

Functional genomics analysis of the effects of co-inhibition of the malarial S-adenosylmethionine decarboxylase/ornithine decarboxylase

by

Anna Catharina van Brummelen

Submitted in partial fulfilment of the requirements for the degree
Philosophiae Doctor

in the Faculty of Natural and Agricultural Sciences
Department of Biochemistry
University of Pretoria
Pretoria
South Africa

SUPERVISOR: Dr. Lyn-Marie Birkholtz
Department of Biochemistry, University of Pretoria, South Africa

CO-SUPERVISOR: Prof. Abraham I Louw
Department of Biochemistry, University of Pretoria, South Africa

CO-SUPERVISOR: Prof. Manuel Llinás
Department of Molecular Biology, Princeton University, USA

October 2008

DECLARATION:

I, Anna Catharina van Brummelen 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:



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Soli Deo gloria

ACKNOWLEDGEMENTS

The completion of this dissertation was made possible by the following people whom I would like to thank with all my heart:

My supervisor, Dr. Lyn-Marie Birkholtz, for your guidance, encouragement, support and faith in me.

Prof. Braam Louw, for your support, guidance and for giving me the opportunity to enrol for a PhD in Biochemistry.

Prof. Manuel Llinás, for the opportunity to do research in your laboratory, for stimulating discussions and long distance guidance via email.

Staff from the University of Pretoria, specifically Jaco de Ridder, Loveness Dzikiti, Fourie Joubert, Nicky Olivier and Sandra van Wyngaardt. Thank you for all your kindness and support, as well as assistance with experimental work and data analyses.

Fellow students and friends from the University of Pretoria, specifically Pieter Burger, Katherine Clark, Jandeli Niemand, Shaun Reeksting, Christiaan Stutzer, Gordon Wells and Marni Williams. It was pleasure to get to know all of you. Thank you for everything!

CSIR staff, specifically Bridget Crampton and Stoyan Stoychev, for your help with analyses.

Fellow students, technicians and friends from Princeton University, specifically Ilsa Leon, Kellen Olszewski and Daniel Wilinski. Thank you for making me feel welcome, also for teaching me to do microarrays and all your hard work with the metabolomics.

My husband, Roy van Brummelen, for all the times we had to go to the lab in the middle of the night, your unconditional love and support.

My parents, Johannes and Adéle Retief and my family. Thank you for all your love, continuous support, endless prayers and encouragement all my life.

SUMMARY

Polyamines are ubiquitous components of all living cells and their depletion usually causes growth arrest or cytostasis, a strategy employed for treatment of West-African trypanosomiasis. In the malaria parasite, *Plasmodium falciparum*, polyamine biosynthesis is regulated by the uniquely bifunctional protein, S-adenosylmethionine decarboxylase/ornithine decarboxylase (PfAdoMetDC/ODC). The unique nature of this protein could provide a selective mechanism for antimalarial treatment.

To validate polyamine depletion and specifically PfAdoMetDC/ODC, as drug target for antimalarial therapeutic intervention, polyamine biosynthesis was completely restrained via the inhibition of both catalytic sites of PfAdoMetDC/ODC with DFMO and MDL73811. The physiological effects during the resulting cytostasis were studied with a comprehensive functional genomics approach. The study was preceded by various assays to determine the treatment dosage that would result in complete cytostasis, without non-specific chemical cytotoxicity. The results obtained revealed that the cytostatic mechanism with growth arrest of the treated parasites and normal progression of the untreated controls require special consideration for basic comparisons of response in terms of the assay methodology used and data analysis. This is particularly important when studying a multistage organism such as *P. falciparum*, which constantly develops and change during the intraerythrocytic developmental cycle, such that growth arrest compared to normal progression would result in significant differences merely due to stage. This critical principle was kept in mind throughout the investigation and was applied to the relative quantification of RNA, proteins and metabolites via a relative time zero approach as opposed to the standard parallel time point comparison.

Three independent functional genomics investigations, namely transcriptomics, proteomics and metabolomics were conducted, in which highly synchronised 3D7 parasite cultures were treated during the schizont stage and parasites were sampled during a time course at three time points (just before and during cytostasis). Transcriptome analysis revealed the occurrence of a generalised transcriptional arrest just prior to the growth arrest. To our knowledge this is the first time that transcriptional arrest as the preceding mechanism of cytostasis due to polyamine depletion, was demonstrated. However, despite the transcriptional arrest, the abundance of 538 transcripts was differentially affected and included three perturbation-specific compensatory transcriptional responses: the increased abundance of the transcripts for lysine decarboxylase and ornithine aminotransferase (OAT) and the decreased abundance of that for S-adenosylmethionine synthetase (AdoMet synthetase). Pearson correlations indicated more subtle effects of the perturbation on the proteome and even more so on the metabolome where homeostasis was generally maintained, except downstream to the enzymatic blockade at PfAdoMetDC/ODC. The perturbation-specific compensatory roles of OAT in the

regulation of ornithine and AdoMet synthetase in the regulation of AdoMet were confirmed on both the protein and metabolite levels, confirming their biological relevance.

The results provide evidence that *P. falciparum* respond to alleviate the detrimental effects of polyamine depletion via the regulation of its transcriptome and subsequently the proteome and metabolome, which supports a role for transcriptional control in the regulation of polyamine and methionine metabolism within the parasite. The study concludes that polyamines are essential molecules for parasite survival and that PfAdoMetDC/ODC is a valid target for antimalarial drug development.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	i
SUMMARY	ii
TABLE OF CONTENTS	iv
LIST OF EQUATIONS	ix
LIST OF FIGURES.....	x
LIST OF TABLES.....	xii
LIST OF ABBREVIATIONS.....	xiii

CHAPTER 1

1.1	HISTORY OF MALARIA	1
1.2	MALARIA AS GLOBAL HEALTH PROBLEM.....	1
1.3	THE PARASITE'S LIFE CYCLE.....	3
1.4	HUMAN MALARIA SPECIES.....	5
1.5	THE PATHOGENESIS AND CLINICAL PRESENTATION OF MALARIA.....	6
1.6	ANTIMALARIAL VACCINES.....	8
1.7	ANTIMALARIAL THERAPEUTICS	9
1.7.1	Quinoline and related antimalarials	10
1.7.2	Artemisinin and derivatives	12
1.7.3	Antifolates	13
1.8	ANTIMALARIAL DRUG TARGETS.....	14
1.9	POLYAMINE METABOLISM.....	15
1.9.1	The biological importance of polyamines	15
1.9.2	The biosynthesis of polyamines	16
1.10	MOLECULAR ASPECTS OF MALARIA	19
1.10.1	Sequenced <i>Plasmodium</i> genome data	19
1.10.2	<i>Plasmodium</i> transcriptome data	21
1.10.3	The <i>P. falciparum</i> proteome and interactome	23
1.10.4	The <i>P. falciparum</i> metabolome	24
1.10.5	Gene regulation in <i>P. falciparum</i>	25
1.10.6	Manipulation of the <i>Plasmodium</i> genome	26
1.11	FUNCTIONAL GENOMICS FOR DRUG DISCOVERY AND TARGET VALIDATION	26

1.12	RESEARCH OBJECTIVES.....	27
	Papers:	28
	Conference proceedings:	29

CHAPTER 2

2.1	INTRODUCTION	30
2.1.1	Polyamine biosynthesis inhibitors	30
2.1.2	Antimalarial drug sensitivity methods	32
2.2	MATERIALS AND METHODS	34
2.2.1	<i>In vitro</i> cultivation of asexual <i>P. falciparum</i> cultures	34
2.2.2	Sorbitol synchronisation of growth stage	35
2.2.3	Parasite growth and drug sensitivity assays	36
2.2.3.1	Drug treatment and plate storage until analysis	36
2.2.3.2	Indirect parasitaemia quantitation assays	37
2.2.3.2.1	Lactate dehydrogenase (Malstat) assay	37
2.2.3.2.2	Histidine-rich protein II (HRPII) ELISA	38
2.2.3.3	Direct parasitaemia quantitation assays	39
2.2.3.3.1	FACS analysis	39
2.2.3.4	Concentration-response curves	39
2.2.3.5	Propidium iodide (PI) membrane integrity assay	40
2.3	RESULTS	41
2.3.1	Validation of signal to inoculum linearity	41
2.3.2	Concentration-response curves and IC ₅₀ s	44
2.3.3	DFMO and MDL73811 interaction during PfAdoMetDC/ODC co-inhibition	49
2.3.4	PI membrane integrity assay of PfAdoMetDC/ODC co-inhibition	49
2.4	DISCUSSION.....	50

CHAPTER 3

3.1	INTRODUCTION	54
3.1.1	Transcriptional profiling of perturbed <i>P. falciparum</i> compared to other organisms	54
3.1.2	Transcriptomics methodologies, experimental design and data analysis	55
3.2	MATERIALS AND METHODS	59
3.2.1	Ensuring the correct treatment dosage for the transcriptomics investigation	59
3.2.1.1	Growth morphology studies	59
3.2.1.2	Radio-labelled substrate assays	59

3.2.2 Drug treatment for the transcriptomics investigation	60
3.2.3 RNA isolation	60
3.2.4 cDNA synthesis	61
3.2.5 Oligonucleotide array spotting and slide post-processing	62
3.2.6 Cy dye cDNA labelling	63
3.2.7 Oligonucleotide array hybridisation, washing and scanning	63
3.2.8 Data analysis	64
3.2.8.1 Exploratory data analysis	64
3.2.8.2 Differential transcript abundance analysis	64
3.2.8.2.1 Linear models for microarray data (LIMMA) analysis	64
3.2.8.2.2 EDGE time course analysis	65
3.2.8.3 Additional data analysis	65
3.2.9 Real-time PCR validation of differential transcript abundance data	66
3.3 RESULTS	66
3.3.1 Ensuring the correct treatment dosage for the functional genomics investigations	66
3.3.2 Transcriptomics sampling, RNA isolation and cDNA synthesis	68
3.3.3 Oligonucleotide microarray analysis	70
3.3.4 Exploratory data analysis	71
3.3.4.1 Hierarchical clustering of data with those of other perturbations	71
3.3.4.2 Hierarchical clustering of data related to polyamine and methionine metabolism	75
3.3.4.3 Phase-ordering and correlation calculations	75
3.3.5 Differential transcript abundance analysis	77
3.3.5.1 Data normalisation	77
3.3.5.2 LIMMA data analysis	80
3.3.5.3 EDGE time course analysis	84
3.3.6 GO assignment of differentially affected transcripts	84
3.3.7 Finding adjacently located genes with differentially affected transcripts	85
3.3.8 PfAdoMetDC/ODC-interactome data comparisons	87
3.3.9 Real-time PCR validation of differential transcript abundance data	89
3.4 DISCUSSION.....	92
3.5 RAW DATA AND SUPPLEMENTARY WEBSITE.....	100

CHAPTER 4

4.1 INTRODUCTION	101
4.1.1 Evidence of post-transcriptional regulation in <i>P. falciparum</i>	101

4.1.2	Integrative biology from <i>Plasmodium</i> functional genomics data	102
4.1.3	Proteomics methodologies	103
4.1.4	Metabolomics methodologies	105
4.2	MATERIALS AND METHODS	106
4.2.1	Proteomics	106
4.2.1.1	Protein extraction and quantitation	107
4.2.1.2	Iso-electric focussing (IEF)	107
4.2.1.3	Two-dimensional polyacrylamide gel electrophoresis (2D-GE)	108
4.2.1.4	Gel scanning and data analysis	108
4.2.1.5	Spot excision, destaining and trypsin digestion for protein identification	109
4.2.2	Metabolomics	110
4.2.2.1	Metabolite extraction and polyamine derivatisation	110
4.2.2.2	LC-MS/MS metabolite analysis	111
4.2.2.3	Metabolomics data analysis	111
4.2.3	Decarboxylase activity assays	112
4.2.3.1	LDC induction in <i>E. coli</i> as assay positive control	112
4.2.4	Methylation status determination	113
4.2.4.1	CpG island analysis of the differential transcript abundance list	113
4.2.4.2	Global methylation assays	113
4.2.4.2.1	gDNA isolation	113
4.2.4.2.2	Methylation negative and positive controls	113
4.2.4.2.3	Restriction-enzyme digestion to assess gDNA methylation	114
4.2.4.2.4	South-Western immunoblotting	114
4.3	RESULTS	115
4.3.1	Proteomics analysis of PfAdoMetDC/ODC co-inhibited <i>P. falciparum</i>	115
4.3.1.1	Differential protein abundance analysis and protein identification	117
4.3.1.2	Perturbation-specific compensatory mechanisms confirmed in the proteome	121
4.3.2	Metabolomics analysis of PfAdoMetDC/ODC co-inhibited <i>P. falciparum</i>	122
4.3.2.1	Perturbation-specific compensatory mechanisms confirmed in the metabolome	123
4.3.3	Compensatory LDC induction during polyamine depletion investigated further	127
4.3.5	gDNA Methylation status investigation	128
4.3.5.1	CpG island analysis of the differential transcript abundance list	128
4.3.4.2	Global methylation assays	128
4.3.4.2.1	Methylation-sensitive restriction endonucleases	129
4.3.4.2.2	South-Western immunoblotting	129
4.4	DISCUSSION.....	130

CHAPTER 5

CONCLUDING DISCUSSION..... 136

REFERENCES 142

APPENDIX A

APPENDIX B

APPENDIX C

APPENDIX D

APPENDIX E

LIST OF EQUATIONS

Equation no.	Title of Equation	Page no.
2.1	Percentage response	37
2.2	Concentration-response curve four-parameter logistic equation	40
3.1	Gene expression log ₂ -ratio	57
3.2	Enzyme total activity.....	60
3.3	Fluorescent dye labelling efficiency	63
4.1	Iso-electric focussing volt hours (constant).....	108
4.2	Iso-electric focussing volt hours (gradient)	108

LIST OF FIGURES

Figure no.	Title of Figure	Page no.
1.1	Malaria geographical distribution and chloroquine resistance	2
1.2	Giemsa-stained thin smears depicting the life cycle of 3D7 <i>P. falciparum</i>	4
1.3	Currently used antimalarial drugs.....	11
1.4	Chemical structures of the natural polyamines	15
1.5	Polyamine metabolism in mammalian cells.....	16
1.6	Polyamine metabolism in <i>Plasmodium</i>	18
1.7	Composite diagram of polyamine levels and biosynthetic enzyme transcript levels.....	18
1.8	The 48 h IDC transcriptomes of 3D7, Dd2 and HBR <i>P. falciparum</i>	22
1.9	The proposed experimental layout for the application of transcriptome and proteome analysis to drug-challenged malaria parasites	27
2.1	Chemical structures of DFMO, MDL73811, MAOBA and MAOEA.....	31
2.2.	Sigmoidal concentration-response curve used by GraphPad Prism 4.0 software to calculate the median inhibitory concentration (IC ₅₀).....	40
2.3	HRPII ELISA optimisation	42
2.4	LDH assay validation	42
2.5	FACS versus microscopy.....	43
2.6	Typical FACS images.....	44
2.7	Concentration-response curves of chloroquine.....	44
2.8	Concentration-response curves of cytostatic compounds	45
2.9	Parasite stage-specific levels of HRPII and LDH activity.....	47
2.10	Transcript levels of LDH and three histidine-rich proteins during the IDC	48
2.11	Concentration-response curves of MDL73811, DFMO and the combination	49
2.12	Giemsa-stained thin smears of chicken erythrocytes and 3D7 <i>P. falciparum</i> -infected human erythrocytes	50
3.1	Six designs of microarray time course experiments	57
3.2	Giemsa-stained thin smears of untreated <i>P. falciparum</i> and cultures treated with 5 mM DFMO, 5 µM MDL73811 or the combination.....	67
3.3	Total activity of AdoMetDC and ODC based on the release of ¹⁴ CO ₂	68
3.4	Transcriptomics sampling times	69
3.5	The total RNA yield and denaturing agarose/formaldehyde electrophoresis.....	69
3.6	Typical 70-mer oligonucleotide spotted arrays.....	70
3.7	Hierarchical data clustering between genes of the PfAdoMetDC/ODC co-inhibition data	72
3.8	Hierarchical data clustering between arrays of the PfAdoMetDC/ODC co-inhibition data and the Llinás perturbation data.....	73
3.9	A tight cluster containing several polyamine pathway transcripts.....	74
3.10	“Biphasic” segregation of the expression/peak abundance of polyamine and methionine metabolism transcripts	75
3.11	Phaseogram depicting the transcriptional profiles of untreated versus PfAdoMetDC/ODC co-inhibition data and Pearson correlation between the PfAdoMetDC/ODC co-inhibition data	76
3.12	Red and green background images of a typical array (111_TAt33)	78
3.13	MA-plots from a typical array (125_UTBt33) before and after data transformation.	79
3.14	Print-tip boxplots from a typical array (116_TBt25) before and after data transformation	79
3.15	Boxplots of log ₂ -ratios (M) and intensities (A) across all arrays post-normalisation.	80
3.16	Red/Green density plots of all the arrays before and after data transformation	80
3.17	Transcript profiles of PfAdoMetDC/ODC, lysine decarboxylase and ornithine aminotransferase	8



	3	
3.18	EDGE output in the form of a histogram	84
3.19	Functional classification of transcripts with increased and decreased differential abundance	85
3.20	Eleven gene cluster from chromosome 10	86
3.21	The relative constant transcription profile of the putative cyclophilin (PFE0505w) in the PfAdoMetDC/ODC co-inhibition data and in the IDC transcriptome	89
3.22	A real-time PCR plot obtained for a five-part cDNA dilution series of the putative cyclophilin	90
3.23	Melting curve analysis of the amplification product of cyclophilin	90
3.24	A standard curve of the putative cyclophilin (PFE0505w)	91
3.25	Polyamine and methionine metabolism (differentially affected transcripts indicated)	95
4.1	The master image and actual images of the three best 2D-GE technical replicates used for analysis of UT _{t1} versus T _{t1} , T _{t2} and T _{t3}	116
4.2.	A typical gel (UTt1_84404) indicating the 41 spots with differential abundance	117
4.3	MALDI-Q-TOF MS/MS protein identification of LDH as an example	119
4.4	A typical gel (UTt1_84404) with an enlarged view of AdoMet synthetase and OAT over the time course.	121
4.5	Metabolite profiles compared to relative t ₀ of putrescine, spermidine, 5-methylthioinosine, ornithine and AdoMet	124
4.6	Metabolite profiles of treated parasites directly compared to the parallel untreated controls for putrescine, spermidine, 5-methylthioinosine, ornithine and AdoMet	126
4.7	Lack of measurable LDC activity of untreated and DFMO/MDL73811-treated parasite lysates after incubation with L-[14C]-lysine.	127
4.8	Gel electrophoresis of digested and undigested gDNA to assess methylation after PfAdoMetDC/ODC co-inhibition.	129
4.9	South-Western blot of 5mC in <i>P. falciparum</i> gDNA	130
4.10	Polyamine and methionine metabolism (differentially affected transcripts, proteins and metabolites indicated)	133

LIST OF TABLES

Table no.	Title of Table	Page no.
1.1	Antimalarial therapeutics and combinations	10
2.1	IC ₅₀ values obtained with three different drug sensitivity assays	46
2.2	PI assay and FACS analysis of PfAdoMetDC/ODC co-inhibited <i>P. falciparum</i>	50
3.1	Real-time PCR primer information	66
3.2	Pearson correlation within the PfAdoMetDC/ODC co-inhibition transcript data	77
3.3	Biological functions of a subset of the transcripts differentially affected according to LIMMA as a result of PfAdoMetDC/ODC co-inhibition	81
3.4	Adjacent gene clusters with decreased abundance transcripts	86
3.5	IDC mRNA expression profiles of the eleven gene cluster from chromosome 10	87
3.6	Interactome data comparisons	88
3.7	Microarray data validation with real-time PCR.....	92
4.1	Iso-electric focussing step-and-hold programme	108
4.2	Correlation of the 2D-GE data across replicates groups	115
4.3	Identification and characterisation of a subset of proteins with differential abundance.....	120
4.4	Pearson correlation of the metabolite data	122
4.5	Metabolites with differential abundance (i.e. fold changes of more than 2 in either direction) in treated and untreated samples (relative to comparison).....	123
4.6	Metabolites with differential abundance (i.e. fold changes of more than 2 in either direction) after PfAdoMetDC/ODC co-inhibition (parallel time point comparison).....	125
4.7	Geecee-count analysis of the genes encoding the 538 differentially affected transcripts	128

ABBREVIATIONS

¹ H-NMR	Proton nuclear magnetic resonance
2D	Two-dimensional
2D-DIGE	Two-dimensional difference gel electrophoresis
2D-GE	Two-dimensional gel electrophoresis
2D-NMR	Two-dimensional nuclear magnetic resonance
2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
5mC	5-Methyl-2-deoxycytosine
5mC(P)	5-Methylcytidine
6mA	6-Methyl-2-deoxyadenine
A	Adenosine or average signal intensities (MA plot)
AcN	Acetonitrile
AdoHcy	S-adenosylhomocysteine
AdoMet	S-adenosylmethionine
AdoMet synthetase	S-adenosylmethionine synthetase
AdoMetDC	S-adenosylmethionine decarboxylase
AMA1	Apical membrane antigen 1
APAD	3-acetyl pyridine adenine dinucleotide
ApiAP2	Apicomplexan Apetala2
ATP	Adenosine triphosphate
BC	Before Christ
bp	Base pair
BSA	Bovine serum albumin
C	Cytidine
CD36	Cluster determinant 36
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CO	Carbon monoxide
CpG	Cytosine Guanine dinucleotide with connecting phosphodiester bond
CPM	Counts per minute
CSA	Chondroitin sulphate A
C _t	Cycle threshold of the real-time amplification curve
Cys	Cysteine
DALY	Disability adjusted life years
dATP	Deoxyadenosine triphosphate
DAVID	Database for annotation, visualization and integrated discovery
dCTP	Deoxycytidine triphosphate
dcAdoMet	Decarboxylated S-adenosylmethionine
DDT	Dichlorodiphenyltrichloroethane
DELI	Double-site enzyme-linked LDH immunodetection
DEPC	Diethyl pyrocarbonate
DFMO	DL-a-difluoromethylornithine
dGTP	Deoxyguanosine triphosphate
DHFR	Dihydrofolate reductase



DHFR/TS	Dihydrofolate reductase/thymidylate synthase
DHPS	Dihydroopteroate synthase
DHPS/PPPK	Dihydroopteroate synthase/dihydroxymethylpterin pyrophosphokinase
DIGE	Difference gel electrophoresis
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
DPM	Disintegrations per minute
DTT	Dithiothreitol
dTTP	Deoxythymidine triphosphate
dUTP	Deoxyuridine triphosphate
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
EtOH	Ethanol
FACS	Fluorescence activated cell sorting
F-MES	Modified Falkow (medium)
FTICR	Fourier transform ion cyclotron resonance
G	Guanosine
GABA	Gamma-aminobutyrate or 4-aminobutyrate
gDNA	Genomic DNA
gff	General feature format
GO	Gene ontology
hpi	Hours post-invasion
HPLC	High-performance liquid chromatography
HRP	Histidine-rich proteins
hrp ^a	Horseradish peroxidase
hrp-conjugate	Anti-mouse horseradish peroxidase-conjugated secondary antibody
HRPII	Histidine- and alanine-rich protein 2
IC ₅₀	Median inhibitory concentration
ICAT	Isotope-coded affinity tags
IDC	Intraerythrocytic developmental cycle
IEF	Iso-electric focusing
IFN	Interferons
IL	Interleukin
IPG	Immobilized pH gradient
iTRAQ	Isobaric tags for relative and absolute quantitation
KEGG	Kyoto Encyclopedia of Genes and Genomes
LB	Luria-Bertani (broth)
LC	Liquid chromatography
LC-ESI/MS	Liquid chromatography/electron spray ionization mass spectrometry
LDC	Lysine decarboxylase
LDH	Lactate dehydrogenase
LIMMA	Linear models for microarray data (software)
LOWESS	Locally weighted scatterplot smoothing



M	Log ₂ -ratios of transcript abundance
m/z	Mass/charge ratio
MALDI	Matrix assisted laser desorption/ionization
MAOBA	5'-Deoxy-5'-[N-methyl]-N-[2-(aminooxy)buthyl]amino]adenosine
MAOEA	5'-Deoxy-5'-[N-methyl]-N-[2-(aminooxy)ethyl]amino]adenosine
MDL73811	5'-{[(Z)-4-amino-2-butenyl]methylamino}-5'-deoxyadenosine
MDR1	Multidrug-resistance type 1 protein
MeOH	Methanol
MIAME	Minimum information about a microarray experiment
MOPS	3-(N-morpholino)propanesulfonic acid
MPMP	Malaria Parasite Metabolic Pathways
Mr	Molecular weight
mRNA	Messenger RNA
mRNP	Messenger ribonucleoprotein complexes
MS	Mass spectrometer/spectrometry
MS/MS	Tandem mass spectrometry
MSP1	Merozoite surface protein 1
MSRE	Methylation-sensitive restriction endonucleases
MudPIT	Multidimensional protein identification technology
NBT	Nitroblue tetrazolium
NMR	Nuclear magnetic resonance
NO	Nitric oxide
OAT	Ornithine aminotransferase
OAT _{met}	Methylated ornithine amino transferase DNA
ODC	Ornithine decarboxylase
ORF	Open reading frame
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pdx1	Pyridoxal-5'-phosphate synthase
PES	Phenazine ethosulphate
PEXEL	<i>Plasmodium</i> export element
PfAdoMetDC/ODC	<i>P. falciparum</i> S-adenosylmethionine decarboxylase/ornithine decarboxylase
PfCRT	<i>P. falciparum</i> chloroquine-resistance transporter
PfEMP1	Erythrocyte membrane protein 1
PI	Propidium iodide
pI	Iso-electric point
PlasmoDB	<i>Plasmodium</i> database
PLP	Pyridoxal-5'-phosphate
PLS	Partial Least Squares
PMF	Peptide mass fingerprint/fingerprinting
PMT	Photon multiplier tube (fluorescent scanners)
ppm	Parts per million
PUMAdb	Princeton University Microarray database
Q	Quadropole
Q-TOF	Quadropole-time-of-flight mass spectrometer/spectrometry
r	Pearson correlation
R	Correlation coefficient of the regression line of data plotted on the same graph



<i>Rifin</i>	Repetitive interspersed family (genes)
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SAGE	Serial analysis of gene expression
SDS	Sodium-dodecylsulphate
SERCA	Sarcoplasmic reticulum calcium-dependent ATPase
SRM	Single reaction monitoring (mass spectrometry)
SSC	Saline sodium citrate
SSP	Standard spot numbers
<i>Stevor</i>	Subtelomeric variable open reading frame (genes)
T	Thymidine or treated (sample)
t_0	Time zero
t_1	Time point 1
t_2	Time point 2
t_3	Time point 3
TAE	40 mM Tris, 20 mM glacial acetic acid, 1 mM EDTA (buffer)
T_m	Melting temperature
TNF	Tumour necrosis factor
TOF	Time-of-flight
tRNA	Transfer RNA
U	Units
UT	Untreated (sample)
UV	Ultraviolet
V	Volts
<i>var</i>	Variant (genes)
Vh	Volt hours
VTS	Vacuolar transport signal
WHO	World Health Organisation

a. HRP is the customary abbreviation for horseradish peroxidase, but to distinguish from the abbreviated histidine rich protein, lowercase characters (hrp) were used.