IN SILICO PREDICTION AND BIOCHEMICAL VALIDATION OF PROTEIN TARGETS IN *Anopheles gambiae*

ADEDEJI, EUNICE OLUWATOBILOBA
(17PCP01674)

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BY

ADEDEJI, EUNICE OLUWATOBILOBA
(17PCP01674)
B.Sc Biochemistry, University of Ibadan, Ibadan
M.Sc Biochemistry, University of Ibadan, Ibadan

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) IN BIOCHEMISTRY IN THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY, OTA, OGUN STATE, NIGERIA

DECEMBER, 2022

ACCEPTANCE

This is to attest that this thesis is accepted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Biochemistry in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Nigeria.

Miss. Adefunke F. Oyinloye
(Secretary, School of Postgraduate Studies) **Signature and Date**

Prof. Akan B. Williams
(Dean, School of Postgraduate Studies) **Signature and Date**

DECLARATION

I, **ADEDEJI, EUNICE OLUWATOBILOBA (17PCP01674)** declare that this research was carried out by me under the supervision of Prof. Olubanke O. Ogunlana of the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Nigeria and Dr. Segun A. Fatumo of the Department of Non-communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom. I attest that the thesis has not been presented either wholly or partially for the award of any degree elsewhere. All sources of data and scholarly information used in this thesis are duly acknowledged.

ADEDEJI, EUNICE OLUWATOBILOBA

Signature and Date

CERTIFICATION

We certify that this thesis titled "***IN SILICO* PREDICTION AND BIOCHEMICAL VALIDATION OF PROTEIN TARGETS IN *Anopheles gambiae***" is an original research work carried out by **ADEDEJI, EUNICE OLUWATOBILOBA (17PCP01674)** in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria under the supervision of Prof. Olubanke O. Ogunlana and Dr. Segun A. Fatumo. We have examined and found this work acceptable as part of the requirements for the award of Doctor of Philosophy (Ph.D) degree in Biochemistry.

Prof. Olubanke O. Ogunlana
(Supervisor) **Signature and Date**

Dr. Segun A. Fatumo
(Co-Supervisor) **Signature and Date**

Prof. Israel S. Afolabi
(Head of Department) **Signature and Date**

Prof. God'swill N. Anyasor
(External Examiner) **Signature and Date**

Prof. Akan B. Williams
(Dean, School of Postgraduate Studies) **Signature and Date**

DEDICATION

This work is dedicated to God Almighty, the source of my life, strength, and wisdom. In Him alone I have my being.

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TABLE OF CONTENTS

CONTENT	PAGES
COVER PAGE	i
TITLE PAGE	ii
ACCEPTANCE	iii
DECLARATION	iv
CERTIFICATION	v
DEDICATION	vi
ACKNOWLEDGEMENTS	vii
TABLE OF CONTENTS	x
LIST OF TABLES	xiv
LIST OF FIGURES	xvi
LIST OF APPENDICES	xix
LIST OF ABBREVIATIONS	xx
ABSTRACT	xxiii
CHAPTER ONE	1
INTRODUCTION	1
1.1. Background to the Study	1
1.2. Statement of the Problem	9
1.3. Research Questions	10
1.4. Research Hypothesis	10
1.5. Aim and Objectives	11
1.5.1. Aim	11
1.5.2. Specific objectives	11
1.6. Justification of the Study	11
1.7. Scope of the Study	13
CHAPTER TWO	15
LITERATURE REVIEW	15
2.1. Life Cycle of *Anopheles* Mosquitoes	15
2.2. *Plasmodium* Infection and Immune Response in *Anopheles* Mosquito	17
2.2.1. Innate immune system of *Anopheles*	17
2.2.1.1. Immune signalling pathways	19
2.2.1.2. Phagocytosis and melanisation	21
2.3. *Anopheles* Metabolic Proteins in Malaria Transmission, Prevention and Control	23
2.3.1. Metabolic proteins as insecticide targets	26
2.3.2. Metabolic proteins and insecticide resistance	31
2.3.2.1. Metabolic resistance	32
2.3.2.2. Target site mechanism	38
2.3.2.3. Cuticular resistance	39
2.3.3. Metabolic proteins, blood digestion, immune response and *Plasmodium* parasite development in *Anopheles*	40
2.3.3.1. Metabolic proteins and blood digestion in *Anopheles*	40

2.3.3.2. Metabolic proteins and immune response to *Plasmodium* infection in *Anopheles* 45
2.3.3.3. Role of metabolic proteins in *Plasmodium* parasite development 47
2.3.3.4. Role of metabolic proteins in fecundity 50
2.3.4. Metabolic proteins and vector control using insecticides: the way forward 52
2.3.4.1. Identifying novel insecticide targets 52
2.3.4.2. Generation of organism-target specific and selective insecticides 56
2.3.4.3. Combining two or more insecticidal agents in a single product 59
2.3.4.4. Combining insecticide with synergists 63
2.3.4.5. Combining insecticides with transmission-blocking agents 68
2.3.4.6. Combining insecticides with sterilants 69
2.3.5. Vector control strategies: genetic modification of metabolism for population replacement or suppression 74
2.4. Genome-scale Metabolic Network Reconstruction 75
2.4.1. Methods for predicting essential reactions in a metabolic network 77
2.5. Experimental Validation of Computationally Predicted Targets 78
2.5.1. Gene knockout techniques 78
2.5.2. Gene knockdown techniques 81
2.5.2.1. Morpholinos for gene knockdown 81
2.5.2.2. RNA interference for gene knockdown by injection 82
2.5.3. Chemical Inhibition 84
2.6. Gaps Identified in Literature 86

CHAPTER THREE 88
MATERIALS AND METHODS 88
3.1. Materials 88
3.1.1. Software, Databases, and Web-servers 88
3.1.2. Equipment 88
3.1.3. Kits, Chemicals, Competent cells and Reagents 89
3.1.4. Organisms 89
3.1.5. Mice 90
3.2. Methods 90
3.2.1. Central metabolic model reconstruction and prediction of targets in *An. gambiae* 90
3.2.1.1. Draft metabolic network reconstruction and manual curation 90
3.2.1.2. Compartmentalisation 91
3.2.1.3. Conversion to BiGG model 92
3.2.1.4. Chokepoint and PUPLZ 92
3.2.1.5. Mapping of transcriptomics datasets 93
3.2.1.6. Selection of genes using prediction from leave-one-organism-out cross-validation (LOOOCV) model 94
3.2.2. RNAi Experiments 94
3.2.2.1. Rearing of *An. gambiae* 94
3.2.2.2. Mice handling 95
3.2.2.3. Primer design and handling 95
3.2.2.4. RNA extraction for dsRNA synthesis 95
3.2.2.5. cDNA synthesis for dsRNA preparation 96
3.2.2.6. DNA extraction from *E. coli* for LacZ template amplification 98

3.2.2.7. PCR amplification of target template from cDNA for dsRNA synthesis 99
3.2.2.8. Agarose gel electrophoresis 103
3.2.2.9. Purification of PCR products 103
3.2.2.10. Gel extraction of PCR products 103
3.2.2.11. Determination of efficiency of qPCR primers 104
3.2.2.12. dsRNA synthesis 109
3.2.2.13. dsRNA injections 111
3.2.2.14. Knockdown efficiency of dsRNA 111
3.2.2.15. Effect of gene knockdown on mosquito survival 112
3.2.2.16. *P. berghei* infection in mice 112
3.2.2.17. Effect of gene knockdown on *Plasmodium berghei* infection in mosquitoes 113
3.2.3. Chemical inhibition experiments 113
3.2.3.1. Sample preparation for biochemical assay 114
3.2.3.2. Determination of Trehalose and Glucose concentration 115
3.2.3.3. Protein determination 117
3.2.3.4. Quantitative RT- PCR 120
3.3. Methods of Statistical Analysis 122

CHAPTER FOUR 123
RESULTS 123
4.1. *In silico* Studies 123
4.1.1. Metabolic model generation and curation 123
4.1.2. Prediction of essential genes 131
4.1.3. Mapping of transcriptomics datasets 131
4.1.4. Selection of genes for experimental validation 141
4.2. RNAi Experiments 145
4.2.1. Primer efficiency determination 145
4.2.2. RNAi by injection of 69 nL of 3 µg/µL dsRNA 146
4.2.3. RNAi by injection of 138 nL of 5 µg/µL dsRNA 153
4.2.4. Effect of knockdown of Arginase or Trehalase on survival of mosquitoes and *P. berghei* development 161
4.2.5. Effect of knockdown of HSP, 2Elf and 1Elf on survival of *An. gambiae* 167
4.3. Validamycin Inhibition 172
4.3.1. Effect of validamycin treatment on mosquito survival 172
4.3.2. Effect of validamycin treatment on pupation of mosquitoes 172
4.3.3. Protein determination 179
4.3.4. Glucose determination 179
4.3.5. Trehalose determination 179
4.3.6. Effect of treatment on expression of selected genes 184

CHAPTER FIVE 189
DISCUSSION 189
5.1. *In silico* Studies 189
5.2. RNAi Experiments 195
5.3. Validamycin Inhibition 201

CHAPTER SIX 205
CONCLUSION AND RECOMMENDATIONS 205
6.1. Summary 205
6.2. Conclusion 206
6.3. Contributions to Knowledge 206
6.4. Recommendations 206
6.5. Limitations to the Study 207

REFERENCES 208
APPENDICES 244

LIST OF TABLES

TABLES	LIST OF TABLES	PAGES
2.1	Classes of insecticides, their resistance mechanisms and associated proteins in *Anopheles* species	34
2.2	Efficacy of alphacypermethrin and chlorfenapyr mixture in insecticide-treated nets	61
2.3	Efficacy of pyrethoids and synergist mixture in insecticide-treated nets	65
2.4	Possible *Anopheles*' metabolic proteins for vector control strategies based on their role in malaria transmission	71
2.5	Gaps identified in literature	86
3.1	Reaction mixture preparation for cDNA synthesis (PrimeScript RT reagent kit)	97
3.2	Primer sequences for dsRNA synthesis	100
3.3	PCR reaction mix preparation (DreamTaq Green DNA Polymerase)	102
3.4	cDNA serial dilution preparation for primer efficiency determination	105
3.5	Real-time PCR reaction component for primer efficiency determination	106
3.6	Real-time PCR cycling conditions	107
3.7	Primer sequences for qPCR	108
3.8	dsRNA synthesis reaction mix preparation	110
3.9	Trehalose determination protocol	116
3.10	Preparation of diluted bovine serum albumin (BSA) standards	119
3.11	Primer sequences for Validamycin inhibition experiments	121
4.1	Network statistics of curated *An. gambiae* central metabolic model	125
4.2	Subsystem division of metabolic model	127
4.3	Essential reactions and their corresponding genes in *An. gambiae* central metabolic network	133
4.4	Predicted genes differentially expressed in guts and haemocytes of *P. berghei* infected mosquitoes	138

4.5	Efficiency of qPCR primers	145
4.6	Percentage knockdown in target genes 72 h after dsRNA injection	160

LIST OF FIGURES

FIGURES	LIST OF FIGURES	PAGES
2.1	Life cycle of *Anopheles* mosquito	16
2.2	Mosquito innate immunity	22
2.3	Role of *Anopheles* metabolic proteins in malaria transmission and control	24
2.4	Conserved catalytic serine residue in acetylcholinesterase (AChE) targeted by insecticides in diverse organisms	29
2.5	Paraoxon binds conserved residues in both humans' and mosquitoes' acetylcholinesterase (AChE), hence toxic to humans	30
2.6	Conserved unpaired cysteine residue in the acetylcholinesterase (AChE) of disease vectors for selective insecticide design	57
2.7	Ways of manipulating metabolic proteins of *Anopheles* for vector control strategies	70
4.1	Summary of genome-scale metabolic model draft reconstruction for *An. gambiae*	124
4.2	Percentage contribution of reactions to metabolic pathways	126
4.3	Venn diagram showing essential gene prediction using Chokepoint and PUPLZ	132
4.4(a-c)	Metabolic fate of 3-hydroxy-L-kynurenine	142
4.5	Metabolism of Arginine	143
4.6(a-b)	Arginase and NOS expression	144
4.7	Gel image of 4 sets of dsRNA template for 3HKT	147
4.8	Relative gene expression of 3HKT in *An. gambiae* 72 h after dsRNA injection	148
4.9	Gel image of 3 sets of dsRNA template for Trehalase	149
4.10	Relative gene expression of Trehalase in *An. gambiae* 72 h after dsRNA injection	150
4.11	Relative gene expression of 3HKT in sugar-fed and blood-fed females 24 h after a blood meal	151

4.12	Relative gene expression of Trehalase in sugar-fed and blood-fed females 24 h after a blood meal	152
4.13	Gel image of dsRNA for HSP, Arg, 2Elf, Tre, 1Elf and LacZ	154
4.14	Relative gene expression of arginase at 24, 48, 72 and 96 h after dsRNA injection in *An. gambiae* G3	155
4.15	Relative gene expression of trehalase at 24, 48, 72 and 96 h after dsRNA injection	156
4.16	Relative gene expression of HSP at 24, 48, 72 and 96 h after dsRNA injection in *An. gambiae* G3	157
4.17	Relative gene expression of 3HKT at 24, 48, 72 and 96 h after dsRNA injection in *An. gambiae* G3	158
4.18	Relative gene expression of 1Elf, 2Elf at 72 h after dsRNA injection in *An. gambiae* G3	159
4.19	Percentage survival of female *An. gambiae* mosquitoes blood-fed at 48 h after knockdown of Trehalase or Arginase	162
4.20	Relative gene expression of arginase in not injected blood-fed (BF) *An. gambiae* G3 compared to sugar-fed (SF) 24 h after blood-feeding	163
4.21	Relative gene expression of arginase at 72 h after dsRNA injection in blood-fed (BF) *An. gambiae* G3	164
4.22	Relative gene expression of trehalase at 72 h after dsRNA injection in sugar-fed (SF) and blood-fed (BF) *An. gambiae* G3	165
4.23	*P. berghei* oocytes count on day 10 post-infection in female *An. gambiae* blood-fed with parasitised mice at 48H after knockdown of Arginase	166
4.24	Percentage survival of female *An. gambiae* after knockdown of HSP, 1Elf or 2Elf	168
4.25(a-c)	Percentage survival of control mosquitoes and HSP-knockdown mosquitoes	169
4.26(a-c)	Percentage survival of control mosquitoes and 2Elf-knockdown mosquitoes	170
4.27	Proposed mechanism by which elongation factor 2 causes death in *An. gambiae*	171

4.28	Percentage survival of L3 *An. gambiae* larvae exposed to Validamycin A	173
4.29	Percentage survival of 2-day old L1 *An. gambiae* larvae exposed to Validamycin A	174
4.30	LC_{50} concentration of Validamycin A	175
4.31	Dose-response curve	176
4.32	Percentage pupation of validamycin treated L3 *An. gambiae* larvae	177
4.33	Percentage pupation of 2-day old L1 *An. gambiae* larvae exposed to Validamycin	178
4.34	Protein Standard Curve	180
4.35	Protein concentration of L3 larvae 24 h after validamycin exposure	181
4.36	Glucose concentration of L3 larvae 24 h after validamycin exposure	182
4.37	Trehalose concentration of L3 larvae 24 h after validamycin exposure	183
4.38	Expression levels of selected genes in pupae following treatment in L3 larvae	185
4.39	Effect of exogenous trehalose on metabolism in *An. gambiae*	186
4.40	Expression levels of selected genes in L3 24 hours after Validamycin A treatment	187
4.41	Proposed mechanism by validamycin A retards pupae development in *An. gambiae*	188

LIST OF APPENDICES

APPENDICES	LIST OF APPENDICES	PAGES
Appendix 1	CHREC ethical permit certificate	244
Appendix 2	Alignment of the amino acid sequences of AChE from 13 animal species	246
Appendix 3	Chemical preparation	250
Appendix 4	Selected pictures of activities in laboratories	252
Appendix 5	Statistical analysis flow chart for gene expression studies	256
Appendix 6	*Anopheles gambiae* central metabolic model	257

LIST OF ABBREVIATIONS

1Elf	Elongation factor 1-alpha
2Elf	Elongation factor 2
3HKT	3-hydroxykynurenine transaminase
ABC transporters	ATP-binding cassette transporters
AChE	Acetylcholinesterase
AgAQP3	*An. gambiae*'s Aquaporin 3
AgaCA	*An. gambiae*'s β-class carbonic anhydrase
AgTreT	*An. gambiae*'s Trehalose transporter
AMP	Antimicrobial peptides
An. gambiae	*Anopheles gambiae*
Arg	Arginase
APL1	*Anopheles Plasmodium* responsive leucine-rich repeat protein 1
ATP	Adenosine triphosphate
BiGG reconstruction	Biochemical, genetic and genomic reconstruction
Cas	Clustered regularly interspaced palindromic repeats associated protein
CEs	Carboxylesterases
CLIPs	Clip domain serine proteases
CRISPR	Clustered regularly interspaced palindromic repeats
crRNA	CRISPR RNA
CTLs	C-type lectin-like proteins
CYP 450s	Cytochrome P450s
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
dsRNA	Double-stranded RNA
FBA	Flux Balance Analysis
FREP 1	Fibrinogen related proteins 1
GABA	Gamma-aminobutyric acid

GILT	gamma-interferon–inducible lysosomal thiol reductase
GLUT	Glucose transporter
GPCRs	G protein-coupled receptors
GPR	Gene-protein-reaction
gRNA	Guide RNA
GSMM or GEM	Genome-scale metabolic network model
GSTs	Glutathione S-transferases
HNH	Histidine asparagine histidine
HR	Homology directed repair
HSP	Heat shock 70kDa protein 1/8
IAPs	Inhibitor of apoptotic proteins
ILPs	Insulin-like peptides
IRS	Indoor residual spraying
ITNs	Insecticide-treated nets
KMO	Kynurenine 3-monooxygenase
LLINs	Long-lasting insecticidal nets
LRIM1	Leucine-rich repeat immune protein 1
LRRs	Leucine-rich repeat proteins
mRNA	messenger RNA
MRTC	Malaria Research and Training Center
NHEJ	Non homologous end joining
NO	Nitric oxide
NOS	Nitric oxide synthase
NOX5	NADPH oxidase 5
P. berghei	*Plasmodium berghei*
P. falciparum	*Plasmodium falciparum*
PAH	Phenylalanine-4-hydroxylase
PAM	Protospacer adjacent motif
PAMP	Pathogen-associated molecular patterns
Pbf	Post-blood-feeding
Pbm	Post blood meal
PCR	Polymerase chain reaction
PDB	Protein data bank

PO	Phenoloxidase
PRR	Pattern recognition receptors
PULPZ	Percentage of unreached products larger than zero
QPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
RNAi	RNA interference
RT-PCR	Real-time polymerase chain reaction
RuvC	Resistance to ultraviolet C
SBML	Systems Biology Markup Language
sgRNA	Single guide RNA
siRNA	small interfering RNA
TALEN	Transcription activator-like effector nucleases
T. cinnabarinus	*Tetranychus cinnabarinus*
TEPs	Thioester-containing proteins
trancRNA	Trans-activating CRISPR RNA
Tre	Trehalase
tRNA	Transfer RNA
XA	Xanthurenic acid
YFP	Yellow florescent dye
ZFN	Zinc finger nucleases

ABSTRACT

Malaria, an endemic disease in sub-Saharan Africa, is transmitted by female *Anopheles* mosquitoes. The major malaria vector control strategy has remained the use of insecticides. However, resistance in *Anopheles* to all classes of existing insecticides motivates the identification of novel targets for malaria vector control. *Anopheles* metabolic proteins represent a repertoire of possible targets, however, finding potential targets using experimental methods alone is a tall order. This study aimed to identify and modulate genes genes central for *An. gambiae*'s survival or *Plasmodium berghei* clearance in the mosquito using *in silico* and biochemical techniques. A multi-compartment central metabolic model of *An. gambiae* was constructed using Reconstruction, Analysis and Visualization of Metabolic Networks (RAVEN) toolbox, manual curation was performed, and essential genes were predicted using chokepoint and percentage of unreached products larger than zero criteria. Experimental validation of selected genes was carried out using RNA interference (RNAi) and chemical inhibition methods. Double-stranded RNA (138 nL of 5 µg/µL) for selected genes and LacZ was injected into respective groups of between 2 to 4 days old female *An. gambiae* mosquitoes. Seventy (70) female mosquitoes per treatment were injected for survival experiments, and survival was monitored from day 2 post-injection until the death of all mosquitoes. Two hundred (200) female mosquitoes per treatment group (LacZ and Arginase) were injected for experiments involving infection with *P. berghei*. Parasite infection was performed 48 h after injection, and oocyte count was determined. Validamycin was administered at varying concentrations to third-stage (L3) larvae and first-stage (L1) larvae, then biochemical parameters and effect on gene-expression were investigated. Appropriate statistical analyses were carried out on the results. The central metabolic model consisted of 570 reactions, 471 metabolites, and 833 genes distributed across cytoplasm, mitochondria, and extracellular compartments. Three genes out of the 106 genes predicted from the network were selected, and three other non-metabolic genes for experimental validation in *An. gambiae* G3 mosquitoes based on established literature alluding to their essentiality. The six genes validated were Trehalase, Arginase, 3-hydroxykynurenine transaminase (3HKT), Heat shock 70kDa protein (HSP), elongation factor 2 (2elf), and elongation factor 1-alpha. Knockdown of HSP and 2elf resulted in a significant reduction ($p<0.05$) in the percentage survival of mosquitoes compared to control groups. Similarly, knockdown of arginase led to a marked reduction ($p<0.05$) in the number of oocytes count per midgut compared to the control group. In addition, larval treatment with validamycin A, an inhibitor of trehalase resulted in marked larval death at 48 h in a dose-dependent manner and retarded development of mosquitoes. Lethal concentration 50 (LC_{50}) was 167.1 ppm in L3 larvae and 30.71 ppm in L1 larvae. Treatment with validamycin increased trehalose concentration at all concentrations considered and expression of insulin-like peptide2 at 500 ppm, 24 h after validamycin A exposure. This study suggests trehalase as a possible larvicide target, revealed the importance of HSP and 2elf for mosquito survival, and arginase for parasite development. These may serve as potential targets for vector control. Further studies to identify suitable inhibitors for these targets is recommended.

Keywords: Anopheles, Elongation factor, Heat shock, Plasmodium, Trehalase, Validamycin, Vector control.