Molecular modeling elucidates parasite-specific features of polyamine pathway enzymes of *Plasmodium falciparum*

by

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The first principle is that you must not fool yourself – and you are the easiest person to fool. So you have to be very careful about that. After you've not fooled yourself, it's easy not to fool other scientists. You just have to be honest in a conventional way after that.

– Richard Feynman

Declaration

I, Gordon Andreas Wells, declare that the thesis/dissertation, which I hereby submit for the degree *Philosophiae Doctor* at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE:..... DATE:.....

Abbreviations and Nomenclature

ADMET	Absorption, Distribution, Metabolism, Excretation and Toxicity
AdoMetDC	*S*-adenosylmethionine decarboxylase
Ama x_a::Ama y_b	Salt-bridge between Ama x of monomer a and Ama y of monomer b
Ama *xyz*	Amino acid *position*
αn	Alpha/3_{10} helix n
βn	Beta strand n
ATP	Adenosine tri-phosphate
AZ	Antizyme
Å	Ångström
BMIC	Bio-Medical Informatics Centre
CAPRI	Critical Assesment of PRedicted Interactions
CHPC	Centre for High Perfomance Computing, Cape Town, SA
CJ	Conjugate gradients
COM	Centre of Mass
CSIR	Council for Scientific and Industrial Research, SA
DDT	Dichlorodiphenyltrichloroethane
DFMO	*alpha*-Difluoromethylornithine
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthase
EC	Enzyme Commission
EGEE	Enabling Grids for E-sciencE

eIF5A	Eukaryotic initiation factor 5A
IRS	Indoor residual spraying
ITN	Instectiside treated nets
MD	Molecular dynamics
NAP	Nuclear Aggregates of Polyamines
NMR	Nuclear Magnetic Resonance
ODC	Ornithine Decarboxylase
PAO	Polyamine oxidase
PDB	Protein Data Bank
Pf Arg	*Plasmodium falciparum* arginase
*Pf*AdoMetdcDC	*Plasmodium falciparum* AdoMetDC
*Pf*ODC	*Plasmodium falciparum* ODC
PLP	Pyridoxal 5'-phosphate
QSAR	Quantitative Structure Activity Relationship
RMSD	Root Mean Square Deviation
RP	Residue Pairing Potential
SSAT	Spermine/spermidine N^1-acetyltransferase
TIM	Triose-phosphate isomerase
TIP3	Transferable Intermolecular Potential

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Summary

Malaria remains a debilitating disease, especially in developing countries of the tropics and sub-tropics. Increasing drug resistance and the rising cost of drug development calls for methods that can cost-effectively identify new drugs. The proteins of the malaria causing *Plasmodium* parasites often exhibit unique features compared to their mammalian counterparts. Such features invite discovery of parasite-specific drugs.

In this study computational methods were applied to understand unique structural features of enzymes from the *Plasmodium* polyamine biosynthesis pathways. Molecular modeling of *P. falciparum* arginase was used to explore the structural metal dependency between enzyme activity and trimer formation. This dependency is not observed in the mammalian host. A novel inter-monomer salt-bridge was discovered between Glu 295 and Arg 404 that helps mediate the structural metal dependency. Removal of the active site metal atoms promoted breaking of the Glu 295_a::Arg 404_b interaction during simulation. The involvement of this salt-bridge was further confirmed by site-directed mutagenesis of the recombinantly expressed enzyme and subsequent simulation of the mutants *in silico*. Mutations designed to break the salt-bridge resulted in decreased enzyme activity and oligomerisation. Furthermore, simulation of the mutants indicated potential loss of metal co-ordination within the active site. The interface around Glu 295_a::Arg 404_b could thus serve as a novel therapeutic target.

In *Plasmodium* the usually separate activities *S*-adenosylmethionine decarboxylase and ornithine decarboxylase occur in a single bifunctional enzyme. Previous studies have established the importance of complex formation and protein-protein interactions for correct enzyme functioning. Disturbing these interactions within the complex may therefore have inhibitory potential. In the second aspect of this study the potential quarternary structure of AdoMetDC/ODC was studied by homology modeling of the domains followed by protein-protein docking. The results from five *Plasmodium* species suggest that one face of each domain is favoured for complex formation. The predicted faces concur with existing experimental results, suggesting the direct involvement of *Plasmodium*-specific inserts in maintaining complex formation. Further fine-grained analysis revealed potentially conserved residue pairs between AdoMetDC/ODC that can be targeted during experimental follow-up.

In both aspects of this study computational methods yielded useful insights into the parasite-specific features of polyamine biosynthesis enzymes from *Plasmodium*. Exploitation of these features may lead to novel parasite-specific drugs. Furthermore, this study highlights

the importance of simulation and computational methods in the current and future practise of Science.