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Supplementary Information for

PETase mutations studied with DFT/MM molecular dynamics simulations

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Energy minimizations: all minimizations were run with the steepest descent method. In the first step, we applied harmonic restraints on the enzyme and the substrate, keeping the solvation waters, counterions, and hydrogens free. In the second step, only the enzyme was restrained. The mutated residues were free in the third step, with the remaining system restrained. In the fourth step, only the backbone of the protein was fixed, and finally, the full optimization was carried out with no constraints applied to the system.

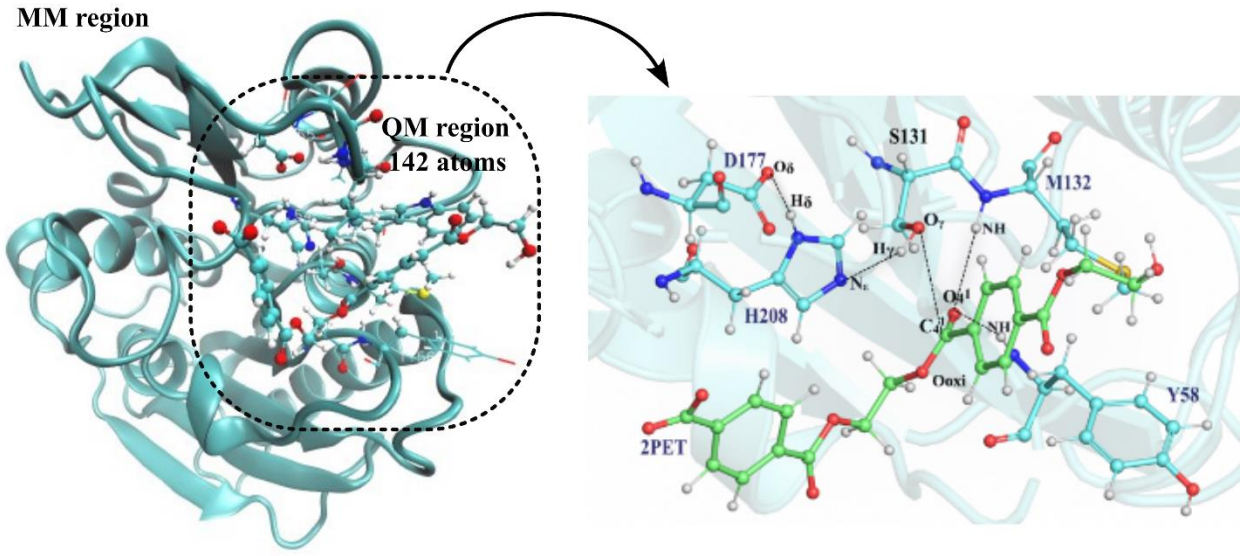

Figure S1. The reactant state of the acylation step. Distances are given in Table S1.

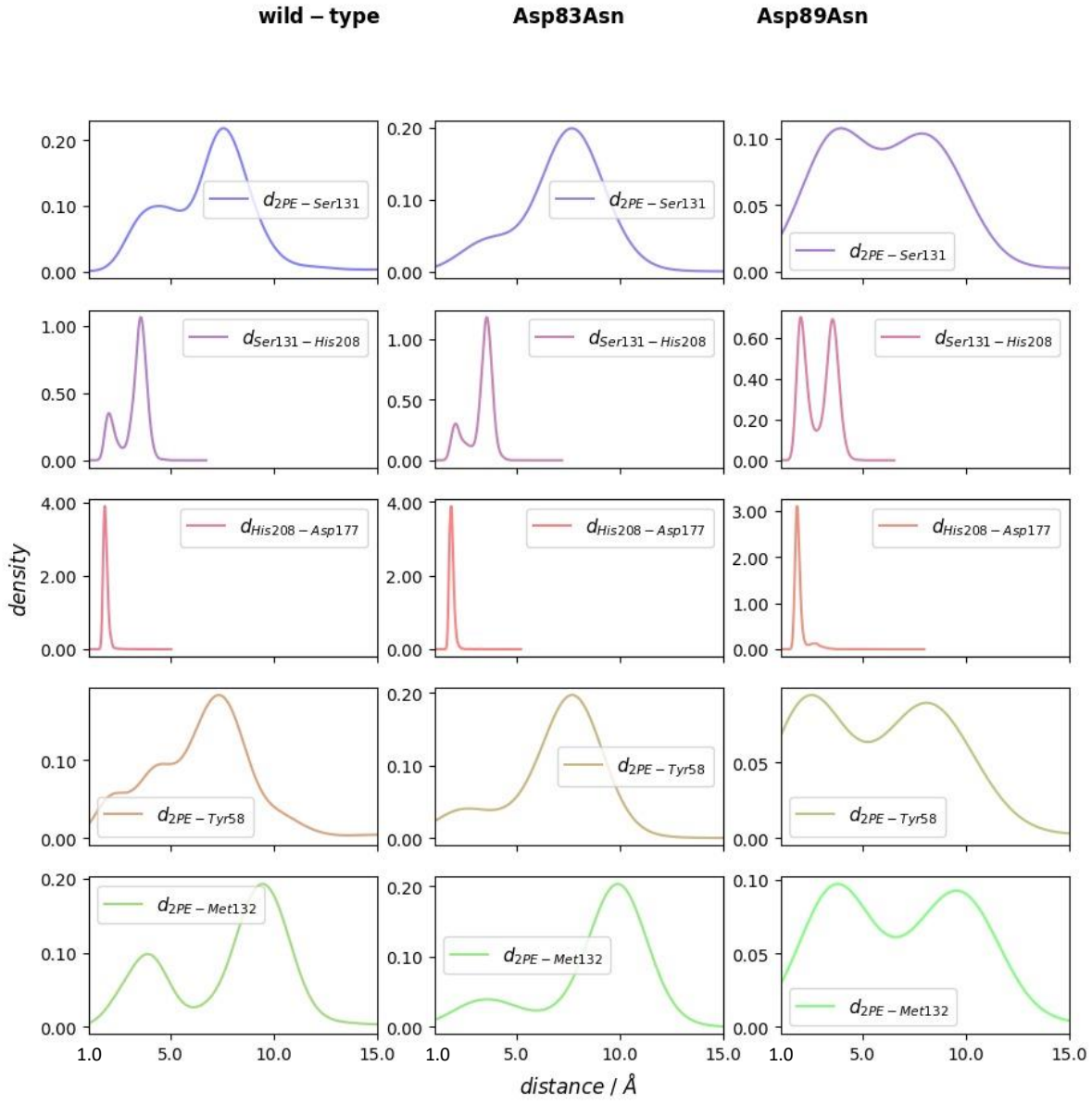

Figure S2. After MD simulation, catalytic distances were obtained for the wild type and each PETase mutant system. The important residues of PETase consist of the catalytic triad (Ser131, Asp177, and His208) and residues forming the oxyanion hole. The oxyanion hole is constructed by the amine groups of Tyr58 and Met132.

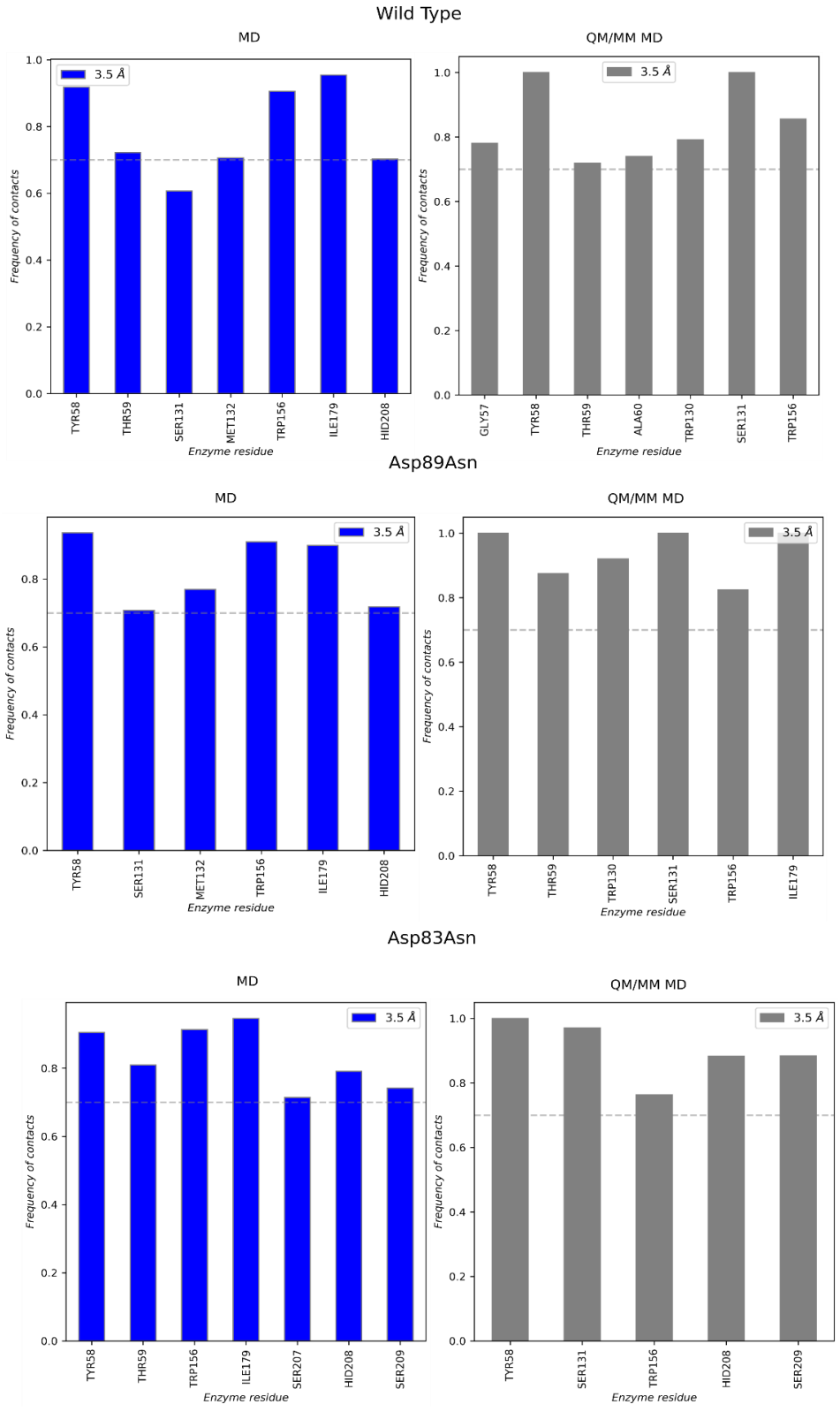

Figure S3. Enzyme residues with the most contacts with the substrate (>70%) during the MD and QM/MM MD simulations: residues within 3.5 Å interact closely with the substrate and contribute the most for substrate binding.

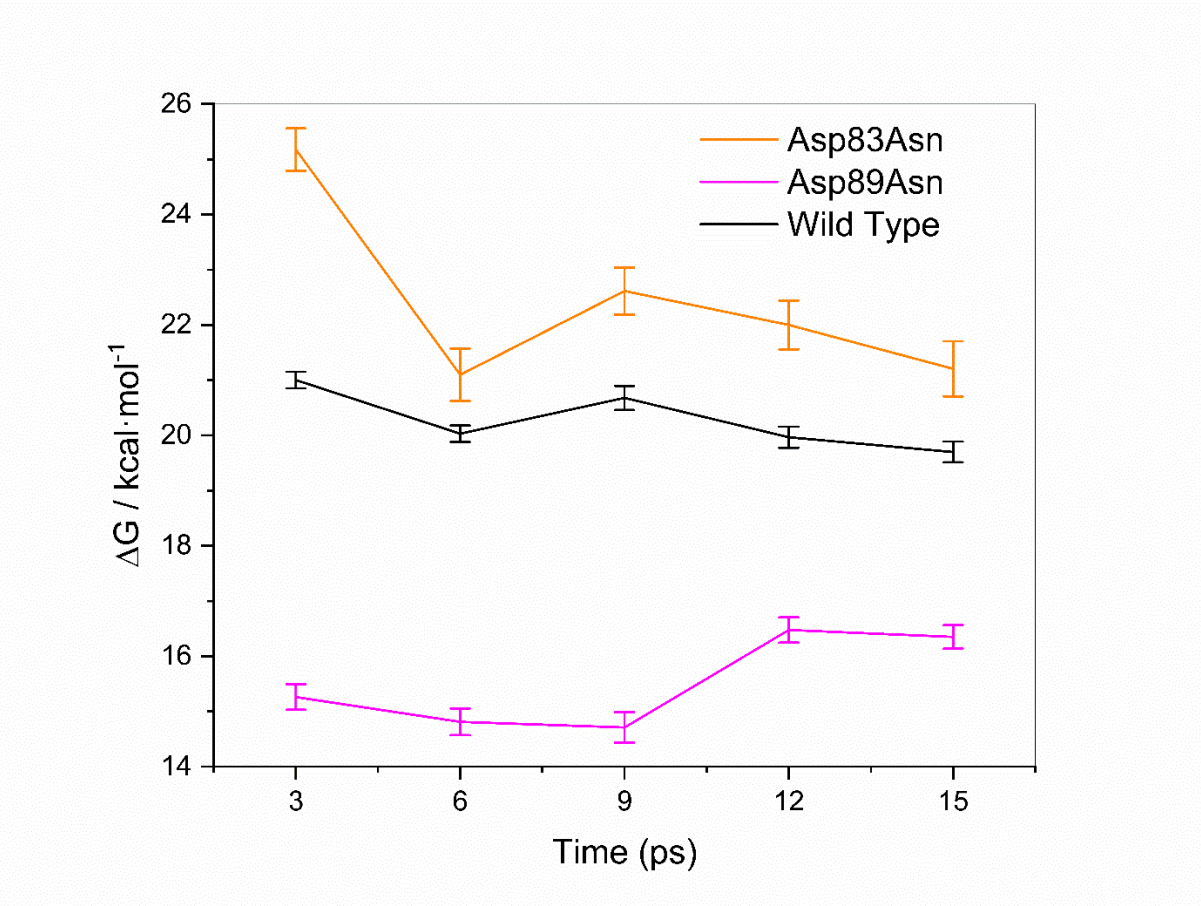

Figure S4. Variation of the Gibbs energy barriers at every 3 ps/window dataset along the umbrella sampling QM/MM MD simulations ran for the wild-type and two mutant systems. The first 3 ps of each window were considered an equilibration period. The respective standard deviations are plotted as error bars; these were calculated with the WHAM package by Grossfield and coworkers after calculation of the correlation time for each of the independent umbrella sampling simulations.

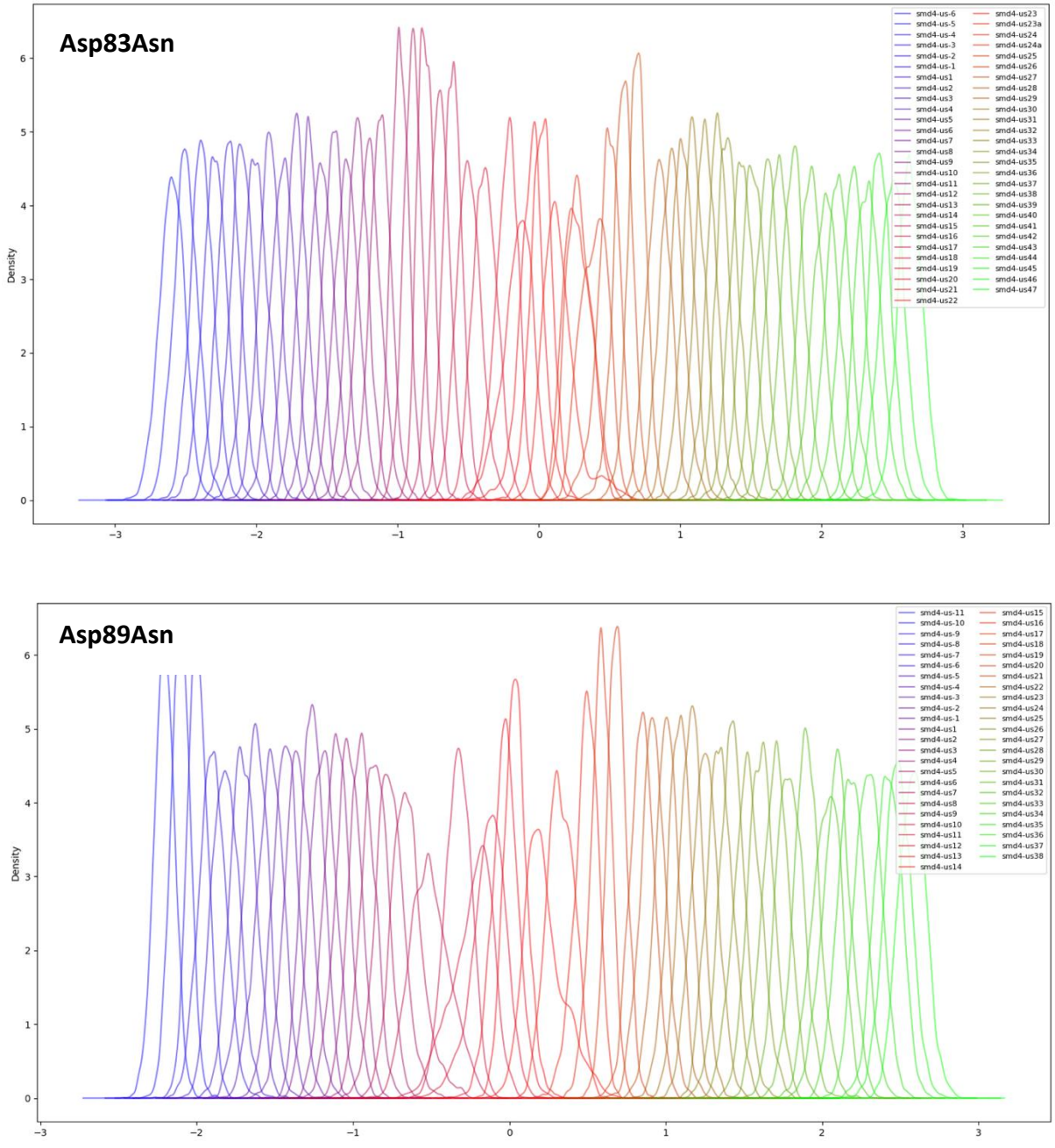

Figure S5. Density distributions for the collective variable RC = $d_{break} - d_{nuc}$, for each umbrella simulation window during the 15 ps/window.

Table S1. Information about the wild type and mutated systems in QM/MM MD calculations

System	#Atoms	# QM atoms	Cell dimensions	# US Windows	Reaction Coordinate
Wild type	34821	142	26.14Å x 24.91Å x 24.14Å	47	ξ(1.37 – 4.78 Å)
Asp83Asn	34821	142	24.0Å x 25.11Å x 26.47Å	55	ξ(1.41 – 3.82 Å)
Asp89Asn	34821	142	26.64Å x 19.88Å x 25.54Å	49	ξ(1.37 – 2.59 Å)

Table S2a. Average distances obtained for the reactants of the simulated systems. Distances are represented in Å as average ± standard deviation – these were computed using a bootstrapping scheme where 200 samples were generated based on the correlation time of each distance. Atom names are given in Figure S1 and in Scheme 1.

Distances	WT	Asp83Asn	Asp89Asn
Ser131 O_γ - C_4^1	3.30 ± 0.13	3.37 ± 0.12	3.29± 0.11
Ser131 H_γ - His208 N_ε	1.76 ± 0.15	1.75 ± 0.15	1.72± 0.14
His208 H_δ - Asp177 O_δ	1.62 ± 0.14	1.81 ± 0.48	1.58 ± 0.14
Tyr58 NH - O_4^1	2.68 ± 0.55	2.87 ± 0.61	2.28 ± 0.38
Met132 NH - O_4^1	3.07 ± 0.42	2.86 ± 0.34	3.05 ± 0.36
C_4^1 - O_{oxi}	1.374 ± 0.040	1.368 ± 0.036	1.368 ± 0.038
Ser131 O_γ - H_γ	1.021 ± 0.037	1.024 ± 0.037	1.026 ± 0.038
O_{oxi} - Ser131 H_γ	3.78 ± 0.29	3.90 ± 0.30	3.95 ± 0.32

Table S2b. Average distances obtained for the transition states of the simulated systems. Distances are represented in Å as average ± standard deviation – these were computed using a bootstrapping scheme where 200 samples were generated based on the correlation time of each distance.

Distances	WT	Asp83Asn	Asp89Asn
Ser131 O_γ - C_4^1	1.49 ± 0.05	1.476 ± 0.048	1.462 ± 0.045
Ser131 H_γ - His208 N_ε	1.27 ± 0.25	1.24 ± 0.26	1.36 ± 0.27
His208 H_δ - Asp177 O_δ	1.39 ± 0.23	1.34 ± 0.28	1.45 ± 0.24
Tyr58 NH - O_4^1	2.05 ± 0.21	2.17 ± 0.37	2.06 ± 0.22

Met132 NH - O_4^1	2.19 ± 0.26	2.13 ± 0.24	2.17 ± 0.22
C_4^1 - O_{oxi}	1.71 ± 0.13	1.75 ± 0.13	1.83 ± 0.15
Ser131 O_γ - H_γ	2.13 ± 0.38	2.29 ± 0.37	2.48 ± 0.25
O_{oxi} - Ser131 H_γ	1.83 ± 0.63	1.72 ± 0.56	1.36 ± 0.36

Table S2c. Average Distances Obtained for the Product. Distances are represented in Å as average ± standard deviation – these were computed using a bootstrapping scheme where 200 samples were generated based on the correlation time of each distance.

Distances	WT	Asp83Asn	Asp89Asn
Ser131 O_γ - C_4^1	1.380 ± 0.038	1.377 ± 0.037	1.373 ± 0.037
Ser131 H_γ - His208 N_ε	5.8 ± 1.5	6.1 ± 1.6	3.6 ± 1.7
His208 H_δ - Asp177 O_δ	1.68 ± 0.15	1.65 ± 0.17	1.60 ± 0.14
Tyr58 NH - O_4^1	2.88 ± 0.83	2.82 ± 0.93	2.35 ± 0.40
Met132 NH - O_4^1	3.42 ± 0.72	2.72 ± 0.68	2.72 ± 0.65
C_4^1 - O_{oxi}	3.90 ± 0.11	3.89 ± 0.13	3.77 ± 0.12
Ser131 O_γ - H_γ	4.98 ± 0.55	4.93 ± 0.64	4.07 ± 0.61
O_{oxi} - Ser131 H_γ	0.999 ± 0.023	1.001 ± 0.026	1.000 ± 0.033