Supporting Information

Antimicrobial Peptide Designing and Optimization employing Large-Scale Flexibility Analysis of Protein-Peptide Fragments

Neeraj Kumar[1,2†], Damini Sood[2†], Ravi Tomar[2], Ramesh Chandra*[2]

[†]Equal Contributing Authors (Co-First Author)

Author affiliations:

1- SRM University, Delhi-NCR Campus, Modi Nagar, Uttar Pradesh, India
2- Department of Chemistry, University of Delhi, Delhi-110007, India.

***Correspondance:**
Prof. Ramesh Chandra
Drug Discovery & Development Laboratory,
Department of Chemistry,
University of Delhi-110007, India
Email: rameshchandragroup@gmail.com

Three dimensional structure retrieval of mutant penicillin-binding protein 5 and assessment

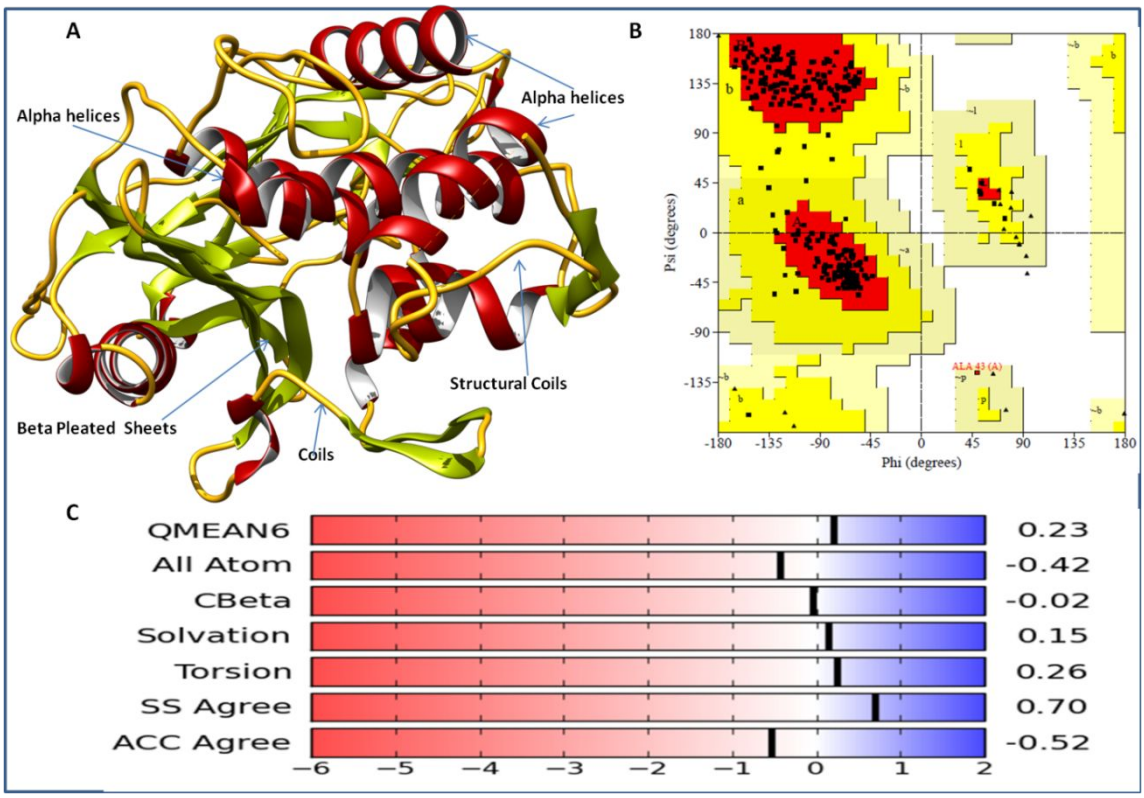

Figure S1: Diagram is depicting **(A)** Crystal structure of the mutant PBP5 protein in newcartoon view. Showing the alpha helices in dark red color with its interior in gray color, beta pleated sheets in green color and random coils in dark yellow color. **(B)** Ramachandran plot of mutant PBP5 protein. (**C**) Various physiochemical properties of mutant PBP5 crystal structure (All atoms, solvation energy, torsion, solvent accessibility) lying in the acceptable region (light blue to blue).

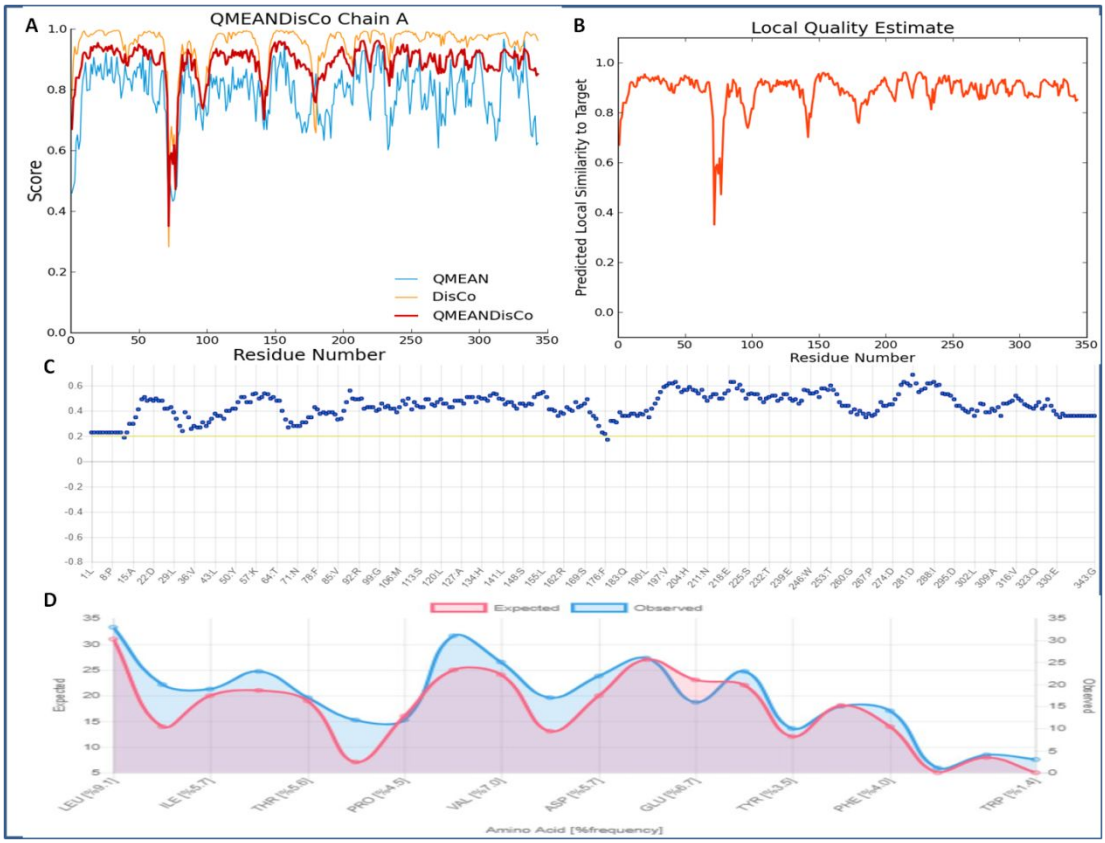

Figure S2: (A) Diagram depicting the accuracy of assessment of crystal structure of mutant PBP5 by QMEANDisco. QMEAN values (blue) indicating the higher model score of PBP5 than experimental structures on average by QMEANDisco **(B)** The local quality plot showing the high quality with a score more than 0.6 value, range expected for high-quality structures. (**C**) Verify3D web interfaces also confirmed the good quality of the structure. (**D**) The structural residues lie in the similar range of experimentally defined large database of structures.

Bacterial resistance study of AMP20 with Mutant PBP5 protein

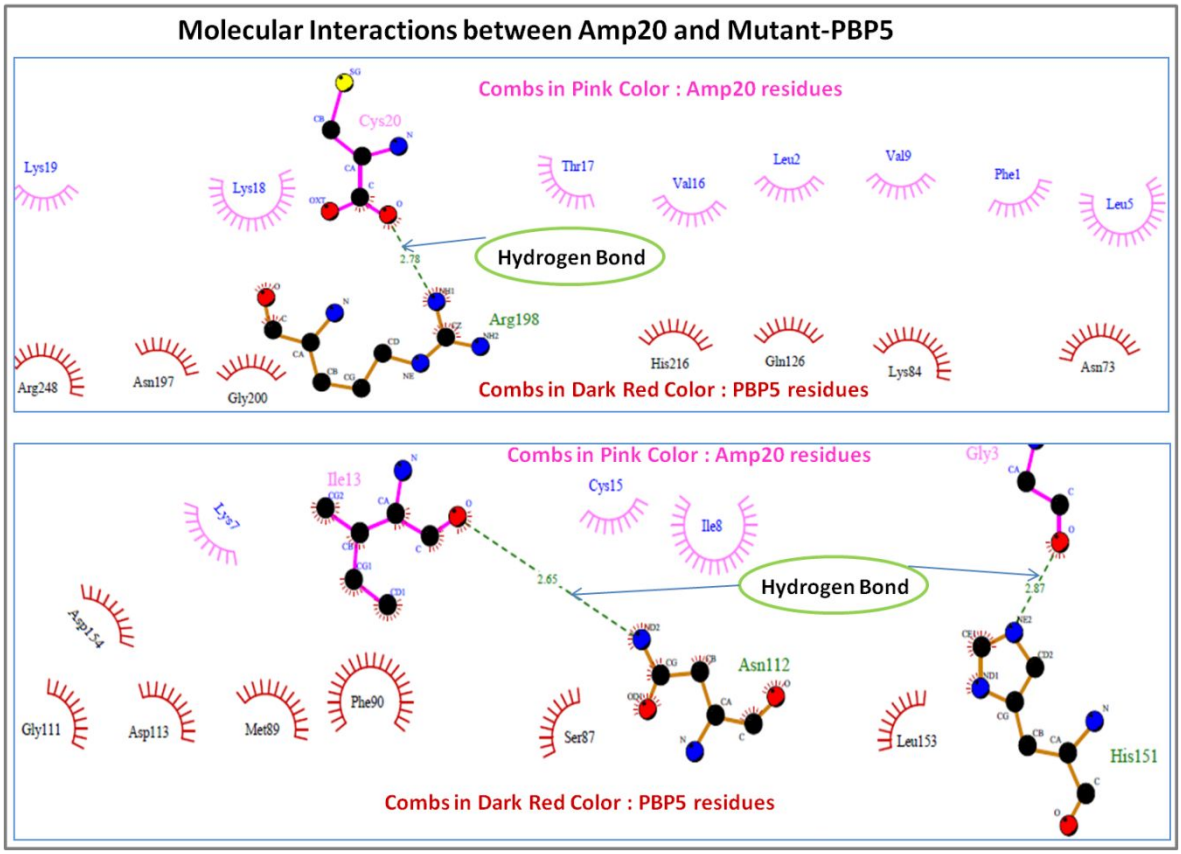

Figure S3: Depiction of molecular interactions of AMP20 with mutant PBP5 protein. Involved hydrophobic interactions are shown in the combs in pink color (AMP20) and dark red combs (mutant PBP5) and two hydrogen bonds are shown in the green color of bond length 2.78 and 2.65 Å.

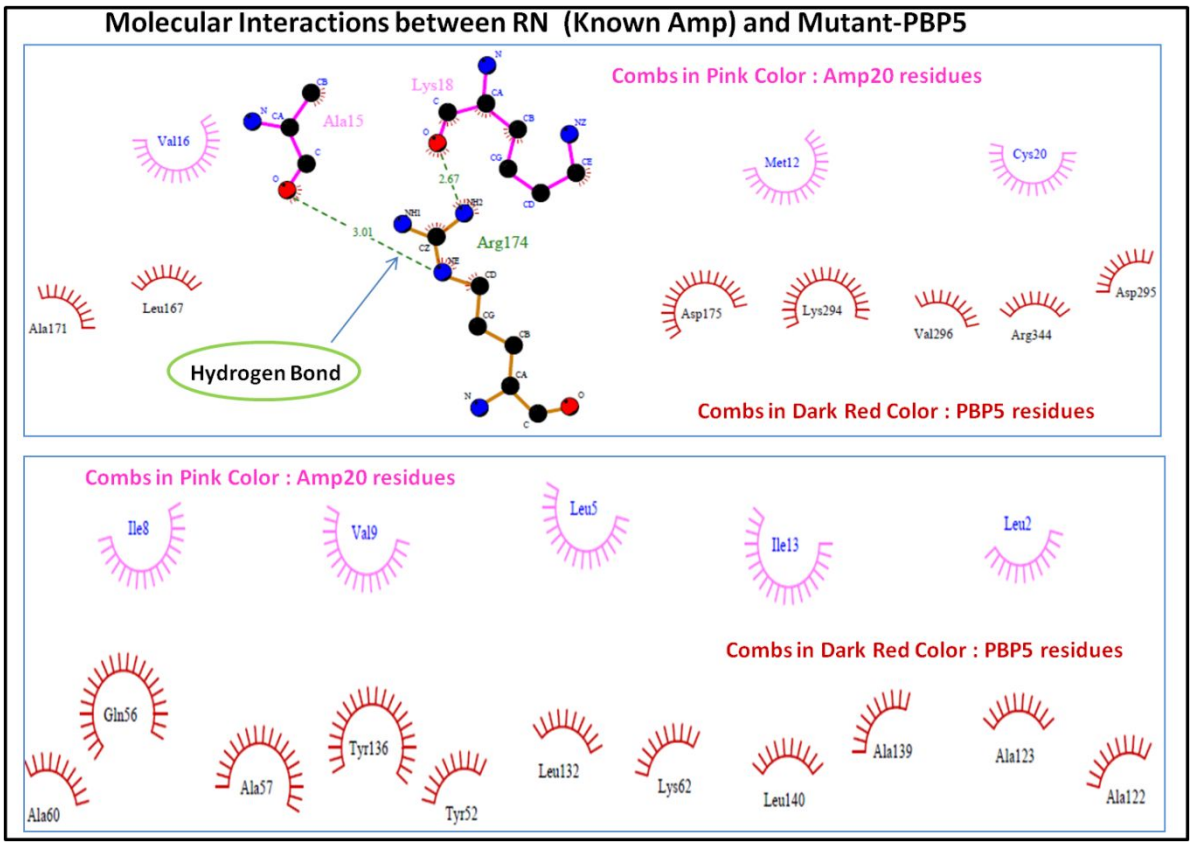

Figure S4: Depiction of molecular interactions of ranalexin (reported antibacterial peptide) with mutant PBP5 protein. Involved hydrophobic interactions are shown in the combs in pink color (AMP20) and dark red combs (PBP5 structural) and hydrogen bond is shown in the green in color of bond length 3.01 Å.

Dynamic stability of AMP with mutant PBP5 protein

We have also analyzed the binding of AMP20 with mtPBP5. We found the AMP20-mtPBP5 system has the minimal deviation within a range of ~0.18 Å to ~ 0.071 Å during the molecular dynamics simulation run. We have also observed the less variation in atomic fluctuation profile of the complex, in the range of ~0.8 Å to ~12.1 Å. There were no or very fewer deviations at position 105 of mtPBP5 protein, Compactness of the complex was also studied and showed that Rg had small deviation of Rg in range of 23.58Å to 23.57 Å, with average Rg value of the complex 23.5619Å, lesser than the mutant unbound protein 23.591 Å and showing the higher compactness of Amp20 bound mutant PBP5.

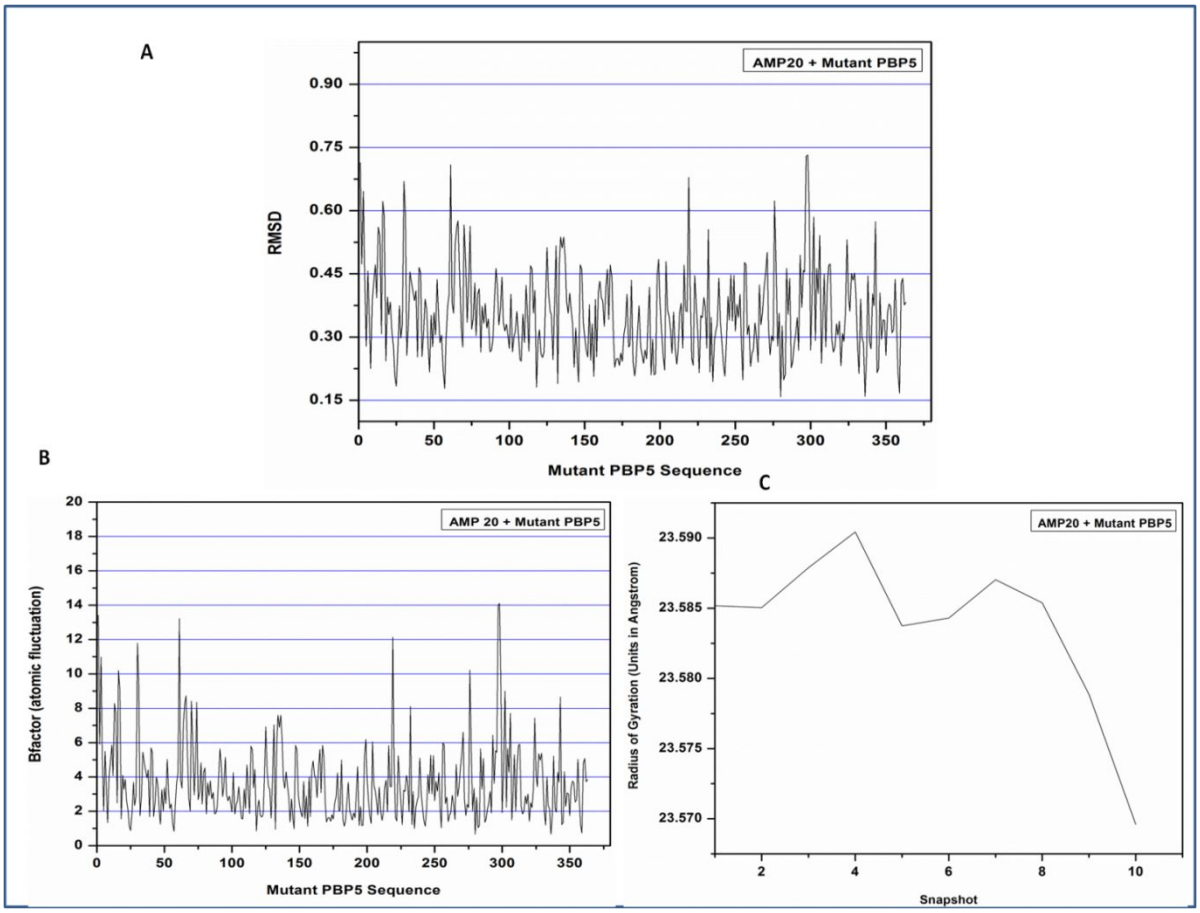

Figure S5: Molecular dynamics simulation plot of the AMP20-mtPBP5 complex; **(A)** The plot depicts the RMSD structural deviation per residue of the complex and showing the minimal deviation in the complex system. **(B)** Atomic fluctuation per residue of the target protein of mutant PBP5; overall indicating the with minimal deviation. **(C)** The radius of gyration plot investigation showing the compactness of protein complex till 10 snapshots. Compactness of protein receptor showing the high flexibility during the interaction with AMP20.

Peptide tertiary structure refinement

Table S1: Peptide tertiary structure refinement parameter calculations using the GalaxyRefine server.

Model	GDT-HA	RMSD	MolProbity	Clash Score	Poor rotamers	Rama favoured
Initial	1.0000	0.000	1.742	6.6	0.0	94.4
MODEL 1	0.9625	0.663	1.346	6.3	0.0	100.0
MODEL 2	0.9750	0.670	1.930	6.3	5.9	100.0
MODEL 3	0.9125	0.692	1.930	6.3	5.9	100.0
MODEL 4	0.9625	0.687	1.246	6.3	0.0	100.0
MODEL 5	0.9500	0.788	2.083	9.4	5.9	100.0