THE *PLASMODIUM* GLUTATHIONE S-TRANSFERASE
Bioinformatics Characterization and Classification into the Sigma Class

Emilee E. Colón-Lorenzo, Adelfa E. Serrano
Department of Microbiology and Medical Zoology, University of Puerto Rico School of Medicine, San Juan, Puerto Rico

Hugh B. Nicholas Jr, Troy Wymore, Alexander J. Ropelewski
Pittsburgh Supercomputing Center, Pittsburgh, PA, U.S.A.

Ricardo González-Méndez
Department of Radiological Sciences, University of Puerto Rico School of Medicine, San Juan, Puerto Rico

Keywords: Malaria, Plasmodium, Glutathione S-transferase, Bioinformatics analysis, Structural alignment, GST classification, Structural modeling.

Abstract: Malaria is a global health problem caused by *Plasmodium* parasites. Glutathione S-transferase (GST) is involved in the conjugation of glutathione to drugs and toxic compounds. It is postulated that GST plays an important role in the development of drug resistance. The three-dimensional (3D) structure of *Plasmodium falciparum* GST (*Pf*GST) has been solved and previous work indicates that the *Pf*GST cannot be assigned to any of the known GST classes. We performed sequence analyses, structural modeling and alignment of GSTs from *Plasmodium* to known structures of the GST from other organisms to classify *Pf*GST into a GST family. Sequence alignments using ClustalW, motif analysis using MEME, and phylogenetic analysis using MEGA4, of *Plasmodium* GSTs and 38 other GST sequences were done. The alignments and motifs show a close relationship to the alpha and sigma class of GSTs. The phylogenetic analysis places the *Plasmodium* GSTs in the sigma class. A comparison of *Pf*GST with known structures of GSTs reveals high structural similarity to the sigma class GST, in particular within the H-site and C-terminus of the protein. These findings allow *Pf*GST to be classified into the sigma class GSTs. These data may open new avenues for the development of novel antimalarials.

1 INTRODUCTION

Malaria is one of the most devastating diseases in the world caused by parasites of the genus *Plasmodium*. It is estimated that more than 350 million cases of malaria infections and, over one million deaths occur annually (WHO, 2005). The inappropriate and indiscriminate use of drugs has led to the development of drug resistance in parasites (Russell, 2004; Whitty *et al.*, 2002; Wongsrichanalai *et al.*, 2002). Efficient and cost effective alternatives to presently used drugs are not yet available. A comprehensive understanding of parasite development and drug resistance will enable the development of more effective drug therapies to combat this disease.

Glutathione S-transferases (GSTs) are a family of detoxification enzymes found in most organisms that conjugate reduced glutathione (GSH) with toxic electrophilic organic compounds and drugs. GSTs have been subdivided into different classes based on their primary structure, immunological properties and substrate specificities (Winayanuwattikun and Ketterman, 2005). The GST classes are widespread and are present in a variety of organisms. These classes include the following: alpha, sigma, mu, pi, theta, zeta and omega classes (Torres-Rivera and Landa, 2008). Additionally, there are organism-specific classes which include several GSTs which are found only in certain kingdoms or phyla: lambda, phi, and tau in plants; delta, epsilon in

E. Colón-Lorenzo E., E. Serrano A., B. Nicholas Jr H., Wymore T., J. Ropelewski A. and González-Méndez R. (2010).
THE PLASMODIUM GLUTATHIONE S-TRANSFERASE - Bioinformatics Characterization and Classification into the Sigma Class.
In *Proceedings of the First International Conference on Bioinformatics*, pages 173-180
DOI: 10.5220/0002747401730180

insects; and beta in prokaryotes. Furthermore, the kappa class is a unique mitochondrial GST.

Altered GSH mediated detoxification is one of the proposed underlying mechanisms for the development of malaria drug resistance. A marked increased in both GSH levels and GSH-related enzymatic activity was reported in *P. berghei* and *P. falciparum* lines resistant to chloroquine (Dubois *et al.,* 1995; Meierjohann *et al.,* 2002; Srivastava *et al.,* 1999b). Recent results showed that the glutathione biosynthesis is essential for the parasite's life cycle and mosquito transmission (Vega-Rodríguez *et al.,* 2009). These are some of the reasons why GST is considered a very promising drug target for the design of antimalarial drugs.

The genomes of *P. falciparum* (*Pf*), *P. vivax* (*Pv*), *P. knowlesi* (*Pk*) and *P. y. yoelii* (*Py*) have been sequenced (Carlton *et al.*, 2008) and revealed that the parasite harbors only one GST. In addition, the three-dimensional (3D) structure of *Pf*GST has been solved (Burmeister *et al*., 2003; Fritz-Wolf *et al*., 2003; Perbandt *et al.*, 2004). Detailed structural comparison to representative structures of the alpha, mu, and pi classes indicate that the *Pf*GST cannot be assigned to any of the above GST classes (Fritz-Wolf *et al.*, 2003; Deponte and Becker, 2005). It was found that *Pf*GST adopts the canonical GST fold and is enzymatically active as a homodimer. All the known structures of GSTs reveal a similar overall fold: a homodimer where each monomer contains an N-terminal α/β-domain with βαβαββα topology and a C-terminal α-helical domain. The active site of GST is located between the two domains. The active site possesses two binding sites: the G-site, which binds reduced GSH, and the H-site, which can accommodate a variety of substrates. In *Pf*GST, the N-terminal domain possesses the G-site similar to that of the alpha, mu, and pi classes studied. The C-terminal domain shields the H-site. Also, the H-site in *Pf*GST differed from the other GSTs studied. But more importantly, *Pf*GST only has 5 amino acid residues after alpha helix 8 and cannot form the required structural elements of the alpha, mu, and pi classes (Fritz-Wolf *et al.*, 2003).

Here we report bioinformatics sequence analyses and structural modeling of GSTs from *Plasmodium*. We performed a phylogenetic analysis of *Plasmodium spp*. sequences and 38 other GST sequences. We have also analyzed the 3D structure of *Pf*GST to classify the protein into a GST family. Our results using *Pf*GST suggest that the *Plasmodium* GSTs are a unique family related to the sigma class. This work is significant for the future design of specific inhibitors for *Plasmodium* GSTs which may lead to the development of novel antimalarials.

2 MATERIALS AND METHODS

2.1 Alignment of the *Plasmodium* GSTs

The GST sequences recovered from *Plasmodium spp*. and used for subsequent sequence analysis are listed in Table 1 in the Appendix. The amino acid sequences were aligned using ClustalW program (Higgins *et al.*, 1994) with the default parameters. The alignment was visualized using GeneDoc program (Nicholas *et al.*, 1997) and some manual editing was made to produce the final alignment.

2.2 Classification of *P. falciparum* GST by Alignment and Phylogenetic Analysis

We performed a BLAST search with *Pf*GST (Q8MU52) as the query sequence, using the iProClass database (Wu *et al*., 2003). The BLAST search was carried out using the default parameters. We selected five members of each of the GST classes (alpha, sigma, pi, mu, delta, tau and theta) except for the zeta class where three sequences were chosen. Since *Plasmodium* is an eukaryotic organism, we focused on sequences from mammals, plants and insects, excluding prokaryotic GSTs. The GST sequences used are listed in Table 1 in the Appendix. The multiple sequence alignment was performed using ClustalW (Higgins *et al.*, 1994). The Multiple Entropy for Motif Elicitation (MEME) program was used to find 20 conserved motifs using 'zero or more occurrences per sequence' pattern for selection of motifs (Bailey *et al.*, 1994). The alignment and motifs were visualized with GeneDoc (Nicholas *et al.*, 1997). The multiple sequence alignment was trimmed manually and then used to perform the phylogenetic analysis. The phylogenetic analysis was done using the MEGA4 program (Tamura *et al.*, 2007) using the neighbor-joining algorithm and a bootstrapped data set of 100 replicates. The program FigTree (http://tree.bio.ed.ac.uk/software/figtree) was used to visualize the consensus tree from the bootstrap analysis.

2.3 Classification of *P. falciparum* GST by Structural Alignment

Structural alignment of *P. falciparum* GST (1Q4J) with a representative member of each of the GST classes - alpha, sigma, pi and mu - was performed using the MultiSeq feature in VMD (Roberts *et al.*, 2006, Humphrey *et al.*, 1996). The 3D structures were obtained from the Protein Data Bank (PDB) (Berman *et al.*, 2000) and are listed in Table 2 in the Appendix. The sigma GST from *Onchocerca volvulus* (2HNL) and the human alpha GST (1PKZ) were used to perform the structural alignments with *Pf*GST (shown in Figure 4). The detailed structural superpositions were carried out with VMD, specifically in the N-terminal domain (G-site), C-terminal domain (H-site), and α-helix at the C-terminus of the proteins. Close-up views of the H-site, G-site and α-helix in the C-terminus were done to facilitate the analysis of the 3D structure of the proteins.

3 RESULTS AND DISCUSSION

3.1 Alignment of the *Plasmodium* GSTs

The multiple sequence alignment of sequences from *Plasmodium spp.* GST is shown in Figure 1. The sequences used in the alignment are listed in Table 1 in the Appendix. The alignment of *Plasmodium spp.* GSTs revealed a significant degree of sequence identity ranging from 80 to 87%.

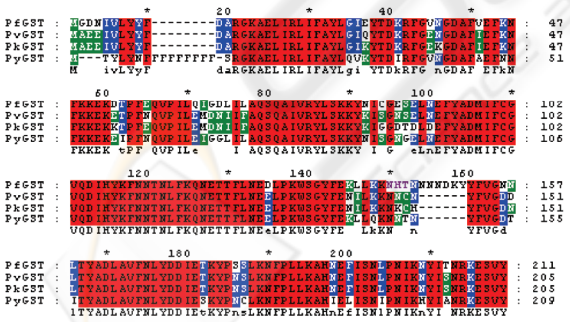

Figure 1: Multiple sequence alignment of the Plasmodium GSTs. Amino acids with a 100% identity are shaded in red, 99-80% identity are in blue and 79-60% identity in green.

The GST sequences are highly conserved in all four species of *Plasmodium*.

3.2 Classification of *P. falciparum* GST by Alignment and Phylogenetic Analysis

A BLAST search in iProClass was performed using GST from *P. falciparum* (Q8MU52). We selected five sequences of each GST classes except for zeta class where we selected only three sequences. A total of 38 GST sequences were obtained and are listed in Table 1 in the Appendix. The multiple sequence alignment was done using the program ClustalW and the MEME program was used to find 20 conserved motifs (Figure 2). The multiple sequence alignment and motif analyses show that the *Plasmodium* GSTs appear to be highly related to the alpha and sigma families of GST. The alignment was trimmed manually and was used to perform the phylogenetic analysis (Figure 3). The phylogenetic tree indicates that *Plasmodium spp.* GSTs analyzed are members of the sigma class of GSTs.

3.3 Classification of *P. falciparum* GST by Structural Alignment

The three-dimensional structure superimposition makes possible the classification of *P. falciparum* glutathione S-transferase in a specific GST class. The three-dimensional structures of GST enzymes from various classes (alpha, sigma, mu and pi) were compared to the *Pf*GST 3D structure (1Q4J) by structural alignment using the MultiSeq feature in the VMD program. The 3D structures used in the structural alignments are listed in Table 2 in the Appendix. Structural alignments of *Pf*GST 3D structure (1Q4J) with alpha and sigma 3D structures are presented in Figure 4.

Figure 4A shows the structural alignment of *Pf*GST (1Q4J) with sigma GST from *Oncocherca volvulus* (2HNL). A close-up view showing the α-helix of the C-terminus is represented in Figure 4B. Figure 4C shows the structural alignment of *Pf*GST (1Q4J) with the alpha GST from human (1PKZ). A close-up view showing the C-terminus is represented in Figure 4D. Analysis of the 3D structural alignment of *Pf*GST with the sigma class GST structure shows high structural similarity in the C-terminus (Figure 4B). The 3D structural alignment of *Pf*GST and the alpha GST demonstrates a good alignment, but the C-terminus shows low similarity (Figure 4C). In addition, we can see that *Pf*GST does not have the extended helix in the C-terminus that is distinctive of the alpha class of GST (Figure 4D). Structural alignments of *Pf*GST 3D structure (1Q4J) with mu and pi 3D structures were performed showing a low

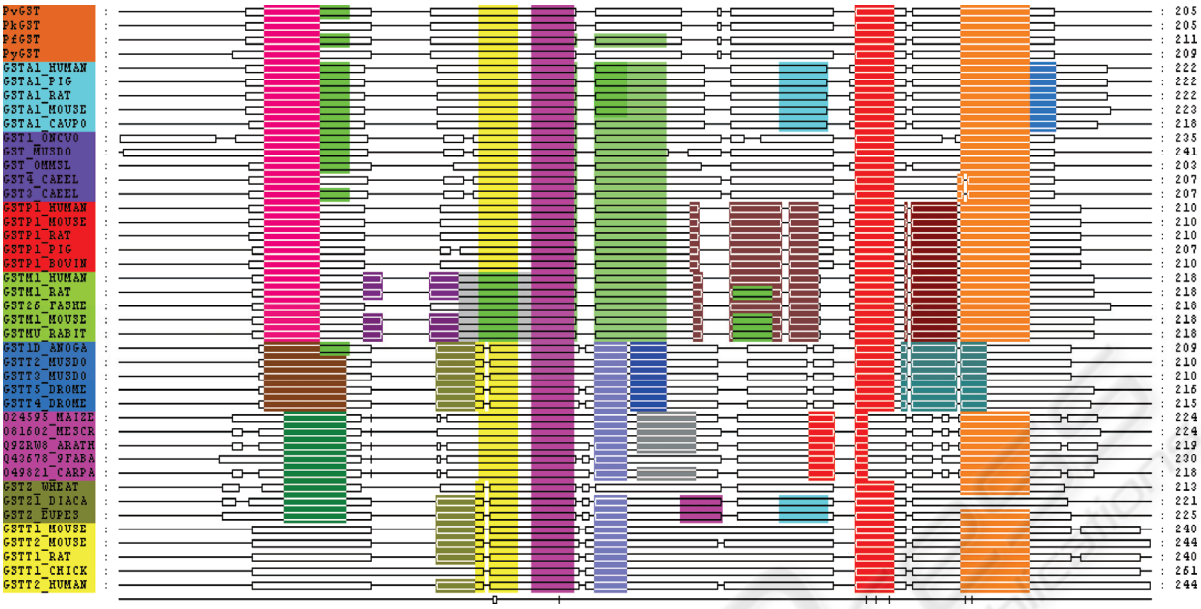

Figure 2: Schematic sequence alignment of four Plasmodium GSTs and 38 GST sequences from eight known GST classes. Double lines represent where amino acids are present and single lines represent gaps in the alignment. MEME motifs are identified by different colors and adjusting edges of MEME motifs was the major alignment adjustment. Plasmodium sequences are colored in orange, alpha sequences in cyan, sigma sequences in purple, pi sequences in red, mu sequences in green, delta sequences in blue, tau sequences in magenta, zeta sequences in olive green and theta sequences in yellow on the left side of the figure.

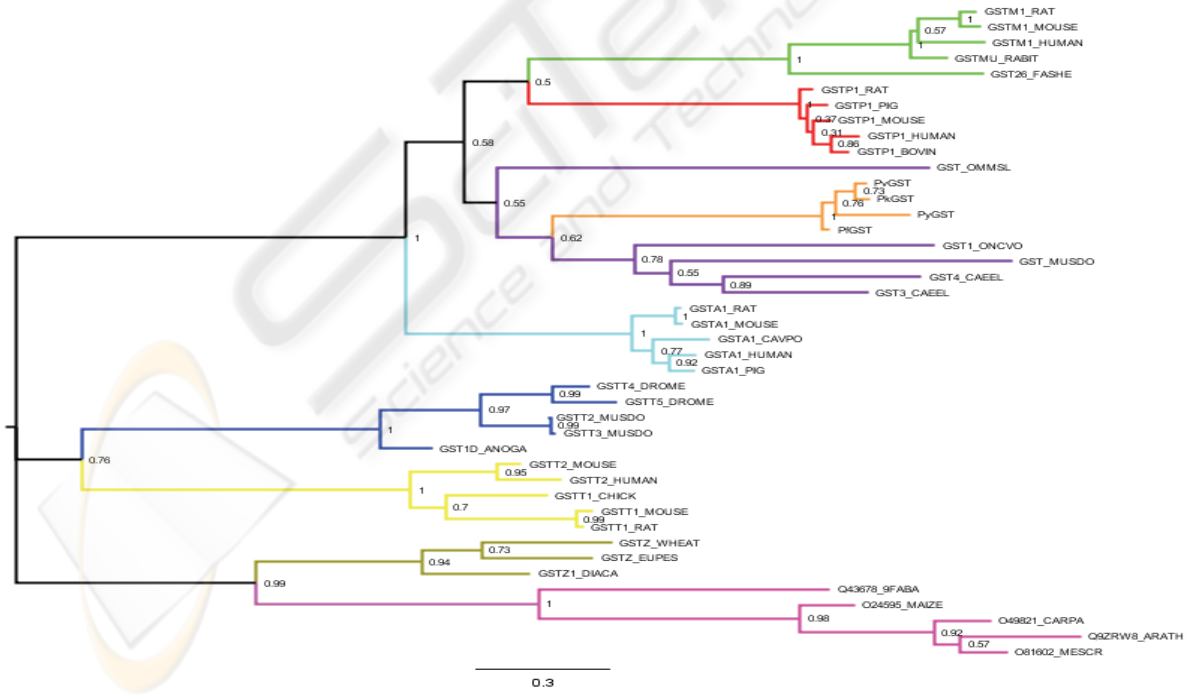

Figure 3: Phylogenetic tree for GST sequences with the fraction of the bootstrap. Plasmodium (orange), sigma (purple), alpha (cyan), pi (red), mu (green), delta (blue), tau (magenta), zeta (olive green) and theta (yellow).

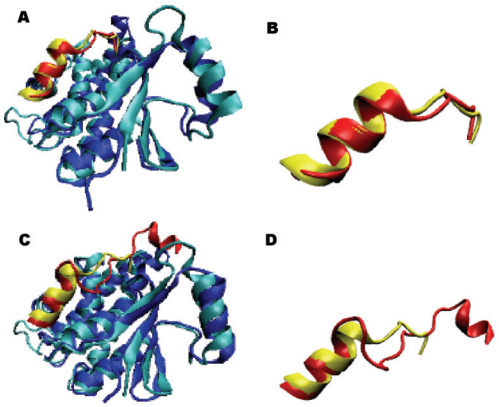

Figure 4: Structural alignment of P. falciparum GST with sigma and alpha class GST structure. (A) Structural alignment of PfGST (cyan) and a sigma class GST (blue); (B) Close-up view showing the C-terminus of PfGST (yellow) and sigma GST (red); (C) Structural alignment of PfGST (cyan) and an alpha class GST (blue); (D) Close-up view showing the C-terminus of PfGST (yellow) and alpha GST (red).

structural similarity (data not shown). An important feature of the C-terminus of alpha class GST is an alpha helix close to the active site. This helix is an essential element of the GST alpha class (Nilsson *et al.*, 2002). Based on this, we performed an analysis of this area of the protein using structural alignments. Fritz-Wolf *et al*. (2003) described the *Pf*GST having a different length C-terminus - only has 5 amino acid residues after alpha helix 8 - and therefore could not form the required structural elements from mammalian GSTs as part of what makes them not classifiable. Our observation is that *Pf*GST aligned well with the sigma class GST from *O. volvulus*. We find that using common structural features and MEME patterns GSTs have a very consistent C-terminus length within each class and that is one of the features that helped clarify the classification of the *Plasmodium* sequences as a sigma class in both the structural alignment (Figure 4) and the sequence alignment (Figure 2).

Furthermore, analysis of the 3D structure of *Pf*GST in the N-terminal domain (G-site) and C-terminal domain (H-site) was performed. Structural alignment of the N-terminal domain, that contains the G-site, reveals that *Pf*GST shares a common backbone fold with the sigma (2HNL) GST from *O. volvulus* and alpha (1PKZ) GST from human. Both structure alignments show a similar binding mode for S-hexylglutathione (GSH derivative). Close views of the N-terminal domain are shown (Figures 5A and 5C, respectively). Structural alignment of the C-terminal domain, that contains the H-site, shows that *Pf*GST shares a common backbone with sigma (2HNL) GST, while alpha (1PKZ) GST is different, specifically in the C-terminus (Figures 5B and 5D, respectively). Generally, the H-site of GST enzymes is more variable than the G-site due to the great number of secondary structures (Fritz-Wolf *et al.,* 2003). These findings are in agreement with Fritz-Wolf *et al*. (2003).

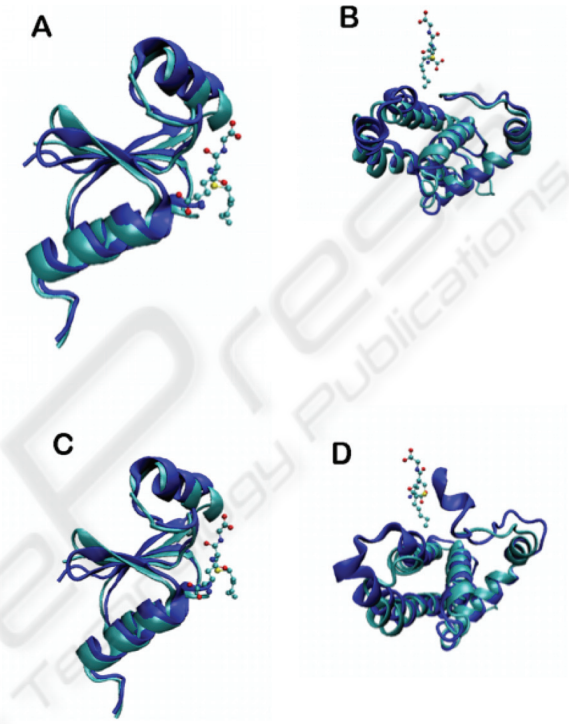

Figure 5: Structural comparison of GST structures from P. falciparum GST with a sigma GST from O. volvulus and an alpha GST from human. (A) Structural alignment of N-terminal (G-site) of PfGST (cyan) and a sigma class GST (blue); (B) Structural alignment of C-terminal (H-site) of PfGST (cyan) and a sigma class GST (blue); (C) Structural alignment of N-terminal (G-site) of PfGST (cyan) and a alpha class GST (blue); (D) Structural alignment of C-terminal (H-site) of PfGST (cyan) and a alpha class GST (blue). The S-hexylglutathione ligand is shown as a ball-and-stick model.

A comprehensive analysis of the 3D structure of *Pf*GST in the H-site, G-site and C-terminus of the proteins was done and established that these regions have high similarity with the sigma class GST from *O. volvulus* (2HNL). The structural alignments support the results obtained with the phylogenetic tree. These results allow us to classify the *Plasmodium* GSTs as members of the sigma class of GSTs.

4 CONCLUSIONS

The GST sequences of four *Plasmodium* species were compared using multiple sequence alignments and found to be highly conserved. When aligned with members of each of the GST classes used in this study our results show that the *Plasmodium* GSTs appear to be highly related to the alpha and sigma families of GSTs. Using phylogenetic analysis we found that *Plasmodium* GST are members of the sigma class. The 3D structure of *Pf*GST was compared, using structural alignments, with members of several GST classes. We determined that *Pf*GST has a high degree of similarity with the sigma class in the H-site, G-site and C-terminus of the protein. Structural analysis and phylogenetic analysis of *Pf*GST revealed that this enzyme represents a unique clade within the sigma class of GSTs.

This work contributes to a better understanding of the structure and classification of *Plasmodium* GSTs. These results have the potential to enhance our knowledge of the relevance of GST to *Plasmodium* drug resistance. It has been recently demonstrated that 7-nitro-2,1,3-benzoxadiazole derivatives are a new class of suicide inhibitors of GST that accumulate in tumor cells and evade the extrusion mechanisms mediated by the multidrug resistance associated protein pumps (MRP) (Ricci *et al.,* 2005). Studies in human cancer cells in-vitro showed that the GST inhibitor 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol (NBDHEX) has high antiproliferative activity and helped overcome MRP mediated drug resistance (Federici *et el.,* 2009; Filomeni *et al.,* 2008). In-silico studies will be undertaken to evaluate NBDHEX's potential as an antimalarial drug. These studies could support the use of this GST inhibitor in *Plasmodium*.

We expect to use the results presented here for further studies using molecular biology and genetics approaches in order to study the involvement of GST in the *Plasmodium* life cycle and mosquito transmission. These studies should be useful to find alternative strategies for malaria control.

ACKNOWLEDGEMENTS

RGM, EECL, HBN, TW, AJP and this project were partially supported by NIGMS grant T36-GM008789 and acknowledge use of the Pittsburgh Supercomputing Center's National Resource for Biomedical Supercomputing resources funded through NIH NCRR grant P41-RR06009. AES and EECL were partially supported by NIGMS/MBRS award GM08224 and from the RCMI award G12RR03051 of the NCRR/NIH.

REFERENCES

Bailey, T.L., Elkan, C., 1994. "Fitting a mixture model by expectation maximization to discover motifs in biopolymers", Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology, pp. 28-36, AAAI Press, Menlo Park, California.

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., Bourne, P.E., 2000. The Protein Data Bank Nucleic Acids Research, 28 pp. 235-242.

Burmeister, C., Perbandt, M., Betzel, Ch., Walter, R. D., Liebau, E., 2003. Crystallization and preliminary X-ray diffraction studies of the glutathione S-transferase from *Plasmodium falciparum*. Acta Crystallogr. D Biol. Crystallogr. 59, 1469–1471.

Carlton, J.M., Adams, J.H., Silva, J.C., *et al.*, 2008. Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. Nature 455:757-763.

Deponte, M., Becker, K., 2005. Glutathione S-transferase from malarial parasites: structural and functional aspects. Methods Enzymol. 401:240–252.

Dubois, V.L., Platel, D.F., Pauly, G., and Tribouley-Duret, J. 1995. *Plasmodium berghei:* Implication of intracellular glutathione and its related enzyme in chloroquine resistance *in vivo*. Exp. Parasitol. 81: 117-124.

Federici, L., Sterzo, C.L., Pezzola, S., Matteo, A.D., Scaloni, F., Federici, G., Caccuri, A.M. 2009. Structural basis for the binding of the anticancer compound 6-(7-Nitro-2,1,3-Benzoxadiazol-4-Ylthio)Hexanol to human glutathione S-transferases. Cancer Research 69:20.

Filomeni G., Turella, P., Dupuis, M.L., Forini, O., Ciriolo, M.R., Cianfriglia, M., Pezzola, S., Federici, G., Caccuri, A.M. 2008. 6-(7-Nitro-2,1,3-Benzoxadiazol-4-Ylthio)Hexanol, a specific glutathione S-transferase inhibitor, overcomes the multidrug resistance (MDR)-associated protein 1-mediated MDR in small cell lung cancer. Molecular cancer therapeutics 7(2).

Fritz-Wolf, K., Becker, A., Rahlfs, S., Harwaldt, P., Schirmer, R. H., Kabsch, W., Becker, K., 2003. X-ray structure of glutathione S-transferase from the malarial parasite *Plasmodium falciparum*. Proc. Natl. Acad. Sci. USA 100, 13821–13826.

Higgins, D., Thompson, J., Gibson, T., Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673-4680.

Humphrey, W., Dalke, A., Schulten, K., 1996. VMD - Visual Molecular Dynamics. J. Molec. Graphics, vol. 14, pp. 33-38

Meierjohann, S., Walter, R.D., Muller, S. 2002. Regulation of intracellular glutathione levels in erythrocytes infected with chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*. Biochem J. Dec 15: 368, 761-8.

Nicholas, K.B., Nicholas, H.B. Jr,, Deerfield, D.W. II, 1997. GeneDoc: Analysis and Visualization of Genetic Variation. *EMBNEW NEWS* 4:14.

Nilsson, L. O., Edalat, M., Pettersson, P. L., Mannervik, B., 2002. Aromatic residues in the C-terminal region of glutathione transferase A1-1 influence rate-determining steps in the catalytic mechanism. Biochimica et Biophysica Acta 29: 1598(1-2):199-205.

Perbandt, M., Burmeister, C., Walter, R. D., Betzel, C., Liebau, E., 2004. Native and inhibited structure of a Mu class-related glutathione S-transferase from *Plasmodium falciparum*. J. Biol. Chem. 279, 1336–1342.

Rambaut, A., 2006. FigTree: Tree figure drawing tool, version 1.0. Available from http://tree.bio. bio.ed.ac.uk/software/figtree/, Institute of Evolutionary Biology, University of Edinburgh.

Ricci, G., De Maria, F., Antonini, G., Turella, P., Bullo, A., Stella, L., Filomeni, G., Federici, G., Caccuri, A.M. 2005. 7-Nitro-2,1,3-benzoxadiazole derivatives, a new class of suicide inhibitors for glutathione S-transferases. The Journal of Biological Chemistry 280:28, 26397-26405.

Roberts, E., Eargle, J., Wright, D., Luthey-Schulten, Z., 2006. MultiSeq: unifying sequence and structure data for evolutionary analysis. BMC Bioinformatics. Aug 16;7:382.

Rusell, S., 2004. The economic burden of illness for households in developing countries: a review of studies focusing on malaria, tuberculosis, and human immunodeficiency virus/acquired immunodeficiency syndrome. Am J Trop Med Hyg.71 (2 Suppl):147-55.

Srivastava, P., Puri, S.K., Kamboj, K.K., and Pandey, V.C. 1999. Glutathione S-transferase activity in malarial parasites. Trop. Med. Int. Health 4:251-254.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596-1599.

Torres-Rivera, A., Landa, A., 2008. Glutathione transferases from parasites: A biochemical view. Acta Tropica 105: 99–112.

Vega-Rodríguez, J., Franke-Fayard, B., Dinglasan, R.R., Janse, C.J., Pastrana-Mena, R., Waters, A.P., Coppens, I., Rodríguez-Orengo, J.F., Jacobs-Lorena, M., Serrano, A.E. 2009. The glutathione biosynthetic pathway of *Plasmodium* is essential for mosquito transmission. PLoS Pathogens 5:2.

Winayanuwattikun, P., Ketterman, A.J., 2005. An electron-sharing network involved in the catalytic mechanism is functionally conserved in different glutathione transferase classes. The Journal of Biological Chemistry.

Whitty, C.J., Rowland, M., Sanderson, F., Mutabingwa, T.K., 2002. Science, medicicne, and the future: Malaria. BMJ 325(7374):1221-1224.

WHO, 2005. Global malaria situation. http://www.who. int/globalatlas/autologin/malria_login.asp

Wongsrichanalai, C., Pickard, A.L., Wernsdorfer, W.H., Meshnick, S.R. 2002. Epidemiology of drug-resistant malaria. Lancet Infect Dis. 4:209-18.

Wu, C.H., Huang, H., Nikolskaya, A., Hu, Z., Barker, W.C., 2004. The iProClass integrated database for protein functional analysis. Comput Biol Chem. Feb;28(1):87-96.

APPENDIX

Table 1: Glutathione S-transferase sequences used in the alignments.

Protein name	Organism	Family class	Accession number	Protein length (aa)
*Pf*GST	*Plasmodium falciparum*	sigma	Q8MU52	211
*Pv*GST	*Plasmodium vivax*	sigma	Q0ZS46	205
*Pk*GST	*Plasmodium knowlesi*	sigma	B3LAI5	205
*Py*GST	*Plasmodium yoelii*	sigma	Q7REH6	209
GSTA1_HUMAN	*Homo sapiens*	alpha	P08263	222
GSTA1_RAT	*Rattus norvegicus*	alpha	P00502	222
GSTA1_MOUSE	*Mus musculus*	alpha	P13745	223
GSTA1_PIG	*Sus scrofa*	alpha	P51781	222
GSTA1_CAVPO	*Cavia porcellus*	alpha	P81706	218
GST1_ONCVO	*Oncocherca volvulus*	sigma	P46434	235
GST_MUSDO	*Musca domestica*	sigma	P46437	241
GST_OMMSL	*Ommastrephes sloanei*	sigma	P46088	203
GST4_CAEEL	*Caenorhabditis elegans*	sigma	Q21355	207
GST3_CAEEL	*Caenorhabditis elegans*	sigma	O16116	207
GSTP1_HUMAN	*Homo sapiens*	pi	P09211	210
GSTP1_MOUSE	*Mus musculus*	pi	P19157	210
GSTP1_RAT	*Rattus norvegicus*	pi	P04906	210
GSTP1_PIG	*Sus scrofa*	pi	P80031	207
GSTP1_BOVIN	*Bos taurus*	pi	P28801	210
GSTM1_HUMAN	*Homo sapiens*	mu	P09488	218
GSTM1_RAT	*Rattus norvegicus*	mu	P04905	218
GST26_FASHE	*Fasciola hepatica*	mu	P30112	218
GSTM1_MOUSE	*Mus musculus*	mu	P10649	218
GSTMU_RABIT	*Oryctolagus cuniculus*	mu	P46409	218
GST1D_ANOGA	*Anopheles gambiae*	delta	Q93113	209
GSTT2_MUSDO	*Musca domestica*	delta	P46431	210
GSTT3_MUSDO	*Musca domestica*	delta	P46432	210
GSTT5_DROME	*Drosophila melanogaster*	delta	Q9VG95	216
GSTT4_DROME	*Drosophila melanogaster*	delta	Q9VG96	215
O24595_MAIZE	Zea mays	tau	O24595	224
O81602_MESCR	*Mesembryanthemum crystallinum*	tau	O81602	224
Q9ZRW8_ARATH	*Arabidopsis thaliana*	tau	Q9ZRW8	219
Q43678_9FABA	*Vigna radiate*	tau	Q43678	230
O49821_CARPA	*Carica papaya*	tau	O49821	218
GSTZ-WHEAT	*Triticum aestivum*	zeta	O04437	213
GSTZ1_DIACA	*Dianthus caryophyllus*	zeta	P28342	221
GSTZ_EUPES	*Euphorbia esula*	zeta	P57108	225
GSTT1_MOUSE	*Mus musculus*	theta	Q64471	240
GSTT2_MOUSE	*Mus musculus*	theta	Q61133	244
GSTT1_RAT	*Rattus norvegicus*	theta	Q01579	240
GSTT1_CHICK	*Gallus gallus*	theta	P20135	261
GSTT2_HUMAN	*Homo sapiens*	theta	P30712	244

Table 2: Glutathione S-transferase sequences used for the structural alignments.

Protein name	Organism	Family class	PDB code
*Pf*GST	*Plasmodium falciparum*	sigma	1Q4J
GST1_ONCVO	*Oncoherca volvulus*	sigma	2HNL
DmGST	*Drosophila melanogaster*	sigma	1MOU
GSTA1_HUMAN	*Homo sapiens*	alpha	1PKZ
GSTM1_HUMAN	*Homo sapiens*	mu	1GTU