Electronic Supplementary Information for:

Cation-π and Hydrophobic Interaction Controlled PET Recognition in Double Mutated Cutinase – Identification of a Novel Binding Subsite for Better Catalytic Activity

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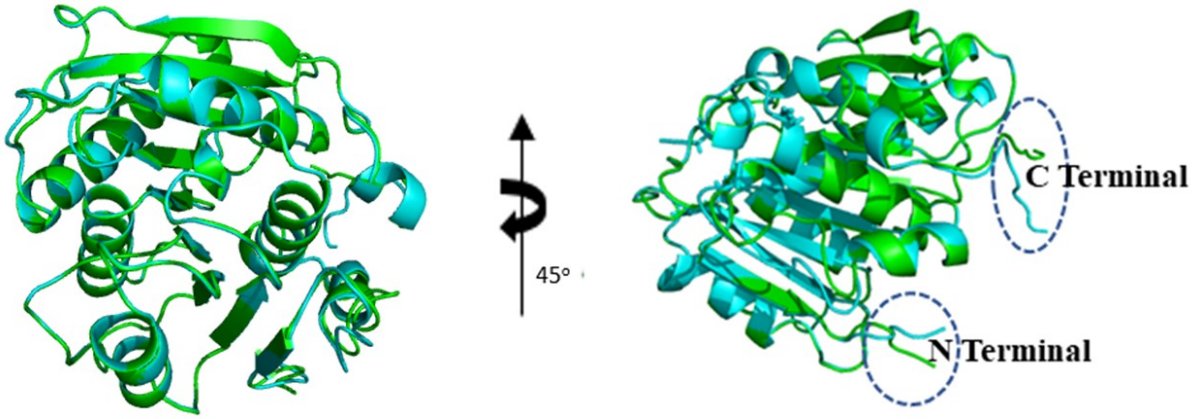
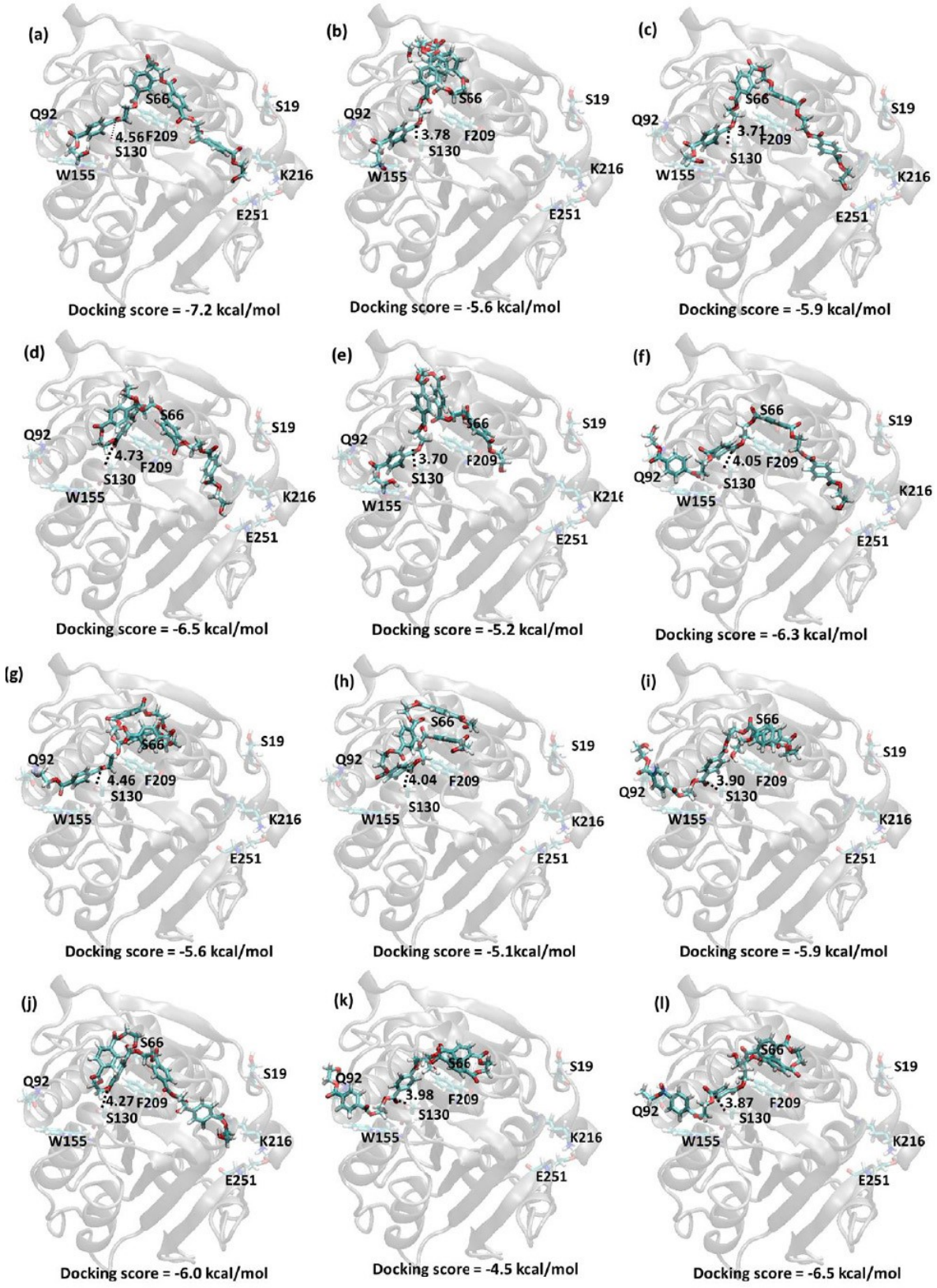


Figure S1. Superimposed average structures of WT *TfCut2* (green) and DM *TfCut2* (cyan) in cartoon representation for 1μs Molecular Dynamics simulation of proteins without PET. The RMSD of the average structure of DM *TfCut2* with respect to WT *TfCut2* is 1.33 Å. The superimposed structures in first image are rotated to get a clear image of the fluctuation at the ends. The end residues with maximum fluctuation are indicated by dotted circles.


(a)
S66
S19
Q92
4.56
F209
S130
W155
K216
E251
Docking score = -7.2 kcal/mol
(b)
S66
S19
Q92
3.78
F209
S130
W155
K216
E251
Docking score = -5.6 kcal/mol
(c)
S66
S19
Q92
3.71
F209
S130
W155
K216
E251
Docking score = -5.9 kcal/mol
(d)
S66
S19
Q92
4.73
F209
S130
W155
K216
E251
Docking score = -6.5 kcal/mol
(e)
S66
S19
Q92
3.70
F209
S130
W155
K216
E251
Docking score = -5.2 kcal/mol
(f)
S66
S19
Q92
4.05
F209
S130
W155
K216
E251
Docking score = -6.3 kcal/mol
(g)
S66
S19
Q92
4.46
F209
S130
W155
K216
E251
Docking score = -5.6 kcal/mol
(h)
S66
S19
Q92
4.04
F209
S130
W155
K216
E251
Docking score = -5.1kcal/mol
(i)
S66
S19
Q92
3.90
F209
S130
W155
K216
E251
Docking score = -5.9 kcal/mol
(j)
S66
S19
Q92
4.27
F209
S130
W155
K216
E251
Docking score = -6.0 kcal/mol
(k)
S66
S19
Q92
3.98
F209
S130
W155
K216
E251
Docking score = -4.5 kcal/mol
(l)
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S19
Q92
3.87
F209
S130
W155
K216
E251
Docking score = -6.5 kcal/mol

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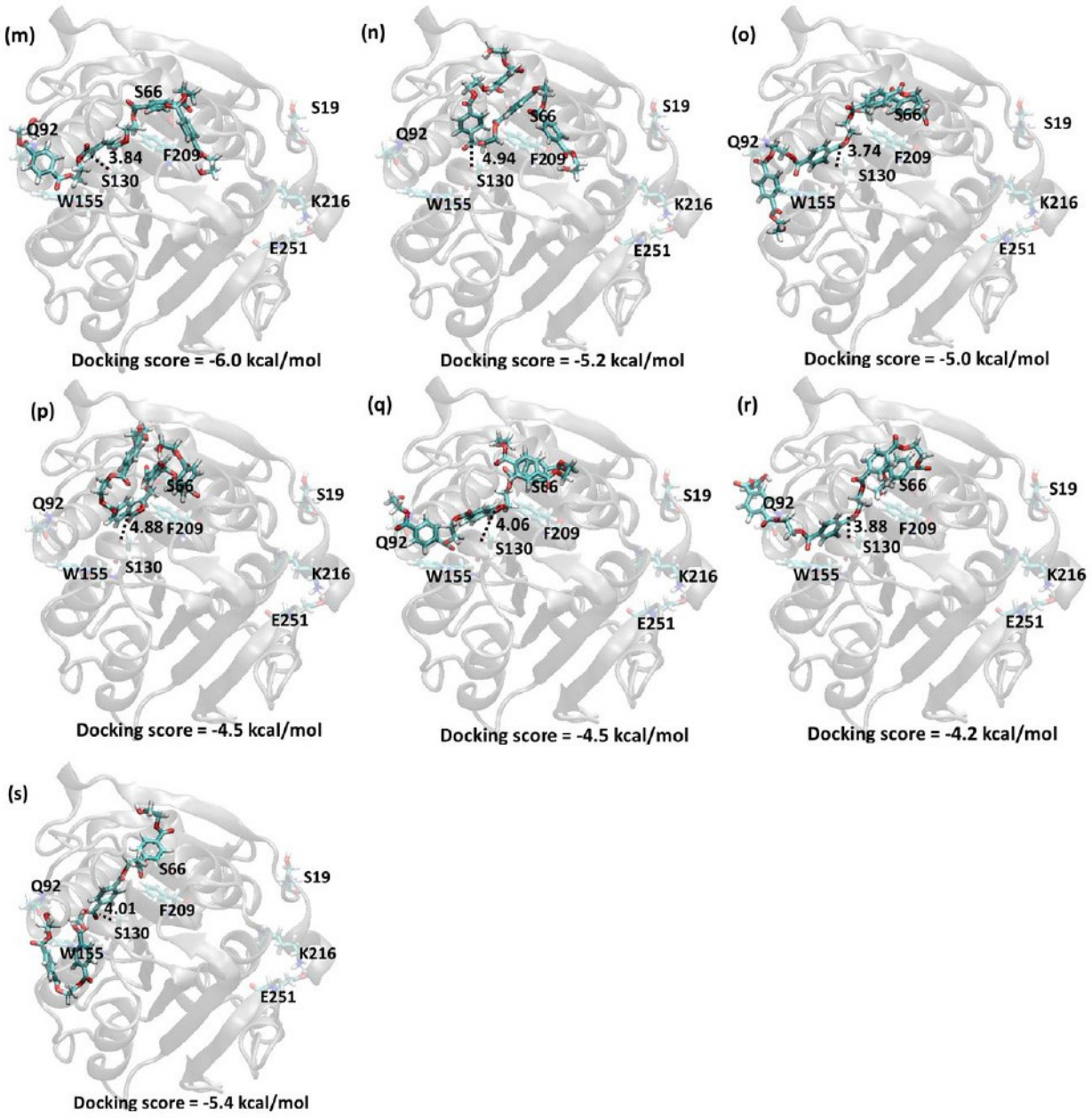
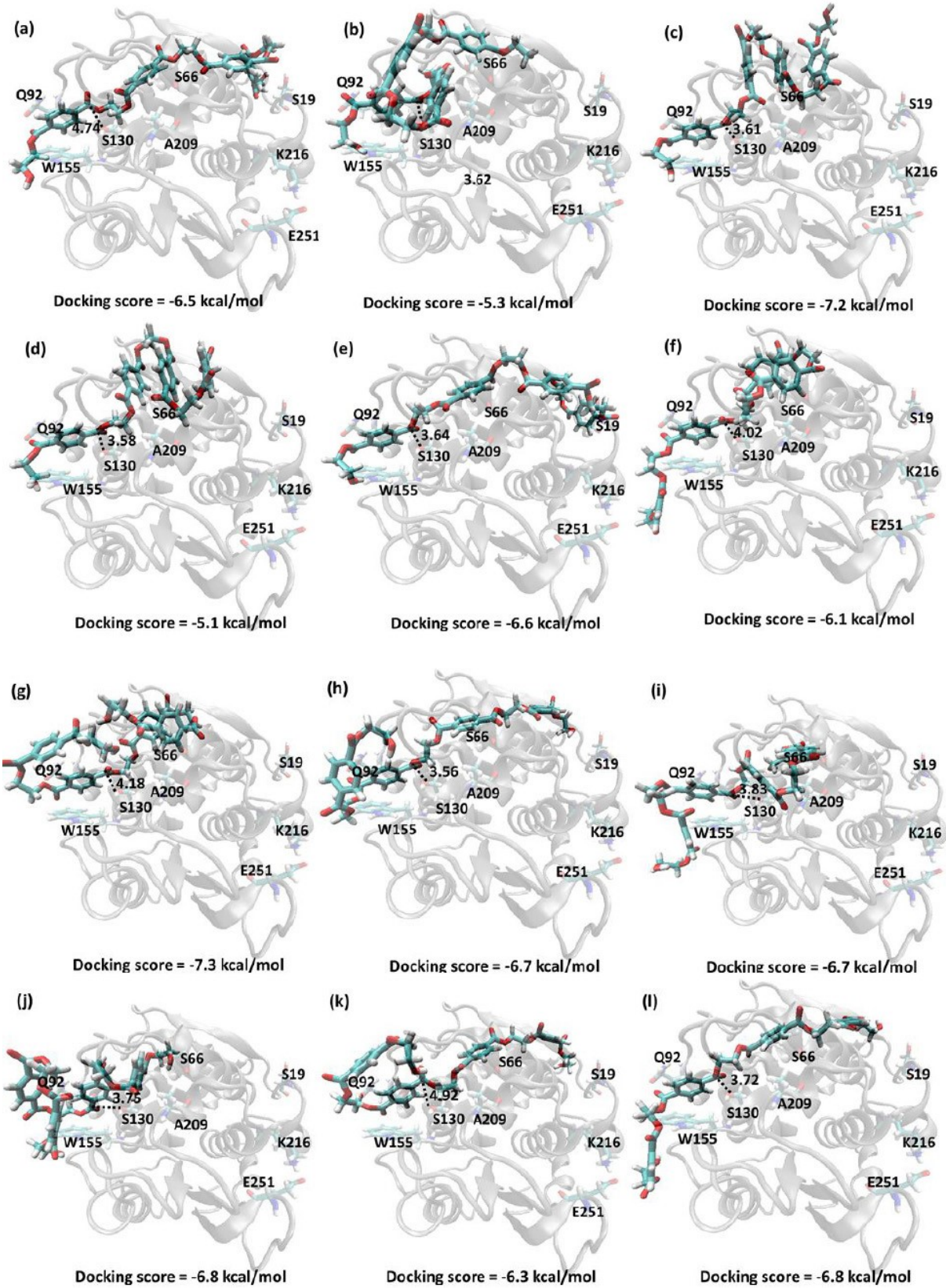


Figure S2. PET conformers docked into WT *TfCut2* which are chosen for further MD studies.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S19
4.74
S130
A209
K216
W155
E251
Docking score = -6.5 kcal/mol
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S66
S19
A209
S130
K216
W155
3.62
E251
Docking score = -5.3 kcal/mol
(c)
S66
Q92
S19
3.61
S130
A209
W155
K216
E251
Docking score = -7.2 kcal/mol
(d)
S66
Q92
S19
3.58
A209
S130
W155
K216
E251
Docking score = -5.1 kcal/mol
(e)
Q92
S66
S19
3.64
S130
A209
W155
K216
E251
Docking score = -6.6 kcal/mol
(f)
Q92
S66
S19
4.02
S130
A209
W155
K216
E251
Docking score = -6.1 kcal/mol
(g)
S66
Q92
S19
4.18
A209
S130
W155
K216
E251
Docking score = -7.3 kcal/mol
(h)
S66
Q92
S19
3.56
A209
S130
W155
K216
E251
Docking score = -6.7 kcal/mol
(i)
S66
Q92
S19
3.83
S130
A209
W155
K216
E251
Docking score = -6.7 kcal/mol
(j)
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Q92
S19
3.75
S130
A209
W155
K216
E251
Docking score = -6.8 kcal/mol
(k)
S66
Q92
S19
4.92
S130
A209
W155
K216
E251
Docking score = -6.3 kcal/mol
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S19
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A209
S130
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K216
E251
Docking score = -6.8 kcal/mol

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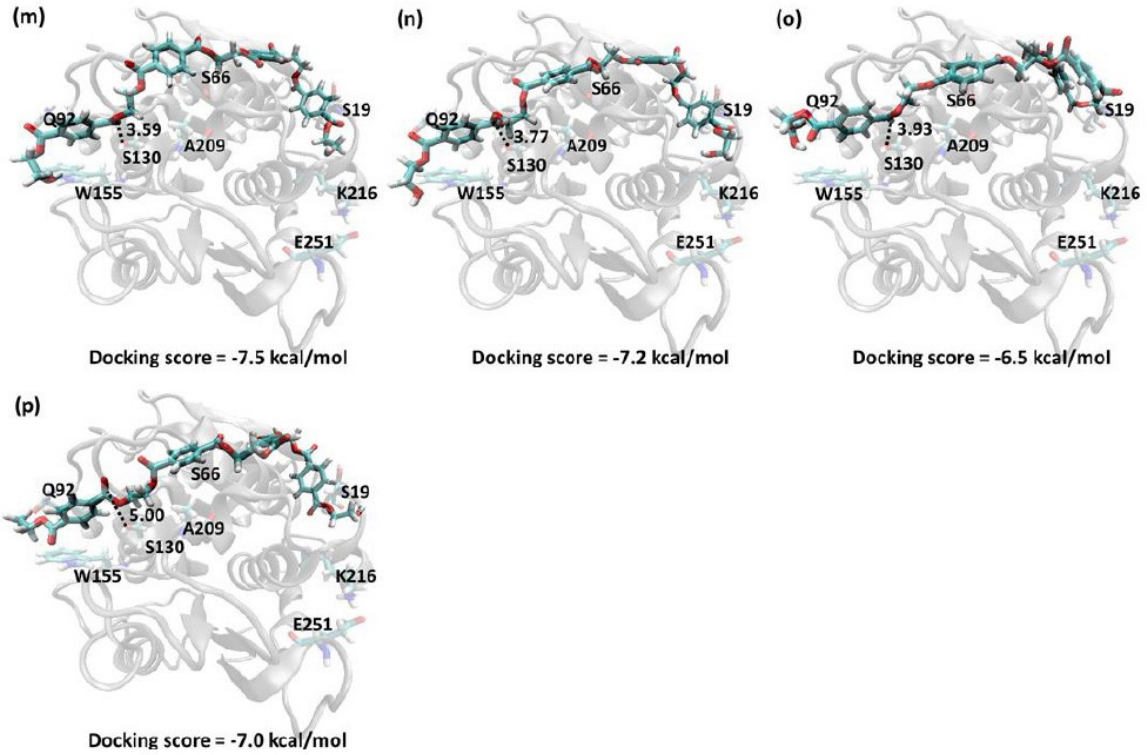


Figure S3. PET conformers docked into DM *TfCut2* which are chosen for further MD studies.

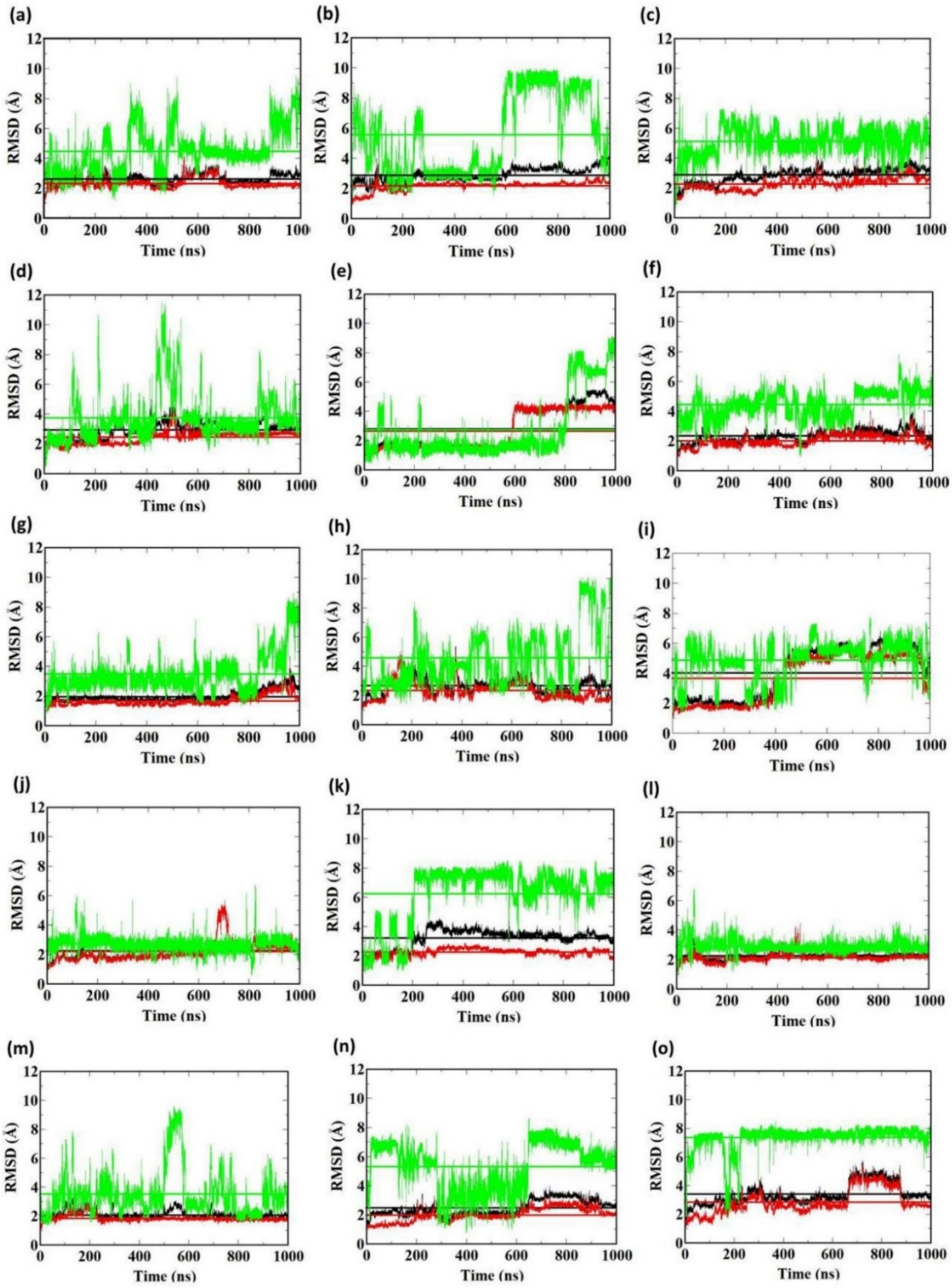


Figure S4. Root mean square deviations (RMSD) of the 15 different conformers of WT *TfCut2* (a to o) of all the non-hydrogen atoms with respect to the starting structure over time for 1µs Molecular Dynamics Simulations. RMSD of WT *TfCut2*-PET system, WT *TfCut2 only*, and PET only are indicated by black, red and green respectively. The average RMSD is indicated by a line parallel to the x-axis.

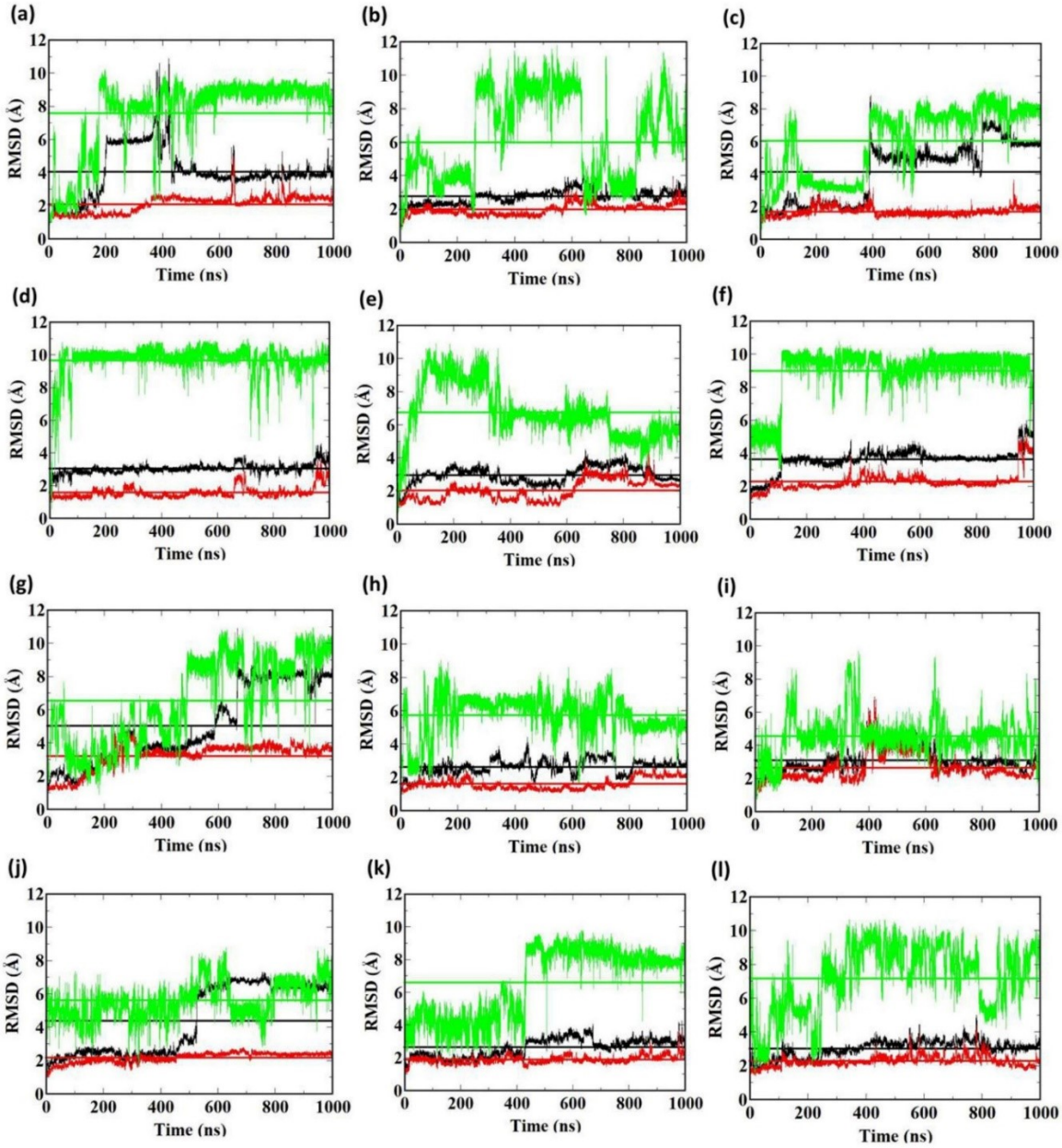


Figure S5. Root mean square deviations (RMSD) of the 12 different conformers of DM *TfCut2* (a to l) of all the non-hydrogen atoms with respect to the starting structure over time for 1µs Molecular Dynamics Simulations. RMSD of DM *TfCut2*-PET system, DM *TfCut2 only*, and PET only are indicated by black, red and green respectively. The average RMSD is indicated by a line parallel to the x-axis.

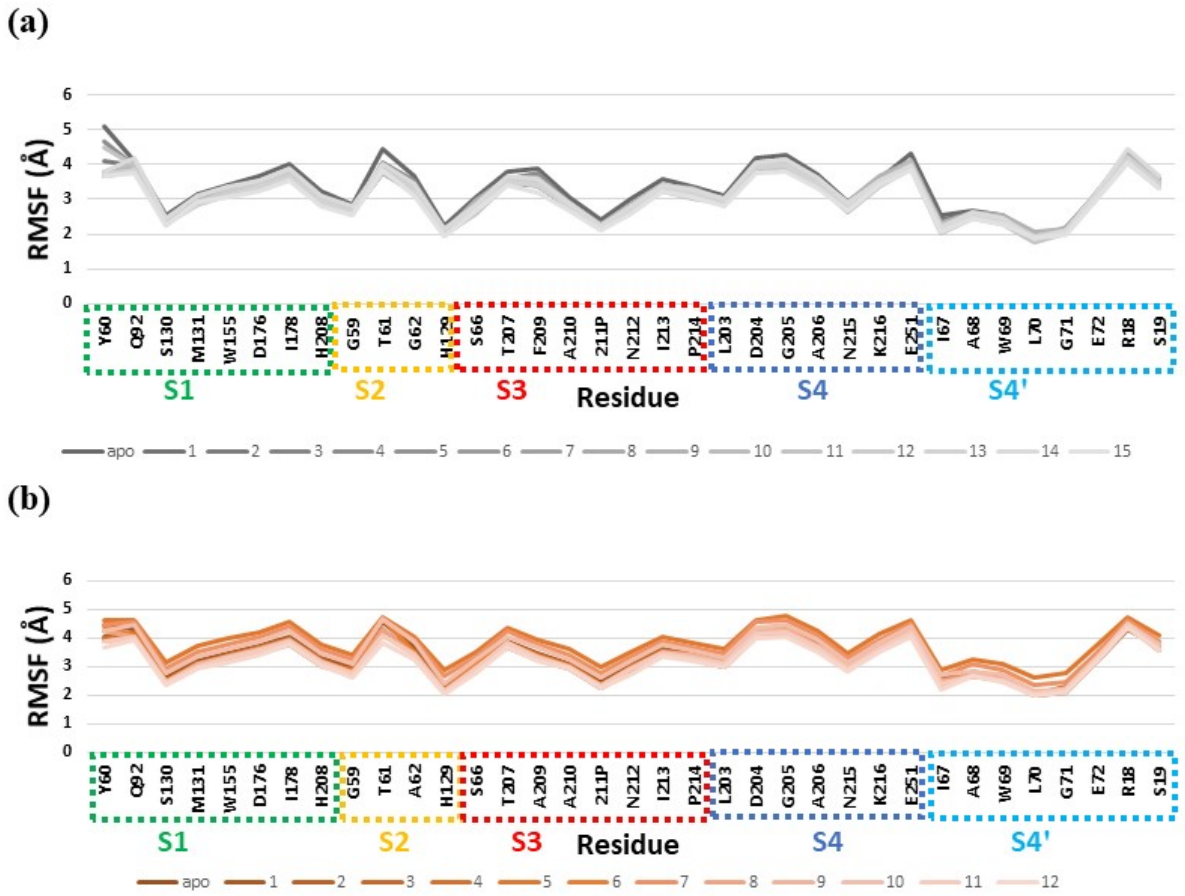


Figure S6. Root mean square fluctuation (RMSF) in Å for binding cleft residues labelled subsite wise in (a) WT *TfCut2*-PET (b) DM *TfCut2*-PET The dotted boxes in X axis are colored according to the color code of their respective subsites.

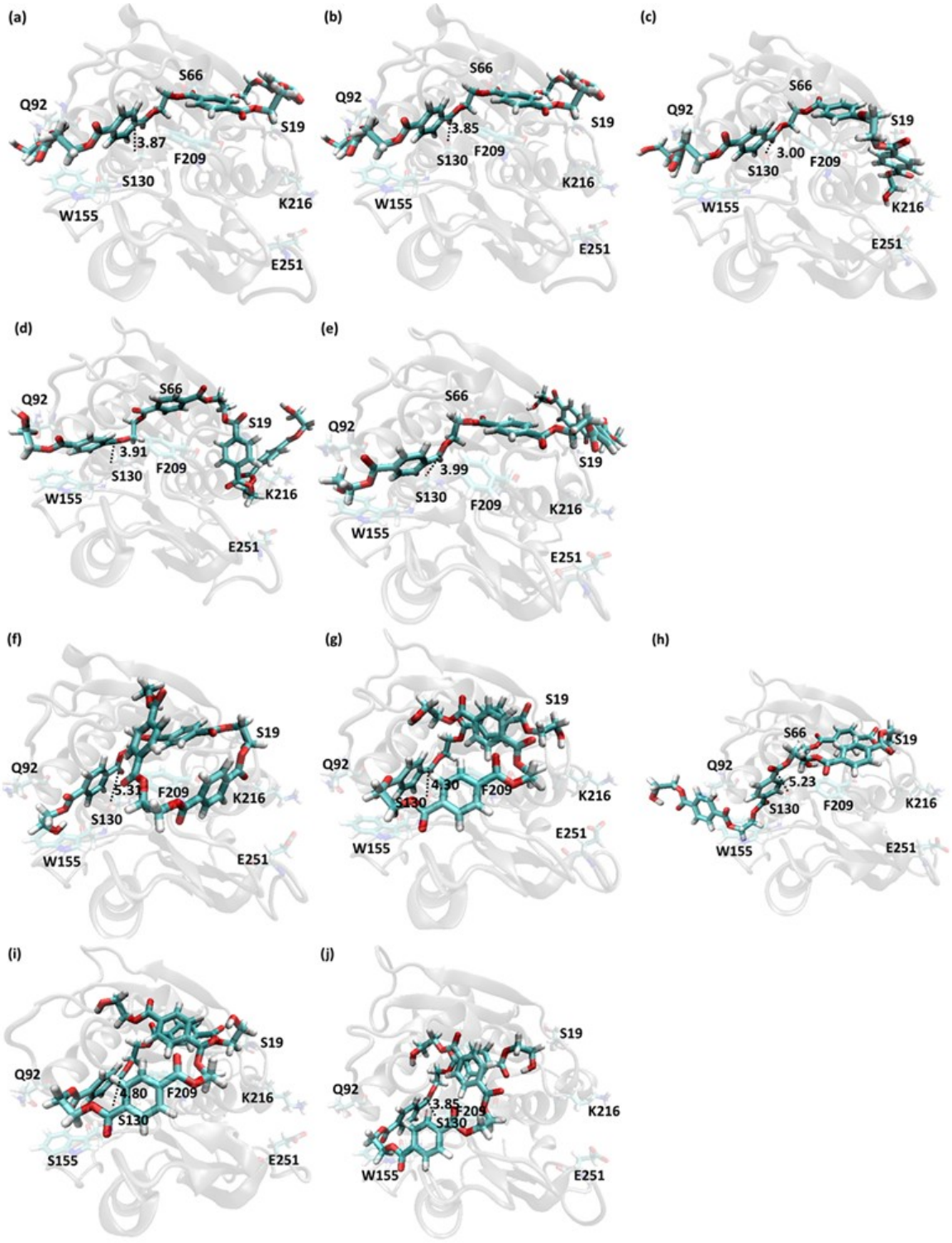


Figure S7. The top5 energy extended (a to e) and least 5 energy folded (f to j) reference structures of WT *TfCut2* obtained after clustering. The contact plot and energy analysis are performed on these model structures.

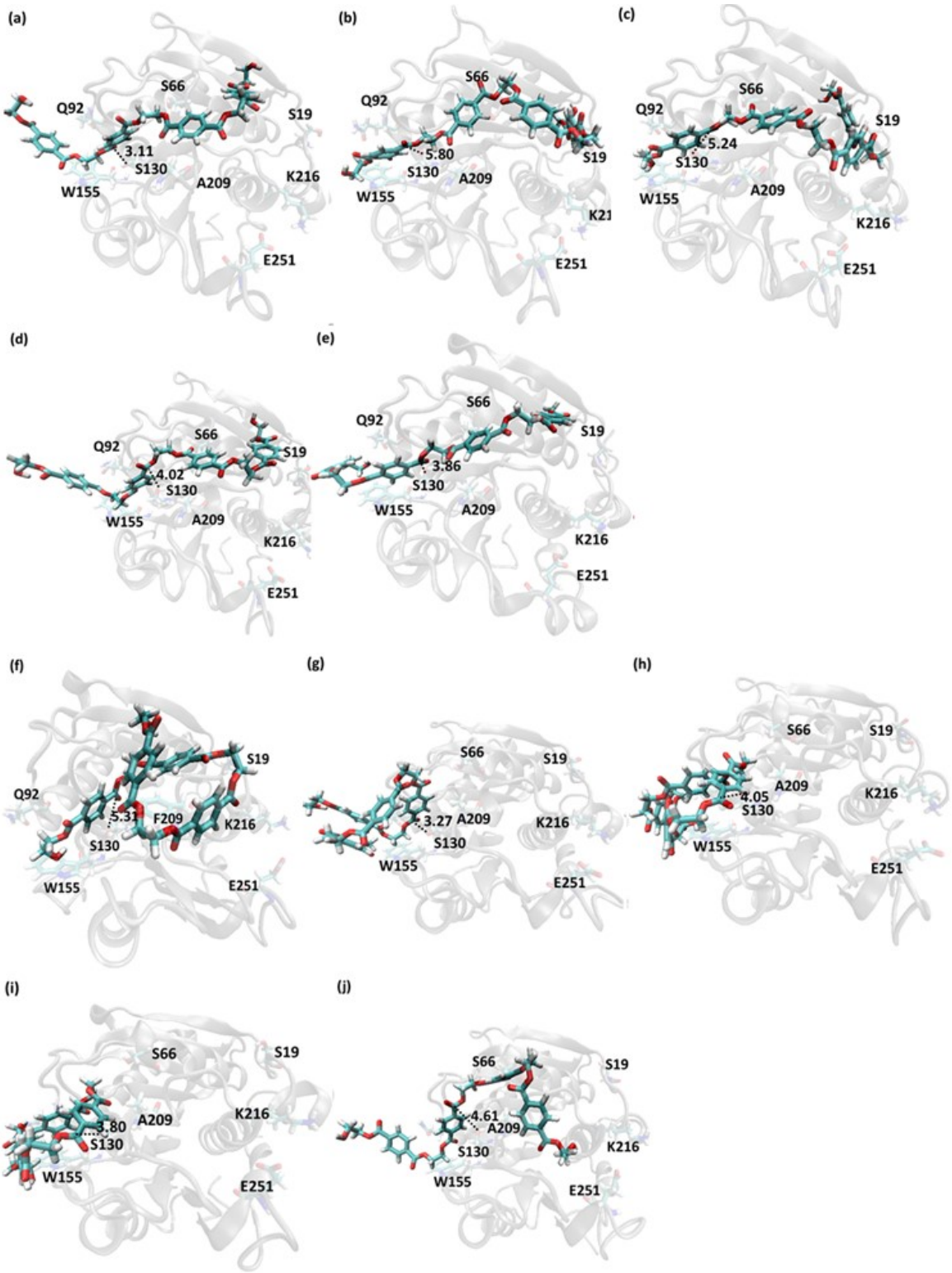


Figure S8. The top5 energy extended (a to e) and least5 energy folded (f to j) reference structures of DM *TfCut2* obtained after clustering. The contact plot and energy analysis are performed on these model structure.

(a)

(b)

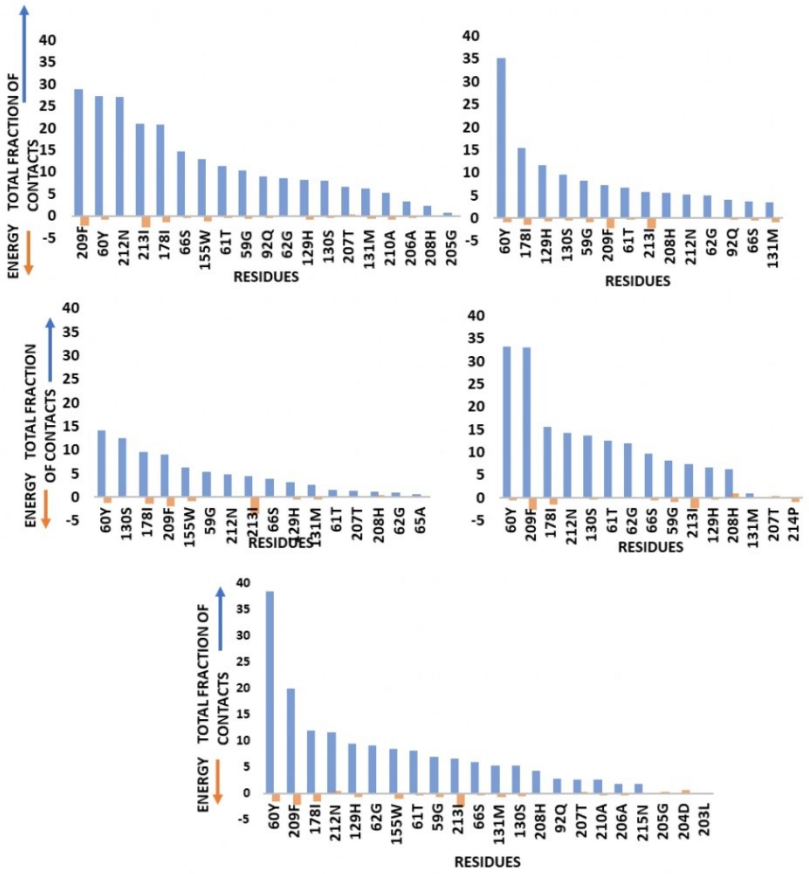
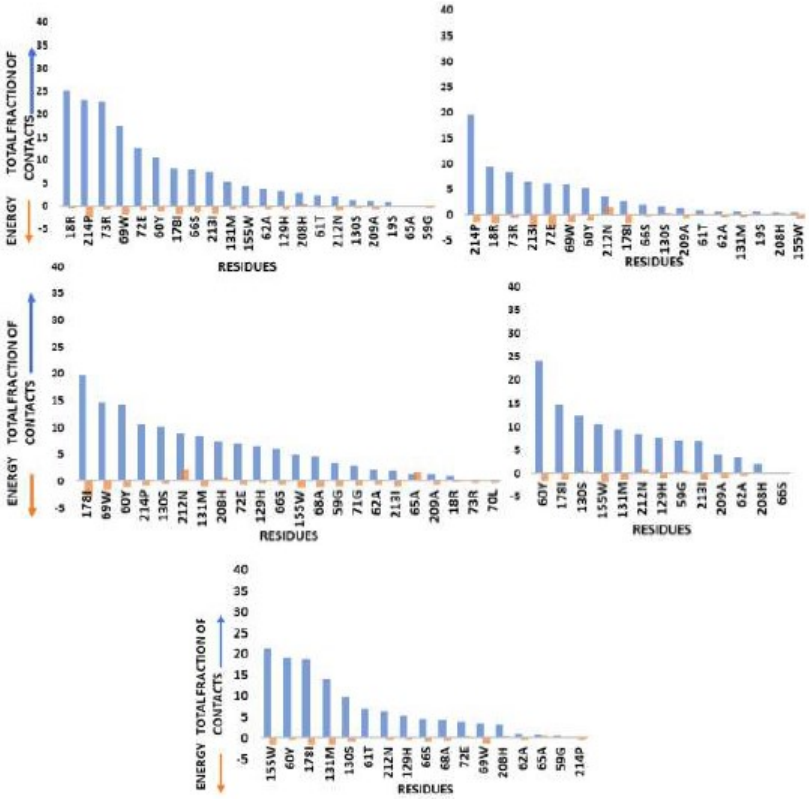


Figure S9. Total fraction of contacts on +Y axis and binding energy using MM-PBSA per-residue analysis (kcal/mol) in -Y axis for the (a) WT *TfCut2* PET extended conformer (b) WT *TfCut2* PET folded conformer The amino acid residues are given in x axis.

(a)



(b)

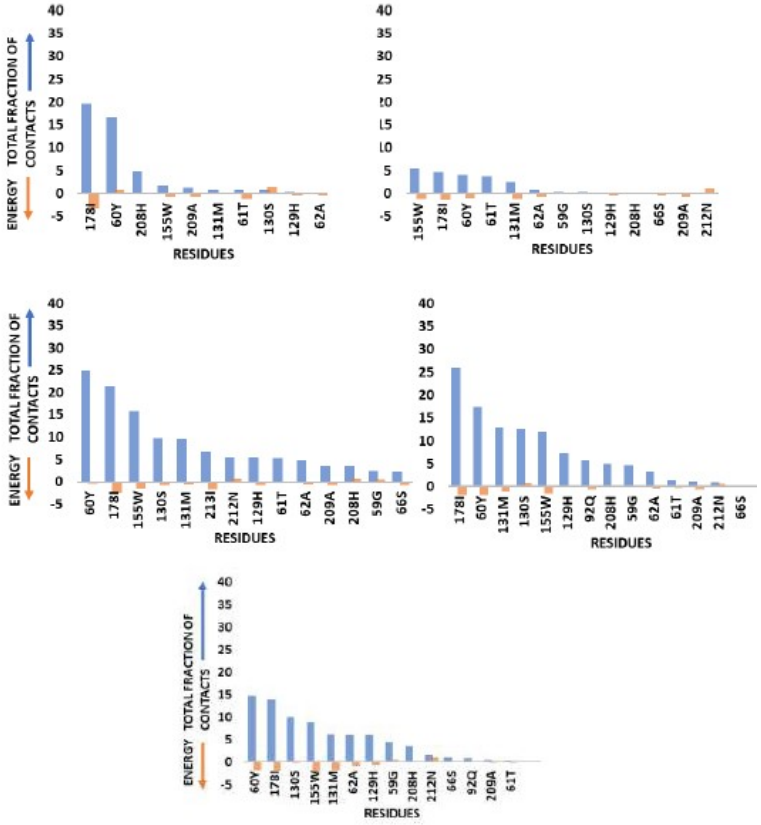


Figure S10. Total fraction of contacts on +Y axis and MM-PBSA per-residue binding energy (kcal/mol) in -Y axis for the (a) DM *TfCut2* extended PET conformer (b) DM *TfCut2* folded PET conformer. The amino acid residues are given in X axis.

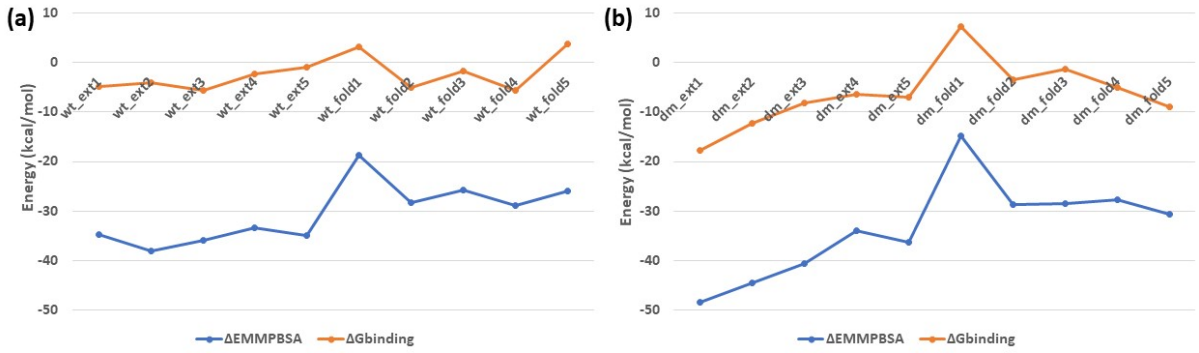


Figure S11. ΔE_{MMPBSA} and $\Delta G_{binding}$ of extended and folded conformers of PET with (a) WT *TfCut2* (b) DM *TfCut2*. Entropy contribution has been calculated using normal mode analysis.

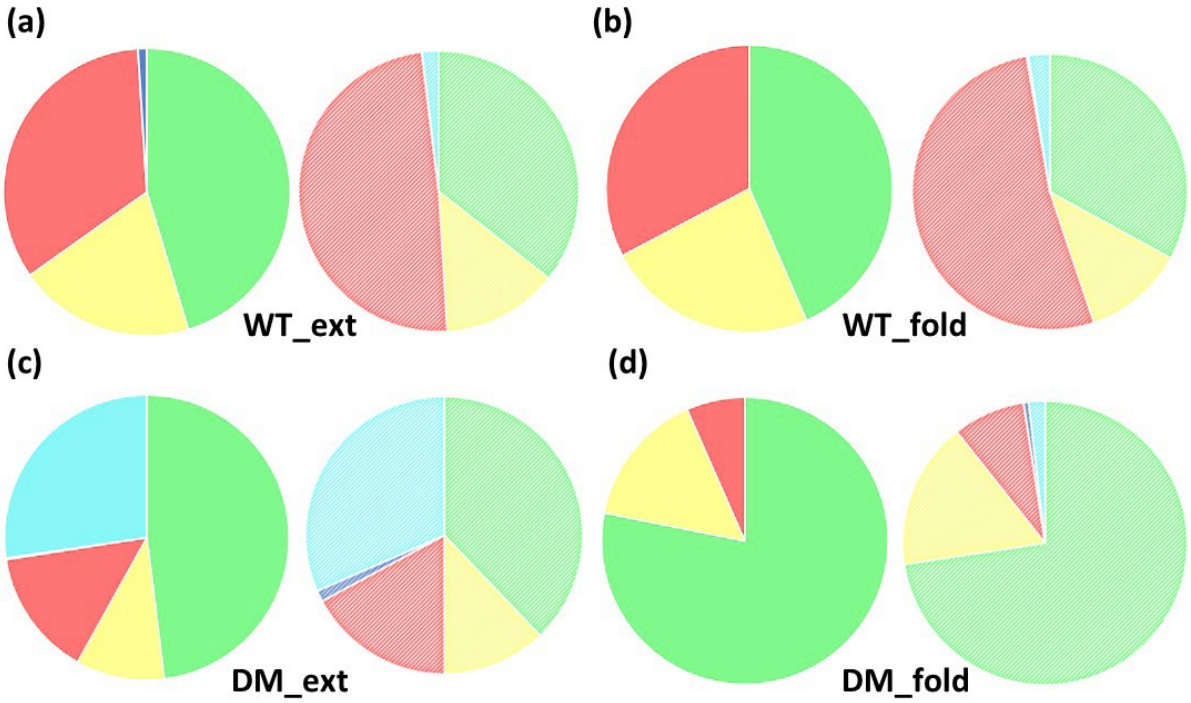


Figure S12. Contact plot and average MM-PBSA per residue binding energy analysis of (a) extended WT *TfCut2* (b) folded WT *TfCut2* (c) extended DM *TfCut2* and (d) folded DM *TfCut2*. The pie chart for contact and energy analysis are indicated with solid and shaded portions respectively.

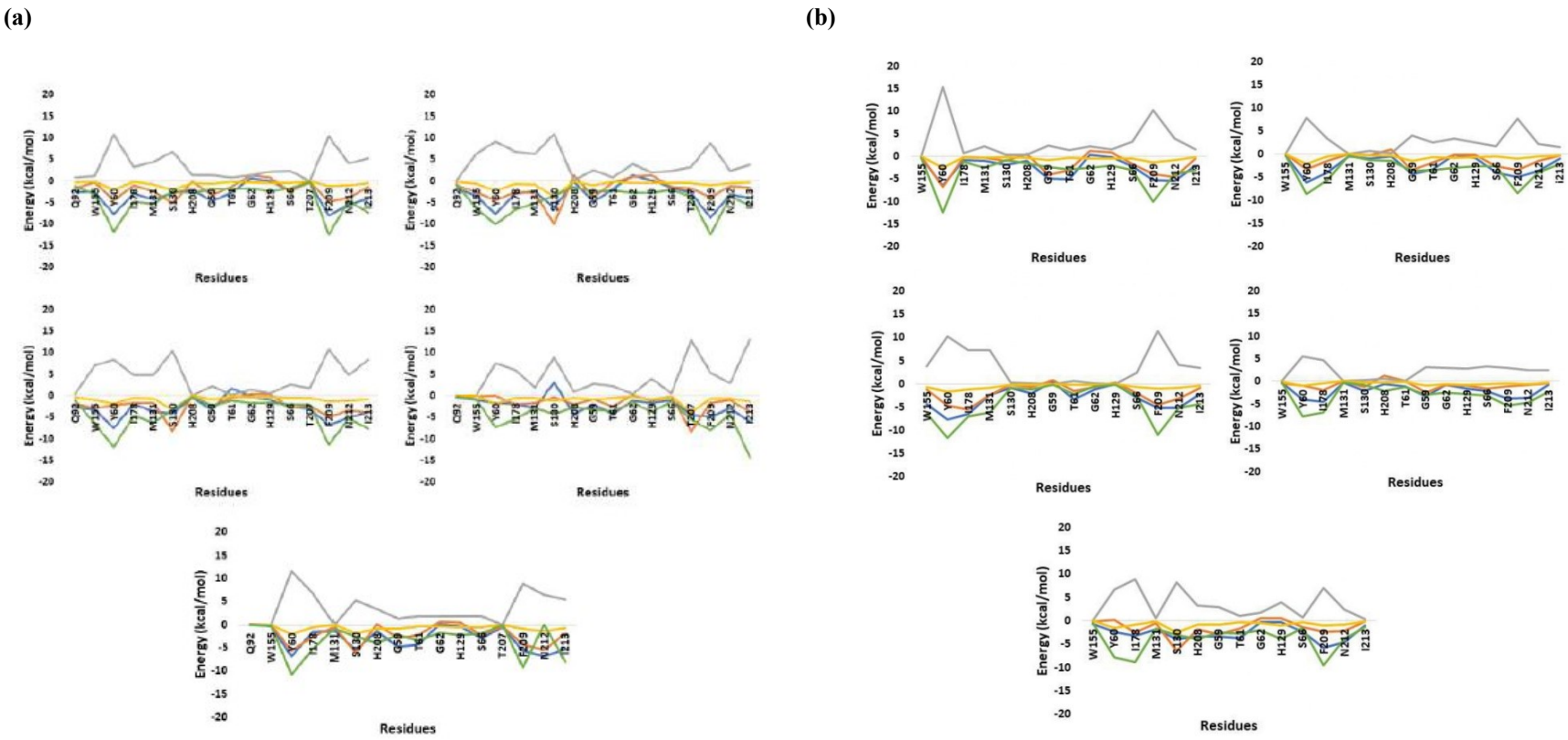
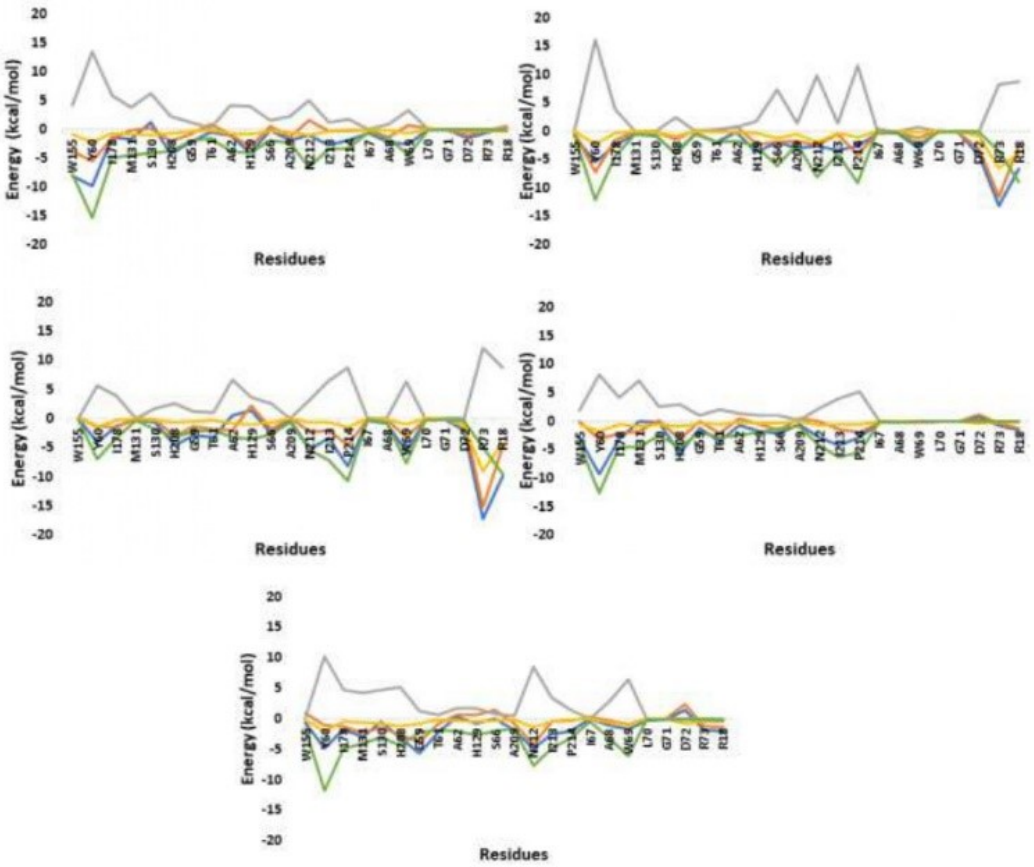


Figure S13. Energy decomposition of aminoacid residues using SAPT0 analysis for (a) WT *TfCut2* extended PET conformer and (b)WT *TfCut2* folded PET conformer. The total energy is indicated by blue. Red, grey, yellow and green respectively indicates the electrostatic, exchange, induction and dispersion components.

(a)

(b)


Energy (kcal/mol)
Residues

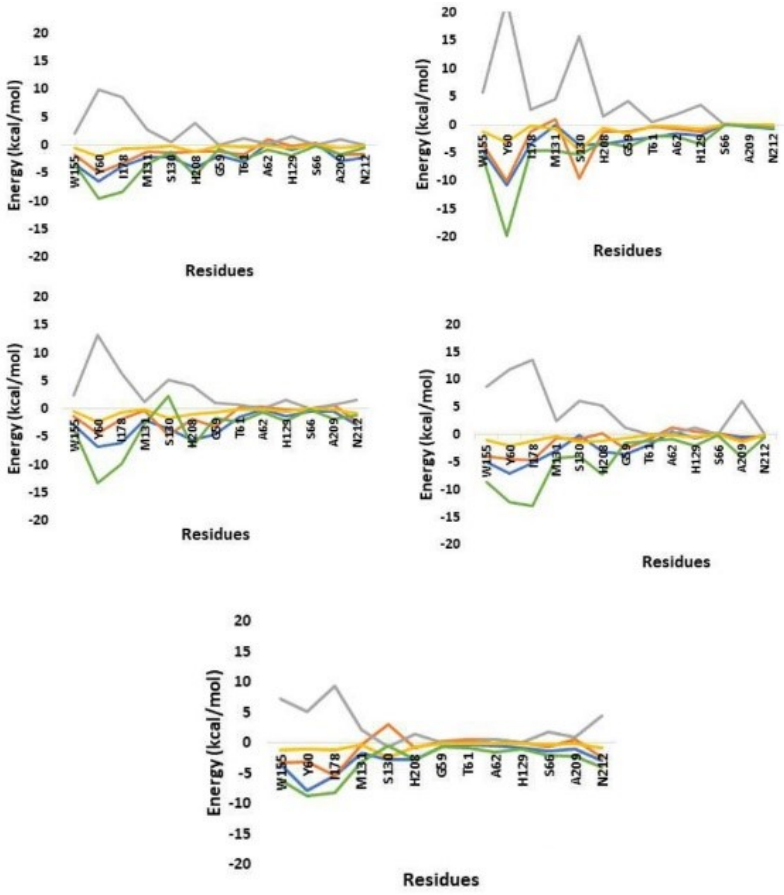


Figure S14. Energy decomposition of amino acid residues of (a) DM *TfCut2* extended PET conformer and (b) DM *TfCut2* folded PET conformer using SAPT0 analysis with total energy indicated by blue. Red, grey, yellow and green respectively indicates the electrostatic, exchange, induction and dispersion component

Supplementary Tables S1-S4

Table S1. Interaction energy of model PET (extended conformer) with WT *TfCut2* calculated using Grimme DFT-D3(BJ) dispersion interaction at the B3LYP/6-31+g(d) level of theory by using Gaussian09 software package.

Residue	**Interaction Energy (kcal/mol)**				
W155	-3.11	-0.79	-0.29	-2.53	-3.53
Y60	-6.77	-1.36	-6.5	-6.29	-6.2
I178	-2.23	-2.12	-1.72	-3.44	-3.64
M131	-3.97	-3.17	-1.43	-4.54	-2.97
S130	-3.83	1.77	-5.69	-3.06	-5.71
H208	-0.39	-3.51	-0.94	-1.91	-0.16
G59	-3.39	-2.27	-5.06	-4.55	-4.84
T61	-1.77	-4.88	-4.74	-3.04	-2.32
G62	-0.75	-1.46	-0.42	-0.39	0.18
H129	-1.2	-2.14	-0.51	-0.56	-0.18
S66	-2.45	-1.2	-2.57	-2.21	-1.77
T207	-1.84	-3.92	-0.43	-0.44	-2.86
F209	-5.72	-4.4	-5.01	-6.94	-7.06
N212	-4.92	-3.51	-6.56	-5.52	-3.15
I213	-4.53	-7.34	-4.5	-5.08	-4.56

Table S2. Interaction energy of model PET (folded conformer) with WT *TfCut2* calculated using Grimme DFT-D3(BJ) dispersion interaction at the B3LYP/6-31+g(d) level of theory by using Gaussian09 software package.

Residue	**Interaction Energy (kcal/mol)**				
W155	-0.31	-0.58	-0.22	-4.59	-0.26
Y60	-5.79	-2.65	-5.78	-7.48	-4.31
I178	-1.01	-4.27	-4.62	-6.3	-5.08
M131	-1.45	-1.85	-0.31	-2.6	-0.2
S130	-2.02	-4.21	-0.93	-0.95	-2.14
H208	-0.93	-2.85	-0.36	-1.71	-0.66
G59	-4.85	-3.1	-4.67	0.39	-3.64
T61	-5.22	-4.27	-3.62	-3.41	-1.38
G62	-0.72	-1.1	-1.04	-1.04	-1.45
H129	-0.17	-0.88	-1.16	-0.03	-1.9
S66	-2.4	-2.3	-4.2	-3	-2.4
F209	-5.12	-5.21	-4.68	-4.49	-3.78
N212	-4.87	-4.59	-4.17	-4.85	-4
I213	-2.4	-1.1	-1.4	-2.5	-0.9

Table S3. Interaction energy of model PET (extended conformer) with DM *TfCut2* calculated using Grimme DFT-D3(BJ) dispersion interaction at the B3LYP/6-31+g(d) level of theory by using Gaussian09 software package.

Residue	**Interaction Energy (kcal/mol)**				
W155	-7.9	-0.8	0	-3	-0.5
Y60	-9.7	-5.3	-4.3	-9	-5.1
I178	-1.9	-2.4	-1.6	-3.3	-1.9
M131	-2	-0.6	-0.2	-0.2	-2.9
S130	0.5	-0.9	-1.2	-0.6	-0.8
H208	-3.5	-3.5	-3.9	-4.4	-2.3
G59	-2.4	-0.4	-2.9	-1.6	-5.2
T61	-0.8	-2.4	-3.2	-3.9	-2.5
A62	-1.7	-0.7	0.2	-1.3	-0.2
H129	-3.8	-3.5	0.8	-1.9	-0.9
S66	-0.7	-3.2	-2.8	-2.1	-0.8
A209	-2.1	-2.9	-0.5	-0.7	-2
N212	-2.2	-3.6	-5.4	-3.2	-5.4
I213	-2.9	-3.6	-3.6	-4.6	-2.9
P214	-2	-3.3	-7.5	-3.2	-2.5
I67	-0.7	-0.4	-0.4	-0.3	-0.1
A68	-2.2	-0.2	-0.7	-0.3	-1.4
W69	-3	-2.8	-5.2	-0.7	-2.3
L70	-0.1	0.1	-0.5	-0.1	-0.3
G71	-0.1	-0.2	-0.1	0	-0.1
E72	-1.58	-4.14	-1.96	0.4	-1.6
R73	-0.6	-12.6	-16.9	-0.7	-1.8
R18	0.1	-7.8	-11.2	-1.5	-2.1

Table S4. Interaction energy of model PET (folded conformer) with DM *TfCut2* calculated using Grimme DFT-D3(BJ) dispersion interaction at the B3LYP/6-31+g(d) level of theory by using Gaussian09 software package.

Residue	**Interaction Energy (kcal/mol)**				
W155	-2.5	-4.4	-3.4	-4.2	-3.6
Y60	-5.4	-8.6	-5.4	-6.6	-7.6
I178	-6.9	-3.9	-4.6	-6.2	-6
M131	-2.2	-0.8	-2.6	-3.5	-2.1
S130	-3.4	-3.6	-2.3	-0.6	-2.7
H208	-5.9	-2.8	-4.2	-3.8	-2.3
G59	-4.3	-3.2	-1.6	-3.5	-0.8
T61	-1.8	-2	-3.2	-1.9	-0.4
A62	-0.3	-1.7	0	0.2	-0.9
H129	-1.8	-2.2	-0.7	-0.8	-1
S66	-0.4	0.1	0.2	0.1	-1.5
A209	-1	-0.4	-2.7	-0.7	-1.4
N212	-2.8	-0.8	-1.9	-0.6	-2.6

Supplementary Movie Captions

Movie S1. The trajectory of DM *TfCut2*-PET systems showing the S1 residues Trp155(violet), Ile178 (pink) and Tyr60 (green) interacting with PET (cyan licorice). This trajectory is part of 1μs simulation with each frame shown corresponding to an interval of 10 ns.

Movie S2. The trajectory of WT *TfCut2*-PET systems showing the binding of PET (cyan licorice) and π-π interactions of phenyl ring of PET with Phe209 (S3). The residues at the end of the active site are shown in green licorice representations with Phe209 being shown in pink. This trajectory is part of 1μs simulation with each frame shown corresponding to an interval of 10 ns.

Movie S3. The trajectory of DM *TfCut2*-PET systems showing the interaction of mutated residue Ala209 (pink licorice) with PET (cyan licorice). This trajectory is part of 1μs simulation with each frame shown corresponding to an interval of 10 ns. The residues at the end of the active site are shown in green licorice representations.

Movie S4. The trajectory of DM *TfCut2*-PET systems showing the binding of PET to the S3 subsite residues Asn212 (violet) and Ile213 (pink). This trajectory is part of 1μs simulation with each frame shown corresponding to an interval of 10 ns.

Movie S5. The trajectory of DM *TfCut2*-PET systems showing the cation-π interactions of Arg18 of S4′ subsite with phenyl ring of PET. The residues at the end of the active site are shown in green licorice representations with Arg73 being shown in pink. This trajectory is part of 1μs simulation with each frame shown corresponding to an interval of 1 ns.

Supporting Files

Some of the coordinate files and the corresponding parameter files of the WT and DM *TfCut2*-PETase system are given in the link below:

https://drive.google.com/drive/folders/1_w0TuPm-eGpmoUMFS7Hrtv9MFyzX3ou?usp=sharing