The Resting State of $P450_{cam}$: A QM/MM study

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Supporting Information

Preparation of the System. Initial coordinates were obtained from the crystal structure of the ferric substrate complex (PDB code 1DZ4, Brookhaven Protein Database) (Schlichting, I.; Berendzen, J. ; Chu, K.; Stock, A.M.; Maves, S.A.; Benson, D.A.; Sweet, R.M.; Ringe, D.; Petsko, G.A.; Sligar, S.G. *Science* **2000**, *287*, 1615). No ordered water molecules in the active site are present in this species. Only one monomer from the crystallographic dimer was used, which corresponds to the A chain of the PDB structure, where the first 10 residues are unresolved. These 10 residues were omitted in the calculations. 310 associated crystal water molecules (chain Z) were included in the model. The coordinates of camphor and the TRIS buffer molecule were deleted. The potassium ion in the binding site is present in the simulations.

The positions of 5 water molecules in the active site were taken from X-ray data by Poulos et al. (Poulos, T. L.; Finzel, B. C.; Howard, A. J. *Biochemistry* **1986**, *25*, 5314. PDB code 1PHC, Brookhaven Protein Database). The relative position of this water cluster was determined by aligning the backbone atoms of the two crystal structures with a least squares fit. Another water molecule was added as the sixth ligand to iron. ESEEM studies indicate that the conformation of the ligand is upright, with the plane spanned by the water molecule perpendicular to the porphyrin plane (Goldfarb, D.; Bernardo, M.; Thomann, H.; Kroneck, P. M. H.; Ullrich, V. *J. Am. Chem. Soc.* **1996**, *118*, 2686). The initial

geometry of the aqua-heme complex was determined by reference to gas phase QM calculations at the DFT level (B3LYP/LACVP,6-31G). Protein hydrogen positions were built with CHARMM at pH7 (see paper for details).

Force Field Parameters. The force field parameters for the axial water ligand were taken from the standard TIP3P parameter set (oxygen: type OT, charge -0.834e; hydrogen: type HT, charge 0.417e). The sulfur atom of the proximal cysteine was assigned an atomic charge of -0.07e. All other parameters were used as obtained from the CHARMM22 library (ref. 20 of the paper).

Preparatory Force Field Calculations. To relax the structure, a series of classical force field calculations was carried out using the CHARMM program (Brooks, B. R.; Burccoleri, R. E.; Olafson, B. D.; States D. J.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187), version 27a2. In these simulations, the coordinates of the entire heme unit, the oxygen atoms of the 5 water molecules in the active site, the proximal cysteine (Cys357), and the outer 8 Å of the solvent were kept fixed. The details of these simulations are completely analogous to the procedure described in ref 21 of the paper, and are shortly summarized as follows:

- An initial geometry optimization included 3600 steps of adopted basis Newton-Raphson (ABNR) mimization to remove close contacts. Heavy atom positions of the enzyme were assigned harmonic constraints [100 kcal/(mol $Å^2$) for the backbone and 50 kcal/(mol $Å^2$) for the side chains] that were scaled down by a factor of 0.65 every 80 steps.

- Molecular Dynamics (NVT): 15 ps heating dynamics (0 $\rightarrow$ 300 K), 200 ps equilibration dynamics (300K), timestep 1 fs, SHAKE constraints (van Gunsteren, W. F.; Berendsen, H. H. C. *Mol. Phys.,* **1977**, *34*, 1311) for bonds to hydrogen atoms.

- Several snapshot structures obtained from the equilibration trajectory were optimized by 5000 steps of ABNR minimization, respectively. The final GRMS was always below 0.003 kcal/(mol $Å^2$)).

Table S1: MM energy minimization from selected snapshots of the equilibration trajectory (coordinates of heme, Cys357, and outer 8 Å of solvent layer fixed): potential energy (kcal/mol) and RMS deviation (Å) of backbone atom (N, C^{α}, C) positions with respect to the X-ray structure (1DZ4) after least square fit alignment.

	Prot1			Prot2		
snap	time / ps	E / kcal mol^{-1} [a]	RMSD / Å	time / ps	E / kcal mol^{-1} [a]	RMSD / Å
0 [b]	0	-62807.88394	0.335	0	-62811.262b10	0.335
1	60	-65421.99644	0.708	50	-66447.91348	0.757
2	120	-65395.40585	0.710	150	-66945.82037	0.915
3	160	-65412.18251	0.762	175	-66805.36913	0.830
4	190	-65450.66363	0.808	195	-67784.90956	0.798
5	200	-65524.51552	0.812	200	-67470.07154	0.816

[a] CHARMM22 force field potential energy (kcal/mol). [b] The X-ray structure after an initial MM optimization, before the MD run.

Table S1 summarizes the results of the energy minimizations of the snapshot structures taken from the two MD trajectories of Prot1 and Prot2. The first structure (snapshot 0) corresponds to the MM optimized geometry before the MD in both cases. We measured the root mean square (RMS) deviation of the backbone atom positions in the minimized structures with respect to the X-ray geometry (1DZ4). Obviously, "snapshot 0" exhibits the smallest RMS deviations of (Prot1/Prot2) 0.335/0.335 Å, whereas all structures extracted from the MD show rather similar values, varying from 0.708 to 0.812 (in Prot 1) and from 0.757 to 0.830 (in Prot2).