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# An Insight into Structural and Functional Characteristics of *Plasmodium falciparum* Farnesyltransferase (PfFT) 3d7: Comparative Modeling and Docking Studies

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## Abstract

A three dimensional model was developed for *Plasmodium falciparum* Farnesyltransferase (PfFT) that has not been solved empirically by X-ray crystallography or NMR. Homology modeling was used for the structure prediction using farnesyltransferase protein 2ZIR 2.4 Å (Rat), and 1JCQ 2.3 Å (Human) a moderate-resolution X-ray crystallography structures. The former showed 36% and the latter, 35% sequence identity with the target PfFT protein. The 3D-model was generated by using modeler in Discovery studio 2.5. The energy refinement was also been carried out in the modeler protocol. The validation study showed 88.2% of the residues (750) to be in the favourable region. In addition binding affinities of some inhibitors were investigated against the modeled protein using GOLD. The results showed that Arg-551, Tyr-824, Tyr-600 were found to be among the interacting residues of PfFT with many of the inhibitors. Our findings could be helpful for the design of new and more potent PfFT inhibitors. .

**Keywords:** *Plasmodium falciparum* Farnesyltransferase (PfFT); Homology modelling; Docking

## Introduction

Farnesyltransferase catalyzes the transfer of a farnesyl residue from farnesylpyrophosphate (FPP) to the thiol of a cysteine side chain of proteins, which carry at the C-termini the so-called CAAX-sequence. C represents a cysteine which side chain is farnesylated; A, amino acids which normally, but not necessarily, carry aliphatic side chains, and X mostly methionine or serine (Fu and Casey, 1999; Wittinghofer and Waldmann, 2000; Bell, 2000). Farnesyltransferase has been identified in different parasites pathogenic to humans, for example in *Plasmodium falciparum* (Chakrabarti et al., 1998; Chakrabarti et al., 2002). *P. falciparum* is a target of particular importance, which is responsible for 300-500 million clinical cases every year, and 1-3 million deaths (Sachs and Malaney, 2002; Ridley, 2002).

In eukaryotic cells, protein farnesyltransferase (PFT) transfers a 15-carbon farnesyl group from farnesyl pyrophosphate (FPP) to the C-terminals of selected proteins which include Ras GTPase, whereas protein geranylgeranyltransferase-1 (PGGT-1) transfers a 20-carbon geranylgeranyl moiety to the g-subunits of heterotrimeric Gproteins (Yokoyama et al., 1992). The inhibition of these enzymes in mammalian cells is exploited in cancer chemotherapy (Lobell et al., 2001). The genome sequence data of *Plasmodium falciparum* indicates the absence of PGGT-1 in the parasite (www.plasmodb.org). This has led to the investigation of P. falciparum PFT (PfFT) as potential target for the development of new antimalarials (Ohkanda et al., 2001; Wiesner et al., 2004; Carrico et al., 2004; Glenn et al., 2005; Ryckebusch et al., 2005; Kettler et al., 2005; Glenn et al., 2006).

Homology modeling has been recently used in the generation of reasonable 3D models of various lipoxygenases which were in turn successfully used in various structure based drug design strategies (Aparoy et al., 2008; Kenyon et al., 2006; Reddy et al., 2008). The root mean square deviation (RMSD) of the model with respect to Cα atoms of the template was measured using the Combinatorial Extension (CE) method (Shindyalov and Bourne, 1998). In this study, we attempt to construct a model structure for human PfFT protein by taking the crystal structure of farnesyltransferase protein 1JCQ 2.3A (Human) and 2ZIR 2.4A (Rat) as templates, which are having same catalytic dominies and analyse the template protein structures by ramachandran plot in Discovery studio 2.5 and found zero residues in disallowed region. 3D-profiles verification, Procheck and alignment of structures to calculate RMSD (Aparoy et al., 2008) were performed as validation methods. The 3D structure of PfFT is subsequently used to carry out the binding site studies and to perform docking studies against the modeled protein with different active inhibitors (available in supplementary information) which were collected from literature (Bendale et al., 2007).

## Materials and Methods

### Identification of templates

All molecular simulations were performed on the HP workstation using Discovery studio 2.5, 2009 software from Accelrys, USA. The amino acid sequence of PfFT 3D7 was obtained from the protein sequence databank in the Swiss-prot or Uniprot_KB (Q8IHP6) at the site www.expasy.org (Gardner et al., 2002). The BLAST program against the Protein Data Bank (PDB) available at NCBI was used to select a template structure for homology modeling of PfFT 3D7. The crystal structure of farnesyltransferase protein 1JCQ 2.3 Å (Human) and 2ZIR 2.4 Å (Rat) were obtained from the PDB (Berman et al.,

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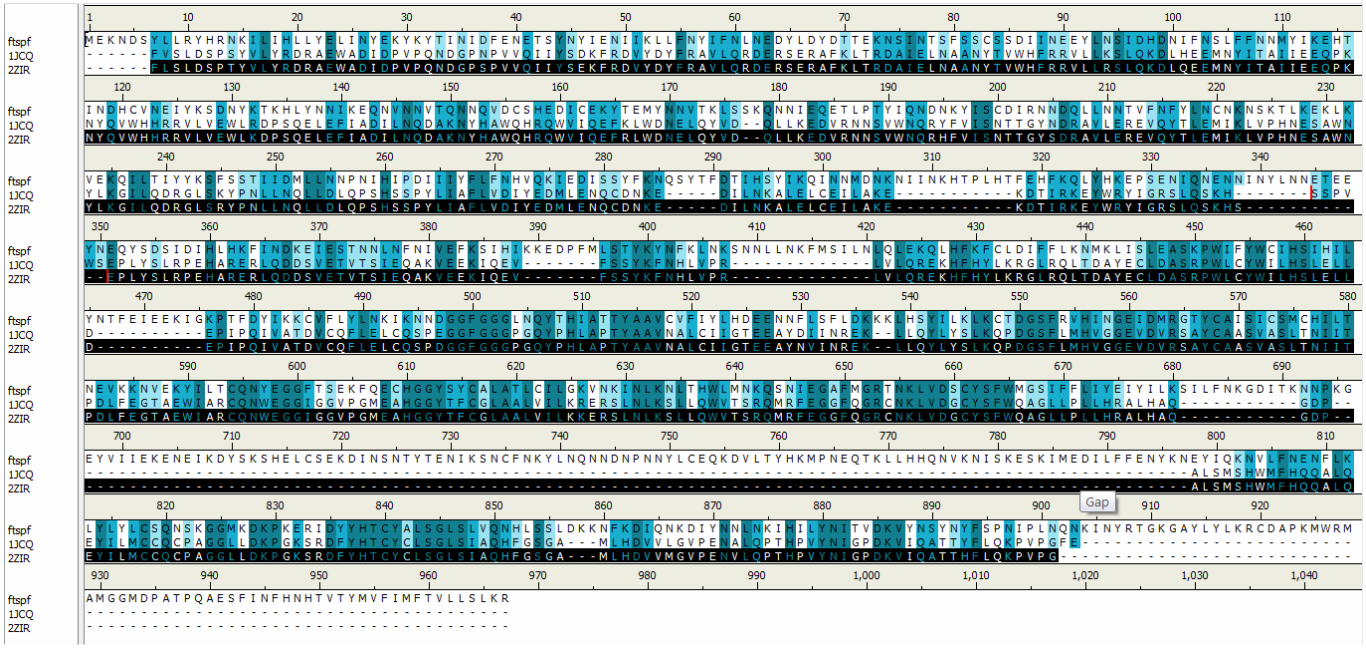


**Figure 1:** Sequence alignment of PfFT 3D7 and templates.

2002) database. From BLAST results 1JCQ showed 88% sequence identity and 2ZIR showed 68% sequence identity with target protein PfFT 3D7.

## Model construction

The first requirement in the construction of the PfFT structural model is the multiple sequence alignment between these templates and the target sequences. The sequence alignment is based on identifying structurally conserved regions (SCR) common to the template and target. The multiple sequence alignment was performed in the Discovery studio 2.5 sequence analysis protocol called "Align multiple sequences" (Wolf et al., 2009; Yona et al., 2000; Smith et al., 1994; Galtier et al., 1996). The three sequences, the two of templates and PfFT were given as input for the protocol which aligns them and gives the output as shown in the Figure1. The side chain refinement for the residues is done on the basis of the protein structure health report using the "side chain refinement" protocol.

Discovery studio 2.5 (MODELER) is used to construct the protein model for the target sequence, under protein modeling protocol cluster (Blundell et al., 1988; John and Sali, 2003; Fiser and Sali, 2003) implemented on HP systems with Linux (Redhat OS) work station. The output of 'align multiple sequences' given as input for this protocol to copy the coordinates of structurally conserved regions of template into the query sequence (PfFT 3D7). Three models were generated and the model showing the least RMS deviation with respect to trace (Cα atoms) of the crystal structure of the templates, 2ZIR and 1JCQ was saved for further refinement and validation. Energy refinement of the 3 models were done after they are built (Sali and Blundell, 1994; Greer, 1980) and this BFGS (Broyden-Fletcher-Goldfarb-Shanno) energy refinement method gives best conformation to the model. Ligand from 2ZIR copied into the query structure (Homology user guide). After the energy refinement the newly built model that is refined with all its conserved regions should be now refined at the loop regions (Fiser and Sali, 2003).

## Model validation

The quality of the refined PfFT structure Figure 4 obtained was checked with Profile-3D. The Profile-3D tests the validity of hypothetical protein structures by measuring the compatibility of the hypothetical structure with its own amino acid sequence (Profile-3D user guide).

The stereo chemical quality of the protein structures is examined by a Ramachandran plot created using the PROCHECK (Laskowski et al., 1993) program. The number of residues that are in the allowed or disallowed regions of the Ramachandran plot in Figure 2 determines the quality of the model. Alignment of the modeled structure with the reference or template structures separately was performed as another validation method to test the reliability of the modeled structure. Two main issues need to be addressed to obtain an optimal structure alignment here. The first issue is the global optimization problem i.e., a large number of combinations of residues equivalent in 3D space has to be searched to determine the optimal solution. The second issue is the determination of a target function i.e., whether the alignment is optimized to align either the largest number of residues, or for the lowest RMSD. The similarity of a pair of proteins is calculated based on the coordinates of their Cα atoms.

## Docking studies

The binding site in the new model was identified and subsequently docked with some active inhibitors for studying receptor-ligand interactions. The docking studies were carried out with GOLD (Jones et al., 1997; Jones et al., 1995) (Genetic Optimization for Ligand Docking) which is a genetic algorithm for docking flexible ligands into protein binding sites. The modeled receptor protein is prepared by minimizing only the added hydrogens on side chain and backbone of the receptor molecule. The CHARMm force field is applied to the model and the hydrogens are minimized. An Input Site Sphere is specified for GOLD to determine the binding cavity. Input inhibitors that were selected from literature were given for docking. The scoring function used by GOLD, is defined by the following energy terms (Verdonk et al., 2003).

$$\text{GOLD Fitness} = S_{hb\_ext} + S_{vdw\_ext} + S_{hb\_int} + S_{vdw\_int}, \quad (1)$$

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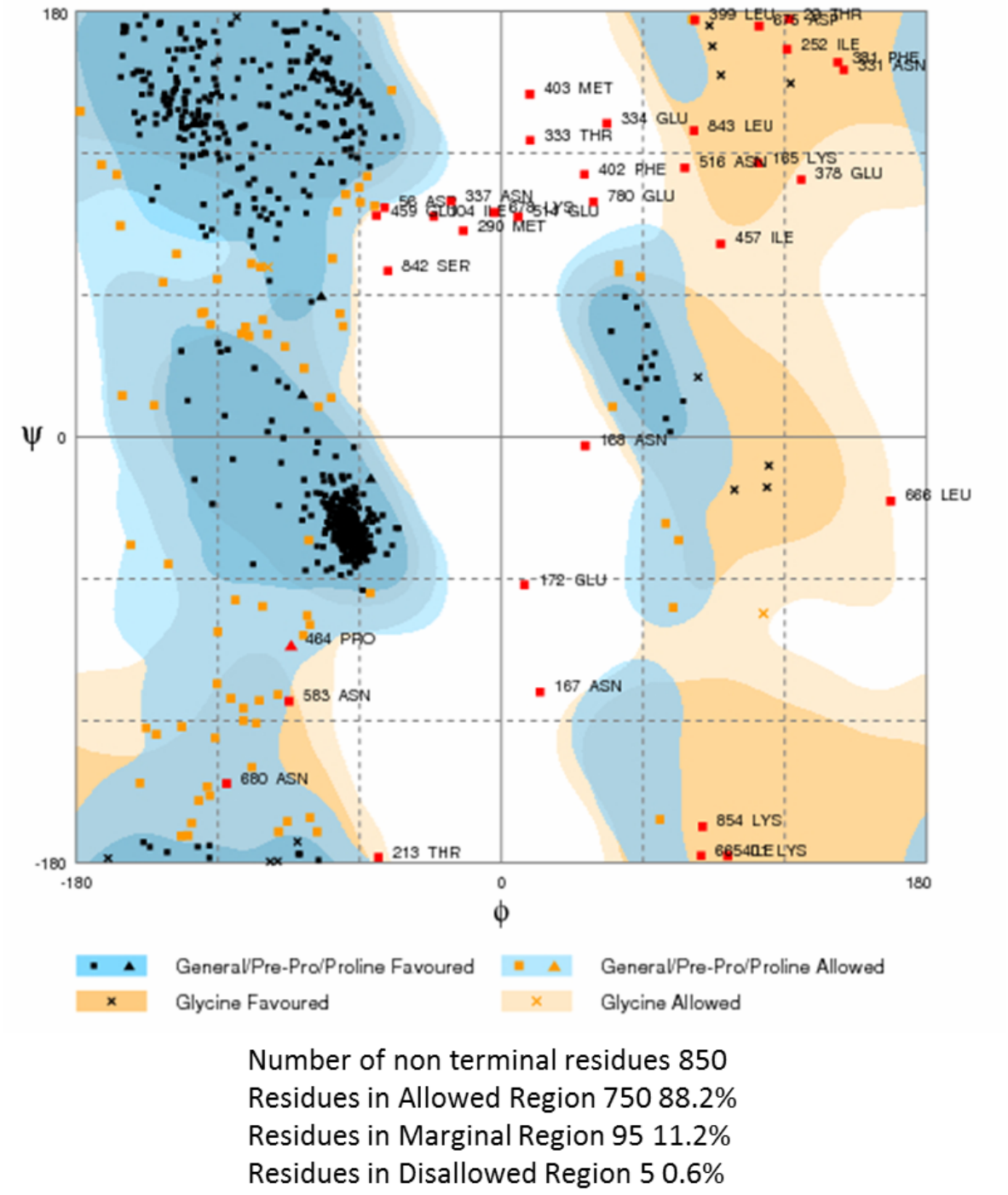


**Figure 2:** Ramachandran plot of modeled protein PfFT 3D7.

Where $S_{hb\_ext}$ is the protein-ligand hydrogen-bond score and $S_{vdw\_ext}$ is the protein-ligand van der Waals score. $S_{hb\_int}$ is the contribution to the Fitness due to intramolecular hydrogen bonds in the ligand this term is switched off in all calculations presented in this work (this is the GOLD default, and generally gives the best results); $S_{vdw\_int}$ is the contribution due to intramolecular strain in the ligand.

## Results and Discussion

The search for the best template for modelling of PfFT was carried out using blast against PDB. The rat protein farnesyltransferase (PDB id. 2ZIR) showed 36% identity and 55% similarity while human protein farnesyltransferase (PDB id. 1JCQ) showed 35% identity and 55% similarity. The multiple sequence alignment showed 18% identity and 36.5% similarity Figure 1.The initial model of PfFT was generated using modeler in Discovery studio 2.5. the initial model was also energy refined during generation itself by selecting the option 'true' in 'refine loops' parameter. The generated model was again subjected to loop refinement which will refine all the main chain confirmations

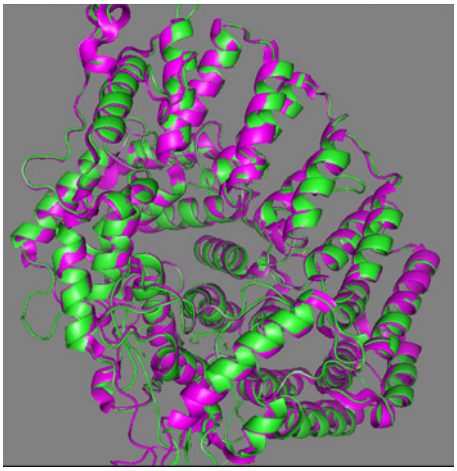

Figure 3a: Alignment between PfFT 3D7 (Green) and 1JCQ (Magenta).

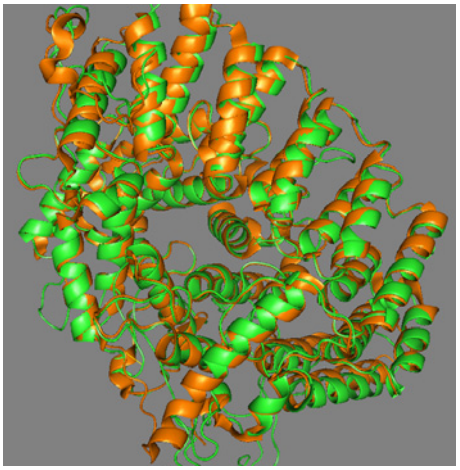

**Figure 3b:** Alignment between PfFT 3D7 (Green) and 2ZIR (Orange).

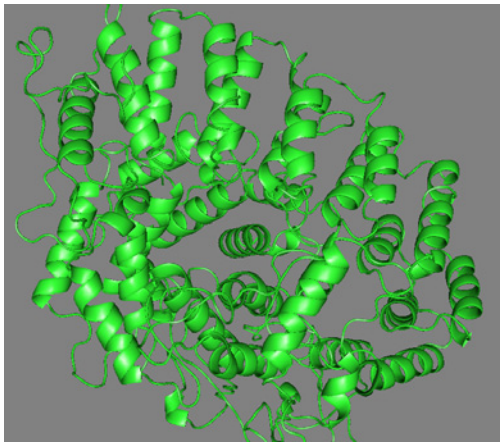

**Figure 4:** Alignment between PfFT 3D7 (Green) and 2ZIR (Orange).

of those residues lying in the structurally variable regions or loops. Later, the main chain part of loop refined model was subjected to side chain refinement so that side chain conformations of those residues lying in the structurally variable regions or loops were also refined. After initial validation, the 3D-profiles resulting score of the modeled protein was observed as 255.8 that lie between expected high score of 408.5 and expected low score of 183.836. The Ramachandran plot of the PfFT satisfied the tests with 88.2% of the residues in the most favoured regions, 11.2% in additional allowed regions and 0.6% in disallowed region, which are (> 10 Å) far from our binding site.

The compatibility between the active site of the PfFT homology model, and the 3D-structures of the templates were checked by superimposing the backbones of the three enzymes. The superimposition of modeled PfFT to that of 1JCQ and 2ZIR gives root-mean-square deviation (*rmsd*) values equal to 0.46 Å and 1.07 Å. Figure 3a, Figure 3b shows the alignments of modeled protein with templates.

Evaluation of the docking results was based on protein-ligand complementarity considering steric properties as well as calculated potential interaction energy in the complex and ligand intramolecular energy. All compounds interact with the PfFT active site through hydrogen bonds with the residues Arg-551, Tyr-824 and Tyr-600. Table 1 lists High active compounds with their docking scores. Gly 559, Trp 652, Tyr 554 and Cys 603 were some of the important amino acids present in the active site of the target. Figure 5a and Figure 5b

| Compound | ED50 | Docking score |
|---|---|---|
| 1 | 1.8 | 80.09 |
| 2 | 3 | 72.19 |
| 3 | 6.2 | 67.47 |
| 4 | 9 | 75.21 |
| 5 | 6 | 81.57 |
| 6 | 9 | 80.09 |
| 7 | 10 | 75.96 |
| 8 | 10 | 78.43 |
| 9 | 11.5 | 73.76 |
| 10 | 12 | 74.36 |

**Table 1:** High active compounds with docking score.

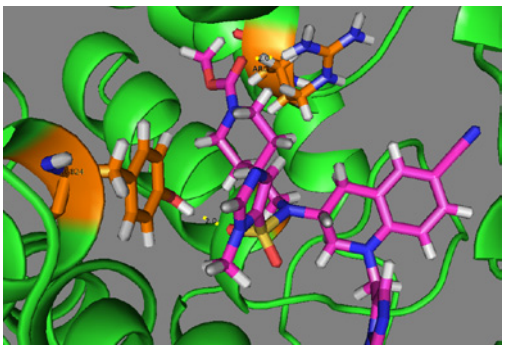

**Figure 5a:** Compound 1 shows H-bonds with Tyr-600,Tyr-824.

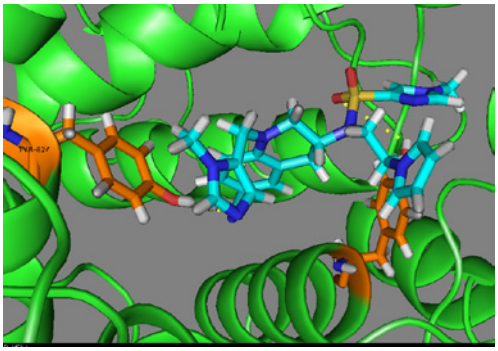

**Figure 5b:** Compound 2 shows h-bonds with Arg-551, Tyr-824

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show the docking poses of compound 1 and compound 2 with the modeled protein.

## Conclusion

Even though the identity and similarity are low, the Modeled protein was found to be evidently reliable after running procheck and by finding out main chain RMSD between the target and template protein. The main aim is the identification, exploitation and analysis of new molecular drug targets at structural level. This computational approach will lead to the discovery and structural development of novel drug targets for PfFT 3D7.

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