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Insights from the docking and molecular dynamics simulation of the Phosphopantetheinyl transferase (PptT) structural model from *Mycobacterium tuberculosis*

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Abstract:
A great challenge is posed to the treatment of tuberculosis due to the evolution of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* in recent times. The complex cell envelope of the bacterium contains unusual structures of lipids which protects the bacterium from host enzymes and escape immune response. To overcome the drug resistance, targeting "drug targets" which have a critical role in growth and virulence factor is a novel approach for better tuberculosis treatment. The enzyme Phosphopantetheinyl transferase (PptT) is an attractive drug target as it is primarily involved in post translational modification of various types-I polyketide synthases and assembly of mycobactin, which is required for lipid virulence factors. Our *in silico* studies reported that the structural model of *M.tuberculosis* PptT characterizes the structure-function activity. The refinement of the model was carried out with molecular dynamics simulations and was analyzed with root mean square deviation (RMSD), and radius of gyration (Rg). This confirmed the structural behavior of PptT in dynamic system. Molecular docking with substrate coenzyme A (CoA) identified the binding pocket and key residues His93, Asp114 and Arg169 involved in PptT-CoA binding. In conclusion, our results show that the *M.tuberculosis* PptT model and critical CoA binding pocket initiate the inhibitor design of PptT towards tuberculosis treatment.

Key words: PptT, *Mycobacterium tuberculosis*, I-TASSER, Molecular dynamics simulations.

Background:
The epidemic disease tuberculosis (TB) affects world population drastically and is responsible for 1.5 million deaths and 8 million new cases per year **[1]**. The bacterium, *Mycobacterium tuberculosis,* causing tuberculosis in *Homo sapiens* can persist in the host and escape from an intact immune response. Available drugs for tuberculosis often cause side effects in immunodeficient patients in long-term use. The bacterium survives these drugs and gives rise to multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacterial mutants causing serious health issues and hurdle in tuberculosis treatment **[2].**

The cell envelope of *Mycobacterium tuberculosis* contains unusual lipids with low cell permeability and is a major reason for bacterial intrinsic drug resistance **[3].** The pathogenicity of bacteria is due to mycolic acids, long chain fatty acids and lipids containing methyl branched fatty acids. The lipids synthesis involves multifunctional enzymes namely polyketide synthases (PKS) and two fatty acid synthase (FAS) systems. The

attachment of 49-phosphopantetheine group from Coenzyme A (CoA) is catalyzed by phosphopantetheinyl transferase (PPTase) enzymes which convert from its inactive apo form to functional holo form **[4]**. Two PPTases in mycobacteria namely AcpS and PptT are highly conserved and activates specific protein substrate in bacterium **[5]**. The enzyme PptT is found to be a novel drug target and is mainly involved in the assembly of mycobactin which is required for virulence, by activating two non-ribosomal synthethases (NRPS) namely MbtB and MbtE. PptT plays a critical role in development of *Mycobacterium tuberculosis* by providing synthesis of components that is needed for the growth and also others factors involved in virulence **[6].**

Methodology:

Protein structural modeling

The target protein sequence of a drug target *Mycobacterium tuberculosis* PptT of length 227 amino acids was downloaded from UniprotKB database (Accession no: O33336). PptT belongs to the transferase family and its molecular function was holo-[acyl-carrier-protein] synthase activity and magnesium ion binding. In the absence of experimental three dimensional structure of *M.tuberculosis* PptT and due to the low sequence homology of PptT in P-BLAST against PDB database, structural modeling of drug target PptT was performed in I-TASSER server based on *ab-initio*/threading method **[7].** I-TASSER (Iterative Threading ASSembly Refinement) is an automated protein prediction server and also the most accurate prediction server. The server predicted five models of target protein and the best model was selected based on C-score. The best model was validated with estimated TM score and RMSD.

Validation of PptT model

M.tuberculosis PptT model was validated by PROCHECK and ProSA server. Stereochemical property of PptT model was analyzed by Ramachandran plot in PROCHECK using SAVES server **[8]**. ProSA server determines the quality index of native structure compared with experimental structures by Z-score **[9]**. Z-score for the given query model is calculated by referring the Z-scores of all experimentally determined protein chains in current PDB. The result was displayed in a plot with two colors representing the groups of structures from X-ray and NMR.

Molecular dynamics simulation

The refinement of PptT model was carried out by molecular dynamics simulation using GROMACS 4.5.4 **[10]**. Topology of the model was performed using GROMOS96 43a1 force field. Solvation of system was in cubic box size of 1.0nm and 2.0 nm between any two periodic images of a protein to provide aqueous environment. Eight chlorine ions were added. Energy minimization was done with short range interactions and constrains using LINCS algorithm and SETTLE algorithm. Two equilibration phases was performed with ensemble NVT with 300 K and coupling constant of 0.1 ps and ensemble NPT with 1 bar and coupling constant of 5 ps. Final MD was run for duration of 10 ns. The plotting of trajectory files was done in Xmgrace tool.

Substrate docking

The substrate binding pocket was identified with molecular docking carried out with *M. tuberculosis* PptT model and CoA in PatchDock server. Algorithm of PatchDock molecular docking is based on shape complementarity principles. The docking results were evaluated with parameters like score, area and atomic contact energy. The ligand was applied with transformation which includes rotational angles and transitions. 3D structure of CoA was downloaded from PDB of *Bacillus subtilis* PptT (PDB code: 1QR0). The docking result of top PptT-CoA complex was visualized in PyMOL to view the hydrogen bond interactions.

Results & Discussion:

The structural modeling of *M.tuberculosis* PptT in I-TASSER server predicted five models based on threading templates. The model with less C-score -2.98 was selected as the best structure. The model accuracy was estimated by TM score of 0.68. The three dimensional structure of PptT was visualized in PyMOL as shown in **(Figure 1a).** The predicted model is proper in its folding with functional domain ACPS which confirmed with Pfam and the experimental structure of *Bacillus subtilis* PptT (1QR0). The secondary elements of *M.tuberculosis* PptT composed of six α helices and six β strands and attain a new fold comprising of a pseudo 2-fold symmetry with two halves of roughly identical size. The comparison of *M.tuberculosis* and *B.subtilis* PptT shows that two strands were absent and the strands changed to loop region was observed. The protein contains two domains with a 'N' terminal domain range from Met1 to Gly103 and a 'C' terminal domain range from Arg104 to Leu227. The substrate CoA tends to bind the active pocket in the region of interface between the two halves of PptT. Each halves contains anti-parallel beta sheets connected with loops forming a barrel like structure.

Validation of the model was carried out with PROCHECK with construction of Ramachandran plot. The results showed the predicted model with 82% core region and four residues in disallowed regions seen in loops. Equal number of glycine and proline residues occupied all regions of plot. Stereochemical property of PptT model was properly validated with phi-psi angles. Quality index of the model was obtained from ProSA server and Z-score of -5 confirmed the mode near to the NMR experimental structures.

The refinement of the model was done with 10 ns molecular dynamics simulations in GROMACS and trajectories were analyzed with RMSD (g_rms), Rg (g_gyrate) and total energy (g_energy). RMSD plot was constructed in order to define the final structure conformation in dynamic system. RMSD with 0.4 nm showed that the structure was less deviated from the starting structure and was well equilibrated during 10 ns as shown in **(Figure 1b).** The radius of gyration (Rg) explained the overall dimension of protein in system and Rg plot showed the slight change in dimension with maximum value of 1.85 nm which occurred during 4 ns to 6 ns. After 6 ns, the protein equilibrated well and attained proper dimension at the end of the simulation as shown in **(Figure 1c).** The total energy of the simulations was -6.48E+05 which showed the structural stability of PptT model. MD simulations results showed that the PptT model was refined well and explained the structural behavior.

Substrate docking of *M.tuberculosis* PptT with CoA was performed in PatchDock. The docking results showed top twenty complexes and the first PptT-CoA complex was selected

as best with a high score of 5388, area of 595.20 and atomic contact energy of -37.14. The transformation on the ligand was rotational angles of -0.07 0.66 -0.99 and transitions of 6.38 -31.19 -83.63. The complex was visualized in PyMOL for the hydrogen bond interactions between the CoA atoms and PptT residues atoms shown in **(Figure 1d).** The results showed that the binding pocket occur in the interface of two domains in PptT with bend conformation near to beta 2 and beta 3 strands and involvement of binding residues His 93, Asp114 and Arg 169. The interacted atoms and bond length was measured and listed in **Table 1(see supplementary material).** His 93 residue was critical in the substrate binding with two hydrogen bonds with CoA and located in beta 2 strands. The conserved residue His was also observed in *B.subtilis* PptT-CoA binding which confirmed the importance of the sequence conservation among the phosphopantetheinyl transferase superfamily. Thus, the substrate binding study of *M.tuberculosis* PptT was significantly important in the function and can be a target for inhibitor design.

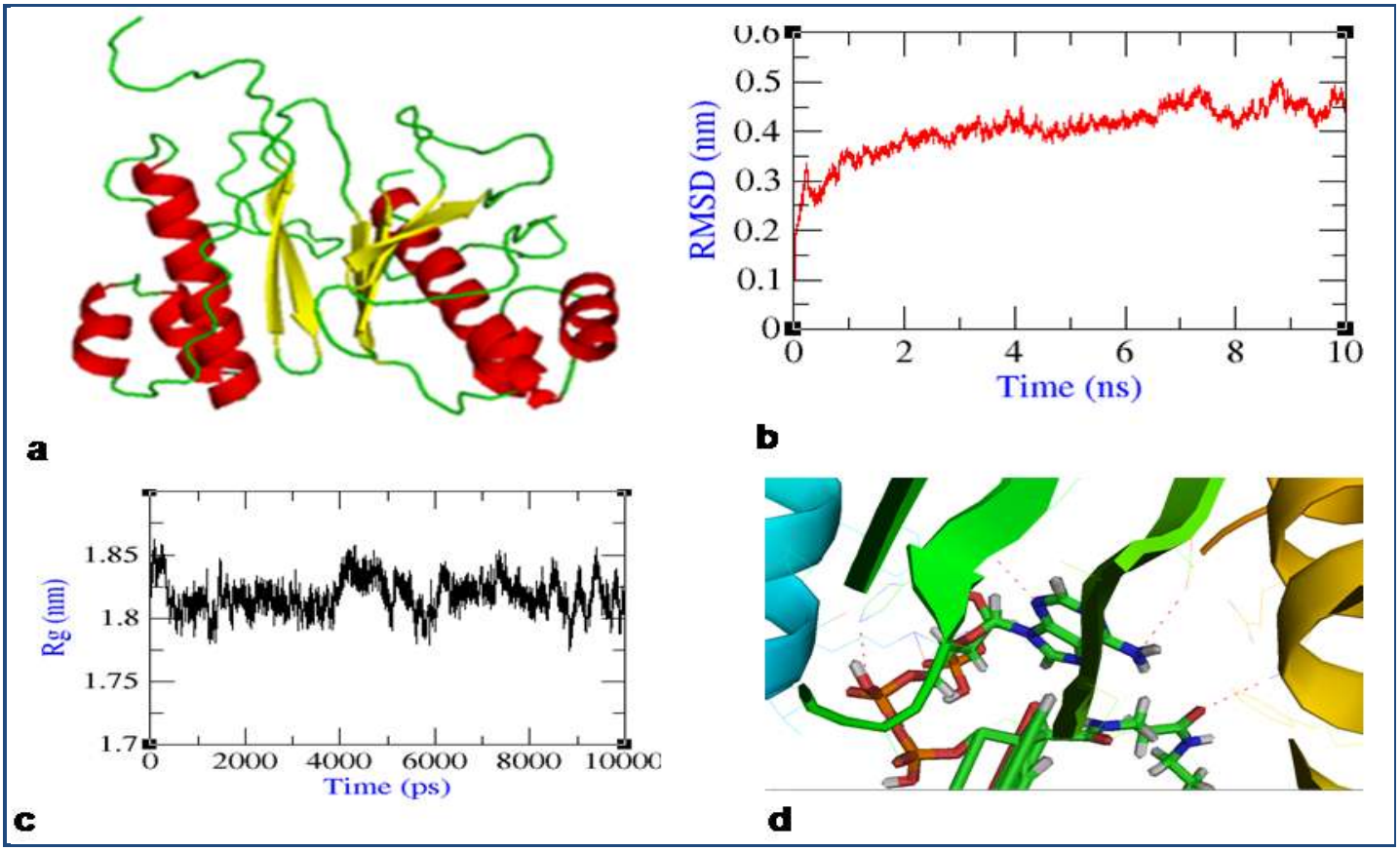

Figure 1: Structural analysis results of *M.tuberculosis* PptT **a)** three dimensional model of PptT using *ab-initio*/threading method from I-TASSER server. Color scheme: Helices in red, strands in yellow and loops in green; **b)** Root means square deviation (RMSD) plot of PptT model from MD simulations of duration 10 ns in GROMACS. **c)** Radius of gyration (Rg) of PptT model from MD trajectory; **d)** PptT-CoA binding interaction from PatchDock based on molecular docking. CoA molecule represent in stick model and protein in cartoon model. Hydrogen bonds represented in pink dashed lines.

Conclusion:

The importance of enzyme PptT in *M.tuberculosis* leads to a suitable drug target for tuberculosis treatment. Computational modeling of PptT determined the validated three dimensional structural models and is observed with native conformation of the phosphopantetheinyl transferase superfamily. Comparison of *M.tuberculosis* PptT structure model with template *B.subtilis* PptT shows that the active structural elements of the enzyme are present to perform the transferase function. MD simulations shows that the PptT model is properly refined and structure is stable with less RMSD value and overall dimension of the protein is maintained during simulations with standard Rg value. The total energy of the model confirmed the stable conformation of the PptT model in the course of 10 ns simulations.

Substrate binding was critical in the function of phosphopantetheinyl transferase superfamily with CoA. The results shows that the binding pocket is the same like *B.subtilis* PptT binding and is involved with bend conformation. *M. tuberculosis* PptT binding site residues His 93, Asp114 and Arg 169 are involved and His 93 is conserved with *B.subtilis* PptT substrate binding. Even though there is less sequence homology with *B.subtilis* PptT, critical residues are conserved in structural level to perform the function. This proves the nature of PptT in structure-function mechanism related to different members with varying sequences. In summary, the structural model of *M. tuberculosis* PptT is more reliable and well studied with transferase function. The substrate binding pocket reported can be useful in inhibitor design against *M. tuberculosis* PptT and may be useful in overcoming multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains.

References:
[1] http://www.who.int/tb/dots/treatment.
[2] Almeida Da Silva PE & Palomino JC, *J Antimicrob Chemoth.* 2011 **66**: 1417 [PMID: 21558086]
[3] Brennan PJ, *Tuberculosis*. 2003 **83**: 91 [PMID: 12758196].
[4] Walsh CT *et al. Curr Opin Chem Biol*. 1997 **1:** 309 [PMID: 9667867]
[5] Chalut C *et al. Proc Natl Acad Sci USA*. 2006 **103**: 8511 [PMID: 16709676]
[6] Leblanc C *et al. PLoS Pathog*. 2012 **8**: 12 [PMID: 23308068]
[7] Roy A *et al. Nature Protoc.* 2010 **5**: 725 [PMID: 20360767]
[8] http://nihserver.mbi.ucla.edu/SAVES
[9] https://prosa.services.came.sbg.ac.at/prosa.php.
[10] Berendsen HJC *et al. Phys Comm.* 1995 **91**: 43

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Supplementary material:

Table 1: Hydrogen bond interactions between *M.tuberculosis* PptT and CoA from PatchDock

Sl.No	Ligand atom	Protein atom	Bond length (Å)
1	H	NE2 (HIS93)	2.3
2	N	CA (HIS93)	3.9
3	N	OD2 (ASP114)	3.2
4	O	NH1 (ARG169)	3.3