Structure determination and identification of inhibitors against FmtA of *Staphylococcus aureus*

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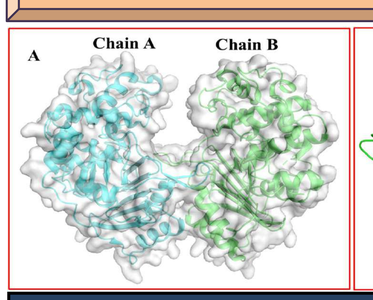
ABSTRACT

Staphylococcus aureus is one of the main causes of infections in hospitals and populations, which causes mild skin infections to various life-threatening diseases like meningitis, pneumonia, and toxic shock syndrome. Teichoic acids are known to be involved in necessary for virulence, cell division, antibiotic resistance, and pathogenesis. In FmtA, two of the conserved motifs: SXXK and Y(S)XN are necessary for binding and catalysis of teichoic acid. We determined the crystal structure of FmtA and structural comparison of FmtA with penicillin recognizing proteins (PRPs) such as D, D-endopeptidase, D, L endopeptidase, D-amino acid amidase (DAA), D-amino peptidase (DAP) and D esterase revealed that FmtA consists of an all α-helical domain and α/β domain sandwiched together. In FmtA, the absence of a long loop I, interactions between Loop I and Ω-Loop, the folding of Loop II over the active site, and the tilting of β12 and β13 strands results in the formation of solvent-exposed and enlarged active site. Our study showed that Ser127 acts as a nucleophile, Lys130 performs the acylation/deacylation, and Tyr211 plays a vital role in the binding of the substrate in FmtA-WTA complex. Our analysis discloses that the esterase activity of FmtA reflects an extension of the catalytic range of the PRPs core structure. Further, we have screened active compounds and the binding affinity was confirmed using molecular docking. Molecular dynamics simulation results illustrated that binding of identified compounds with FmtA results in the formation a higher stable FmtA-inhibitor(s) complexes as compared to FmtA-TA complex. Further, in-vitro binding assays will be performed to check the inhibition of novel esterase for the inhibition of *S. aureus*.

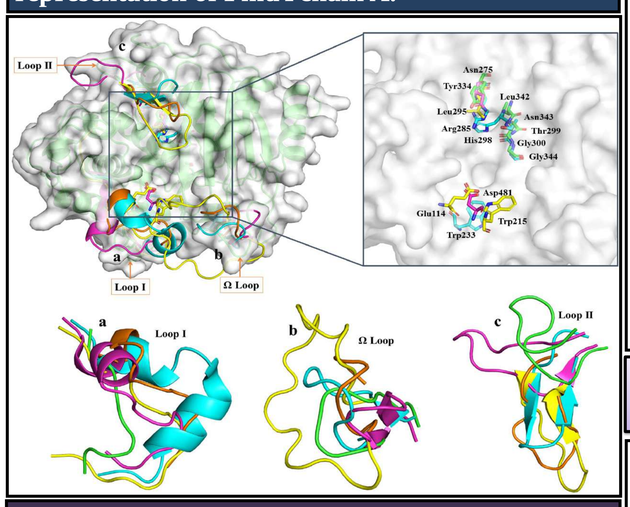
MATERIALS AND METHODS

- ❖ FmtA protein was purified and crystallized.
- ❖ X-Ray diffraction data was collected and processed using HKL2000 and solved by molecular replacement.
- ❖ BALBES, an automated MR pipeline, provided reasonable phases.
- ❖ Buccaneer and AutoBuild generated models were used for chain extension.
- ❖ Model quality was assessed by Molprobity.
- ❖ Virtual screening of FmtA.
- ❖ Molecular Docking to check the binding affinity of identified molecules.
- ❖ MD simulation to confirm the stability of FmtA-inhibitor(s) complexes.

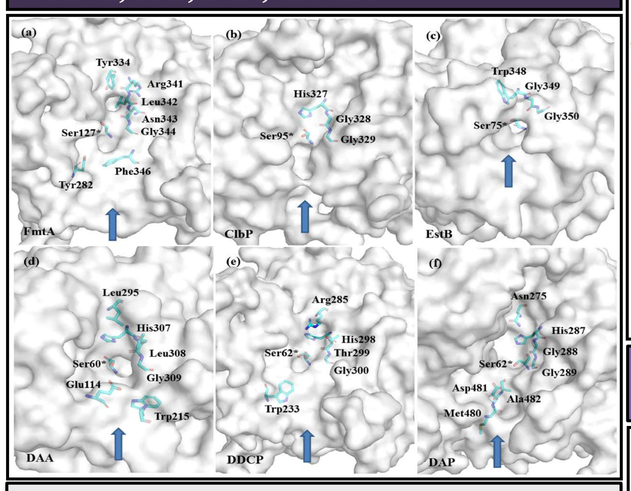
RESULTS



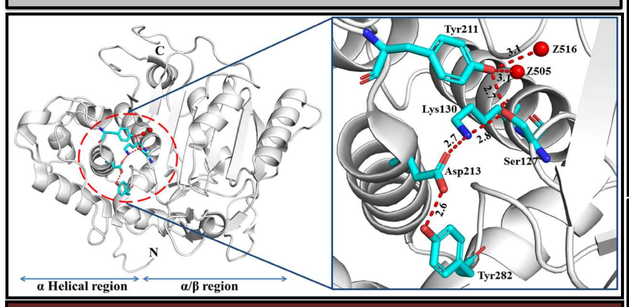
FmtA structure: A) Surface view and B) Cartoon representation of FmtA chain A.



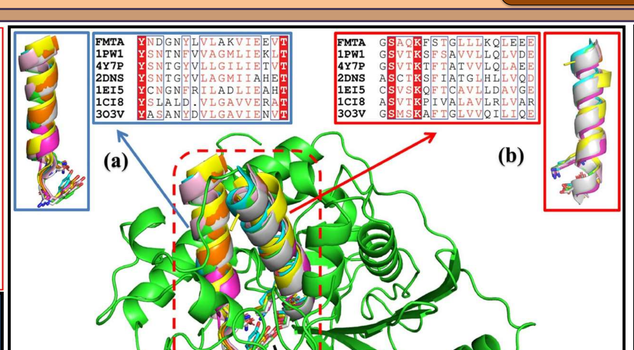
FmtA with Loop I, Loop I, Ω Loop, and Loop II of DDCP, DAP, DAA, and ADP.



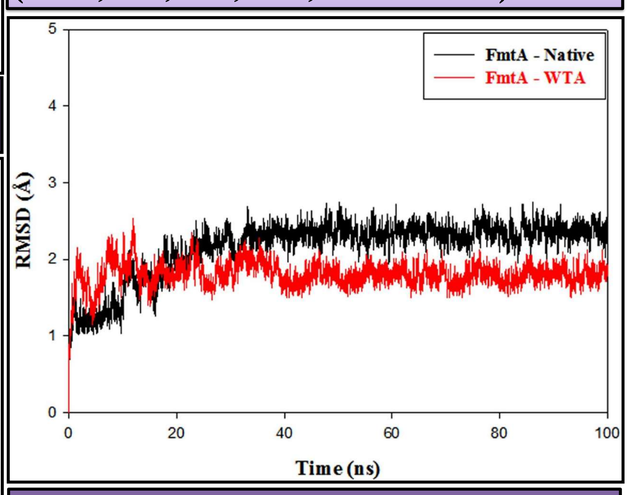
Snapshots of active-site surfaces of FmtA and PRPs.



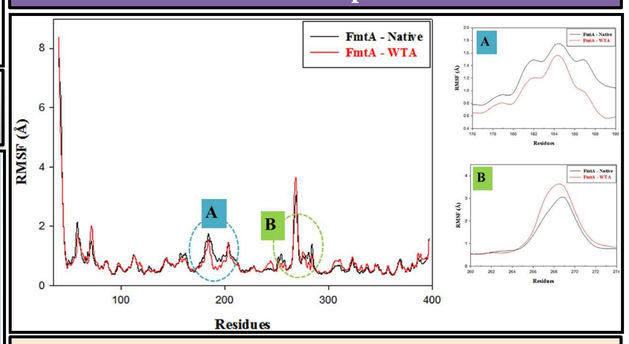
Active site of FmtA.



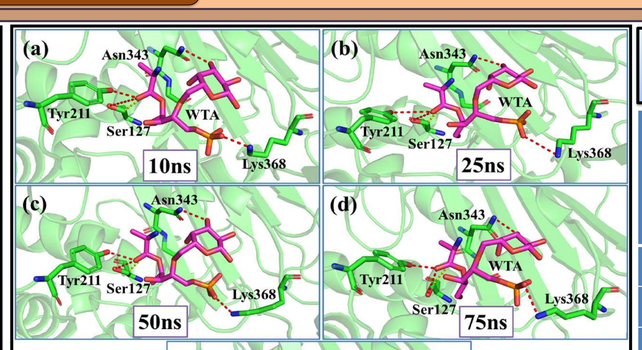
Structural comparison of FmtA with other PRPs (DDCP, ADP, DAP, DAA, EstB and ClbP).



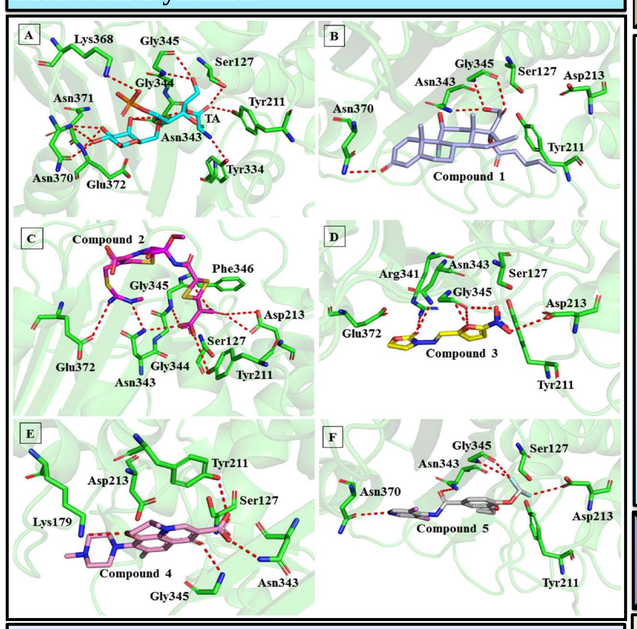
Root Mean Square Deviation (RMSD) of FmtA-Native and FmtA-WTA complex.



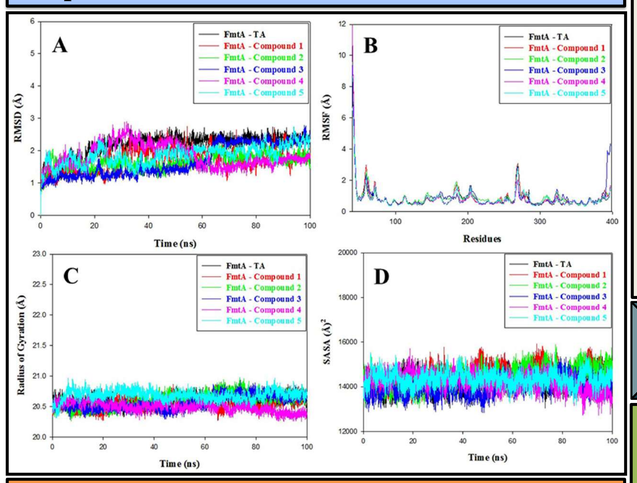
Root Mean Square Fluctuation (RMSF) of FmtA-Native & FmtA-WTA complex. Blue and green dotted circles show the rmsf of Loop I and Ω Loop, respectively



Conformations of WTA at different stages of Molecular Dynamics.



Interactions of teichoic acid (TA) and identified compounds with FmtA.



MD results of FmtA-TA and FmtA-inhibitor(s) complexes.

Binding affinity of teichoic acid and identified compounds with FmtA.

S.No.	Compound	Binding affinity	
		AutoDock Vina	AutoDock Tools
1	Teichoic Acid	-6.4	-5.2
2	Compound 1	-7.5	-7.4
3	Compound 2	-8.5	-9.5
4	Compound 3	-7.2	-7.1
5	Compound 4	-7.9	-7.2
6	Compound 5	-7.2	-7.6

SIGNIFICANCES

- ➢ Crystal structure of FmtA, a novel esterase of *S. aureus* is solved.
- ➢ Loop I, Ω-Loop and Loop II impact the active site of FmtA and makes it shallow and surface exposed.
- ➢ Active site serine act as nucleophile while lysine act as acylation and deacylation in D-alanylation of WTA.
- ➢ Tyrosine hold the active site serine and WTA in FmtA-WTA complex.
- ➢ Novel potent molecules against FmtA were identified.

IMPORTANT REFERENCES

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