Dhananjay Kumar, Anshul Sarvate, Deblina Dey, Lakshmi Sahitya U, Kumar Gaurav Shankar, K. Kasturi / International Journal of Engineering Research and Applications (IJERA) ISSN: 2248-9622 www.ijera.com
Vol. 3, Issue 1, January-February 2013, pp.023-033

Molecular Modeling and Simulation Studies of Acyl CoA Synthetaseof Mycobacteriumleprae

Dhananjay Kumar[*], Anshul Sarvate[1], Deblina Dey[1], Lakshmi Sahitya U[2], Kumar Gaurav Shankar[3], K. Kasturi[2]

*(Department of Bioinformatics, Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Pune, Maharashtra
1(Department of Bioinformatics, Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Pune, Maharashtra).
2(Department of Biotechnology, AcharyaNagarjuna University, Guntur, A. P.)
3(Department of Computer Science, JNU, India)

ABSTRACT

Leprosy or Hansen's disease is caused by an obligate intracellular pathogen i.e. *Mycobacterium leprae*. Leprosy is a granulomatous disease of peripheral nerves and mucosa of the upper respiratory tract. This infectious disease results in Leprosy reactions that cause irreversible nerve damage and disabilities. The organism requires minimal set of functional genes for its survival. Most of the genes are involved in biosynthetic and metabolic pathways, so the product of these genes can be aimed for the novel drug target. Acyl CoA Synthetase is an enzyme that participates in fatty acid biosynthesis. The activation of fatty acids by Acyl-CoA Synthetase is the need of de novo lipid biosynthesis, fatty acid catabolism and remodeling of biological membranes. Therefore by emphasizing this protein as a drug target can help in the identification of novel drugs to cure leprosy. A well organized research comprising of analogue based drug design and molecular dynamics plays a major role in obtaining the lead molecules. The bacteria have developed resistance against many of the drugs available in the market. Therefore identification of the novel drug target and potent drug can be helpful in better prevention of the disease.

Keywords- docking, homology modeling, leprosy, M.leprae, molecular, molecular dynamics, ramachandran plot.

I Brief Introduction of Leprosy

Leprosy was originated over 5000 years ago, almost going back to the Neolithic times[50]. Though remaining disfiguring strip conditions were perplexed with leprosy, deformities symptomatic of the disease now illustrious to be caused by *Mycobacterium leprae* are recognizable in umpteen archaeological finds[24]. One of the most famous example is the skull of Robert the Bruce that shows the artist symptom in systemic leprosy of nasal septum collapse. By gothic times, synchronous archeological relic shows that - at least in few societies - leprosy was diagnosed conservatively and thus mostly accurately[18]. Historically, the bulky numbers of bacterium in the tissues of lepromatous patients no doubtfulness led to the aetiologic agent. Thus, *Mycobacteriumleprae*, existence suggests that it is one of the first bacterium to be determined[24].In 1873 first convincing association of a microorganism with a hominian disease, Armauer Hansen[27], unconcealed the leprosy bacillus in skin biopsies but failed to culture *Mycobacteriumleprae*. A century afterwards the nine banded armadillo[33], was victimized as a replacement host enabling huge quantities of the bacillus which has been kept apart for biochemical and physiological studies[59]. Consequent efforts to corroborate procreation in synthetic media acquired have been equally futile, although metabolic activity can be sensed[21].

Leprosy is one of the oldest filmed diseases, relic a serious health problem though prevalence has been low extensively by 1947, as dapsone (4, 4¢-diaminodiphenylsulphone) was discovered. At that time it became the only effective, but exclusive weakly bactericidal, anti-leprosy drug. The figure of cases of leprosy worldwide remained at roughly 11 million finished to the early-1980s but by then, dapsone resistant strains of *M. leprae* had enlarged to appraising levels. The imperative comeback by the World Health Organization to this problem was to acquire multi-drug regimens against *M. leprae*. Since the treatment now included apace antiseptic medicine, rifampicin and also the treatment had good coverage, so the number of cases as expected drops down[71]. At one stage dominance of leprosy was around 3 million, though incidence (i.e. rate of appearance of new cases) remains as high as before multi-drug therapy was introduced[53] and immunization with BCG[34,47], the incidence of disease remains bedevilment with more than 690,000 new person reported annnualy[72]. The most main usage in the leprosy check in the penultimate millennium has been the launching of multi-drug therapy (MDT) [54] in 1982; recommendation of the WHO study group[10]. Freely visible long-term multi-drug therapy that combines rifampicin, clofazimine

and dapsone effectively targets the bacterium patch minimizing the process of drug-resistant strains[1]. In 1991 WHO starts to destroy leprosy as a public health problem which dramatically minimize the global disease prevalence, suggesting a persistent unknown reservoir. In 2006, 259,017 new cases were reported, out of this 54% of these new reported cases occurred in India, 1 of 118 countries that has achieved voiding status (downed as figure < 1 case/10,000 assemblage). Brazil, Democratic Republic of the Congo, Mozambique and Nepal acquire not achieved execution and invoice for 23% of new cases[73].

The Rational drug use is in this overview incidental to the medical therapeutic view received at the WHO conference of 1985 in Nairobi[2]: Rational use of drug requires that forbearing obtain medications appropriate to their clinical needs, in doses that grapple their own requirements, for a passable phase of instance and at the smallest outlay to them and their community[44]. Leprosy was endemic in Norway (amongst a few, stray parts of Europe) in Hansen's day. Later, it has become more demonstrating in tropical countries, peculiarly - but not solely - in poor local societies. Of the 122 countries where leprosy was considered disease in 1985, 110 score now reached the end of expelling at the state direct by 2003 and leprosy relic a semipublic upbeat job only in 12 countries[22].

1.1. Description of the Condition

The bacilli of leprosy are likely spread through tiny droplets from the nose or mouth from infected and raw individuals[48]. Tissue infected with the leprosy bacilli, *M. leprae*, contains up to 166 pg of a peculiar phenolic glycolipid for each mg dry wt. of *M. Leprae*[51,11]. Leprosy can happen in varied clinical forms, parasitic on the greeting of the vector scheme. Some of the persons with a few skin patches and the merchandise of bacilli are relatively slender; this is classified as paucibacillary (PB) leprosy[48]. Remaining people with many skin patches and a superior assort of bacilli in their body and are classified as multibacillary (MB) leprosy[64,74].
Actually, leprosy is immunologically important[25] and humans are the only famous hosts applicable to the coefficient of leprosy; so the World Health Organization (WHO) currently recommends a 6 and 12-month handling programme for paucibacillary leprosy and multibacillary leprosy, respectively. To stop such kind of disease novel drug targets are required in prescript to the organization of new drugs against antibacterial tender pathogens. Generally, a target should cater enough selectivity, yielding a drug which is precise or highly selective against the pathogen with respect to the human host[3]. Moreover, the target should be intrinsic for growing and viability of the pathogen at least under the stipulation of infection[45]. While studying about the different drug targets we found a long-chain protein (i.e. Acyl-CoA Synthetase) which is essential for fatty acid degradation, phospholipids remodeling, and the creation of interminable Acyl-CoA esters that regulates many physiological processes[60,19]. These membrane-bound enzymes act on non-polar hydrophobic substrates, fatty acids, generating Acyl-CoA Synthetase, primal reactive intermediates in lipid synthesis pathways that are water-soluble as advantageously as powerful detergents[28,65,35,66,37,75]. The structure of these membrane proteins has not been solved for the mammalian Acyl CoA Synthetase but homology to a bacterial form, whose structure has been determined, points at peculiar structural features that are consequential for these enzymes across species[60,28,61,4,5].
In clinical studies, noteworthy advance has been made concerning the immunology and immunopathology of leprosy, the genetics of human resistance, mechanisms of nerve unhealthiness, and chemotherapy. In nearly all of these areas, nevertheless, leprosy remains poorly comprehended compared to different leading bacterial diseases[14] and remains a clinically cardinal disease to this day.

1.2. Causes

The body's immune response to the antigens of the leprosy bacilli may create chances of inflammation in the skin and nerves, known as reactions. There are 2 different types of reactions: type 1 reaction or reversal reaction (RR) and type 2 reactions or erythema nodosumleprosum (ENL). Reactions may occur during multidrug therapy or it can also occur before/after the multidrug therapy and also they are the primary cause of nerve damage and impairment in leprosy[67,76,38]. Nerve damage occurs very slowly and oftentimes it remains unnoticed or may be it recognizes at a very late stage. So it shows the symptoms of a reaction which forces people to seek help[31,49].

1.3. Impact

Leprosy is most importantly a disabling disease. The WHO estimates around two to three million people all over the world because of disabilities to leprosy[77]. Usually people suffered from leprosy, especially because of those visual deformities and disabilities, dread discrimination and stigmatization. These people may have faced intense social and also some kind of psychological problems[29,55,39].

II Target Identification

Leprosy is an unceasing bacterial disease of the skin and nerves in the hands and feet and, in some cases, the lining of the nose[62]. Leprosy can be escalating, causing eternal damage to the skin,

nerves, limbs, and eyes[56]. The clinical symptoms of leprosy diversify but most of all it damages the skin, nerves, and secretion membranes[78]. The resultant of the muse shows that Acyl-CoA Synthetase protein sequence containing 579 amino acid residues with ID NO (Q9CD78) which plays a vital role in lipid metabolism[58]. It belongs to ATP dependent AMP-binding enzyme family. It activates fatty acids, which functions as signaling molecules and are a structural element of membranes[6]. It has one AMP binding domain **(Fig 1)**[58] since, deletion and decay of the gene of Acyl-CoA Synthetase causes removal of numerous grave metabolic activities[12] and it can also be used as a good target to have a good control of that disease.

III Sequence homology and Conserved domain search

Acyl-CoA Synthetase protein describes the evolutionary relationship with Long Chain Fatty Acid CoA Synthetase[40]. It has been found that both are homologous and further the structural properties of Fatty Acyl CoA can be used as a reference for the study of Acyl CoA. The Pfam[65] results demonstrate AMP-binding domain (40-491), which helps, in catalytic commotion of the protein. This part consists of SER/Thr/Gly colorful area and can be analyzed again by a conserved Pro-Lys-Gly triplet. **(Fig. 1)**[58] the enzymes family consist of Acetyl CoA Synthetase, luciferase and different added intimately kindred Synthetase[58]**.**

Fig.1- showing the domain of target protein[58]

Pfam[23] database search revealed one AMP-binding domain. **(Fig 2)**[46] shows multiple sequence alignment of *M.tb* FadD13 with *E. coli*, *FadD*and*ttLC-FACS*, which reveals that there are 3 conserved regions: out of them 2 are ATP-AMP binding domains, residues from 163– 173 are referred as P-motif, 300–306 called as A-motif and one fatty-acid binding domain, residues 375–399 known as FACS signature motif. These domains are conserved within the super family of adenylate forming enzymes. The predicted model for *M. tb*FadD13consists of 2 domains—**a]** a large N-terminal domain (residues 1–395) and **b]** a small C-terminal domain **(402–503)** which are further connected by a six-amino acid peptide linker, i.e. the L motif (residues **396– 401).** Secondary structure of the model was analyzed by iMolTalk[15], which describes that the structure contains 12 α-helices, eight 3_{10} helices and 26 ß-strands **(Fig.3a,3b).** This particular protein represents to the family of adenylate-forming enzymes and also shows the presence of an A-motif **(adenine-binding site; residues 300– 306**) and P-motif **(phosphate-binding site; residues 163–173)** which forms the AMP/ATP binding domain, as it is demonstrated by Q-Site Finder[41]. An additional conserved region of 25-amino acid long segment, a fatty-acid binding region **(residues 375–399; FACS signature motif)**, which is similar to the family of FACS, and CASTp[16] has been used to predict the given binding region.[46]

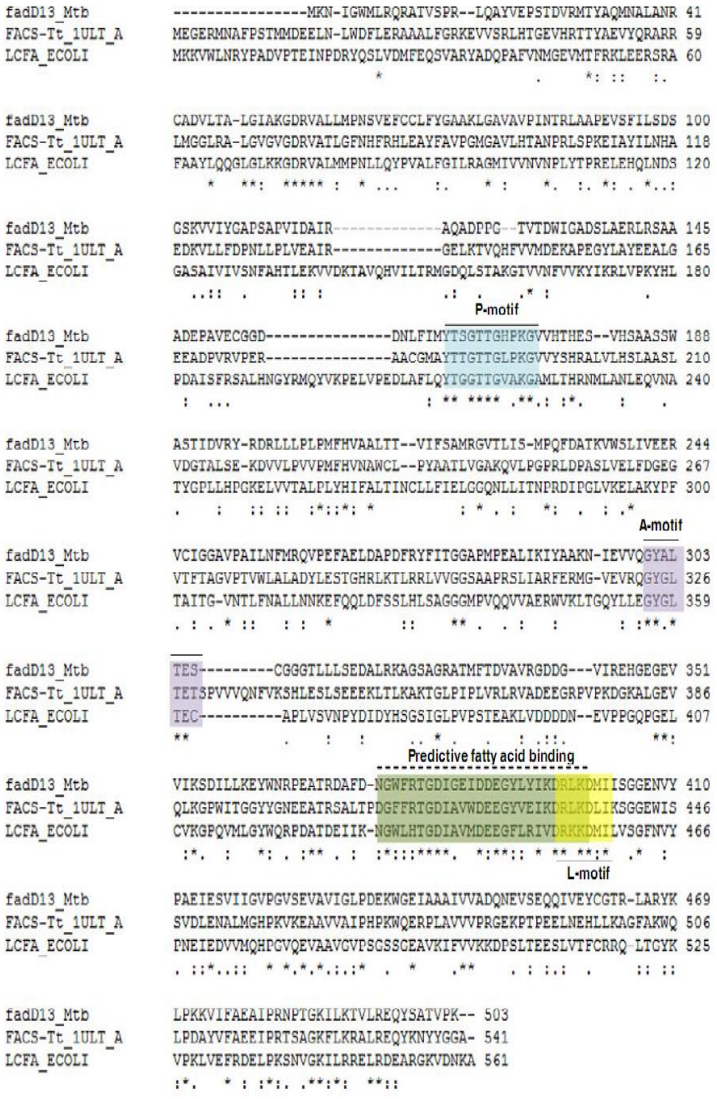

Fig.2- Multiple sequence alignment of *M. tb* adD13 with *E. coli* fadD and *ttLC-FACS.* The identical residues in the aligned sequences are indicated with an asterisk (*). P-motif is phosphate-binding site colored in blue, A-motif is adenine-binding site colored in purple, L-motif is linker motif colored in yellow and fatty acid binding site is indicated in green. [46]

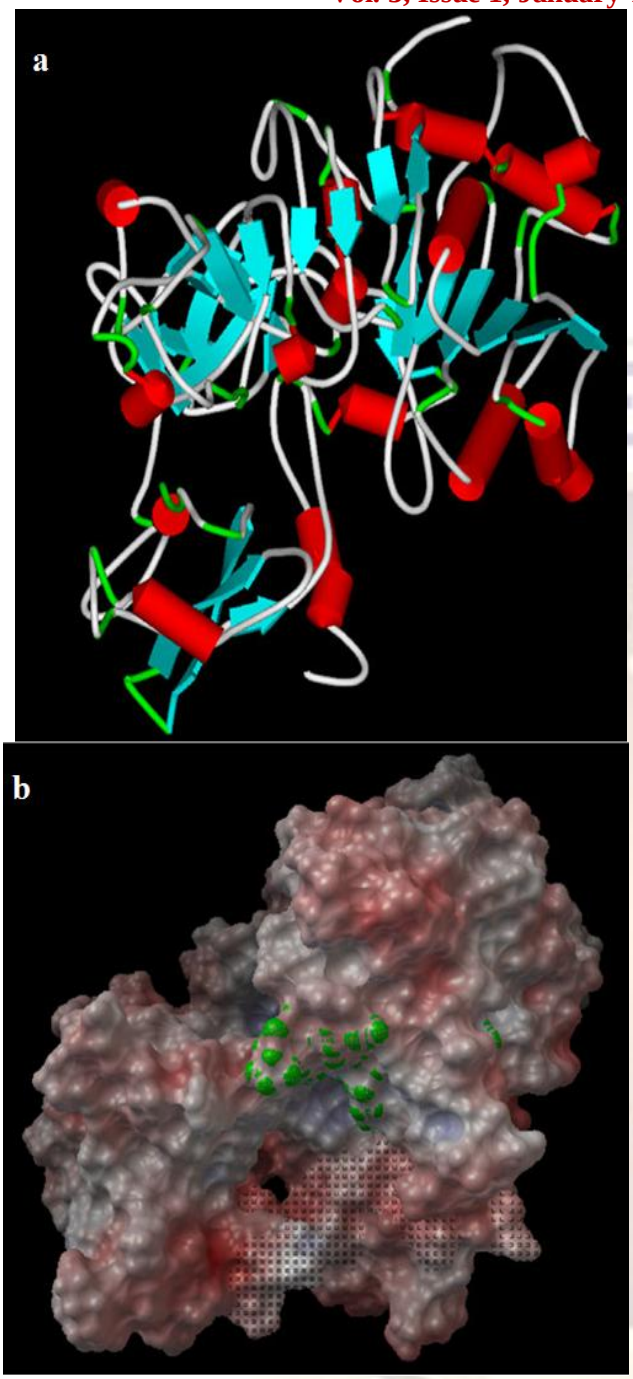

Fig.3-Three-dimensional model of *M. tb* FadD13 a) Schematic representation of *M. tb* FadD13. Red colour cylinders represent α-helix and blue arrows represent β-sheets. N and C terminals are represented in white colour.[46] b) Electrostatic potential surface map of the protein with the A-motif, P-motif and fatty-acid binding site. Positive potentials are shown in blue, negative potentials in red, neutral in white and ligand in green.[46]

IV Homology Modeling

The vital aspiration of homology modeling is to predict a structure from its sequence with an accuracy which will be equivalent to the most excellent results achieved experimentally[20]. This would let the users to carefully use the rapidly generated ***in-silico*** protein models in all the contexts where today only experimental structures provide a solid basis: structure-based drug design, analysis of protein function, interactions, antigenic behavior, and rational design of proteins with increased stability or novel functions. In adding together, protein modeling is the merely way to obtain structural information if any how experimental techniques fail, sometimes due to proteins are simply too large for NMR analysis and cannot be crystallized for X-ray diffraction[58]. For homology modeling first the target sequence was retrieved from the database (Table1), then BLAST-p was performed against Protein Data Bank (PDB) and the highest scoring entry (high bit score and low e value) was taken as template **(Table1).** Finally the protein model was generated using modelingsoftwares**(Table1).**

Table-1 – The targets, templates and softwares used for Homology Modeling[46,58]

Target	Sequence ID	Template	Software	Reference
Acyl CoA Synthetase	Q9CD78 (Swiss-Prot)	1V26	Swiss Modeller	SuhanyaRamamoorthi, S. Venkatesh
Fatty Acyl CoA	CAA16147	1ULT	Rokky-P	NidhiJatana et. al

4.1.SWISS-MODEL

(http://swissmodel.expasy.org) is an automated comparative modeling server basically to predict the three dimensional (3D) protein structures[32]. SWISSMODEL provides several levels of user interaction through its World Wide Web interface: in the 'first approach mode' only an amino acid sequence of a protein is submitted to build a 3D model[68]. Template selection, alignment and model building are done completely automated by the server. The reliability of SWISS-MODEL is continuously evaluated in the EVA-CM project. ROKKY-P[57] a server for De novo structure prediction by the simfold energy function with the multi-canonical ensemble fragment assembly. According to the result generated from various protein structure evaluation servers, model 3 generated by Rokky-P was found as the best model **(Table 2)**[46].

Table No.2 - Quality assessment of the models obtained by various protein structure prediction servers[46]

Structure prediction server	**PROCHECK[a]**	**Verify 3D[b]**	**WHAT IF[c]**
Modeller	65.4%	43.06%	-4.822
Prime	79.10%	89.27%	-3.270
SWISS-MODEL	83.10%	93.83%	-2.121
Rokky-P-Model 1	86.60%	87.50%	-0.387
PHYRE	88.3%	87.42%	-1.549
Rokky-P-Model 5	90.50%	76.59%	-0.619
Rokky-P-Model 2	90.50%	88.69%	-0.143
Rokky-P-Model 4	90.80%	86.71%	-0.143
Rokky-P-Model 3	**91.50%**	**88.49%**	**0.266**

[a]Percentage of residues in the most favoured region
[b]Percentage of residues having 3D-1D score >0.2
[c]Ramchandran Z-score, Z-values above 4.0 and below -4.0 are very uncommon

V Structure Visualization:-

As an ensue of Swiss-PDB Viewer, we can predict the 3-dimensional structure of the protein Acyl CoA Synthetase**(Fig. 4)** based on the homologous protein structure Long Chain Fatty Acid CoA Synthetase whose 3-dimensional structure is already known either with the help of X-ray Crystallography or NMR. The protein contains the AMP binding site as template.[58]

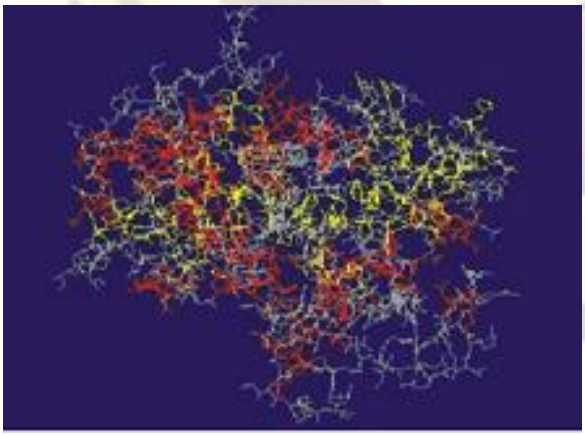

Fig.4- Shows the predicted structure of the protein Acyl-CoA Synthetase (Red – α helix, Yellow - ß Sheet) [58]

VI Evaluation and assessment of generated model:-

The modeled protein which is built on the basis of 1v26 B-Chain protein describes 83.2% of residue in the most favoured region of Ramachandran plot **(Fig. 5)**[58] when it was evaluated with a tool called Structure Analysis and Verification Server[47] it shows that this very structure could be used as a good target model for the design of drug.[58]

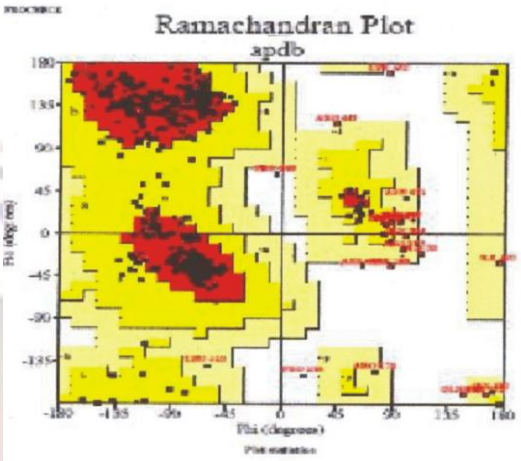

Fig.5- Shows the Ramachandran plot of the protein[58]

Ramachandran plot of the given model describes that 99.8% of the residues lie in the allowed region as shown in Fig. 6 with only 1 residue is available in disallowed region for the same structure. The VERIFY-3D[42] analysis is used to show the compatibility 3D-1D score >0.2 to be 99.40% corresponding to acceptable side chain environments. ProQ[79] also gave a very good LGScore of 6.03 and a most importantly MaxSub of 0.17 for the model while ERRAT[13] showed the overall quality factor to be 79.59% for the model. The 'what-if quality[70] report' results summarized in **(Table 3)** indicate that the best sophisticated model showed a Z-score of -2.16 which shows that it is a suitable range for a valid structure. The Z-score of < =-5.0 denotes a poorly refined molecule[46]**.**

Table 3- What-if quality report (Z-score) for the initial model of FadD13 before performing the MD simulation and for the final model of *M. tb* FadD13 refined by the MD simulation[46]

	Backbone-backbone contacts	Backbone-side chain contacts	Side chain-backbone contact	Side chain-side chain contacts	Z-score for all contacts
Initial model	−1.25	−3.02	−2.7	−3.3	−3.3
Refined model	−2.24	−0.92	−1.6	−0.8	−2.1

What-if *Fine packing qQualitycControl*report. Average values of the Z-score for all contacts of the protein can be read as follows: -5.0≤Z-score (guaranteed wrong structure) <−3.0≤Z-score (probably good structure) <−2.0≤Z-score (good model)

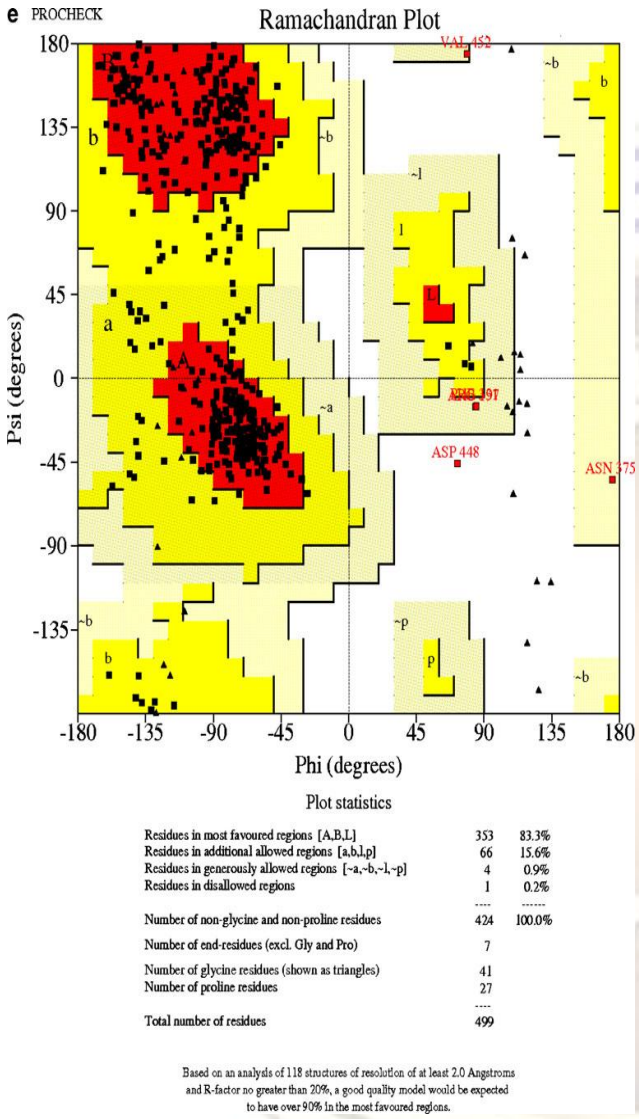

Fig.6 – Shows the Ramachandran plot of the final model of the protein[46]

VII Active Site Prediction

Binding site was characterized by using Q-Site Finder[43] and CASTp[16] and these were validated by using the information on binding sites in other homologous proteins.[8,30].

Putative Active Sites with Spheres, universally known as PASS[52] is used to predict the active site as shown in the **(Fig 7),** which could be used as the possible docking site for the newly developed ligand.[58]

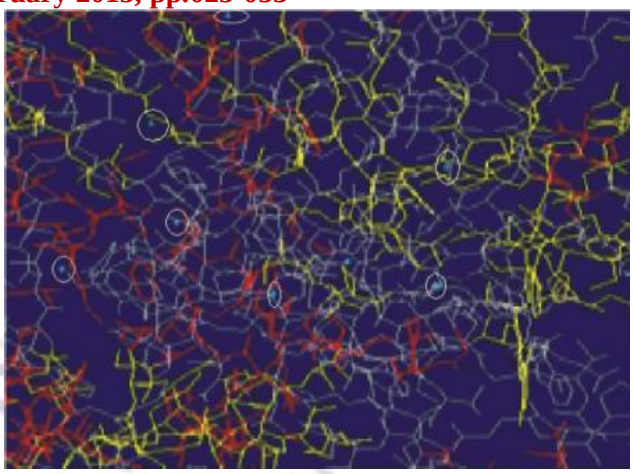

Fig.7-Blue Colour circled Spots represents the predicted Active Site in the target protein[58]

VIII Molecular Docking

A drug named as 4 - ((4 - amino 3 chlorophenyl) sulphonyl) phenyl amine **(Fig 8)** has been generated using the NCI Enhanced Server[26], as an analogue of the first line drug, dapsone and it has also been predicted that the drug have Anti-Myobacterial action which could serve as the ligand. This particular drug was also analyzed by effectuation of Christopher Lipinski's rule-of-five[69], which confirms that the designed ligand has the properties and structural features that make molecules much or less like a drug.[58]

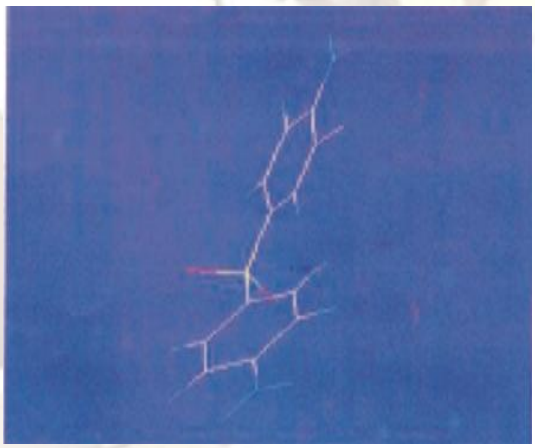

Fig.8- Shows the generated ligand 4 - ((4 amino 3-chlorophenyl) sulphonyl) phenylanmine[58]

The ensue provided by Hex[17] shows a quite fine docking between the ligand and the target protein and they are interpreted on the basis of binding distance which is measured to be 5.034 Angstrom between the ligand and the active site **(Fig 9)** with respect to the Tyrosine while Dapsone shows the distance of 6.052 Angstrom with respect to the same amino acid. Hence this proves that reduction in distance between the target protein and ligand increases the docking effect between the target protein and its respective ligand.[58]

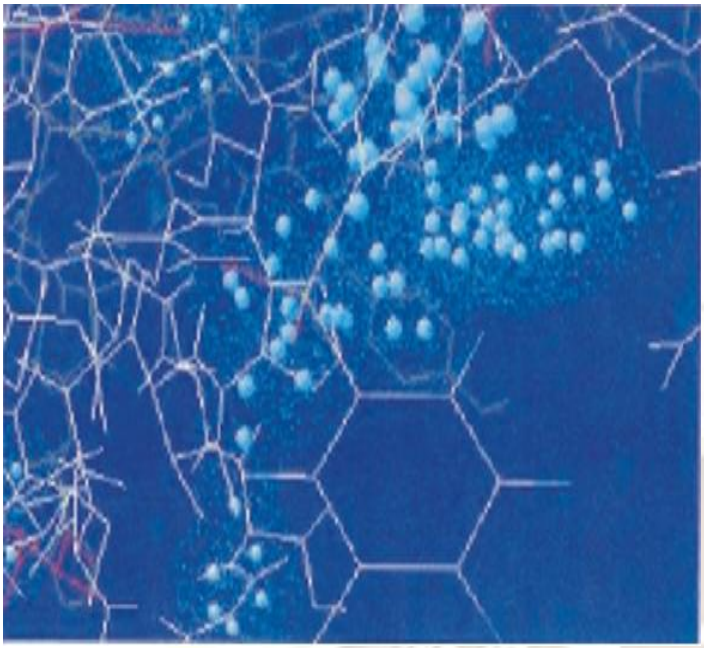

Fig.9-Showing the docked ligand to the target protein[58]

Many of the substrates like ATP, CoA and various fatty acids were docked to *M. tb***FadD13** by the help of IFD (Induced Fit Docking) protocol of Schrödinger[63]. ATP and CoA gave the best XP Gscore, in terms of kcal/mol. The fatty acids binded to M. tb FadD13 in an order of decreasing binding: cerotic acid>lignoceric acid>palmitic acid>capric acid according to their scores. M. tb FadD13 has higher affinity for very long chain fatty acids especially cerotic (26:0) and lignoceric (24:0) acid as compared to palmitic (16:0) / capric (10:0) acid as also observed through experimental studies[36]. Docking had also been carried out with other different ligands in the following order: ATP, fatty acid (lignoceric acid) followed by CoA **(Fig. 10a)** and after that the docked complex was further refined using Desmond 2.2[9]. The key amino acids interacting with the substrates were identified as: Gly[166], Lys[172], Thr[304], Glu[305], Thr[485],Lys[487] forming hydrogen bonds with ATP, Tyr[362] and Asp[371] with fatty acid and Thr[167], Thr[168] ,His[170] and Tyr[362] with CoA as analyzed by LIGPLOT[80]**(Fig. 10b)**.[46]

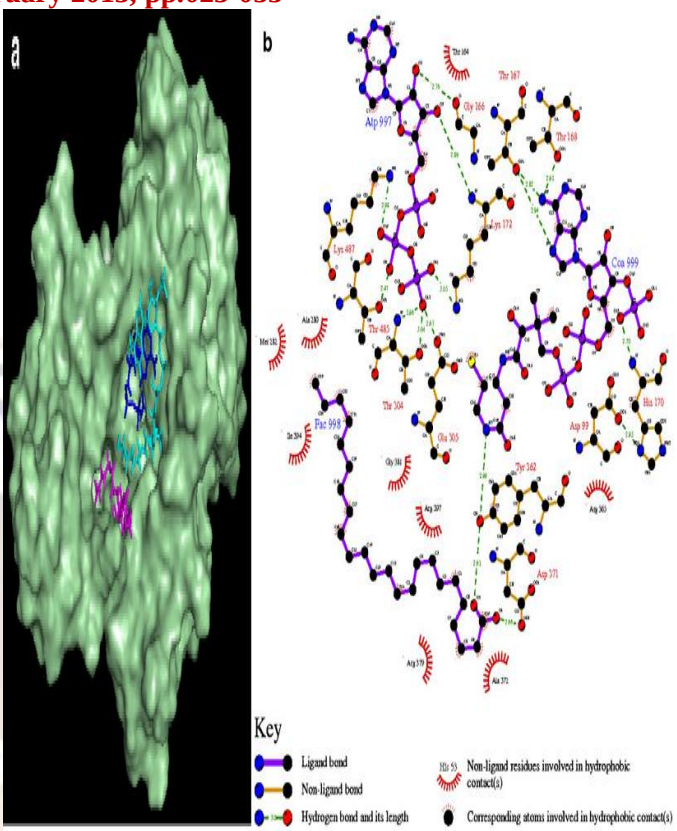

Fig.10- Docking of multiple ligands (ATP, fatty acid and CoA) to *M. tb*FadD13 by using induced fit docking. A) *M. tb*FadD13 docked with ATP, lignoceric acid (24:0) and CoA with lignoceric acid shown in pink colour, ATP in purple and CoA in blue. B) Ligplot showing the protein-ligand interactions in *M. tb* FadD13 complexed with ATP, lignoceric acid and CoA. ATP is represented by Atp 997, lignoceric acid by Faa 998 and CoA by Coa 999.[46]

IX Molecular Dynamics

As the results have obtained from different protein structure evaluation servers, out of them model 3 generated by Rokky-P was preferred as the concluding model **(Table 2)**. Desmond 2.0[9] was used for the further molecular dynamics simulation of the final model for a period of 12 ns. Frames were collected after every 1 ns, energy minimized and after that it was evaluated with various protein-evaluation servers. The total energy reaches equilibrium by 10 ns as shown by the stratagem of the total energy versus MD. After a small rearrangement from the initial conformation, the structure is relatively stable during the whole MD as shown by the RMSD map analysis during the 12 ns MD simulation **(Fig 11a)**. Ultimately the final model obtained was evaluated by the ProSA[81] program which examines, whether the interaction of each residue with the remainder of the protein is maintained in a favorable manner **(Figure 11b)** shows that ProSA[81] of the desired model gave a Z

score within the acceptable range **(-10 to 10, good ProSA scores are negative and depend on length of protein)**. **Figure 11c** shows that the energy remains negative for almost all amino acid residues indicating the acceptability of the predicted model.[46]

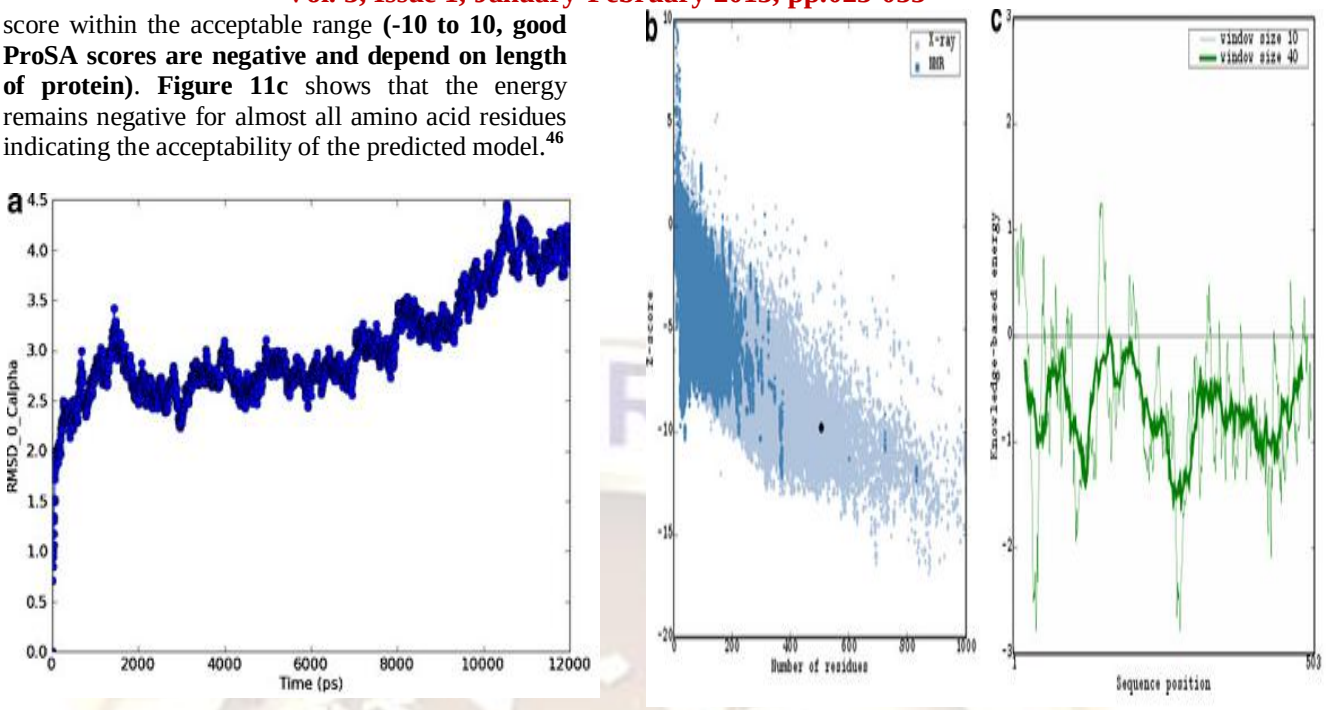

Fig.11- Analysis of the final model after molecular dynamics simulation a) RMSD plot of the MD simulation as a function of timescale.[46] b) z-plot of final model generated by ProSA. The z-plot shows only chains with less than 1000 residues and a z-score $\leq$ 10. The z-score of *M. tb* FadD13 is highlighted as large dot c) Energy plot of the final model obtained by ProSA.[46]

X Conclusion

The protein structure of Acyl CoA Synthetase of *Mycobacterium leprae* is predicted, this protein can be taken as drug target because it is responsible in fatty acid metabolism. Acyl CoA Synthetase shows homology with Fatty Acyl CoA Synthetase of *Mycobacterium tuberculosis* therefore we used this protein to assign its structural and functional properties to our target protein. Both of the proteins were modelled using different softwares and then validated. In Ramachandran plot both of them shows almost similar number of residues in the most favoured region. Multiple sequence alignment of FACS of *M. tuberculosis* with *E. coli* and *T. thermophilus* shows presence of conserved motifs, namely P-motif, L-motif, A-motif and fatty acid binding site; this indicates presence of these motifs in Acyl CoA Synthetase also. The active site of Acyl CoA Synthetase was predicted and also the derivative of dapsone was generated and was docked with Acyl CoA Synthetase, which shows more binding affinity of Acyl CoA Synthetase towards the newly formed dapsone derivative as compared to dapsone.

The FACS was docked with ATP, CoA and various fatty acids. The multiple ligands docking were also done in an order starting from ATP, fatty acid (lignoceric acid) and then by CoA, and as an outcome interacting residues were obtained. Finally MD simulation was performed to refine and validate the generated model. We hope that the validated model of the protein presented in this study will be a step forward towards the designing and development of novel drug targets against leprosy.

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