Supporting Information

Structural Insights from Molecular Dynamics Simulations of Tryptophan 7-Halogenase and Tryptophan 5-halogenase

Jon Ainsley[1], Adrian J. Mulholland[2], Gary W. Black[1], Olivier Sparagano[3], Christo Z. Christov[1,4,], Tatyana G. Karabencheva-Christova[1,4,*]*

[1]Department of Applied Sciences, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, NE1 8ST, United Kingdom

[2]Centre for Computational Chemistry, School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, United Kingdom

[3]Vice-Chancellor's Office, Coventry University, Alan Berry Building, Priory Street, Coventry, CV1 5FB, United Kingdom

[4]Department of Chemistry, Michigan Technological University, Houghton, 49931, MI, USA

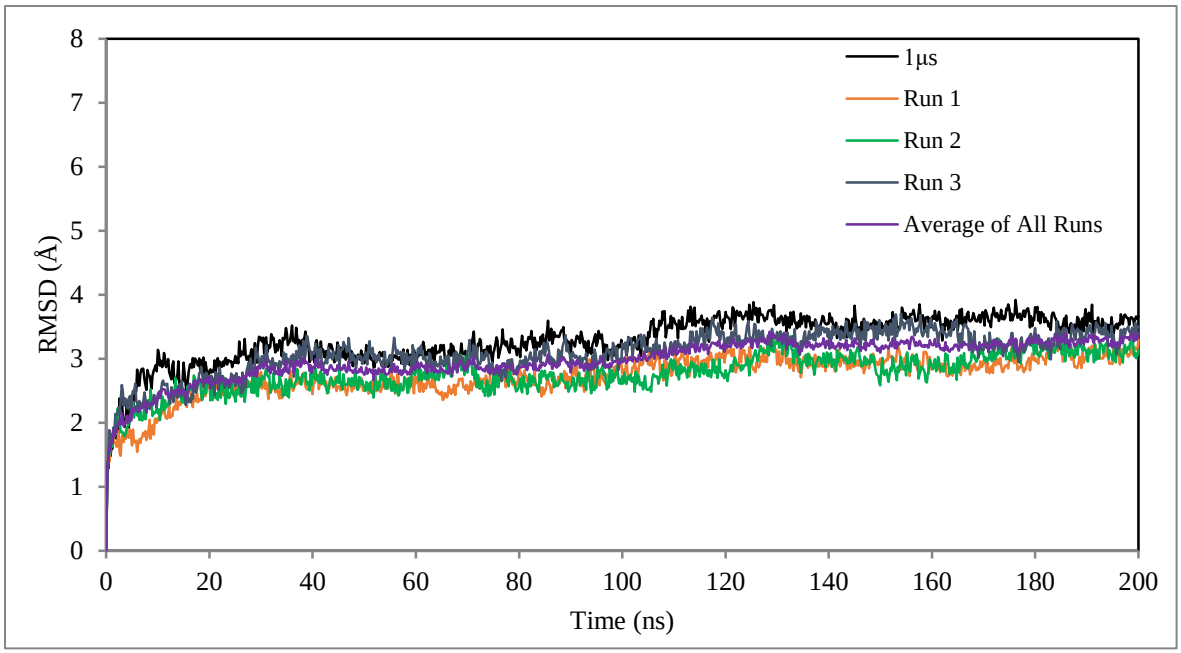

Figure S1: RMSD of C-alfa atoms for four individual MD trajectories and for an average trajectory.

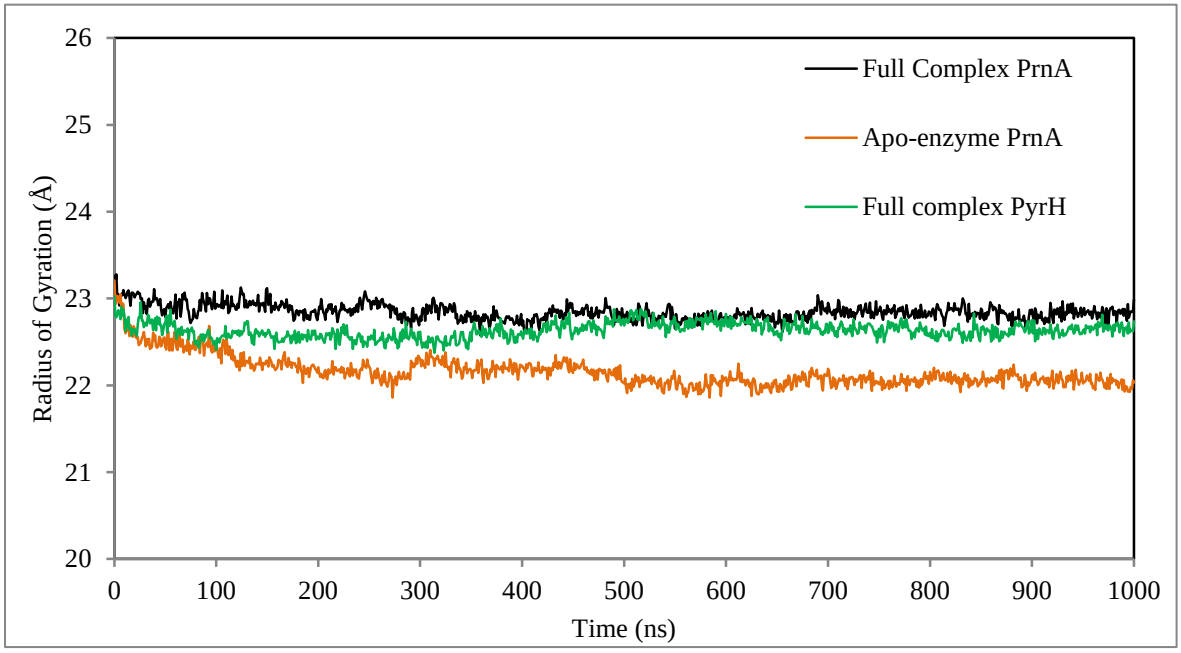

Figure S2: Radius of gyration for full complex PrnA in black, apoenzyme PrnA in red, and full complex PyrH in orange.

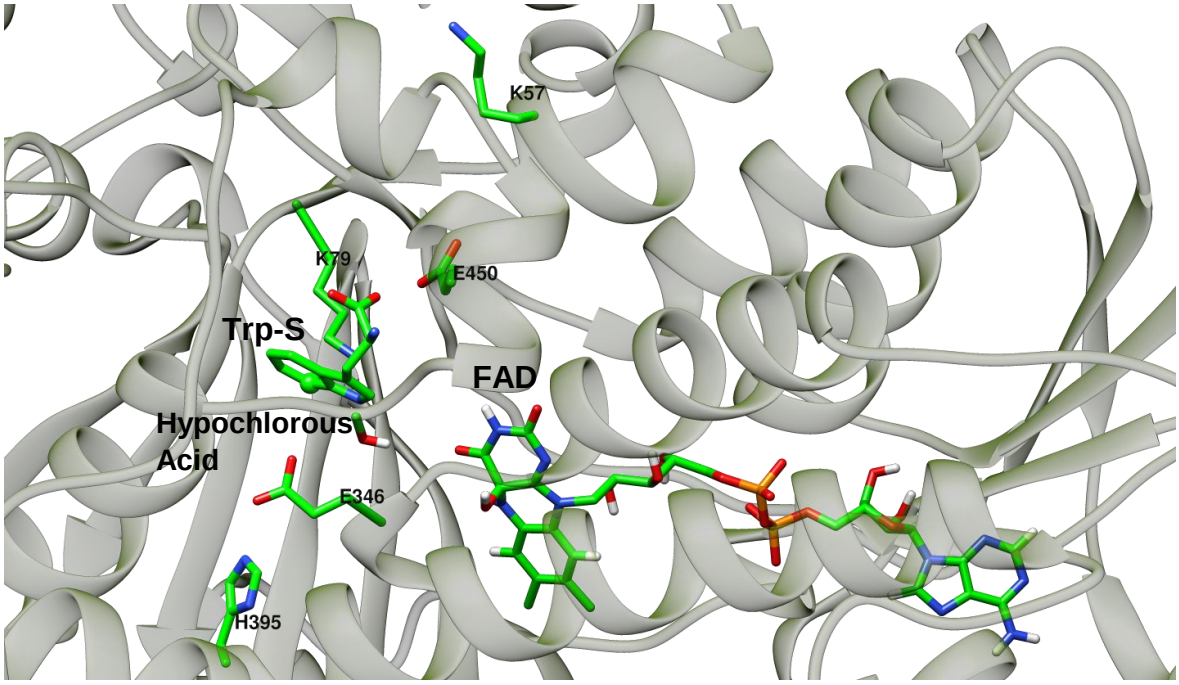

Figure S3: Electrostatic interactions around the Trp-S binding site in PrnA.

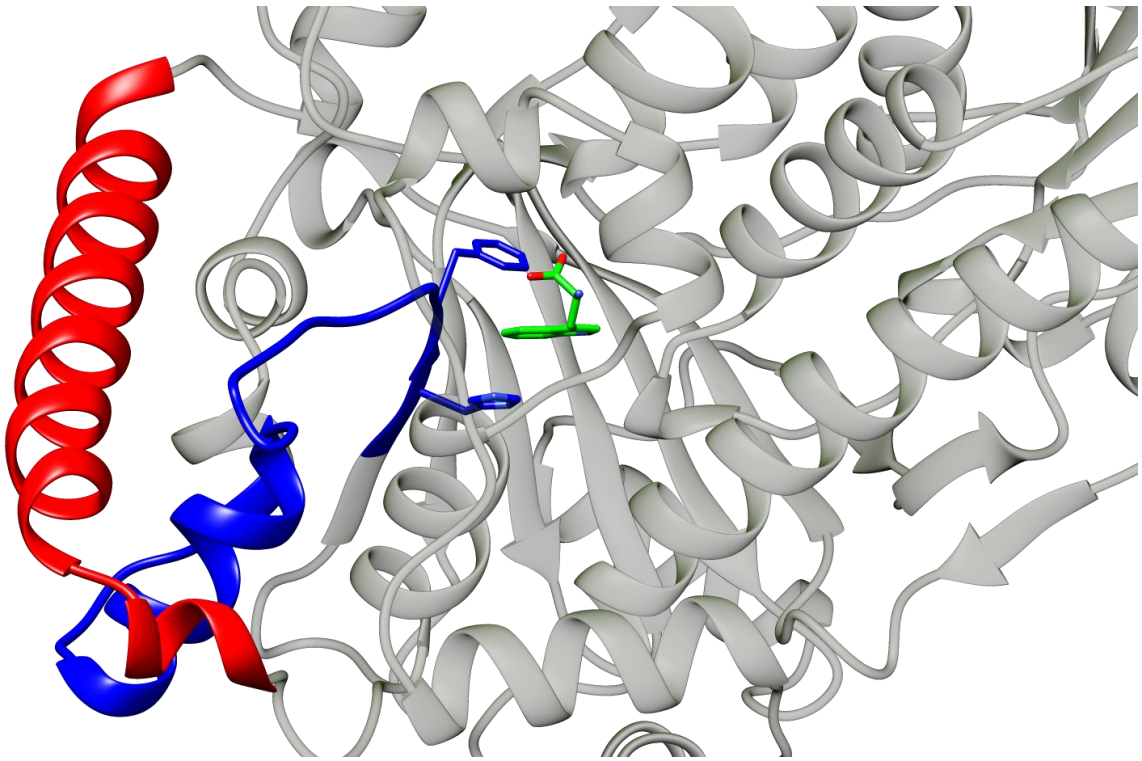

Figure S4: Loop-helix region containing the tryptophan binding residues H101 and F103 in (shown in blue) correlates with 475-505 helix (shown in red), Trp-S is shown in green.

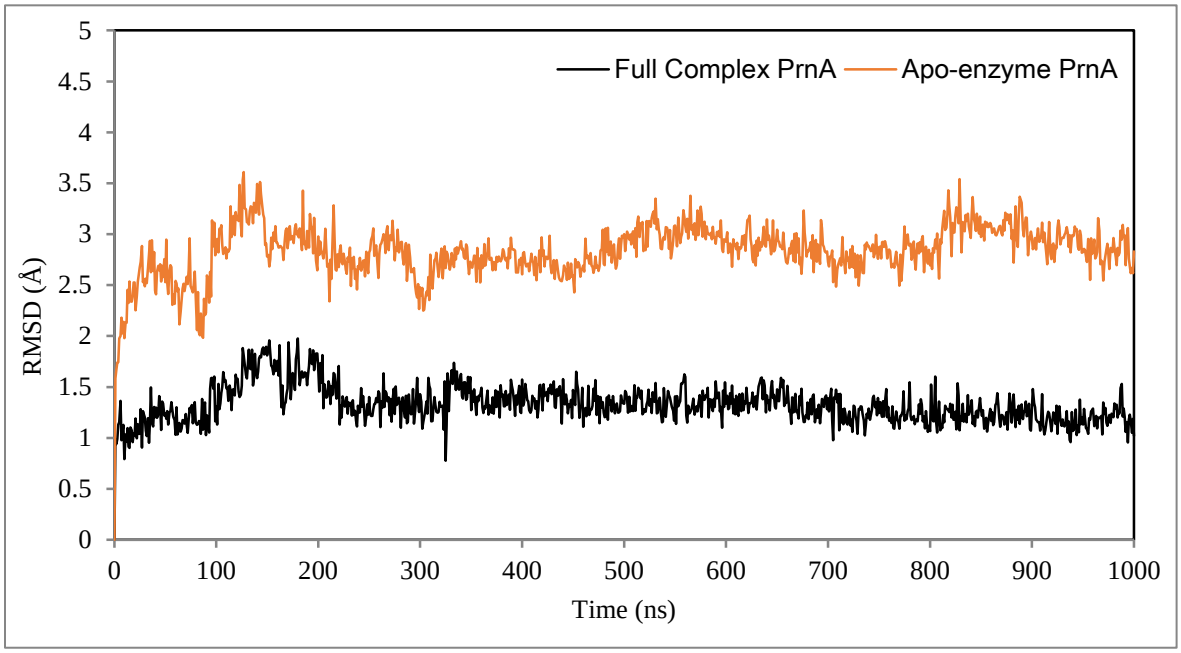

Figure S5: RMSD of the FAD binding strap consisting of residues 39-54 in the PrnA full complex and PrnA Apo-enzyme.

Table S1: Distances between the Centers of Mass of hydrophobic sidechains and the Centers of Mass of FAD in the PrnA full complex and the crystal structure.

Benzene moiety of flavin	Distance in crystal structure (Å)	Averaged Distance in the MD trajectory (Å)
V47	4.7	5.3
W274	5.9	6.5
F341	5.4	8.5
P344	4.9	5.4
Heterocyclic moiety of flavin		
I350	5.3	9.7
P344	5.7	5.2
Adenine ring moiety of FAD		
I42	4.6	4.7
L223	6.0	8.4
R221*	6.0	5.4

* indicates about a cation-π interaction.

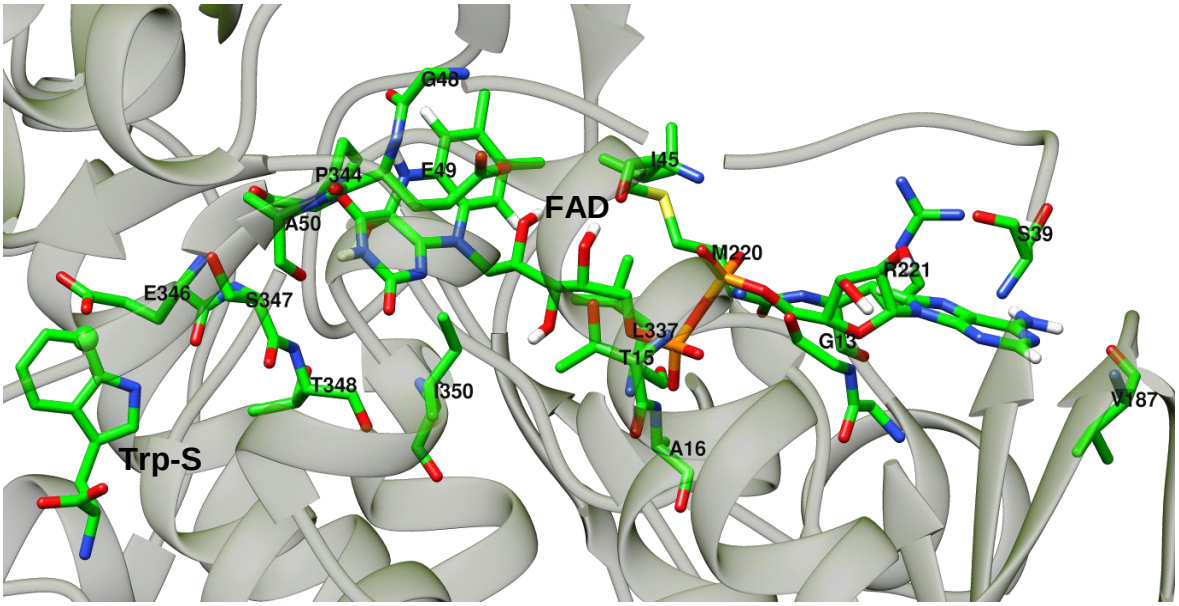

Figure S6: Hydrogen bonding interactions of FAD within the PrnA.

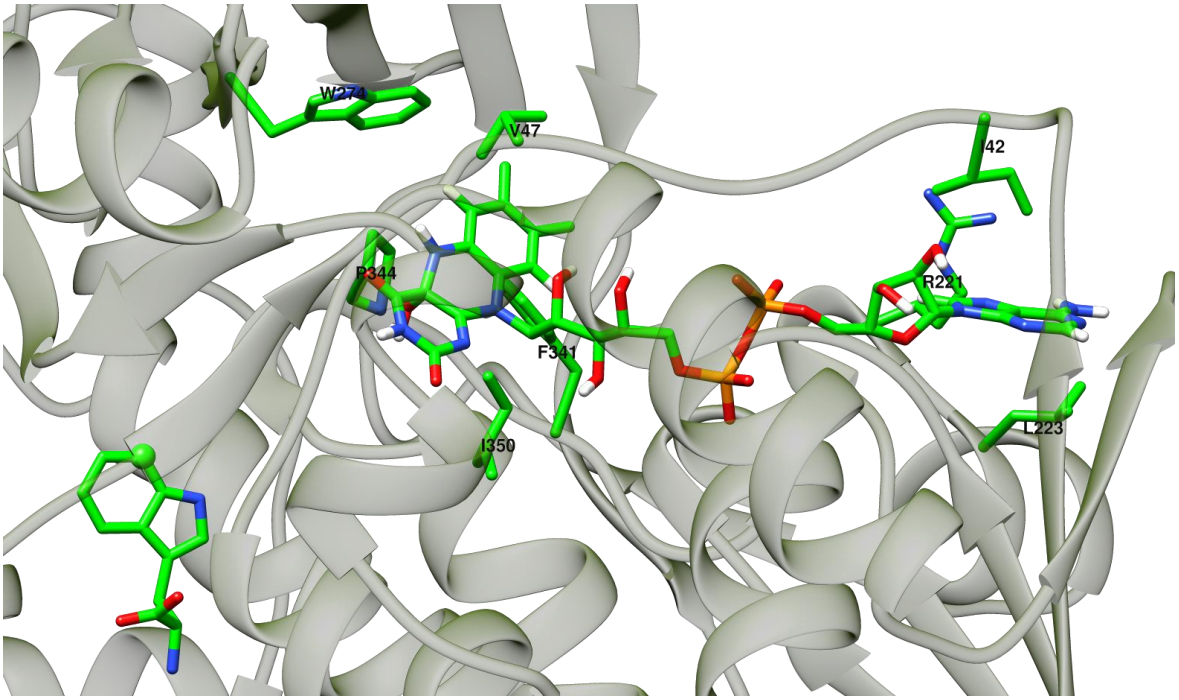

Figure S7: Hydrophobic and cation-π interactions of FAD within the PrnA.

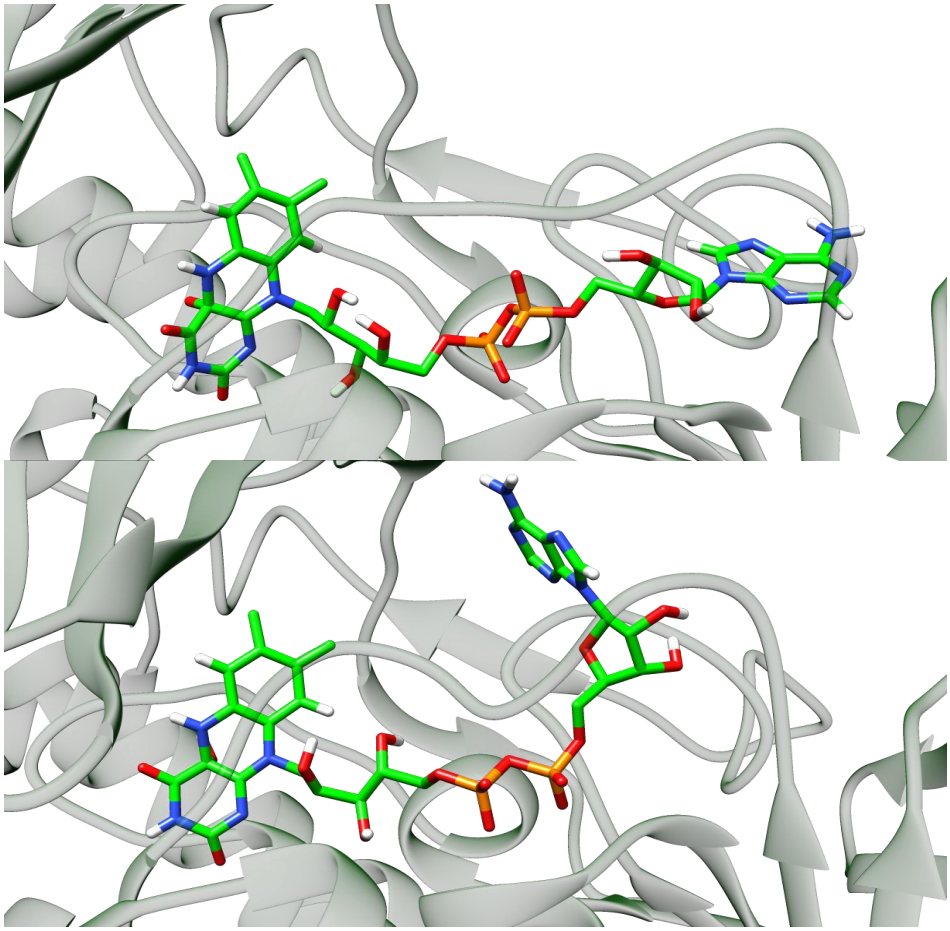

Figure S8: Top image shows the linear conformation of FAD found in the PrnA and PyrH crystal structures. Bottom image shows the bent conformation adopted by FAD after the equilibration phase of the MD simulation.

Table S2: Hydrogen bonding interactions between FAD and the protein in the PrnA full complex MD simulation and crystal structure.

residue name and number	Atom type of FAD binding residue	Atom type of FAD atom in pdb	Distance in crystal structure (Å)	Average distance in trajectory (Å)	% of simulation time <=3.5Å
G12	O	O2B	7.0	3.6	52.3
T15	N	O2A	7.1	3.4	69.6
A16	N	OBC	3.0	3.2	87.8
A50	N	N3	3.4	3.5	62.9
A50	N	O4	3.1	2.8	99.9
A50	O	N3	2.8	3.3	68.7
A50	N	O4	3.0	2.8	99.4
A50	O	N3	2.6	3.3	70.6
M220	N	N1A	11.3	3.4	90.7
R221	NH2	N7A	5.1	4.0	50.9
R221	NE	N7A	4.0	3.7	52.3
L337	N	O2P	2.7	3.2	84.7
P344	O	n/a	n/a	3.5	55.6
E346	O	n/a	n/a	3.3	76.4
S347	OG	O4	4.8	2.6	99.9
T348	N	O2	4.8	3.4	62.9
I350	N	O2	2.9	2.9	99.3

Distances are measured from donor to acceptor atom. n/a measurements are FAD atoms that do not exist in the crystal structure due to its modification to hydroxy-FAD.

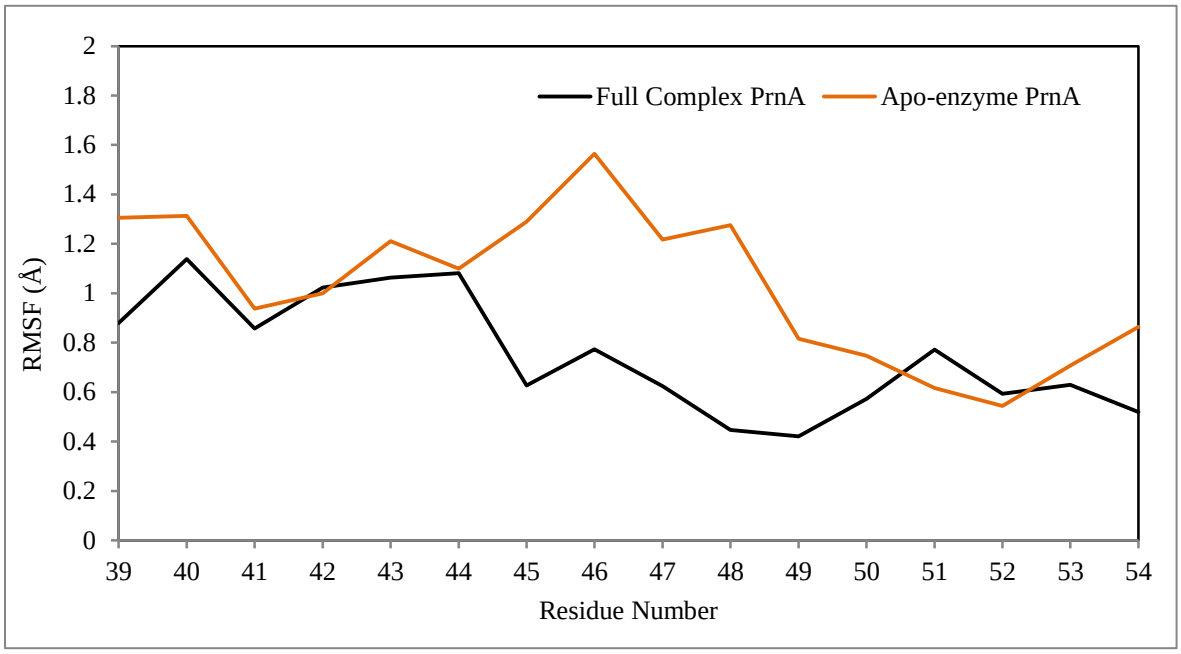

Figure S9: RMSF of the FAD binding strap region of PrnA.

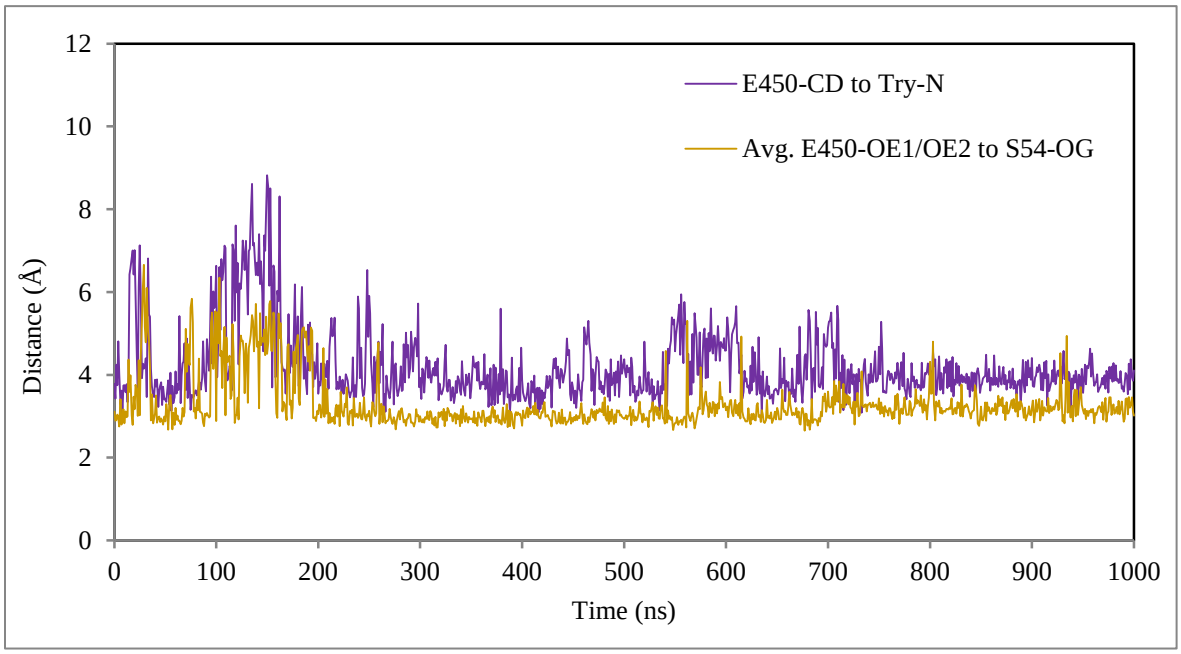

Figure S10: An electrostatic interaction between the carboxylate of E450 and the substrate tryptophan amino group and the hydrogen bonding between the sidechain of E450 and the sidechain of S45.

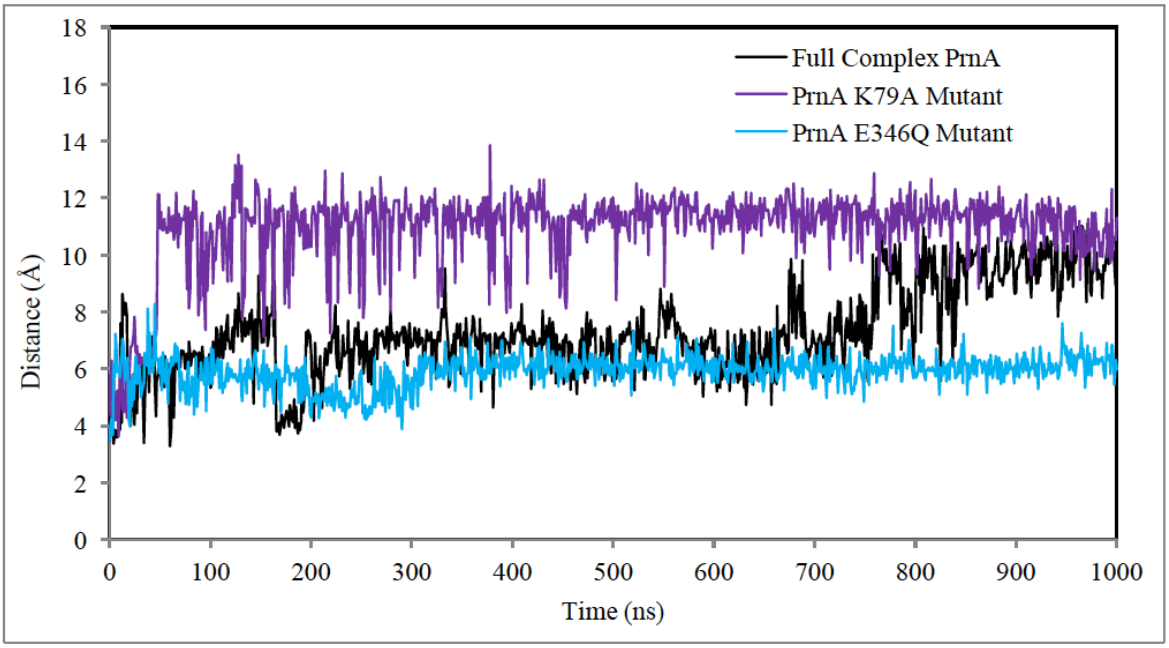

Figure S11: Distance between Lys79 NZ and hypochlorous acid Cl for the PrnA full complex, K79A mutant and E346Q mutant simulations.

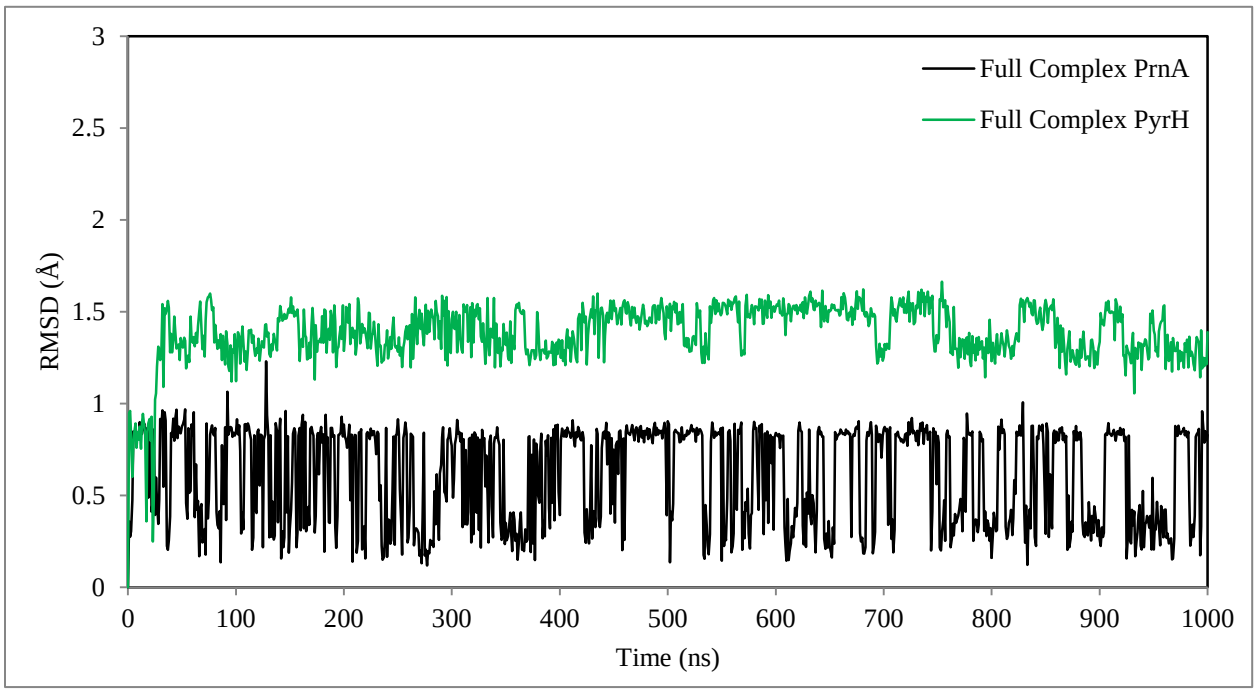

Figure S12: RMSD of the heavy atoms of tryptophan in the PrnA full complex and PyrH full complex MD simulations.

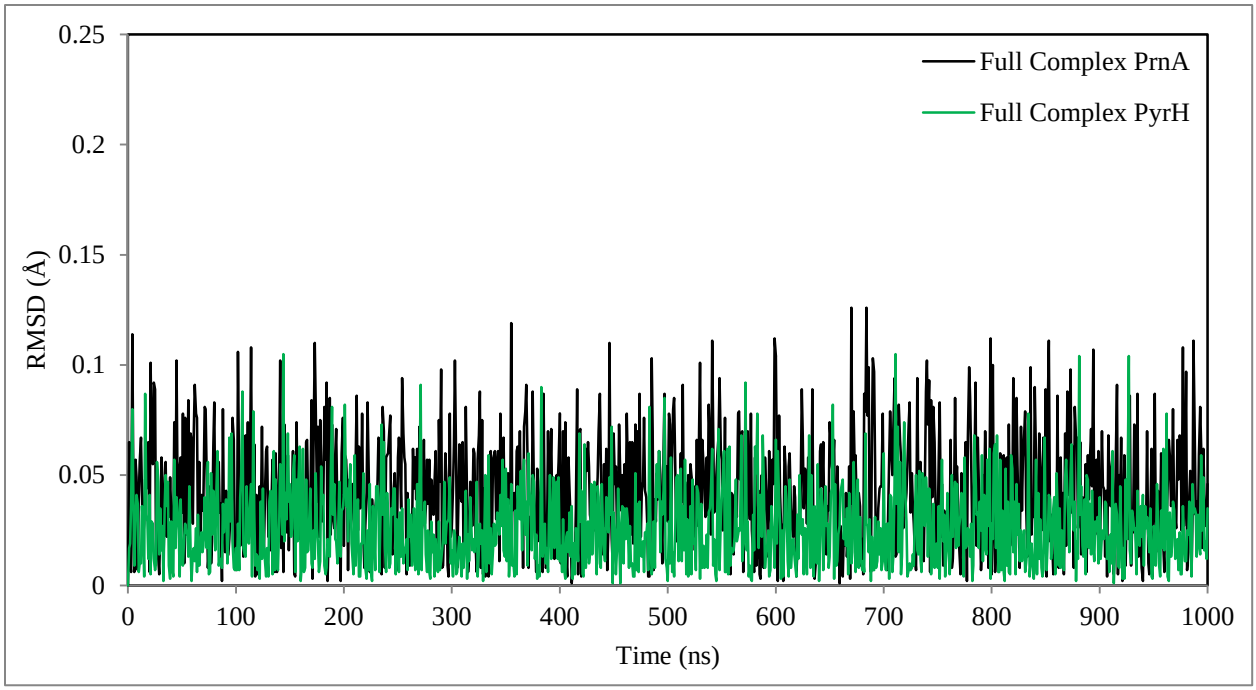
0.25
0.2
0.15
0.1
0.05
0
RMSD (Å)
0
100
200
300
400
500
600
700
800
900
1000
Time (ns)
Full Complex PrnA
Full Complex PyrH

Figure S13: RMSD of hypochlorous acid in the PrnA full complex and PyrH full complex MD simulations.

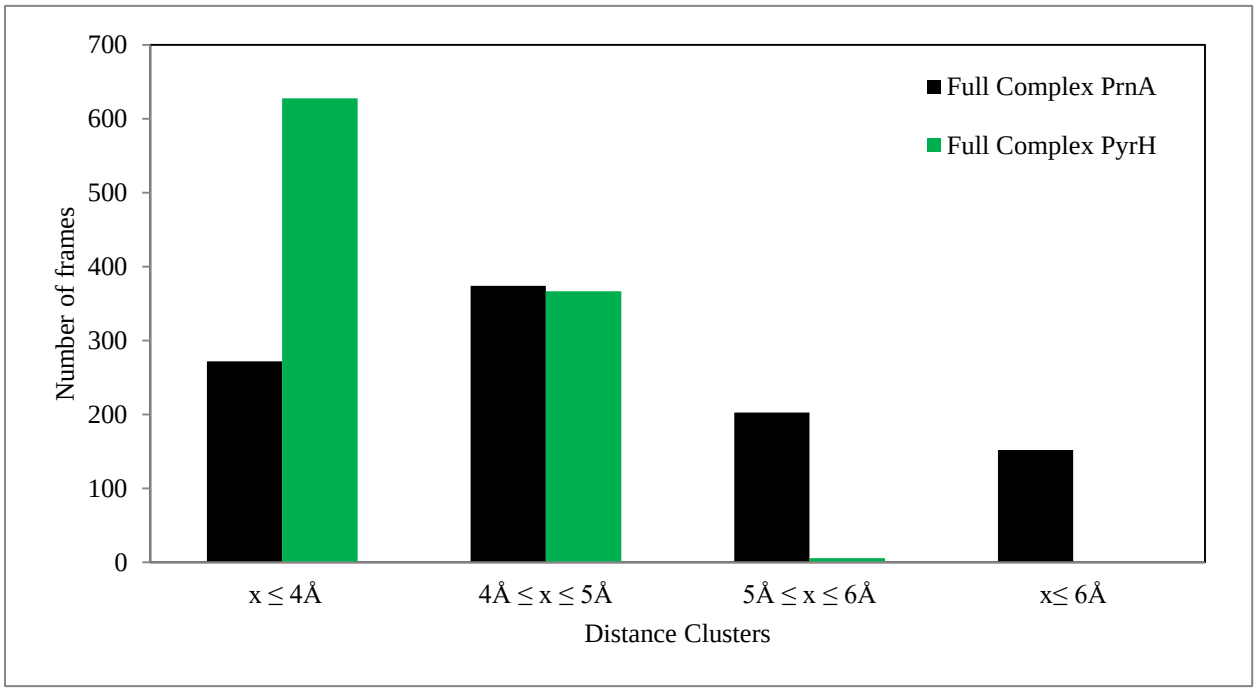

Figure S14: A bar chart showing the clustering of distances between the substrate tryptophan CZ2/CZ3 atom and the hypochlorous acid Cl atom, for both the PrnA full complex and PyrH full complex MD simulations.

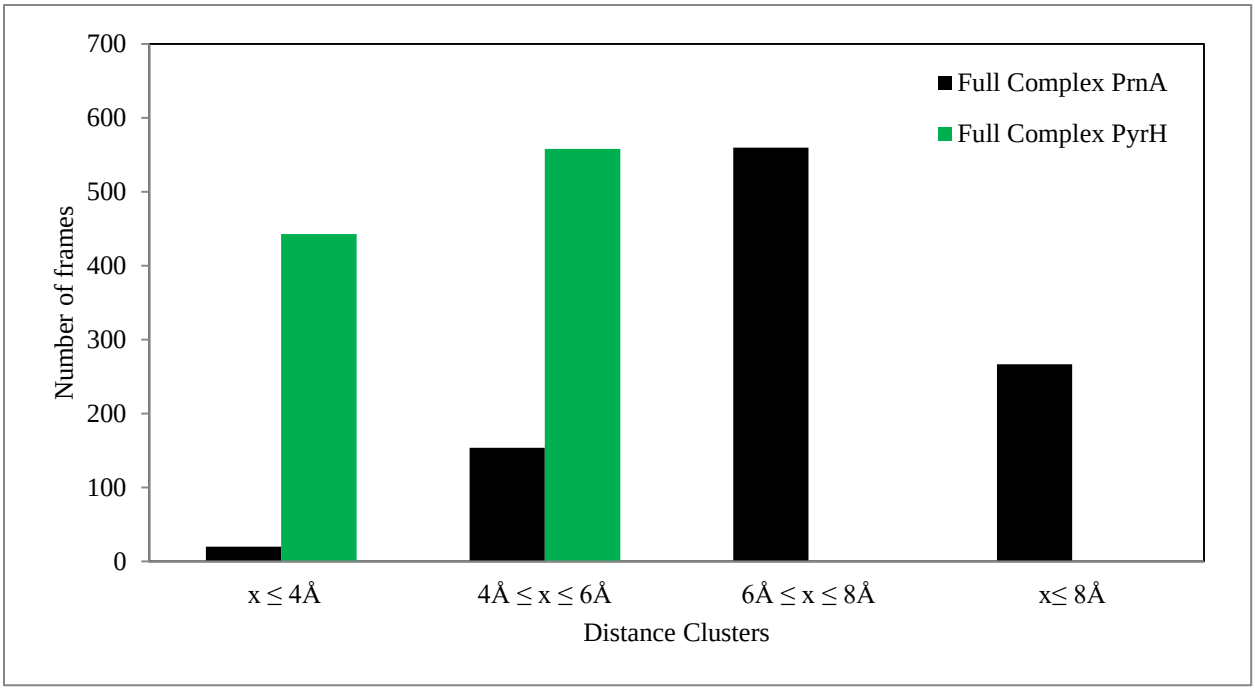

Figure S15: A bar chart showing the clustering of distances between the Lys79/75 NZ atom and the hypochlorous acid Cl atom, for both the PrnA full complex and PyrH full complex MD simulations.

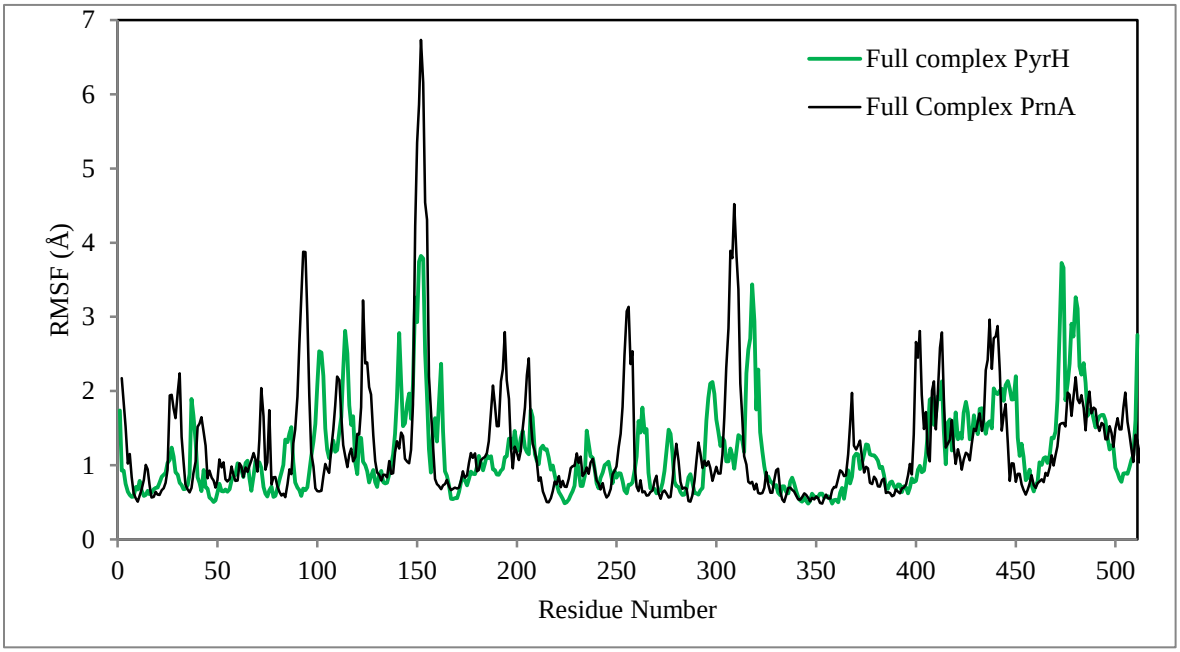

Figure S16: RMSF of the PrnA full complex and PyrH full complex MD simulations.

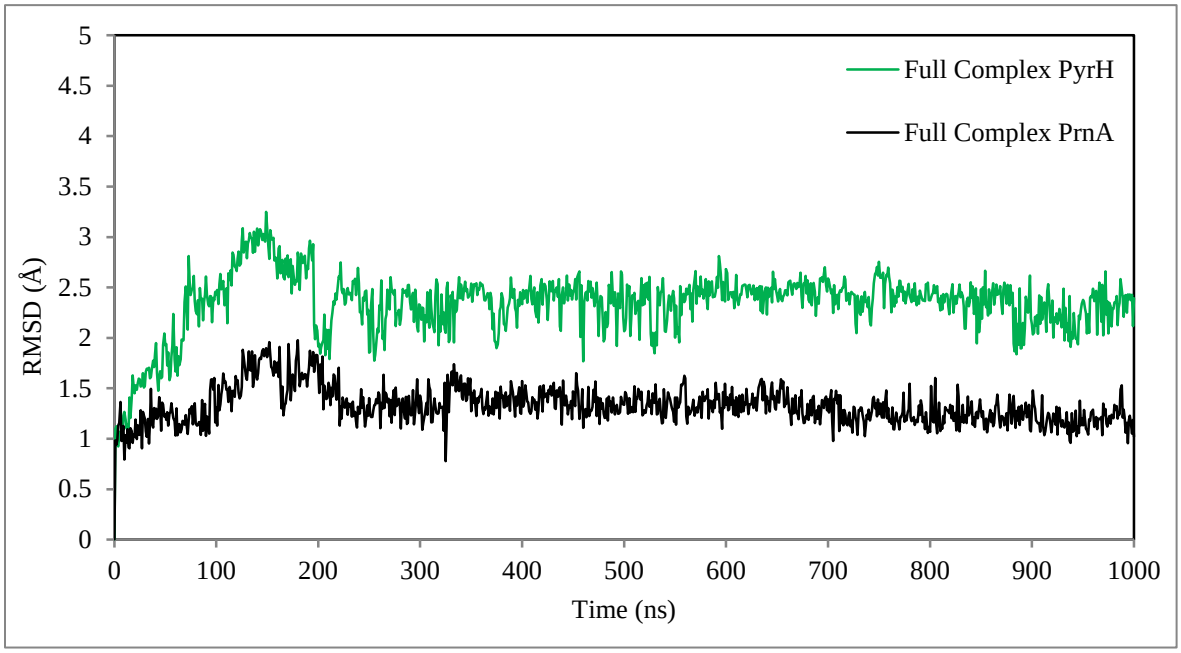

Figure S17: RMSD of the strap region (residues 37-50) of PyrH with the RMSD of the strap region (residues 39-54) of PrnA.

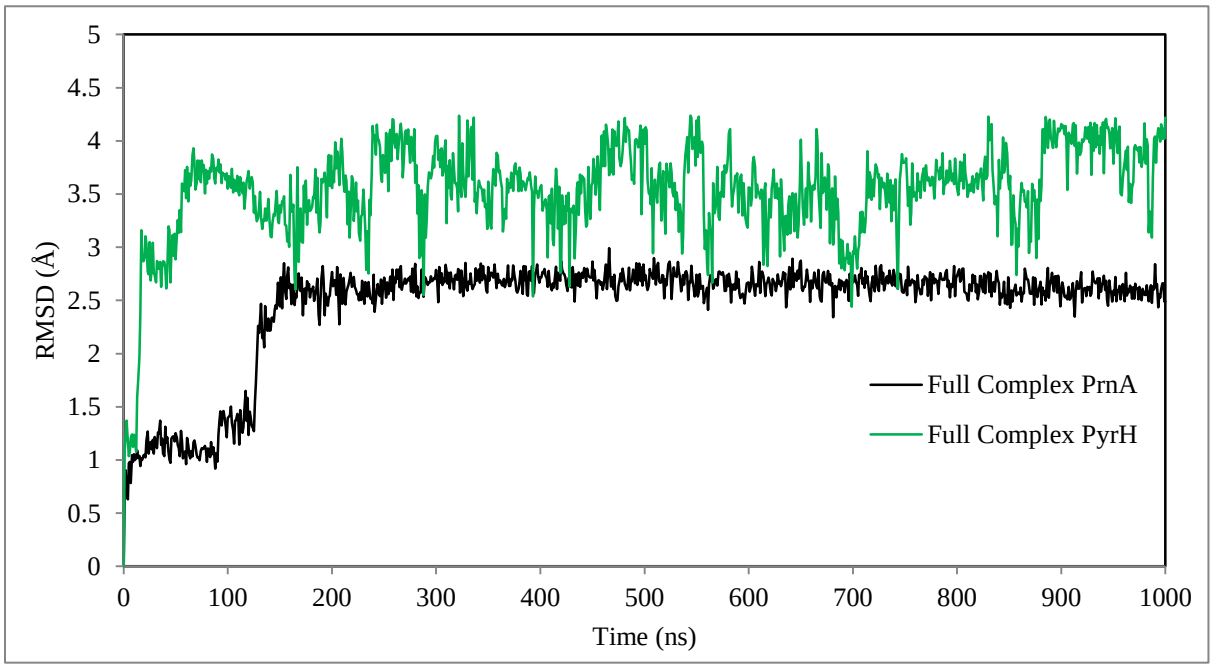

Figure S18: RMSD of FAD in the PrnA full complex and PyrH simulations.

Table S3: Hydrogen bond distances in the tryptophan binding site of PyrH for the MD simulation and crystal structure.

Residue name and number	Atom type	Residue name and number	Atom type	% of simulation <=3.5Å	Average distance	% of simulation time <=3.5Å	Distance in crystal structure (Å)
H92	NE2	E354	OE1	45.3	4.0	45.3	4.4
H92	NE2	E354	OE2	51.0	3.9	51.0	4.9
E354	N	E354	OE1	64.3	3.4	64.3	4.4
E354	N	E354	OE2	68.3	3.4	68.3	5.0
H403	NE2	E354	OE1	99.7	2.8	99.7	4.6
H403	NE2	E354	OE2	99.9	2.8	99.9	2.6
E354	O	HYP	O1	99.6	2.7	99.6	2.4
A47	O	K75	NZ	96.3	3.0	96.3	5.4
T270	OG1	K75	NZ	73.3	3.3	73.3	4.5
S355	OG	K75	NZ	85.0	3.2	85.0	2.6
Hyp	O	K75	NZ	72.2	3.4	72.2	3.1
A47	O	S355	OG	46.8	3.6	46.8	4.4
S50	OG	TRY	O	96.6	2.9	96.6	2.8
S50	OG	TRY	OXT	97.3	2.9	97.3	2.7

S50	N	TRY	O	54.2	3.6	54.2	2.6
S50	N	TRY	OXT	47.6	3.7	47.6	4.5
P93	O	TRY	NE1	41.1	3.6	41.1	3.2
F94	O	TRY	NE1	40.2	3.6	40.2	6.2
F164	N	TRY	O	39.6	4.2	39.6	6.1
F164	N	TRY	OXT	45.4	4.0	45.4	7.3
F164	O	TRY	N	91.6	3.2	91.6	7.9
S455	OG	TRY	NE1	52.2	3.5	52.2	4.1

Measurements were made between the donor and acceptor atoms.

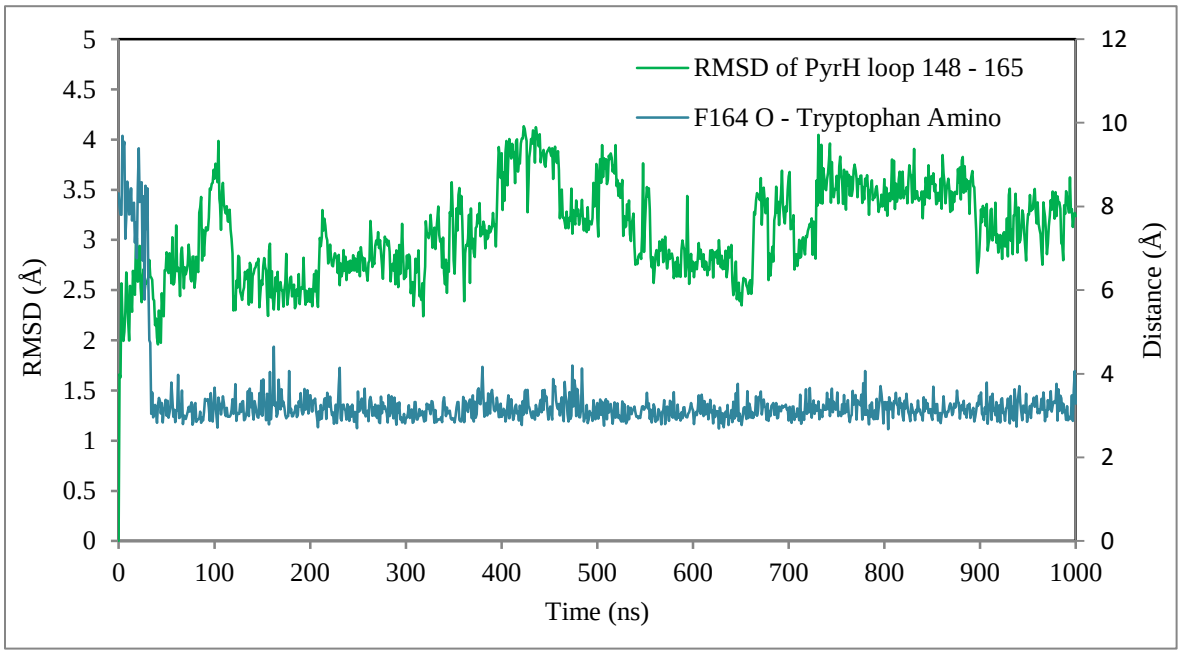

Figure S19: A plot showing the relationship between the RMSD of the flexible loop region spanning residues 148-165 in the PyrH simulation and the hydrogen bonding interaction between F164 and the substrate tryptophan.

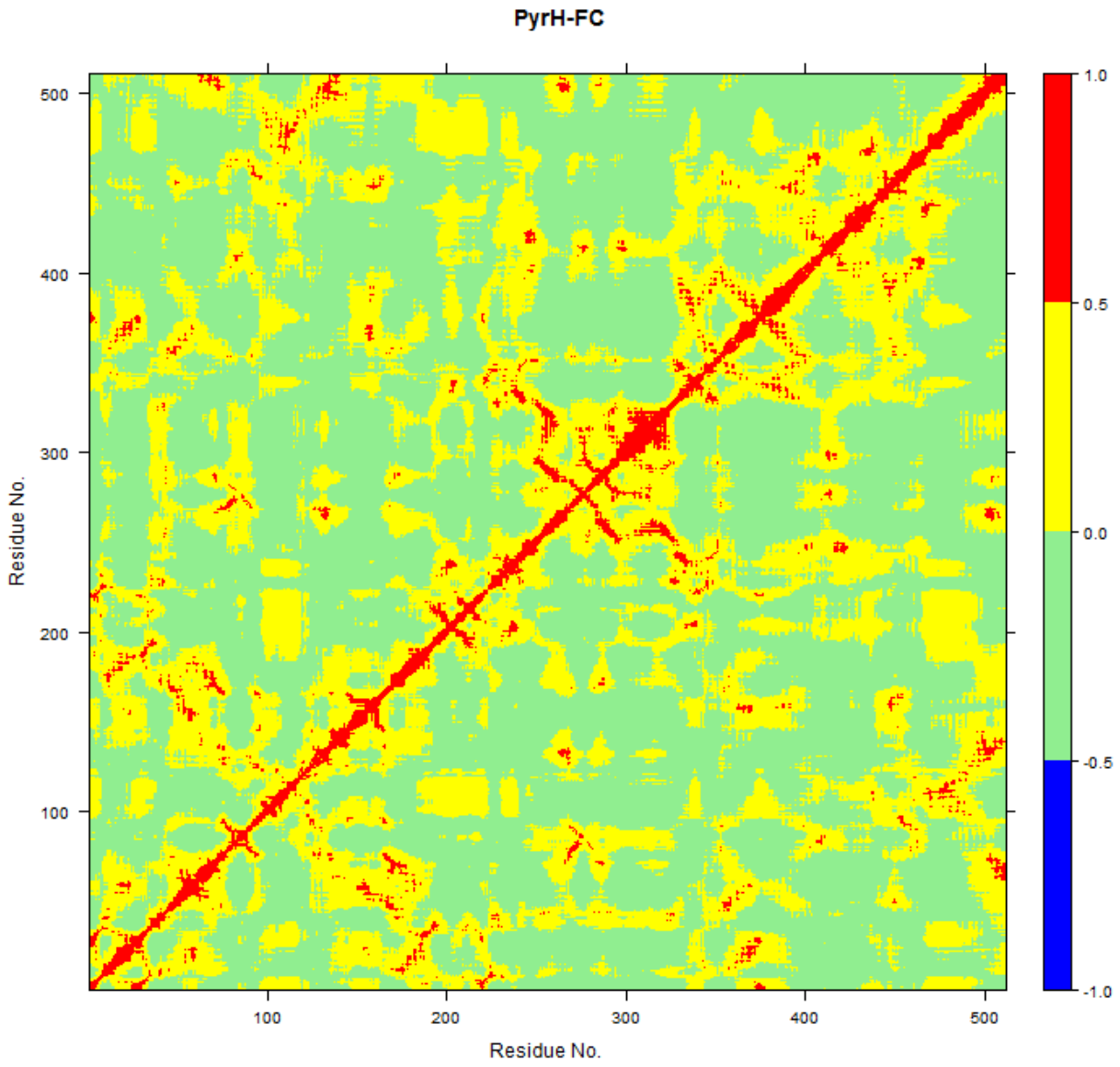

Figure S20: The DCCA plot of the PyrH simulation**.**

Table S4: Distances between Centres of Mass of hydrophobic sidechains with the indole ring of tryptophan in PyrH.

Residue name and number	Average distance (100-1000ns) (Å)	Distance in crystal structure (Å)
F49	5.3	5.8
I78	7.6	6.7
H92	5.8	4.2
F94	3.7	4.0
Y454	6.6	6.7

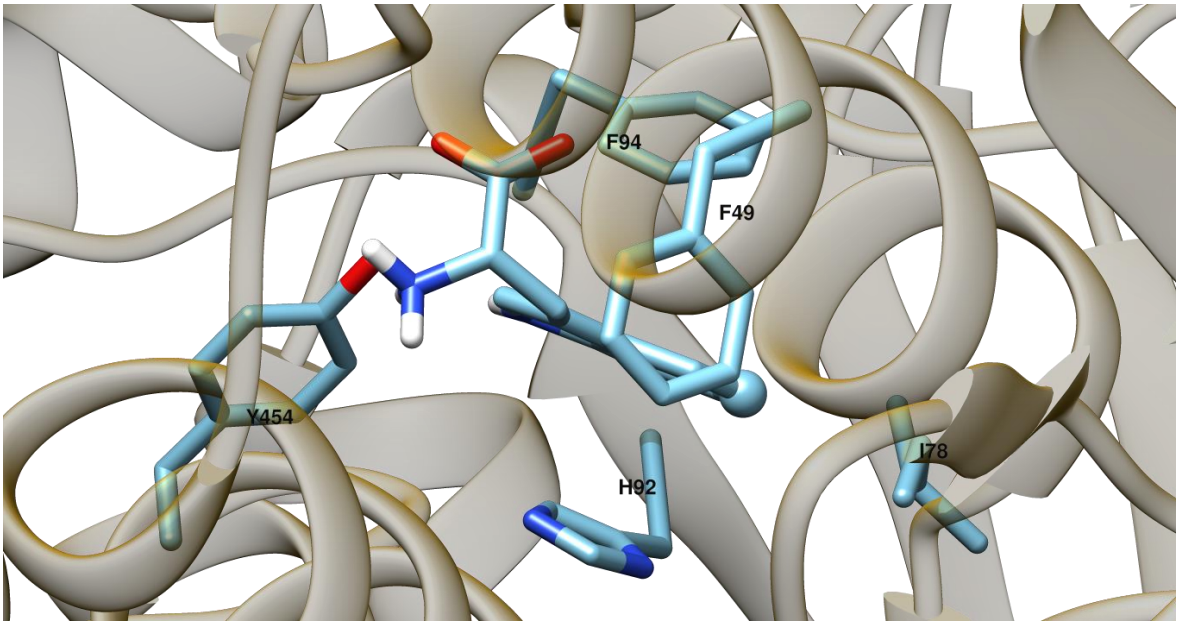

Figure S21: Hydrophobic interactions in the Trp-S binding site of PyrH.

Table S5: Electrostatic interactions in the tryptophan binding site of PyrH.

PyrH ES Residue name and number	Atom type	Residue name and number	Atom type	Average distance in MD (Å)	Distance in crystal structure (Å)
H403	NE2	E354	CD	3.1	3.4
E452	CD	Trp	N	3.9	5.4
R96	CZ	E452	CD	7.5	4.1

Measurements are made between the centers of the charged groups.

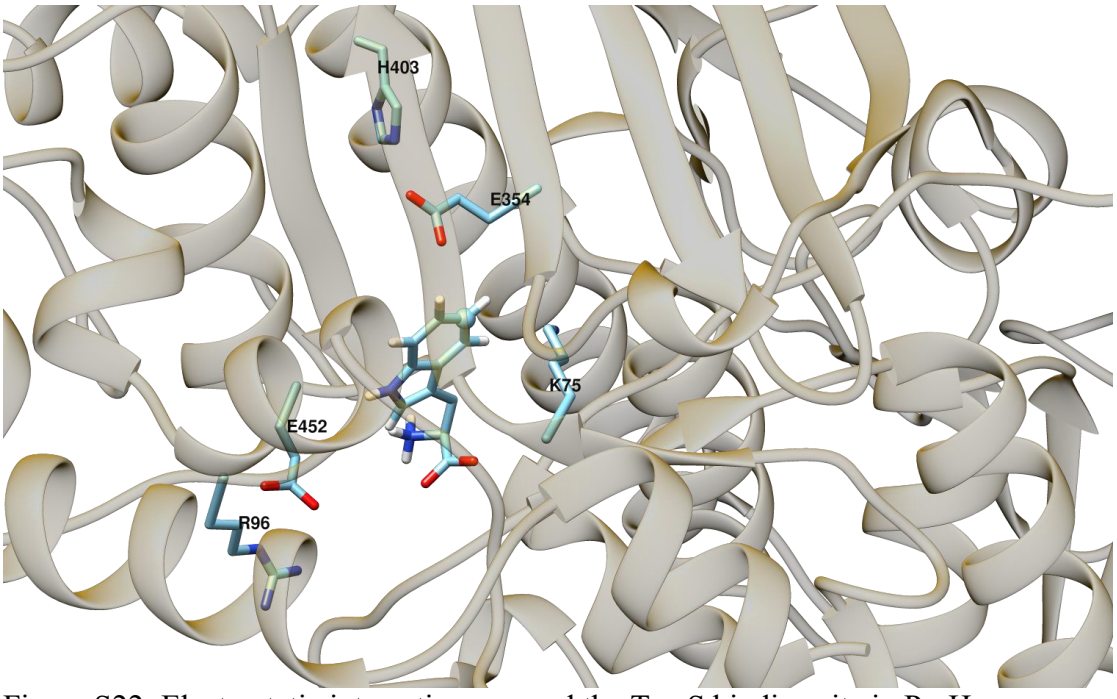

Figure S22: Electrostatic interactions around the Trp-S binding site in PyrH.

Table S6: Hydrogen bond distances of FAD in PyrH. Measurements were made between the donor and acceptor atoms.

Residue name and number	Atom type	PyrH H-bonding FAD atom in pr.gro	PyrH H-bonding FAD atom in pdb	Distance in crystal structure (Å)	Average distance in 100-1000ns trajectory (Å)	% of simulation time (100-1000ns) with distance <=3.5Å
G10	N	O3B	O3'	3.1	6.6	0.0
G11	N	O2*	O2B	6.3	2.6	99.8
T12	OG1	OAC	OBC	5.5	3.5	60.3
T12	OG1	O4'	OAG	4.0	7.5	0.0
A13	N	OAB	O2P	4.0	2.6	100.0
A13	N	OAZ	O2A	7.7	3.7	42.2
A13	N	O1P	OAC	3.5	4.5	23.4
G14	N	OAC	OBC	4.0	2.7	100.0
G14	N	OBA	O3P	7.4	3.0	97.8
S36	N	O3*	O3B	5.0	3.2	95.1
S36	N	N3	N3A	3.5	3.4	65.2

S36	N	O2B	O2'	3.6	7.0	0.0
N38	N	O2*	O2B	6.6	3.9	50.0
V39	O	O2B	O2'	2.9	5.2	7.0
I42	N	OBA	O3P	3.2	3.4	73.4
I42	N	O1A	OBA	3.4	7.1	0.0
I42	N	O2A	OAZ	2.8	5.5	0.3
V44	O	O2'	OAK	2.6	5.1	0.9
V44	O	O4'	OAG	3.4	7.4	0.0
A47	N	NBJ	N3	3.1	3.5	70.4
A47	N	OBI	O2	4.3	4.2	42.3
A47	N	O4	OBL	3.5	5.0	0.0
A47	O	N3	NBO	2.9	6.5	0.0
V195	N	N1A	N1	3.0	13.4	0.0
V195	O	N6A	N6	3.4	13.9	0.0
R229	NE	N1	N1A	7.1	3.3	85.9
L345	N	O2P	OAB	2.9	7.5	0.0
I358	N	O2	OBI	2.7	2.7	100.0

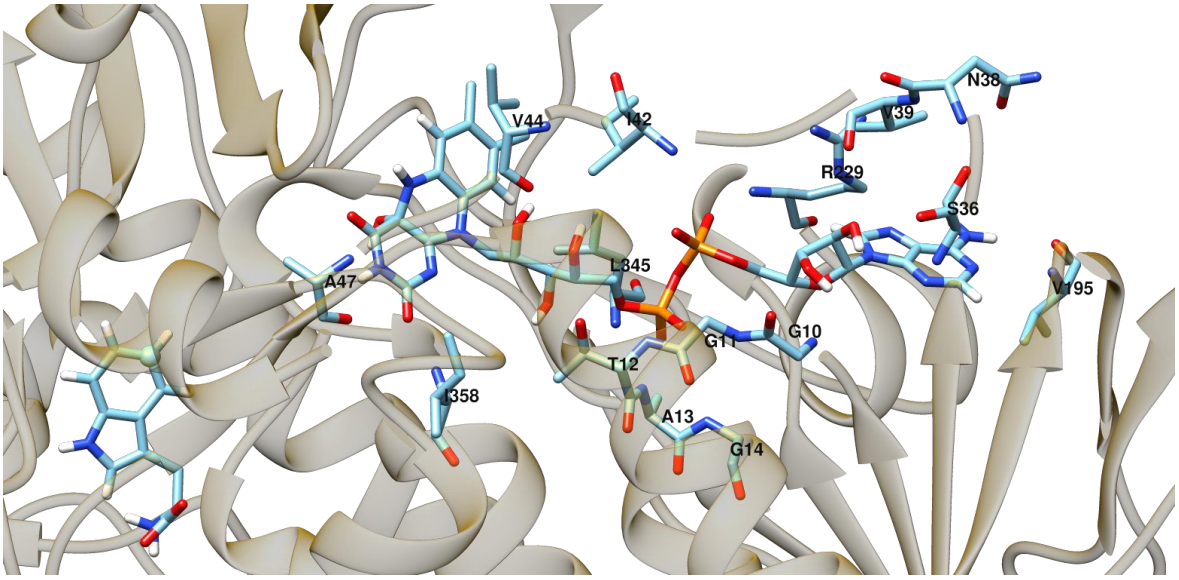

Figure S23: Hydrogen bonding interactions around tha FAD binding site in PyrH.

Table S7: Distances between Centers of Mass of hydrophobic sidechains and Centres of Mass of hydrophobic moieties of FAD in PyrH.

PyrH FAD Hydrophobic contacts	Distance in crystal structure (Å)	Average distance in trajectory 100-1000ns (Å)
Adenine moiety of Flavin COM		
V39	5.1	5.9
I42	12.8	5.6
V195	5.7	12.8
R229*	6.0	5.4
L231	5.9	9.8
Benzene moiety of Flavin COM		
V44	4.5	7.0
W281	5.4	6.1
L345	6.4	5.9
F349	4.5	5.1
P352	4.7	5.9
Heterocyclic moiety of Flavin COM		

P352	5.3	6.3
I358	5.3	5.3

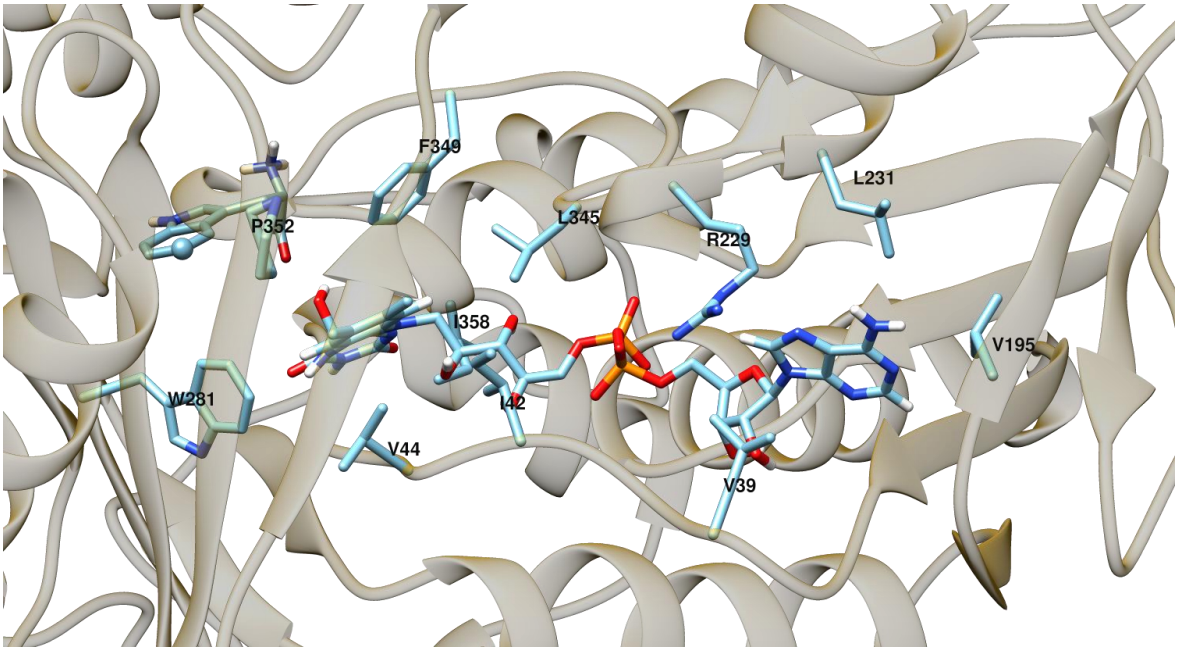

Figure S24: Hydrophobic interactions in the FAD binding site of PyrH.