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Comparative Analysis of Novel Targets for Antimalarial Drugs: Structural and Mechanistic Insights about Plasmodium falciparum Enzymes

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Abstract- Plasmodium falciparum, the causative agent of severe human malaria. The dominance of resistant strains has compelled to the discovery and development of new and different modes-of-action. Current plasmodial drug discovery efforts remains lack far-reaching set of legitimated drug targets. Prerequisite of these targets (or the pathways in which they function) is that they prove to be crucial for parasite survival. Thioredoxin Reductase is a flavoprotein that catalyzes the NADPH-dependent reduction of thioredoxin. It plays an important role in maintaining the redox environment of the cell. A third redox active group transfers the reducing equivalent from the apolar active site to the surface of protein. This group is a second redox active disulfide in thioredoxin reductase. The vital importance of the thioredoxin redox cycle (encompassing NADPH, thioredoxin reductase and thioredoxin) is stressed by the confirmation that thioredoxin reductase is indispensable for the survival of intraerythrocytic P. falciparum. Cytosolic Plasmodium falciparum Spermidine synthase linked with the polyamine metabolism is a potential target for antimalarial chemotherapy due to the vital role of spermidine in the activation of the eukaryotic translation initiation factor 5A, cell proliferation and the mechanism of the aminopropyltransferase action of Spermidine Synthase. Methyl Erythritol 4-Phosphate (MEP)/Rohmer pathway is assumed to have specific inhibitors designed against enzymes of this pathway with less toxicity and fewer side effects. 2C-Methyl-d-Erythritol 2, 4 – Cyclodiphosphate Synthase (MECPS), catalyzes the formation of isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate. All three enzymes represents as promising drug targets for rational drug designing.

Index Terms- Plasmodium falciparum, Thioredoxin Reductase, Homology Modeling, Structure Validation

I. INTRODUCTION

Malaria is life threatening disease caused by group of organisms Apicomplexa, differentiated by presence of four-membrane relict plastid. This deadly disease affects poorest population of about 107 countries [1, 2].From recent estimates it has been indicated that there are 300-500 million clinical cases death toll rises between 1.5-2.7 million occurs worldwide annually. 90% of the death occurs in tropical Africa. Out of 1.4 billion people, 1.2 billon people are of South East Region live in malaria prone area [16]. The sufferings are due to massive loss of productive man hours. The violent cycle of malaria and poverty continues in its most grave form in the developing nations where the poorest of poor cannot afford costly medication[1].Causative agent of human malaria is intracellular parasites of the genus *Plasmodium* spread by *Anopheles gambiae* mosquitoes. There are four species of human infecting *Plasmodium*. Out of these *P.falciparum* is the most deadly [3].Eradication of malaria became very difficult in the battle against this parasite due to drug resistant *Plasmodium falciparum* [1].

Thioredoxin reductase is a part of family of glutathione reductase-like homodimeric flavoenzymes [6]. *Plasmodium* possesses two chief NADPH-dependent redox systems consisting whole glutathione system [7,17, 18] and thioredoxin system with wide range of antioxidant defence mechanism, major antioxidant redox-enzyme is Thioredoxin reductase [7,19,20]. An entire Thioredoxin system comprises of thioredoxin reductase (TrxR), various thioredoxins and thioredoxin-dependent peroxidases (TPx) [7, 17, 21-23]. Malaria parasites are prone to disruption of the redox equilibrium at the time of erythrocytic life stages [7].Thioredoxin include the reduction of nucleotides to deoxy-nucleotides and alteration of transcription factors such as NF-kB [8, 24-26].

Plastid is the organelle which is crucial for the survival of these parasites and advantage is it consists of various pathways such as fatty acid, heme and isoprenoid biosynthesis [27] which is uniquely present in bacteria, plant and apicomplexan unlike humans [28-29]. Plasmodium utilizes plastidial methylerythritol 4-phosphate pathway (MEP) for isoprenoid biosynthesis. To stop the multi-drug resistance and spreading of *Plasmodium* strains various enzymes of this pathway such as 1-deoxy-D-xylulose-5-phosphate synthase, 1-deoxy-D-xylulose-5-phosphate reductoisomerase and **2C-methyl-D-erythritol 2, 4-cyclodiphosphate (MECP) synthase** [2, 30-31].

Plasmodium falciparum **spermidine synthase** (PfSpdSyn) belongs to the huge protein family of aminopropyltransferase. PfSpdSyn enzyme has many features; it makes less amount of spermidine found in the parasite, that increases DNA-polymerase activity six folds [35] and plays key role in modification and activation of the eukaryotic translation initiation factor eIF5A [36-39].Since PfSpdSyn is related to polyamine metabolism, polyamine biosynthesis results in depletion of spermidine due to

accumulation of unmodified eIF5A. Molecular and biochemical characterizations supported in determination of PfSpdSyn specific inhibitors. Importance of this enzyme is due to its product spermidine [5, 40].

II. Why These Enzymes Are Selected As Drug Targets?

In high-Mr TrxR C-terminal redox-active centre of the mammalian enzyme consist of selenocysteine-cysteine pair (Se-CysCys) while the Plasmodium TrxR consists a CysXXXXCys motif. The C-terminal sequences are SGASILQAGCUG in thioredoxin reductase from human placenta [58, 59] and AAKGGCGGGKCG in thioredoxin reductase from P.falciparum [60].

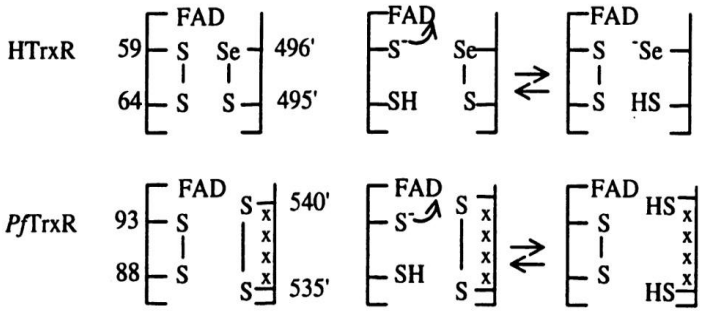

Fig.1 HTrxR, human thioredoxin reductase; and Pf TrxR, *Plasmodium falciparum* thioredoxin reductase. Residues having Numbers without primes come from one subunit while those with primes come from the other; the break at the bottom also symbolizes the two polypeptide chains. The curved arrow indicates charge transfer from the donor thiolate to the Acceptor FAD [8].

This chemical structure difference can be exploited for designing specific inhibitors, as shown in fig 1. There is noteworthy difference between the active sites of parasite and host proteins [9]. In mammalian Spermidine Synthase to some extent putrescine can be replaced by spermidine as aminopropyl acceptor whereas P.falciparum Spermidine Synthase has the potential to catalyse the formation of spermine. This functional difference is may be due to structural differences [3, 47]. MEP pathway is absent in human hosts due to this reason this pathway protein MECP Synthase signifies wonderful drug target [2].

III. Pathways and Systems Related to These Enzymes

Thioredoxin System for Thioredoxin Reductase:

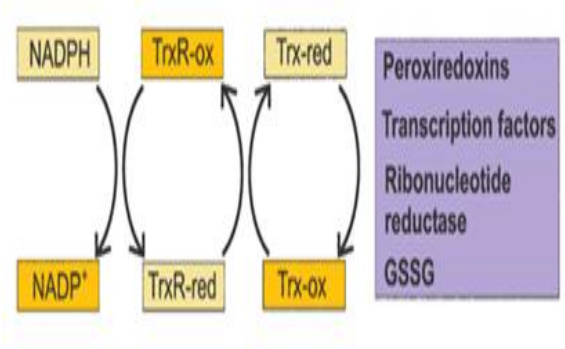

Fig.2 TrxR-ox, oxidized form of thioredoxin reductase; TrxR-red, reduced form of thioredoxin reductase; Trx-ox, Oxidized from of thioredoxin; Trx-red, reduced from of thioredoxin [9].

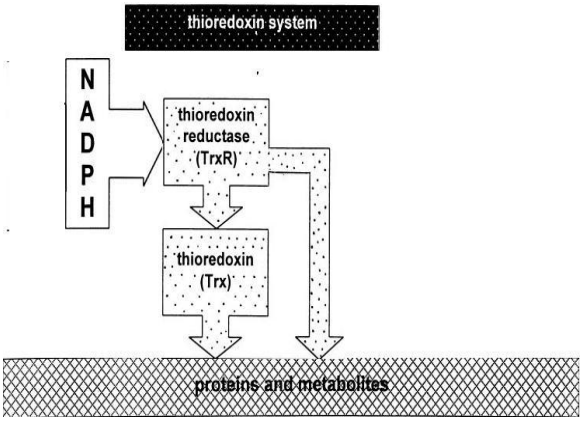

Fig.3 Intracellular disulphide- reducing system; the arrows are indicating the flow of reducing equivalents Originating from NADPH [6].

The thioredoxin redox cycle consists cascade of redox active proteins that shuttles reducing equivalents from NADPH to an acceptor molecule. There are acceptors such as ribonucleotide reductase and transcription factors. Thioredoxin reduces peroxiredoxins and GSS.G. This system is indispensable for the survival of *Plasmodium falciparum* [9] as in fig.2. Low molecular weight compounds and in fact proteins are included in broad substrate spectrum of high molecular weight TrxRs. [6, 10]. Question arises that the variety of TrxR substrates would choose which of the redox centre for reduction. In this context it depends upon the size of substrate, its charge and polarity. According to the catalytic mechanism it has been suggested that larger substrates react at the C-terminal redox active site whereas small compounds sometimes uses shortcut via the flavin /the internal catalytic cysteines [6] as in fig.3.

Two or more thioredoxins and TrxRs those act in different cell compartments are described in fig 4[10].

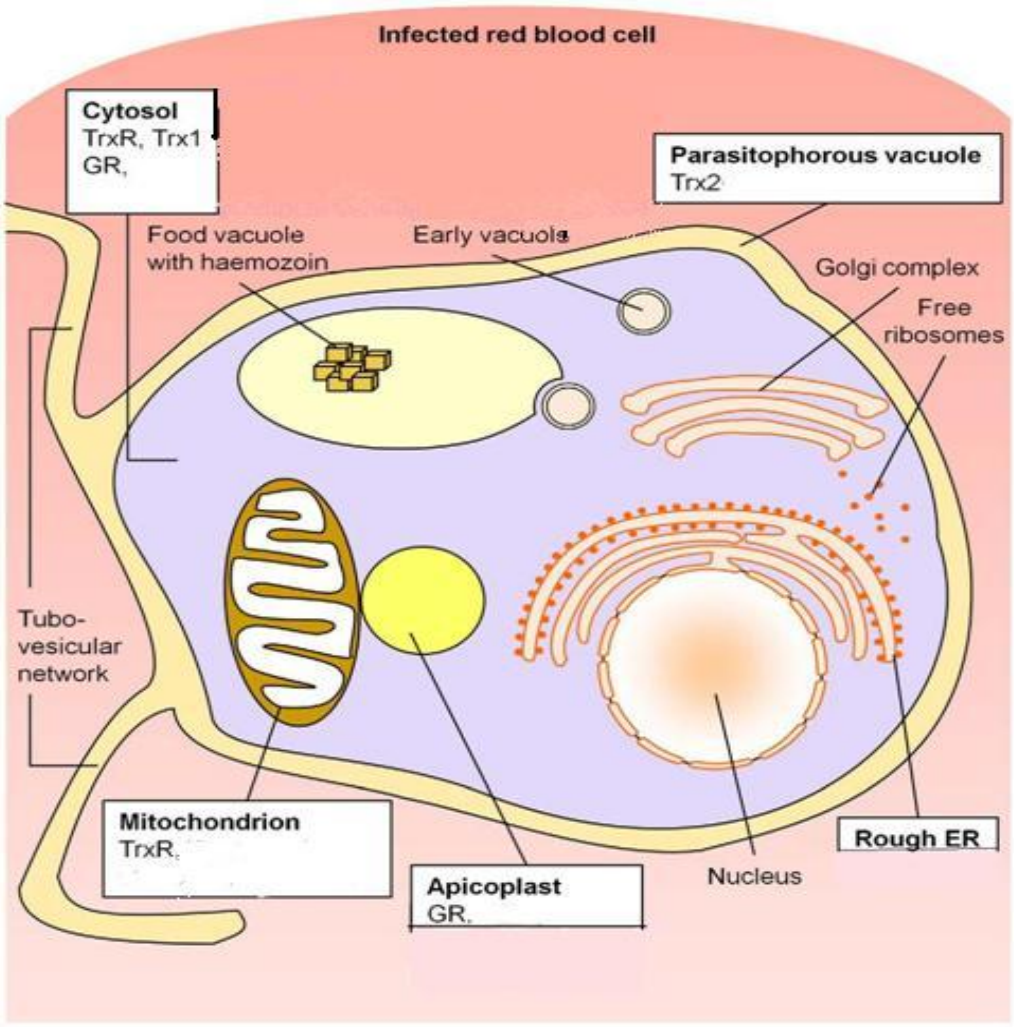

Fig.4 Subcellular compartmentation of cellular redox metabolism: Glutathione reductase and Thioredoxin Reductase in malaria parasite [7].

Polyamine biosynthesis pathway for Spermidine Synthase:

The polyamine biosynthetic pathway is responsible for the metabolism of plentiful amines essential for parasite growth, proliferation and differentiation. Polyamine biosynthesis acts as a potential parasite metabolic target. Polyamines are crucial and unique such as aliphatic amines consist of spermidine, putrescine and spermine. At the time of cell proliferation and differentiation this biosynthesis of polyamines is at the peak as polyamines are the key factor for growth and differentiation processes of pro- and eukaryotes [3, 5]. The highlighted part in fig 5 shows the role of spermidine synthase. It also plays an essential role in the stabilization of DNA and RNA, phospholipids, and numerous *in vivo* proteins.

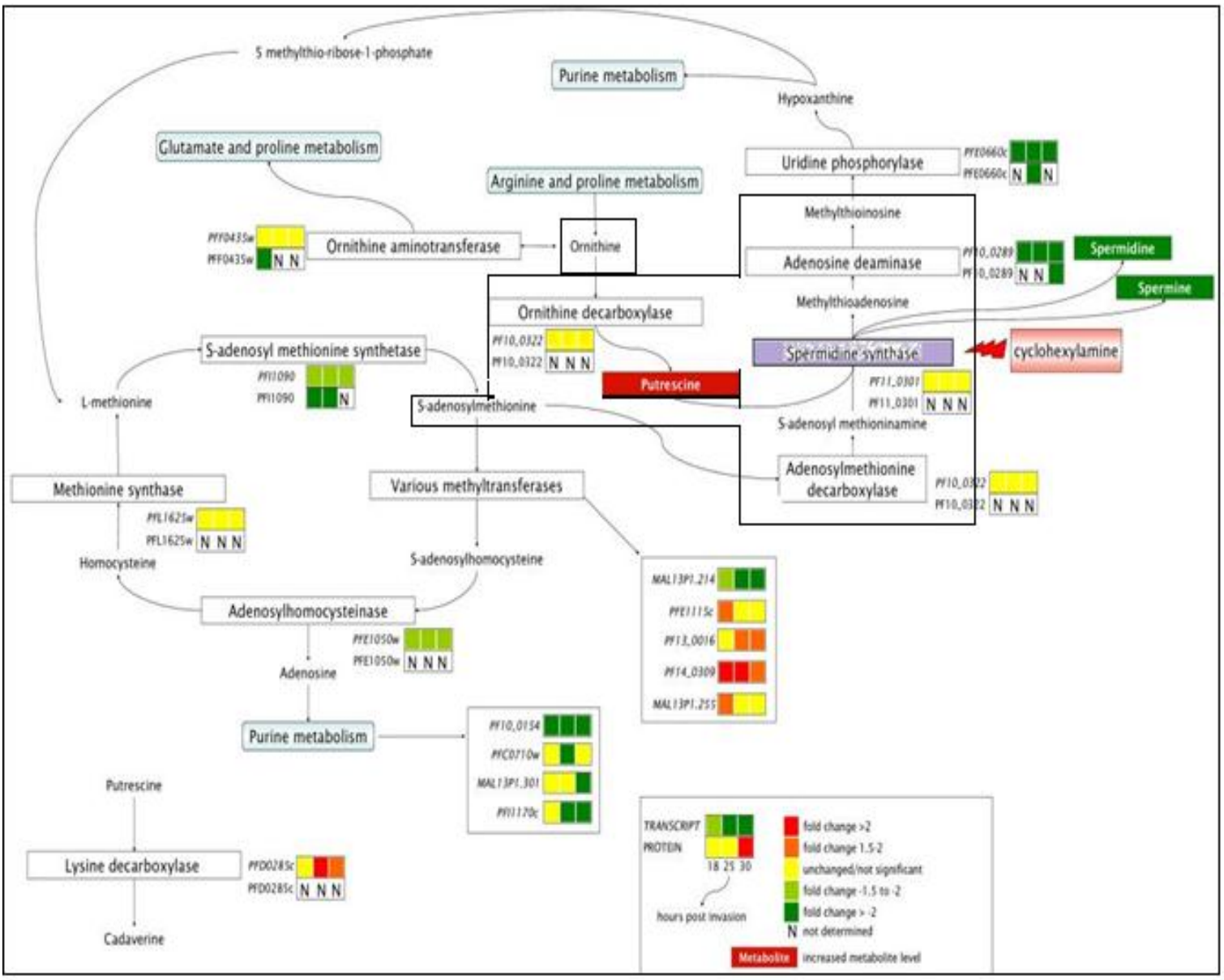

Fig.5 Role of Spermidine Synthase (purple) in polyamine biosynthetic pathway [11].

Ornithine decarboxylase (ODC) facilitates the decarboxylation of ornithine to yield putrescine, which provides as a scaffold for supplementing aminopropyl moiety from S-adenosylmethionine (dcAdoMet).The product of S-adenosylmethionine decarboxylase (AdoMetDC) catalyses to produce spermidine and Spermine. Due to spermidine and spermine synthase catalyzes spermine to produce 5'-methylthioadenosine (MTA) i.e. by-product of both the reaction [5] (fig.5).

Methyl Erythritol 4-phosphate (MEP) Pathway for Methylerythritol 2, 4-Cyclodiphosphate (MECP) Synthase:

There are two different pathways to carry out isoprenoid biosynthesis [30]:

- Mevalonate pathway [31] and
- Plastidial Methylerythritol 4-phosphate (MEP) pathway/ Rohmer pathway [32-34]

Plasmodium makes use of MEP pathway solely and this statement is supported by the studies of including inhibition of mevalonate pathway. MEP pathway is mevalonate independent pathway for isoprenoid synthesis in chloroplast of plants, eubacteria and apicomplexa [2, 55, 57].

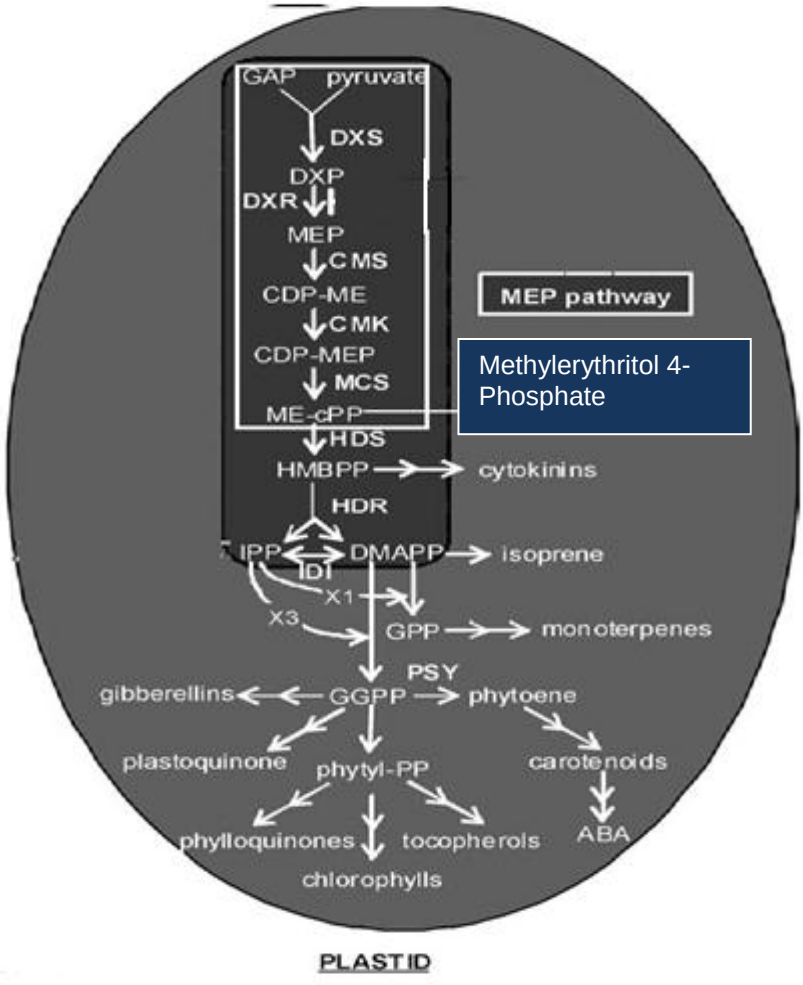

Fig.6 MEP Pathway; DXP, deoxyxylulose 5-phosphate; DXS, DXP synthase (EC 4.1.3.37); DXR, DXP Reductoisomerase (EC 1.1.1.267); GAP, Glyceraldehyde 3 - phosphate; CDP- ME, 4 - diphosphocytidyl – Methylerythritol; CDP-MEP, CDP-ME 2 - Phosphate; CMK, CDP-ME kinase (EC 2.7.1.148); CMS, CDP-ME Synthase (EC 2.7.7.60); MCS, ME-cPP synthase (EC 4.6.1.12); ME-cPP, methylerythritol 2, 4-cyclodiphosphate; ABA, abscisic acid; HBMPP hydroxymethylbutenyl 4-diphosphate; HDR, HMBPP reductase (EC 1.17.1.2); HDS, HMBPP synthase (EC 1.17.4.3); IDI, IPP isomerise (EC 5.3.3.2); IPP, isopentenyl diphosphate; GGPP, Geranylgeranyl diphosphate; GPP, geranyl diphosphate; PSY, phytoene synthase [12].

Initially the reaction of MEP pathway has been started by condensation of (hydroxyethyl) thiamine obtained from pyruvate with the C1 aldehyde group of Glyceraldehyde 3-phosphate (GAP) to produce deoxyxylulose 5-phosphate (DXP) catalyzed by deoxyxylulose 5-phosphate synthase (DXS).

In the next step the enzyme DXP reductoisomerase (DXR) reduces DXP intramolecular rearrangement takes place to produce methylerythritol 4-phosphate (MEP) which is the first committed precursor of plastid isoprenoids [12]. MEP is associated with CTP to generate 4-diphosphocytidyl-2C-methyl-D-erythritol (CDP-ME) [49,50] and pyrophosphate in a reaction mediated by 4-diphosphocytidyl-2C-methylerythritol synthetase.CDP-ME is phosphorylated by an ATP dependent 4-(cytidine 5' - diphospho)-2C-methylerythritol kinase [51] to produce CDP-ME-2-phosphste (CDP-ME2P) [55,56,52-54].

In the last stage CDP-ME2P is transformed to MECP and CMP catalyzed by MECP Synthase [4].Hence, MEP is converted to Methylerythritol 2, 4-cyclodiphosphate in three enzymatic steps [12]. All the steps are described in fig 6.

Reactions catalyzed by Thioredoxin Reductase, Spermidine Synthase and 2C-Methyl-d-Erythritol 2, 4-Cyclodiphosphate Synthase

Thioredoxin Reductase (TrxR) :

$$NADPH + TrxS_2 + H^+ \xrightarrow{\text{TrxR}} NADP^+ + Trx\,(SH)_2$$

Thioredoxin Reductase

Fig.7 Thioredoxin Reductase catalyzes electron transfer from NADPH to the disulphide of the substrate generates a Selenolthiol which is active site in the reduction of Trx [13].

Spermidine Synthase:

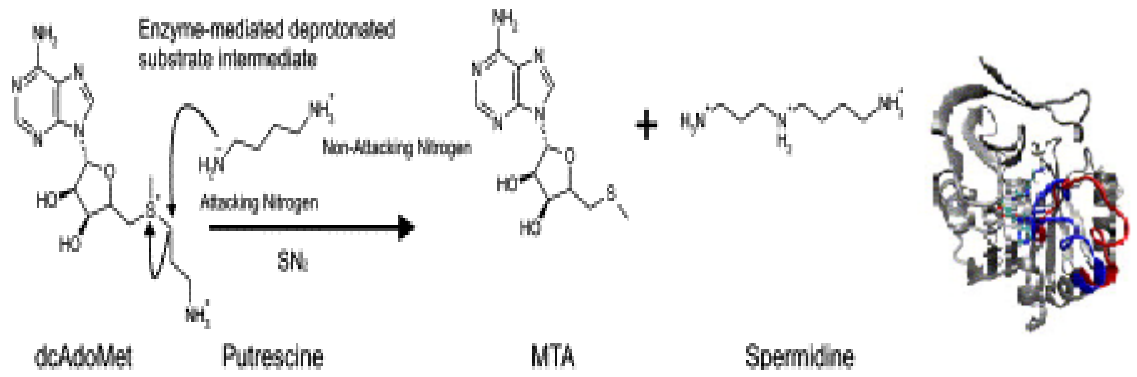

Fig.8 Mechanism of action for Spermidine Synthase. Attacking nitrogen of putrescine leads to nucleophilic attack on Electrophilic carbon of dcAdoMet due to deprotonation of the attacking nitrogen catalyzed by the enzyme Spermidine Synthase [5].

2C-methyl-d-erythritol 2, 4-cyclodiphosphate (MECP) synthase:

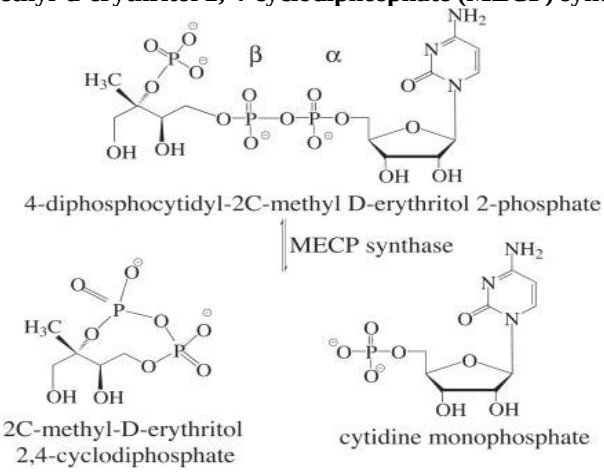

Fig.9 Nucleophilic attack by the ME2P phosphate of CDP-ME2P, 4- diphosphocytidyl – methylerythritol2-phosphate; The phosphate generate a pentacoordinate transition State, also stabilized by metal ion coordination and in the second stage then collapses to release Cytidine monophosphate (CMP) and the Cyclodiphosphate products i.e. 2C-methyl-D-erythritol 2,4-cyclodiphosphate [4].

Comparative Studies and Analysis of the enzymes:
Multiple Sequence Alignments:

Thioredoxin Reductase

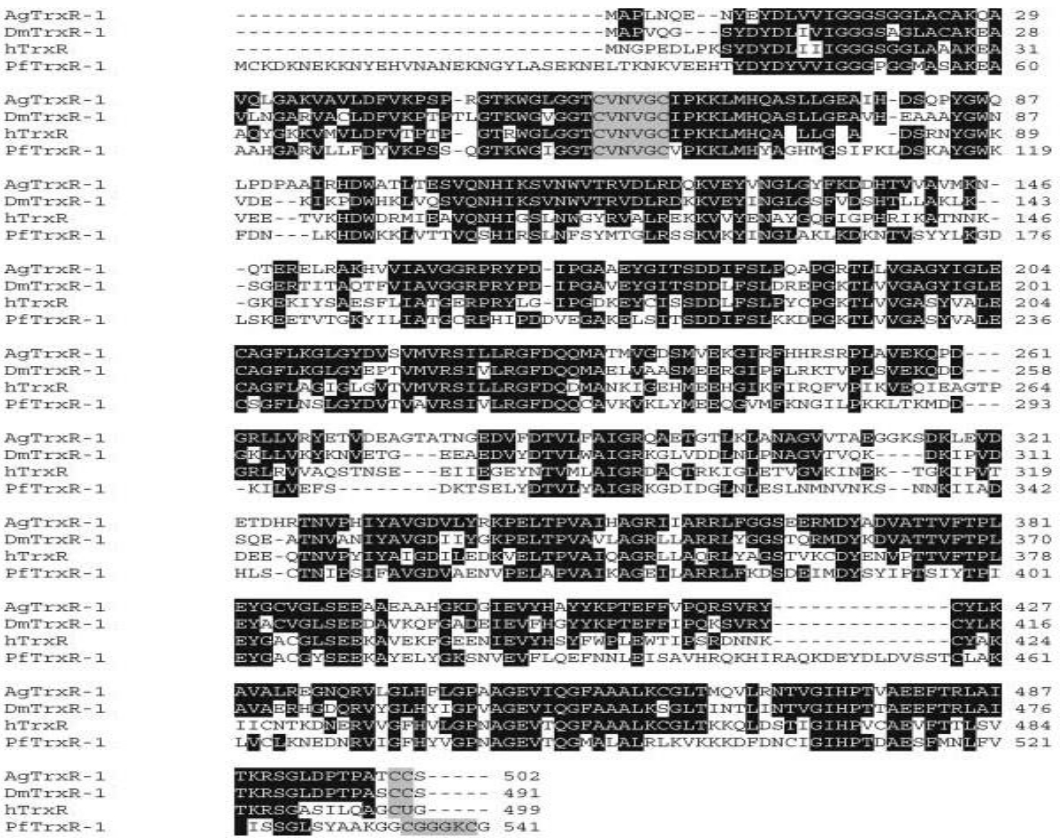

Fig.10 Multiple Sequence Alignment of high molecular mass Thioredoxin Reductase (TrxR) using online tool CLUSTALW [14].

Organism name	Accession Numbers from NCBI	Identity (%) with *Anopheles gambiae*	Reference
Plasmodium falciparum (PfTrxR-1)	CAA60574	45	Holger Bauer, Stephan Gromer, Andrea Urbani et.al,2003
Drosophila melanogaster (DmTrxR-1)	AAG25639	69	
Homo sapiens (hTrxR)	AAB35418	52	

Table 1: Comparison of percentage identity among *P.falciparum, D.melanogaster* and Human with respect to *A.gambiae* [14].

Spermidine Synthase:

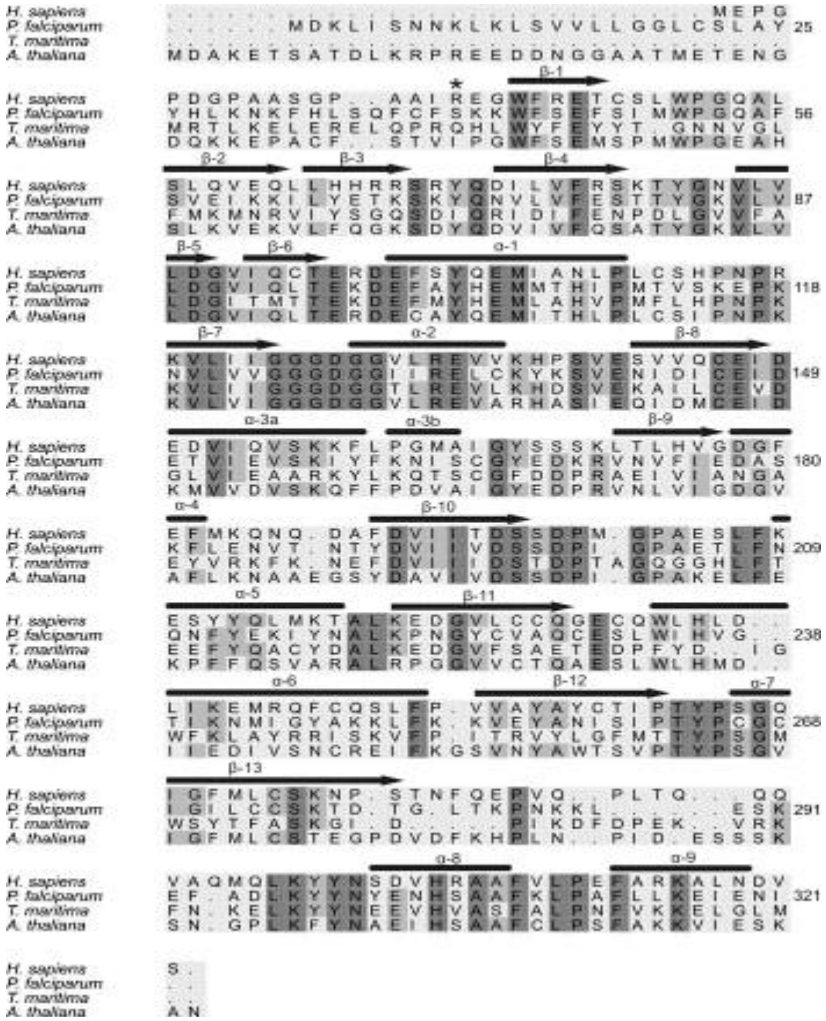

Fig.11 T-coffee package is used for Protein sequences alignment of P.falciparum, Arabidopsis thaliana, Thermatoga maritima, Humans Spermidine Synthase [5].
In fig.11 cylinders represent helices and arrows represent β - strands.

Arabidopsis thaliana and *Thermatoga maritima* are used as templates for homology modeling. Light gray and dark gray shaded amino acid regions represent conserved regions denoting 50% - 80% and higher than 80% respectively. Numbering is used in indication of amino acid sequence of PfSpdSyn. * indicates the starting of homology model. It has significant sequence identity (49%) with the Spermidine synthase of *Arabidopsis thaliana* and (32%) of *Thermatoga maritima* [5].

MECP Synthase:

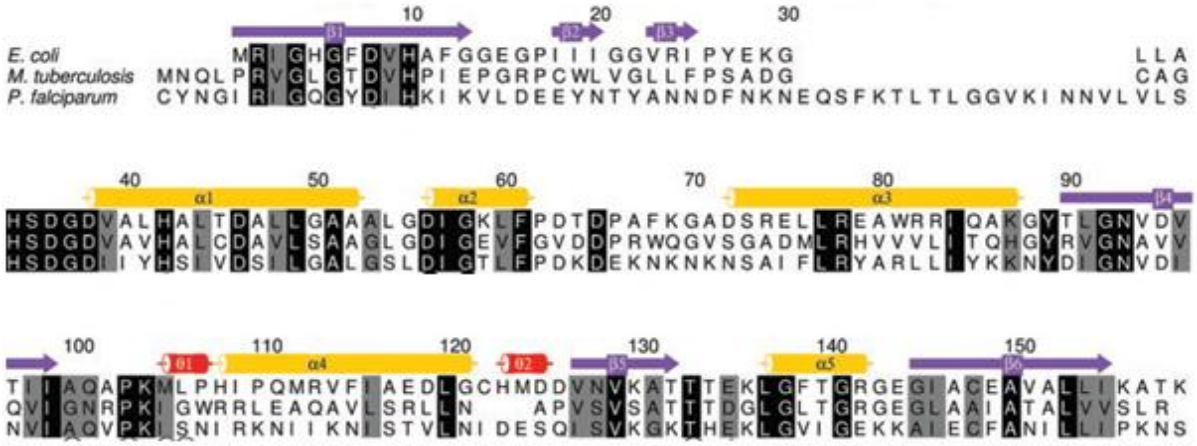

Fig.12 Alignment of MECP Synthase sequences from Escherichia coli, Mycobacterium tuberculosis and P.falciparum (β-sheet, purple; α -helix, gold; θ-helix, red)[4].

Organism name	Identity (%) with *Escherichia coli*	Reference
Plasmodium falciparum	35	Lauris E. Kemp, Charles S. Bond, and William N. Hunter, February 27,2002
Mycobacterium leprae	40	

Table 2. Calculated percentage identities of Plasmodium falciparum and Mycobacterium leprae with respect to E.coli

The three enzymes combinely reveal a sequence identity of about 20%.

Model Building Aided By Homology Modeling:

Homology Modeling has been carried out for obtaining the structure of other enzymes of *Plasmodium falciparum.* An overview of modeling approach can be recapitulated as follows: it consists of four steps:

i) Sets of models are derived
ii) Best models are selected on the basis of relative objective function values from the generated models
iii) molecular dynamics
iv) final validation step[1].

Target – Template Alignment: Refer table 3

Target	Template	Software	Identity	References
Thioredoxin Reductase	Crystal structure of TrxR type 2 of mouse (PDB ID: 1ZDL: A chain)	ClustalX	43.1%	Amit K. Banerjee, Neelima Arora &U.S.N. Murty,September 2009
Spermidine Synthase	Crystal structure of *A.thaliana* (PDB-ID:IXJ5)	T-Coffee Package	49%	Pieter B. Burgera, Lyn-Marie Birkholtz et al, February 2007
	Crystal structure of *A. thaliana* (PDB-ID:IXJ5)	MALIGN Script	47%	Duvvuru Muni Rajasekhara Reddy, December 23, 2006
MECP Synthase	Crystal structure of *P. vivax* (PDB ID: 3B6N_A)	ClustalX	60%	Neelima Arora, Amit Kumar Banerjee, U.S.N Murty, *Electronic Journal of Biology*, 2010, Vol. 6(2): 52-57

Table 3 Templates selected for Homology modeling.

Structural alignment of target and template: Shown in fig.13

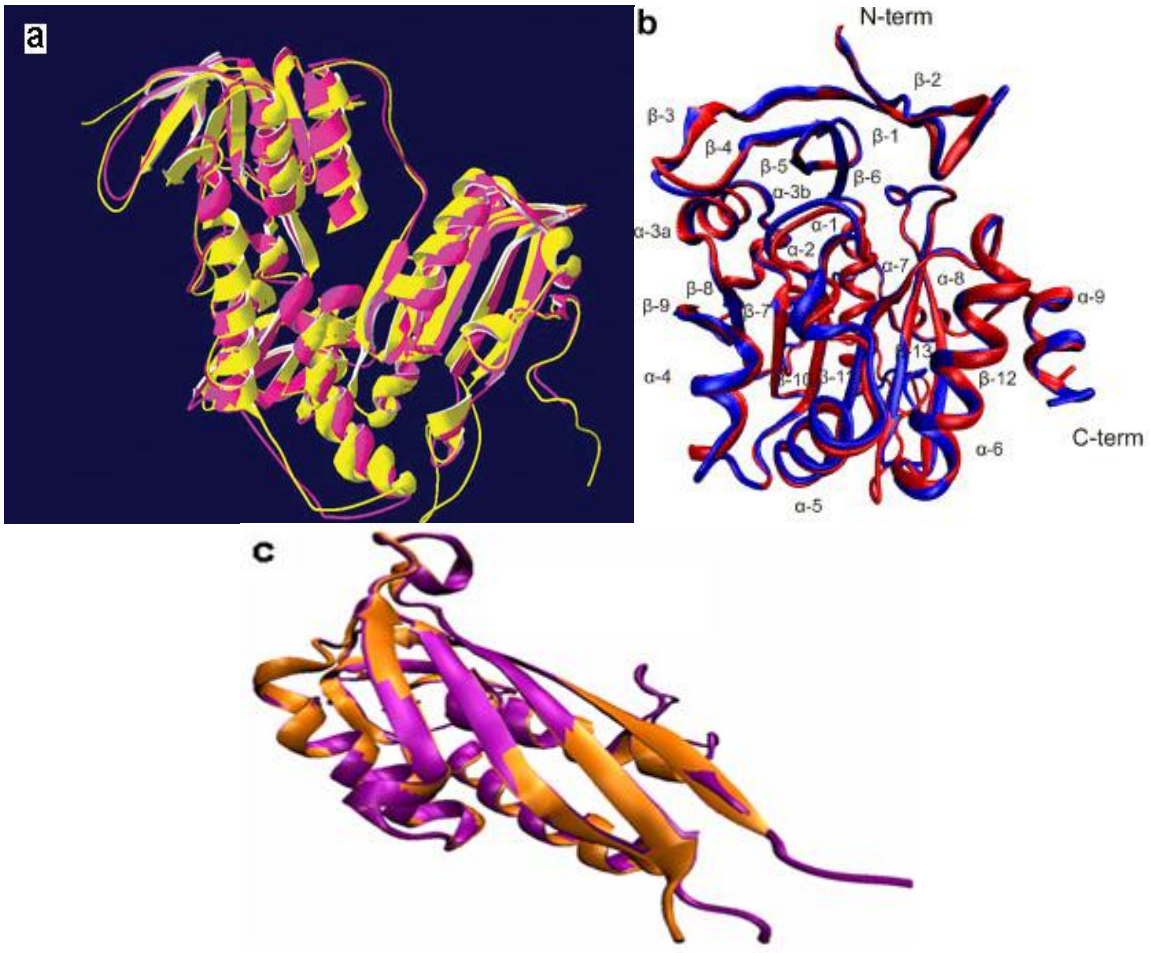

Fig.13 Superimposition of target and template a) target (Yellow) and template (Rose) of TrxR [1] b) target (Red) and Template 2HTE (Blue) of PfSpdSyn [5] c) target (Purple) and template (Orange) of MECP Synthase.

IV. HOMOLOGY MODELING

MODELLER is software used for homology modeling which utilizes target - template alignment for resolving tertiary model of the protein. This program performs command based modeling [2] shown in fig.14 and refer table 3.

Proteins	Software	Number of models generated by modeller	Value of MODELLER objective function	References
Thioredoxin Reductase	MODELLER 9v3	20	3204.0662	Amit Kumar Banerjee, Neelima Arora & U.S.N. Murty, September 2009
Spermidine Synthase	MODELLER 7v7[48]	25	967.8491	Duvvuru Muni Rajasekhara Reddy, December 23,2006
MECP Synthase	MODELLER 9v7 [46]	10	1964.3772	Neelima Arora, Amit Kumar Banerjee, U.S.N Murty, electronic Journal of Biology, 2010, Vol. 6(2): 52-57

Table 3 Evaluation of models generated using MODELLER

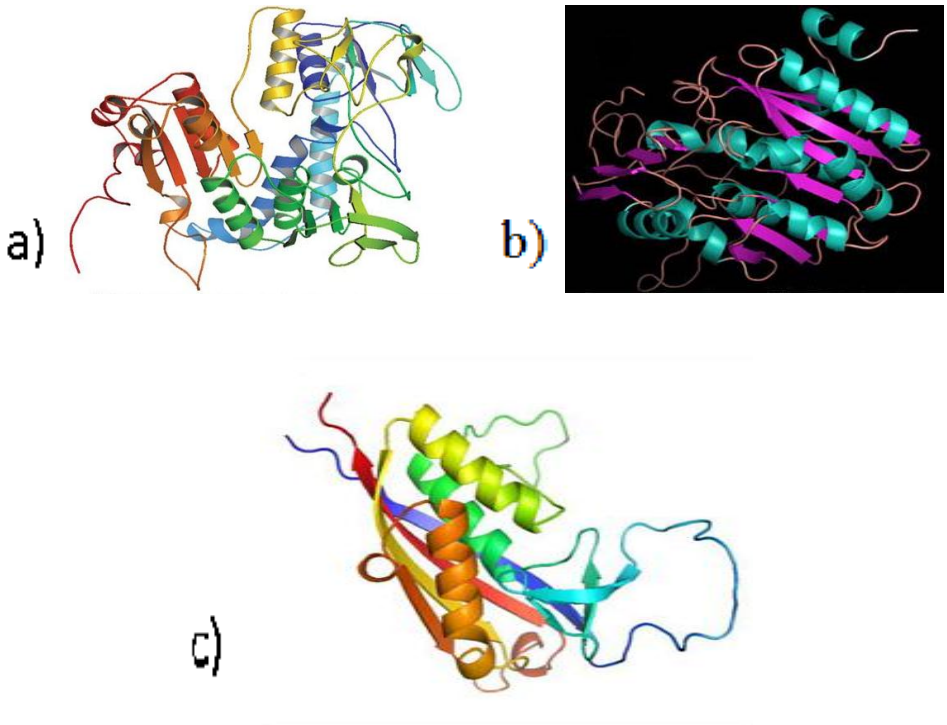

Fig.14 Computationally modelled 3D Structure of Thioredoxin Reductase [1], Spermidine Synthase[3], MECP Synthase[2] Using MODELLER software.

V. Structure Validation

The geometry of the model is assessed with Ramachandran's Plot calculations using **PROCHECK** and **RAMPAGE** servers. These servers consider backbone phi and psi dihedral angles for stereochemical evaluation. Root Mean Square Deviation (RMSD) value is calculated to indicate the close homology between backbone atoms of the template and the model [2] refer table 5 and fig.15.

Protein	Server	Property	Values	RMSD value	References
Thioredoxin Reductase	PROCHECK	Most Favoured Regions	78.3%	1.29Å	Amit Kumar Banerjee, Neelima Arora & U.S.N. Murty,September 2009
		Additionally Allowed Regions	17.6%		
		Generally Allowed Regions	3.2%		
		Disallowed Regions	0.9%		
Spermidine Synthase	PROCHECK	Most Favoured Regions	91.3%	0.4Å	Duvvuru Muni Rajasekhara Reddy,December 23, 2006
		Additionally Allowed Regions	6.4%		
		Generally Allowed Regions	2.3%		
		Disallowed Regions	-		
MECP Synthase	RAMPAGE	Most Favoured Regions	95%	0.5Å	Neelima Arora, Amit Kumar Banerjee, U.S.N Murty, electronic Journal of Biology, 2010, Vol. 6(2): 52-57
		Allowed Regions	5%		

Table 5 Distribution of Psi and Phi angles using RAMPAGE and PROCHECK servers

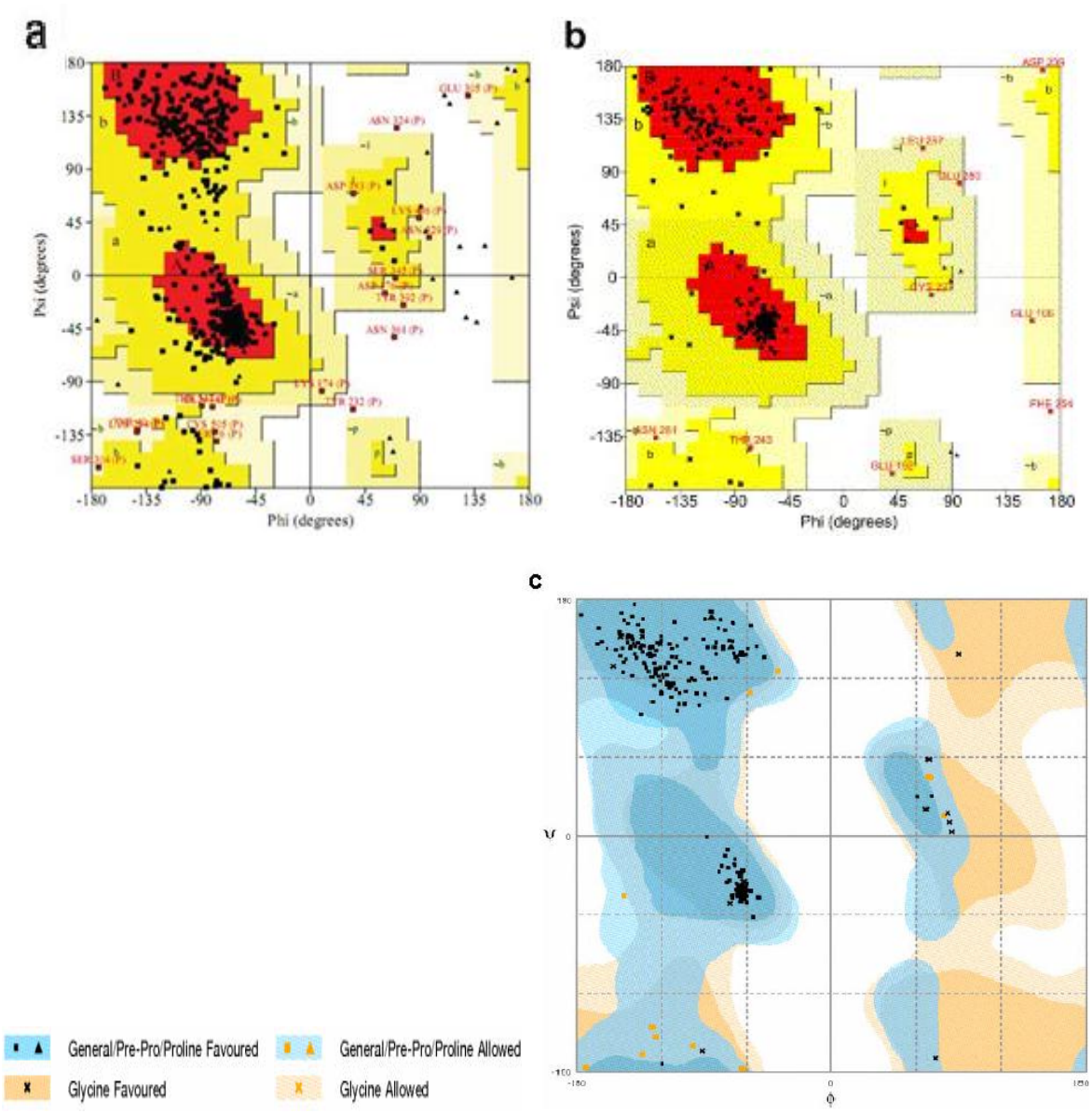

Fig.15 Ramachandran Plots showing the distribution of Phi and Psi Angles of TrxR[1], PfSpdSyn[5]and MECP Synthase[2] models

Active Site Analysis: Refer table 6

Thioredoxin Reductase

The final best model that has been selected, possible binding sites were searched using the **CASTp** server [15, 41].91 possible binding sites has been determined Area of active site was predicted 666.2 and volume of 791.2 [1] (fig.16).

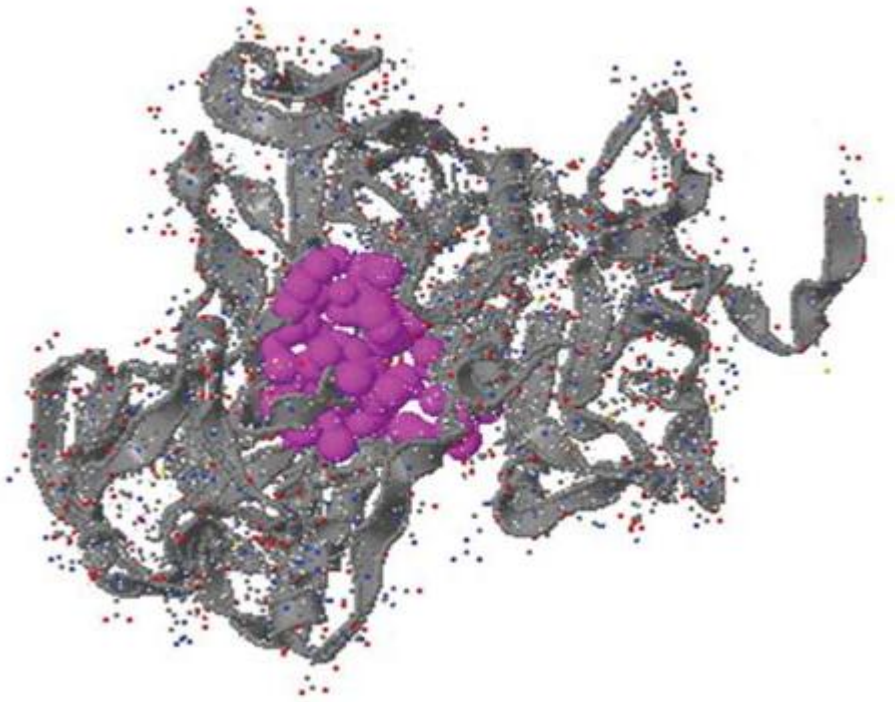

Fig.16 Predicted active site of Thioredoxin reductase generated model.

VI. SPERMIDINE SYNTHASE

S-adenosyl-1,8-diamino-3-thiooctane(AdoDATO) is substrate analogue. Models of PfSpdSyn generated by modeller with and without AdoDATO [45] consist of active site residues interact with AdoDATO shows high conservation. AdoDATO is converted *Insilco* into two moieties dcAdoMet and putrescine.

In the model of PfSpdSyn model, the binding sites for putrescine and dcAdoMet were clear. The dcAdoMet binding cavity is denoted by the residues enclosing the adenosyl fragment of AdoDATO on the other hand the residues surrounding the polyamine part denotes putrescine binding cavity. Putrescine binding cavity consists of central hydrophobic region flanked by two negatively charged regions suggested by [Korolev et al. and Shirahata et al.] [42, 43].Composition of this region is Trp_{51},Val_{91},Tyr_{102},Ile_{235},Tyr_{246},Pro_{247} and Ile_{269}.The two electron donating regions are composed of Gln_{93},Tyr_{102},Asp_{196},Ser_{197},Gln_{229} and Glu_{231},Asp_{199} and His_{236} respectively. Eight hydrogen bonds were discovered between PfSpdSyn model and dcAdoMet. Hydrogen bonds were formed between dcAdoMet and Asp_{127}, Asp_{178}, Ala_{179}, Asp_{196}, His_{103} and Pro_{203} provokes two hydrogen bonds through water molecules 12 and 13 and further hydrogen bond with water molecule 11, which again forms hydrogen bond with Asp_{127}.Asp_{127} together with His_{103} and Asp_{196} forms hydrogen bonds with the aminopropyl chain of dcAdoMet. It has been suggested that these three hydrogen bonds are crucial to orient the aminopropyl chain so that nucleophilic attack by putrescine [43] on an electrophillic carbon can be feasible [5] (fig.17).

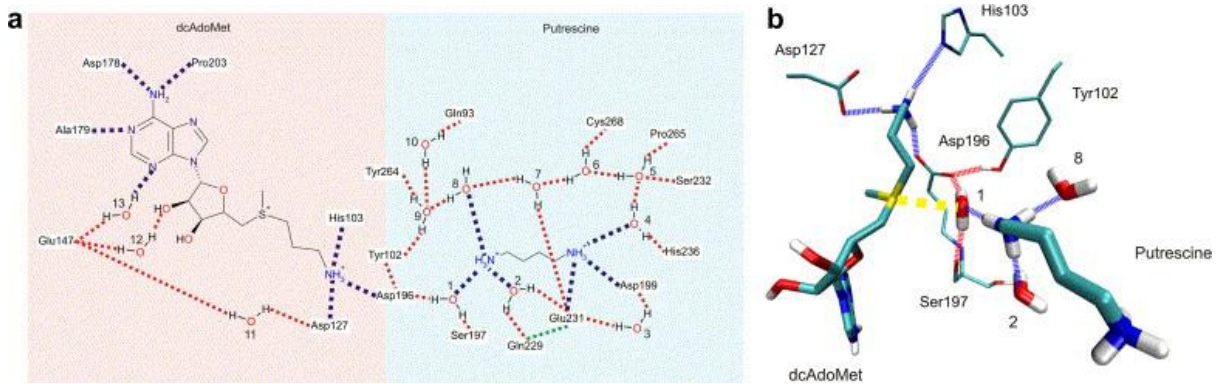

Fig.17 a) A 2D representation of the interactions between PfSpdSyn and its substrates. The dcAdoMet and Putrescine

Binding cavity is denoted in the apricot shaded area to the left and blue area to the right respectively. Water Molecules assume to attach and orient putrescine (via hydrogen bonds denoted by dashed red lines) and dashed Blue lines denote hydrogen bonds with nitrogen atoms. A protein-protein interaction hydrogen bond between Gln_{229} and Glu_{231} is denoted by green colour. b) A 3D representation of most important interaction for substrate Binding and catalysis. Dashed lines in red and blue denotes hydrogen bonds with the substrates. The polar Interaction between water molecule 1 and the positively charged sulphur of dcAdoMet is denoted by Yellow Colour [5].

VII. MECP Synthase

Q-Site Finder software has been employed for determining binding sites. It has predicted 10 binding sites from the modelled structure. Among all these active sites largest site with volume of 233 cubic Å has been selected [2].

CDP and two metal ions Zn^{2+} and Mn^{2+} makes a homotrimer which helps to get details about protein ligand interaction and analysis of active site. The homotrimer consist of three active sites present in a cleft formed by two subunits whose residues interact with CDP (Refer fig.18). The active site formation is mainly due to C-terminal section of α1 and the turn directing into and the N-terminal region of alpha1 along with the short α2 of one subunit. These fragments interact with one of the metal ions (Zn), the ribose and diphosphate of CDP. The base interacts with the N terminus of alpha4 and C terminus of beta5 i.e. from partner subunit. Residues from the partner subunit is denoted by prime ('). The cytosine of CDP is located in an aliphatic pocket created by the side chains of Ala-100', Lys-104', Met-105', Leu-106',Ala-131', and Thr-133' and forms four hydrogen bonds with main atoms(fig.5).The base amine N4 gives hydrogen bonds to the carbonyl group of cis-Pro-103' and Ala-100' while N3 and O2 are acceptors for such interactions with the amides of Met-105' and Leu106' respectively. The hydroxyl groups of ribose form direct hydrogen-bonding interactions with the carboxylate of Asp-56 and the amide of Gly-58(Fig.5), in addition solvent-mediated interactions with Asp-46 O δ2 and carbonyl of Ala-131' are also observed. The side chain of Asp-56 is positioned by interaction with the amides of Gly-58, Lys-59, and Ala-131'and it provides an attachment for ribose. One alpha-phosphate phosphoryl oxygen interacts with Thr-133' by accepting two hydrogen bonds from the amide and hydroxyl groups while the other free alpha-phosphate oxygen atom directs Mn^{2+}.The beta-phosphate supplies oxygen ligands for both Zn^{2+} and Mn^{2+} and a solvent mediated interaction with Thr-132' O γ [4].

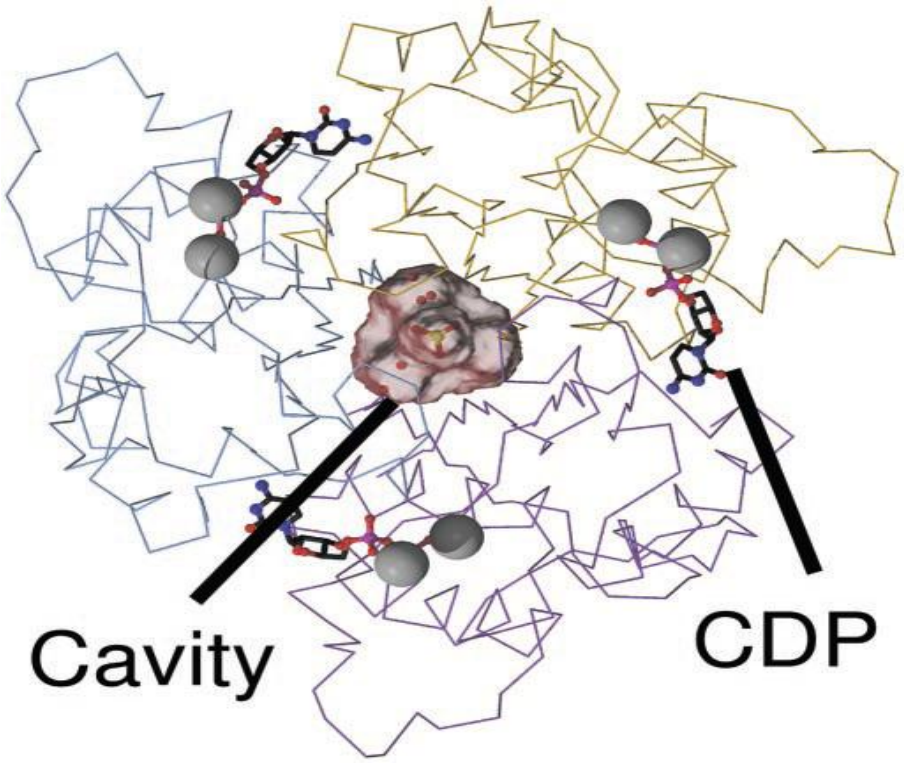

Fig.18 Hydrophobic intersubunit cavity with red semitransparent surface with trimer which is a predicted active site for MECP Synthase.

Protein	Binding Site Residues of Active Site	References
Thioredoxin	Pro_{51},Thr_{87},Val_{91},Gly_{92},Cys_{94},Lys_{96},Cys_{194},Ser_{212},Phe_{216}, Glu_{236},Val_{233},Tyr_{232},Ser_{231},Cys_{237},Ala_{313},Ile_{314},Gly_{315},	Amit Kumar Banerjee, Neelima Arora & U.S.N.

Reductase	Arg_{316},Gly_{356},Asp_{357},Pro_{363},Glu_{364},Leu_{365},Ala_{366},Pro_{367}, Ala_{369}, Pro_{394} Ser_{396},Ile_{397},Tyr_{398}, Gly_{483} and Gln_{487}.	Murty, September 2009.
Spermidine Synthase	Trp_{51},Val_{91},Tyr_{102},Ile_{235},Tyr_{246},$Pro_{247}Ile_{269}$,Gln_{93},Tyr_{102}, Asp_{196},Ser_{197},Gln_{229}, Glu_{231},Asp_{199} and His_{236} [43, 44]	Pieter B. Burgera, Lyn-Marie Birkholtz et al, February 2007.
MECP Synthase	Val_{179},Ile_{180},Ala_{181},Gln_{182},Val_{183},Pro_{184},Lys_{185},Ile_{186}, Ser_{187}, Arg_{190},Val_{210},Lys_{211},Gly_{212},Lys_{213} and Thr_{214}	Neelima Arora, Amit Kumar Banerjee, U.S.N Murty, electronic Journal of Biology, 2010, Vol. 6(2): 52-57.

Table 6 Binding Site analysis of all the three enzymes.

VIII. SUMMARY

Malaria is the main cause of death rate attributable to a communicable disease. Antimalarial drug resistance seems to be the greatest force against ruthless battle of malaria. Resistance against antimalarial drugs increasing and widening its prospects to the unaffected areas also. Due to this fact it convinces to explore more novel drugs. Comparative protein modeling is very much helpful in rational drug designing. In the shortage of experimental data, at the model building only the known crystal structure of homologous protein is reliable to gain structural information. Three-dimensional model of all the target enzymes was constructed. Generated models were further assessed by various structure validation methods which give affirmation about the correctness of the model. The enzymes of redox system of the Plasmodium species are assumed to be interesting potential targets whose inhibition affects several vulnerable points in redox mechanism. Polyamine biosynthetic pathway is also targeted as this pathway also houses some important enzymes such as Spermidine Synthase from *Plasmodium falciparum*. The model is believed to provide some clue to design inhibitor specific to the enzyme for the treatment of malaria and will help in locating active sites and conformations.MEP pathway consists of various good drug targets which can be selected as future antimalarial therapeutics. MECPS is one of the promising and attractive drug targets. The models generated will provide insight about its structure. Model will provide a base for clarifying structure function relationship and paves way towards *Insilco* drug designing.

REFERENCES

[1] Amit Kumar Banerjee, Neelima Arora & U.S.N. Murty, Structural model of the Plasmodium falciparum Thioredoxin reductase: a novel target for antimalarial drugs J Vector Borne Dis 46, September 2009, pp. 171–183.

[2] Neelima Arora, Amit Kumar Banerjee, U.S.N Murty, Homology model of 2C-Methyl-d-erythritol 2, 4- cyclodiphosphate (MECP) synthase of Plasmodium falciparum 3D7. Electronic Journal of Biology, 2010, Vol. 6(2): 52-57.

[3] Duvvuru Muni Rajasekhara Reddy, Comparative protein modeling of Spermidine Synthase from Plasmodium falciparum: A potential target for anti-malarial drug Therapy, December 23, 2006.

[4] Lauris E. Kemp, Charles S. Bond, and William N. Hunter, Structure of 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase: An essential enzyme for isoprenoid biosynthesis and target for antimicrobial drug development, February 27, 2002.

[5] Pieter B. Burger, Lyn-Marie Birkholtz, Fourie Joubert, Nashya Haider, Rolf D.Walter and Abraham I. Louw, Structural and mechanistic insights into the action of Plasmodium falciparum spermidine synthase openUP – February 2007.

[6] Katja Becker, Stephan Gromer, R. Heiner Schirmer and Sylke MuÈ ller, minireview, Thioredoxin reductase as a pathophysiological factor and drug target, Eur. J. Biochem. FEBS 2000.

[7] Sebastian Kehr, Nicole Sturm, Stefan Rahlfs, Jude M. Przyborski, Katja Becker, Compartmentation of Redox Metabolism in Malaria Parasites, December 2010 | Volume 6 | Issue 12 | e1001242.

[8] Charles H. Williams Jr1, L. David Arscott1, Sylke MuÈ ller, Brett W. Lennon, Martha L. Ludwig, minireview, Thioredoxin reductase Two modes of catalysis have evolved. Eur. J. Biochem. FEBS 2000.

[9] Sylke Muller, microreview, Redox and antioxidant systems of the malaria parasite Plasmodium falciparum, Molecular Microbiology (2004) 53(5), 1291–1305.

[10] Stefan M. Kanzok, R. Heiner Schirmer, Ivana Tu rbachova, Rimma Iozef, and Katja Becker, The Thioredoxin System of the Malaria Parasite Plasmodium falciparum GLUTATHIONE REDUCTION REVISITED, THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 275, No. 51, Issue of December 22, pp. 40180–40186, 2000.

[11] John VW Becker1, Linda Mtwisha1, Bridget G Crampton, Stoyan Stoychev, Anna C van Brummelen, Shaun Reeksting, Abraham I Louw, Lyn-Marie Birkholtz and Dalu T Mancama, Plasmodium falciparum spermidine synthase inhibition results in unique perturbation-specific effects observed on transcript, protein and metabolite levels, *BMC Genomics* 2010.

[12] Manuel Rodrı´guez-Concepcio, Early steps in isoprenoid biosynthesis: Multilevel regulation of the supply of common precursors in plant cells, Phytochemistry Reviews (2006) 5: 1–15 Springer 2006.

[13] Tatyana Sandalova,Liangwei Zhong,Ylva Lindquist ,Arne Holmgren , and Gunter Schneider, Three-dimensional structure of a mammalian thioredoxin reductase: Implications for mechanism and evolution of a selenocysteine-dependent enzyme, National Institutes of Health, Bethesda, MD, and approved June 15, 2001.

[14] Holger Bauer, Stephan Gromer, Andrea Urbani, Martina Schnolzer, R. Heiner Schirmer and Hans-Michael Muller, Thioredoxin reductase from the malaria mosquito Anopheles gambiae. Comparisons with the orthologous enzymes of Plasmodium falciparum and the human host, Eur. J. Biochem. 270, 4272–4281 (2003) FEBS 2003.

[15] Mamatha DM, Nagalakshmamma K, Dev VA, Rajesh, Sheerin VS. Protein modeling of apical membrane antigen-1(AMA-1) of Plasmodium cynomolgi. Afr J Biotechnol 2007; 6 (22): 2628–32.

[16] Shiv Lal, G.S. Sonal, P.K. Phukan, Report, Status of Malaria in India, Journal ofIndian Academy of Clinical Medicine _ Vol. 5 _ No. 1.

[17] Becker K, Koncarevic S, Hunt NH (2005) Oxidative stress and antioxidant defense in malarial parasites. In: Sherman IW, ed. Molecular Approaches to Malaria.Washington, DC: ASM Press. pp 365–383.

[18] Farber PM, Arscott LD, Williams CH, Jr., Becker K, Schirmer RH (1998) Recombinant Plasmodium falciparum glutathione reductase is inhibited by the antimalarial dye methylene blue. FEBS Lett 422: 311–314.

[19] Rahlfs S, Fischer M, Becker K (2001) Plasmodium falciparum possesses a classical glutaredoxin and a second, glutaredoxin-like protein with a PICOT homology domain. J Biol Chem 276: 37133–37140.

[20] Deponte M, Becker K, Rahlfs S (2005) Plasmodium falciparum glutaredoxin-like proteins. Biol Chem 386: 33–40.

[21] Akoachere M, Iozef R, Rahlfs S, Deponte M, Mannervik B, et al. (2005) Characterization of the glyoxalases of the malaria parasite Plasmodium falciparum and comparison with their human counterparts. Biol Chem 386: 41–52.

[22] Nickel C, Rahlfs S, Deponte M, Koncarevic S, Becker K (2006) Thioredoxin networks in the malaria parasite Plasmodium falciparum. Antioxid Redox Signal 8:1227–1239.

[23] Muller S (2004) Redox and antioxidant systems of the malaria parasite Plasmodium falciparum. Mol Microbial 53: 1291–1305.

[24] Holmgren, A. (1989) Minireview: thioredoxin and glutaredoxin systems. J. Biol.Chem. 264, 13963±13966.

[25] Hirota, K., Murata, M., Sachi, Y., Nakamura, H., Takeuchi, J., Mori, K. & Yodoi, J.(1999) Distinct roles of thioredoxin in the cytoplasm and in the nucleus ± a two-step mechanism of redox regulation of transcription factor NF-kB. J. Biol. Chem. 274, 27891±27897.

[26] Gromer, S., Schirmer, R.H. & Becker, K. (1999) News and views on thioredoxin reductases. Redox Report 4, 221±228.

[27] Roos D.S., Crawford M.J., Donald R.G., Fraunholz M., Harb O.S., He C.Y.Kissinger J.C., Shaw M.K., Striepen B. (2002) Mining the Plasmodium genome database to define organellar function: what does the apicoplast do? Philos Trans R Soc Lond B Biol Science, 357: 35–46.

[28] Ralph S.A., D'Ombrain M.C., McFadden G.I. (2001) The apicoplast as an antimalarial drug target. Drug Resist Update, 4: 145–151.

[29] McFadden G.I., Roos D.S. (1999) Apicomplexan plastids as drug targets. Trends in Microbiology, 7(8): 328-333.

[30] Kemp L.E., Bond C.S., Hunter W.N. (2002) Structure of 2C-methyl-D-erythritol 2,4 Cyclodiphosphate synthase: an essential enzyme for isoprenoid biosynthesis and target for antimicrobial drug development. Proc. Natl Acad. Sci. USA, 99: 6591– 6596.

[31] Bloch K. (1992) Sterol molecule: structure, biosynthesis and function. Steroids,57(8): 378-383.

[32] Rohmer M., Knani M., Simonin P., Sutter B., Sahm H. (1993) Isoprenoid biosynthesis in bacteria: a novel pathway for the early steps leading to isopentenyl diphosphate. Biochem. J, 295: 517-524.

[33] Eisenreich W., Schwarz M., Cartayrade A., Arigoni D., Zenk M.H., Bacher A.(1998) The deoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms. Chem. Biol, 5: R221–R233.

[34] Boucher Y., Doolittle W.F. (2000) The role of lateral gene transfer in the evolution of isoprenoid biosynthesis pathways. Mol. Microbiol, 37: 703–716.

[35] U. Bachrach and L.A. Abu-Elheiga, Eur. J. Biochem. 191 (1990), p. 633.

[36] T.L. Byers, B. Ganem and A.E. Pegg, Biochem. J. 287 (1992), p. 717.

[37] T.L. Byers, R.S. Wechter, R.H. Hu and A.E. Pegg, Biochem. J. 303 (1994), p. 89.

[38] S. Müller, E. Liebau, R.D. Walter and R.L. Krauth-Siegel, Trends Parasitol. 19(2003), p. 320.

[39] A. Kaiser, A. Gottwald, W. Maier and H.M. Seitz, Parasitol. Res. 91 (2003), p. 508.

[40] N. Haider, M. Eschbach, S. de Souza Dias, T. Gilberger, R.D. Walter and K. Luersen, Mol. Biochem. Parasitol. 142 (2005), p. 224.

[41] Mamatha DM, Nagalakshmamma K, Dev VA, RajesSheerin VS. Protein modeling of apical membrane antigen-1(AMA-1) of Plasmodium cynomolgi. Afr J Biotechnol 2007; 6 (22): 2628–32.

[42] A. Shirahata, N. Takahashi, T. Beppu, H. Hosoda and K.. Samejima, Biochem. Pharmacol. 44 (1991), p. 205.

[43] S. Korolev, Y. Ikeguchi, T. Skarina, S. Beasley, C. Arrowsmith, A. Edwards, A. Joachimiak, A.E. Pegg and A. Savchenko, Nat. Struct. Biol. 9 (2002), p. 27.

[44] Y. Ikeguchi, M.C. Bewley and A.E. Pegg, J. Biochem. (Tokyo) 139 (2006), p. 1.

[45] A. Sali and T.L. Blundell, J. Mol. Biol. 234 (1993), p. 779.

[46] Sali A., Blundell T.L. (1993) Comparative protein modelling by satisfaction of spatial restraints. J. Mol. Biol, 234(3): 779–815.

[47] N. Haider, et al., Mol Biochem Parasitol., 142:224 (2005) [PMID: 15913804].

[48] A. Sali & T. L. Blundell, J. Mol. Biol., 234:779 (1993) [PMID: 8254673].

[49] Rohdich, F., Wungsintaweekul, J., Fellermeier, M., Sagner, S., Herz, S., Kis, K., Eisenreich, W., Bacher, A. & Zenk, M. N. (1999) Proc. Natl. Acad. Sci. USA 96, 11758–11763.

[50] Richard, S. B., Bowman, M. E., Kwiatkowski, W., Kang, I., Chow, C., Lillo, A. M.Cane, D. E. & Noel, J. P. (2001) Nat. Struct. Biol. 8, 641–647.

[51] Kuzuyama, T., Takagi, M., Kaneda, K., Watanabe, H., Dairi, T. & Seto, H. (2000) Tetrahedron Lett. 41, 2925–2928.

[52] Rohdich, F., Eisenreich, W., Wungsintaweekul, J., Hecht, S., Schuhr, C. A. & Bacher, A. (2001) Eur. J. Biochem. 268, 3190–3197.

[53] Herz, S., Wungsintaweekul, J., Schuhr, C. A., Hecht, S., Luttgen, H., Sagner, S.Fellermeier, M., Eisenreich, W., Zenk, M. H., Bacher, A., et al. (2000) Proc. Natl Acad. Sci. USA 97, 2486–2490.

[54] Takagi, M., Kuzuyama, T., Kaneda, K., Watanabe, H., Dairi, T. & Seto, H. (2000) Tetrahedron Lett. 41, 3395–3398.

[55] Campos, N., Rodriguez-Concepcion, M., Sauret-Gueto, S., Gallego, F., Lois, L.-M.& Boronat, A. (2001) Biochem. J. 353, 59– 67.

[56] Rohdich, F., Kis, K., Bacher, A. & Eisenreich, W. (2001) Curr. Opin. Chem. Biol. 5,535–540.

[57] Freiberg, C., Wieland, B., Spaltmann, F., Ehlert, K., Brotz, H. & Labischinski, H. (2001) J. Mol. Microbiol. Biotechnol. 3, 483–489.

[58] Gladyshev, V.N., Jeang, K.T. & Stadtman, T.C. (1996) Selenocysteine, identified as the penultimate C-terminal residue in human T-cell thioredoxin reductase, corresponds to TGA in the human placental gene. Proc. Natl Acad. Sci. USA 93, 6146±6151.

[59] Gasdaska, P.Y., Gasdaska, J.R., Cochran, S. & Powis, G. (1993) Cloning and sequencing of a human thioredoxin reductase. FEBS Lett. 373, 5±9.

[60] MuÈller, S., Gilberger, T.-W., FaÈrber, P.M., Becker, K., Schirmer, R.H. & Walter, R.D. (1996) Recombinant putative glutathione reductase of Plasmodium falciparum exhibits thioredoxin reductase activity. Mol. Biochem. Parasitol. 80, 215±219.

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