

Supporting Information

Experimental methods:

The catalase samples (in the resting ferric form) from beef liver were prepared with a buffer of 50 mM KH_2PO_4 with the pH adjusted to 7.0 at 25°C using 1M KOH, approaching physiological conditions. Details about the set-up have been discussed in details before.[1-5] The sample temperature was varied from 5°C up to 30°C. Higher temperatures (up to 37 °C) showed no changes in the spectra. To minimize radiation damage, the sample was flown in a cell equipped with a soft X-ray transparent silicon nitride (Si_3N_4) membrane window (500x500 nm²), ensuring its renewal every ~5 msec for a beam focus of 20x40 μm^2 . The obtained spectra were normalized dividing by the current obtained from the refocus mirror of the BESSY II U41-PGM beamline just before the light enters the experimental setup.

Multiplet calculations:

Briefly, the theory starts off with the Hamiltonian of the metal atom, taking into account all Coulomb interactions as well as the spin-orbit coupling between the atomic orbitals.[6-8] To describe the local symmetry, ligand field parameters are added. The local symmetry of the iron sites in all haem species (either five-coordinated pyramidal or six-coordinated planar) is similar to a D_{4h} symmetry[9,10] and can be started with a regular O_h symmetry.[11] In O_h symmetry, the 3d orbitals are split into t_{2g} (d_{xz} , d_{xy} , d_{yz}) and e_g (d_z^2 , $d_{x^2-y^2}$) classes with an energy difference of $10Dq$. In lowering the symmetry from O_h to a distorted D_{4h} , the orbitals further decompose into b_{2g} (d_{xy}), e_g (d_{xz} , d_{yz}), a_{1g} (d_z^2) and b_{1g} ($d_{x^2-y^2}$) and two more parameters (Ds and Dt) are introduced. The calculated spectra are broadened with a Lorentzian and a Gaussian, to describe the lifetime and instrumental broadening, respectively. The ligand field parameters ($10Dq$, Ds and Dt) are adjusted to obtain the best fit to the experimental XAS spectra, however Ds and Dt hardly influence them and can be set to zero.

Covalent mixing of the metal valence d-orbitals with the ligand valence p-orbitals is simulated using a charge-transfer model, which in the case of LMCT adds a $d^{n+1}\underline{L}$ configuration above the d^n ground state. The $d^{n+1}\underline{L}$ configuration is set at an energy Δ above the d^n configuration, and these two states are coupled by configuration interaction (CI), represented by the mixing term $T_i = \langle d^n | h | d^{n+1}\underline{L} \rangle$, where h is the molecular Hamiltonian and T_i is proportional to metal-ligand overlap for each of the i symmetry blocks. For a donor ligand system, the ground and LMCT states are written as linear combinations of $|3d^n\rangle$ and $|3d^{n+1}\underline{L}\rangle$ wave functions, while the L-edge excited state is a linear combination of $|2p^5 3d^{n+1}\rangle$ and $|2p^5 3d^{n+2}\rangle$ wave functions. The coefficients of these linear combinations are functions of T and Δ for the ground state and T' and Δ' for the excited state, where $\Delta' = \Delta + U - Q$ (also called the charge transfer energy) with U being the 3d-3d electron repulsion (so-called Hubbard energy) and Q the 2p-3d repulsion (core hole energy). Ligand field, T and Δ are allowed to vary in the final state fits (i.e., decrease) but have

little effect on the covalent mixing observed from the fits, as was also found in ref. [12]. Δ' and U-Q affect the energy position of the L_3 and L_2 -edges (Figure S2). In order to include back-bonding (MLCT) in addition to σ -donation, it is necessary to introduce a third state, Δ_p , above the d^n configuration. The ground-state wave function is now a linear combination of three configurations, $3d^{n-1}\bar{L}$, $3d^n$, and $3d^{n+1}\underline{L}$. In introducing MLCT parameters, U-Q has to be changed in order to maintain a good agreement with experimental spectra. This optimization was performed by switching off the MLCT mixing parameters and varying only Δ' and U-Q (Figure S2). Further details for the three configuration simulations, including both LMCT and MLCT, are given in refs [13] and [12]. For the present purposes, the only parameters of relevance are the ligand field, Δ , Δ' , U-Q and the mixing terms T_i .

Table S1 reproduces the parameters we used for methaemoglobin in our previous contribution [5], and those optimized here for catalase without and with the MLCT contributions. Figures S1 shows the effect of the $T(a_{1g})$ LMCT and $T(e_g)$ LMCT contributions on the spectra. Note that both influence the energy splitting of the doublets at both edges. Figure S2 shows the effect of U-Q on the spectra, with and without MLCT contributions. It mainly affects the pre-edge feature at the L_3 -edge. Finally, figure S3 shows the influence of π back-donation on the spectra. The best agreement with the experimental spectrum is obtained by considering π back-donation from d_{xy} , d_{xz} and d_{yz} metal orbitals to the ligand. If only d_{xy} ($T(b_{2g})$ MLCT=0.7 and $T(e_g)$ MLCT=0) or $d_{xz,yz}$ ($T(b_{2g})$ MLCT=0 and $T(e_g)$ MLCT=0.7) orbitals are taken into account, the intensity of the pre-edge peak increases and the overall accordance with experiment decreases.

	Methaemoglobin	Catalase (LMCT)	Catalase (LMCT+MLCT)
Ground state Configuration	64% d^5 + 36% $d^6\bar{L}$	63% d^5 + 37% $d^6\bar{L}$	3% $d^4\bar{L}^-$ + 62% d^5 + 35% $d^6\bar{L}$
10Dq (eV)	1.3	1.0	1.0
Δ (eV)	0	0	0
Δ_p (eV)	0	0	-2.0
U-Q	-2.0	-2.0	-1.0
$T(b_{2g})$ MLCT	0	0	0.7
$T(e_g)$ MLCT	0	0	0.7
$T(b_{1g})$ LMCT	4.0	4.0	4.0
$T(a_{1g})$ LMCT	3.2	2.8	2.8
$T(b_{2g})$ LMCT	1.0	1.0	1.0
$T(e_g)$ LMCT	1.5	2.0	2.0

Table S1. Ground state configurations and selected charge transfer multiplet parameters used to simulate experimental the XA spectra of haemoglobin and catalase in a D_{4h} symmetry. 10Dq is the cubic crystal

field. Δ and Δ' are the charge transfer energies defined as $\Delta = E(3d^6\bar{L}) - E(3d^5)$ and $\Delta_p = E(3d^4\bar{L}) - E(3d^5)$. Q is the core hole potential and U the Hubbard energy accounting for 3d-3d correlations. $T(b_1)$, $T(a_1)$, $T(b_2)$ and $T(e)$ are the mixing parameters to consider in D_{4h} symmetry. MLCT and LMCT are abbreviations of metal-to-ligand and ligand-to-metal charge transfer respectively.

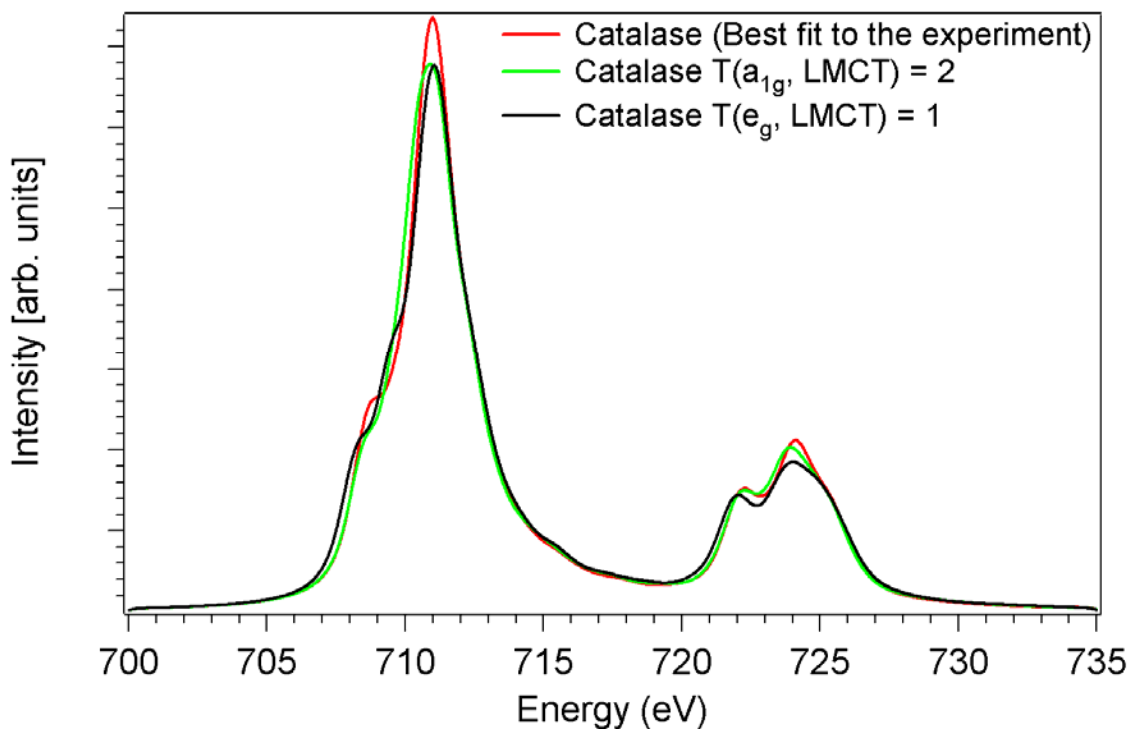


Figure S1: Effect of the reduction of the parameters $T(a_{1g}, \text{LMCT})$ (green) and $T(e_g, \text{LMCT})$ (black). $T(a_{1g}, \text{LMCT})$ corresponds to the mixing between ligand orbitals and d_{z^2} metal orbital while $T(e_g, \text{LMCT})$ corresponds to the mixing between ligand orbitals and d_{xz}/d_{yz} metal orbitals. The best simulation for catalase is plotted in red.

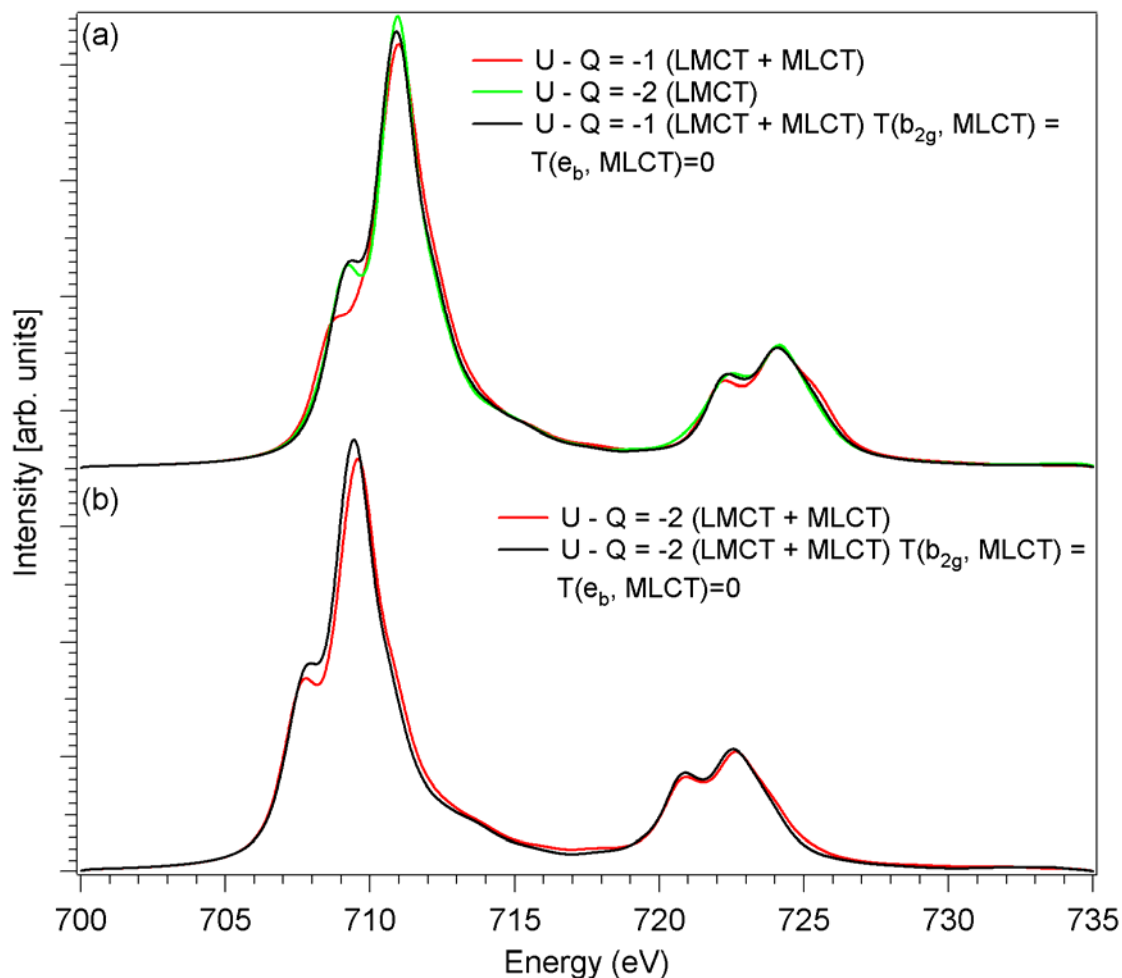


Figure S2: Simulated Fe L-edge absorption spectra obtained for (a) $U-Q=-1$ eV considering LMCT and MLCT (red), $U-Q=-2$ eV considering LMCT only (green), $U-Q=-1$ eV considering LMCT and MLCT in the particular case $T(b_{2g}, \text{MLCT})=T(e_g, \text{MLCT})=0$ (black) and (b) $U-Q=-2$ eV considering LMCT and MLCT (red) and $U-Q=-2$ eV considering LMCT and MLCT in the particular case $T(b_{2g}, \text{MLCT})=T(e_g, \text{MLCT})=0$ (black). Q is the core hole energy and U the Hubbard energy accounting for 3d-3d correlation effects.

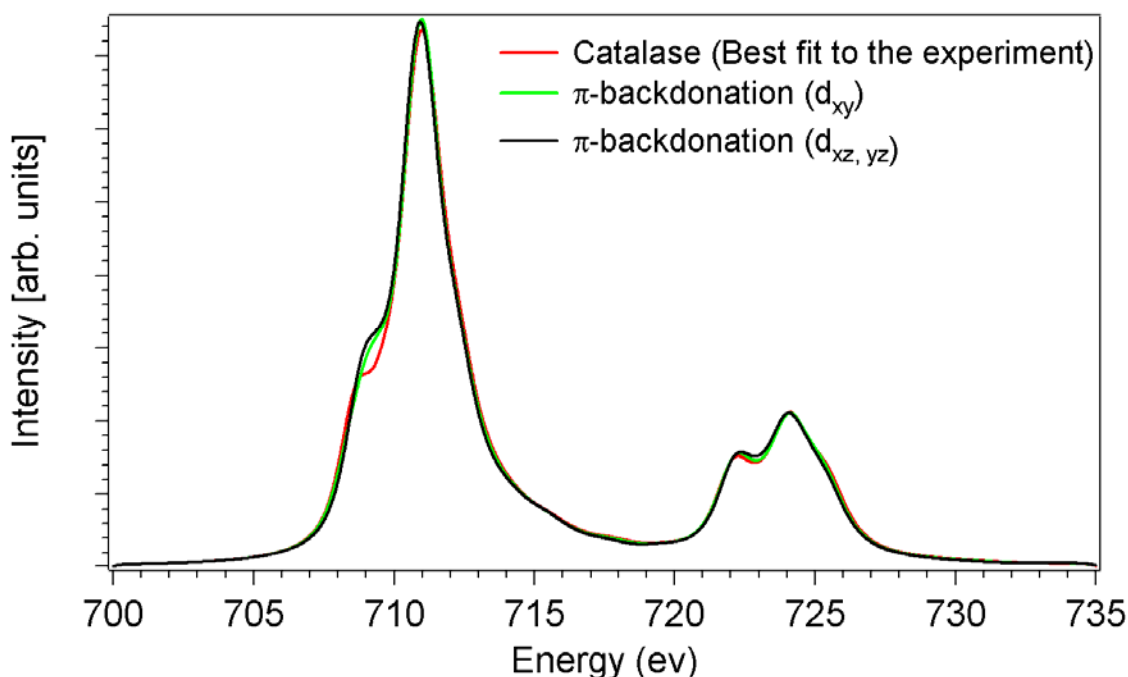


Figure S3: Effect of π back-donation considering the mixing between ligand orbitals and the d_{xy} metal orbital only ($T(b_{2g})$ MLCT=0.7 and $T(e_g)$ MLCT=0 - in green) and the mixing between ligand orbitals and the $d_{xz,yz}$ metal orbitals only ($T(b_{2g})$ MLCT=0 and $T(e_g)$ MLCT=0.7 - in black). The best simulation for catalase is plotted in red.

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