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Isochorismate Synthase (MenF) - 3D Prediction in Mycobacterium Tuberculosis: A Potential Drug Target

Leeladhar Sammatur, Anuradha, C.M., Srinivasulu Cheemanapalli, Madhusudana P. and Suresh Kumar Chitta*

Department of Biotechnology, Sri Krishnadevaraya University, Ananapuramu, India

*Corresponding author: chitta34c@gmail.com / suresh.chitta@skuniversity.ac.in

ABSTRACT

In fastest Developing countries, disease burden make an unhealthy and undeveloped. It is still challenge to prevent Tuberculosis (TB) even though lot of preventable and curable measurements taken up by both state and central governments in India. In this context we are focused to explore the new 3D drug target structurally and functionally by using bioinformatics tool. We searched the new drug target by comparative search analysis in KEGG data base (previous work not published). One of the new drug target Isochorismatesynthase (MenF) was selected and performed sequence alignment, Homology Modeling, validation and performed secondary structure analysis. Moduller results showed that the 3D-Model is accurate and accepted model. Validation of 3D model is confirmed that, there is 99.03% amino acids are in allowed and generously allowed region of Ramachandran plot, this can be useful for further steps in the insilico research. ProsA analysis provides the local and over all model quality within the Z scores limits. Pdb sum analysis provided the main chain, side chain and active site residues. This clearly explains the utilization of bioinformatics tools in the process of drug development to young researchers.

Keywords: Tuberculosis, isochorismatesynthase, homology modeling, Ramachandran plot

Mycobacterium tuberculosis (Mtb), the main causative organism of tuberculosis (TB), is a successful pathogen that overcomes the numerous challenges presented by the immune system of the host (Dye *et al.,* 1999). More adults die due to TB every year than AIDS and malaria together (Corbett *et al.,* 2003). It is difficult to kill Mtb for a number of reasons such as its slow growth, dormancy, complex cell envelope, intracellular pathogenesis and genetic homogeneity. Person with active TB disease will infect on average between 10 to 15 people every year. But people infected with TB bacilli will not necessarily become sick with the disease. The immune system "walls off" the TB bacilli which, protected by a thick waxy coat, can lie dormant for years and when the immune system is weakened, the chances of becoming sick are greater. Until 50 years ago, there were no medicines to cure TB. Now, strains that are resistant to a single drug have been documented in every country surveyed; what is more, strains of TB resistant to all major anti-TB drugs have emerged. A particularly dangerous form of drug-resistant TB is multidrug-resistant TB (MDR-TB), which is defined as the disease caused by TB bacilli resistant to at least isoniazid and rifampicin, the two most powerful anti-TB drugs. TB is generally treatable, it requires extensive chemotherapy (up to two years of treatment) with second-line anti-TB drugs which are more costly than first-line drugs, and which produce adverse drug reactions that are more severe, though manageable. The emergence of extensively drug-resistant (XDR) TB, particularly in settings where many TB patients are also infected

with HIV, poses a serious threat to TB control, and confirms the urgent need to strengthen basic TB control and to apply the new WHO guidelines for the programmatic management of drug-resistant TB.The complete genome sequence of *Mycobacterium tuberculosis* H37Rv provides an opportunity for a more focused and planned approach towards the identification of new drug targets. An important advantage of this analysis is the possibility of identifying a novel target that is present in many bacteria and subsequently designing a drug that could be active against a wide range of bacteria.

In addition, availability of human genome sequence can help in eliminating the potential drug targets that have close human homologues. Thus, the possibilities of using complete genome sequences for target identification are virtually unlimited (Madhusudan *et al.*, 2012; Sharmila Anishetty, *et al.*, 2005). The Mtbgenome revealed the presence of a large number and variety of putative protease genes, some encoding potential proteins to metabolic pathways for survival of bacterium (Van Rie, and Enarson, 2006). This includes a family of Ubiquinone and other terpenoid-quinone biosynthesis pathways (Madhusudan *et al.*, 2014), which share a high degree of importance and are constitutively expressed in *Mycobacterium tuberculosis* H37Rv for ubiquinone biosynthesis (Madhusudan *et al.*, 2014). The gene for the isochorismate synthase (MenF) is present in the genome of this organism. Therefore, MenF was selected for further analysis (Chopra, *et al.*, 2003, Klebe, G., 2000). TheMenF from Mtb H37rv catalyzes the conversion of chorismate to isochorismate, Fig. 1, which is subsequently converted by a sequentialenzymemati steps to menaquinone. General acid – general base catalysis has been proposedfor isochorismatesynthesis [EC:5.4.4.2] in all enzymatic reactions.

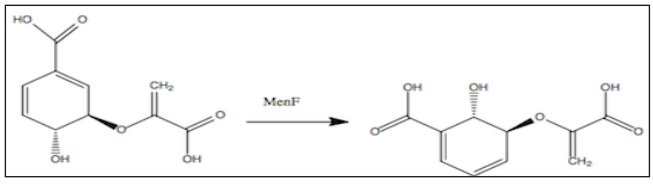


Fig. 1: Isochorismate synthase reaction (MenF)

Thus, isochorismate production from chorismate by the ICS enzymesis the result of general acid – general base catalysis with a lysine as the base and a glutamic acid as the acid, in reverse protonation states. Fig. 2. In the present study has been conducted with the development of a 3D model of Mtb-MenF and Energy minimization, Evolutionary analysis of Mtb-MenF sequence with other species of bacteria, annotate the 3-D structure of Mtb-MenF potential drug target and 3D model Verification and Validation of Mtb-MenF. Mtb-MenF play important role in biosysnthesis of Ubiquinone, it play a crucial role in conversation of chorismate to isochorismate.

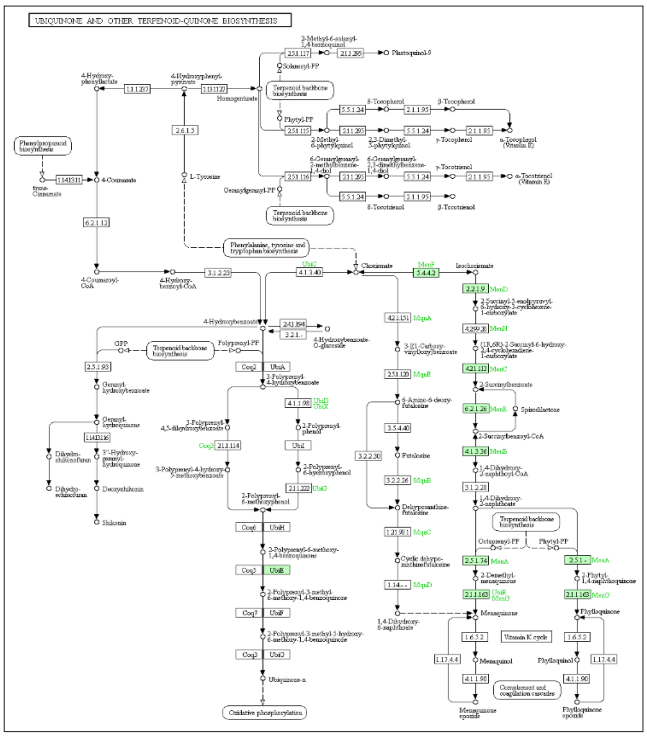


Fig. 2: Ubiquinone and terpenoidquinine biosynthesis pathway (enzyme MenFshown in circle)

MATERIALS AND METHODS

In present work all the calculations were carried out with high frequency computational analysis such as molecularmodeling,energyminimizations,Secondary structure, side chain and main chai parameters with Hi-end server (Pentium IV 3.4 MHzs, AMD Athlon 64 bit, Dual processor with 1GB RAM) manufactured

by HCL Corporation, Pondicherry, India was used. MODELLER is a computer program used, by which a set of geometrical criteria are used to create a probability density function for the location of each atom in the protein. The method relies on an input sequence alignment between the target amino acid sequence to be modeled and a template protein whose structure has been solved (Sali and Blundll 1993; Sali 1995a, 1995b). Pymol is an open-source, molecular visualization system, which is well suited to produce high quality 3D images of small molecules and biological macromolecules such as proteins. According to the author, almost a quarter of all published images of 3D protein structures in the scientific literature were made using PyMOL. (http://www.delanoscientific. com/). Clustal-W: Available on line at EMBL server is widely used for pairwise/ multiple sequence alignment computer program (Thompson *et al.,* 1997).

All these databases are available online through the Entrez search engine. (www.ncbi.nlm.nih.gov/). The Protein Data Bank (PDB) is used for retrieval of 3-D structural data of template proteins and nucleic acids (www.rcsb.org/pdb). SWISS-Prot (Expasy) server is used for protein sequence. Swiss-Prot strives to provide reliable protein sequences associated with a high level of annotation (such as the description of the function of a protein, its domains structure, post-translational modifications, variants, etc.), a minimal level of redundancy and high level ointegration with other databases. (http://expasy.org/sprot/). (http://www.ebi.ac.uk/Tools/clustalw/index.html). The PDBsum is a pictorial database that provides an at-a-glance overview of the contents of each 3D structure deposited in the Protein Data Bank (PDB). This server provides cleft and groves on the surface of protein molecules deposited in protein data bank. Pdbsum can also give ligand binding site with Ligplot graphs in two dimensional appearances (Laskowski *et al*., 2005). (http://www.ebi.ac.uk/pdbsum/). ProSA program (Wiederstein *et al*., 2007) which exploitsthe advantages of interactive web-based applications for thedisplay of Z-scores and energy plots that highlight potentialproblems spotted in protein structures. In particular, the qualityscores of a protein are displayed in the context of all known protein structures and problematic parts of a structure are shown and highlighted in a 3D molecule viewer. The service specifically addresses the needs encountered in the validation of protein structures obtained from X-ray analysis, NMR spectroscopy and theoretical calculations. (http://prosa.services.came .sbg.ac.at).

The most important point in any homology modeling study, besides the choice of the reference, is the alignment of the sequences. The greatest attention was thus paid to the careful construction of a robust alignment. Sequence analysis was carried out through retrieving the sequences from NCBI and from KEGG databases. Sequence homology search was conducted through the blast-P program available at NCBI. Homology modeling of target sequence needs a template crystal structure coordinates which were obtained by performing blast-P at NCBI with selection of database as PDB at (www.ncbi.nlm.nih.gov/blast.html.) (Domagalski, M.J. *et al*., 2008). The coordinates of selected crystal structures of sequence similar structures of target protein were obtained from PDB and used for prediction of 3D structure of target protein using MODELLER 9v18.

In order to identify conserved and variable regions of the sequences and in determining the most robsi gap arrangement, pairwise sequence alignment of homologous proteins of the target sequence Clustal-W (Chenna *et. al*., 2003) with appropriate parameters were used as per the specified instructions. The Clustal-X alignment file of the selected sequences was used for the basic parameter for further creating the phylogenetic tree with target, as query sequence.

Mtb-MenF Homology modeling

The 3-D homology models of given target protein sequence was predicted using crystal structural coordinates (Blundell *et al.,* 1996) of templates on the basis of sequence alignment. All steps of homology modeling and refinement were carried out through MODELLER 9v18 using base line commands specified by software supplier (Sali and Blundell, 1993). The

method described below is used in the present study to predict the 3D models of Mtb-MenF enzyme. There are three kinds of input files are required to perform homology modeling using MODELLER. They are PDB atom files with coordinates for the templates, the alignment file with alignment of the template structures with the target sequence, and finally PY file, a MODELLER command file that instructs MODELLER what to do.

Each atom file is named as code.pdb where code is a short protein code, preferably the PDB code. The atom file contains the only protein co-ordinates without hetero atoms while modeling target protein. One of the formats for the alignment file is related to the PIR data base format which is the preferred format for homology modeling by MODELLER. The .PY file contains commands for MODELLER. A sample steering file is to produce one model of sequence.

A number of intermediary files were created as the program proceeds. After 10 minutes, the final protein model is written to file protein .B999901. A log file was also created with information about the run, as implemented in the 'model' .PY script which also be used for variety of modeling tasks not only for comparative modeling. Input: script file (steering file; alignment file, PDB file(s) for template(s). Output files are .log: long file, .ini : initial conformation for optimization, .rsr: restraints file .sch: VTEM schedule file, .B999????: PDB atom file(s) for the model(s) of the target sequence , .V9999????: Violation profiles for the model(s)

Evaluation of the built 3D protein model

A protein 3D model derived from homology modeling technique may have some sources of errors. It is important, therefore, to have an assessment of structure's quality and to be able to identify regions that may need modifications especially at protein folding and turns. The aim of model evaluation is to determine whether the built model is acceptable and suitable to use for molecular analysis such as docking and dynamics. The accuracy of the comparative built structures were tested using the ENERGY command of the MODELLER program (Sali and Blundell, 1993) and tools like PROCHECK, (Laskowski *et al*., 1993) and ProSa (Sippl, 1993) In addition, the variability of the homology model has been compared by superposition of Cα traces and backbone atoms model and crystal structures, from which the RMSD value for positional differences between equivalent atoms calculated with SPDV (Guex, 1999),which clearly judges the accuracy of model.

PROCHECK

The PROCHECK suite of programs provides a detailed check on the stereochemistry of a protein structure. The stereo chemical parameter checks implemented in PROCHECK are derived from high-resolution protein structures, against which the structure is compared on a residue-by-residue basis. The criteria are Ramachandran plot, peptide bond planarity, C-alpha tetrahedral distortion, non bonded interactions, hydrogen bond energies, and closeness off side chain dihedral angles to ideal values. The reliability of the refined Mtb-MenF, was carried out by program such as PROCHECK (Laskowski *et al*., 1993) and ProSa-web (Sippl, 1993).

Prediction of secondary structure of protein

The prediction of protein secondary structure is a major parrot of the general protein folding problem and the method of obtaining some structural information for any sequence. Secondary structure predication is important in establishing alignments during homology modeling. Secondary structure analysis is carried out through the ProFunc and PDBSUM server (Laskowski *et al.*, 2005), which gives the clear data of protein, alpha helices, sheets, turns, beta hairpins, beta bluges, gamma turns etc., The Mtb-MenF secondary structure prediction was done through PDBSUM online server, which provides complete data about the helices, beta sheets, turns (Laskowski *et al.*, 2005). Motif Scan server used for identity function of motifs or domain present in Mtb-MenFat http://scansite.mit.edu.

RESULTS AND DISCUSSION

Sequence analysis

The Amino acid sequence of Mtb-MenF obtained from Kegg and NCBI comprising of 372 amino acids and bearing a gi. No. 888824 is shown in Fig. 3 in FASTA format.

>ICS MtbH37rv

MSAHVATLHPEPPFALCGPRGTLIARGVRTRYCDVRAAQAALRSG
TAPILLGALPFDVSRPAALMVPDGVLRARKLPDWPTGPLPKVRVA
AALPPPADYLTRIGRARDLLAAFDGPLHKVVLARAVQLTADAPLD
ARVLLRRLVVADPTAYGYLVDLTSAGNDDTGAALVGASPELLVA
RSGNRVMCKPFAGSAPRAADPKLDAANAAALASSAKNRHEHQLV
VDTMRVALEPLCEDLTIPAQPQLNRTAAVWHLCTAITGRLRNISTT
AIDLALALHPTPAVGGVPTKAATELIAELEGDRGFYAGAVGWCDG
RGDGHWVVSIRCAQLSADRRAALAHAGGGIVAESDPDDELEETTT
KFATILTALGVEQ

Fig. 3: Amino acid sequence of MenF

The amino acid sequence of Mtb H37rv-MenF was collected from in FASTA format has been used for sequence alignment search using BlastP tool at NCBI Fig. 4. The blast-P tool was performed by selecting database as PDB.

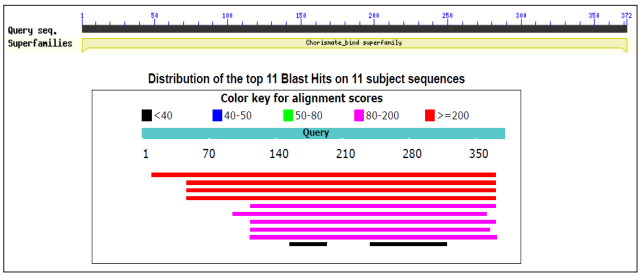


Fig. 4: Blastp result showing top 11 blast his with quirysequency

The MenF protein is highly conserved, shown by the sequence alignment in Fig. 5. The conserved residues are denoted using star, semi conserved with: and non conserved with no indication. Most importantly, the active sites of the proteins are conserved. This is significant because an inhibitor molecule that binds to conserved amino acid of MenF will have a higher probability to inhibit all it.

Fig. 5: Sequence alignment of Mtb-MenF and Bacillus anthrasis species showing the sequence homology

Phylogentic Analysis

MenF is a protease that is thought to be essential for ubiquinone biosynthesis in the life cycle of MtbH37rv. For this reason, the MenF protein sequence was BLASTed against the protein databank protein sequence database to identify homologs in other species. The results showed that the majority of homologs are contained in bacteria. Although similar sequences were found mainly in Bacillus anthraces, some were identified in Steptomysis, *E.coli*, streptococcus and enterobacteria as well as others.

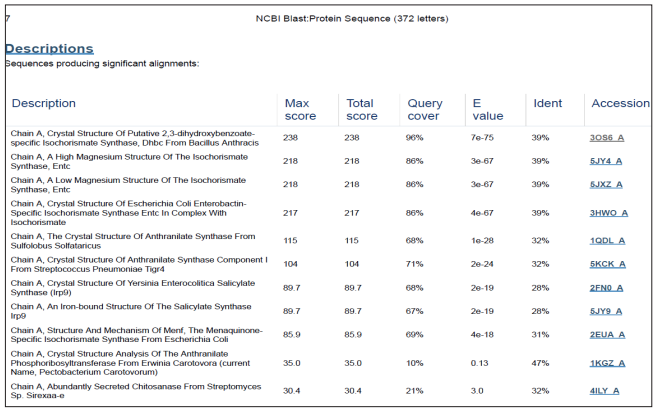


Table 1: Species found to contain homologs of the MenF protein

Table 1 identifies a sampling of the species found to contain homologs to MenF. A phylogenetic tree, Fig. 6 was constructed to examine the evolutionary

distance between the bacterial homologs. The tree was constructed using an implicit alignment between the database sequences. This is based on the alignment of the sequences with the MenF protein. Although the identity between human and Mtb-MenF proteins shows no significant similarity, this result is significant for drug design. It is essential that an inhibitor designed to treat an infection in humans does not interact with human proteins. It can be seen that the active site of the MenF protein (denoted by orange stars) is not conserved in the human homolog, Fig. 7. This will enable the designing of an inhibitor that will only fit the MenF active site.

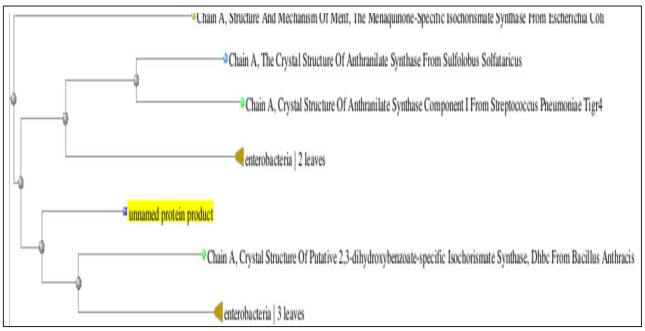


Fig. 6: Phylogenetic tree showing the divergence of menF (Unnnamed) homologs. The protein labeled unknown in yellow highlighting is the submitted menF sequence. The closest match the program made was, in fact, the menF protein in Bacillus Anthraces, seen directly next to the submitted sequence

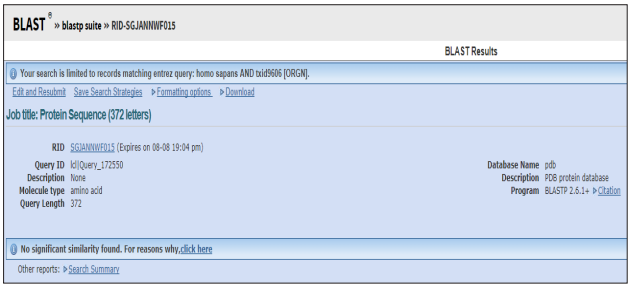


Fig. 7: Identity between human and Mtb-MenF, shows no significant similarity

Homology Modeling

In order to construct the homology model of Mtb-MenF an atom file, alignment file and steering file were generated and run through MODELLER 9v18, nearly 100 runs were set to obtain the apt homology model of Mtb-MenF and further the structural refinement was done using Swiss-PDB Viewer (Guex, 1997). MODELLER 9v18 utilizes the crystal structure coordinates (3os6; Domagalski *et al.*, 2008) as template to generate the final 3D model of Mtb-MenF (Fig. 8a). The superposition of the crystal structures 3os6(Domagalski *et al.*, 2008) on to the Mtb-MenF gave C-alpha Fig. 8b, RMS values of 0.64A^{o} and backbone RMS value 0.62A^{o}; it can thus be characterized as a good theoretical model for further analysis.

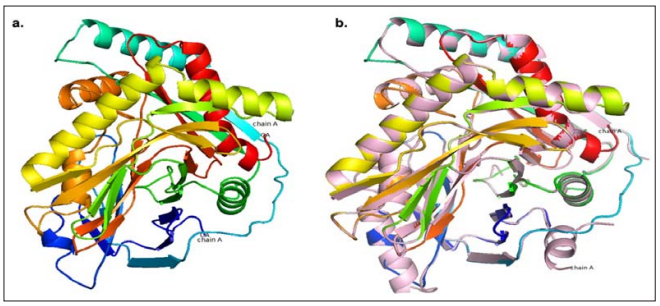


Fig. 8(a): The built 3-D model of Mtb-MenF showing different secondary structure conformations. (b) superimposed model ics is in multicolour template 3os5 is in light pink

Energy minimization (http://igc.ethz.ch/gromos)

The build model of Mtb-MenF was subjected to energy minimization with SPDBV with energy minimization option. The energy of the modeled molecule before and after minimization is compared. Obviously, the energy has been reduced after minimization.

- Energy Before minimization: 2726.033 KJ/mol
- Energy After minimization: -4650.041 KJ/mol

With the energy minimizations program all the angles, bonds torsion angles and electrostatic energy were well calculated under GROMOS96 without reaction field.

Mtb-MenFstereo chemical analysis

PROCHECK, and ProSA-web analysis were used to

perform the stereochemical and structural evaluation of the optimized built 3D model of Mtb-MenF . In order to establish the quality of the final energy minimized of Mtb-MenF, the PROCHECK was used (Laskowski, 1993). The Ramachandran plot constructed for Mtb-MenF showed a good distribution for the Φ/ψ angles of all amino acid residues where, 99.3% of amino acids are in the most favored, generously and additionally allowed regions with only 0.7% of amino acids in disallowed regions Fig. 9.

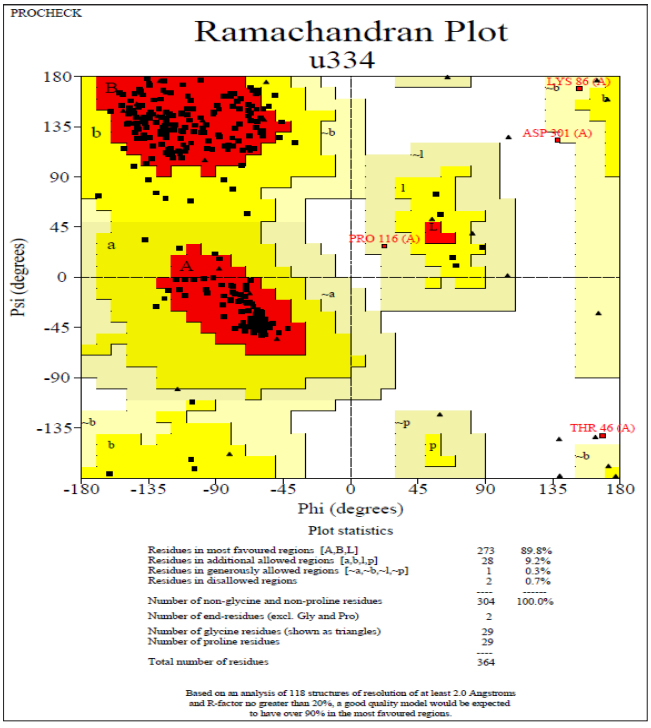


Fig. 9: Ramachandran plot of MenF

Moreover, the average G-factor which is the measure of the normality degree of the protein's properties is inside permitted values (Laskowski, 1993). The side chain parameters of Mtb-MenF shows that the χ1-gouche minus standard deviation, trans standard deviation, gouche plus standard deviation and pooled standard deviation and χ2- trans deviation are in good agreement with the expected values. Bond lengths and hydrogen bond lengths also did not deviate significantly from standard values. Thus, based on the criteria used in PROCHECK, the Mtb-MycP1 could be characterized as a good structure suitable for molecular docking and dynamics also (Fig. 10).

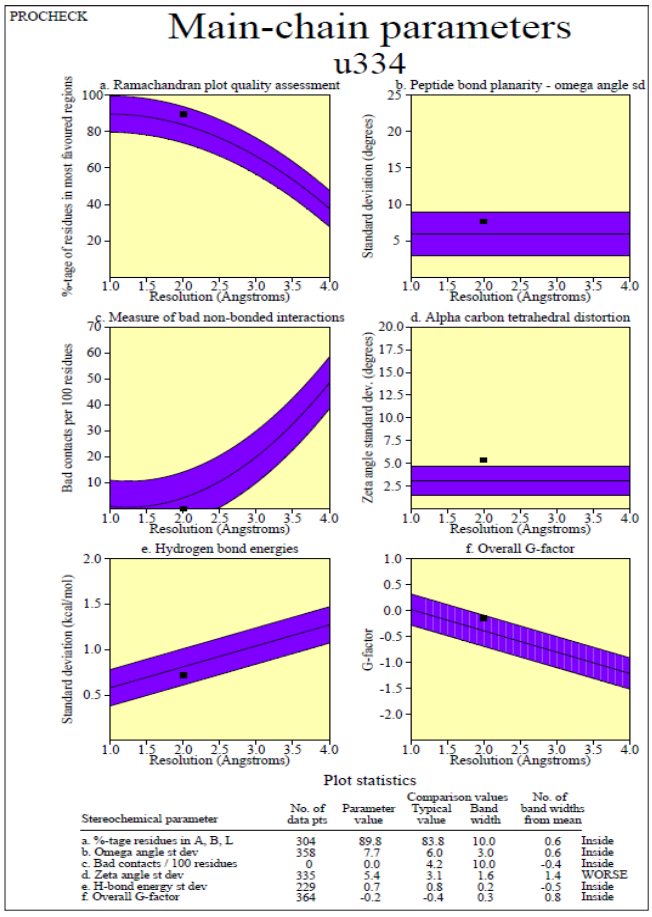


Fig. 10a: The six graphs on the main-chain parameters plot show the MtbMenF model (Solid Square) compares with well-refined structures at a similar resolution. The dark band in each graph represents the results from the well-refined structures. Ramachandran plot, Omega angle standard deviation, Bad contacts, zeta angle, H-bond energy standard deviation, Overall G-factor showing the quality of MtbMenF model

The PROSA and ProSA-web analysis of a protein structure shows the energy graphs having negative values correspond to stable parts of the structure. The ProSA-web analysis of Mtb-MenF showed the energy graphs. The analysis showed the Z scores, -7.92 for Mtb-MenF. (Fig. 11).

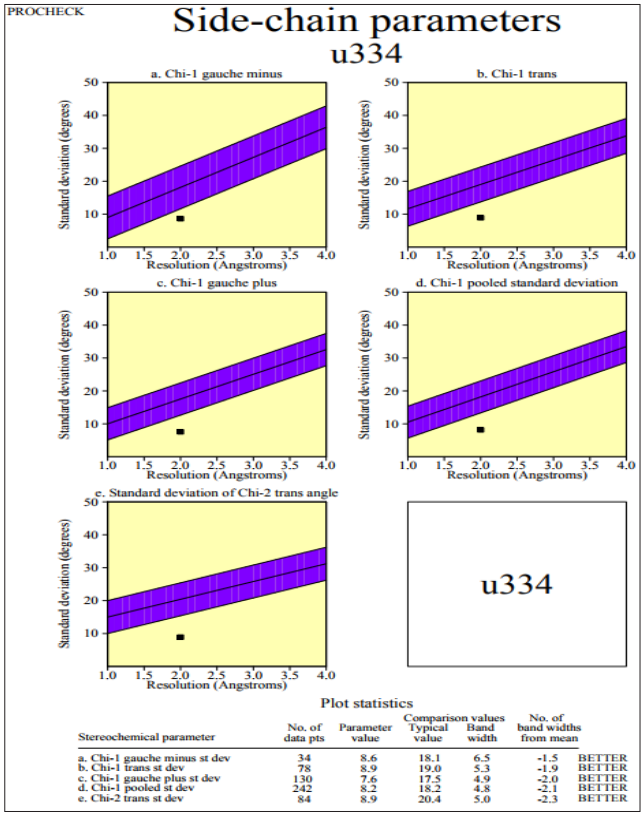


Fig. 10b: The Side chain parameters evaluated for MtbMenF model (solid squares) generated by PROCHECK

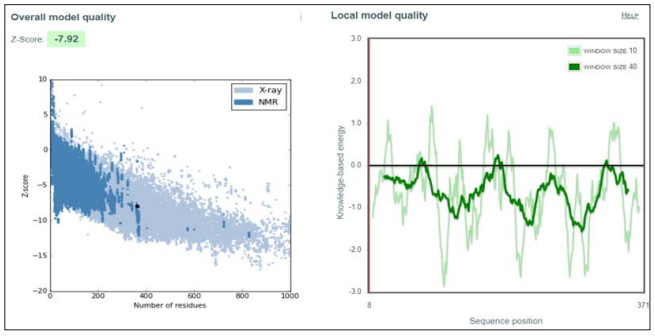
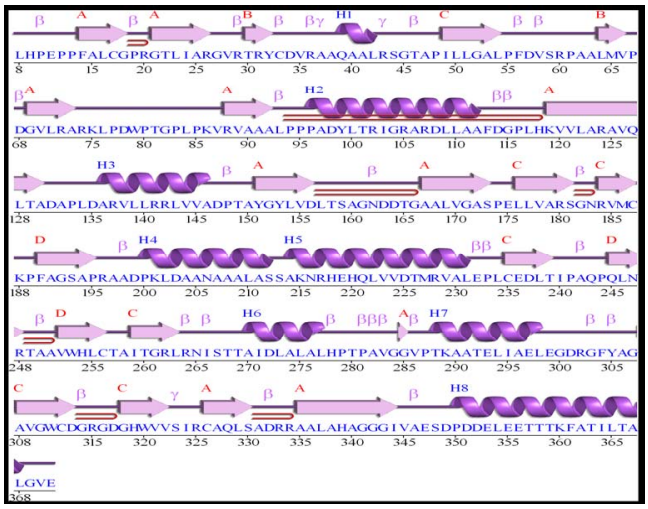


Fig. 11: ProSA-web Z-scores of Mtb-MenF, determined by X-ray crystallography (light blue) or NMR spectroscopy (dark blue) with respect to their length

Secondary structure analysis of Mtb-MenF

The secondary structure characterization of Mtb-MenF carried out through the PDBSUM online server showed various secondary structure elements like helices, strands, turns and loops etc., (Fig. 12). The PDBSUM server data analysis of Mtb-MenF showed 8 helices, seven beta hairpins, 8 beta bulges, 22 strands, four sheets, 5 helix-helix interactions, 32 beta turn and 3 gamma turns.



struc: ces labelled H1, H2, ... and strands by their sheets A, B, ...

Fig. 12: Amino acids involved in different secondary structural conformations in Mtb-MenF analyzed from PDBsum (http://www.ebi.ac.uk/thorntonsrv/databases /pdbsum/)

Cleft analysis of Mtb-MenF

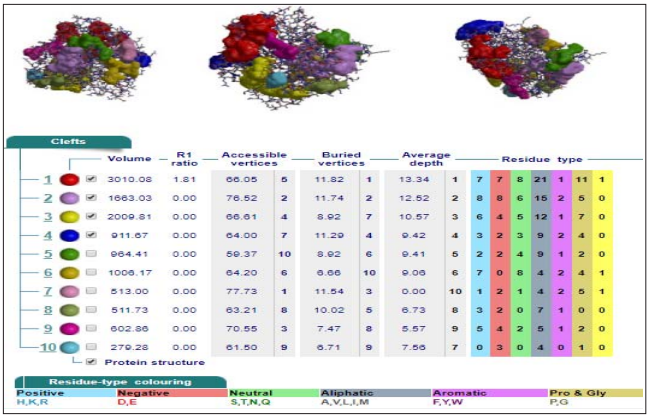


Fig. 13: Binding site, binding surface and cleft parts of MenF

CONCLUSION

Mtb-MenF is involved in biosynthesis of various types of metabolic pathways. Due to unavailability of X-ray/NMR crystallographic structure in PDB, a homology model of MenF has been constructed using the X-ray structures of (PDB code: 3os6) as template, by comparative protein modeling principles. The built model of Mtb-MenF has the correct stereochemistry as gauged from the Ramachandran plot and good three dimensional (3D) structure compatibility as assessed by the *verify-3D* score and PROCHECK. The structurally and functionally important residues of Mtb-MenF have been identified using the crystal structure. The homology model conserves the topological and active features of the Mtb-MenF family of proteins. Homology modeling is a multistep process which converts a linear amino acid sequence of a protein into a 3-D structure. The amino acid sequence of Mtb-MenF (target) was taken from GenBank (NCBI) and subjected to similarity search through BLAST group of programs to identify the structural homolog with highest degree. The most homologous structure is the one with lowest E value among all the hits. The structural homolog with PDB id-3OS6 was selected as the template. The FASTA format of both the target and template was loaded on CLUSTAL-X to obtain the alignment file. Three files that are needed to run MODELLER^R Alignment file, Atom file, Script file (Top file) were prepared as per HELP file of MODELLER^R and modeling was done on hi-end server (optron-64 bit dual process).

After almost three hours a 'b file'(coordinate file) was created in the bin folder by MODELLER^R. This structure was then displayed by dragging the coordinate file on the screen of visualization package PYMOL. The structure showed the presence of alpha-helices, beta-pleated loops this structure was then evaluated for possible errors by PROCHECK. The structure was satisfactory as per the Ramachandran plot constraints. It satisfied the G value as well as other constraint values. The built Mtb-MenFmodel has been found to be satisfactory showing the energy values and Ramachandran plot values indicating the suitability of built model for molecular interactions, i.e. drug and protein interactions using Auto dock Tools, which is believed to provide valuable insights towards the design of an inhibitor for MenF. This is an intangible benefit that can help to design research programs especially in protein targeted drug design. Hence the studies conducted systematically in the present thesis, i.e. the computational design of 3-D structures of MenF and substrates interaction studies will definitely provide highly useful information towards rational design of new tuberculosis drugs through experimental research design for further formulations as per pharmaceutical norms.

CONFLICT OF INTEREST

No conflict of interest by authors

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