Supporting Information: An intermediate conformational state of cytochrome P450cam-CN in complex with putidaredoxin

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Supporting Information Available

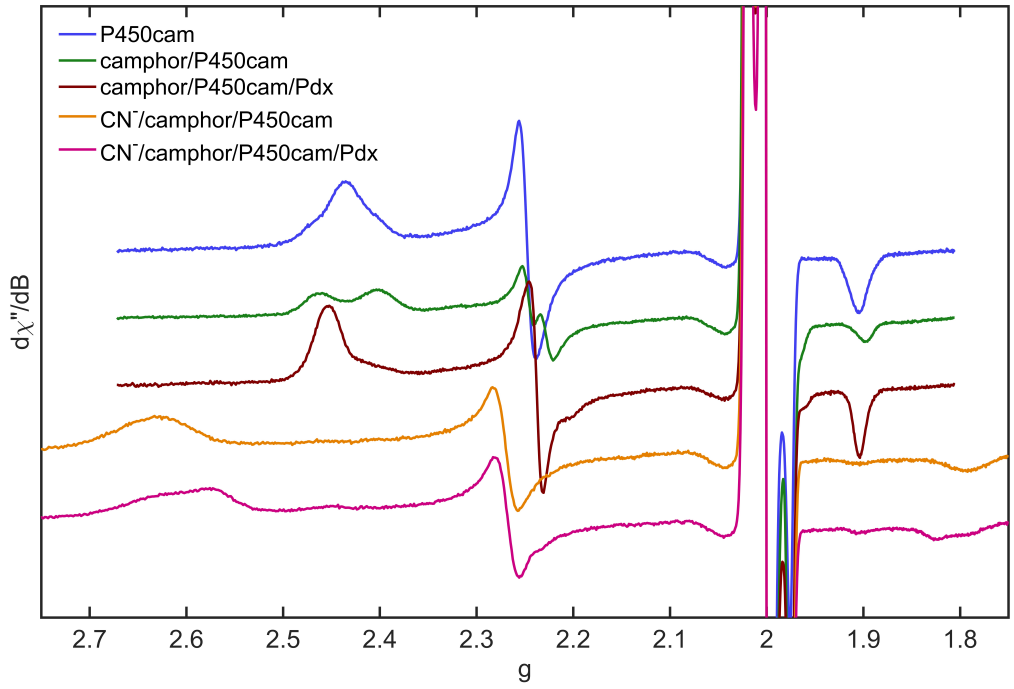

Figure S1: CW X-band EPR spectra in the low-spin region for 4MT-labeled ferric P450cam in different states, P450cam (blue), camphor/P450cam (green), camphor/P450cam/Pdx (brown), CN^-/camphor/P450cam (orange), and CN^-/camphor/P450cam/Pdx (magenta) complexes, at 50K and 0.2 mW microwave power.

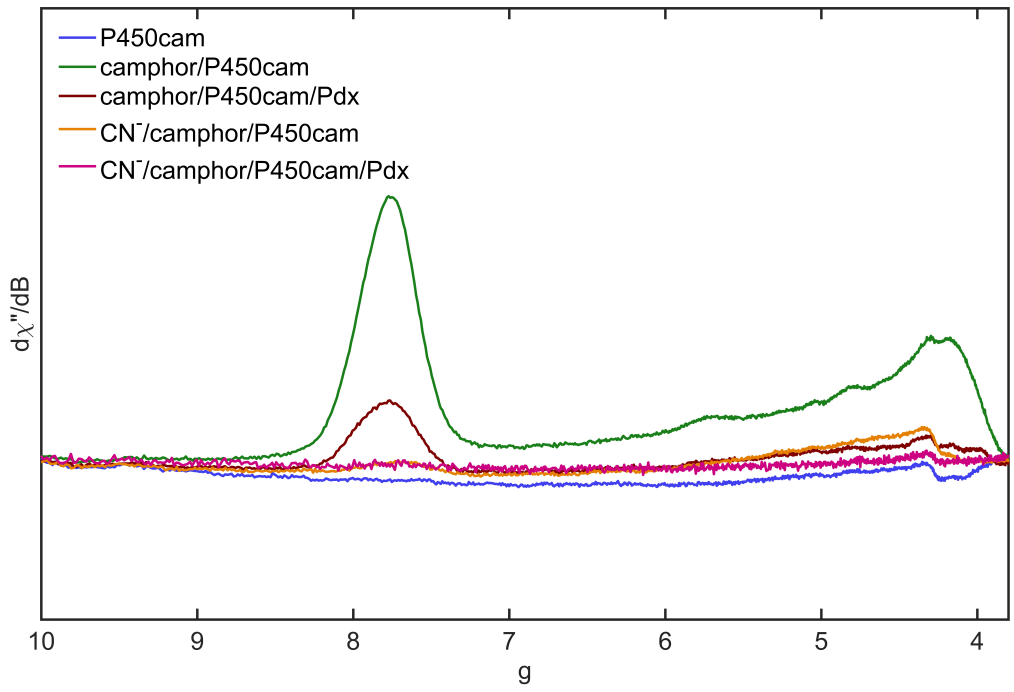

Figure S2: CW X-band EPR spectra in the high-spin region for 4MT-labeled ferric P450cam in different states, P450cam (blue), camphor/P450cam (green), camphor/P450cam/Pdx (brown), CN^-/camphor/P450cam (orange), and CN^-/camphor/P450cam/Pdx (magenta) complexes, at 15K and 2.0 mW microwave power.

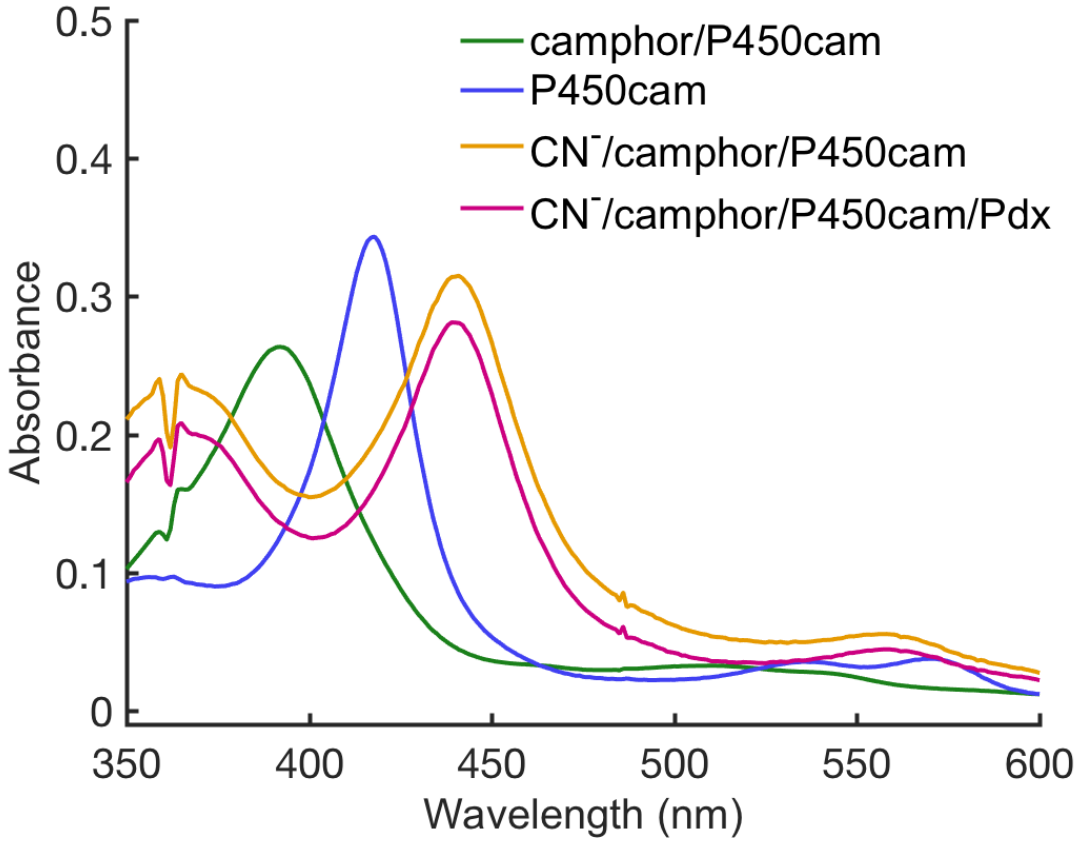

Figure S3: UV/Vis spectra at 25 °C of 4MT-labeled ferric P450cam for camphor/P450cam (green), P450cam (blue), CN^-/camphor/P450cam (orange), and CN^-/camphor/P450cam/Pdx (magenta) complexes.

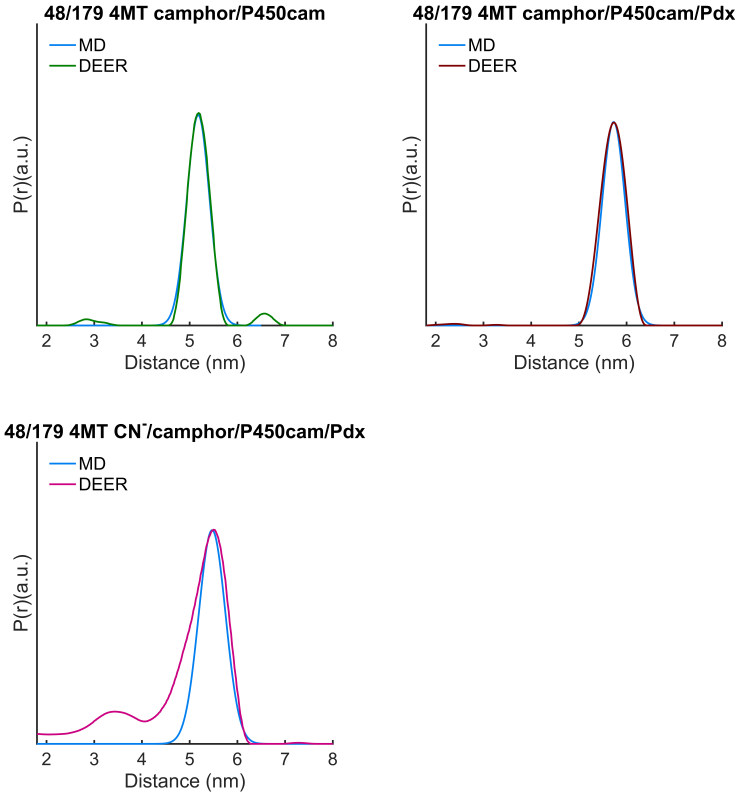

Figure S4: Comparison of distance distributions predicted by MD (blue) with the observed DEER distributions for spin-labeled forms of camphor/P450cam (green), camphor/P450cam/Pdx (brown), and CN^-/camphor/P450cam/Pdx (magenta) complexes. The MD distance was calculated between the nitroxide nitrogen atoms of the 4MT labels.

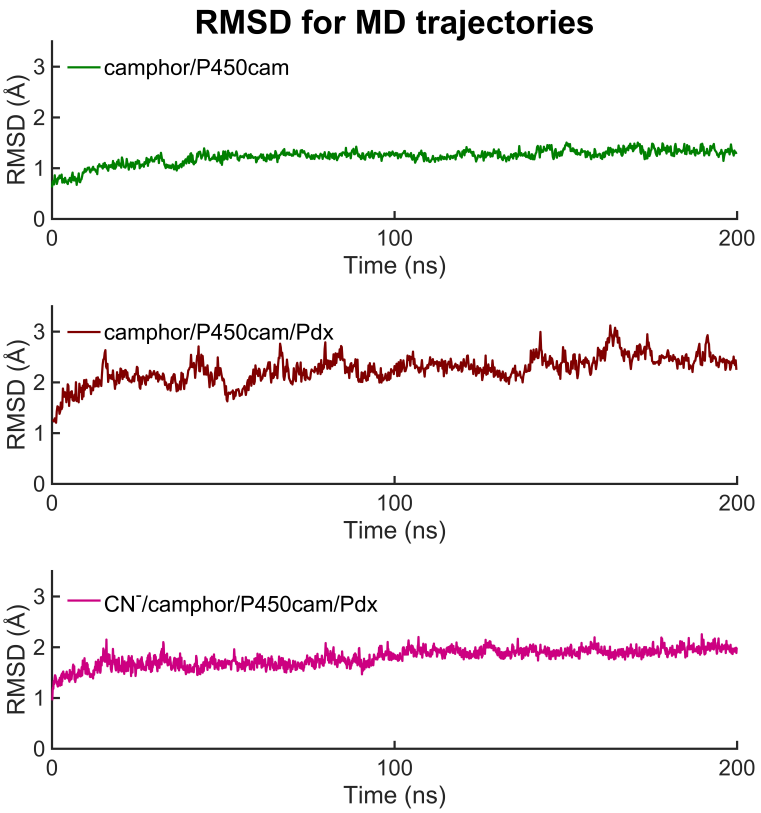

Figure S5: RMSD for 200ns MD trajectories of ferric camphor/P450cam (green), camphor/P450cam/Pdx (brown), and CN^-/camphor/P450cam/Pdx (magenta) complexes.

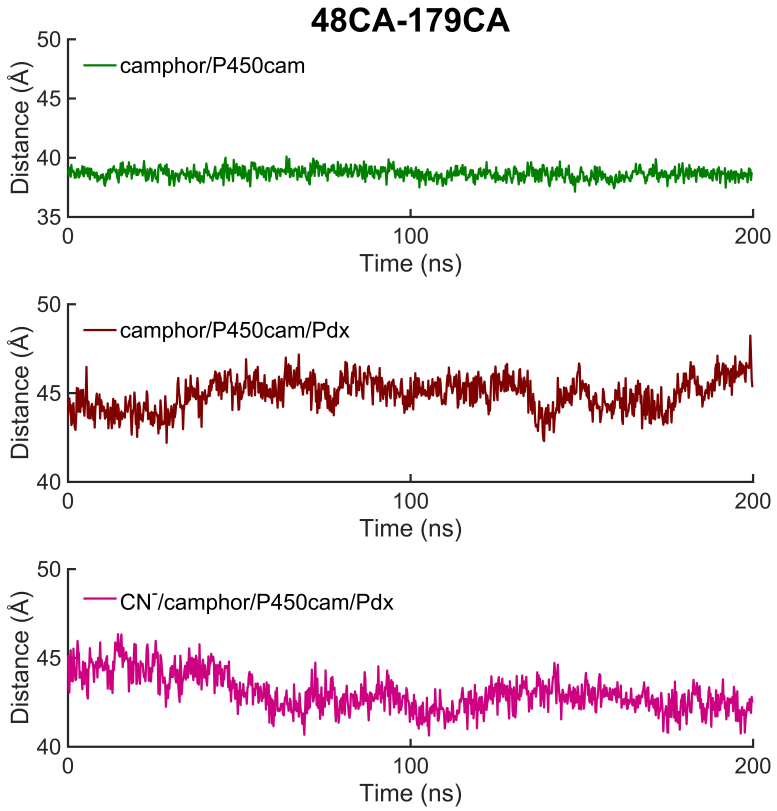

Figure S6: Time evolution from MD trajectories for the distance between the α carbons of the 4MT labeled residues, Ser48 and Tyr179, for camphor/P450cam (green), camphor/P450cam/Pdx (brown), and CN^-/camphor/P450cam/Pdx (magenta) complexes.

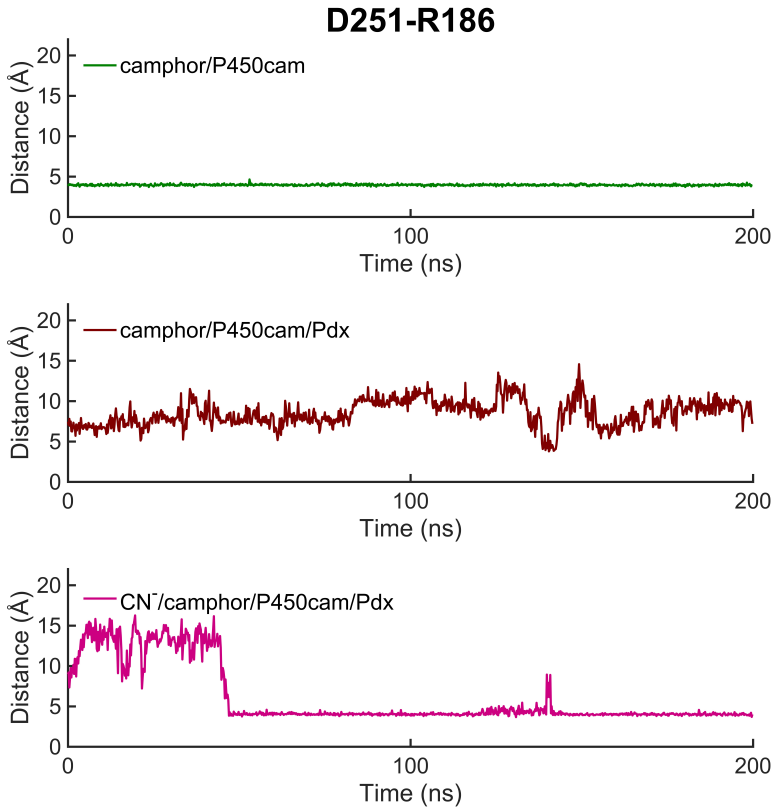

Figure S7: Time evolution from MD trajectories for the distance between Asp251 CG and Arg186 NZ for camphor/P450cam (green), camphor/P450cam/Pdx (brown), and CN^-/camphor/P450cam/Pdx (magenta) complexes.

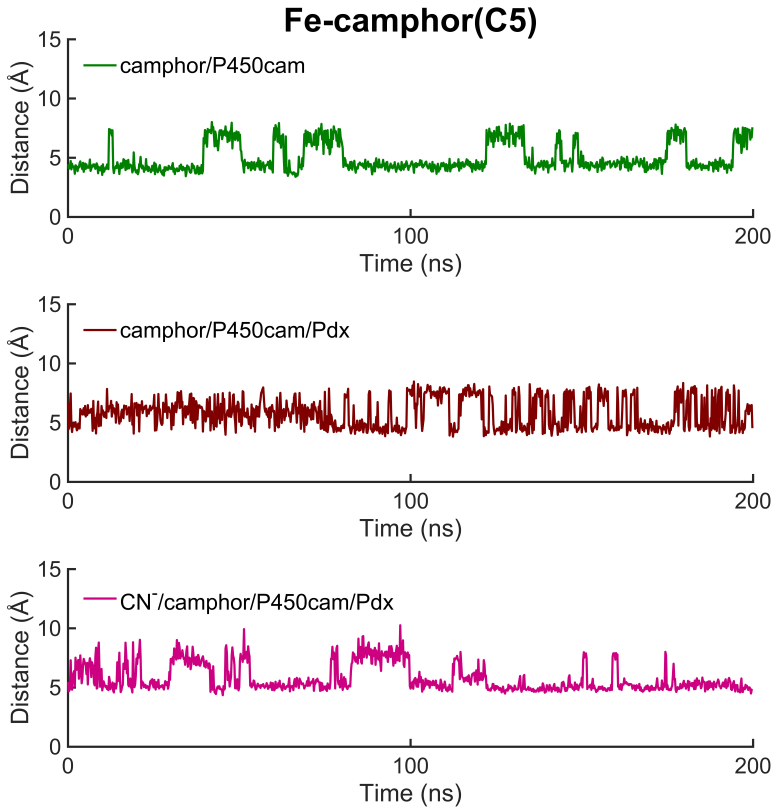

Figure S8: Time evolution from MD trajectories for the distance between heme iron and camphor C5 for camphor/P450cam (green), camphor/P450cam/Pdx (brown), and CN^-/camphor/P450cam/Pdx (magenta) complexes.

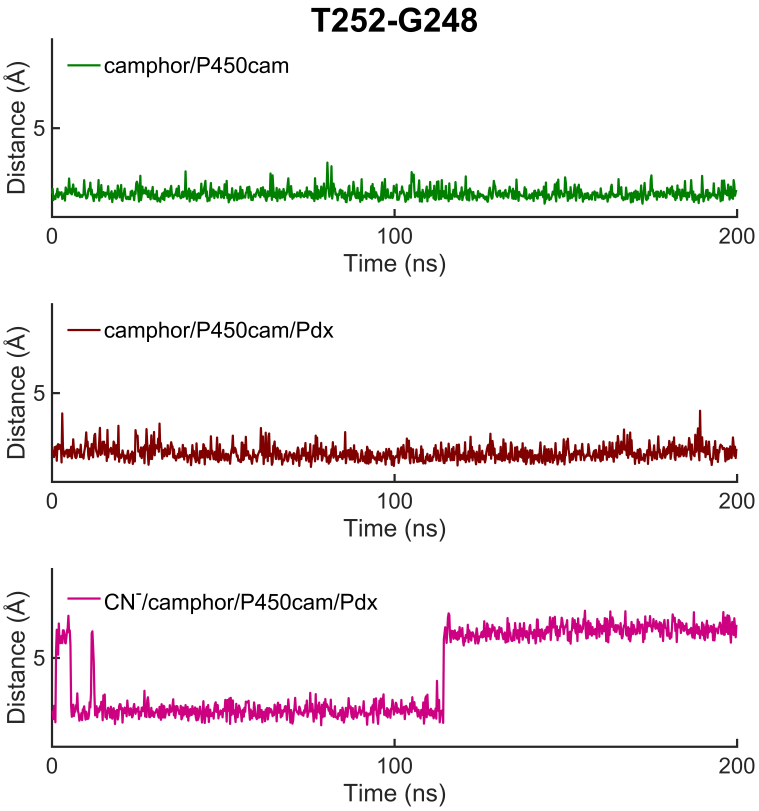

Figure S9: Time evolution from MD trajectories for the distance between the hydroxyl oxygen atom of Thr252 and the carbonyl oxygen atom of Gly248 for camphor/P450cam (green), camphor/P450cam/Pdx (brown), and CN^-/camphor/P450cam/Pdx (magenta) complexes.

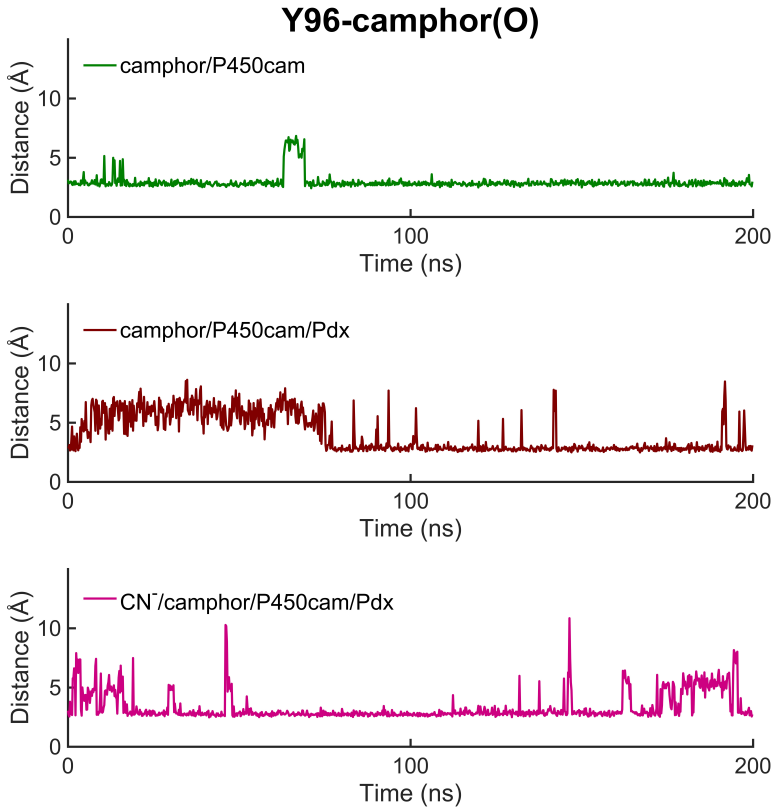

Figure S10: Time evolution from MD trajectories for the distance between the hydroxyl oxygen atom of Tyr97 and the carbonyl oxygen atom of camphor for camphor/P450cam (green), camphor/P450cam/Pdx (brown), and CN^-/camphor/P450cam/Pdx (magenta) complexes.

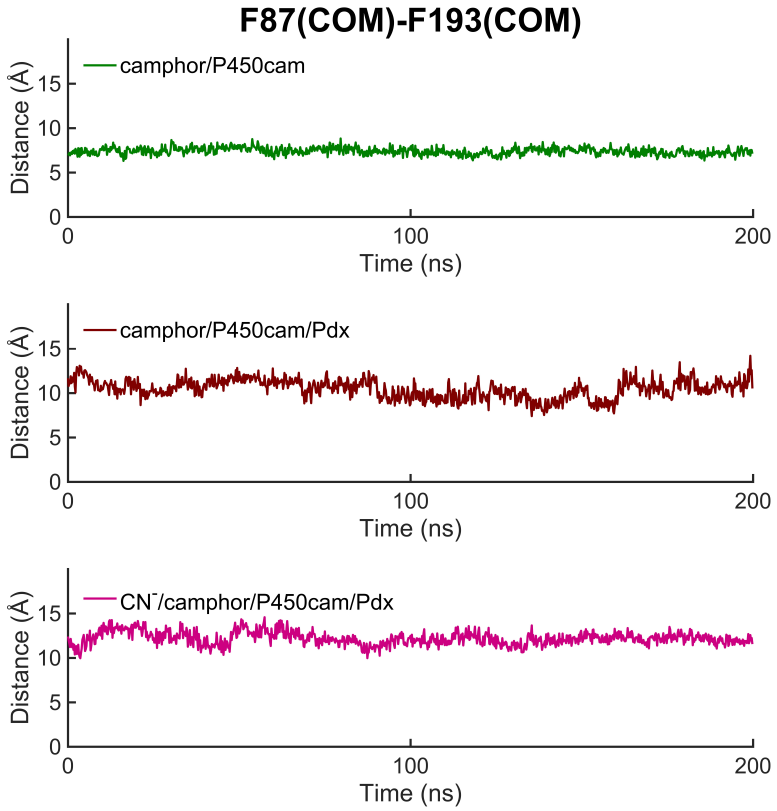

Figure S11: Time evolution from MD trajectories for the distance between the center of mass of Phe87 and Phe193 for camphor/P450cam (green), camphor/P450cam/Pdx (brown), and CN^-/camphor/P450cam/Pdx (magenta) complexes.

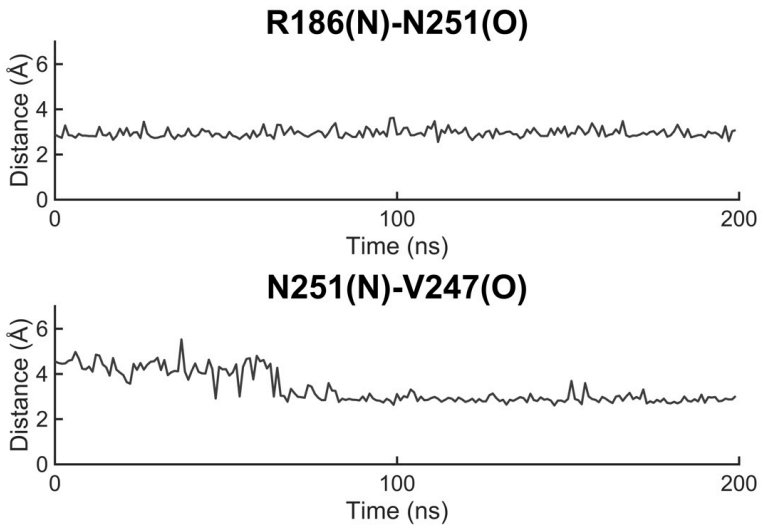

Figure S12: Time evolution from MD trajectories for the distance between Arg186 NH1 and Asn251 OD (top), and for the interaction between Val247 O and Asn251 ND2 (bottom) for CN^-/camphor/P450cam(D251N)/Pdx. For the first 80ns, Asn251 interacts only with Arg186. After 80ns, the Asn251 makes an additional interaction with Val247.

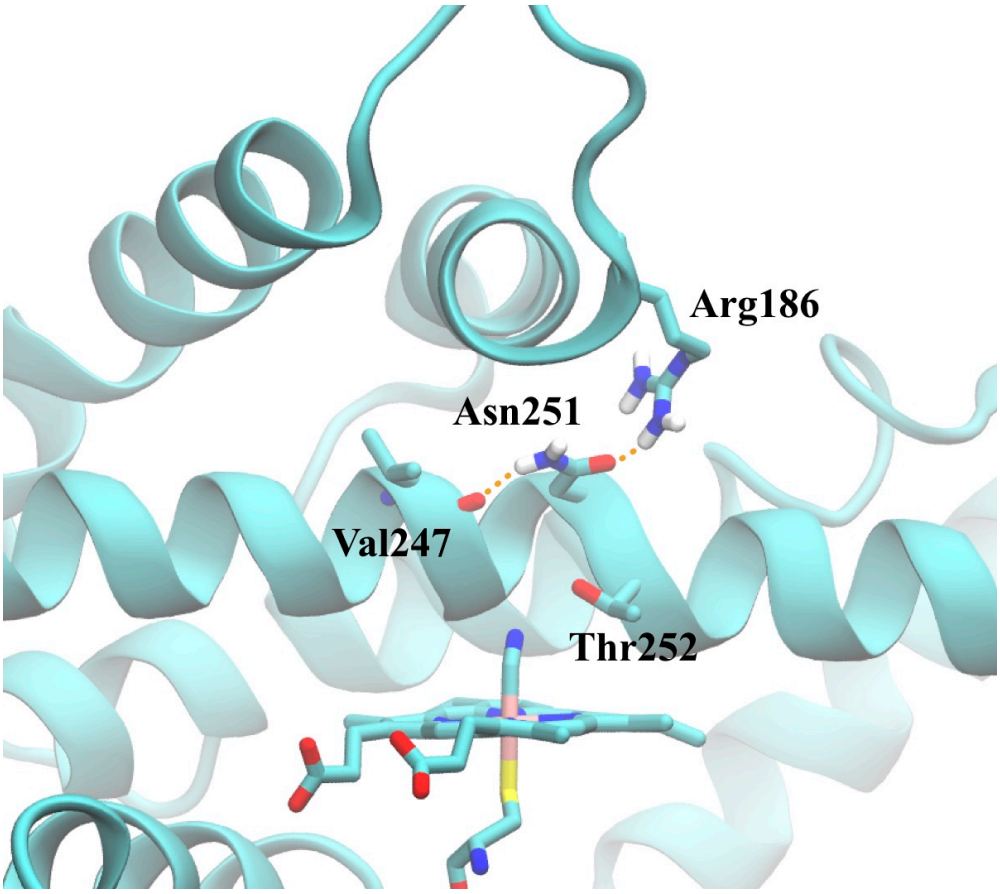

Figure S13: MD snapshot for the CN^-/camphor/P450cam(D251N)/Pdx complex corresponding to the alternative rotamer indicated in Figure S12.

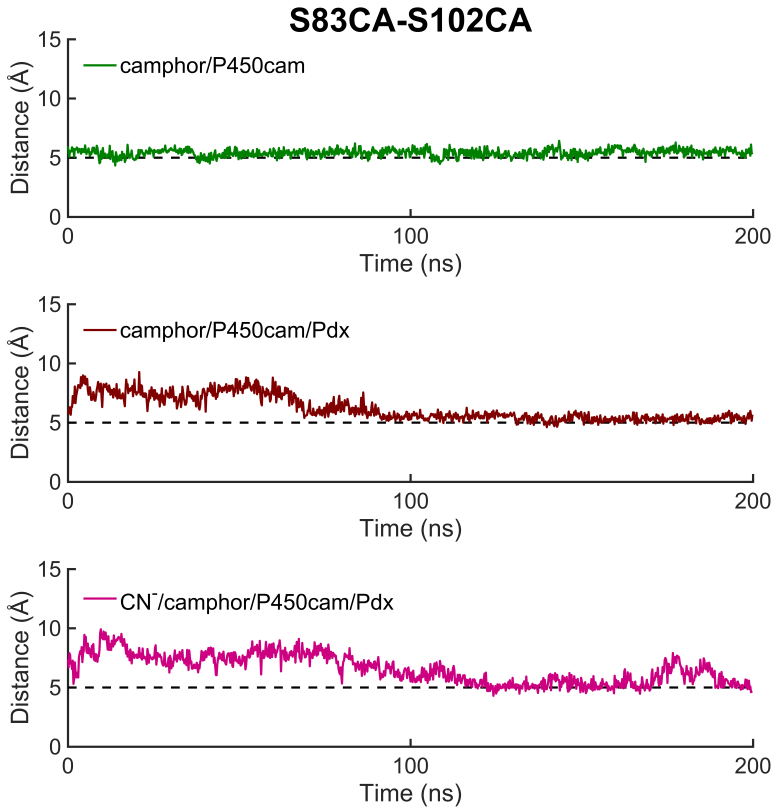

Figure S14: Time evolution from MD trajectories for the distance between Ser83 CA and Ser102 CA for camphor/P450cam (green), camphor/P450cam/Pdx (brown), and CN^-/camphor/P450cam/Pdx (magenta) complexes.

Table S1: NADH consumption rate of P450cam.

Enzyme	NADH consumption rate (μM/s/μM P450cam)
P450cam	9.96± 0.57
4MT-labeled P450cam(C48S, Y179S)	8.36± 0.38
4MT-labeled P450cam(C48S, Y179S, D251N)	0.02± 0.01

P450cam is cytochrome P450 from *Pseudomonas Putida* containing the mutations C334A, C58S, C85S, C136S, C285S.