

Active-Site Hydration and Water Diffusion in Cytochrome P450cam: A Highly Dynamic Process

Yinglong Miao and Jerome Baudry

Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee-Knoxville, Knoxville, Tennessee; and University of Tennessee-Oak Ridge National Laboratory Center for Molecular Biophysics, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Supporting Information

For “Active site hydration and water diffusion in cytochrome P450cam: a highly dynamic process.” by Y. Miao and J. Baudry.

Fig. S1 (a) Superimposed X-ray crystal structures of apo-P450 with six-coordinate low-spin heme iron (blue) and cam-P450 with five-coordinate high-spin heme iron (red). The nine N-terminal residues missing in crystal structures are shown in yellow. (b) K^+ binding site: backbone carbonyl oxygens of Glu84, Gly93, Glu94 and Tyr96, (c) residues with nonstandard protonation states, and (d) schematic representation of MD simulation model showing protein solvated in a host medium of 0.1M KCl.

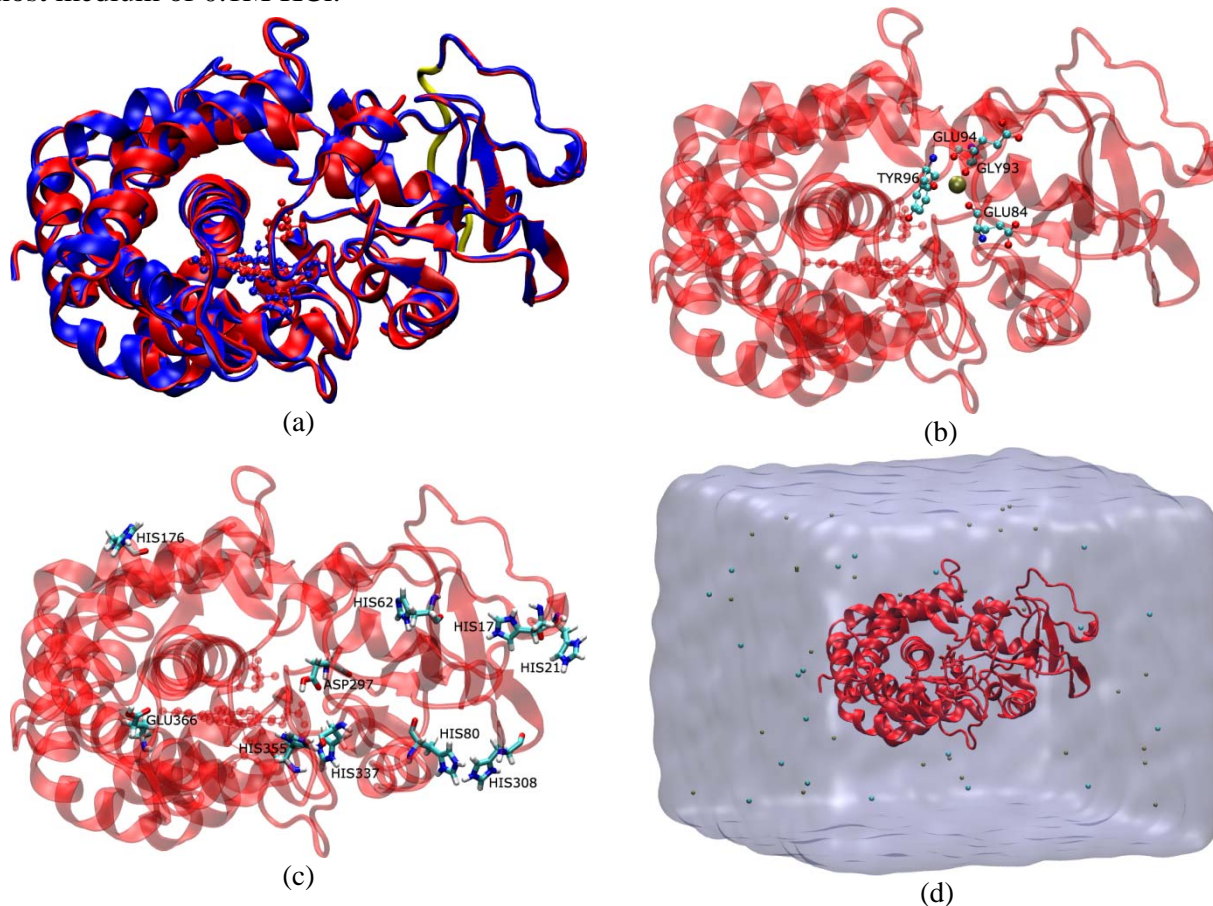
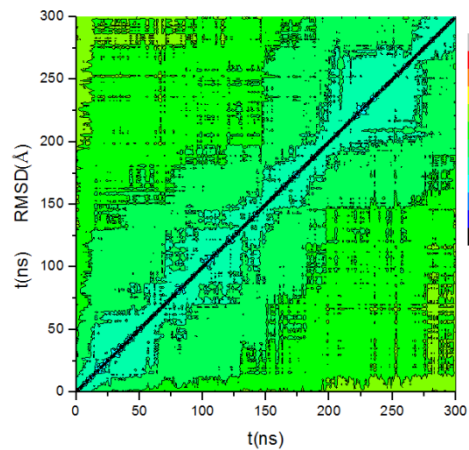
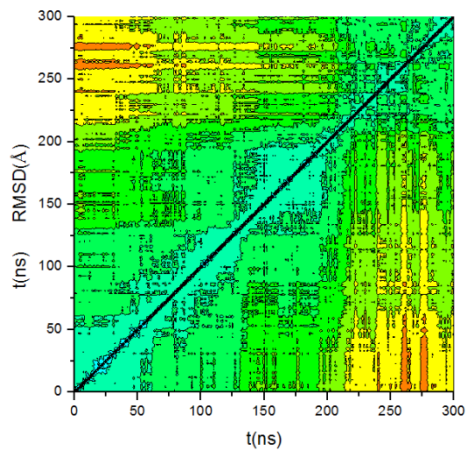


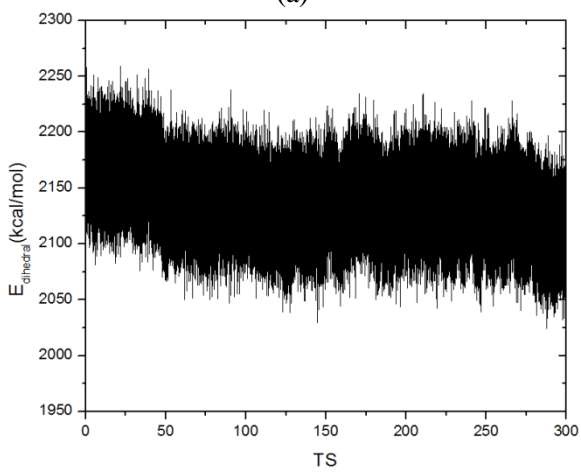
Fig. S2 RMSD between every two trajectory snapshots during MD simulation of (a) apo-P450 and (b) cam-P450. The system dihedral energies of (c) apo-P450 and (d) cam-P450 converge after ~ 100 ns.



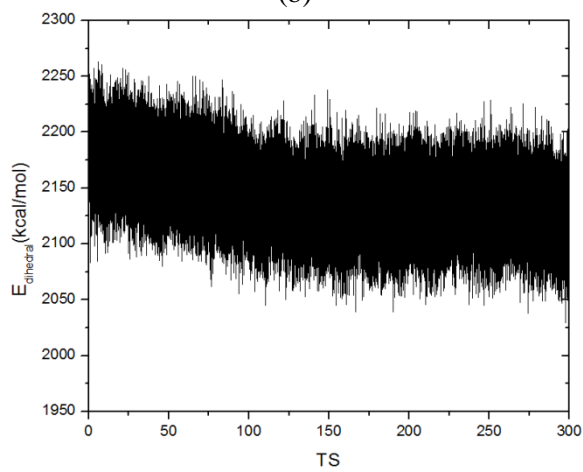
(a)



(b)



(c)



(d)

Fig. S3 Water molecules in the apo-P450 crystal structure within 5 Å of the camphor molecule (as located in the camphor-bound crystal structure) are indicated in blue spheres. Additional water molecules located further away from the binding site but within 13 Å of the heme's iron atoms are displayed in orange (apo-P450) and green (camphor bound).

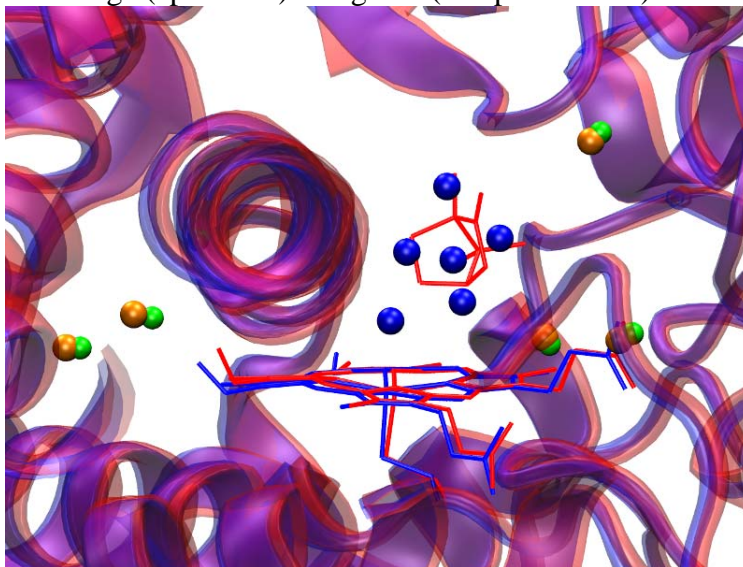
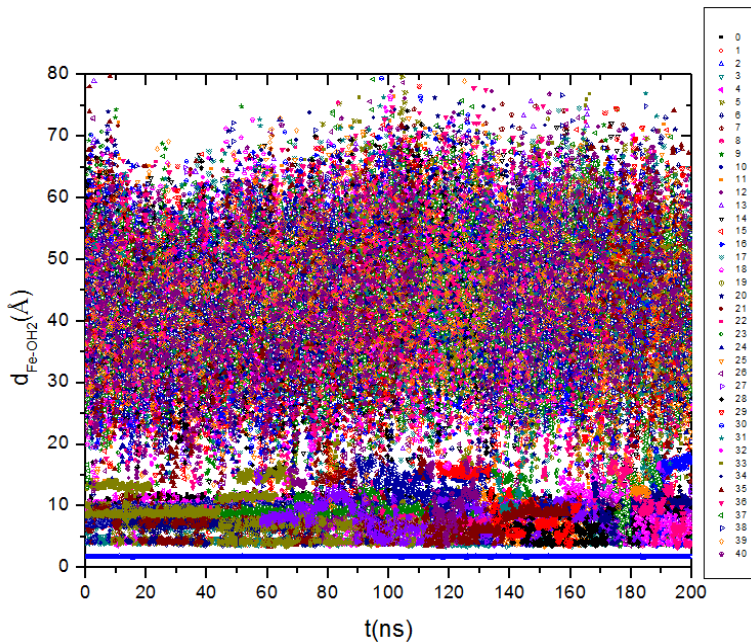
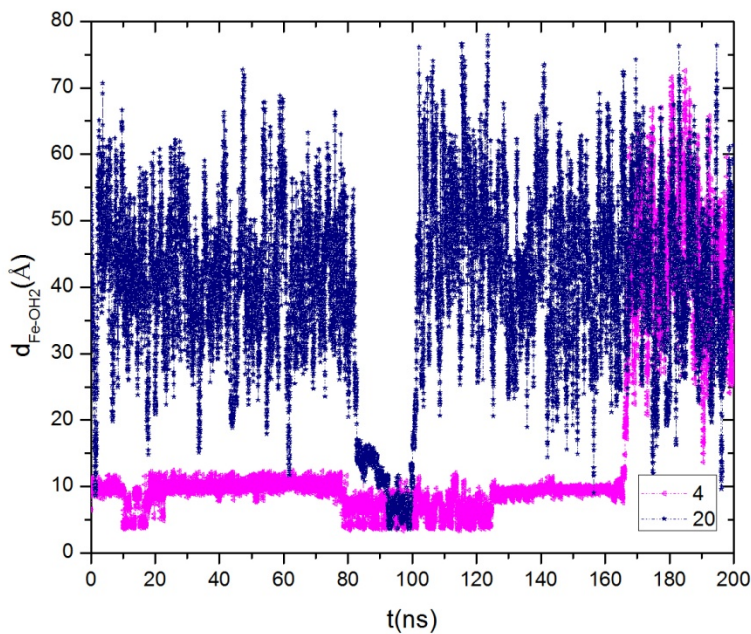


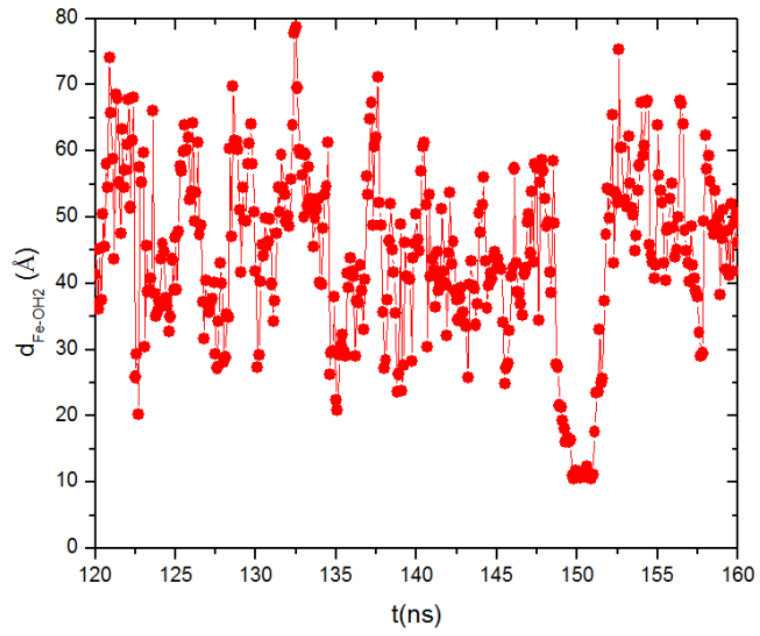
Fig. S4 (a) Time series of the distances between heme's iron and the 41 water molecules that appear in protein active site cavity and 36 of which diffuse through protein channels in the simulation of apo-P450. (b) Among these 36 water molecules, wat4 diffuses fast through channel "2e" during 163.54-166.36 ns (i.e., 2.82 ns) while wat35 diffuses comparatively slower through channel "2d" during 81.92-91.00 ns (i.e., 9.08 ns). (c) Distance between heme's iron and the only water molecule that was observed to diffuse into and out of the distal region of protein active site cavity at ~150 ns in the simulation of cam-P450 and (d) trajectory of this water molecule visualized to diffuse through channel "2a" in the protein.



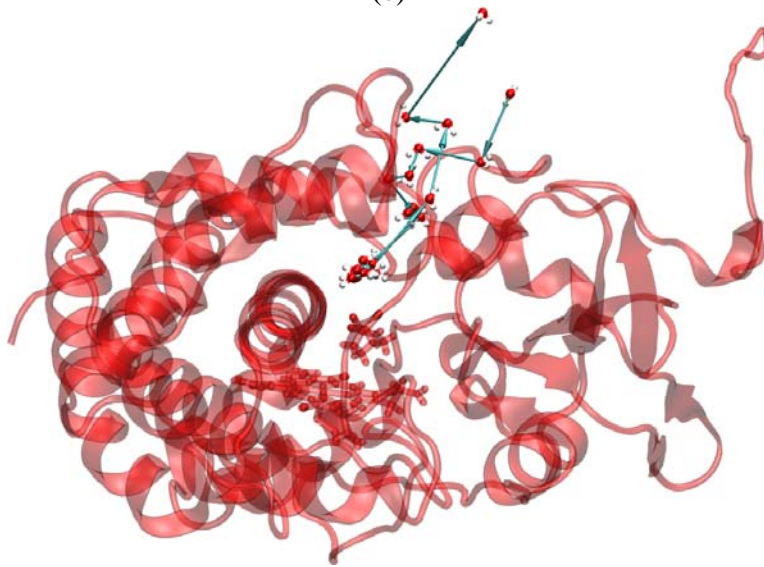
(a)



(b)



(c)



(d)

Fig. S5 (a) Root mean square deviation (RMSD) of atomic positions in camphor between MD trajectory snapshots and the crystal structure. Orientation of camphor with respect to referential residues Tyr96, Thr252, and Gly248 in the protein active site in (b): X-ray crystal configuration, (c): at 50ns (RMSD=3.86Å) and (d): (RMSD=2.39Å).

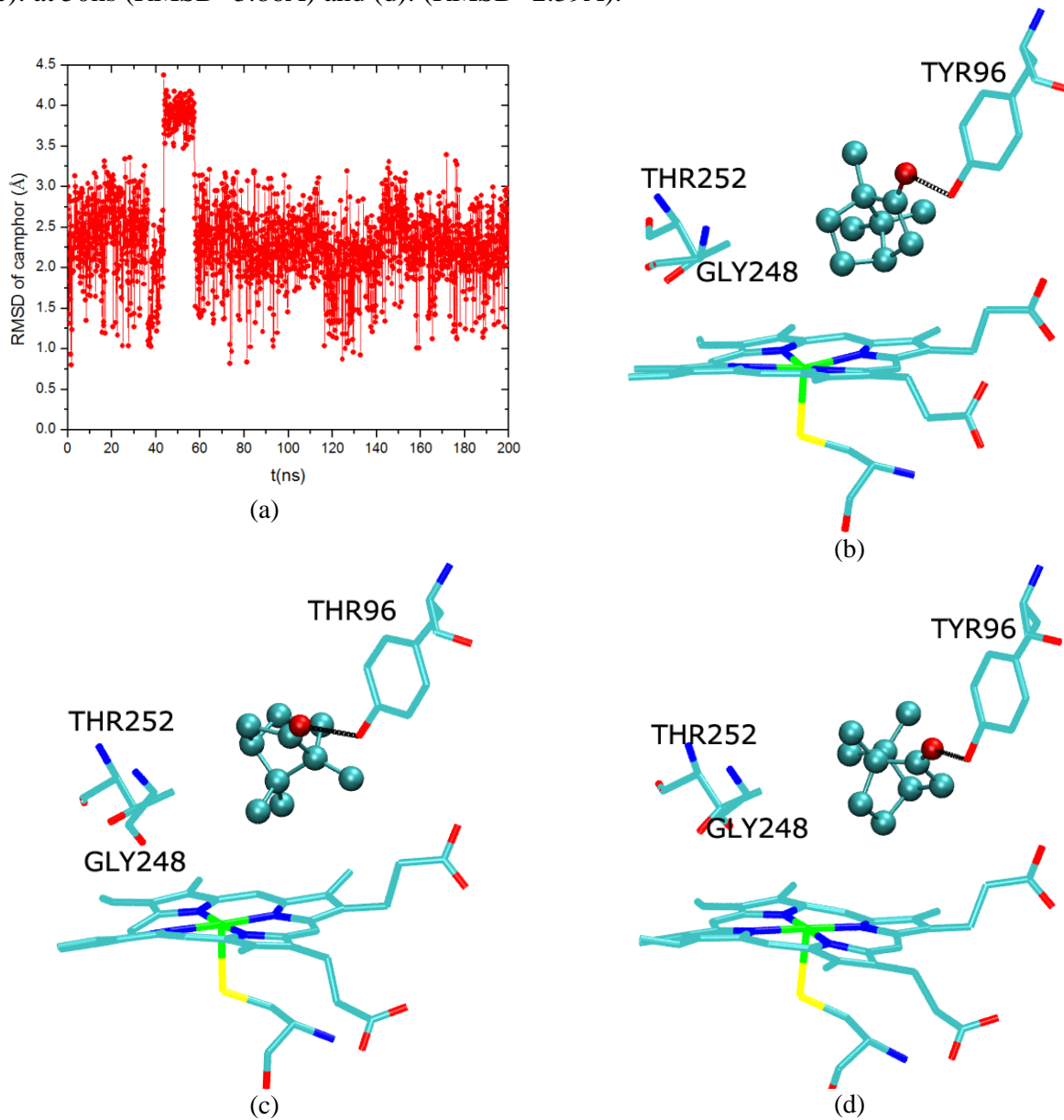
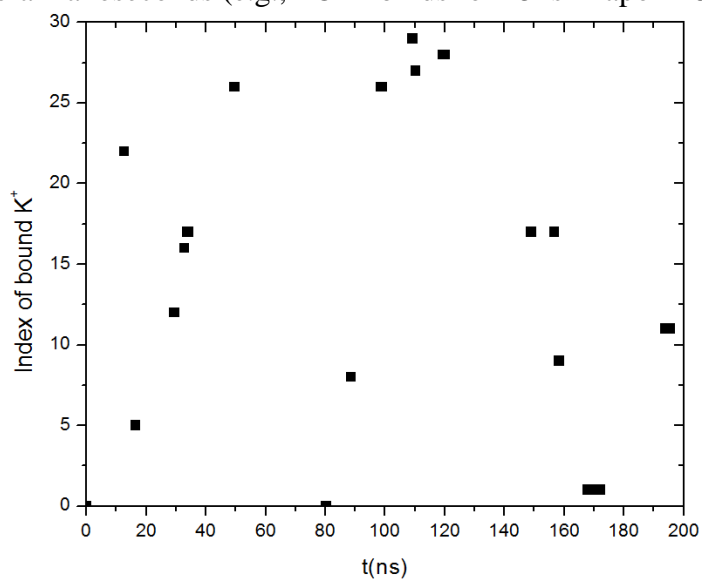
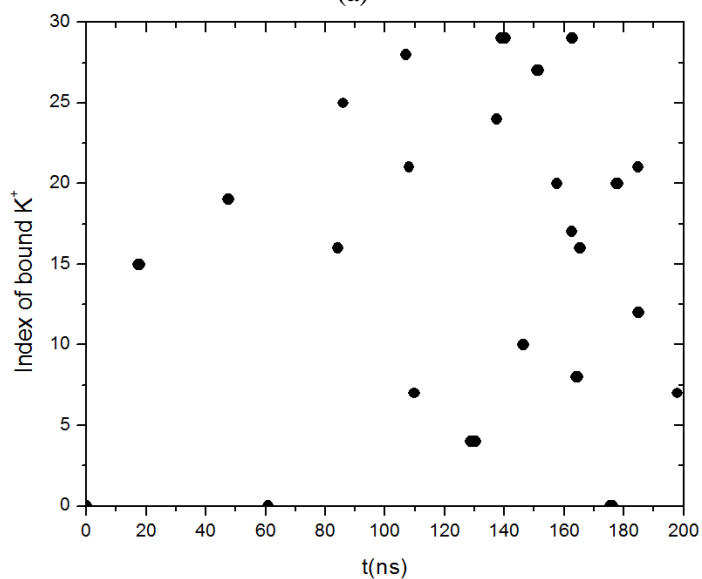


Fig. S6 Occupancy of K^+ ions found within 3 angstroms of the K-binding site in (a): apo-p450 (a total of 14 K^+ ions) and (b): cam-P450 (17 K^+ ions) during the 100-300ns MD simulation. K^+ ions were observed to diffuse out and into the K^+ binding site in both simulations with a few of them binding for several nanoseconds (e.g., POT1 binds for ~5ns in apo-P450).



(a)



(b)

Fig. S7 Free energy isosurfaces of protein hydration in the camphor-free MD trajectory.

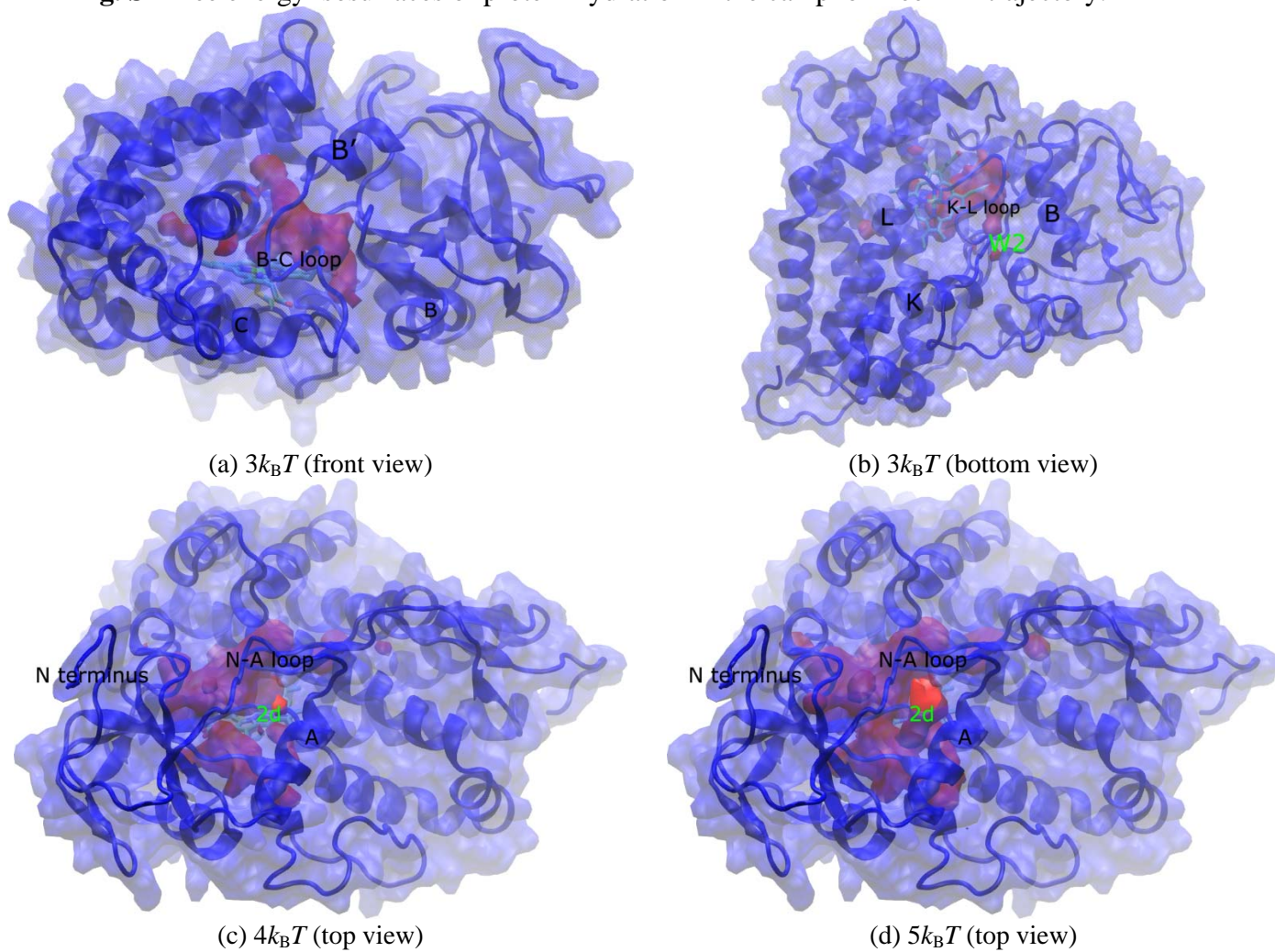
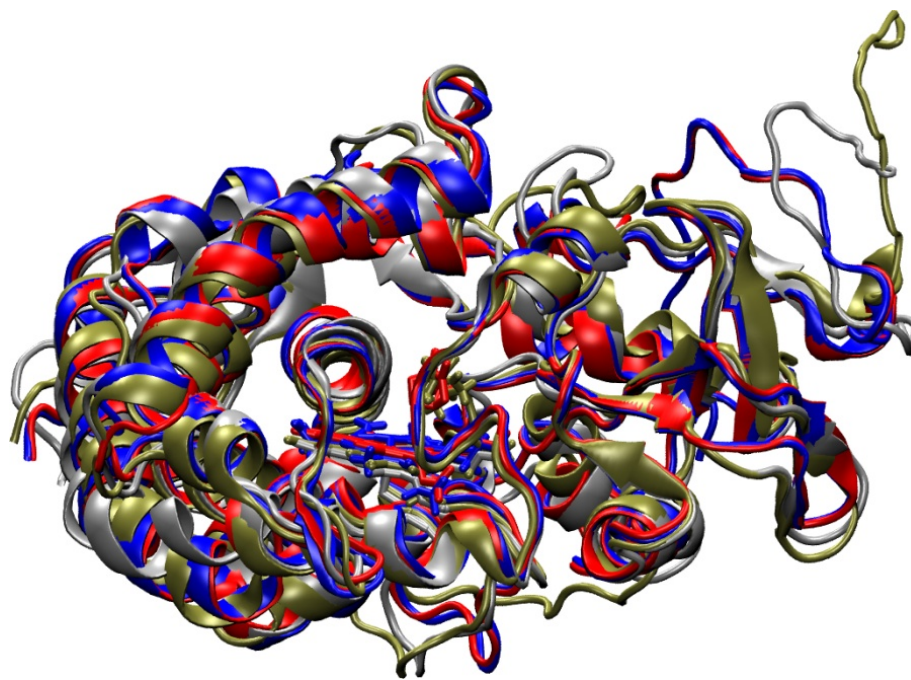
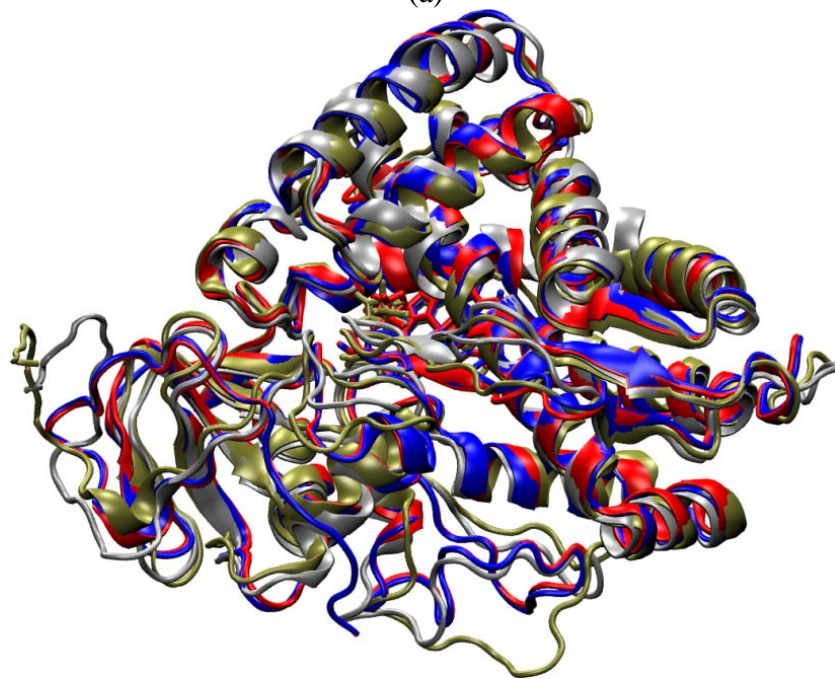


Fig. S8 Average structures of apo-P450 (silver) and cam-P450 (tan) calculated from 100-300 ns MD trajectories, and X-ray crystal structures (blue for apo-P450 and red for cam-P450): (a) front view looking down the I helix and (b) top view looking down on the heme plane from the distal side.

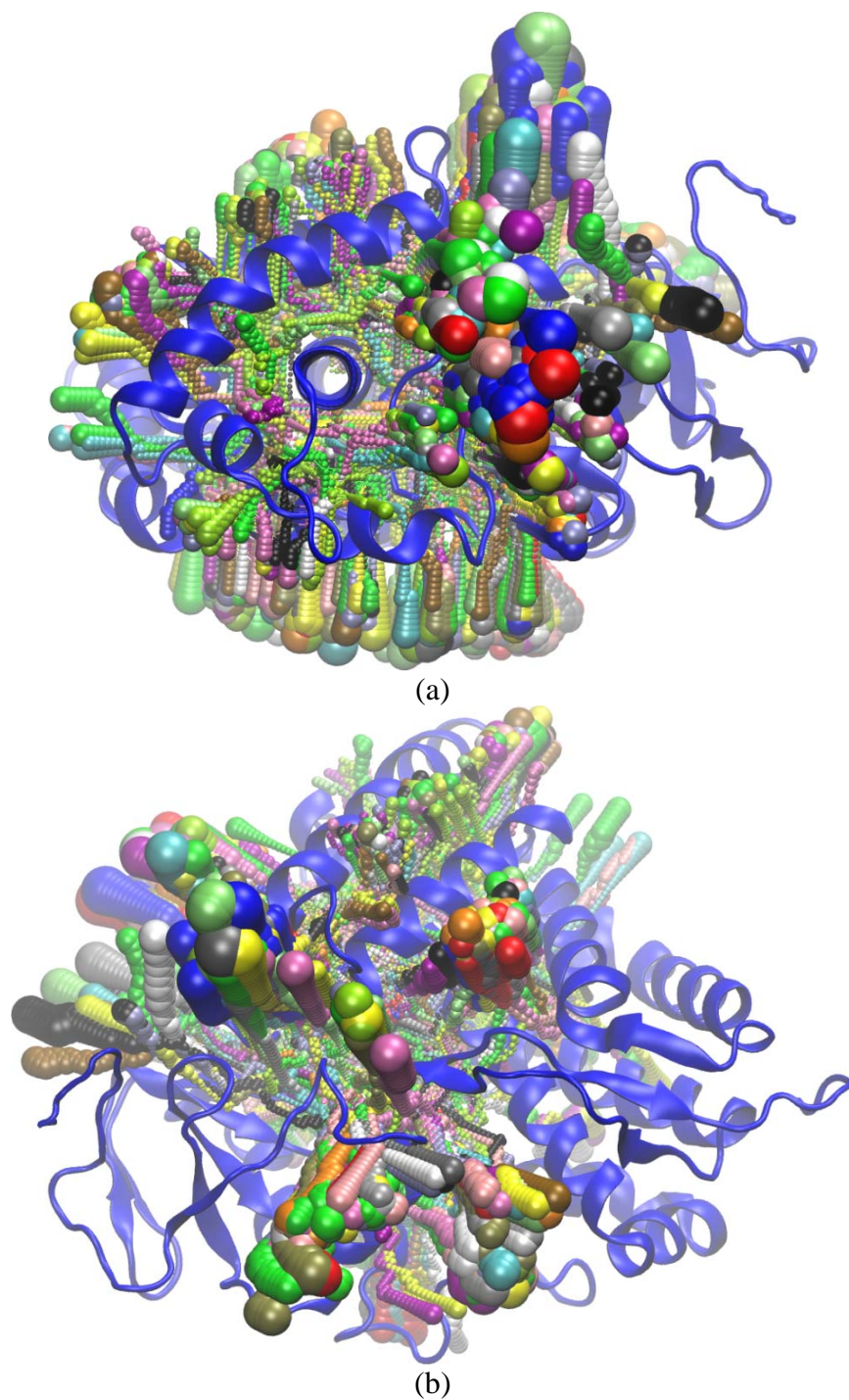


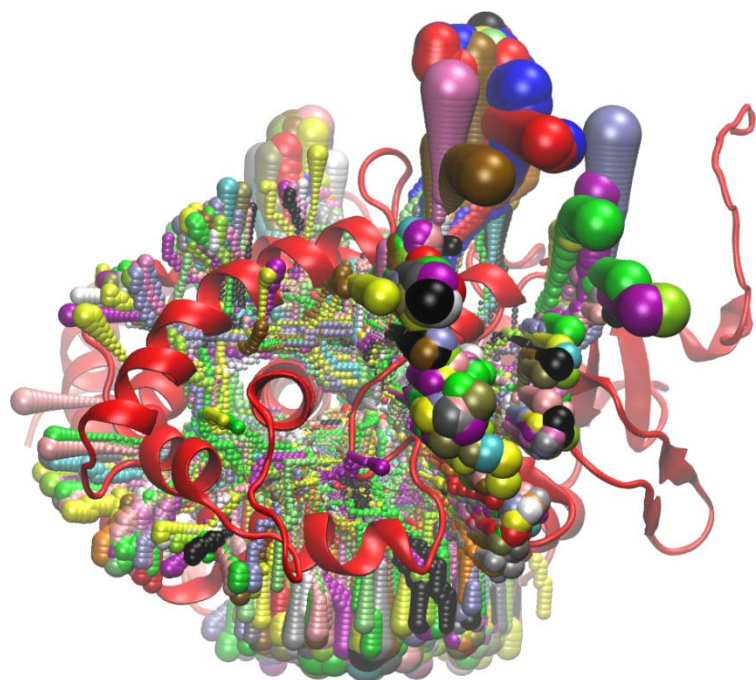
(a)



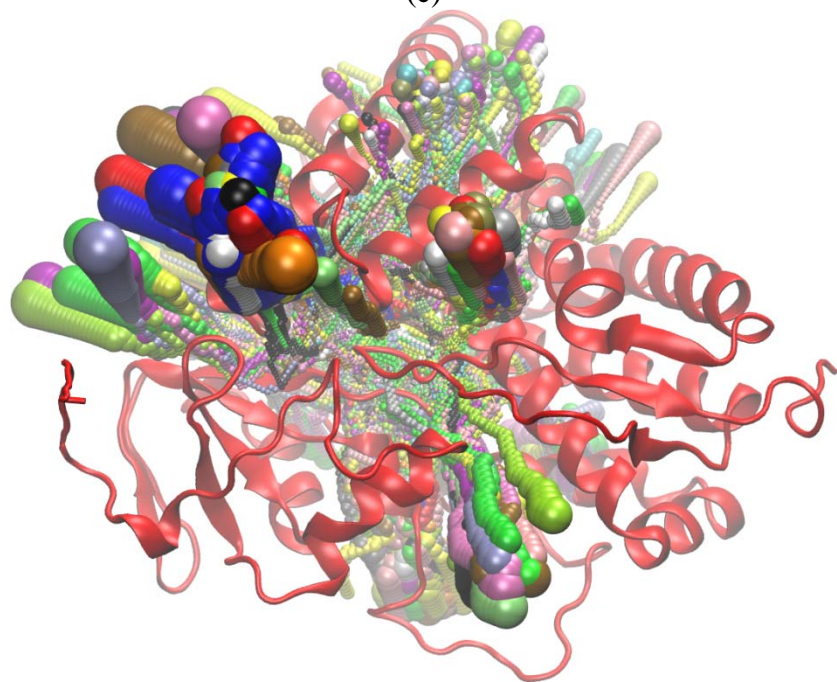
(b)

Fig. S9 CAVAR output channels calculated from the 100-300 ns MD trajectory of P450cam: (a) front view looking down the I helix and (b) top view looking down on the heme plane from the distal side of apo-P450, and similarly (c) front view and (d) top view for calculation in cam-P450.



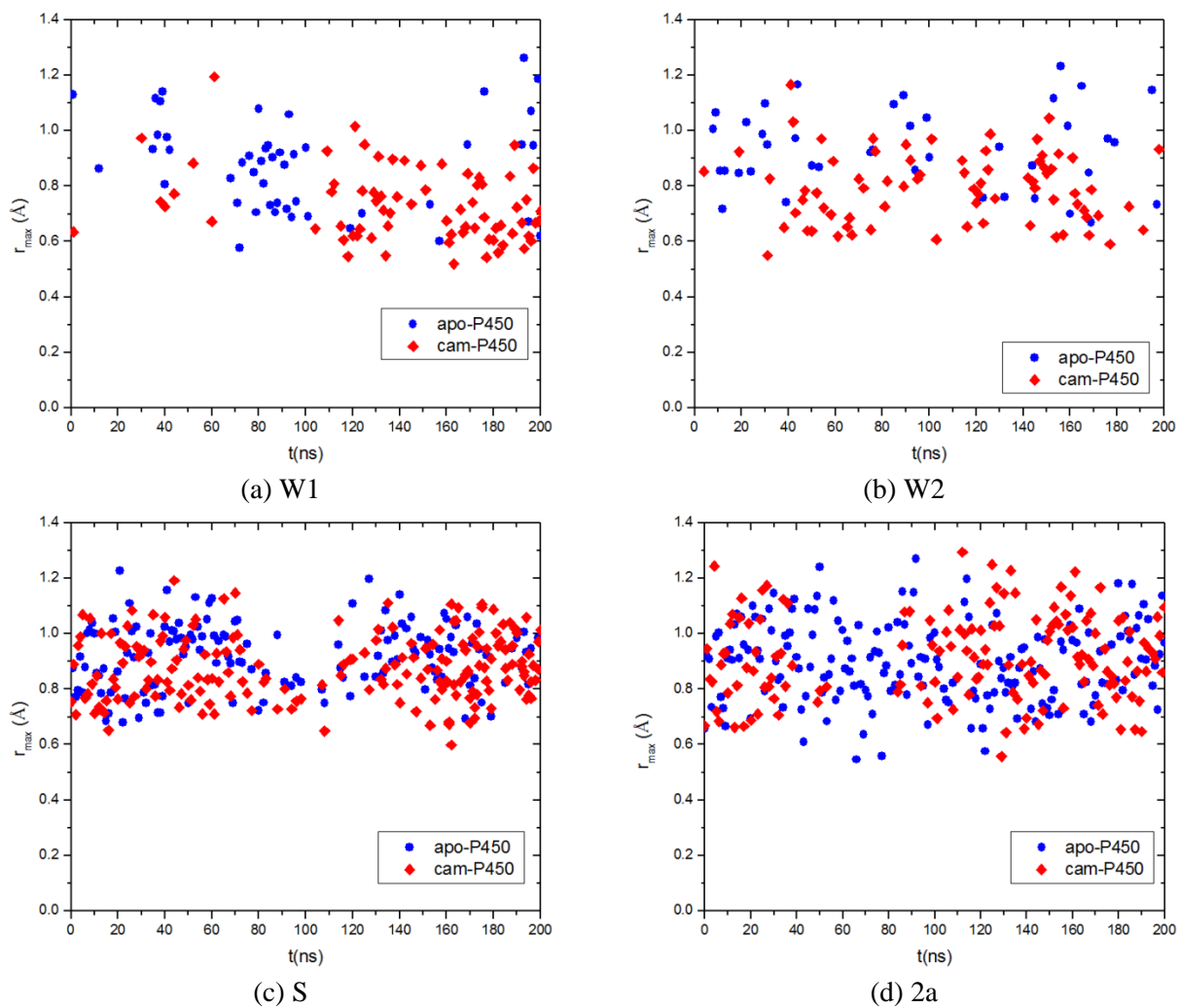


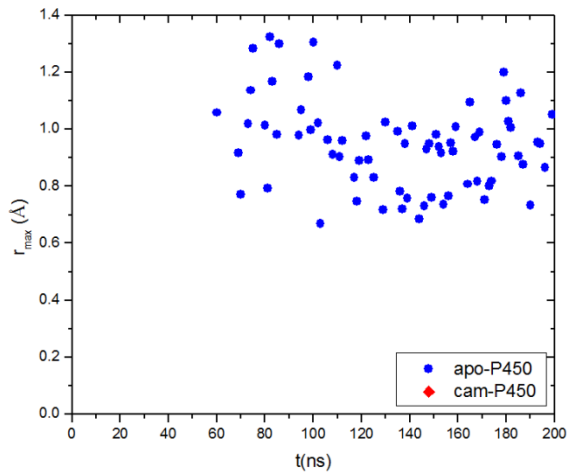
(c)



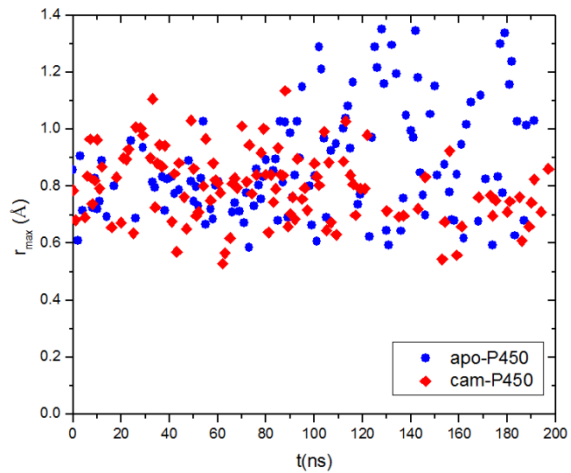
(d)

Fig. S10 Time series of channels' r_{\max} for apo P450cam (blue dots) and camphor-bound P450cam (red diamonds) for channels (a): "W1", (b): "W2", (c): "S", (d): "2a", (e): "2d", (f): "2e", (g): "2f" and (h): "Pdx2". Channels "2d" and "2f" do not appear in the camphor-bound P450cam simulation.

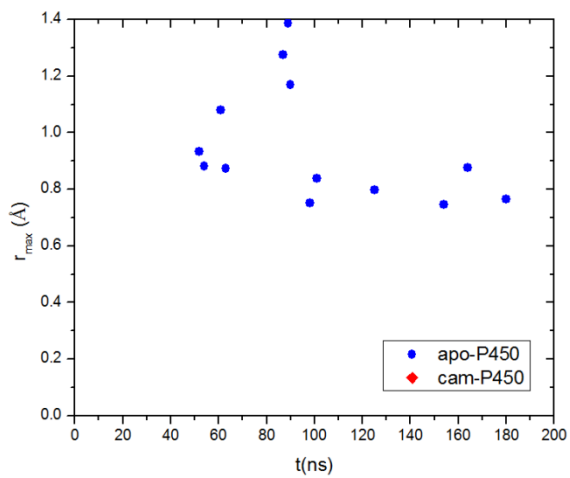




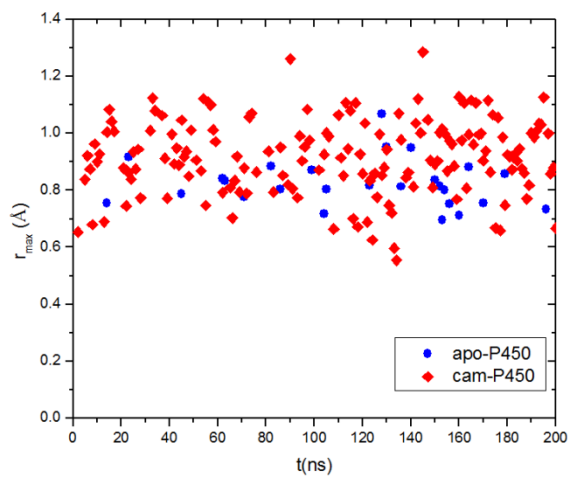
(e) 2d



(f) 2e



(g) 2f



(h) Pdx2

Fig. S11 (a): Distribution of the 41 unique water molecules that were identified to appear in the protein active site cavity in apo-P450 simulation and CAVER channels, (b): same, for the cam-P450 simulation. The channel spheres are represented with half of their radii for clarity and camphor is shown in vdW spheres.

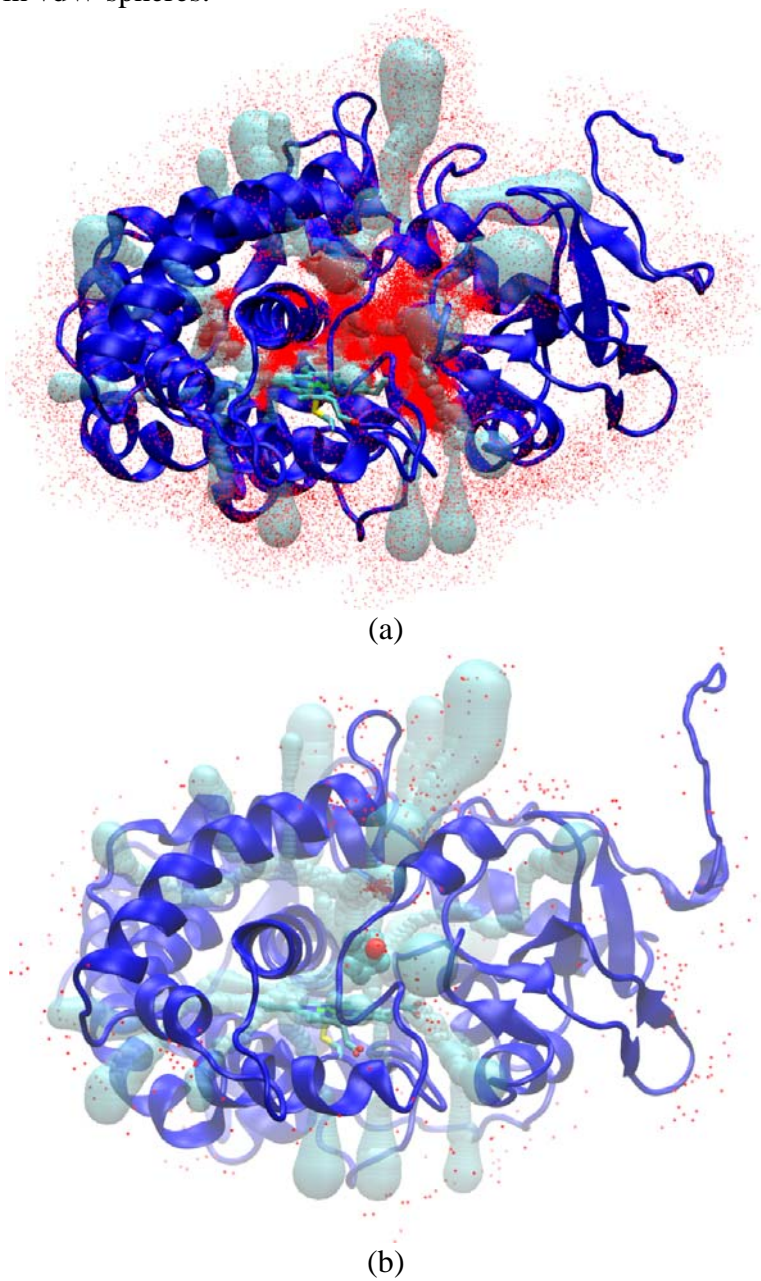


Table S1 Residues lining channel “W1” in (a) apo-P450 and (b) cam-P450 listed according to their corresponding scaled reaction coordinate along the channel with bin=1.5Å.

RC	residue-list
0	TYR96 VAL295 HEM417
0.04	TYR96 THR101 LEU244 HEM417
0.09	PHE87 TYR96 THR101 ASP297 HEM417
0.13	PHE87 TYR96 THR101 VAL295 ASP297 HEM417
0.17	PRO86 PHE87 TYR96 ASP297 HEM417
0.22	PRO86 PHE87 TYR96 ASP297 HEM417
0.26	SER83 PRO86 TYR96 THR101 ARG299 HSP355
0.3	SER83 PRO86 TYR96 THR101 ARG299
0.35	SER83 PRO86 TYR96 THR101 ARG299
0.39	SER83 THR101 ARG299 HSP355
0.43	SER83 ARG299 HSP355 HEM417
0.48	SER82 SER83 ASP104 ARG299 HSP355
0.52	SER82 SER83 ASP104 ARG299 HSP355
0.57	ASP104 ARG299 SER354 HSP355
0.61	ASP77 TYR78 PHE81 SER82 ARG299 SER354
0.65	ASP77 TYR78 PHE81 SER82 ASP104
0.7	ASP77 TYR78 ARG79 PHE81 ASP104
0.74	TYR78 ASP104 PRO105 SER354
0.78	TYR78 ARG79 ASP104 PRO105
0.83	TYR78 ARG79 ASP104 PRO105 PRO106 GLU107
0.87	TYR78 ARG79 PRO105 PRO106 GLU107
0.91	TYR78 ARG79 PRO105 PRO106 GLU107
0.96	TYR78 ARG79 PRO106 GLU107
1	ARG79 PRO106 GLU107

(a) apo-P450

RC	residue-list
0	PHE87 TYR96 LEU244 GLY248 HEM417 CAM422
0.05	PHE87 TYR96 ASP297 ILE395 HEM417 CAM422
0.09	PRO86 PHE87 TYR96 LEU244 ASP297 ILE395 HEM417 CAM422
0.14	PRO86 PHE87 TYR96 THR101 ASP297 HEM417 CAM422
0.18	PRO86 TYR96 THR101 CAM422
0.23	SER83 PRO86 PHE87 TYR96 THR101 ARG299 HEM417
0.27	SER83 PRO86 THR101 ASP297 ARG299 HEM417 CAM422
0.32	SER83 PRO86 THR101 HSP355 HEM417
0.36	SER83 THR101 GLN108 ARG299 HSP355 HEM417
0.41	SER83 ARG299 HSP355 HEM417

0.45	SER83 GLN108 ARG299 SER354 HSP355 HEM417
0.5	SER82 SER83 ARG299 HSP355
0.55	PHE81 GLN108 ARG299 SER354 HSP355
0.59	TYR78 PHE81 SER83 ARG299 SER354 HSP355
0.64	TYR78 PHE81 SER82 SER83 ASP104 ARG299 SER354 HSP355
0.68	TYR78 ARG79 PHE81 SER82 SER83 ASP104 SER354 HSP355
0.73	TYR78 SER82 SER83 THR101 MET103 ASP104 PRO105 GLN108 SER354 HSP355 HEM417
0.77	TYR78 SER82 SER83 MET103 ASP104 PRO105 GLN108 SER354
0.82	TYR78 ARG79 MET103 ASP104 PRO105 PRO106 GLU107 GLN108 SER354
0.86	TYR78 ARG79 ASP104 PRO105 PRO106 GLU107 GLN108 ARG109 THR302
0.91	TYR78 ARG79 ASP104 PRO105 PRO106 GLU107 THR302
0.95	TYR78 ARG79 ASP104 PRO105 PRO106 GLU107 ARG109 THR302
1	TYR78 ARG79 PRO105 PRO106 GLU107 ARG109

(b) cam-P450

Table S2 Residues lining channel “W2” in (a) apo-P450 and (b) cam-P450 listed according to their corresponding scaled reaction coordinate along the channel.

RC	residue-list
0	VAL295 HEM417
0.06	PHE87 VAL295 ASP297 HEM417
0.11	VAL295 ASP297 HEM417
0.17	PHE87 VAL295 ASP297 HEM417
0.22	PHE87 VAL295 ASP297 GLN322 HEM417
0.28	TYR75 ASP297 GLN322 HEM417
0.33	ASP297 GLN322 HEM417
0.39	TYR75 ASP297 GLN322 GLY351 HEM417
0.44	TYR75 GLN322 HSE352
0.5	TYR75 GLN322 THR348 GLY351 HSE352
0.56	TYR75 GLN322 THR348
0.61	ILE71 ARG72 TYR75 GLN322 SER325 THR348 HSE352
0.67	ILE71 ARG72 TYR75 THR348 HSE352
0.72	ILE71 ARG72 TYR75 GLU76 SER325 THR348 HSE352
0.78	ARG72 TYR75 GLU76 SER346 THR348 HSE352
0.83	ARG72 GLU76 SER346 THR348 HSE352
0.89	ARG72 GLU76 SER346 THR348 HSE352
0.94	ARG72 GLU76 SER346 HSE352
1	GLU76 SER346

(a) apo-P450

RC	residue-list
0	PHE87 THR185 VAL295 ILE395 VAL396 HEM417 CAM422
0.05	PHE87 VAL295 ILE395 VAL396 HEM417 CAM422
0.1	VAL295 ASP297 ILE395 HEM417 CAM422
0.15	PHE87 VAL295 ASP297 GLN322 ILE395 HEM417 CAM422
0.2	VAL295 ASP297 HEM417 CAM422
0.25	TYR75 VAL295 ALA296 ASP297 GLN322 HEM417
0.3	TYR75 VAL295 ALA296 ASP297 GLN322 GLY351 HEM417
0.35	TYR75 ASP297 LEU320 GLN322
0.4	TYR75 VAL295 ALA296 ASP297 LEU320 GLN322
0.45	TYR75 VAL295 ALA296 GLN322 THR348 GLY351 HSE352
0.5	TYR75 VAL295 ALA296 GLN322 THR348 GLY351 HSE352
0.55	TYR75 LEU320 PRO321 GLN322 THR348
0.6	ILE71 ARG72 TYR75 LEU320 PRO321 GLN322 SER325 THR348 HSE352
0.65	ILE71 ARG72 TYR75 PRO321 GLN322 SER325 THR348 HSE352

0.7	ILE71 ARG72 TYR75 PRO321 GLN322 SER325 THR348 GLY351 HSE352
0.75	ARG72 GLN322 SER325 THR348 HSE352
0.8	ARG72 GLN322 SER325 GLY326 SER346 HSE347 THR348 HSE352
0.85	ARG72 SER325 GLY326 SER346 HSE347 THR348 HSE352
0.9	ARG72 ARG290 SER325 GLY326 LEU327 ASP328 ARG330 ASN332 SER346 HSE347 THR348 HSE352
0.95	ARG72 SER325 GLY326 LEU327 ASP328 GLU329 ARG330 GLU331 ASN332 SER346 HSE347
1	ARG72 ARG290 GLY326 LEU327 ASP328 GLU329 ARG330 GLU331 ASN332 LYS344 VAL345 SER346 HSE347

(b) cam-P450

Table S3 Residues lining channel “S” in (a) apo-P450 and (b) cam-P450 listed according to their corresponding scaled reaction coordinate along the channel.

RC	residue-list
0	TYR96 VAL247 VAL295 VAL396
0.05	PHE87 TYR96 VAL247 ILE395 VAL396
0.1	THR185 VAL247 ILE395 VAL396
0.15	THR185 VAL247 GLY248
0.2	THR185 VAL247
0.25	THR181 THR185 VAL247
0.3	THR181 THR185 ASP251 VAL396
0.35	THR181 ASP182 THR185 ARG186 ASP251 LYS392 VAL396
0.4	THR181 ASP182 GLN183 ARG186 ASP251
0.45	THR181 ASP251
0.5	LYS178 TYR179 THR181 ASP182 GLN183 ASP251
0.55	LYS178 ASP182 ARG186 ASP251
0.6	LYS178 ASP182 ARG186 LYS392
0.65	LYS178 TYR179 ASP182 GLN183
0.7	LYS178 TYR179 ASP182 GLN183
0.75	LYS178 TYR179 ASP182 GLN183 ASP188 LYS392 GLN400
0.8	LYS178 TYR179 ASP182 GLN183 ASP188 LYS392 GLN400
0.85	LYS178 TYR179 GLN183 ASP188 LYS392 GLN400
0.9	TYR179 ASP188 LYS392 GLN400
0.95	TYR179 ASP188 GLN400
1	TYR179 ASP188 GLN400

(a) apo-P450

RC	residue-list
0	THR185 PHE193 LEU244 VAL247 GLY248 CAM422
0.04	MET184 THR185 ARG186 ASP251 THR252 ILE395 VAL396 CAM422
0.09	MET184 THR185 ARG186 VAL247 GLY248 LEU250 ASP251 THR252 CAM422
0.13	THR181 MET184 THR185 VAL247 GLY248 LEU250 ASP251
0.17	THR181 MET184 THR185 PHE193 VAL247 LEU250 ASP251
0.22	THR181 ASP182 MET184 THR185 ARG186 PHE193 VAL247 ASP251
0.26	THR181 ASP182 GLN183 MET184 THR185 ARG186 PHE193 ASP251 THR252
0.3	THR181 ASP182 GLN183 MET184 THR185 ARG186 PRO187 ASP251 VAL396
0.35	LYS178 TYR179 LEU180 THR181 ASP182 GLN183 MET184 THR185 ARG186 MET191 LEU250 ASP251
0.39	LYS178 TYR179 LEU180 THR181 ASP182 GLN183 MET184 THR185 ARG186 MET191 LEU250 ASP251

0.43	GLU156 LYS178 TYR179 ASP182 GLN183 ARG186 LEU250 ASP251
0.48	GLU156 LYS178 TYR179 ASP182 GLN183 ARG186 SER190 MET191 VAL254 ASN255
0.52	GLU156 LYS178 TYR179 ASP182 GLN183 VAL254
0.57	LYS178 ASP182 GLN183 THR185 ARG186 PRO187 ASP188 ASP251 THR252 VAL254 ASN255
0.61	LYS178 ASP182 GLN183 ARG186 PRO187 ASP188 LYS392 GLN400
0.65	ARG186 PRO187 ASP188 ASN255 LYS392 VAL396 VAL399 GLN400
0.7	LYS178 ASP182 GLN183 ARG186 PRO187 ASP188 GLY189 MET191 LYS392 VAL399 GLN400
0.74	LYS178 TYR179 ASP182 GLN183 ARG186 ASP188 GLY189 SER190 MET191 LYS392 GLN400
0.78	LYS178 ASP188 GLY189 SER190 ILE389 LYS392 GLN400
0.83	TYR179 ASP182 GLN183 ASP188 GLY189 SER190 MET191 LYS392 GLN400
0.87	TYR179 GLN183 ASP188 GLY189 SER190 LYS392 GLN400
0.91	TYR179 ASP188 GLY189 SER190 LYS392 GLN400
0.96	ASP188 GLY189 SER190 ILE389 GLN400
1	ASP188 SER190

(b) cam-P450

Table S4 Residues lining channel “2a” in (a) apo-P450 and (b) cam-P450 listed according to their corresponding scaled reaction coordinate along the channel.

RC	residue-list
0	PHE87 TYR96 ILE395
0.06	PHE87 TYR96 THR185 VAL247 ILE395
0.11	PHE87 TYR96 MET184 THR185 VAL247 ILE395
0.17	TYR96 MET184 THR185 PHE193 ILE395
0.22	PHE87 TYR96 MET184 THR185 PHE193 ILE395
0.28	PHE87 TYR96 GLN183 MET184 THR185 PHE193 ILE395
0.33	PHE87 ALA92 TYR96 GLN183 MET184 THR185 PHE193 ILE395
0.39	TYR29 PHE87 TYR96 GLN183 MET184 PRO187 PHE193 ILE395
0.44	TYR29 ALA92 TYR96 GLN183 PRO187 PHE193
0.5	GLU91 ALA92 TYR96 GLN183 PRO187 PHE193
0.56	GLU91 GLN183 PRO187 THR192 PHE193
0.61	GLN183 PRO187 MET191 THR192 PHE193
0.67	GLU91 PRO187 GLY189 SER190 MET191 THR192 PHE193
0.72	GLU91 GLY189 SER190 MET191 THR192
0.78	GLU91 GLY189 SER190 MET191 THR192
0.83	GLU91 GLY189 SER190
0.89	GLY189 SER190 MET191 THR192
0.94	GLY189 SER190 MET191
1	GLY189 SER190 MET191

(a) apo-P450

RC	residue-list
0	PHE87 TYR96 MET184 THR185 PHE193 VAL247
0.05	PHE87 MET184 THR185 PHE193 ILE395 VAL396 CAM422
0.1	PHE87 ALA92 MET184 THR185 PRO187 PHE193 ILE395
0.15	PHE87 TYR96 MET184 THR185 PRO187 PHE193
0.2	PHE87 ALA92 TYR96 MET184 THR185 PRO187 PHE193 ILE395
0.25	PHE87 ALA92 ALA95 TYR96 MET184 PHE193 ILE395
0.3	PHE87 PRO89 ALA92 ALA95 TYR96 MET184 THR185 PRO187 PHE193 ILE395
0.35	GLU91 ALA92 GLU94 ALA95 MET184 THR185 PRO187 THR192 PHE193
0.4	PHE87 GLU91 ALA92 GLU94 ALA95 THR185 PRO187 THR192 PHE193
0.45	TYR29 PHE87 PRO89 ARG90 GLU91 ALA92 GLU94 PRO187 THR192 ILE395
0.5	TYR29 PRO89 ARG90 GLU91 ALA92 GLU94 PRO187 ASP188 THR192
0.55	GLU91 ALA92 GLU94 PRO187 THR192 PHE193
0.6	PRO89 GLU91 GLU94 PRO187 ASP188 GLY189 THR192
0.65	TYR29 ASN59 PRO89 ARG90 GLU91 GLU94 PRO187 ASP188 GLY189 THR192
0.7	GLU91 ASP188 GLY189 SER190 THR192

0.75	ASN59 PRO89 GLU91 ASP188 GLY189 SER190
0.8	ALA9 ASN10 ASN59 GLU91
0.85	ASN8 ALA9 ASN10 LEU11 GLU91 GLY189
0.9	GLN6 SER7 ASN8 ALA9 ASN10 LEU11
0.95	GLN6 SER7 ASN8 ALA9 ASN10 LEU11 GLY189 SER190
1	THR1 ILE5 GLN6 SER7 ASN8 ALA9 ASN10 LEU11 GLY189 SER190

(b) cam-P450

Table S5 Residues lining channel “2d” in apo-P450 listed according to their corresponding scaled reaction coordinate along the channel.

RC	residue-list
0	GLY248 VAL295 ASP297 HEM417
0.03	GLY248 VAL295 HEM417
0.06	GLY248 THR252 VAL295 HEM417
0.09	VAL295 HEM417
0.12	THR252 LEU294 VAL295 HEM417
0.15	THR252 LEU294 VAL295 HEM417
0.18	LEU294 VAL295 ASP297 GLN322
0.21	SER293 LEU294 VAL295 GLN322
0.24	SER293 LEU294 VAL295 GLN322
0.26	SER293 LEU294 VAL295 GLN322
0.29	SER293 LEU294 VAL295 GLN322 MET323
0.32	SER293 VAL295 ALA296 ASP297 PRO321 GLN322 MET323
0.35	TRP42 VAL295 ALA296 PRO321 GLN322 MET323
0.38	TRP42 VAL295 ALA296 ASP297 PRO321
0.41	TRP42 VAL295 ALA296 ASP297 PRO321
0.44	TRP42 PHE87 ALA296
0.47	MET28 TRP42 ALA296
0.5	MET28 TYR29 TRP42 ALA296 GLY394
0.53	MET28 TYR29 VAL38 TRP42 SER393 GLY394
0.56	MET28 TYR29 VAL38 TRP42
0.59	PHE26 MET28 VAL38 TRP42 MET323
0.62	PHE26 MET28 VAL38 TRP42 MET323
0.65	PHE24 PHE26 ASP27 MET28 VAL38 ALA41 TRP42 VAL44
0.68	PHE26 ASP27 MET28 GLY37 VAL38 ALA41 TRP42
0.71	PHE26 ASP27 MET28 TYR29 PRO31 VAL38 ALA41 SER393
0.74	ASP27 MET28 PRO31 GLY37 VAL38 ALA41 SER393
0.76	PHE26 ASP27 MET28 TYR29 ASN30 PRO31
0.79	ASP27 TYR29 ASN30 PRO31 SER32 ASN33 LEU34 SER35 ALA36 GLY37 VAL38 ALA41 SER393
0.82	ASP27 ASN30 PRO31 SER32 ASN33 LEU34 SER35 ALA36 ALA41
0.85	PHE26 ASP27 ASN30 PRO31 SER32 ASN33 LEU34 SER35 ALA41
0.88	ASP25 PHE26 ASP27 ASN30 PRO31 SER32 ASN33 LEU34 ALA41
0.91	THR1 GLN6 PHE24 ASP25 PHE26 ASP27 PRO31 SER32 ASN33 LEU34 SER35 ALA41 VAL44
0.94	THR1 PHE24 ASP25 PHE26 SER32 ASN33 LEU34 VAL44
0.97	THR1 GLN6 PHE24 ASP25 PHE26 SER32 ASN33 LEU34 VAL44
1	THR1 THR2 GLU3 THR4 ILE5 GLN6 PHE24 ASP25 ASN33 LEU34 VAL44 SER48

Table S6 Residues lining channel “2e” in (a) apo-P450 and (b) cam-P450 listed according to their corresponding scaled reaction coordinate along the channel.

RC	residue-list
0	TYR96 PHE98 THR101 ARG240 LEU244
0.07	PHE87 TYR96 PHE98 THR101
0.14	PRO86 PHE87 TYR96 THR101
0.21	PRO86 TYR96 ASP97 PHE98 THR101 SER102
0.29	PRO86 TYR96 ASP97 PHE98 THR101 SER102 ARG240
0.36	GLU84 PRO86 PHE87 TYR96 ASP97 THR101 SER102
0.43	GLU84 TYR96 ASP97 SER102
0.5	GLU84 TYR96 ASP97 SER102
0.57	GLU84 ASP97 ARG240
0.64	GLU84 ASP97 ARG240
0.71	THR1 GLU84
0.79	ARG240
0.86	THR1 GLU84
0.93	THR1
1	THR1

(a) apo-P450

RC	residue-list
0	PRO86 PHE87 TYR96 PHE98 LEU244 ASP297 ILE395 CAM422
0.07	PHE87 TYR96 THR101 ARG240 LEU244 ASP297 HEM417 CAM422
0.13	PRO86 PHE87 TYR96 THR101 ASP297 HEM417 CAM422
0.2	PRO86 PHE87 TYR96 PHE98 THR101 HEM417 CAM422
0.27	SER83 PRO86 PHE87 TYR96 THR101 SER102 MET103 HEM417
0.33	SER83 PRO86 PHE87 TYR96 PHE98 THR101 SER102 ARG240
0.4	SER83 GLU84 CYS85 PRO86 PHE87 TYR96 THR101 SER102
0.47	GLU84 CYS85 PRO86 ARG90 GLY93 TYR96 THR101 SER102 MET103 ASP104 GLN108
0.53	GLU84 ARG90 GLY93 TYR96 ASP97 PHE98 ILE99 THR101 SER102 MET103 ARG240
0.6	GLU84 CYS85 ARG90 GLU94 TYR96 ASP97 SER102 MET103
0.67	GLU84 CYS85 ARG90 GLU94 TYR96 SER102 MET103 ASP104 GLN108
0.73	GLU84 ARG90 GLU94 TYR96 ASP97 SER102 MET103 ASP104 GLN108
0.8	GLU84 GLU94 ASP97 ILE99 SER102 MET103 ASP104 GLN108 ARG240
0.87	GLU84 ASP97 ILE99 MET103 ASP104 GLU107 GLN108 PHE111 ARG240
0.93	GLU84 MET103 ASP104 GLU107 GLN108 PHE111 ARG240
1	GLU84 MET103 ASP104 GLU107 ARG109 GLN110 PHE111

(b) ca-P450

Table S7 Residues lining channel “2F” in apo-P450 listed according to their corresponding scaled reaction coordinate along the channel.

RC	residue-list
0	VAL247 GLY248 VAL295 HEM417
0.03	VAL247 GLY248 THR252 HEM417
0.06	GLY248 THR252 VAL295 HEM417
0.09	THR252 VAL295 HEM417
0.12	THR252 LEU294 VAL295 HEM417
0.15	LEU294 VAL295 HEM417
0.18	LEU294 VAL295 GLN322
0.21	SER293 LEU294 VAL295 ASP297 GLN322
0.24	SER293 LEU294 VAL295 ALA296 ASP297 GLN322
0.27	SER293 LEU294 VAL295 ALA296 GLN322
0.3	VAL295 ALA296 GLN322 MET323
0.33	VAL295 ALA296 ASP297 LEU320 PRO321 GLN322
0.36	TRP42 ALA296 ASP297 LEU320 PRO321
0.39	TRP42 PHE87 ALA296 ASP297
0.42	MET28 TRP42 ALA296
0.45	MET28 TRP42 ALA296 MET323
0.48	MET28 VAL38 TRP42
0.52	PHE26 MET28 VAL38 TRP42 SER393 GLY394
0.55	MET28 TYR29 ALA296 GLY394 SER397
0.58	MET28 TYR29 GLY394
0.61	MET28 TYR29 ASN30 SER393 GLY394 SER397
0.64	MET28 TYR29 VAL38 SER393 GLY394 SER397
0.67	VAL38 LYS392 SER393 SER397
0.7	PRO31 ALA36 GLY37 VAL38 TRP42 HSE391 LYS392 SER393 SER397
0.73	MET28 PRO31 ALA36 GLY37 VAL38 HSE391 LYS392 SER393
0.76	TYR29 ASN30 PRO31 SER35 ALA36 LYS392 SER393
0.79	TYR29 ASN30 PRO31 LEU34 SER35 ALA36 LYS392 SER393
0.82	TYR29 ASN30 PRO31 ASN33 LEU34 SER35 ALA36 ASP188 LYS392
0.85	TYR29 ASN30 PRO31 SER32 ASN33 LEU34 SER35 ASP188 LYS392
0.88	ASN30 PRO31 SER32 ASN33 ASP188 LYS392
0.91	ASN30 PRO31 SER32 ASN33 LEU34 ASP188 GLY189
0.94	ASN30 SER32 ASN33 LEU34 ASP188 GLY189
0.97	SER32 ASN33 LEU34 ASP188 GLY189 SER190
1	GLY189 SER190