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Three-dimensional structure of *Plasmodium falciparum* knob associated heat shock protein 40 predicted by homology modeling method

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Abstract
Malaria remains one of the world's widespread diseases and is responsible for enormous worldwide mortality. Increasing emergence of resistant *Plasmodium* strains impedes the efforts and strategies made to control and eradicate the disease. Cell surface structures termed knobs comprise pathogenesis related protein complexes which facilitate the presentation of major virulence factor 'PfEMP1' (*Plasmodium falciparum* erythrocyte membrane protein 1) on the erythrocyte membrane. A knob associated Hsp40 (heat shock protein 40) 'PFB0090c' is implicated in chaperoning knob assembly which underscore its importance in parasite biology. The present study aims to propose a three dimensional structure of PFB0090c. A homology model of complete conserved region including J domain, G/F region and C terminal substrate binding domain was derived based on multiple templates. We show that use of multiple templates increases the quality of the generated model and structure is not biased to a particular template used in homology modeling. The proposed structure of PFB0090c was evaluated for its quality using Verify 3D, Errat and Rampage programs. Since Hsps are considered probable anti-malarial drug targets, our proposed model for PFB0090c may help in structure based drug designing against lethal malaria.

Keywords: Malaria, *Plasmodium falciparum*, Heat shock proteins, homology modeling, PFB0090c

1. Introduction
Malaria is a major human health concern caused by protozoan *Plasmodium*. Among all species of human malaria parasites viz. *P. falciparum, P. vivax, P. malariae P. Ovale*, and *P. knowlesi*, *P. falciparum* is the major cause of severe malaria, and is responsible for high mortality rates associated with the disease [1]. The ability of intraerythrocytic *P. falciparum* to interact with endothelial receptors mediates the process of cytoadherence which is the key cause for the pathology of the disease [2]. PfEMP1 encoded by Var genes is the major virulent factor known to interact with endothelial receptors [2]. Extensive remodelling of host cell enables the survival of the parasite intracellularly, and promotes the pathophysiology of the disease [3]. Host cell remodelling includes modifications in the infected erythrocytes that are mediated by several exported proteins collectively known as secretome/exportome [4]. Formation of electron-dense protrusions termed 'knobs' at the surface of iRBCs is one of the key characteristic features of remodelling [5]. Knobs are mainly comprised of knob associated histidine-rich protein (KAHRP) that facilitates the presentation of PfEMP1 to the surface of iRBCs [5]. Gene knock out studies revealed that in addition to KAHRP, a type IV Hsp40 (PF10_0381), and a member of PHIST family (PFD1170c) are required for knob formation [3].
The life cycle of malarial parasite alternates between cold-blooded mosquito and warm-blooded human hosts. During its transmission to human hosts, the parasite faces heat shock (increase of more than 10 ºC) and is further exposed to temperature fluctuations during the febrile episodes that are the clinical hallmark of the disease [6]. Regular fever bouts have been shown to promote the growth and development of asexual intra-erythrocytic stages of the parasite [7]. It also enhances the ability of infected erythrocytes to adhere to vascular endothelium and promote the pathogenesis of the disease [8]. In order to cope with adverse conditions, the parasite extensively remodels the host cell that helps it to adapt and survive in the stressful environment. The Pf genome encodes numerous chaperones including members of heat shock protein families of ~40kDa, ~ 60kDa, ~70kDa and ~90kDa that are upregulated in response to stress caused by parasite infection [6]. As proteins tend to misfold/denature in response to stress, heat shock proteins function as molecular chaperones to facilitate their

folding in native conformations. Additionally, heat shock proteins are suggested to play cytoprotective role in the development of parasite in the human host [9]. Proteome analysis and loss of function mutant study revealed molecular chaperones specifically proteins of Hsp40 family to form a part of malaria exportome [6, 10-12]. Apart from providing thermo-protection using their chaperone function [13], Hsps are also implicated in other processes crucial for parasite survival. This includes inter organelle protein trafficking, protein export to the host cell and regulation of parasite pathogenesis [11, 14, 15], underscoring their importance in parasite biology. Hsp40s also denoted as DnaJ in prokaryotes are characterized by the presence of J domain containing the His-Pro-Asp (HPD) tripeptide that forms the signature motif of this family [16]. They are classified into four types/subfamilies (Type I-IV) based on their domain organisation [17].

The present study aims to propose a three dimensional structure for complete conserved region of a type II Hsp40 'PFB0090c' using homology modeling. A previous report suggests the role of this protein in chaperoning knob assembly [18], making it an attractive anti-malarial drug target. Here, we have compared the scores of verify 3D, errat and Rampage programs for the models generated using basic and advanced modeling service of Modeller. The model with the highest scores was selected to provide an insight into PFB0090c's three dimensional structure. The proposed structure of PFB0090c may help in using this protein as anti-malarial drug target.

2. Material and methods

2.1 Homology modeling

The Fasta sequence of PFB0090c was retrieved from PlasmoDB database [19]. Blastp against the RCSB Protein Databank was used to search for suitable template for modelling PFB0090c [20]. Different approaches were adopted to get a good quality model for complete conserved region of PFB0090c (88 to 408 amino acids) using Modeller9.14 [21]. A user can give 5 different modelling requests to the server. These include basic modeling, advanced modeling, iterative modeling, difficult modeling, modeling with cryo-EM. Here, we have used basic and advanced modeling for building a reliable homology model of PFB0090c. Basic modeling was performed using the structure of *Thermus Thermophilus* DnaJ as a template (PDB ID: 4J80) whereas in advanced modeling, multiple templates (PDB ID's 4J80, 2EJ7 3LZ8, 2Q2G) were used.

2.2 Model validation

With the aim of proposing a reliable model of PFB0090c, we used Verify 3D, Errat and Rampage programs for assessing the quality of the models [22-24].

3. Results and Discussion

3.1 3D structure prediction and quality assessment

Based on the result of Blastp for PFB0090c's sequence against PDB, we found a single template of Thermus Thermophilus Dnaj (PDB id 4J80; 28% identity out of 77% query cover) for the complete conserved region which includes J domain, G/F region and substrate binding domain. Using this as template, Basic modeling was performed using mod9.14 to build the model for PFB0090c (amino acid sequence range: 88 to 408). The quality of the obtained model was assessed using verify 3D and Errat and rampage programs [22-24]. Based on the scores obtained, we found that model structure obtained using basic modeling was not of good quality owing to its low verify 3D (79.75%) and Errat (27.476) scores. Verify 3D score indicates the compatibility of an atomic model (3D) with its own amino acid sequence (1D). A good quality model must have more than 80% residues to have average 3D-1D score>=0.2 [22]. The errat score depicts the overall quality of the model. For the good quality model, the generally accepted range is > 50 [23].

We next used advanced modeling service of Modeller which uses multiple templates. We first selected three templates (PDB ID's: 4J80, 2Q2G, 2EJ7) for modeling PFB0090c based on percentage of query cover and sequence similarity (Table1). Structure obtained using these templates have verify 3D score and Errat score of 73.52% and 42.949 respectively, so it was not considered as a good quality model. We then selected one more template (PDB ID: 3LZ8) and used all four templates (PDB ID's: 4J80, 2Q2G, 2EJ7, 3LZ8) for building the model. This time verify 3D and Errat scores of the model were 84.42% and 60.133 respectively. The stereo chemical property was validated through Ramachandran plot obtained from RAMPAGE server [24]. The Ramachandran plot showed 95% of the residues in the favoured region. Verify 3D, Errat and Ramachandran plots for this model are represented in Fig. 1. Considering these scores, we selected this model for proposing a reliable tertiary structure for the complete conserved region of PFB0090c. Comparison of Verify 3D, Errat and Rampage scores for models obtained using different templates is given in Table 2.

Table 1: Templates used for modeling PFB0090c with their PDB id's, percentage of query cover and sequence similarity

Source organism	PDB ID	% Query cover	% sequence similarity
Crystal Structure Of Dimerization Domain Of Hsp40 From Cryptosporidium Parvum Cgd2_1800	2Q2G	41	53
EPR-X-RAY diffraction structure of Thermus Thermophilus Dnaj	4J80	77	28
Solution structure of the DnaJ domain of the human protein Hcg3	2EJ7	18	56
Structure of a Putative Chaperone Dnaj from Klebsiella Pneumoniae Subsp. Pneumoniae	3LZ8	63	26

Table 2: Verify 3D, Errat and Ramachandran scores for the validation of predicted models using different templates. Validation scores in bold represent the best model selected for PFB0090c.

	Verify 3D	Errat	Rampage (Favoured)
Basic modeling with 4J80 as template	79.75%	27.476	91.0% 91.6
Advanced modeling with 3 templates: 4J80, 2Q2G, 2EJ7	73.52	42.949	90.6%
Advanced modeling with 4 templates: 4J80, 2Q2G, 2EJ7, 3LZ8	84.42	60.133	95%

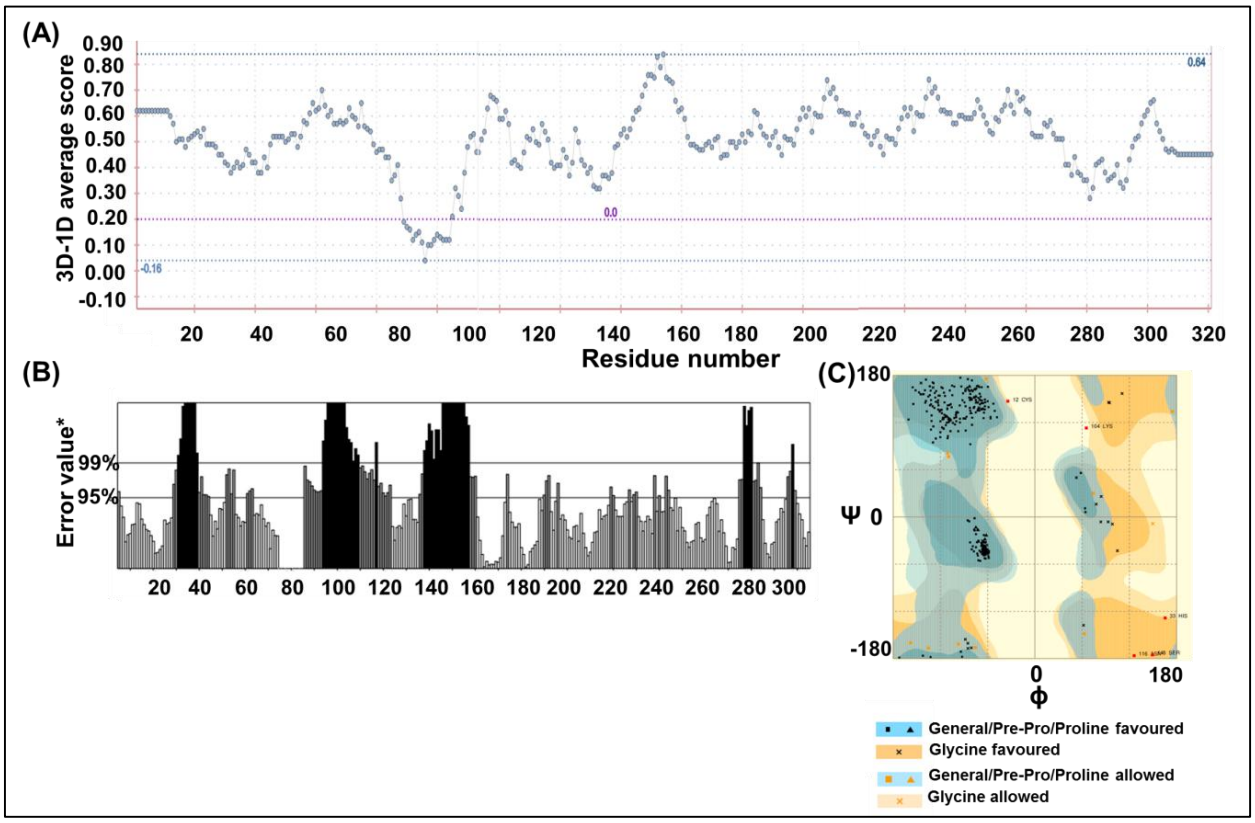

Fig 2: Verify 3D (A), Errat (B) and Ramachandran plots (C) of modelled PFB0090c.

The modelled PFB0090c was observed to be folded into α helices, β strands and loops (Fig. 2). J domain consists of four α helices. HPD motif that forms the major binding site for Hsp70 lies in the loop region between helix II and helix III (Fig. 2). The C terminal substrate binding region consists of two domains which are folded into β strands (Fig. 2).

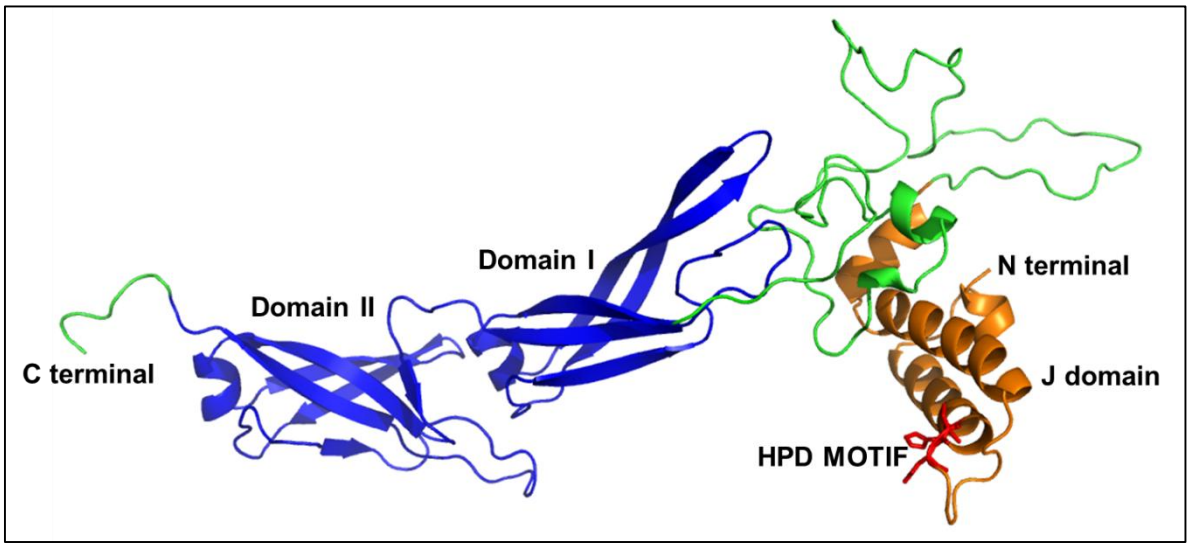

Fig 2: Cartoon representation of homology model of PFB0090c. J domain (orange) comprises four alpha helices while HPD (red), the signature motif of Hsp40 proteins is shown in sticks. G/F region (green) mainly consists of loops. C terminal region carry two domains (blue) consisting of beta strands.

4. Conclusion

The present study proposes a reliable structure for complete conserved region of PFB0090c using homology modelling. We used basic and advanced modelling function of Modeller for its structure prediction. The best model for PFB0090c was build using advanced modeling where four templates (PDB ID's: 4J80, 2Q2G, 2EJ7, 3LZ8) were used. It was considered as the most accepted model based on its verify 3D, Errat and Rampage scores. Since Hsps play a critical role in survival of malaria parasite in the human host, and are possible drug targets, our proposed structure for PFB0090c may form the basis for structure-based drug design against lethal malaria.

5. Acknowledgements

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6. Competing interests

Authors declare that they have no competing interests.

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