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A SYSTEMATIC STUDY ON THE STRUCTURE AND FUNCTION OF PLASMODIUM FALCIPARUM TRIOSEPHOSPHATE ISOMERASE AS A THERAPEUTIC OPTION IN ANTIMALARIAL DRUG DESIGN

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Abstract

The most virulent of all the malarial parasites happens to be *Plasmodium falciparum* and is the major causative agent of malaria which is a global public health problem. Research towards the development of new antimalarial drugs and drug targets has been intensified because of the building up of drug resistance observed in Plasmodium falciparum. In order to detect *Plasmodium falciparum* metabolic enzymes as potential targets for drugs, efforts was intensified to predict the 3D structure and ligand binding site of the parasitic enzyme. The focus on glycolytic enzymes in the malaria parasite results from the observation that in the asexual stage of the parasite in the human red blood cells, the energy requirements of the organism is almost exclusively met by glycolysis. Moreover, the fact that there appears to be a significant difference between *Plasmodium falciparum* TIM and human (*Homo sapiens*) TIM makes it a suitable candidate for drug therapy.

Here, we present a computational analysis of the *Plasmodium falciparum* TIM which has been annotated using the database of the National Centre for Biotechnology Information (NCBI). Various web-based bioinformatics tools have been used to determine the characteristics of the enzyme such as its physiochemical properties, disease causing region, secondary and tertiary structures, conserved domains and ligand binding sites.

The outcome of the sub-cellular localization prediction of the TIM of Plasmodium falciparum confirmed it to be a cytoplasmic enzyme with a score of 99.8%. The alignment with its HsTIM ortholog produced a sequence identity of 43.5%. The tertiary structure comparison with the *Hs*TIM also predicted functional similarity, while 7 ligand/drug binding sites were detected. Two disease causing regions were also detected and the antigenicity site prediction revealed unique sites that remain non-homologous to the *Hs*TIM.

Detection of binding sites, enzyme stability and structure prediction from this experiment will aid molecular docking studies for therapeutic drug designing against *Plasmodium falciparum*. The antigenicity site prediction coupled with the detection of the disease causing region is also useful in the development of potent malaria vaccines.

Keywords: *Plasmodium falciparum; Resistance; Alignment, Ortholog; Antigenicity*

Introduction

According to the 2015 World Health Organization report, malaria is the most serious and widely spread disease that affects mankind with an estimated 438,000 yearly deaths [1]. There was a noticeable decrease in the rate of infection in the year 2000[1] but an increase was observed as against 2014's 198 million cases [2]. Over 75% of the sub-Saharan cases of malaria were due to *Plasmodium falciparum*, but the case is different in other malarial countries where some other less virulent species of Plasmodium predominates. Most cases of malarial deaths are as a result of *Plasmodium falciparum* infection [3]. Today, malaria is being regarded as one of the most common poverty related diseases and the death rate has a result of malaria has been on the increase since inhabitants of most African villages have limited access to adequate treatment [4].

Plasmodium falciparum remains the most virulent of all the malaria causing species of *plasmodium* [5-8]. The female anopheles mosquito is responsible as the vector for the transmission of *Plasmodium falciparum* which is a protozoan parasite. The most dangerous form of this disease, otherwise known as the malignant [9] or *falciparum* [10] malaria is caused by this species of *Plasmodium* [11]. Before *Plasmodium falciparum* is being transmitted, the parasite stays in the salivary gland of the vector. At this point, the parasite is in its sporozoite stage. The vector takes its blood meal as it injects a little amount of saliva into the wound created on the skin. Antihemostatic and anti-inflammatory enzymes are contained in the saliva of the mosquito and these block the process of blood clotting and as well inhibit reactions for pain. 5 to 200 sporozoites are being released into the host's blood stream at every bite blood and they proceed to infect the human host [12]. It takes only very few minutes for the sporozoites in the infected blood to circulate to the liver cells.

In many stages of the life cycle of the malarial parasite, it exhibits a very fast growth rate. This is as result of the acquisition of nutrients, and the metabolism of such biological molecules for reproduction and survival [13]. The concept that the metabolism of the parasite in relationship to that of the host can be intertwined is a clear one. This is as a result of the intimacy in the relationship between the host and parasite [16]. The complex parasitic life cycle which involves both the vertebrate and invertebrate hosts, including differential locations in each of the hosts further contributes to the complications experienced between the host and parasite.

The development of novel therapeutic procedures which exploits the parasite's metabolic uniqueness can be achieved by a clearer understanding of the metabolism of the parasite [13, 15]. The primary source of energy of the *Plasmodium falciparum* parasite is glucose and this is actively fermented by the parasite's blood stage. Also, the metabolic process of converting glucose to lactate as found in other organisms is the same as that of the *Plasmodium falciparum* [16]. The utilization of blood glucose by the parasite is 75 times more that the rate of utilization of the blood glucose as observed in uninfected Red Blood Cells [14, 17]. The reaction of glycolysis as observed in the parasite is at a very high rate and lactate becomes the end product of the conversion of glucose by the parasite i.e an approximate value 85 percent of the glucose used up by the parasite is being converted to lactate [36].

In this study, a bioinformatics investigation of the structure and function of the *Plasmodium falciparum* Triosephosphate isomerase was directed at the prediction of potential drug and vaccine target sites.

Materials and Methods

Sequence retrieval

The TIM amino acid sequence of *P. falciparum* was obtained from the National Center for Biotechnological Information database (NCBI) [18]. The protein is assigned an accession number of AAA18799.1. The physicochemical properties, subcellular localization, secondary structure, three-dimensional (3D) structures and antigenicity sites were all predicted utilizing a wide range of computational servers and databases.

Physiological–biochemical characterization

The physicochemical characterization, molecular weight, isoelectric point (pI), total number of negative and positive residues, aliphatic index, extinction coefficient, instability index, and grand average hydropathicity (GRAVY) of the TIM protein

[21] were all predicted utilizing the Expasy Protparam server [19].

Subcellular localization and signal peptide

Identification of the protein subcellular localization of the protein was done using the Psort (http://psort.nibb.ac.jp/form2.html) [21, 22, 23].

Prediction of the protein secondary structure

The CFSSP secondary structure prediction server [24] was utilized in the prediction of the secondary structure of Plasmodium falciparum TIM protein [25, 26]. The properties of the secondary structure of a protein include the alpha helix, beta pleated sheets, beta turns etc. Identification and comparison of conserved domain.

The conserved domain prediction was done using the Molecular Evolutionary Genetics Analysis (MEGA) [27] offline bioinformatics tool to detect the conserved domain and to show any domain similarity between the PfTIM and the HsTIM

3D structure prediction

Phyre2, a web-based software [28], was used in predicting the 3-dimentional structure of the *Pf*TIM and *Hs*TIM [29].

Active site prediction

The 3DLigandSite automated software was used in predicting the active site of the Plasmodium falciparum Triosephosphate isomerase [30].

Prediction of antigenicity site

The Immunomedicine group antigenicity prediction online server was used in the prediction of the protein antigenicity site. This server operates by predicting integral segments of the amino acid sequence which probably possess antigenic properties by eliciting an immune response [31]. This server is reported to have an accuracy of approximately 75%.

Prediction of the disease causing regions The globplot online server [32] was used in predicting the protein disease causing regions.

Results

Physical and chemical characteristics

The amino acid sequence of *Plasmodium falciparum* triosephosphate isomerase (Accession number AAA18799.1) contained 248 amino acids and a molecular weight of 27934.70Da. The pI was predicted to be 6.01. The mammalian, yeast, and E. coli protein half-lives were estimated respectively to be 30, >20, and >10 h, with an instability coefficient of 31.37. This is a characteristic of a stable protein. The GRAVY (Grand Average of Hydrophobicity) of the *Plasmodium falciparum* Triose phosphate isomerase is – 0.315 and it has an extinction coefficient of 21,680. Therefore, the Triosphosphate isomerase is a stable and weakly acidic enzyme. Subcellular localization.

The Triosephosphate isomerase of Plasmodium falciparum was predicted to be localized 99.8% in the cytoplasm. The prediction detected no signal peptide.

Disease causing region prediction

GlobPlot identified 2 disease causing regions. The result which was shown in Fig. 1 shows the disease causing regions ranges from amino acid number 26-33 (SFNNLDFD) and 64-74 (QNVSKFGNGSY).

Multiple sequence alignment

The sequence alignment in this figure is targeted at indicating the Triose phosphate isomerase protein conserved domains and variable sites in both human and the plasmodium parasite. The *Plasmodium falciparum* and *Homo sapien* Triose phosphate isomerase amino acid sequences were obtained from the NCBI database with accession number AAA18799.1 and CAA49379.1 respectively. The positions of the amino acids were numbered beside the sequences. The sequence alignment results show a high level of sequence identity.

Amino acid composition

The results show a high concentration of amino acids with hydrophobic side chains. These amino acids include glycine, alanine, leucine, isoleucine, phenylalanine etc.

Secondary structure prediction

The secondary structure analysis reveals that *Plasmodium falciparum* Triosephosphate isomerase consists a total of 195 α-helix residues which makes up 78.6% of the structure, 106 beta pleated sheet

residues, making up 42.7% of the structure and the beta turns, a total of 35 residues making up 14.1% of the structure.

Tertiary structure prediction

The tertiary structures were predicted by PHYRE2 using a total of 246 amino acid residues (99% of the sequence). Modelling was done with a 100% confidence by the single highest scoring template. The results show a high level of similarity between the structures.

Ligand binding sites prediction

The 3D ligand automated software showed the 7 active sites of Plasmodium falciparum Triosephospateisomerase with metallic heterogens to which drugs can bind.

Antigenicity sites prediction

The tools used predicated several antigenicity sites. *Plasmodium falciparum* Triosephosphate isomerase possesses 12 antigenicity sites. This sites were carefully studied in order to detect regions that are non homologous to the human Triosephosphateisomerase. The amino acids contained in these regions are localized at the following positions; 32-33, 35-38, 41-42, 44-48, 50-51, 53-54, 56-57, 87-90, 92-94, 120, 122-125, 127, 138, 140-141, 143-145, 147, 149-151, 153, 158-159, 161, 167, 179, 183, 188, 190-192, 194, 202-203, 204-205, 207, 216, 218-219,221-222, 223 and 238-239.

Discussion

The pI of the *P. falciparum* TIM by the biochemical characterization analysis has predicted the protein to be slightly acidic with a value of 6.01 [33]. The hydrophobicity scale produces descriptive value for the relative hydrophobicity of residues of amino acids. The more positive or negative the value, the more hydrophobicity or hydrophilicity of the sequences located in that protein region [34].The GRAVY calculator used in predicting the hydrophobicity assigned to the protein a value of -0.31.

The instability index describes the estimated level of protein stability in a test tube. A protein is said to be stable if its instability index is smaller than 40 and unstable if its instability index is greater than 40 [35, 36]. The P. falciparum TIM is therefore a stable protein with an instability value of 31.37. The time taken for half the quantity of a cellular protein to disappear after it has been synthesized in the cell gives definition to the protein's half-life. ProtParam server uses the "N-end rule". This rule finds the relationship between the protein's half-life and its N-terminal residue [37, 38]. The N-end rule started with the observation that the determination of the stability in vivo of any protein depends greatly on the N-terminal residue of the protein from [39, 40, 41]. Methionine (Met) is the N-terminal amino acid of the amino acid sequence hence, its estimated half-life in the mammalian cell is 30 hours. The extinction coefficient is the quantity of light absorbed by a protein at a specific wavelength. Studies have shown the possibility of estimating the molar extinction coefficient of a protein by the application of the knowledge if the composition of its amino acid [42]. The extinction coefficient was predicted to be 21680 M-1 cm-1.

The multiple sequence alignment with the HsTIM revealed the variable sites of the P. falciparum TIM amino acid sequence. These regions are potential target sites for therapeutic agents [43, 44]. The sub-cellular localization prediction confirmed TIM to be a cytoplasmic enzyme which catalyzes the conversion of glyceraldehydes-3-phosphate to dihydroxyacetone phosphate [45, 46]. The secondary structure prediction showed a high percentage helix and sheet, contributing to the stability and conservation of the cytoplasmic enzyme 47, 52]. A biomolecule functions more as an intricate three-dimensional structure, contrary to its linear polymer. The occurrence of mutation is at the sequence level, but its effects are exhibited at the level of functional level, and the tertiary structure of the protein is directly related to its function [48, 49]. The tertiary structure comparison of the P. falciparum and *Homo sapien* TIM suggests a functional similarity between the two enzymes [50, 51]. The ordered and disordered state of the amino acid sequence were instrumental in the prediction of the disease causing regions of the enzyme [37]. Two of these regions were detected in the P. falciparum TIM and the amino acid sequence displayed in the result. These regions can also serve as a potential target for therapeutic agents. Antigenicity is the property of being able to induce a specific immune response [52].

The *P. falciparum* TIM antigenicity sites were displayed in the antigenicity table but a conscious effort was made to compare these regions with its *Hs*TIM ortholog in order to detect the non homologous regions to avoid the killing of normal human cells. This region can be considered also as an ideal target for drugs and in the development of vaccines [53]. 7 ligand binding sites were detected from the *P. falciparum* TIM. They include; the 2nd ALA, 3rd ARG, 4th LYS, 10th ASN, 98th ARG, 102nd PHE, 171th GLY. This vital information makes the *P. falciparum* TIM a unique drug target in the treatment of malaria while the development of suitable enzyme inhibitors is made easier [54]. We have a strong believe that this bioinformatics analysis will be helpful in the development and design of new vaccines and drugs Against the *P. falciparum* TIM.

Conclusion

The TIM sequence of *the P. falciparum was obtained from* the NCBI database and various bioinformatics tools were used to determine the numerous characteristics of the enzyme such as the secondary structure, physiochemical properties, the 3-dimensional structure, conserved domain, disease causing region, ligand binding sites etc. The 3-dimensional structure shows 7 distinct sites for the binding of ligands. Two disease causing regions were also detected with the presence of many antigenicity sites. The non homologous antigenicity sites can serve as potential target for drug or vaccines against*P. falciparum* TIM.

With the specific analysis of parameters in this study, potential anti-malarial drugs and vaccines can be designed to target the *Plasmodium falciparum* Triosephosphate isomerase and passed through further laboratory experiments in future.

Abbreviations

TIM; Triosephosphate isomerase
HsTIM; *Homo sapien* Triosephospate isomerase
GRAVY; Grand Average Hydropathicity
CFSSP; Chou and Fasman Secondary Structure Prediction Server

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Table 1. Antigenicity site

There are 12 antigenic determinants in your sequence

n	Start Position	Sequence	End Position
1	32	FDPSKLDVVVFPVSVHY	48
2	50	HTRKLLQS	57
3	87	NIEYVIIG	94
4	120	NLKAVVCFG	128
5	138	KTIEVITKQVKAFVDL	153
6	158	DNVILAYEPLWA	169
7	179	EQAQLVHK	186
8	188	IRKIVKD	194
9	202	NQIRILYG	209
10	216	NCSSLIQ	222
11	227	DGFLVGN	233
12	236	LKESFVDII	244

Figure 1. Disease causing region graph

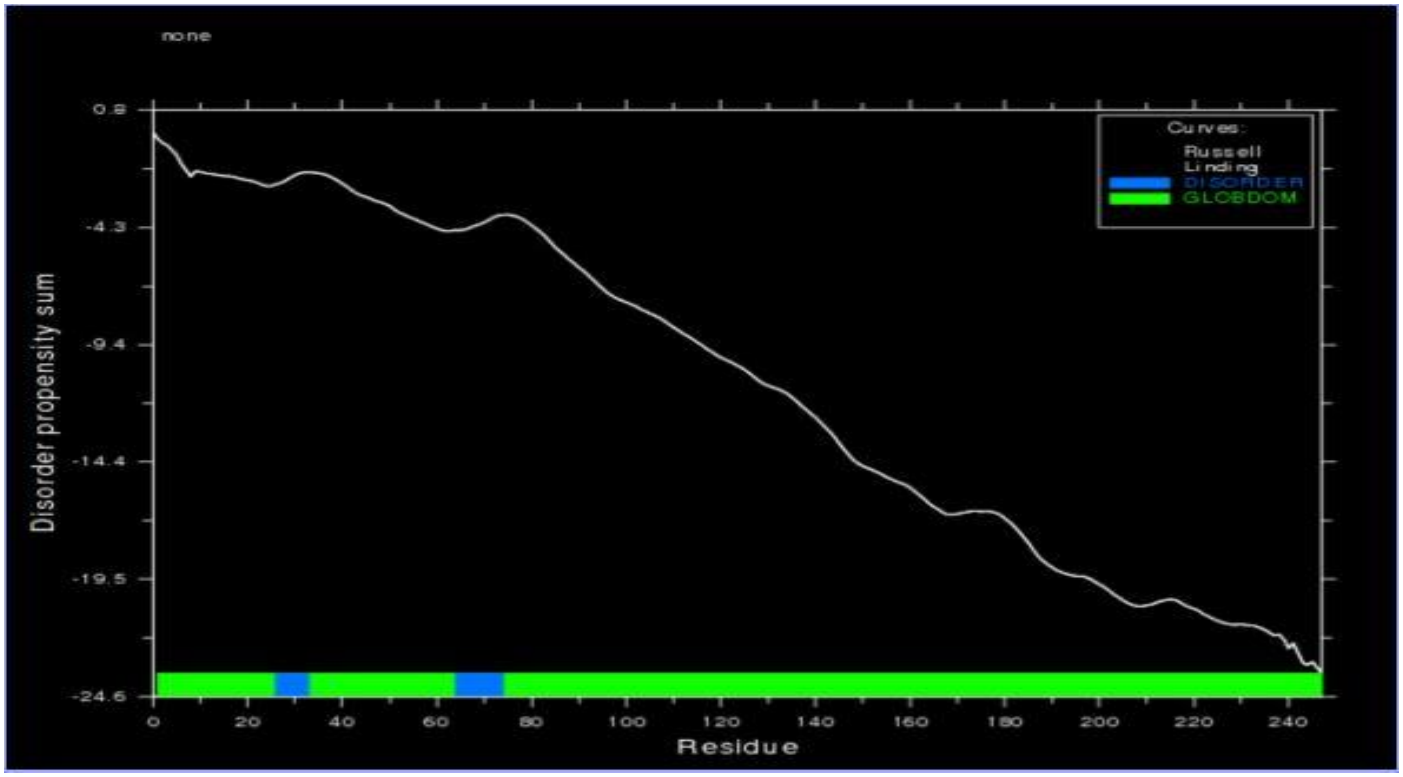

Figure 2: Multiple sequence alignment identical amino acids are shown in red color while green color show strongly similar amino acids and the blue color indicates amino acids that are weakly similar.

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CLUSTAL 2.0.10 multiple sequence alignment


AAA18799.1      MA--RKYFVAANWKCNGTLESIKSLTNSFNNLDFDPSKLDVVVFPVSVHYDHTRKLLQSK 58
CAA49379.1      MAPSRKFFVGGNWKMNGRKQSLGELIGTLNAAKV-PADTEVVCAPPTAYIDFARQKLDPK 59
                **  **:**..*** **  :*: .* .::*  .. *:. :**  * :.: *.:*: *:.*

AAA18799.1      FSTGIQNVSKFGNGSYTGEVSAEIAKDLNIEYVIIGHFERRKYFHETDEDVREKLQASLK 118
CAA49379.1      IAVAAQNCYKVTNGAFTGEISPGMIKDCGATWVVLGHSERRHVFGESDELIGQKVAHALA 119
                ::.. **  *. **::***:*. : ** .  :*::** ***: * *:** : :*:  :*

AAA18799.1      NNLKAVVCFGESLEQREQNKTIEVITKQVKAFVDLIDNFDNVILAYEPLWAIGTGKTATP 178
CAA49379.1      EGLGVIACIGEKLDEREAGITEKVVFEQTKVIADNVKDWSKVVLAYEPVWAIGTGKTATP 179
                :.* .:.*:**.*::** . * :*: :*.*.::* :.::.:*:*****:**********

AAA18799.1      EQAQLVHKEIRKIVKDTCGEKQANQIRILYGGSVNTENCSSLIQQEDIDGFLVGNASLKE 238
CAA49379.1      QQAQEVHEKLRGWLKSNVSDAVAQSTRIIYGGSVTGATCKELASQPDVDGFLVGGASLKP 239
                :*** **:::*  :*.. .:  *:. **:*****.  .*..* .* *:******.****

AAA18799.1      SFVDIIKSAM 248
CAA49379.1      EFVDIINAKQ 249
                .*****::
```

Figure 3: Amino acid composition graph

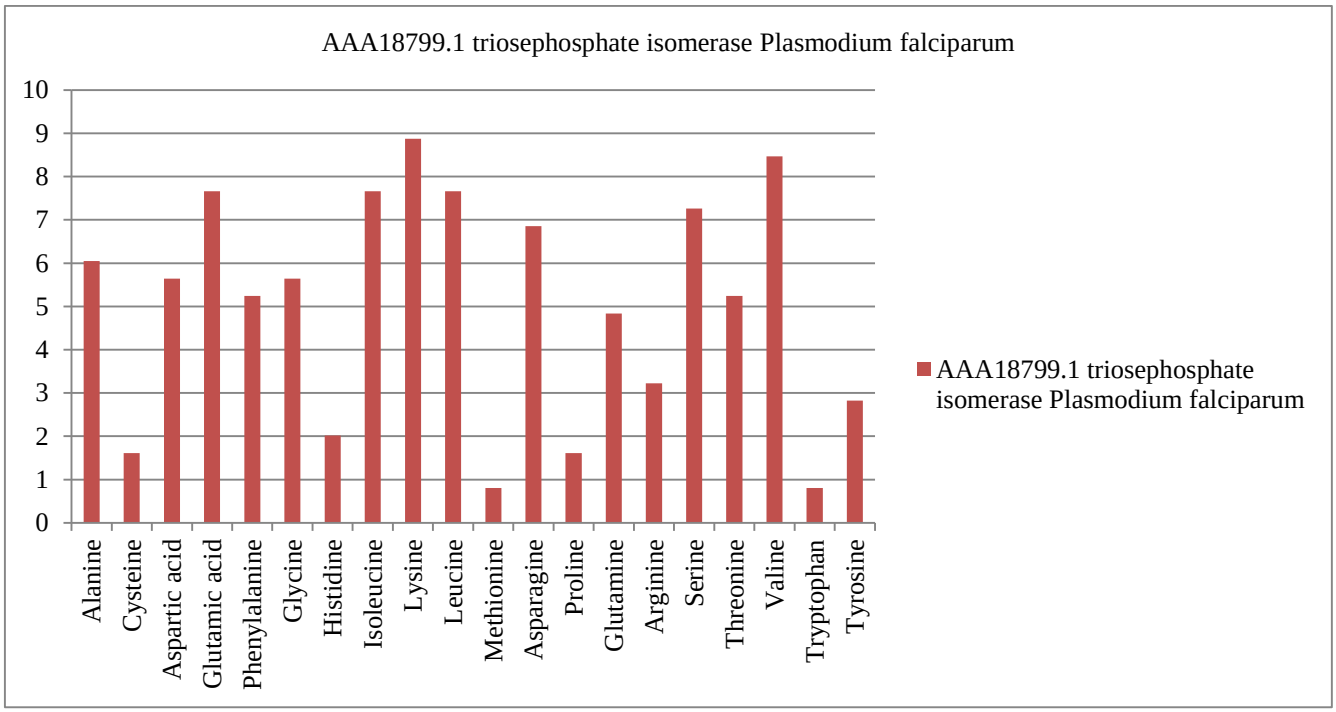

Figure 4: Secondary structure

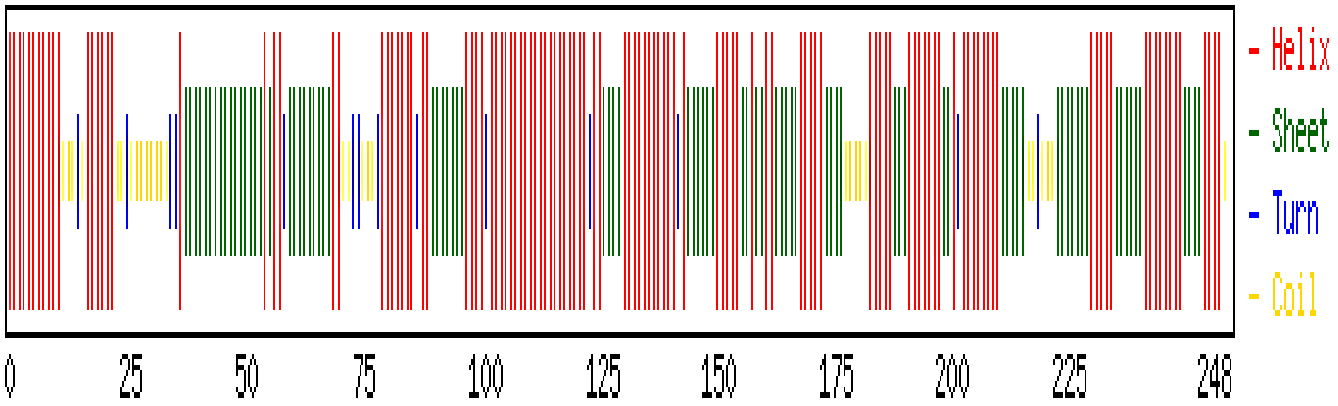

Figure 6: Plasmodium TIM tertiary structure

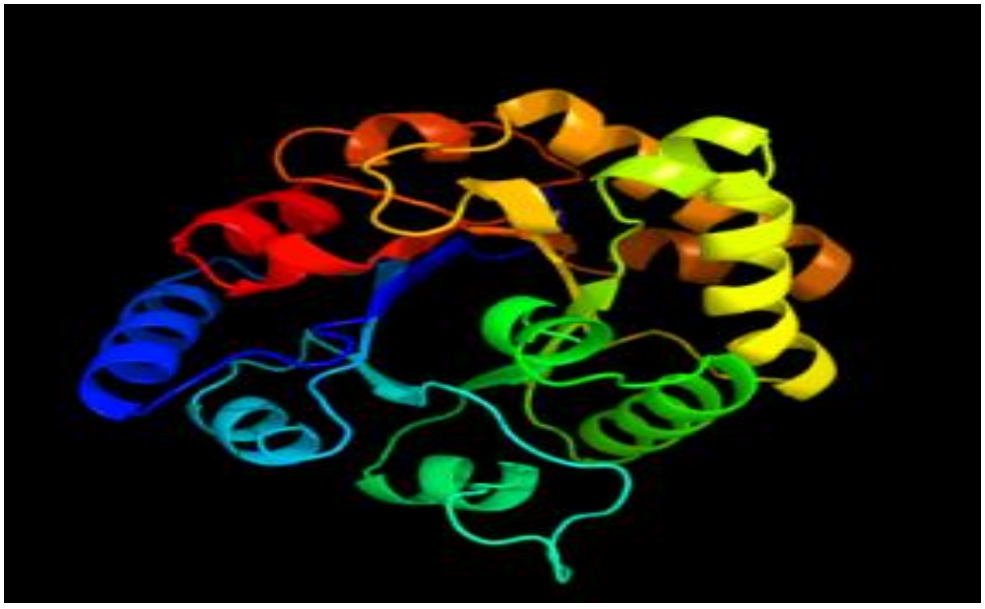

Figure 7: Human TIM tertiary structure

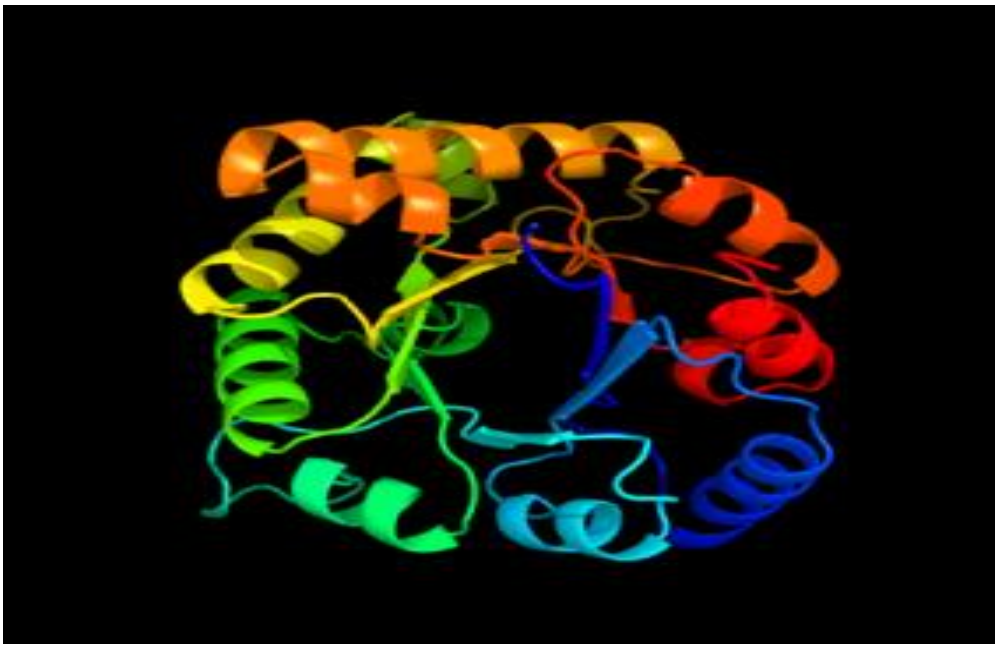

Figure 8: Three-dimensional (3D) ligand binding site. The blue, green and ash colors indicate the ligand binding site, metallic heterogens and non-ligand binding site respectively

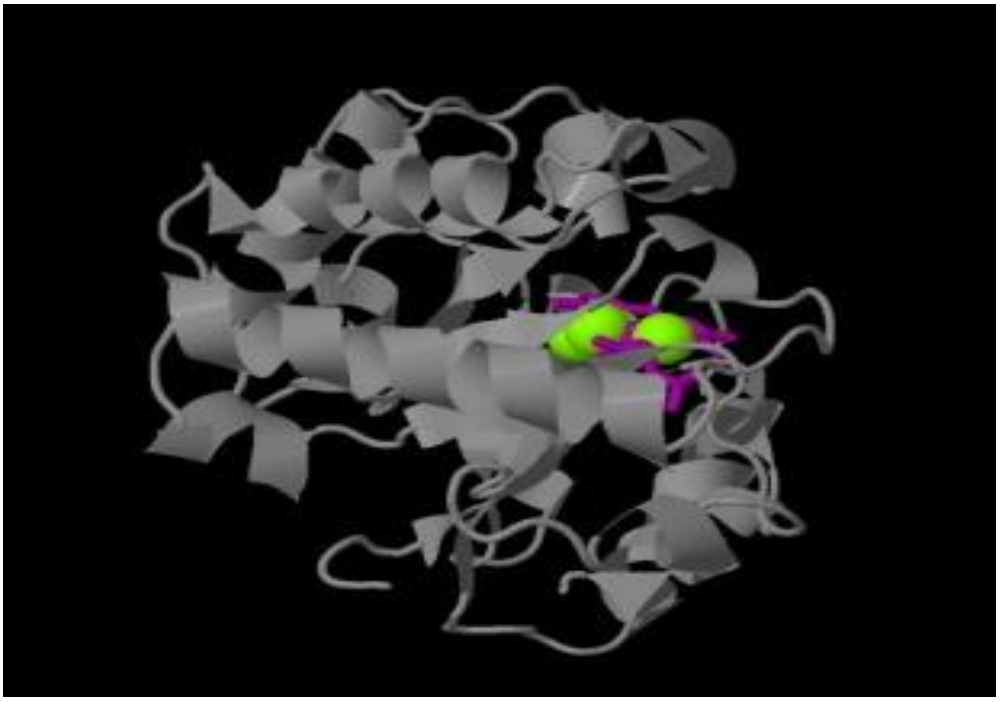

Figure 9: Antigenicity sites graph