

# The strictly conserved Cys76 plays a crucial role in the conformational stability of reduced Px III

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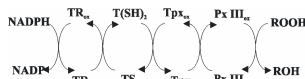
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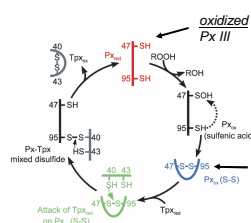
**Introduction:** In trypanosomes and leishmania are the causative agents of several tropical diseases. The parasites lack catalases and selenocysteine-containing glutathione peroxidases. Instead, cysteine-containing glutathione peroxidase-type (Px I, Px II, and Px III) enzymes are – together with 2-Cys-peroxiredoxins - responsible for hydroperoxide reduction acting both as trypanredoxin peroxidases. Px III belongs to a subgroup of glutathione peroxidase-type enzymes, in which the catalytic selenocysteine is replaced by a cysteine. In classical selenocysteine glutathione peroxidases, the reduction of the peroxide is achieved through attack by the selenocysteine, which subsequently is reduced by glutathione. In the cysteine homologues, a second resolving cysteine takes over the part of the glutathione and forms an intermediate disulfide bridge. In *T. brucei*, the glutathione peroxidase-type enzymes proved to be essential. Unravelling their mechanism could provide a basis to control the parasite.

## Reaction scheme of Px III

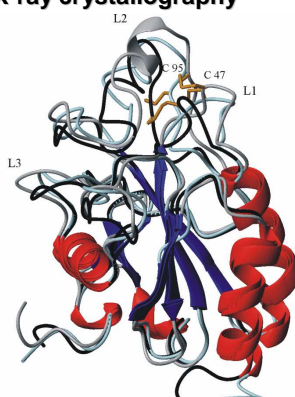
Trypanosomes and leishmania have a unique thiol redox metabolism that is based on trypanothione [N1,N8-bis(glutathionyl)spermidine] and the flavoenzyme trypanothione reductase.



With NADPH as primary electron source, the reducing equivalents flow via trypanothione and trypanredoxin (Tpx), a distant relative of the thioredoxin protein family, onto the peroxidases, which then reduce the hydroperoxide substrates.



## Structure determination by NMR and X-ray crystallography



— oxidized C76S Px III, X-ray structure  
— oxidized C76S Px III NMR-structure  
— reduced C76S Px III, NMR structure

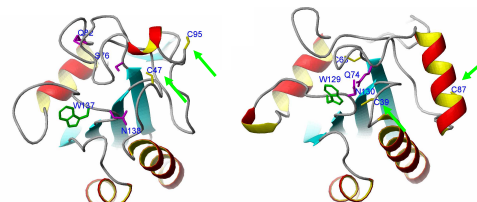
The two catalytically active cysteine residues Cys47 on loop L1 and Cys95 on loop L2 are depicted in orange.

The reduced and the oxidized form of C76S mutant of Px III are almost identical.

## Comparison of the reduced structures of C76S Px III with Px II

NMR structure of C76S Px III (Melchers et al., 2008)

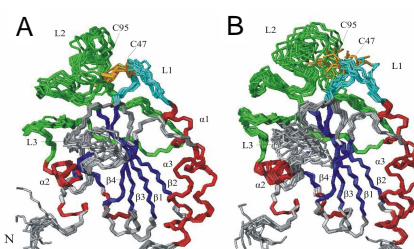
Crystal structure of Px II (Alpey et al., 2008)



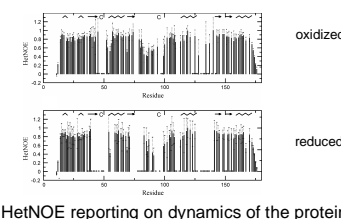
Contrary to our results, the crystal structure of Px II published by the group of Fairlamb (right) shows the presence of a catalytic triad in the reduced form (W129, Q74, C39) and an additional helix like the crystal structures of classical glutathione peroxidases (Alpey 2008). Note the large distance between the Cysteines in Px II (green arrows).

→ Difference attributable to method or mutation C76S?

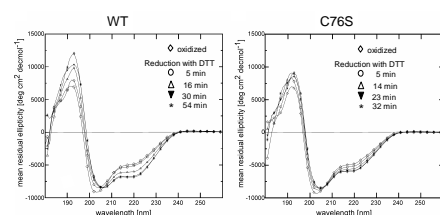
**The loop L2 harbouring Cys95 shows conformational exchange and has little NOE contacts under both conditions.**



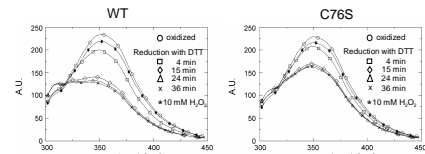
NMR structure ensemble (A) oxidized and (B) reduced protein. Residues showing conformational exchange are depicted in green.



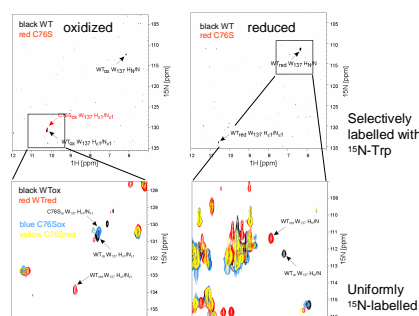
## Comparison of WT and C76S mutant



CD spectra reveal different increase in helicity upon reduction.



Trp fluorescence spectra show that the environment of Trp137 changes differently upon reduction.



<sup>15</sup>N-HSQC spectra corroborate that Trp137 is different in WT and C76S mutant

→ Cys76 appears to influence the equilibrium between different conformations.

**Conclusions:** Cys76 was regarded as unimportant for the catalytic reaction (Schlecker et al., 2007). It is, however, conserved throughout the entire family of selenocysteine and cysteine glutathione peroxidases. Our results show that it plays a critical role for the reduced enzyme in assuming different conformational states. This implies that the catalytic triad may only be required as short-lived intermediate during catalysis and that Cys76 influences the equilibrium between different conformations.

**References:** Schlecker T, Comini MA, Melchers J, Ruppert T, Krauth-Siegel RL. (2007) *Biochem J*. 405:445-54.; Melchers J, Diechtierow M, Fehér K, Sinning I, Tews I, Krauth-Siegel RL, Muhle-Goll C. (2008) *J Biol Chem* 283:30401-11. Alpey MS, König J, Fairlamb AH. (2008) *Biochem J*. 414:375-81.; Muhle-Goll C, Füller F, Ulrich AS, Krauth-Siegel RL. (2010) *FEBS Lett*. 584:1027-32.