## Contents

### Chapter 1

1. **An overview of malaria parasite biology**  
   1.1 Introduction  
   1.2 A brief history of malaria  
   1.3 Classification  
   1.4 Life cycle of *Plasmodium*  
   1.5 Developmental stages of *Plasmodium*  
   1.5.1 The Pre-erythrocytic Stage of *Plasmodium*  
   1.5.2 The erythrocytic stage of *Plasmodium*  
   1.5.2.1 Invasion of merozoites into erythrocytes  
   1.5.2.2 Food vacuole formation and haemoglobin degradation.  
   1.5.2.3 Schizont maturation and egress  
   1.5.3 Gametocytogenesis and sexual stages development in *Plasmodium*  
   1.5.3.1 Gamete to ookinete transition  
   1.5.3.2 Ookinete to oocyst transition  
   1.5.3.3 Oocyst Differentiation and sporozoite formation  
   1.5.3.4 Sporozoite motility and invasion of Salivary gland  
   1.6 Antimalarial drugs  
   1.6.1 Artemisinin derivatives  
   1.6.2 Aminoquinolines  
   1.6.2.1 Chloroquine  
   1.6.2.2 Amodiaquine  
   1.6.2.3 Primaquine  
   1.6.3 Aryl amino alcohol  
   1.6.3.1 Quinine  
   1.6.3.2 Mefloquine  
   1.6.3.3 Lumefantrine  
   1.6.4 Antifolate drugs
1.6.4.1 Sulfadoxine/pyrimethamine
1.6.4.2 Proguanil and Chlorproguanil
1.6.5 Atovaquone
1.6.6 Antibiotics used for treating malaria
1.7 Malaria Vaccine
1.7.1 Pre-erythrocytic vaccines (Liver-stage Vaccine)
1.7.2 Attenuated sporozoites: a promising whole organisms approach
1.7.3 Genetically attenuated sporozoites
1.7.4 Erythrocytic stage vaccine (Blood stage vaccine)
1.7.5 Sexual stage vaccine (Transmission-blocking vaccine)
1.8 Metabolic pathways as drug target in \textit{Plasmodium}
1.8.1 Hemoglobin Metabolisms in \textit{Plasmodium} as drug target
1.8.2 Apicoplast as drug target in \textit{Plasmodium}
1.8.3 Mitochondria as drug target in \textit{Plasmodium}
1.8.4 Kinases as drug target in \textit{Plasmodium}
1.8.5 Transporters as drug targets in \textit{Plasmodium}

\textbf{Chapter 2} \hspace{1cm} 48–56

2 \textit{De novo} heme biosynthetic pathway in malaria parasite
2.1 Heme
2.2 Heme Biosynthesis: Shemin and C$_5$-Pathway
2.2.1 Synthesis of $\delta$-aminolevulinate ‘the committed precursor’
2.2.2 Conversion of ALA into Heme
2.3 Compartmentalization of Heme-Biosynthetic Pathway
2.4 Heme biosynthetic pathway in malaria parasite
2.4.1 Features of Heme Biosynthetic Enzymes in \textit{Plasmodium}

\textbf{Present study} \hspace{1cm} 57

\textbf{Specific objectives of the study} \hspace{1cm} 57

\textbf{Chapter 3} \hspace{1cm} 59–81

3 Contribution of host heme versus \textit{de novo} biosynthetic heme in asexual stage development of malaria parasite
3.1 Introduction
3.2 Material and Methods 61
3.2.1 *In vitro* culture of *Plasmodium falciparum* 61
3.2.2 Cryopreservation and revival of asexual stage *Plasmodium falciparum* 62
3.2.3 Synchronization of *Plasmodium falciparum* 62
3.2.4 Preparation of free parasites 62
3.2.5 Maintenance and propagation of *Plasmodium berghei* 63
3.2.6 Cryopreservation and revival of *Plasmodium berghei* 63
3.2.7 Drugs used during *P. berghei* growth and maintenance 63
3.2.8 Isolation of parasite genomic DNA 64
3.2.9 Generation of *P. berghei* ALAS and FC knockouts 64
3.2.10 *In vitro* radiolabeling experiments 66
3.2.11 Preparation of parasite mitochondria and food vacuole 67
3.2.12 Extraction of total heme and hemozoin-heme 67
3.2.13 Other procedures 68
3.2.14 Statistical analysis 68
3.3 Results 69
3.3.1 The role of parasite-synthesized heme during the intraerythrocytic stages of *P. berghei* 69
3.3.2 Radiolabeling of hemoglobin-heme and tracing its path in the parasite 72
3.3.3 The role of parasite biosynthetic heme in *P. falciparum* cultures 77
3.4 Discussion 79

**Chapter 4** 82–92

4 Essentiality of *de novo* heme biosynthesis in the sexual and liver stages of malaria parasite 82
4.1 Introduction 83
4.2 Materials and Methods 84
4.2.1 Maintenance and propagation of *Plasmodium berghei* 84
4.2.2 Drugs used during *P. berghei* growth and maintenance 84
4.2.3 *In vitro* ookinete formation in *P. berghei* Wild type and Knockout parasites 84
4.2.4 Exflagellation assay 85
4.2.5 Rearing of *Anopheles stephensi* mosquitoes 85
4.2.6 Observation of *P. berghei* infection in *An. Stephensi* 85
4.2.6.1 Determination of ookinete 85
4.2.6.2 Determination of Oocyst 86
4.2.6.3 Determination of Sporozoite 86
4.2.7 Isolation of sporozoite and counting 86
4.2.8 *P. berghei* infection studies in *Anopheles stephensi* 87
4.2.9 Sporozoite infections in mice 87
4.2.10 Statistical analysis 88
4.3 Results 88
4.3.1 The role of parasite biosynthetic heme in the mosquito stages 88
4.3.2 The role of parasite biosynthetic heme in liver stage development 90
4.4 Discussion 91

**Chapter 5** 93–102

5 *De novo* heme biosynthetic pathway gene knockout parasite as genetically attenuated sporozoite vaccine 93

5.1 Introduction 94
5.2 Materials and Methods 95
5.2.1 Maintenance and propagation of *Plasmodium berghei* 95
5.2.2 Rearing of *Anopheles stephensi* mosquitoes 95
5.2.3 Sporozoite isolation and counting 95
5.2.4 *P. berghei* ALAS knockout and *PbWT* infection studies in *Anopheles stephensi* 96
5.2.5 Immunizing mice with *PbALASKO* sporozoites and examining liver stage protection 96
5.2.6 Quantification of CD8\(^+\) memory cell response 96
5.3 Results 97
5.3.1 *PbALASKO* sporozoites as genetically attenuated sporozoite vaccine 97
5.4 Discussion 100

6 References 103–154

7 List of Publications 155