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Current Opinion in Chemical Biology

Reactive sulfur species (RSS): persulfides, polysulfides, potential, and problems

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Sulfur is a crucial element in biology due to its unique properties and wide range of accessible oxidation states. This reactivity gives rise to the generation of reactive sulfur species (RSS), which have emerged as a diverse class of small molecules and functional groups with important roles in chemical biology and bioinorganic chemistry. In this review, we focus on basic properties of simple RSS, highlight recent insights into the interconnectivity of RSS with reactive nitrogen species, and discuss recent advances in methods for RSS detection and measurement. Finally, we highlight key reactivity considerations that must be taken into account when working with RSS and interpreting the outcomes of labeling methods for RSS and related species.

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Current Opinion in Chemical Biology 2018, **49**:1–8

This review comes from a themed issue on **Bioinorganic chemistry**

Edited by **Kyle M Lancaster**

For a complete overview see the Issue and the Editorial

Available online 19th September 2018

https://doi.org/10.1016/j.cbpa.2018.08.012

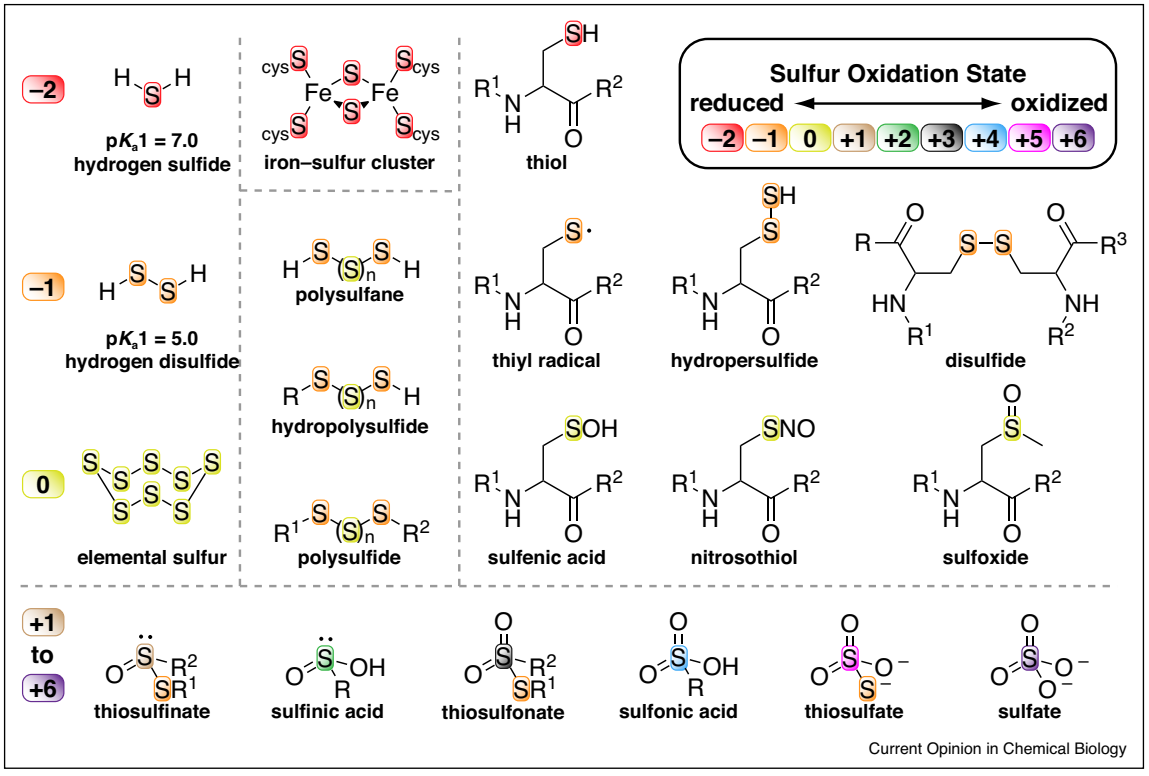
Introduction

Sulfur-containing compounds, known for their foul smell and toxicity, are essential to life due to the roles of reactive sulfur species (RSS) in cell signaling, redox homeostasis, and metabolic regulation [1••,2]. For example, hydrogen sulfide (H_2S) was likely a primary energy source for early life on Earth during the putative 'iron–sulfur world' [3]. In this anoxic era, RSS not only provided reducing equivalents for energy generation but also contributed to discrete iron–sulfur cluster formation due to the metallophilicity and well-defined coordination geometries of RSS. These ancient cofactors persist in modern biology as catalysts in important biosynthetic reactions, electron transporters in mitochondria, as well as many other functions [4,5].

Even after the appearance of dioxygen in Earth's atmosphere during the great oxidation event (~2.5 B years ago), sulfur has remained crucial to life due to its unique properties, including its wide range of accessible oxidation states ranging from –2 to +6 (Figure 1) [6]. RSS in the most reduced state (S^{2-}), such as H_2S and thiols, are good nucleophiles, especially when deprotonated, and are also good reducing agents ($E^{\circ\prime}(HS_2^-, H^+/2HS^-) = -0.230$ V [7•], $E^{\circ\prime}$(cystine/cysteine) = −0.245 V [8], both vs NHE). Note: In this review, 'H_2S' will be used as a generic term to encompass the equilibrium mixture of H_2S (~30%), HS^- (~70%), and S^{2-} (<0.1%) that exist in equilibrium in aqueous solutions at physiological pH. These S^{2-} species can undergo a one electron oxidation to the S^{1-} state to produce sulfhydryl ($HS^•$) or thiyl radicals ($RS^•$), which can recombine to form hydrogen disulfide (HSSH), related hydrodisulfides/persulfides (RSSH), or disulfides (RSSR). This redox chemistry is crucial for cellular redox homeostasis, which is in part maintained by the γ-Glu-Cys-Gly tripeptide glutathione (GSH) and its oxidized disulfide GSSG [2]. Further sulfur oxidation to the S^0 state generates species such as polysulfides, sulfenic acids (RSOH), or elemental sulfur (S_8) [9]. The term 'sulfane sulfur,' now common for describing many S^0-containing species, has the requirement that the S atom has 6 valence electrons, can tautomerize to a thiosulfoxide form (e.g., RSSH to RS(S)H), and is covalently bonded to two or more sulfur atoms (e.g., $RS(S)_nSR$) or to a S atom and an ionizable hydrogen (e.g., R-SSH). Further oxidized species, such as sulfinic acids (RSO_2H) and sulfonic acids ($RS(O)_2OH$), also play important roles in biology but are beyond the scope of this review.

The diverse redox landscape of RSS provides simple ways in which key chemical properties of RSS such as nucleophilicity, electrophilicity, pK_a, and bond strengths can be modulated by interactions with other redox-active systems, thus making RSS ideal for many roles in biological signaling. For example, H_2S, along with carbon monoxide (CO) and nitric oxide (NO), is now recognized as an endogenously-produced gaseous signaling molecule (gasotransmitter) [1••]. At low concentrations, H_2S exerts beneficial cardiovascular effects including cytoprotection, anti-inflammation, angiogenesis, and vasodilation [3]. The chemical mechanisms of H_2S signaling remain an active area of research, but three mechanisms that have received significant attention include cross-reactivity with NO, per/polysulfide formation, and reactions with metalloenzymes [3,10]. In this review, we will focus on

Figure 1



Sulfur oxidation states of selected, common, biologically-relevant RSS. This figure is adapted in part from Ref. [6].

recent advancements in RSS chemistry, studies at the interface of reactive sulfur/nitrogen species, as well as key considerations and challenges associated with RSS labeling and measurement.

NO/H_2S crosstalk

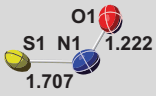
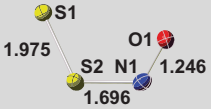
The biological interdependence of H_2S and NO in angiogenesis, vasodilation, and vascular remodeling suggests that, in addition to working together cooperatively, NO and H_2S might form hybrid S/N species that account for this observed 'cross-talk' [11–13]. Of such species, thionitrous acid (HSNO) and perthionitrite ($SSNO^-$) have emerged as two prominent hybrid species, although the activity, stability, and bioavailability of these species has also generated controversy [14,15,16•,17–19]. Drawing parallels to nitrosothiols, Filipovic and coworkers originally proposed HSNO as a primary S/N hybrid species, which was supported by mass spectrometry (MS), Fourier transform infrared (FTIR), and UV–visible (UV–vis) measurements [14] as well as thorough corroboration with other experimental and computational work [20,21]. By contrast, Feelisch and coworkers failed to observe HSNO by MS or ^{15}N nuclear magnetic resonance (NMR) spectroscopy when monitoring the reaction of H_2S with nitrosothiols in buffered aqueous solution, but instead observed transient formation of HSNO en route to $SSNO^-$ formation by UV–vis spectroscopy [15,16•,17]. Complicating stability considerations, Filipovic and coworkers later demonstrated that the PNP^+ (*bis*(triphenylphosphine)iminium) salt of $SSNO^-$ is not stable in water and readily reacts with thiols in organic solutions, suggesting that $SSNO^-$ generation may likewise be unstable in such biological contexts [18,19]. Further analysis of the characterization and stability of such species is provided in a recent review by Olabe and coworkers [10]. Adding a further level of complexity, HSNO and $SSNO^-$ have also been shown to interconvert through polysulfide-mediated reactions [1••,16•,22]. Despite these seemingly contradictory reports, one unifying theme is that both HSNO and $SSNO^-$ display environment-dependent stability and can be interconverted by other RSS species. An attractive hypothesis is that HSNO and $SSNO^-$ are merely easily-observable components of a more complicated pool of S/N hybrid species that can favor different speciation, and thus different reactivity pathways, under different conditions or in response to different stimuli (Table 1).

Persulfides and polysulfides

Polysulfides are known products of NO/H_2S crosstalk, and H_2S can react with certain RSS to form persulfides and polysulfides [1••]. Additionally, growing evidence suggests that H_2S signaling is connected to the post-translational oxidative modification of cysteine residues to form cysteine

Table 1

Summary of characterization data for HSNO, $SSNO^-$, and RSNO species

	HSNO	$SSNO^-$	RSNO
Proposed mechanism of formation [10,18]	$RSNO + H_2S \longrightarrow RSH + HSNO$	$HSNO + H_2S \longrightarrow HS_2^- + H^+ + HNO$ $RSNO + HS_2^- \longrightarrow RSH + SSNO^-$	
Solid-state structure (PNP^+ salt, Å)	 (SNO^-) [23]	 [18]	
S–N BDE (calcd, kcal mol^{-1})	27.7 (HSNO) 36.2 (SNO^-) [19]	16.0 (HSSNO) 22.1 ($SSNO^-$) [19]	~33 (Cys-NO) [24,25] 23.3–32.4 (Alkyl) [26]
ESI-MS (*m/z*)	63.9902 (63.9852, +, calcd) [14]	93.9427 (93.9427, –, calcd) [16•]	
UV–vis (λ_{max}, nm (ε_{max}, $M^{-1}\,cm^{-1}$))	338 (H_2O) [14] 323 (SNO^-, MeCN) [27]	409 [28], 412 [16•] (H_2O) 442 (MeCN) [18] 448 (3125, Acetone) [19]	335 (850), 543 (17) (Cys-NO, H_2O) [29,30] 336 (770), 544 (15) (GSNO, H_2O) [29,31]
^{15}N NMR (δ, ppm)	322 (D_2O) [14] 314 (Acetone-d_6) [19]	322 (D_2O) [10] 354 (Acetone-d_6) [19] 332 (THF-d_8) [22]	Cys-NO: 728 (3:1 D_2O:CD_3CN) [32] GSNO: 768 (D_2O) [33]
FTIR (ν_{NO}, cm^{-1})	1568 (H_2O) [14] 1596 (*cis*, Ar matrix) 1569 (*trans*, Ar matrix) [34]	1304 (KBr) [18]	Cys-NO: 1490 (ATR) GSNO: 1520 (ATR) [35]

persulfides [36,37]. This process has often been referred to as 'sulfhydration' in the literature but is more accurately termed 'persulfidation' because there is no hydration event involved in the underlying chemistry. In addition, we emphasize that although persulfidation is often associated with H_2S reactivity, H_2S cannot directly persulfidate cysteine residues because the sulfur atoms in both H_2S and cysteine are in the fully reduced −2 oxidation state. Thus, persulfidation requires an oxidation event to generate a source of S^{-1}.

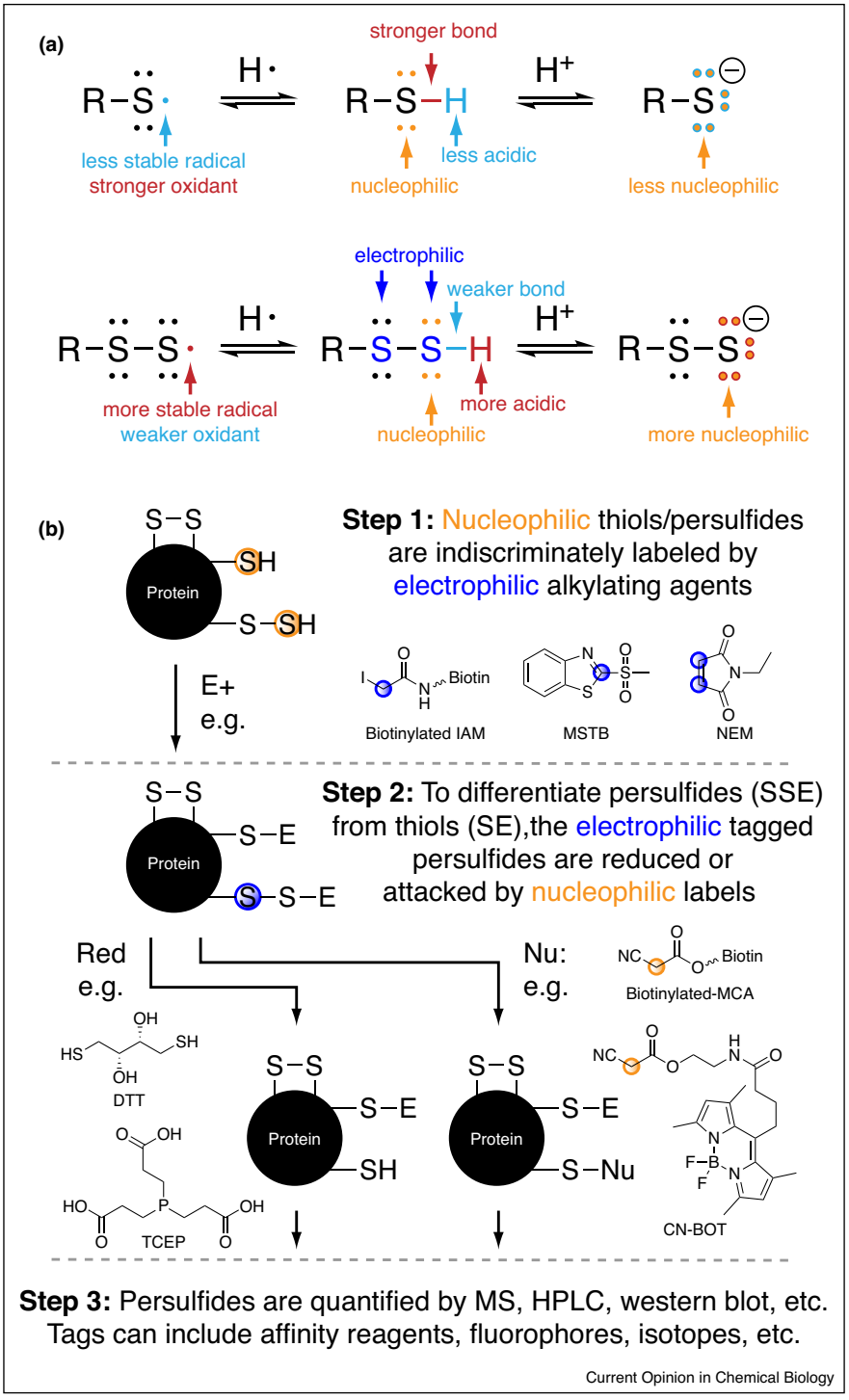
Once formed, persulfides are better nucleophiles, acids, and reductants than the corresponding thiols [38]. For example, deprotonated RSS^- anions are more nucleophilic than analogous RS^- thiolate anions due to the α-effect, which stems from lone-pair lone-pair repulsion between the adjacent sulfur atoms [39]. Unlike thiols, which are exclusively nucleophilic, persulfides are ambiphilic. Both sulfur atoms are electrophilic in neutral protonated persulfides, whereas in deprotonated anionic persulfides the terminal sulfur atom is nucleophilic and the non-terminal sulfur atom is electrophilic. Similarly, RSSH species are more acidic than RSH species by ~2 pK_a units and the S–H bond in persulfides is weaker than in thiols, making $RSS^•$ species more stable than $RS^•$ radicals. Consequently, persulfides are better one-electron reductants than thiols or H_2S ($E^{\circ\prime}(RSS^•/RSS^-) = 0.68$ V; $E^{\circ\prime}(RS^•, H^+/RSH) = 0.96$ V; $E^{\circ\prime}(S^{•-}, H^+/HS^-) = 0.91$ V) [1••]. The oxidative persulfidation of thiol residues results, therefore, in the formation of better reductants, which suggests a possible role of persulfides as protective modifications to preserve thiol fidelity under conditions of oxidative stress.

Echoing the parallels between thiol and persulfide reactivity, most aspects of inorganic polysulfide reactivity, including nucleophilicity, acidity, and redox promiscuity, are also enhanced in comparison to H_2S (Figure 2a) [38]. Thus, the greater reactivity of per/polysulfides over H_2S has spurred the intriguing hypothesis that such per/polysulfides are actually responsible for RSS biochemical signaling [38]. Although the enhanced reactivity in comparison to H_2S makes such species more versatile signaling agents, it also makes understanding their reactivity more challenging. For example, inorganic polysulfides rapidly undergo disproportionation and dissociation reactions to form complicated mixtures of species in organic solution, and available sources of Na_2S_2, Na_2S_3, and Na_2S_4 rapidly generate equilibrium mixtures in aqueous solution [40,41]. Prominent strategies of tagging polysulfide species with alkylating agents are discussed below, and we refer interested readers to recent in-depth reviews on polysulfide detection by Xian [42] and Banerjee [1••].

Tagging methods for per/polysulfides

A current challenge in expanding our understanding of the biological roles of per/polysulfide species is the development, application, and interpretation of reliable methods for their detection and quantification. Most fluorescent probes, trapping methods, and tagging assays for per/polysulfides make use of the presence of both nucleophilic and electrophilic sulfur atoms to enable selective detection (Figure 2b). We note that the reagents and detection methods for per/polysulfide detection have evolved significantly in the past few years and that conclusions from individual studies should be analyzed carefully on the basis of which methods were used.

Figure 2



(a) Differences in chemical properties between thiol and persulfide species. **(b)** Schematic depicting selected labeling methods for measuring per/polysulfide species.

The initial report demonstrating protein persulfidation used a modified biotin switch method for persulfide analysis, which was based on the incorrect assumption that persulfides would not react with the electrophilic thiol-labeling reagent *S*-methylmethanethiosulfonate (MMTS). More useful modifications of this method have focused two-step 'tag-switch' assay in treatment with an electrophilic trapping agent, such as methylsulfonyl

benzothiazol (MSBT), to label both thiol and persulfide residues. The key second step is cleavage of the electrophilic disulfides formed from persulfide labeling with a nucleophilic labeling reagent, such as biotinylated methyl cyanate (MCA), which enables differentiation of the labeled thiol and persulfide residues after standard pull-down methods [43,44]. Importantly, MCA and related cyanoacetic acid-derived nucleophilic labeling reagents are unreactive towards other electrophilic RSS such as nitrosothiols. This protocol has been further developed to use nucleophilic fluorescent tags, which allow visualization of persulfidated residues in fixed cells by fluorescence microscopy [45•].

As a whole, exploiting the ambiphilic nature of persulfides allows for the selective detection of persulfides, even after initial indiscriminate electrophilic labeling of thiols and persulfides because the redox and electrophilic properties of the resultant disulfide atoms allow for further differentiation [1••,46]. Building from this two-step persulfide detection approach, Hatzoglou [47], Pfeilschifter [48], and Nagy [49] have all recently reported methods that utilize an initial global thiol and persulfide alkylation step, followed by a reduction step to differentiate the labeled thiols and persulfides. Although the identity of the electrophilic and reductive reagents vary between these assays, these approaches appear to provide positive improvements that enable persulfide measurement by different affinity tags, gel electrophoresis, and common MS methods.

Challenges in measuring polysulfide distributions and the Curtin–Hammett principle

Many of the above methods for per/polysulfide labeling and quantification rely on alkylation methods to 'freeze' per/polysulfide species within complex product distributions, and this snapshot is then used to draw conclusions [42,50–53]. It is well established, however, that many of these sulfur-rich RSS are in a dynamic equilibrium and rapidly interconvert [40,41]. This interconversion is particularly prevalent for polysulfides, as polysulfide interconversion is significantly faster than the electrophilic labeling, which means that such systems are under Curtin–Hammett control. Importantly, the observed polysulfide distributions in these systems reflect the relative difference in activation barriers for electrophilic labeling of the rapidly-interconverting polysulfides rather than the actual distribution of polysulfides in the system.

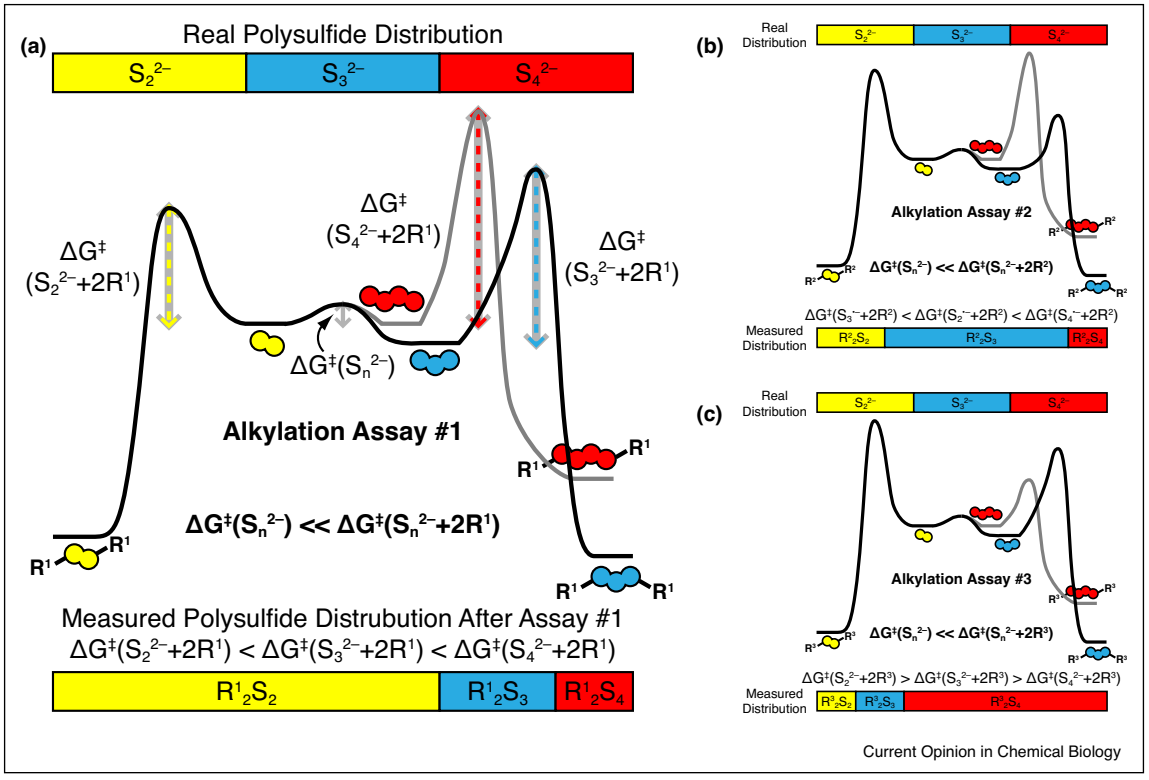
Recent work by Nagy and coworkers using complementary analytical methods confirmed experimentally that the electrophilic labeling of polysulfides is indeed under Curtin–Hammett control [54••]. For example, when cysteine and glutathione hydropolysulfides were generated *in situ* and quenched with alkylating agents such as iodoacetamide (IAM), *N*-ethylmaleimide (NEM), and monobromobimane (MBB), only short polysulfide adducts were observed with NEM labeling whereas longer chain adducts were formed with IAM or MBB. Similarly, alkylation of inorganic polysulfides in aqueous solutions gave significantly different distributions of capped polysulfides, with NEM labeling affording only short (R-$S_{<4}$-R) species, whereas IAM labeling of identical solutions provided longer-chain (R-$S_{<7}$-R) adducts. Furthermore, treatment of IAM-capped polysulfides with NEM resulted in polysulfide cleavage, which confirmed recent reports that NEM may react directly with polysulfur chains [55], thus highlighting that organic polysulfides, either generated under the labeling conditions or present in the initial solutions, are likely modified directly by the labeling reagents.

Further supporting the premise that polysulfide alkylation is under Curtin–Hammett control, the observed distribution of alkylated polysulfides were compared to real-time polysulfide distribution by exploiting the fact that different polysulfides exhibit different UV–vis absorbance maxima in organic solution (e.g.: in acetone $S_2^{\bullet-}$ (426 nm), $S_3^{\bullet-}$ (610 nm), and $S_4^{\bullet-}$ (490 nm) [54••]) [56–58]. Different mixtures of inorganic polysulfides were generated by treating S_8 with varying equivalents of NBu_4SH [54••]. Although the UV–vis spectra demonstrated that different polysulfide distributions were present in solution, electrophilic trapping of these different distributions with benzyl chloride [59] resulted in identical alkylated product distributions when measured by NMR spectroscopy. Similar results were observed in ESI-MS studies of aqueous solutions of polysulfides quenched by IAM, where $S_3^{\bullet-}$ was favored in the absence of IAM but $S_2^{\bullet-}$ was favored after the IAM quench. Taken together, these complementary studies confirm that these common alkylating protocols operate under Curtin–Hammett control, meaning that the barriers of interconversion between the polysulfide species are significantly lower than that of any reaction between polysulfides and the trapping reagent (Figure 3). These results suggest that reporting the total sulfane sulfur content, obtained by adding all of the per/polysulfide species measured, is a more robust observation rather than reporting exact per/polysulfide distributions.

Conclusions and future directions

Reactive sulfur species offer an exciting class of molecules with broad-reaching activity in chemical biology and redox biochemistry. The enhanced chemical reactivity of per/polysulfides, when compared to the parent thiols and H_2S, draws parallels to the classical exchange between Annie Oakley and Frank Butler in Irving Berlin's *Annie Get Your Gun*: 'Anything you can do I can do better; I can do anything better than you.' Although the heightened activity of RSS provides an attractive platform for sulfur-related signaling, key chemical needs remain in understanding this complex

Figure 3



Representation of hypothetical reaction coordinate diagrams for electrophilic labeling of polysulfide distributions under Curtin–Hammett control. Under these conditions, the measured distributions do not reflect true equilibrium distributions because the rate-determining step is the labeling rather than the equilibration event. Therefore, the observed labeled polysulfide distributions reflect the activation barrier for labeling different polysulfides rather than the actual distributions of polysulfides. *Note*: the distributions shown in this figure are illustrative and do not reflect actual polysulfide distribution data.

biological activity. Basic investigations into per/polysulfide formation, interconversion, stability, and reactivity with other (in)organic molecules under physiologically-relevant conditions are needed. In addition, new insights into the differential reactivity of small molecule and protein per/polysulfides, as well as reactivity differences in solvent-accessible versus buried RSS, are paramount. Furthermore, expanded and refined chemical methods for detecting, quantifying, and/or delivering RSS will enable new investigations and insights not possible with currently-available technologies. Finally, we stress that interpretations of biological outcomes of RSS chemistry and signaling need to be made in the context of the limitations of the experimental methods and tools used in elucidating new activities.

Conflict of interest statement

Nothing declared.

Acknowledgments

Our work related to the bioorganic and bioinorganic chemistry of reactive sulfur species is supported by the NSF (CHE-1454747) and the Dreyfus Foundation.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as

- • of special interest
- •• of outstanding interest

1. •• Filipovic MR, Zivanovic J, Alvarez B, Banerjee R: **Chemical biology of H_2S signaling through persulfidation**. *Chem Rev* 2018, **118**:1253-1337.
An exhaustive review on the chemical and biological properties of H_2S and persulfides.

2. Gruhlke MC, Slusarenko AJ: **The biology of reactive sulfur species (RSS)**. *Plant Physiol Biochem* 2012, **59**:98-107.

3. Olson KR, Straub KD: **The role of hydrogen sulfide in evolution and the evolution of hydrogen sulfide in metabolism and signaling**. *Physiology* 2016, **31**:60-72.

4. Braymer JJ, Lill R: **Iron–sulfur cluster biogenesis and trafficking in mitochondria**. *J Biol Chem* 2017, **292**:12754-12763.

5. Cardenas-Rodriguez M, Chatzi A, Tokatlidis K: **Iron–sulfur clusters: from metals through mitochondria biogenesis to disease**. *J Biol Inorg Chem* 2018, **23**:509-520.

6. Mishanina TV, Libiad M, Banerjee R: **Biogenesis of reactive sulfur species for signaling by hydrogen sulfide oxidation pathways**. *Nat Chem Biol* 2015, **11**:457-464.

7. • Koppenol WH, Bounds PL: **Signaling by sulfur-containing molecules. Quantitative aspects**. *Arch Biochem Biophys* 2017, **617**:3-8.

Presents thermodynamic properties of the conversion between various RSS, and comments on the feasibility of such species to be relevant to biological signaling.

8. Millis KK, Weaver KH, Rabenstein DL: **Oxidation/reduction potential of glutathione**. *J Org Chem* 1993, **58**:4144-4146.

9. Pan J, Carroll KS: **Persulfide reactivity in the detection of protein S-sulfhydration**. *ACS Chem Biol* 2013, **8**:1110-1116.

10. Marcolongo JP, Zeida A, Slep LD, Olabe JA: **Thionitrous acid/thionitrite and perthionitrite intermediates in the "crosstalk" of NO and H_2S**. In *Inorganic Reaction Mechanisms.* Edited by van Eldik R, Hubbard CD. Academic Press; 2017:277-309. Advances in Inorganic Chemistry, vol 70.

11. Hosoki R, Matsuki N, Kimura H: **The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide**. *Biochem Biophys Res Commun* 1997, **237**:527-531.

12. Zhao W, Zhang J, Lu Y, Wang R: **The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener**. *EMBO J* 2001, **20**:6008-6016.

13. Whiteman M, Li L, Kostetski I, Chu SH, Siau JL, Bhatia M, Moore PK: **Evidence for the formation of a novel nitrosothiol from the gaseous mediators nitric oxide and hydrogen sulphide**. *Biochem Biophys Res Commun* 2006, **343**:303-310.

14. Filipovic MR, Miljkovic J, Nauser T, Royzen M, Klos K, Shubina T, Koppenol WH, Lippard SJ, Ivanovic-Burmazovic I: **Chemical characterization of the smallest S-nitrosothiol, HSNO; cellular cross-talk of H_2S and S-nitrosothiols**. *J Am Chem Soc* 2012, **134**:12016-12027.

15. Cortese-Krott MM, Fernandez BO, Santos JL, Mergia E, Grman M, Nagy P, Kelm M, Butler A, Feelisch M: **Nitrosopersulfide ($SSNO^-$) accounts for sustained NO bioactivity of S-nitrosothiols following reaction with sulfide**. *Redox Biol* 2014, **2**:234-244.

16. • Cortese-Krott MM, Kuhnle GG, Dyson A, Fernandez BO, Grman M, DuMond JF, Barrow MP, McLeod G, Nakagawa H, Ondrias K *et al.*: **Key bioactive reaction products of the NO/H_2S interaction are S/N-hybrid species, polysulfides, and nitroxyl**. *Proc Natl Acad Sci U S A* 2015, **112**:E4651-4660.
Reviews the chemistry and characterization of nitrosothiols, HSNO, and SSNO–.

17. Cortese-Krott MM, Butler AR, Woollins JD, Feelisch M: **Inorganic sulfur–nitrogen compounds: from gunpowder chemistry to the forefront of biological signaling**. *Dalton Trans* 2016, **45**:5908-5919.

18. Wedmann R, Zahl A, Shubina TE, Durr M, Heinemann FW, Bugenhagen BE, Burger P, Ivanovic-Burmazovic I, Filipovic MR: **Does perthionitrite $SSNO^-$ account for sustained bioactivity of NO? A (bio)chemical characterization**. *Inorg Chem* 2015, **54**:9367-9380.

19. Wedmann R, Ivanovic-Burmazovic I, Filipovic MR: **Nitrosopersulfide ($SSNO^-$) decomposes in the presence of sulfide, cyanide or glutathione to give HSNO/SNO^-: consequences for the assumed role in cell signalling**. *Interf Focus* 2017, **7**:20160139.

20. Ivanova LV, Anton BJ, Timerghazin QK: **On the possible biological relevance of HSNO isomers: a computational investigation**. *Phys Chem Chem Phys* 2014, **16**:8476-8486.

21. Nava M, Martin-Drumel MA, Lopez CA, Crabtree KN, Womack CC, Nguyen TL, Thorwirth S, Cummins CC, Stanton JF, McCarthy MC: **Spontaneous and selective formation of HSNO, a crucial intermediate linking H_2S and nitroso chemistries**. *J Am Chem Soc* 2016, **138**:11441-11444.

22. Bailey TS, Henthorn HA, Pluth MD: **The intersection of NO and H_2S: persulfides generate NO from nitrite through polysulfide formation**. *Inorg Chem* 2016, **55**:12618-12625.

23. Victor E: *Reactions of S-nitrosothiols with biomimetic iron complexes and other transition metals*. Massachusetts Institute of Technology; 2014. PhD thesis.

24. Baciu C, Gauld JW: **An assessment of theoretical methods for the calculation of accurate structures and S–N bond dissociation energies of S-nitrosothiols (RSNOs)**. *J Phys Chem A* 2003, **107**:9946-9952.

25. Koppenol WH: **Nitrosation, thiols, and hemoglobin: energetics and kinetics**. *Inorg Chem* 2012, **51**:5637-5641.

26. Bartberger MD, Mannion JD, Powell SC, Stamler JS, Houk KN, Toone EJ: **S–N dissociation energies of S-nitrosothiols: on the origins of nitrosothiol decomposition rates**. *J Am Chem Soc* 2001, **123**:8868-8869.

27. Seel F, Kuhn R, Simon G, Wagner M: **PNP-perthionitrit und PNP-monothionitrit/PNP-perthionitrite and PNP-monothionitrite**. *Z Naturforsch B* 1985, **40**:1607-1617.

28. Seel F, Wagner M: **Über die umsetzung von sulfiden mit stickstoffmonoxid in wäßrigen lösungen**. *Z Anorg Allg Chem* 1988, **558**:189-192.

29. Szaciłowski K, Stasicka Z: ***S*-nitrosothiols: materials, reactivity and mechanisms**. *Prog React Kinet Mech* 2001, **26**:1-58.

30. Komiyama T, Fujimori K: **Kinetic studies of the reaction of S-nitroso-L-cysteine with L-cysteine**. *Bioorg Med Chem Lett* 1997, **7**:175-180.

31. Goldstein S, Czapski G: **Mechanism of the nitrosation of thiols and amines by oxygenated NO solutions: the nature of the nitrosating intermediates**. *J Am Chem Soc* 1996, **118**:3419-3425.

32. Kun W, Yongchun H, Wei Z, Ksebati MB, Ming X, Cheng J-P, Wang PG: **^{15}N NMR and electronic properties of S-nitrosothiols**. *Bioorg Med Chem Lett* 1999, **9**:2897-2902.

33. Gao Y, Dai Y, Wu G: **Solid-state ^{15}N and ^{17}O NMR studies of S-nitrosothiols**. *J Phys Chem B* 2017, **121**:7311-7317.

34. Nonella M, Huber JR, Ha TK: **Photolytic preparation and isomerization of thionyl imide, thiocyanic acid, thionitrous acid, and nitrogen hydroxide sulfide in an argon matrix: an experimental and theoretical study**. *J Phys Chem* 1987, **91**:5203-5209.

35. Kogelheide F, Kartaschew K, Strack M, Baldus S, Metzler-Nolte N, Havenith M, Awakowicz P, Stapelmann K, Lackmann J-W: **FTIR spectroscopy of cysteine as a ready-to-use method for the investigation of plasma-induced chemical modifications of macromolecules**. *J Phys D: Appl Phys* 2016, **49**:084004.

36. Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK, Barrow RK, Yang G, Wang R, Snyder SH: **H_2S signals through protein S-sulfhydration**. *Sci Signal* 2009, **2**:ra72.

37. Nagy P: **Mechanistic chemical perspective of hydrogen sulfide signaling**. In *Methods in Enzymology*. Edited by Cadenas E, Packer L. Academic Press; 2015:3-29. Hydrogen Sulfide in Redox Biology, Part A, vol 554.

38. Fukuto JM, Ignarro LJ, Nagy P, Wink DA, Kevil CG, Feelisch M, Cortese-Krott MM, Bianco CL, Kumagai Y, Hobbs AJ *et al.*: **Biological hydropersulfides and related polysulfides — a new concept and perspective in redox biology**. *FEBS Lett* 2018, **592**:2140-2152.

39. Jencks WP, Carriuolo J: **Reactivity of nucleophilic reagents toward esters**. *J Am Chem Soc* 1960, **82**:1778-1786.

40. Steudel R: **Inorganic polysulfides S_n^{2-} and radical anions $S_n^{\cdot-}$**. In *Elemental Sulfur and Sulfur-Rich Compounds II.* Edited by Steudel R. Springer; 2003:127-152.

41. Chivers T, Elder PJ: **Ubiquitous trisulfur radical anion: fundamentals and applications in materials science, electrochemistry, analytical chemistry and geochemistry**. *Chem Soc Rev* 2013, **42**:5996-6005.

42. Liu H, Radford MN, Yang CT, Chen W, Xian M: **Inorganic hydrogen polysulfides: chemistry, chemical biology and detection**. *Br J Pharmacol* 2018 http://dx.doi.org/10.1111/bph.14330.

43. Zhang D, Macinkovic I, Devarie-Baez NO, Pan J, Park CM, Carroll KS, Filipovic MR, Xian M: **Detection of protein S-sulfhydration by a tag-switch technique**. *Angew Chem Int Ed* 2014, **53**:575-581.

44. Park C-M, Macinkovic I, Filipovic MR, Xian M: **Use of the "tag-switch" method for the detection of protein *S*-sulfhydration**. In *Methods in Enzymology*. Edited by Cadenas E, Packer L. Academic Press; 2015:39-56. Hydrogen Sulfide in Redox Biology, Part B, vol 555.

45. • Wedmann R, Onderka C, Wei S, Szijarto IA, Miljkovic JL, Mitrovic A, Lange M, Savitsky S, Yadav PK, 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.
Uses an advancement in 'tag-switch' detection of persulfides to identify the thioredoxin system as a regulator of intracellular persulfidation.

46. Park CM, Weerasinghe L, Day JJ, Fukuto JM, Xian M: **Persulfides: current knowledge and challenges in chemistry and chemical biology**. *Mol Biosyst* 2015, **11**:1775-1785.

47. Gao XH, Krokowski D, Guan BJ, Bederman I, Majumder M, Parisien M, Diatchenko L, Kabil O, Willard B, Banerjee R *et al.*: **Quantitative H_2S-mediated protein sulfhydration reveals metabolic reprogramming during the integrated stress response**. *Elife* 2015, **4**:e10067.

48. Longen S, Richter F, Kohler Y, Wittig I, Beck KF, Pfeilschifter J: **Quantitative persulfide site identification (qPerS-SID) reveals protein targets of H_2S releasing donors in mammalian cells**. *Sci Rep* 2016, **6**:29808.

49. Doka E, Pader I, A: Johansson K, Cheng Q, Ballago K, Prigge JR, Pastor-Flores D, Dick TP, Schmidt EE *et al.*: **A novel persulfide detection method reveals protein persulfide- and polysulfide-reducing functions of thioredoxin and glutathione systems**. *Sci Adv* 2016, **2**:e1500968.

50. Kamyshny A, Goifman A, Gun J, Rizkov D, Lev O: **Equilibrium distribution of polysulfide ions in aqueous solutions at 25 °C: a new approach for the study of polysulfides' equilibria**. *Environ Sci Technol* 2004, **38**:6633-6644.

51. Filpponen I, Guerra A, Hai A, Lucia LA, Argyropoulos DS: **Spectral monitoring of the formation and degradation of polysulfide ions in alkaline conditions**. *Ind Eng Chem Res* 2006, **45**:7388-7392.

52. Kimura Y, Toyofuku Y, Koike S, Shibuya N, Nagahara N, Lefer D, Ogasawara Y, Kimura H: **Identification of H_2S_3 and H_2S produced by 3-mercaptopyruvate sulfurtransferase in the brain**. *Sci Rep* 2015, **5**:14774.

53. Miyamoto R, Koike S, Takano Y, Shibuya N, Kimura Y, Hanaoka K, Urano Y, Ogasawara Y, Kimura H: **Polysulfides (H_2S_n) produced from the interaction of hydrogen sulfide (H_2S) and nitric oxide (NO) activate TRPA1 channels**. *Sci Rep* 2017, **7**:45995.

54. •• Bogdandi V, Ida T, Sutton TR, Bianco C, Ditroi T, Koster G, Henthorn HA, Minnion M, Toscano JP, van der, Vliet A *et al.*: **Speciation of reactive sulfur species and their reactions with alkylating agents: do we have any clue about what is present inside the cell?** *Br J Pharmacol* 2018 http://dx.doi.org/10.1111/bph.14394.
Establishes through multiple corraborative experiments that current methods for alkylating per/polysulfide species operate under Curtin–Hammett control. Thus, measured specific polysulfide distributions derived from such assays do not reflect real-time polysulfide distributions.

55. Akaike T, Ida T, Wei FY, Nishida M, Kumagai Y, Alam MM, Ihara H, Sawa T, Matsunaga T, Kasamatsu S *et al.*: **Cysteinyl-tRNA synthetase governs cysteine polysulfidation and mitochondrial bioenergetics**. *Nat Commun* 2017, **8**:1177.

56. Clark RJH, Cobbold DG: **Characterization of sulfur radical anions in solutions of alkali polysulfides in dimethylformamide and hexamethylphosphoramide and in the solid state in ultramarine blue, green, and red**. *Inorg Chem* 1978, **17**:3169-3174.

57. Paris J, Plichon V: **Electrochemical reduction of sulfur in dimethylacetamide**. *Electrochim Acta* 1981, **26**:1823-1829.

58. Chivers T, Drummond I: **Characterization of the trisulfur radical anion $S_3^{\bullet-}$ in blue solutions of alkali polysulfides in hexamethylphosphoramide**. *Inorg Chem* 1972, **11**:2525-2527.

59. Ahrika A, Robert J, Anouti M, Paris J: **Nucleophilic substitution of alkyl halides by electrogenerated polysulfide ions in N,N-dimethylacetamide**. *Acta Chem Scand* 1999, **53**:513-520.