Supplementary Information for

## The Extraordinary Catalytic Ability of Peroxiredoxins: a Combined Experimental and QM/MM Study on the Fast Thiol Oxidation Step

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## Measurement of the thermodynamic parameters of MtAhpE oxidation by $H_2O_2$

The rate constants of MtAhpE oxidation by H<sub>2</sub>O<sub>2</sub> at different temperatures were determined by taking advantage of the decrease in the protein intrinsic fluorescence intensity that occurs upon enzyme oxidation as previously described.<sup>1</sup> MtAhpE was reduced by incubation with 1 mM dithiotreitol for 30 minutes and 4°C. Excess reductant was removed by gel filtration using a HiTrap column (Amersham Bioscience). Reduced MtAhpE (1 $\mu$ M) was rapidly mixed with excess concentrations of H<sub>2</sub>O<sub>2</sub> (8, 20, 40, 60 μM) in sodium phosphate (0.1 M) buffer plus 0.1 mM diethylene triamine pentaacetic acid (dtpa), pH 7.4, using an Applied Photophysics SX-20 stopped-flow spectrofluorimeter (mixing time of  $\leq 1.2$ ms) at different temperatures (ranging from 10°C to 37°C), and following the total fluorescence intensity decrease ( $\lambda_{ex}$ = 295 nm). Observed rate constants were determined by fitting experimental data to single exponential curves. From the slope of the plots of observed rate constants versus H<sub>2</sub>O<sub>2</sub> concentrations, the second-order rate constants for the reaction between the reduced enzyme and H<sub>2</sub>O<sub>2</sub> at pH 7.4 and different temperatures were calculated. In this reaction, the thiolate of MtAhpE and the protonated peroxide are the reactive species. Since the pK<sub>a</sub> values of the peroxidatic cysteine residue in MtAhpE was measured as 5.2 at 25°C, and the pK<sub>a</sub> values of H<sub>2</sub>O<sub>2</sub> is 11.7, more than 99 % of the thiol will be as thiolate and of the peroxide will be protonated at pH 7.4,<sup>1</sup> and thus, the rate constants measured at pH 7.4 are equal to the pH-independent rate constants, since according to the following equation:

$$k_{RS^{-}} = k_{app}^{pH} \left( \frac{K_a^{RSH} + \left[ \mathbf{H}^+ \right]}{K_a^{RSH}} \right) \left( \frac{\mathbf{H}^+}{\mathbf{H}^+} + K_a^{H_2O_2} \right) \quad (eq.1)$$

where  $k_{RS^{-}}$  corresponds to the real or pH-independent rate constant,  $k_{app}^{pH}$  to the apparent or pHdependent rate constant,  $K_{a}^{RSH}$  is the acidity constant of the peroxidatic thiol in *Mt*AhpE and  $K_{a}^{H_2O_2}$  the acidity constant of the first hydrogen peroxide proton dissociation. Activation energy (E<sub>a</sub>) of the reaction was obtained from the fitting of the data thus obtained to Arrhenius equation. Activation enthalpy ( $\Delta H^{\#}$ ) and entropy ( $\Delta S^{\#}$ ) were obtained from the slope and intercepts of the Eyring plot, respectively, using the Eyring equation.

## Details of the classical molecular dynamics simulations

The crystal structure of *Mt*AhpE dimer (PDBid: 1XXU)<sup>2</sup> was used as starting structure. Cys<sub>P</sub> was configured on its unprotonated form. The system was immersed in a pre-equilibrated octahedral box of 25 Å in radius with TIP3P water molecules.<sup>3</sup> All used residue parameters correspond to the *parm99* Amber force field,<sup>4</sup> except for the  $H_2O_2$  moiety, which were developed by a standard procedure: partial charges were computed using the restricted electrostatic potential (RESP) recipe and DFT electronic structure calculations with the PBE functional and *dzvp* basis set. Equilibrium distances and angles, as well as force constants, were computed using the same methods and basis set used for computed charges. All simulations were performed using periodic boundary conditions with a 15 Å cutoff and particle mesh Ewald (PME) summation method for treating the electrostatic interactions. The hydrogen bond lengths were kept at their equilibrium distance by using the SHAKE algorithm, while temperature and pressure were kept constant with a Langevin thermostat and barostat, respectively, as implemented in the AMBER12 program.<sup>4</sup>

In the case of apo-*Mt*AhpE, the equilibration protocol consisted of (i) slowly heating the whole system from 0 to 300 K for 20 ps at constant volume, with harmonic restraints of 80 kcal mol<sup>-1</sup> Å<sup>-2</sup> for all  $C_{\alpha}$  atoms and (ii) pressure equilibration of the entire system simulated for 1 ns at 300 K with the same restrained atoms. After these two steps, an unconstrained 50 ns MD long simulation at constant temperature (300 K) was performed.

The docking of the peroxide in the enzyme's active-site was done converting a selected water molecule into a  $H_2O_2$  molecule. After performing the same equilibration protocol described above, a 20 ns long MD was performed restraining the S-O<sub>A</sub> distance to any value smaller than 6 Å.

## Details of the QM/MM molecular dynamics simulations

QM-MM simulations were carried out using our own developed code (for details on the QM-MM scheme see references 5 and 6) with similar protocols and parameters as were used previously.<sup>7,8</sup> This

scheme was constructed by partitioning the system into a QM and a MM subsystems. Considering a configuration of N<sub>c</sub> atoms in the MM subsystem with coordinates and partial charges {R<sub>1</sub>, q<sub>1</sub>} and N<sub>q</sub> atoms in the QM subsystem with coordinates and nuclear charges { $\tau_a$ ,  $z_a$ }, the ground state, Born-Oppenheimer potential energy surface is written as:

$$E[\{R_{i}\},\{\tau_{a}\}] = E_{QM} + E_{QM-MM} + E_{MM} \quad (eq.2)$$

where the first term is a purely QM piece given by the standard Kohn-Sham expression. The second term in eq 2 is the coupling term between QM and MM subsystems, which is given by

$$E_{QM-MM} = \sum_{l=1}^{N_c} q_l \int \frac{\rho(r)}{|r-R_l|} dr + \sum_{l=1}^{N_c} \sum_{a=1}^{N_q} \left[ \upsilon_{LJ}(|R_l-\tau_a|) + \frac{q_l z_a}{|R_l-\tau_a|} \right]$$
(eq. 3),

where  $v_{LJ}$  is the Lennard-Jones potential between the classical and quantum part of the system and  $\rho(r)$  is the electron density of the QM subsystem.

The QM system consisted in the  $H_2O_2$  molecule and  $Cys_{45}$  and  $Arg_{116}$  sidechains (see Figure 3). For the QM region, computations were performed at the generalized gradient approximation (GGA) level, using the *PBE* combination of exchange and correlation functionals, with a *dzvp* basis set for the expansion of the one-electron orbitals. The electronic density was also expanded in an auxiliary basis set and the coefficients for the fitting were computed by minimizing the error in the Coulomb repulsion energy.

To explore reaction's free energy and mechanism, we employed an umbrella sampling scheme, choosing as reaction coordinate the difference between the  $O_A-O_B$  and the S- $O_A$  distances (see Figure 3), which was sampled from -1.7 to 1.4 Å, divided in 32 simulation windows, using harmonic potential constants of 200 kcal mol<sup>-1</sup> Å<sup>-2</sup>. Initial configurations were generated from preliminary 100 ps classical equilibration runs in which the solute was treated classically as a rigid moiety, followed by a 2 ps QM/MM MD. For each window, QM/MM MD simulations were run for at least 10 ps. We employed the Verlet algorithm to integrate Newton's equations with a time step of 1 fs. During the simulations, the temperature was held constant at 300 K using the Langevin thermostat.

All dynamics visualizations and molecular drawings were performed with VMD 1.9.1.9



Figure S1. Rate constant temperature dependence of the reduction of  $H_2O_2$  by *Mt*AhpE at pH 7.4.  $k_{obs}$  (s<sup>-1</sup>) as a function of  $H_2O_2$  concentration at T=9.5, 17, 25, 31 and 37°C. Figure corresponds to one independent experiment that was repeated three times with almost identical results.



Figure S2. Radial correlations functions of  $Cys_P$  with water oxygen atoms, calculated from MDs of the apo-*Mt*AhpE (solid black line) and the Michaelis complex with  $H_2O_2$  (solid red line). The corresponding integrated values are also shown (dashed lines, right y-axis).



Figure S3. Representative structures of the ps.  $H_2O_2$ , S atom from Cys<sub>45</sub> and Arg<sub>116</sub> sidechain are represented as balls, while sidechains of Thr<sub>42</sub>, Glu<sub>48</sub> and Pro<sub>135</sub> and water molecules are represented by sticks. Atoms are colored according to their type: cyan, carbon; red, oxygen; blue, nitrogen; yellow, sulfur. Relevant hydrogen bonds are indicated by dashed black lines.



Figure S4. Charge evolution during the reaction. Mulliken charges (e) of S atom (solid line),  $O_A$  atom (dashed line),  $O_B$  atom (pointed line) are plotted vs. reaction coordinate (Å).

Table S1. Relevant structural parameters of the *rs*, *ts* and *ps*. Values as average and standard deviation (in brackets) calculated for the QM/MM dynamics within the region of interest. Distances in angstroms, angles and dihedrals in degrees.

structural parameter	reactant state	transition state	product state
distance (S- $O_A$ )	3.08 (0.05)	2.31 (0.05)	1.67 (0.04)
distance ( $O_A$ - $O_B$ )	1.48 (0.03)	1.91 (0.05)	2.76 (0.05)
angle (S- $O_A$ - $O_B$ )	94.1 (8.3)	161.6 (6.2)	114.2 (8.1)
distance $(O_B-H_t)$	1.94 (0.05)	2.00 (0.12)	1.01 (0.03)
dihedral ( $O_A$ -S- $N_2$ - $N_3$ )	-1.4 (12.1)	8.1 (10.9)	-73.9 (11.1)
distance (S-N <sub>2</sub> )	3.38 (0.18)	3.28 (0.15)	4.58 (0.20)
distance (S-N <sub>3</sub> )	3.32 (0.13)	3.48 (0.21)	3.43 (0.15)
distance (S-O <sub>alcohol group Thr42</sub> )	3.01 (0.09)	3.07 (0.13)	4.19 (0.36)
distance $(O_A-N_3)$	3.81 (0.28)	3.22 (0.48)	2.58 (0.19)
distance $(O_B-N_3)$	3.20 (0.27)	4.03 (0.70)	4.68 (0.24)
distance ( $O_A$ - $N_{amide Cys45}$ )	3.08 (0.05)	2.31 (0.05)	1.67 (0.04)
distance ( $N_2$ - $O_{carboxilic Glu48}$ )	3.99 (0.24)	3.85 (0.24)	3.77 (0.44)
distance (N <sub>2</sub> -O <sub>Pro135</sub> )	2.80 (0.10)	2.78 (0.10)	2.85 (0.12)

**3D** Animation Specifications (Zeida\_etal.mpg). The animation was prepared putting together representative pictures from each one of the 32 simulation windows described above. Active-site microenvironment and hydrogen peroxide are shown. Colors: sulfur, yellow; oxygen, red; carbon, cyan; nitrogen, blue; hydrogen, white.

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