

**RNA sequencing analysis of *Plasmodium falciparum*  
extracellular vesicles**

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**Declaration**

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

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**Abstract**

*Plasmodium falciparum* is the most lethal malaria parasite. Blood stage *P. falciparum* parasites secrete extracellular vesicles (EVs), which contain a variety of bioactive molecules and can transfer them to host and/or parasite recipient cells. The RNA content of *P. falciparum* EVs, which could potentially affect biological processes within the host or mediate communication within the parasite population, has not been previously characterised. In my MPhil project, I described transcripts that are preferentially enriched in PfEVs relative to the whole parasite transcriptome.

*P. falciparum* culture supernatants were collected at multiple developmental time windows from both a long-term laboratory isolate and two short-term lab-adapted clinical isolates. EVs were purified using preparative ultracentrifugation and imaged using sectioned transmission electron microscopy. PfEV-cDNA libraries were generated using the dUTP method and sequenced. Read mapping was done using HISAT2, while differential expression analysis and gene ontology were performed using the R/Bioconductor packages ‘*edgeR*’ and ‘*ClusterProfiler*’ respectively.

Sectioned transmission electron microscopy imaging showed PfEVs to have a dense lumen surrounded by a lipid bilayer. The size range of the PfEVs was 40 - 200 nm with median size of 100 nm. Analysis of the EV-RNA using an Agilent Bioanalyzer pico RNA chip showed that EV-RNA was in the size range of 25 - 4000bp, peaking at 500bp with little or no traces of ribosomal RNA. I identified 958 transcripts enriched in PfEVs secreted by late stage parasites relative to the total parasite RNA profile as a whole, and fewer than 300 for the other parasite time windows. Gene ontology showed that the transcripts enriched in PfEVs released by mature parasites expressed proteins involved in regulation of cellular processes. There were no overrepresented gene ontology terms for the early and mid-parasite time windows.

Conclusively, PfEVs are enriched in transcripts that express regulatory proteins such as RNA binding proteins and transcription factors, as well as the exported family of *P. falciparum* proteins. In other systems, EV-RNA is translated in recipient cells and can activate cytosolic immune sensors. Therefore, further studies are required to shed light on the role of PfEV-RNA in host-parasite interaction.

## Acronyms and Abbreviations

ABCA	ATP-binding cassette transporter 1
AMA1	Apical Membrane Antigen 1
AP2	Apetala 2 domain
APiAP2	Apicomplexa Apetala 2 domain
BAM	Binary alignment/Map
BED	Browser Extensible Data
Cav-1	Caveolin-1
CCM	Culture conditioned medium
cDNA	complementary deoxyribonucleic acid
CGNP	Category gene network plot
CSP	Circumsporozoite protein
dUTP	deoxy-uridine triphosphate
<i>edgeR</i>	Empirical Analysis of Digital Gene Expression Data in R
ELV	exosome like vesicles
ENA	European Nucleotide Archive
ESCRT	Endosomal sorting complex required for transport
ETRAMP	Early transcribed membrane protein
evDEGs	extracellular vesicle differentially enriched genes
EVs	extracellular vesicles
EXP	exported proten

FPKM	fragments per kilobase of exons per million reads
gDNA	genomic deoxyribonucleic acid
GECO	gametocyte erythrocyte cytosolic protein
GEST	gamete egress and traversal protein
GEXP	gametocyte exported protein
GFF3	General Feature Format 3
GIG	gametocytogenesis implicated protein
GM3	monosialodihexosylganglioside
GO	Gene Ontology
GRA	dense granule
GTF	Gene transfer format
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HISAT	Hierarchical Indexing for Spliced alignment of Transcripts
HSP	heat shock protein
IFN	interferon
IL	interleukin
IMC	inner membrane complex
iRBC	infected-red blood cell
ISEV	International Society of Extracellular Vesicles
KAHRP	knob associated histidine rich protein
KEMRI	Kenya Medical Research Institute

LacCer	lactosylceramide
MAF1	repressor of RNA polymerase III transcription
MAHRP1	membrane associated histidine rich protein 1
MC-2TM	Maurer's Cleft two transmembrane proteins
MCs	Maurer's Cleft
mRNA	messenger ribonucleic acid
MSP1	Merozoite Surface protein1
MVs	Microvesicles
MVBs	Multi-vesicular bodies
ncRNA	non-coding RNA
NHSBT	National Health Insurance Blood and Transplant
PBS	Phosphate buffered saline
PC	Phosphatidylserine
PEG	Polyethylene glycol
PfEVs	<i>Plasmodium falciparum</i> extracellular vesicles
PfPTP1	PfEMP1 trafficking protein1
PHIST	<i>Plasmodium</i> helical interspersed subtelomeric protein
PI	Phosphatidylinositol
PIC	Pre-initiation complex
PLGA	poly-lactic-co-glycolic acid
PS	Phosphatidylserine



PTEX	<i>Plasmodium</i> translocon of exported proteins
PUF1	gene encoding a mRNA binding protein
PV	Parasitophorous vacuole
PVM	Parasitophorous Vacuole Membrane
Rap2	Rhoptry associated protein 2
RBCs	red blood cells
REXP1	ring exported protein 1
RhopH2	Rhoptry high molecular weight protein 2
RIFIN	repetitive interspersed family of proteins
RIN	RNA integrity number
ROS	reactive oxygen species
RPMI 1640	Roswell Park Memorial Institute 1640 culture medium
rRNA	ribosomal ribonucleic acid
RSEM	RNA-Seq by Expectation Maximization
RTS,S,	a malaria vaccine
SAM	Sequence Alignment/Map
SBP1	Spectrin binding protein 1
SMN	Survival motor neuron like protein
SNARE	Soluble NSF Attachment Protein REceptor"
STEVAR	Subtelomeric variable open reading frame protein family
STING	Stimulator of interferon genes

**x**

TEM	Transmission electron microscopy
TNF $\alpha$	Tumor necrosis factor alpha
TSG101	Tumor susceptibility gene 101
TVN	Tubulovesicular network
VCAM-1	Vascular cell adhesion protein 1
VPS	Vacuolar protein sorting complex
WSI	Wellcome Sanger Institute

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