

# Transpersulfidation or H<sub>2</sub>S Release? Understanding the Landscape of Persulfide Chemical Biology

Kaylin G. Fosnacht,<sup>a</sup> Jyoti Sharma,<sup>b</sup> Pier Alexandre Champagne,<sup>b,\*</sup> and Michael D. Pluth<sup>a,\*</sup>

<sup>a</sup>Department of Chemistry and Biochemistry, Materials Science Institute, Knight Campus for Accelerating Scientific Impact, Institute of Molecular Biology, University of Oregon, Eugene, Oregon, USA

<sup>b</sup>Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, New Jersey, USA

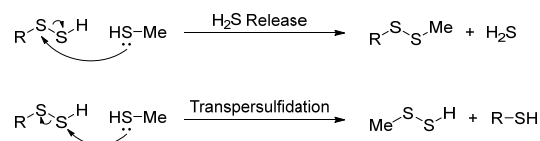
**Keywords:** *Persulfide, transpersulfidation, hydrogen sulfide, reactive sulfur species*

**ABSTRACT:** Persulfides (RSSH) are biologically important reactive sulfur species that are endogenously produced, protect key cysteine residues from irreversible oxidation, and are important intermediates during different enzymatic processes. Although persulfides are stronger nucleophiles than their thiol counterparts, persulfides can also act as electrophiles in their neutral, protonated form in specific environments. Moreover, persulfides are electrophilic at both sulfur atoms, and reaction with a thiolate can lead to either H<sub>2</sub>S release with disulfide formation or alternatively result in transpersulfidation. Despite the broad acceptance of these reaction pathways, the specific properties that control whether persulfides react through the H<sub>2</sub>S releasing or transpersulfidation pathway remains elusive. Herein, we use a combined computational and experimental approach to directly investigate the reactivity between persulfides and thiols to answer these questions. Using DFT calculations, we demonstrate that increasing steric bulk or electron withdrawal near the persulfide can shunt persulfide reactivity through the transpersulfidation pathway. Building from these insights, we use a persulfide donor and TME-IAM trapping agent to experimentally monitor and measure transpersulfidation from a bulky penicillamine-based persulfide to a cysteine-based thiol, which to the best of our knowledge is the first direct observation of transpersulfidation between low molecular weight species. Taken together, these combined approaches highlight how the properties of persulfides are directly impacted by local environments, which has significant impacts in understanding the complex chemical biology of these reactive species.

## INTRODUCTION

Reactive sulfur species (RSS) play critical roles in biological chemistry, including roles in redox homeostasis and small molecule signaling pathways. This important class of compounds encompasses a wide array of oxidation states, with molecules containing sulfur atoms in the -2 to +6 oxidation state including such as H<sub>2</sub>S, persulfides (RSSH), polysulfides (RSS<sub>n</sub>SR), sulfenic acids (RSOH), and sulfate (SO<sub>4</sub><sup>2-</sup>).<sup>1-3</sup> These redox active species are involved in diverse biological functions across all kingdoms of life, and the fundamental chemistry by which RSS exert action is a rapidly-expanding area of investigation. Building from the discovery that endogenous H<sub>2</sub>S participates in various mammalian signaling pathways in the late 1990's, significant effort has expanded our understanding of how H<sub>2</sub>S and other RSS are intertwined in complex pathways associated with RSS generation, translocation, and action.<sup>4-5</sup> More recent work has further clarified that many cellular signaling processes initially attributed to H<sub>2</sub>S alone are likely due to persulfides.<sup>6-8</sup> Similar to H<sub>2</sub>S, persulfides are generated endogenously through both enzymatic and non-enzymatic processes, and cellular persulfide levels are generally found in the low micromolar range, although elevated glutathione persulfide levels have also been found in specific environments, such as brain tissue.<sup>9-14</sup> Persulfides protect essential protein Cys residues from irreversible oxidation, are soluble sources of redox-labile sulfur, and are versatile intermediates in

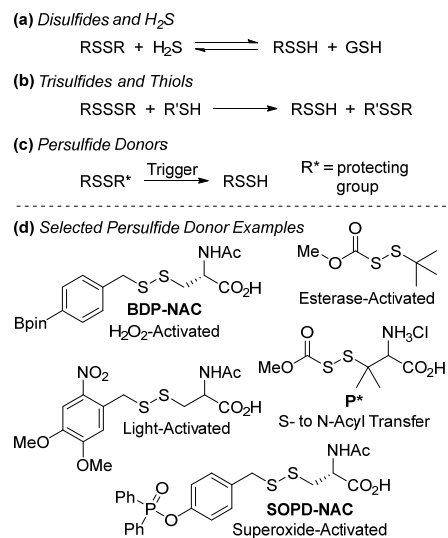
important cellular processes.<sup>15-17</sup> For example, persulfide intermediates have been observed during processes including Fe-S cluster formation, sulfur homeostasis maintenance by thioredoxin, sulfur transfer by 3-mercaptopyruvate sulfur transferase, prevention against Tau hyperphosphorylation, as well as other systems.<sup>18-24</sup>



**Scheme 1.** Comparison of attack at the sulfenyl sulfur to generate a disulfide and H<sub>2</sub>S and at the sulfhydryl sulfur to undergo a transpersulfidation reaction. RSS are shown in their neutral form for simplicity.

Despite the broad importance of persulfide chemistry, understanding the fundamental chemistry of this functional group remains challenging because both the local environment and protonation state directly impact persulfide reactivity. For example, although other RSS such as H<sub>2</sub>S or thiols exclusively function as nucleophiles, persulfides are both nucleophilic and electrophilic with an additional propensity to participate in one electron chemistry. Persulfides are more acidic than the corresponding thiol by 1–4 pK<sub>a</sub> units (e.g. pK<sub>a</sub> GSH = 8.94, GSSH = 5.45), and are also significantly better nucleophiles than thiols due to the α-effect.<sup>25-26</sup> In

their neutral protic form, persulfides are electrophilic at either sulfur atom and reactivity with nucleophiles such as thiolates, cyanide, sulfite, phosphines, and amines has been observed previously.<sup>27-30</sup> More specifically, reaction with a thiolate at the inner sulfenyl sulfur (RSSH) results in disulfide formation and H<sub>2</sub>S release whereas reaction at the outer sulfhydryl sulfur (RSSH) results in sulfur transfer, or transpersulfidation, to the incoming nucleophile (Scheme 1). Both of these reaction manifolds are common in RSS chemical biology, with select reported H<sub>2</sub>S donors generating an intermediate persulfide and transpersulfidation observed during enzymatic processes.<sup>31-32</sup> Despite the increasing literature examples of this bifurcated reactivity between persulfides and thiols, approaches to control or understand these pathways remain elusive.



**Figure 1.** Methods employed to generate persulfides *in situ* including (a) reaction of disulfides with H<sub>2</sub>S, (b) reaction of trisulfides with thiols, or (c) persulfide donors activated by a specific trigger. (d) Examples of persulfide donors that are activated by hydrogen peroxide, esterase, light, S- to N-acyl transfer, or superoxide.

To further understand the reactivity and biological roles of persulfides, different approaches have been developed to generate persulfides. Methods used to isolate low molecular weight (LMW) model persulfides have been helpful for exploring fundamental persulfide reactivity, however the organic solvent environments used in these investigations does not map to persulfide reactivity in aqueous systems.<sup>7, 33-40</sup> Alternatively, persulfides can be produced *in vitro* through the upregulation of persulfide-producing enzymes or by exogenous treatment of enzyme substrates.<sup>9, 41</sup> Other approaches to generate LMW persulfides include the treatment of oxidized thiols with H<sub>2</sub>S, disulfides with H<sub>2</sub>S, or trisulfides with thiols, although such reactions are reversible and typically generate RSS mixtures (Figure 2a–b).<sup>12, 42-45</sup> Another commonly used approach is the use of persulfide donors, also known as persulfide prodrugs or protected persulfides, that release persulfides when activated by specific conditions or stimuli.<sup>46</sup> Examples include persulfide donors activated by hydrolysis, nucleophiles, enzymes, or light (Figure 2c–d).<sup>47-51</sup> Although persulfide donors provide an important approach for generating and investigating the biological impacts of persulfides, the specific reactivity of

the structurally-diverse released persulfides are generally not investigated directly and may depend on the characteristics of the released persulfides. Expanding the fundamental understanding of structural impacts on persulfide stability, persistence, and reactivity would significantly advance our understanding of whether released persulfides participate primarily in H<sub>2</sub>S release, transpersulfidation, or a mixture of both pathways.

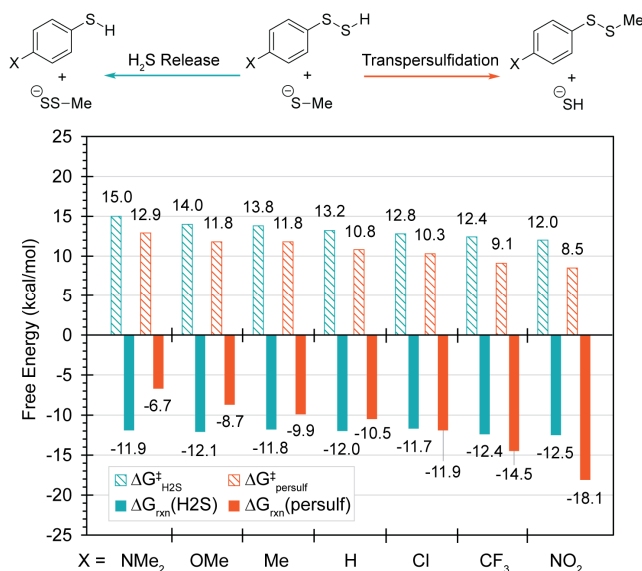
Initial insights into persulfide persistence were obtained during prior work from our lab using an esterase-activated persulfide donor where the sterically bulky *tert*-butyl persulfide was more persistent in buffer than the less bulky ethyl or benzyl persulfides.<sup>51</sup> Other reports have postulated similar impacts of bulk on persulfide reactivity, but experimental investigations into this hypothesis are primarily lacking.<sup>33, 52</sup> With the goal of bridging this major gap in knowledge, we report here a combined computational and experimental approach to examine how both electronic and steric components influence persulfide properties and can be used to tune H<sub>2</sub>S release versus transpersulfidation pathways. Importantly, computational investigations are a key component of this approach because they allow for specific molecular entities to be investigated without unwanted interference from other generated RSS and also because they allow for motifs to be investigated that would not be possible experimentally due to cross reactivity, synthetic challenges, or persulfide stability issues. Using this approach, we demonstrate here how the electronic structure of persulfides influences the linear free energy relationship for both the thermodynamic and kinetic preferences for H<sub>2</sub>S release versus transpersulfidation. Moreover, translating this approach to the persulfide local steric environment, we use these computational insights to experimentally monitor and measure transpersulfidation from a bulky penicillamine-based persulfide and a cysteine-based thiol, which to the best of our knowledge is the first direct observation of transpersulfidation between low molecular weight species.

## RESULTS AND DISCUSSION

To overcome the experimental limitations of working with a broad array of functionalized persulfides, we used computational approaches to investigate the fundamental energetics of persulfide reaction manifolds. Importantly, this approach allows for individual reaction pathways to be probed directly without potential interference from competing pathways and also removes the need to synthesize these otherwise unstable molecules. In addition, computational investigations can be used to identify target systems that can be prepared experimentally to validate the predicted reactivity from computational approaches.

**Computational Investigation of Persulfide Reactivity with Thiols.** To investigate how persulfide electron density impacts the intrinsic energy landscape of persulfides for competing pathways of H<sub>2</sub>S release and transpersulfidation, we first investigated a series of phenyl persulfides substituted with electron donating or withdrawing groups in the *para* position. DFT calculations were performed at the  $\omega$ B97X-D/aug-cc-pV(T+d)Z/SMD(H<sub>2</sub>O) level of theory, which provides accurate results for nucleophilic substitution reactions involving S–S bonds.<sup>53-54</sup> To investigate the intrinsic energy landscape of persulfides for competing pathways of H<sub>2</sub>S release and transpersulfidation, we used methyl

thiolate as a model nucleophile to simplify possible conformations and protonation states. In each system we calculated the thermodynamic ( $\Delta G_{\text{rxn}}$ ) and activation ( $\Delta G^\ddagger$ ) free energy for both the  $\text{H}_2\text{S}$  release ( $\Delta G_{\text{rxn}}(\text{H}_2\text{S})$ ,  $\Delta G_{\text{H}_2\text{S}}^\ddagger$ ) and transpersulfidation pathways ( $\Delta G_{\text{rxn}}(\text{persulf})$ ,  $\Delta G_{\text{persulf}}^\ddagger$ ). When comparing the energetics of these systems (Figure 2), in all cases transpersulfidation is kinetically favored over  $\text{H}_2\text{S}$  release, and the selectivity for transpersulfidation over  $\text{H}_2\text{S}$  release increases as electron withdrawing groups are added to the persulfide. For example, transpersulfidation is kinetically favored over  $\text{H}_2\text{S}$  release by 2.1 kcal mol<sup>-1</sup> for the electron-donating  $\text{NMe}_2$  persulfide, versus by 3.5 kcal mol<sup>-1</sup> for electron-withdrawing  $\text{NO}_2$  persulfide. Interestingly, transpersulfidation for electron-poor persulfides is more exergonic than  $\text{H}_2\text{S}$  release, which we attribute to the different basicities of the aryl thiolate products.



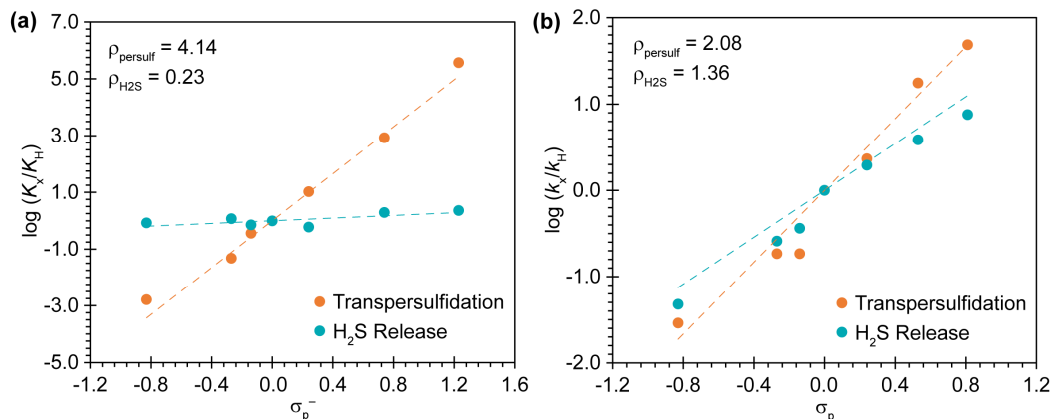
**Figure 2.** Activation and reaction free energies (kcal mol<sup>-1</sup>) for  $\text{H}_2\text{S}$  release or transpersulfidation of substituted aryl persulfides with methanethiolate.

Using the reaction energetics obtained in Figure 2, we next used this data to investigate the Hammett linear free energy relationship to better understand the factors influencing both the kinetic and thermodynamic preferences for transpersulfidation and  $\text{H}_2\text{S}$  release. We converted the

$\Delta G_{\text{rxn}}(\text{H}_2\text{S})$  and  $\Delta G_{\text{rxn}}(\text{persulf})$  values to  $K_{\text{eq}}$  values at 298 K to generate the Hammett plot in Figure 3a. Because the negative charge of the product thiolate can be stabilized through resonance, we used  $\sigma_p^-$  values in our analysis.<sup>55</sup> Electronic substitution has a major impact on transpersulfidation ( $\rho = 4.14$ ), which is likely driven by the different  $\text{p}K_{\text{a}}$  values of the resultant aryl thiolate products. By contrast, there is only minimal dependence upon electronic substitution for  $\text{H}_2\text{S}$  release ( $\rho = 0.23$ ). We attribute this lower sensitivity to the lack of charge buildup in the disulfide product formed after  $\text{H}_2\text{S}$  release. The significant difference in  $\rho$  values for the  $\text{H}_2\text{S}$  release and transpersulfidation pathways shows that the thermodynamic favorability for transpersulfidation can be greatly favored by including electron withdrawing groups on the persulfide.

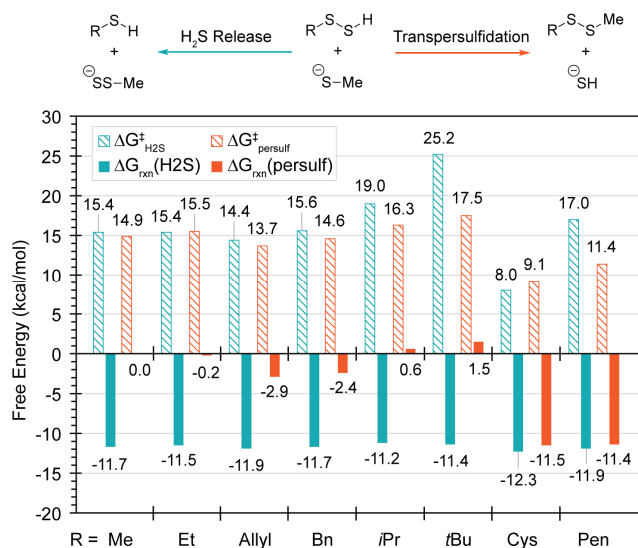
To complement these thermodynamic insights, we also used  $\Delta G_{\text{H}_2\text{S}}^\ddagger$  and  $\Delta G_{\text{persulf}}^\ddagger$  to generate a Hammett plot to understand how electronic structure impacts the barriers for these competing reaction pathways (Figure 3b). For the transition state structures we found the best correlation with  $\sigma_p$  values, which suggests a lower degree of overall charge delocalization in the transition state.<sup>55</sup> The Hammett plot for both reaction pathways is consistent with partial negative charge character on the sulfonyl sulfur, with a larger charge buildup for the transpersulfidation reaction, which is consistent with the aryl thiol acting as a leaving group. The transpersulfidation pathway ( $\rho = 2.08$ ) shows a greater sensitivity to negative charge buildup than the  $\text{H}_2\text{S}$  release pathway ( $\rho = 1.36$ ), which again highlights that transpersulfidation can be favored by inclusion of electron withdrawing substituents.

Having demonstrated the impact of electronic donating and withdrawing substituents on persulfide reactivity, we next investigated the energy landscape for the competing  $\text{H}_2\text{S}$  and transpersulfidation reactions of alkyl persulfides with different steric profiles (Me, Et, allyl, Bn, *i*Pr, *t*Bu). We first compared  $\Delta G^\ddagger$  for  $\text{H}_2\text{S}$  release and transpersulfidation for alkyl persulfides with increasing steric bulk (Figure 4). Our expectation was that more steric bulk on the persulfide would increase the barrier for attack by  $\text{MeS}^-$ . Consistent with this expectation, we observed an increase in  $\Delta G^\ddagger$  for both reaction pathways going from primary (Et) to secondary (*i*Pr) to tertiary (*t*Bu) persulfides. This increase is more



**Figure 3.** Hammett linear free energy relationship of the (a) thermodynamic and (b) kinetic preferences for transpersulfidation and  $\text{H}_2\text{S}$  release.

significant for the H<sub>2</sub>S releasing pathway because the thiolate needs to attack the sulfenyl sulfur atom, which is more impacted by the steric hinderance of the alkyl group. Indeed, all aliphatic primary persulfides (Me, Et, allyl, Bn) have similar barriers for both pathways, which is supported by prior computational work with MeSSH that showed little preference for nucleophilic attack on either sulfur atom.<sup>56</sup> Increasing in steric bulk, both *i*PrSSH and *t*BuSSH show lower barriers for transpersulfidation over H<sub>2</sub>S release by 2.7 and 7.7 kcal mol<sup>-1</sup>, respectively. Taken together, these data support that primary persulfides function as both H<sub>2</sub>S and transpersulfidation reagents, whereas bulkier persulfides have a significant kinetic preference for transpersulfidation over H<sub>2</sub>S release. More broadly, this chemistry is consistent with prior experimental work with primary persulfides, such as diallyl trisulfide, which is a naturally occurring polysulfide from garlic and alliums that is well established to release H<sub>2</sub>S in the presence of thiols through the intermediate release of the allyl persulfide followed by subsequent reaction with a thiol to release H<sub>2</sub>S.<sup>57-60</sup>

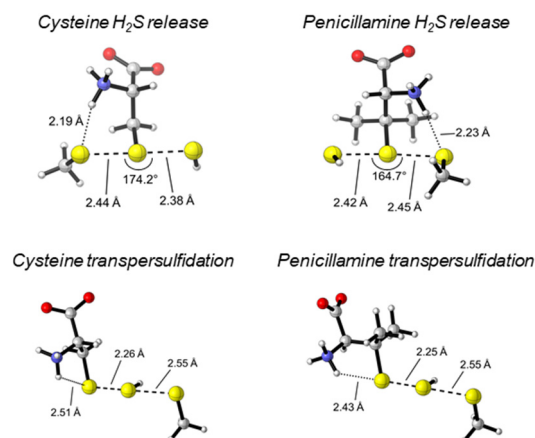


**Figure 4.** Activation and reaction free energies (kcal mol<sup>-1</sup>) for H<sub>2</sub>S release or transpersulfidation of alkyl persulfides with methyl thiolate.

The steric effects are also apparent when comparing the reactivity of penicillamine and cysteine persulfides. Although ΔG<sup>‡</sup><sub>persulf</sub> values are similar for both persulfides (11.4 vs. 9.1 kcal mol<sup>-1</sup>), the bulkier penicillamine persulfide has a much larger ΔG<sup>‡</sup><sub>H<sub>2</sub>S</sub> than cysteine persulfide (17.0 vs. 8.0 kcal mol<sup>-1</sup>). This difference is due to the tertiary carbon next to the sulfenyl sulfur atom, which distorts the S<sub>N</sub>2-like transition state angle from the near linear 174° for cysteine persulfide to a much more bent 165° for the penicillamine derivative (Figure 5). These results are especially interesting, as penicillamine persulfides are commonly used as cysteine analogues in persulfide donors due to the increased stability of the donor itself. Although these structures are commonly used interchangeably, the steric bulk of the penicillamine alters the reactivity of the persulfide considerably from that of cysteine persulfide.

To further probe this system with more biologically-relevant nucleophiles, we also investigated the reaction of cysteine persulfide with methyl thiolate, cysteine thiolate, and

a bulky penicillamine-based thiolate as the nucleophiles (Figure S5). We found that as the nucleophile increases in size, the preference for the H<sub>2</sub>S releasing pathway over transpersulfidation is eroded. For example, the cysteine thiolate nucleophile results in a 0.3 kcal mol<sup>-1</sup> kinetic preference for transpersulfidation over H<sub>2</sub>S release, which increases to 0.6 kcal mol<sup>-1</sup> for the penicillamine-based system. Although increased nucleophile steric bulk does affect reaction preference for transpersulfidation over H<sub>2</sub>S release, persulfide steric bulk has a much larger effect on reaction energetics.

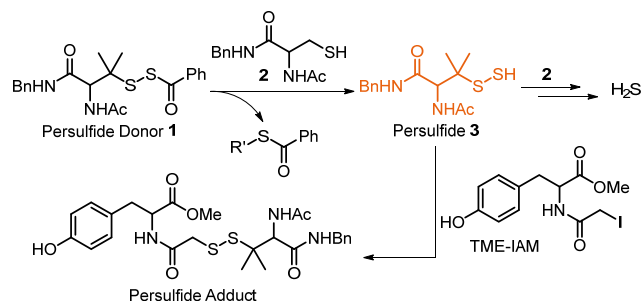


**Figure 5.** Computed H<sub>2</sub>S release and transpersulfidation transition structures (TSs) for cysteine (Cys) and penicillamine (Pen) systems.

*Experimental Transpersulfidation Observation and Measurement.* After demonstrating through computational methods that transpersulfidation between persulfides and thiols can be influenced directly by persulfide steric bulk, we next aimed to translate these DFT results into experimental solution reactivity. As mentioned previously, the reversible reaction between LMW persulfides and thiols resulting in H<sub>2</sub>S release and disulfide formation is well established, and adaptations of this chemistry have been used to generate GSSH *in situ* by the reaction of GSSG with H<sub>2</sub>S.<sup>26</sup> Further dissecting the direct reaction of a persulfide with a thiol, either H<sub>2</sub>S release and disulfide formation or transpersulfidation can occur. Prior work on this reaction has attributed the favorable release of H<sub>2</sub>S to the leaving group ability of H<sub>2</sub>S (pK<sub>a</sub> = 6.98) versus a thiol such as GSH (pK<sub>a</sub> = 8.94), with transpersulfidation being accessible in specific environments within proteins.<sup>32, 61</sup> Indeed, transpersulfidation has primarily been observed between proteins or between a protein and LMW species in select systems, although this reactivity is also hypothesized to occur more broadly during other cellular processes.<sup>62-65</sup> One report did observe an increase in GSSH with increased cysteine persulfide production through enzymatic means in cell lysates, but it is unclear if the increase in GSSH is due to direct reaction of CysSSH with GSH or through other pathways.<sup>9</sup> Expanding beyond these systems, to the best of our knowledge the direct transpersulfidation between LWM persulfides and thiols has not previously been directly observed experimentally.

For probe this question directly, we built from the computational insights from above by modifying a penicillamine-based acyl perthiol donor platform reported by Xian

in 2013.<sup>66</sup> These acyl perthiols react with Cys or GSH to form an intermediate persulfide, which subsequently reacts further with excess thiol to release H<sub>2</sub>S (Scheme 2). In addition, these systems demonstrated good activity against myocardial ischemia-reperfusion injury in mouse models, demonstrating the efficacy of this system in more complex environments.

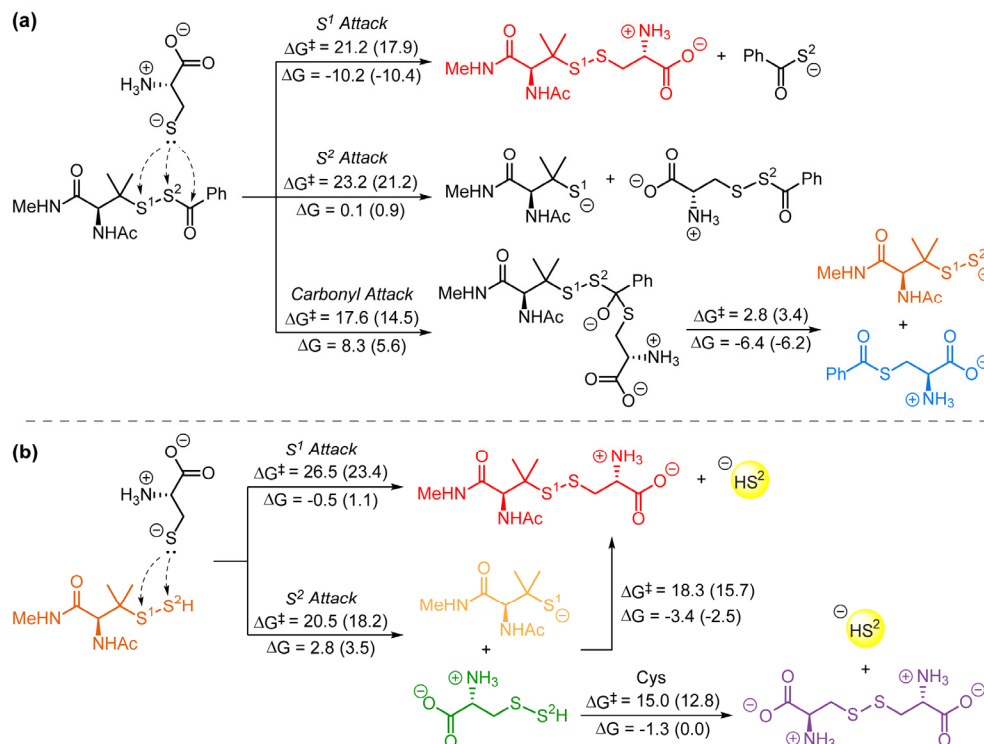


**Scheme 2.** Reactivity of the penicillamine-based acyl perthiol donor platform. Persulfide donor **1** with thiol **2** releases persulfide **3** that further react to release H<sub>2</sub>S or can be trapped by treatment with trapping agent TME-IAM to form a stable persulfide adduct.

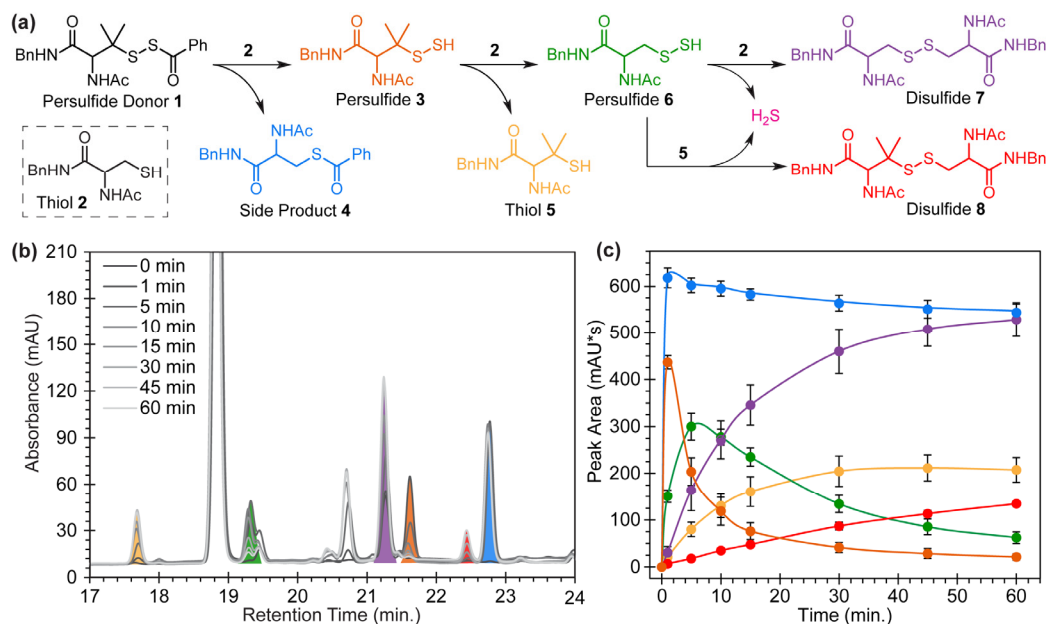
To better understand the specific reactivity of the tertiary persulfide intermediate formed in this reaction, we modified both the parent donor platform and thiol reactant with benzyl groups to improve HPLC detection. With this modified system, reaction of acyl perthiol **1** with *N*-acetyl cysteine derived **2** results in release of the analogous penicillamine-based persulfide **3** (Scheme 3). Since persulfides have a short persistence in solution due to their reaction with other thiols or persulfides, we used a persulfide trapping

agent to form a stable persulfide adduct to aid in analysis. After screening different trapping agents, we chose to use the iodoacetamide derivative *N*-iodoacetyl L-tyrosine methyl ester (TME-IAM), which has been used previously to trap and stabilize persulfides and other sulfhydryl species in complex RSS solutions.<sup>67</sup> Specifically, we used TME-IAM to trap and measure both penicillamine-derived persulfide **3** as well as the associated transpersulfidation product **6** by HPLC (*vide infra*)

Before beginning experimental studies, we confirmed that the expected reactivity of the persulfide donor scaffold was not significantly altered by the presence of the other functional groups on the penicillamine backbone. Specifically, we investigated the attack on different electrophilic sites including thiol attack at the carbonyl carbon and also at the sulfenyl sulfur atom (S<sup>1</sup>) or sulfhydryl sulfur atom (S<sup>2</sup>) of the perthiol (Figure 6a). DFT calculations were performed using a deprotonated cysteine as the model nucleophile and simplifying the structure of **1** by replacing the benzyl group with a methyl group. The values shown below were obtained with the ωB97X-D or M06-2X methods, using the aug-cc-pV(T+d)Z basis set and SMD solvation model for water, with the M06-2X results being represented in parentheses for energy tabulations below. These investigations demonstrated that nucleophilic attack by cysteine thiolate at the carbonyl carbon is kinetically favored by 3.6 (3.4) kcal mol<sup>-1</sup> over attack at the S<sup>1</sup> site and by 5.6 (6.7) kcal mol<sup>-1</sup> over attack at the S<sup>2</sup> site (Figure 6a). Additionally, we hypothesized that the bulky persulfide released from the persulfide donor would prefer transpersulfidation with the less bulky cysteine rather than direct H<sub>2</sub>S release, whereas the less bulky cysteine persulfide could then react through the



**Figure 6.** Computationally derived energetics of the reaction between the persulfide donor scaffold and cysteine thiolate at either the S<sup>1</sup>, S<sup>2</sup>, or carbonyl carbon position and (b) the generated penicillamine-based persulfide with cysteine thiolate at either the S<sup>1</sup> or S<sup>2</sup> positions. Calculations were performed at the ωB97X-D/aug-cc-pVDZ/SMD(water) // ωB97X-D or (M06-2X)/aug-cc-pV(T+d)Z/SMD(water) level of theory. Free energies (in kcal mol<sup>-1</sup>) of reaction are relative to the preceding intermediate.



**Figure 7.** (a) Proposed reaction pathway of the persulfide donor **1** with excess **2**. After treatment with the TME-IAM trapping agent, persulfides, thiols, and other hydrosulfide species form stable adducts. (b) Stacked HPLC chromatograms of the TME-IAM trapping experiment with persulfide donor **1** (100  $\mu$ M), **2** (1.0 mM), and TME-IAM (10 mM) and (c) peak area plotted of identified compounds over 60 minutes.

H<sub>2</sub>S releasing pathway. To investigate this question directly, we compared the activation barriers for these two processes from the generated persulfide intermediate. We found that cysteine thiolate attack at the S<sup>2</sup> site, which results in transpersulfidation and formation of cysteine persulfide, was kinetically favored by 6.0 (5.2) kcal mol<sup>-1</sup> over the H<sub>2</sub>S releasing pathway occurring through attack at S<sup>1</sup>. Although this reaction is reversible, with a positive free energy of reaction of 2.8 (3.5) kcal mol<sup>-1</sup>, H<sub>2</sub>S release from cysteine persulfide by reaction with a cysteine thiolate is the most kinetically favored (15.0 (12.8) kcal mol<sup>-1</sup>) pathway of cysteine persulfide reactivity (Figure 6b).

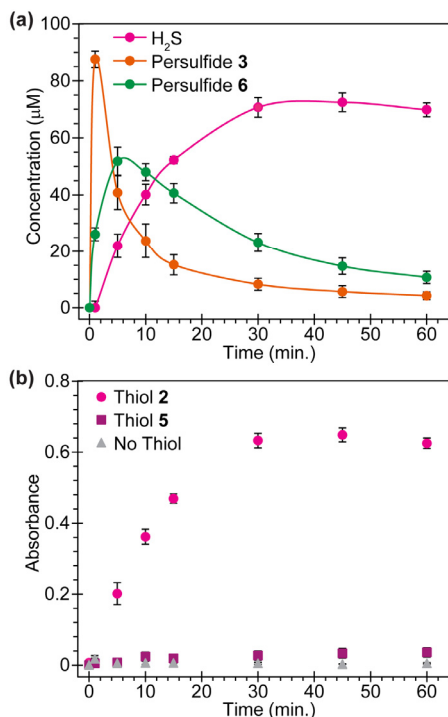
To test these computational predictions experimentally, persulfide donor **1** (100  $\mu$ M) was added to a solution containing **2** (1.0 mM, 10 equiv.) in PBS buffer (10 mM, pH 7.4, 10% MeCN). Aliquots of the resultant solution were removed at specified timepoints, quenched with TME-IAM (10 mM, 100 equiv.), and analyzed by HPLC (Figure 7 and S6). Treatment with excess TME-IAM was required to measure persistent persulfides and other RSS in solution and also trap unreacted thiols that could perturb RSS equilibria. Authentic samples of trapped persulfide analogues **S4** and **S5** and side product **4** were prepared to allow for HPLC quantification (Figure S7–S12) and disulfides **7** and **8** were prepared *in situ* to confirm peak identity (Figure S13). Trapped persulfide identity was additionally confirmed by treatment of a 5-minute experiment timepoint with tris(2-carboxyethyl)phosphine (TCEP) to ablate peaks correlated with trapped persulfides as well as HPLC purification of hypothesized persulfide adduct peaks followed by MS analysis (Figure S14 and S15).

Through HPLC analysis, we showed that donor **1** was fully activated within the first minute of the experiment, with the appearance of both persulfide **3** and the expected side product **4** (Figure 7). The concentration of persulfide **3**

then diminishes over time with concomitant growth of persulfide **6** formed from transpersulfidation, which reaches a maximum concentration at 5 minutes. Further evidence of transpersulfidation includes the appearance of tertiary thiol **5** produced during transpersulfidation. In addition, disulfides **7** and **8** are observed later in the reaction due to the reaction of persulfide **6** with either thiol **2** or **5**. Overall, this experiment shows that the more bulky penicillamine-based persulfide **3** undergoes a transpersulfidation reaction with the less bulky cysteine-based **2** to produce persulfide **6** before releasing H<sub>2</sub>S.

Having demonstrated direct experimental evidence for transpersulfidation, we next wanted to measure H<sub>2</sub>S release, which should be generated during reaction of persulfide **6** with a thiol (Figure 7a). H<sub>2</sub>S release was measured using the methylene blue H<sub>2</sub>S assay under the same conditions used for the persulfide trapping experiments. Treatment of persulfide donor **1** (100  $\mu$ M) with excess **2** resulted in 86  $\mu$ M of H<sub>2</sub>S release (86%), which reached a maximum at 45 minutes (Figure 8). Interestingly, although the HPLC trapping experiments showed full persulfide donor activation at 1 minute, no H<sub>2</sub>S was observed at early reaction time points. This result suggests that H<sub>2</sub>S release is more efficient from the less bulky persulfide and that efficient H<sub>2</sub>S release does not occur until transpersulfidation from the tertiary to the primary persulfide occurs. As expected, when persulfide donor **1** was treated with **5** instead of **2** minimal H<sub>2</sub>S release was observed, which is consistent with the decreased nucleophilicity of the bulkier tertiary thiol **5** (Figure 8b). Furthermore, in the absence of thiol, persulfide donor **1** did not release H<sub>2</sub>S, which confirms that this donor is stable in solution until activated by a thiol. These results are particularly important in the design of persulfide donors with tertiary persulfides, such as those derived from penicillamine, because these platforms likely primarily function as

transpersulfidation agents and the observed H<sub>2</sub>S release is due to subsequent transpersulfidation to primarily thiols like Cys or GSH. In addition, the increased pK<sub>a</sub> of penicillamine (~10.5) when compared to primary thiols like Cys (8.5) or GSH (~8.9) also means that more of the tertiary persulfide is in the neutral, protonated, and electric form at physiological pH, which further preferences reactivity through the transpersulfidation manifold



**Figure 8.** (a) Hydrogen sulfide release vs. persulfide 3 and 6 release from persulfide donor 1 (100 μM) when treated with 2 (1.0 mM) compared over 60 minutes. Persulfide 3 and 6 were quantified with a calibration curve of the persulfide adduct analogues. (b) Hydrogen sulfide release from persulfide donor 1 (100 μM) when treated with 2 (1.0 mM) or 5 (1.0 mM) monitored at 670 nm by the methylene blue H<sub>2</sub>S assay over 60 minutes in PBS buffer (10 mM, pH 7.4).

## CONCLUSIONS

Using a combined computational and experimental approach, we demonstrated the persulfide characteristics that govern transpersulfidation versus H<sub>2</sub>S release. Our computational work demonstrated that the reaction of electrophilic persulfides with thiolates can be modulated by both steric and electronic modifications. Inclusion of electron withdrawing groups on *p*-substituted aryl persulfides increased both the kinetic and thermodynamic preference for transpersulfidation over H<sub>2</sub>S release. Persulfides with limited steric bulk can react through either the H<sub>2</sub>S releasing or the transpersulfidation pathways, whereas bulkier tertiary persulfides favor transpersulfidation over H<sub>2</sub>S release. We confirmed this computational prediction using solution-based experimental transpersulfidation measurements from a tertiary penicillamine-based persulfide to generate a cysteine-based persulfide. H<sub>2</sub>S release from the system was confirmed to derive from the resultant and less-hindered cysteine-based persulfide, which is likely the main H<sub>2</sub>S

releasing pathway for these types of penicillamine-based persulfide donors. Overall, this work revises the prior assumption that persulfides primarily react to release H<sub>2</sub>S rather than undergoing transpersulfidation followed by subsequent H<sub>2</sub>S release. These results also suggest that the preference for transpersulfidation can be influenced by the electronic and steric components of persulfide, which is particularly relevant for applications of synthetic persulfide donors. Taken together, we anticipate that these results will not only help guide the design of future persulfide donors and bioanalytical trapping approaches, but also positively contribute to our fundamental understanding of biological persulfide reactivity within complex systems.

## AUTHOR INFORMATION

### Corresponding Authors

a.\* E-mail: [pluth@uoregon.edu](mailto:pluth@uoregon.edu)

b.\* E-mail: [pier.a.champagne@njit.edu](mailto:pier.a.champagne@njit.edu)

### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

## ACKNOWLEDGMENT

We thank support from the NSF (CHE-2004150 to MDP), ACS Petroleum Research Fund (61891-DNI4 to PAC), and Beetham Family Fellowship Program (KGF). Calculations were performed on the Lochness cluster at NJIT.

## ABBREVIATIONS

DFT, density functional theory; RSS, reactive sulfur species; LMW, low molecular weight; TME-IAM, N-iodoacetyl L-tyrosine methyl ester; HPLC, high-performance liquid chromatography; PBS, phosphate buffer saline; TCEP, tris(2-carboxyethyl)phosphine; MS, mass-spectrometry.

## REFERENCES

- (1) Giles, G. I.; Jacob, C., Reactive Sulfur Species: An Emerging Concept in Oxidative Stress. *Biol. Chem.* **2002**, 383, 375-388.
- (2) Gruhlke, M. C. H.; Slusarenko, A. J., The Biology of Reactive Sulfur Species (Rss). *Plant Physiol. Biochem.* **2012**, 59, 98-107.
- (3) Mishanina, T. V.; Libiad, M.; Banerjee, R., Biogenesis of Reactive Sulfur Species for Signaling by Hydrogen Sulfide Oxidation Pathways. *Nat. Chem. Biol.* **2015**, 11, 457-464.
- (4) Abe, K.; Kimura, H., The Possible Role of Hydrogen Sulfide as an Endogenous Neuromodulator. *J. Neurosci.* **1996**, 16, 1066-1071.
- (5) Wang, R., Two's Company, Three's a Crowd: Can H<sub>2</sub>S Be the Third Endogenous Gaseous Transmitter? *FASEB J.* **2002**, 16, 1792-1798.
- (6) Ono, K.; Akaike, T.; Sawa, T.; Kumagai, Y.; Wink, D. A.; Tantillo, D. J.; Hobbs, A. J.; Nagy, P.; Xian, M.; Lin, J., et al., Redox Chemistry and Chemical Biology of H<sub>2</sub>S, Hydropersulfides, and Derived Species: Implications of Their Possible Biological Activity and Utility. *Free Radic. Biol. Med.* **2014**, 77, 82-94.
- (7) Filipovic, M. R.; Zivanovic, J.; Alvarez, B.; Banerjee, R., Chemical Biology of H<sub>2</sub>S Signaling through Persulfidation. *Chem. Rev.* **2018**, 118, 1253-1337.
- (8) Rohwerder, T.; Sand, W., The Sulfane Sulfur of Persulfides Is the Actual Substrate of the Sulfur-Oxidizing Enzymes from *Acidithiobacillus* and *Acidiphilium* Spp. *Microbiology* **2003**, 149, 1699-1710.
- (9) Ida, T.; Sawa, T.; Ihara, H.; Tsuchiya, Y.; Watanabe, Y.; Kumagai, Y.; Suematsu, M.; Motohashi, H.; Fujii, S.; Matsunaga, T., et al., Reactive Cysteine Persulfides and S-Polythiolation Regulate Oxidative Stress and Redox Signaling. *Proc. Natl. Acad. Sci.* **2014**, 111, 7606-7611.

- (10) Griffiths, K.; Ida, T.; Morita, M.; Lamb, R. J.; Lee, J. J.; Frenneaux, M. P.; Fukuto, J. M.; Akaike, T.; Feelisch, M.; Madhani, M., Cysteine Hydropersulfide Reduces Lipid Peroxidation and Protects against Myocardial Ischaemia-Reperfusion Injury - Are Endogenous Persulfides Mediators of Ischaemic Preconditioning? *Redox Biol.* **2023**, *60*, 102605.
- (11) Akaike, T.; Ida, T.; Wei, F.-Y.; Nishida, M.; Kumagai, Y.; Alam, M. M.; Ihara, H.; Sawa, T.; Matsunaga, T.; Kasamatsu, S., et al., Cysteinyl-Trna Synthetase Governs Cysteine Polysulfidation and Mitochondrial Bioenergetics. *Nat. Commun.* **2017**, *8*, 1177.
- (12) Benchoam, D.; Cuevasanta, E.; Möller, Matías N.; Alvarez, B., Persulfides, at the Crossroads between Hydrogen Sulfide and Thiols. *Essays Biochem.* **2020**, *64*, 155-168.
- (13) Bianco, C. L.; Akaike, T.; Ida, T.; Nagy, P.; Bogdandi, V.; Toscano, J. P.; Kumagai, Y.; Henderson, C. F.; Goddu, R. N.; Lin, J., et al., The Reaction of Hydrogen Sulfide with Disulfides: Formation of a Stable Trisulfide and Implications for Biological Systems. *Br. J. Pharmacol.* **2019**, *176*, 671-683.
- (14) Kimura, Y.; Koike, S.; Shibuya, N.; Lefer, D.; Ogasawara, Y.; Kimura, H., 3-Mercaptopyrivate Sulfurtransferase Produces Potential Redox Regulators Cysteine- and Glutathione-Persulfide (Cys-Ssh and Gssh) Together with Signaling Molecules H<sub>2</sub>S<sub>2</sub>, H<sub>2</sub>S<sub>3</sub> and H<sub>2</sub>S. *Sci. Rep.* **2017**, *7*, 10459.
- (15) Yang, C. T.; Devarie-Baez, N. O.; Hamsath, A.; Fu, X. D.; Xian, M., S-Persulfidation: Chemistry, Chemical Biology, and Significance in Health and Disease. *Antioxid. Redox Signal.* **2020**, *33*, 1092-1114.
- (16) Kasamatsu, S.; Nishimura, A.; Morita, M.; Matsunaga, T.; Abdul Hamid, H.; Akaike, T., Redox Signaling Regulated by Cysteine Persulfide and Protein Polysulfidation. *Molecules* **2016**, *21*, 1721.
- (17) Millikin, R.; Bianco, C. L.; White, C.; Saund, S. S.; Henriquez, S.; Sosa, V.; Akaike, T.; Kumagai, Y.; Soeda, S.; Toscano, J. P., et al., The Chemical Biology of Protein Hydropersulfides: Studies of a Possible Protective Function of Biological Hydropersulfide Generation. *Free Radic. Biol. Med.* **2016**, *97*, 136-147.
- (18) Parent, A.; Elduque, X.; Cornu, D.; Belot, L.; Le Caer, J.-P.; Grandas, A.; Toledano, M. B.; D'Autréaux, B., Mammalian Frataxin Directly Enhances Sulfur Transfer of Nfs1 Persulfide to Both IscU and Free Thiols. *Nat. Commun.* **2015**, *6*, 5686.
- (19) Bridwell-Rabb, J.; Fox, N. G.; Tsai, C.-L.; Winn, A. M.; Barondeau, D. P., Human Frataxin Activates Fe-S Cluster Biosynthesis by Facilitating Sulfur Transfer Chemistry. *Biochemistry* **2014**, *53*, 4904-4913.
- (20) Dóka, É.; Pader, I.; Biró, A.; Johansson, K.; Cheng, Q.; Ballagó, K.; Prigge, J. R.; Pastor-Flores, D.; Dick, T. P.; Schmidt, E. E., et al., A Novel Persulfide Detection Method Reveals Protein Persulfide- and Polysulfide-Reducing Functions of Thioredoxin and Glutathione Systems. *Sci. Adv.* **2016**, *2*, e1500968.
- (21) Dóka, É.; Ida, T.; Dagnell, M.; Abiko, Y.; Luong, N. C.; Balog, N.; Takata, T.; Espinosa, B.; Nishimura, A.; Cheng, Q., et al., Control of Protein Function through Oxidation and Reduction of Persulfidated States. *Sci. Adv.* **2020**, *6*, eaax8358.
- (22) Wedmann, R.; Onderka, C.; Wei, S.; Szijártó, I. A.; Miljkovic, J. L.; Mitrovic, A.; Lange, M.; Savitsky, S.; Yadav, P. K.; Torregrossa, R., et al., Improved Tag-Switch Method Reveals That Thioredoxin Acts as Depersulfidase and Controls the Intracellular Levels of Protein Persulfidation. *Chem. Sci.* **2016**, *7*, 3414-3426.
- (23) Yadav, P. K.; Yamada, K.; Chiku, T.; Koutmos, M.; Banerjee, R., Structure and Kinetic Analysis of H<sub>2</sub>S Production by Human Mercaptopyrivate Sulfurtransferase. *J. Biol. Chem.* **2013**, *288*, 20002-20013.
- (24) Giovannazzo, D.; Bursac, B.; Sbodio, J. I.; Nalluru, S.; Vignane, T.; Snowman, A. M.; Albacarys, L. M.; Sedlak, T. W.; Torregrossa, R.; Whiteman, M., et al., Hydrogen Sulfide Is Neuroprotective in Alzheimer's Disease by Sulfhydrating Gsk3 $\beta$  and Inhibiting Tau Hyperphosphorylation. *Proc. Natl. Acad. Sci.* **2021**, *118*, e2017225118.
- (25) Portillo-Ledesma, S.; Sardi, F.; Manta, B.; Tourn, M. V.; Clippe, A.; Knoop, B.; Alvarez, B.; Coitiño, E. L.; Ferrer-Sueta, G., Deconstructing the Catalytic Efficiency of Peroxiredoxin-5 Peroxidative Cysteine. *Biochemistry* **2014**, *53*, 6113-6125.
- (26) Benchoam, D.; Semelak, J. A.; Cuevasanta, E.; Mastrogiovanni, M.; Grassano, J. S.; Ferrer-Sueta, G.; Zeida, A.; Trujillo, M.; Möller, M. N.; Estrin, D. A., et al., Acidity and Nucleophilic Reactivity of Glutathione Persulfide. *J. Biol. Chem.* **2020**, *295*, 15466-15481.
- (27) Kawamura, S.; Otsuji, Y.; Nakabayashi, T.; Kitao, T.; Tsurugi, J., Aralkyl Hydrodisulfides. IV. The Reaction of Benzyl Hydrodisulfide with Several Nucleophiles. *J. Org. Chem.* **1965**, *30*, 2711-2714.
- (28) Tsurugi, J.; Nakabayashi, T.; Ishihara, T., Aralkyl Hydrodisulfides. I. III. The Reaction with Tertiary Phosphines. *J. Org. Chem.* **1965**, *30*, 2707-2710.
- (29) Tsurugi, J.; Abe, Y.; Nakabayashi, T.; Kawamura, S.; Kitao, T.; Niwa, M., Aralkyl Hydrodisulfides. XI. Reaction with Amines. *J. Org. Chem.* **1970**, *35*, 3263-3266.
- (30) Pan, J.; Carroll, K. S., Persulfide Reactivity in the Detection of Protein S-Sulfhydration. *ACS Chem. Biol.* **2013**, *8*, 1110-1116.
- (31) Levinn, C. M.; Cerda, M. M.; Pluth, M. D., Activatable Small-Molecule Hydrogen Sulfide Donors. *Antioxid. Redox Signal.* **2019**, *32*, 96-109.
- (32) Cuevasanta, E.; Möller, M. N.; Alvarez, B., Biological Chemistry of Hydrogen Sulfide and Persulfides. *Arch. Biochem. Biophys.* **2017**, *617*, 9-25.
- (33) Bailey, T. S.; Pluth, M. D., Reactions of Isolated Persulfides Provide Insights into the Interplay between H<sub>2</sub>S and Persulfide Reactivity. *Free Radic. Biol. Med.* **2015**, *89*, 662-667.
- (34) Bailey, T. S.; Zakharov, L. N.; Pluth, M. D., Understanding Hydrogen Sulfide Storage: Probing Conditions for Sulfide Release from Hydrodisulfides. *J. Am. Chem. Soc.* **2014**, *136*, 10573-10576.
- (35) Böhme, H.; Zinner, G., Über Darstellung Und Eigenschaften Von Alkyl-Hydro-Polysulfiden. *Justus Liebigs Ann. Chem.* **1954**, *585*, 142-149.
- (36) Tsurugi, J.; Abe, Y.; Kawamura, S., Synthesis of 2-Substituted Ethyl Hydrodisulfides. *Bull. Chem. Soc. Japan* **2006**, *43*, 1890-1892.
- (37) Chatterji, T.; Keerthi, K.; Gates, K. S., Generation of Reactive Oxygen Species by a Persulfide (BnssH). *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3921-3924.
- (38) Heimer, N. E.; Field, L.; Neal, R. A., Biologically Oriented Organic Sulfur Chemistry. 21. Hydrodisulfide of a Penicillamine Derivative and Related Compounds. *J. Org. Chem.* **1981**, *46*, 1374-1377.
- (39) Tsurugi, J.; Kawamura, S.; Horii, T., Aryl Hydrodisulfides. *J. Org. Chem.* **1971**, *36*, 3677-3680.
- (40) Li, K.; Zakharov, L. N.; Pluth, M. D., Taming the Dichalcogenides: Isolation, Characterization, and Reactivity of Elusive Perselenide, Persulfide, Thioselenide, and Selenosulfide Anions. *J. Am. Chem. Soc.* **2023**, *145*, 13435-13443.
- (41) Yadav, P. K.; Martinov, M.; Vitvitsky, V.; Seravalli, J.; Wedmann, R.; Filipovic, M. R.; Banerjee, R., Biosynthesis and Reactivity of Cysteine Persulfides in Signaling. *J. Am. Chem. Soc.* **2016**, *138*, 289-299.
- (42) Cuevasanta, E.; Lange, M.; Bonanata, J.; Coitiño, E. L.; Ferrer-Sueta, G.; Filipovic, M. R.; Alvarez, B., Reaction of Hydrogen Sulfide with Disulfide and Sulfenic Acid to Form the Strongly Nucleophilic Persulfide. *J. Biol. Chem.* **2015**, *290*, 26866-26880.
- (43) Vasas, A.; Dóka, É.; Fábán, I.; Nagy, P., Kinetic and Thermodynamic Studies on the Disulfide-Bond Reducing Potential of Hydrogen Sulfide. *Nitric Oxide* **2015**, *46*, 93-101.
- (44) Francoleon, N. E.; Carrington, S. J.; Fukuto, J. M., The Reaction of H<sub>2</sub>S with Oxidized Thiols: Generation of Persulfides and Implications to H<sub>2</sub>S Biology. *Arch. Biochem. Biophys.* **2011**, *516*, 146-153.
- (45) Zhang, D.; Macinkovic, I.; Devarie-Baez, N. O.; Pan, J.; Park, C.-M.; Carroll, K. S.; Filipovic, M. R.; Xian, M., Detection of Protein S-Sulfhydration by a Tag-Switch Technique. *Angew. Chem. Int. Ed.* **2014**, *53*, 575-581.
- (46) Dillon, K. M.; Matson, J. B., A Review of Chemical Tools for Studying Small Molecule Persulfides: Detection and Delivery. *ACS Chem. Biol.* **2021**, *16*, 1128-1141.
- (47) Powell, C. R.; Dillon, K. M.; Wang, Y.; Carrazzone, R. J.; Matson, J. B., A Persulfide Donor Responsive to Reactive Oxygen Species: Insights into Reactivity and Therapeutic Potential. *Angew. Chem. Int. Ed.* **2018**, *57*, 6324-6328.
- (48) Chaudhuri, A.; Venkatesh, Y.; Das, J.; Gangopadhyay, M.; Maiti, T. K.; Singh, N. D. P., One- and Two-Photon-Activated Cysteine Persulfide Donors for Biological Targeting. *J. Org. Chem.* **2019**, *84*, 11441-11449.
- (49) Artaud, I.; Galardon, E., A Persulfide Analogue of the Nitrosothiol Snap: Formation, Characterization and Reactivity. *ChemBioChem* **2014**, *15*, 2361-2364.
- (50) Wang, Y.; Dillon, K. M.; Li, Z.; Winckler, E. W.; Matson, J. B., Alleviating Cellular Oxidative Stress through Treatment with Superoxide-Triggered Persulfide Prodrugs. *Angew. Chem. Int. Ed.* **2020**, *59*, 16698-16704.
- (51) Fosnacht, K. G.; Cerda, M. M.; Mullen, E. J.; Pigg, H. C.; Pluth, M. D., Esterase-Activated Perthiocarbonate Persulfide Donors Provide Insights into Persulfide Persistence and Stability. *ACS Chem. Biol.* **2022**, *17*, 331-339.

- (52) Kawamura, S.; Nakabayashi, T.; Kitao, T.; Tsurugi, J., Aralkyl Hydrodisulfides. VI. The Reaction of Benzhydryl Hydrosulfide with Several Nucleophiles. *J. Org. Chem.* **1966**, *31*, 1985-1987.
- (53) Sharma, J.; Champagne, P. A., Benchmark of Density Functional Theory Methods for the Study of Organic Polysulfides. *J. Comp. Chem.* **2022**, *43*, 2131-2138.
- (54) Sharma, J.; Champagne, P. A., Mechanisms of the Reaction of Elemental Sulfur and Polysulfides with Cyanide and Phosphines. *Chem. - Eur. J.* **2023**, *29*, e202203906.
- (55) Ritchie, C. D.; Sager, W. F., An Examination of Structure-Reactivity Relationships. In *Progress in Physical Organic Chemistry*, 1964; pp 323-400.
- (56) Saund, S. S.; Sosa, V.; Henriquez, S.; Nguyen, Q. N. N.; Bianco, C. L.; Soeda, S.; Millikin, R.; White, C.; Le, H.; Ono, K., et al., The Chemical Biology of Hydropersulfides (RssH): Chemical Stability, Reactivity and Redox Roles. *Arch. Biochem. Biophys.* **2015**, *588*, 15-24.
- (57) Benavides, G. A.; Squadrito, G. L.; Mills, R. W.; Patel, H. D.; Isbell, T. S.; Patel, R. P.; Darley-Usmar, V. M.; Doeller, J. E.; Kraus, D. W., Hydrogen Sulfide Mediates the Vasoactivity of Garlic. *Proc. Natl. Acad. Sci.* **2007**, *104*, 17977-17982.
- (58) Amagase, H., Clarifying the Real Bioactive Constituents of Garlic. *J. Nutrition* **2006**, *136*, 716S-725S.
- (59) Liang, D.; Wu, H.; Wong, M. W.; Huang, D., Diallyl Trisulfide Is a Fast H<sub>2</sub>S Donor, but Diallyl Disulfide Is a Slow One: The Reaction Pathways and Intermediates of Glutathione with Polysulfides. *Org. Lett.* **2015**, *17*, 4196-4199.
- (60) Cai, Y.-R.; Hu, C.-H., Computational Study of H<sub>2</sub>S Release in Reactions of Diallyl Polysulfides with Thiols. *J. Phys. Chem. B* **2017**, *121*, 6359-6366.
- (61) Hughes, M. N.; Centelles, M. N.; Moore, K. P., Making and Working with Hydrogen Sulfide: The Chemistry and Generation of Hydrogen Sulfide in vitro and Its Measurement in vivo: A Review. *Free Radic. Biol. Med.* **2009**, *47*, 1346-1353.
- (62) Huang, G.-T.; Yu, J.-S. K., Enzyme Catalysis That Paves the Way for S-Sulphydration via Sulfur Atom Transfer. *J. Phys. Chem. B* **2016**, *120*, 4608-4615.
- (63) Landry, A. P.; Moon, S.; Kim, H.; Yadav, P. K.; Guha, A.; Cho, U.-S.; Banerjee, R., A Catalytic Trisulfide in Human Sulfide Quinone Oxidoreductase Catalyzes Coenzyme a Persulfide Synthesis and Inhibits Butyrate Oxidation. *Cell Chem. Biol.* **2019**, *26*, 1515-1525.e1514.
- (64) Cuevasanta, E.; Reyes, A. M.; Zeida, A.; Mastrogiiovanni, M.; De Armas, M. I.; Radi, R.; Alvarez, B.; Trujillo, M., Kinetics of Formation and Reactivity of the Persulfide in the One-Cysteine Peroxiredoxin from Mycobacterium Tuberculosis. *J. Biol. Chem.* **2019**, *294*, 13593-13605.
- (65) Pedre, B.; Dick, T. P., 3-Mercaptopyrivate Sulfurtransferase: An Enzyme at the Crossroads of Sulfane Sulfur Trafficking. *Biol. Chem.* **2021**, *402*, 223-237.
- (66) Zhao, Y.; Bhushan, S.; Yang, C.; Otsuka, H.; Stein, J. D.; Pacheco, A.; Peng, B.; Devarie-Baez, N. O.; Aguilar, H. C.; Lefer, D. J., et al., Controllable Hydrogen Sulfide Donors and Their Activity against Myocardial Ischemia-Reperfusion Injury. *ACS Chem. Biol.* **2013**, *8*, 1283-1290.
- (67) Kasamatsu, S.; Ida, T.; Koga, T.; Asada, K.; Motohashi, H.; Ihara, H.; Akaike, T., High-Precision Sulfur Metabolomics Innovated by a New Specific Probe for Trapping Reactive Sulfur Species. *Antioxid. Redox Signal.* **2021**, *34*, 1407-1419.

