

Supplementary information

Impact of Mutation on the Structural Stability and Conformational Landscape of Inhibitor-resistant TEM β -lactamase: A High-performance Molecular Dynamics Simulation Study

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Figure S1: Multiple sequence alignment of the wild type (1ZG4) and three IRT variant; AJC64564.1 (pUE184TEM), AJC64567.1 (pUE203TEM), and AJC64568.1 (pUE210TEM). The amino acid position (substitutions at amino acid residues 69, 84, 165, 184, 262, 276) is marked according to the Amber classification of Class A β -lactamases.

1ZG4	MSIQHFRVALIPFFAAFCLPVFAHPETLVVKVDAEDQLGARVGYIELDLNSGKILESF
AJC64567.1	MSIQHFRVALIPFFAAFCLPVFAHPETLVVKVDAEDQLGARVGYIELDLNSGKILESF
AJC64564.1	MSIQHFRVALIPFFAAFCLPVFAHPETLVVKVDAEDQLGARVGYIELDLNSGKILESF
AJC64568.1	MSIQHFRVALIPFFAAFCLPVFAHPETLVVKVDAEDQLGARVGYIELDLNSGKILESF *****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

1ZG4	EERFPMMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVREL
AJC64567.1	EERFPMMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVREL
AJC64564.1	EERFPMLSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVREL
AJC64568.1	EERFPMMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVREL *****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

1ZG4	CSAAITMSDNTAANLLLTTIGGPKELETAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTM
AJC64567.1	CSAAITMSDNTAANLLLTTIGGPKELETAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTM
AJC64564.1	CSAAITMSDNTAANLLLTTIGGPKELETAFLHNMGDHVTRLDRGEPELNEAIPNDERDTTM
AJC64568.1	CSAAITMSDNTAANLLLTTIGGPKELETAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTM *****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

1ZG4	PVAMATTLRKLLTGELLTLASRQQLDWMEADKVAGPLLRSALPAGWFIADKSGAGERGS
AJC64567.1	PVAMATTLRKLLTGELLTLASRQQLDWMEADKVAGPLLRSALPAGWFIADKSGAGERGS
AJC64564.1	PAAMATTLRKLLTGELLTLASRQQLDWMEADKVAGPLLRSALPAGWFIADKSGAGERGS
AJC64568.1	PAAMATTLRKLLTGELLTLASRQQLDWMEADKVAGPLLRSALPAGWFIADKSGAGERGS *****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

1ZG4
AJC64567.1
AJC64564.1
AJC64568.1

RGRIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW
RGRIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW
RGRIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW
RGRIAALGPDGKPSRIVVIYTTGSQATMDERSRQIAEIGASLIKHW

262 276

Figure S2: Replica plots for 100 ns simulation time frame shown for mutant proteins A) pUE184TEM B) pUE203TEM C) pUE210TEM (displayed in replica 1 black, replica 2 red, replica 3 blue). All replica has a very similar pattern to overall simulation time.

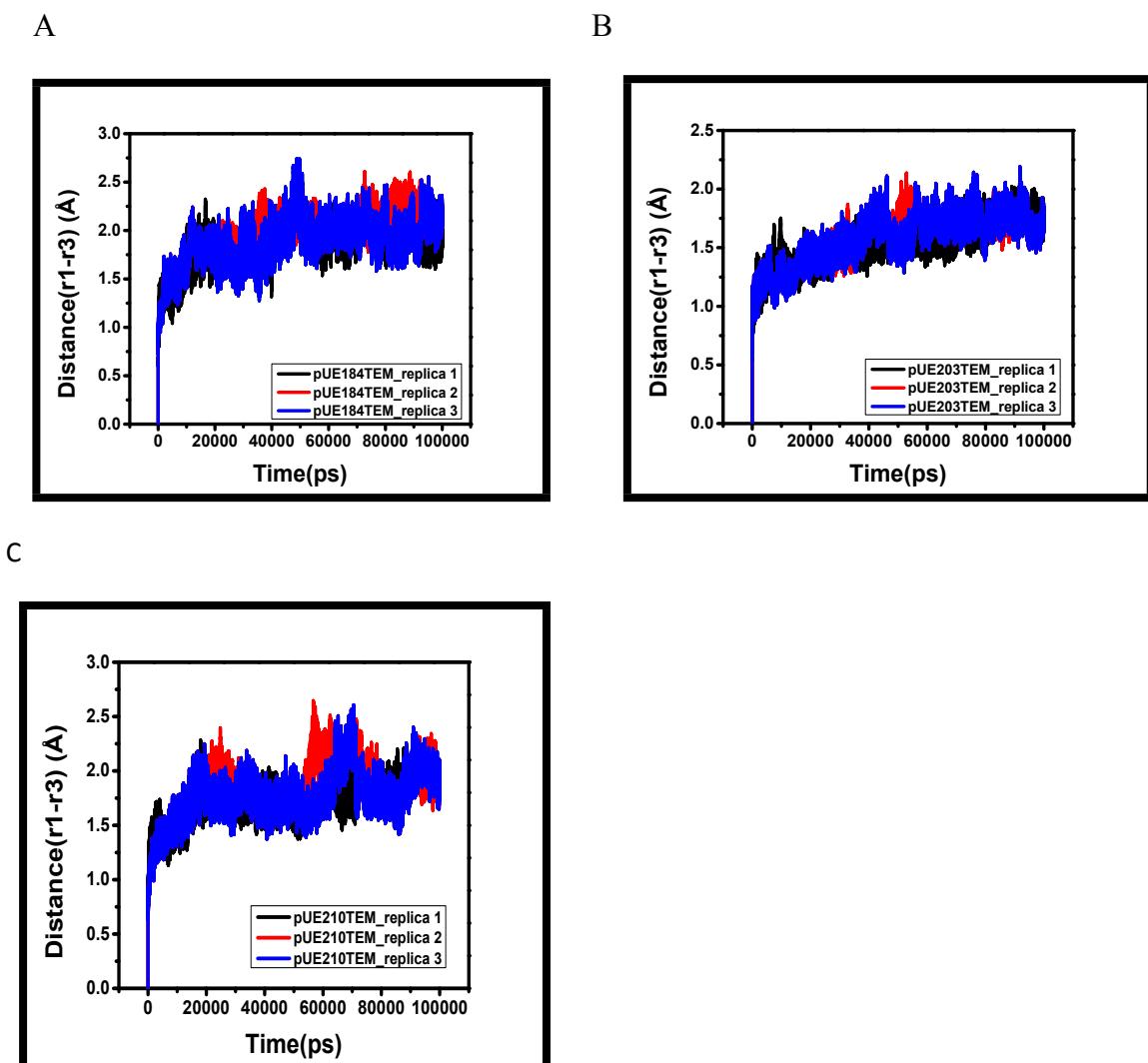
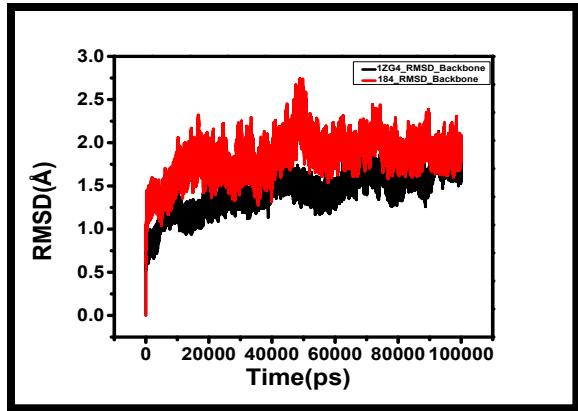
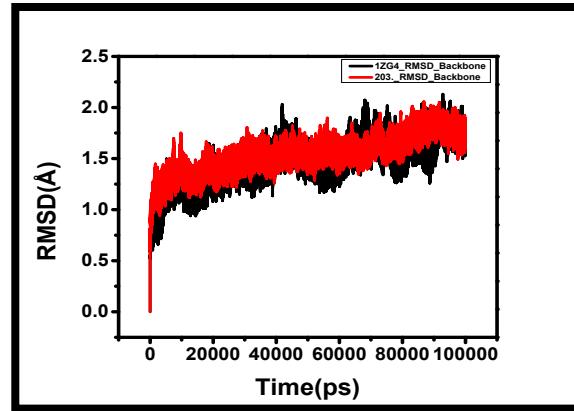


Figure S3: C- α backbone RMSD plots for 100 ns simulation time frame shown for the wild type protein 1ZG4 (displayed in black) in comparison with mutant proteins A) pUE184TEM B) pUE203TEM C) pUE210TEM (displayed in red).

A



B



C

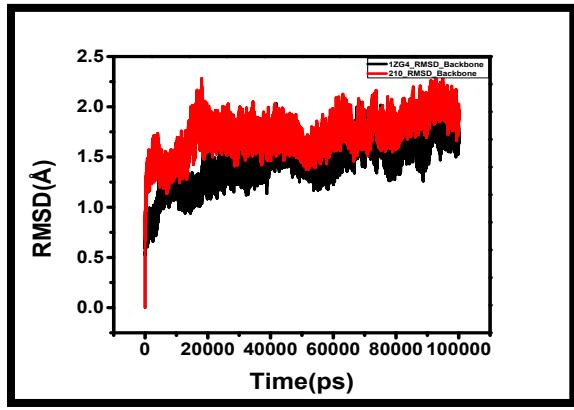
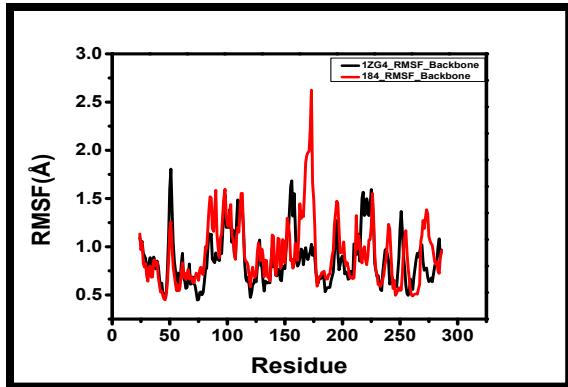
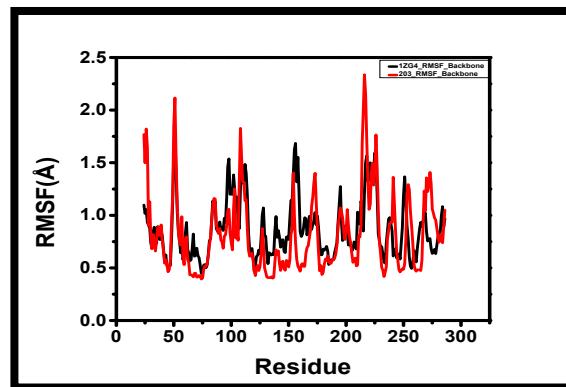


Figure S4: RMSF plots for the backbone C- α atoms for 100 ns of the simulation shown for the wild type protein 1ZG4 (displayed in black) in comparison with mutant proteins a) pUE184TEM b) pUE203TEM c) pUE210TEM (displayed in red).

A



B



C

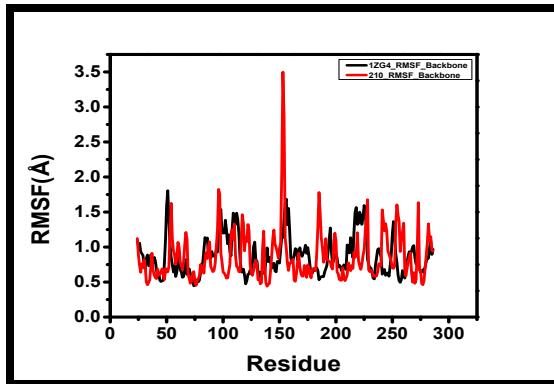
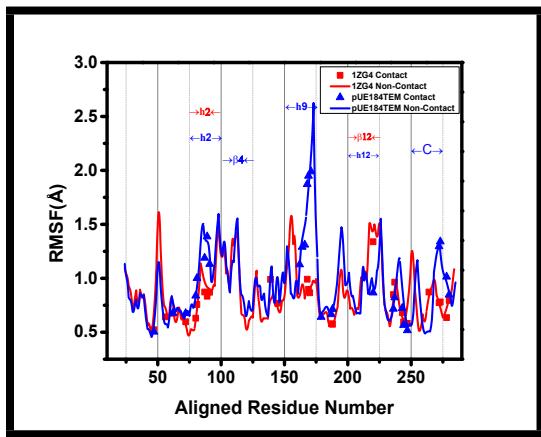
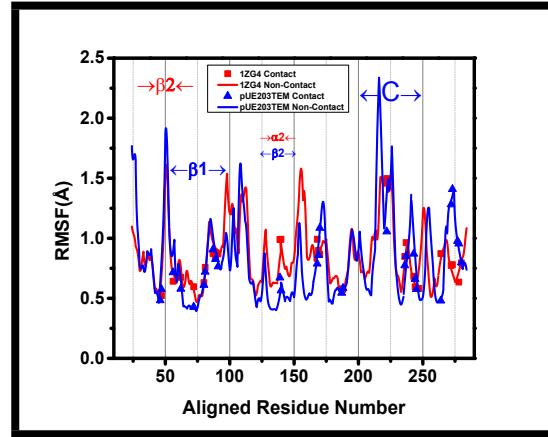


Figure S5: RMSF plots for contact and non-contact residue mobility shown for the wild type protein 1ZG4 (displayed in red) in comparison with mutant proteins A) pUE184TEM B) pUE203TEM C) pUE210TEM (displayed in blue). Contact residues are shown as solid symbols in the plot and arrow indicating opposite side (\longleftrightarrow) means contact residue are highly mobile and an arrow indicating same side ($\rightarrow\leftarrow$) means contact residue are less mobile

A



B



C

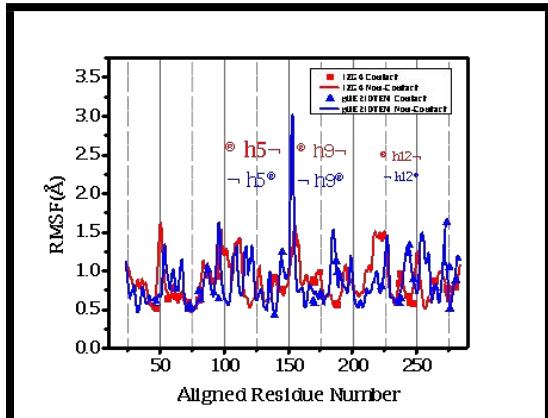
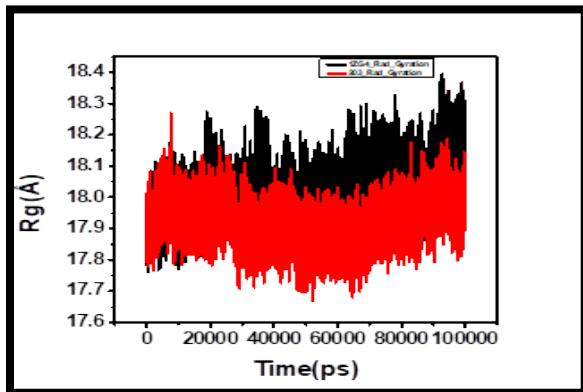
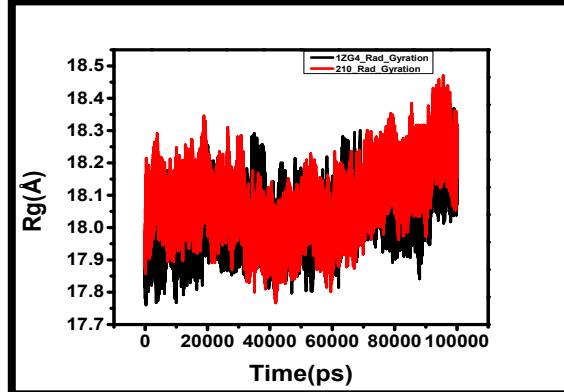


Figure S6 : Radius of gyration for the backbone C- α atoms for 100 ns of the simulation shown for the wild type protein 1ZG4 (displayed in black) in comparison with mutant proteins a) pUE184TEM b) pUE203TEM c) pUE210TEM (displayed in red).

A



B



C

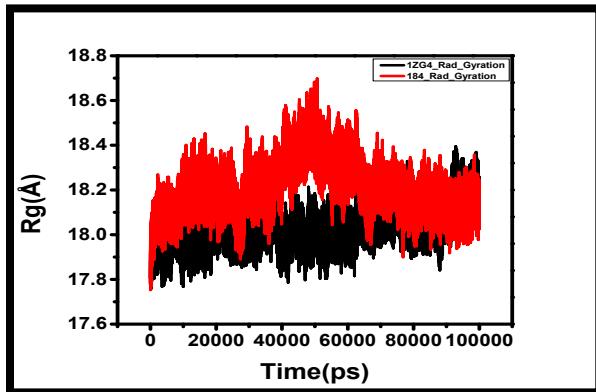
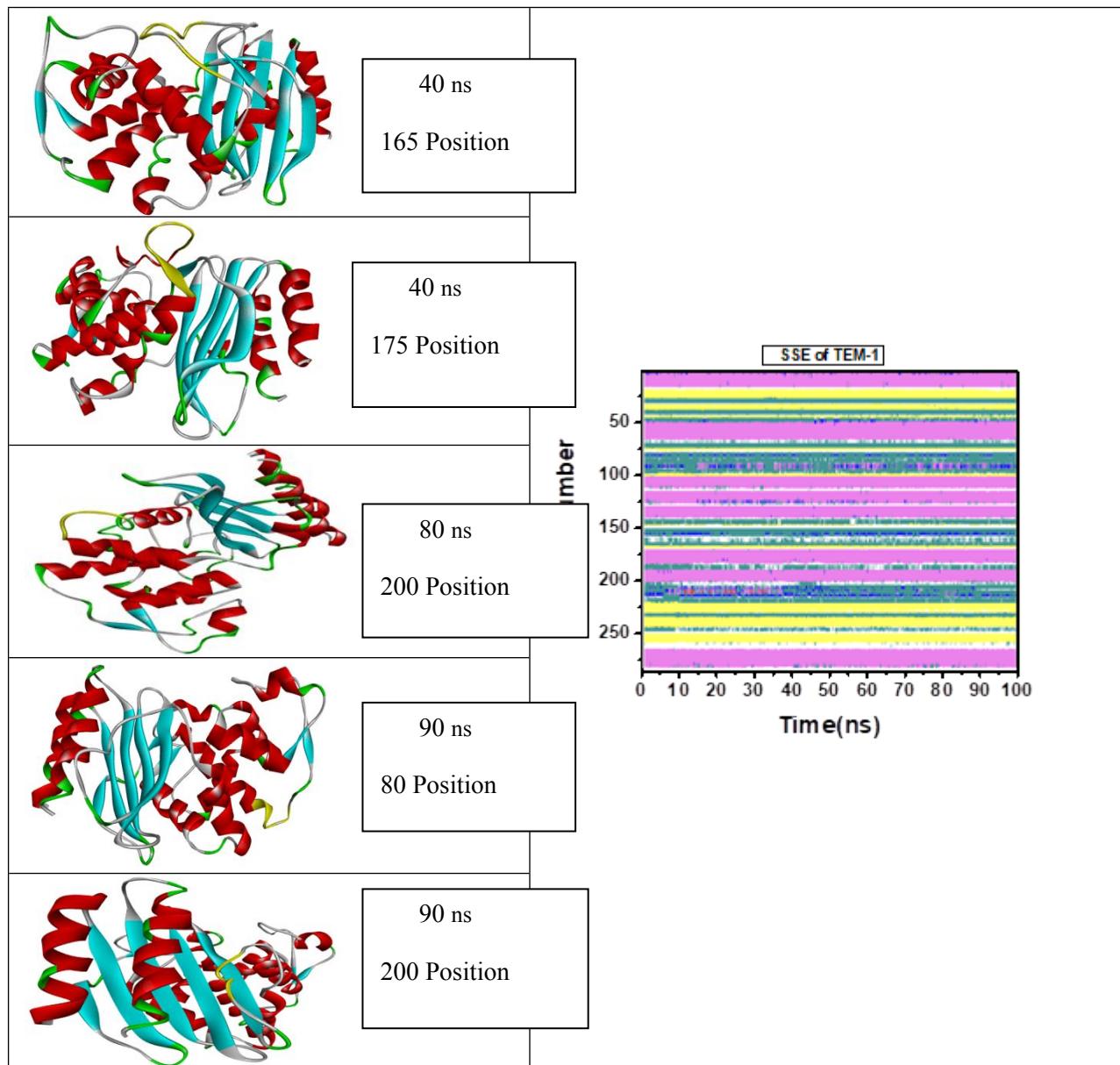
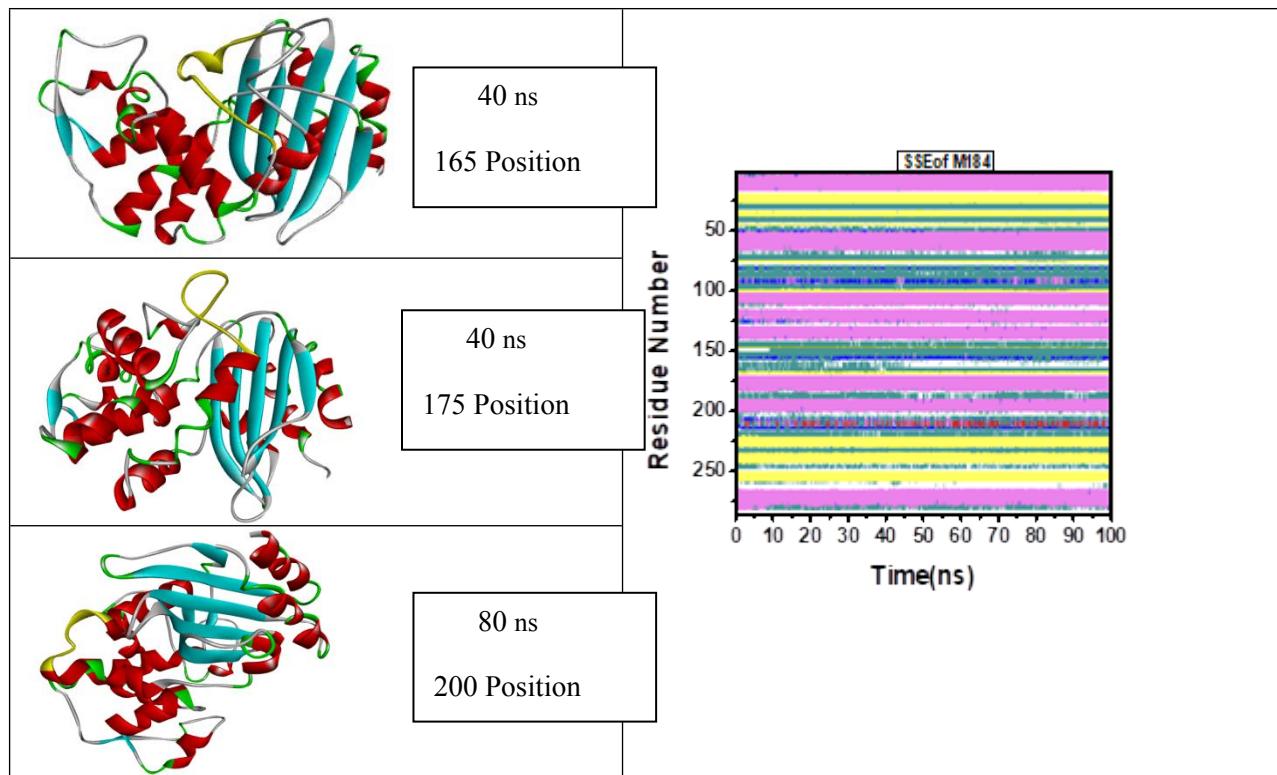


Figure S7: Projection of SSEs throughout the MD simulation trajectory for A)1ZG4 and mutant protein B) pUE184TEM, C) pUE203TEM, D) pUE210TEM) along with comparative snapshots of the simulation trajectory for different nanosecond timescale. Mutated amino acids are marked in yellow in the ribbon diagram of the modeled proteins. SSE composition represented by different color codes from helices; pink, turns; green, 3-10 helix; blue, β -strands; yellow, coils; white.

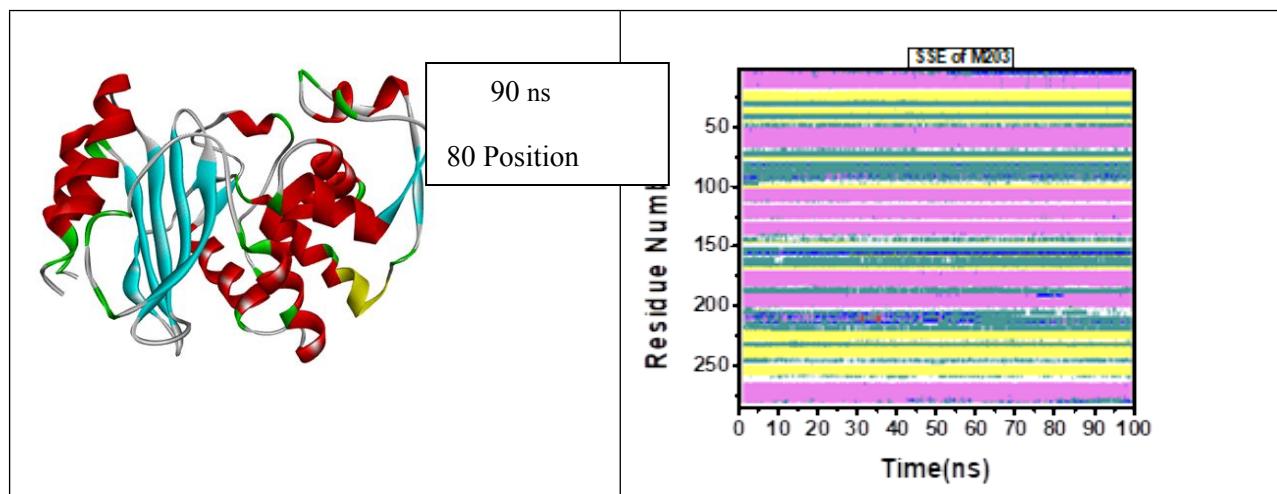
A



B



C



D

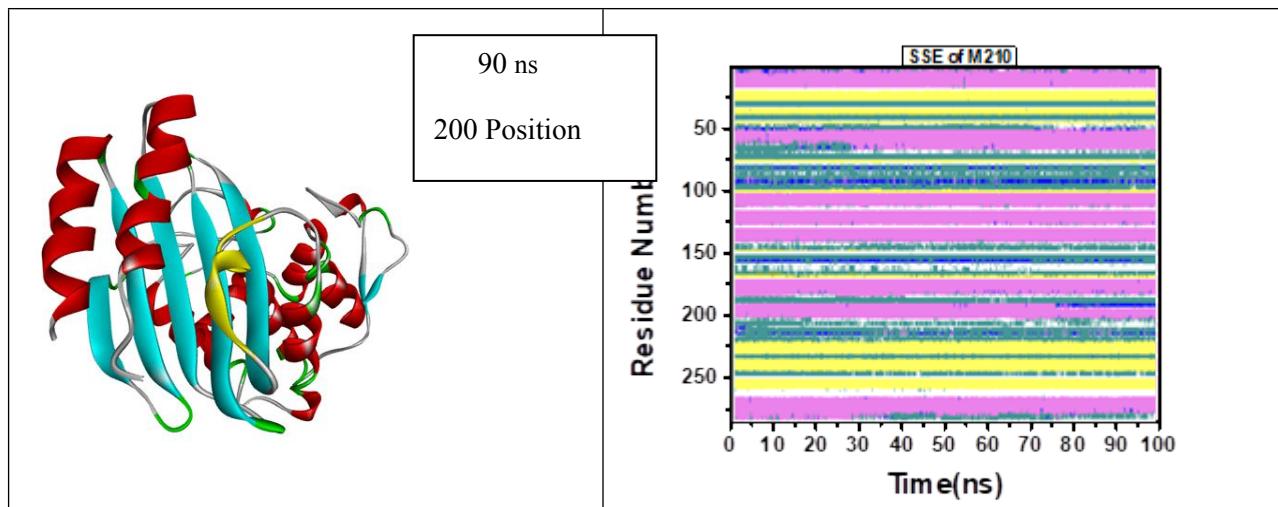


Table S1: Average MD RMSF values for all contact residues with respect to all residues (Standard deviations parenthesis) and enrichment factor (EF) of wild type (1ZG4) and three IRT variants; pUE184TEM, pUE203TEM, pUE210TEM

Protein	RMSF (Å)		Contacts	
	All Residues	All Contacts	EF	p-value
	Average	Average		
1ZG4	0.87(0.27)	0.77(0.178)	0.105	0.048
pUE184TEM	0.9358(0.325)	1.024(0.424)	1.27	0.041
pUE203TEM	0.8276(0.371)	0.7721(0.2352)	1.14	0.03
pUE210TEM	0.865(0.391)	0.833(0.2912)	1.06	0.032