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MOLECULAR MODELING OF *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX*DIHYDROPTEROATE SYNTHASE

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ABSTRACT

Sequence study of DHPS from Plasmodium vivax and Plasmodium falciparum revealed new alterations at a number of locations, some of which were at or close to the Sulphadoxine binding site. In order to determine the structure of these proteins and determine any deviations from the known structures of homologous proteins, a study was conducted. Molecular docking was used to better examine the binding of sulphadoxine. Many software programmes for elementary level homology modelling are freely accessible online, including Python 2.4, PyMOL (DeLano Scientific LLC, USA; www.delanoscientific.com), Deep View - SPDBV, and Swiss - Model (online or downloadable). Some commercial softwares, like INSIGHT II, Accelrys, etc., can be used for advanced level modelling, which incorporates threading or ab - initio predictions. We solely employed the entry-level tools for this study. The obtained models of P. vivax DHPS in its wild-type and mutant forms did not exhibit any obvious differences. The altered residues F365L and D459A were found in the helix area, a good distance from the primary binding cavity. The sulfadoxine molecule's docking test similarly revealed no connection between these alterations and the drug binding. In conclusion, our investigation describes the preliminary impact of new mutations on drug binding. These findings require further investigation utilising an appropriate experimental design and superior computer software, such as threading or a more sophisticated ab-initio approach.

KEYWORDS: *Plasmodium Vivax, Plasmodium Falciparum, Molecular Docking, Dihydropteroate Synthase*

Introduction

Sulfadoxine-pyrimethamine (S-P), often known as Fansidar, is a common malaria treatment due to the rapid expansion of chloroquine-resistant Plasmodium falciparum malaria in many nations. The first sulfa medicines (sulfones and sulfonamides) were used to treat malaria in the 1930s. Numerous clinical investigations over the following ten years revealed that these medications were typically successful against Plasmodium falciparum malaria but much less so against Plasmodium vivax malaria (Achari et al.,2007). Sequence study of DHPS from Plasmodium vivax and Plasmodium falciparum revealed new alterations at a number of locations, some of which were at or close to the Sulphadoxine binding site. Based on the discrepancies found, structural analysis of these proteins was needed to determine whether the amino acid mutations had altered any of the protein's active regions. In order to determine the structure of these proteins and determine any deviations from the known structures of homologous proteins, a study was conducted. Molecular docking was used to better examine the binding of sulphadoxine (Baca et al.,2000).

Experimental Approach to Conformation Determination

X-ray crystallography and NMR spectroscopy are two well-known methods for determining molecule conformation. The first of the two, X-ray crystallography, crystallises the entire protein molecule to an extremely high purity level in order to examine the crystal dimensions using X-Ray diffraction. The approach is particularly helpful for any structural conformation because of the high specificity it offers. Only short peptides can benefit from NMR spectroscopy because it produces a spectrum that becomes difficult to interpret as polypeptide size grows. Despite being incredibly beneficial, both procedures need a lot of time, effort, money, and purity. Protein structure modelling theoretical techniques have been developed to address this issue. These methods employ currently available X-ray crystallographic structures to attempt to anticipate the structural relationships between proteins and these crystal structures. This is based on the fact that around one-third of all sequences in the current database are clearly related to at least one known crystal structure (by homology and similarity) (Bartelloni et al.,2007).

Homology Modeling

A method known as homology or comparative modelling uses an alignment to one or more known protein X-ray crystal structures to estimate the three-dimensional structure of a given protein sequence (Target) (Template). This is based on the observation that, assuming the evolutionary distance is small, structural and functional similarity can be assumed even if the sequence identity between the Target and the Template is between 25 and 30 percent (Bawijo et al.,2008). This presumption is based on the observation that protein folds change during evolution far less than amino acid sequences do (due to insertions or mutations), and can thus be preserved even when sequence similarity is minimal (Camargo et al.,2007). The biological functions of two proteins may be closely related if they have the same folds, twists, or loops. Although theoretical modelling has limits and cannot replace a high-resolution structure determination, it can provide valuable insight into the general fold and the characteristics of the protein surface, which justifies its growing significance as a potent tool in structural biology.

Tools for Homology Modeling

Online Swiss-Model or downloadable Deep View - SPDBV (Camargo et al., 2007), PyMOL (DeLano Scientific LLC, USA; www.delanoscientific.com), and Python 2.4 are only a few examples of free software for elementary level homology modelling. Some commercial softwares, like INSIGHT II, Accelrys, etc., can be used for advanced level modelling, which incorporates threading or ab - initio predictions. We solely employed the entry-level tools for this study.

An interactive molecular graphics application for viewing and understanding protein structures is called DeepView - the Swiss-PdbViewer (or SPDBV). New protein structures can also be modelled in conjunction with existing protein structures from Swiss-Model, a server for automated comparative protein modelling maintained at http://www.expasy.org/swissmod. Additionally, molecular visualisations, viewing, and analysis of protein structures are done using PyMOL and Python. Only a Python environment is necessary for PyMOL to function. All of

these programmes can be used to analyse and create protein structures in an appealing way and support protein database structures files.

Additionally, MODELLER is utilised for comparative or homology modelling of three-dimensional protein structures (Cheng et al.,2007). When a sequence to be modelled is aligned with known related structures provided by the user, Modeller automatically creates a model that includes all non-hydrogen atoms. It can perform de novo modelling of loops in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, comparison of protein structures, etc. Comparative protein structure modelling is implemented by satisfying spatial restraints. Both SPDBV and PyMOL model viewers support viewing and studying modeller files.

Molecular Docking

A technique known as docking in the field of molecular modelling predicts the preferred orientation of one molecule to another when they are linked together to form a stable complex. The strength of association or binding affinity between two molecules can be predicted using, for example, scoring functions, once the preferred orientation is known.

The orientation in which small molecule therapeutic candidates connect to their protein targets is routinely predicted using docking to determine the small molecule's affinity and activity. Therefore, docking is crucial to the logical design of pharmaceuticals. The goal of molecular docking is to reduce the free energy of the entire system by achieving an optimal conformation for the protein and ligand as well as their relative orientation. The community of molecular docking is particularly fond of two methods. One method depicts the protein and the ligand as complementary surfaces using a matching methodology. The second method simulates the real docking procedure while computing the pairwise interaction energies between the ligand and protein. The goal of this work was to employ homology models to predict the resistance patterns

of the parasite population by analysing these genes for previously known mutations or any other unique alterations (Darlow et al.,2002).

Tools for Molecular Docking

The molecular docking was performed using Molegro Virtual Docker (Molegro Bioinformatics Solution, Denmark; http://www.molegro.com/). It is an integrated environment for studying and predicting how ligands interact with macromolecules. The identification of ligand binding modes is done by iteratively evaluating a number of candidate solutions (ligand conformations) and estimating the energy of their interactions with the macromolecule. The highest scoring solutions are returned for further analysis. MVD requires a three-dimensional structure of both protein and ligand (usually derived from X-ray/NMR experiments or homology modeling). MVD performs flexible ligand docking, so the optimal geometry of the ligand will be determined during the docking (Chin et al.,2006).

MATERIALS & METHODS

From northern India, many blood samples containing P. vivax were collected and frozen at 20°C. During the years 2015 to 2020, blood samples from malaria cases with a clinical diagnosis were taken by skilled medical professionals. This region, which is located in India's northwest, experiences erratic outbreaks of Plasmodium vivax and Plasmodium falciparum malaria, particularly following the rainy season. A sample of 2 to 5 ml of infected blood was taken with the patients' informed agreement, placed in a 16 percent acid citrate dextrose solution, and shipped in a cold chain (4°C) to our lab. Before DNA extraction, collected blood samples were kept at -20°C. After the patient's blood had frozen, a technique for separating genomic DNA had been previously disclosed (Chin et al.,2006). The QIAGEN RNeasy kit was utilised to isolate total RNA. Samples of P. vivax from Maesod, Thailand (de Bwin et al.,2002),

Molecular Modeling and Docking

Software's used:

- Swiss – Prot database (http://www.expasy.ch/)
- Modeller 9v3 (Sali Lab)

- Swiss – Pdb Viewer v3.7b2 (Glaxo Wellcome Experimental Research)
- Python 2.4/ Pymol 0.99 (DeLano Scientific LLC, USA)
- Molegro Virtual Docker (MolegroApS, Denmark).

To perform homology modeling, first the X – ray crystallographic template structures were identified using the First Approach Mode at Swiss Modeling website (http://www.swissmodeling.expasy.ch/). The structures showing approximately 27 – 30% identity with target sequence and having resolution of approximately 2Aº were selected and used for developing homologue model by Modeller 9v3. Models for both wild type and mutant protein sequences were developed. The obtained models were analyzed for changes in helix, beta sheets or loops.

For the molecular docking of ligand on the enzyme, Molegro Virtual Docker was used. The obtained homologue model of enzyme was analysed first for available binding cavities. Identified individual cavities were used for docking of ligand. Affinity of ligand to enzyme binding site was analysed by visible interactive H – bonds on the ligand.

Results

Dihydropteroate Synthase (DHPS) structures for P. falciparum and P. vivax were created using homology modelling by Modeller 9v3, and the resulting structures were used to dock the medication sulfadoxine to see how mutations affected its binding.

***P. falciparum* DHPS**

Using the Swiss Model First Approach Mode (http://expasy.hcuge.ch/sprot/prosite.html), the whole sequence of P. falciparum PPPK - DHPS was submitted for protein homologue search.Only 390 - 710 amino acid residues out of the 711 amino acid sequence had a match. The early region of the PPPK (1–389) did not match any existing structure, but the DHPS region was

the only one to match an X-ray crystal structure.

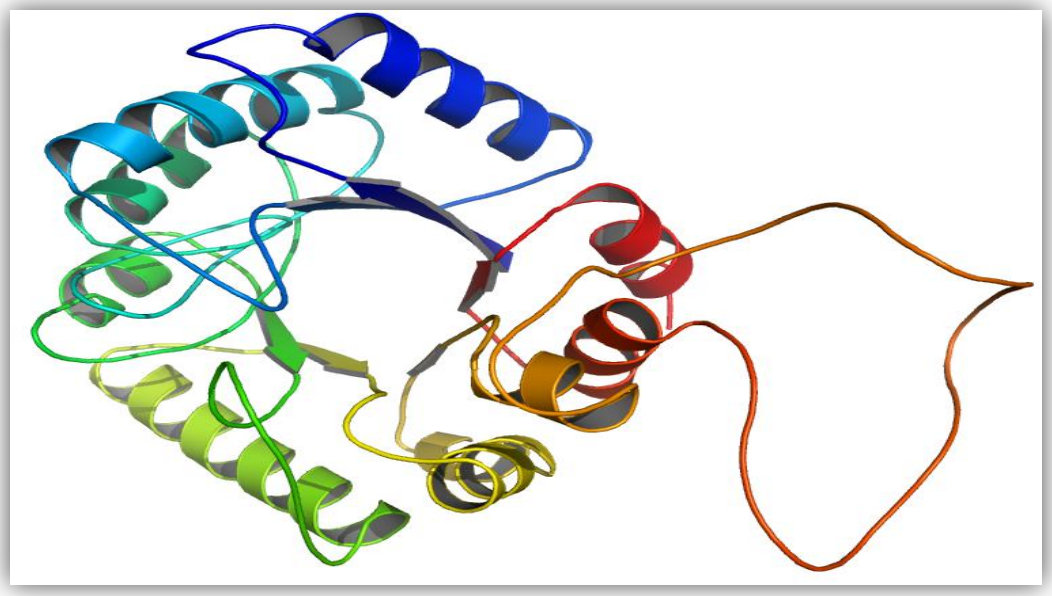

Figure 1: DHPS structure of wild type *P. falciparum*

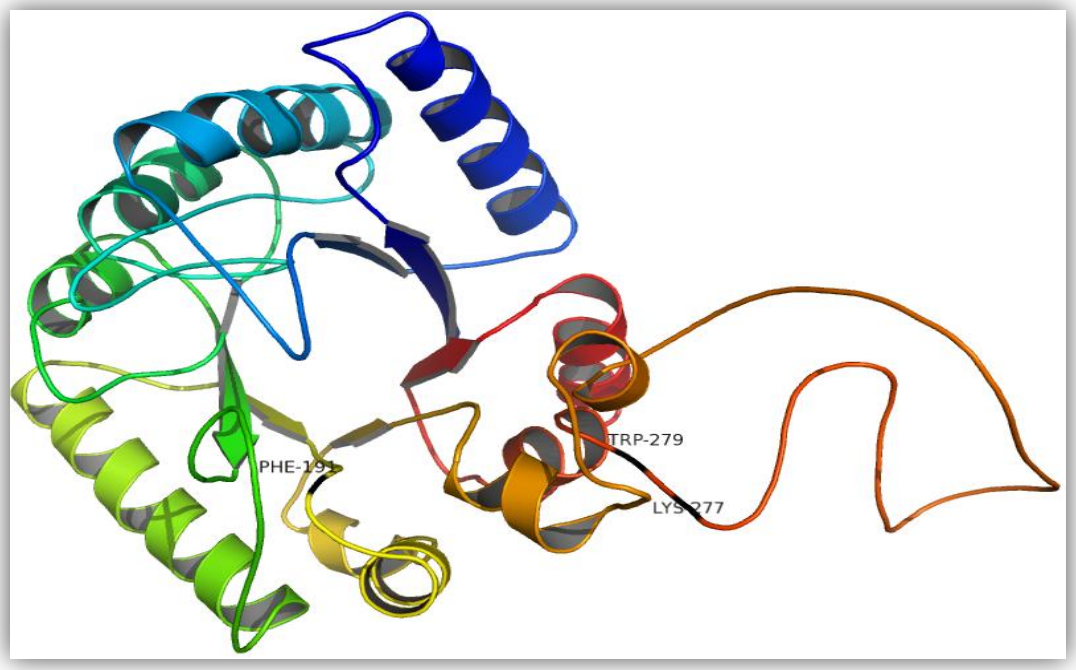

Figure 2: DHPS structure of mutant *P. falciparum* (Residue nos. 191, 277 and 279 corresponds to 587, 666 and 668 respectively from original *Pf*DHPS sequence)

The E. coli DHPS template structure, with a 2.0 Ao resolution crystal structure and a 27 percent amino acid identity to the target P. falciparum sequences, was the closest template structure identified by the BLAST search of the ExPDB database (http://swissmodel.expasy.org/SM Blast.html). The structures for the P. falciparum Dihydropteroate Synthase (DHPS) proteins for the wild type (A437S587N666C668), single mutant (A437F587N666C668 and A437S587N666W668), and double mutant (A437S587K666W668) were created using Modeller 9v3 using the X-Ray crystal structure of the E.coli DHPS as The template structure and the alignment of the target sequence with the structures made up the input. For both the wild type and mutant P. falciparum DHPS, a total of 500 iterations were assigned. The structure with the highest score out of 500 structures obtained as output was chosen for analysis (Fig. 1, 2). Both the wild-type and mutant structures of P. falciparum were stacked in order to study the precise impact of these changes (Fig. 3). The N666K and C668W mutations were present at the beginning of the large loop area, whereas the S587F mutation was found on a helix that was far from the Sulfadoxine binding site. The simple N666K mutation in the modified structure caused only a tiny alteration in the loop, however the C668W mutation caused the huge loop to significantly change or bend. The structure with the two double mutations above displayed a significant bend in the large loop. Additionally, a little helix was seen to form in the mutant structure (Fig. 4).

A molecular docking analysis was also carried out to examine the impact on the binding of Sulfadoxine molecules to the altered structure. The Sulphadoxine molecular structure was acquired for this via Pubchem (www.pubchem.ncbi.nlm.nih.gov). Possible active site/binding cavities were found in both the P. falciparum DHPS wild type (Fig. 5) and mutant (Fig. 6) structures to execute the docking of the Sulphadoxine molecule with DHPS. In the huge loop area of the wild type P. falciparum DHPS, a large cavity could be seen. However, the mutant structure revealed a distinct division in this binding cavity, revealing one large cavity split into two.

The molecular docking analysis showed five best options with minimized energy. The best among these was chosen and analysed. On docking the Sulphadoxine molecule to the *P.*

falciparum DHPS wild type structure, 3 probable interactive H – bonds were visible near the binding site at the amino acid 666 (Fig. 7). A similar analysis was carried out using *P. falciparum* DHPS mutant structure. Though the drug molecule alignment near the mutated active site was observed but instead of 3 interactive H- bonds only one bond was observed in the mutant form (Fig.8).

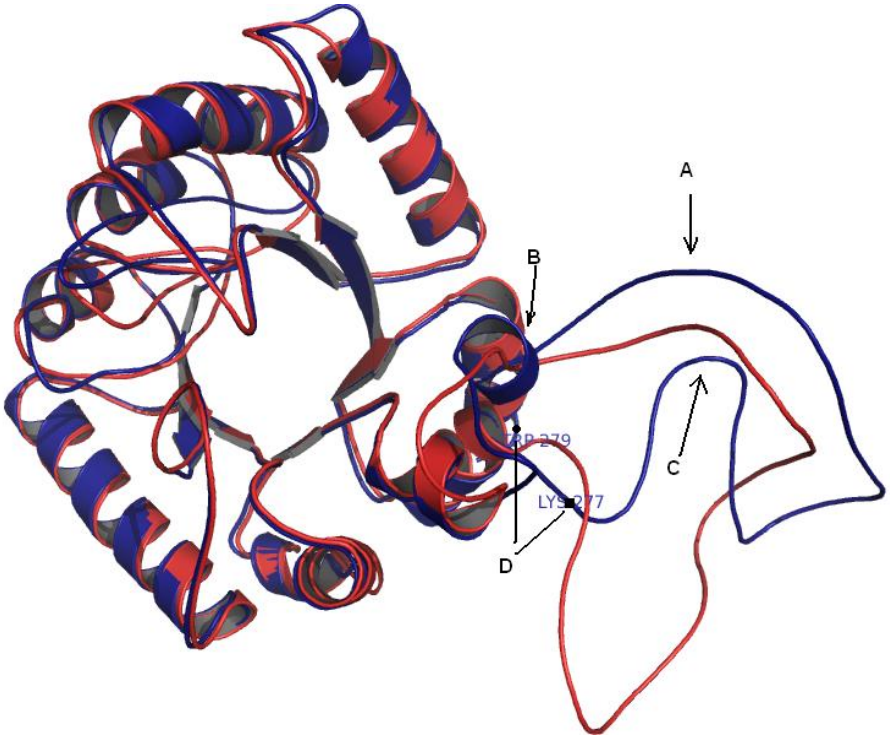

Figure 3: *Pf*DHPS wild type (red) and mutant (blue) structures aligned. A = The big loop region showing variations. B = A small helix developed in the mutant structure. C = Bend in the big loop. D = Locations of Novel mutations N666K (LYS277) and C668W (TRP279) in the loop. (Actual numbers of residues should be considered with addition of a value of 389 to match with standard *Pf*DHPS sequence)

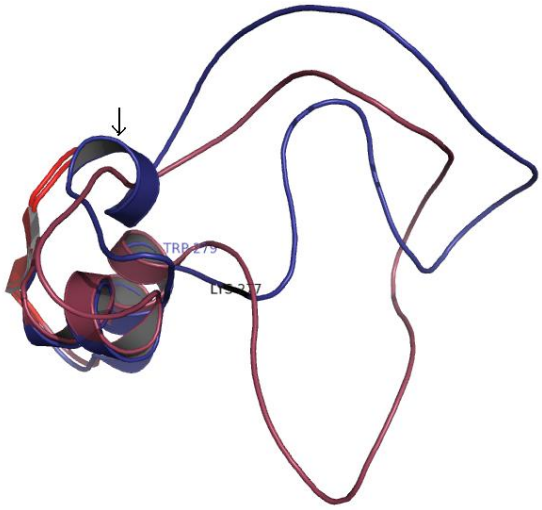

Figure 4: Big loop region of *Pf*DHPS wild type (red) and mutant (blue) structures. Arrow showing the small helix in *Pf*DHPS mutant

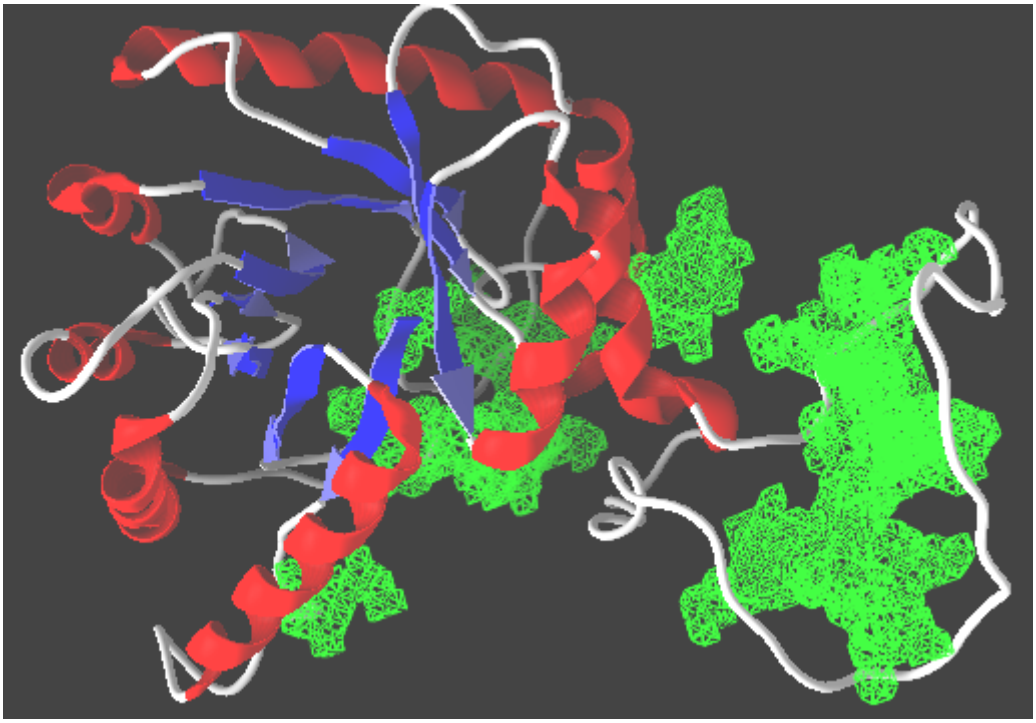

Figure 5: Structure of wild type *P. falciparum* DHPS with binding cavities (green)

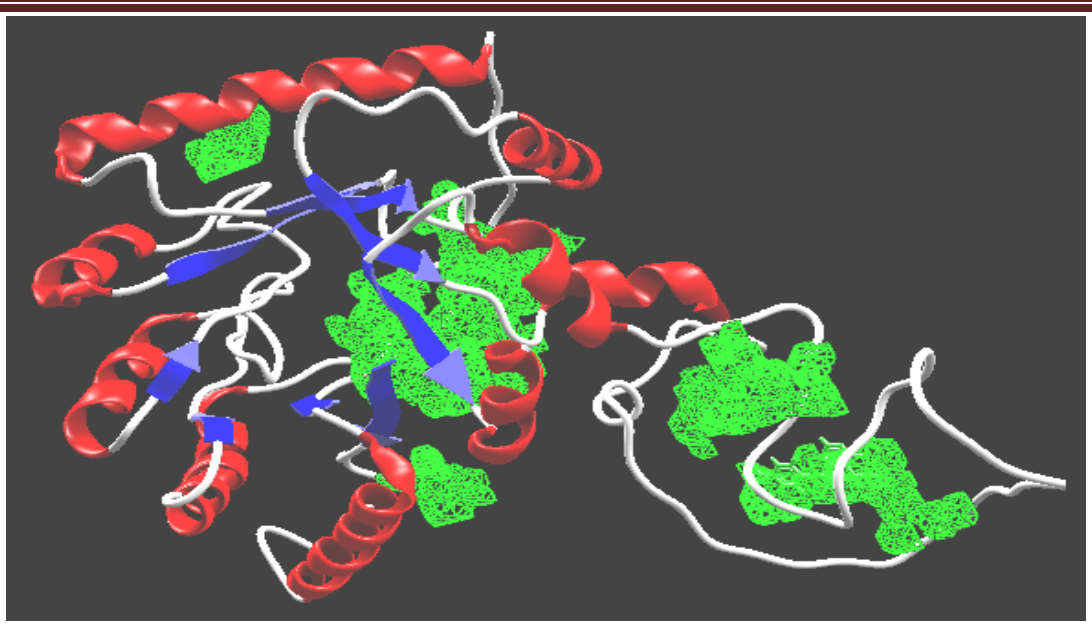

Figure 6: Structure of mutant *P. falciparum* DHPS with binding cavities (green)

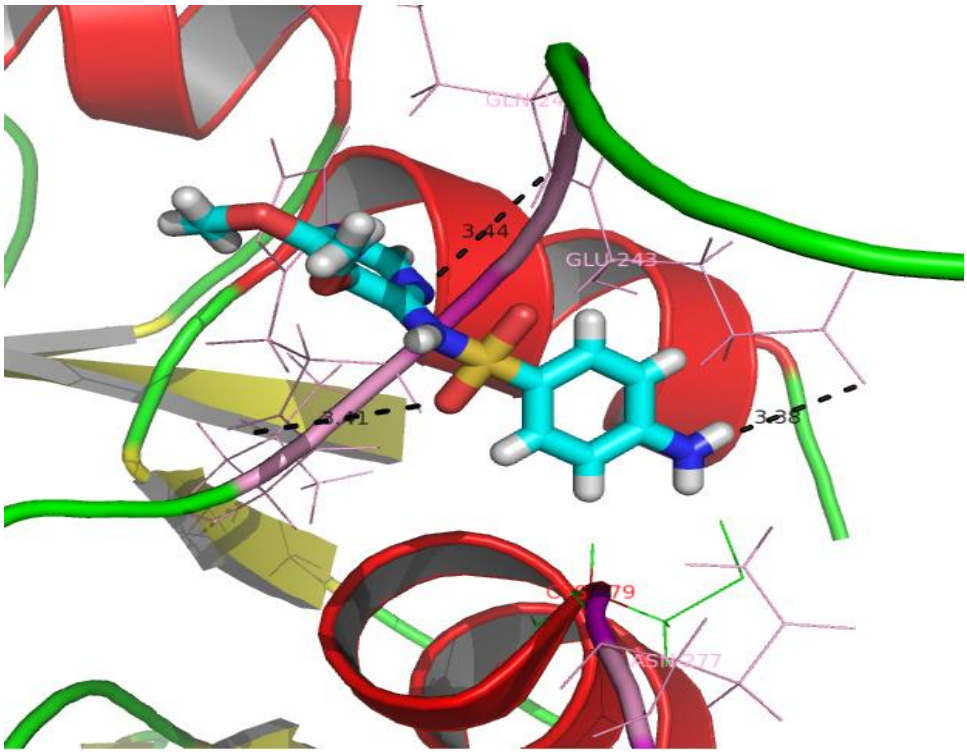

Figure 7: Sulfadoxine molecule docked with wild type *Pf*DHPS near the binding site 666 (277 in figure). The drug molecule is showing 3 polar interactions with residues other than that at 666.

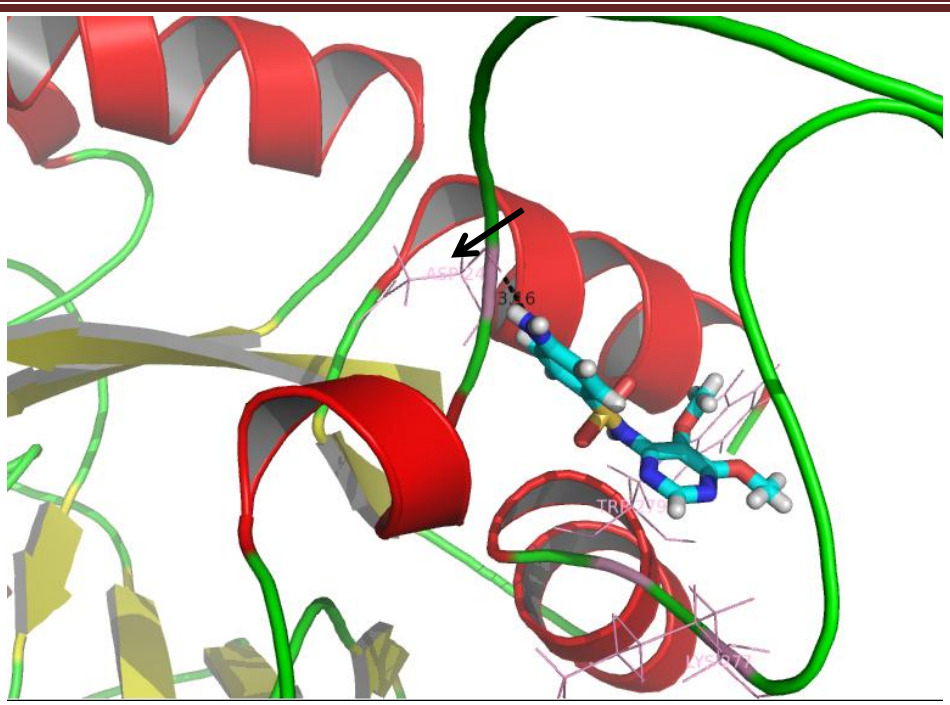

Figure 8 Sulfadoxine molecule docked with mutant type *Pf*DHPS near the binding site 666 (277 in figure). Arrow indicates the single polar interaction.

***P. vivax* DHPS**

Similar to *P. falciparum*¸ homology modeling for *P. vivax* DHPS wild type and mutants was also performed. In the DHPS region a similarity match could be obtained only for 258 (331 – 588) amino acid residues. The closest template structure found with the BLAST search of ExPDB database (http://swissmodel.expasy.org/SM_ Blast. html) was of *Bacillus anthracis* DHPS, with 2.0 A^{o} resolution crystal structure and shared 30% amino acid identity to the target *P.vivax* sequences (Fig.9).

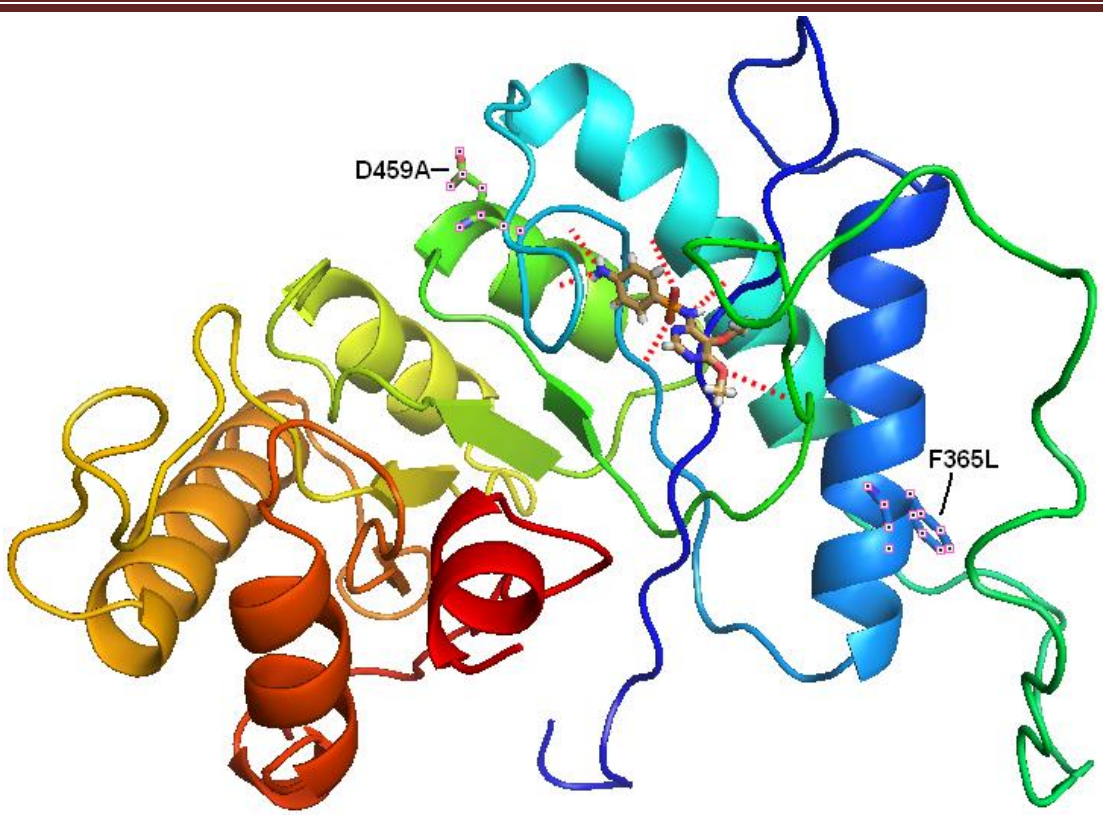

Figure 9: Structure of mutant *P. vivax* DHPS showing sites of novel mutations and the drug molecule interactions

The obtained models of wild type and mutant *P. vivax* DHPS did not show any marked difference. The changed residues F365L and D459A were present in helix region which was decently away from main binding cavity. A docking trial of the sulfadoxine molecule also showed no relation of these mutations with the drug binding.

Discussion

Homology modelling of the specific protein is one of the most useful techniques for describing the structural changes brought on by recognised new mutations in the protein sequences. More details about these molecules are revealed by further research of the drug binding characteristics

using molecular docking of the drug to these predicted structures. So, for both P. falciparum and P. vivax, we created models for wild-type and mutant DHPS (showing new mutations), and then molecularly docked the Sulfadoxine molecule to these structures.

The S587F mutation was located on the helix far from the binding site and had no effect on the structure of the enzyme. Contrarily, insert 2 of wild type PfDHPS has the expected binding residue 666N for sulfadoxine (Doberstyn et al., 2009). (de Beer et al., 2006). According to several investigations, P. falciparum bifunctional enzymes need parasite-specific inserts to function properly (Eskandarian et al .,2002). Therefore, changes to this and the nearby residues may have an impact on how this medication binds to the DHPS enzyme. When the derived structures of wild and mutant PfDHPS (having mutations at codons 666 and 668) were superimposed, the large loop region underwent a modification or bend. Although the shift from Asparagine (N) to Lysine (K) is not significant because both amino acids are positively charged and aliphatic in nature, the change from Cysteine (C) to Tryptophan (W) is significant because tryptophan is an aromatic amino acid while asparagine is an aliphatic amino acid. The addition of a larger aromatic ring and side chain to the structure in the latter instance may be the main reason for the loop's change.

The maximum likelihood of a drug molecule binding to codon 666 was revealed by analyses of various docking options for the drug molecule to the enzyme. This mutation (N666K) caused the drug molecule to move away from the binding site, indicating either poor or no binding to the site. The decrease from three to one interactive H-bonds also pointed to a potentially unstable drug molecule binding. Parameters for energy minimization were taken into consideration. Additionally, the drug molecule may not be able to bind to the enzyme as effectively as in wild type due to the mutant P. falciparum DHPS's splitting of the large cavity into two.

Similar to wild-type P. vivax DHPS, mutant P. vivax DHPS, both had large predicted Sulfadoxine binding sites at positions 382-384, 552, 688, 691, and 713. (Findlay et al.,2001). We noticed novel mutations at amino acid sites 364, 459, 601 and 617. Due to the difficulty of obtaining a homologous model for the entire P. vivax DHPS, only two mutations—F365L and

D459A—could be studied. Both mutation sites are rather far from the predicted binding sites and do not exhibit any changes to the enzyme's or the binding cavities' structural characteristics. As the other two unique mutations are located in the repeat area of P. vivax DHPS and previous research by Harinasvta et al. (2007) suggests that this region may not play any part in the drug binding, these mutations were not given as much attention.

CONCLUSION: In conclusion, the purpose of this study was to investigate, on a preliminary level, the impact that new mutations have on drug binding. There is a need for additional research to be carried out in order to validate these findings by utilizing an appropriate experimental setting and improved computational tools, such as threading or a more advanced ab – initio method.

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