

Supporting information for:

Evidence of a Thermodynamic Ramp for Hole-Hopping to Protect a Redox Enzyme from Oxidative Damage

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Discussion of the protein environment around Tyr34

Redox reactions involving Tyr residues are frequently coupled to proton transfer reactions when a base is present near the phenolic proton.^{S1} The nearest potential proton acceptor for Tyr34 is a histidine residue (His30). However, the distance between the phenolic oxygen and the nearest nitrogen (N_δ) of the imidazole ring of His30 is ≈ 5 Å in the crystallographic structure while in typical proton transfer reactions the donor–acceptor distance is within a regular hydrogen bond (HB) distance (≈ 3 Å).^{S2} In addition, the crystal structure shows that

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N_ϵ of His30 is at HB distance from the phenolic oxygen of another Tyr residue not involved in the hole transport chain (Tyr166) and that the phenolic oxygen of Tyr34 is at HB distance from the side-chain nitrogen of Gln143. Thus, the protein environment does not promote the interaction between Tyr34 and His30. It has to be noted that if His30 could serve as proton acceptor for the oxidized Tyr (TyrOH⁺), this could affect the thermodynamic ramp for the hole transfer. In fact, the oxidation potential of the [TyrO[•]...⁺H - His]/[TyrO - H... His] couple has been estimated to be $\approx 0.15\text{-}0.25$ V (i.e., $\approx 15\text{-}25$ kJ/mol) lower than the one for the TyrOH⁺/TyrOH couple.^{S3,S4} Such a difference is comparable to the oxidation potential difference between Tyr34 and Trp161 (see Table 1 in the main text) and thus a proton-coupled ET reaction for Tyr34 oxidation could make the hole transfer between Tyr34 and Trp161 thermodynamically disadvantaged. However, the hole transfer along the chain could be in any case kinetically favoured. For the proton to be transferred at the large 5 Å distance between O(Tyr34) and N_δ (His30) a semiclassical diffusion-like path would in fact be required, which is expected to yield a slow proton transfer.^{S2,S5} In contrast, a rough estimate of the kinetic rate of the hole transfer between Tyr34 and Trp161 using the square barrier tunneling model, suggests a very fast reaction. According to such a model,^{S6} the electron transfer rate k_{ET} is $k_{ET} = 10^{13} \exp[-\beta(R - R_0)] \exp[-(\Delta G^0 + \lambda)^2 / (4\lambda k_B T)]$ with β the exponential distance decay parameter, R the edge-to-edge distance and R_0 the contact distance between donor and acceptor, ΔG^0 the standard free energy change for the electron transfer reaction, λ the reorganization energy and k_B the Boltzmann constant. With $\Delta G^0 = -\Delta(\Delta A^0)$ for the Tyr34-Trp161 hole transfer (see Table 1 in the main text), $R = 5$ Å and using for the other quantities the values suggested by Gray and Winkler^{S6} (i.e., $\beta = 1.1$ Å⁻¹, $R_0 = 3$ Å, $\lambda = 0.8$ eV ≈ 80 kJ/mol) we obtain that ET between Trp161 and Tyr34 occurs on the time scale of hundreds of picoseconds. Thus, the protein environment around Tyr34 suggests that hole hopping is faster than a potential proton loss from the oxidized Tyr34, preserving the protective role of the whole chain.

The MD-PMM approach to compute oxidation potentials

In MD-PMM calculations, similarly to QM/MM procedures,^{S7-S17} a portion of the system to be treated at electronic level is pre-defined (the quantum center, QC), with the rest of the system described at a classical atomistic level exerting an electrostatic effect on the QC. With a relatively low computational cost, the MD-PMM can be applied to a very large set of molecular configurations, hence providing the dynamical coupling of electronic properties with classical degrees of freedom. Indeed, the phase space sampling is provided by fully classical molecular dynamics (MD) and thus a statistically relevant sampling of the QC/environment configurations can be achieved, which is necessary for a proper description of functional properties in dynamical, complex systems. For each configuration of the whole system generated from the MD simulation, the effect of the external environment on the QC eigenstates is included by building and diagonalizing the perturbed electronic Hamiltonian matrix \tilde{H} constructed in the basis set of the unperturbed Hamiltonian eigenstates of the QC. Indicating with \mathcal{V} and \mathbf{E} the perturbing electric potential and field, respectively, exerted by the environment on the QC:

$$\tilde{H} \simeq \tilde{H}^0 + \tilde{I}q_T\mathcal{V} + \tilde{Z}_1 \quad (1)$$

$$[\tilde{Z}_1]_{j,j'} = -\mathbf{E} \cdot \langle \phi_j^0 | \hat{\boldsymbol{\mu}} | \phi_{j'}^0 \rangle \quad (2)$$

where q_T , $\hat{\boldsymbol{\mu}}$ and ϕ_j^0 are the QC total charge, dipole operator and unperturbed electronic eigenfunctions, respectively, \tilde{I} is the identity matrix and the angled brackets indicate integration over the electronic coordinates. The diagonalization of \tilde{H} provides a set of eigenvectors and eigenvalues representing the QC perturbed electronic eigenstates and energies. More details on the method can be found in the original articles.^{S18,S19}

For the more specific task of calculating oxidation potentials, the perturbed electronic

ground-state energies are calculated for the QC reduced and oxidized chemical states, providing the perturbed electronic ground-state energy shift. The (Helmholtz) free energy change ΔA^0 associated to the electron/hole transfer reaction, corresponding to the reduction/oxidation potential, can be calculated using the following equation:^{S20}

$$\begin{aligned}\Delta A^0 &= -k_B T \ln \langle e^{-\beta \Delta \mathcal{U}_{ox \rightarrow red}} \rangle_{ox} = k_B T \ln \langle e^{-\beta \Delta \mathcal{U}_{red \rightarrow ox}} \rangle_{red} \\ &\approx -k_B T \ln \langle e^{-\beta \Delta \varepsilon_{ox \rightarrow red}} \rangle_{ox} = k_B T \ln \langle e^{-\beta \Delta \varepsilon_{red \rightarrow ox}} \rangle_{red}\end{aligned}\quad (3)$$

In the above equation $\Delta \mathcal{U}_{ox \rightarrow red(red \rightarrow ox)}$ is the whole system energy change upon reduction (oxidation), $\Delta \varepsilon_{ox \rightarrow red(red \rightarrow ox)} = \varepsilon_{red} - \varepsilon_{ox}(\varepsilon_{ox} - \varepsilon_{red})$, with ε_{red} and ε_{ox} representing the perturbed ground-state electronic energy of the *red* (reduced) and *ox* (oxidized) chemical states, respectively, and the approximation $\Delta \mathcal{U}_{ox \rightarrow red(red \rightarrow ox)} \approx \Delta \varepsilon_{ox \rightarrow red(red \rightarrow ox)}$ is used, *i.e.*, the environment internal energy change associated to the QC reduction is disregarded being exactly zero within the present description.^{S20} ε_{red} and ε_{ox} are the perturbed electronic ground-state energies evaluated at each MD frame via the MD-PMM approach. The subscripts of the angle brackets indicate that averaging can be performed in either the oxidized or reduced ensemble, *i.e.*, with the perturbing environment configurations obtained by classical MD simulations performed with the QC in either the reduced or the oxidized chemical state. Although Eq. 3 is based on an exact relation in principle, given the sampling problems of finite-time simulations, the best estimate of the oxidation free energy is obtained by averaging the values provided by the estimates obtained from the reduced and oxidized ensembles MD simulations.^{S21} However, in the present case we are not interested in an exact estimate of the absolute value of the oxidation potentials of the Tyr/Trp residues, but rather in an estimate of the shifts between such oxidation potentials along the chain. For this reason, we have here used only the reduced simulation ensemble, *i.e.*, a long but unique simulation with all the Tyr/Trp residues in the reduced (neutral) state. In addition, the simulation was

performed with the active site in its oxidized state (Mn^{3+}). Such a choice mimics indeed a condition of potential oxidative stress, in which the active site might release a hole to the nearest Tyr/Trp residues in the reduced state.

Quantum chemical calculations

In order to apply the MD-PMM procedure to compute the oxidation potential of the residues involved in the 5 Å distance cutoff chain (Tyr34, Trp161, Trp123 and Trp125), indole (tryptophan side chain) and phenol (tyrosine side chain) are selected as QCs. Two sets of quantum chemical calculations are carried out on each chemical species in vacuo: in their neutral and charged state (cationic phenol and indole). Neutral and cationic phenol and indole were optimized in vacuum at the DFT(B3LYP)/6-31+G(d) level. Time-Dependent Density Functional Theory calculations^{S22} on each chemical species provided then the gas-phase energies of the unperturbed states and the expected values of all the ground to excited unperturbed transition dipole moments and excited state dipole moments necessary for the MD-PMM procedure to be applied. The calculations are performed with Becke’s three parameters exchange and Lee, Yang and Parr correlation functionals (B3LYP)^{S23,S24} in conjunction with the 6-31+G(d) basis set. Such a level of theory was already successfully used for the modeling of charge transfer processes involving Tyr and Trp residues.^{S25}

MD simulation

The crystallographic structure of the tetrameric assembly of MnSOD2 (Pdb code 1N0J^{S26}) is used as starting structure for the MD simulation. The AMBER99 force field parameters^{S27} are adopted for the protein and all the force-field parameters for the oxidized active site (Mn^{3+}) are taken from Neves et al.^{S28} The structure was solvated in a periodic rhombic dodecahedral box filled with TIP3P water molecules.^{S29} After a solute optimization and a subsequent solvent relaxation, the protein was gradually heated from 50 K to 300 K using

100 ps MD simulations. The trajectory was then propagated for 100 ns in the NVT ensemble with isokinetic temperature coupling^{S30} keeping the temperature constant at 300 K. Periodic boundary conditions and a non-bond pairlist cutoff of 9.0 Å were used and the long-range electrostatic interactions were treated with the particle mesh Ewald method.^{S31} The bond lengths were fixed^{S32} and a time step of 2 fs for numerical integration of the equations of motion was used. The simulations were performed with the GROMACS software package.^{S33} Configurations of the whole system generated from the MD simulation and collected every ps were used to calculate the perturbed electronic ground-state energies via the MD-PMM approach.

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