H₂S and reactive sulfur signaling at the host-bacterial pathogen interface

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Bacterial pathogens that cause invasive disease in the vertebrate host must adapt to host efforts to cripple their viability. Major host insults are reactive oxygen and reactive nitrogen species as well as cellular stress induced by antibiotics. Hydrogen sulfide (H₂S) is emerging as an important player in cytoprotection against these stressors, which may well be attributed to downstream more oxidized sulfur species termed reactive sulfur species (RSS). In this review, we summarize recent work that suggests that H₂S/RSS impacts bacterial survival in infected cells and animals. We discuss the mechanisms of biogenesis and clearance of RSS in the context of a bacterial H₂S/RSS homeostasis model and the bacterial transcriptional regulatory proteins that act as “sensors” of cellular RSS that maintain H₂S/RSS homeostasis. In addition, we cover fluorescence imaging- and MS–based approaches used to detect and quantify RSS in bacterial cells. Last, we discuss proteome persulfidation (S-sulfuration) as a potential mediator of H₂S/RSS signaling in bacteria in the context of the writer-reader-eraser paradigm, and progress toward ascribing regulatory significance to this widespread post-translational modification.

Infectious disease is a global and significant threat to human health. There is an increasingly urgent need to develop new antimicrobial strategies to combat these increasingly drug-resistant and life-threatening pathogens (1, 2). One important approach to do this is to understand bacterial adaptation to the myriad of host immune responses that have evolved to clear bacterial infections. For example, transition metal homeostasis (metallostasis) (3) effectively controls the metalation status of the proteome (4, 5). Upon infection, the host actively disrupts metallostasis by restricting access to intoxicating cells with metals (9, 10) to limit bacterial growth. Pathogens, in turn, adapt by employing specialized transcriptional regulators, metallosensors, that sense metals and regulate the expression of genes encoding proteins that collectively maintain bioavailable metal in a range compatible with physiological needs (Fig. 1A, top) (11–14). In an analogous fashion, bacteria encode specialized transcriptional regulators that sense oxidized or “reactive” sulfur species (RSS), derived from hydrogen sulfide (H₂S) (15–20). As cellular concentrations of RSS rise, RSS sensors turn on the expression of genes that encode enzymes that reduce cellular loads of H₂S/RSS to avoid H₂S toxicity and overpersulfidation of the metabolome and proteome (Fig. 1A, bottom). These RSS sensors, like metallosensors, control H₂S/RSS homeostasis, allowing bacterial cells access to these molecules at low concentrations to meet physiological needs. In the infected host, H₂S and RSS are derived from host cell metabolism, from commensal bacteria in polymicrobial communities, or from the pathogen itself. Recent studies that build on prior work (21) suggest that bacterial H₂S biogenesis may well be a clinically important adaptive response during infections (22–26).

A second feature that is common to metallostasis and H₂S/RSS homeostasis, beyond the sensors themselves, is the concept of speciation (Fig. 1B). In metallostasis, speciation defines the metallome, or all coordination complexes, both small molecule and protein, and oxidation states of all transition metals in the cell (Fig. 1B, top). Metallosensors surveil the cytoplasm for some specific feature of the metallome (e.g. zinc in exchange-labile complexes) and alter gene expression upon metal binding. In H₂S/RSS homeostasis, speciation is defined by the components of the RSS pool, which encompasses organic and inorganic molecules containing sulfur in oxidation states higher than H₂S, many of which contain sulfur-bonded or “sulfane” sulfur (Fig. 1B, bottom) (27). Analogous to a metallosensor, known RSS sensors specifically surveil the cytoplasm for a particular feature of the RSS pool, in this case sulfane sulfur (15–20).

In this review, we summarize the biogenesis and clearance of H₂S/RSS and the potential role these molecules play in bacterial infections. In addition, we discuss the molecular mechanisms of RSS sensors that maintain H₂S/RSS homeostasis in bacteria. Elucidation of how H₂S/RSS are leveraged in bacteria at the host-pathogen interface relies on the development of molecular tools to identify, detect, and quantify H₂S and RSS as well as small-molecule probes to generate these species in vitro or in vivo. Last, we discuss recent efforts to detect and understand the regulatory significance of protein persulfidation (S-sulfuration) in bacteria.

Hydrogen sulfide and reactive sulfur species in bacteria

H₂S is an electron-rich molecule historically well-known to drive photosynthesis (28) and energy metabolism in sulfide-oxidizing and sulfate- or sulfite-reducing microorganisms (29, 30). In 2011, Nudler and co-workers (21) reported that endogenously synthesized H₂S or application of exogenous sulfide salts protected multiple bacterial pathogens against a broad array of mechanistically distinct antibiotics when grown in culture. This initial report, despite few insights into a possible mechanism, suggested that H₂S might have beneficial properties in human disease–causing microorganisms and has thus inspired
considerable research over the last 10 years. These bacteria endogenously synthesize H$_2$S utilizing bacterial homologs of the mammalian reverse transsulfuration pathway via “side” reactions catalyzed by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (31–34) or from cysteine catabolism to 3-mercaptopyruvate (3-MP) via cysteine aminotransferase (CAT) (35). 3-MP is then converted to pyruvate and H$_2$S by 3-MP sulfurtransferase (3MST) (36) via the intermediacy of a protein persulfide, E-SSH (Fig. 2A). Bacteria generally encode either 3MST or CBS/CSE, and it was recently demonstrated that L-cysteine desulfhydrases and cysteine desulfurases also contribute to H$_2$S biogenesis in Escherichia coli (39, 40). In addition, two groups recently reported the discovery of a glycolyl enzyme from Bilophila wadsworthia that catalyzes C=S bond cleavage in the catabolism of tissue-abundant taurine and the analogous alcohol isethionate (2-hydroxyethanesulfonate) (41, 42). This reaction produces sulfite (SO$_3^-$), which is reduced to H$_2$S by a dissimilatory sulfite reductase, thus defining a novel pathway for H$_2$S production by gut microbiota.

With a sulfur oxidation state of “−2”, H$_2$S and organic thiols (e.g. cysteine or GSH) are in their most reduced forms and can only function as cellular reductants (27, 43). RSS harbor higher sulfur oxidation states, ranging from “−1” to “+6” (Fig. 1B, bottom). The organic thiol persulfide (hydropersulfide, RSSH) is of particular interest because of its “Janus” character and can function as either a nucleophile when deprotonated (RSS$^-$) or an electrophile when protonated (RSSH$^+$). Due to a considerably lower pK$_a$ than the corresponding thiol, the anionic form predominates at physiological pH (43–45). Persulfides also have enhanced nucleophilicity compared with their corresponding thiolate because of the α-effect (46), which increases the reactivity of the terminal sulfur atom because of unpaired electrons in the adjacent atom.

Persulfides readily react with oxidants such as hydrogen peroxide (H$_2$O$_2$) and peroxynitrite (44, 47) and are superior one-electron reductants to thiols and H$_2$S as reviewed elsewhere (48–50). Their Janus character, enhanced nucleophilicity, and superior reducing capabilities make RSSH, along with organic polysulfides and their inorganic counterparts, potent antioxidants (Fig. 1B, bottom) (43, 51, 52). These properties may well be responsible for many of the beneficial traits attributed to H$_2$S, including protection against oxidative stress and antibiotics in the infected host (21, 26, 47).

Recent work by several groups reveals significant physiological overlap or cross-talk between H$_2$S/RSS and reactive oxygen (ROS) and reactive nitrogen (RNS) species. Oxidation of RSS results in the production of inorganic sulfur-containing molecules sulfite, thiosulfate, and sulfate (Fig. 2B) (51). ROS can also drive the formation of low-molecular weight (LMW) thiol disulfides (RSSR) and sulfinic acids (RSSH), a major physiological marker of H$_2$O$_2$ reactivity, which reacts with HS$^-$ to form...
organic RSS (Fig. 2B). H₂S and nitric oxide (NO·) intersect via nitroxylic (HNO), and incubation of bacterial cells with a nitroxylic donor, Angeli’s salt, results in an increase in cellular levels of RSS in *Staphylococcus aureus* (53) possibly via thionitrous acid (HSNO) or nitrosopersulfide (SSNO−) formation (Fig. 2B) (54–56). In addition, polysulfides can by synthesized from incubation of RSH with sodium nitrite (NO/C02) to form organic nitrosothiols (RSNO), which readily react with HS− at acidic pH to form a mixture of RS−S−SR, consistent with proposed H₂S/NO cross-talk (57). Last, protein persulfidation (S-sulfuration) is now a widely recognized post-translational modification (PTM) believed to function in H₂S signaling alongside, and possibly interconverting with, other thiol modifications, including S-thiolation (RSSR), S-nitrosation (RSNO), or oxidation to sulfenic, sulfinic (RSO2H), and sulfonic acids (RSO3H) (Fig. 2C) (43, 58–60). It is important to note that the chemistry presented here between H₂S/RSS and ROS/RNS can potentially occur on both small-molecule and protein thiols (Fig. 2, B and C). Furthermore, the onslaught of host-generated ROS and RNS at sites of infection suggests this chemical cross-talk may be biologically relevant in the infected host (61–64).

**Physiological conditions for the production, regulation, and signaling of H₂S/RSS in bacteria**

**H₂S and the gut microbiome**

The gut microbiome is a complex, nutrient-rich environment and host to well over 100 bacterial species (65, 66). The microorganisms that inhabit this niche are a significant endogenous source of sulfur-containing compounds and H₂S, the
latter estimated to range from ~0.2 to 2.4 mM (Fig. 3A) (67). Methionine catabolism to cysteine via the reverse transsulfuration pathway (Fig. 2A) and the catabolism of organic sulfonates, notably taurine, are known to be catalyzed by gut microbiota (41, 42, 68–72). This niche is also home to sulfate-reducing bacteria that are responsible for significant production of H2S (73, 74), in addition to the reduction of tetrathionate and thiosulfate to H2S that also occurs in the gut (75). H2S produced by the gut microbiota is oxidized by gastrointestinal epithelial cells to thiosulfate and tetrathionate (76, 77); these molecules in turn are utilized by the microbiota as electron acceptors, resulting in a symbiotic relationship derived from interconversion of sulfur species (Fig. 3A) (72). Perturbation of this symbiotic relationship results in the accumulation of H2S now linked to several gut-derived diseases (78–80). Interestingly, gut inflammation caused by the pathogen Salmonella enterica serovar Typhimurium results in increased production of tetrathionate from the oxidation of thiosulfate by gut inflammation–derived ROS, which this bacterium uses as an alternate electron acceptor, thus providing a growth advantage in this niche (81).

**H2S at the host-pathogen interface**

There are two major physiological conditions within the infected host where H2S and downstream RSS may enhance bacterial survival in what is otherwise a hostile microenvironment. These are (i) resistance against myriad oxidative stressors and antibiotic challenge and (ii) H2S/RSS-dependent regulation of biofilm dynamics. The host immune system produces diverse ROS, including O2 (82–84), hydrogen peroxide (H2O2) (85), and hydroxy radical (OH·) (86) to combat bacterial infections (87). Antibiotics are also thought to induce generalized oxidative stress (88–91), although this has been widely debated in recent years (92, 93). The first documentation that H2S impacted antibiotic resistance in E. coli was published 47 years ago (94). Renewed interest came in 2011 when it was demonstrated that bacterially derived H2S conferred resistance to a broad range of antibiotics in several bacterial pathogens (21). Bacterially derived NO· has also been found to provide protection against antibiotics by the same group (95) perhaps because of H2S/NO· cross-talk, which has only been investigated in recent years (Fig. 2B) (53–56). It was not until 2014 that RSS were shown to function as antioxidants in mammalian cells (47), which may partly explain H2S-enhanced bacterial resistance to antibiotics (21). RSS have been detected in a number of bacterial pathogens (17, 20, 53, 96) and are the subject of ongoing work to better understand the role of RSS in the bacterial response to the host immune system.

Recent work has investigated the mechanism by which H2S/RSS might confer antibiotic resistance and protection against ROS and sulfide toxicity (Fig. 3B). In E. coli, increased H2S results in a respiratory flux switch from that of the primary cytochrome bo oxidase to the alternate cytochrome bd oxidase, a copper-free enzyme that is far less susceptible to inhibition by H2S (26, 97). Although cytochrome bd oxidase does not pump protons, it still enables aerobic metabolism and robust growth. This respiratory switch in response to H2S may well occur in several other bacterial pathogens, including Acinetobacter baumannii (20) and Mycobacterium tuberculosis; in the latter case, low levels of H2S enhance the respiration, energy production, and survival of M. tuberculosis in infected mice (25). Further studies are required to establish the generality of this adaptive response across a wider range of organisms. Others have postulated that H2S-mediated cytoprotection occurs via sequestration of the prooxidant free Fe(II) (23, 98); however, this remains incompletely understood.

A number of recent reports have described a potential role of H2S at the host-pathogen interface beyond protection against
antibiotic and oxidative stress (22, 24, 25, 99). In infected macrophages and in mice, *Helicobacter pylori* was found to induce the expression of the host transsulfuration pathway enzyme CSE (Fig. 2A), resulting in increased cystathionine production that enhances *H. pylori* survival in these models (99). Any connection of cystathionine to host or bacterially derived H2S was not elucidated in this work. *S. aureus* and *E. coli* strains lacking enzymes involved in H2S biogenesis are more readily cleared in infected macrophages and are less resistant to leukocyte-mediated killing in a burn-infection model (22). In addition, *E. coli* strains lacking the H2S-generating enzyme 3MST, when challenged with antibiotics, give rise to a suppressor mutation that recovers H2S biogenesis via up-regulation of the single-domain sulfurtransferase PspE (24). Together, these studies suggest that H2S biogenesis reduces the efficacy of antibiotics and that up-regulation of H2S may be a clinically important adaptive response during infections. These studies support the proposal that H2S functions as an infection-relevant antioxidant or pro-antioxidant, in the latter case, as a precursor to oxidized RSS (47).

Whereas H2S/RSS-dependent regulation of biofilm dynamics remains largely unknown, recent studies suggest a potential connection. Biofilms are often polymicrobial communities that assemble on both abiotic (e.g. catheters and implants) and biotic (e.g. cells and cell debris) surfaces while conferring increased resistance to antibiotics (100–102). Cells near the base of biofilm structures are often nutrient-poor, and some reside at anoxic/anoxic boundary. Low-O2 (hypoxic) conditions can also result from increased O2 consumption by host immune cells to produce superoxide anion (O2·−) (103, 104). In these low-O2 regions, bacteria respire via reduction of nitrate (NO3−), producing NO as a pathway to nitrous oxide (N2O) and dinitrogen (N2) (105). These nitrogen-containing species have been reported to lead to biofilm dissemination of *S. aureus* (106) and *P. aeruginosa* (107, 108), consistent with an impact on biofilm dynamics.

Redox homeostasis is also implicated in proper biofilm formation in *P. aeruginosa* (109), whereas cysteine and GSH-deficient uropathogenic *E. coli* exhibit deregulated biofilm formation that is restored upon the addition of exogenous thiols (110). Although the connection between biofilm regulation and H2S/RSS homeostasis is largely speculative at this point, H2S has been detected in cystic fibrosis sputum, a complex biofilm (111), and H2S has been found to promote formation of biofilms by intestinal microbiota while reducing the proliferation of planktonic bacterial cells (112). We recently characterized the biofilm growth–associated repressor, BigR, in *A. baumannii* as an RSS sensor (20, 113), as previously characterized in plant pathogens (114–116). That work also identified two transcriptional regulators in *A. baumannii* known or projected to be involved in biofilm regulation that were characterized by significantly increased protein persulfidation mediated by exogenous sulfide (20). Whereas these studies suggest that H2S/RSS homeostasis impacts biofilm dynamics, more studies are needed to better understand this connection at a mechanistic level.

### Biogenesis and clearance of organic RSS in bacteria

The endogenous production of H2S in bacteria suggests that more oxidized forms of sulfur may be present in cells and formed via enzymatic and possibly nonenzymatic mechanisms (Fig. 4, A–C). The extent to which these pathways, particularly nonenzymatic routes, contribute to RSS pools in bacteria is not known and may well differ among organisms. Emerging evidence in mammalian systems demonstrates the role of ferric (FeIII)-heme in the oxidation of H2S, which reduces the FeIII to FeII and forms the one-electron oxidized radical HS• upon dissociation (Fig. 4A) (117–121). Recent work reveals that this mechanism is used to reactivate enzymes requiring a catalytically active ferrous heme from the inactive ferric state, formed during turnover (121). Additionally, HS• can be formed by the reaction of H2S with superoxide radical anion O2•− or with cysteine-coordinated Zn(II) sites in proteins (122). FeII-heme has also been shown to result in formation of thiosulfate and hydro polysulfide species in mammalian systems (Fig. 4A) (117, 118). Formation of organic thyl radical, RS•, and related organic polysulfide species may occur via similar chemistry, supported by recent work using an LMW thiol for the reactivation of a catalytically active ferrous heme (Fig. 4B) (121). The extent to which heme-based biogenesis of RSS occurs in bacteria is not yet known.

An important enzymatic route to the biogenesis of RSS in bacteria is the sulfide:quinone oxidoreductase (SQR) (76, 123–125). SQR catalyzes the two-electron oxidation of H2S to sulfane sulfur fixed as organic and inorganic per- and polysulfides (Fig. 4B), concomitant with reduction of the quinone pool (126). This enzyme may well provide a source of electrons for the alternative cytochrome bd oxidase in organisms that encode this alternate oxidase, analogous to that observed for SQRS with complex III/IV when the concentration of H2S is low (Fig. 3B) (76). In organisms (e.g. *Enterococcus faecalis*) that do not appear to encode an SQRS but where RSS have been detected and quantified (17), the mechanism of RSS biogenesis is not known, and may well suggest a role for nonenzymatic or as yet uncharacterized enzymatic processes in these organisms. In addition to SQRS, recent work in *A. baumannii* reveals that 3MST may also contribute to pools of LMW persulfides, although there are clearly other contributors (20).

### Sulfurtransferases

Major structural classes of sulfurtransferases (STRs) adopt either a rhodanese or TusA (tRNA 2-thiouridine–synthesizing protein A)-like fold (126) and harbor an active-site cysteine that is known or projected to function in interdomain or intermolecular persulfide transfer, termed transsulfuration (Fig. 4C) (127). Although rhodanese domains were originally believed to function in cyanide (CN−) detoxification by forming thiocyanide (SCN−) (128), it is well-established that Fe–S cluster bio- genesis, molybdenum cofactor biosynthesis, 2-thiouridine synthesis, and thiamine pyrophosphate biosynthesis are known or proposed to use STRs as persulfide transfer catalysts (129–133). Such “targeted” transsulfuration reactions require specific, albeit likely transient, interactions between donor and
acceptor and an exposed active site, as described for TSTD1 and thioredoxin in colon epithelial cells (134).

“Orphan” STRs, which we define as not yet connected to any biosynthetic pathway, particularly those regulated by RSS sensors in bacteria, may well play roles in sulfide detoxification or assimilation (17, 135), but their biological functions remain enigmatic. This remains a significant challenge in the field. RSS sensor–regulated STRs are often kinetically characterized in vitro as sulfurtransferases from a thiosulfate donor to a CN− acceptor; however, their physiological donors and acceptors, whether they be small molecules or proteins, have generally not been identified, and any role in targeted transsulfuration has not been established (Fig. 4C). Recently, a single cysteine peroxiredoxin (a major H2O2-detoxifying enzyme) characterized by a long-lived sulfenylated intermediate was shown to rapidly react with H2S to form a protein persulfide, which participated in persulfide transfer to a thiol acceptor (136). This suggests that peroxiredoxins may function in transsulfuration, but this requires further investigation (Fig. 4C). Similarly, the extent to which small-molecule RSS species, particularly those containing sulfane sulfur, participate in transsulfuration reactions with each other, LMW thiols, or even protein thiols is largely unknown (Fig. 4C).

**RSS clearance enzymes**

In addition to the biogenesis of RSS, a number of bacterial enzymes have been characterized that function in their clearance (Fig. 4D). A well-known player in the clearance of organic persulfides is persulfide dioxygenase (PDO), which harbors a mononuclear, nonheme FeII center (76, 137–140). In bacteria, PDOs have been characterized as single or multidomain enzymes, and the presence of additional domains appears to impact the distribution of products. All PDOs, regardless of their domain organization, use molecular oxygen to oxidize the terminal sulfur of an RSSH substrate to sulfite, which, for a single-domain PDO, is the final product. Some PDOs have an appended STR domain, and these have been designated PDO-rhodanese fusion proteins (PRFs) (Fig. 4D). In the case of the PRF characterized in *Burkholderia phytofirmans*, the C-terminal rhodanese domain generates the GSH persulfide substrate that the PDO domain then oxidizes to sulfite (138). In contrast, the multidomain PRF CstB from *S. aureus* oxidizes two equivalents of persulfide substrate to thiosulfate as the final product; the C-terminal rhodanese domain also possesses transsulfuration and thiosulfate transferase activity (139). In contrast to the oxidative chemistry of PDOs, *E. faecalis* encodes a CoA disulfide reductase-rhodanese homology domain fusion protein.
(CoADR-RHD) that specifically reduces CoA persulfide to form the reduced thiol and H2S and is thus a CoA persulfide reductase (CoAPR) (Fig. 4D) (17, 141–143).

**Regulatory sensing of RSS in bacteria**

The discovery of endogenous H2S production and pathways for the biogenesis and clearance of RSS in bacteria requires a mechanism to establish cellular H2S/RSS homeostasis. This is mediated by RSS sensors (Fig. 1A, bottom). We and others have discovered and characterized structurally diverse transcriptional regulators that react with RSS to drive transcriptional derepression or activation of genes encoding common sulfide detoxification or oxidation enzymes described above. These RSS sensors are widespread and have been identified in both Gram-positive and Gram-negative organisms. They include CstR from *S. aureus* (15, 16) and *E. faecalis* (17), SqrR from *Rhodobacter capsulatus* (18, 113), the SqrR homolog BigR from *Xylella fastidiosa* (114–116) and *A. baumannii* (20), and FisR from *Cupriavidus pinatubonensis* (19) and *A. baumannii* (20).

**CstR**

The CsoR-like sulfurtransferase repressor, CstR, is a member of the CsoR (copper-sensitive operon repressor) family of transcriptional repressors (144) and was first discovered in *S. aureus* (15, 16). *S. aureus* CstR regulates the cst operon encoding a multidomain STR (CstA), a PRF (CstB), and a type II SQR, rather analogous to the well-studied mitochondrial sulfide multidomain STR (CstA), a PRF (CstB), and a type II SQR, human pathogen *E. faecalis* where (126). Recently, we characterized CstR from a second human pathogen *E. faecalis* (17, 113). It is interesting to note that PigS and its regulon are part of the larger PigP’ regulon involved in the biosynthesis of the antibiotic prodigiosin (Pig), thus implying a regulatory connection between antibiotic biosynthesis and H2S/RSS homeostasis. The ArsR-family RSS sensors that have been functionally characterized behave analogously to CstR, functioning as repressors in the reduced state and dissociating from the DNA upon reaction with sulfane sulfur-containing RSS, to readily form nearly exclusively tetrasulfide (SqrR) and pentasulfide (BigR) bridges, respectively (Fig. 5B, right) (113). It is interesting to note that PigS and its regulon are part of the larger PigP’ regulon involved in the biosynthesis of the antibiotic prodigiosin (Pig), thus implying a regulatory connection between antibiotic biosynthesis and H2S/RSS homeostasis.

Recent work from our laboratory utilized SqrR as a model diethiol transcriptional regulator to investigate the structural and reactivity features that govern its oxidant selectivity and specificity (113). Indeed, SqrR is specific for sulfane sulfur and only forms a disulfide when treated with potent diazirene electrophilic TMAD (diamide), but not with more common cellular oxidants including GSH disulfide or H2O2. Whereas this low reactivity toward cellular oxidants can be partially explained by the relatively high apparent pKa of the diethiol pair, the high selectivity toward RSS is enforced by structural features of SqrR in various oxidation states. These structures reveal a high energetic barrier to form the disulfide because of large rearrangements that must occur in order to form the disulfide; in addition, the disulfide is not on pathway to form the major tetrasulfide product. In contrast, formation of the tetrasulfide does not require large structural rearrangements; on the contrary, this linkage results in the collapse of the diethiol pocket that completely shields the tetrasulfide linkage from solvent. This study demonstrates that SqrR-like diethiol-based repressors achieve high RSS specificity from the conformational landscape of the protein ensemble, which favors installation of a PTM that minimizes local structural frustration (113). It will be interesting to determine whether these principles apply to other structural classes of RSS sensors or if there are additional determinants that dictate their specificity.

**SqrR and homologs**

The sulfide:quinone reductase repressor, SqrR, was originally characterized in the photosynthetic bacterium *R. capsulatus* and is responsible for the regulation of 45% of all sulfide-responsive genes in this organism, including an SQR (18). SqrR is a member of the arsenic repressor (ArsR) superfamily (146) in striking structural contrast to CstR. SqrR adopts the ArsR family α1-α2-α3-α4-β1-β2-α5 “winged-helical” dimeric fold, where one Cys in the α2 helix and one Cys in the α5 helix from the same subunit create a pair of diethiol RSS-sensing sites on the dimer (Fig. 5B, middle) (113). Other RSS-responsive ArsR family repressors include the biofilm growth-associated repressor BigR, characterized in *X. fastidiosa* (114–116) and *A. baumannii* (20), and PigS characterized in *Serratia* spp. (147). *A. baumannii* BigR regulates a secondary RSS detoxification system that includes a second PDO and two transmembrane proteins proposed to be involved in the transport of sulfur-containing molecules (20, 147). Although PigS has not been functionally characterized as an RSS sensor, it regulates several enzymes known to function in H2S/RSS clearance, including a single-domain PDO and a CoAPR encoded by *coaP* (Fig. 5B, left) (147). In contrast to CstR, these (poly)sulfur bridges are formed within a subunit, and although other linkages are made, they are far less abundant compared with the mixture of products found in CstR (146).

**FisR**

A third structural class of RSS-sensing transcriptional regulators first characterized in *C. pinatubonensis* (19), and more
recently in *A. baumannii* (20), is FisR (Fis family transcriptional regulator). In both bacteria, FisR transcriptionally activates the expression of a PDO and SQR and a putative sulfite/sulfonate effluxer, TauE (148), in only *A. baumannii* (Fig. 5C, left). In contrast to CstR and SqrR-like RSS sensors, FisR is a canonical σ54-dependent transcriptional activator that harbors an N-terminal regulatory domain, a central AAA+ ATPase domain, and a C-terminal DNA-binding domain (Fig. 5C, middle) (149). In *C. pinatubonensis* FisR, reaction with inorganic RSS appears to result in the formation of di- and tetrasulfide cross-links between two cysteine residues in the regulatory domain, which in turn stimulates the ATPase activity of the central AAA+ domain, which likely activates hexameric assembly and promoter melting by σ54-RNA polymerase (Fig. 5C, right) (19). In *A. baumannii* FisR, these cysteines are not present, and as a result, H2S/RSS is likely sensed using an alternate mechanism (20), which includes heme-based (117–121) and mononuclear, nonheme Fe-based RSS-sensing regulatory models (150–152).

**Chemical tools for generation, detection, and quantification of RSS**

To understand the role of H2S and RSS in signaling at the host-pathogen interface, tools must be available that allow for the generation, detection, and quantification of these species *in vivo*. The type and number of molecular probes used for the generation of H2S and RSS have substantially increased over the past several years, and they are now being used in bacteria to provide critical insights into H2S signaling in these organisms (22, 25, 26). Fluorescence-based probes provide rapid and sensitive detection of H2S or sulfane sulfur with several options now commercially available. In addition, recent efforts to quantify H2S and RSS in complex cellular mixtures have provided
new insights into this process. As many of these molecular tools have been extensively reviewed elsewhere (153–159), here we provide only a summary of the available approaches, while pointing out specific challenges to their use.

**H$_2$S and RSS donors**

H$_2$S donors largely fall into three main classes. These are hydrolysis-based, thiol-activated, or caged COS donors (Fig. 6A). Hydrolysis-based donors function over a wide range of pH, with GYY4137 as a widely used and commercially available donor employed by several groups to study the mechanism of H$_2$S cytoprotection in bacteria (22, 25, 160). Concerns over the relatively slow release rates have led to second-generation GYY4137 derivatives, including JK-2, that more efficiently release H$_2$S at physiological pH (161). The use of hydrolysis-based donors requires careful consideration of the pH dependence and kinetics of H$_2$S release, and many donors are not commercially available. Thiol-dependent H$_2$S donors are attractive tools because of their use of typically cell-abundant cellular reducing thiols, including GSH and cysteine (Fig. 6A). However, some of these probes are quite slow, require high concentrations of thiol, or are activated by a specific thiol (154). As the type and concentrations of thiols in bacteria have only recently been investigated and only in a small sampling of bacteria (17, 20, 53, 96), this potentially limits the broad...
applicability of such probes in bacteria. In addition, the use of cellular thiols to activate compounds may disrupt the cellular thiol/disulfide redox balance, leading to physiological impacts not specifically due to H₂S release. Furthermore, some thiol-activated donors proceed through a highly reactive persulfide intermediate, making it difficult to attribute a physiological impact to the persulfide intermediate or to H₂S itself. Last, COS-based donors have been designed with various release mechanisms and rely on an endogenous carbonic anhydrase to produce H₂S in high catalytic yields from probe-dependent release of COS (Fig. 6A) (162). The use of these compounds, like other enzyme-activated H₂S donors (26), obviously relies on broad cell permeability and constitutive expression of a carbonic anhydrase (163), neither of which has been systematically investigated in bacteria.

Whereas H₂S donors have been extensively developed over the last 10 years, RSS donors typically found in the form of persulfide donors are comparatively less so (153, 158). For in vitro chemical reactivity studies, we and others have relied on in situ thiol persulfide generation from the reaction of excess H₂S with a disulfide, resulting in a mixture containing the thiol persulfide that is not easily separated from the remaining reactants of H₂S, disulfide, and thiol (45). Although in situ thiol persulfide generation may also result in formation of the more stable trisulfide species (164), we observed no reaction of SqrR (Fig. 5B) with cysteine trisulfide (113). Furthermore, whereas this work also revealed somewhat faster kinetics of SqrR with the in situ generated persulfide versus a persulfide donor analog of S-nitrosoacetyl-penicillamine (SNAP), the SNAP analog persulfide donor has the added benefit that it is soluble in aqueous solution and spontaneously generates the persulfide species after S to N carbonyl transfer (Fig. 6B) (165). In addition, Wang and co-workers (166, 167) recently developed a series of esterase-sensitive persulfide donors, including GSH persulfide, whose physiological effects have thus far only been studied in mammalian cells. These cell-permeable persulfide donors generate persulfide species under physiological conditions with minimal disruption to cellular redox status (i.e. they are not thiol-activated, an important feature).

ROS-activated H₂S and persulfide donors have also garnered recent attention (Fig. 6B) (154, 158) because these types of probes are particularly useful for studying ROS/H₂S cross-talk, which is likely relevant at sites of infection. These donors are specific for H₂O₂ activation and react readily to generate H₂S or a persulfide species. Recently, two groups have developed H₂O₂-specific persulfide-generating probes that exhibit greater cytoprotective effects in cells challenged with oxidative stress than H₂S only-generating probes, such as GYY4137 (168, 169). This finding is consistent with the idea that RSS rather than H₂S per se are important effector molecules of H₂S signaling and cytoprotection and merits further study in bacterial cells.

Detection of H₂S/RSS

Although historically many groups have utilized methylene blue (170) or lead acetate (21) paper strips to measure H₂S in growing bacterial cells, the latter is not quantitative, and both methods are unable to detect RSS. More recently, several groups have developed sensitive fluorescent probes for detection of H₂S and RSS, and this has been comprehensively reviewed elsewhere (155, 157, 159). Of these tools, the WSP and SSP series of fluorescent molecules that detect H₂S and sulfane sulfur, respectively, are most commonly used in both mammalian and bacterial cells (Fig. 6C) (24, 77). When used by themselves, these probes cannot be used to perform absolute quantitation of H₂S/RSS; however, one group recently coupled SSP4 cell labeling with MS to quantitate sulfane sulfur, thus establishing the possibility of making these optical methods quantitative (171). This approach, however, would only allow for quantitation of total sulfane sulfur without molecule-specific identification. Regardless of their inability to provide absolute quantitation, these probes provide a rapid and simple readout for production of H₂S/RSS appropriate for comparing bacterial strains and growth conditions. In addition, the SSP series provide a rapid means for quantifying sulfane sulfur from enzymatic activity in vitro, a strategy recently used to measure RSS biogenesis and clearance activities of catalase and superoxide dismutase, respectively (172, 173).

Quantification of H₂S/RSS in cell lysates

Isotope-dilution LC–MS–based methods to detect and quantify H₂S and RSS have considerable advantages over optical imaging modalities, including the ability to perform molecular identification and absolute quantitation; however, these methods are not easily applied to living bacterial cells and therefore rely on post-cell growth sample work-up and chemical derivatization of RSS. We and others have traditionally employed electrophilic trapping by monobromobimane (MBB) with quantitation originally via fluorescence detection and more recently via the addition of isotopically labeled internal standards necessary for LC–MS quantitation (Fig. 6D) (17, 20, 47, 53, 96). It was recently shown that quantitation of H₂S by MBB is sensitive to small variations in pH, alkylation time, and temperature, thus emphasizing the importance of controlling reaction conditions when comparing samples (174). Bogdani and co-workers (175) also showed that the electrophilicity of the alkylation agent also impacts quantitation of RSS to varying degrees. N-Ethylmaleimide, the most electrophilic agent tested, resulted in cleavage of per- and polysulfide chains, whereas β-(4-hydroxyphenyl)ethyl iodoacetamide (HPE-IAM) (Fig. 6D) showed little to no cleavage under the same experimental conditions. MBB has an intermediate impact resulting in slightly lower yields of per- and polysulfide species relative to HPE-IAM but exhibited significantly better quantitation than N-ethylmaleimide. As a result, many groups now use HPE-IAM for quantification of RSS (175–177).

A more general consideration of electrophile-based trapping approaches for RSS quantitation is that these methods provide only a snapshot of cellular RSS speciation, particularly in light of our incomplete understanding of sulfane sulfur “scrambling” that will conspire against any method of quantitative analysis. Better understanding of this will provide complementary information to quantitative techniques and valuable insights on the lifetime and speciation of RSS in cells.
H2S signaling via protein S-sulfuration

PTMs of cysteine thioles are known to impact signaling and play regulatory roles in proteins (43, 178–180). Persulfidation of cysteine residues can occur via reduction of nitrosated or sulfenylated protein thiols, while also protecting these latter thiols from overoxidation (59, 60). Persulfidation may also function in H2S signaling as a regulatory modification by introducing altered chemistry or “blocking” of active-site thiols, thiols in a regulatory or allosteric domain, or those found in transcriptional regulators, as shown for the virulence regulator MgrA in S. aureus (96). However, the relevance and regulatory nature of this PTM remain largely unexplored in bacteria, leaving large gaps in our understanding of protein persulfidation as a regulatory modification in H2S/RSS signaling. For example, persulfidation of the active-site thiol in the cell-abundant glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been reported by several groups; however, there are conflicting reports to whether this modification activates (58) or inhibits (96, 181) the activity of this enzyme. Furthermore, the same active site thiol in GAPDH is the target of many other thiol modifications, including S-nitrosation (182), S-sulfonylation (183), and S-thiolation (184). To better understand the role of protein persulfidation in H2S/RSS signaling, it becomes important to understand how this modification is installed and removed from proteins and to identify regulatory targets of this PTM.

It is instructive to discuss thiol-based PTMs, including persulfidation, S-sulfonylation, or S-nitrosation, in the “writer, reader, eraser” paradigm, which represents the formation, signal transduction, and removal of PTMs (Fig. 7A). The writer refers to the process(es) that install the PTM to the protein target, such as S-nitrosoglutathione for protein S-nitrosation (185). The reader includes PTM-dependent interacting partners, whether it be a protein or small molecule, thus transducing the signal in response to the PTM (e.g. S-nitrosation of procaspase-3 promotes its interaction with acid sphingomyelinase and prevents apoptosis) (186). Last, the eraser refers to the proteins or small molecules responsible for removing the PTM after signal transduction has occurred. In S-nitrosation, several denitrosylases have been characterized, including the thioredoxin system (187); other enzymes reduce S-nitrosoglutathione or S-nitroso-CoA directly to sulfenamides, thus lowering the steady-state pools of these small-molecule NO donors (188–190). For bacterial protein persulfidation, the writers and erasers are perhaps slightly better understood, whereas we have little to no knowledge of the readers. We note also that writers and erasers are typically restricted to enzymes (e.g. kinase-phosphatase and/or acetylase-deacetylase pairs), but here we explicitly consider nonenzymatic pathways as well.

The writers of protein persulfidation may include nonenzyme-catalyzed transsulfuration with RSS or direct reaction of H2S with more oxidized cysteines or, enzymatically, via transsulfuration by STRs or other enzymes (Fig. 7A, left). Although...
the reactivity of STRs strongly suggests a role in protein trans-sulfuration, little is known regarding the rates of these reactions, specificity, and physiological impact of many STRs. These small-molecule and protein writers can also potentially function in reverse, as the erasers, by removing the sulfane sulfur in a process that is not fully understood. In S. aureus, the cst operon-encoded STR CstA was shown to facilitate the removal of the persulfide moiety on the cysteine desulfurase SufS in support of the idea that STRs can also catalyze the removal of protein persulfides (135). In addition, the thioredoxin (Trx)/thioredoxin reductase cascade may be largely responsible for the removal of protein persulfides for which there is now evidence (Fig. 7A, right) (191, 192). The characterization of two thioredoxins in S. aureus that have significant activities on protein persulfides relative to disulfides versus the canonical TrxA certainly suggests a role for Trx in this process, as well as the possibility that bacteria encode specific thioredoxin-like proteins for this purpose (96). Furthermore, a thioredoxin-based proteomic profiling strategy was used to identify potential cellular targets for these persulfide-reducing thioredoxins in S. aureus (193). Analogous strategies might be applied to STRs in an effort to identify protein targets that could function as donors or acceptors in trans-sulfuration reactions.

Detection of global proteome persulfidation

To evaluate proteome persulfidation, a number of methods have been developed over the past several years to identify candidate targets of this PTM. The first method proposed for detection of protein persulfides relies on S-methylmethane-thiosulfonate to selectively methylate protein thiols over persulfides, followed by persulfide capture by biotin–HPDP (N-[6-(biontinamido)-hexyl]-3’-(2-pyridyldithio)propionamide) and enrichment by streptavidin beads (Fig. 7B) (58). However, the selectivity of S-methylmethanethiosulfonate toward thiols over persulfides has been challenged and shown not to be sufficient for general application (194). Several groups then developed approaches that rely on derivatizing both protein thiols and persulfides with a biotinylated alkylating agent (96, 192, 195, 196). After enrichment with streptavidin, the mixed disulfide that characterizes only persulfide-containing peptides or proteins is selectively reduced, alkylated, and identified by LC–MS/MS. Enrichment of whole proteins versus peptides is far more prone to artifacts (192, 197) and should be avoided. Enriching peptides rather than proteins will also tend to minimize the presence of false positives that derive from endogenous disulfide bonds in the lysate, although this may be expected to be low for cytoplasmic lysates, given the high reducing capacity of the cytoplasm (96, 195, 196).

Several “tag-switch” approaches have also been developed for identifying protein persulfides (191, 197–200). These methods do not rely on the reduction of mixed disulfides from alkylated persulfides but instead exploit the unique reactivity of the mixed disulfide toward a specific nucleophile. The first tag-switch method reported utilized an electrophilic blocking reagent, methylsulfonylbenzo-thiazole, to react with both protein thiols and persulfides (199). These residues when blocked have very different reactivities toward nucleophiles (i.e. thiol adducts are unreactive, whereas persulfide adducts as mixed disulfides retain significant reactivity). The persulfide adduct is then reacted with a cyanoacetate-based reagent (e.g. CN-biotin) to “switch” the tag on these residues (Fig. 7B) (47, 198, 199). Several cyanoacetate-based reagents have been developed, including fluorescent adducts, and each relies on its high specificity toward the mixed disulfide of protein persulfides over protein disulfides (191). Most recently, Zivanovic et al. (200) reported a new tag-switch workflow where 4-chloro-7-nitrobenzofuran (201, 202) is used to label thiols, persulfides, sulfenic acids, and free amines, followed by selective switching of the persulfide-derived mixed disulfide by dimedone, a reagent commonly used to detect sulfenylated cysteines (Fig. 7B) (203, 204). The advantage of this workflow is that the availability of many derivatized dimesones makes it possible to modify the workflow to fit the application, be it fluorescence imaging, streptavidin enrichment, or click chemistry–based enrichment approaches and downstream MS (205).

Although these workflows are powerful and are expected to be broadly applied, none achieve direct detection of protein persulfides. A recent study by Fu et al. (206) approached this challenge by leveraging the inherent pKa difference between persulfides and thiols in their development of low-pH quantitative thiol reactivity profiling (QTRP) (Fig. 7C). QTRP incorporates an initial alkylation step in low-pH conditions, where most thiols are protonated and unreactive, thus allowing selective alkylation of persulfides (206). Use of isotopically labeled reagents permits estimation of the fraction persulfide versus thiol for all Cys-containing peptides. An important shortcoming of this method is that pKa estimations for thiols and persulfides (44) are not generally known on a proteome scale and may well vary widely. Thus far, QTRP has only been applied in mammalian cells; it would be of interest to determine how many proteins can be detected with high fractional persulfidation levels in less complex bacterial proteomes, which may aid in the identification of regulatory persulfides, characterized by high relative quantitation of persulfide versus thiol.

Finally, regardless of the workflow used to detect bacterial proteome persulfidation, these studies seem to suggest that the proteome may function as a “sink” for sulfane sulfur, given that upward of ~15% of the proteins in a bacterial proteome are persulfidated (at some fraction) in a way that is not greatly impacted by the addition of exogenous sulfide to growing cells (20, 96). Taken at face value, this suggests that most of these proteome persulfidation events are unlikely to be regulatory, consistent with a lack of strongly compelling persulfidation consensus sequence motif, implying that these cysteines may be persulfidated at random (20, 206, 207). The challenge we now face is not in the detection of sites of persulfidation in a proteome but to distinguish those regulatory sites from those that are persulfidated collaterally on solvent-accessible, non-conserved, and highly reactive cysteines. This is a key aspect of the “writer, reader, eraser” paradigm of H2S/RSS signaling that is thus far largely unexplored. We would expect regulatory persulfidation events to be found on conserved cysteines in enzymes that catalyze committed steps or define metabolic hubs or branch points in a metabolic pathway(s) (195), in transcriptional regulators (111), as allosteric modulators, or in...
enzyme active sites (e.g. GAPDH) (58, 96, 181). Indeed, a subset of these regulatory cysteines may be subject to other PTMs, which would increase the biological complexity of the regulatory response.

Future outlook

Whereas studies of the chemical biology of H2S and RSS have greatly expanded in recent years, our molecular understanding of the underlying mechanisms that drive regulatory signaling and cytoprotection of bacterial cells against antibiotic and host assault remains poorly defined. The high reactivity and potentially facile interconversion of RSS represents a particularly challenging analytical problem. Recent advances in methodologies used to quantify RSS will allow us to further elucidate their roles as potent oxidants (47), modulators of antibiotic efficacy (21), and the “currency” of H2S signaling, the latter analogous to LMW organic nitrilotriols in S-nitrosation–based signaling pathways (185, 188–190).

Recent studies have begun to establish the physiological importance of H2S/RSS biogenesis in bacteria at the host-pathogen interface and the central role played by distinct classes of transcriptional regulators that act as molecular sensors that survey cellular H2S/RSS required, in some cases, to retain full pathogen virulence (17, 96). We do not yet fully understand the mechanisms that make these sensors specific for RSS relative to other oxidants (16), although these data are beginning to appear (113). Finally, although global S-sulfuration profiling has successfully mapped hundreds of sites of protein persulfidation in many different contexts and systems, the next major challenge is to identify and characterize regulatory sites of protein persulfidation in bacteria, like that recently found in human aquaporin-8, a gated H2O2 transmembrane channel (208). These thiolos represent physiologically relevant targets of reversible persulfidation that impact enzyme activity, a metabolic pathway(s) or gene expression program (195), beyond simple detoxification and homeostasis of H2S/RSS. We suspect that as yet uncharacterized “orphan” STRs that are regulated by RSS sensors (17, 135) and perhaps cell-abundant peroxiredoxins (136) and thioredoxins may be excellent candidates as writers and/or erasers in H2S/RSS signaling (Fig. 7A), where specific protein-protein interactions may well “direct traffic” in the dynamic flow of regulatory sulfur in cells. Addressing these challenges will better strengthen the foundation of H2S/RSS signaling as a bona fide signaling process in bacterial cells in the infected host, thus representing new physiology prime for the development of novel antimicrobial interventions.

Conflict of interest—The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations—The abbreviations used are: RSS, reactive sulfur species; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; 3-MP, 3-mercaptoppyruvate; CAT, cysteine aminotransferase; 3MST, 3-MP sulfuratransferase; ROS, reactive oxygen species; RNS, reactive nitrogen species; LMW, low-molecular weight; PTM, post-translational modification; SQR, sulfide:quinone oxidoreductase; STR, sulfuratransferase; PDO, persulfide dioxygenase; PRF, PDO- rhodanese fusion protein; SNAP, S-nitrosoacyl-penicillamine; MBB, monobromobimane; HPE-IAM, β-(4-hydroxyphenyl)ethyl iodoacetamide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; QTRP, quantitative thiol reactivity profiling.

Note added in proof—In a recent paper, Ng et al. (2020) Front. Microbiol. 11, 1875 showed that two multidrug-resistant strains, including one clinical isolate, of Acinetobacter baumannii, do not biosynthesize appreciable hydrogen sulfide and that exogenous NaHS appears to potentiate the killing efficacy of a number of diverse antibiotics, in contrast to nearly all other findings discussed here. Interestingly, neither A. baumannii strain used by Ng et al. encodes 3-mercaptoppyruvate sulfuratransferase (3MST) or RSS sensors FisR or BigR (see Fig. 5) or FisR- and BigR-regulated genes as described in A. baumannii ATCC 17978 (20).

References

1. Lusti-Narasimhan, M., Pessoa-Silva, C. L., and Temmerman, M. (2013) Moving forward in tackling antimicrobial resistance: WHO actions. Sex. Transm. Infect. 89, iv57–iv59 CrossRef Medline
2. Chang, H. H., Cohen, T., Grad, Y. H., Hanage, W. P., O’Brien, T. F., and Lipsitch, M. (2015) Origin and proliferation of multiple-drug resistance in bacterial pathogens. Microbiol. Mol. Biol. Rev. 79, 101–116 CrossRef Medline
3. Ma, Z., Jacobsen, F. E., and Giedroc, D. P. (2009) Coordination chemistry of bacterial metal transport and sensing. Chem. Rev. 109, 4644–4681 CrossRef Medline
4. Waldron, K. J., Rutherford, J. C., Ford, D., and Robinson, N. J. (2009) Metallloproteins and metal sensing. Nature 460, 823–830 CrossRef Medline
5. Wang, Y., Weisenhorn, E., MacDiarmid, C. W., Andreini, C., Bucci, M., Taggart, J., Banci, L., Russell, J., Coon, J. J., and Eide, D. J. (2018) The cellular economy of the Saccharomyces cerevisiae zinc proteome. Metallomics 10, 1755–1776 CrossRef Medline
6. Zygiel, E. M., and Nolan, E. M. (2018) Transition metal sequestration by the host-defense protein calprotectin. Annu. Rev. Biochem. 87, 621–643 CrossRef Medline
7. Damo, S. M., Kehl-Fie, T. E., Sugitani, N., Holt, M. E., Rathi, S., Murphy, W. J., Zhang, Y., Betz, C., Hench, L., Fritz, G., Skar, E. F., and Chazin, W. J. (2013) Molecular basis for manganese sequestration by calprotectin and roles in the innate immune response to invading bacterial pathogens. Proc. Natl. Acad. Sci. U. S. A. 110, 3841–3846 CrossRef Medline
8. Cunden, L. S., and Nolan, E. M. (2018) Bioinorganic explorations of Zn(II) sequestration by human S100 host-defense proteins. Biochemistry 57, 1673–1680 CrossRef Medline
9. Sheldon, J. R., and Skar, E. P. (2019) Metals as phagocyte antimicrobial effectors. Curr. Opin. Immunol. 60, 1–9 CrossRef Medline
10. Xu, Z., Wang, P., Wang, H., Yu, Z. H., Au-Yeung, H. Y., Hirayama, T., Sun, H., and Yan, A. (2019) Zinc excess increases cellular demand for iron and decreases tolerance to copper in Escherichia coli. J. Biol. Chem. 294, 16978–16991 CrossRef Medline
11. Capdevila, D. A., Edmonds, K. A., and Giedroc, D. P. (2017) Metallochaperones and metalloregulation in bacteria. Essays Biochem. 61, 177–200 CrossRef Medline
12. Capdevila, D. A., Wang, J., and Giedroc, D. P. (2016) Bacterial strategies to maintain zinc metalloastasis at the host-pathogen interface. *J. Biol. Chem.* **291**, 20858–20868 CrossRef Medline

13. Jordan, M. R., Wang, J., Capdevila, D. A., and Giedroc, D. P. (2020) Multimetal nutrient restriction and crosstalk in metalloastasis systems in microbial pathogens. *Curr. Opin. Microbiol.* **55**, 17–25 CrossRef Medline

14. Osman, D., Martini, M. A., Foster, A. W., Chen, J., Scott, A. J. P., Morton, R. J., Steed, J. W., Lurie-Luke, E., Huggins, T. G., Lawrence, A. D., Warren, M., I. Chivers, P. T., and Robinson, N. J. (2019) Bacterial sensors define intracellular free energies for correct enzyme metalation. *Nat Chem Biol.* **15**, 241–249 CrossRef Medline

15. Grossoehme, N., Kehl-Fie, T. E., Ma, Z., Adams, K. W., Cowart, D. M., Scott, R. A., Skaar, E. P., and Giedroc, D. P. (2011) Control of copper resistance and inorganic sulfur metabolism by paralogous regulators in *Staphylococcus aureus*. *J. Biol. Chem.* **286**, 13522–13531 CrossRef Medline

16. Luebke, J. L., Shen, J., Bruce, K. E., Kehl-Fie, T. E., Peng, H., Skaar, E. P., and Giedroc, D. P. (2014) The CsoR-like sulfurtransferase repressor (CsrR) is a persulfide sensor in *Staphylococcus aureus*. *Mut. Microbiol.* **94**, 1343–1360 CrossRef Medline

17. Shen, J., Walsh, B. J. C., Flores-Mireles, A. L., Peng, H., Zhang, Y., Zhang, Y., Trinidad, J. C., Hultgren, S. J., and Giedroc, D. P. (2018) Hydrogen sulfide sensing through reactive sulfur species (RSS) and nitroxyl (HNO) in *Enterococcus faecalis*. *ACS Chem. Biol.* **13**, 1610–1620 CrossRef Medline

18. Shimizu, T., Shen, J., Fang, M., Zhang, Y., Hori, K., Trinidad, J. C., Bauer, C. E., Giedroc, D. P., and Masuda, S. (2017) Sulfide-responsive transcriptional repressor SgrR functions as a master regulator of sulfide-dependent photosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 2355–2360 CrossRef Medline

19. Li, H., Li, J., Lu, C., Xia, Y., Xin, Y., Liu, H., Xun, L., and Liu, H. (2017) FisR activates e^8-dependent transcription of sulfoxide-oxidizing genes in *Cupriavidus pinatubonensis* JIMP134. *Mut. Microbiol.* **105**, 373–384 CrossRef Medline

20. Walsh, B. J. C., Wang, J., Edmonds, K. A., Peng, H., Zhang, Y., Trinidad, J. C., Skaar, E. P., and Giedroc, D. P. (2020) The response of *Acinetobacter baumannii* to hydrogen sulfide reveals two independent persulfide sensing systems and a connection to biofilm regulation. *mBio* **11**, e01254-20 CrossRef Medline

21. Shatalin, K., Shatalina, E., Mironov, A., and Nudler, E. (2011) H2S biogenesis by human cystathionine γ-lyase leads to the novel sulfur metabolites lantionine and homolantionine and is responsive to the grade of hyperhomocysteinemia. *J. Biol. Chem.* **284**, 11601–11612 CrossRef Medline

22. Singh, S., and Banerjee, R. (2011) P.L.-dependent H2S biogenesis. *Biochim. Biophys. Acta* **1814**, 1518–1527 CrossRef Medline

23. Singh, S., Padovani, D., Wu, W., Singh, S., Vitvitsky, V., and Banerjee, R. (2009) H2S biogenesis by human cystathionine γ-lyase leads to the novel sulfur metabolites lantionine and homolantionine and is responsive to the grade of hyperhomocysteinemia. *J. Biol. Chem.* **284**, 11601–11612 CrossRef Medline
64. Yang, Y., Bazhin, A. V., Werner, J., and Karakhanova, S. (2013) Reactive oxygen species in the immune system. *Int. Rev. Immunol.* **32**, 249–270 CrossRef Medline

65. Ley, R. E., Peterson, D. A., and Gordon, J. I. (2006) Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* **124**, 837–848 CrossRef Medline

66. Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D. R., Li, J., Xu, J., Li, S., Li, D., *et al.* MetaHIT Consortium, (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–66 CrossRef Medline

67. Macfarlane, G. T., Gibson, G. R., and Cummings, J. H. (1992) Comparison of fermentation reactions in different regions of the human colon. *J. Appl. Bacteriol.* **72**, 57–64 CrossRef Medline

68. Riedijk, M. A., Stoll, B., Chacko, S., Schierbeek, H., Sunehag, A. L., van Goudoever, J. B., and Burris, D. G. (2007) Methionine transmethylation and transsulfuration in the piglet gastrointestinal tract. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 3408–3413 CrossRef Medline

69. Shoveller, A. K., Brunton, J. A., House, J. D., Pencharz, P. B., and Ball, R. O. (2003) Dietary cysteine reduces the methionine requirement by an equal proportion in both parenterally and enterally fed piglets. *J. Nutr.* **133**, 4215–4224 CrossRef Medline

70. Stegink, L. D., and Besten, L. D. (1972) Synthesis of cysteine from methionine in normal adult subjects: effect of route of alimentation. *Science* **178**, 514–516 CrossRef Medline

71. Zlotkin, S. H., Bryan, M. H., and Anderson, G. H. (1981) Cysteine supplementation to cysteine-free intravenous feeding regimes in newborn infants. *Am. J. Clin. Nutr.* **34**, 914–923 CrossRef Medline

72. Barton, L. L., Ritz, N. L., Fauque, G. D., and Lin, H. C. (2017) Sulfur cycling and the intestinal microbiome. *Dig. Dis. Sci.* **62**, 2241–2257 CrossRef Medline

73. Gibson, G. R., Macfarlane, G. T., and Cummings, J. H. (1988) Occurrence of sulphate-reducing bacteria in human faeces and the relationship of disimilatory sulphate reduction to methanogenesis in the large gut. *J. Appl. Bacteriol.* **65**, 103–111 CrossRef Medline

74. Macfarlane, G. T., Cummings, J. H., and Macfarlane, S. (2007) Sulphate-reducing bacteria and the human large intestine. In *Sulphate-reducing Bacteria: Environmental and Engineered Systems* (Barton, L. L., and Hamilton, W. A., eds) pp. 503–522, Cambridge University Press, Cambridge, UK

75. Barrett, E. L., and Clark, M. A. (1987) Tetrahydrobiopterin production and reduction of hydrogen sulfide from thiols. *Microbiol. Rev.* **51**, 192–205 CrossRef Medline

76. Libiad, M., Yadav, P. K., Vitvitsky, V., Martinov, M., and Banerjee, R. (2014) Organization of the human mitochondrial hydrogen sulfide oxidation pathway. *J. Biol. Chem.* **289**, 30901–30910 CrossRef Medline

77. Libiad, M., Vitvitsky, V., Bostelaar, T., Bak, D. W., Lee, H. J., Sakamoto, N., Fearon, E., Lyssiotis, C. A., Weerapan, E., and Banerjee, R. (2019) Hydrogen sulfide perturbs mitochondrial bioenergetics and triggers metabolic reprogramming in colon cells. *J. Biol. Chem.* **294**, 12077–12090 CrossRef Medline

78. Pitcher, M. C., and Cummings, J. H. (1996) Hydrogen sulphide: a bacterial toxin in ulcerative colitis? *Gastroenterology* **101**, 914–917 CrossRef Medline

79. Attene-Ramos, M. S., Wagner, E. D., Plewa, M. J., and Gaskins, H. R. (2006) Evidence that hydrogen sulfide is a genotoxic agent. *Mol. Cancer Res.* **4**, 9–14 CrossRef Medline

80. Xu, G. Y., Winston, J. H., Shenoy, M., Zhou, S., Chen, J. D., and Pasricha, P. (2009) The endogenous hydrogen sulfide producing enzyme cystathionine-β synthase contributes to vesicular hypertensitivity in a rat model of irritable bowel syndrome. *Mol. Pain* **5**, 44 CrossRef Medline

81. Winter, S. E., Thienennitz, P., Winter, M. G., Butler, B. P., Huseby, D. L., Crawford, R. W., Russell, J. M., Bevins, C. L., Adama, L. G., Tsolis, R. M., Roth, J. B., and Bäumler, A. J. (2010) Gut inflammation provides a respiratory electron acceptor for *Salmonella*. *Nature* **467**, 426–429 CrossRef Medline

82. Babior, B. M., Kipnes, R. S., and Curnutte, J. T. (1973) Biological defense mechanisms: the production by leukocytes of superoxide, a potential bactericidal agent. *J. Clin. Invest.* **52**, 741–744 CrossRef Medline

83. Goldstein, I. M., Roos, D., Kaplan, H. B., and Weissmann, G. (1975) Complement and immunoglobulins stimulate superoxide production by human leukocytes independently of phagocytosis. *J. Clin. Invest.* **56**, 1155–1163 CrossRef Medline

84. Johnston, R. B., Jr., Keele, B. B., Jr., Misra, H. P., Lehmyer, J. E., Webb, L. S., Baehner, R. L., and Ralagopolan, K. V. (1975) The role of superoxide
anion generation in phagocytic bacterial activity: studies with normal and chronic granulomatous disease leukocytes. *J. Clin. Invest.* **55**, 1357–1372 CrossRef Medline

85. Root, R. K., Metcalf, J., Oshino, N., and Chance, B. (1975) H₂O₂ release from human granulocytes during phagocytosis. I. Documentation, quantitation, and some regulating factors. *J. Clin. Invest.* **55**, 945–955 CrossRef Medline

86. Tauber, A. L., and Babiö, B. M. (1977) Evidence for hydroxyl radical production by human neutrophils. *J. Clin. Invest.* **60**, 374–379 CrossRef Medline

87. Mundy, H., Björkstén, B., Svancara, C., Ohman, L., and Dahlgren, C. (1991) Extracellular release of reactive oxygen species from human neutrophils upon interaction with *Escherichia coli* strains causing renal scarring. *Infect. Immun.* **59**, 4168–4172 CrossRef Medline

88. Albesa, I., Becerra, M. C., Battán, P. C., and Páez, P. L. (2004) Oxidative stress involved in the antibacterial action of different antibiotics. *Biochem. Biophys. Res. Commun.* **317**, 605–609 CrossRef Medline

89. Kohanski, M. A., Dwyer, D. J., Hayete, B., Lawrence, C. A., and Collins, J. J. (2007) Inhibition of staphylococcal biofilm formation by nitrite. *J. Bacteriol.* **189**, 7911–7919 CrossRef Medline

90. Klare, W., Das, T., Ibugo, A., Buckle, E., Manefield, M., and Manos, J. (2016) Glutathione-disrupted biofilms of clinical *Pseudomonas aeruginosa* strains exhibit an enhanced antibiotic effect and a novel biofilm transcriptome. *Antimicrob. Agents Chemother.* **60**, 4539–4551 CrossRef Medline

91. Hufnagel, D. A., Price, J. E., Stephenson, R. E., Kelley, J., Benoit, M. F., and Chapman, M. R. (2018) Thiol starvation induces redox-mediated disruption of *Escherichia coli* biofilm components. *J. Bacteriol.* **200**, e00389-17 CrossRef Medline

92. Cowley, E. S., Kopf, S. H., LaRiviere, A., Ziebis, W., and Newman, D. K. (2015) Pediatric cystic fibrosis sputum can be chemically dynamic, anoxic, and extremely reduced due to hydrogen sulfide formation. *mBio* **6**, e00767-15 CrossRef Medline

93. Motta, J.-P., Flanigan, K. L., Agbor, T. A., Beatty, J. I., Blacker, R. W., Workentine, M. L., Da Silva, G. J., Wang, R., Buret, A. G., and Wallace, J. L. (2015) Hydrogen sulfide protects from colitis and restores intestinal microbiota biofilm and mucus production. *Inflamm. Bowel Dis.* **21**, 1006–1017 CrossRef Medline

94. Barbosa, R. L., and Benedetti, C. E. (2007) BigR, a transcriptional repressor from plant-associated bacteria, regulates an operon implicated in biofilm growth. *J. Bacteriol.* **189**, 6185–6194 CrossRef Medline

95. Guimarães, B. G., Barbosa, R. L., Soprano, A. S., Campos, B. M., de Souza, T. A., Tonoli, C. C. C., Leme, A. F. P., Murakami, M. T., and Benedetti, C. E. (2011) Plant pathogenic bacteria utilize biofilm growth-associated repressor (BigR), a novel winged-helix redox switch, to control hydrogen sulfide detoxification under hypoxia. *J. Bacteriol.* **193**, 26148–26157 CrossRef Medline

96. de Lira, N. P. V., Pauletti, B. A., Marques, A. C., Perez, C. A., Caserta, R., de Souza, A. A., Vercesi, A. E., Paes Leme, A. F., and Benedetti, C. E. (2013) Hydrogen sulfide production and utilization in the reverse transsulfuration pathway. *J. Biol. Chem.* **288**, 8310–8320 CrossRef Medline

97. Vitvitsky, V., Yadav, P. K., Kurthen, A., and Banerjee, R. (2015) Sulfide oxidation by a noncanonical pathway in red blood cells generates thiosulfate and poly sulfides. *J. Biol. Chem.* **290**, 8310–8320 CrossRef Medline

98. Bostelaar, T., Vitvitsky, V., Kumutima, J., Lewis, B. E., Yadav, P. K., Brudno, T. C., Filipovic, M., Lehnert, N., Stemmler, T. L., and Banerjee, R. (2016) Hydrogen sulfide oxidation by myoglobin. *Am. J. Chem. Soc.* **138**, 8476–8488 CrossRef Medline

99. Ruetz, M., Kumutima, J., Lewis, B. E., Filipovic, M. R., Lehnert, N., Stemmler, T. L., and Banerjee, R. (2017) A distal ligand mutes the interaction of hydrogen sulfide with human neuroglobin. *J. Biol. Chem.* **292**, 6512–6528 CrossRef Medline
120. Vitvitsky, V., Mijúkovic, J. L., Bostelaar, T., Adhihari, B., Yadav, P. K., Steiger, A. K., Torregrossa, R., Pluth, M. D., Whitman, M., Banerjee, R., and Filipovic, M. R. (2018) Cytochrome c reduction by H2S potentiates sulfide signaling. ACS Chem. Biol. 13, 2300–2307 CrossRef Medline

121. Nelp, M. T., Zheng, V., Davis, K. M., Stiefel, K. J. E., and Groves, I. T. (2019) Potent activation of indoleamine 2,3-dioxygenase by polysulfides. J. Am. Chem. Soc. 141, 15288–15300 CrossRef Medline

122. Lange, M., Ok, K., Shimberg, G. D., Bursac, B., Markó, L., Ivanovic-Burmazovic, I., Michel, S. L. I., and Filipovic, M. R. (2019) Direct zinc finger protein persulfidation by H2S is facilitated by Zn(2). Angew. Chem. Int. Ed. Engl. 58, 7997–8001 CrossRef Medline

123. Shen, J., Peng, H., Zhang, Y., Trinidad, I. C., and Giedroc, D. P. (2016) Staphylococcus aureus sap encodes a type II sulfide:quione oxidoreductase and impacts reactive sulfur speciation in cells. Biochemistry 55, 6524–6534 CrossRef Medline

124. Duz, A., Töth, A., Németh, B., Balogh, T., Kós, P. B., and Rákthely, G. (2018) A novel enzyme of type VI sulfide:quinone oxidoreductases in purple sulfur photosynthetic bacteria. Appl. Microbiol. Biotechnol. 102, 5133–5147 CrossRef Medline

125. Landry, A. P., Moon, S., Kim, H., Yadav, P. K., Guha, A., Cho, U.-S., and Banerjee, R. (2019) A catalytic trisulfide in human sulfide quinone oxidoreductase catalyzes coenzyme a persulfide synthesis and inhibits butyrate oxidation. Cell Chem Biol. 26, 1515–1525 CrossRef Medline

126. Walsh, B. J., Brito, J. A., and Giedroc, D. P. (2020) Hydrogen sulfide signaling and enzymology. in Comprehensive Natural Products III (Liu, H.-W., and Begley, T. P., eds), Vol 4, pp 430–288, 5426–35812 CrossRef Medline

127. Mueller, E. G. (2006) Trafficking in persulfides: delivering sulfur in bio- and enzymology. Protein interactions.

128. Dahl, J. U., Urban, A., Bolte, A., Sriyabhaya, P., Donahue, J. L., Nimtz, M., and Giedroc, D. P. (2015) Conformational analysis and chemical reactivity of the multido- main persulfide dioxygenase-sulfurtransferase involved in hydrogen sulfide detoxification. Biochemistry 54, 4542–4554 CrossRef Medline

129. Wallen, J. R., Mallett, T. C., Boles, W., Parsonage, D., Furdui, C. M., Karplus, P. A., and Claiborne, A. (2009) Crystal structure and catalytic properties of Bacillus anthracis CoADR-RHD: implications for flavin-linked sulfur trafficking. Biochemistry 48, 9650–9667 CrossRef Medline

130. Studholme, D. J., and Dixon, R. (2003) Domain architectures of Bacillus anthracis H16, a sulfite exporter in Shewanella loihica PV-4: implications of peroxide resistance for the mechanism of sulfite reductase. Biochemistry 42, 9080–9086 CrossRef Medline

131. Weinitschke, S., Denger, K., Cook, A. M., and Smits, T. H. M. (2007) The sulfite exporter in Clostridium beijerinckii e02782-19: implications for the mechanism of H2S-driven electron transport. FEMS Microbiol. Rev. 31, 130–143 CrossRef Medline

132. Studyholme, D. J., and Dixon, R. (2003) Domain architectures of high-affinity sulfite transporters. J. Bacteriol. 185, 1757–1767 CrossRef Medline

133. D’Autreaux, B., Tucker, N. P., Dixon, R., and Spiro, S. (2005) A non-haem iron centre in the transcription factor NorR senses nitric oxide. Nature 437, 769–772 CrossRef Medline

134. Tucker, N. P., D’Autreaux, B., Youssafzai, F. K., Fairhurst, S. A., Spiro, S., and Dixon, R. (2008) Analysis of the nitric oxide-sensing non-heme iron center in the NorR regulatory protein. J. Biol. Chem. 283, 1076–1085 CrossRef Medline

135. Yang, B., Nie, X., Xiao, Y., Gu, Y., Jiang, W., and Yang, C. (2020) Ferrus-iron-activated transcriptional factor AdhR regulates redox homeostasis in Clostridium beijerinckii. Appl. Environ. Microbiol. 86, e02782-19 CrossRef Medline

136. Bora, P., Chauhan, P., Pardeshi, K. A., and Chakrapani, H. (2018) Small molecule generators of biologically reactive sulfur species. RSC Adv. 8, 27359–27374 CrossRef Medline

137. McCoy, J. G., Bingman, C. A., Bitto, E., Holdorf, M. M., Makaroff, C. A., and Phillips, G. N., Jr. (2006) Structure of an ETHE1-like protein from Arabidopsis thaliana. Acta Crystallogr. D Biol. Crystallogr. 62, 964–970. CrossRef Medline

138. Motl, N., Skiba, M. A., Kabil, O., Smith, J. L., and Banerjee, R. (2017) Structural and biochemical analyses indicate that a bacterial persulfide dioxygenase-rhodanese fusion protein functions in sulfur assimilation. J. Biol. Chem. 292, 14026–14038 CrossRef Medline

139. Shen, J., Keithly, M. E., Armstrong, R. N., Higgin, K. A., Edmons, K. A., and Giedroc, D. P. (2015) Staphylococcus aureus CstA is a novel multido- main persulfide dioxygenase-sulfurtransferase involved in hydrogen sulfide detoxification. Biochemistry 54, 4542–4554 CrossRef Medline
154. Levin, C. M., Cerda, M. M., and Pluth, M. D. (2020) Activatable small-molecule hydrogen sulfide donors. Antioxid. Redox Signal. 32, 96–109 CrossRef Medline

155. Lin, Y. S., Chen, W., Xian, M., and Chang, C. J. (2017) Chemical probes for molecular imaging and detection of hydrogen sulfide and reactive sulfur species in biological systems. Chem. Soc. Rev. 44, 4596–4618 CrossRef Medline

156. Powell, C. R., Dillon, K. M., and Matson, J. B. (2018) A review of hydrogen sulfide (H2S) donors: chemistry and potential therapeutic applications. Biochem. Pharmacol. 149, 110–123 CrossRef Medline

157. Takano, Y., Echizen, H., and Hanaoka, K. (2017) Fluorescent probes and selective inhibitors for biological studies of hydrogen sulfide- and polysulfide-mediated signaling. Antioxid. Redox Signal. 27, 669–683 CrossRef Medline

158. Xu, S., Hamsath, A., Neill, D. L., Wang, Y., Yang, C. T., and Xian, M. (2019) Strategies for the design of donors and precursors of reactive sulfur species. Chemistry 25, 4005–4016 CrossRef Medline

159. Jiao, X., Li, J., Niu, J., Xie, X., Wang, X., and Tang, B. (2018) Small-molecule fluorescent probes for imaging and detection of reactive oxygen, nitrogen, and sulfur species in biological systems. Anal. Chem. 90, 533–555 CrossRef Medline

160. Li, L., Whiteman, M., Guan, Y. Y., Neo, K. L., Cheng, Y., Lee, S. W., Zhao, Y., Baskar, K., Tan, C. H., and Moore, P. K. (2008) Characterization of a novel, water-soluble hydrogen sulfide-releasing molecule (GYY4137): new insights into the biology of hydrogen sulfide. Circulation 117, 2351–2360 CrossRef Medline

161. Kang, J., Li, Z., Organ, C. L., Park, C.-M., Yang, C.-T., Pacheco, A., Wang, D., Lefer, D. J., and Xian, M. (2016) pH-controlled hydrogen sulfide release for myocardial ischemia-reperfusion injury. J. Am. Chem. Soc. 138, 6356–6369 CrossRef Medline

162. Levin, C. M., Cerda, M. M., and Pluth, M. D. (2019) Development and application of carbonyl sulfide-based donors for H2S delivery. Acc. Chem. Res. 52, 2725–2731 CrossRef Medline

163. Supuran, C. T., and Capasso, C. (2017) An overview of the bacterial carbonic anhydrases. Metabolites 7, 56 CrossRef Medline

164. Bianco, C. L., Akaike, T., Ida, T., Nagy, P., Bogdándi, V., Toscano, J. P., Kumagai, Y., Henderson, C. F., Goddu, R. N., Lin, J., and Fukuto, J. M. (2019) The reaction of hydrogen sulfide with disulfides: formation of a stable trisulfide and implications for biological systems. Br. J. Pharmacol. 176, 671–683 CrossRef Medline

165. Artaud, I., and Galardon, E. (2014) A persulfide analogue of the nitrosothiol SNAP: formation, characterization and reactivity. ChemBiochem 15, 2361–2364 CrossRef Medline

166. Yuan, Z., Zheng, Y., Yu, B., Wang, S., Yang, X., and Wang, B. (2018) Esterase-sensitive glutathione persulfide donor. Org. Lett. 20, 6364–6367 CrossRef Medline

167. Zheng, Y., Yu, B., Li, Z., Yuan, Z., Organ, C. L., Trivedi, R. K., Wang, S., Lefer, D. J., and Wang, B. (2017) An esterase-sensitive prodrug approach for controllable delivery of persulfide species. Angew. Chem. Int. Ed. Engl. 56, 11749–11753 CrossRef Medline

168. Bora, P., Chauhan, P., Manna, S., and Chakrapani, H. (2018) A vinyl-boronate ester-based persulfide donor controllable by hydrogen peroxide, a reactive oxygen species (ROS). Org. Lett. 20, 7916–7920 CrossRef Medline

169. Powell, C. R., Dillon, K. M., Wang, Y., Carrazone, R. J., and Matson, J. B. (2018) A persulfide donor responsive to reactive oxygen species: insights into reactivity and therapeutic potential. Angew. Chem. Int. Ed. Engl. 57, 6324–6328 CrossRef Medline

170. Moste, R. R. (1975) Hydrogen sulfide determination by the methylene blue method. Anal. Chem. 47, 1204–1205 CrossRef Medline

171. Bibi, S.-I., Luck, B., Zukunft, S., Wittig, J., Chen, W., Xian, M., Papapetrou, A., Hu, J., and Fleming, I. (2018) A selective and sensitive method for quantification of endogenous polysulfide production in biological samples. Redox Biol. 18, 295–304 CrossRef Medline

172. Olson, K. R., Gao, Y., Arif, F., Arora, K., Patel, S., DeLeon, E. R., Sutton, T. R., Feelsch, M., Cortese-Krott, M. M., and Straub, K. D. (2018) Metabolism of hydrogen sulfide (H2S) and production of reactive sulfur species (RSS) by superoxide dismutase. Redox Biol. 15, 74–85 CrossRef Medline
JBC REVIEWS: \( \text{H}_2\text{S}/\text{RSS} \) homeostasis and reactive sulfur species in bacteria

191. Wedmann, R., Onderka, C., Wei, S., Szijártó, I. A., Miljkovic, J. L., Mitrovic, A., Lange, M., Savitsky, S., Yadav, P. K., Torregrossa, R., Harrer, E. G., Harrer, T., Ishii, I., Gollasch, M., Wood, M. E., et al. (2016) Improved tag-switch method reveals that thioredoxin acts as depersulfidase and controls the intracellular levels of protein persulfidation. *Chem. Sci.* 7, 3414–3426 CrossRef Medline

192. Dóka, E., Pader, I., Biró, A., Johansson, K., Cheng, Q., Ballagó, K., Prigge, J. R., Richter, F., Köhler, Y., Wittig, I., Beck, K.-F., and Pfeilschifter, E. G., Harrer, T., Ishii, I., Gollasch, M., Wood, M. E., et al. (2016) A novel persulfide detection method reveals protein persulfide- and polysulfide-reducing functions of thioredoxin and glutathione systems. *Sci. Adv.* 2, e1500968 CrossRef Medline

193. Peng, H., Zhang, Y., Trinidad, J. C., and Giedroc, D. P. (2018) Thioredoxin profiling of multiple thioredoxin-like proteins in *Staphylococcus aureus*. *Front. Microbiol.* 9, 2385 CrossRef Medline

194. Pan, J., and Carroll, K. S. (2013) Persulfide reactivity in the detection of protein \( \text{S} \)-sulphydration. *ACS Chem. Biol.* 8, 1110–1116 CrossRef Medline

195. Gao, X. H., Krokowski, D., Guan, B. J., Bederman, I., Majumder, M., Parisien, M., Diatchenko, L., Kabil, O., Willard, B., Banerjee, R., Wang, B., Bebek, G., Evans, C. R., Fox, P. L., Gerson, S. L., et al. (2015) Quantitative \( \text{H}_2\text{S} \)-mediated protein sulfhydration reveals metabolic reprogramming during the integrated stress response. *Elife* 4, e10067 CrossRef Medline

196. Longen, S., Richter, F., Köhler, Y., Wittig, I., Beck, K.-F., and Pfeilschifter, J. (2016) Quantitative persulfide site identification (qPers-SID) reveals protein targets of \( \text{H}_2\text{S} \) releasing donors in mammalian cells. *Sci. Rep.* 6, 29808 CrossRef Medline

197. Kouroussis, E., Adhikari, B., Zivanovic, J., and Filipovic, M. R. (2019) Measurement of protein persulfidation: improved tag-switch method. in *Vascular Effects of Hydrogen Sulfide: Methods and Protocols* (Belotowsky, J., ed) pp. 37–50, Springer, New York

198. Park, C.-M., Macinkovic, I., Filipovic, M. R., and Xian, M. (2015) Use of the “tag-switch” method for the detection of protein \( \text{S} \)-sulphydration. *Methods Enzymol.* 555, 39–56 CrossRef Medline

199. Zhang, D., Macinkovic, I., Devarie-Baez, N. O., Pan, J., Park, C.-M., Carroll, K. S., Filipovic, M. R., and Xian, M. (2014) Detection of protein \( \text{S} \)-sulphydration by a tag-switch technique. *Angew. Chem. Int. Ed. Engl.* 53, 575–581 CrossRef Medline

200. Zivanovic, J., Kouroussis, E., Kohl, J. B., Adhikari, B., Bursac, B., Schott-Roux, S., Petrovic, D., Miljkovic, J. L., Thomas-Lopez, D., Jung, Y., Miler, M., Mitchell, S., Milosevic, V., Gomes, J. J., Benhar, M., et al. (2019) Selective persulfide detection reveals evolutionarily conserved antiaging effects of \( \text{S} \)-sulphydration. *Cell Metab.* 30, 1152–1170.e13 CrossRef Medline

201. Bernal-Perez, L. F., Prokai, L., and Ryu, Y. (2012) Selective N-terminal fluorescent labeling of proteins using 4-chloro-7-nitrobenzofurazan: a method to distinguish protein N-terminal acetylation. *Anal. Biochem.* 428, 13–15 CrossRef Medline

202. Ellis, H. R., and Poole, L. B. (1997) Novel application of 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole to identify cysteine sulfenic acid in the AhpC component of alkyl hydroperoxide reductase. *Biochemistry* 36, 15013–15018 CrossRef Medline

203. Pauelsen, C. E., and Carroll, K. S. (2013) Cysteine-mediated redox signaling: chemistry, biology, and tools for discovery. *Chem. Rev.* 113, 4633–4679 CrossRef Medline

204. Furdui, C. M., and Poole, L. B. (2014) Chemical approaches to detect and analyze protein sulfenic acids. *Mass Spectrom. Rev.* 33, 126–146 CrossRef Medline

205. Pan, J., and Carroll, K. S. (2014) Chemical biology approaches to study protein cysteine sulphydration. *Biopolymers* 101, 165–172 CrossRef Medline

206. Fu, L., Liu, K., He, J., Tian, C., Yu, X., and Yang, J. (2019) Direct proteomic mapping of cysteine persulfidation. *Antioxid. Redox Signal.* 10.1089/ars.2019.7777 CrossRef Medline

207. Yang, J., Gupta, V., Carroll, K. S., and Liebler, D. C. (2014) Site-specific mapping and quantification of protein \( \text{S} \)-sulphenylation in cells. *Nat. Commun.* 5, 4776 CrossRef Medline

208. Bestetti, S., Medrano-Fernandez, I., Galli, M., Ghitti, M., Bienert, G. P., Musco, G., Orsi, A., Rubartelli, A., and Sittia, R. (2018) A persulfidation-based mechanism controls aquaporin-8 conductance. *Sci. Adv.* 4, eaar5770 CrossRef Medline