SUPPORTING TEXT

SI Methodology

Quantum Chemical Calculations

The QM/MM method (1-6)[1] dissects the total enzyme into two subsystems: the active center is one subsystem, and the rest of the protein constitutes the second subsystem. The active center is described by a QM method, in the present case density functional theory (DFT) in the unrestricted Kohn-Sham formalism using the B3LYP hybrid functional. The rest of the system (see below) is treated by MM using a force field calibrated for proteins. The two subsystems are allowed to interact by electrostatic and van der Waals terms, such that the QM subsystem adapts its electronic structure and charge distribution to the field exerted by the protein environment. In the present study, we apply the electrostatic embedding scheme that incorporates the MM charges into the one-electron Hamiltonian of the QM procedure, while the dangling bonds at the QM/MM boundary are capped with hydrogen link atoms in the framework of the charge shift method (7). The Chemshell software (8) is used to perform the QM/MM calculations by integrating the TURBOMOLE package (9) for QM, and the DL-POLY program (10) for MM using the CHARMM22 force field (11). For QM/MM geometry optimization, we used the HDLC optimizer (12). The QM subsystem is calculated by using an effective core potential on iron and the double zeta LACVP basis set (13, 14), coupled with the 6-31G basis-set for all other atoms; hereafter this basis set will be referred to as B1. The results obtained with this basis set were tested by other calculations made with a larger basis set: polarization, and diffuse functions were added on oxygen, nitrogen and sulfur. We will refer to this basis set as B2.

[1] ?/Au: Only one reference is permitted per number, so references had to be renumbered.

To prepare suitable initial structures for the QM/MM calculations, we started from the experimental x-ray crystal structure of CPO obtained as described and modified as indicated above, and built a complete model of the solvated enzyme by adding the missing hydrogen atoms and a 16-Å-thick water solvent layer. The complete system consists of 18,342 atoms, including 12,738 atoms in the solvent. The system was subsequently relaxed by performing pure force field energy minimization and short molecular dynamics (MD) simulations, using the CHARMM22 force field. During these classical force field calculations, the coordinates of the entire heme unit and the coordinating cysteine (Cys-29) were kept fixed.

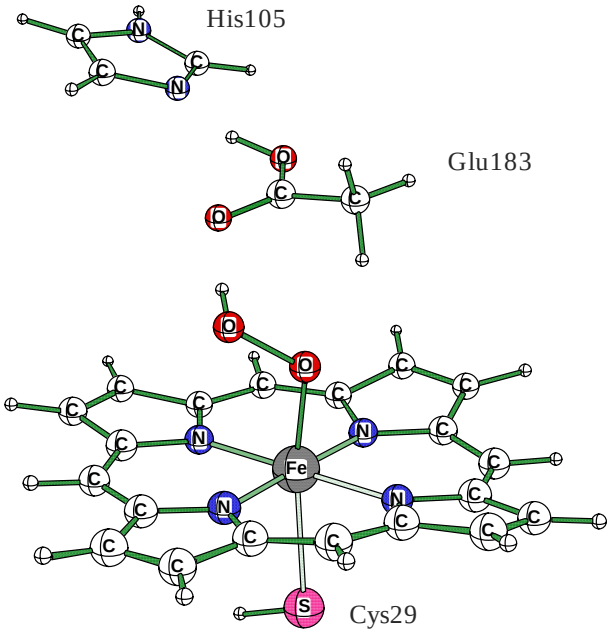


Fig. 6. The model used for the calculations of the QM subsystem in the QM/MM calculations. Shown is the QM region used during the QM/MM calculations. The heme was described by porphine without the propionate, vinyl and methyl substituents. The cysteine proximal ligand was replaced by a SH group, as in previous work on P450 (15, 16). In addition, the QM subsystem involved the two residues, His-105 and Glu-183, the latter of which interacts with the dioxygen ligand in the distal side of the heme; Glu-183 was modeled by an acetic group and His-105 by a doubly protonated imidazole. These two residues interact strongly with each other through a hydrogen bond (OH---N). Finally, during the QM/MM calculations, the

region subjected to QM/MM optimization involved the following residues: Hem-29 (comprising the porphine and the SH groups), Ala-31, Leu-32, Phe-103, His-105, Glu-183, Phe-186, ACET (an acetic acid in the channel), and W226.

QM/MM Scan Energies

Scan along the O-H coordinates at the UB3LYP/B1:CHARMM level in the doublet state.

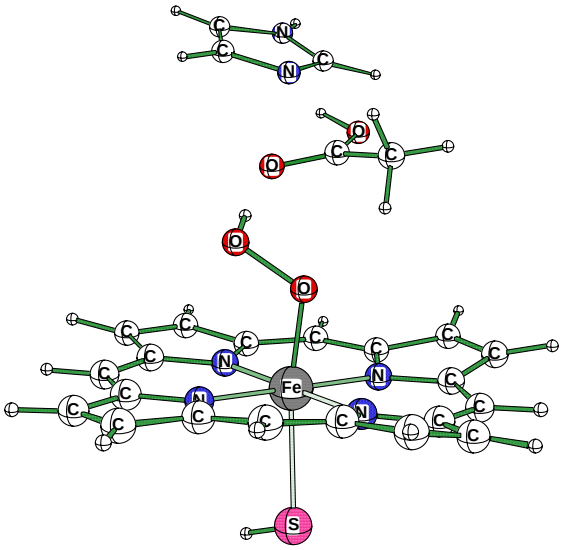
Distance	E(QM)	E(QM/MM)	ΔE(QM)	ΔE(QM/MM)
0.999	-2116.381981	-2187.181722	0.00	0.00
1.049	-2116.380386	-2187.180134	1.00	1.00
1.099	-2116.376162	-2187.176424	3.65	3.32
1.149	-2116.370714	-2187.171121	7.07	6.65
1.199	-2116.364504	-2187.16514	10.97	10.40
1.249	-2116.358512	-2187.159145	14.73	14.17
1.299	-2116.352985	-2187.153615	18.20	17.64
1.349	-2116.349731	-2187.149237	20.24	20.38
1.399	-2116.346996	-2187.146145	21.95	22.32
1.449	-2116.34492	-2187.14371	23.26	23.85
1.499	-2116.34435	-2187.142194	23.61	24.80
1.549	-2116.343273	-2187.140732	24.29	25.72
1.599	-2116.342319	-2187.139383	24.89	26.57
1.649	-2116.341399	-2187.138076	25.47	27.39
1.699	-2116.340366	-2187.136677	26.11	28.27
1.749	-2116.339412	-2187.135346	26.71	29.10

Scan along the O-H coordinates at the UB3LYP/B1:CHARMM level in the quartet state.

Distance	E(QM)	E(QM/MM)	ΔE(QM)	ΔE(QM/MM)
1.008	-2116.361706	-2187.161248	12.72	12.85
1.058	-2116.360669	-2187.160111	13.37	13.56
1.108	-2116.359685	-2187.157707	13.99	15.07
1.158	-2116.357686	-2187.155067	15.25	16.73
1.208	-2116.355805	-2187.152704	16.43	18.21
1.258	-2116.354207	-2187.150733	17.43	19.45
1.308	-2116.352921	-2187.14907	18.24	20.49
1.358	-2116.35186	-2187.147635	18.90	21.39
1.408	-2116.350798	-2187.146301	19.57	22.23
1.458	-2116.349829	-2187.145068	20.18	23.00
1.508	-2116.348856	-2187.143854	20.79	23.76
1.558	-2116.347897	-2187.142537	21.39	24.59
1.608	-2116.346815	-2187.141163	22.07	25.45
1.658	-2116.345823	-2187.139783	22.69	26.32
1.708	-2116.344623	-2187.138274	23.44	27.26
1.758	-2116.343282	-2187.136708	24.28	28.25

Coordinates of the QM region for the species A, B, C and D

A



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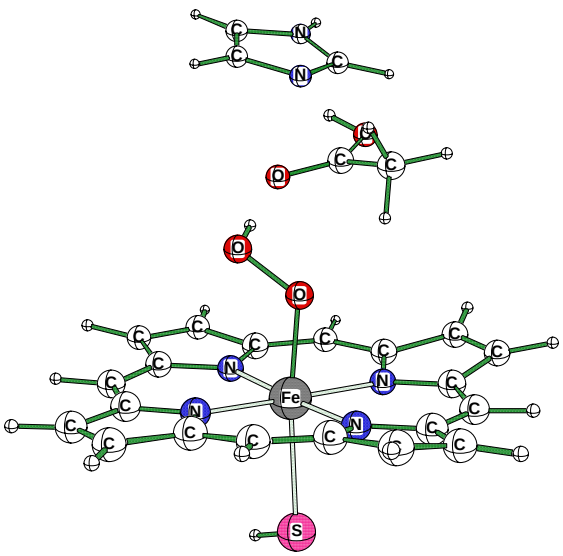
B



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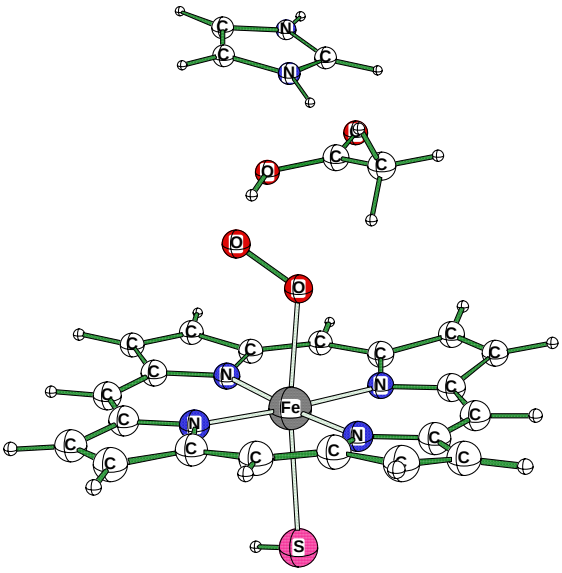
C



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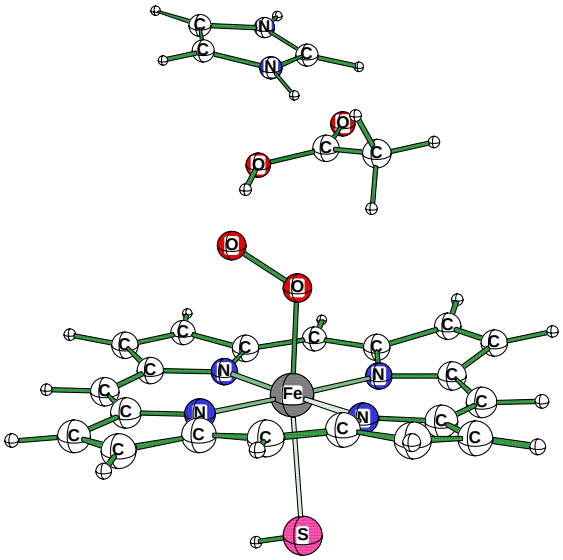

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QM/MM Scan Energies with B2

Scan along the O-H coordinates at the UB3LYP/B2:CHARMM level in the doublet state.

Distance	E(QM)	E(QM/MM)	ΔE(QM)	ΔE(QM/MM)
0.982	-2116.592278	-2187.393294	0.00	0.00
1.032	-2116.590413	-2187.391437	1.17	1.17
1.082	-2116.585632	-2187.38689	4.17	4.02
1.132	-2116.579308	-2187.380693	8.14	7.91
1.182	-2116.572301	-2187.373754	12.54	12.26
1.232	-2116.565448	-2187.366875	16.84	16.58
1.282	-2116.5594	-2187.360466	20.63	20.60
1.332	-2116.553874	-2187.355005	24.10	24.03
1.382	-2116.550166	-2187.34913	26.43	27.71
1.432	-2116.548821	-2187.34722	27.27	28.91
1.482	-2116.547864	-2187.345909	27.87	29.73
1.532	-2116.546672	-2187.344647	28.62	30.53
1.582	-2116.546142	-2187.343576	28.95	31.20
1.632	-2116.545267	-2187.342425	29.50	31.92
1.682	-2116.544386	-2187.341239	30.05	32.66
1.732	-2116.54348	-2187.340087	30.62	33.39

Scan along the O-H coordinates at the UB3LYP/B2:CHARMM level in the quartet state.

Distance	E(QM)	E(QM/MM)	ΔE(QM)	ΔE(QM/MM)
0.982	-2116.5782	-2187.375395	8.84	11.23
1.032	-2116.5763	-2187.373621	10.00	12.35
1.082	-2116.5721	-2187.369241	12.69	15.09
1.132	-2116.5658	-2187.363271	16.64	18.84
1.182	-2116.5589	-2187.356757	20.96	22.93
1.232	-2116.5529	-2187.350896	24.74	26.61
1.282	-2116.55	-2187.347039	26.51	29.03
1.332	-2116.547	-2187.344068	28.41	30.89
1.382	-2116.5447	-2187.341574	29.83	32.45
1.432	-2116.5465	-2187.341064	28.72	32.77
1.482	-2116.5454	-2187.339676	29.41	33.65
1.532	-2116.5443	-2187.338334	30.13	34.49
1.582	-2116.5432	-2187.337028	30.81	35.31
1.632	-2116.5422	-2187.335683	31.44	36.15
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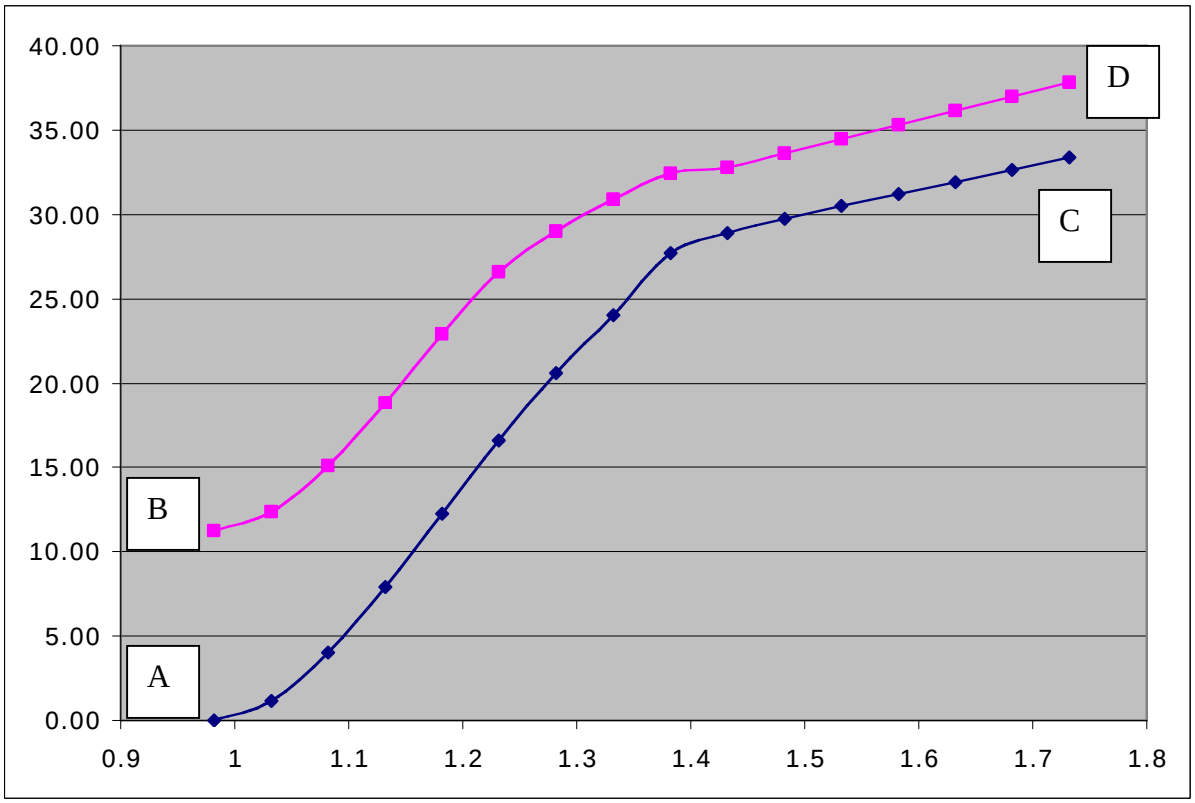
1.732	-2116.5399	-2187.333017	32.89	37.82



QM/MM (UB3LYP/B2:CHARMM) energy profiles between the Fe-OOH^- (A and B) and Fe-O_2^{2-} (C and D) structures of CPO, in the doublet (blue line)and quartet (pink line) states.

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[2] ?/Au: Please check references carefully. They had to be redone to conform to PNAS style.
[3] ?/Au: Please confirm authors. The first 10 are required.

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