Predictive Methods for Computational Metalloenzyme Redesign - A Test Case with Carboxypeptidase A

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Supplemental Information

Native peptidase/peptide			
	ES	TS	EI
TPSS	0.0	13.8	5.3
BP86	0.0	13.9	7.4
B3LYP	0.0	15.8	6.2
TPSSh	0.0	13.3	3.7
V243R_FpepD mutant peptidase/peptide			
	ES	TS	EI
TPSS	0.0	17.9	10.1
BP86	0.0	13.0	9.8
B3LYP	0.0	21.9	13.6
TPSSh	0.0	21.6	13.7
V243K_FpepE mutant peptidase/peptide			
	ES	TS	EI
TPSS	0.0	28.6	18.9
BP86	0.0	17.2	19.0
B3LYP	0.0	29.7	19.2
TPSSh	0.0	27.4	15.0

Table S1: For native and two mutants (V243R_FpepD, V243K_FpepE) Single points of stationary points with the TPSS, BP86, B3LYP, and TPSSh functional and def2-TZVPP basis set for all atoms from structures geometrically optimized with TPSS/def2-SVP (H,C,N,O) and def2-TZVPP (Zn^{2+}). Energies are in kcal/mol.

Native peptidase/peptide			
	ES	TS	EI
H69	0.05011	0.0461	0.05171
E72	-0.84435	-0.86923	-0.88227
R127	0.93467	0.9008	0.94108
H196	0.06622	0.07172	0.07382
V243	0.00026	0.00017	6E-05
E270	-0.84731	-0.58525	-0.55422
Peptide	-0.97994	-1.24874	-1.52329
Wat	-0.06968	0.00425	0.23132
Zn	1.69001	1.68024	1.66181
V243R_FpepD mutant peptidase/peptide			
	ES	TS	EI
H69	0.06817	0.06071	0.04592
E72	-0.83326	-0.8564	-0.84521
R127	0.93307	0.91007	0.92049
H196	-0.03165	-0.0145	-0.0183
R243	0.9049	0.9134	0.8666
E270	-0.84356	-0.5694	-0.57727
Peptide	-1.84552	-2.0743	-2.26343
Wat	-0.01874	0.00563	0.20675
Zn	1.66653	1.6524	1.66447

Table S2: Natural population (NPA) charges of residues in native and V243R_FpepD mutant peptidase/peptide system calculated with TPSSh functional and def2-TZVPP basis set for all atoms.

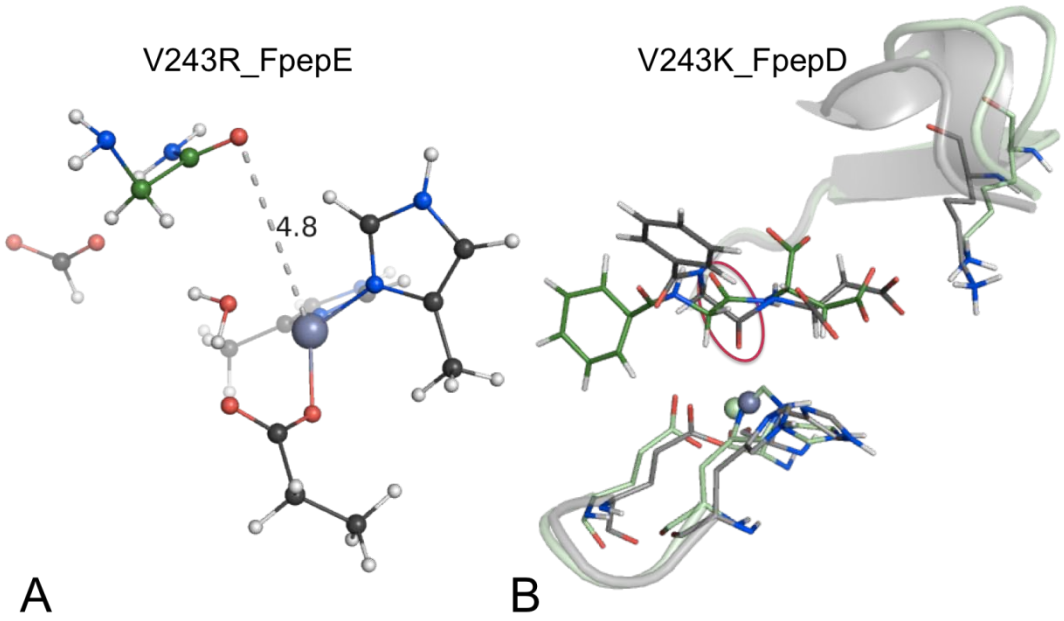

Figure S1: (A) Loose coordination of the V243R_FpepE mutant eliminates this mutant as a potential redesign candidate. The average carbonyl and zinc distance is 4.8 ± 0.5 Å. (B) The mutant peptide in the V243K_FpepD completely flips around in the binding pocket and looses the key carbonyl-zinc interaction. A comparison of the first structure (gray protein) and final structure (green protein) with the pink circle highlighting the carbonyl that looses coordination with the zinc upon flipping of the substrate.

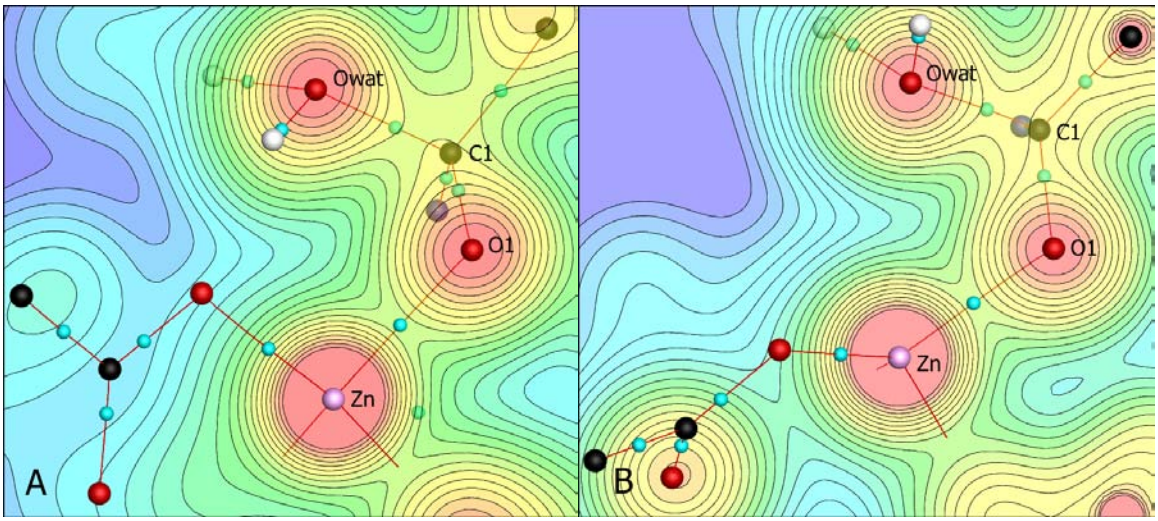

Figure S2: Bond paths and critical points for the both the native (A) and mutant (B) product states (EI).