Structural basis for a distinct catalytic mechanism in *Trypanosoma brucei* tryparedoxin peroxidase

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Supplemental Results

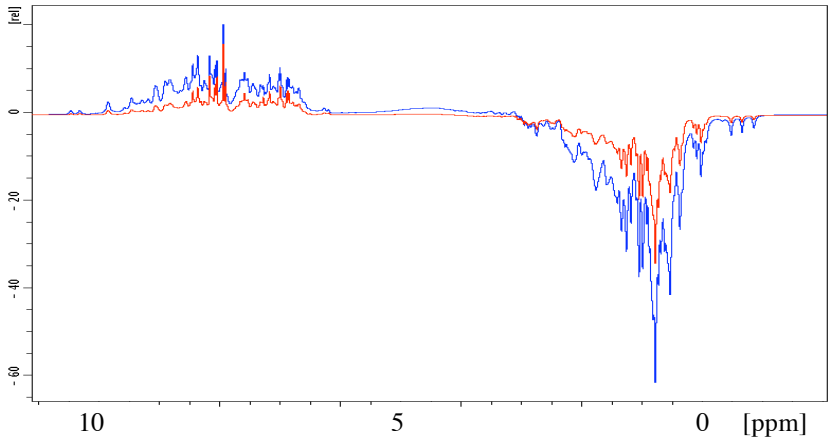

Figure S1. ^{1}H T2 spin-echo relaxation measurements
Px III was measured at 21 ^{o}C in 50 mM sodium phosphate, 100 mM KCl, pH 6.8 with a translational diffusion time of 8 µs (blue) or 12 ms (red). The ratio between the two spectra is 0.43. A further spectrum with 8 ms translational diffusion time was also obtained (not shown). The ^{1}H-T2 was estimated from these spectra to be 14.1 +/- 1.5 ms. The protein concentration was 1 mM.

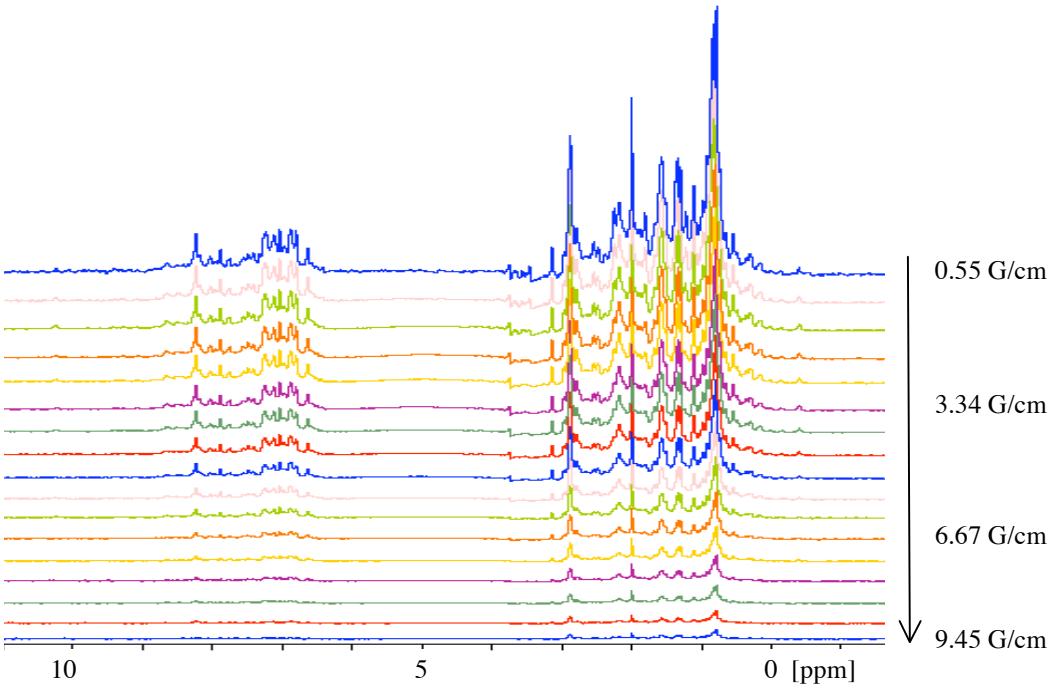

Figure S2. Pulsed-field-gradient (PFG) diffusion experiment
Stack plot of signal attenuation of Px III obtained using the water-SLED experiment (1). The diffusion coefficient was obtained from a fit of the normalized resonance intensities:
$I(G) = I(0)\exp[-(\gamma\delta G)^2(\Delta - \delta/3)D_s]$.
The PFG duration δ was 7 ms, the diffusion time (Δ) was 125 ms, and the PFG strength (G) varied from 0.55 to 9.45 G/cm. The diffusion coeffient D_s of 0.953 +/- 0.008 * 10^{-6} cm^2s^{-1} is typical for a monomeric protein at 1 mM concentration at 21° C, the buffer condition are the same as in Fig. S1 (Ref. 1, supplemental material).

1. Altieri, A. S., Hinton, D. P. and Byrd, R. A. (1995) *J. Am. Chem. Soc.,***117**, 7566-7561

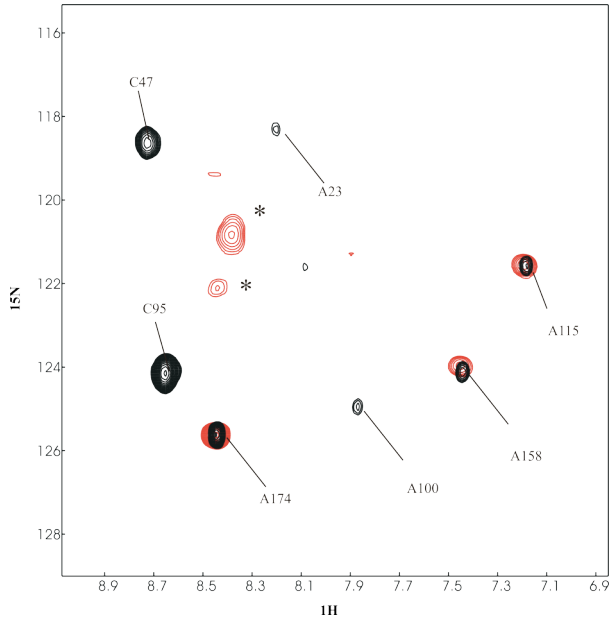

Figure S3 Overlay of the $^{1}H^{15}N$-HSQC spectra of oxidized (black) and reduced (red) selectively ^{15}N-Cys labelled Px III

T. brucei Px III was selectively labelled with ^{15}N-Cys; more signals occur, however, because Cys is metabolically converted into Ala in *E. coli*. The H_N-resonances of oxidized and reduced Px III are shown in black and red, respectively. In contrast to the signals of Ala^{115}, Ala^{158} and Ala^{174}, the resonances of Cys^{47} and Cys^{95} disappear or shift strongly to the position of the two signals marked by an asterisk. The identity of the resonances of the redox active cysteines in the oxidized protein was proven by a ^{15}N-edited NOESY, whereas for reduced Px III the signals that possibly represent the Cys resonances were too weak for further analysis.

Removal of DTT from reduced Px III led to the reappearance of the spectrum of oxidized Px III.

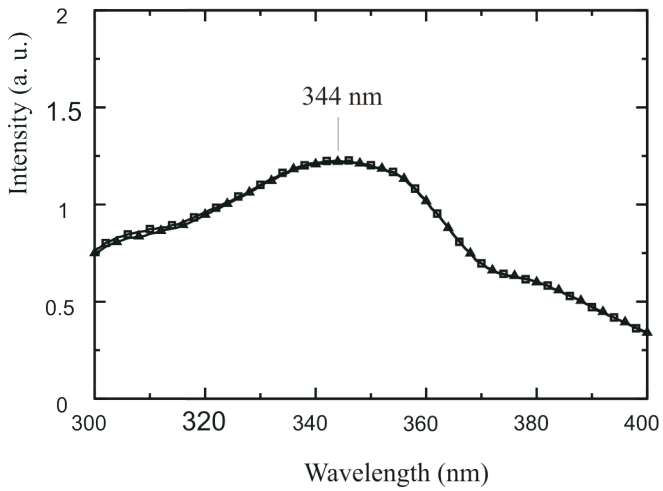

Figure S4. **Fluorescence emission spectra of (□) and reduced (Δ) Px III.**

The spectra were recorded at room temperature in a 1 x 1 cm cuvette on an Aminco-Bowman Series 2 Luminescence Spectrometer (SLM-Aminco Inc., Northampton, MA). The solutions contained 30 µM Px III in 50 mM sodium phosphate, 100 mM KCl, pH 6.8. The reduced sample was obtained by 10 min treatment with 2 mM DTT prior to recording the spectrum. The excitation was set to 290 nm. Emitted light was detected at 90° between 300 and 400 nm. For both redox states of the protein maximum emission was detected at 344 nm. a. u., arbitrary units.

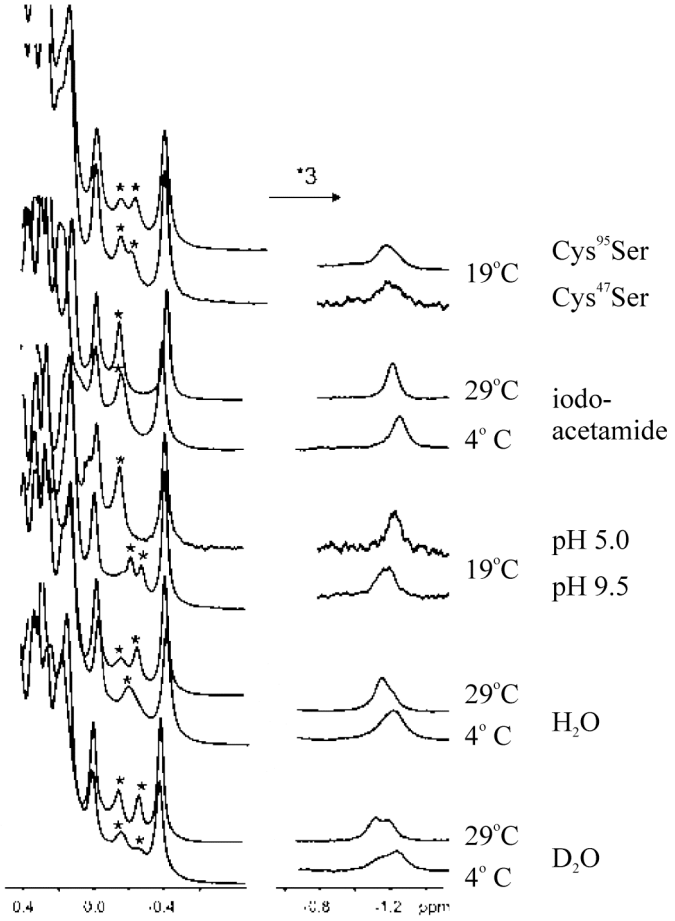

Figure S5 Doubling of resonances in the reduced form of *T. brucei* peroxidase III
The aliphatic region between 0.5 and -1.5 ppm of 1D ^{1}H spectra of PX III under various conditions is shown. The section between -0.6 and -1.5 ppm is magnified by a factor of 3 to achieve better clarity. The pH was 6.8, if not otherwise stated.

2D ^{1}H-^{1}H NOESY analysis revealed that in the reduced form of Px III several resonances are doubled indicating the presence of two different conformations. To study the underlying process to more detail we recorded a series of 1D NMR experiments between 4 and 29 ^{o}C and at different buffer conditions. As an example, the well-isolated resonances of Hγ12 of Ile^{163} (frequencies between -1.1 and -1.3 ppm) and Hγ21 of Val^{110} (frequencies marked by asterixes) are shown. Low temperatures shifted the equilibrium towards one conformation whereas at elevated temperatures the other one was populated to a higher extent. Exchange of H_2O to D_2O likewise influenced the ratio of the two populations suggesting a kinetic isotope effect, as did a buffer exchange from neutral to either acidic (pH 5.0) or basic (pH 9.5) pH values. At acidic pH, only one population was visible that probably corresponded to the conformation populated at low temperature, whereas at basic pH two populations were present. Taken together, these effects strongly advocated for a protonation/deprotonation event.
Carboxamidomethylation of both cysteine residues yielded a single resonance coinciding with the form at pH 5.0, thus demonstrating that deprotonation of one or both cysteines was the source of the two conformations. In order to reveal whether both cysteines were involved in the two conformations, the Ser^{47} and Ser^{95} mutants were analyzed. Comparison with the other spectra indicated that in the spectrum of the Ser^{47} mutant one conformation dominated whereas the Ser^{95} mutant again showed both conformations.

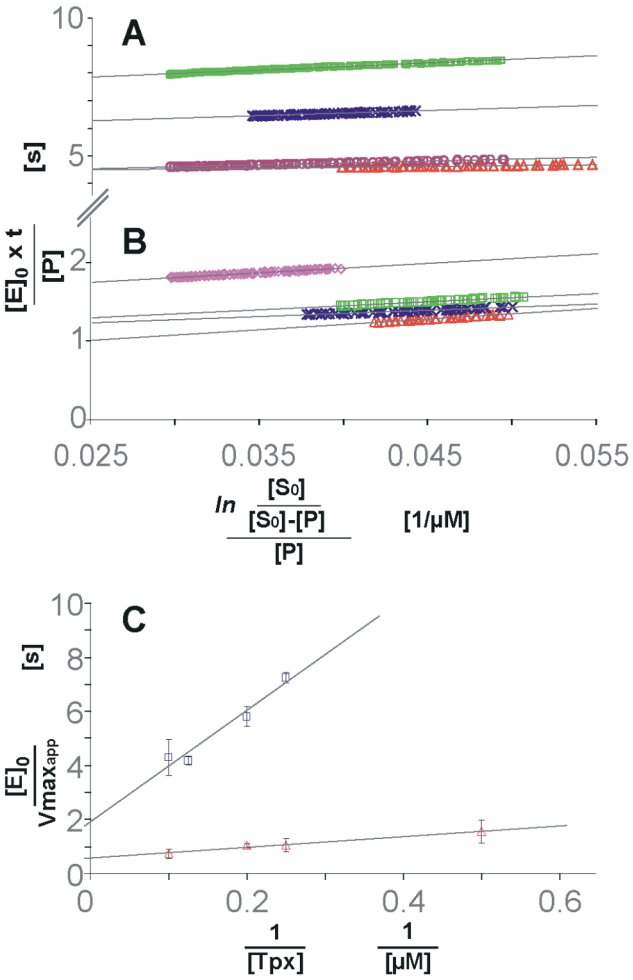

Figure S6 Kinetic analysis of H_2O_2 reduction by wildtype and Glu^{97} Px III.
A and *B*, primary plots of Glu^{97} and wt Px III, respectively. The activities were measured at a fixed concentration of 100 µM trypanothione and five different concentrations of Tpx: 10 µM (Δ), 8 µM (○), 5 µM (x), 4 µM (□) and 2 µM (◊). The ordinate with $[E]_0$ x t/($[S]_0$ −[S]) and ($[S]_0$ −[S]) = [P] represents $[E]_0/v$ since *v* corresponds to [P]/t. The abscissa is equivalent to $1/[H_2O_2]$. The intersection with the ordinate therefore gives the $[E]_0/V$max value for the respective Tpx concentration. The parallel slopes give Φ_1 with $k_1' = 1/\Phi_1$ and are in accordance with a ping-pong mechanism. *C*, secondary plot for Glu^{97} (□) and wt (Δ) Px III. The reciprocal velocities for infinite hydroperoxide concentrations obtained from A and B are plotted against the reciprocal concentrations of Tpx. The slope gives Φ_2 with $k_2' = 1/\Phi_2$, the intersection with the ordinate Φ_0 with $k_{cat} = 1/\Phi_0$.