern the precise amount of H<sub>2</sub>S delivered by these salts. Furthermore, a 10 μM aqueous solution of NaSH has a half-life of only 0.5 min due to volatilization of H<sub>2</sub>S,<sup>260</sup> and the H<sub>2</sub>S generated from intravenously injected Na<sub>2</sub>S is rapidly exhaled.<sup>261</sup> Additionally, toxic side effects from these salts may arise from their rapid release rate of H<sub>2</sub>S in aqueous solution, which fails to mimic endogenous H<sub>2</sub>S dynamics. Motivated by these limitations, researchers have developed slow-release H<sub>2</sub>S donors that produce this gas on biologically relevant time scales. A number of different small-molecule sulfide donors have been recently reported.<sup>257,258,262-264</sup> These include simple molecules that undergo uncontrolled hydrolysis,<sup>174,175,265-267</sup> in addition to organic H<sub>2</sub>S-releasing compounds that are selectively activated by light,<sup>268-272</sup> enzymatic activity,<sup>273-276</sup> thiols,<sup>277-284</sup> and reactive oxygen species (ROS).<sup>285-288</sup> In contrast to the prevalence of inorganic CO- and NO-releasing molecules,<sup>289-294</sup> an overwhelming majority of H<sub>2</sub>S donors are organic molecules. Herein, recent efforts towards the development of metal-based small-molecule H<sub>2</sub>S donors are described.

### 5.2 Tetrathiomolybdate

Tetrathiomolybdate ( $[\text{MoS}_4]^{2-}$ ; TM) is a commonly used reagent in organic chemistry as a sulfur transfer reagent and reductant.<sup>295,296</sup> This ion is prepared by the reaction of the oxymolybdate anion ( $[\text{MoO}_4]^{2-}$ ) and  $\text{H}_2\text{S}$  in basic aqueous solution.<sup>297</sup> Historically, TM has received significant attention for its ability to inhibit Cu<sup>I</sup>-trafficking proteins through the formation of sulfur-bridged Cu-Mo clusters.<sup>298</sup> This orally available complex has been widely used to treat disorders associated with improper copper metabolism, such as Wilson's disease.

Although TM has been long known to release  $\text{H}_2\text{S}$  under acidic<sup>299</sup> or high temperature<sup>300,301</sup> conditions, recent work has demonstrated that this compound, and to a lesser extent its congener tetrathiotungstate ( $[\text{WS}_4]^{2-}$ , TT), produce this gas via hydrolysis under biological conditions.<sup>302-305</sup> TM generates  $\text{H}_2\text{S}$  via hydrolysis over a period of hours in buffered aqueous solution<sup>302</sup> and interacts with biological thiols, such as glutathione, to produce other reactive persulfide and polysulfide species.<sup>303</sup> The  $\text{H}_2\text{S}$  generated through TM hydrolysis was shown to prevent  $\text{H}_2\text{O}_2$ -induced oxidative stress and preserve cellular function in vitro.<sup>302</sup> When administered intravenously, TM significantly reduces infarct size in mice subjected to either myocardial or cerebral ischemia<sup>304</sup> and prevents oxidative damage and loss of functional activity in preclinical stroke models.<sup>305</sup> A recent report suggested that  $\text{H}_2\text{S}$  produced by TM hinders its anticancer activity in A549 adenocarcinoma cells by enhancing cell growth at low concentrations and upregulating  $\text{H}_2\text{S}$  producing enzymes.<sup>306</sup>

Mechanistic studies suggest that the protective effects of TM arise from its ability to decrease mitochondrial ROS levels<sup>304</sup> and improve antioxidant enzyme activity,<sup>305</sup> which are consistent with the therapeutic effects reported for  $\text{H}_2\text{S}$ .<sup>307</sup> Further mechanistic studies have suggested that TM and TT are transported into cells by anion exchange protein-1 (AE-1).<sup>303</sup> Despite the promise of TM as a biologically relevant  $\text{H}_2\text{S}$  donor, it was reported that TM obtained from different commercial sources produced variable amounts  $\text{H}_2\text{S}$ ,<sup>304</sup> highlighting the difficulty of obtaining these simple compounds in analytically pure forms. Additionally, the  $\text{H}_2\text{S}$  release from TM is uncontrollable; once the complex is placed in aqueous solution,  $\text{H}_2\text{S}$  is spontaneously generated and the release cannot be targeted to a specific location in vivo. It would be advantageous to develop agents that produce  $\text{H}_2\text{S}$  selectively in response to biorthogonal stimuli, allowing for selective and controlled  $\text{H}_2\text{S}$  production in complex biological systems.

### 5.3 Light-Activated Donors

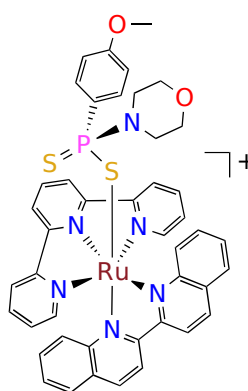
Light-activated  $\text{H}_2\text{S}$ -releasing agents have been recognized as promising tools for studying the biological and therapeutic properties of this gas.<sup>262,263</sup> Photoactivatable donors allow for localized and noninvasive delivery of  $\text{H}_2\text{S}$  in vitro and offer exciting possibilities for delivery in vivo. Upon irradiation with a specific wavelength of light, the donor undergoes photoinduced decomposition to cleave a photoactivated protecting group and release an  $\text{H}_2\text{S}$  producing moiety. The first example of a photocaged  $\text{H}_2\text{S}$  donor consisted of a *bis*-orthonitrobenzyl protected geminal-dithiol (*gem*-dithiol).<sup>268</sup> The nitrobenzyl protecting groups are removed upon reaction with 365 nm light to produce an unstable geminal-dithiol, which undergoes rapid hydrolysis in aqueous solution to produce  $\text{H}_2\text{S}$ . Other photoactivated  $\text{H}_2\text{S}$  donors containing *gem*-dithiol, thiobenzaldehyde, and ketoprofenate moieties have also been reported.<sup>257,268-270</sup>

The vast majority of these photoactivated compounds require ultraviolet (UV) light ( $\lambda \leq 400$  nm) for activation. This requirement limits the applicability of these donors in vivo given that these wavelengths ineffectively penetrate biological tissue

and can give rise to phototoxicity.<sup>308</sup> As such, researchers have sought to develop light-activated donors that function at lower energy wavelengths.

By virtue of their favorable photophysical properties (see eibc0174)<sup>309,310</sup> metal compounds have been widely used for light-activated delivery of CO and NO.<sup>310-314</sup> By contrast, photoactivated H<sub>2</sub>S donors based on inorganic systems have not been reported until recently. An early example of a metal-based H<sub>2</sub>S release platform evoked polyethylene glycol functionalized LiYF<sub>4</sub>:Yb/Tm upconverting nanoparticles (UPNCs) conjugated to a caged *gem*-dithiol compound. Under near infrared (NIR) irradiation, the UPNCs emit UV light, which then unmasks the protected *gem*-dithiol to produce H<sub>2</sub>S. This strategy was shown to deliver H<sub>2</sub>S both in living cells and ex vivo in a pork skin model.<sup>315</sup> A complementary nanoparticle-based system consisting of a singlet oxygen (<sup>1</sup>O<sub>2</sub>) photosensitizer and 1,3-diphenylisobenzothiophene (DPBT) encapsulated in artificial polymersomes has also been reported. The photosensitizer, a Pt<sup>II</sup> porphyrin complex or biscyclometalated Ir<sup>III</sup> compound, generates <sup>1</sup>O<sub>2</sub> upon irradiation with visible light, which subsequently reacts with DPBT to produce H<sub>2</sub>S.<sup>316</sup> These early studies have demonstrated how the photochemical properties of metal complexes can be leveraged to develop selective H<sub>2</sub>S donors that can be activated by light irradiation.

The characterization and biological activity of a red light-activated H<sub>2</sub>S-releasing Ru<sup>II</sup> complex was recently reported.<sup>317</sup> In this study, coordination of GYY4137<sup>173,174</sup> to a ruthenium photocage (**38**, Figure 15) suppresses the spontaneous hydrolysis-driven H<sub>2</sub>S release from this compound. Compound **38** produces H<sub>2</sub>S in living cells upon irradiation with red (631 nm) light and protects H9c2 cardiomyoblast cells against an in vitro model of ischemic reperfusion injury. Compound **38** is the first example of an inorganic small-molecule H<sub>2</sub>S donor that is activated by irradiation with red light; a BODIPY-based thiocarbamate compound was reported as the first NIR light-activated organic H<sub>2</sub>S donor in the same year.<sup>272</sup> This work highlights how transition metal complexes may serve as viable light-activated H<sub>2</sub>S donors that can operate in biologically relevant settings.



**38**

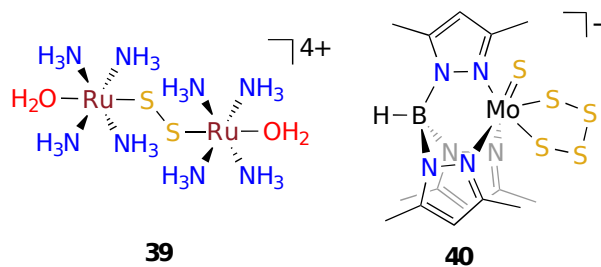
**Figure 15.** Chemical structure of compound **38**.

#### 5.4 Reduction-Activated Donors

The redox chemistry of transition metal ions offers unique opportunities for redox-responsive delivery of biologically relevant molecules. The redox environment

of biological tissue or cells can vary dramatically depending on the specific cell type, organelle, or conditions. In the presence of low  $O_2$  levels, a state known as hypoxia, the cellular environment becomes highly reducing as cells and tissue lose the ability to maintain redox balance.<sup>318</sup> In this context several inorganic complexes containing metals such as Ru, Os, Pt, Cu, and Co have been developed as redox-activated prodrugs,<sup>319-323</sup> which release cytotoxic anticancer agents upon reduction that occurs in hypoxic cells. A recent report has shown that a dinuclear persulfide ( $\mu-S_2^{2-}$ ) bridged ruthenium compound (**39**, Figure 16) produces  $H_2S$  selectively upon reduction.<sup>324</sup> The ability of this complex to produce  $H_2S$  upon reduction was leveraged to deliver this gasotransmitter to hypoxic cells, making **39** the first example of a hypoxia-activated  $H_2S$  donor. Notably, mechanistic studies revealed that the reduction process does not proceed through production of  $H_2S_2$  like many organic persulfide compounds,<sup>325-330</sup> but rather directly produces  $H_2S$ . Lastly, this complex was shown to preserve cell viability in H9c2 cardiomyoblast cells subjected to an in vitro model of ischemic reperfusion injury. Given that the redox chemistry of transition metal ions is highly dependent on the nature of the supporting ligands, persulfide-bridged metal complexes offer exciting opportunities for development of tunable  $H_2S$  donors that can deliver this gas under a wide span of biologically relevant redox potentials.

Recently, a  $Mo^{IV}$  tetrasulfido complex (**40**, Figure 16) was reported to undergo a two-electron reduction to produce  $HS_2^-$  and a  $Mo^{VI}$  trisulfide species.<sup>331</sup> The reductive release of  $HS_2^-$  by compound **40** was only demonstrated in organic solvent or aqueous organic mixtures, but this study demonstrates the potential of this class of compounds for the development of inorganic complexes for delivery of  $H_2S$  and other RSS to biological systems.



**Figure 16.** Redox-active metal complexes that produce RSS upon reduction.

## 6. OUTLOOK AND CONCLUSIONS

Studies on the biological activity of  $H_2S$  and related RSS have highlighted diverse reactivity of these species with metal-containing biomolecules. This immense diversity and the complex nature of these reactions have made it difficult to obtain intimate mechanistic understanding of these processes. In some cases, such as CcO, model compounds that mimic the active site of the enzyme or proteins have greatly increased understanding of the reactivity between  $H_2S$  and these biomolecules. The biological targets of  $H_2S$  and its derived RSS have only just begun to be elucidated and a number of biologically relevant metal-containing biomolecules, such as ZF proteins, are still not well studied. Investigation of the biological activity of  $H_2S$  is further complicated by its propensity to form reactive polysulfides and persulfides, which possess biological activity distinct from that of  $H_2S$ .<sup>332,333</sup>

The utility of inorganic complexes for H<sub>2</sub>S detection and delivery in biological environments has only recently been realized. Inorganic complexes display favorable photophysical properties compared to organic fluorophores and offer exciting opportunities for electrochemiluminescence-based H<sub>2</sub>S sensors. Furthermore, reduction-activated and coordination-activated sensors have shown promise for the development of reversible H<sub>2</sub>S sensors that could potentially be used to monitor biological H<sub>2</sub>S dynamics over extended periods of time.

Inorganic complexes have only recently begun to be investigated for their use as H<sub>2</sub>S donors for delivery of biologically relevant concentrations of this gas in vitro. The rich photochemical and redox properties of coordination compounds offer exciting opportunities for the development of novel H<sub>2</sub>S donors for use as tools for studying the role of H<sub>2</sub>S and related RSS in biological processes and disease.

## 7. RELATED ARTICLES

eibc0215.pub2; eibc2724; eibc0174; eibc0056; eibc0097; eibc0099; eibc0100; eibc0253; eibc2067; eibc2068; eibc2489; eibc0213

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