Supplementary

Role of conserved residues in catalytic activity of NDM-1: an approach of site directed mutagenesis and molecular dynamics

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Running title: Generation of Mutants in NDM-1

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Total supplementary figures: 6

Total supplementary tables: 3

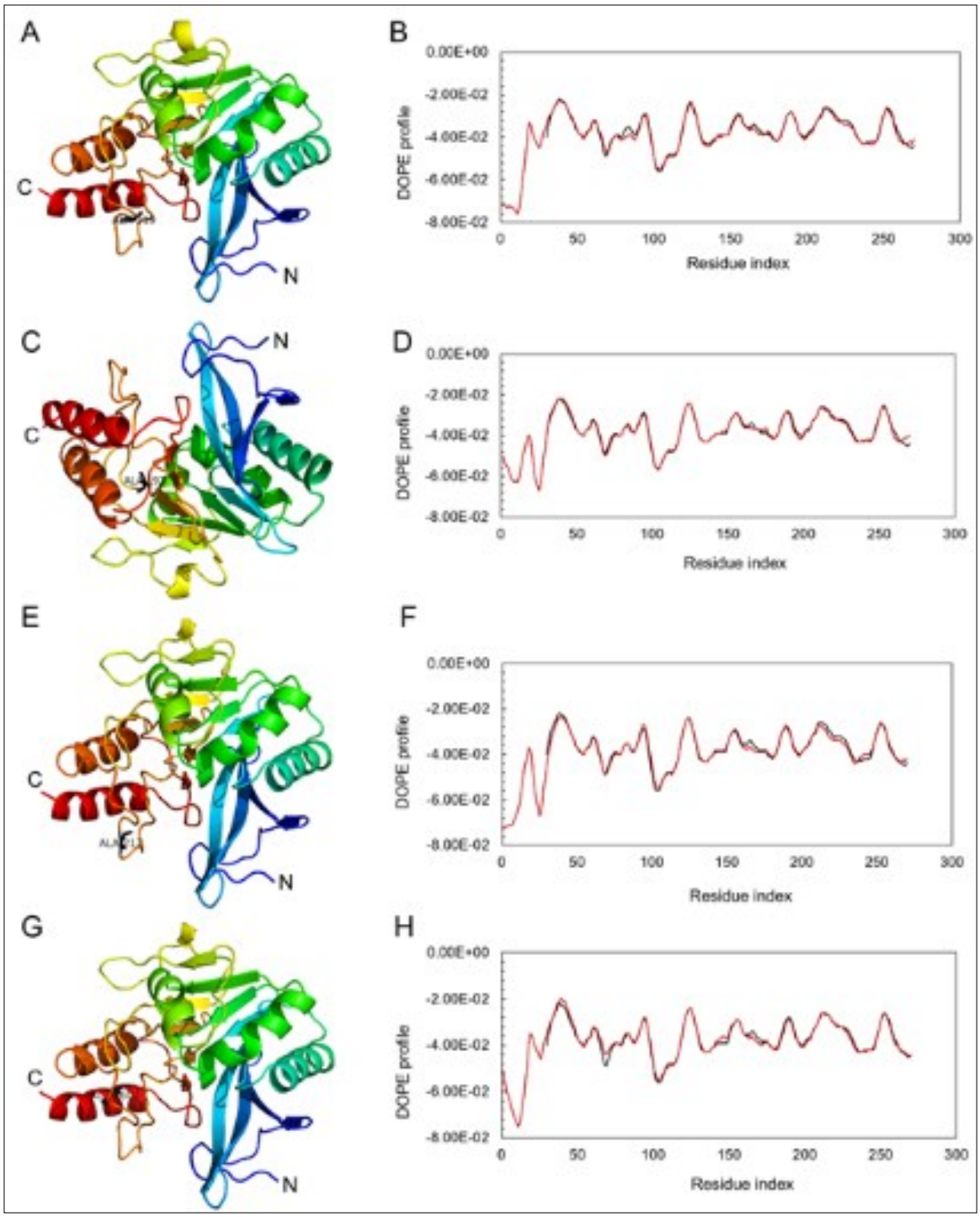


Fig. S1. Tertiary models and DOPE energy profile of mutant proteins. (A) Tertiary structure of G219A, (B) DOPE energy profile of G219A model, (C) Tertiary structure of N193A, (D) DOPE energy profile of N193A model, (E) Tertiary structure of S217A, (F) DOPE energy profile of S217A model, (G) Tertiary structure of T262A and (H) DOPE energy profile of T267A model. Template and models are shown in black and red lines, respectively.

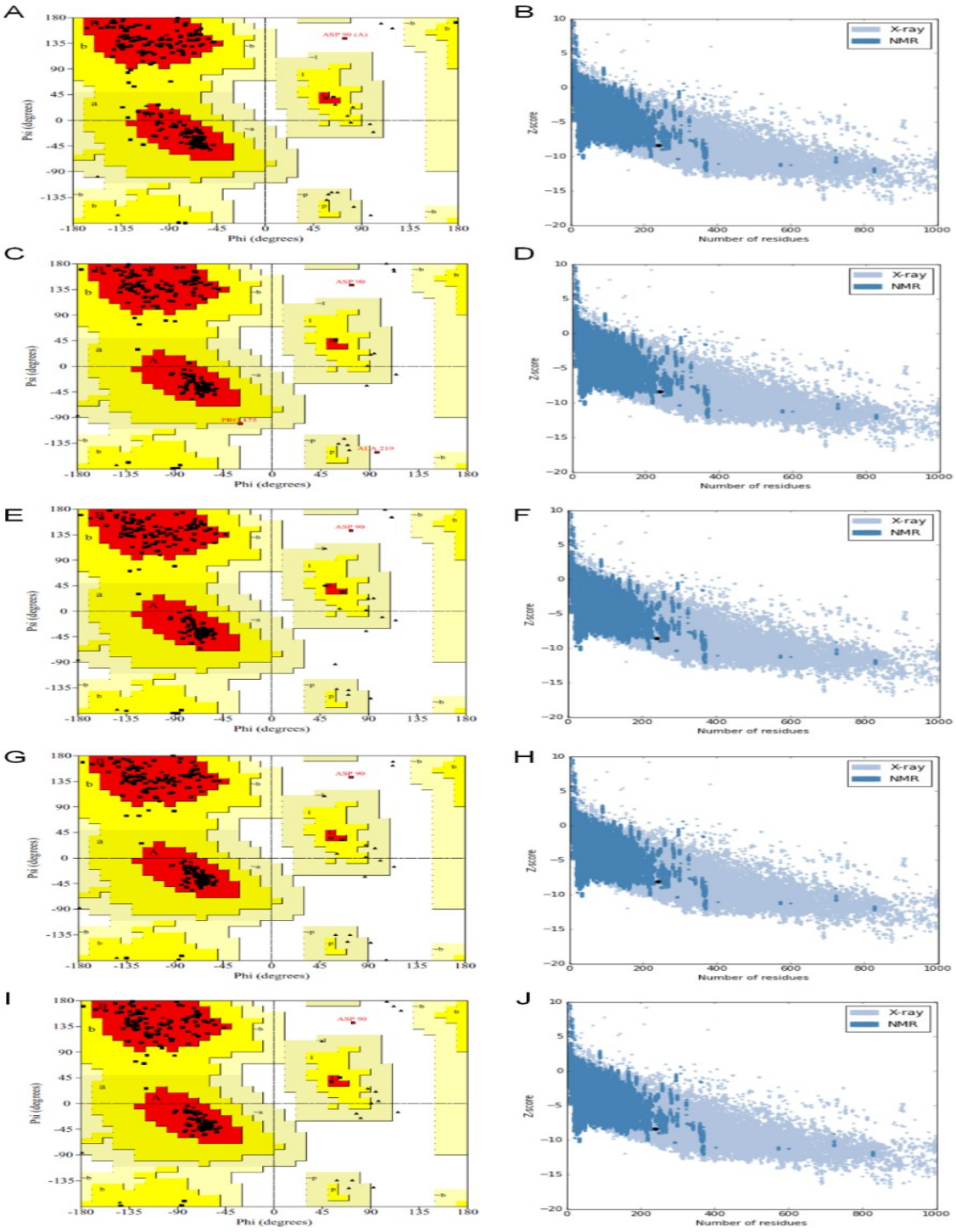
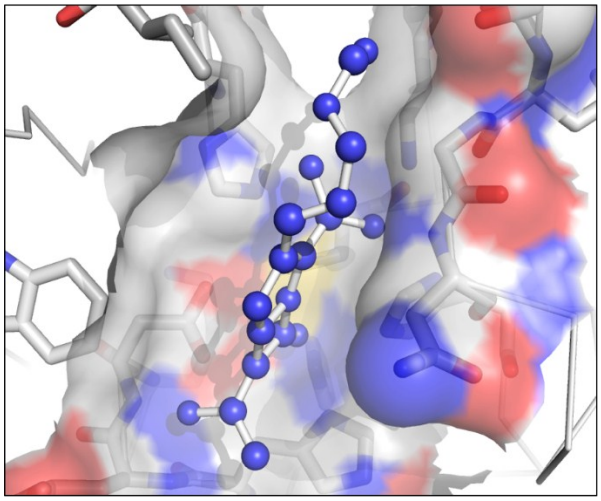


Fig S2. Model validation and assessment. (A) Ramachandran plot of wildtype protein, (B) ProSA plot of wildtype protein, (C) Ramachandran plot of G219A mutant protein, (D) ProSA plot of G219A mutant protein, (E) Ramachandran plot of N193A mutant protein, (F) ProSA plot of N193A mutant protein, (G) Ramachandran plot of S217A mutant protein, (H) ProSA plot of

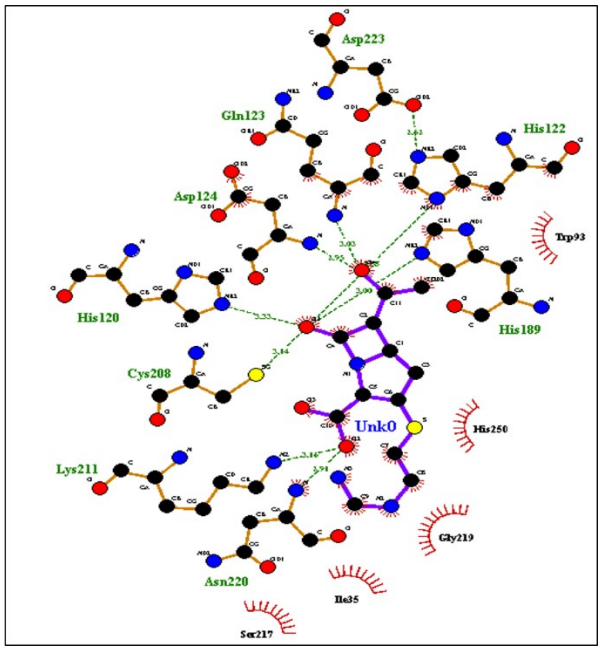
S217A mutant protein, (I) Ramachandran plot of T262A mutant protein and (J) ProSA plot of T262A mutant protein.

Wildtype -Imipenem complex

A

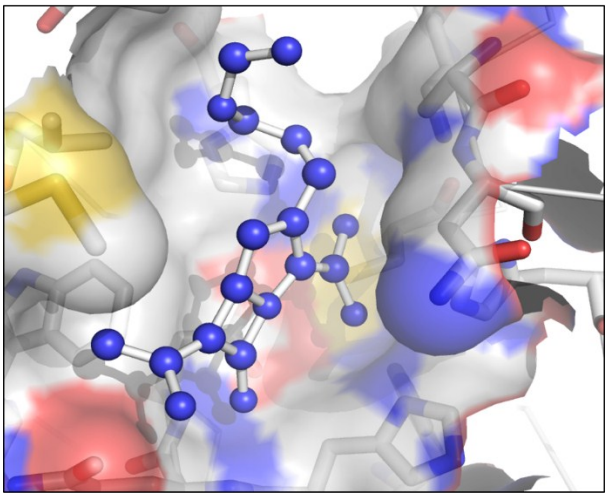


A*



G219A-Imipenem complex

B



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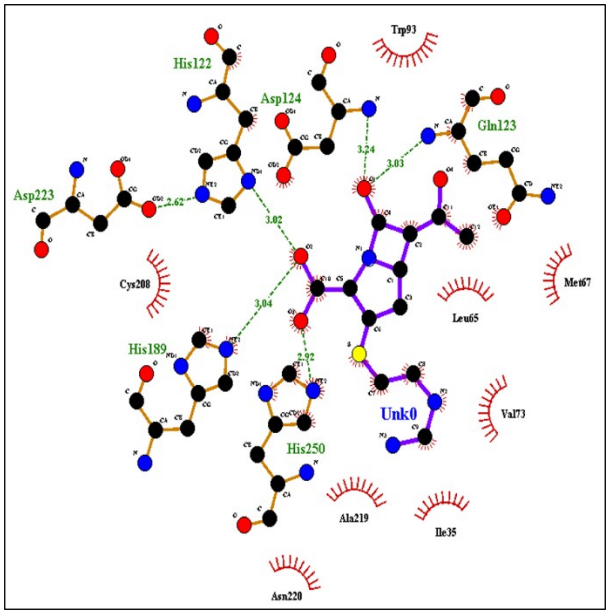
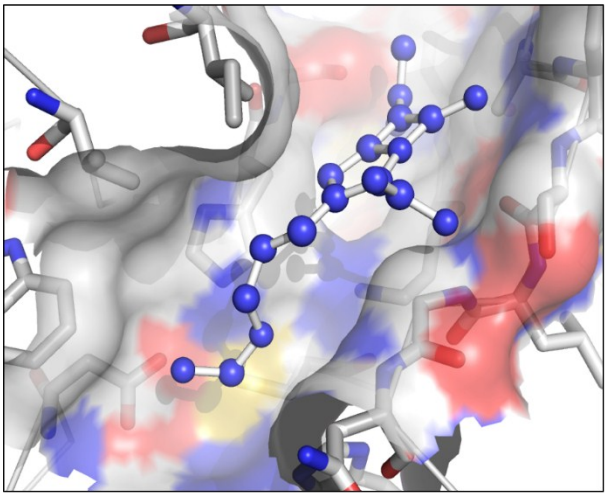


N193A-Imipenem complex

C



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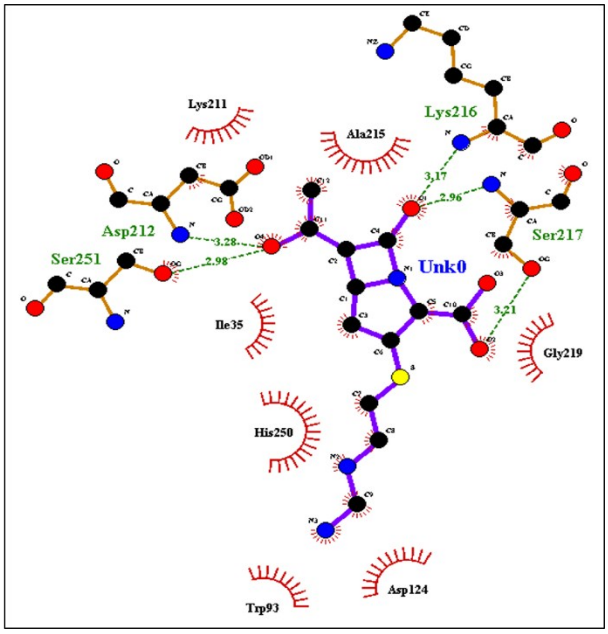
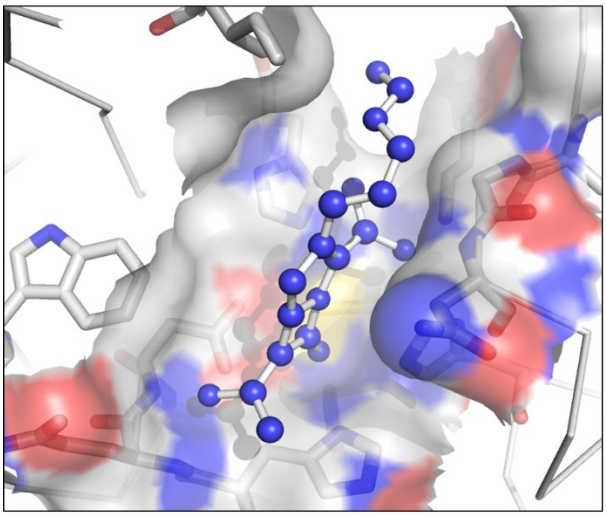
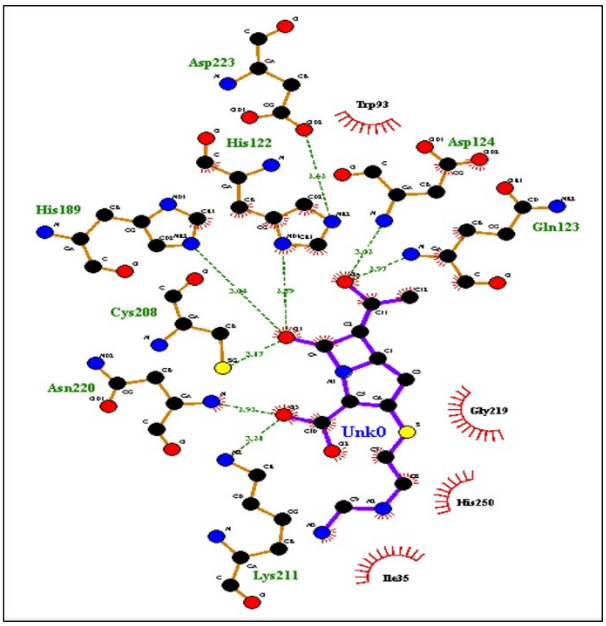


S217A-Imipenem complex

D

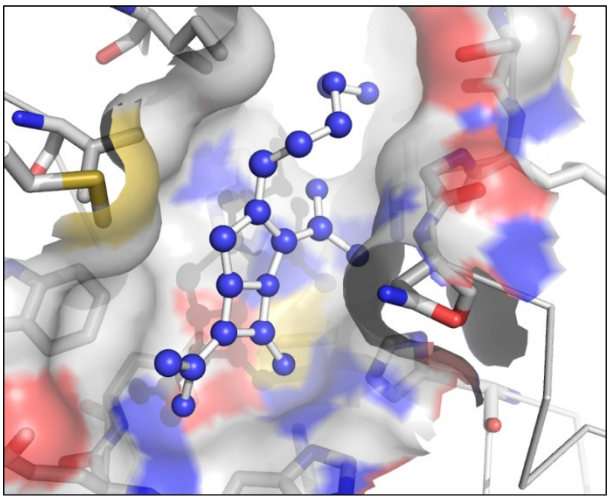


D*



T262A-Imipenem complex

E



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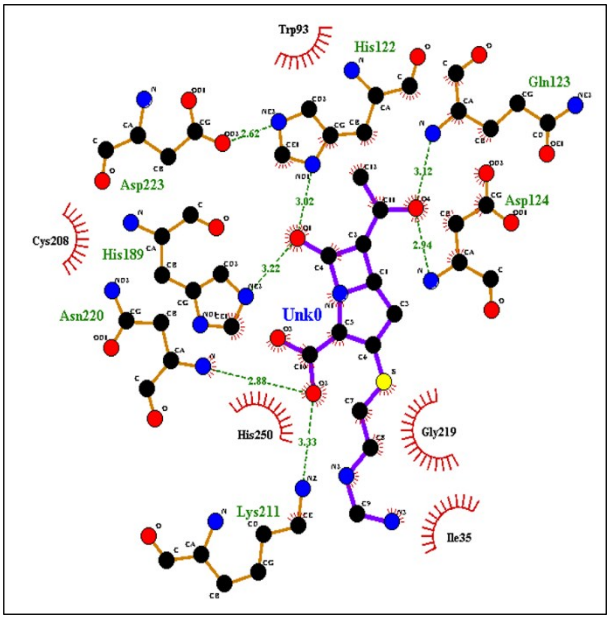
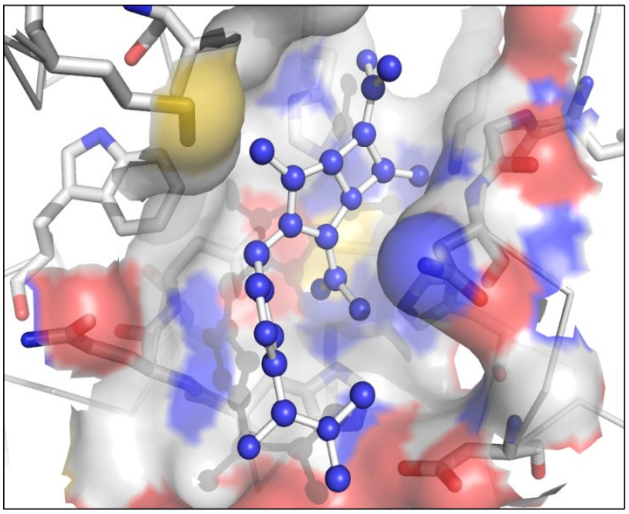


Wildtype -Meropenem complex

F



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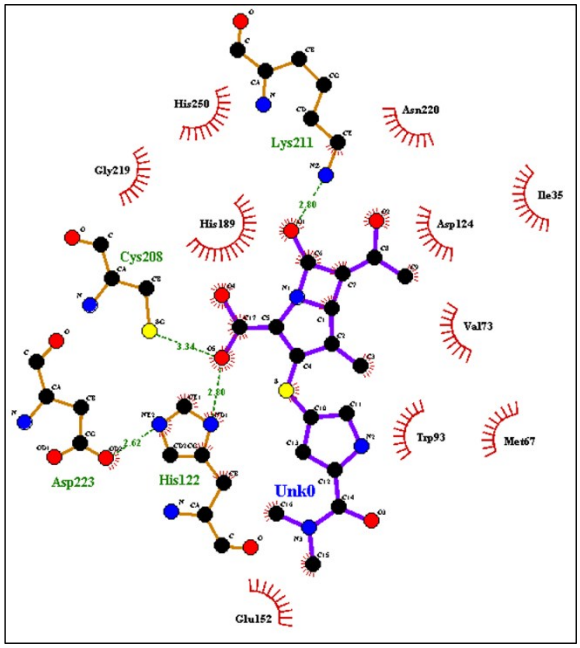
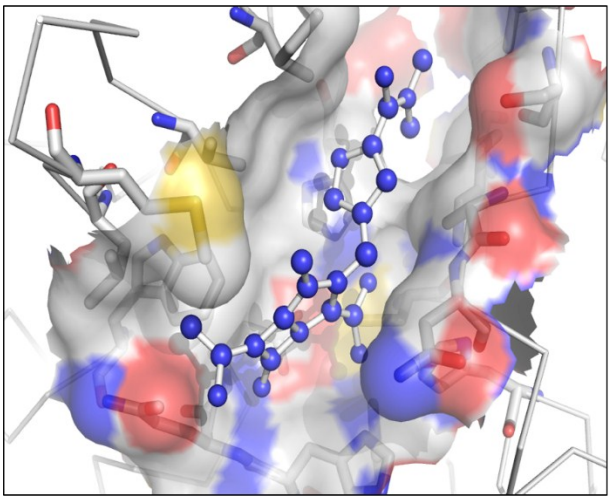


G219A-Meropenem complex

G



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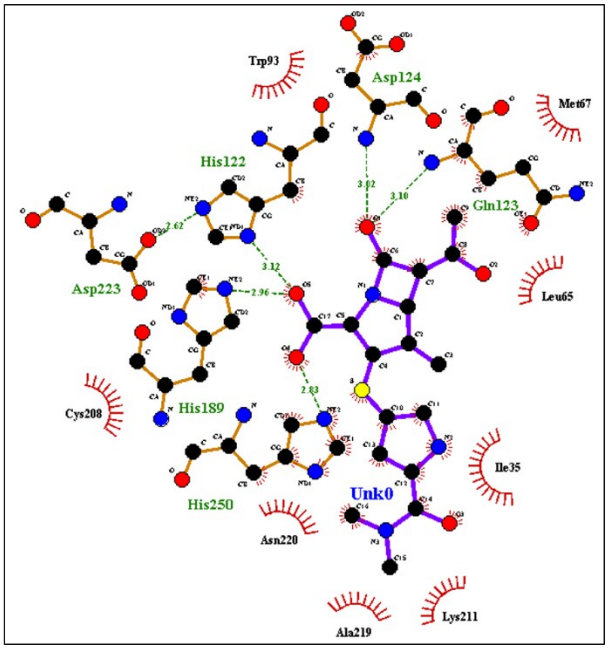
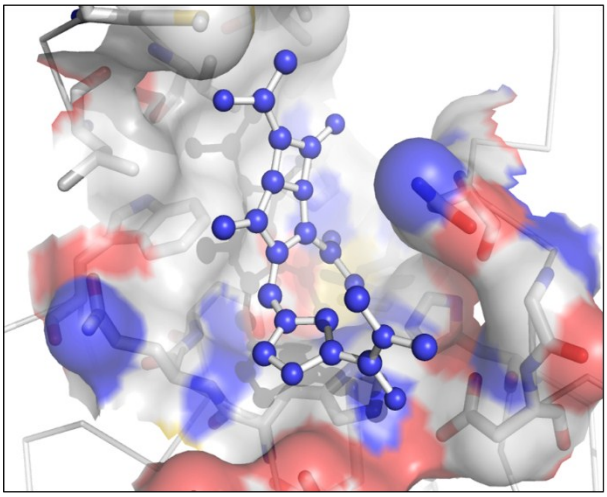


N193A-Meropenem complex

H



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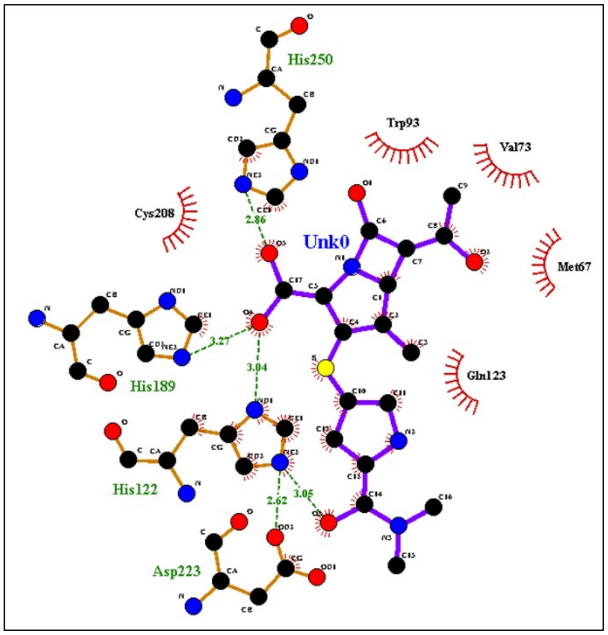
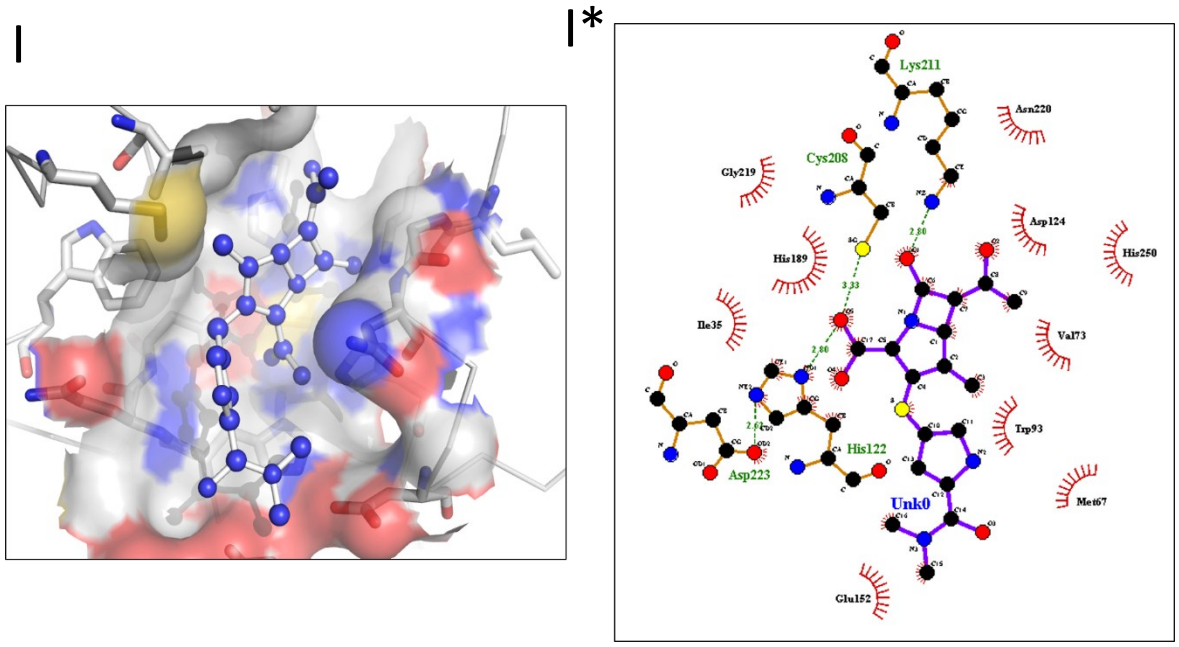


S217A-Meropenem complex



T262A-Meropenem complex

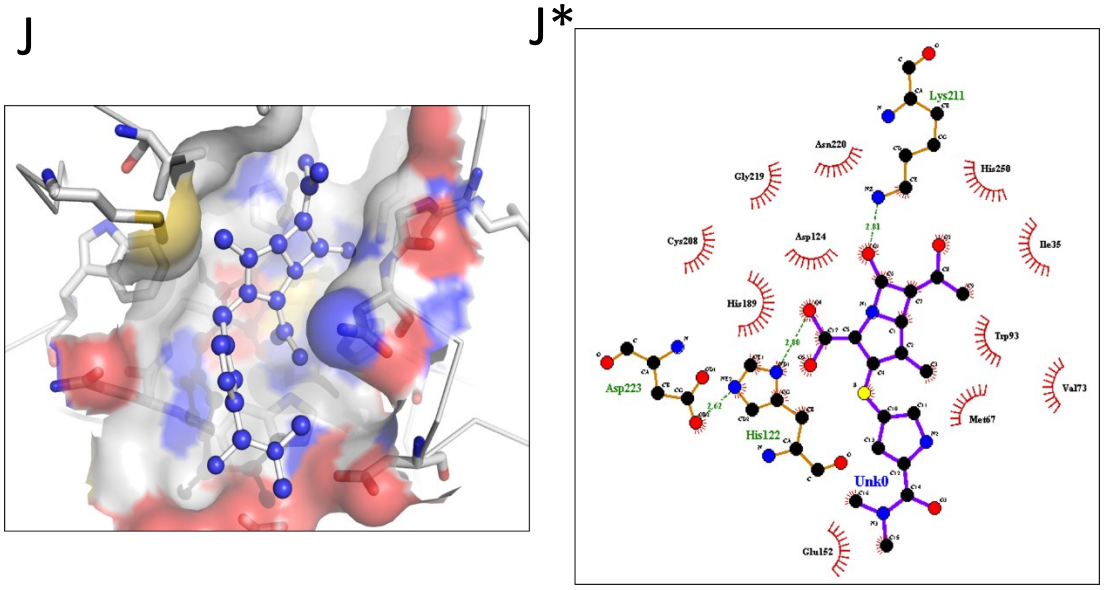


Fig. S3. 3D and 2D interaction maps of protein-ligand complexes. 3D and 2D plots were depicted in PyMOL and LigPlot+, respectively.

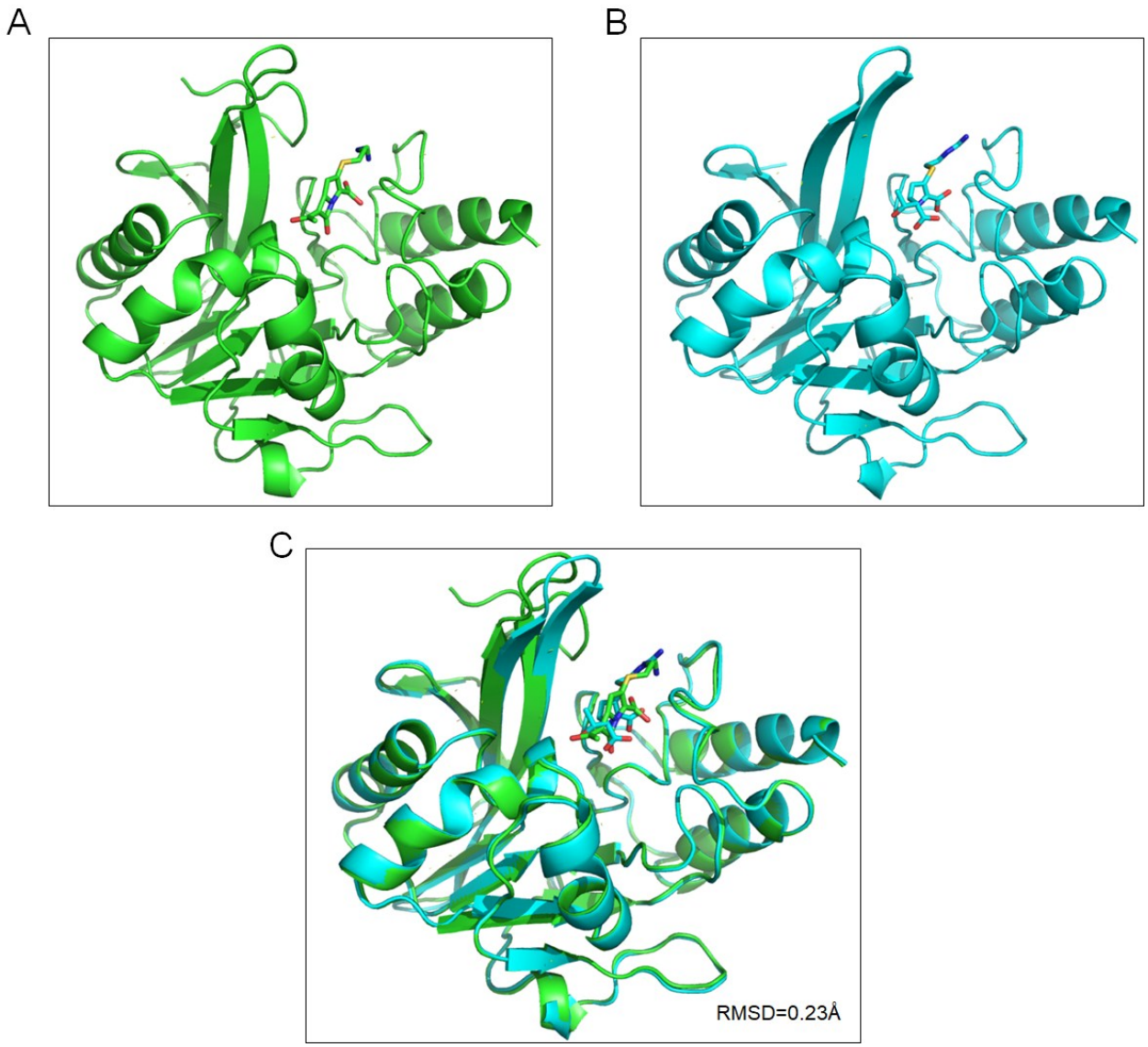


Fig. S4 Super-imposing the current docking complex and x-ray protein-drug complex. (A) 3D structure of protein-ligand complex. (B) 3D structure of protein-ligand complex obtained from X-ray study. (C) Super-imposing of docking and x-ray protein-ligand complex. Protein-ligand docking and X-ray complex structure were shown in green and cyan cartoon mode, respectively.

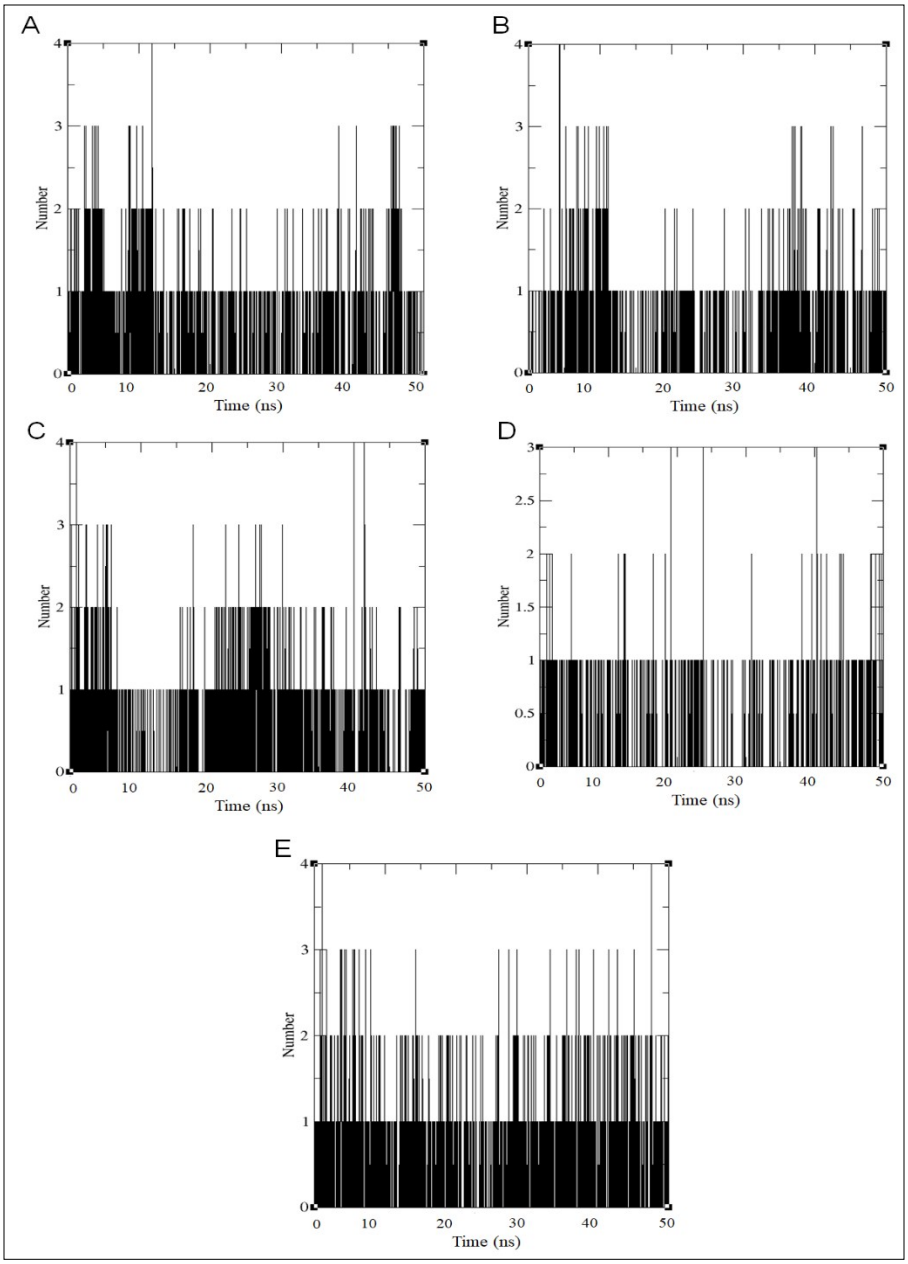


Fig. S5. Hydrogen bond analysis of protein-imipenem complex during MD simulation. (A) H-bond between wildtype and imipenem complex, (B) H-bond between G219A and imipenem complex, (C) H-bond between N193A and imipenem complex, (D) H-bond between S217A and imipenem complex and (E) H-bond between T262A and imipenem complex.

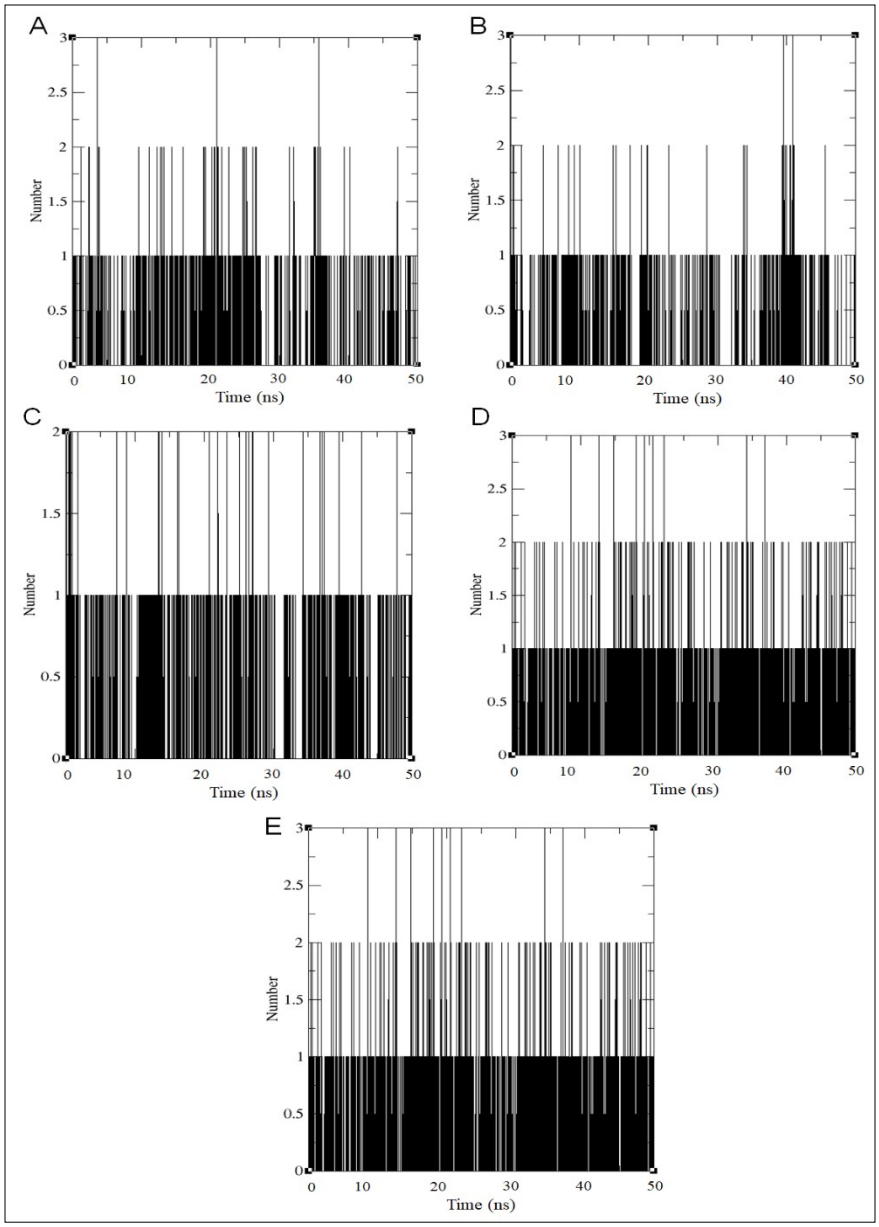


Fig. S6. **Hydrogen bond analysis of protein-meropenem complex during MD simulation.** (A) H-bond between wildtype and meropenem complex, (B) H-bond between G219A and meropenem complex, (C) H-bond between N193A and meropenem complex, (D) H-bond between S217A and meropenem complex and (E) H-bond between T262A and meropenem complex.

Table S1. Primer Sequence

S.NO.	Primer	SEQUENCE (5'-3')
1	**NDM-1 F**	**GAGCGGGCATATGCCGGGTGAAATCC**
2	**NDM-1 R**	**AGTAGTAAGCTTATCAGCGCAGCTTGTCGGCC**
3	**T262AF**	**CAATCACTCATGCGGCCCGCATG**
4	**T262AR**	**CATGCGGGCCGCATGAGTGATTG**
5	**N193AF**	**CACCAGTGACGCTATCACCGTTG**
6	**N193AR**	**CAACGGTGATAGCGTCACTGGTG**
7	**S217AF**	**GCAAGGCCAAGGCGCTCGGCAATC**
8	**S217AR**	**GATTGCCGAGCGCCTTGGCCTTGC**
9	**G219AF**	**CAAGTCGCTCGCCAATCTCGGTG**
10	**G219AR**	**CACCGAGATTGGCGAGCGACTTG**

Table S2. DOPE score of top models.

Models	DOPE score
G219A	-28500.313
N193A	-28339.381
S217A	-28385.564
T260A	-28279.775

Table S3. Quality assessment of generated model along with Native

	Ramachandran plot statistics					
	Favoured region	**Allowed region**	**Disallowed region**	**Verify 3D**	**Overall quality**	**ProSA (z-score)**
Wildtype	92.6	6.9	0.5	100	93.4	-8.37
G219A	94.1	4.9	1	100	93.5	-8.35
N193A	94.1	5.4	0.5	100	93.9	-8.6
S217A	94.6	4.9	0.5	99.59	90.5	-8.09
T262A	94.1	5.4	0.5	99.59	94.4	-8.4