This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2005)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2005 Term

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1-cys Peroxiredoxin

By

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For 77:222, Spring 2005
24 Mar 2005

Are Prx, TSA and TPx the same?

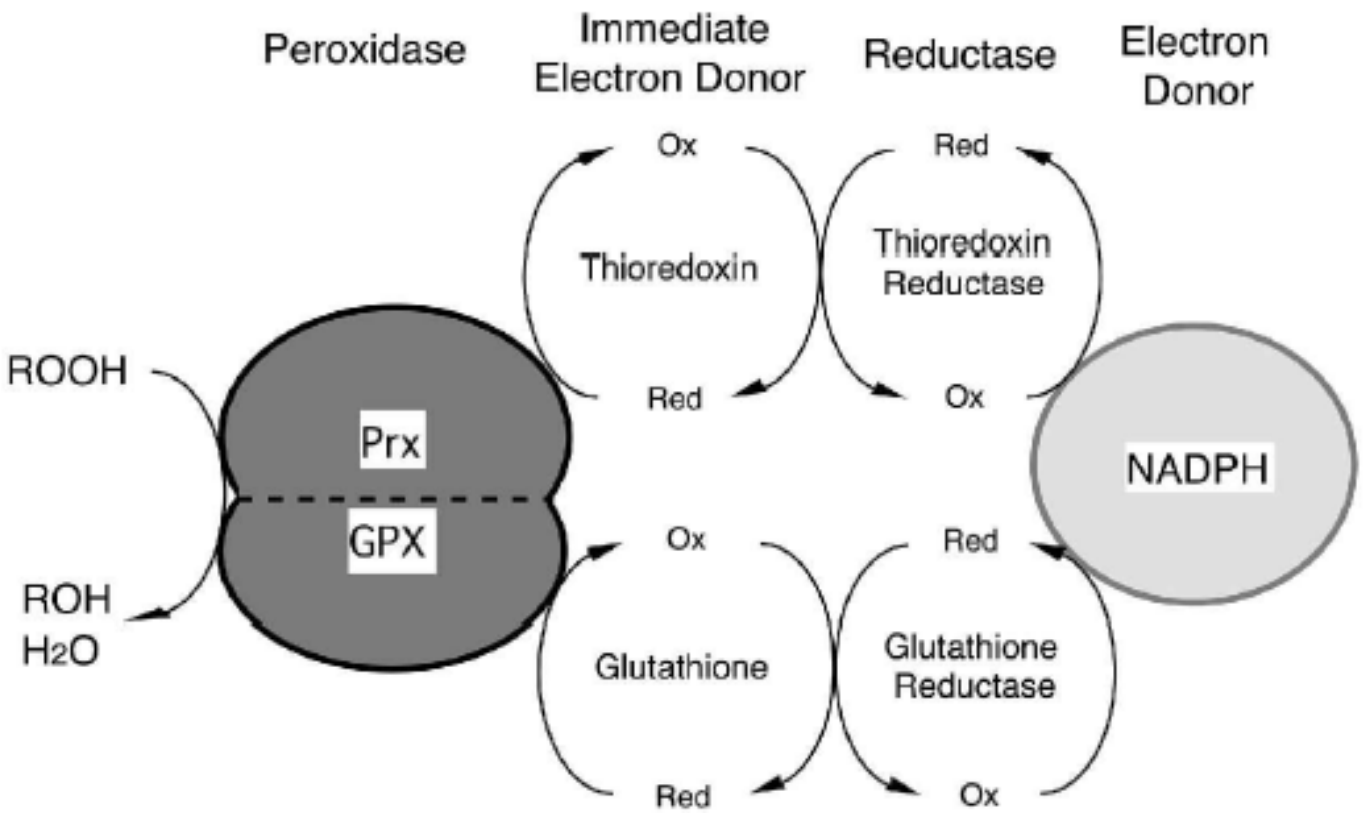
- Peroxiredoxins (Prx), are a superfamily of nonseleno-proteins that catalyze the thiol-dependent reduction of peroxides.

- TSA (thiol-specific antioxidant). Provides protection against oxidative damage caused by the thiol-containing oxidation system (Fe^{3+} , O_2, and RSH)

- TPx (thioredoxin peroxidase) is another name reflecting that Trx is the immediate electron donor. Not all Prxs use Trx as electron donors (GSH).

- *J. Biol. Chem.* 1994 **269**, 27670-27678
- *J. Biol. Chem.* 1998 **263**, 4704–4711.

An enzymatic reaction common to Prx family members is the reduction of hydroperoxides



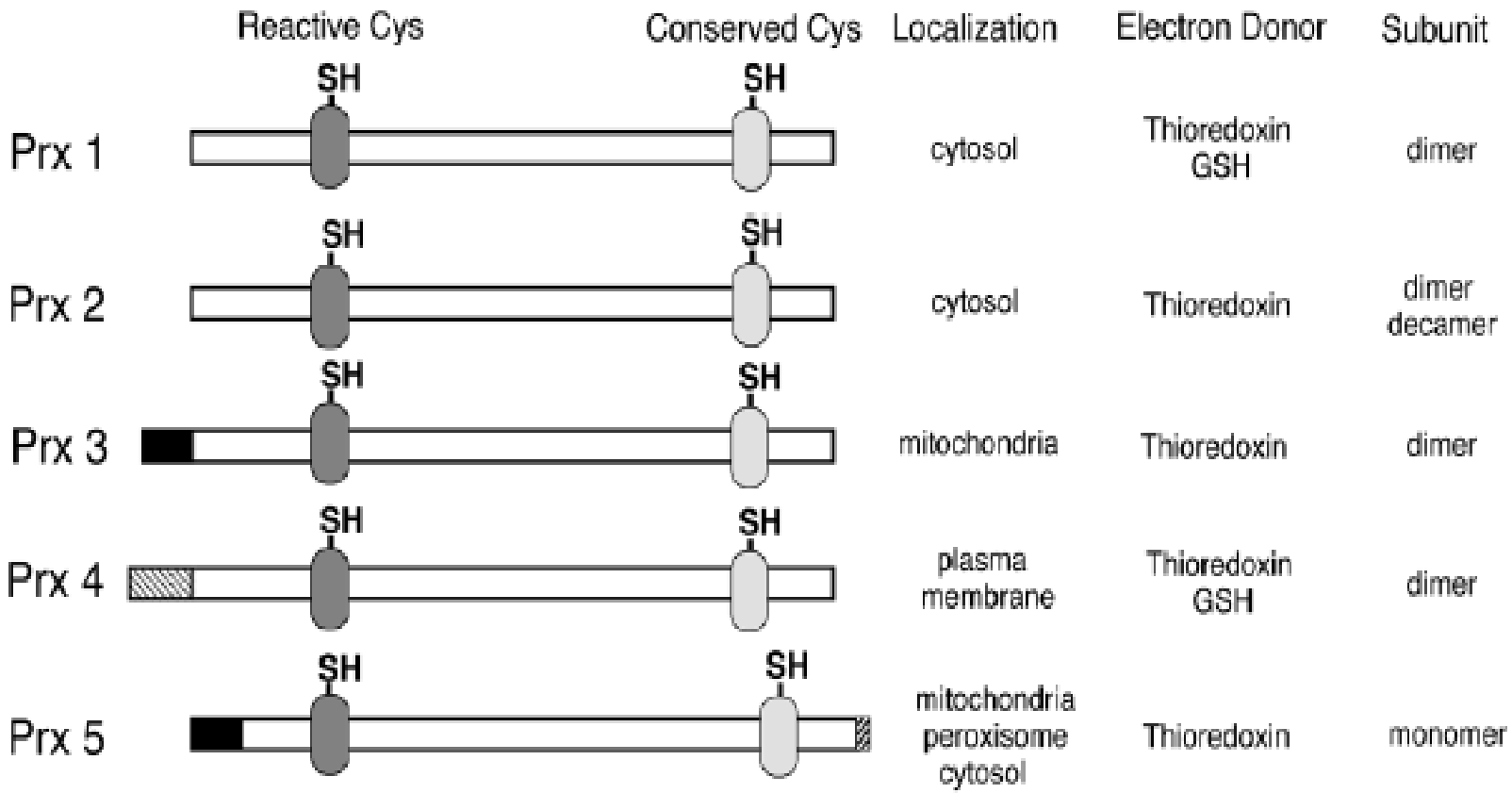
The peroxidase reaction of Prx and GPx in co-ordination with either Trx/Trx reductase or GSH/GSSG-reductase system. Peroxidase activity of Prx and GPx can utilize electrons from Trx/Trx reductase and/or GSH/GSH reductase, although activity of GPx is higher than Prx.

Redox Report 2002 **7**: 123-130

Peroxiredoxins (Prx)

- They share a common reactive Cys residue in the N-terminal region that corresponds to Cys 47 of the yeast Prx.
- Peroxiredoxins are capable of serving as a peroxidase and involve thioredoxin and/or glutathione as the electron donor.
- They have been divided into two subgroups according to the number of their conserved cysteine residue(s), namely the one- and two-cysteine groups.

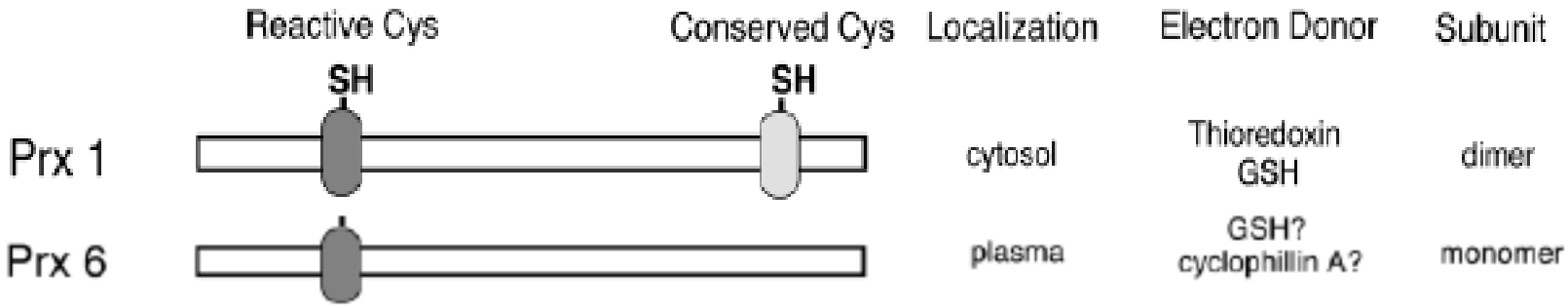
Schematic representation of 5 mammalian Prxs (2-Cys Prxs).



Positions of essential Cys for peroxidase activity are indicated as SH. Prx3 and Prx5 have mitochondrial import signals at their N-termini. Prx5 has a peroxisomal localization signal at its C-terminus. Prx4 has a signal peptide for secretion at the N-terminus.

Redox Report 2002 **7**: 123-130

Schematic representation of 1-Cys Prx (Prx 6).

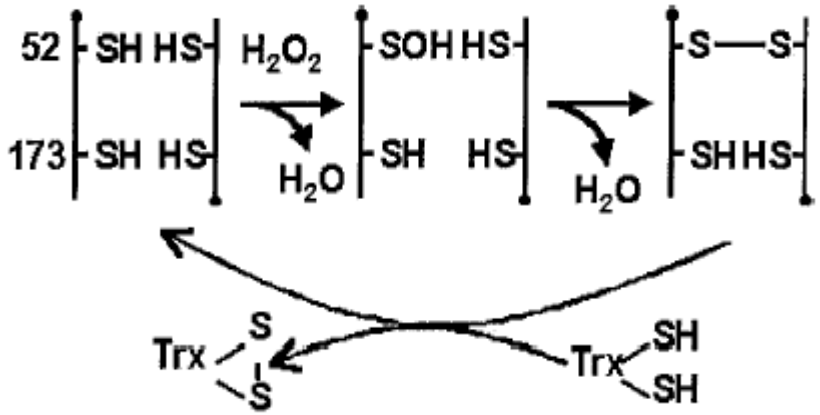


Positions of essential Cys for peroxidase activity are indicated as SH.

1-cysPrx, a member of the peroxiredoxin family that contains a single conserved cysteine residue, reduces a broad spectrum of hydroperoxides.

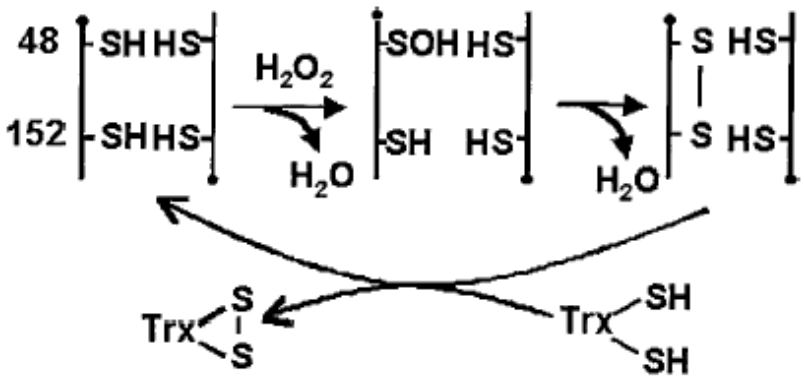
Redox Report 2002 **7**: 123-130

Proposed mechanisms for the peroxidase reaction of 2-Cys Prx



Reaction mechanism of mammalian Prx I–IV.

The conserved N- and C-terminal Cys residues that are separated by 121 amino acid residues. An **intermolecular** disulfide is formed and the disulfide is subsequently reduced by Trx, could not be achieved by glutathione (GSH) or glutaredoxin.



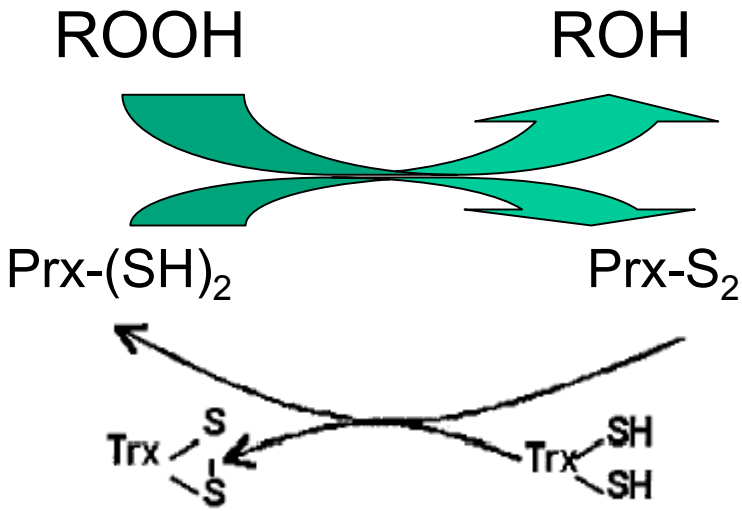
Reaction mechanism of mammalian Prx V

Cys48 is the site of oxidation by peroxides, and then oxidized Cys48 reacts with the sulfhydryl group of Cys152 to form an **intramolecular** disulfide linkage

The disulfide formed by Prx V is reduced by Trx, but not by glutaredoxin or GSH.

IUBMB Life, 2001 **52**: 35–41

Proposed mechanisms for the peroxidase reaction of 2-Cys Prx



Prx I–V each contain two conserved cysteine residues and utilize thioredoxin as the redox cofactor, reducing H_2O_2 and organic hydroperoxides into corresponding alcohols.

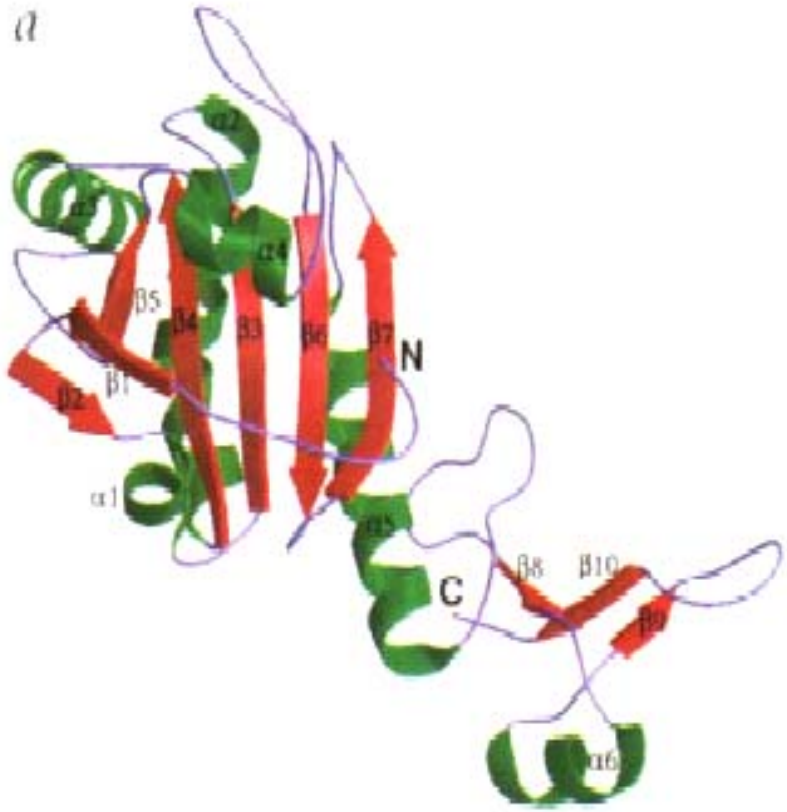
1-Cys peroxiredoxin

- 1-Cys peroxiredon (1-cysPrx) is widely expressed in tissues; it is enriched in lung and especially in Clara and alveolar type II epithelial cells. This protein has been shown to catalyze the reduction of hydroperoxides, including phospholipid hydroperoxides.

- It does not use thioredoxin as reductant. It uses glutathione (GSH) or cyclophilin A as an electron donor.

- This enzyme also has phospholipase A_2 activity.

Crystal structure of 1-cys Prx (monomer)



A ribbon diagram of a human 1-Cya Prx (C91S-hORF6) monomer.

α-helices—green. β-strands – red

Nature Structural Biology. 1998 **5**:400-406

Crystal structure of 1-cys Prx (dimer)

- A stereo ribbon diagram of the C91S-hORF6 dimer. Monomers A and B are drwan in red and blue respectively.
- Panels were prepared using MOLSCRIPT and RASTER-3D.

Nature Structural Biology. 1998 **5**:400-406

Distribution of the Prx isoforms in various rat tissues.

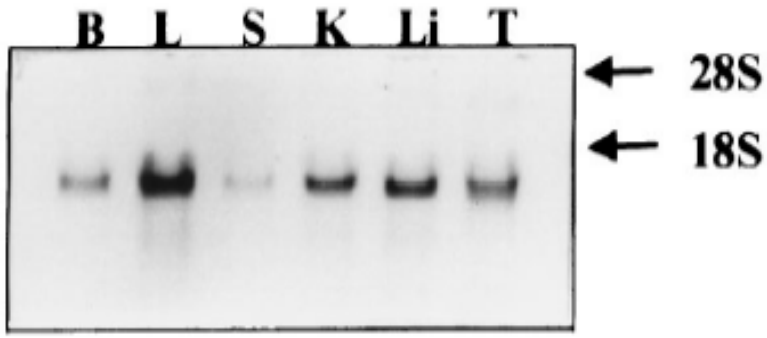
	1-Cys Prx
Tissues	Prx VI
Placenta	0.2
Thymus	0.03
Testicle	1.0
Thyroid	0.2
Pancreas	0.03
Adrenal	0.3
Brain	1.7
Hypothalamus	0.7
Spleen	0.03
Lung	1.7
Kidney	0.3
Liver	0.3
Heart	0.3

Data are expressed as micrograms of Prx per milligram of soluble protein

1-Cys Prx was detected more in the lung and brain than in other tissues

IUBMB Life, 2001 **52**: 35–41

Northern blot analysis of the 1-Cys Prx gene.

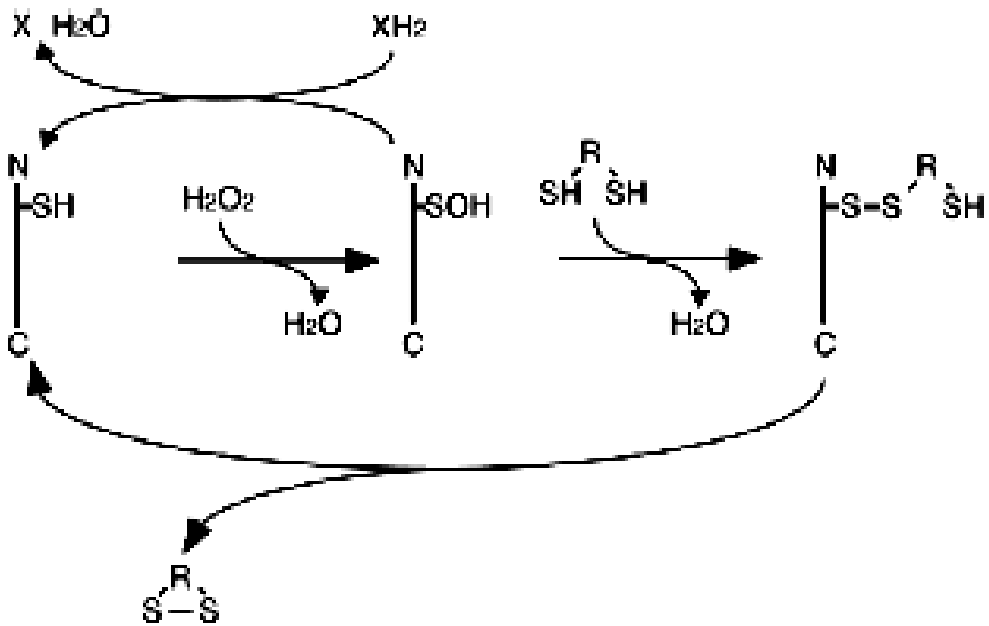


- Ten micrograms of total RNA were loaded on the 1% agarose gel in the presence of 6.7% formaldehyde and transferred to a nylon membrane. The 1-Cys Prx transcript was detected by hybridizing the blot with a DIG-labeled
- 1-Cys Prx cDNA fragment cloned from the mouse brain library. Different levels of the 1.5-kb 1-Cys Prx transcripts are observed in the adult tissues (B, brain; L, lung; S, spleen; K, kidney; Li, liver; T, testis)

It shows that the murine 1-Cys Prx gene is transcribed into about 1.5-kb mRNA and is ubiquitously expressed in the adult with some tissue variability, but 1-Cys Prx was detected more in the lung than in other tissues.

Gene 1999; **234**: 337–344.

Proposed mechanisms for the peroxidase reaction of mammalian 1-Cys Prx.



SH, sulfhydryl.

SH–R–SH indicates dithiol compounds such as Trx.

XH means an unknown electron donor.

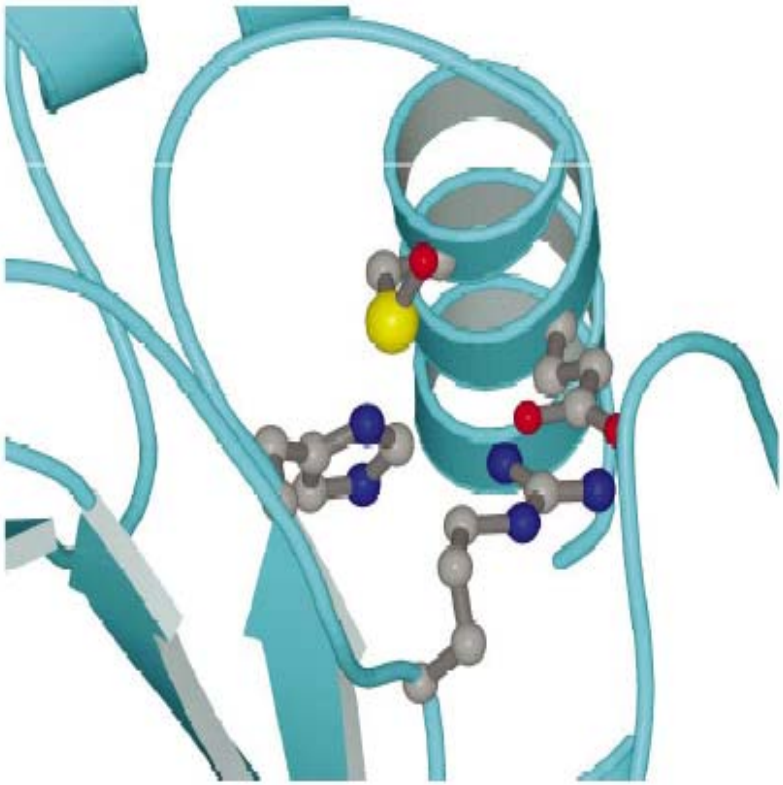
Cys47–SH is the site of oxidation in 1-Cys Prx

the existence of Cys-SOH (cysteine sulfenic acid) has been conclusively demonstrated in the x-ray crystal structure of the oxidized native 1-Cys Prx.

J Biol Chem 2000; **275**: 20346–20354.

Redox Report 2002 **7**: 123-130

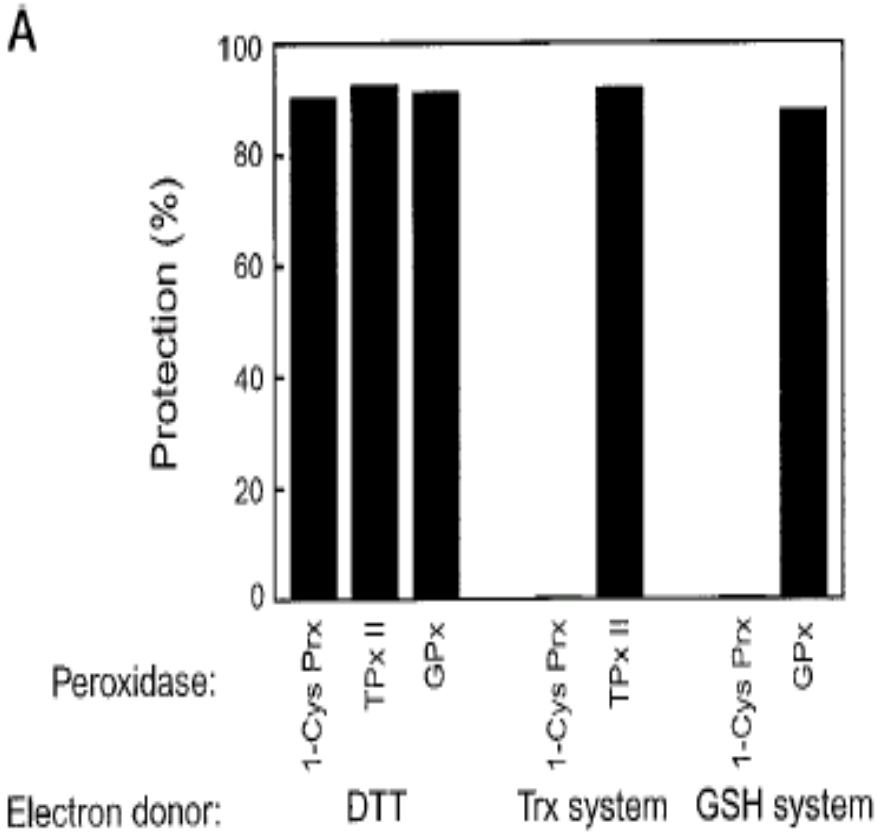
Cysteine sulfenic acids at the active sites of 1-Cys Prx



- Shown are X-ray crystallographic structures of human peroxiredoxin VI (1prx at 2.0 Å).
- Depicted as ribbon diagrams for the protein backbones, and side chain and cofactor atom colors of yellow = S, red = O, blue = N, gray = C, and pink = P.

Annu. Rev. Pharmacol. Toxicol. 2004. **44**:325–47

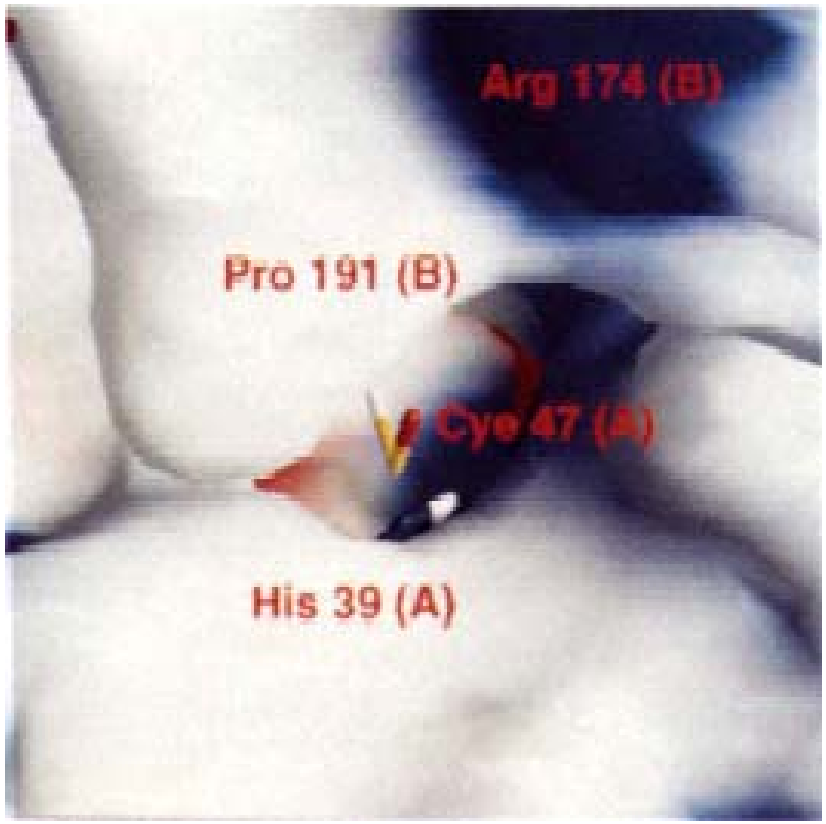
Evaluation of Trx and GSH as electron donors to 1-Cys Prx.



- The Cys-SOH of oxidized 1-Cys Prx can be reduced by nonphysiological thiols such as dithiothreitol (DTT) but not by Trx
- The physiological electron donor (or donors) that supports the peroxidase activity of 1-Cys Prx remains to be identified
- These results suggest that neither Trx nor GSH can efficiently reduce oxidized 1-Cys Prx.

J Biol Chem, 1998 **273**: 6303-6311

The entrance of the active site pocket



The solvent accessible surface of the reactive site pocket region are displayed for dimeric forms of C91S-hORF6. The entrance becomes narrower in the dimer. The location of the residues of the other monomer above the entrance (Pro191(B) and Arg (B)) are indicated on the surface.This figure was generated using GRASP.(Red, negative charged region; Blue, positive charged region)

Nature Structural Biology. 1998 **5**:400-406

The diagrammatic scheme of the 1-Cys Prx gene structure

Exon number	Exon size (bp)	Intron size (kb)
1	128	3.4
2	157	3.5
3	147	0.45
4	144	2.0
5	840	

The murine brain Prx6 gene consists of five exons and four introns.

The exons (filled boxes) and the introns (lines) are drawn to scale. The arrow indicates the transcription direction.

Gene 1999; **234**: 337–344.

Phospholipase A2 (PLA2) activity of 1-Cys Prx

- An **a**cidic Ca^{2+}-**i**ndependent **p**hospho**l**ipase **A(2)** (aiPLA(2)). It demonstrates maximal activity in acidic medium, and accordingly, it was named as aiPLA2.

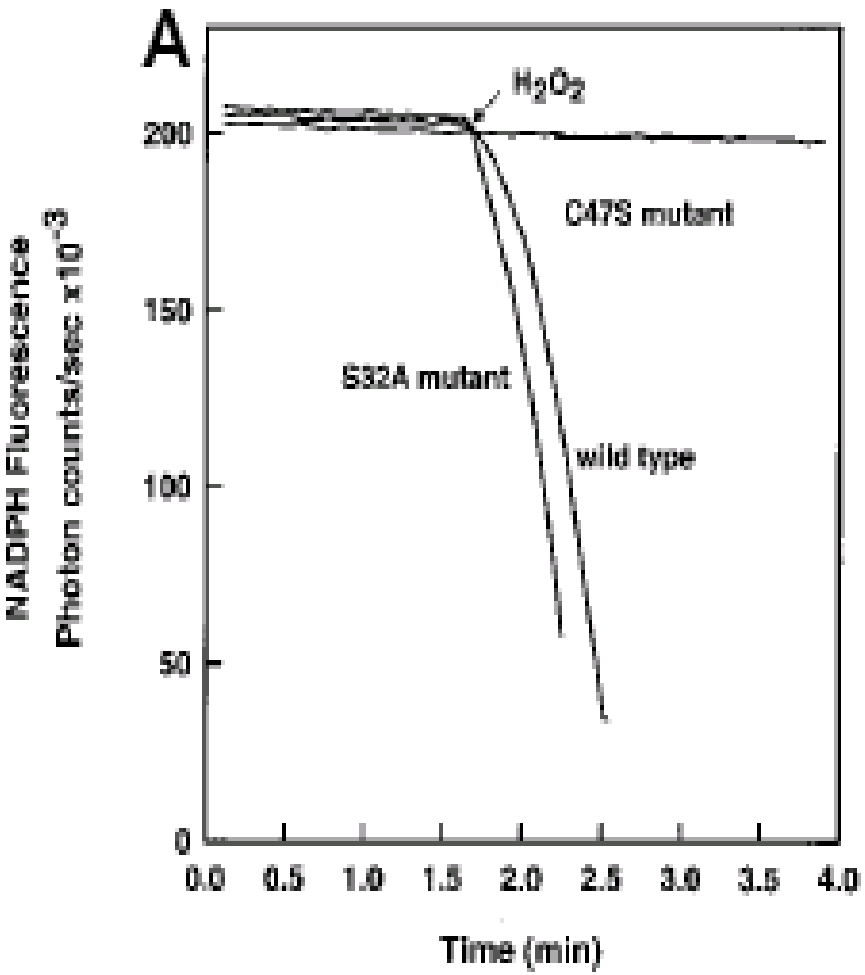
  J Biol Chem 1997; **272**: 2542–2550.

- The translation product of wild type 1-Cys peroxiredoxin clone expressed in wheat germ showed significant NSGPx activity (measured with H_2O_2); it also hydrolyzed DPPC at pH 4 in the absence of Ca^{2+} to liberate free fatty acid, thus exhibiting aiPLA2 activity (measured with [^{3}H]DPPC).

DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine; bp, base pair(s)

J Biol Chem 2000; **275**: 28421–28427

Peroxidase activities of purified human wild type, S32A, and C47S 1-Cys peroxiredoxin.



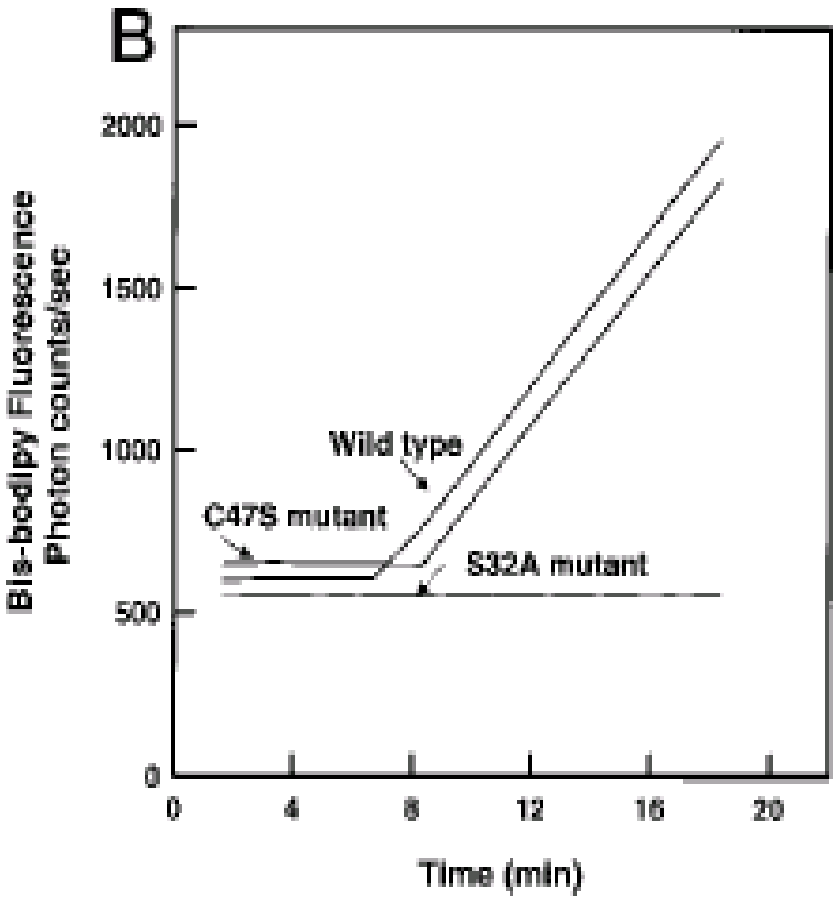
Assay was measured by fluorescence measurement at 460 nm (340 nm excitation) recorded as photon counts. H_2O_2 was substrate. Decreasing fluorescence indicates NADPH oxidation coupled to reduction of H_2O_2

Mutagenesis of Cys47 totally abolished NSGPx activity while PLA2 activity in the mutant remained at the wild type level.So Cys47 is critical site for NSGPx activity.

NSGPx, non-selenium glutathione peroxidase.

J Biol Chem 2000; **275**: 28421–28427

aiPLA2 activities of purified human wild type, S32A, and C47S 1-Cys peroxiredoxin.



Assay of aiPLA2 activity by bodipy fluorescence measurement at 520 nm (490 nm excitation) recorded as photon counts.Use bis-bodipy PC as substrate. Increasing fluorescence indicates the liberation of bodipy fatty acid from bis-bodipy PC resulting in fluorescence dequenching.

The S32A mutation abolished PLA2 activity but did not affect Peroxidase activity.The result show that Ser32 is critical for the hydrolase activity of the enzyme.

J Biol Chem 2000; **275**: 28421–28427

Summary

- 1-Cys peroxiredoxin is a bifunctional (“moonlighting”) enzyme with two distinct active sites. Cys 47 is critical for NSGPx (nonseleno GPx) activity and Ser 32 is critical for the hydrolase activity.

- The bifunctional catalytic properties of 1-Cys peroxiredoxin are compatible with a simultaneous role for the protein in the regulation of phospholipid turnover as well as in protection against oxidative injury.