Supporting Information

QM/MM Study of the Second Proton Transfer in the Catalytic Cycle of the D251N Mutant of Cytochrome P450cam

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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 geometry optimization

B2: TZVP all for single-point calculations

Asn-C-I: Mechanism I in the Asn251 channel (coupling reaction): direct proton transfer from Thr252 to O2 followed by O1-O2 bond cleavage, then proton transfer from Wat901 to Thr252, and finally proton transfer from Asn251 to Wat901.

Asn-C-II: Mechanism II in the Asn251 channel (coupling reaction): O1-O2 bond cleavage, then proton transfer from Thr252 to O2, proton transfer from Wat901 to Thr252, and finally proton transfer from Asn251 to Wat901.

Asn-C-III: Mechanism III in the Asn251 channel (uncoupling reaction): direct proton transfer from Thr252 to O1 followed by O-Fe bond cleavage, then proton transfer from Wat901 to Thr252, and finally proton transfer from Asn251 to Wat901.

Asn-C-IV: Mechanism IV in the Asn251 channel (uncoupling reaction): O1-Fe cleavage, then proton transfer from Thr252 to O1, proton transfer from Wat901 to Thr252, and finally proton transfer from Asn251 to Wat901.

QM regions:

I. QM1: Iron-porphine (without heme side chains), sulfur atom of Cys357, axial HO_2 moiety, the $C^{\gamma 2}H_3$-$C^{\beta}H$-$O^{\gamma 1}H$ unit of Thr252, Wat901, WatS (only in model III), the $C^{\beta}H_2$-C^{γ} (=$O^{\delta 1}$)(-$N^{\delta 2}H_2$) unit of Asn251.

II. QM2: QM1 + ($CH_3CH_2NHC(NH_2)_2$) of Arg186 residue.

III. QM3: QM2 + Cys357, CO group of Leu356, and NH-CH unit of Leu358.

2. Computed energies, spin densities, and partial charges

- **D251N no flip model – mechanism II**

Table S1: Computed energies in Asn-C-II.

	Basis set	Cpd0	TS1	IC1	TS2	IC2	TS3	PC
QM/MM	B1	0.00	14.01	13.87	21.86	10.95	24.55	9.52
	B2	0.00	14.34	13.37	23.00	8.99	25.03	6.40

Table S2: Computed spin densities and Mulliken charges for doublet states of reactant, intermediates, and product in Asn-C-II.

			Fe	**OH[a]**	**O1**	**SH**	**Por**	**Thr252[b]**	**Asn251[c]**	**Wat901[d]**
Cpd0	B1	Spin	0.997	-0.003	0.096	-0.024	-0.065	---	---	---
		Charge	0.484	-0.087	-0.318	-0.454	-0.551	0.016	-0.136	---
	B2	Spin	1.024	-0.003	0.084	-0.024	-0.081	---	---	---
		Charge	0.340	-0.097	-0.231	-0.573	-0.392	0.017	-0.106	0.041
TS1	B1	Spin	1.284	-0.640	0.435	-0.047	-0.088	-0.006	---	---
		Charge	0.468	-0.071	-0.304	-0.474	-0.406	-0.012	-0.139	---
	B2	Spin	1.438	-0.640	0.435	-0.047	-0.088	-0.002	---	---
		Charge	0.234	-0.196	-0.228	-0.583	-0.122	-0.033	-0.113	0.041
IC1	B1	Spin	1.339	-0.736	0.550	-0.053	-0.097	-0.004	---	---
		Charge	0.462	-0.119	-0.347	-0.484	-0.400	-0.013	-0.138	0.046
	B2	Spin	1.439	-0.693	-0.432	-0.055	-0.119	---	---	---
		Charge	0.236	-0.179	-0.248	-0.591	-0.122	-0.030	-0.109	0.043
TS2	B1	Spin	1.470	-0.537	0.256	-0.079	-0.126	-0.084	---	-0.002
		Charge	0.485	0.552	-0.292	-0.440	-0.059	-0.004	-0.133	0.045
	B2	Spin	1.597	-0.351	-0.100	-0.075	-0.149	-0.117	---	-0.002
		Charge	0.197	-0.153	-0.176	-0.546	0.018	-0.237	-0.126	0.022
IC2	B1	Spin	1.345	-0.007	0.754	-0.024	-0.086	-0.989	---	-0.005
		Charge	0.472	0.004	-0.488	-0.502	-0.437	-0.213	-0.121	0.071
	B2	Spin	1.431	-0.003	0.699	-0.027	-0.117	-0.985	0.002	0.002
		Charge	0.286	-0.011	-0.443	-0.615	-0.201	-0.189	-0.091	0.059
TS3	B1	Spin	1.327	-0.001	0.780	-0.080	-0.325	-0.739	0.001	0.003
		Charge	0.493	-0.011	-0.424	-0.466	-0.213	0.353	0.093	0.163
	B2	Spin	1.411	-0.001	0.730	-0.083	-0.346	-0.718	0.001	0.006
		Charge	0.266	-0.008	-0.364	-0.573	0.113	0.266	0.147	0.139
PC	B1	Spin	1.204	-0.030	0.914	-0.166	-0.940	-0.001	---	---
		Charge	0.465	-0.010	-0.368	-0.430	0.277	-0.276	-0.807	-0.020
	B2	Spin	1.310	-0.002	0.838	-0.187	-0.955	-0.002	---	---
		Charge	0.261	-0.015	-0.314	-0.511	0.538	-0.309	-0.775	-0.075

[a] OH species bound or next to FeO unit, protonated in IC2, TS3 and PC. [b] Thr252 residue is deprotonated in IC2. [c] Asn251 is deprotonated in PC. [d] Wat901 is deprotonated in IC2.

Table S3: Computed energies in Asn-C-II in the presence of Arg186 (QM2).

	Basis set	Cpd0	IC1	IC2	PC
QM/MM	B1	0.00	14.78	11.58	8.19

Table S4: Computed spin densities and Mulliken charges for doublet states of reactant, intermediates, and product in Asn-C-II in the presence of Arg186 (QM2).

			Fe	OH[a]	O1	SH	Por	Thr252[b]	Asn251[c]	Wat901[d]	Arg186
Cpd0	B1	Spin	0.998	-0.003	0.094	-0.024	-0.065	---	---	---	---
		Charge	0.485	-0.087	-0.316	0.453	-0.550	0.016	-0.019	0.046	0.880
IC1	B1	Spin	1.339	-0.721	0.534	-0.052	-0.095	-0.004	---	---	---
		Charge	0.463	-0.125	-0.342	-0.484	-0.400	-0.020	-0.021	0.047	0.880
IC2	B1	Spin	1.334	-0.004	0.765	-0.024	-0.086	-0.992	0.001	0.005	---
		Charge	0.471	-0.020	-0.486	-0.502	-0.438	0.005	-0.012	0.073	0.887
PC	B1	Spin	1.205	-0.008	0.913	-0.166	-0.939	-0.004	---	---	---
		Charge	0.465	-0.013	-0.369	-0.429	0.277	-0.091	-0.652	-0.007	0.817

[a] OH species bound or next to FeO unit, protonated in IC2, TS3 and PC. [b] Thr252 residue is deprotonated in IC2. [c] Asn251 is deprotonated in PC. [d] Wat901 is deprotonated in IC2.

- **D251N flip model – mechanism II**

Table S5: Computed energies in Asn-C-II.

	Basis set	Cpd0	TS1	IC1	TS2	IC2	TS3	PC
QM/MM	B1	0.00	13.99	9.03	17.62	12.41	21.09	19.15
	B2	0.00	14.52	8.48	17.23	10.36	20.92	17.73

Table S6: Computed spin densities and Mulliken charges for doublet states of reactant, intermediates, and product in Asn-C-II.

			Fe	OH[a]	O1	SH	Por	Thr252[b]	Asn251[c]	Wat901[d]
Cpd0	B1	Spin	0.998	-0.003	0.095	-0.025	-0.065	---	---	---
		Charge	0.487	-0.081	-0.324	-0.461	-0.554	-0.006	-0.060	-0.060
	B2	Spin	1.024	-0.003	0.083	-0.024	-0.081	---	---	---
		Charge	0.341	-0.089	-0.237	-0.578	-0.396	0.004	-0.048	0.004
TS1	B1	Spin	1.285	-0.665	0.444	-0.048	-0.085	-0.006	---	---
		Charge	0.477	0.417	0.307	-0.478	-0.389	-0.010	-0.062	-0.009
	B2	Spin	1.437	-0.663	0.399	-0.052	-0.115	-0.006	---	---
		Charge	0.238	-0.193	-0.252	-0.585	-0.125	-0.034	-0.051	0.004
IC1	B1	Spin	1.486	-0.980	0.625	-0.029	-0.110	0.008	---	---
		Charge	0.499	-0.023	-0.550	-0.460	-0.363	-0.027	-0.067	---
	B2	Spin	1.570	-0.967	0.561	-0.033	-0.135	0.004	---	---
		Charge	0.273	-0.071	-0.465	-0.572	-0.079	-0.041	-0.055	0.010
TS2	B1	Spin	1.317	-0.302	0.800	-0.088	-0.502	-0.234	---	---
		Charge	0.486	-0.220	-0.460	-0.475	-0.073	-0.236	-0.072	-0.026
	B2	Spin	1.396	-0.392	0.741	-0.097	-0.554	-0.093	---	---
		Charge	0.276	-0.294	-0.400	-0.574	0.220	-0.186	-0.059	-0.013
IC2	B1	Spin	1.344	-0.001	0.760	-0.064	-0.285	-0.765	---	---
		Charge	0.482	-0.030	-0.473	-0.486	-0.261	-0.360	-0.061	-0.008
	B2	Spin	1.430	-0.003	0.705	-0.074	-0.318	-0.793	---	---
		Charge	0.281	-0.024	-0.417	-0.589	0.010	-0.372	-0.053	0.004
TS3	B1	Spin	1.296	-0.004	0.816	-0.119	-0.645	-0.219	0.002	-0.123
		Charge	0.474	-0.013	-0.431	-0.452	0.056	-0.067	-0.082	-0.093
	B2	Spin	1.388	-0.003	0.756	-0.142	-0.709	-0.153	0.002	-0.136
		Charge	0.269	-0.047	-0.373	-0.540	0.341	-0.159	-0.079	-0.118
PC	B1	Spin	1.290	-0.001	0.825	-0.132	-0.691	-0.009	-0.004	-0.288
		Charge	0.468	-0.064	-0.421	-0.444	-0.087	-0.088	-0.088	-0.425
	B2	Spin	1.387	-0.008	0.760	-0.154	-0.753	-0.010	-0.004	-0.232
		Charge	0.262	-0.063	-0.360	-0.529	0.039	-0.123	-0.075	-0.482

[a] OH species bound or next to FeO unit, protonated in IC2, TS3 and PC. [b] Thr252 residue is deprotonated in IC2. [c] Asn251 is deprotonated in PC. [d] Wat901 is deprotonated in IC2.

Table S7: Computed energies in Asn-C-II in the presence of Arg186 (QM2).

	Basis set	Cpd0	IC1	IC2	PC
QM/MM	B1	0.00	9.07	12.29	18.83

Table S8: Computed spin densities and Mulliken charges for doublet states of reactant, intermediates, and product in the Asn-C-II in the presence of Arg186 (QM2).

			Fe	OH[a]	O1	SH	Por	Thr252[b]	Asn251[c]	Wat901[d]	Arg186
Cpd0	B1	Spin	0.998	-0.003	0.095	-0.025	-0.065	---	---	---	---
		Charge	0.487	-0.082	-0.323	-0.460	-0.554	0.006	-0.008	-0.011	0.870
IC1	B1	Spin	1.486	0.979	0.624	-0.028	-0.111	-0.008	---	---	---
		Charge	0.497	-0.023	-0.550	-0.459	-0.363	-0.027	0.061	-0.005	0.869
IC2	B1	Spin	1.339	-0.009	0.766	-0.066	0.306	-0.730	---	0.006	---
		Charge	0.480	-0.026	-0.470	-0.484	-0.241	-0.186	0.063	-0.005	0.870
PC	B1	Spin	1.289	0.006	0.827	-0.135	-0.709	-0.018	-0.002	-0.263	---
		Charge	0.468	-0.064	-0.420	-0.441	0.104	-0.115	0.040	-0.435	0.864

[a] H_2O refers to IC2, TS3 and PC, and OH to other species, [b] Thr252 residue is not protonated in IC2, [c] Asn251 is not protonated in PC, [d] OH refers to IC2, and H_2O to other species.

- **D251N flip with WatS model – mechanism II**

Table S9: Computed energies in the Asn-C-II.

	Basis set	Cpd0	TS1	IC1	TS2	PC
QM/MM	B1	0.00	14.03	11.76	12.08	7.64
	B2	0.00	13.91	12.43	12.87	6.70

Table S10: Computed spin densities and Mulliken charges for doublet states of reactant, intermediates, and product in the Asn-C-II.

			Fe	OH[a]	O1	SH	Por	Thr252[b]	Asn251[c]	Wat901[d]
Cpd0	B1	Spin	1.041	-0.003	0.062	-0.034	-0.065	---	---	---
		Charge	0.502	-0.076	-0.366	-0.456	-0.540	0.019	-0.020	0.034
	B2	Spin	1.063	-0.003	0.055	-0.033	-0.081	---	---	---
		Charge	0.361	-0.070	-0.309	-0.572	-0.370	0.015	-0.043	0.026
TS1	B1	Spin	1.330	-0.638	0.391	-0.052	-0.091	-0.004	---	---
		Charge	0.472	-0.055	-0.329	-0.471	-0.379	-0.013	-0.021	0.033
	B2	Spin	1.486	-0.630	0.326	-0.057	-0.121	-0.004	---	---
		Charge	0.232	-0.238	-0.255	-0.579	-0.082	-0.019	-0.044	0.029
IC1	B1	Spin	1.498	-0.984	0.620	-0.035	-0.111	0.012	---	---
		Charge	0.514	-0.050	-0.555	-0.453	-0.353	-0.012	-0.020	0.033
	B2	Spin	1.580	-0.953	0.555	-0.043	-0.148	-0.008	----	---
		Charge	0.261	-0.123	-0.444	-0.560	-0.055	-0.016	-0.044	0.027
TS2	B1	Spin	1.281	-0.249	0.837	-0.109	-0.788	-0.005	---	---
		Charge	0.470	-0.469	-0.425	-0.460	0.218	-0.088	0.020	-0.076
	B2	Spin	1.371	-0.102	0.779	-0.137	-0.897	-0.012	---	---
		Charge	0.260	-0.396	-0.359	-0.541	0.483	-0.317	-0.058	-0.051
PC	B1	Spin	1.260	-0.004	0.862	-0.177	-0.939	---	---	-0.248
		Charge	0.473	-0.055	-0.398	-0.413	0.301	-0.227	-0.050	-0.543
	B2	Spin	1.359	-0.006	0.792	-0.196	-0.955	---	---	-0.246
		Charge	0.254	-0.057	-0.335	-0.498	0.565	-0.067	-0.061	-0.583

[a] OH species bound or next to FeO unit, protonated in IC2, TS3 and PC. [b] Thr252 residue is deprotonated in IC2. [c] Asn251 is deprotonated in PC. [d] Wat901 is deprotonated in IC2.

Table S11: Computed energies in Asn-C-II in the presence of Arg186 (QM2).

	Basis set	Cpd0	IC1	PC
QM/MM	B1	0.00	11.91	6.07

Table S12: Computed spin densities and Mulliken charges for doublet states of reactant, intermediates, and product in the Asn-C-II in the presence of Arg186 (QM2).

			Fe	OH[a]	O1	SH	Por	Thr252[b]	Asn251[c]	Wat901[d]	Arg186
Cpd0	B1	Spin	1.043	-0.003	0.061	-0.036	-0.066	---	---	---	---
		Charge	0.502	-0.076	-0.368	-0.315	-0.539	0.019	0.058	0.027	0.827
IC1	B1	Spin	1.500	-0.984	0.619	-0.035	0.112	0.012	---	---	---
		Charge	0.514	-0.051	-0.556	-0.451	-0.352	-0.011	-0.057	0.022	0.822
PC	B1	Spin	1.264	-0.001	0.858	-0.180	-0.940	-0.005	---	---	---
		Charge	0.474	-0.033	-0.400	-0.409	0.305	-0.099	0.027	-0.528	0.786

[a] OH species bound or next to FeO unit, protonated in IC2, TS3 and PC. [b] Thr252 residue is deprotonated in IC2. [c] Asn251 is deprotonated in PC. [d] Wat901 is deprotonated in IC2.

- **D251N no flip model – mechanism III**

Table S13: Computed energies in Asn-C-III.

	Basis set	Cpd0	TS1	PC
QM/MM	B1	0.00	29.91	21.14

Table S14: Computed spin densities and Mulliken charges for doublet states of reactant, intermediates, and product in Asn-C-III.

			Fe	OH	O1	SH	Por	Thr252	Asn251
Cpd0	B1	Spin	0.997	-0.003	0.096	-0.024	-0.065	---	---
		Charge	0.484	-0.087	-0.318	-0.454	-0.551	0.016	-0.136
TS1	B1	Spin	1.213	---	0.005	-0.117	-0.079	-0.006	---
		Charge	0.612	0.184	-0.292	-0.334	-0.382	-0.099	-0.058
PC	B1	Spin	1.372	-0.004	-0.001	-0.156	-0.209	-0.001	---
		Charge	0.417	-0.084	-0.473	-0.244	-0.258	-0.188	-0.178

- **D251N flip model – mechanism III**

Table S15: Computed energies in Asn-C-III

	Basis set	Cpd0	TS1	PC
QM/MM	B1	0.00	30.43	25.30

Table S16: Computed spin densities and Mulliken charges for doublet states of reactant, intermediates, and product in Asn-C-III.

			Fe	OH	O1	SH	Por	Thr252	Asn251
Cpd0	B1	Spin	0.998	-0.003	0.095	-0.025	-0.065	---	---
		Charge	0.487	-0.081	-0.324	-0.461	-0.554	-0.006	-0.060
TS1	B1	Spin	1.228	---	-0.001	-0.123	-0.082	-0.009	---
		Charge	0.610	0.156	-0.282	-0.337	-0.390	-0.055	-0.034
PC	B1	Spin	1.337	-0.004	-0.002	-0.156	-0.207	-0.004	---
		Charge	0.427	-0.079	-0.457	-0.252	-0.263	-0.231	-0.092

- **D251N flip with WatS model - mechanism III**

Table S17: Computed energies in Asn-C-III.

	Basis set	Cpd0	TS1	PC
QM/MM	B1	0.00	22.56	19.88

Table S18: Computed spin densities and Mulliken charges for doublet states of reactant, intermediates, and product in Asn-C-III.

			Fe	**OH**	**O1**	**SH**	**Por**	**Thr252**	**Asn251**
Cpd0	B1	Spin	1.041	-0.003	0.062	-0.034	-0.065	---	---
		Charge	0.502	-0.076	-0.366	-0.456	-0.540	0.019	-0.020
TS1	B1	Spin	1.773	-0.166	-0.284	-0.127	-0.301	0.006	---
		Charge	0.400	0.352	-0.422	-0.419	-0.345	0.055	0.007
PC	B1	Spin	1.440	-0.003	-0.004	-0.178	-0.255	---	---
		Charge	0.413	-0.028	-0.572	-0.255	-0.216	0.144	-0.029

- **D251N no flip model – mechanism IV**

Table S19: Computed energies in Asn-C-IV.

	Basis set	Cpd0	TS1	IC1	TS2	PC
QM/MM	B1	0.00	27.05	24.16	25.26	21.43

Table S20: Computed spin densities and Mulliken charges for doublet states of reactant, intermediates, and product in Asn-C-IV.

			Fe	**OH**	**O1**	**SH**	**Por**	**Thr252**	**Asn251**
Cpd0	B1	Spin	0.997	-0.003	0.096	-0.024	-0.065	---	---
		Charge	0.484	-0.087	-0.318	-0.454	-0.551	0.016	-0.136
TS1	B1	Spin	1.886	-0.243	-0.633	-0.046	-0.151	0.002	---
		Charge	0.479	0.308	-0.133	-0.528	-0.590	0.041	-0.130
IC1	B1	Spin	1.935	-0.227	-0.522	-0.013	-0.180	0.007	---
		Charge	0.417	0.116	-0.297	-0.567	-0.573	-0.007	-0.136
TS2	B1	Spin	1.806	-0.183	-0.363	-0.110	-0.236	0.007	---
		Charge	0.394	0.248	-0.396	-0.442	-0.432	0.077	-0.096
PC	B1	Spin	1.458	-0.013	-0.014	-0.174	-0.256	---	---
		Charge	0.400	-0.027	-0.569	-0.261	-0.236	-0.152	-0.172

- **D251N flip model – mechanism IV**

Table S21: Computed energies in Asn-C-IV.

	Basis set	Cpd0	TS1	IC1	TS2	PC
QM/MM	B1	0.00	26.58	20.81	28.67	25.61

Table S22: Computed spin densities and Mulliken charges for doublet states of reactant, intermediates, and product in Asn-C-IV.

			Fe	OH	O1	SH	Por	Thr252	Asn251
Cpd0	B1	Spin	0.998	-0.003	0.095	-0.025	-0.065	---	---
		Charge	0.487	-0.081	-0.324	-0.461	-0.554	-0.006	-0.060
TS1	B1	Spin	1.902	-0.230	-0.634	-0.034	-0.140	0.001	---
		Charge	0.487	0.328	-0.141	-0.546	-0.608	0.061	-0.061
IC1	B1	Spin	1.942	-0.249	-0.539	0.002	-0.161	0.005	---
		Charge	0.416	0.135	-0.271	-0.579	-0.591	-0.034	-0.066
TS2	B1	Spin	1.625	-0.087	-0.120	-0.162	-0.246	-0.027	---
		Charge	0.395	0.085	-0.473	-0.332	-0.331	-0.105	-0.078
PC	B1	Spin	1.436	-0.001	-0.001	-0.171	-0.261	-0.001	---
		Charge	0.394	-0.080	-0.483	-0.253	-0.223	-0.213	-0.086

- **D251N flip with WatS model – mechanism IV**

Table S23: Computed energies in Asn-C-IV.

	Basis set	Cpd0	TS1	IC1	TS2	PC
QM/MM	B1	0.00	23.23	21.32	22.94	21.02

Table S24: Computed spin densities and Mulliken charges for doublet states of reactant, intermediates, and product in Asn-C-IV.

			Fe	OH	O1	SH	Por	Thr252	Asn251
Cpd0	B1	Spin	1.041	-0.003	0.062	-0.034	-0.065	---	---
		Charge	0.502	-0.076	-0.366	-0.456	-0.540	0.019	-0.020
TS1	B1	Spin	1.911	-0.182	-0.572	-0.043	-0.176	0.002	---
		Charge	0.474	0.211	-0.200	-0.547	-0.592	0.056	-0.018
IC1	B1	Spin	1.903	-0.194	-0.437	-0.049	-0.229	-0.006	---
		Charge	0.417	0.086	-0.358	-0.534	-0.502	-0.013	-0.021
TS2	B1	Spin	1.660	-0.082	-0.174	-0.160	-0.254	0.001	---
		Charge	0.420	0.034	-0.537	-0.359	-0.340	-0.028	-0.013
PC	B1	Spin	1.449	-0.005	-0.006	-0.176	-0.261	---	---
		Charge	0.419	-0.031	-0.575	-0.263	-0.213	-0.147	-0.035

3. Optimized geometries and relatives energies of mechanisms III and IV

- **D251N no flip model – mechanism III**

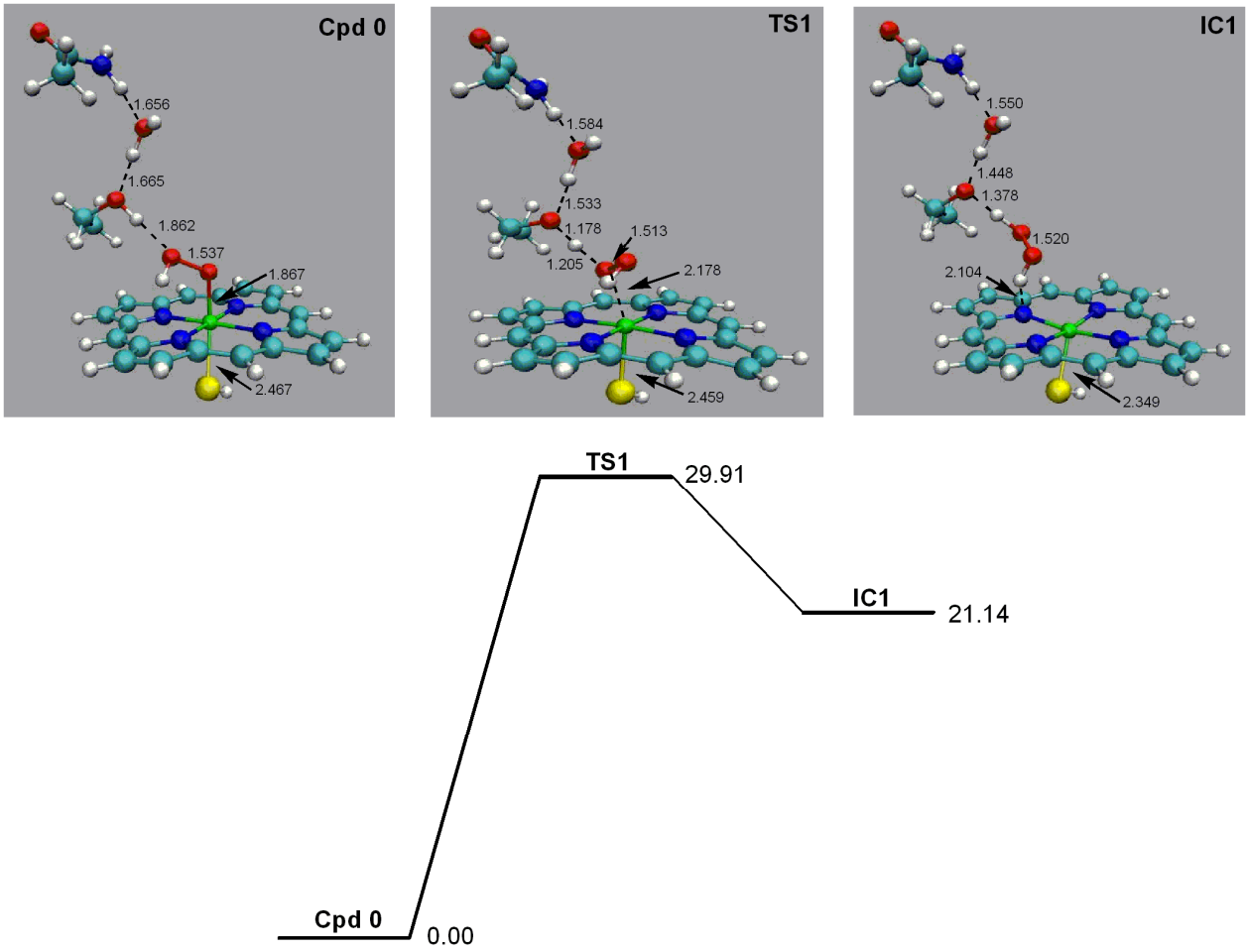

Figure S1. (a): Optimized geometries of Cpd 0, TS1, and PC (UB3LYP/B1/CHARMM) for the no flip model. Only the QM region is shown. (b): Energy profile of mechanism III. Energy in kcal/mol relative to Cpd 0.

- **D251N flip model – mechanism III**

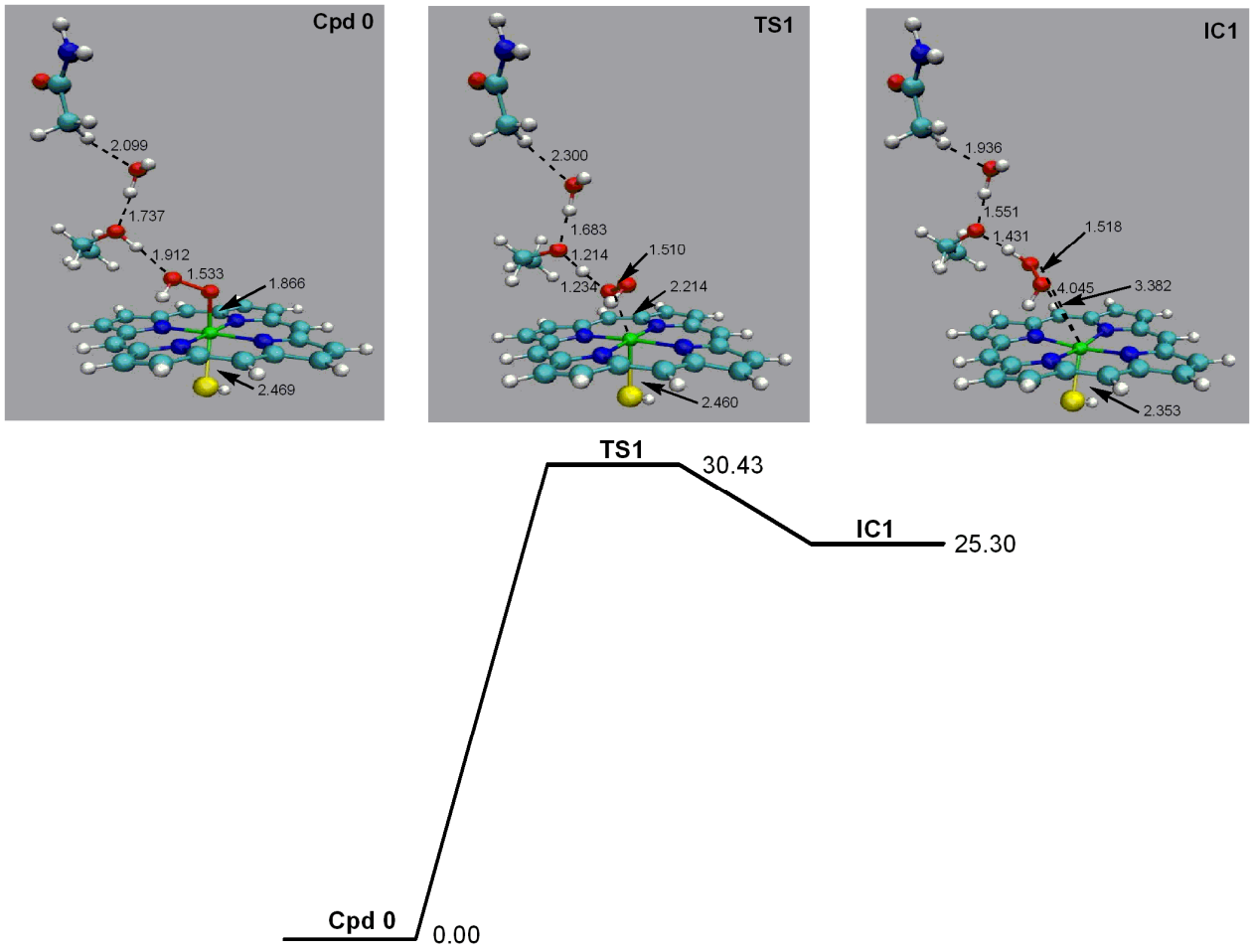

Figure S2. (a): Optimized geometries of Cpd 0, TS1, and PC (UB3LYP/B1/CHARMM) for the flip model. Only the QM region is shown. (b): Energy profile of mechanism III. Energy in kcal/mol relative to Cpd 0.

- **D251N flip with WatS model – mechanism III**

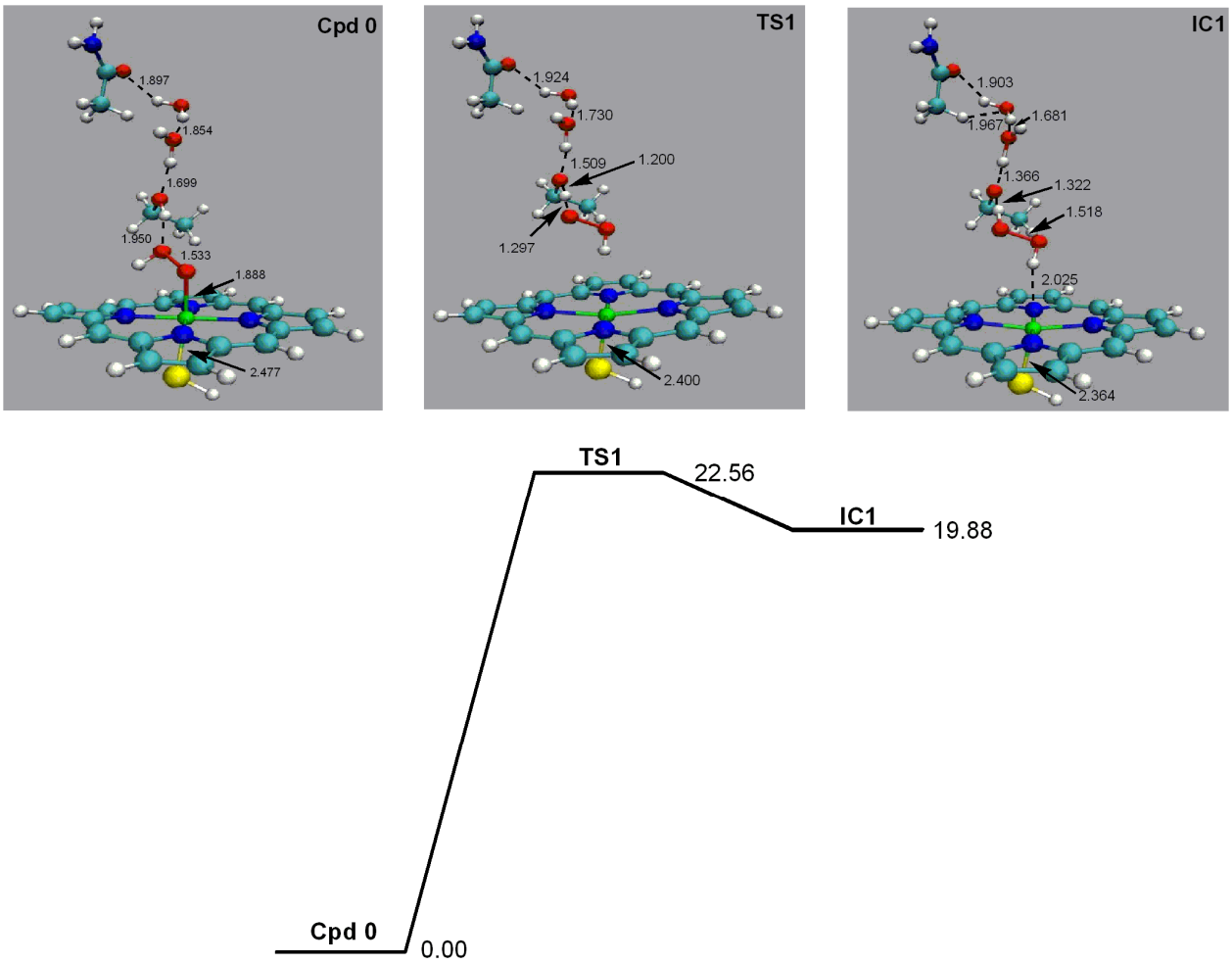

Figure S3. (a): Optimized geometries of Cpd 0, TS1, and PC (UB3LYP/B1/CHARMM) for the flip with WatS model. Only the QM region is shown. (b): Energy profile of mechanism III. Energy in kcal/mol relative to Cpd 0.

- **D251N no flip model – mechanism IV**

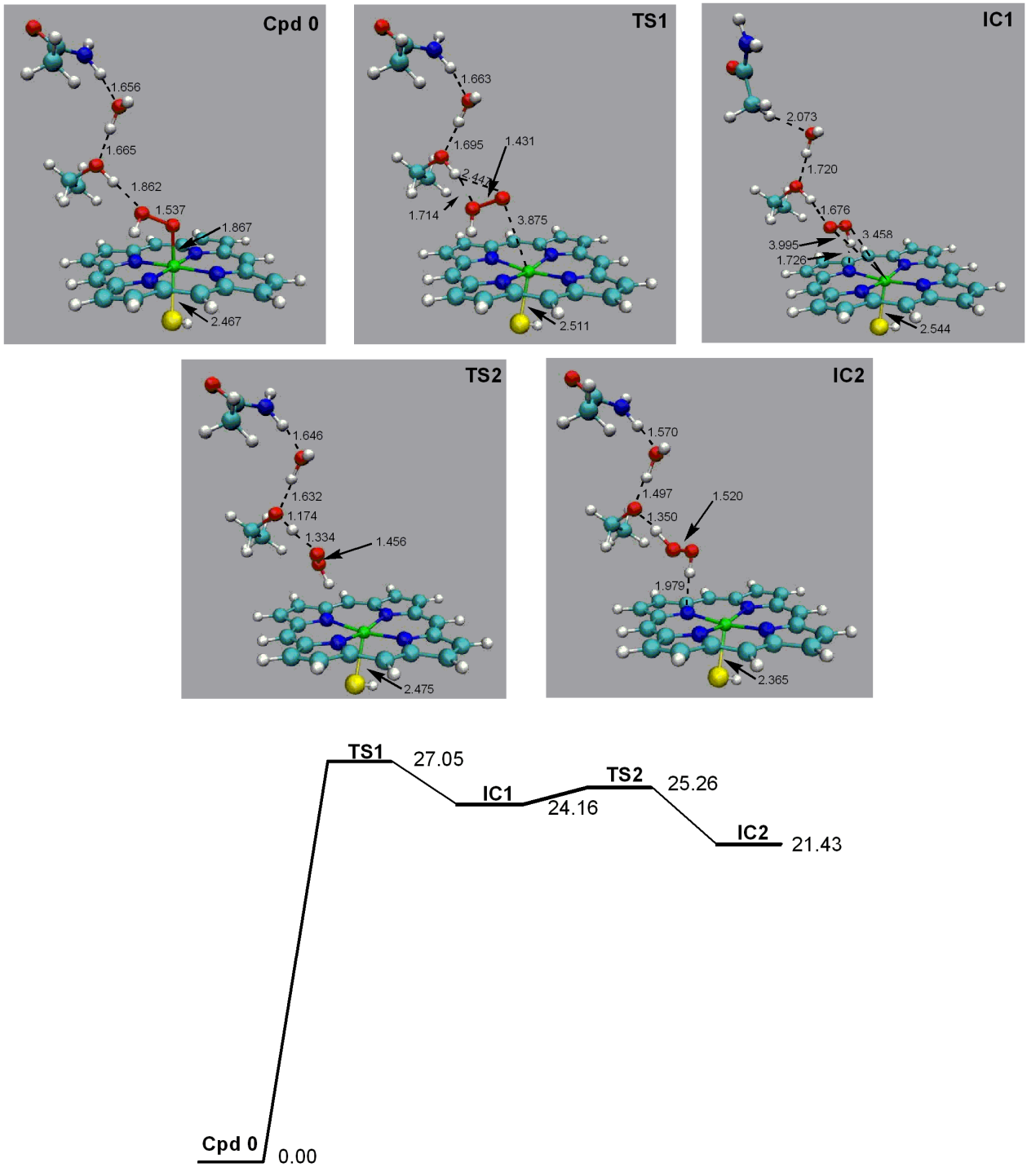

Figure S4. (a): Optimized geometries of Cpd 0, TS1, IC1, TS2, and PC (UB3LYP/B1/CHARMM) for the no flip model. Only the QM region is shown. (b): Energy profile of mechanism IV. Energy in kcal/mol relative to Cpd 0.

- **D251N flip model – mechanism IV**

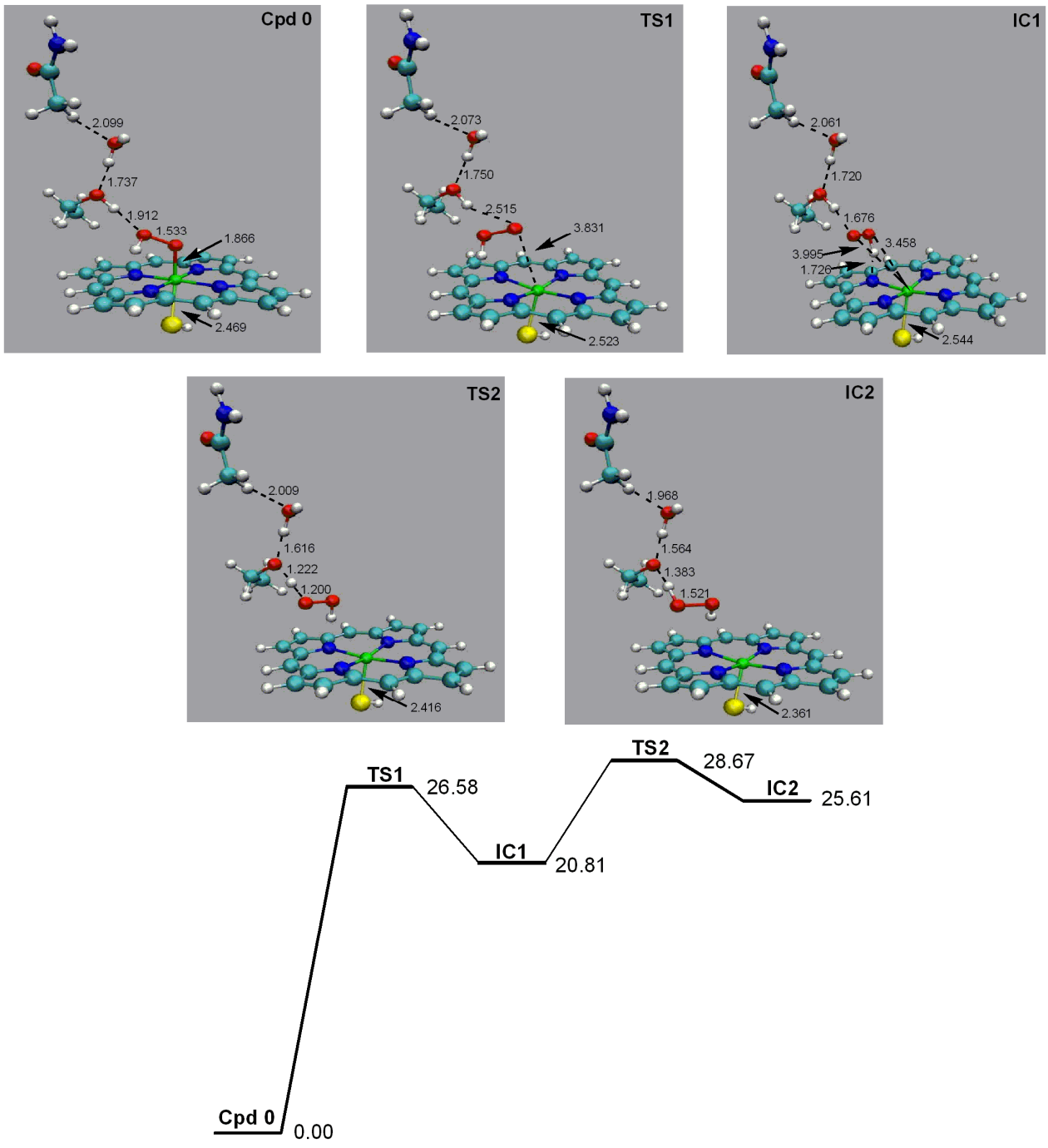

Figure S5. (a): Optimized geometries of Cpd 0, TS1, IC1, TS2, and PC (UB3LYP/B1/CHARMM) for the flip model. Only the QM region is shown. (b): Energy profile of mechanism IV. Energy in kcal/mol relative to Cpd 0.

- **D251N flip with WatS model – mechanism IV**

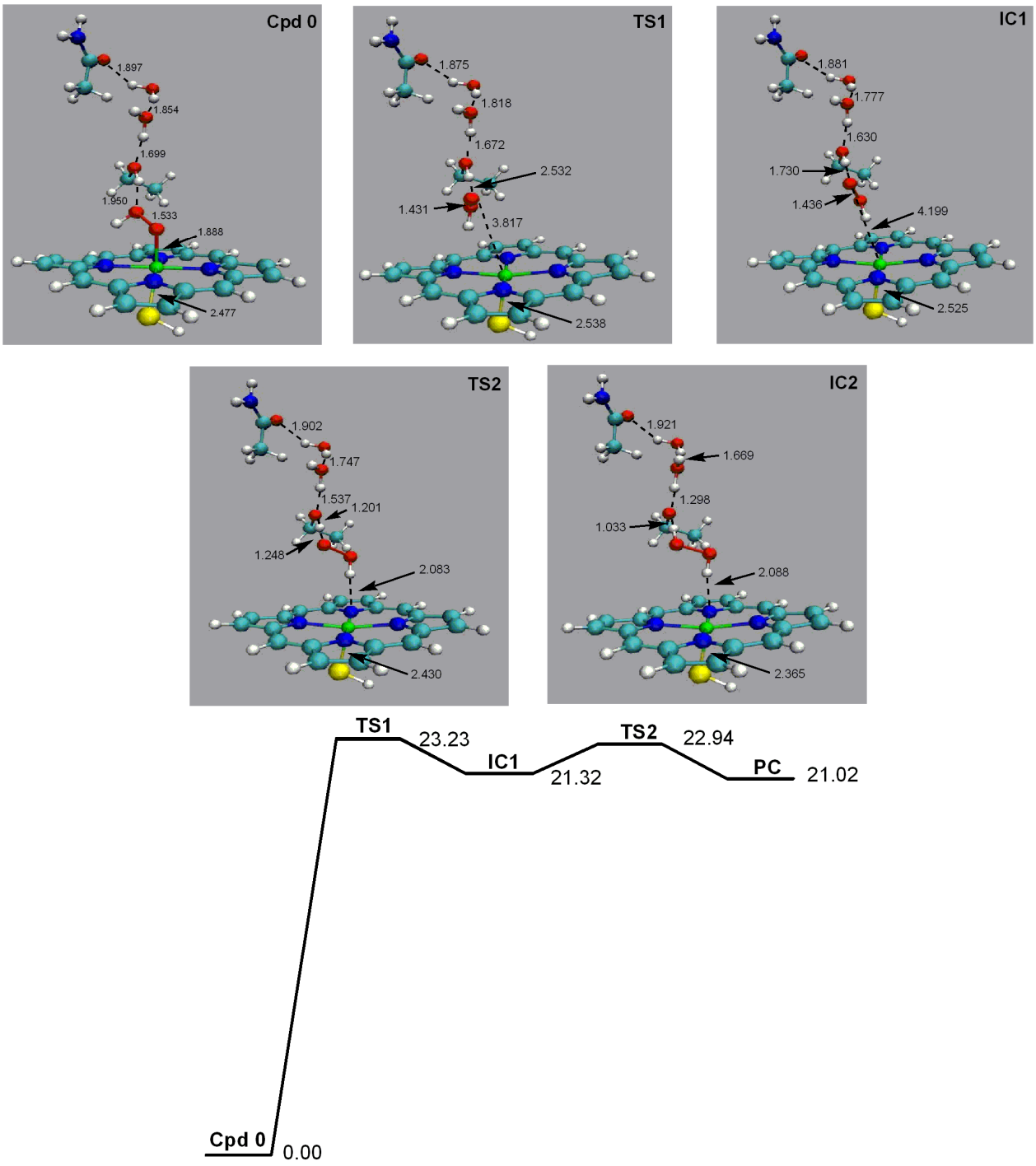

Figure S6. (a): Optimized geometries of Cpd 0, TS1, IC1, TS2, and PC (UB3LYP/B1/CHARMM) for the flip with WatS model. Only the QM region is shown. (b): Energy profile of mechanism IV. Energy in kcal/mol relative to Cpd 0.

- **QM region QM2 – mechanism II**

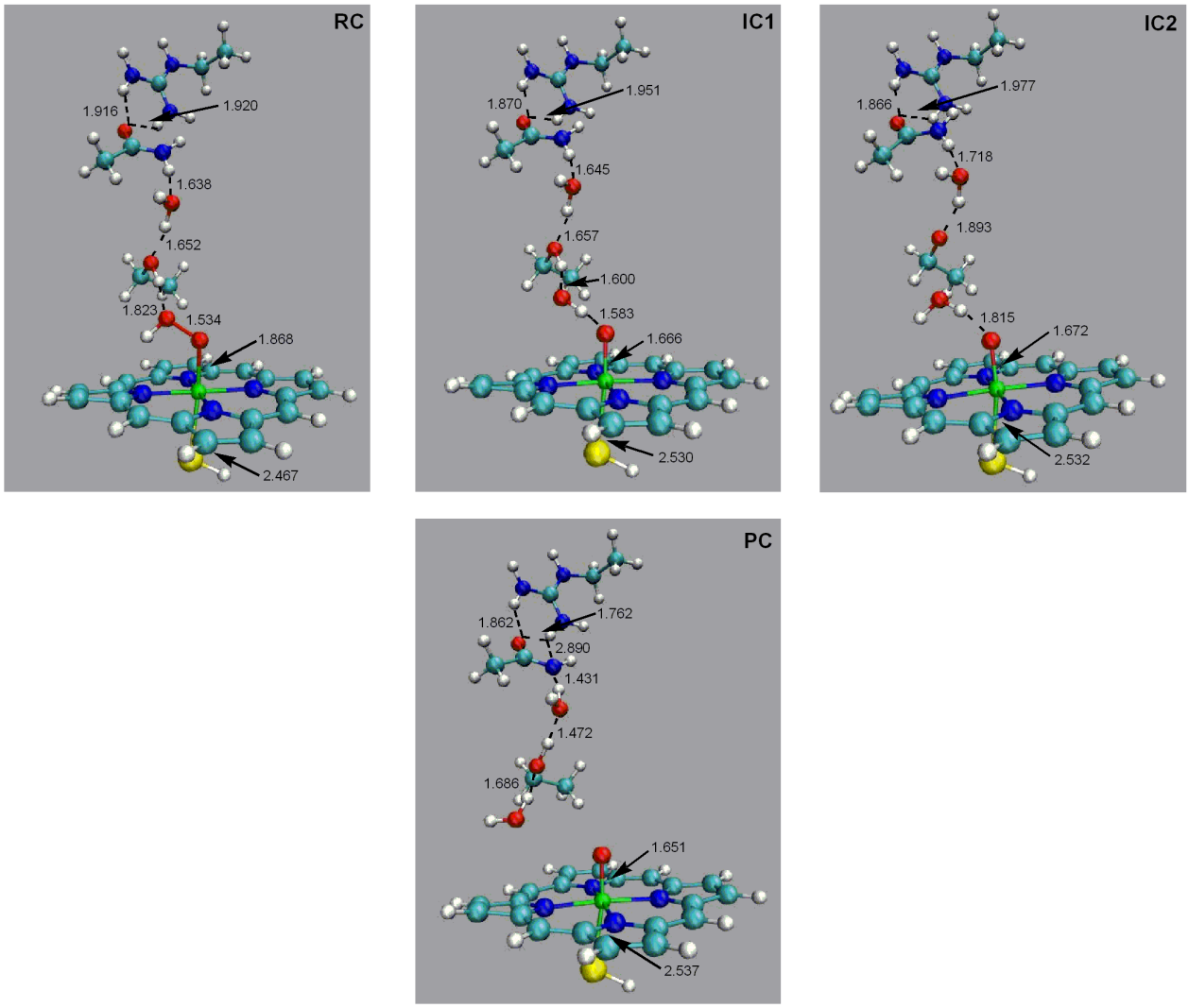

Figure S7. Optimized geometries of Cpd 0, IC1, IC2, and PC of mechanism II (UB3LYP/B1/CHARMM) for the no flip model with QM region QM2. Only the QM region is shown.

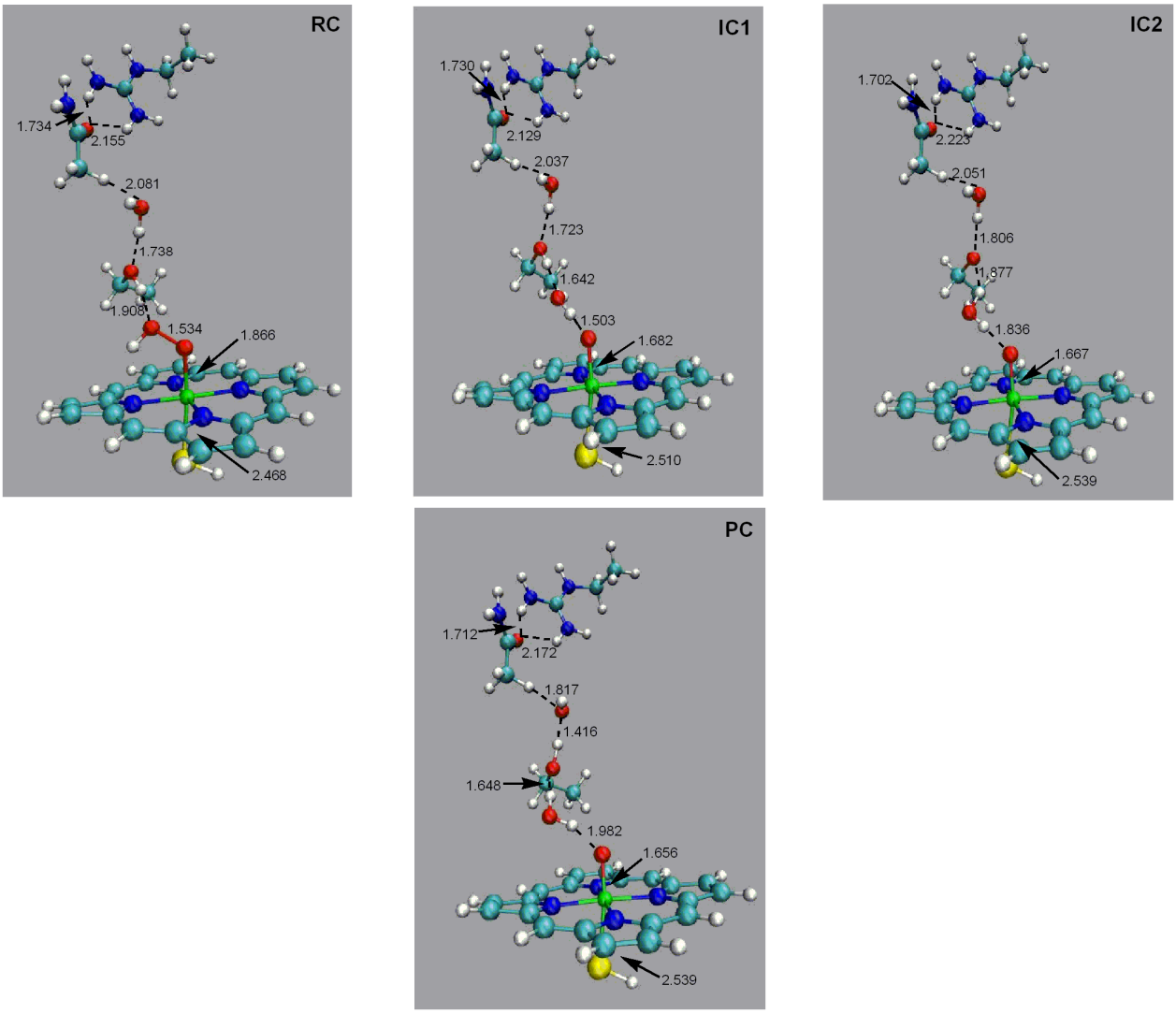

Figure S8. Optimized geometries of Cpd 0, IC1, IC2, and PC of mechanism II (UB3LYP/B1/CHARMM) for the flip model with QM region QM2. Only the QM region is shown.

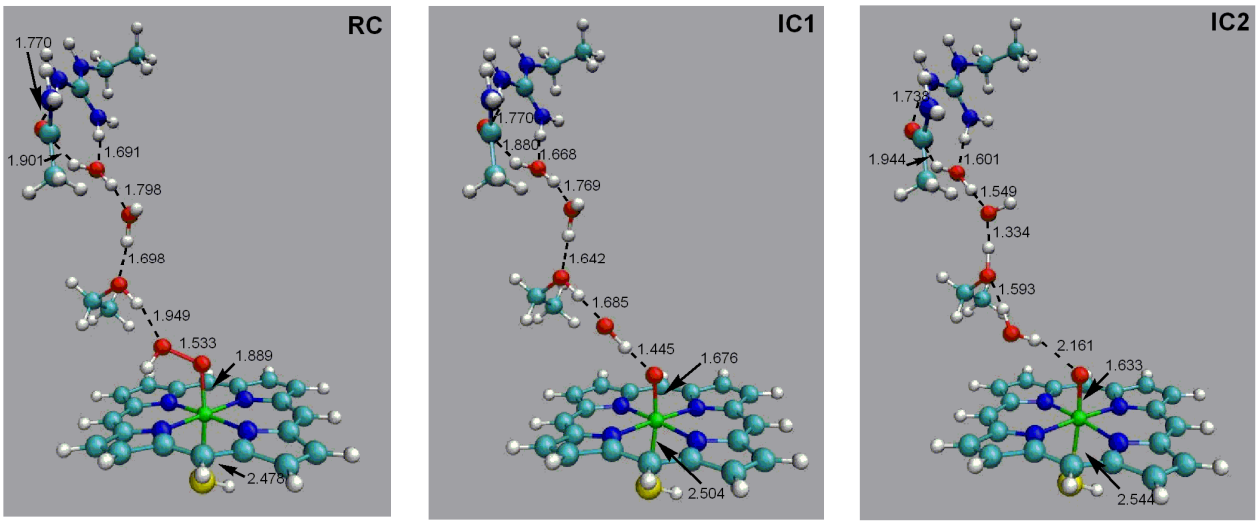

Figure S9. Optimized geometries of Cpd 0, IC1, IC2, and PC of mechanism II (UB3LYP/B1/CHARMM) for the flip with WatS model with QM region QM2. Only the QM region is shown.

4. QM/MM energy profiles (UB3LYP/B1/CHARMM) from path calculations

- **D251N no flip model**

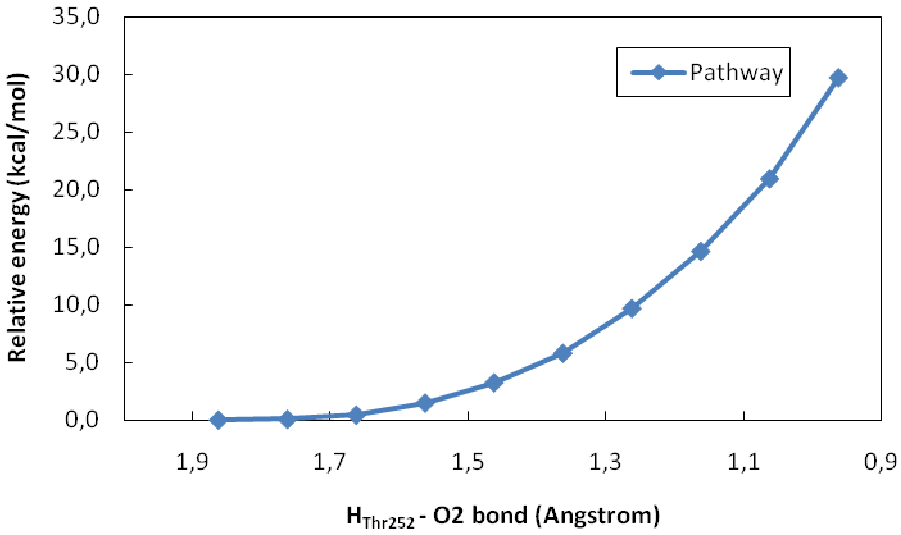

Figure S10. Energy profile for the first step of mechanism I (direct proton transfer from Thr252 to O2) for the no flip model. Energies are given in kcal/mol relative to Cpd 0 (RC).

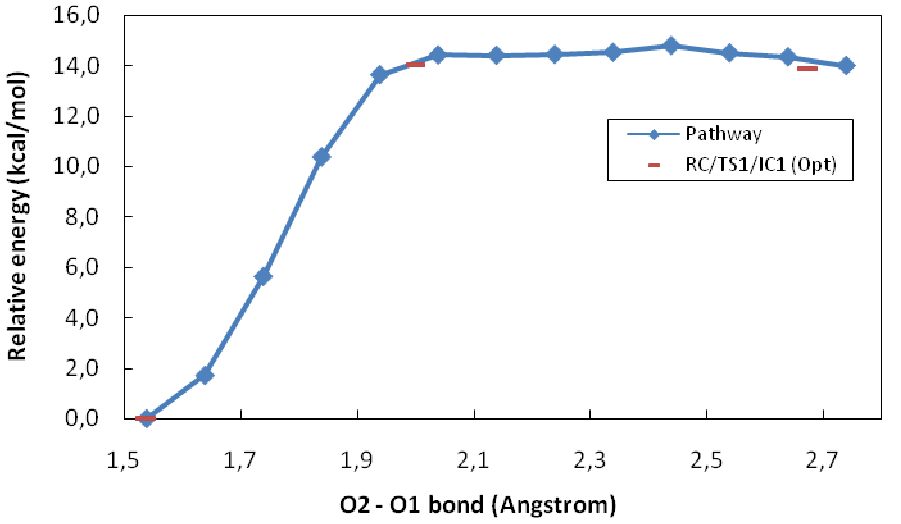

Figure S11. Energy profile for the first step of mechanism II (O1 – O2 bond cleavage) for the no flip model. Energies are given in kcal/mol relative to Cpd 0 (RC).

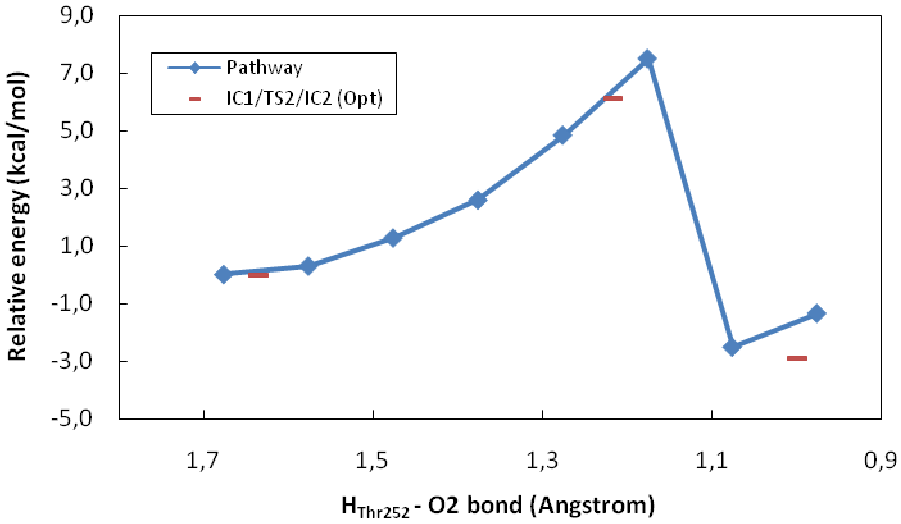

Figure S12. Energy profile for the second step of mechanism II (proton transfer from Thr252 to O2) for the no flip model. Energies are given in kcal/mol relative to IC1.

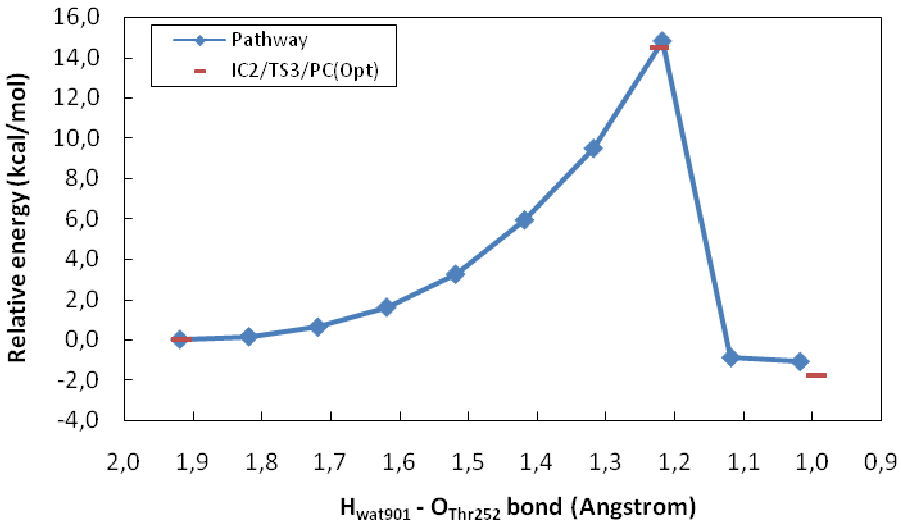

Figure S13. Energy profile for the third step of mechanism II (proton transfer from Wat901 to O_{Thr252}) for the no flip model. Energies are given in kcal/mol relative to IC2.

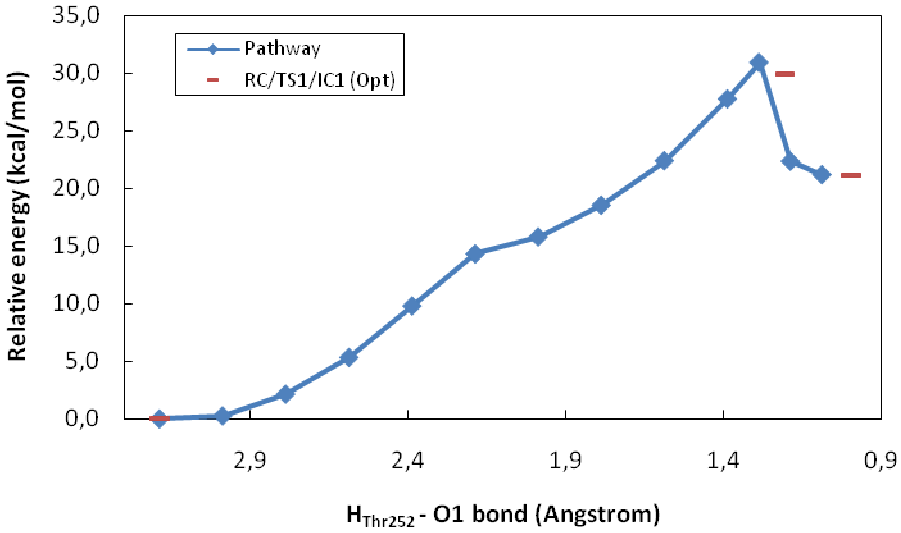

Figure S14. Energy profile for the first step of mechanism III (direct proton transfer from Thr252 to O1) for the no flip model. Energies are given in kcal/mol relative to Cpd 0 (RC).

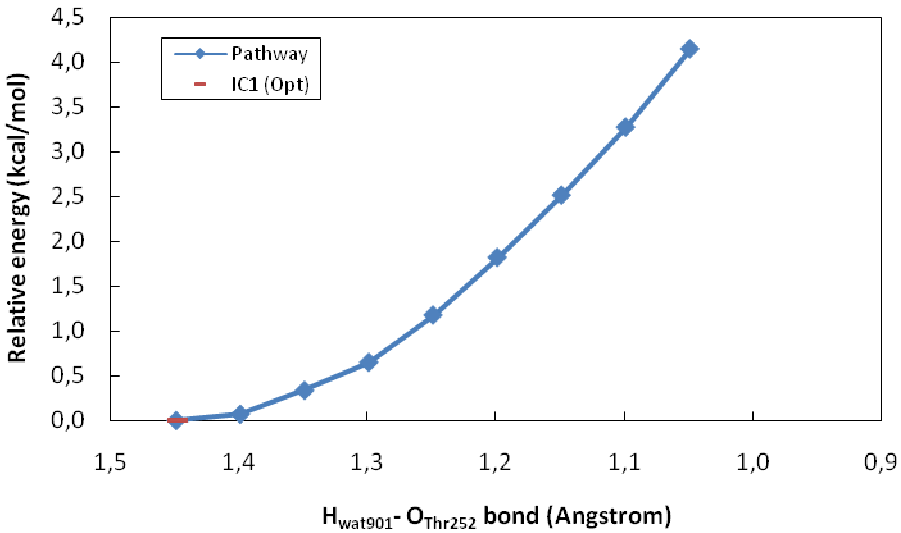

Figure S15. Energy profile for the second step of mechanism III (proton transfer from Wat901 to O_{Thr252}) for the no flip model. Energies are given in kcal/mol relative to IC1.

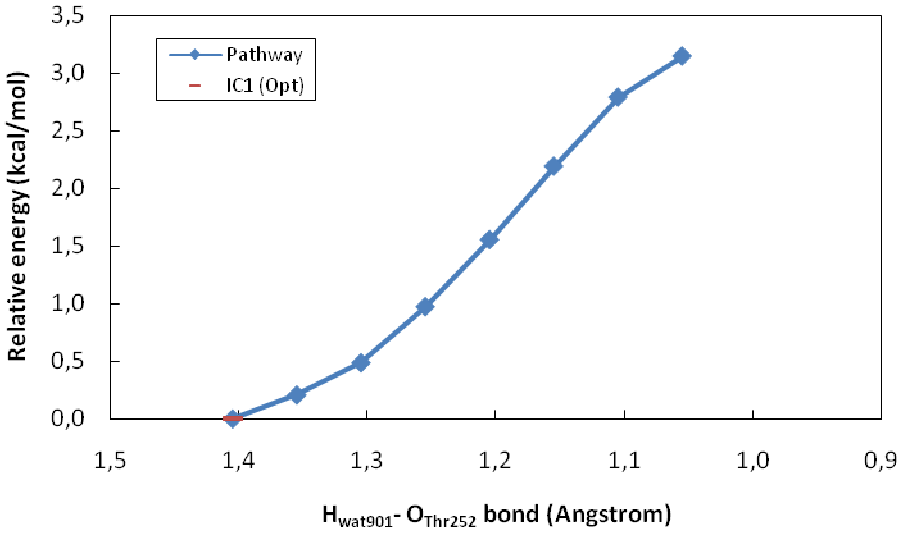

Figure S16. Energy profile for the second step of mechanism III (proton transfer from Wat901 to O_{Thr252}) for the no flip model with the QM region QM2. Energies are given in kcal/mol relative to Cpd 0 (RC).

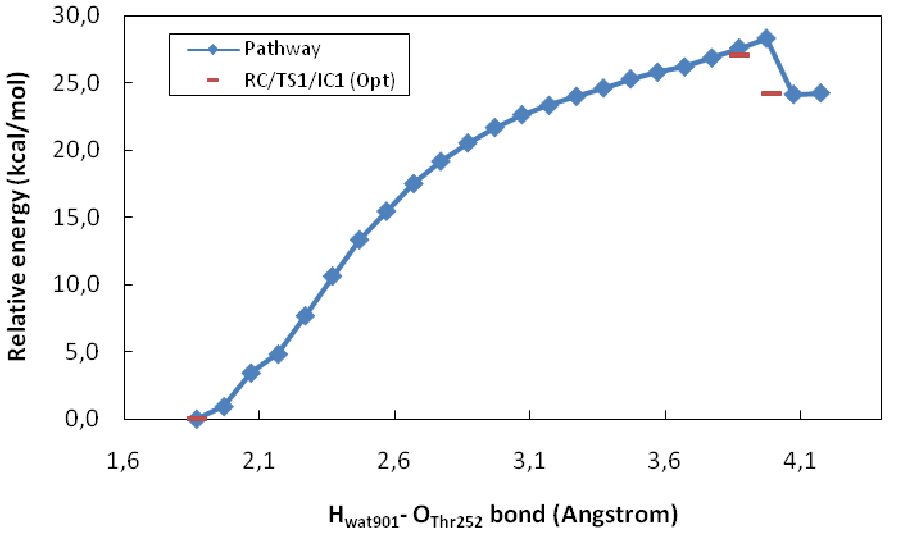

Figure S17. Energy profile for the first step of mechanism IV (Fe – O1 bond cleavage) for the no flip model. Energies are given in kcal/mol relative to Cpd 0 (RC).

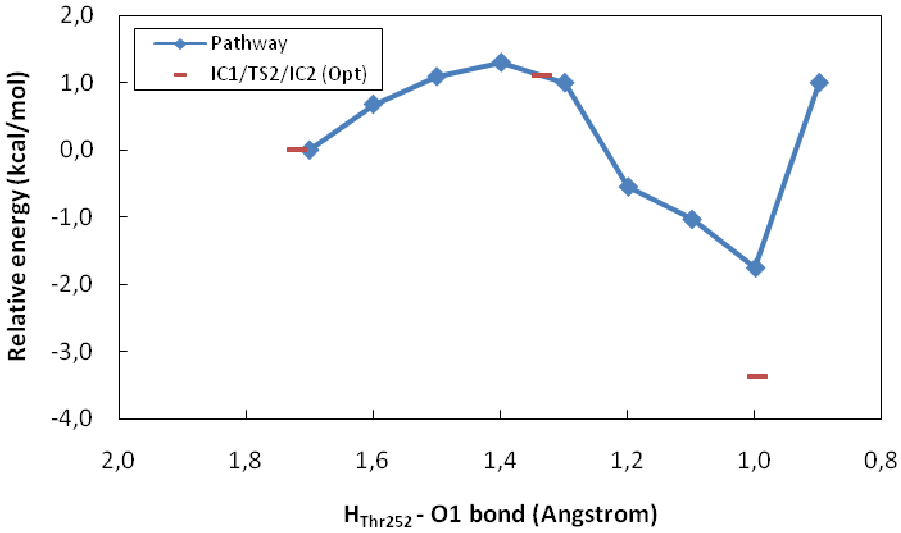

Figure S18. Energy profile for the second step of mechanism IV (proton transfer from Thr252 to O1) in the no flip model. Energies are given in kcal/mol relative to IC1.

- **D251N flip model:**

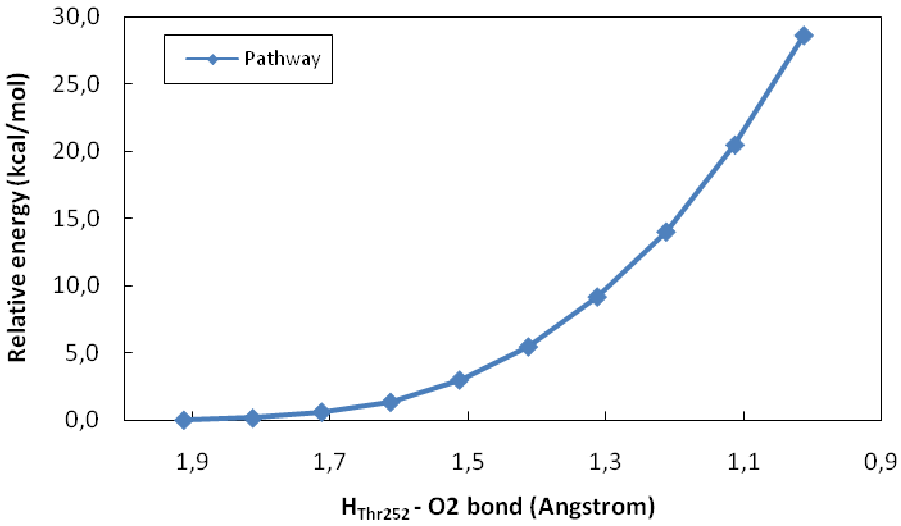

Figure S19. Energy profile for the first step of mechanism I (direct proton transfer from Thr252 to O2) for the flip model. Energies are given in kcal/mol relative to Cpd 0 (RC).

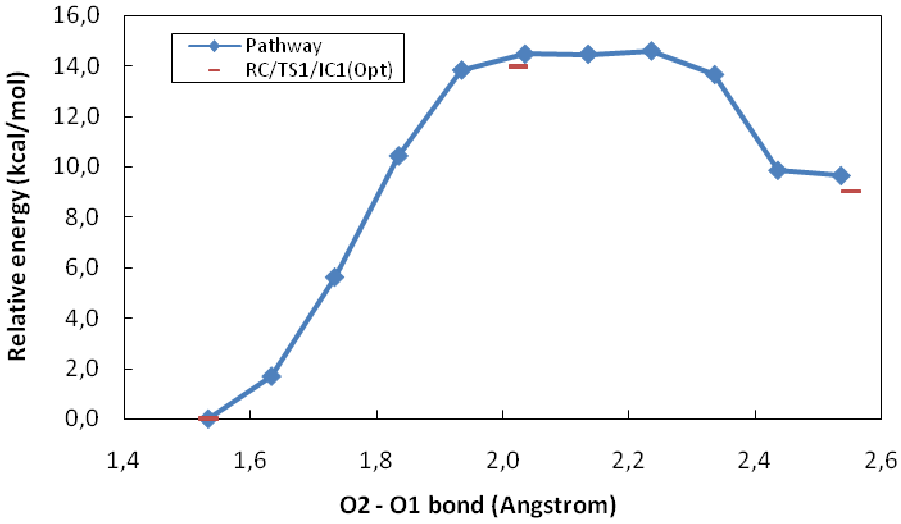

Figure S20. Energy profile for the first step of mechanism II (O1 – O2 bond cleavage) for the flip model. Energies are given in kcal/mol relative to Cpd 0 (RC).

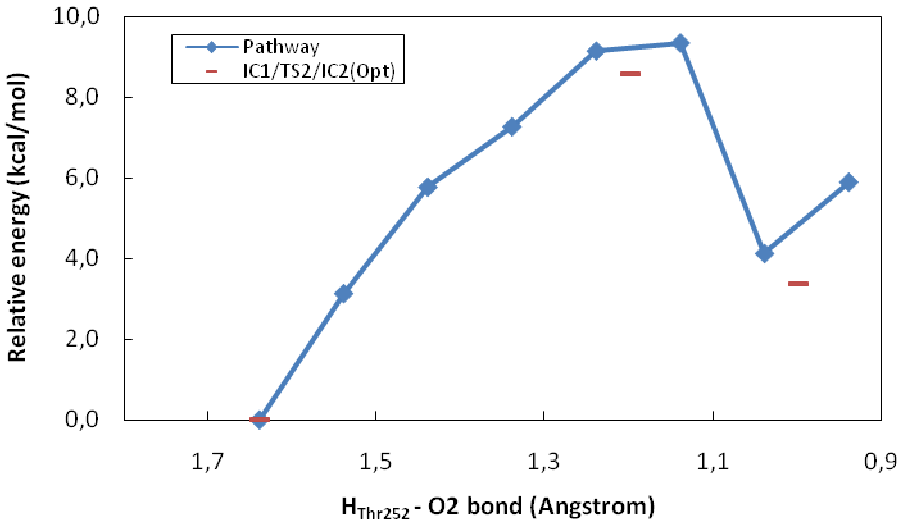

Figure S21. Energy profile for the second step of mechanism II (proton transfer from Thr252 to O2) for the flip model. Energies are given in kcal/mol relative to IC1.

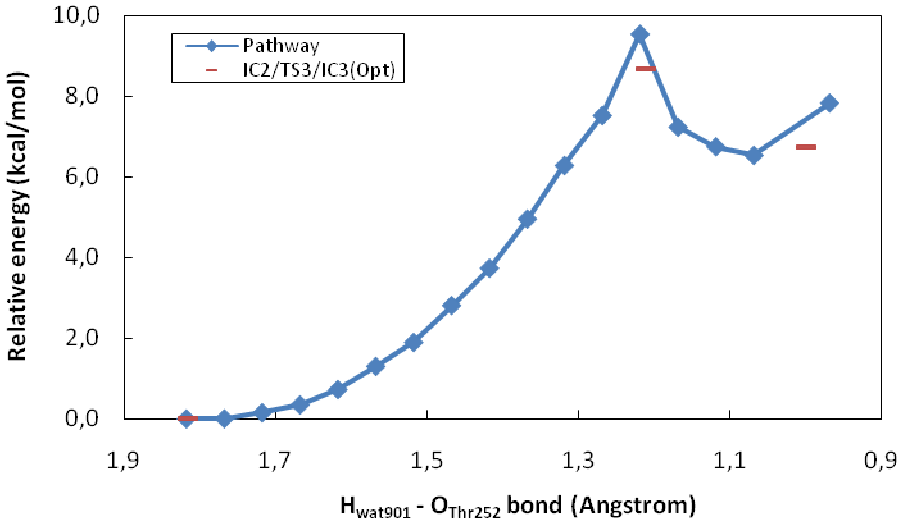

Figure S22. Energy profile for the third step of mechanism II (proton transfer from Wat901 to O_{Thr252}) for the flip model. Energies are given in kcal/mol relative to IC2.

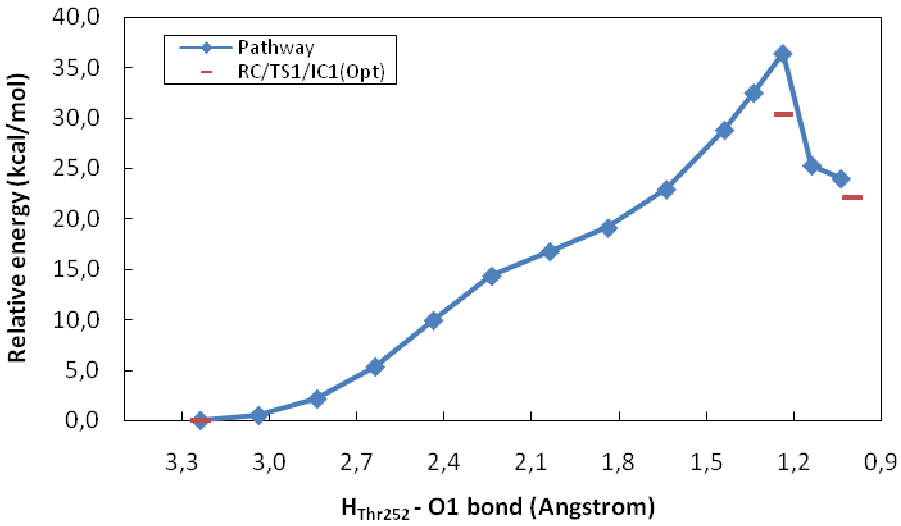

Figure S23. Energy profile for the first step of mechanism III (direct proton transfer from Thr252 to O1) for the flip model. Energies are given in kcal/mol relative to Cpd 0 (RC).

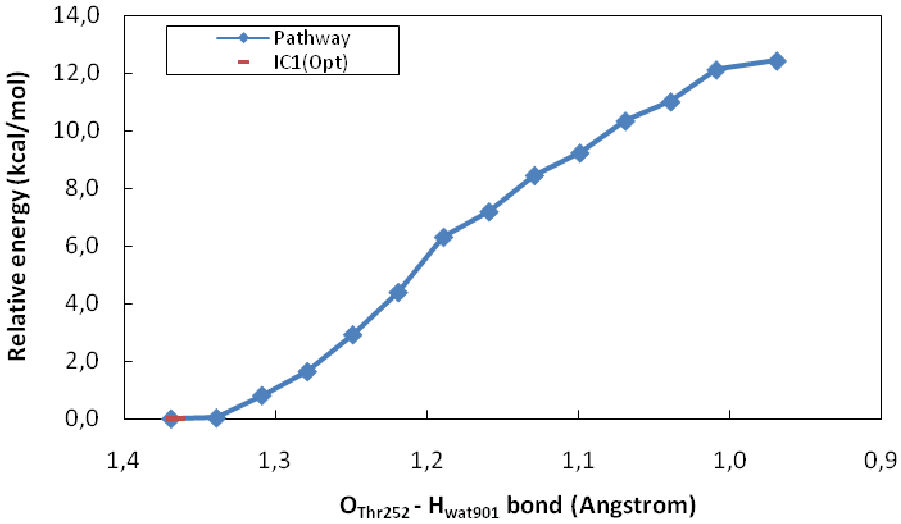

Figure S24. Energy profile for the second step of mechanism III (proton transfer from Wat901 to O_{Thr252}) for the flip model. Energies are given in kcal/mol relative to IC1.

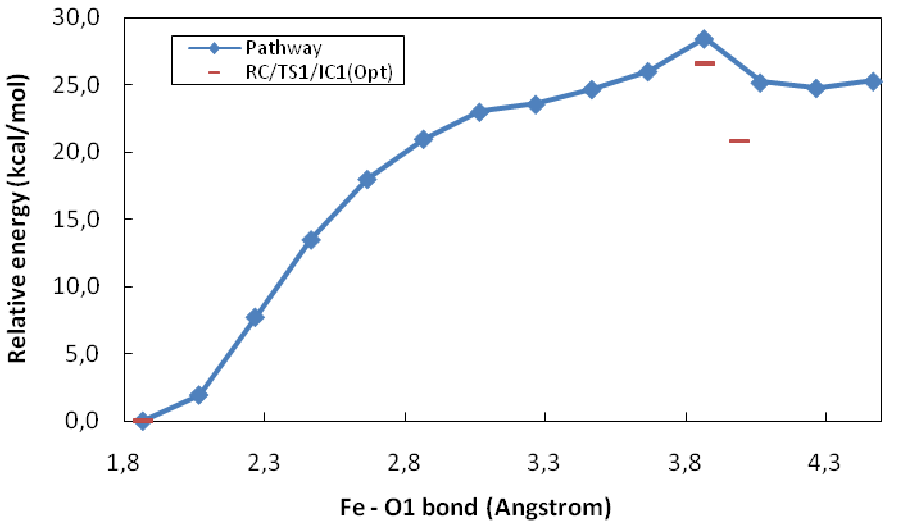

Figure S25. Energy profile for the first step of mechanism IV (Fe – O1 bond cleavage) for the flip model. Energies are given in kcal/mol relative to Cpd 0 (RC).

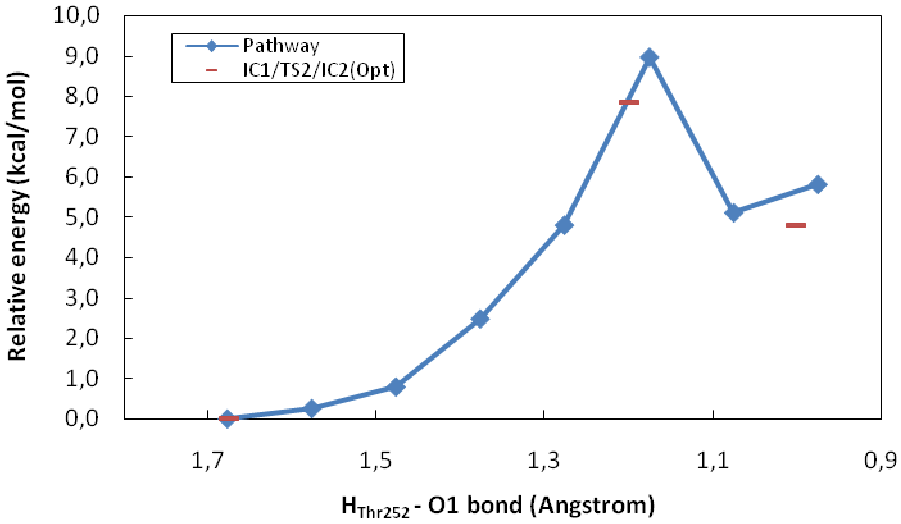

Figure S26. Energy profile for the second step of mechanism IV (proton transfer from Thr252 to O1) for the flip model. Energies are given in kcal/mol relative to IC1.

- **D251N flip with WatS model**

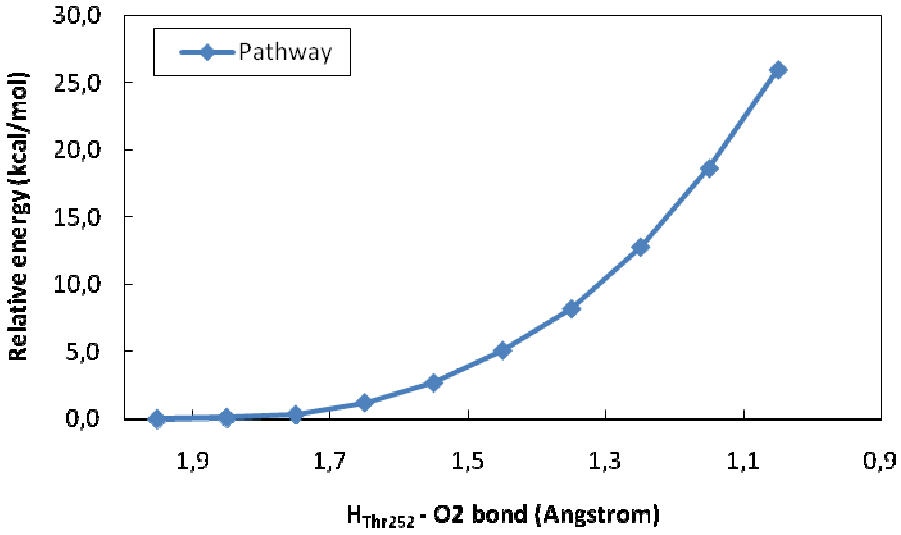

Figure S27. Energy profile for the first step of mechanism I (direct proton transfer from Thr252 to O2) for the flip with WatS model. Energies are given in kcal/mol relative to Cpd 0 (RC).

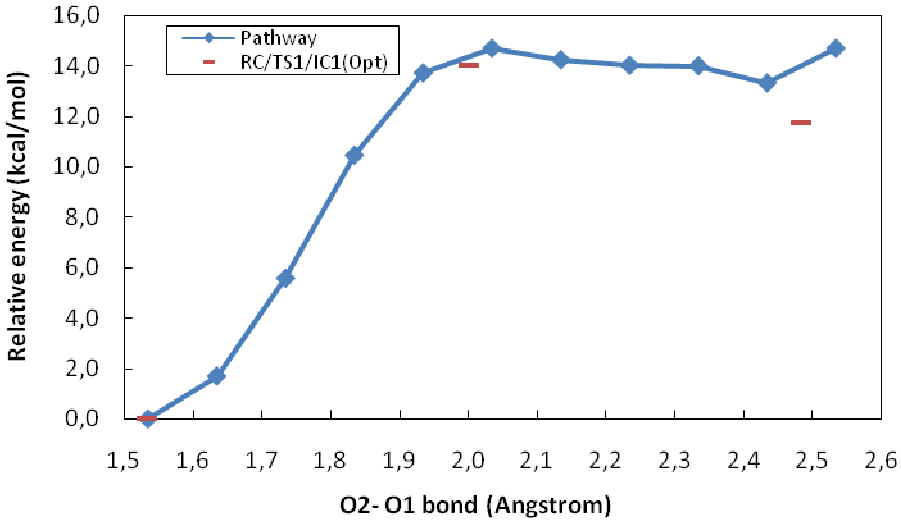

Figure S28. Energy profile for the first step of mechanism II (O1 – O2 bond cleavage) for the flip with WatS model. Energies are given in kcal/mol relative to Cpd 0 (RC).

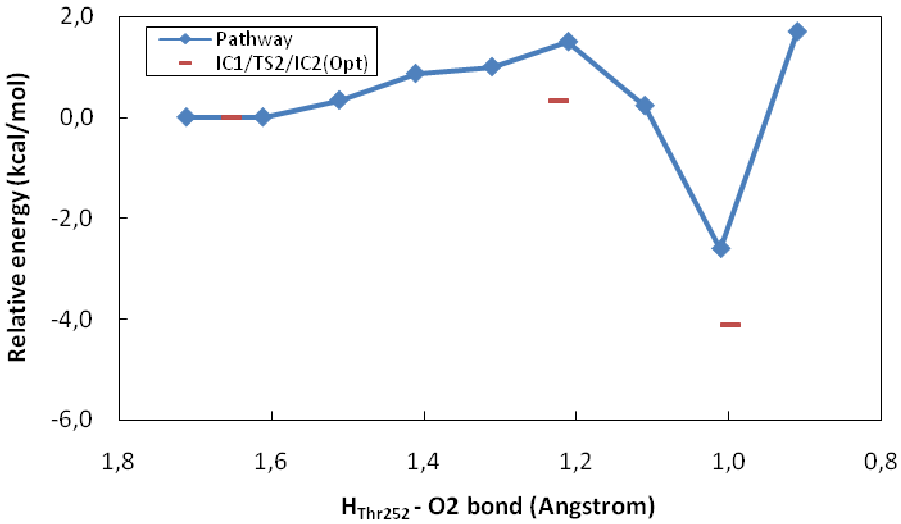

Figure 29. Energy profile for the second step of mechanism II (proton transfer from Thr252 to O2) for the flip with WatS model. Energies are given in kcal/mol relative to IC1.

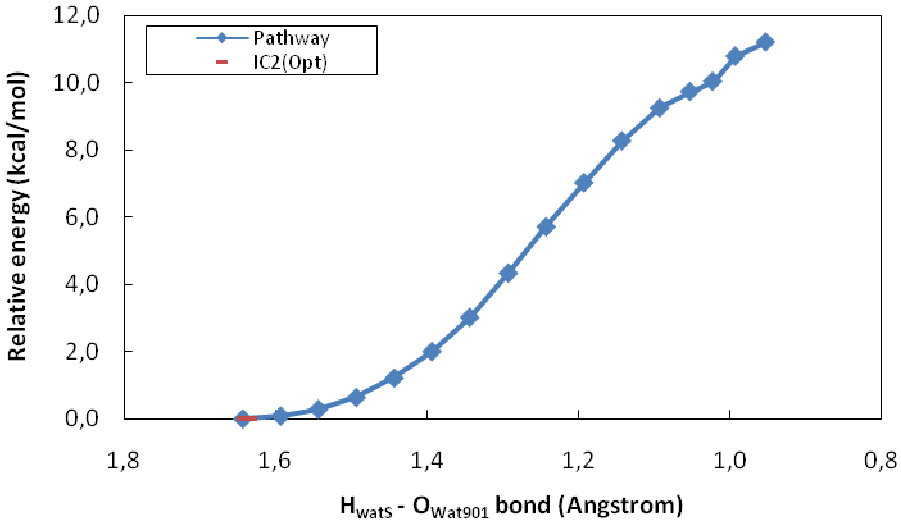

Figure S30. Energy profile for the third step of mechanism II (proton transfer from WatS to O_{wat901}) for the flip with WatS model. Energies are given in kcal/mol relative to IC2.

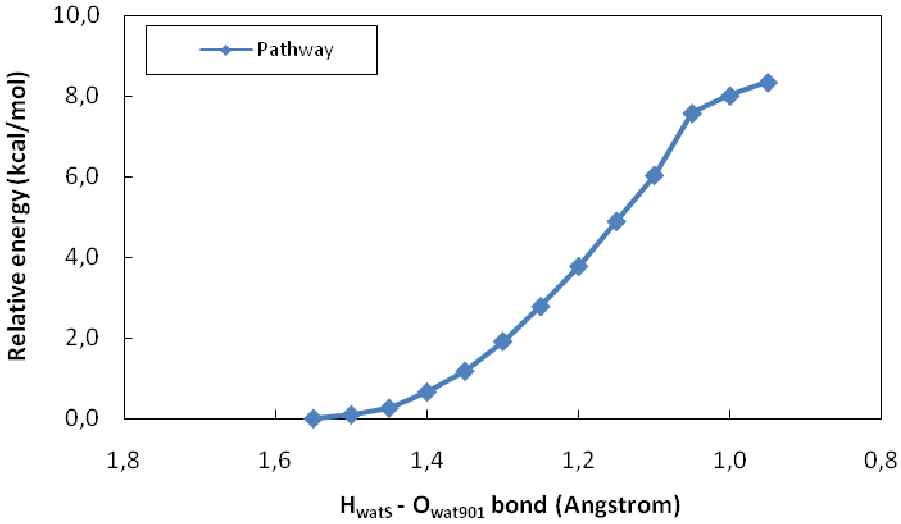

Figure S31. Energy profile for the third step of mechanism II (proton transfer from WatS to O_{wat901}) for the flip with WatS model with QM region QM2. Energies are given in kcal/mol relative to IC2.

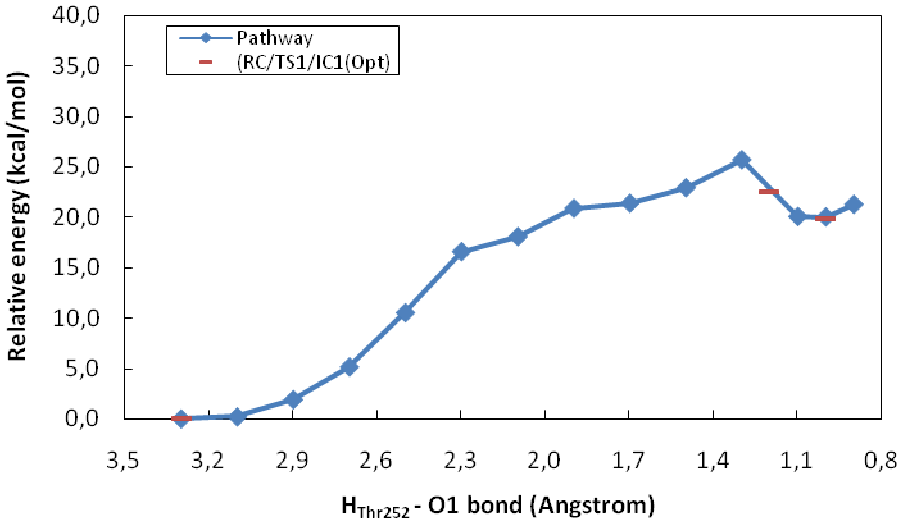

Figure S32. Energy profile for the first step of mechanism III (direct proton transfer from Thr252 to O1) for the flip with WatS model. Energies are given in kcal/mol relative to Cpd 0 (RC).

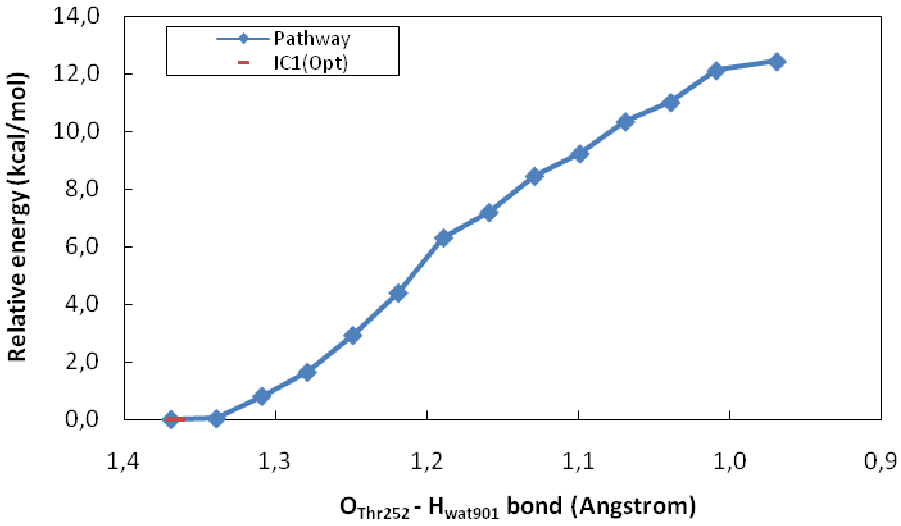

Figure S33. Energy profile for the second step of mechanism III (proton transfer from Wat901 to O_{Thr252}) for the flip with WatS model. Energies are given in kcal/mol relative to IC1.

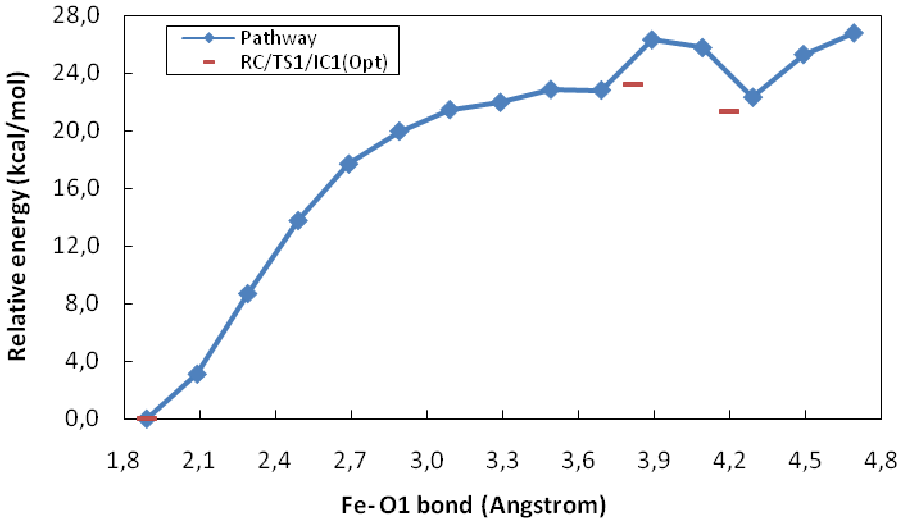

Figure S34. Energy profile for the first step of mechanism IV (Fe – O1 bond cleavage) for the flip with WatS model. Energies are given in kcal/mol relative to Cpd 0 (RC).

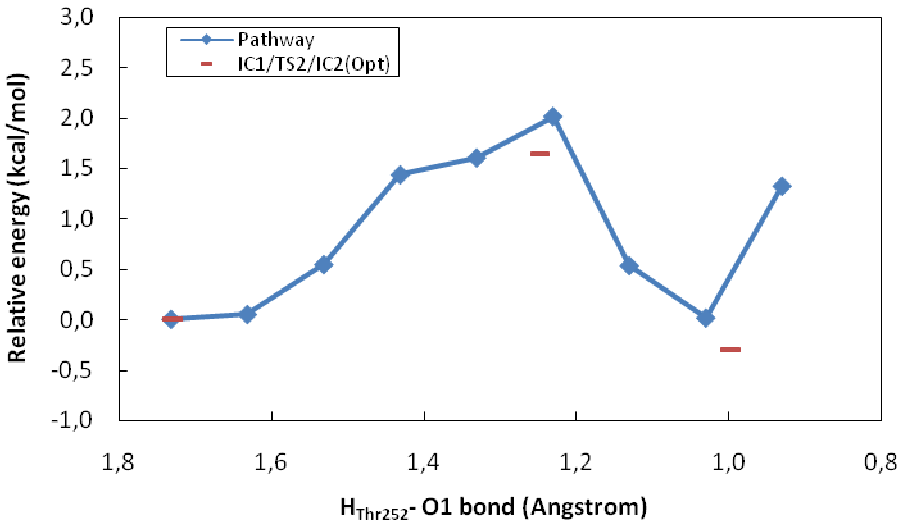

Figure S35. Energy profile for the second step of mechanism IV (proton transfer from Thr252 to O1) for the flip with WatS model. Energies are given in kcal/mol relative to Cpd 0 (RC).

5. Computed relative energies of mechanism II

Table S25: Computed relative energies (kcal/mol) of mechanism II for no flip, flip and flip with WatS models using basis sets B1 and B2 (with respect to Cpd 0).

No Flip model	Basis set	RC	TS1	IC1	TS2	IC2	TS3	PC
E(QM-ee)	B1	0.00	13.75	13.17	20.21	8.70	21.85	-3.52
	B2	0.00	14.08	12.67	21.35	6.74	22.33	-6.64
E(MM)	B1	0.00	0.26	0.70	1.65	2.25	2.70	13.04
	B2	0.00	0.26	0.70	1.65	2.25	2.70	13.04
E(QM/MM)	B1	0.00	14.01	13.87	21.86	10.95	24.55	9.52
	B2	0.00	14.34	13.37	23.00	8.99	25.03	6.40
E(QM-gas)	B1	0.00	18.57	15.24	23.82	12.63	28.41	14.42
Flip model	Basis set	RC	TS1	IC1	TS2	IC2	TS3	PC
E(QM-ee)	B1	0.00	12.84	7.78	17.36	11.25	19.99	12.03
	B2	0.00	13.37	7.23	16.97	9.20	19.82	10.61
E(MM)	B1	0.00	1.15	1.25	0.26	1.16	1.10	7.12
	B2	0.00	1.15	1.25	0.26	1.16	1.10	7.12
E(QM/MM)	B1	0.00	13.99	9.03	17.62	12.41	21.09	19.15
	B2	0.00	14.52	8.48	17.23	10.36	20.92	17.73
E(QM-gas)	B1	0.00	18.22	12.91	21.01	15.87	23.22	15.28
Flip + WatS model	Basis set	RC	TS1	IC1	TS2	PC		
E(QM-ee)	B1	0.00	12.48	9.96	10.48	-0.88		
	B2	0.00	12.36	10.63	11.27	-1.82		
E(MM)	B1	0.00	1.55	1.80	1.60	8.52		
	B2	0.00	1.55	1.80	1.60	8.52		
E(QM/MM)	B1	0.00	14.03	11.76	12.08	7.64		
	B2	0.00	13.91	12.43	12.87	6.70		
E(QM-gas)	B1	0.00	16.43	10.82	15.72	15.32		

E(QM/MM) refers to the total QM/MM energy of the system. E(QM-ee) denotes the contributions from the QM calculation for the QM region (including the electrostatic interactions with the MM point charges). E(MM) represents the contributions from the MM calculation for the MM region 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.

It is obvious that the relative QM/MM energies are dominated by the QM contributions. Except for PC, the MM contributions to the relative energies are typically of the order of 1-2 kcal/mol. The comparison between the E(QM-ee) and E(QM-gas) values shows that the electrostatic QM/MM interactions generally stabilize all species relative to RC, typically by 3-5 kcal/mol (more so for PC in the no flip and flip+WatS models).

The largest MM contributions are found for PC in the no flip model. To check for conceivable rearrangements, we have reoptimized the structure of PC starting from TS3 and confirmed that TS3 and PC are connected by a direct path on the potential energy surface (as traced during careful reoptimization).

6. MD simulation of the water network in the product of the flip with WatS model

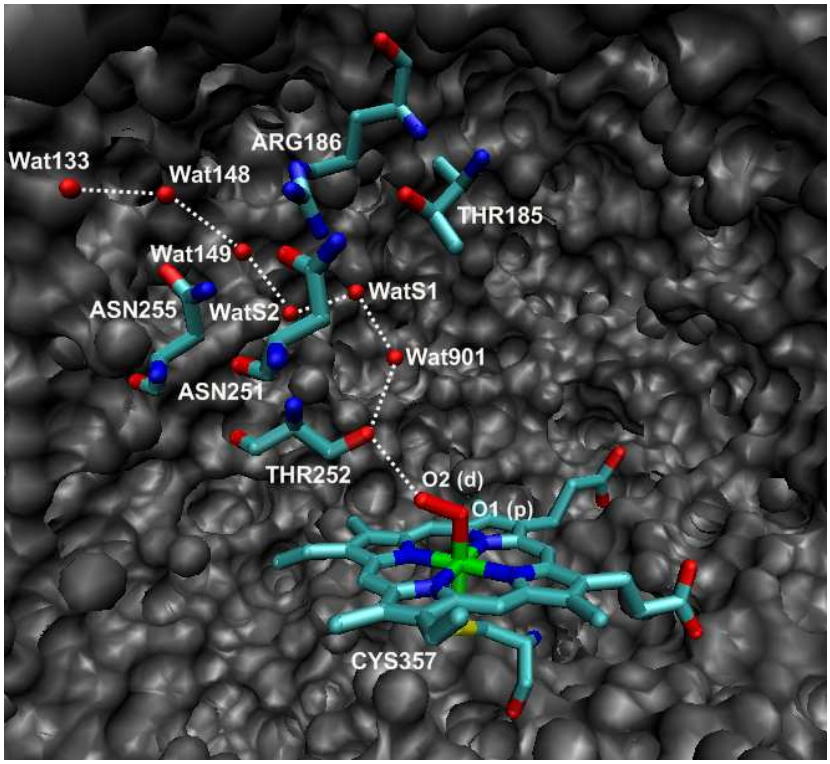

Figure S36. Active-site environment and hydrogen-bonded water network in the product species (PC) of the flip with WatS model. Red spheres indicate water molecules that connect the Thr252 residue with the bulk solvent.

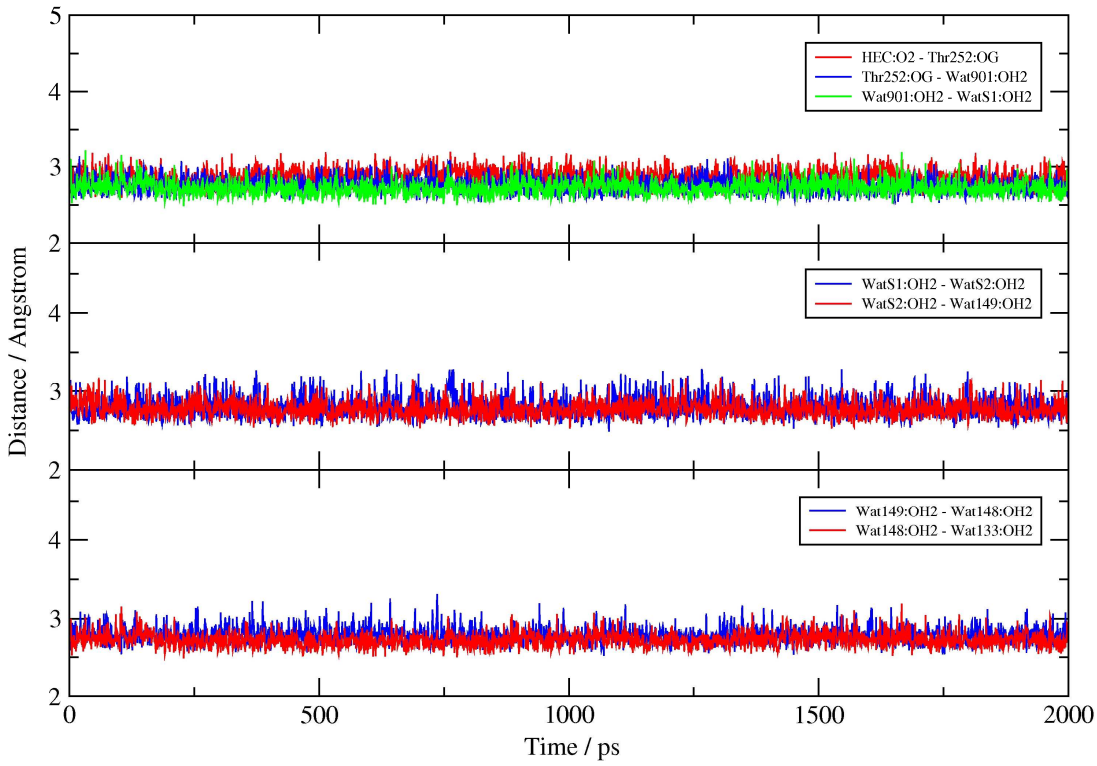

Figure S37. Monitoring the mobility of the water molecules that form a possible proton transfer channel in the D251N mutant (for atom labels see Figure S36, Hec is the porphine-FeO2 unit).

Table S26: Average values and standard deviations of the distances between HEC:O2 and Thr252:OG, Thr252:OG and Wat901:OH2, Wat901:OH2 and WatS1:OH2, WatS1:OH2 and WatS2:OH2, WatS2:OH2 and Wat149:OH2, Wat149:OH2 and Wat148:OH2, and Wat148:OH2 and Wat133:OH2, during 2 ns of MD simulation (in Å). See Figures S36 and S37.

		Average distance	Standard deviation
D251N	Hec:O2-Thr252:OG	2.864	0.114
	Thr252:OG - Wat901:OH2	2.753	0.099
	Wat901:OH2 – WatS1:OH2	2.724	0.100
	WatS1:OH2 – WatS2:OH2	2.799	0.141
	WatS2:OH2 – Wat149:OH2	2.773	0.113
	Wat149:OH2 – Wat148:OH2	2.785	0.111
	Wat148:OH2 – Wat133:OH2	2.724	0.100

7. Computed spin densities and partial charges for the intermediate IC2

Table S27: Computed spin densities and Mulliken charges for the doublet state of intermediate IC2 in Asn-C-II (no flip model) with different functionals, basis sets, and QM regions from single-point calculations at the B3LYP/B1/MM geometry.

		Fe	H2O[a]	O1	Por	Thr252[b]	Asn251	Wat901[d]
B3LYP/B1	Spin	1.345	-0.007	0.754	-0.086	-0.989	---	-0.005
QM1	Charge	0.472	0.004	-0.488	-0.437	-0.213	-0.121	0.071
B3LYP/B2	Spin	1.431	-0.003	0.699	-0.117	-0.985	0.002	0.002
QM1	Charge	0.286	-0.011	-0.433	-0.201	-0.189	-0.091	0.059
BHLYP/B1	Spin	1.012	-0.005	1.032	-0.025	-1.000	0.001	0.006
QM1	Charge	0.755	0.003	-0.478	-0.681	0.006	-0.098	0.059
BHLYP/B2	Spin	1.057	-0.003	1.004	-0.043	-0.997	0.001	0.002
QM1	Charge	0.424	0.011	-0.371	-0.381	0.009	-0.076	0.047
BLYP/B1	Spin	1.250	0.027	0.756	-0.197	-0.800	-0.001	-0.003
QM1	Charge	0.295	0.019	-0.433	-0.202	-0.169	-0.144	0.072
BLYP/B2	Spin	1.333	0.008	0.683	-0.216	-0.752	0.001	0.002
QM1	Charge	0.215	0.024	-0.394	-0.016	-0.212	-0.133	0.054
B3LYP/B1	Spin	1.269	-0.006	0.831	-0.088	-0.989	---	0.005
gas phase, QM1	Charge	0.453	-0.003	-0.448	-0.619	0.004	-0.108	0.056
B3LYP/B2	Spin	1.339	-0.004	0.756	-0.111	-0.981	---	0.002
gas phase, QM1	Charge	0.243	-0.001	-0.403	-0.723	-0.004	-0.083	0.042
B3LYP/B1	Spin	1.334	-0.004	0.765	-0.086	-0.992	0.001	0.005
QM2[c]	Charge	0.471	-0.020	-0.486	-0.438	0.114	-0.012	0.073
B3LYP/B1	Spin	1.343	-0.004	0.762	-0.085	-0.993	0.001	----
QM3 [c]	Charge	0.418	0.008	-0.485	-0.416	0.006	-0.012	0.077
B3LYP/B2	Spin	1.414	-0.001	0.708	-0.121	-0.999	---	0.001
QM3 [c]	Charge	0.275	0.004	-0.429	-0.199	-0.021	-0.075	0.062

[a] Protonated OH species next to FeO unit. [b] Thr252 residue is deprotonated. [c] Geometry reoptimized at the B3LYP/MM level using the given basis set and QM region. [d] Wat901 is deprotonated

Table S28: Computed spin densities and Mulliken charges for the doublet state of intermediate IC2 in Asn-C-II (flip model) with different functionals, basis sets, and QM regions.

		Fe	H2O[a]	O1	Por	Thr252[b]	Asn251[c]	Wat901[d]
B3LYP/B1	Spin	1.344	-0.001	0.760	-0.235	-0.765	---	---
QM1	Charge	0.482	-0.030	-0.473	-0.261	-0.360	-0.061	-0.008
B3LYP/B2	Spin	1.430	-0.003	0.705	-0.318	-0.793	---	---
QM1	Charge	0.281	-0.024	-0.417	0.010	-0.372	-0.053	0.004
BHLYP/B1	Spin	1.029	-0.001	1.079	-0.088	-1.009	---	-0.008
QM1	Charge	0.776	-0.072	-0.482	-0.681	0.031	-0.047	0.004
BHLYP/B2	Spin	1.101	-0.001	1.058	-0.102	-1.013	---	-0.004
QM1	Charge	0.436	-0.020	-0.363	-0.357	0.005	-0.042	0.002
BLYP/B1	Spin	1.299	-0.025	0.732	-0.305	-0.623	---	0.005
QM1	Charge	0.311	-0.013	-0.432	-0.084	-0.257	-0.074	-0.015
BLYP/B2	Spin	1.392	-0.011	0.654	-0.389	-0.605	---	0.008
QM1	Charge	0.204	-0.002	-0.370	-0.040	-0.311	-0.071	-0.007
B3LYP/B1	Spin	1.251	-0.007	0.861	-0.181	-0.861	---	0.007
gas phase, QM1	Charge	0.488	-0.024	-0.417	-0.557	-0.158	-0.053	-0.019
B3LYP/B2	Spin	1.307	-0.004	0.791	-0.191	-0.819	---	0.003
gas phase, QM1	Charge	0.314	-0.018	-0.332	-0.353	-0.068	-0.094	-0.020
B3LYP/B1	Spin	1.301	-0.003	0.713	-0.099	-0.832	---	0.003
QM2 [c]	Charge	0.263	-0.001	-0.365	-0.271	-0.177	-0.032	-0.010
B3LYP/B1	Spin	1.339	0.009	0.766	-0.306	-0.730	---	0.006
QM3 [c]	Charge	0.480	-0.026	-0.470	-0.241	-0.186	-0.063	0.005
B3LYP/B2	Spin	1.434	-0.002	0.703	-0.273	-0.786	---	0.004
QM3 [c]	Charge	0.257	-0.022	-0.419	-0.053	-0.179	0.027	0.011

[a] Protonated OH species next to FeO unit. [b] Thr252 residue is deprotonated. [c] Geometry optimized at the B3LYP/MM level using the given basis set and QM region. [d] Wat901 is deprotonated

Table S29: Spin densities and Mulliken charges of the initial guess and the optimized wave function of the doublet state of intermediate IC2 at the B3LYP/B1 level of theory in the gas phase (QM1 region).

	Initial guess				Converged wave function			
	No Flip		Flip		No Flip		Flip	
	Charge	Spin	Charge	Spin	Charge	Spin	Charge	Spin
Thr252[a]	-0.917	-0.006	-0.894	-0.007	0.005	-0.995	-0.158	-0.763
Fe	0.533	1.192	0.540	1.186	0.453	1.270	0.489	1.250
O1	-0.457	0.879	-0.445	0.885	-0.448	0.838	-0.416	0.862
Por	-0.214	-0.468	-0.228	-0.460	-0.615	-0.087	-0.561	-0.086

.[a] Thr252 residue is deprotonated

8. Overview figure showing all relevant residues.

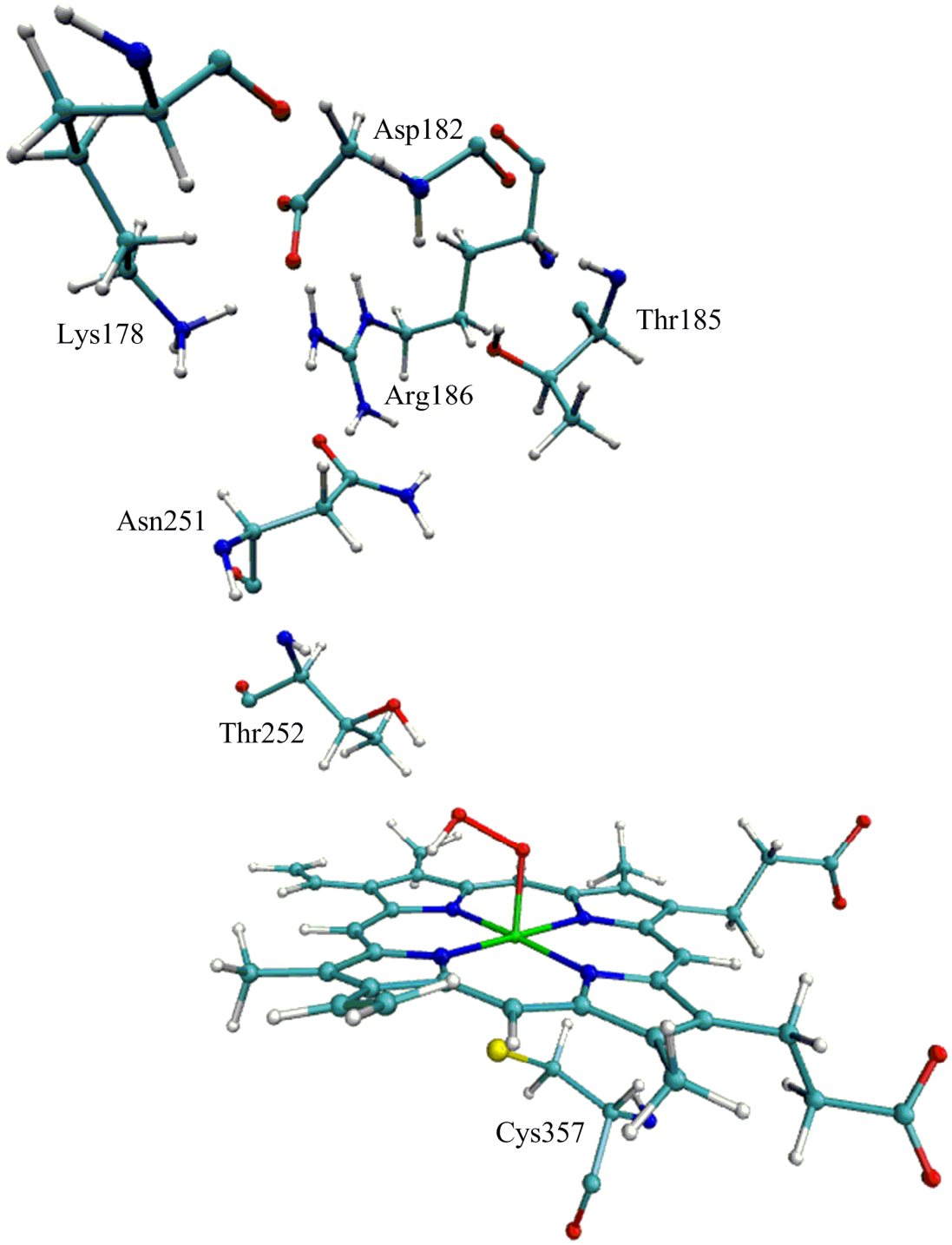

Figure S38. Overview of important residues in the active site of cytochrome P450cam.

9. QM/MM energy profiles (UB3LYP/B1/CHARMM) from path calculations of concerted proton transfer processes

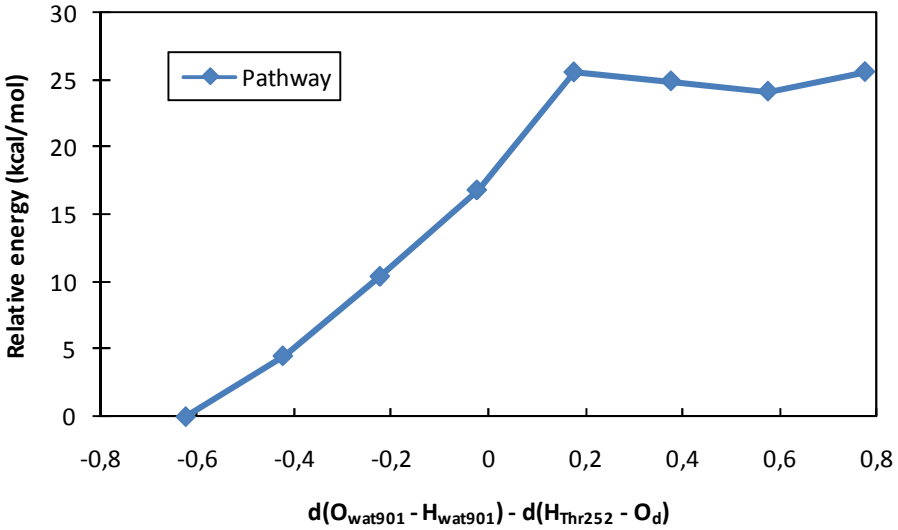

Figure S39. Energy profile for the concerted proton transfer of mechanism II (proton transfer from Thr252 to O_d and from Wat901 to Thr252) for the no flip model. Energies are given in kcal/mol relative to IC1 (which is 13.9 kcal/mol higher in energy than Cpd 0).

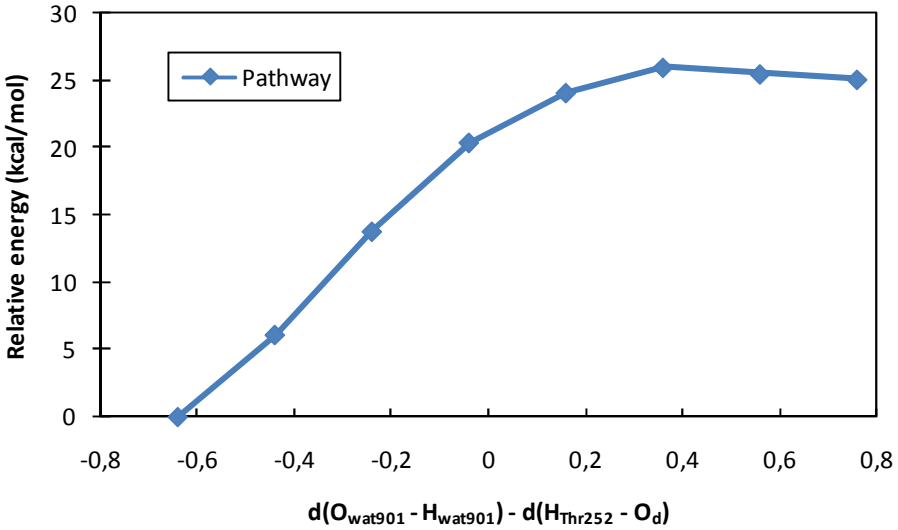

Figure S40. Energy profile for the concerted proton transfer of mechanism II (proton transfer from Thr252 to O_d and from Wat901 to Thr252) for the flip model. Energies are given in kcal/mol relative to IC1 (which is 9.0 kcal/mol higher in energy than Cpd 0).