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Research Article

STRUCTURAL MODEL OF THE ALPHA PHOSPHOGLUCOMUTASE: A PROMISING TARGET FOR THE TREATMENT OF MYCOBACTERIUM TUBERCULOSIS

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ABSTRACT

Objective: Tb is considered to be growing menaces in various countries especially Africa and South East Asia. In 2011, 8.7 million people fell ill with TB, out of which a total of about 1.4 million people died. The children being affected in large, increases the severity of Tb. It remains to be the leading cause of death of people infected with HIV. The growing multidrug resistant strains of bacteria affecting the population has increase since 2010. The search for potential targets for fighting Tb has identified different pathways for drug development against tuberculosis. One such pathway identified, an enzyme alpha phosphoglucomutase involved in bacterial polysaccharide capsule formation, important for bacterial virulence and infection. The absence of X-ray crystallographic structure of alpha phosphoglucomutase resulted in the modelling of this potential target.

Methods: Homology modelling of was performed by modeller9.1, the multiple sequence alignment was carried out selecting three different relevant templates. Model evaluation was performed using Ramachandran plot and ERRAT plot, further RMSD with the template obtained using Pymol.

Results: Stereochemical evaluation of protein by Ramachandran plot indicated a good quality model with 99.8% residues in the most favoured and allowed regions. The model was compared with the suitable template by superimposing the structures, RMSD was determined to be 0.202Å, the further analysis by ERRAT program gave a score of 95.733, both indicating a good quality model.

Conclusion: The various results obtained, conclude the reliability of modelled protein which can be further used for Denovo design of inhibitors against the target.

Keywords: Alpha Phosphoglucomutase, Mycobacterium tuberculosis, lipopolysaccharide capsule, Bacterial virulence, Multidrug resistance, Comparative modelling.

INTRODUCTION

The risk of Tb affecting the population is increasing in a very dramatic manner. The emergence of multidrug resistance is considered to be a crucial factor behind this shocking fact [1]. As per WHO in 2011, this entirely preventable and treatable disease caused death of around 1.4 million people out of which about 64,000 of them were children, from all around the world [2]. It is the top three causes of death among women of age group 15 to 44. There are estimated 65000 people with MDR-TB in 2010. Mycobacterium tuberculosis is considered to the most deadly as the chances of spreading of the infection is extremely high, mainly effecting population with weak immune system especially HIV infected people [1]. The fact which gives hope being the development and ongoing research activities for the development of various effective therapy and treatment strategies which exceptionally was able to save around 7 million lives all over the world since 1995 [2].

Mycobacterium Tb alpha phosphoglucomutase is an important enzyme for disease causing bacteria. Alpha Phosphoglucomutase (EC 5.4.2.2), enzyme belong to the class of isomerases which performs the intramolecular transfer of phosphate group residue to the substrate, a reversible process that contributes to glycolysis. The reaction involves glucose 1, 6-diphosphate intermediate [3]. The action of glycogen phosphorylase produces Glucose-1-phosphate, which is then further converted to glucose-6-phosphate by PGM [4]. The Glucose-6-phosphate thus formed participates in either the glycolytic pathway or the pentose phosphate pathway. The enzyme also performs the interconversion of 1-phosphate and 6-phosphate isomers of alpha-D-hexoses, and the interconversion of alpha-D-ribose 1-phosphate and 5-phosphate. The PGM-catalyzed reverse reaction which results in the formation of glucose-1-phosphate undergoes conversion to UDP-glucose, an important precursor for the formation of different exo polysaccharide present in the bacterial capsule. Many of these lipopolysaccharides are responsible for the bacterial virulence and provide adequate resistance to the disease causing bacteria [5].

MATERIAL & METHODS

Homology modelling [6] was carried out using modeller 9.10 version. The primary sequence of the alpha Phosphoglucomutase (Accession No: NP_217584) of *Mycobacterium tuberculosis* was obtained from the public domain protein sequence database of NCBI. The characterization of the sequence was carried out using the online Expasy-ProtParam tool [7]. Alpha Phosphoglucomutase have 547 amino acid residues and the estimated molecular weight is 58265.6 with an isoelectric point (pI) of 5.54, it is the pH at which the protein carries no net charge. The instability index is resolved to be 24.78 indicating the protein to be stable. The GRAVY index value of -0.105 provides information about the hydrophilic nature. The reliable data obtained from ConSeq [8] server explores the nature of residues.

Homology modelling of Mycobacterium Tb alpha phosphoglucomutase: Comparative modelling or homology modelling is the method usually followed when the crystalline structure of the target is not known. In homology modelling the target designing is carried out by four stages: template selection, sequence alignment, model generation followed by refinement and model evaluation.

Template selection: The search for similarity was performed using online tool PSI-BLAST [9] against PDB [10] database keeping default parameters like E-value threshold 10, word size 3 and Blosum 62 Matrix. The aim was to identify high-resolution X-ray crystallography structures as template for performing multiple sequence alignment with maximum percentage identity and query coverage. Bacterial phosphoglucomutase (PDB: 3NA5: A chain) with resolution of 1.70 gave identity of 58% and query coverage of 99%, Putative Phosphoglucomutase from Thermus Thermophilus Hb8 (PDB: 2ZOF: A chain) with resolution of 2.52 gave identity 56% and query coverage of 96%, Salmonella typhimurium phosphoglucomutase (PDB: 2FUV: A chain) with resolution of 2.00 gave identity 58% and query coverage of 98% respectively with the template protein.

Target-template alignment*:* A target and multiple templates sequence were aligned using Clustal Omega [11, 12] employing Blosum scoring matrix with a gap penalty of 10 (Fig. 1).This improved version of programme gives better alignment accuracy. The generated alignment was further used for the construction of targeted protein.

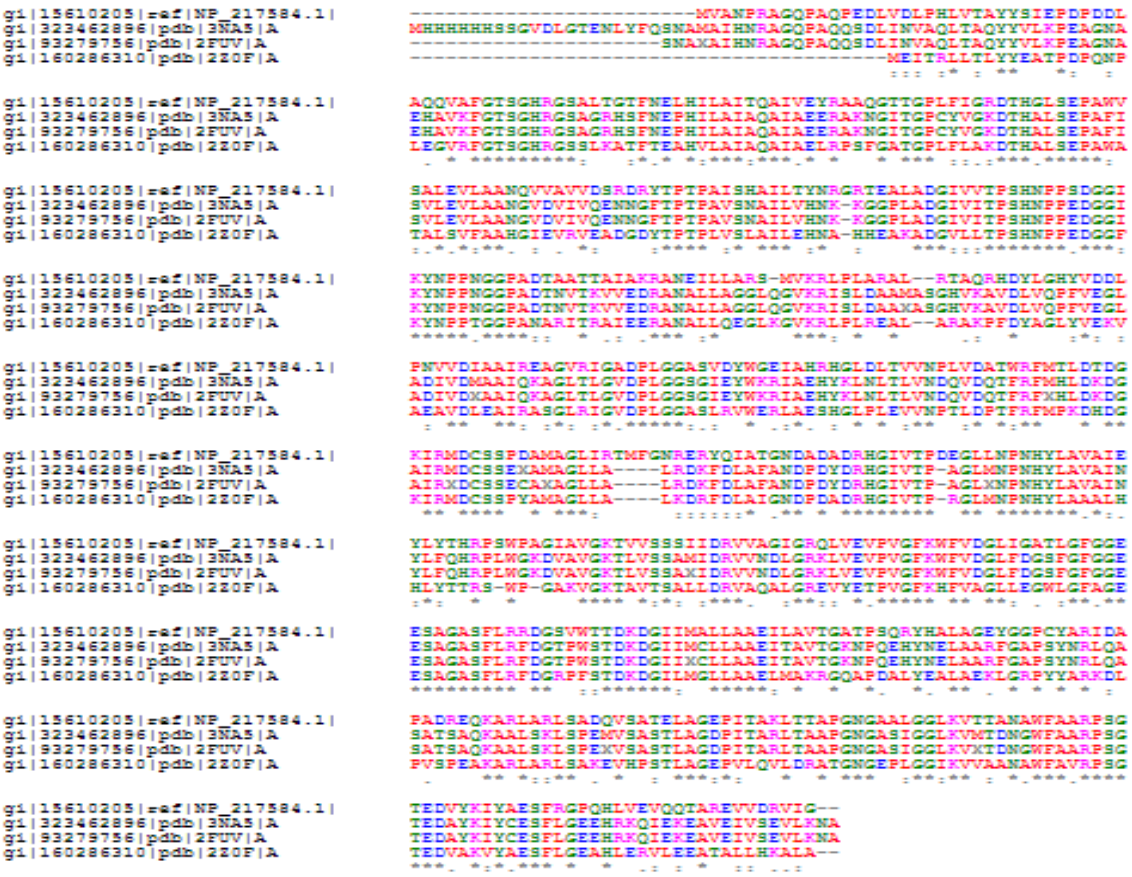


Fig. 1*:* Multiple sequence alignment between *Mtb* phosphoglucomutase and template 3NA5_ A chain, 2ZOF_A, 2FUV_A

Generation of model: The model generation was carried out using MODELLER 9.10 [13, 14], which works on probability density functions (PDFs) generating fine quality three dimensional homology model of the target protein based on multiple sequence alignment of the selected templates.

MODELLER performs comparative protein structure modelling by satisfaction of spatial restraints [13, 14]. It can be used for various tasks, such as de novo modelling of loops in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple sequence alignment of proteins and comparison of protein structures. The comparison is carried out by MODELLER to infer distance and angle constraints from a template structure further use with energy terms for building the protein model. The final model selection was carried out using the least functional value, further the model was subjected to refinement and validation.

Prediction of Secondary structure: The secondary structures prediction of the alpha phosphoglucomutase was carried out using different methods Discrimination of protein secondary structure class (DSC) [15], (DPM) [16] , Self-optimized prediction method with alignment (SOPMA) [17] , Hierarchical neural network (HNN) [18], PHD [19] ,GOR4 [20], , Predator [21], CONCORD [22], SIMPA96 [23], Sec.Cons [24] , PSIPRED [25,26].

Intrinsic disorder prediction: In homology modelling it is very important to identify regions of imperfection, for this various programs were selected and used for the protein analysis. The tools such as DisEMBL [27], Globplot [28], Regional order neural network (RONN) [29] and Protein disorder prediction system (PRDOS) [30] performed the check to report back problematic regions, applying different algorithms. The further study of results gave common regions of defect.

Structure validation: The evaluation of the modelled protein is an essential part of homology modelling. Thus the stereo chemical quality evaluation was carried out using various online programs such as WHATIF [31], PROCHECK [32], WHATCHECK [33], ERRAT [34], ProsaII [35] and VERIFY 3D [36].The further investigation was proceeded for the identification of the active site.

Active site identification*:* The Computed Atlas of Surface Topology of Proteins [37] gives reliable information regarding the surface of proteins, such structural information helps in identifying and characterizing protein active sites, binding sites and functional residues of the pockets. The adequate information of such pockets within the interior of proteins is obtained by measuring the concave surface regions of the three dimensional structures. The major function of the area and volume determination is carried out by solvent accessible surface model (Richards surface) and molecular surface model (Connolly surface).

RESULTS & DISCUSSION

The sequence of alpha phosphoglucomutase was used for the protein sequence blast PSI-BLAST [38]. The selected templates were subjected to multiple sequence alignment along with the target sequence. Model generation was further carried out using Modeller9.10. From the developed models, initial selection was carried out by considering the least objective function value, Ramachandran plot and ERRAT score.

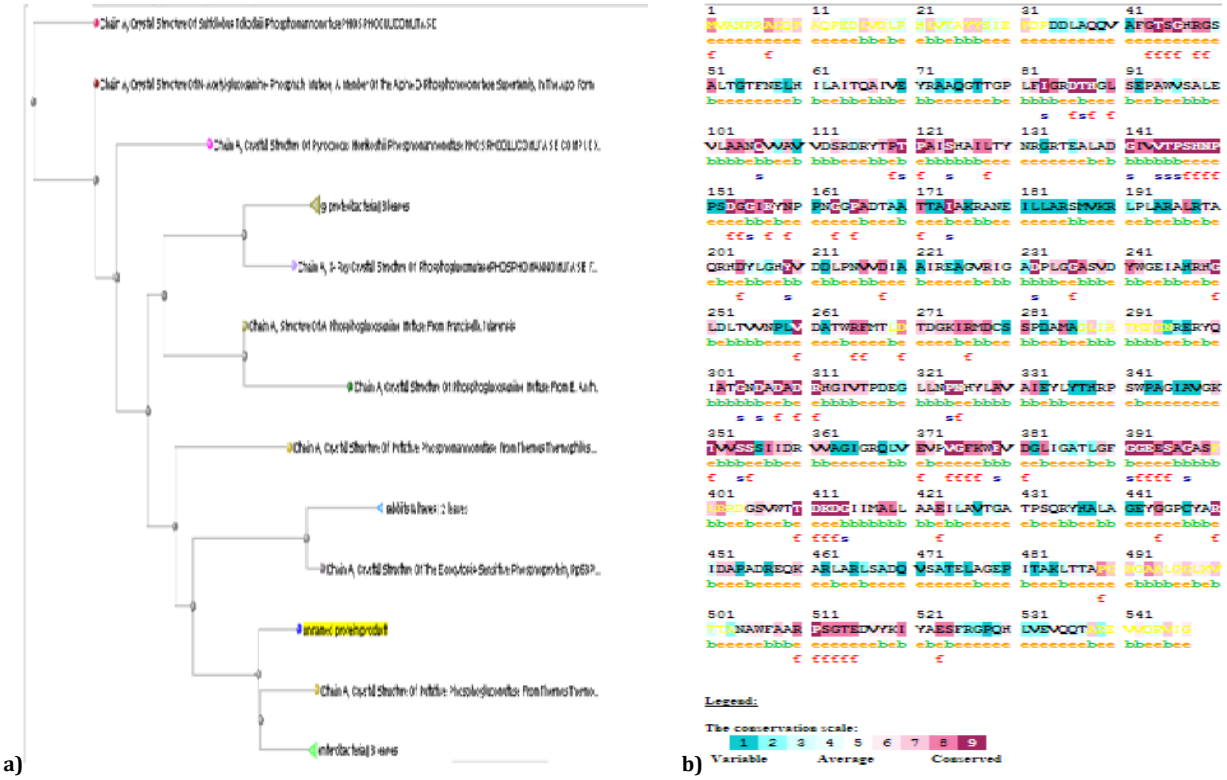


Fig. 2: (a) Phylogenetic tree obtained using clustal omega (b) Conservation scores of amino acids on a scale varying from 0–9.

Homology modelling: Homology modelling is performed by following the basic four steps (1) An Initial multiple sequence alignment. (2) Model generation using MODELLER 9.10. (3) Selection based on relative objective function values (4) Final validations of the generated models. Model with least objective function of -52065.828125 was selected for further analysis.

Secondary structure prediction*:* On analysing the protein using various secondary structure prediction tools, the presence of random coil is obtained to be dominating in the structure, followed by alpha helix and extended strand. The presence of beta turn was shown by SOPMA. The analysis indicated the absence of various other secondary structures such as 3_{10} helix, Pi helix, Beta bridge, Beta turn, Bend region, ambigous states.

Intrinsic disorder identification in protein: On analysing the result using different servers the common regions of disorder was identified to be 2-10, 45- 48, 274-278, 243-260, 295-321,387-396 (Fig. 3).

Structure validation: The steriochemical validation of model was carried out with Ramachandran's plot. Psi and Phi dihedral angles is used for the Stereochemical evaluation of backbone of the protein revealing that 93.5, 5.9, 0.7 and 0.0% of residues were falling within the most favoured regions, additionally allowed regions, generously allowed regions and none in disallowed regions respectively of Ramachandran's plot.

Ramachandran plot analysis showed that several residues ARG 290, ARG-185, SER 147 were placed in energetically less favoured regions of the plot. Remaining residues are in the favourable regions, which state that the selected structure is feasible for further studies. Totally, 99.4% of the residues are in the most favoured and allowed regions. The G-factor of 0.12 computed in PROCHECK, indicates an acceptable protein environment.

Similarly, WHATCHECK revealed the RMS Z-score various parameters such as bond lengths (0.951), bond angles (1.190), omega angle restraints (0.659) tight, side chain planarity (0.328) tight, improper dihedral distribution (1.140), the obtained result showed the positive value for all indicating adequate quality of the modelled protein . The structural average packing was -0.742 which is in the allowed range for better quality model.

Table 1: Secondary structure prediction by various programs

Secondary structure	Alpha helix (Hh)	Extended strand (Ee)	Beta turn (Tt)	Random coil(Cc)
DPM(%)	32.91	11.52	3.47	52.10
DSC(%)	35.65	11.70	0.00	52.65
HNNC(%)	38.03	16.09	0.00	45.89
PHD(%)	38.94	11.52	0.00	49.54
GOR4(%)	36.75	15.17	0.00	48.08
PSIPRED(%)	30.35	12.98	0.00	56.67
SOPMA(%)	40.95	19.74	8.41	30.90
SIMPA96(%)	33.82	16.64	0.00	49.54
Sec.Cons(%)	33.09	10.97	0.00	45.89
Predictprotein(%)	35.65	15.36	0.00	48.99

Table 2: Prediction of protein disorder by various programs

Sever	Disorder	Disorder by REM465	Disorder by loop/coil definition	Disorder by HOTLOOP'S definition
RONN	2-12, 41-48,141-178,187-191, 308-311, 455-484,486 - 490			
PRDOS	1-10, 43-50, 243- 260, 543- 547			
GLOBPROT		3- 13, 45-52, 79-88,143-168, 231-238,274-280, 308-321, 387-396, 445-450, 485- 496		
DISEMBL		1-12	1-55, 73-94, 111-123, 133-169, 204-216, 226-239, 246-261, 268-283, 295-325, 337-348, 385-412, 428-435, 441-454, 472-516,523-530	1-17, 303-311, 317-324

Low packing Z score was shown by ASN 257, PRO 146. WHATIF program identified the RMSD Z-Score of various parameters used for the verification of structure quality indicates backbone-backbone contacts (–0.22), backbone sidechain contacts (–1.71), sidechain-backbone contacts (–1.20), sidechain-sidechain contacts (–2.41) (Fig. 5).

Table 3: Global quality scores

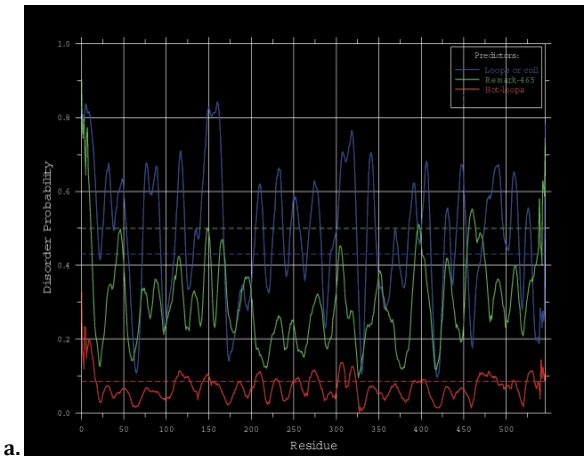
Program	*Verify3D*	*ProsaII(-ve)*	*ProsaII (-ve)*	*Procheck (phi-psi)3*	*Procheck (all)3*	*MolProbity Clashscore*
Raw score	0.46	0.12	0.12	0.12	0.06	80.61
Z-score1	0.00	0.79	0.79	0.79	0.35	-12.31

Moreover, the structural integrity of final model was reflected by a Z-Score of –1.54 was obtained for the model which is well within the quality control value of 2.0, indicating the fine quality of the model. Abnormal packing was exhibited by a number of residues such as ARG 48, ARG 526, MET 292, HIS 338, PHE 293, HIS 249, ARG 114, ARG 298, ARG 465, ARG 403, ARG 248. Inside/outside RMS Z-score is 0.995, RMS Z score for bond angle which is 1.19 which is in the normal range. The results obtained from the evaluation using WHATIF indicate that the homology modelled structure is very reasonable. The detailed study of the modelled protein alpha phosphoglucomutase was carried out using the online sever protein structure validation suite (PSVS) which includes PROCHECK, VERIFY 3D, ProsaII.

The negative value for ProsaII score is given by good quality model, our protein so modelled here exhibited negative score. The modelled protein have a score of 0.46 for the analysis by Verify 3D indicating a good quality model. Analyzing the statistics of nonbonded interactions between different atoms, a score of greater than 50 is normally acceptable. The generated model gave an ERRAT score of 95.733(Fig. 4).

Determination of RMSD of the protein: The model obtained was further refined and used for analysis. The root-mean-square deviation (RMSD) was obtained using pymol (38). The RMSD value between the backbone atoms of the template Bacterial phosphoglucomutase (PDB: 3NA5: A chain) and the homology modelled protein is 0.202 Å again indicating a close homology with selected template. Therefore, the modelled structure has a reliable conformation for further analysis.

Active site identification: Further investigation of the protein active sites with the CAST P server helped in the identification of 88 pockets. The CAST P study revealed the area to be around 2648.6 and volume covered to be 8082.6. The detailed results indicates the presence of the following amino acids in the active site 1MET,2VAL,36LEU,37ALA,39GLN,40VAL,41ALA,42PHE,43GLY,44THR,45SER,46GLY,47HIS,48ARG,49GLY,50SER,52LEU, 53THR,55THR,145THR,147SER,148HIS,149ASN,150PRO,152SER,153ASP,157GLYS,157LYS,162ASN,165PRO,166ALA,167ASP,168THR,171THR,175ALA,176LYS,179ASN,270ASP,271THR,272ASP,274LYS,276ARG,276ARG,278ASP,280SER,281SER,282PRO,306ASP,308ASP,310ASP,311ARG,323ASN,324PRO,325ASN,326HIS,351THR,353VAL,355SER,357ILE,373PRO,374VAL,375GLY,375LY,376PHE,376PHE,377LYS,378TRP,380VAL,381ASP,393GLU,394GLU,395SER,397GLY,411ASP,412LYS,436TYR,446PRO,448TYR,449ALA,450AR,452ASP,457ARG,460LYS,461ALA,464ALA,465ARG,493ALA,494ALA,495LEU,496GLY,510ARG,511PRO,512SER,513GLY,514THR,515GLU,516ASP,517VAL,519LYS,521TYR,523GLU (Fig. 6).



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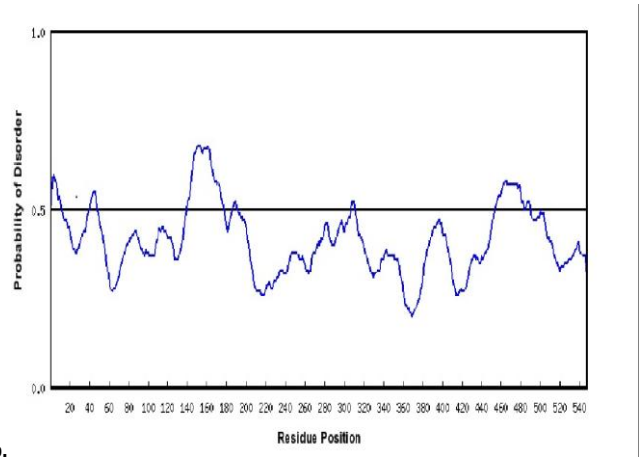

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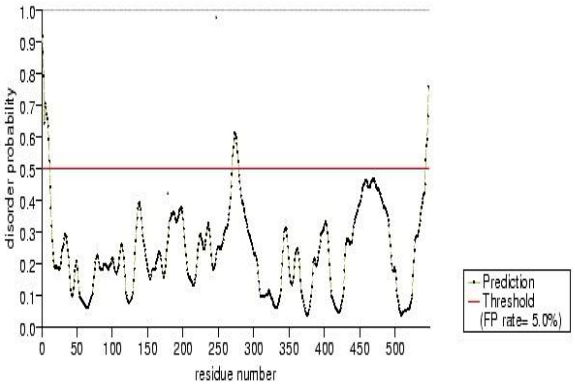

c. prdos

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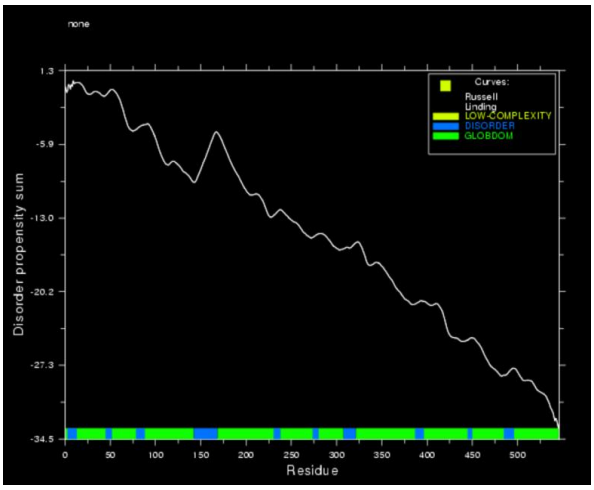


Fig. 3: Intrinsic disorder profile of the protein (a) DISEMBLE (b) RONN (c) PRDOS (d) GLOBPRO

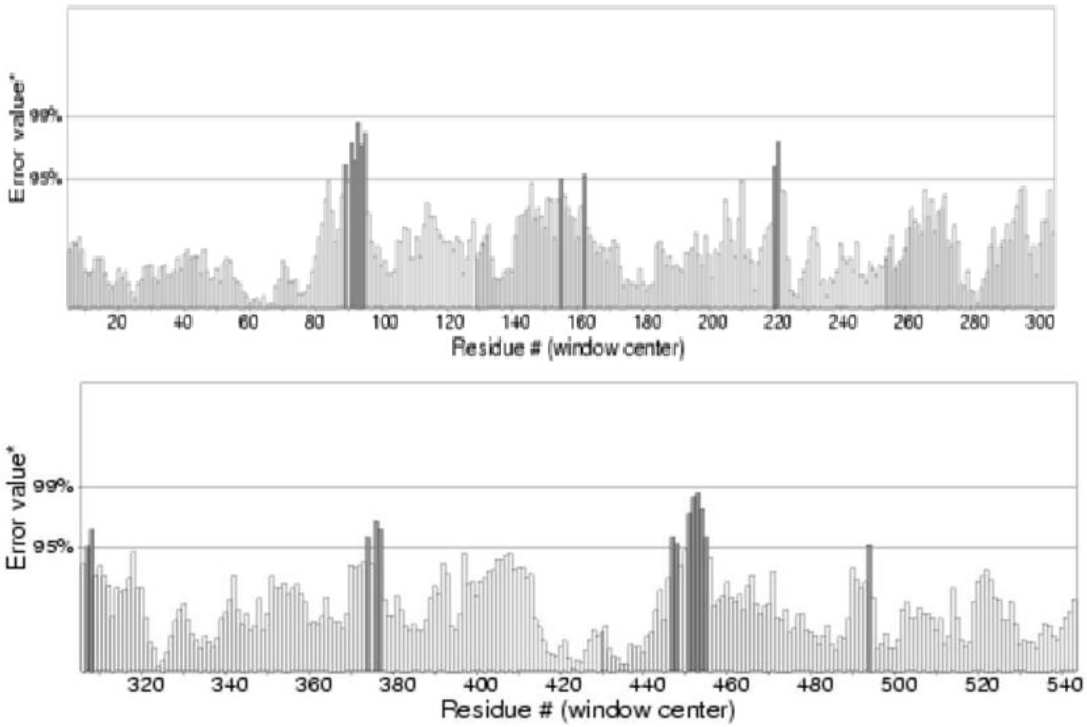


Fig. 4: ERRAT plot of modelled Mtb Alpha phosphoglucomutase

CONCLUSION

Due to the constant efforts by different organisations, spread of infection and mortality rate due to tuberculosis has decreased, but it still remain to be a major issue around the world. Development of multiple drug resistance and constant mutation to the virulent strains of mycobacterium species has urged the importance of research in this field. The inhibition of various pharmaceutical companies towards the research for tropic disease treatment has led to lack of efficient drugs in the market.

The important reason to explore more into their detailed mechanism of life cycle and pathways will provide us with more relevant facts, possible ways to fight Tb. The absence of structural information of such potential target is still a major challenge for drug development.

Comparative protein modelling is an efficient method for carrying out research in such situation. The modelled protein structure of alpha phosphoglucomutase of mycobacterium tuberculosis is evaluated using various reliable programs showed promising results. The structure provides reliable information about the enzyme, which can be further studied for the development of new therapy for the efficient treatment of the disease.

The Url of various programs used in this project:

1. PDB: http://www.rcsb.org/pdb/home/home.do
2. Expasy-ProtParam tool: http://web.expasy.org/protparam/
3. Consurf server: http://consurf.tau.ac.il/verify.php
4. Tool PSI-BLAST: http://www.ebi.ac.uk/Tools/sss/psiblast/
5. Tool Clustal Omega: http://www.ebi.ac.uk/Tools/msa/clustalo

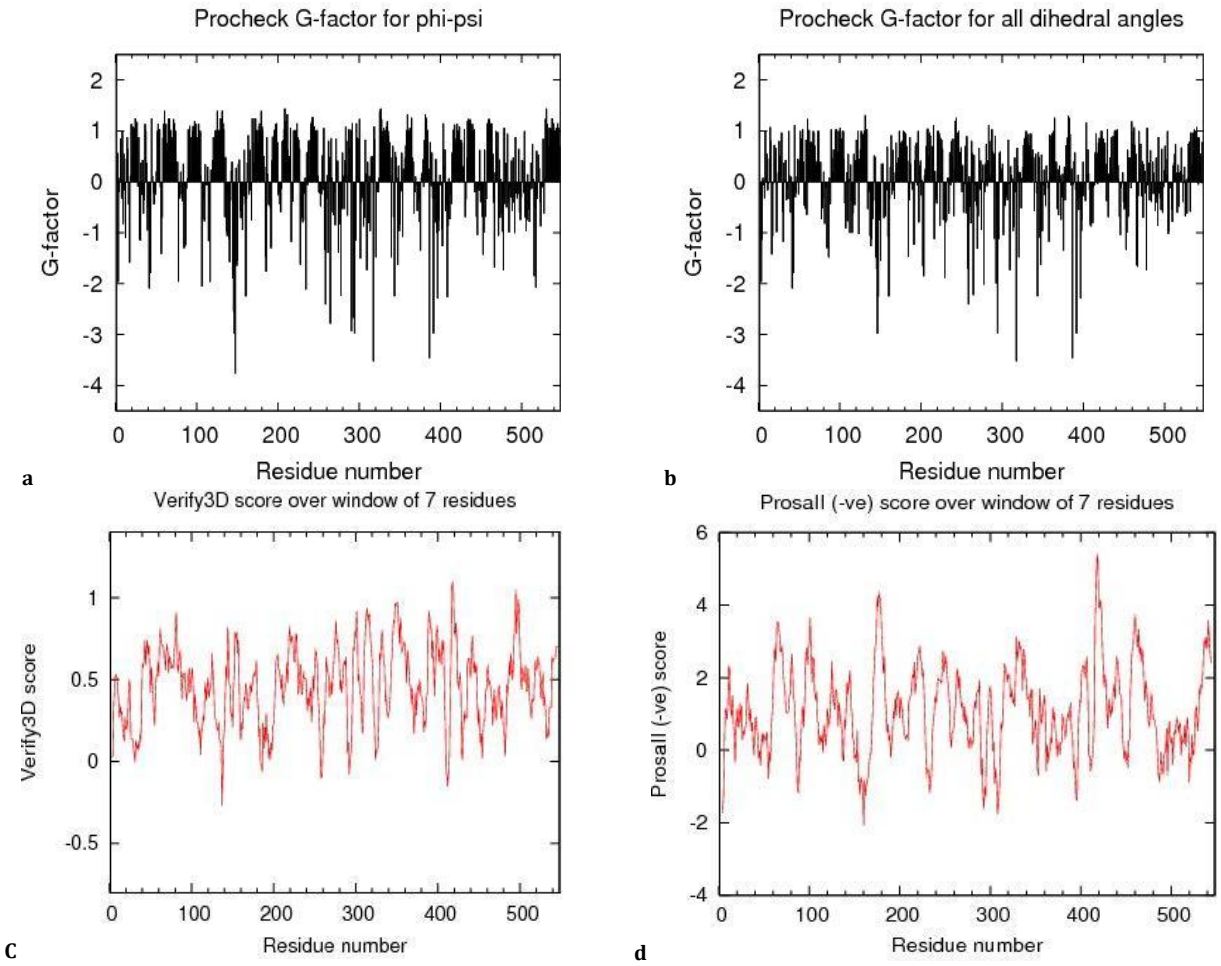
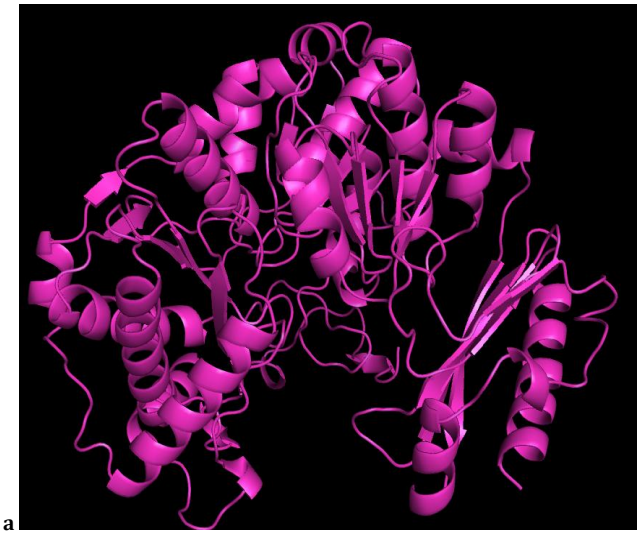
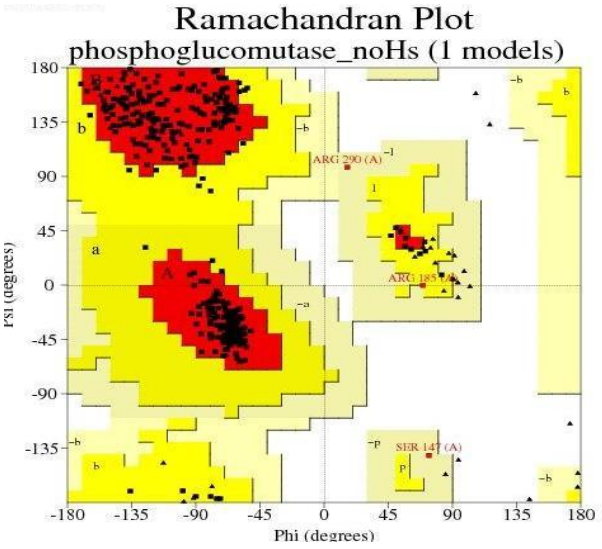
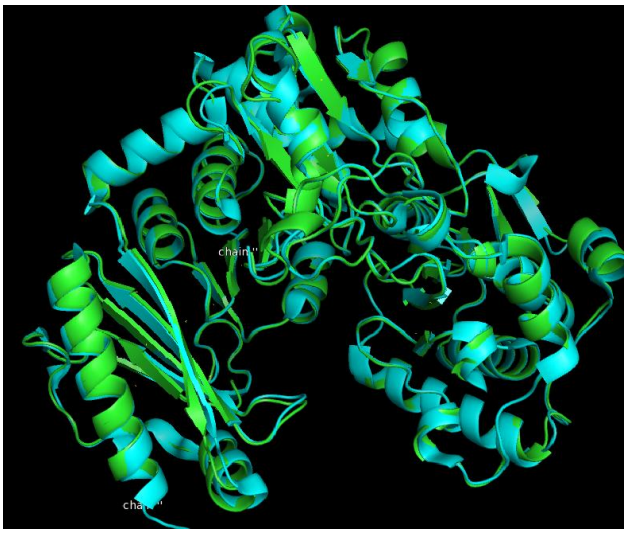


Fig. 5: Quality check profile of modelled protein (a) Procheck G-factor for phi-psi(b) Procheck G-factor for all dihedral angles (c) Verify3D profile (d) Prosa11(-ve) profile

1. Modeller 9.10: http://www.salilab.org/modeller/
2. Secondary structure prediction tool: http://genamics.com/expression/strucpred.htm
3. Intrinsic disorder prediction
4. Globplot: http://globplot.embl.de/
5. Prdos: http://prdos.hgc.jp/cgi-bin/top.cgi
6. RONN: http://www.strubi.ox.ac.uk/RONN
7. Protein analysis tool kit: http://bioserv.cbs.cnrs.fr/htbinpost/pat/new/wpat.pl?dir=example_1&tool=simpa96
8. CASTP: http://sts.bioengr.uic.edu/castp/
9. Dissemble: http://dis.embl.de/







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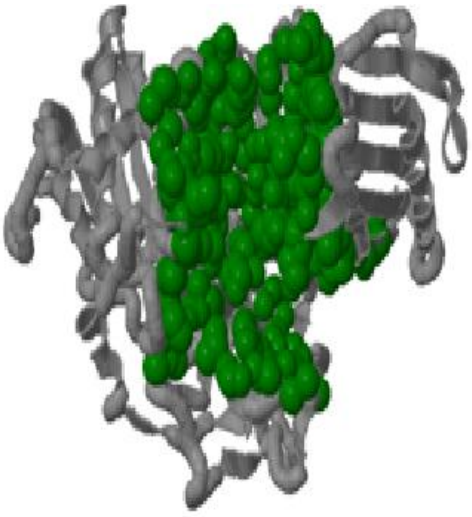

d

Fig. 6: (a)structure of modelled protein Mtb alpha phosphoglucomutase (b)Ramachandran plot for the modelled protein (c)Superimposition of target (green) and template(blue) (d) protein active site predicted by CASTp

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