Electronic Supplementary Material (ESI) for RSC Advances.

Conformational Flexibility Influences Structure-Function Relationships in TyrosylProtein Sulfotransferase - 2

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Table S1: The MD setup for the wild type TPST-2 and the experimental mutants

S.No	MD-setup	Occurrence	Ions	Timescale (ns)
1	Wt-TPST-2	wild type	Na+ (10)	100
2	R78A	loop between β3-α1 (5' PSB motif)	Na+ (12)	100
3	E99A	loop between β4-α2	Na+ (8)	100
4	K158A	β5	Na+ (12)	100
5	S285A	α13	Na+ (10)	100
6	T198A	βe	Na+ (10)	100
7	R101A	loop between β4-α2	Na+ (12)	100
8	R105A	α2	Na+ (12)	100
9	R122A	α3	Na+ (12)	100

Table S2: Average properties of wild type TPST-2 and mutant's structures computed for RMSD and RMSF for the TPST-2

	RMSD C α (Å)								RMSF	
Name	All		Alpha helices		Beta sheets		Loops		RMSF > 1Å (%)	RMSF < 1Å (%)
	μ	σ^2	μ	σ^2	μ	σ^2	μ	σ^2		
WT	2.72	0.27	2.25	0.25	1.73	0.14	3.2	0.66	57	43
R78A	2.9	0.45	3.09	0.39	2.39	0.27	3.66	0.47	67	33
E99A	2.68	0.39	2.94	0.34	2.55	0.21	3.40	0.48	55	45

R101A	2.54	0.28	2.91	0.29	2.07	0.23	3.21	0.24	78	22
R105A	3.01	0.31	3.08	0.26	2.51	0.29	3.76	0.45	65	35
R122A	2.35	0.25	2.67	0.27	1.83	0.19	2.82	0.18	67	33
K158A	2.98	0.35	3.04	0.29	2.66	0.28	3.64	0.36	81	19
S285A	3.73	0.43	3.85	0.45	2.28	0.35	4.77	0.60	78	28
T198A	3.60	0.43	3.54	0.38	2.65	0.22	4.63	0.74	75	25

μ = mean, σ^2 =standard deviation

Table S3: Local structural RMSF of wild type and mutants of TPST-2. The region is chosen to increase or decrease if there is an average change in RMSF of > 0.3 Å in at least 50% of its residue

Name	Increased		Decreased	
	Residue span	Name	Residue span	Name
E99A	114-120 , 340-350	α3, α16	149-153, 201-210,256-261	α4-β5, βe α7-α8, α10-α11
K158A	75-85, 111-131, 184-194 ,200-230, 237-247,276-289, 340-350	5'PBS, α2α3α3-α4, 3'PBS, βeα8α8-β7, β7α9, α12-α13, α16	148-153, 328-336	α4-β5, α15-α16
R78A	105-120,174-176	α2α3, α6-β6	149-154, 200-203,	α4-β5, βe
S285A	65-70, 97-108, 132-148, 265-295	β2-β3, β4-α2α2, α4 α12α12-α13α13-α14	149-154, 200-205, 331-335	α4-β5, βe, α15-α16
R101A	102-118, 135-148, 162-176, 243-250, 265-270, 277-283	α2α3, α4α4-β5 ,α5α6, α10, α12, α12-α13	149-152, 303-308, 329-335	α4-β5, α13-α14, α15-α16
R105A	65-70 188-200, 277-290	β2-β3, α7βe, α12-α13α13	149-154, 259-262, 302-309, 330-337	α4-β5, α10-α11, α13-α14,α15-α16
R122A	170-174, 308-310	α6-β6, α13-α14	149-152, 259-262, 330-337	α4-β5, α11, α15-α16
T198A	115-130, 164-176, 228-232, 261-270	α13, α5α6, α8-β7, α11α12	149-152, 200-205, 301-308,330-336	α4-β5, βe, α13-α14, α15-α16

* Regions underlined are the loops between the alpha helix and beta sheets.

Table S4: Hydrogen bond profile of cofactor binding site of wild type TPST-2 for Protomer A

Donner	Acceptor	Distance(Å)	Probability %
S79 (bb)	OBC-sulfate	2.78	95.1
G80 (bb)	OBC-Sulfate	2.71	97.3
T81 (sc)	OBE-Sulfate	2.6	96.1
T82 (sc)	OBD-sulfate	2.59	98.8
T82 (bb)	OBD-sulfate	2.75	99.1
L83 (bb)	OAG- phosphate	2.83	98.8
S285 (sc)	OAF- phosphate	2.67	95.5
S191 (sc)	3'PBS-OAB	3.1	66.9
N294 (bb)	N7-ring	3.04	89.9

S79 (bb)	OBC-Sulfate	2.78	95.1
G80 (bb)	OBC-Sulfate	2.71	97.3
T81 (sc)	OBE-Sulfate	2.6	96.1
T82 (sc)	OBD-sulfate	2.59	98.8
T82 (bb)	OBD-sulfate	2.75	99.1
L83 (bb)	OAG- phosphate	2.83	98.8
S285 (sc)	OAF- phosphate	2.67	95.5
V289 (bb)	S285 (bb)	3.5	28.0

Table S5: Important substrate and catalytic site interactions of wild type TPST-2 averaged over 100ns

Substrate Residue Interactions		Distance (Å)	Catalytic site Residue Interactions		Distance (Å)
Y1006 (sc)	E99 (sc)	3.16	R78 (sc)	PAPS (OAD)	3.0
Y1006 (sc)	P77 (sc)	4.2	R78 (sc)	Q288 (sc)	4.1
Y1006 (bb)	T198 (bb)	3.5	R78 (sc)	S285 (sc)	3.7
Y1006 (bb)	A200 (bb)	3.5	R78 (bb)	Y1006 (sc)	5.8
Y1006 (sc)	I199 (sc)	4.1	S285 (sc)	PAPS (OAF)	2.6
Y1006 (sc)	P160 (sc)	3.9	S285 (sc)	PAPS (OAD)	4.8
D1005 (bb)	T198 (sc)	3.1	K158 (sc)	OBE sulfate	3.6
D1005 (bb)	R101 (sc)	3.5	K158 (sc)	Y1006 (sc)	4.6
D1005 (sc)	R101 (sc)	4.3	E99 (sc)	K158 (sc)	2.7
D1005 (sc)	R122 (sc)	5.2	Y1006 (sc)	PAPS sulfate	6.1
E1004 (bb)	R101 (sc)	3.5			
F1003 (sc)	R101(sc)	4.3			
D159 (sc)	R101(bb)	3.2			
E1007 (sc)	R105 (sc)	3.6			
R122 (sc)	E99 (sc)	4.0			

Table S6: The substrate angle for wild type and mutants for Cα atoms of 1004, 1005 and 1006 residues of substrate peptide

Name	Angle (mean)
WTFC	95.9°
R78A	93.5°
E99A	103.5°
K158A	94.5°
S285A	90.0°
R101A	95.3°
R105A	96.1°
R122A	91.8°

T198A	93.1°

Table S7: The hydrogen bond profile for substrate binding in the wild type TPST-2

Donor	Acceptor	Distance (Å)	Probability (%)
Y1006 (bb)	T198 (bb)	3.5	86.6
A200 (bb)	Y1006 (bb)	4	63.6
Y1006 (sc)	E99 (sc)	3.1	99.9
T198 (sc)	D1005 (bb)	3.1	79.1
T198 (sc)	D1005 (sc)	3.5	59.6
R101 (sc)	E1004 (bb)	3.4 (70-ns)	25.6
R101 (sc)	D1005 (bb)	0.38	0.5

Table S8: The cluster and minimized structure distances of mutant E99A and wild type TPST-2

	Cluster Analysis				Initial crystal structure with hydrogen
	Wild-type		E99A mutant		Wild type
Residue	Cluster 1 (A)	Cluster 2 (A)	Cluster 1 (A)	Cluster 2 (A)	Protomer (A)
E99 (sc) - R101(sc)	7.74	7.58	4.13	7.23	7.31
E99 (sc) - K158 (sc)	3.77	4.14	4.38	4.20	3.52
E99 (sc) –Y1006 (oh)	2.80	2.61	10.64	8.72	2.36
Y1006(oh) - sulfate	5.85	6.47	7.92	3.64	4.56
R78(bb) - Y1006(oh)	4.64	5.56	4.25	3.23	4.91
K158 (sc) - sulfate	5.87	5.65	5.34	4.77	3.80
K158 (sc) – Y1006 (oh)	4.44	4.60	9.49	6.47	4.35

Note: oh = hydroxyl group of substrate Y1006

Table S9: The cluster analysis and minimized structure distances of mutant T198A and wild type TPST-2

	Cluster Analysis				Initial crystal structure with hydrogen
	Wild type		Mutant T198A		Wild Type
Residue	Cluster 1 (A)	Cluster 2 (A)	Cluster 1 (A)	Cluster 2 (A)	Protomer (A)
T198 (bb) –Y1006(bb)	1.83	1.89	2.72	2.83	**2.09**
T198 (sc) – D1005 (sc)	2.38	2.33	3.84	4.15	**3.92**
T198(sc) – D1005 (bb)	3.05	2.75	5.15	5.35	2.89
A200(bb) - Y1006 (bb)	2.23	4.34	2.70	2.72	1.81
I199 (sc) - Y1006 (sc)	3.77	3.65	3.63	4.34	4.17

Table S10: The cluster analysis and minimized structure distances of mutant R101 and wild type TPST-2

	Cluster Analysis				Initial crystal structure with hydrogen
	Wild type TPST-2		K158A mutant		Wild type
Residue	Cluster 1 (A)	Cluster 2 (A)	Cluster 1 (A)	Cluster 2 (A)	Protomer (A)
R101 (sc)-E1004 (bb)	2.92	3.18	6.12	6.93	2.83
R101 (sc)-D1005 (bb)	3.48	2.98	4.91	5.68	2.99
R101 (sc) - D1005 (sc)	6.17	3.69	9.10	9.47	4.38

Table S11: The cluster analysis of S285A mutant and the wild type TPST-2

	Cluster Analysis				Initial crystal structure with hydrogen
	Wild type TPST-2		S285A mutant		Wild Type
Residue	Cluster 1 (A)	Cluster 2 (A)	Cluster 1 (A)	Cluster 2 (A)	Protomer (A)
S285 (sc) - bridge-oxygen of cofactor	3.70	4.10	5.92	6.70	3.96
S285(sc)- phosphate close to sulphate	2.52	2.83	4.77	5.55	2.55
S285(sc) - Oxygen OAF of phosphate close to sulphate	1.57	1.75	3.72	4.53	2.57
S285 (sc) – OAF of cofactor	3.35	2.88	2.29	3.24	5.64

Table S12: The cluster analysis of K158A mutant and the wild type TPST-2

Cluster Analysis					Initial crystal structure with hydrogen
	Wild type TPST-2		K158A mutant		Wild type
Residue	Cluster 1 (A)	Cluster 2 (A)	Cluster 1 (A)	Cluster 2 (A)	Protomer (A)
K158 (sc) – Y1006 (oh)	4.44	4.60	10.53	11.20	4.35
K158 (sc) - sulfate group (OBE)	6.09	5.97	11.84	10.18	4.60
K158 (sc) – bridge oxygen of cofactor	7.08	6.91	12.03	11.26	4.89

Table S13: The Cluster Analysis of E99A and the wild type TPST-2

	Cluster Analysis				Initial crystal structure with hydrogen
	Wild-type TPST-2		E99A mutant		Wild type
Residue	Cluster 1 (A)	Cluster 2 (A)	Cluster 1 (A)	Cluster 2 (A)	Protomer (A)
E99 (sc) - R101 (sc)	7.74	7.58	4.13	7.23	7.31
E99 (sc) - K158 (sc)	3.77	4.14	4.38	4.20	3.52
E99 (sc) - Y1006 (oh)	2.80	2.61	10.64	8.72	2.36
Y1006 (oh) - sulfate	5.85	6.47	7.92	3.64	4.56
R78 (sc) -Y1006 (oh)	4.64	5.56	4.25	3.23	4.91
K158 (sc) - sulfate	5.87	5.65	5.34	4.77	3.80
K158 (sc) - Y1006 (oh)	4.44	4.60	9.49	6.47	4.35

Table S14 The average properties of the multiple runs of the wild type TPST-2 for 100 ns

Name	Mean (Å)	S.D (Å) within each trajectory	S.E.M (Å)	S.D (Å) In respect to the average trajectory	E99 (sc)-Y1006(OH) Å	Y1006(OH)-PAPS(sulphur) Å	Substrate angle
Run 1	2.73	0.24	0.004	0.22	3.1	6.1	95.9°
Run 2	2.81	0.15	0.002	0.21	3.4	6.5	95.5°
Run 3	2.40	0.23	0.004	0.21	3.5	6.7	94.7°
Averaged	*2.65*	*0.16*	*0.002*		*3.3*	*6.4*	*95.3°*
200ns (extension of Run 1)	2.90	0.34	0.003		3.6	6.3	96.0°

Table S15 The averaged properties of E99A mutant for multiple run trajectories for 100 ns run

Name	Mean (Å)	S.D (Å)	S.E.M (Å)	A99(sc) – Y1006(sc0	A99(sc) – K158 (sc)	Substrate angle

Run 1	2.42	0.25	0.006	9.5	4.3	103.5°
Run 2	2.99	0.30	0.006	8.2	4.0	98.9°
Averaged all	2.7	0.27	0.006	8.8	4.1	101.2°

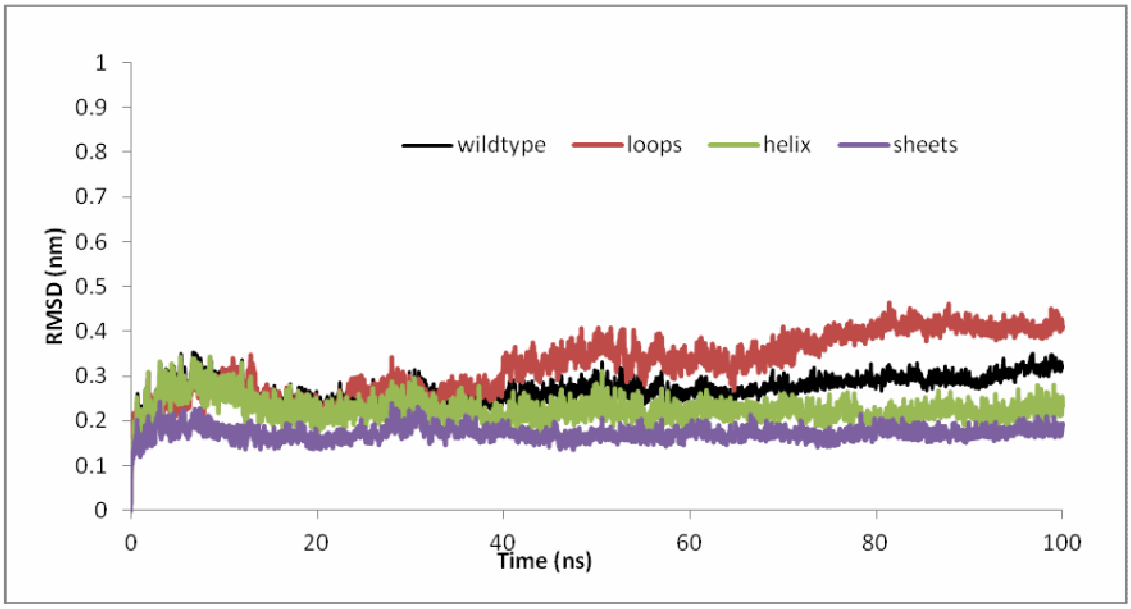


Figure S1 The secondary structure RMSDs of wild type TPST-2. This includes alpha helix, beta sheets and loops for 100ns.

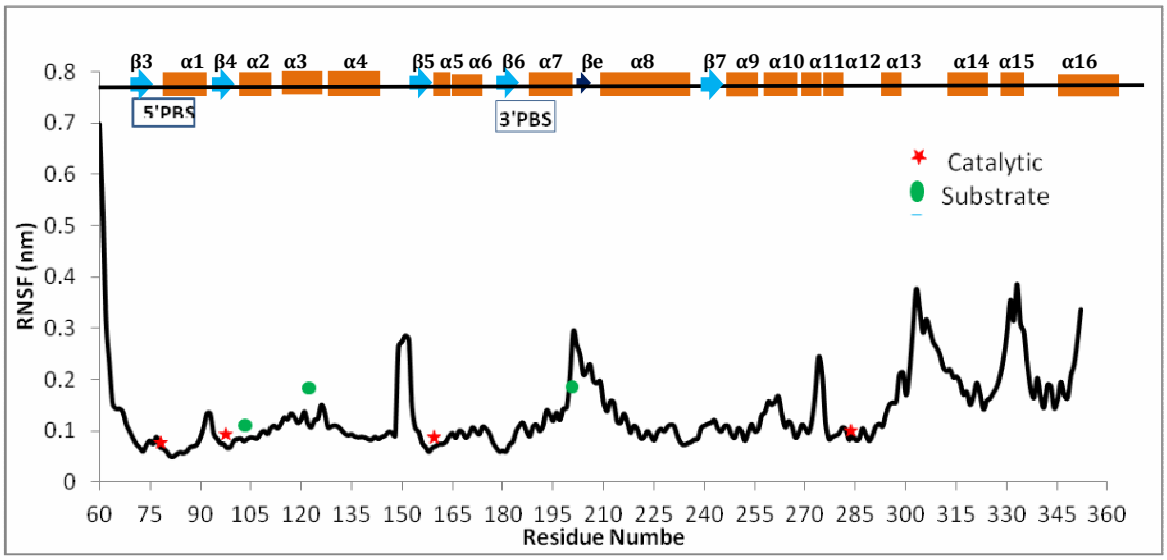


Figure S2 the RMSF analysis of protomer A of wild type TPST-2. The 5'PBS and 3'PBS are the cofactor binding sites and βe is the substrate binding residues in the wild type TPST-2. The catalytic residues are represented in red colour star and the substrate binding residues are in green circle representation.

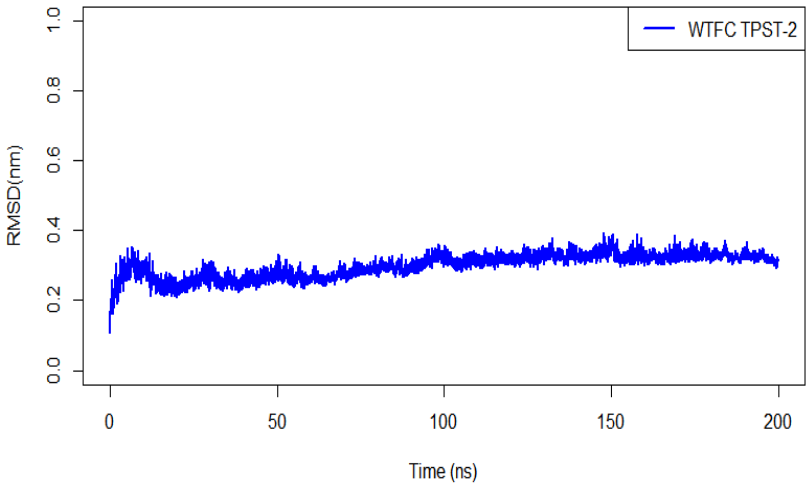


Figure S3 The RMSD trajectory of all C α atoms of wild type TPST-2 (WT FC) for 200ns

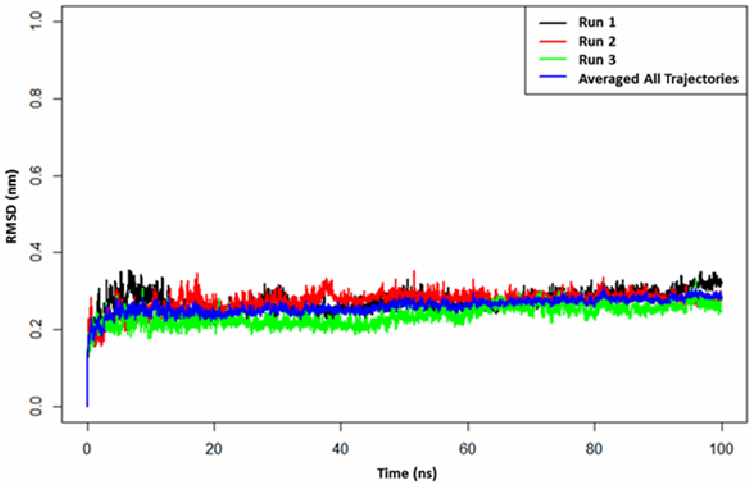


Figure S4 The RMSD of wild type TPST-2. The average trajectory is shown in blue colour from run 1, run 2 and run 3

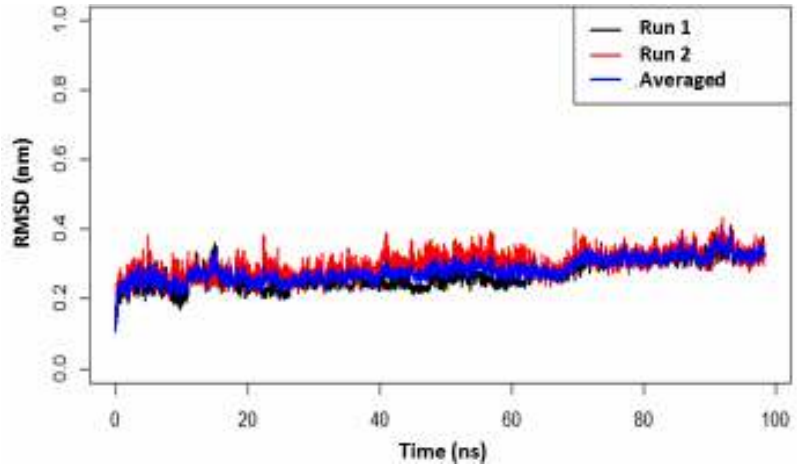


Figure S5 The replica runs of the E99A mutant along with the average run (100ns) for two trajectories.

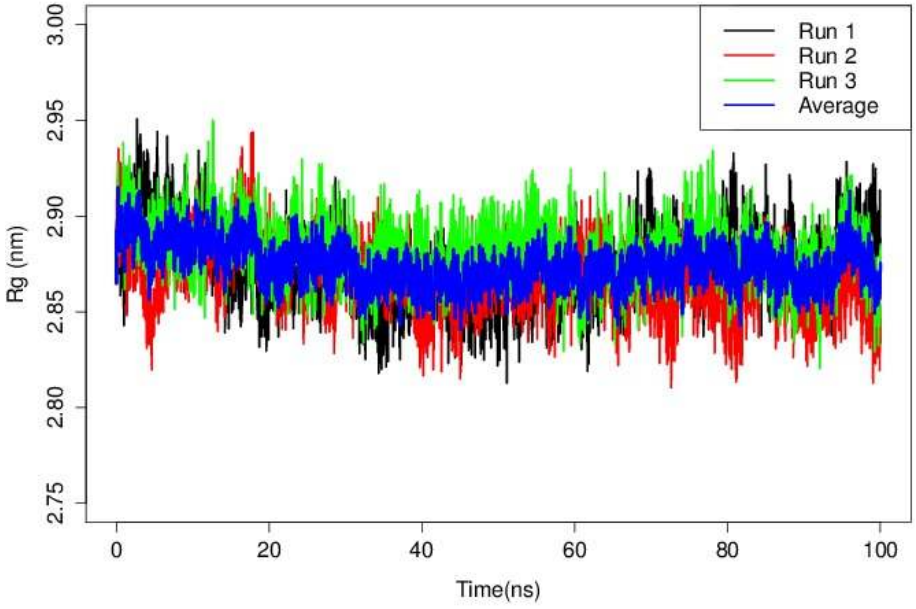


Figure S6 The Radius of Gyration (Rg) of WTFC for 100 ns. The average value of Rg from the independent runs has been plotted in blue colour as function of simulation time.

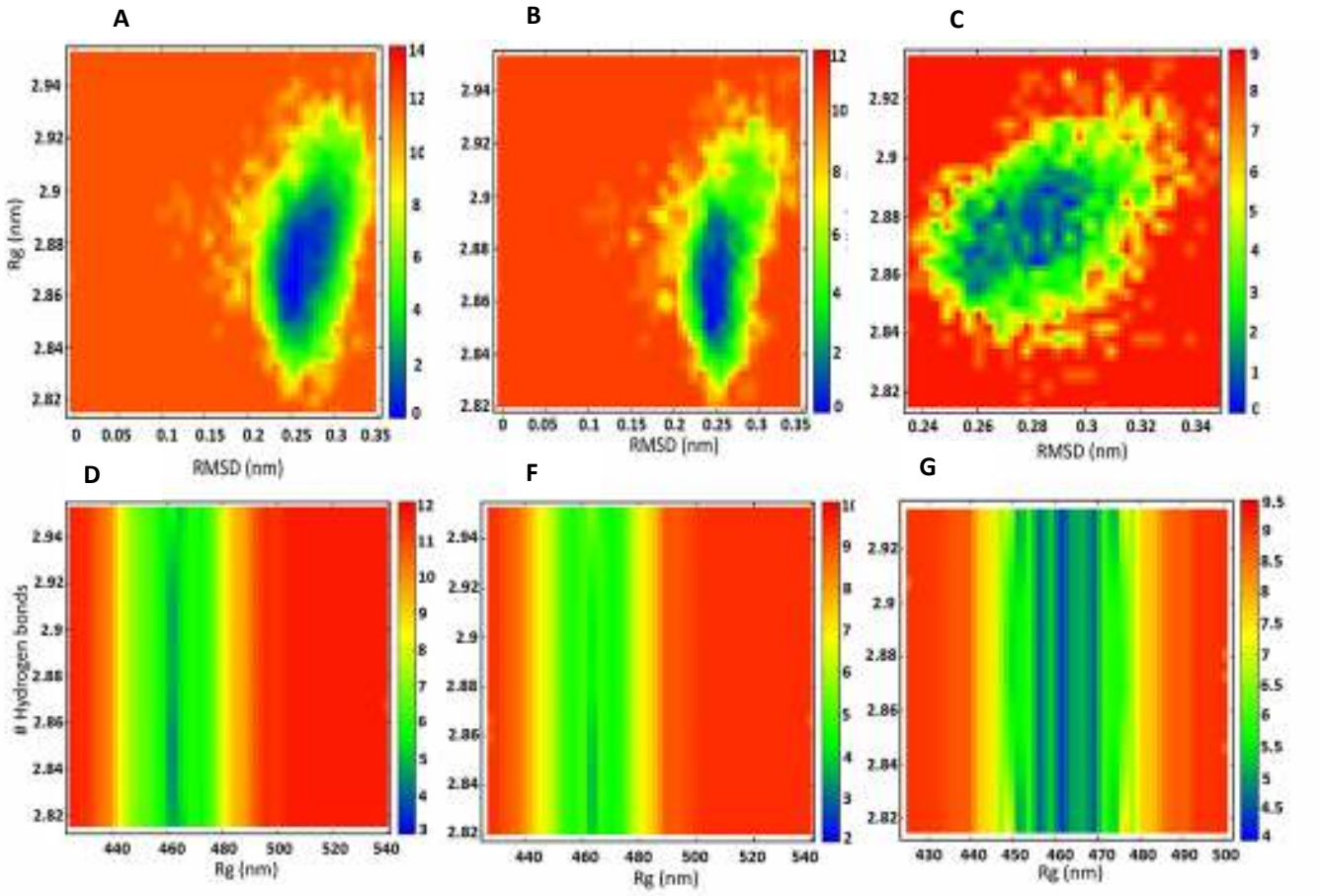


Figure S7 Figure S5 The free energy landscape (FEL) sampled from 100 ns trajectory for wild type TPST2 (WTFC) at 300 K. The Gibbs free energy is estimated from probability distribution of the system with respect to Radius of gyration (Rg), Root Mean Square Deviation (RMSD) and protein-protein hydrogen bonds. (A) RMSD vs Rg from 0-100 ns, (B) RMSD vs Rg from 0-50 ns, (C) RMSD vs Rg from 50-100 ns, (D) Rg vs protein-protein hydrogen bonds from 0-100 ns, (E) Rg vs protein-protein hydrogen bonds from 0-50 ns, (F) Rg vs protein-protein hydrogen bonds from 50-100ns. The FEL was obtained using g_sham option in Gromacs.

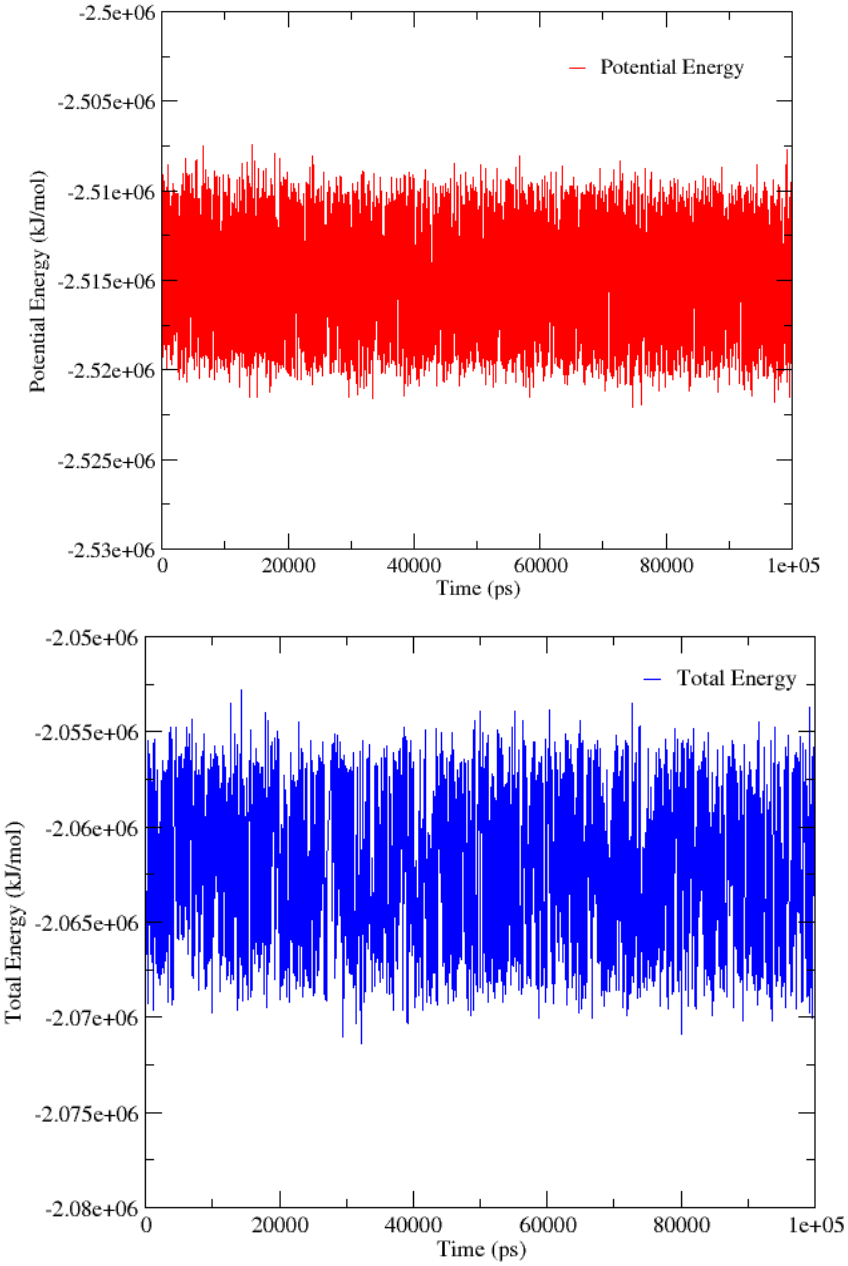


Figure S8 The plot of total and potential energy of WTFC vs simulation time for 100 ns.

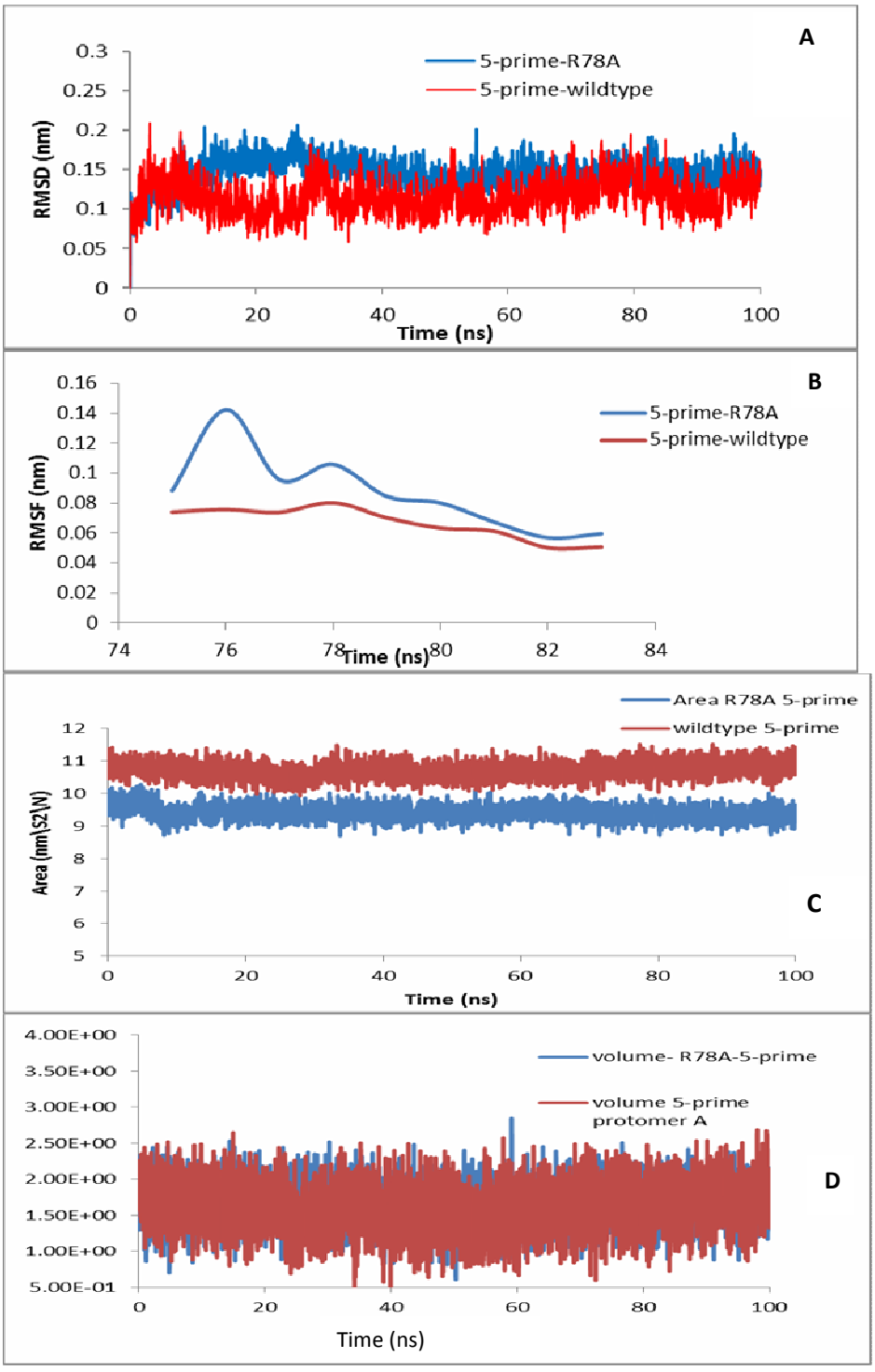


Figure S9 The 5'PBS binding region of the wild type TPST-2 compared against R78A mutant;(A) The RMSD of 5'PBS region, (B) RMSF of 5'PBS region, (C) The SASA of 5'PBS region and (D) volume of 5'PBS region of wild type TPST-2 and the R78A mutant

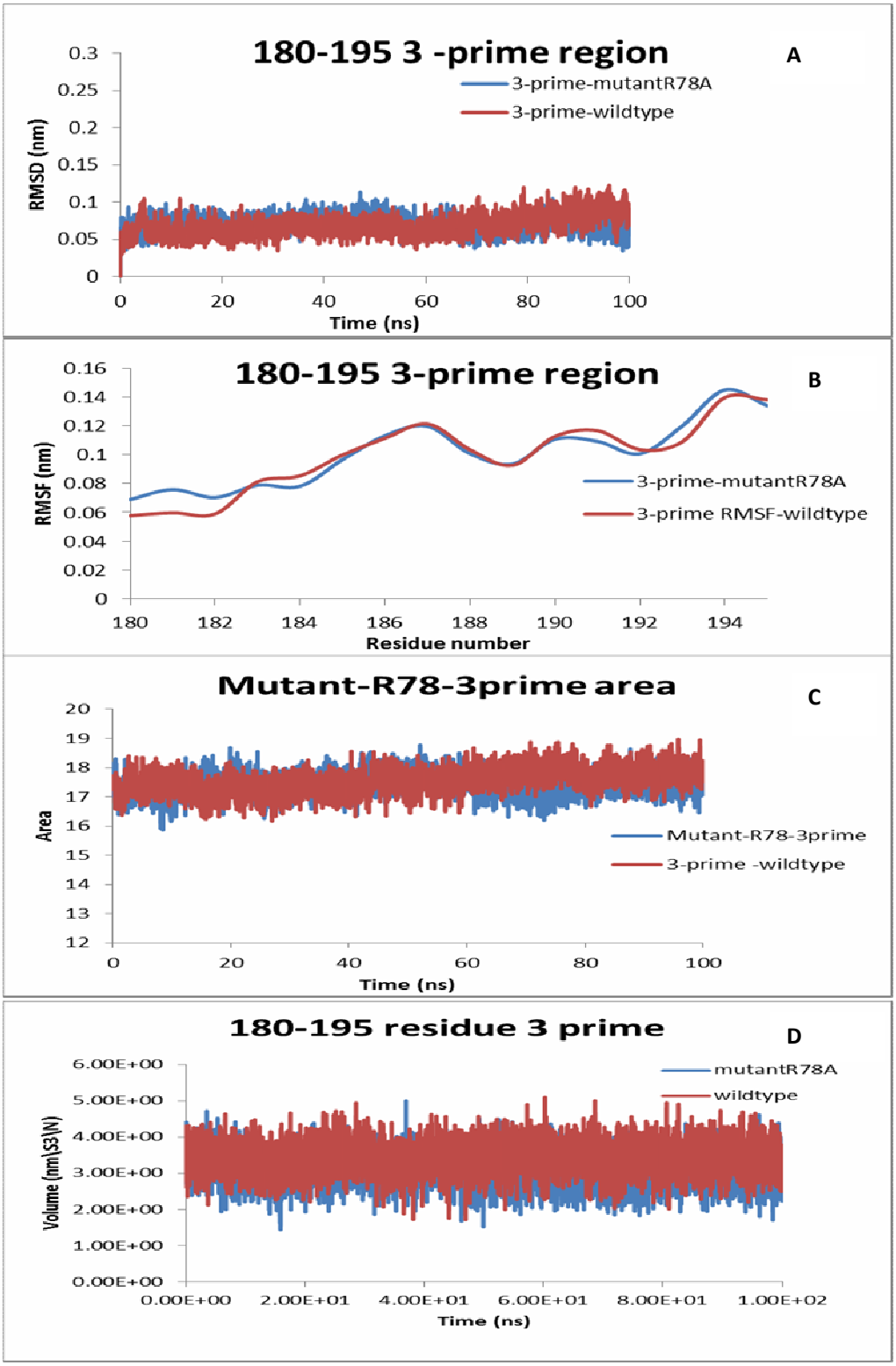


Figure S10 The 3'PBS binding region of the wild type TPST-2 compared against R78A mutant**.** (A) The RMSD of 3'PBS region, (B) RMSF of 3'PBS region, (C) The SASA of 3'PBS region and (D) volume of 3'PBS region of wild type TPST-2 and the mutant R78A.

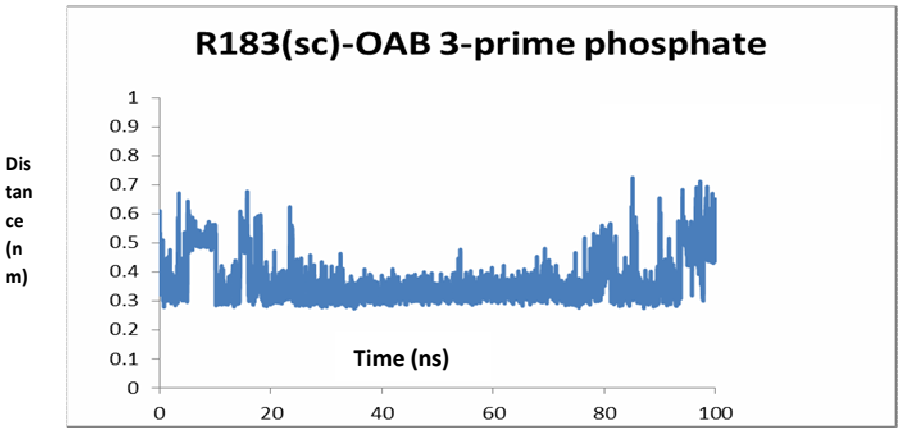


Figure S11 The electrostatic interactions of the side chain of R183 with OAB oxygen of 3'PBS region of wild type TPST-2

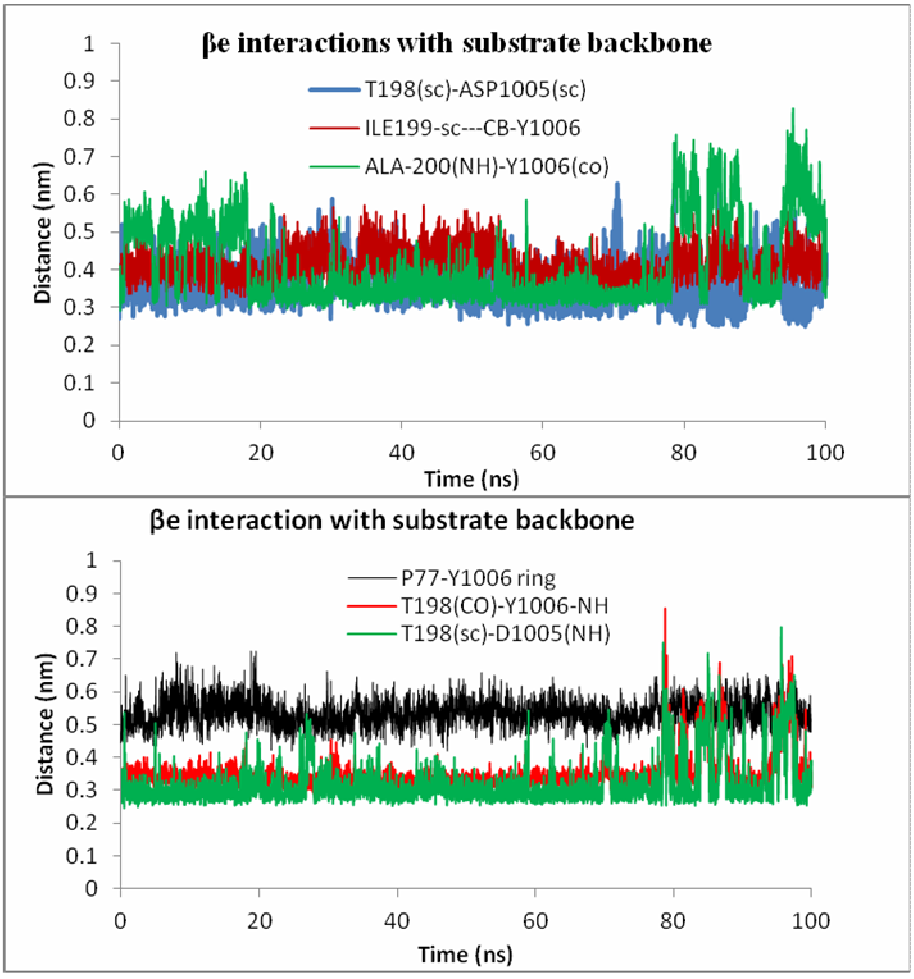
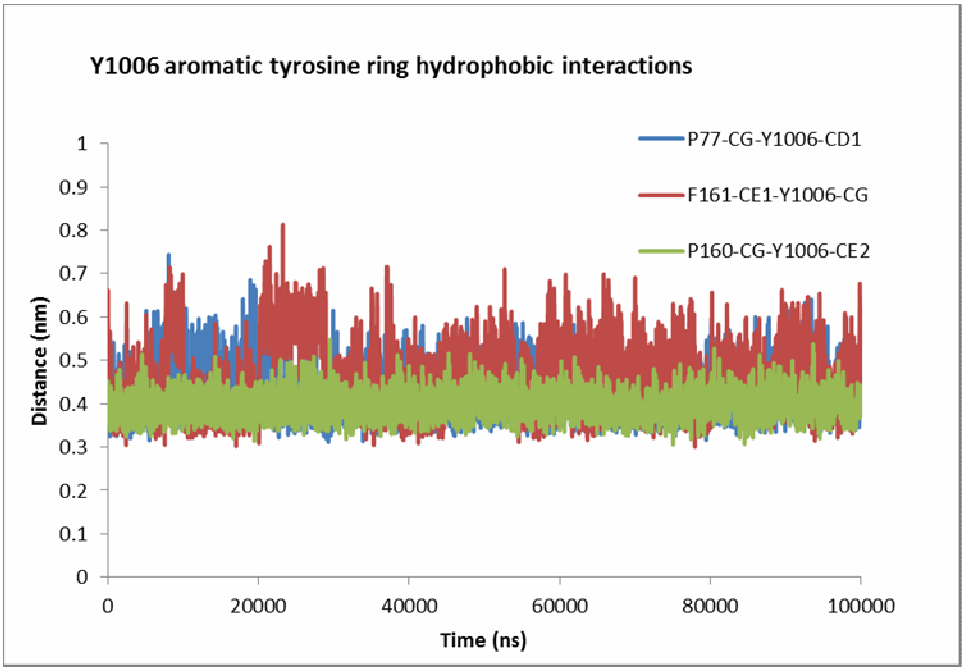


Figure S12 The interactions of wild type TPST-2 with the substrate tyrosine Y1006



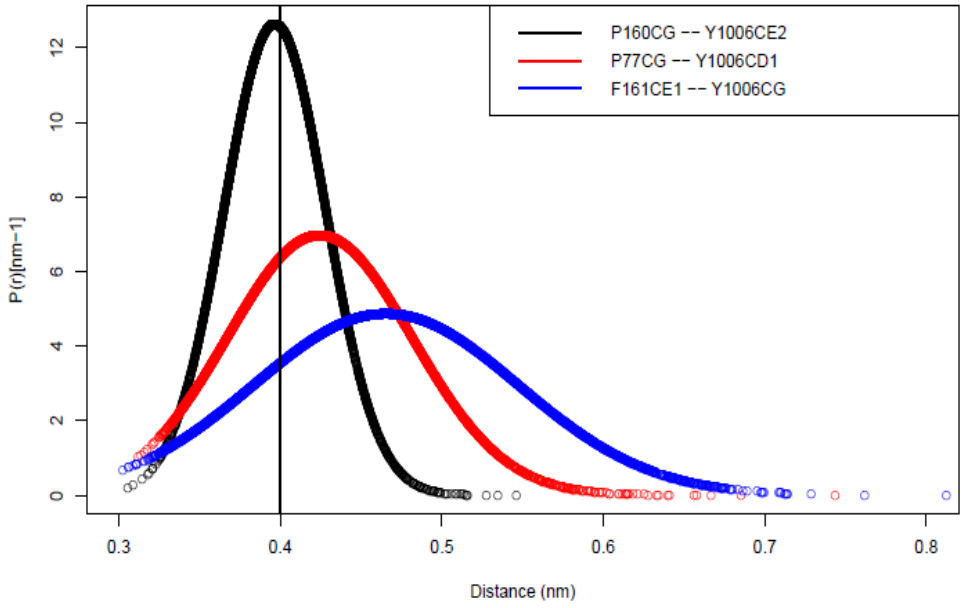


Figure S13 The hydrophobic interactions of wild type TPST-2 with Y1006 aromatic ring of substrate**.** The normalized distribution of wild type TPST-2 with Y1006 aromatic ring of substrate.

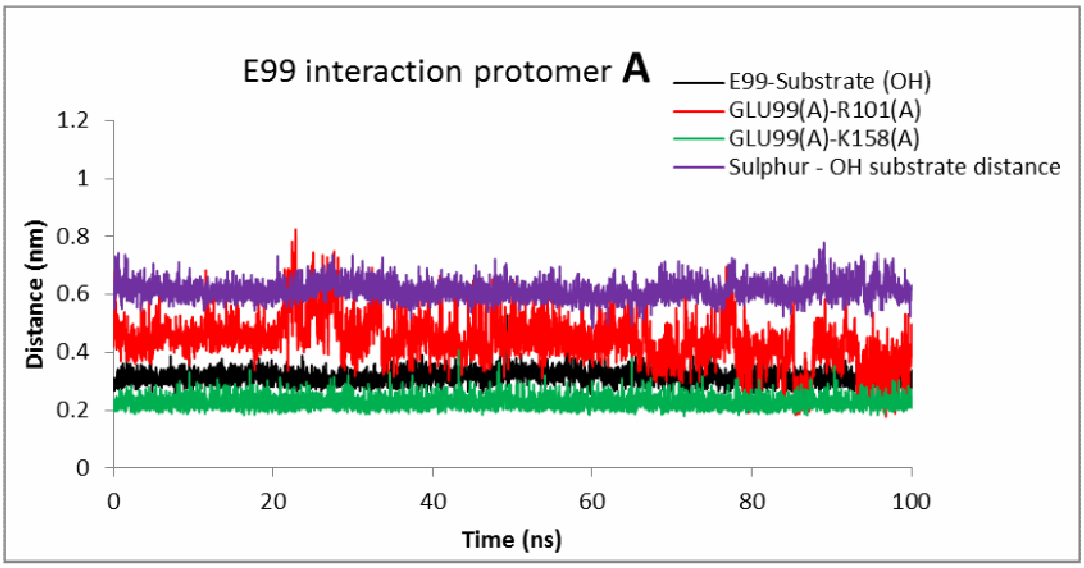


Figure S14 The E99 residue side chain interactions of wild type TPST-2

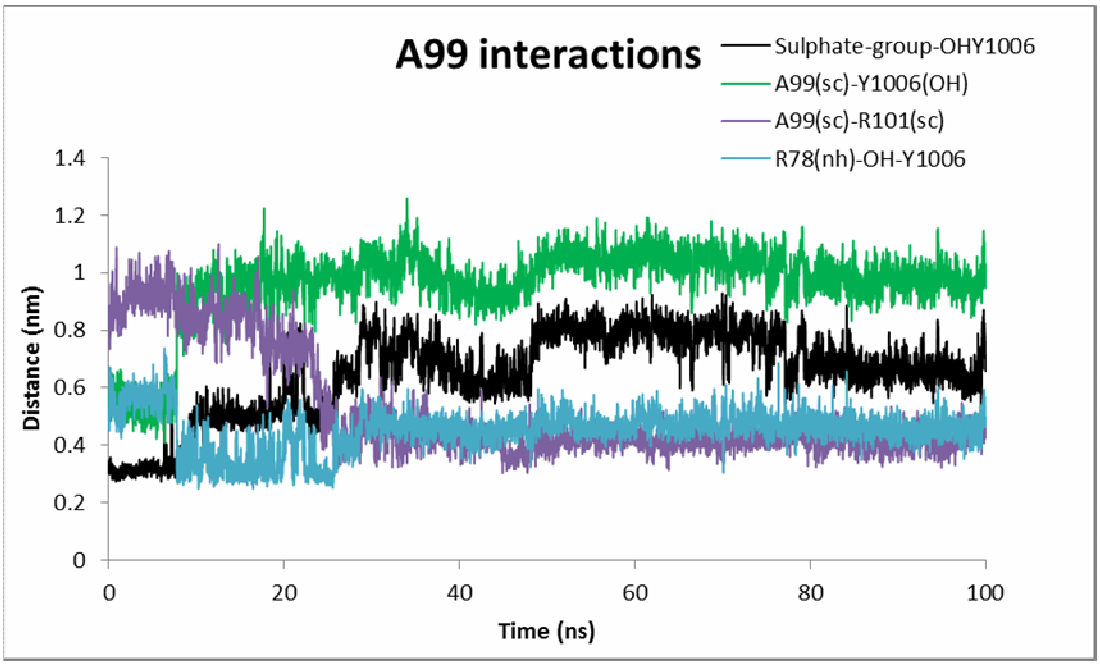


Figure S15 The E99A mutant interactions for 100 ns trajectory

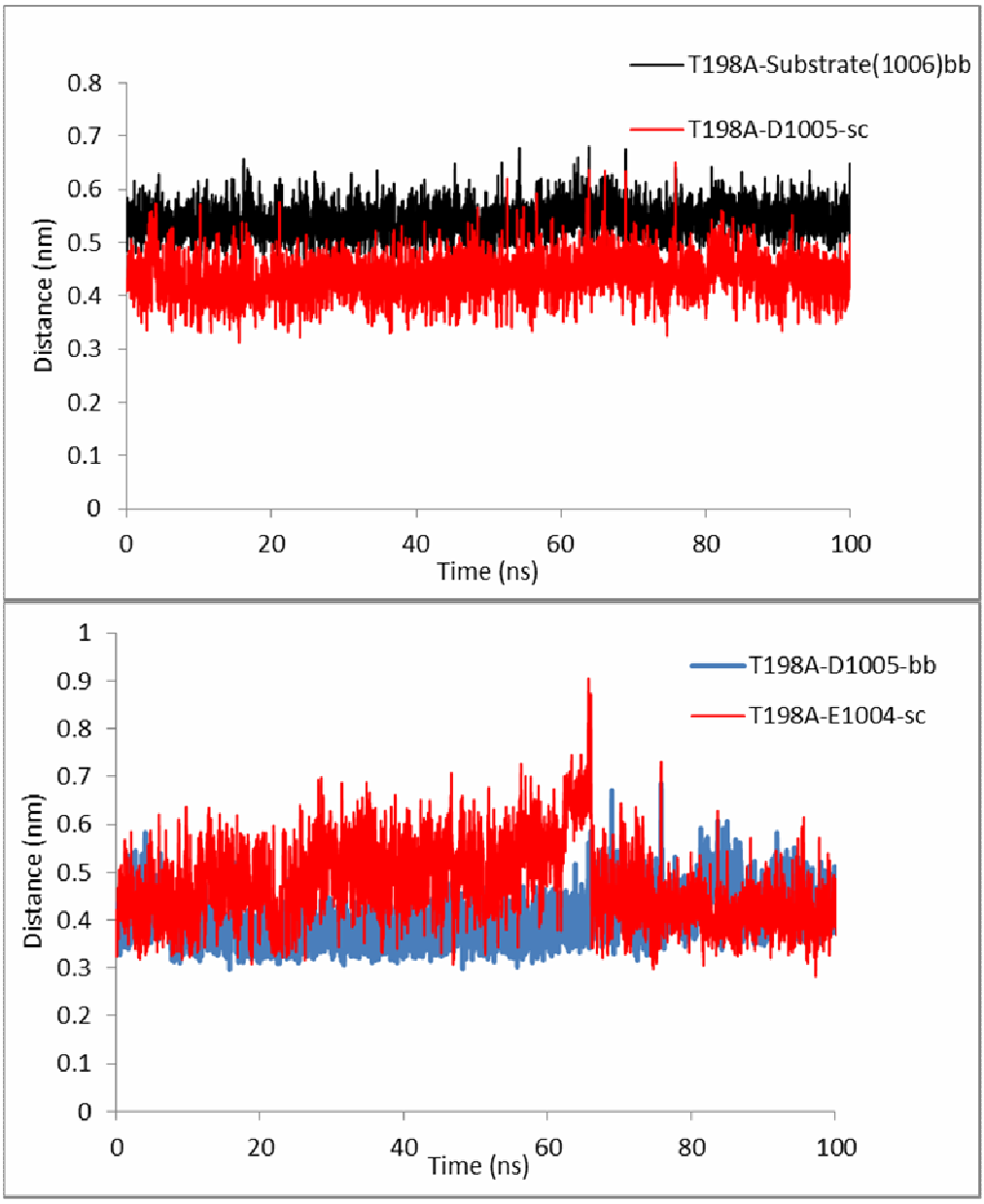


Figure S16 The T198A mutant interactions with residue D1005 and E1004 sidechain and backbone

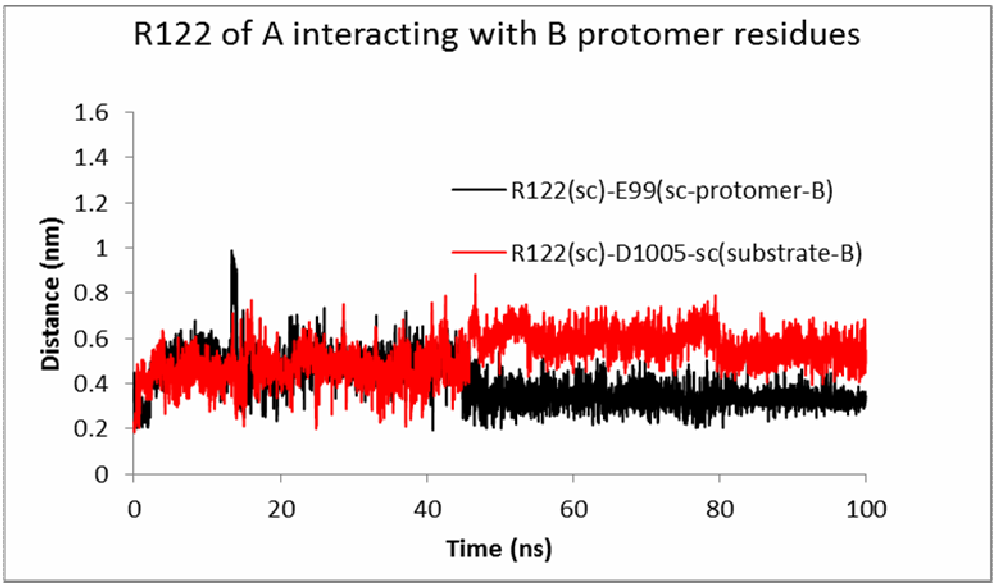


Figure S17 The electrostatic interactions between the side chain of R122 and E99 and D1005 of Wild type TPST-2.

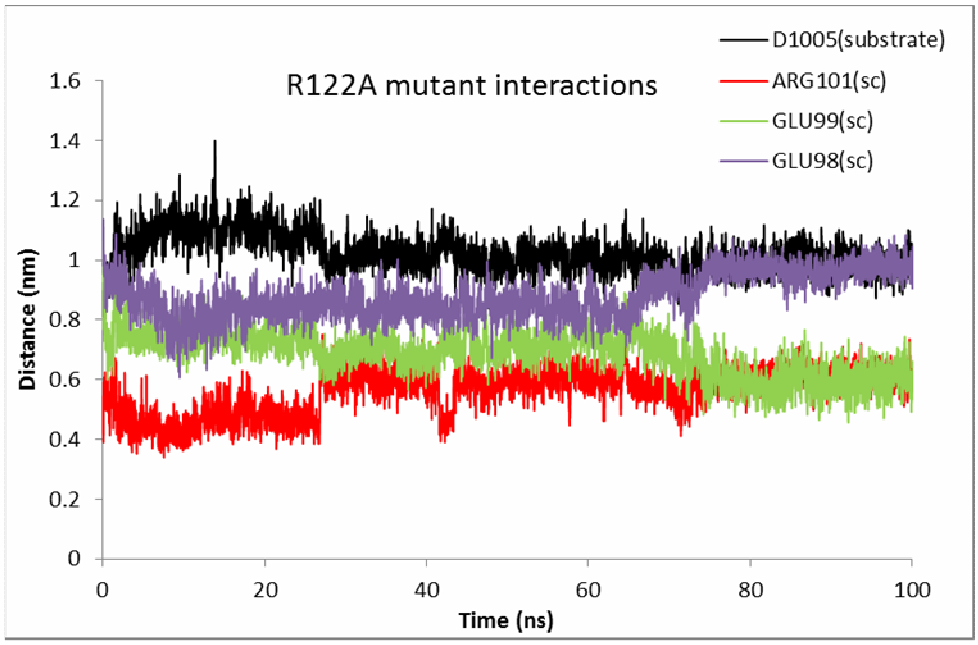


Figure S18 The electrostatic interaction of R122A mutant and the D1005, E99, E98 and R101 residues

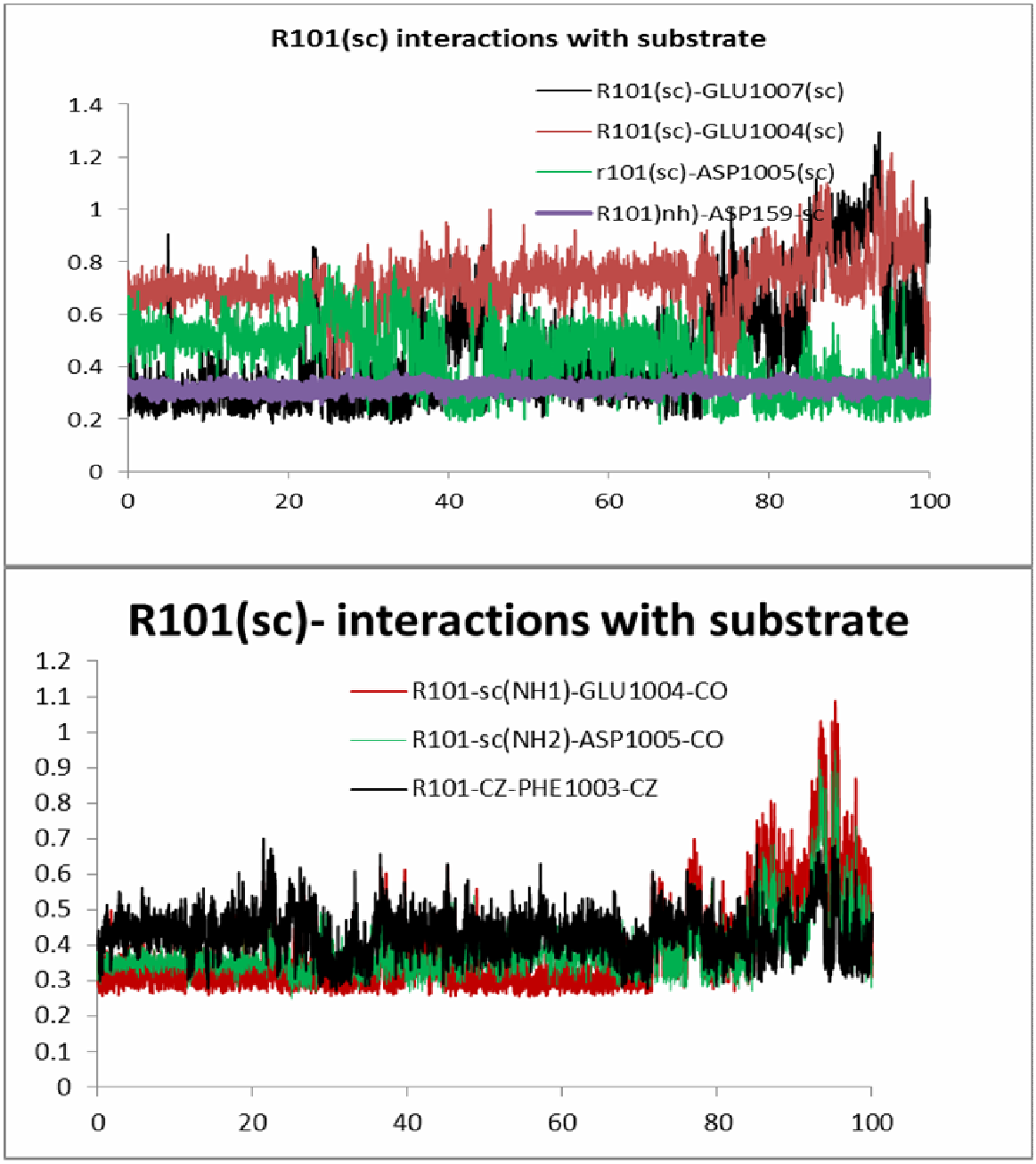


Figure S19 The R101 interactions of wild type TPST-2 with the substrate peptide residues

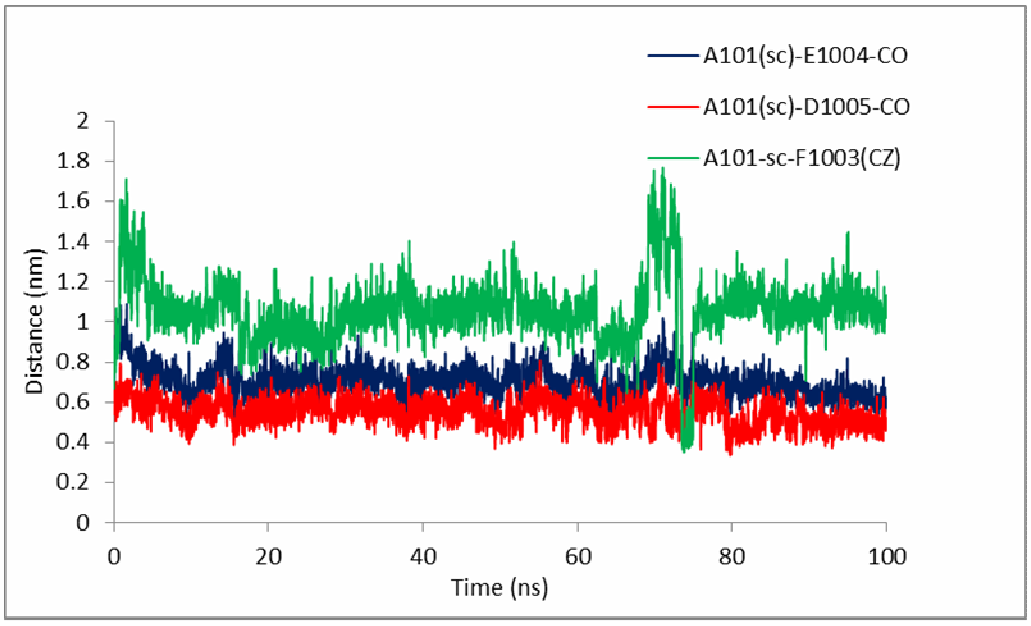


Figure S20 The interactions of R101A mutant with the substrate peptide

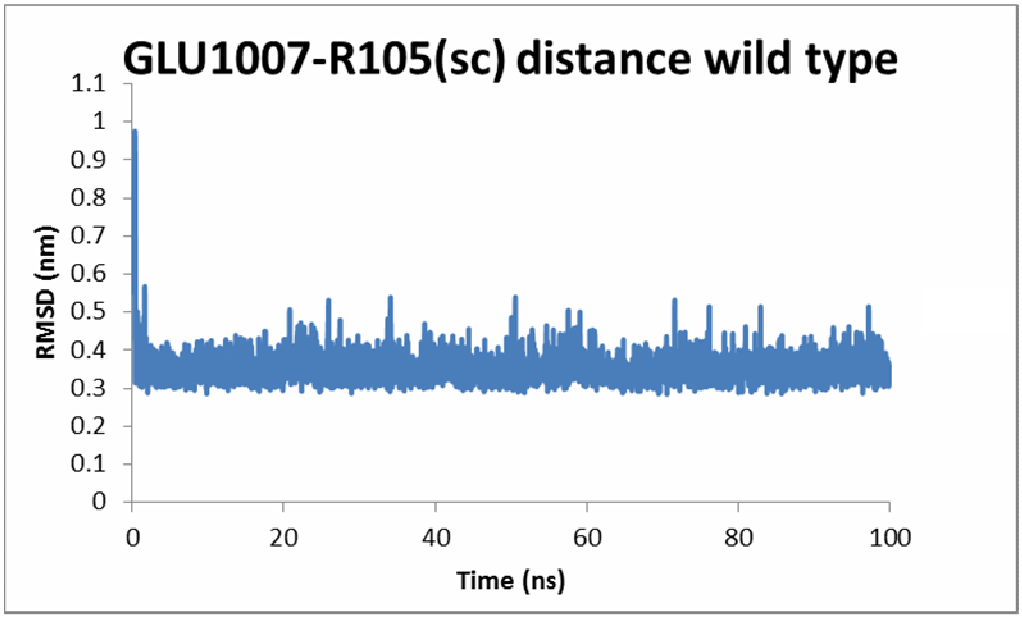


Figure S21 R105 residue of wild type TPST-2 interactions with substrate residue

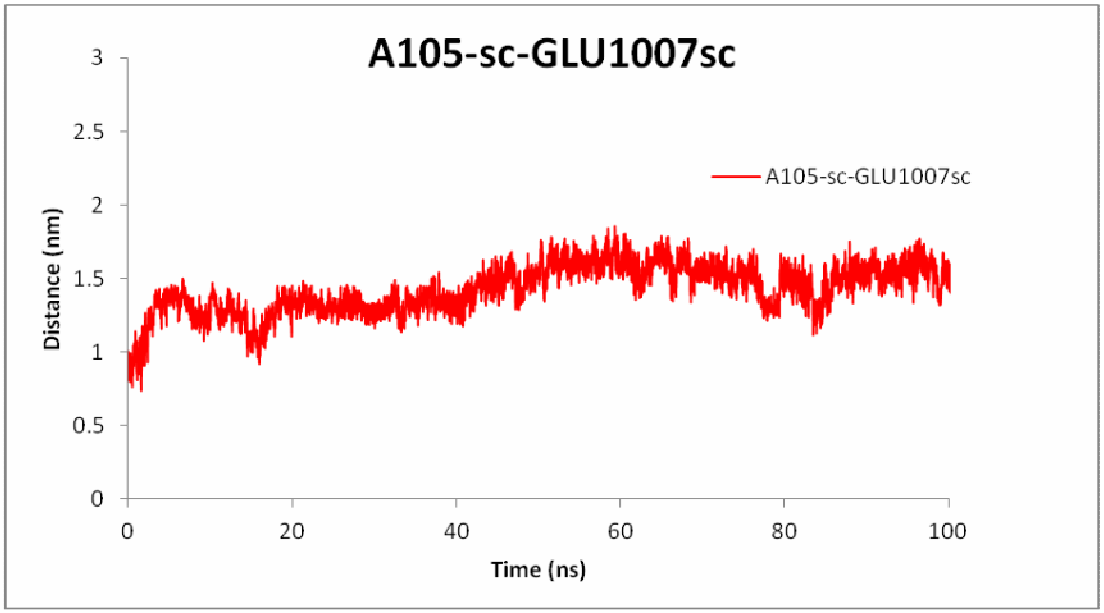


Figure S22 The distance between theA105 residue and GLU1007 in R105A mutant

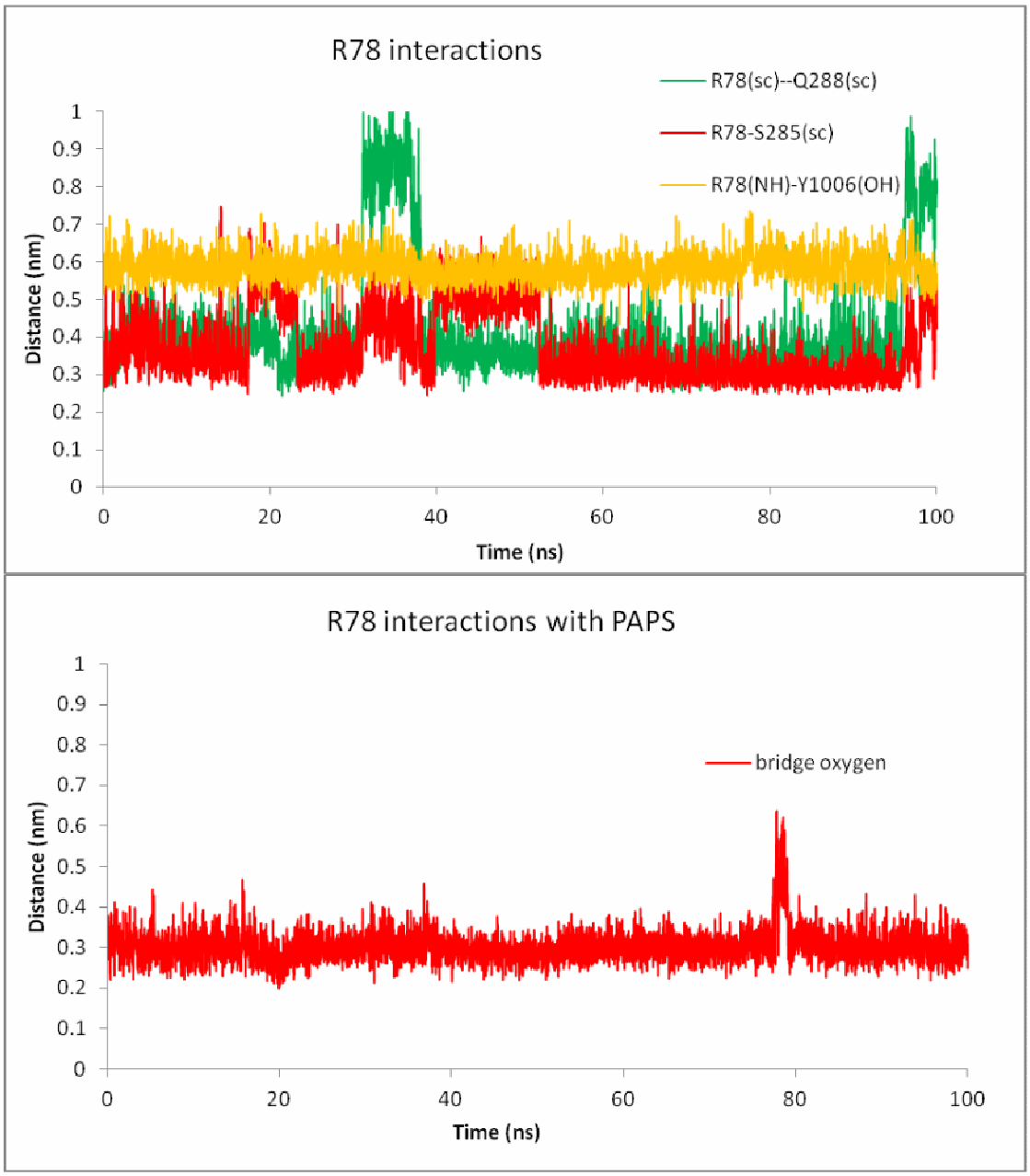


Figure S23 R78 residue of wild type TPST-2 interactions with the cofactor PAPS and substrate Y1006 backbone. The R78 of wild type TPST-2 interacting with the bridge oxygen of the cofactor PAPS

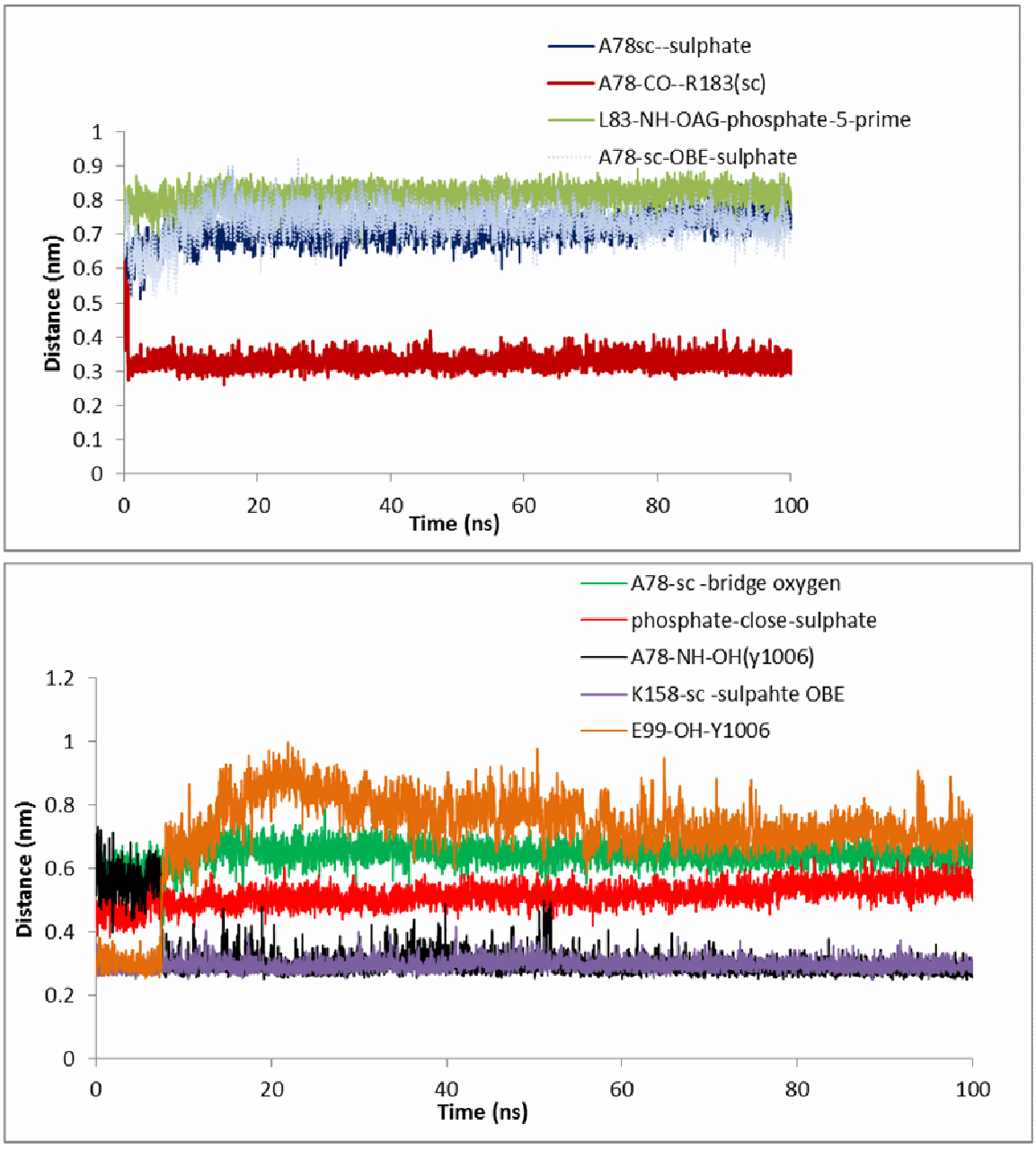


Figure S24 The interaction of R78A mutant for 100ns trajectory

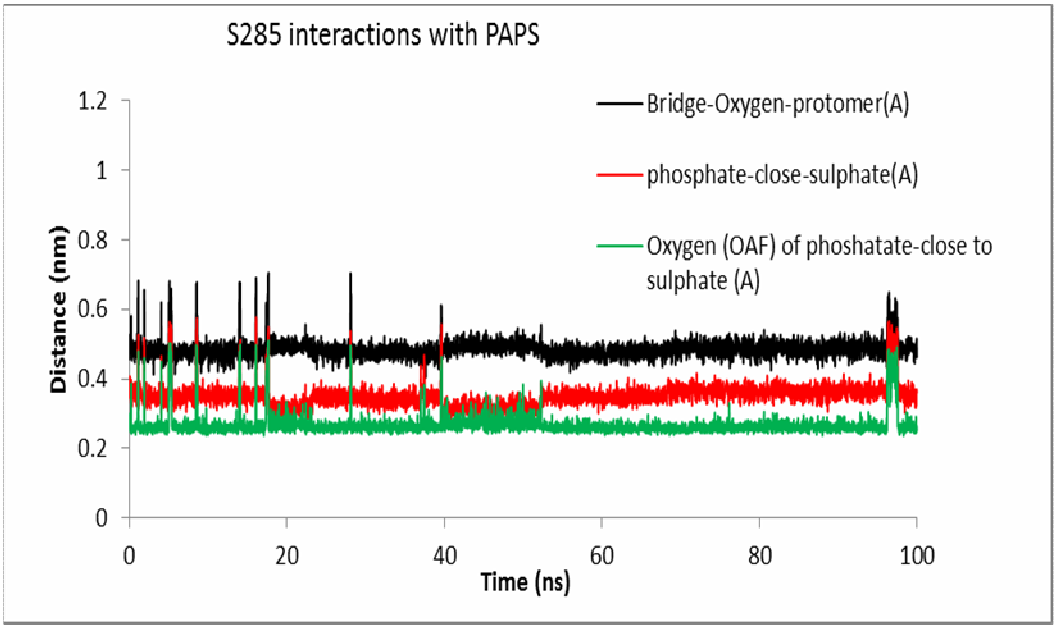


Figure S25 Interactions of S285 side chain in wild type TPST-2

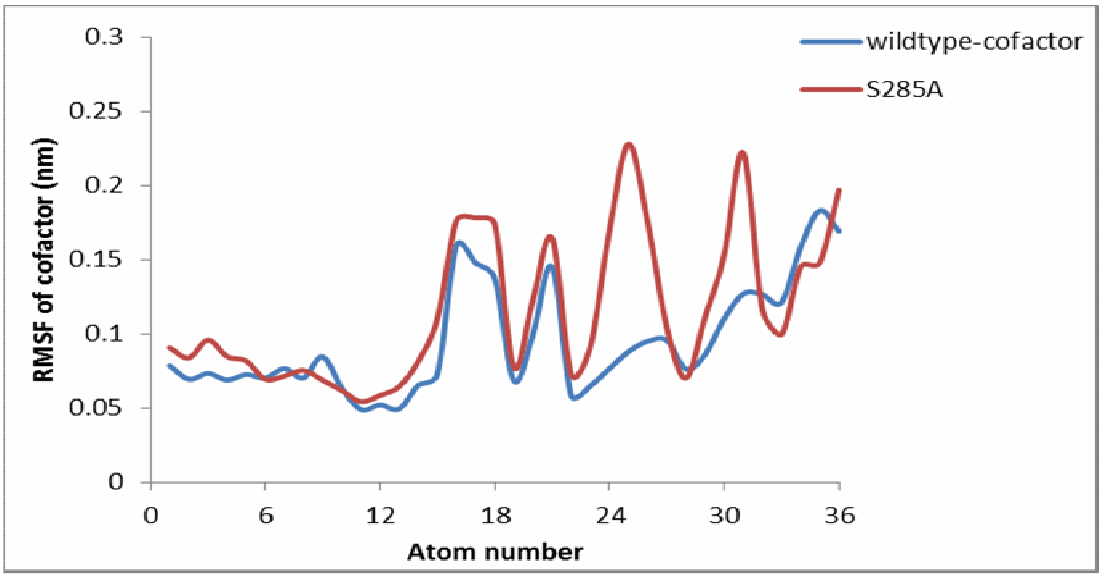


Figure S26 The RMSF of the cofactor of the mutant S285A and the wild type TPST-2

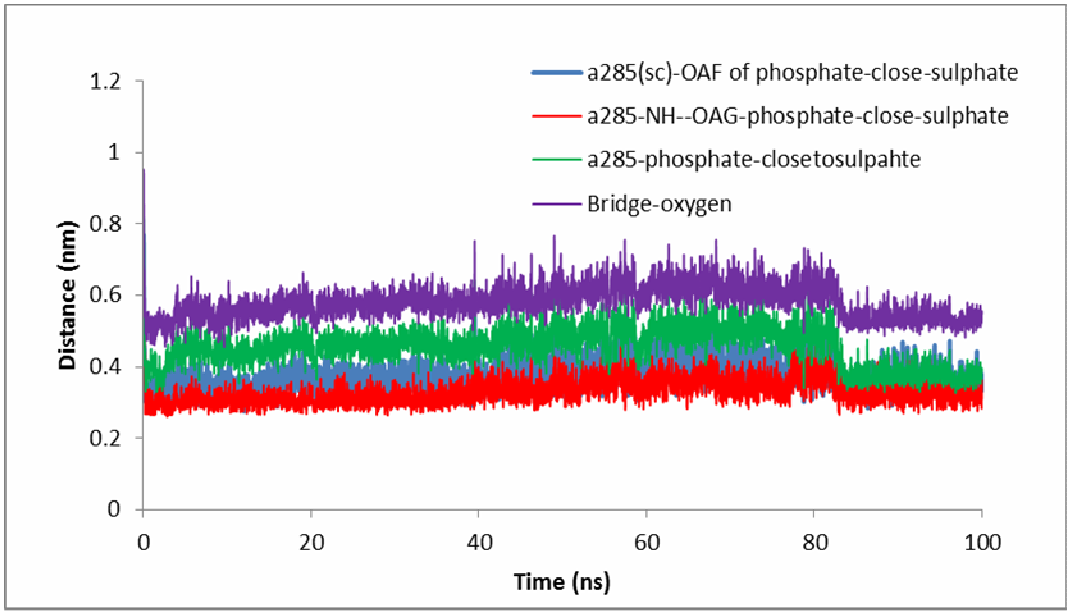


Figure S27 The interaction of A285 in S285A mutant for 100 ns trajectory

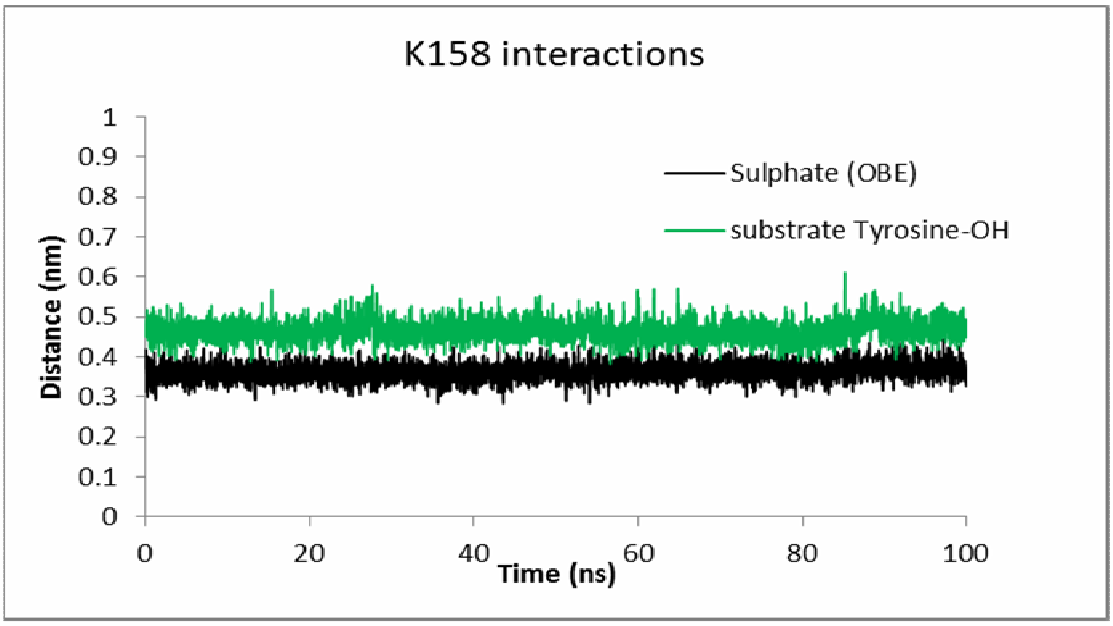


Figure S28 The interactions of K158 residue in wild type TPST-2 for 100 ns trajectory

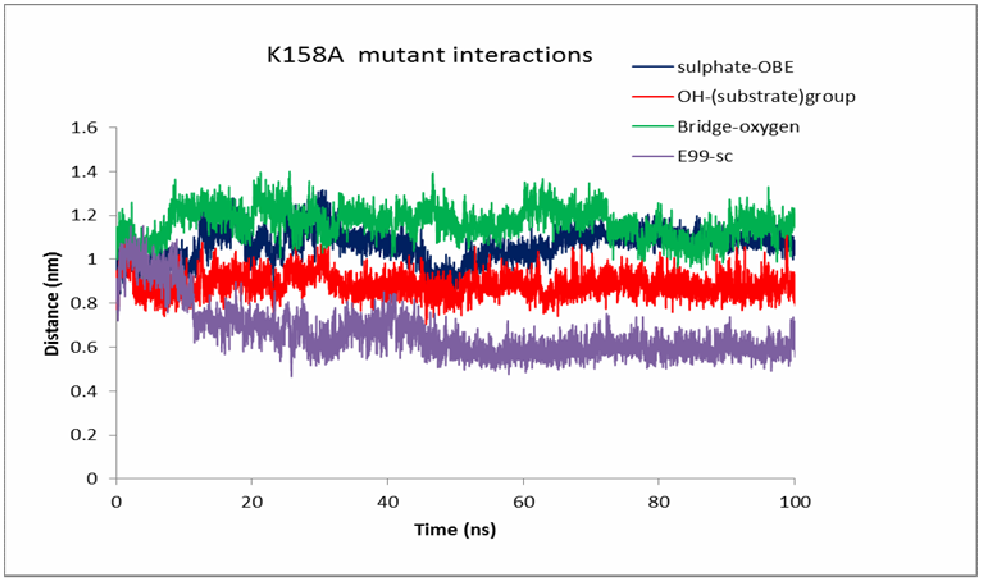


Figure S29 The K158A mutant interactions with the substrate and cofactor for 100 ns trajectory

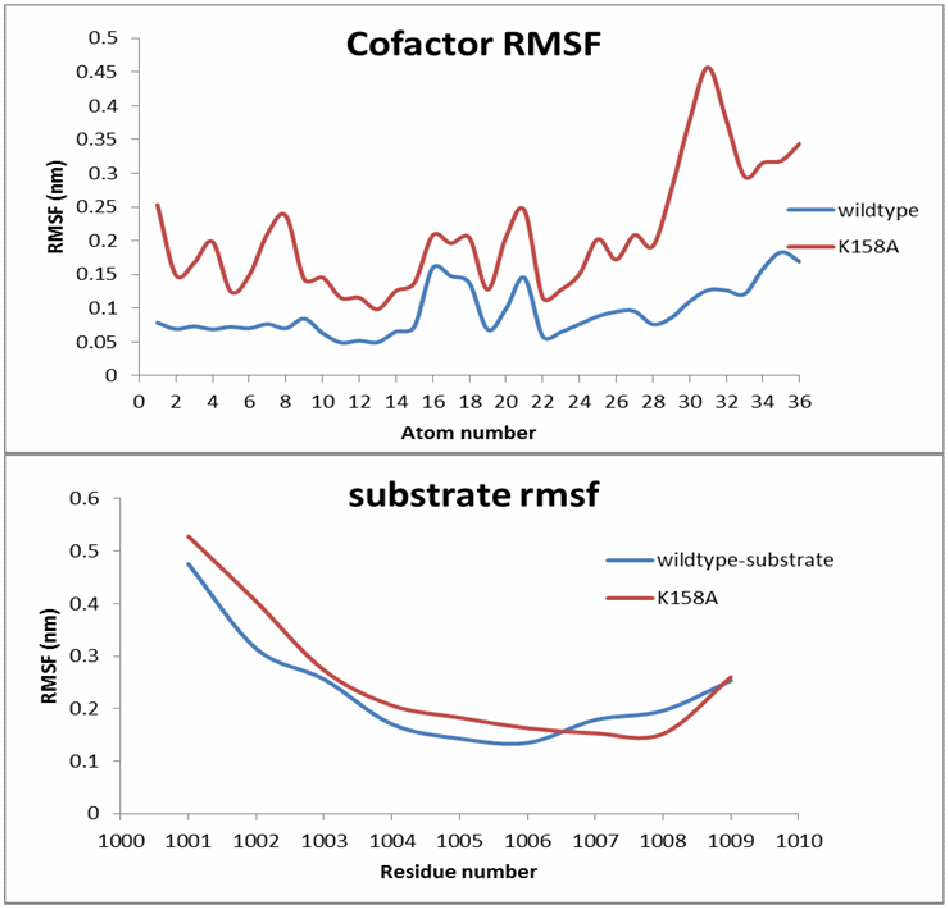


Figure S30 The RMSF of the cofactor and substrate of the K158A mutant and wild type TPST-2

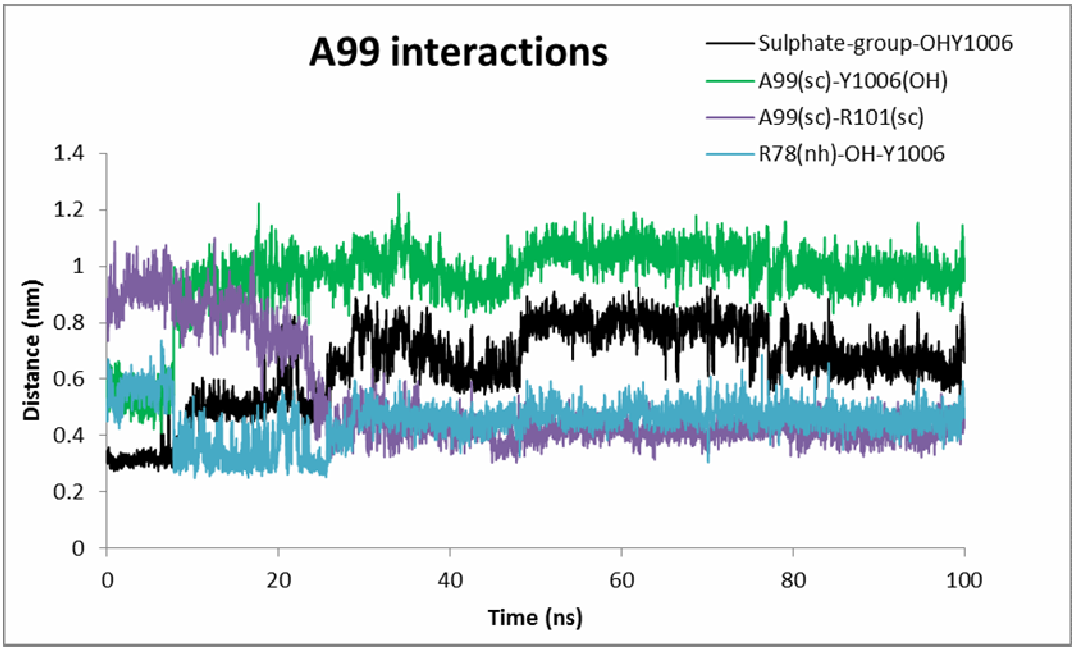


Figure S31 The A99 interaction of Mutant E99A for 100 ns trajectory

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