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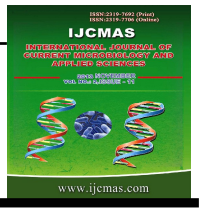

Original Research Article

3D Structure Prediction with Functional site identification of Fatty Acid Retinal binding (FAR) protein: A Target against Filarial Fight

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Keywords

FAR-Fatty Acid Retinal binding protein); Homology modeling; Ligand; Modeller; *Wuchereria bancrofti.*

A B S T R A C T

Lymphatic filariasis is a major and common tropical issue yet to be resolved. Unfortunately being the alarming situation; till date no satisfactory results have been observed in terms of its prevention and cure. Different preventive and curative approaches of bioinformatics are being in progress against this dreadful disorder. Drug designing and protein modeling is of course a basic need for new interventions where still better research is in demand. Hence adopting the bioinformatics approach towards filariasis protein target identification an initial and most important part is commenced by targeting FAR protein because of its strong conservation of the biochemical activities with significant role. Protein three dimensional (3D) structures are functionally very important at proteomic level. Thus in the current study 3D structure of FAR protein of *Wuchereria bancrofti* is modeled through comparative modeling by using Swiss model software. The resulting model consists of 87.8% of the residue falling in the most favoured regions. Prediction of functional site is important and related step hence in current course of work we also predicted putative functional site residues. Molecular model of FAR protein documented in this study may provide a valuable aid for designing an inhibitor or potential therapeutic aid against filariasis in near future.

Introduction

Lymphatic Filariasis a parasitic and infectious tropical disease, caused by thread-like parasitic nematode such as *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* (Anand *et al*., 2011; Gnanasekar *et al*., 2004; Lalitha *et al*., 1998; Dabir *et al*., 2006) is a second leading cause of long term problems with permanent disability (Ottesen *et al*., 1997). Filariasis is endemic in tropical regions of Asia, Africa, Central and SouthAmerica with more than 120 million people infected and one billion people at risk for infection with little or no mortality rate (Michael and Bundy, 1997). Basic

symptom is clogged lymphatic ducts lead to severe swelling of limbs and genitalia, as well as damage to kidneys and the lymphatic system itself. In the later stages of infection, the disease is characterized by a disfiguring condition known as elephantiasis resulting in physical disability and severe social stigma and psychological distress. Despite the severity of filarial disease and its impediment to progress in developing countries, research in this area is neglected and under-funded (https://www.neb.com/tools-and-resources /feature-articles). Still there are no preventive measures, while only drug treatments such as Ivermectin, Albendazole, and Diethylcarbamazine are available which targets the immature stages but not the long-lived adult worms (Richard *et al*., 2005). Hence there is a tremendous research scope in this area in relation with drug designing.

In current era of advance research technologies in computational approaches opened a new gate way for drug designing which is based on target selection and identification. These approaches are playing a significant role in resolving the solutions of many biological queries like in structural aspect of proteins, Insilico characterization, gene identification, phylogenetic studies, target identification etc. In terms of proteins, 3D structure is most important as it controls the functionality of complete protein. So for in continuation molecular modeling is first and almost important step for prediction of 3D structure of protein which is targeted for future study. In current course of study FAR protein is selected and targeted as it is evident through literature that it has strong conservation of the biochemical activities between the different parasite species, with different post-translational modifications which may relate to the biology of the larvae of pathogen (Garofalo *et al*., 2002). As this protein is present only in worm, not reported in human hence it can be used as a potential drug candidate against filarial disease (Altschul *et al*., 1990). It is well known that proteomic studies based on structural proteomics are playing a significant role in deciphering the many related issues with novel drug designing and therapeutic up gradations. Structural aspect of protein in 3D form is a very important and preliminary step in advance approaches towards therapeutic research based on proteomic approaches. Keeping it in current course of work, 3D structural prediction of FAR protein is being done by different computational approaches. 3D model structure of FAR protein of *Wuchereria bancrofti* is generated along with putative functional site prediction during the study by using comparative modeling approach.

Materials and Methods

Sequence Retrieval and Template selection

For modeling study our query is Fatty Acid Retinal Binding (FAR) protein sequence (Accession No AAL33794.1) from Gene Bank that is 159 residue long mature peptide for which template protein is 2w9y (A).

Physicochemical characterization

Number of hydrogen bonds, helices, strands, turns and theoretical Isoelectric point (pI), Molecular weight, Number of positive and negatively charged residues, Extinction coefficient, Instability Index, Aliphatic Index and Grand average hydropathicity (GRAVY) of the FAR protein were analyzed by using the

Expasy's protparam server (Gasteiger *et al*., 2005). (Table2).

Modeling of the structure

Comparative modeling was performed by Swiss model to generate putative 3D model of targeted protein. The Swiss model performs modeling via sequence alignments for selection of the putative template protein to generate the 3D model of query sequence (Arnold *et al*., 2006; Schwede *et al*., 2003; Guex *et al*., 1997). CHIMERA is used for energy minimization as well as for the visualization of predicted model (Thomas *et al*., 2005). Verification and validation of predicted 3D structure was done by QMEAN Server, PROCHECK, Ramachandran plot and visualization of predicted structure is progressed by Chimera tool.

Active site prediction

Structure based functional site prediction is corrected out by using the PROFUNC and Q-SITE FINDER software's (Laskowski *et al*., 2005; Alasdair and Richard, 2005)

Result and Discusison

Modeling of the structure

3D Model of our query protein (FAR) was generated by the Swiss model and visualization is done by Chimera. It is used to generate the best 3D model structures of query protein (A/C no AAL33794.1) based on template protein 2w9y (A) with good sequence identity and X-ray resolution and The model that satisfies, all the validation criteria based on the following programs as PROCHECK, CHIMERA is presented in (Fig.1). The Ramachandran plot analysis (Fig.2) and Residue properties (Fig.3) of the FAR protein performed by PROCHECK. The Ramachandran plot analysis (Fig.2) and Residue properties (Fig.3) (by PROCHECK) of the FAR protein model structure showed that of the 87.8% of the residues Ψ / Φ angles falling in the most favoured regions, 11.5% in the additional allowed regions and 0.0% Residues in generously allowed regions. Only 0.8% residues in the disallowed regions were present.

Quality assessment of the model via QMEAN Z score of (-1.04) revealed that the QMEAN4 score is a composite score consisting of a linear combination of 4 statistical potential terms (estimated model reliability between 0-1) (Benkert *et al*., 2011). The pseudo-energies of the contributing terms are given below together with their Z-scores with respect to scores obtained for high-resolution experimental structures of similar size solved by X-ray crystallography (Fig.4) (Table.1). This score gives authenticity about good quality of model generated in this study. Energy minimization was performed by Chimera which optimized the model structure FAR from initial energy -6782.240582 KJ/mol to final energy -8324.893009 KJ/mol. The result of comparative structural analysis shows that this model is the best structural model for FAR protein of *wuchereria bancrofti* because it shows maximum residues (87.8%) in the favoured region with minimised energy -8324.893009 having -1.04 QMEAN Z score.

Active site residues

Functional site prediction is integral part of this study as it is a basic priority during

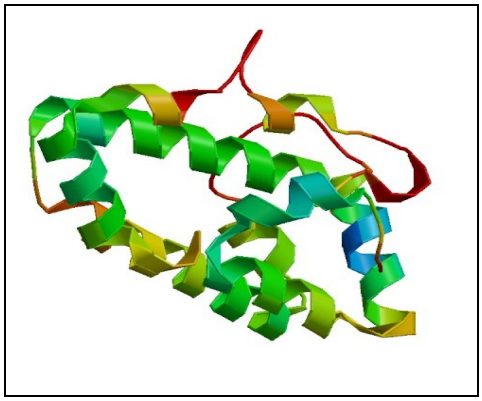
Table.1 The pseudo-energies of the contributing terms with their Z-scores with respect to scores obtained for high-resolution experimental structures of similar size solved by X-ray crystallography**.**

Scoring function term	Raw score	Z-score
C_beta interaction energy	-45.35	-1.47
All-atom pairwise energy	-3949.25	-0.51
Solvation energy	-15.19	0.38
Torsion angle energy	-20.37	-1.49
QMEAN4 score	0.698	-1.04

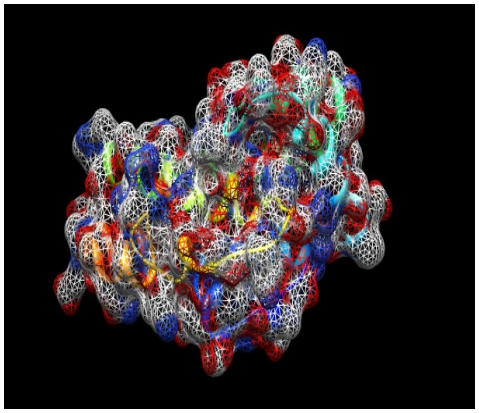
Table.2 Physicochemical parameters of FAR protein

Name of Protein	Isoelectric point(pI)	Molecular Weight (MW)	Negatively charged residues (-R)	Positively charged residues (+R)	Extinction coefficient (EC)	Instability index (II)	Aliphatic index (AI)	GRAVY
FAR protein	6.20	18031.6	29	28	5960	45.16	89.12	-0.543

Figure 1: Modelled 3D structure of FAR protein showing (A) Helices and sheets (B) Hydrophobicity surface.



(A)



(B)

Figure.2 Ramachandran plot of the Ψ/Φ distribution of the FAR model as obtained by PROCHECK. Showing 83.3% residues are in most favored regions and 13.3% additional & 2.7% generously allowed regions

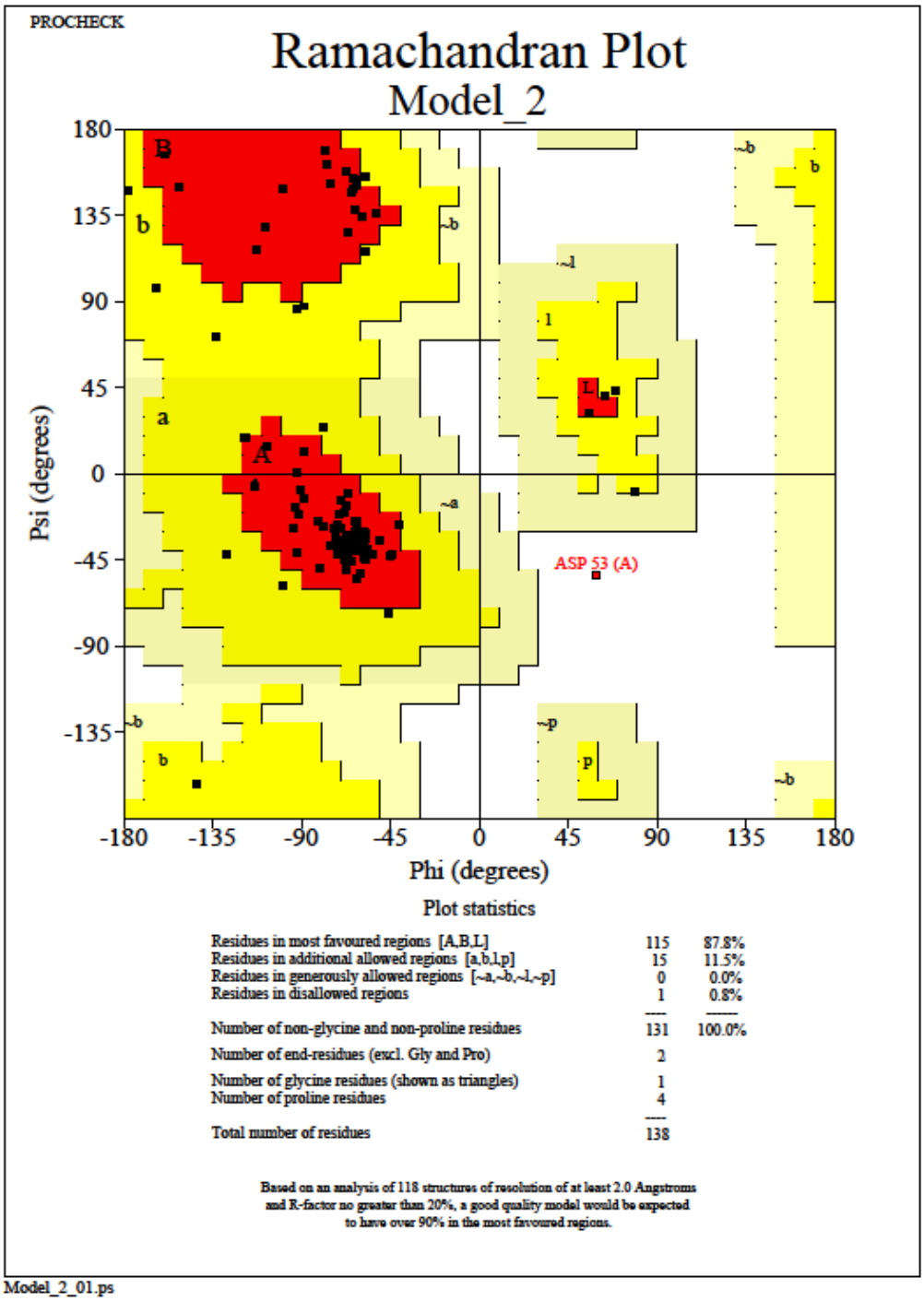
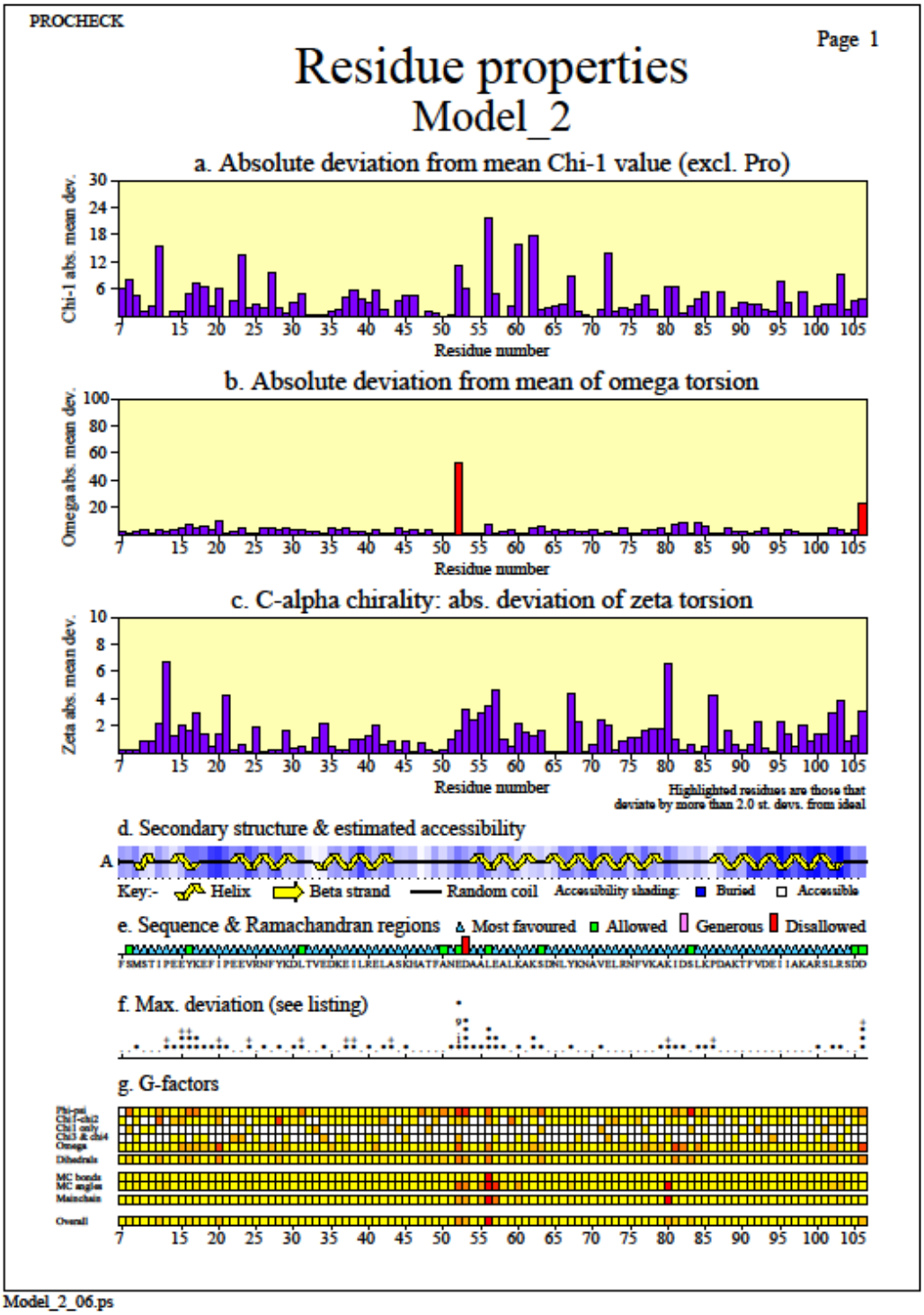
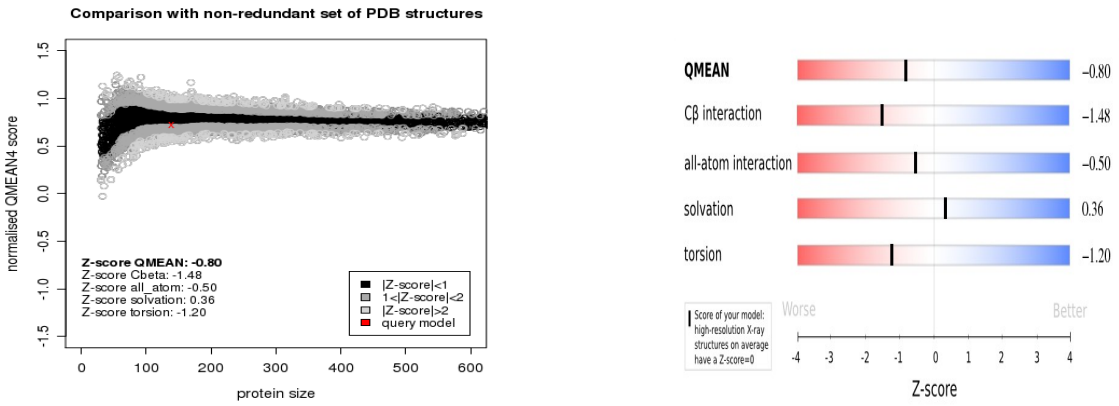


Figure.3 Residue properties of FAR model as obtained by PROCHECK



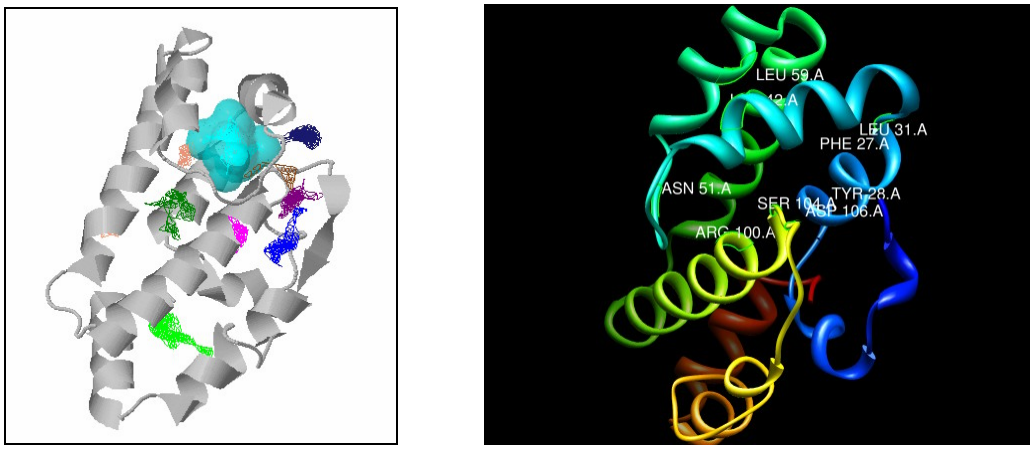
Model_2_06.ps

Figure.4 Estimated absolute model quality of FAR protein (A) Score components of FAR protein (B) developed by swiss model



(A) **(B)**

Figure.5 Showing predicted active sites by Q-SITE FINDER (A) Active site residues of FAR protein by chimera (B).



(A) **(B)**

protein ligand interaction. Servers such as Profunc and Q site finder which illustrated the putative active sites and active site residues in the model structure are used for this analysis. These servers analysis resulted the following putative functional site residues of the targeted Fatty Acid Rational Binding Protein are ASN51, PHE27, TYR28, LYS29, ALA55, LEU59, ARG100, LEU31, SER101, ASP106 and LEU42 (Fig.5).

Insilico study of protein is helpful in almost all research fields including protein structure prediction. The very basic assumption of protein biology i.e. structure to function analysis reveals that these structural information may give us a

prediction about the functionality of proteins. This structural based functional site prediction method enumerates the putative amino acid residues from our protein models. It illustrates that these above mentioned identified residues are involved in making ligand binding site pockets is indicative that these residues play active role in drug/ligand binding activity. Model development and functional site prediction of this protein will give great focus on understanding the role of amino acids and helps in deciphering the involvement of these residues during mechanism of protein ligand interaction. A molecular model of FAR protein of filarial disease as predicted in this study may provide a valuable aid in selecting any inhibitor or better ligand molecule against filariasis disease and could play a significant role as a future target for drug designing. This predictive approach may become applicable for novel drug developmental studies against this lymphatic disorder (Manisha *et al*., 2012). Till the date only symptomatic drugs are available for filarial disorder which is sometime showing severe side effects also. Hence it is apt to say that there is a need for better drug molecule against this dreadful disorder. Such kind of research orientations and these novel findings can give a new light of fight against this disease.

Generated model can be strongly and effectively used to analyze structural information and can be further implemented in future drug designing. This model further may provide new insights regarding the biology of the protein with respect to its function and can give new hopes and insights in relation to new aspects of drug designing against frightened filiariasis.

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