Review

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# Peroxiredoxins: guardians against oxidative stress and modulators of peroxide signaling

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**Peroxiredoxins (Prxs) are a ubiquitous family of cysteine-dependent peroxidase enzymes that play dominant roles in regulating peroxide levels within cells. These enzymes, often present at high levels and capable of rapidly clearing peroxides, display a remarkable array of variations in their oligomeric states and susceptibility to regulation by hyperoxidative inactivation and other post-translational modifications. Key conserved residues within the active site promote catalysis by stabilizing the transition state required for transferring the terminal oxygen of hydroperoxides to the active site (peroxidatic) cysteine residue. Extensive investigations continue to expand our understanding of the scope of their importance as well as the structures and forces at play within these critical defense and regulatory enzymes.**

## Oxidative stress defenses and the recently recognized importance of Prxs

Prxs are ubiquitous enzymes that have emerged as arguably the most important and widespread peroxide and peroxynitrite scavenging enzymes in all of biology [1,2]. Discovered to be widely distributed peroxidases in the mid-1990s [3], the role of Prxs was long overshadowed by well-known oxidative stress defense enzymes such as catalase and glutathione peroxidase (Gpx). However, refined kinetics measurements now imply that Prxs reduce more than 90% of cellular peroxides [2,4]. Helping awaken interest in Prxs were several developments in the early 2000s. It was shown that as little as ~100 μM hydrogen peroxide caused rapid inactivation of human PrxI by hyperoxidation during catalytic turnover [5] and this sensitivity was shown to be due to conserved structural features within many eukaryotic Prxs [6]. The seemingly paradoxical finding that a peroxidase would be so easily inactivated by its own substrate led to the development of the 'floodgate' hypothesis [6], which posits that Prx inactivation enables peroxide-mediated signaling in eukaryotes, a phenomenon now known to regulate many normal cellular functions [7]. Prx hyperoxidation was also shown to be reversible *in vivo* [8] and the enzyme responsible for this 'resurrection' was identified and named sulfiredoxin (Srx)

## Glossary

**Arrhenius analysis:** determination of the activation energy ($E_a$) for a chemical reaction by measuring the reaction rate at multiple temperatures and fitting the experimental data to the Arrhenius equation for how a rate constant ($k$) depends on absolute temperature ($T$): $k = Ae^{-Ea/RT}$, where $R$ is the universal gas constant and $A$ is a factor accounting for the fraction of substrate molecules that have the kinetic energy to react. The Eyring equation gives the temperature dependence of the entropy ($\Delta S^{\ddagger}$) and the enthalpy ($\Delta H^{\ddagger}$) of formation: $k = \frac{RT}{Nh} e^{\left(\frac{\Delta S^{\ddagger}}{R}\right)} e^{\left(\frac{-\Delta H^{\ddagger}}{RT}\right)}$, where $N$ is Avogadro's number and $h$ is Plank's constant.

**Dismutation of superoxide:** a chemical process whereby one molecule of superoxide donates one electron to another molecule of superoxide, generating one molecule each of oxygen and hydrogen peroxide. This process can be accelerated by the enzyme superoxide dismutase but is also quite rapid in the absence of enzyme.

**Double displacement/ping-pong kinetics:** a mechanism in which the enzyme first reacts with one substrate to release a product and give a modified, intermediate form of the enzyme, then reacts with the second substrate to return to its original state and release a second product. A double displacement reaction gives a characteristic family of parallel lines when the concentrations of both substrates are varied and the initial rates are plotted on a double reciprocal, Lineweaver–Burk plot [97].

**Henderson–Hasselbalch equation:** an equation relating the negative log of the hydrogen ion ($H^+$) concentration (pH) to the ratio of the concentrations of deprotonated ($A^-$, the conjugate base) to protonated (HA, the conjugate acid) forms of a chemical species for which the acid dissociation constant ($K_a$), and therefore the negative log of the $K_a$ ($pK_a$), is known [97]:

$$pH = pK_a + log\frac{[A^-]}{HA} \quad [I]$$

Used here, HA represents the thiol group (protonated Cys) and $A^-$ represents the thiolate group (deprotonated Cys).

**Michaelis complex:** the enzyme–substrate complex formed before any chemical change.

**Oxidizing equivalent:** a species that has the propensity to accept one or two electrons depending on the reaction in question.

**Quantum mechanics–molecular mechanics (QM–MM) studies:** a computational method for simulating enzyme reactions. A small part of the system directly participating in the chemical reaction (typically some atoms of the substrate and enzyme) is simulated using QM, which analyzes the electronic changes involved in the making and breaking of bonds. The remainder of the system is simulated using a simpler and less computationally expensive MM force field that treats atoms as fixed quantities [98].

**Redox relay:** the transfer of redox state from one molecule to another, typically in the context of this review by the transfer of two electrons at a time between thiols, disulfides, or sulfenic acid centers. These transfers often occur via a transient mixed-disulfide intermediate linking the two centers during thiol–disulfide interchange [99].

**Second-order rate constant:** a rate constant which, when multiplied by the concentrations of two reactants in a bimolecular chemical reaction, yields the rate of the reaction. The typical units for a second-order rate constant are $M^{-1}\,s^{-1}$.

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[9]. Powerful additional evidence that Prxs are crucial to proper cell regulation is that Prx I knockout mice develop severe hemolytic anemia as well as lymphomas, sarcomas, and carcinomas by 9 months of age [10].

Prxs have attracted the attention of cancer researchers not only for their apparent function as tumor suppressors (or in some circumstances promoters [11]) but also because they have elevated expression levels in various cancer tissues and immortalized cell lines. High Prx levels have been associated with the resistance of tumors and cancer-derived cell lines toward certain chemo- and radiotherapies [12–14]. Additional links of Prxs with disease are their abnormal nitration in early Alzheimer's disease patients [15] and a role in promoting inflammation associated with ischemic brain injury [16]. Additionally, that pathogens rely on their Prxs to evade host immune systems makes them promising targets for the development of novel antibiotics [14,17]. Such roles for Prxs in disease motivate continued exploration of their physiological roles as well as the molecular mechanisms at play in Prx enzymatic function and regulation. This review focuses on the state of our understanding of the biochemical and structural mechanisms involved in catalysis and hyperoxidation sensitivity across this widespread group of enzymes. Further, the potential mechanisms through which Prxs may regulate cell signaling are discussed and a series of open questions in Prx biology and chemistry are posed.

## The catalytic prowess of Prxs

Prxs are cysteine-based peroxidases that do not require any special cofactors for their activity. During their catalytic cycle, a peroxidatic Cys ($C_P$) thiolate ($C_P$-$S^-$) contained within a universally conserved PxxxTxxC motif (with T in some Prxs replaced by S) attacks a hydroperoxide substrate and is oxidized to a $C_P$-sulfenic acid ($C_P$-SOH), and then frequently to an inter- or intrasubunit disulfide, before being reduced (via a mixed disulfide with a reductant) to reform the thiolate (Figure 1). As alluded to earlier, Prxs were long thought to be ~1000 times slower than the historically better-known catalase and glutathione peroxidases (Gpxs). However, by developing sensitive spectral assays in which disulfide reduction was not rate limiting, it was shown (e.g., for the model bacterial Prx AhpC from *Salmonella typhimurium* and human PrxII) that the $k_{cat}/K_M$ for $H_2O_2$ for some Prxs is as high as $10^7$–$10^8$ $M^{-1}$ $s^{-1}$ [18–20].

Also important in catalysis are conformational changes between a fully folded (FF) conformation in which $C_P$ can react with peroxide and a locally unfolded (LU) conformation in which the $C_P$ is exposed and can form a disulfide with the so-called 'resolving' Cys ($C_R$) present in many Prxs. In the $C_P$-$S^-$ and $C_P$-SOH forms, the FF and LU conformations rapidly equilibrate [21–25], but in disulfide forms Prxs become locked in the LU conformation (Figure 1). As proposed in 2003 [6], stabilization of the FF conformation should effectively promote further oxidation of $C_P$ by peroxide because of the greater opportunity to react with a second molecule of peroxide within the active site. The $C_P$-SOH form of those Prxs having a highly stabilized FF conformation does more readily form a Cys-sulfinate ($C_P$-$SO_2^-$), rendering the Prx inactive

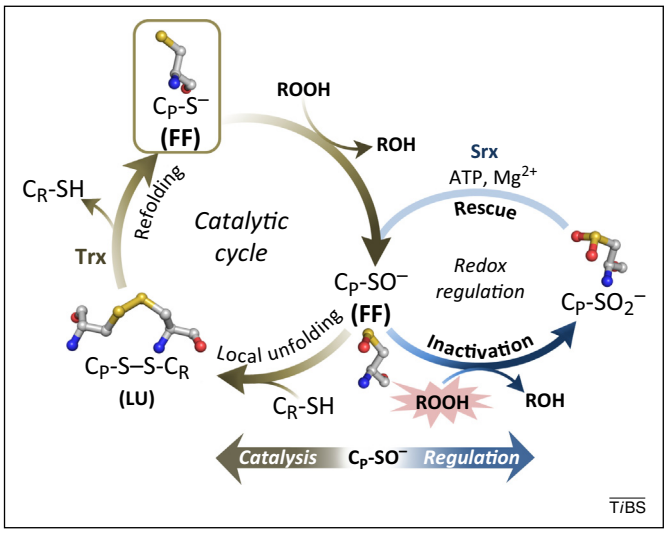


**Figure 1**. The catalytic and regulatory cycles of 2-Cys peroxiredoxins (Prxs). Shown in brown is the normal Prx cycle with the structure of the peroxidatic Cys ($C_P$) residue shown for each redox state (carbons are colored gray, nitrogens blue, oxygens red, and sulfurs yellow; hydrogens are not shown for simplicity). The $C_P$ thiolate ($RS^-$) in the fully folded (FF) active site is first oxidized by the peroxide to form the sulfenic acid (R-SOH) or sulfenate ($R\text{-}SO^-$) (computational approaches suggest stabilization of the $C_P$ as the sulfenate, but the true protonation state remains uncertain). This sulfenate, which must undergo a conformational change to become locally unfolded (LU), then forms a disulfide bond with the resolving Cys ($C_R$) in 2-Cys Prxs. Reductive recycling by thioredoxin (Trx) or a Trx-like protein or domain (e.g., tryparedoxin, N-terminal domain of bacterial AhpF) then restores the thiolate in the FF active site for another catalytic cycle. Shown in blue is the redox-linked regulatory cycle of predominantly eukaryotic Prxs, wherein the $C_P$ sulfenate becomes further oxidized, in the presence of high peroxide levels, to the inactive sulfinate ($R\text{-}SO_2^-$). In some organisms and Prx isoforms, the active enzyme is restored by the ATP-dependent activity of sulfiredoxin (Srx).

(Figure 1, redox regulation cycle) [25,26]. In many eukaryotes, including humans, the enzyme Srx catalyzes the repair of hyperoxidized Prxs in the Prx1 group (see next section), restoring their activity [14,27]. Given that Prxs are abundant and highly reactive with cellular peroxides and peroxynitrite and have activity that can be regulated, they are well suited to play roles not just in oxidant defense but also in redox sensing and signaling and perhaps even in the recovery of oxidatively damaged proteins [28–33]. The various physiological roles of Prxs can be better understood in the context of structural and functional features that are shared between certain members. Thus, we describe next how Prxs are classified into evolutionary subfamilies and how these different subfamilies vary in phylogenetic distribution, cellular localization, oligomerization, conformation change, and susceptibility to hyperoxidative inactivation.

## Functional and structural subdivisions of Prxs

Prxs exist in six evolutionary subfamilies (Prx1, Prx5, Prx6, Tpx, PrxQ, and AhpE) that vary in oligomeric states and interfaces and in the locations of the resolving Cys [34]. In general, Prx1 subfamily enzymes – typically doughnut-shaped decamers – are the most highly expressed, constituting 0.1–1% of the soluble protein in the cell [35]. These are the Prxs that have been commonly referred to as 'typical 2-Cys' Prxs [36], as they were the original type of '2-Cys Prx' (having both a $C_P$ and $C_R$) discovered [3]. In these proteins, the $C_R$ is near the C terminus of the second chain of a dimer, thus forming an intersubunit disulfide bond during the