



## ACKNOWLEDGEMENTS

# Molecular characterization of the hexose transporter (PfHT1) of *Plasmodium falciparum* in *Xenopus laevis* oocytes

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## LIST OF ABBREVIATIONS

1-DOG	1-deoxy-D-glucose
2,5-AHM	2,5-anhydro-D-mannitol
2-DOG	2-deoxy-D-glucose
3-OMG	3- <i>O</i> -methyl-D-glucose
6-DOG	6-deoxy-D-glucose
A	Adenine
AMA	Apical merozoite antigen
ATB-BMPA	2- <i>N</i> -4-(1-azi-2,2,2-trifluoroethyl) benzoyl-1,3-bis(D-mannos-4-yloxy)-2-propylamine
ATP	Adenosine triphosphate
BFA	Brefeldin A
bp	Base pair
C	Cytosine
C5-DMB-ceramide	<i>N</i> -[5-(5,7-dimethylBODIPY)-1-pentanoyl]-D-erythro-sphingosine
C6-NBD-cer	<i>N</i> -[7-(4-nitrobenzo-2-oxa-1,3-diazole)] amino-caproyl sphingosine
C6-NBD-Sm	C6-NBD-sphingomyelin
CD36	Cluster determinant 36
CHO	Chinese Hamster Ovaries
CO <sub>2</sub>	Carbon dioxide
CTP	Cytidine triphosphate
DEPC	Diethyl pyrocarbonate
DHFR-TS	Dihydrofolate reductase-thymidylate synthase
DHODase	Dihydroorotate dehydrogenase



DNA	Deoxyribonucleic acid
DOG	Deoxy D-glucose
DPM	Decay per minute
EDTA	Ethylenediaminetetraacetic acid
EM	Erythrocyte membrane
ER	Endoplasmic reticulum
FT-IR	Fourier transform infrared
G	Guanine
GFP	Green fluorescent protein
GLUT1	Mammalian glucose transporter 1
GPI	Glycosylphosphatidylinositol
Grp	Glucose-regulated protein
GTP	Guanosine triphosphate
HDEL	His-Asp-Glu-Leu
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HIF-1	Hypoxia-induced factor 1
ICAM-1	Intercellular adhesion molecule-1
IFN- $\gamma$	Gamma interferon
IL	Interleukin
INOS	Nitric oxide synthetase
IPTG	Isopropyl-D-galactoside
IRBC	Infected red blood cell
KAHRP	Knob associated histidine rich protein
KDEL	Lys-Asp-Glu-Leu
$K_i$	Half-maximal inhibition constant for carrier transport
$K_m$	Affinity or Michaelis constant

LB Broth	Luria-Bertani Broth
LDH	Lactate dehydrogenase
M	Molar
MFS	Major facilitator superfamily
MHC	Major histocompatibility complex
MS-222	3-Aminobenzoic Acid Ethyl Ester or Tricaine
MSA	Merozoite surface antigen
RESA	Ring-infected erythrocyte surface antigen
N	Asparagine
NBMPR	6-((4-nitrobenzyl)thio)-9- $\beta$ -D-ribofuranosylpurine
NO	Nitric oxide
NPP	New permeation pathways
SDS-PAGE	SDS polyacrylamide gel electrophoresis
O <sub>2</sub>	Oxygen
ORF	Open reading frame
SOD	Superoxide dismutase
<i>P.</i>	<i>Plasmodium</i>
Pb(ec)	<i>P. berghei</i> erythrocyte cytoplasm located protein
Pb(em)	<i>P. berghei</i> erythrocyte membrane located protein
pCMBS	<i>p</i> -chloromercuribenzenesulfonate
PCR	Polymerase Chain Reaction
PfEMP1	<i>P. falciparum</i> erythrocyte membrane protein 1
PfENT1	<i>P. falciparum</i> encoded nucleoside transporter 1
PfERC	<i>P. falciparum</i> ER-located calcium binding protein
PfERD2	<i>P. falciparum</i> ERD2
PfHRP	<i>P. falciparum</i> histidine rich protein
PfHT1	<i>P. falciparum</i> hexose transporter 1
PfNT1	<i>P. falciparum</i> encoded nucleoside transporter 1
PM	Plasma membrane
PNK	T4 Polynucleotide Kinase
PPM	Parasite plasma membrane
PPPK-DHPS	Dihydropteroate synthase-2-amino-4-hydroxy-6-hydroxymethyl-dihydropteridine pyrophosphokinase





PV	Parasite vacuole
PVM	Parasite vacuole membrane
Q	Glutamine
WHO	World Health Organisation
RBC	Red blood cell
RBCM	Red blood cell membrane
RESA	Ring-infected erythrocyte surface antigen
RNA	Ribonucleic acid
SDEL	Ser-Asp-Glu-Leu
SDS	Sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
sERA	Secondary ER of Apicomplexa
SERP	Serine rich protein
SOD	Superoxide dismutase
SP	Sulfadoxine-pyrimethamine
T	Thymine
TAE	Tris-acetate-EDTA buffer
TBE	Tris-borate-EDTA buffer
TBS	Tris buffered saline
THT	Trypanosome hexose transporter
T <sub>m</sub>	Melting temperature
TNF	Tumour necrosis factor
Tris	2-amino-2-(hydroxymethyl)-1,3-propanediol
TVM	Tubulovesicular membrane
TVN	Tubulovacuolar network
UTP	Uridine triphosphate
UTR	Untranslated region
UV	Ultraviolet



var	Variant
v/v	Volume/ volume
w/v	Weight/ volume
WHO	World Health Organisation

X-gal 5-bromo-4-chloro-indolyl- $\beta$ -D-galactoside

Malaria is both an acute and chronic disease caused by protozoa of the genus *Plasmodium*. Four species cause human malaria namely *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. The protozoa are transmitted to humans by a male mosquito of the *Anopheles* genus. The parasite's sexual stage cycle is completed in the mosquito on the cycle of development. The mosquito then infects humans and the cycle repeats. (Malaria, Union Naval Hospital, 2014)

## 1.2 Historical review

Malaria is a very old disease and historians claim it is thought to have existed since Malaria probably appeared in Africa 250,000 years ago, through the migration of the Mediterranean storm birds and south East Asia. In the past, it was common in the Nile valley, the Amazon basin and the West Indies. It is derived from the Latin word "malum" and "aria" which means "bad air". It was also known as "Roman fever". The unusual, periodic stages of waters led the Romans to begin drainage programs, the first when Augustus (Malaria, an United Resource, 2001; Bradley, 1946)

The malarial parasite was first detected in fresh blood smears by the physician in the 1330s and in 1789 Laveran, working in Algeria, defined the protozoan cause of malaria. It wasn't until 1897 that the Dutchman discovered the mosquito as the vector for the disease. The significance of the parasite was recognized by the malaria field until in 1892 when an improved method of staining blood smears was developed so the morphology of the parasite could be studied. This led to the identification of different stages such as the ring forms, trophozoites, schizonts and merozoites. This was however unobtainable through the use of light microscopy, and only until the

## SUMMARY

Malaria kills up to 3 million people and affects a further 500 million people annually. Parasite multidrug resistance to most antimalarial drugs is a growing concern. There is therefore a need to develop new and effective drugs against existing validated targets, as well as a need to identify new targets. Recently the *P. falciparum* hexose transporter (PfHT1) was isolated and cloned. This protein has potential as a drug target since the malaria parasite depends solely on glucose provided by the host for its energy.

Studies on the transporter have revealed several differences between it and the human glucose transporter, GLUT1, which is significant towards identifying species-specific drug targets. These include PfHT1's ability to transport glucose at a higher affinity ( $K_m = 1.0 \pm 0.2$  mM) than GLUT1 ( $K_m = 2.3$  mM, 2.6 mM). Also, PfHT1 can transport fructose at a relatively high affinity ( $K_m = 11.5 \pm 1.6$  mM), whereas GLUT1 cannot ( $K_m > 50$  mM). PfHT1 forms interactions with glucose at positions C-3 and C-4, whereas GLUT1 forms interactions with glucose at positions C-1 and C-3 with a lesser contribution from positions C-4 and C-6. It is also known from the literature that PfHT1 helix 5 Q169 is important for fructose transport.

In this study interactions formed between glucose and PfHT1 helices 5 and 7 were investigated using point mutagenesis. Also the importance of PfHT1 helices 7-12 and the C-terminal tail were investigated using PfHT1/ GLUT1 chimaeric proteins. From the results of this study the helix 7 302SGL motif may be important for interactions with glucose at C-1 and C-5, with a lesser contribution from C-3 and C-6. PfHT1 helix 5 may form interactions with glucose at C-2, C-3, C-5 and C-6. Chimaeras investigated in this study were not functional. However, the importance of the presence of at least 7 amino acids directly after and belonging to transmembrane helix 12 could be investigated as a result of these studies.

Further information into important amino acids for PfHT1-substrate interactions is still required. These studies are pioneering towards understanding the differences that exists between the glucose transporters of the malaria parasite and its human host. The information obtained point the way to further studies on the potentially crucial helices 5 and 7 of PfHT1 that could be targeted by future antimalarial drugs.

## OPSOMMING

Tot soveel as 3 miljoen mense sterf jaarliks aan malaria en 'n verdere 500 miljoen word geaffekteer. Die weerstand wat die malaria parasiet teen beskikbare medisyne toon is kommerwekkend. Daar bestaan dus 'n behoefte vir die ontwikkeling van nuwe en effektiewe middels teen beproefde teikens vir die vernietiging van die parasiet asook die karakterisering van nuwe teikens. Die *P. falciparum* heksose transporteerder is onlangs geïsoleer en gekloneer. Hierdie proteïen het die potensiaal om gebruik te kan word as medisinale teiken aangesien die malaria parasiet afhanklik is van die gasheer as bron van glukose vir energie.

Vorige studies het aangetoon dat daar heelwat verskille is tussen die parasiet en die menslike glukose transporteerder GLUT1 wat 'n bydrae kan lewer tot die identifisering van moontlike spesies-spesifieke medisinale teikens. PfHT1 het die vermoë om glukose teen 'n hoër affiniteit te transporteer ( $K_m = 1.0 \pm 0.2$  mM) as GLUT1 ( $K_m = 2.3$  mM,  $2.6$  mM). Verder kan PfHT1 fruktose teen 'n relatief hoë affiniteit transporteer ( $K_m = 11.5 \pm 1.6$  mM) terwyl GLUT1 dit nie kan doen nie ( $K_m > 50$  mM). PfHT1 reageer met glukose by C-3 en C-4 terwyl GLUT1 reageer met C-1 en C-3, met kleiner bydraes deur C-4 en C-6. Uit die literatuur is dit bekend dat PfHT1 heliks 5 Q169 belangrik is vir fruktose transport.

Die interaksies tussen glukose en PfHT1 heliks 5 en 7 is in hierdie studie ondersoek deur gebruik te maak van puntmutasies. Die noodsaaklikheid van PfHT1 heliks 7-12 en die C-terminaal stert vir heksose transport was ondersoek deur gebruik te maak van PfHT1/ GLUT1 "chimaera" proteïene. Uit die resultate van die studie blyk dit dat die 302SGL motief in heliks 7 belangrik kan wees vir interaksies met glukose by posisies C-1 en C-5 en tot 'n mindere mate met posisies C-3 en C-6. PfHT1 heliks 5 mag moontlik interreageer met glukose by posisies C-2, C-3, C-5 en C-6. Die "chimaeras" wat ondersoek was is nie funksioneel nie. Die moontlike belangrikheid van die teenwoordigheid van ten minste 7 aminosure onmiddellik na en as deel van die transmembraan heliks 12 kon aangetoon word as 'n gevolg van hierdie studie.





Verdere inligting oor die essensiële aminosure vir PfHT1-substraat interaksies word steeds benodig om die verskille wat bestaan tussen die glukose transporteerder van die malaria parasiet en die menslike gasheer uit te wys. Die inligting wat uit hierdie studie verkry is baan die weg vir verdere studies op helikse 5 en 7 van PfHT1 wat potensieël baie belangrik kan wees as teikens vir toekomstige malaria teenmiddels.