Redox Biochemistry and Chemistry 10 (2024) 100039

Contents lists available at ScienceDirect

Redox Biochemistry and Chemistry

journal homepage: www.journals.elsevier.com/redox-biochemistry-and-chemistry

Reaction of peroxynitrite with thiols, hydrogen sulfide and persulfides

Madia Trujillo [a,b], Ernesto Cuevasanta [b,c], Lucía Turell [b,d], Dayana Benchoam [b,d], Gerardo Ferrer-Sueta [b,c], Ari Zeida [a,b], Celia Quijano [a,b], Sebastián Carballal [a,b], Rafael Radi [a,b], Beatriz Alvarez [b,d,*]

[a] Departamento de Bioquímica, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
[b] Centro de Investigaciones Biomédicas (CEINBIO), Universidad de la República, Montevideo, Uruguay
[c] Laboratorio de Fisicoquímica Biológica, Instituto de Química Biológica, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay
[d] Laboratorio de Enzimología, Instituto de Química Biológica, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

ARTICLE INFO

Keywords:
Peroxynitrite
Thiols
Oxidation
Nitrogen dioxide
Carbonate radical
Hydrogen sulfide
Persulfide
Sulfenic acid
Thiyl radical

ABSTRACT

Three decades of research on the biochemistry of peroxynitrite (ONOOH/$ONOO^-$) have established that this stealthy oxidant is formed in biological systems, and that its main targets are carbon dioxide (CO_2), metalloproteins and thiols (RSH). Peroxynitrous acid reacts directly with thiols (precisely, with thiolates, RS^-), forming sulfenic acids (RSOH). In addition, the free radicals derived from peroxynitrite, mainly carbonate radical anion ($CO_3^{\bullet-}$) and nitrogen dioxide ($NO_2^\bullet$) formed from the reaction of peroxynitrite anion with CO_2, oxidize thiols to thiyl radicals ($RS^\bullet$). These two pathways are under kinetic competition. The primary products of thiol oxidation can follow different decay routes; sulfenic acids usually react with other thiols forming disulfides, while thiyl radicals can react with oxygen, with other thiols and with other reductants such as ascorbic acid. Peroxynitrite is also able to oxidize hydrogen sulfide (H_2S/HS^-) and persulfides ($RSSH/RSS^-$). Among the different biological thiols, peroxiredoxins stand out as main peroxynitrite reductases due to their very high rate constants of reaction with peroxynitrite together with their abundance. Rooted in kinetic concepts, evidence is emerging for the role of peroxiredoxins in peroxynitrite detoxification, with potential implications in diseases in which peroxynitrite is involved.

1. Peroxynitrite is a fleeting biological oxidant

Peroxynitrite (ONOOH/$ONOO^-$)[1] caught the attention of biomedical researchers in the beginning of the 1990s [1,2], although it had been known to chemists for decades. In the following ~35 years, peroxynitrite formation in biological systems was established and its biochemistry elucidated, contributing to evidence roles for peroxynitrite as cytotoxic effector and signaling mediator.

Peroxynitrite is mainly formed in biological systems through the diffusion-controlled reaction between superoxide ($O_2^{\bullet-}$) and nitric oxide ($NO^\bullet$) radicals [1,3] (Equation (1) and Fig. 1).

$$NO^\bullet + O_2^{\bullet-} \rightarrow ONOO^- \quad (1)$$

Other potential sources have been proposed [4,5], but their contribution appears to be minimal. On kinetic grounds, the reaction between superoxide and nitric oxide is unavoidable even when these radicals can undergo alternative reactions and their concentration is low [6,7].

Peroxynitrous acid is a weak acid with a pK_a of 6.8, and thus both base ($ONOO^-$) and acid (ONOOH) exist in comparable concentrations close to neutral pH. While ONOOH is relatively unstable and decays spontaneously even in inert chemical environments, its conjugate base $ONOO^-$ is relatively stable and can be stored in alkaline aqueous solutions.

The biological decay of peroxynitrite is dominated by a number of selenol and thiol peroxidases (RSe^-/RS^- in Fig. 1), that have extremely fast reactions with ONOOH and can vastly outcompete almost any other intracellular target [8–11]. As depicted in Fig. 1, the only potential

* Corresponding author. Laboratorio de Enzimología, Instituto de Química Biológica, Facultad de Ciencias, Universidad de la República, Iguá 4225, 11400, Montevideo, Uruguay.
E-mail address: beatriz.alvarez@fcien.edu.uy (B. Alvarez).

[1] The term "peroxynitrite" is used in this text for the mixture of peroxynitrite anion ($ONOO^-$) and its conjugated acid, peroxynitrous acid (ONOOH, pK_a 6.8). The IUPAC recommended names are oxoperoxonitrate (1−) and hydrogen oxoperoxonitrate, respectively.

https://doi.org/10.1016/j.rbc.2024.100039
Received 9 April 2024; Received in revised form 9 July 2024; Accepted 8 August 2024
Available online 8 August 2024

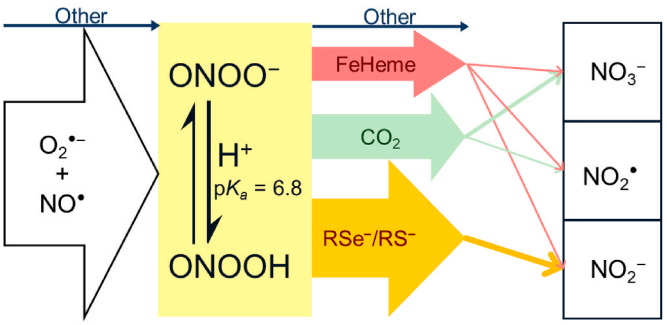

Fig. 1. Main source and sinks of peroxynitrite. Biological formation of peroxynitrite is mostly due to the reaction between superoxide and nitric oxide radicals. The consumption of peroxynitrite is diverse and dependent on the biological compartment involved. The width of the arrows gives a rough idea of the prevalence of each reaction. Thiol and selenol peroxidases (RS^- and RSe^-) are the preferred targets in most intracellular spaces, whereas CO_2 appears as a prevailing consumer in extracellular milieu. In some specialized compartments, rich in heme proteins, the reaction with heme iron can be a competitive target. There are other routes for peroxynitrite consumption, but they cannot compete kinetically with the prevailing reactions shown.

competitors of the peroxidases are carbon dioxide (CO_2), that reacts with $ONOO^-$ [12], and several iron heme proteins that can reach very high concentration in specific cells and cellular compartments [13–15]. There are numerous other potential routes of peroxynitrite consumption, from spontaneous homolysis [1] to reaction with low molecular weight (LMW) thiols [2,16] and a long list of reductants [17]. However, most of these reactions cannot compete kinetically with the reactions with thiol and selenol peroxidases, carbon dioxide and heme proteins. The reactions of peroxynitrite with the peroxidases will be discussed below in Sections 4 and 7, and that with carbon dioxide will be discussed in Sections 4 and 8. The reactions with heme proteins are quite diverse and beyond the scope of this article; the reader is referred to Ref. [17] for a detailed discussion. In any case, these three main routes of decay are fast, implying that peroxynitrite never accumulates in biological systems beyond very low steady-state concentrations, estimated in the low nanomolar range [18].

The main nitrogen-containing products of peroxynitrite reactions are nitrate (NO_3^-, isomerization), nitrite (NO_2^-, two-electron reduction), and nitrogen dioxide ($NO_2^{\bullet}$, homolytic cleavage or one-electron reduction). Nitrogen dioxide can cause further oxidation and nitration of different biomolecules. In addition, the reaction of peroxynitrite with carbon dioxide forms the oxidizing carbonate radical anion ($CO_3^{\bullet-}$) as well as nitrogen dioxide [19], and the reaction with some heme proteins can form oxidizing species such as oxo-ferryl compounds (Fe(IV)═O), sometimes accompanied by porphyrin or amino acid radicals [13–15]. Thus, the reaction with carbon dioxide and certain heme proteins diverts part of the oxidizing properties to secondary species that can exert downstream effects.

The low steady-state concentrations of peroxynitrite in biological systems complicate the study of its biochemistry. Another drawback is that its existence is very hard to trace. Most species derived from peroxynitrite can be formed from other sources. For example, nitrogen dioxide can be formed from the oxidation of nitrite with heme peroxidases and from nitric oxide autooxidation. Therefore, extremely careful and detailed studies need to be made before invoking peroxynitrite as effector in any given process or condition; the studies should include the modulation of the production or consumption of the superoxide and nitric oxide precursors. Protein tyrosine nitration is a good approximation to a peroxynitrite footprint, and as such has been used for a long time, although peroxynitrite-independent nitration pathways also exist [17]. In recent years, boronic acid probes are increasingly being employed to assess peroxynitrite formation in biological systems. While they were initially conceived for the detection of hydrogen peroxide, they react with peroxynitrite with rate constants 10^6 times higher [20,21]. Further discussions about peroxynitrite detection can be read in Refs. [22,23].

2. Thiols ionize to nucleophilic thiolates

Most biological thiols (RSH/RS^-)[2] are derived from the amino acid cysteine, and the most interesting ones are cysteine-containing proteins. To describe the reactions of biological thiols, it is extremely important to remember that we are actually describing the chemistry of thiolates (RS^-). When protonated, thiols (RSH) are relatively unreactive and nonpolar but their conjugate bases (RS^-) can be strong nucleophiles. So, we are dealing with a very concise group of molecules (deprotonated derivatives of cysteine) that have wide ranges of reactivity in several dimensions.

The most obvious dimension resides in the acid-base properties of the thiols, since deprotonation is a requisite for reactivity. Cysteine as a free amino acid has a pK_a of 8.3, but when forming part of proteins, and especially when being part of enzyme active sites, the range of pK_as is very wide, at least from 2.5 in the case of pig glutaredoxin [24] to 10.5 in *Escherichia coli* cDsbD [25], *i.e.*, eight orders of magnitude variations in acidity, since pK_a is a logarithmic expression. The pK_a of the thiol/thiolate pair causes a species distribution with pH that affects the measured values of reaction rate constants. Thus, in the literature there are values of "apparent rate constants" that are measured at a specific pH and consider the concentration of all species (thiol + thiolate), and "pH-independent rate constants" that are not measured but extrapolated and that only consider the concentration of thiolate. The usual procedure to obtain pH-independent rate constants (k_{RS}-) consists in measuring apparent rate constants (k_{pH}^{app}) at several values of pH and extrapolating the value of the rate constant to an extreme (very often hypothetical) pH value in which all thiol is deprotonated. The main use of pH-independent rate constants is to compare the reactivity of different thiolates towards one substrate, a comparison that cannot be carried out using apparent rate constants at a particular pH. For example, pH-independent rate constants can be used to assess the effect of the molecular environment on the reactivity of a thiolate, independently of the availability of the thiolate at a given pH.

A second dimension refers to the nucleophilicity of thiolates. Thiolates are nucleophilic since they have the potential to donate a pair of electrons to an electrophile to form a new covalent bond. Nucleophilicity is revealed by reaction rates; the faster the rate constant with a certain electrophile, the stronger the nucleophile. Thiolates are good nucleophiles because of their negative charge and the relatively low electronegativity, large size and polarizability of sulfur.

Finally, an additional dimension of reactivity refers to the selectivity of the nucleophilicity. Each catalytic cysteine residue in the active site of an enzyme specializes in only one type of electrophile. For instance, the catalytic cysteines in thioredoxins and glutaredoxins specialize in disulfides as electrophiles, those of peptidases specialize in the carbonyl of a peptide bond, and those of peroxidases have hydroperoxides as favored electrophiles. This dimension of electrophile specialization can be further divided into more specific sets, as not all disulfides, peptide bonds or hydroperoxides are equally good substrates within their sets. Of course, cross-reactions exist but their rate constants are significantly smaller [26].

[2] Thiols (RSH), also called sulfhydryls or mercaptans, are in equilibrium with the thiolate anions (RS^-); for example, in the case of the amino acid cysteine, the pK_a is 8.3.

3. Early work evidenced that the oxidation of thiols by peroxynitrite can occur through two- and one-electron pathways

The study of the reaction of peroxynitrite with thiols was the first to establish a second-order reaction between peroxynitrite and a biomolecule [2]. Indeed, the reaction was characterized as a two-electron redox process between peroxynitrous acid and thiolate to yield nitrite and the corresponding sulfenic acid (RSOH) as products [2,16] (Equation (2)).

$$ONOOH + RS^- \rightarrow NO_2^- + RSOH \quad (2)$$

This reaction was initially studied on the amino acid cysteine and the cysteinyl residue in bovine serum albumin using fast kinetic spectrophotometric techniques (*i.e.*, stopped-flow spectrophotometry), taking advantage of the characteristic 302 nm absorbance of peroxynitrite anion ($ONOO^-$). Indeed, peroxynitrite was consumed within a fraction of a second and followed second-order reaction kinetics in the presence of thiols, with apparent rate constants of 5900 M^{-1} s^{-1} and 2600–2800 M^{-1} s^{-1} for cysteine and bovine serum albumin, respectively, at pH 7.4 and 37 °C [2]. The early work demonstrated that under physiologically relevant conditions, peroxynitrite reacted with "typical" thiols with rate constants about 1000 times higher than hydrogen peroxide (H_2O_2).

In the following years, the reaction of peroxynitrite with the tripeptide glutathione was characterized, reporting an apparent second order rate constant of 1360 M^{-1} s^{-1} at pH 7.4 and 37 °C [16,27], fully compatible with the previously reported values [2]. It was also evidenced that, among the 20 amino acids, the only ones that are able to react directly with peroxynitrite are cysteine, methionine and tryptophan, and that cysteine is the one that reacts the fastest [28]; the other amino acids, including tyrosine, react with secondary species derived from peroxynitrite. Also, peroxynitrite was shown to cause thiol oxidation in non-mammalian and mammalian cells [29,30], the reversibility of which (*i.e.*, by addition of 1,4-dithiothreitol, DTT) was progressively jeopardized as the level of the added oxidant increased. Indeed, under mild conditions, the sulfenic acid initially formed usually reacts with another thiol forming a disulfide (RSSR), which can be reduced back to thiols with suitable reductants, while excess peroxynitrite can lead to the formation of other products that are not easily reduced. In addition, using isolated organelles, peroxynitrite was shown to inactivate mitochondrial (*e.g.*, succinate dehydrogenase [31,32]) and sarcoplasmic reticulum enzymes (*e.g.*, sarcoplasmic reticulum Ca^{2+}-ATPase [33]) *via* thiol oxidation, observations that were later recapitulated in cells and tissues [34,35].

At the end of the first decade of studies on peroxynitrite-mediated thiol oxidation, a key discovery was reported showing that bacterial peroxiredoxins elicited "peroxynitrite reductase" activity through an efficient catalytic process involving the two-electron oxidation of an active site cysteine [36]. The rate constant of the reaction of peroxynitrite with this cysteine was 1.51 × 10^6 M^{-1} s^{-1} (pH 6.75, room temperature), for the alkyl hydroperoxide reductase C (AhpC) from *Salmonella typhimurium*. This value is over 1000-fold higher than with LMW thiols, suggesting the existence of endogenous enzymatic peroxynitrite decomposition systems. The observation was later extended to other microbial and mammalian enzymes. The reactions of the peroxiredoxins with peroxynitrite kinetically outcompete the reactions of other reductants, including glutathione [37,38]. They turned out to be so relevant biologically, that they represent major pathways for peroxynitrite consumption at various sub-cellular compartments, including the cytosol and mitochondria (see Sections 4, 7 and 12 on thiol peroxidases).

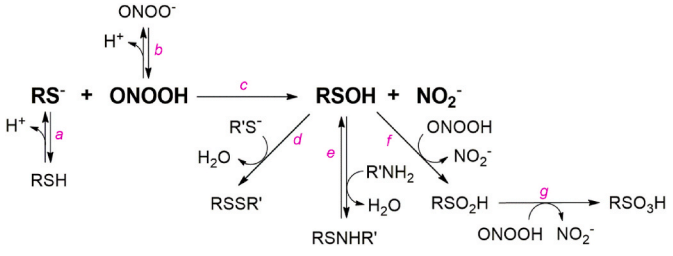

Fig. 2. Direct reaction of peroxynitrite with thiols and main decay pathways of sulfenic acid. The conjugate base of the thiol, the thiolate (*a*, variable pK_a) reacts with peroxynitrous acid (*b*, pK_a 6.8) forming sulfenic acid and nitrite (*c*). Sulfenic acid can decay by reacting with another thiolate forming a disulfide (*d*), with an amine forming a sulfenamide (*e*) or, once deprotonated to sulfenate (not shown), by further oxidation to sulfinic (*f*) and sulfonic (*g*) acids. Protons are sometimes omitted for clarity.

Revealing the second-order reaction of peroxynitrite with thiols (Equation (2)) was of key relevance to shift the focus of an earlier and seminal work that suggested that the oxidative capacity of peroxynitrite towards biomolecules was due to the proton-catalyzed homolysis to hydroxyl radical ($HO^•$) and nitrogen dioxide ($NO_2^•$) [1], which occurs with an apparent first-order rate constant of 1.13 s^{-1} at pH 7.4 and 37 °C [27] (Equation (3)). The discovery of the direct reaction of peroxynitrite with thiols paved the way, experimentally and mechanistically, to identify the preferential targets of the oxidant under biologically-relevant conditions which depend on second-order reactions that largely outcompete the homolytic pathway (reviewed in Ref. [18]).

$$ONOOH \rightarrow 0.7\ HNO_3 + 0.3\ HO^• + 0.3\ NO_2^• \quad (3)$$

While the direct reaction of peroxynitrite with thiols initially yields the two-electron oxidation product of a thiol, the sulfenic acid (Equation (2)), early electron paramagnetic resonance (EPR)-spin trapping studies showed that under relatively low thiol concentrations (micromolar levels), the fraction of peroxynitrite that homolyzes to hydroxyl and nitrogen dioxide radicals (Equation (3)) can promote the one-electron oxidation of thiols to the corresponding thiyl radicals (Equation (4)) [39–41].

$$HO^•/NO_2^• + RS^- \rightarrow HO^-/NO_2^- + RS^• \quad (4)$$

Even though this proton-catalyzed homolytic process is not quantitatively relevant under biological conditions, these initial observations served as the foundation to assess peroxynitrite-mediated oxidation of thiols to thiyl radicals under situations that favor the formation of one-electron oxidants, such as in the presence of carbon dioxide (see Sections 4 and 8 on oxidation to thiyl radicals), situations that also lead to the formation of 3-nitrotyrosine.

4. The two pathways for thiol oxidation by peroxynitrite are under kinetic competition

The oxidation of thiols by peroxynitrite can occur *via* two main pathways. First, peroxynitrite can react directly with thiols and oxidize them by two-electrons to sulfenic acids (Fig. 2). Second, the free radicals derived from peroxynitrite can oxidize thiols by one-electron, forming thiyl radicals (Fig. 3).

These two pathways are under kinetic competition. In biological scenarios, the direct reaction of peroxynitrite with thiols depends on the product of the rate constant (apparent rate constant at the appropriate pH and temperature) multiplied by the concentration of the thiol (total sum of thiol + thiolate). Table 1 shows second-order rate constants for the reactions of peroxynitrite with selected thiols, and comprehensive compilations can be found in Refs. [17,42,43]. The rate constants have apparent values between 10^3 and 10^8 M^{-1} s^{-1} at pH 7.4, depending on the particular thiol. The total intracellular concentration of thiols is in the tens of millimolar range. Glutathione, the most abundant LMW thiol, is 2–17 mM, and mostly reduced [44,45], but its rate constant with peroxynitrite is only 1.4 × 10^3 M^{-1} s^{-1}. In contrast, some thiol peroxidases have rate constants with peroxynitrite that are in the upper limit (10^5–10^8 M^{-1} s^{-1}) and can be present at relatively high concentrations in particular compartments; for example, the concentration of the

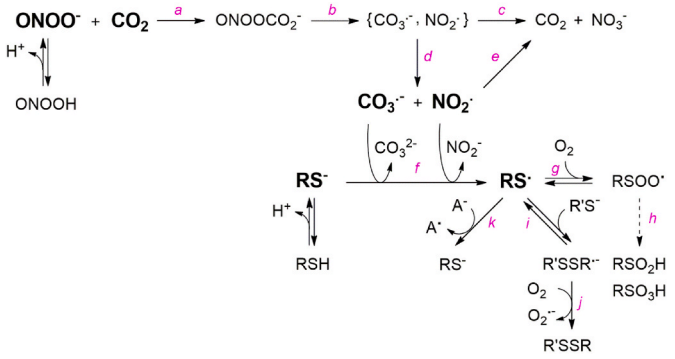

Fig. 3. Thiol oxidation by carbonate and nitrogen dioxide radicals and main decay pathways of thiyl radicals. Peroxynitrite anion reacts with carbon dioxide to form the putative adduct, $ONOOCO_2^-$ (*a*), which homolyzes to a geminate radical pair consisting of carbonate radical and nitrogen dioxide within a solvent cage (*b*). The radicals can recombine inside the cage giving carbon dioxide and nitrate in 67 % yield (*c*), while 33 % can diffuse out of the cage (*d*). Carbonate radical and nitrogen dioxide can recombine (*e*) or oxidize thiolate to thiyl radical (*f*). The main fates of thiyl radical are the reaction with oxygen (*g, h*), with another thiolate forming a reducing radical ($R'SSR^{\bullet -}$) that reacts with oxygen giving superoxide (*i, j*), or the oxidation of different compounds (represented by A^-), such as ascorbate (*k*). Protons are sometimes omitted for clarity. Dashed arrows indicate processes that occur in more than one reaction.

peroxiredoxin, Prdx2, is ~240 μM in red blood cells [46], while Prdx3 concentration is ~100 μM in the mitochondrial matrix of bovine adrenal cells [47]. Hence, the reaction of peroxynitrite with thiol peroxidases is one of the kinetically preferred decay pathways, and will be further discussed below in Section 7.

The one-electron oxidation of thiols by peroxynitrite is secondary to the formation of free radicals. The homolysis of peroxynitrous acid to hydroxyl radical and nitrogen dioxide (Equation (3)) is slow (1.13 s^{-1} at pH 7.4 and 37 °C [27]) and is unlikely to be significant in biological systems. In contrast, the reaction of peroxynitrite anion with carbon dioxide can indeed occur given the relatively high rate constant (4.6 × 10^4 M^{-1} s^{-1} at pH 7.4 and 37 °C [59]) and carbon dioxide concentration (1–2 mM). This reaction leads to the formation of carbonate radical and nitrogen dioxide in 33 % yield, both of which can oxidize thiols to thiyl radicals (Fig. 3). It starts with the formation of a short-lived adduct, $ONOOCO_2^-$, which homolyzes to a geminate radical pair within a solvent cage. The carbonate and nitrogen dioxide radicals can recombine inside the cage giving carbon dioxide and nitrate in 67 % yield, while 33 % of the radicals can diffuse and react with targets such as thiols. In the absence of targets, carbon dioxide can be considered to catalyze the isomerization of peroxynitrite to nitrate [60].

In addition to the formation of one-electron oxidants from the reaction of peroxynitrite with carbon dioxide, the reaction with some transition metal centers can also lead to the formation of oxidizing metal derivatives, such as the oxo-ferryl Compounds I and II of hemeperoxidases [13].

Importantly, the one-electron oxidation of thiols to thiyl radicals can trigger oxygen-dependent chain reactions that can amplify the initial oxidation event [41]. The reactions of thiyl radicals are further described in Sections 8 and 9.

5. Peroxynitrous acid reacts with thiolates giving sulfenic acid and nitrite

The relatively high rate constants of the reactions of peroxynitrite with thiols together with the high concentrations of thiols in biological systems translate into thiols being highly relevant targets of peroxynitrite.

These reactions are second-order processes involving protonated peroxynitrous acid (ONOOH) and deprotonated thiolates (RS^-) as reacting species. Indeed, early in the 1990s, the study of the pH-dependency study of the reaction with cysteine showed that the kinetics had a bell-shaped profile [2], reflecting the acid-base equilibria of peroxynitrous acid and the thiolate, as evidenced in later studies [16].

To understand and compare the efficiency and the specialization of peroxynitrite reactions with different thiols, the pH distribution of thiols/thiolates needs to be considered, as mentioned in Section 2 above. In addition, the pH distribution of peroxynitrous acid/peroxynitrite needs to be considered as well, since only peroxynitrous acid reacts with thiolates. To do that, the experimental apparent rate constants (k_{pH}^{app}) can be fitted to a two-pK_a function (Equation (5)), where the pH-independent rate constant (k_{RS^-}) represents the actual rate constant between thiolate and peroxynitrous acid.

$$k_{pH}^{app} = k_{RS^-}\left(\frac{[H^+]}{K_a^{ONOOH} + [H^+]}\right)\left(\frac{K_a^{SH}}{K_a^{SH} + [H^+]}\right) \quad (5)$$

As shown in Table 1, the apparent rate constants with peroxynitrite are in the 10^3 M^{-1} s^{-1} range at physiological pHs for different

Table 1
Rate constants of peroxynitrite reactions with selected thiols and persulfides.

	k_{pH}^{app} (M^{-1} s^{-1})	Conditions	pK_a	k_{RS^-} (M^{-1} s^{-1})	Ref.
Thiols					
Cysteine	4.5×10^3	pH 7.4, 37 °C	8.3	2.9×10^5	[2]
Cysteine ethyl esther	6.8×10^3	pH 7.4, 37 °C	6.6	5.8×10^4	[16]
Glutathione	1.4×10^3	pH 7.4, 37 °C	8.8	2.6×10^5	[16,27]
Human serum albumin	3.8×10^3	pH 7.4, 37 °C	8.1	1.1×10^5	[28,48]
AhpC [a]	$(1.2–1.5) \times 10^6$	pH 6.75, RT	<5	$(2.4–3.0) \times 10^6$	[36]
Human Prdx2 [a]	1.4×10^7	pH 7.4, 25 °C	4.8	7×10^7	[10,49]
Human Prdx3 [a]	1×10^7	pH 7.8, 25 °C	ND [b]	ND [b]	[50]
Human Prdx5 [a]	7×10^7	pH 7.8, 25 °C	5.2	7×10^8	[51,52]
*Mt*AhpE [a]	1.9×10^7	pH 7.4, 25 °C	5.2	9.5×10^7	[53]
Poplar GPx5 [a]	1.4×10^6	pH 7.4, 25 °C	5.2	7×10^6	[54]
*Xf*Ohr [a]	2×10^7	pH 7.4, 25 °C	5.2	1×10^8	[11]
Hydrogen sulfide	6.65×10^3	pH 7.4, 37 °C	7.0	5.6×10^4	[55]
Persulfides					
Glutathione	1.25×10^5	pH 7.2, 37 °C	5.5	4.7×10^5	[56]
Human serum albumin	1.2×10^4	pH 7.4, 20 °C	ND [b]	ND [b]	[57]
*Mt*AhpE [a]	$\sim 10^4$	pH 7.4, 25 °C	ND [b]	ND [b]	[58]

[a] AhpC is akyl hydroperoxide reductase C from different bacteria; Prdx2, Prdx3 and Prdx5 are peroxiredoxins 2, 3 and 5, respectively; *Mt*AhpE is *Mycobacterium tuberculosis* alkyl hydroperoxide reductase E; GPx5 is glutathione peroxidase 5; *Xf*Ohr is *Xylella fastidiosa* organic hydroperoxide resistance protein.
[b] ND is not-determined.

biologically relevant LMW thiols, such as free cysteine or glutathione. These values translate into pH-independent rate constants of ~10^5 M^{-1} s^{-1}. In contrast, the pH-independent rate constants with hydrogen peroxide are in the 1–10 M^{-1} s^{-1} range (compilation in [42]).

The accumulated kinetic and thermodynamic evidence regarding the reactions between thiols and peroxynitrite is consistent with typical concerted S_N2 reactions (substitution, nucleophilic, bimolecular reactions) [2,16,27,61]. Molecular modeling helped to understand the mechanistic basis of the process, suggesting that the reaction occurs *via* the nucleophilic attack of the thiolate's sulfur atom on the outer oxygen atom of the protonated peroxide group, yielding nitrite anion as the leaving group (Fig. 4) [56,61]. The free energy barrier is mostly due to the appreciable electronic reorganization together with the changes in the solvation structure, associated with the alignment of the sulfur and oxygen atoms at the transition state (Fig. 4). The good leaving group nature of the nitrite anion contributes deeply to the relative low value of this barrier when comparing with other, slower-reacting hydroperoxides, such as hydrogen peroxide, in which the leaving group is hydroxide (OH^-) [61,62]. Nevertheless, the protein microenvironment of the thiolate can alter the reactivity towards the different hydroperoxides, as in the case of some specialized thiol peroxidases that react faster with hydrogen peroxide than with peroxynitrite [10]. It is noteworthy that a very similar S_N2 mechanism was suggested for the reaction of persulfides (RSSH) with peroxynitrite [56], and also for selenols as discussed in the work by Messias *et al.* published in this same special issue.

As expected from other typical S_N2 reactions, the reactivity of LMW thiolates towards peroxynitrous acid correlates with the basicity, *i.e.*, the pH-independent rate constants are higher for those thiolates whose conjugated acids have high pK_as. Remarkably, the reactivity of many protein thiolates does not correlate with basicity, suggesting that the environment of the thiolate in particular proteins can modulate nucleophilicity independently of basicity, contributing to the selectivity of the nucleophilicity mentioned above [26,52].

Finally, one aspect that may cause confusion in the reaction of peroxynitrous acid with thiolates is that the range of rate constants is relatively narrow because unspecialized thiolates also react relatively fast with peroxynitrous acid. For instance, the cysteine thiolate residue of human serum albumin has a pH-independent rate constant of 1.1 × 10^5 M^{-1} s^{-1} [28,63], while the most specific peroxidase studied so far (human Prdx5) has a pH-independent rate constant of 7 × 10^8 M^{-1} s^{-1} [52]. Other peroxides have much wider ranges of rate constants with thiolates, for instance, those of hydrogen peroxide span 1–10^8 M^{-1} s^{-1} (compilations of rate constants in Refs. [42,43]).

6. Sulfenic acid is a transient product of thiol oxidation

As previously mentioned, the two-electron oxidation product of a thiol is a sulfenic acid (RSOH). This oxidation can be performed not only by peroxynitrite but also by hydrogen peroxide (H_2O_2), peroxymonocarbonate (HCO_4^-), organic hydroperoxides (ROOH), hypo

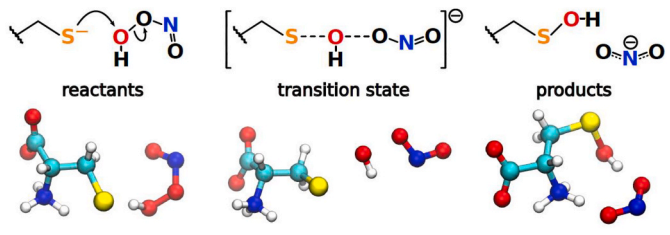

Fig. 4. Molecular mechanism of peroxynitrite reduction by thiols. Top: schematic representation of reactants, transition state and products during the S_N2 type reaction between a generic thiolate and peroxynitrous acid, highlighting the characteristic linearity of the transition state. Bottom: molecular representations of the species described above (for the thiolate of cysteine) obtained from molecular modeling [61].

(pseudo)halous acids (HOX, *e.g.* HOCl) or haloamines (R_2NX) [42,64].

Sulfenic acids are usually short-lived intermediates although, in some cases, the protein microenvironment protects them from further reactions. They can engage in reversible and irreversible reactions, and this particular feature enables them to be part of the catalytic mechanism of several enzymes, to regulate protein function and to act as signal transducers [65,66]. Sulfenic acids can display both nucleophilic and electrophilic reactivities. They can participate in one of various reactions, leading to a wide variety of products (Fig. 2). First, protonated sulfenic acids react with LMW or protein thiolates leading to the formation of disulfides (pathway *d* in Fig. 2). If the thiol is glutathione, then the protein gets glutathionylated, a well-known posttranslational modification that protects the cysteine from further oxidation and is also involved in the catalytic mechanisms of some enzymes such as glutathione peroxidases, glutaredoxins and glutathione reductase [67,68]. The reaction with protein thiols leads to the formation of intra or intermolecular disulfides, and this reaction is on the basis of the catalytic mechanisms of several enzymes such as peroxiredoxins [49,69]. Sulfenic acids can also react as electrophiles reversibly with an amine (RNH_2) or, more frequently, with an amide of the peptide backbone, leading to the formation of a cyclic sulfenamide (sulfenylamide) (pathway *e* in Fig. 2) [70–72]. In the presence of oxidants, including peroxynitrite [73], the deprotonated sulfenate (RSO^-) can be further oxidized to sulfinic (RSO_2H) and then to sulfonic (RSO_3H) acids in reactions often called over or hyperoxidation (pathways *f* and *g* in Fig. 2). These oxidation reactions are irreversible except in the case of several typical 2-Cys peroxiredoxins where this modification can be reverted by sulfiredoxin, an ATP-dependent oxidoreductase [74]. Recently, it has been proposed that this enzyme is also able to reduce sulfinic acids formed in other proteins [75]. Because a sulfenic acid can behave both as an electrophile and as a nucleophile, two sulfenic acids can react with each other, yielding a thiosulfinate (RS(O)SR). This reaction occurs as part of the catalytic cycle of sulfiredoxins [76], however, it is not a common pathway of sulfenic acid decay due to steric constraints and competing reactions. In addition, ascorbate can reduce sulfenic acids to thiols [77,78]. In the last years, it has also been shown that sulfenic acid can react with hydrogen sulfide (H_2S), leading to the formation of a persulfide (RSSH). This reaction has been proposed as a mechanism of transduction of the signals triggered by hydrogen sulfide [57,58,79].

Many of the reaction pathways described above for the decay of sulfenic acid were observed and kinetically characterized in the sulfenic acid formed in human serum albumin [28,57,63,80–83]. This sulfenic acid is particularly stable due to the absence of neighbor thiols and to the location in a crevice that protects from intermolecular reactions [82]. However, in general, the elusive nature of sulfenic acid has challenged its detection *in vitro* and *in vivo*. Several probes and the associated analytical methods have been proposed to aid in the detection of sulfenic acid (reviewed in Refs. [84–86]). Importantly, a probe to detect sulfenic acid has to kinetically outcompete the reaction pathways described above. This is particularly critical in cells, where thiols are highly abundant. Additionally, the probe has to distinguish sulfenic acid from other cysteine oxidation products such as disulfides, sulfenylamides and persulfides [87–90]. Last, it has to be borne in mind that several oxidants can form sulfenic acid from thiols. Thus, further evidence would be needed to associate the detection of sulfenic acid with a particular oxidant.

7. Thiol-dependent peroxidases are efficient peroxynitrite reductases

Different families of thiol-dependent peroxidases catalyze the two-electron reduction of peroxynitrite to nitrite, through a bisustratic reaction with a ping-pong kinetic mechanism. The oxidizing part of the catalytic cycle consists of a S_N2 type reaction, in which peroxynitrous acid and the thiolate of a critical cysteine residue of the peroxidase, named the peroxidatic cysteine (Cys_P), are the reactive species

(Equation (6)) [8,17].

$$P\text{-}Cys_pS^- + ONOOH \rightarrow P\text{-}Cys_pSOH + NO_2^- \quad (6)$$

The sulfenic acid formed at Cys_P then follows different routes depending on the particular peroxidase. In most cases, there is a resolution step involving the reaction of the sulfenic acid with another cysteine, the resolving cysteine of the protein, to form a disulfide. This step is followed by the reduction of the disulfide to the original thiol by thioredoxin/thioredoxin reductase or similar protein systems [49,91,92]. Importantly, the sulfenic acid or the disulfide, transiently formed in the peroxiredoxins, can potentially oxidize a target protein, usually with the intermediacy of protein-protein interactions. This last enables peroxiredoxins to channel the oxidation event to a different signal partner in redox relay sequences (reviewed in Refs. [69,93]).

Peroxiredoxins are ubiquitously expressed thiol-dependent peroxidases notable due to their high catalytic efficiency and abundance [8,94,95]. The peroxynitrite reductase activity of peroxiredoxins was first described for alkyl hydroperoxide reductase C (AhpC) from different bacteria [36], and later confirmed for several members of the family, including examples from all the six subfamilies in which peroxiredoxins are classified [36,96,97]. The apparent rate constants for the reduction of peroxynitrite by peroxiredoxins are in the 10^5–10^8 M^{-1} s^{-1} range at physiological pH [36] (Table 1 and compilations in Refs. [17,42,43]). Furthermore, oxidized peroxiredoxins are also rapidly reduced by their reducing partners, ensuring that in most studied cell types, the enzyme is predominantly in the reduced state under basal, non-stressed conditions [92,98]. Combined with the high concentration in which peroxiredoxins are usually expressed, the kinetic data indicate that they should be considered as main intracellular targets for peroxynitrite formed in different biological systems [8,17]. Indeed, the role of peroxiredoxins in the reduction of peroxynitrite has been demonstrated either at a cellular and at a whole organism level (see Section 12).

Peroxynitrite can not only oxidize but also hyperoxidize the Cys_P in peroxiredoxins, through the reaction of the sulfenic acid in the oxidized enzyme with a second molecule of oxidant, resulting in sulfinic acid formation in Cys_P and the consequent enzyme inactivation (Equation (7)) [10,50,99].

$$P\text{-}Cys_pSO^- + ONOOH \rightarrow Cys_pSO_2H + NO_2^- \quad (7)$$

In the case of human peroxiredoxins 1 and 2 (Prdx1 and Prdx2), the apparent rate constants for these reactions are 2.8×10^5 and 3.5×10^4 M^{-1} s^{-1} at pH 7.3, respectively [91]. As indicated in Section 6, hyperoxidation of some peroxiredoxins can be enzymatically reverted through reactions catalyzed by sulfiredoxins. In addition, peroxynitrite-derived radicals can lead to other post-translational modifications in these proteins, such as tyrosine nitration and dimerization [10,50]. Indeed, Prdx3 was one of the nitrated proteins detected in a mouse model of diabetes disease [100]. Notably, nitration of Prdx2 by peroxynitrite promotes changes in protein structure that cause an increase in its peroxidase activity and enhance its resistance to hyperoxidation [101,102].

In addition to peroxiredoxins, other thiol-dependent peroxidases, such as cysteine-based glutathione peroxidases (GPx), catalyze the reduction of peroxynitrite. Some glutathione peroxidases contain a peroxidatic selenocysteine instead of Cys_P, and are therefore selenol- instead of thiol-dependent. Glutathione peroxidases share with peroxiredoxins the thioredoxin fold, and thus, both are members of the thioredoxin superfamily. Poplar GPx5, a thiol-dependent glutathione peroxidase, and mammalian GPx1, which is a selenol-dependent enzyme, reduce peroxynitrite with rate constants of $\sim 10^6$ M^{-1} s^{-1} at physiological pH [54,103]. The oxidized forms of the enzymes are then usually reduced by thioredoxin or glutathione in thiol- or selenol-dependent enzymes, respectively [104]. The role of GPx1 in peroxynitrite detoxification *in vivo* remains unclear [105,106].

Importantly, the bacterial organic hydroperoxide resistance protein (Ohr), which is structurally unrelated to the former peroxidases, also catalyzes the reduction of peroxynitrite and uses dihydrolipoamide as electron donor. The rate constant of Cys_P oxidation in *Xylella fastidiosa* Ohr by peroxynitrite is 2×10^7 M^{-1} s^{-1} at pH 7.4 [11].

Considering that the thiol pK_a values in peroxidases are usually low (<6.4), thiolate availability is >90 %, while peroxynitrite, with a pK_a of 6.8, is only ~20 % protonated at physiological pH. Thus, pH-independent rate constants of peroxynitrous acid reactions with Cys_P thiolates in peroxidases are ~5-fold higher than those reported at pH 7.4 (Equation (6)), in the 10^6–10^8 M^{-1} s^{-1} range (Table 1), and, differently from LMW thiolates, they do not correlate with thiolate basicity [26,52]. The aspects underlying the modulation of acidity, reactivity and specificity in peroxidases are only now starting to be unraveled on a case by case basis (reviewed in Ref. [107]).

8. The free radicals derived from peroxynitrite can oxidize thiols to thiyl radical

As described above, peroxynitrous acid reacts directly with thiolates present in LMW compounds, peptides and proteins. Besides this two-electron oxidation reaction, an additional reaction pathway has been described, involving the one-electron oxidation of thiolates by strong oxidants derived from peroxynitrite. The main one-electron oxidants that peroxynitrite can give rise to are nitrogen dioxide and carbonate radical. In addition, peroxynitrite can generate other one-electron oxidants such as metal-center derived oxidants (following the reaction with particular metalloproteins), and, to a minor extent, hydroxyl radical (following the proton-catalyzed homolysis, Equation (3)). The reactions of thiols with nitrogen dioxide and carbonate radical are summarized herein.

Nitrogen dioxide is a reddish-brown free radical that can be formed from peroxynitrite mainly through the one-electron reduction of peroxynitrite or through the reaction of peroxynitrite with carbon dioxide. In addition, there are other routes for nitrogen dioxide formation, such as the autooxidation of nitric oxide or the oxidation of nitrite by hemeperoxidases/hydrogen peroxide [17]. The one-electron reduction potential of the $NO_2^{\bullet}/NO_2^-$ couple is 1.04 V/SHE [108], and nitrogen dioxide is able to react fast with thiolates. For example, the rate constant for the reaction between nitrogen dioxide and free cysteine is 5×10^7 M^{-1} s^{-1} at pH 7.4 [109].

Carbonate radical anion is formed from the reaction of peroxynitrite with carbon dioxide. This reaction leads to the formation of carbonate radical and nitrogen dioxide in 33 % yield, and is described above in Section 4 and Fig. 3. Carbon dioxide (not bicarbonate nor carbonate) and peroxynitrite anion were identified as the reacting species [12], and the product carbonate radical was confirmed by EPR [19,60,110]. There are other possible routes for carbonate radical formation, for example, the peroxidase-like activity of CuZn SOD in the presence of hydrogen peroxide and carbon dioxide/bicarbonate [111,112]. With a one-electron reduction potential for the $CO_3^{\bullet-}$, H^+/HCO_3^- couple of 1.78 V/SHE at pH 7 [113], carbonate radical is a very strong oxidant. In addition, it is able to react fast with thiolates. For example, the rate constant with free cysteine is 4.6×10^7 M^{-1} s^{-1} at pH 7, and increases at alkaline pH [114].

These peroxynitrite-derived oxidants can react with thiols forming thiyl radicals, and cysteinyl and glutathionyl radical as well as albumin thiyl radical have been detected by EPR-spin trapping studies [39–41,113,115]. Kinetic competition determines whether thiols are the targets of nitrogen dioxide and carbonate radical instead of other reductants or other protein residues, particularly aromatic ones. In this regard, tyrosine residues are other relevant targets for these strong one-electron oxidants. Tyrosines do not react directly with peroxynitrite. They can react with one-electron oxidants forming tyrosine phenoxyl radicals, which can couple with nitrogen dioxide resulting in the formation of 3-nitrotyrosine derivatives (reviewed in Ref. [17]).

The one-electron reduction potential of the thiyl radical/thiol couple ($RS^{\bullet}$, H^+/RSH) is 0.92 V/SHE at pH 7.4 in the case of glutathione [116].

This indicates that only strong one-electron oxidants are able to oxidize thiols to thiyl radicals, and that, once formed, thiyl radicals are themselves strong one-electron oxidants, able to oxidize other biomolecules.

9. The main fates of thiyl radical are reactions with oxygen, thiols and other reductants like ascorbate

Once formed, thiyl radicals can react with different molecules depending on the kinetic competition between potential targets. Their fate will depend not only on the rate constants, but on the target concentration. The reaction between two thiyl radicals renders the disulfide (Equation (8)). Although it is diffusion-controlled, this reaction is unlikely to be significant because it is order two in the free radicals, which achieve very low steady-state concentrations [117]. Likewise, the reaction with nitric oxide, which is likely to be present in systems where peroxynitrite is formed, albeit at very low concentrations, forms nitrosothiols, although this reaction is unlikely to constitute a preferential decay pathway (Equation (9)). In contrast, oxygen, thiols and ascorbate are among the main targets (Fig. 3) [117].

$$RS^{\bullet} + RS^{\bullet} \rightarrow RSSR \quad (8)$$

$$RS^{\bullet} + NO^{\bullet} \rightarrow RSNO \quad (9)$$

Oxygen (O_2) reacts reversibly with thiyl radical (Equation (10), reactions *g* and *h* in Fig. 3). The forward and reverse rate for glutathionyl radical are 2×10^9 M^{-1} s^{-1} and 6×10^5 s^{-1}, respectively [118].

$$RS^{\bullet} + O_2 \rightleftharpoons RSOO^{\bullet} \quad (10)$$

The product is thioperoxyl radical, $RSOO^{\bullet}$, which is an oxidizing radical that can participate in the propagation of chain reactions. Thioperoxyl radical can rearrange to sulfonyl radical ($RS(O)O^{\bullet}$) and undergo oxygen atom transfer with a thiol giving sulfinyl radical ($RSO^{\bullet}$) and sulfenic acid [117]. The sulfonyl radical can be reduced to sulfinic acid (RSO_2H) or react with another oxygen to form the species $RS(O_2)OO^{\bullet}$, ultimately forming sulfonic acid (RSO_3H) [119]. Additionally, sulfinic acid can be oxidized by carbonate radical and nitrogen dioxide to sulfonyl radical [120].

Studies of thiol oxidation by peroxynitrite revealed a bell-shaped profile for oxygen consumption as a function of cysteine concentration, both for the free amino acid [41] and the cysteinyl residue in bovine serum albumin [121]. This profile supports the existence of two competing reaction pathways for peroxynitrite. At low thiol concentrations, peroxynitrite-derived radicals are formed through homolysis and can be increasingly scavenged by thiols, resulting in thiyl radical formation and oxygen consumption, while at high thiol concentrations, the direct reaction of the oxidant with the thiolate prevails, and oxygen consumption decreases [41].

Most mammalian cells are exposed to 4–6 % oxygen. In order to estimate the molar concentration of oxygen in cells, the partial pressure, the solubility in the solution, as well as the temperature, must be taken into account [122–124]. Air-equilibrated solutions (exposed to 21 % oxygen) have an oxygen concentration of approximately 181–191 μM at 37 °C, therefore oxygen concentrations in cells are estimated to be in the μM range [123,124]. At these physiological concentrations of oxygen, thiolates and other reductants likely outcompete oxygen for reaction with thiyl radical.

Thiols, actually thiolates, constitute preferential targets for thiyl radical. The reaction is reversible and fast, with pH-independent rate constants 6×10^8 M^{-1} s^{-1} for the forward reaction and 2×10^5 s^{-1} for the reverse reaction (for glutathione) [117,125,126], and favored by the high concentrations of thiols in biological systems. The disulfide anion radical ($RSSR^{\bullet-}$) formed is a reducing radical that reacts fast with oxygen (5×10^8 M^{-1} s^{-1} [127]) forming superoxide radical, which can then be detoxified with superoxide dismutase (Equations (11)–(13), reactions *i* and *j* in Fig. 3). Supporting this, oxygen consumption during peroxynitrite reaction with cysteine is inhibited by superoxide dismutase [41]. This pathway is considered a radical sink for thiyl radicals and other radicals formed intracellularly [128].

$$RS^{\bullet} + RS^{-} \rightleftharpoons RSSR^{\bullet-} \quad (11)$$

$$RSSR^{\bullet-} + O_2 \rightarrow RSSR + O_2^{\bullet-} \quad (12)$$

$$2\,O_2^{\bullet-} + 2\,H^{+} \rightarrow O_2 + H_2O_2 \quad (13)$$

Given the millimolar intracellular level of glutathione, this tripeptide is a likely target for thiyl radicals. Its thiol has a pK_a of 8.9 [129] and at physiological pH approximately 2 % will be ionized.

Ascorbate is another possible target for thiyl radical, since it reacts with a rate constant of 6×10^8 M^{-1} s^{-1} at pH 7 [130], forming ascorbyl radical ($Asc^{\bullet}$) and regenerating the thiolate (Equation (14), reaction *k* in Fig. 3). Ascorbate is present at concentrations around 50 μM in blood and 500 μM in some tissues, thus it is a likely target *in vivo* [131]. The thioperoxyl radical can also be reduced by ascorbate (Equation (15)) [131]. The reaction with ascorbate has been proposed as another possible radical sink for thiyl and other radicals. The somehow redundant ascorbate and superoxide pathways for radical detoxification probably coexist, and their relative weight is influenced by pH, oxygen, ascorbate and glutathione concentration in each compartment and condition [128,131].

$$RS^{\bullet} + Asc^{-} \rightarrow RS^{-} + Asc^{\bullet} \quad (14)$$

$$RSOO^{\bullet} + Asc^{-} \rightarrow RSOO^{-} + Asc^{\bullet} \quad (15)$$

Thiyl radicals can undergo reversible hydrogen atom/electron transfer with amino acids, peptides and proteins (Equation (16)). This is particularly relevant with aromatic residues. As an example, the reaction of glutathionyl radical with a Gly-Tyr peptide has a rate constant of 5.8×10^6 M^{-1} s^{-1} [132]. The reverse reaction (reaction of a tyrosine phenoxyl radical with glutathione) has a similar rate constant, and Equation (16) has an equilibrium constant close to unity, so thiols can also reduce tyrosyl radical back to tyrosine [133]. This repair has proved particularly significant in the case of the *Trypanosoma cruzi* cytosolic iron superoxide dismutase (FeSOD), in which a cysteine residue close to the active site protects from peroxynitrite-dependent inactivation by avoiding the nitration of an active-site tyrosine. Intramolecular electron transfer from the cysteine repairs the intermediate tyrosine phenoxyl radical to tyrosine, preventing nitration [134].

$$RS^{\bullet} + TyrOH \rightleftharpoons RSH + TyrO^{\bullet} \quad (16)$$

Thiyl radicals in proteins can also react reversibly with amino acid (AA)C–H bonds forming carbon-centered radicals $(AA)C^{\bullet}$. The reactions can proceed between residues of different molecules, with rate constants of 10^3–10^5 M^{-1} s^{-1}, or within the same molecule, with rate constants around 10^5 and 10^6 s^{-1} for the forward and reverse reactions, respectively [135,136]. Reversible transfers between thiyl radical and proximal carbons forming α-mercaptoalkyl and β-mercaptoalkyl radicals have been observed in cysteine, glutathione and ribonucleotide reductase (Cys175). Mercaptoalkyl radicals can react with oxygen forming peroxyl radicals ($(AA)COO^{\bullet}$) [135,136], and oxygen has been shown to play a role in radical propagation reactions involved in peroxynitrite oxidation of bovine serum albumin [121].

Since thiyl radicals can participate in intramolecular hydrogen atom/electron transfers, they can induce damage at sites distant from the initial point of attack. Competing reactions of the thiyl radical with glutathione or ascorbate will protect proteins from damage in most cases. However, if the thiyl radical is formed inside the protein, in a site that is not accessible to the reducing agents, these intramolecular electron transfer reactions can result in protein damage [136].

10. Hydrogen sulfide reacts with peroxynitrite

A few decades ago, it was proposed that hydrogen sulfide (H_2S) could have a role as a physiological modulator in mammals [137]. Among several effects, protection against ischemia-reperfusion events and oxidant insults was observed when hydrogen sulfide was administered in biological models [138,139]. The first hypothesis raised to explain the observation was that hydrogen sulfide directly scavenged the oxidants, but this hypothesis was later reevaluated following the study of the corresponding kinetics [140].

To be precise, hydrogen sulfide is not a thiol but is still a sulfur nucleophile. When protonated as H_2S, it is freely diffusible [141,142]. It can deprotonate to form hydrosulfide (HS^-, a better nucleophile) with a pK_a of 7.0 [143]. At pH 7.4 and 37 °C, approximately 19 % of total is found as H_2S and 81 % as HS^-, while the levels of S^{2-} are negligible. Formation of hydrogen sulfide in mammals can be catalyzed by three enzymes involved in sulfur amino acids metabolism: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (MPST). CBS and CSE exhibit substrate and reaction promiscuity, and form hydrogen sulfide from cysteine and/or homocysteine [144,145]. MPST converts 3-mercaptopyruvate (derived from cysteine transamination) into pyruvate, and initially produces a protein persulfide intermediate (MPST-SSH) that can react with thiols, ultimately releasing hydrogen sulfide [146,147]. The gut microbiota constitutes another source of hydrogen sulfide for the host [148,149]. The primary route of hydrogen sulfide consumption in mammals resides in the mitochondria and involves the flavoenzyme sulfide quinone oxidoreductase (SQOR), which transfers the electrons to coenzyme Q_{10} in the electron transport chain and the sulfur to LMW acceptors [150], mainly glutathione, generating glutathione persulfide (GSSH) as the product [151,152]. This oxidation pathway is completed by other enzymatic activities (persulfide dioxygenase, thiosulfate sulfurtransferase, and sulfite oxidase) that transform the outer sulfur of glutathione persulfide to the end products sulfate (SO_4^{2-}) and thiosulfate ($S_2O_3^{2-}$), which can be excreted [150,151].

Hydrogen sulfide reacts with peroxynitrite; precisely, HS^- reacts with ONOOH [140]. This reaction has been proposed to occur through a S_N2 mechanism similar to that of thiolates in which the nucleophilic sulfur can attack the peroxide group to form sulfenic acid (HSOH) and nitrite [55]. The experimentally-determined rate constants show that the reaction is slightly slower than those of LMW thiolates [55,140,153]. At pH 7.4 and 37 °C, a rate constant of 6.65×10^3 M^{-1} s^{-1} was determined (Table 1) [55]. Considering this value and the low nanomolar concentrations that hydrogen sulfide levels can reach in the tissues of mammals [154], the direct scavenging of peroxynitrite by hydrogen sulfide seems unlikely [140], highlighting that other mechanisms probably underlie the beneficial effects of hydrogen sulfide.

An interesting aspect of this reaction is that mixtures of peroxynitrite and hydrogen sulfide produce an intriguing variety of products markedly different from those obtained with thiols [55,153]. While the reaction of peroxynitrite with cysteine produces no colored compounds, hydrogen sulfide generates yellow products with an absorbance maximum at 408 nm. Possible intermediates involved in the reaction are HSOH and HSSH, which are also nucleophiles. It has been hypothesized that a reaction with a second peroxynitrite molecule could generate the colored compounds, which probably contain sulfur and nitrogen [55]. Among the possible products are the isomers $HSNO_2$ and HSONO. Several physiological functions that are still under study have been assigned to small inorganic sulfur-nitrogen compounds [155–157].

In addition to the direct reaction between peroxynitrite and hydrogen sulfide, the free radicals derived from peroxynitrite can also oxidize hydrogen sulfide [140,158]. The initial product formed is sulfanyl radical ($HS^{\bullet}/S^{\bullet-}$), which can lead to oxygen consumption. As in the case of thiols, plots of oxygen consumption as a function of hydrogen sulfide are bell-shaped. Nevertheless, the one-electron oxidation of hydrogen sulfide can lead to a wider range of reactive intermediates and stable products than in the case of thiols [140].

11. Persulfides react with peroxynitrite and are excellent one-electron reductants

When an extra sulfur is added to a thiol, a persulfide ($RSSH/RSS^-$) is obtained. Persulfides retain or enhance some features of the original thiol (*e.g.*, nucleophilicity and reducing character) while showing new properties (electrophilicity, increased acidity, ability to transfer sulfur) [159,160]. Persulfides can be generated through several pathways. Briefly, the cysteines in the active sites of desulfurases or sulfurtransferases can obtain one extra sulfur from substrates like cysteine, 3-mercaptopyruvate or thiosulfate [161–163]. Polysulfides can be donors of sulfurs to exposed cysteines in proteins [164]. CBS and CSE can produce LMW persulfides by cleaving the disulfides of cysteine or homocysteine [165]. The formation of sulfenic acids and disulfides in proteins and LMW compounds allows the generation of persulfides through their direct reaction with hydrogen sulfide [55,58]. Also, radical pathways have been proposed, involving the reaction of the radical $HS^{\bullet}/S^{\bullet-}$ with a thiol in the presence of oxygen [137].

Peroxynitrite is able to react with persulfides, specifically, with ionized persulfides (RSS^-). Similar to the reactions of peroxynitrous acid with thiolates, the products are likely perthiosulfenic acids (RSSOH) and nitrite. The reactions of peroxynitrite with typical LMW persulfides are faster than those with the analogous thiols (Table 1). For example, the persulfide of glutathione reacts with an apparent rate constant of 1.25×10^5 M^{-1} s^{-1} (pH 7.23, 37 °C) with peroxynitrite, which is 97 times higher than the rate constant of the thiol under the same conditions [56]. The increased reactivity of persulfides toward peroxynitrite with respect to thiols has also been observed for some protein persulfides. For instance, persulfidation of the cysteine thiol of human serum albumin increases the rate constant of the reaction with peroxynitrite 4.4-fold at pH 7.4 [57]. However, this behavior could vary in the particular cases of enzymes that use peroxynitrite as substrate. Indeed, persulfidation of the critical cysteine in the peroxiredoxin AhpE lowers its reactivity in comparison with the unmodified reduced protein as a result of alterations in the structure of the selective active site [58].

In addition, LMW ionized persulfides (RSS^-) have higher reactivity with electrophiles than putative thiolates (RS^-) with similar basicity, which is indicative of an alpha effect in persulfides (increased reactivity of a nucleophile when the atom adjacent to the nucleophilic atom has high electron density) [56,166–169]. Particularly, the alpha effect in the reaction of glutathione persulfide with peroxynitrite has a magnitude of 50 [56].

Notably, the oxidation products of persulfides are restored more easily than those of thiols. The reactions of peroxynitrite (or other two-electron oxidants) with thiols produce sulfenic (RSOH), sulfinic (RSO_2H) and sulfonic (RSO_3H) acids, while those with persulfides likely generate perthiosulfenic (RSSOH), perthiosulfinic ($RSSO_2H$) and perthiosulfonic ($RSSO_3H$) acids [170–172]. The products of hyperoxidation of thiols are, in general, irreversible, whereas those of persulfides can be reduced back to thiols, constituting a plausible cellular protection mechanism to prevent irreversible damage under oxidative stress (Fig. 5) [171–175].

Remarkably, persulfides are excellent one-electron reductants. The reactions of persulfides with one-electron oxidants, such as peroxynitrite-derived nitrogen dioxide and carbonate radical, form perthiyl radicals ($RSS^{\bullet}$). These radicals are particularly stable, do not react with oxygen and decay mainly by dimerization to the tetrasulfide (RSSSSR) [176–179], hindering radical propagation reactions. In fact, persulfides have been proposed to inhibit ferroptosis, a type of cell death caused by excessive lipid peroxidation [180,181].

12. Thiol peroxidases are relevant targets for peroxynitrite *in cellula* and *in vivo*

Combined evidence arising from measurements of 3-nitrotyrosine

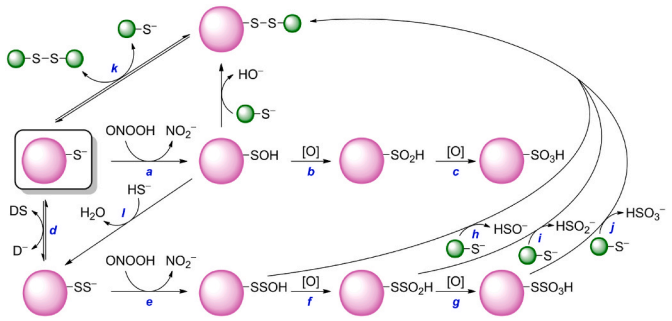

Fig. 5. Formation of persulfides as a protective mechanism for thiols against oxidants. Protein thiols (RSH) can be oxidized to sulfenic (RSOH) (*a*), sulfinic (RSO_2H) (*b*) and sulfonic acids (RSO_3H) (*c*). Sulfinic and sulfonic are unlikely to be restored. When a persulfide (RSSH) is formed (*i.e.*, through the reaction with a sulfur donor, DS) (*d*), the thiol could be protected from irreversible oxidations. Persulfides can be oxidized to perthiosulfenic (RSSOH) (*e*), perthiosulfinic ($RSSO_2H$) (*f*) and perthiosulfonic acids ($RSSO_3H$) (*g*). All of them are able to react with other thiols (R'SH) to form mixed disulfides (RSSR') (*h*, *i*, *j*) that can be reduced to restore the original thiol (*k*). Hydrogen sulfide could also react with sulfenic acids to form persulfides (*l*), which could be reduced to restore the original thiol (*d*, *e*-*k*).

levels and utilization of novel peroxynitrite probes, together with the modulation of the precursor free radicals, superoxide and nitric oxide, have enabled the association of peroxynitrite with diverse physiological and pathological processes, including the control of microbial infections, aging, neurodegenerative diseases, vascular dysfunction, atherosclerosis, cancer, and inflammation (reviewed in Refs. [35,182]). Thiol peroxidases are among the main targets of peroxynitrite, and the relevance of these reactions has been unambiguously demonstrated by experiments involving these enzymes in both cellular and animal models. Some selected examples are provided below.

a) *Trypanosoma cruzi* transformed to overexpress cytosolic or mitochondrial peroxiredoxins had increased ability to detoxify peroxynitrite generated either *in vitro* or by activated macrophages [99]; this favored infection in an animal model [183].
b) Mice with endothelial deletion of the gene that encodes the mitochondrial thioredoxin reductase, and therefore lack the main reducing system for mitochondrial peroxiredoxins, showed increased peroxynitrite steady-state levels, which resulted in disruption of vascular integrity [184].
c) Inhibition of peroxiredoxins using conoidin A or specific deletion of Prdx1 sensitized pancreatic β cells and rat islets to DNA damage and death caused by peroxynitrite [185].
d) In *Pseudomona aeruginosa* treated with a catalase inhibitor, the lack of thiol peroxidase Ohr expression caused increased peroxynitrite-dependent cytotoxicity [11].
e) *Saccharomyces cerevisiae* lacking thiol-dependent peroxidases showed increased cellular levels of peroxynitrite detected by the coumarin boronic acid probe [186].

13. Concluding remarks

Three decades of research on the biochemistry of peroxynitrite have not only established the formation of this stealthy oxidant in biological systems but have also revealed the role of thiols as the main targets of peroxynitrite, as well as of its derived radicals. Among the different biological thiols, thiol peroxidases stand out as main peroxynitrite reductases due to the very high rate constants of reaction with peroxynitrite together with the relatively high concentrations of the enzymes. Rooted in strong thermodynamic and kinetic concepts, solid evidence is emerging for the role of thiol peroxidases in peroxynitrite detoxification, with potential implications in diseases in which peroxynitrite is involved.

Funding

This work was supported in part by grants from Comisión Sectorial de Investigación Científica (CSIC) and Comisión Académica de Posgrado (CAP), Universidad de la República (CSIC I + D 2020 to EC and MT, CSIC I + D 2022 to GFS, SC and LT, CSIC I + D Grupos 2022 to CQ, and EI to RR); Fondo Clemente Estable, National Agency for Investigation and Innovation (ANII), Uruguay (to BA, GFS and SC); and ECOS Sud program (to BA). Additional funding was obtained from Programa de Desarrollo de Ciencias Básicas (PEDECIBA), The Richard Lounsbery Foundation (to RR), and Programa de Alimentos y Salud Humana (PAyS, IDB-R.O.U. 4950/OC-UR).

CRediT authorship contribution statement

Madia Trujillo and Beatriz Alvarez: Writing - review and editing, Writing - original draft, Conceptualization. Ernesto Cuevasanta, Lucía Turell, Dayana Benchoam, Gerardo Ferrer-Sueta, Ari Zeida, Celia Quijano, Sebastián Carballal and Rafael Radi: Writing - review and editing, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J.S. Beckman, T.W. Beckman, J. Chen, P.A. Marshall, B.A. Freeman, Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 1620–1624, https://doi.org/10.1073/pnas.87.4.1620.
[2] R. Radi, J.S. Beckman, K.M. Bush, B.A. Freeman, Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide, J. Biol. Chem. 266 (1991) 4244–4250, https://doi.org/10.1016/S0021-9258(20)64313-7.
[3] N.V. Blough, O.C. Zafiriou, Reaction of superoxide with nitric oxide to form peroxonitrite in alkaline aqueous solution, Inorg. Chem. 24 (1985) 3502–3504, https://doi.org/10.1021/ic00216a003.
[4] R. Smulik, D. Dębski, J. Zielonka, B. Michałowski, J. Adamus, A. Marcinek, B. Kalyanaraman, A. Sikora, Nitroxyl (HNO) reacts with molecular oxygen and forms peroxynitrite at physiological pH, J. Biol. Chem. 289 (2014) 35570–35581, https://doi.org/10.1074/jbc.M114.597740.
[5] M.P. Doyle, J.W. Hoekstra, Oxidation of nitrogen oxides by bound dioxygen in hemoproteins, J. Inorg. Biochem. 14 (1981) 351–358, https://doi.org/10.1016/S0162-0134(00)80291-3.
[6] R. Kissner, T. Nauser, P. Bugnon, P.G. Lye, W.H. Koppenol, Formation and properties of peroxynitrite as studied by laser flash photolysis, high-pressure stopped-flow technique, and pulse radiolysis, Chem. Res. Toxicol. 10 (1997) 1285–1292, https://doi.org/10.1021/tx970160x.
[7] C. Quijano, N. Romero, R. Radi, Tyrosine nitration by superoxide and nitric oxide fluxes in biological systems: modeling the impact of superoxide dismutase and nitric oxide diffusion, Free Radic. Biol. Med. 39 (2005) 728–741, https://doi.org/10.1016/j.freeradbiomed.2005.04.014.
[8] M. Trujillo, G. Ferrer-Sueta, L. Thomson, L. Flohé, R. Radi, Kinetics of peroxiredoxins and their role in the decomposition of peroxynitrite, in: L. Flohé, J.R. Harris (Eds.), Peroxiredoxin Systems, Springer Netherlands, Dordrecht, 2007, pp. 83–113, https://doi.org/10.1007/978-1-4020-6051-9_5.
[9] M. Trujillo, G. Ferrer-Sueta, R. Radi, Kinetic studies on peroxynitrite reduction by peroxiredoxins, in: Methods in Enzymology, Elsevier, 2008, pp. 173–196, https://doi.org/10.1016/S0076-6879(08)01210-X.
[10] B. Manta, M. Hugo, C. Ortiz, G. Ferrer-Sueta, M. Trujillo, A. Denicola, The peroxidase and peroxynitrite reductase activity of human erythrocyte peroxiredoxin 2, Arch. Biochem. Biophys. 484 (2009) 146–154, https://doi.org/10.1016/j.abb.2008.11.017.
[11] T.G.P. Alegria, D.A. Meireles, J.R.R. Cussiol, M. Hugo, M. Trujillo, M.A. De Oliveira, S. Miyamoto, R.F. Queiroz, N.F. Valadares, R.C. Garratt, R. Radi, P. Di Mascio, O. Augusto, L.E.S. Netto, Ohr plays a central role in bacterial responses against fatty acid hydroperoxides and peroxynitrite, Proc. Natl. Acad. Sci. U.S.A. 114 (2017), https://doi.org/10.1073/pnas.1619659114.
[12] S.V. Lymar, J.K. Hurst, Rapid reaction between peroxonitrite ion and carbon dioxide: implications for biological activity, J. Am. Chem. Soc. 117 (1995) 8867–8868, https://doi.org/10.1021/ja00139a027.
[13] R. Floris, S.R. Piersma, G. Yang, P. Jones, R. Wever, Interaction of myeloperoxidase with peroxynitrite. A comparison with lactoperoxidase,

horseradish peroxidase and catalase, Eur. J. Biochem. 215 (1993) 767–775, https://doi.org/10.1111/j.1432-1033.1993.tb18091.x.
[14] A. Denicola, J.M. Souza, R. Radi, Diffusion of peroxynitrite across erythrocyte membranes, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 3566–3571, https://doi.org/10.1073/pnas.95.7.3566.
[15] M. Exner, S. Herold, Kinetic and mechanistic studies of the peroxynitrite-mediated oxidation of oxymyoglobin and oxyhemoglobin, Chem. Res. Toxicol. 13 (2000) 287–293, https://doi.org/10.1021/tx990201k.
[16] M. Trujillo, R. Radi, Peroxynitrite reaction with the reduced and the oxidized forms of lipoic acid: new insights into the reaction of peroxynitrite with thiols, Arch. Biochem. Biophys. 397 (2002) 91–98, https://doi.org/10.1006/abbi.2001.2619.
[17] G. Ferrer-Sueta, N. Campolo, M. Trujillo, S. Bartesaghi, S. Carballal, N. Romero, B. Alvarez, R. Radi, Biochemistry of peroxynitrite and protein tyrosine nitration, Chem Rev 118 (2018) 1338–1408, https://doi.org/10.1021/acs.chemrev.7b00568.
[18] G. Ferrer-Sueta, R. Radi, Chemical biology of peroxynitrite: kinetics, diffusion, and radicals, ACS Chem. Biol. 4 (2009) 161–177, https://doi.org/10.1021/cb800279q.
[19] M.G. Bonini, R. Radi, G. Ferrer-Sueta, A.M.D.C. Ferreira, O. Augusto, Direct EPR detection of the carbonate radical anion produced from peroxynitrite and carbon dioxide, J. Biol. Chem. 274 (1999) 10802–10806, https://doi.org/10.1074/jbc.274.16.10802.
[20] A. Sikora, J. Zielonka, M. Lopez, J. Joseph, B. Kalyanaraman, Direct oxidation of boronates by peroxynitrite: mechanism and implications in fluorescence imaging of peroxynitrite, Free Radic. Biol. Med. 47 (2009) 1401–1407, https://doi.org/10.1016/j.freeradbiomed.2009.08.006.
[21] N. Rios, L. Piacenza, M. Trujillo, A. Martínez, V. Demicheli, C. Prolo, M. N. Álvarez, G.V. López, R. Radi, Sensitive detection and estimation of cell-derived peroxynitrite fluxes using fluorescein-boronate, Free Radic. Biol. Med. 101 (2016) 284–295, https://doi.org/10.1016/j.freeradbiomed.2016.08.033.
[22] C. Prolo, N. Rios, L. Piacenza, M.N. Álvarez, R. Radi, Fluorescence and chemiluminescence approaches for peroxynitrite detection, Free Radic. Biol. Med. 128 (2018) 59–68, https://doi.org/10.1016/j.freeradbiomed.2018.02.017.
[23] M.N. Möller, N. Rios, M. Trujillo, R. Radi, A. Denicola, B. Alvarez, Detection and quantification of nitric oxide-derived oxidants in biological systems, J. Biol. Chem. 294 (2019) 14776–14802, https://doi.org/10.1074/jbc.REV119.006136.
[24] Z.R. Gan, W.W. Wells, Identification and reactivity of the catalytic site of pig liver thioltransferase, J. Biol. Chem. 262 (1987) 6704–6707, https://doi.org/10.1016/S0021-9258(18)48299-3.
[25] D.A.I. Mavridou, J.M. Stevens, S.J. Ferguson, C. Redfield, Active-site properties of the oxidized and reduced C-terminal domain of DsbD obtained by NMR spectroscopy, J. Mol. Biol. 370 (2007) 643–658, https://doi.org/10.1016/j.jmb.2007.04.038.
[26] G. Ferrer-Sueta, Chemical basis of cysteine reactivity and specificity: acidity and nucleophilicity, in: Redox Chemistry and Biology of Thiols, Elsevier, 2022, pp. 19–58, https://doi.org/10.1016/B978-0-323-90219-9.00014-5.
[27] W.H. Koppenol, J.J. Moreno, W.A. Pryor, H. Ischiropoulos, J.S. Beckman, Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide, Chem. Res. Toxicol. 5 (6) (1992) 834–842, https://doi.org/10.1021/tx00030a017.
[28] B. Alvarez, G. Ferrer-Sueta, B.A. Freeman, R. Radi, Kinetics of peroxynitrite reaction with amino acids and human serum albumin, J. Biol. Chem. 274 (1999) 842–848, https://doi.org/10.1074/jbc.274.2.842.
[29] H. Rubbo, A. Denicola, R. Radi, Peroxynitrite inactivates thiol-containing enzymes of trypanosoma cruzi energetic metabolism and inhibits cell respiration, Arch. Biochem. Biophys. 308 (1994) 96–102, https://doi.org/10.1006/abbi.1994.1014.
[30] M.G. Salgo, E. Bermudez, G.L. Squadrito, W.A. Pryor, DNA damage and oxidation of thiols peroxynitrite causes in rat thymocytes, Arch. Biochem. Biophys. 322 (1995) 500–505, https://doi.org/10.1006/abbi.1995.1493.
[31] R. Radi, M. Rodriguez, L. Castro, R. Telleri, Inhibition of mitochondrial electron transport by peroxynitrite, Arch. Biochem. Biophys. 308 (1994) 89–95, https://doi.org/10.1006/abbi.1994.1013.
[32] F.R. Gadelha, L. Thomson, M.M. Fagian, A.D.T. Costa, R. Radi, A.E. Vercesi, Ca2+-Independent permeabilization of the inner mitochondrial membrane by peroxynitrite is mediated by membrane protein thiol cross-linking and lipid peroxidation, Arch. Biochem. Biophys. 345 (1997) 243–250, https://doi.org/10.1006/abbi.1997.0259.
[33] R.I. Viner, A.F. Hühmer, D.J. Bigelow, C. Schöneich, The oxidative inactivation of sarcoplasmic reticulum Ca(2+)-ATPase by peroxynitrite, Free Radic. Res. 24 (1996) 243–259, https://doi.org/10.3109/10715769609088022.
[34] C. Schöneich, R.I. Viner, D.A. Ferrington, D.J. Bigelow, Age-related chemical modification of the skeletal muscle sarcoplasmic reticulum Ca-ATPase of the rat, Mechanisms of Ageing and Development 107 (1999) 221–231, https://doi.org/10.1016/S0047-6374(98)00158-4.
[35] L. Piacenza, A. Zeida, M. Trujillo, R. Radi, The superoxide radical switch in the biology of nitric oxide and peroxynitrite, Physiol. Rev. 102 (2022) 1881–1906, https://doi.org/10.1152/physrev.00005.2022.
[36] R. Bryk, P. Griffin, C. Nathan, Peroxynitrite reductase activity of bacterial peroxiredoxins, Nature 407 (2000) 211–215, https://doi.org/10.1038/35025109.
[37] L. Thomson, A. Denicola, R. Radi, The trypanothione–thiol system in Trypanosoma cruzi as a key antioxidant mechanism against peroxynitrite-mediated cytotoxicity, Arch. Biochem. Biophys. 412 (2003) 55–64, https://doi.org/10.1016/S0003-9861(02)00745-2.
[38] M. Trujillo, H. Budde, M.D. Piñeyro, M. Stehr, C. Robello, L. Flohé, R. Radi, Trypanosoma brucei and trypanosoma cruzi tryparedoxin peroxidases catalytically detoxify peroxynitrite via oxidation of fast reacting thiols, J. Biol. Chem. 279 (2004) 34175–34182, https://doi.org/10.1074/jbc.M404317200.
[39] O. Augusto, R.M. Gatti, R. Radi, Spin-trapping studies of peroxynitrite decomposition and of 3-morpholinosydnonimine N-ethylcarbamide autooxidation: direct evidence for metal-independent formation of free-radical intermediates, Arch. Biochem. Biophys. 310 (1994) 118–125, https://doi.org/10.1006/abbi.1994.1147.
[40] R.M. Gatti, R. Radi, O. Augusto, Peroxynitrite-mediated oxidation of albumin to the protein-thiyl free radical, FEBS (Fed. Eur. Biochem. Soc.) Lett. 348 (1994) 287–290, https://doi.org/10.1016/0014-5793(94)00625-3.
[41] C. Quijano, B. Alvarez, R.M. Gatti, O. Augusto, R. Radi, Pathways of peroxynitrite oxidation of thiol groups, Biochem. J. 322 (Pt 1) (1997) 167–173, https://doi.org/10.1042/bj3220167.
[42] M. Trujillo, B. Alvarez, R. Radi, One- and two-electron oxidation of thiols: mechanisms, kinetics and biological fates, Free Radic. Res. 50 (2016) 150–171, https://doi.org/10.3109/10715762.2015.1089988.
[43] A. Zeida, M. Trujillo, G. Ferrer-Sueta, A. Denicola, D.A. Estrin, R. Radi, Catalysis of peroxide reduction by fast reacting protein thiols: focus review, Chem. Rev. 119 (2019) 10829–10855, https://doi.org/10.1021/acs.chemrev.9b00371.
[44] R.E. Hansen, D. Roth, J.R. Winther, Quantifying the global cellular thiol-disulfide status, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 422–427, https://doi.org/10.1073/pnas.0812149106.
[45] R. Requejo, T.R. Hurd, N.J. Costa, M.P. Murphy, Cysteine residues exposed on protein surfaces are the dominant intramitochondrial thiol and may protect against oxidative damage, FEBS J. 277 (2010) 1465–1480, https://doi.org/10.1111/j.1742-4658.2010.07576.x.
[46] R.B. Moore, M.V. Mankad, S.K. Shriver, V.N. Mankad, G.A. Plishker, Reconstitution of Ca(2+)-dependent K+ transport in erythrocyte membrane vesicles requires a cytoplasmic protein, J. Biol. Chem. 266 (1991) 18964–18968, https://doi.org/10.1016/S0021-9258(18)55157-7.
[47] S. Watabe, H. Kohno, H. Kouyama, T. Hiroi, N. Yago, T. Nakazawa, Purification and characterization of a substrate protein for mitochondrial ATP-dependent protease in bovine adrenal cortex, The Journal of Biochemistry 115 (1994) 648–654, https://doi.org/10.1093/oxfordjournals.jbchem.a124390.
[48] J. Bonanata, L. Turell, L. Antmann, G. Ferrer-Sueta, S. Botasini, E. Méndez, B. Alvarez, E.L. Coitiño, The thiol of human serum albumin: acidity, microenvironment and mechanistic insights on its oxidation to sulfenic acid, Free Radic. Biol. Med. 108 (2017) 952–962, https://doi.org/10.1016/j.freeradbiomed.2017.04.021.
[49] S. Portillo-Ledesma, L.M. Randall, D. Parsonage, J. Dalla Rizza, P.A. Karplus, L. B. Poole, A. Denicola, G. Ferrer-Sueta, Differential kinetics of two-cysteine peroxiredoxin disulfide formation reveal a novel model for peroxide sensing, Biochemistry 57 (2018) 3416–3424, https://doi.org/10.1021/acs.biochem.8b00188.
[50] M.I. De Armas, R. Esteves, N. Viera, A.M. Reyes, M. Mastrogiovanni, T.G. P. Alegria, L.E.S. Netto, V. Tórtora, R. Radi, M. Trujillo, Rapid peroxynitrite reduction by human peroxiredoxin 3: implications for the fate of oxidants in mitochondria, Free Radic. Biol. Med. 130 (2019) 369–378, https://doi.org/10.1016/j.freeradbiomed.2018.10.451.
[51] M. Dubuisson, D. Vander Stricht, A. Clippe, F. Etienne, T. Nauser, R. Kissner, W. H. Koppenol, J.-F. Rees, B. Knoops, Human peroxiredoxin 5 is a peroxynitrite reductase, FEBS (Fed. Eur. Biochem. Soc.) Lett. 571 (2004) 161–165, https://doi.org/10.1016/j.febslet.2004.06.080.
[52] M. Trujillo, A. Clippe, B. Manta, G. Ferrer-Sueta, A. Smeets, J.-P. Declercq, B. Knoops, R. Radi, Pre-steady state kinetic characterization of human peroxiredoxin 5: taking advantage of Trp84 fluorescence increase upon oxidation, Arch. Biochem. Biophys. 467 (2007) 95–106, https://doi.org/10.1016/j.abb.2007.08.008.
[53] M. Hugo, L. Turell, B. Manta, H. Botti, G. Monteiro, L.E.S. Netto, B. Alvarez, R. Radi, M. Trujillo, Thiol and sulfenic acid oxidation of AhpE, the one-cysteine peroxiredoxin from *Mycobacterium tuberculosis* : kinetics, acidity constants, and conformational dynamics, Biochemistry 48 (2009) 9416–9426, https://doi.org/10.1021/bi901221s.
[54] B. Selles, M. Hugo, M. Trujillo, V. Srivastava, G. Wingsle, J.-P. Jacquot, R. Radi, N. Rouhier, Hydroperoxide and peroxynitrite reductase activity of poplar thioredoxin-dependent glutathione peroxidase 5: kinetics, catalytic mechanism and oxidative inactivation, Biochem. J. 442 (2012) 369–380, https://doi.org/10.1042/BJ20111378.
[55] E. Cuevasanta, A. Zeida, S. Carballal, R. Wedmann, U.N. Morzan, M. Trujillo, R. Radi, D.A. Estrin, M.R. Filipovic, B. Alvarez, Insights into the mechanism of the reaction between hydrogen sulfide and peroxynitrite, Free Radic. Biol. Med. 80 (2015) 93–100, https://doi.org/10.1016/j.freeradbiomed.2014.12.017.
[56] D. Benchoam, J.A. Semelak, E. Cuevasanta, M. Mastrogiovanni, J.S. Grassano, G. Ferrer-Sueta, A. Zeida, M. Trujillo, M.N. Möller, D.A. Estrin, B. Alvarez, Acidity and nucleophilic reactivity of glutathione persulfide, J. Biol. Chem. 295 (2020) 15466–15481, https://doi.org/10.1074/jbc.RA120.014728.
[57] E. Cuevasanta, M. Lange, J. Bonanata, E.L. Coitiño, G. Ferrer-Sueta, M. R. Filipovic, B. Alvarez, Reaction of hydrogen sulfide with disulfide and sulfenic acid to form the strongly nucleophilic persulfide, J. Biol. Chem. 290 (2015) 26866–26880, https://doi.org/10.1074/jbc.M115.672816.
[58] E. Cuevasanta, A.M. Reyes, A. Zeida, M. Mastrogiovanni, M.I. De Armas, R. Radi, B. Alvarez, M. Trujillo, Kinetics of formation and reactivity of the persulfide in the one-cysteine peroxiredoxin from Mycobacterium tuberculosis, J. Biol. Chem. 294 (2019) 13593–13605, https://doi.org/10.1074/jbc.RA119.008883.

[59] A. Denicola, B.A. Freeman, M. Trujillo, R. Radi, Peroxynitrite reaction with carbon dioxide/bicarbonate: kinetics and influence on peroxynitrite-mediated oxidations, Arch. Biochem. Biophys. 333 (1996) 49–58, https://doi.org/10.1006/abbi.1996.0363.

[60] O. Augusto, S. Goldstein, J.K. Hurst, J. Lind, S.V. Lymar, G. Merenyi, R. Radi, Carbon dioxide-catalyzed peroxynitrite reactivity - the resilience of the radical mechanism after two decades of research, Free Radic. Biol. Med. 135 (2019) 210–215, https://doi.org/10.1016/j.freeradbiomed.2019.02.026.

[61] A. Zeida, M.C. González Lebrero, R. Radi, M. Trujillo, D.A. Estrin, Mechanism of cysteine oxidation by peroxynitrite: an integrated experimental and theoretical study, Arch. Biochem. Biophys. 539 (2013) 81–86, https://doi.org/10.1016/j.abb.2013.08.016.

[62] A. Zeida, R. Babbush, M.C. González Lebrero, M. Trujillo, R. Radi, D.A. Estrin, Molecular basis of the mechanism of thiol oxidation by hydrogen peroxide in aqueous solution: challenging the S_N2 paradigm, Chem. Res. Toxicol. 25 (2012) 741–746, https://doi.org/10.1021/tx200540z.

[63] M.J. Torres, L. Turell, H. Botti, L. Antmann, S. Carballal, G. Ferrer-Sueta, R. Radi, B. Alvarez, Modulation of the reactivity of the thiol of human serum albumin and its sulfenic derivative by fatty acids, Arch. Biochem. Biophys. 521 (2012) 102–110, https://doi.org/10.1016/j.abb.2012.03.011.

[64] A. Zeida, R. Radi, Thiol oxidation by biologically-relevant reactive species, in: Redox Chemistry and Biology of Thiols, Elsevier, 2022, pp. 99–113, https://doi.org/10.1016/B978-0-323-90219-9.00028-5.

[65] N.O. Devarie-Baez, E.I. Silva Lopez, C.M. Furdui, Biological chemistry and functionality of protein sulfenic acids and related thiol modifications, Free Radic. Res. 50 (2016) 172–194, https://doi.org/10.3109/10715762.2015.1090571.

[66] L. Turell, A. Zeida, M. Trujillo, Mechanisms and consequences of protein cysteine oxidation: the role of the initial short-lived intermediates, Essays Biochem. 64 (2020) 55–66, https://doi.org/10.1042/EBC20190053.

[67] C.L. Grek, J. Zhang, Y. Manevich, D.M. Townsend, K.D. Tew, Causes and consequences of cysteine S-glutathionylation, J. Biol. Chem. 288 (2013) 26497–26504, https://doi.org/10.1074/jbc.R113.461368.

[68] F.T. Ogata, V. Branco, F.F. Vale, L. Coppo, Glutaredoxin: discovery, redox defense and much more, Redox Biol. 43 (2021) 101975, https://doi.org/10.1016/j.redox.2021.101975.

[69] S.F. Villar, G. Ferrer-Sueta, A. Denicola, The multifaceted nature of peroxiredoxins in chemical biology, Curr. Opin. Chem. Biol. 76 (2023) 102355, https://doi.org/10.1016/j.cbpa.2023.102355.

[70] W.S. Allison, L.V. Benitez, C.L. Johnson, The formation of a protein sulfenamide during the inactivation of the acyl phosphatase activity of oxidized glyceraldehyde-3-phosphate dehydrogenase by benzylamine, Biochem. Biophys. Res. Commun. 52 (1973) 1403–1409, https://doi.org/10.1016/0006-291X(73)90657-8.

[71] A. Salmeen, J.N. Andersen, M.P. Myers, T.-C. Meng, J.A. Hinks, N.K. Tonks, D. Barford, Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate, Nature 423 (2003) 769–773, https://doi.org/10.1038/nature01680.

[72] R.L.M. Van Montfort, M. Congreve, D. Tisi, R. Carr, H. Jhoti, Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B, Nature 423 (2003) 773–777, https://doi.org/10.1038/nature01681.

[73] M. Fontana, F. Giovannitti, L. Pecci, The protective effect of hypotaurine and cysteine sulphinic acid on peroxynitrite-mediated oxidative reactions, Free Radic. Res. 42 (2008) 320–330, https://doi.org/10.1080/10715760801999727.

[74] B. Biteau, J. Labarre, M.B. Toledano, ATP-dependent reduction of cysteine–sulphinic acid by S. cerevisiae sulphiredoxin, Nature 425 (2003) 980–984, https://doi.org/10.1038/nature02075.

[75] S. Akter, L. Fu, Y. Jung, M.L. Conte, J.R. Lawson, W.T. Lowther, R. Sun, K. Liu, J. Yang, K.S. Carroll, Chemical proteomics reveals new targets of cysteine sulfinic acid reductase, Nat. Chem. Biol. 14 (2018) 995–1004, https://doi.org/10.1038/s41589-018-0116-2.

[76] X. Roussel, G. Béchade, A. Kriznik, A. Van Dorsselaer, S. Sanglier-Cianferani, G. Branlant, S. Rahuel-Clermont, Evidence for the formation of a covalent thiosulfinate intermediate with peroxiredoxin in the catalytic mechanism of sulfiredoxin, J. Biol. Chem. 283 (2008) 22371–22382, https://doi.org/10.1074/jbc.M800493200.

[77] G. Monteiro, B.B. Horta, D.C. Pimenta, O. Augusto, L.E.S. Netto, Reduction of 1-Cys peroxiredoxins by ascorbate changes the thiol-specific antioxidant paradigm, revealing another function of vitamin C, Proc Natl Acad Sci U S A 104 (2007) 4886–4891, https://doi.org/10.1073/pnas.0700481104.

[78] V. Anschau, G. Ferrer-Sueta, R.L. Aleixo-Silva, R. Bannitz Fernandes, C.A. Tairum, C.C.C. Tonoli, M.T. Murakami, M.A. de Oliveira, L.E.S. Netto, Reduction of sulfenic acids by ascorbate in proteins, connecting thiol-dependent to alternative redox pathways, Free Radic. Biol. Med. 156 (2020) 207–216, https://doi.org/10.1016/j.freeradbiomed.2020.06.015.

[79] I.V. Peshenko, H. Shichi, Oxidation of active center cysteine of bovine 1-Cys peroxiredoxin to the cysteine sulfenic acid form by peroxide and peroxynitrite, Free Radic. Biol. Med. 31 (2001) 292–303, https://doi.org/10.1016/S0891-5849(01)00579-2.

[80] S. Carballal, R. Radi, M.C. Kirk, S. Barnes, B.A. Freeman, B. Alvarez, Sulfenic acid formation in human serum albumin by hydrogen peroxide and peroxynitrite, Biochemistry 42 (2003) 9906–9914, https://doi.org/10.1021/bi027434m.

[81] L. Turell, H. Botti, S. Carballal, G. Ferrer-Sueta, J.M. Souza, R. Durán, B. A. Freeman, R. Radi, B. Alvarez, Reactivity of sulfenic acid in human serum albumin, Biochemistry 47 (2008) 358–367, https://doi.org/10.1021/bi701520y.

[82] L. Turell, M. Steglich, M.J. Torres, M. Deambrosi, L. Antmann, C.M. Furdui, F. J. Schopfer, B. Alvarez, Sulfenic acid in human serum albumin: reaction with thiols, oxidation and spontaneous decay, Free Radic. Biol. Med. 165 (2021) 254–264, https://doi.org/10.1016/j.freeradbiomed.2021.01.039.

[83] L. Turell, H. Botti, S. Carballal, R. Radi, B. Alvarez, Sulfenic acid-a key intermediate in albumin thiol oxidation, J Chromatogr B Analyt Technol Biomed Life Sci 877 (2009) 3384–3392, https://doi.org/10.1016/j.jchromb.2009.03.035.

[84] V. Gupta, K.S. Carroll, Sulfenic acid chemistry, detection and cellular lifetime, Biochim. Biophys. Acta 1840 (2014) 847–875, https://doi.org/10.1016/j.bbagen.2013.05.040.

[85] Y. Shi, K.S. Carroll, Activity-based sensing for site-specific proteomic analysis of cysteine oxidation, Acc. Chem. Res. 53 (2020) 20–31, https://doi.org/10.1021/acs.accounts.9b00562.

[86] C.M. Furdui, L.B. Poole, Chemical approaches to detect and analyze protein sulfenic acids, Mass Spectrom. Rev. 33 (2014) 126–146, https://doi.org/10.1002/mas.21384.

[87] J.M.M. Pople, J.M. Chalker, A critical evaluation of probes for cysteine sulfenic acid, Curr. Opin. Chem. Biol. 60 (2021) 55–65, https://doi.org/10.1016/j.cbpa.2020.07.011.

[88] Y. Shi, K.S. Carroll, Comments on 'A critical evaluation of probes for cysteine sulfenic acid, Curr. Opin. Chem. Biol. 60 (2021) 131–133, https://doi.org/10.1016/j.cbpa.2021.01.004.

[89] E. Bischoff, L. Lang, J. Zimmermann, M. Luczak, A.M. Kiefer, G. Niedner-Schatteburg, G. Manolikakes, B. Morgan, M. Deponte, Glutathione kinetically outcompetes reactions between dimedone and a cyclic sulfenamide or physiological sulfenic acids, Free Radic. Biol. Med. 208 (2023) 165–177, https://doi.org/10.1016/j.freeradbiomed.2023.08.005.

[90] E. Galardon, D. Padovani, Reactivity of persulfides toward strained bicyclo[6.1.0]nonyne derivatives: relevance to chemical tagging of proteins, Bioconjug. Chem. 26 (2015) 1013–1016, https://doi.org/10.1021/acs.bioconjchem.5b00243.

[91] J. Dalla Rizza, L.M. Randall, J. Santos, G. Ferrer-Sueta, A. Denicola, Differential parameters between cytosolic 2-Cys peroxiredoxins, PRDX1 and PRDX2, Protein Sci. 28 (2019) 191–201, https://doi.org/10.1002/pro.3520.

[92] S.F. Villar, L. Corrales-González, B. Márquez de Los Santos, J. Dalla Rizza, A. Zeida, A. Denicola, G. Ferrer-Sueta, Kinetic and structural assessment of the reduction of human 2-Cys peroxiredoxins by thioredoxins, FEBS J. 291 (2024) 778–794, https://doi.org/10.1111/febs.17006.

[93] D. Talwar, T.P. Dick, Thiol peroxidase-based redox relays, in: Redox Chemistry and Biology of Thiols, Elsevier, 2022, pp. 307–320, https://doi.org/10.1016/B978-0-323-90219-9.00007-8.

[94] S.G. Rhee, H.Z. Chae, K. Kim, Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling, Free Radic. Biol. Med. 38 (2005) 1543–1552, https://doi.org/10.1016/j.freeradbiomed.2005.02.026.

[95] L. Flohé, J.R. Harris. Introduction: History of the Peroxiredoxins and Topical Perspectives, Springer, 2007, https://doi.org/10.1007/978-1-4020-6051-9.

[96] L. Soito, C. Williamson, S.T. Knutson, J.S. Fetrow, L.B. Poole, K.J. Nelson, PREX: PeroxiRedoxin classification indEX, a database of subfamily assignments across the diverse peroxiredoxin family, Nucleic Acids Res. 39 (2011) D332–D337, https://doi.org/10.1093/nar/gkq1060.

[97] K.J. Nelson, S.T. Knutson, L. Soito, C. Klomsiri, L.B. Poole, J.S. Fetrow, Analysis of the peroxiredoxin family: using active-site structure and sequence information for global classification and residue analysis, Proteins 79 (2011) 947–964, https://doi.org/10.1002/prot.22936.

[98] A.G. Cox, C.C. Winterbourn, M.B. Hampton, Measuring the redox state of cellular peroxiredoxins by immunoblotting, in: Methods in Enzymology, Elsevier, 2010, pp. 51–66, https://doi.org/10.1016/S0076-6879(10)74004-0.

[99] L. Piacenza, G. Peluffo, M.N. Alvarez, J.M. Kelly, S.R. Wilkinson, R. Radi, Peroxiredoxins play a major role in protecting *Trypanosoma cruzi* against macrophage- and endogenously-derived peroxynitrite, Biochem. J. 410 (2008) 359–368, https://doi.org/10.1042/BJ20071138.

[100] I.V. Turko, L. Li, K.S. Aulak, D.J. Stuehr, J.-Y. Chang, F. Murad, Protein tyrosine nitration in the mitochondria from diabetic mouse heart, J. Biol. Chem. 278 (2003) 33972–33977, https://doi.org/10.1074/jbc.M303734200.

[101] L.M. Randall, B. Manta, M. Hugo, M. Gil, C. Batthyàny, M. Trujillo, L.B. Poole, A. Denicola, Nitration transforms a sensitive peroxiredoxin 2 into a more active and robust peroxidase, J. Biol. Chem. 289 (2014) 15536–15543, https://doi.org/10.1074/jbc.M113.539213.

[102] L. Randall, B. Manta, K.J. Nelson, J. Santos, L.B. Poole, A. Denicola, Structural changes upon peroxynitrite-mediated nitration of peroxiredoxin 2; nitrated Prx2 resembles its disulfide-oxidized form, Arch. Biochem. Biophys. 590 (2016) 101–108, https://doi.org/10.1016/j.abb.2015.11.032.

[103] K. Briviba, R. Kissner, W.H. Koppenol, H. Sies, Kinetic study of the reaction of glutathione peroxidase with peroxynitrite, Chem. Res. Toxicol. 11 (1998) 1398–1401, https://doi.org/10.1021/tx980086y.

[104] M. Maiorino, F. Ursini, V. Bosello, S. Toppo, S.C.E. Tosatto, P. Mauri, K. Becker, A. Roveri, C. Bulato, L. Benazzi, A. De Palma, L. Flohé, The thioredoxin specificity of Drosophila GPx: a paradigm for a peroxiredoxin-like mechanism of many glutathione peroxidases, J. Mol. Biol. 365 (2007) 1033–1046, https://doi.org/10.1016/j.jmb.2006.10.033.

[105] Y. Fu, H. Sies, X.G. Lei, Opposite roles of selenium-dependent glutathione peroxidase-1 in superoxide generator diquat- and peroxynitrite-induced apoptosis and signaling, J. Biol. Chem. 276 (2001) 43004–43009, https://doi.org/10.1074/jbc.M106946200.

[106] X. Wang, Y. Han, F. Chen, M. Wang, Y. Xiao, H. Wang, L. Xu, W. Liu, Glutathione peroxidase 1 protects against peroxynitrite-induced spiral ganglion neuron damage through attenuating NF-κB pathway activation, Front. Cell. Neurosci. 16 (2022) 841731, https://doi.org/10.3389/fncel.2022.841731.

[107] M. Trujillo, C.A. Tairum, M.A. de Oliveira, L.E.S. Netto, Chapter 13 - thiol- and selenol-based peroxidases: structure and catalytic properties, in: B. Alvarez, M. A. Comini, G. Salinas, M. Trujillo (Eds.), Redox Chemistry and Biology of Thiols, Academic Press, 2022, pp. 277–305, https://doi.org/10.1016/B978-0-323-90219-9.00008-X.

[108] D.A. Armstrong, R.E. Huie, W.H. Koppenol, S.V. Lymar, G. Merenyi, P. Neta, B. Ruscic, D.M. Stanbury, S. Steenken, P. Wardman, Standard electrode potentials involving radicals in aqueous solution: inorganic radicals (IUPAC Technical Report), Pure Appl. Chem. 87 (2015) 1139–1150, https://doi.org/10.1515/pac-2014-0502.

[109] E. Ford, M.N. Hughes, P. Wardman, Kinetics of the reactions of nitrogen dioxide with glutathione, cysteine, and uric acid at physiological pH, Free Radic. Biol. Med. 32 (2002) 1314–1323, https://doi.org/10.1016/s0891-5849(02)00850-x.

[110] M.N. Alvarez, G. Peluffo, L. Folkes, P. Wardman, R. Radi, Reaction of the carbonate radical with the spin-trap 5,5-dimethyl-1-pyrroline-N-oxide in chemical and cellular systems: pulse radiolysis, electron paramagnetic resonance, and kinetic-competition studies, Free Radic. Biol. Med. 43 (2007) 1523–1533, https://doi.org/10.1016/j.freeradbiomed.2007.08.002.

[111] S.P.A. Goss, R.J. Singh, B. Kalyanaraman, Bicarbonate enhances the peroxidase activity of Cu,Zn-superoxide dismutase, J. Biol. Chem. 274 (1999) 28233–28239, https://doi.org/10.1074/jbc.274.40.28233.

[112] S.I. Liochev, I. Fridovich, On the role of bicarbonate in peroxidations catalyzed by Cu,Zn superoxide dismutase, Free Radic. Biol. Med. 27 (1999) 1444–1447, https://doi.org/10.1016/S0891-5849(99)00190-2.

[113] M.G. Bonini, O. Augusto, Carbon dioxide stimulates the production of thiyl, sulfinyl, and disulfide radical anion from thiol oxidation by peroxynitrite, J. Biol. Chem. 276 (2001) 9749–9754, https://doi.org/10.1074/jbc.M008456200.

[114] S. Chen, M.Z. Hoffman, Rate constants for the reaction of the carbonate radical with compounds of biochemical interest in neutral aqueous solution, Radiat. Res. 56 (1973) 40, https://doi.org/10.2307/3573789.

[115] J. Vásquez-Vivar, A.M. Santos, V.B.C. Junqueira, O. Augusto, Peroxynitrite-mediated formation of free radicals in human plasma: EPR detection of ascorbyl, albumin-thiyl and uric acid-derived free radicals, Biochem. J. 314 (1996) 869–876, https://doi.org/10.1042/bj3140869.

[116] E. Madej, P. Wardman, The oxidizing power of the glutathione thiyl radical as measured by its electrode potential at physiological pH, Arch. Biochem. Biophys. 462 (2007) 94–102, https://doi.org/10.1016/j.abb.2007.03.002.

[117] P. Wardman, C. Von Sonntag, [3] Kinetic factors that control the fate of thiyl radicals in cells, in: Methods in Enzymology, Elsevier, 1995, pp. 31–45, https://doi.org/10.1016/0076-6879(95)51108-3.

[118] M. Tamba, G. Simone, M. Quintiliani, Interactions of thiyl free radicals with oxygen: a pulse radiolysis study, Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 50 (1986) 595–600, https://doi.org/10.1080/09553008614550991.

[119] M.D. Sevilla, D. Becker, M. Yan, The Formation and structure of the sulfoxyl radicals RSO·, RSOO·, RSO $_2$ ·, and RSO $_2$ OO· from the reaction of cysteine, glutathione and penicillamine thiyl radicals with molecular oxygen, Int. J. Radiat. Biol. 57 (1990) 65–81, https://doi.org/10.1080/09553009014550351.

[120] A. Baseggio Conrado, M. D'Angelantonio, A. Torreggiani, L. Pecci, M. Fontana, Reactivity of hypotaurine and cysteine sulfinic acid toward carbonate radical anion and nitrogen dioxide as explored by the peroxidase activity of Cu,Zn superoxide dismutase and by pulse radiolysis, Free Radic. Res. 48 (2014) 1300–1310, https://doi.org/10.3109/10715762.2014.951839.

[121] A. Aicardo, M. Mastrogiovanni, A. Cassina, R. Radi, Propagation of free-radical reactions in concentrated protein solutions, Free Radic. Res. 52 (2018) 159–170, https://doi.org/10.1080/10715762.2017.1420905.

[122] H. Forstner, E. Gnaiger, Calculation of equilibrium oxygen concentration, in: E. Gnaiger, H. Forstner (Eds.), Polarographic Oxygen Sensors, Springer, Berlin, Heidelberg, 1983, pp. 321–333, https://doi.org/10.1007/978-3-642-81863-9_28.

[123] T.L. Place, F.E. Domann, A.J. Case, Limitations of oxygen delivery to cells in culture: an underappreciated problem in basic and translational research, Free Radic. Biol. Med. 113 (2017) 311–322, https://doi.org/10.1016/j.freeradbiomed.2017.10.003.

[124] A.C. Casella, C. Prolo, J. Pereyra, N. Ríos, L. Piacenza, R. Radi, M.N. Álvarez, Superoxide, nitric oxide and peroxynitrite production by macrophages under different physiological oxygen tensions, Free Radic. Biol. Med. 212 (2024) 330–335, https://doi.org/10.1016/j.freeradbiomed.2023.12.024.

[125] M.Z. Hoffman, E. Hayon, Pulse radiolysis study of sulfhydryl compounds in aqueous solution, J. Phys. Chem. 77 (1973) 990–996, https://doi.org/10.1021/j100627a005.

[126] M.Z. Baker, R. Badiello, M. Tamba, M. Quintiliani, G. Gorin, Pulse radiolytic study of hydrogen transfer from glutathione to organic radicals, Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. (1982), https://doi.org/10.1080/09553008214550691.

[127] W.A. Prütz, J. Butler, E.J. Land, The glutathione free radical equilibrium, GS. + GS−⇌ GSS.−G, mediating electron transfer to FE(III) -cytochrome c, Biophys. Chem. 49 (1994) 101–111, https://doi.org/10.1016/0301-4622(93)E0057-C.

[128] C.C. Winterbourn, Superoxide as an intracellular radical sink, Free Radic. Biol. Med. 14 (1993) 85–90, https://doi.org/10.1016/0891-5849(93)90512-S.

[129] S. Portillo-Ledesma, F. Sardi, B. Manta, M.V. Tourn, A. Clippe, B. Knoops, B. Alvarez, E.L. Coitiño, G. Ferrer-Sueta, Deconstructing the catalytic efficiency of peroxiredoxin-5 peroxidatic cysteine, Biochemistry 53 (2014) 6113–6125, https://doi.org/10.1021/bi500389m.

[130] L.G. Forni, J. Mönig, V.O. Mora-Arellano, R.L. Willson, Thiyl free radicals: direct observations of electron transfer reactions with phenothiazines and ascorbate, J. Chem. Soc., Perkin Trans. 2 (1983) 961–965, https://doi.org/10.1039/P29830000961.

[131] P. Wardman, Evaluation of the "radical sink" hypothesis from a chemical-kinetic viewpoint, J. Radioanal. Nucl. Chem. 232 (1998) 23–27, https://doi.org/10.1007/BF02383706.

[132] W.A. Prütz, J. Butler, E.J. Land, A.J. Swallow, The role of sulphur peptide functions in free radical transfer: a pulse radiolysis study, Int. J. Radiat. Biol. 55 (1989) 539–556, https://doi.org/10.1080/09553008914550591.

[133] L.K. Folkes, M. Trujillo, S. Bartesaghi, R. Radi, P. Wardman, Kinetics of reduction of tyrosine phenoxyl radicals by glutathione, Arch. Biochem. Biophys. 506 (2011) 242–249, https://doi.org/10.1016/j.abb.2010.12.006.

[134] A. Martinez, G. Peluffo, A.A. Petruk, M. Hugo, D. Piñeyro, V. Demicheli, D. M. Moreno, A. Lima, C. Batthyány, R. Durán, C. Robello, M.A. Martí, N. Larrieux, A. Buschiazzo, M. Trujillo, R. Radi, L. Piacenza, Structural and molecular basis of the peroxynitrite-mediated nitration and inactivation of Trypanosoma cruzi iron-superoxide dismutases (Fe-SODs) A and B: disparate susceptibilities due to the repair of Tyr35 radical by Cys83 in Fe-SODB through intramolecular electron transfer, J. Biol. Chem. 289 (2014) 12760–12778, https://doi.org/10.1074/jbc.M113.545590.

[135] C. Schoneich, Thiyl radicals: formation, properties, and detection, Redox Chemistry and Biology of Thiols, 2022, p. 115, https://doi.org/10.1016/B978-0-323-90219-9.00001-7.

[136] C. Schöneich, Thiyl radicals and induction of protein degradation, Free Radic. Res. 50 (2016) 143–149, https://doi.org/10.3109/10715762.2015.1077385.

[137] M.R. Filipovic, J. Zivanovic, B. Alvarez, R. Banerjee, Chemical biology of H2S signaling through persulfidation, Chem Rev 118 (2018) 1253–1337, https://doi.org/10.1021/acs.chemrev.7b00205.

[138] J.W. Elrod, J.W. Calvert, J. Morrison, J.E. Doeller, D.W. Kraus, L. Tao, X. Jiao, R. Scalia, L. Kiss, C. Szabo, H. Kimura, C.-W. Chow, D.J. Lefer, Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 15560–15565, https://doi.org/10.1073/pnas.0705891104.

[139] S. Jha, J.W. Calvert, M.R. Duranski, A. Ramachandran, D.J. Lefer, Hydrogen sulfide attenuates hepatic ischemia-reperfusion injury: role of antioxidant and antiapoptotic signaling, Am. J. Physiol. Heart Circ. Physiol. 295 (2008) H801–H806, https://doi.org/10.1152/ajpheart.00377.2008.

[140] S. Carballal, M. Trujillo, E. Cuevasanta, S. Bartesaghi, M.N. Möller, L.K. Folkes, M. A. García-Bereguiaín, C. Gutiérrez-Merino, P. Wardman, A. Denicola, R. Radi, B. Alvarez, Reactivity of hydrogen sulfide with peroxynitrite and other oxidants of biological interest, Free Radic. Biol. Med. 50 (2011) 196–205, https://doi.org/10.1016/j.freeradbiomed.2010.10.705.

[141] J.C. Mathai, A. Missner, P. Kügler, S.M. Saparov, M.L. Zeidel, J.K. Lee, P. Pohl, No facilitator required for membrane transport of hydrogen sulfide, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 16633–16638, https://doi.org/10.1073/pnas.0902952106.

[142] E. Cuevasanta, A. Denicola, B. Alvarez, M.N. Möller, Solubility and permeation of hydrogen sulfide in lipid membranes, PLoS One 7 (2012) e34562, https://doi.org/10.1371/journal.pone.0034562.

[143] M.N. Hughes, M.N. Centelles, K.P. Moore, 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. 47 (2009) 1346–1353, https://doi.org/10.1016/j.freeradbiomed.2009.09.018.

[144] T. Chiku, D. Padovani, W. Zhu, S. Singh, V. Vitvitsky, R. Banerjee, H2S biogenesis by human cystathionine gamma-lyase leads to the novel sulfur metabolites lanthionine and homolanthionine and is responsive to the grade of hyperhomocysteinemia, J. Biol. Chem. 284 (2009) 11601–11612, https://doi.org/10.1074/jbc.M808026200.

[145] S. Singh, D. Padovani, R.A. Leslie, T. Chiku, R. Banerjee, Relative contributions of cystathionine beta-synthase and gamma-cystathionase to H2S biogenesis via alternative trans-sulfuration reactions, J. Biol. Chem. 284 (2009) 22457–22466, https://doi.org/10.1074/jbc.M109.010868.

[146] N. Shibuya, M. Tanaka, M. Yoshida, Y. Ogasawara, T. Togawa, K. Ishii, H. Kimura, 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain, Antioxid. Redox Signal. 11 (2009) 703–714, https://doi.org/10.1089/ars.2008.2253.

[147] P.K. Yadav, K. Yamada, T. Chiku, M. Koutmos, R. Banerjee, Structure and kinetic analysis of H2S production by human mercaptopyruvate sulfurtransferase, J. Biol. Chem. 288 (2013) 20002–20013, https://doi.org/10.1074/jbc.M113.466177.

[148] G.T. Macfarlane, G.R. Gibson, J.H. Cummings, Comparison of fermentation reactions in different regions of the human colon, J. Appl. Bacteriol. 72 (1992) 57–64, https://doi.org/10.1111/j.1365-2672.1992.tb04882.x.

[149] B. Deplancke, K. Finster, W.V. Graham, C.T. Collier, J.E. Thurmond, H.R. Gaskins, Gastrointestinal and microbial responses to sulfate-supplemented drinking water in Mice, Exp Biol Med (Maywood) 228 (2003) 424–433, https://doi.org/10.1177/153537020322800413.

[150] T.M. Hildebrandt, M.K. Grieshaber, Three enzymatic activities catalyze the oxidation of sulfide to thiosulfate in mammalian and invertebrate mitochondria, FEBS J. 275 (2008) 3352–3361, https://doi.org/10.1111/j.1742-4658.2008.06482.x.

[151] M. Libiad, P.K. Yadav, V. Vitvitsky, M. Martinov, R. Banerjee, Organization of the human mitochondrial hydrogen sulfide oxidation pathway, J. Biol. Chem. 289 (2014) 30901–30910, https://doi.org/10.1074/jbc.M114.602664.

[152] A.P. Landry, D.P. Ballou, R. Banerjee, H2S oxidation by nanodisc-embedded human sulfide quinone oxidoreductase, J. Biol. Chem. 292 (2017) 11641–11649, https://doi.org/10.1074/jbc.M117.788547.

[153] M.R. Filipovic, J. Miljkovic, A. Allgäuer, R. Chaurio, T. Shubina, M. Herrmann, I. Ivanovic-Burmazovic, Biochemical insight into physiological effects of H_2S:

reaction with peroxynitrite and formation of a new nitric oxide donor, sulfinyl nitrite, Biochem. J. 441 (2012) 609–621, https://doi.org/10.1042/BJ20111389.
[154] J. Furne, A. Saeed, M.D. Levitt, Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values, Am. J. Physiol. Regul. Integr. Comp. Physiol. 295 (2008) R1479–R1485, https://doi.org/10.1152/ajpregu.90566.2008.
[155] R. Wedmann, A. Zahl, T.E. Shubina, M. Dürr, F.W. Heinemann, B.E. C. Bugenhagen, P. Burger, I. Ivanovic-Burmazovic, M.R. Filipovic, Does perthionitrite (SSNO–) account for sustained bioactivity of no? A (Bio)chemical characterization, Inorg. Chem. 54 (2015) 9367–9380, https://doi.org/10.1021/acs.inorgchem.5b00831.
[156] M.M. Cortese-Krott, A.R. Butler, J.D. Woollins, M. Feelisch, Inorganic sulfur–nitrogen compounds: from gunpowder chemistry to the forefront of biological signaling, Dalton Trans. 45 (2016) 5908–5919, https://doi.org/10.1039/C5DT05034K.
[157] J.P. Marcolongo, M.F. Venâncio, W.R. Rocha, F. Doctorovich, J.A. Olabe, NO/H2S "crosstalk" reactions. The role of thionitrites (SNO–) and perthionitrites (SSNO–), Inorg. Chem. 58 (2019) 14981–14997, https://doi.org/10.1021/acs.inorgchem.9b01978.
[158] T.N. Das, R.E. Huie, P. Neta, S. Padmaja, Reduction potential of the sulfhydryl radical: pulse radiolysis and laser flash photolysis studies of the formation and reactions of ·SH and HSSH·- in aqueous solutions, J. Phys. Chem. A 103 (1999) 5221–5226, https://doi.org/10.1021/jp9907544.
[159] D. Benchoam, E. Cuevasanta, M.N. Möller, B. Alvarez, Hydrogen sulfide and persulfides oxidation by biologically relevant oxidizing species, Antioxidants 8 (2019) 48, https://doi.org/10.3390/antiox8020048.
[160] D. Benchoam, E. Cuevasanta, M.N. Möller, B. Alvarez, Persulfides and their reactions in biological contexts, in: Hydrogen Sulfide, John Wiley & Sons, Ltd, 2022, pp. 49–76, https://doi.org/10.1002/9781119799900.ch3.
[161] E.G. Mueller, Trafficking in persulfides: delivering sulfur in biosynthetic pathways, Nat. Chem. Biol. 2 (2006) 185–194, https://doi.org/10.1038/nchembio779.
[162] J.-C. Lec, S. Boutserin, H. Mazon, G. Mulliert, S. Boschi-Muller, F. Talfournier, Unraveling the mechanism of cysteine persulfide formation catalyzed by 3-mercaptopyruvate sulfurtransferases, ACS Catal. 8 (2018) 2049–2059, https://doi.org/10.1021/acscatal.7b02432.
[163] P. Horowitz, N.L. Criscimagna, The use of intrinsic protein fluorescence to quantitate enzyme-bound persulfide and to measure equilibria between intermediates in rhodanese catalysis, J. Biol. Chem. 258 (1983) 7894–7896, https://doi.org/10.1016/s0021-9258(20)81997-8.
[164] B.J.C. Walsh, D.P. Giedroc, H2S and reactive sulfur signaling at the host-bacterial pathogen interface, J. Biol. Chem. 295 (2020) 13150–13168, https://doi.org/10.1074/jbc.REV120.011304.
[165] T. Ida, T. Sawa, H. Ihara, Y. Tsuchiya, Y. Watanabe, Y. Kumagai, M. Suematsu, H. Motohashi, S. Fujii, T. Matsunaga, M. Yamamoto, K. Ono, N.O. Devarie-Baez, M. Xian, J.M. Fukuto, T. Akaike, Reactive cysteine persulfides and S-polythiolation regulate oxidative stress and redox signaling, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 7606–7611, https://doi.org/10.1073/pnas.1321232111.
[166] D. Benchoam, E. Cuevasanta, J.V. Roman, R. Banerjee, B. Alvarez, Acidity of persulfides and its modulation by the protein environments in sulfide quinone oxidoreductase and thiosulfate sulfurtransferase, J. Biol. Chem. (2024) 107149, https://doi.org/10.1016/j.jbc.2024.107149.
[167] W.P. Jencks, J. Carriuolo, Reactivity of nucleophilic reagents toward esters, J. Am. Chem. Soc. 82 (1960) 1778–1786, https://doi.org/10.1021/ja01492a058.
[168] J.O. Edwards, R.G. Pearson, The factors determining nucleophilic reactivities, J. Am. Chem. Soc. 84 (1962) 16–24, https://doi.org/10.1021/ja00860a005.
[169] V. Gold, Glossary of terms used in physical organic chemistry, Pure Appl. Chem. 55 (1983) 1281–1371, https://doi.org/10.1351/pac198355081281.
[170] D.E. Heppner, M. Hristova, T. Ida, A. Mijuskovic, C.M. Dustin, V. Bogdándi, J. M. Fukuto, T.P. Dick, P. Nagy, J. Li, T. Akaike, A. van der Vliet, Cysteine perthiosulfenic acid (Cys-SSOH): a novel intermediate in thiol-based redox signaling? Redox Biol. 14 (2018) 379–385, https://doi.org/10.1016/j.redox.2017.10.006.
[171] É. Dóka, T. Ida, M. Dagnell, Y. Abiko, N.C. Luong, N. Balog, T. Takata, B. Espinosa, A. Nishimura, Q. Cheng, Y. Funato, H. Miki, J.M. Fukuto, J.R. Prigge, E.E. Schmidt, E.S.J. Arnér, Y. Kumagai, T. Akaike, P. Nagy, Control of protein function through oxidation and reduction of persulfidated states, Sci. Adv. 6 (2020), https://doi.org/10.1126/sciadv.aax8358 eaax8358.
[172] J. Zivanovic, E. Kouroussis, J.B. Kohl, B. Adhikari, B. Bursac, S. Schott-Roux, D. Petrovic, J.L. Miljkovic, D. Thomas-Lopez, Y. Jung, M. Miler, S. Mitchell, V. Milosevic, J.E. Gomes, M. Benhar, B. Gonzalez-Zorn, I. Ivanovic-Burmazovic, R. Torregrossa, J.R. Mitchell, M. Whiteman, G. Schwarz, S.H. Snyder, B.D. Paul, K.S. Carroll, M.R. Filipovic, Selective persulfide detection reveals evolutionarily conserved antiaging effects of S-sulfhydration, Cell Metab 30 (2019) 1152–1170. e13, https://doi.org/10.1016/j.cmet.2019.10.007.
[173] R. Wedmann, C. Onderka, S. Wei, I.A. Szijártó, J.L. Miljkovic, A. Mitrovic, M. Lange, S. Savitsky, P.K. Yadav, R. Torregrossa, E.G. Harrer, T. Harrer, I. Ishii, M. Gollasch, M.E. Wood, E. Galardon, M. Xian, M. Whiteman, R. Banerjee, M. R. Filipovic, Improved tag-switch method reveals that thioredoxin acts as depersulfidase and controls the intracellular levels of protein persulfidation, Chem. Sci. (2016), https://doi.org/10.1039/C5SC04818D.
[174] R. Greiner, Z. Pálinkás, K. Bäsell, D. Becher, H. Antelmann, P. Nagy, T.P. Dick, Polysulfides link H2S to protein thiol oxidation, Antioxid. Redox Signal. 19 (2013) 1749–1765, https://doi.org/10.1089/ars.2012.5041.
[175] R. Millikin, C.L. Bianco, C. White, S.S. Saund, S. Henriquez, V. Sosa, T. Akaike, Y. Kumagai, S. Soeda, J.P. Toscano, J. Lin, J.M. Fukuto, The chemical biology of protein hydropersulfides: studies of a possible protective function of biological hydropersulfide generation, Free Radic. Biol. Med. 97 (2016) 136–147, https://doi.org/10.1016/j.freeradbiomed.2016.05.013.
[176] S.A. Everett, L.K. Folkes, P. Wardman, K.D. Asmus, Free-radical repair by a novel perthiol: reversible hydrogen transfer and perthiyl radical formation, Free Radic. Res. 20 (1994) 387–400, https://doi.org/10.3109/10715769409145638.
[177] S.A. Everett, P. Wardman, Perthiols as antioxidants: radical-scavenging and prooxidative mechanisms, Meth. Enzymol. 251 (1995) 55–69, https://doi.org/10.1016/0076-6879(95)51110-5.
[178] J.-P.R. Chauvin, E.A. Haidasz, M. Griesser, D.A. Pratt, Polysulfide-1-oxides react with peroxyl radicals as quickly as hindered phenolic antioxidants and do so by a surprising concerted homolytic substitution, Chem. Sci. 7 (2016) 6347–6356, https://doi.org/10.1039/C6SC01434H.
[179] Z. Wu, D.A. Pratt, Radical Substitution provides a unique route to disulfides, J. Am. Chem. Soc. 142 (2020) 10284–10290, https://doi.org/10.1021/jacs.0c03626.
[180] Z. Wu, V.S. Khodade, J.-P.R. Chauvin, D. Rodriguez, J.P. Toscano, D.A. Pratt, Hydropersulfides inhibit lipid peroxidation and protect cells from ferroptosis, J. Am. Chem. Soc. 144 (2022) 15825–15837, https://doi.org/10.1021/jacs.2c06804.
[181] U. Barayeu, D. Schilling, M. Eid, T.N. Xavier da Silva, L. Schlicker, N. Mitreska, C. Zapp, F. Gräter, A.K. Miller, R. Kappl, A. Schulze, J.P. Friedmann Angeli, T. P. Dick, Hydropersulfides inhibit lipid peroxidation and ferroptosis by scavenging radicals, Nat. Chem. Biol. 19 (2023) 28–37, https://doi.org/10.1038/s41589-022-01145-w.
[182] C. Prolo, L. Piacenza, R. Radi, Peroxynitrite: a multifaceted oxidizing and nitrating metabolite, Curr. Opin. Chem. Biol. 80 (2024) 102459, https://doi.org/10.1016/j.cbpa.2024.102459.
[183] M.N. Alvarez, G. Peluffo, L. Piacenza, R. Radi, Intraphagosomal peroxynitrite as a macrophage-derived cytotoxin against internalized trypanosoma cruzi, J. Biol. Chem. 286 (2011) 6627–6640, https://doi.org/10.1074/jbc.M110.167247.
[184] P. Kameritsch, M. Singer, C. Nuernbergk, N. Rios, A.M. Reyes, K. Schmidt, J. Kirsch, H. Schneider, S. Müller, K. Pogoda, R. Cui, T. Kirchner, C. De Wit, B. Lange-Sperandio, U. Pohl, M. Conrad, R. Radi, H. Beck, The mitochondrial thioredoxin reductase system (TrxR2) in vascular endothelium controls peroxynitrite levels and tissue integrity, Proc. Natl. Acad. Sci. U.S.A. 118 (2021) e1921828118, https://doi.org/10.1073/pnas.1921828118.
[185] J.S. Stancill, J.T. Happ, K.A. Broniowska, N. Hogg, J.A. Corbett, Peroxiredoxin 1 plays a primary role in protecting pancreatic β-cells from hydrogen peroxide and peroxynitrite, Am. J. Physiol. Regul. Integr. Comp. Physiol. 318 (2020) R1004–R1013, https://doi.org/10.1152/ajpregu.00011.2020.
[186] A.L. Condeles, F. Gomes, M.A. De Oliveira, L.E. Soares Netto, J.C. Toledo Junior, Thiol peroxidases as major regulators of intracellular levels of peroxynitrite in live Saccharomyces cerevisiae cells, Antioxidants 9 (2020) 434, https://doi.org/10.3390/antiox9050434.