Supplementary Material:

Coupling and Uncoupling Mechanisms in the Methoxy Threonine Mutant of Cytochrome P450cam: A QM/MM Study

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Content

1. Abbreviations and conventions.

Energies are given in kcal/mol, distances in Å, angles in degree, and charges and spin densities in e. Energies (in kcal/mol) refer to the following basis sets:

B1: LACVP (Fe)/6-31G (rest) for optimization (doublet/quartet)

B2: TZVP all for single-point calculations

In Tables S15-S21 of geometrical parameters:

Glu366 channel: W1: Wat523, W2:Wat566, W3: Wat687, and W4: Wat902

Asp251 channel: W1:Wat901

Mechanisms:

Glu-C-I: mechanism I in Glu366 channel (coupling reaction): O^1-O^2 bond cleavage, then proton transfer from methoxy group to O^2 (distal oxygen) followed by proton transfer from Glu366 to methoxy group through a water network.

Glu-C-II: mechanism II in Glu366 channel (coupling reaction): Direct proton transfer from methoxy group to O^2 (distal oxygen) followed by proton transfer from Glu_{366} to methoxy group through a water network.

Glu-C-III: mechanism III in Glu366 channel (uncoupling reaction): O^1-Fe bond cleavage, then proton transfer from methoxy group to O^1 (proximal oxygen) followed by proton transfer from Glu366 to methoxy group through a water network.

Glu-C-IV: mechanism IV in Glu366 channel (uncoupling reaction): Direct proton transfer from methoxy group to O^1 (proximal oxygen) followed by proton transfer from Glu366 to methoxy group through a water network.

Asp-C-I: mechanism I in Asp251 channel (coupling reaction): O^1-O^2 bond cleavage, then proton transfer from methoxy group to O^2 (distal oxygen) followed by proton transfer from Asp251 to methoxy group through one water molecule.

Asp-C-II: mechanism II in Asp251 channel (coupling Reaction): Direct proton transfer from methoxy group to O^2 (distal oxygen) followed by proton transfer from Asp251 to methoxy group through one water molecule.

Asp-C-III: mechanism III in Asp251 channel (uncoupling reaction): O^1-Fe bond cleavage, then proton transfer from methoxy group to O^1 (proximal oxygen) followed by proton transfer from Asp251 to methoxy group through one water molecule.

Asp-C-IV: mechanism IV in Asp251 channel (uncoupling reaction): Direct proton transfer from methoxy group to O^1 (proximal oxygen) followed by transfer from Asp251 to methoxy group through one water molecule.

2. Computed energies.

Glu_{366} Channel:

Table S1. *Computed relative energies in Glu-C-I.*

	Basis set	Cpd0	TS1	IC1	TS2	IC2	TS3	Cpd1
QM/MM	B1	0.00	18.11	10.30	18.48	1.12	17.22	8.01
	B2	0.00	20.65	13.02	21.19	4.54	20.32	12.15

Table S2. *Computed relative energies in Glu-C-II.*

	Basis set	Cpd0	TS1	IC1	TS2	Cpd1
QM/MM	B1	0.00	20.78	0.84	19.96	8.04
	B2	0.00	23.77	2.33	24.48	13.53

Table S3. *Computed relative energies in Glu-C-III.*

	Basis set	Cpd0	TS1	IC1	TS2	IC2	TS3	Fe RS
QM/MM	B1	0.00	30.25	17.74	42.35	40.61	60.74	28.42
	B2	0.00	32.20	20.02	43.79	41.68	63.99	30.00

Table S4. *Computed relative energies in Glu-C-IV.*

	Basis set	Cpd0	TS1c	IC1	TS2	Fe RS
QM/MM	B1	0.00	40.25	37.99	58.25	25.19
	B2	0.00	42.04	36.11	59.64	27.71

Asp_{251} Channel:

Table S5. *Computed relative energies in Asp-C-I.*

	Basis set	Cpd0	TS1	IC1	TS2	IC2	TS3	Cpd1
QM/MM	B1	0.00	18.63	14.43	22.55	11.37	23.01	7.65
	B2	0.00	19.02	15.41	23.61	14.04	25.85	11.29

Table S6. *Computed relative energies in Asp-C-III.*

	Basis set	Cpd0	TS1	IC1	TS2	IC2	TS3	Fe RS
QM/MM	B1	0.00	28.08	25.74	47.57	47.41	58.37	28.83
	B2	0.00	30.01	28.58	50.06	49.00	61.49	30.92

Table S7. *Computed relative energies in Asp-C-IV.*

	Basis set	Cpd0	TS1	IC1	TS2	Fe RS
QM/MM	B1	0.00	52.89	47.55	54.94	26.44
	B2	0.00	55.07	50.74	55.83	29.06

3. Spin densities and Mulliken charges.

Glu_{366} channel:

Table S8. *Computed spin densities and Mulliken charge distributions for doublet states of reactant, intermediates and product in Glu-C-I.*

			Fe	OH[a]	O_1	SH	Por	MeO-Thr_{252}	Glu_{366}
Cpd0	B1	Spin	0.998	-0.001	0.091	-0.022	-0.066	0.000	0.000
		Charge	0.435	-0.042	-0.376	-0.438	-0.535	0.012	-0.123
	B2	Spin	1.027	-0.001	-0.078	-0.023	-0.081	0.000	0.000
		Charge	0.347	-0.011	-0.321	-0.560	-0.434	0.001	-0.113
IC1	B1	Spin	1.399	-0.927	0.714	-0.029	-0.156	0.001	0.000
		Charge	0.465	-0.041	-0.519	-0.462	-0.361	0.014	-0.122
	B2	Spin	1.486	-0.910	0.660	-0.033	-0.202	0.000	0.000
		Charge	0.287	-0.064	-0.465	-0.587	-0.133	-0.003	-0.111
IC2	B1	Spin	1.364	0.009	0.742	-0.027	-0.109	-0.981	0.000
		Charge	0.451	0.029	-0.508	-0.469	-0.415	-0.033	-0.122
	B2	Spin	1.457	0.008	0.684	-0.031	-0.139	-0.981	0.000
		Charge	0.291	0.018	-0.461	-0.595	-0.224	-0.006	-0.111
Cpd1	B1	Spin	1.355	-0.003	0.771	-0.130	-0.731	0.000	-0.273
		Charge	0.453	0.014	-0.470	-0.417	0.143	-0.054	-0.594
	B2	Spin	1.454	0.005	0.706	-0.146	-0.767	0.001	-0.251
		Charge	0.276	0.029	-0.431	-0.518	0.398	-0.052	-0.642

[a] OH species bound to FeO unit, protonated in IC2 and Cpd1.

Table S9. *Computed spin densities and Mulliken charge distributions for doublet states of reactant, intermediates and product in Glu-C-II.*

			Fe	OH[a]	O_1	SH	Por	MeO-Thr_{252}	Glu_{366}
Cpd0	B1	Spin	0.998	-0.001	0.091	-0.022	-0.066	0.000	0.000
		Charge	0.435	-0.042	-0.376	-0.438	-0.535	0.012	-0.123
	B2	Spin	1.027	-0.001	-0.078	-0.023	-0.081	0.000	0.000
		Charge	0.347	-0.011	-0.321	-0.560	-0.434	0.001	-0.113
IC1	B1	Spin	1.373	0.009	0.734	-0.026	-0.102	-0.990	0.000
		Charge	0.452	0.032	-0.512	-0.462	-0.412	-0.042	-0.122
	B2	Spin	1.434	0.005	0.721	-0.040	-0.109	-0.976	0.000
		Charge	0.291	0.020	-0.459	-0.578	-0.252	-0.053	-0.115
Cpd1	B1	Spin	1.353	0.006	0.773	-0.128	-0.679	-0.001	-0.326
		Charge	0.449	0.012	-0.470	-0.409	0.098	-0.047	-0.550
	B2	Spin	1.447	0.005	0.710	-0.138	-0.736	0.000	-0.303
		Charge	0.280	0.031	-0.430	-0.507	0.298	-0.044	-0.614

[a] OH species bound to FeO unit, protonated in IC1 and Cpd1.

Table S10. *Computed spin densities and Mulliken charge distributions for doublet states of reactant, intermediates and product in Glu-C-III.*

			Fe	OOH[a]	SH	Por	MeO-Thr$_{252}$	Glu$_{366}$
Cpd0	B1	Spin	0.998	0.090	-0.022	-0.066	0.000	0.000
		Charge	0.435	-0.418	-0.438	-0.535	0.012	-0.123
	B2	Spin	1.027	-0.079	-0.023	-0.081	0.000	0.000
		Charge	0.347	-0.332	-0.560	-0.434	0.001	-0.113
IC1	B1	Spin	1.963	-0.918	-0.047	-0.091	0.000	0.000
		Charge	0.388	-0.096	-0.602	-0.660	0.037	-0.120
	B2	Spin	1.978	-0.876	0.036	-0.137	0.000	0.000
		Charge	0.626	-0.136	-0.703	-0.787	0.028	-0.109
IC2	B1	Spin	2.000	-0.066	0.080	-0.082	-0.933	0.000
		Charge	0.365	0.009	-0.662	-0.685	0.007	-0.121
	B2	Spin	2.035	-0.039	0.093	-0.133	-0.957	0.000
		Charge	0.596	0.001	-0.768	-0.809	-0.016	-0.111
Fe RS	B1	Spin	1.205	-0.007	-0.096	-0.105	0.003	0.000
		Charge	0.392	0.120	-0.209	-0.315	-0.064	-0.816
	B2	Spin	1.210	0.003	-0.106	-0.104	-0.003	0.000
		Charge	0.413	0.022	-0.332	-0.254	-0.046	-0.826

[a] OOH species bound to Fe atom, protonated in IC2 and Fe RS.

Table S11. *Computed spin densities and Mulliken charge distributions for doublet states of reactant, intermediates and product in Glu-C-IV.*

			Fe	OOH[a]	SH	Por	MeO-Thr$_{252}$	Glu$_{366}$
Cpd0	B1	Spin	0.998	0.090	-0.022	-0.066	0.000	0.000
		Charge	0.435	-0.418	-0.438	-0.535	0.012	-0.123
	B2	Spin	1.027	-0.079	-0.023	-0.081	0.000	0.000
		Charge	0.347	-0.332	-0.560	-0.434	0.001	-0.113
IC1	B1	Spin	2.000	-0.087	0.075	-0.086	-0.904	0.000
		Charge	0.370	-0.102	-0.658	-0.690	0.013	-0.121
	B2	Spin	2.029	-0.057	0.096	-0.142	-0.926	0.000
		Charge	0.644	-0.016	-0.764	-0.855	-0.007	-0.112
Fe RS	B1	Spin	1.204	0.011	-0.113	-0.093	-0.008	0.000
		Charge	0.374	0.130	-0.202	-0.300	0.103	-0.935
	B2	Spin	1.211	0.010	-0.121	-0.095	-0.010	0.000
		Charge	0.424	0.048	-0.319	-0.267	0.122	-0.944

[a] OOH species bound to Fe atom, protonated in IC1 and Fe RS.

Asp_{251} channel:

Table S12. *Computed spin densities and Mulliken charge distributions for doublet states of reactant, intermediates and product in Asp-C-I.*

			Fe	OH[a]	O_1	SH	Por	MeO-Thr_{252}	Asp_{251}
Cpd0	B1	Spin	0.928	-0.002	0.135	0.002	-0.061	0.000	0.000
		Charge	0.427	-0.095	-0.315	-0.415	-0.236	0.036	-0.168
	B2	Spin	0.973	-0.003	0.122	-0.002	-0.076	0.000	0.000
		Charge	0.305	-0.110	-0.223	-0.538	0.149	0.033	-0.166
IC1	B1	Spin	1.357	-0.795	0.747	-0.037	-0.236	-0.003	0.000
		Charge	0.453	-0.125	-0.494	-0.462	0.224	0.010	-0.167
	B2	Spin	1.446	-0.761	0.692	-0.044	-0.284	-0.004	0.000
		Charge	0.280	-0.152	-0.448	-0.581	0.526	0.004	-0.158
IC2	B1	Spin	1.345	-0.039	0.752	-0.022	-0.085	-0.953	0.000
		Charge	0.448	-0.017	-0.500	-0.484	-0.124	0.049	-0.150
	B2	Spin	1.434	-0.026	0.697	-0.026	-0.112	-0.971	0.000
		Charge	0.292	-0.012	-0.460	-0.604	0.350	0.002	-0.132
Cpd1	B1	Spin	1.352	0.004	0.772	-0.158	-0.547	0.000	-0.184
		Charge	0.461	0.012	-0.472	-0.372	0.509	-0.049	-0.757
	B2	Spin	1.446	0.003	0.708	-0.181	-0.587	0.000	-0.144
		Charge	0.257	0.019	-0.423	-0.469	0.986	-0.027	-0.828

[a] OH species bound to FeO unit, protonated in IC2 and Cpd1.

Table S13. *Computed spin densities and Mulliken charge distributions for doublet states of reactant, intermediates and product in Asp-C-III.*

			Fe	OOH[a]	SH	Por	MeO-Thr_{252}	Asp_{251}
Cpd0	B1	Spin	0.928	0.133	0.002	-0.061	0.000	0.000
		Charge	0.427	-0.410	-0.415	-0.236	0.036	-0.168
	B2	Spin	0.973	-0.125	-0.002	-0.076	0.000	0.000
		Charge	0.305	-0.333	-0.538	0.149	0.033	-0.166
IC1	B1	Spin	1.980	-0.969	0.073	-0.084	0.002	0.000
		Charge	0.418	-0.069	-0.626	-0.376	0.048	-0.162
	B2	Spin	1.994	-0.943	0.079	-0.114	0.000	0.000
		Charge	0.649	-0.135	-0.717	-0.200	0.038	-0.151
IC2	B1	Spin	1.993	-0.083	0.090	-0.085	-0.918	0.000
		Charge	0.401	-0.019	-0.651	-0.421	0.059	-0.147
	B2	Spin	2.034	-0.061	0.105	-0.132	-0.939	0.000
		Charge	0.647	-0.030	-0.747	-0.265	0.016	-0.128
Fe RS	B1	Spin	1.439	0.001	-0.162	-0.218	0.000	0.000
		Charge	0.344	-0.028	-0.215	0.155	0.015	-0.933
	B2	Spin	1.373	0.002	-0.159	-0.163	0.000	0.000
		Charge	0.445	-0.043	-0.307	0.401	0.022	-0.962

[a] OOH species bound to Fe atom, protonated in IC2 and Fe RS.

Table S14. *Computed spin densities and Mulliken charge distributions for doublet states of reactant, intermediates and product in Asp-C-IV.*

			Fe	**OOH**[a]	**SH**	**Por**	**MeO-Thr$_{252}$**	**Asp$_{251}$**
Cpd0	B1	Spin	0.928	0.133	0.002	-0.061	0.000	0.000
		Charge	0.427	-0.410	-0.415	-0.236	0.036	-0.168
	B2	Spin	0.973	-0.125	-0.002	-0.076	0.000	0.000
		Charge	0.305	-0.333	-0.538	0.149	0.033	-0.166
IC1	B1	Spin	1.969	-0.001	0.105	-0.072	-1.000	0.000
		Charge	0.378	-0.007	-0.619	-0.403	0.025	-0.158
	B2	Spin	2.012	-0.003	0.115	-0.122	-0.998	0.000
		Charge	0.682	-0.049	-0.722	-0.307	0.015	-0.144
Fe RS	B1	Spin	1.442	0.002	-0.171	-0.207	-0.001	0.000
		Charge	0.389	0.054	-0.229	0.136	-0.087	-0.941
	B2	Spin	1.362	0.001	-0.153	-0.156	0.000	0.000
		Charge	0.467	0.031	-0.324	0.352	-0.036	-0.972

[a] OOH species bound to Fe atom, protonated in IC1 and Fe RS.

4. Selected geometrical parameters.

Glu_{366} Channel:

Table S15. *Geometry parameters for B1 in Glu-C-I.*

	S - Fe	Fe - O_1	O_1 - O_2	O_2-H_2	O_2 – H_{res}	C_{res} – H_{w4}	O_{w4} - H_{w3}	O_{w3} - H_{w2}	O_{w2} - H_{w1}	O_{w1} - H_{Glu366}
RC	2.550	1.854	1.513	0.987	2.158	2.504	1.855	1.856	1.634	1.525
TS1	2.514	1.666	2.414	0.985	2.120	2.466	1.804	1.875	1.636	1.528
IC1	2.510	1.678	2.711	1.018	2.416	2.483	1.814	1.890	1.639	1.530
TS2	2.512	1.673	2.646	1.016	1.318	2.552	1.836	1.829	1.644	1.522
IC2	2.514	1.673	2.702	0.997	0.974	2.507	1.862	1.873	1.636	1.528
TS3	2.507	1.676	2.654	1.000	0.973	1.376	1.303	3.012	1.214	1.175
Cpd 1	2.534	1.665	2.837	0.986	0.983	1.098	1.095	3.160	1.025	1.022

Table S16. *Geometry parameters for B1 in Glu-C-II.*

	S - Fe	Fe - O_1	O_1 - O_2	O_2-H_2	O_2 – H_{res}	C_{res} – H_{w4}	O_{w4} - H_{w3}	O_{w3} - H_{w2}	O_{w2} - H_{w1}	O_{w1} - H_{Glu366}
RC	2.550	1.854	1.513	0.987	2.158	2.504	1.855	1.856	1.634	1.525
TS1	2.499	1.671	2.333	0.986	1.280	2.458	1.857	1.799	1.634	1.527
IC1	2.511	1.673	2.692	0.998	0.973	2.531	1.831	1.870	1.637	1.528
TS2	2.525	1.667	2.859	0.985	0.984	1.223	1.338	1.880	1.314	1.246
Cpd 1	2.529	1.667	2.829	0.986	0.983	1.087	1.015	1.838	1.021	1.014

Table S17. *Geometry parameters for B1 in Glu-C-III.*

	S - Fe	Fe - O_1	O_1 - O_2	O_2-H_2	O_1 – H_{res}	C_{res} – H_{w4}	O_{w4} - H_{w3}	O_{w3} - H_{w2}	O_{w2} - H_{w1}	O_{w1} - H_{Glu366}
RC	2.550	1.854	1.513	3.658	2.158	2.504	1.855	1.856	1.634	1.525
TS1	2.539	2.896	1.391	3.381	2.108	2.489	1.866	1.868	1.636	1.527
IC1	2.580	3.730	1.394	3.770	2.635	2.615	1.912	1.831	1.647	1.535
TS2	2.633	4.192	1.493	1.123	2.017	2.475	1.887	1.843	1.633	1.524
IC2	2.639	4.192	1.510	1.003	1.951	2.464	1.866	1.871	1.639	1.528
TS3	2.618	3.726	1.531	0.993	1.971	1.306	1.240	2.972	1.288	1.374
Fe RS	2.339	2.205	1.510	0.989	1.971	1.093	1.001	2.996	1.046	1.064

Table S18. *Geometry parameters for B1 in Glu-C-IV.*

	S - Fe	Fe - O_1	O_1 - O_2	O_2-H_2	O_1 – H_{res}	C_{res} – H_{w4}	O_{w4} - H_{w3}	O_{w3} - H_{w2}	O_{w2} - H_{w1}	O_{w1} - H_{Glu366}
RC	2.550	1.854	1.513	3.658	2.158	2.504	1.855	1.856	1.634	1.525
TS1	2.621	3.753	1.491	1.134	2.045	2.560	1.860	1.950	1.636	1.525
IC1	2.626	3.680	1.509	1.005	1.963	2.534	1.856	1.958	1.635	1.525
TS2	2.604	3.151	1.513	0.999	2.142	1.201	1.226	1.461	1.360	1.414
Fe RS	2.348	2.147	1.512	0.989	2.349	1.011	1.016	1.812	1.006	1.014

Asp_{251} channel:

Table S19. *Geometry parameters for B1 in Asp-C-I.*

	S - Fe	Fe - O_1	O_1 - O_2	O_2 - H_2	O_1 – H_{res}	C_{res} – H_{w1}	H_{w1} – O_{w1}	O_{w1} - H_{Asp251}
RC	2.416	1.842	1.535	0.987	2.209	2.439	1.009	1.442
TS1	2.487	1.661	2.217	0.984	2.060	2.468	1.009	1.478
IC1	2.510	1.674	2.880	1.004	2.104	2.470	1.011	1.476
TS2	2.511	1.674	2.685	1.014	1.206	2.485	1.001	1.501
IC2	2.521	1.672	2.915	0.983	0.981	2.481	1.014	1.523
TS3	2.511	1.677	2.796	0.996	0.978	1.282	1.999	1.420
Cpd1	2.517	1.666	2.911	0.984	0.975	1.093	2.442	0.994

Table S20. *Geometry parameters for B1 in Asp-C-III.*

	S - Fe	Fe - O_1	O_1 - O_2	O_1 - H_{res}	O_1 – H_{res}	C_{res} – H_w	H_{w1} – O_{w1}	O_{w1} - H_{Asp251}
RC	2.416	1.842	1.535	3.050	2.209	2.439	1.009	1.442
TS1	2.557	3.065	1.402	2.151	2.842	2.493	1.005	1.489
IC1	2.557	3.666	1.384	2.148	3.456	2.492	1.006	1.488
TS2	2.573	4.090	1.511	1.111	3.605	2.508	0.992	1.537
IC2	2.572	4.080	1.511	1.001	1.952	2.498	0.992	1.537
TS3	2.565	3.494	1.509	1.000	1.974	1.300	1.178	1.424
Fe RS	2.344	4.319	1.523	1.016	2.533	1.095	2.105	1.004

Table S21. *Geometry parameters for B1 in Asp-C-IV.*

	S - Fe	Fe - O_1	O_1 - O_2	O_1 - H_{res}	O_2 – H_{res}	C_{res} – H_w	H_{w1}– O_{w1}	O_{w1} - H_{Asp251}
RC	2.416	1.842	1.535	3.050	2.209	2.439	1.009	1.442
TS1	2.442	2.083	1.509	1.051	2.001	2.410	1.031	1.379
IC1	2.523	2.981	1.514	1.010	2.000	2.406	0.998	1.498
TS2	2.565	3.264	1.515	1.005	1.984	1.357	1.143	1.473
Fe RS	2.349	3.630	1.510	0.986	1.938	1.097	2.196	1.004

5. Optimized geometries and energy profiles for hydrogen transfer from ethyl methyl ether to OH and OOH in the gas phase.

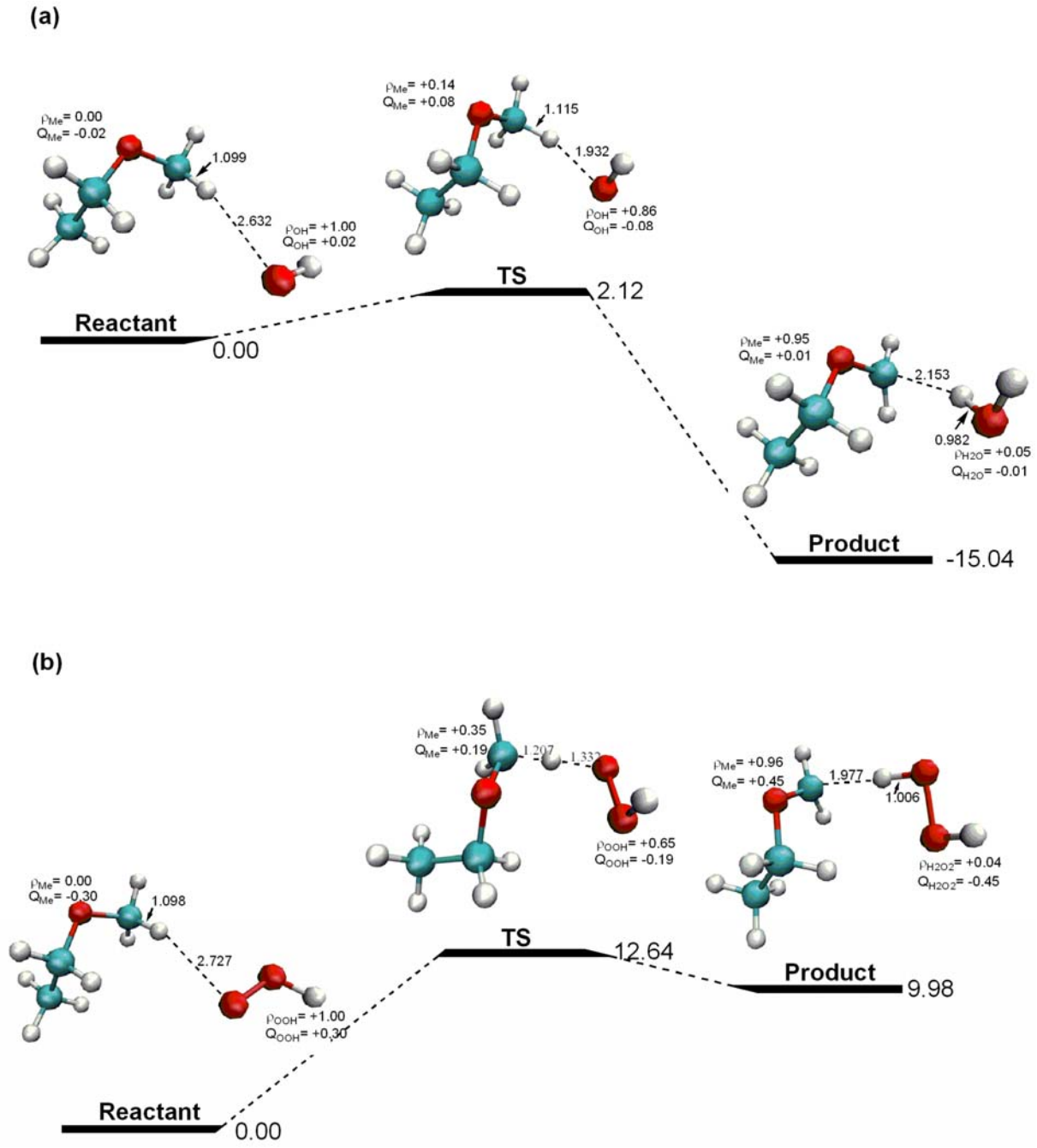

Figure S1. (a) Optimized geometries and energy profiles of reactant, TS1 and product (UB3LYP/6-31+G*) for hydrogen transfer from methyl ethyl ether to the OH radical, (b) optimized geometries and energy profiles of reactant, TS1 and product (UB3LYP/6-31+G*) for hydrogen transfer from methyl ethyl ether to the OOH radical. Relative energies are given in kcal/mol.

6. Molecular dynamics simulations.

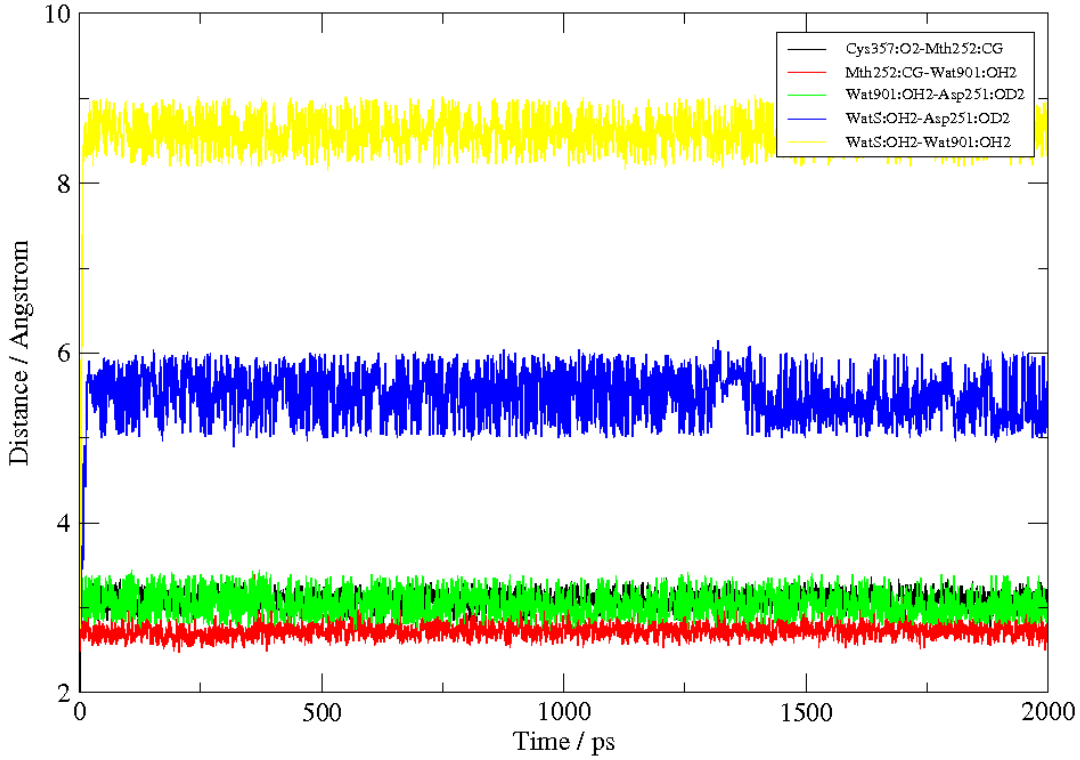

Figure S2. Monitoring the mobility of the water molecule for Thr252MeT mutant in the Asp251 channel (Thr252 has been replaced by methoxy threonine).

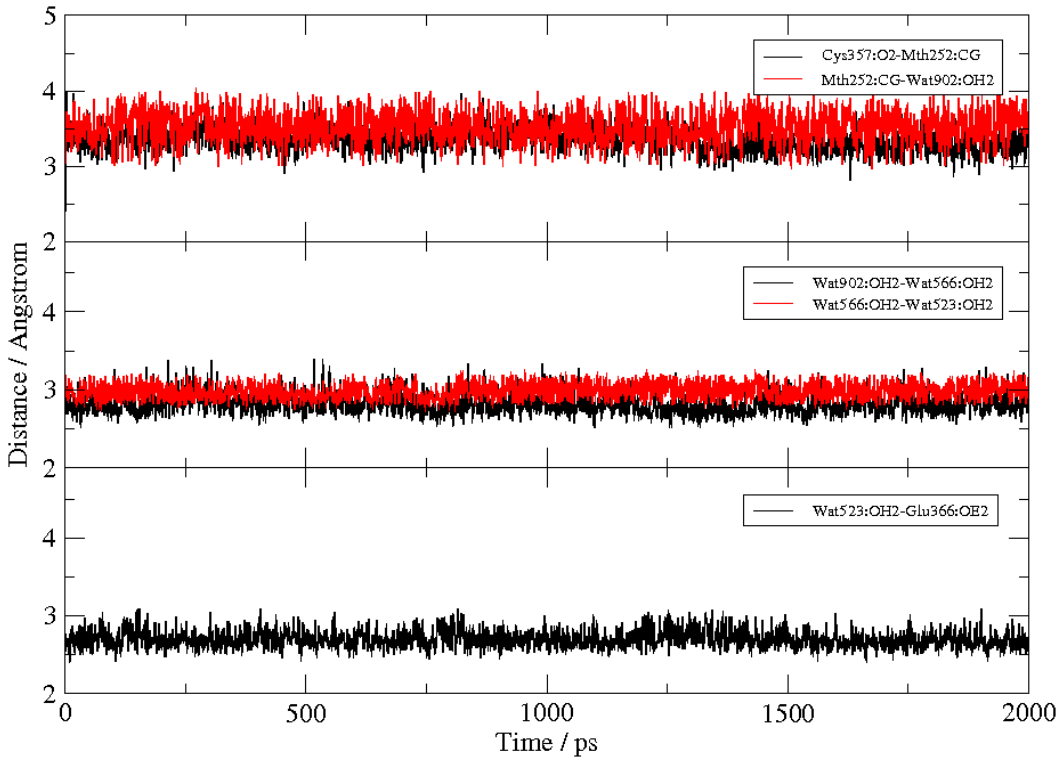

Figure S3. Monitoring the mobility of the water molecules for Thr252MeT mutant in the Glu366 channel (Thr252 has been replaced by methoxy threonine).

7. Energy components for the stationary points in mechanisms I and II.

Table S22: Energy components (kcal/mol) of mechanisms I and II in the Asp251 and Glu366 channels using basis set B1 (with respect to Cpd 0).

Asp-C-I	**RC**	**TS1**	**IC1**	**TS2**	**IC2**	**TS3**	**PC**
E(QM-ee)	0.00	17.46	13.20	20.60	9.53	21.15	1.84
E(MM)	0.00	1.17	1.23	1.95	1.84	1.86	5.81
E(QM/MM)	0.00	18.63	14.43	22.55	11.37	23.01	7.65
E(QM-gas)	0.00	19.72	16.34	25.84	13.34	31.24	9.47
Glu-C-I	**RC**	**TS1**	**IC1**	**TS2**	**IC2**	**TS3**	**PC**
E(QM-ee)	0.00	17.63	9.47	17.54	0.72	15.57	4.06
E(MM)	0.00	0.48	0.83	0.94	0.40	1.65	3.95
E(QM/MM)	0.00	18.11	10.30	18.48	1.12	17.22	8.01
E(QM-gas)	0.00	20.65	13.02	20.77	1.18	20.71	6.21
Glu-C-II	**RC**	**TS1**	**IC1**	**TS2**	**PC**		
E(QM-ee)	0.00	20.26	-0.07	17.83	4.31		
E(MM)	0.00	0.52	0.91	2.13	3.73		
E(QM/MM)	0.00	20.78	0.84	19.96	8.04		
E(QM-gas)	0.00	26.08	1.02	23.48	9.16		

E(QM/MM) refers to the total QM/MM energy of the system. E(QM-ee) denotes the contributions from the QM calculation (including the electrostatic interactions with the MM point charges). E(MM) represents the contributions from the MM calculation and from the remaining QM/MM interactions (bonded and van-der-Waals interactions). E(QM-gas) refers to gas-phase single-point calculations at QM/MM optimized geometries.

8. Computed relative energies for an additional snapshot, mechanism I in the Asp251 channel

Table S23. Computed relative energies (kcal/mol) in Asp-C-I, snapshot 1500 ps.

	Basis set	**Cpd0**	**TS1**	**IC1**	**TS2**	**IC2**	**TS3**	**Cpd1**
QM/MM	B1	0.00	19.25	13.02	21.53	10.45	23.19	6.92

The additional snapshot was drawn after 1500 ps of MD simulation. The QM/MM calculations were performed in complete analogy to those for the first snapshot (see main paper). The geometries of all stationary points were fully optimized. The results given above were obtained using basis B1.

9. Computed relative energies in gas-phase model systems.

Two model systems were used to study the intrinsic hydrogen and proton donor abilities of the Thr and MeT residues at the B3LYP/B1 level. For the wild-type enzyme, the model system encompassed C_2H_5OH (representing Thr252), the OH radical, the FeO porphine system, and SH (representing Cys357). The model system for the Thr252MeT mutant was designed accordingly with $C_2H_5OCH_3$ representing the MeT residue. Both model systems are smaller than the QM regions used in the QM/MM studies (lacking Asp251 and Wat901, see Figure 1 of the main paper) to capture the intrinsic reactivities. In the wild-type model system, proton transfer to the OH radical with concomitant electron transfer from the heme is a barrier-free process making it impossible to compute the corresponding reactant and transition state structures. In the Thr252MeT model system, the hydrogen transfer proceeds over a significant barrier of 4.52 kcal/mol. Compared with the wild-type enzyme, the increase in the barrier due to Thr252MeT mutation must therefore be greater than 4.5 kcal/mol. The molecular structures of reactant, transition state, and product in the model system (shown in Figure S4) are very similar to those in the Thr252MeT enzyme.

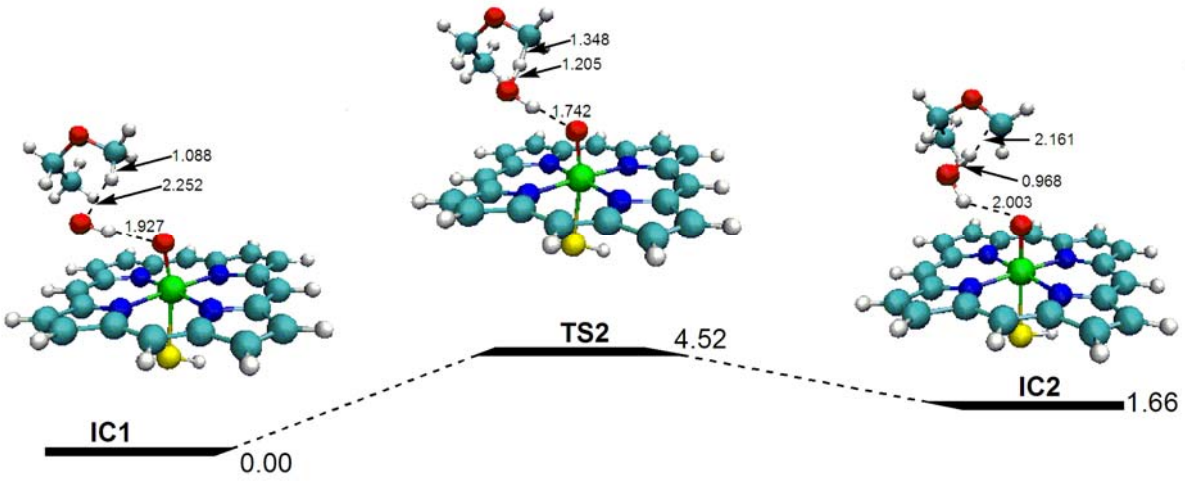

Figure S4. Optimized geometries (Å) and energy profile (kcal/mol) for hydrogen abstraction in the small QM model system of the Thr252MeT mutant computed at the UB3LYP/B1 level. Relative energies are given with respect to IC1.