Chapter 1

General introduction

1.1 An introduction to malaria

1.1.1 Malaria as a major global health problem

Caused by protozoa of the *Plasmodium* genus, malaria is a disease that has plagued humanity since antiquity. Challenges in surveillance make it difficult to quantify the current global burden of malaria; the World Health Organisation (WHO) estimated the number of clinical cases in 2010 to be over 200 million, with over one million being fatal, but it is possible that this is a significant underestimate[136, 359]. Approximately half of the world's population live in areas where malaria is endemic and the disease persists particularly in some of the world's poorest countries. Sub-Saharan Africa bears the largest burden of this severe disease, which has its most devastating effects in young children and represents a major barrier to development[360].

One of the United Nations' Millennium Development Goals was to bring about a decrease in the global incidence of malaria by 2015. This has catalysed truly international research efforts, with an annual investment in excess of $2billion towards combating what was previously a neglected, understudied disease[266]. Progress has been abundant: half of all sub-Saharan African households are now thought to own a protective insecticide treated bednet[360]; annotated genome sequences are available for a growing number of *Plasmodium* isolates[48, 49, 107, 255, 260]; and the world's first malaria vaccine (albeit only partially effective) has been developed[244]. Such efforts are estimated to have prevented a quarter of a billion cases and saved over one million lives[360].

Despite ongoing successes in disease prevention and treatment, there is still a great need to better understand the pathology of malaria, the biology of *Plasmodium* parasites and the dy-

namics of transmission to confront challenges in the future. We still lack an effective vaccine and are encountering a growing number of drug-resistant parasites. Furthermore, it is possible that changes in global climate and human behaviours will bring the threat of malaria to immunologically naïve populations[88].

1.1.2 *Plasmodium* parasites cause malaria

Apicomplexan *Plasmodium* parasites are single-celled eukaryotes, over 200 species of which have been identified. *Plasmodium* species have parasitised birds, reptiles and mammals long before the evolution of humans, with the earliest evidence for their existence dating as far back as 30 million years[267]. Human infection is thought to have originated on multiple occasions from ape-infecting parasites and is thought to have become prevalent around the time of the Agricultural Revolution over 10,000 years ago[191, 194, 195], with *Plasmodium* DNA still detectable in human remains from Ancient Egypt[182]. These protozoan parasites were identified by microscopy as the causative agent of malaria in the late 19th century, and mosquitoes were soon after identified as being responsible for transmitting these parasites to humans[283].

All *Plasmodium* species complete a complex life cycle between a vertebrate and mosquito host, progressing through a series of morphologically-distinct forms, first described over 200 years ago[121](Figure 1.1). Human infection begins when a host is bitten by an infected *Anopheles* mosquito. When the mosquito takes a blood meal, *Plasmodium* sporozoites enter the human host via the skin. These sporozoites migrate to the liver where they replicate asymptomatically to create thousands of merozoites, which are released into the bloodstream. Here the parasites undergo further cycles of asexual reproduction. These cycles begin with the merozoites' invasion of a red blood cell (RBC), within which they replicate. These new merozoites cause lysis of the RBC, such that they are released back into the blood where they rapidly invade a new RBC. A subset of merozoites will develop into gametocytes, which go on to produce micro- and macro-gametes that fuse as part of sexual reproduction in the mosquito host. Fused gametes develop into diploid ookinetes, which are able to cross the mosquito midgut wall and form an oocyst, where sporozoites develop. Once these sporozoites are released they invade the mosquito salivary gland, allowing them to be transmitted to a new human host when the mosquito feeds.

At least five *Plasmodium* species naturally infect humans. *P. falciparum* is responsible for the most severe cases of malaria, and for the majority of fatalities. It is found throughout South East Asia and in parts of South America but is hyperendemic in most of Sub-Saharan Africa,

with as many as 50% of people infected[310]. *P. vivax* is thought to cause the largest number of infections globally, but has been less widely studied, perhaps owing to its lower mortality rate[219]. Whilst *P. vivax* infections do occur in Africa, the majority of clinical cases occur in Central Asia[114]. Infections with *P. malariae,* or one of the two sub-species of *P. ovale* are not known to be as prevalent, but are likely under-reported[44, 322]. These particular parasites have thus received comparatively little attention in the form of research effort. *P. knowlesi* was long-considered a macaque pathogen, but is now recognised to cause disease in people living in the regions of South East Asia where humans and macaques interact[162]. Mixed infections, whereby hosts are simultaneously infected with more than one *Plasmodium* species are also thought to be common[208].

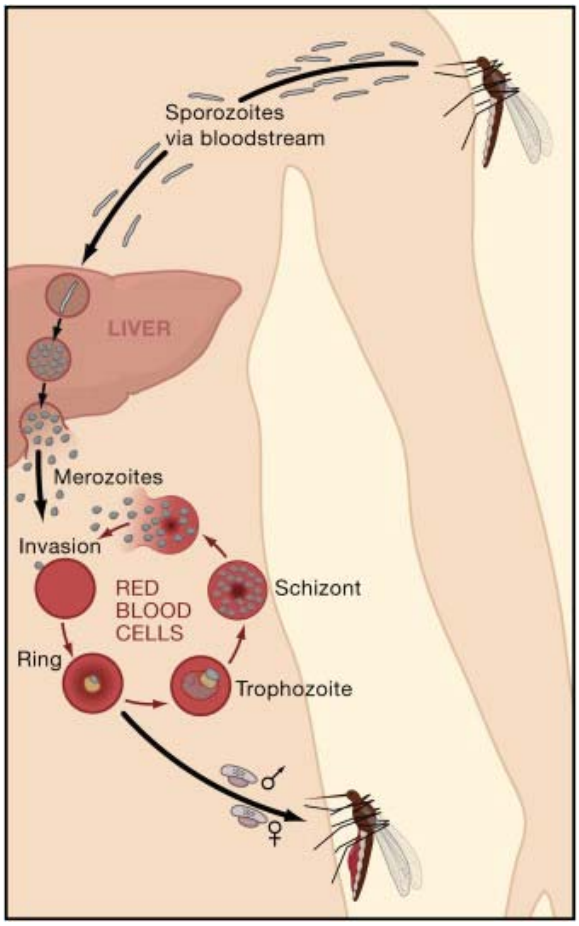

Figure 1.1: **Parasite life cycle in the human host**
Figure from *Invasion of red blood cells by malaria parasites* by Cowman and Crabb (2006)[61]. Reproduced with permission.

1.1.3 *Plasmodium* merozoites invade red blood cells

1.1.3.1 Merozoite structure

The blood-stage merozoite form of the parasite has been the most intensively studied, and is the primary focus of the work described in this thesis. At just over 1µm in length *P. falciparum* merozoites are amongst the smallest known eukaryotic cells. Free merozoites have an elliptical cross section with a projected apical end where secretory organelles - rhoptries, micronemes and dense granules - are located (Figure 1.2). Parasites also possess a highly specialised endomembrane system known as the inner membrane complex (IMC) located just below the plasma membrane. This structure and its connections to the cytoskeleton are thought to play critical roles in determining and maintaining the shape of the merozoite, in cell division, and in mediating its RBC-invasion mechanism[3, 22, 39]. The merozoite surface is covered in a 15-20nm thick filamentous coat[14] comprised primarily of a number of glycosylphosphatidylinositol (GPI)-anchored membrane proteins and peripherally associated proteins[115, 293]. Amongst these integral membrane proteins are those described as MSPs (merozoite surface proteins), including PfMSP1, 2, 4, 5 and 10 and a family of cysteine-rich proteins which are-associated with adhesive properties in *P. falciparum*[221]. These proteins interact with other

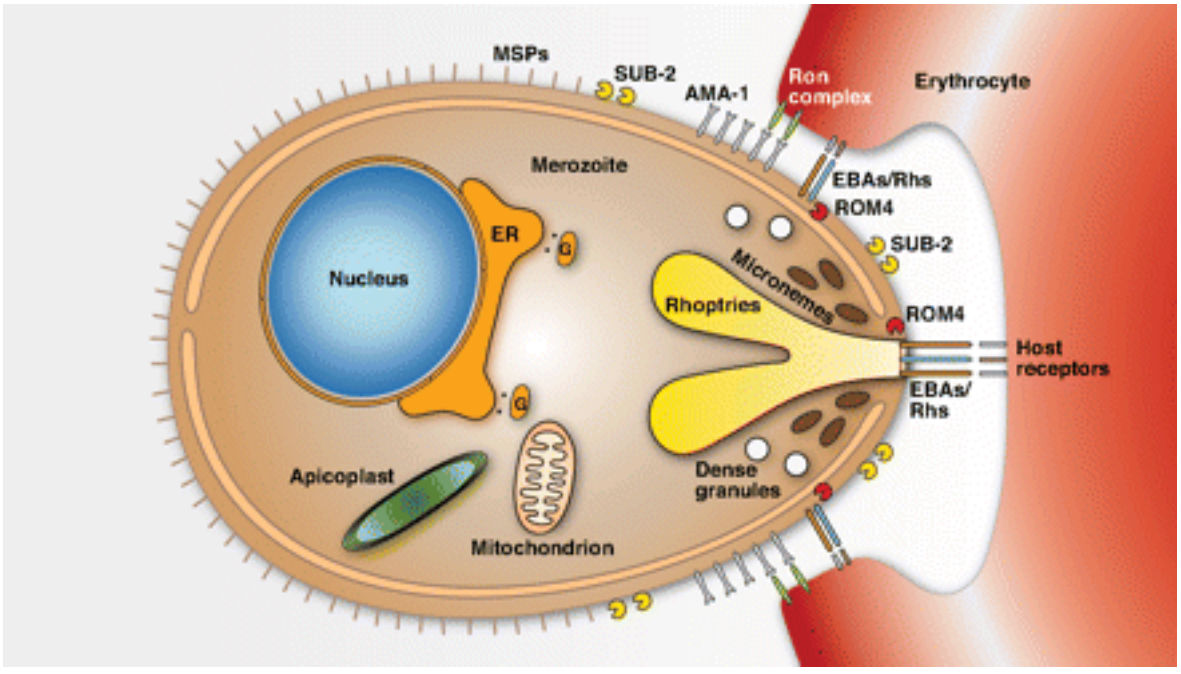

Figure 1.2: **Merozoite structure and RBC invasion ligands**
Figure from *That was then but this is now: malaria research in the time of an eradication agenda* by Kappe *et al.*, (2010)[163]. Reproduced with permission from AAAS.

parasite proteins, including those from the PfMSP3- and PfMSP7-families, which themselves do not possess a GPI anchor or transmembrane domain, to tether them to the merozoite surface. The PfMSP1 and PfMSP2 proteins are thought to comprise about two-thirds of the total protein content of the merozoite surface[115]. PfMSP1 is proteolytically processed such that four associated PfMSP1 peptides derived from a single precursor are present on the merozoite surface[28]. A fragment of each PfMSP6 and PfMSP7 are also peripherally associated with these PfMSP1 fragments to constitute an assembly known as the MSP1 complex[257, 336]. A number of other membrane proteins and peripherally-associated proteins have been identified as more minor components of the merozoite surface, many of which are delivered from *Plasmodium*'s secretory organelles prior to RBC invasion[61, 293].

1.1.3.2 RBC invasion and parasitism

In order to invade a host RBC, the merozoite carries out a number of steps in a defined sequence, which has been elucidated by microscopy[4, 81]. The invasion process involves molecular recognition events which cause the initial physical association between the two cell types. The merozoite then re-orientates such that its apical pole is in contact with the RBC surface, with which a tighter interaction is then made. Powered by its actin-myosin motor complex, the merozoite actively drives entry into the RBC, such that this tight junction moves from the apical to the posterior end of the merozoite[166]. During this invasion process, merozoite surface proteins and those involved in the tight junction are proteolytically cleaved and released into the bloodstream[135]. The final result of this process is that the merozoite ends up in the RBC cytoplasm surrounded in host-derived membrane in what is known as the parasitophorous vacuole. Whilst inside the RBC, the parasite digests a substantial proportion of the host cell's haemoglobin. Some of this haemoglobin consumption provides nutrition for parasite growth but it is thought that it plays a more pivotal role in regulating the osmotic stability of the parasitised RBC (pRBC), so as to prevent lysis before the parasite has undergone asexual reproduction[192]. After merozoites have divided several times, the pRBC, at this point termed a schizont, ruptures, releasing as many as 32 merozoites into the bloodstream. Within a matter of seconds, these merozoites invade new RBCs and the cycle repeats. Merozoites are known to progress through their erythrocytic cycle in synchrony, resulting in waves of RBC rupture, occurring approximately every 48 hours in *P. falciparum* malaria[1]. The mechanism and function of this synchrony is not fully understood; it could potentially be driven by the host responses to infection, or conversely be an adaptation of the parasite to overwhelm

[1] Synchronicity in merozoite invasion/lysis cycles is not observed in all infections, and is less common when the host is simultaneously infected by *Plasmodium* parasites with a diverse range of genotypes[334]

the immune system when merozoites are exposed[127, 176].

Whilst intracellular, *P. falciparum* is known to modify the surface of the pRBC by trafficking a subset of its own proteins to the membrane. Amongst these is PfEMP1[214], deriving from a family of around 60 highly variable proteins, encoded by *var* genes. PfEMP1 is a critical component of 'knob' structures on pRBCs which enable them to adhere to vascular endothelia[62], thus avoiding passage to, and destruction by, the spleen. This process is referred to as cytoadherence or sequestration and is thought to account for the much of increased morbidity associated with *P. falciparum* infections compared with other *Plasmodium* species. PfEMP1 is also thought to mediate platelet-mediated clumping of pRBCs[259] and 'rosetting' whereby pRBCs aggregate with uninfected RBCs[54].

1.1.4 Malaria can have life-threatening sequelae

The first symptoms of infection with *Plasmodium* parasites occur after an incubation period of at least one week. Although malaria is often described as being characterised by a periodic fever in the human host, it usually presents as a combination of symptoms including, but not limited to, fever, chills, headaches, nausea and malaise. In uncomplicated infections these symptoms can be temporarily debilitating but usually resolve within a few weeks. However, a proportion of infections do progress to severe, life-threatening syndromes. Severe Malarial Anaemia (SMA) is defined by the WHO as having detectable parasitaemia and a hematocrit lower than 15%, indicating that RBC counts are at least half healthy levels[248]. SMA is responsible for a large proportion of childhood deaths in malaria-endemic regions[84]. Anaemia results not only from the direct parasite-driven lysis of pRBCs but primarily from the destruction of uninfected RBCs[150], by macrophage-mediated phagocytosis or clearance by the spleen[179]. Respiratory distress, renal failure and septic shock-like symptoms can develop in a subset of cases and are responsible for a small proportion of fatalities.

Certain sequelae are particular to *P. falciparum* malaria. Cerebral malaria, thought to occur in about 1% of clinical *P. falciparum* cases[360], is characterised by neurological impairment and can lead to coma and death. Adherence of pRBCs in the brain is thought obstruct blood flow through cerebral microvasculature, which might deprive the brain of oxygen. At the same time, the pro-inflammatory cytokines produced as part of the anti-parasite immune response are thought to destabilise the blood-brain barrier, exacerbating the neurological impairment[142]. Pregnant women are at particular risk from *P. falciparum* malaria. Parasites express a specific PfEMP1 variant that is able to bind to placental CSA, which causes the adherence of pRBCs to blood vessels in the placenta[344]. This blocks the flow of nutrients

to the developing foetus and as such can negatively affect the outcome of the pregnancy.
Although re-infection is common in malaria-endemic regions, those who recover from *P. falciparum* malaria and remain unexposed do not develop any further symptoms. However, recurrence of disease can be observed many years after exposure to *P. vivax* or *P. ovale,* the parasite remaining dormant in the liver in a form known as the hypnozoite[57].

1.1.5 Natural immunity to malaria provides incomplete protection from *Plasmodium* infections

Immunity to malaria is enigmatic. Even people living in endemic regions are not thought to develop true sterile immunity to malaria. However, provided they are continually exposed, people gradually develop a level of protection against life-threatening disease, even when parasites are detectable in their blood (Figure 1.3). This slow development of immunity is widely considered to be the result of strain-specificity in immune responses[153], such that apparent immunity results from the generation of a repertoire of strain-specific responses to a diverse range of *Plasmodium* parasite infections[70]. This would explain why the majority of deaths occur in infants and why protection from severe disease appears to wane in the absence of continual exposure.

1.1.5.1 Innate and adaptive immune responses

The immunological basis of protection against malaria is still unclear and has been investigated at multiple stages of the parasite lifecycle. Whilst the induction of anti-sporozoite antibodies has been shown to provide protection against disease[165, 269, 301], the sporozoite stage has not been clearly demonstrated to be a target of protective antibody responses in natural infections. Since blood-stage infection is so commonly observed it is often assumed that naturally-induced immune responses against sporozoites are largely ineffective at preventing disease. This might result from the low number of sporozoites transmitted by the mosquito[2], which provides a scarcity of antigen against which the immune system can respond. There is also a body of evidence suggesting that sporozoites drive the suppression of anti-parasite immune response from the moment they are injected into the skin[130].
The mechanisms by which the host controls blood-stage *Plasmodium* parasites have been investigated extensively, however a unified picture of which responses are protective has not yet emerged. Antibody responses have long been recognised as important in controlling infection, since the observation that immunoglobulins prepared from adults living in endemic areas could

[2]Studies in rodent models suggest a median of about 18 sporozoites are injected per bite[212]

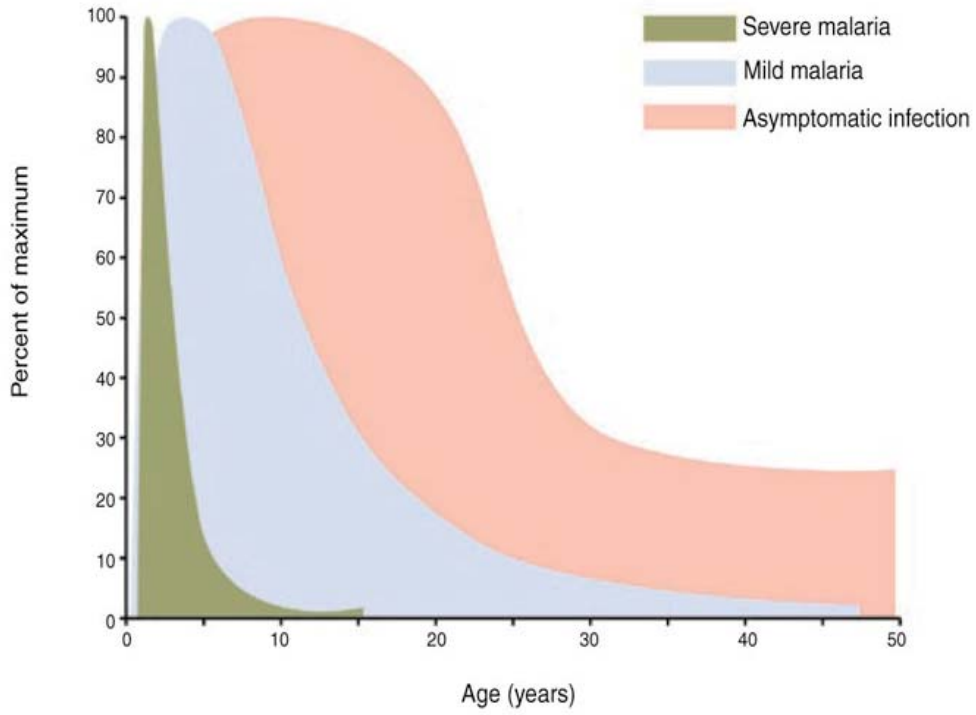

Figure 1.3: **Acquisition of immunity to malaria**
Data collected from a population living in an endemic region of *P. falciparum* transmission show that immunity to severe disease is acquired during infancy, but infections still persists into adulthood.
Figure from *Immunity to malaria: more questions than answers* by Langhorne *et al.*, (2008)[185]. Reproduced with permission.

have a therapeutic effect on children suffering from malaria[58]. A dependence on antibody to limit parasitaemia has been corroborated by studies in B-cell deficient mice, that are unable to clear *P. chabaudi* from the blood[184]. Antibody may function to control infection in several ways; by directly binding to merozoite antigens marking the parasite for antibody-dependent cell mediated killing and phagocytosis[149, 252], by binding to merozoite antigens to prevent them from invading RBCs[29], or by binding to PfEMP1 on pRBCs to induce their clearance from the bloodstream[37]. A broad range of parasite-specific antibodies are detectable in the serum of *P. falciparum*-exposed individuals, but it is still unclear as to which confer protection from severe disease[91]. PfMSP2, PfMSP3, PfMSP7, PfSEA1, PfAMA1, PfGLURP and PfSERA9 are amongst a growing number of candidate proteins against which antibody is thought to protect individuals in specific populations [124, 145, 243, 251, 273, 311]. More thorough analyses of a larger range of candidate antigens will be required to develop a complete picture

of naturally-protective antibody responses.

Antibody-independent mechanisms also play a role in the host immune response to infection by *Plasmodium* parasites. A number of PAMPs (pathogen-associated molecular patterns), including GPI (by which merozoite surface proteins are anchored to the membrane) and haemozoin (produced from the parasites' digestion of RBC haemoglobin), are thought to bind to Toll-like receptors (TLRs), and stimulate both $CD4^+$ and $CD8^+$ T-cells to produce pro-inflammatory cytokines[56, 93, 236]. These cytokines include Interferonγ (IFNγ) and Tumour Necrosis Factor α (TNFα), which are thought to contribute to parasite killing in the early stages of infection[92, 231, 232]. However, the activation of an inflammatory immune response in malaria has more often been associated with a negative outcome for the host, the over-production of cytokines being considered highly immunopathological[199].

1.1.5.2 Genetic resistance to malaria

The human race has evolved alongside *Plasmodium* parasites since antiquity, and its selective pressures have left their mark on the human genome, such that resistance to certain strains of malaria can be afforded by host genetic factors. Notably, a number of blood disorders are commonly associated with protection against malaria. The HbS variant of the HBB gene - which encodes RBC β-globin component of haemoglobin - has arisen on multiple independent occasions and been maintained in malaria-endemic populations, even though homozygotes suffer from life-threatening sickle-cell disease. This risk is balanced against the ten-fold reduced incidence of *P. falciparum* malaria in heterozygotes[1]. A number of other variants in the HBA and HBB globin genes are also found in malaria-endemic regions, again affording a degree of protection from malaria at the expense of sub-optimal haemoglobin functionality[55, 97, 226]. Similarly, a number of RBC enzyme deficiencies are thought to have been maintained in populations as they prevent *Plasmodium* parasites from surviving intracellularly. The most common example is glucose-6-phosphate dehydrogenase (G6PD) deficiency which is observed in African and Mediterranean populations that have evolved with *P. falciparum* infections[198]. Interestingly, it is thought that the parasite has adapted to produce its own G6PD, counteracting the host's once-innate defence[341]. Similarly *P. vivax* transmission in Africa was thought to be restricted by the lack the FY Duffy blood group antigen, which was thought to be required by the parasite for entry into the RBC[222][3]. However it is now recognised that *P. vivax* isolates from Madagascar are able to invade Duffy-negative RBCs indicating that parasites have once again evolved to overcome this restriction[217]. Recent large-scale genome-wide studies linking host genotypes with disease outcomes have helped to clarify the role of these host loci

[3] *P. knowlesi* is also thought to require Duffy antigen to enter RBCs

believed to affect malaria susceptibility and have identified many more host genes that might also contribute[151, 202].

1.1.6 Blood-stage *P. falciparum* parasites are highly adept at immune-evasion

Part of the reason why long-lasting, sterile immunity to malaria is has eluded mankind may lie in the ability of the parasite to overcome the defences of the host immune system. The largely intracellular habitat of the parasite within RBCs shields *Plasmodium* parasites from the onslaught of immune effector cells and molecules present in the blood. RBCs are one of the few cell types that do not present intracellular antigen on their surface via MHC Class I molecules, further hiding parasite-derived antigens from immune surveillance. The spleen mechanically filters damaged RBCs from the blood and thus might be expected to bring about the destruction of pRBCs. However, *P. falciparum*-infected RBCs adhere to endothelia (as described in 1.1.3.2) so as to avoid passage to the spleen. PfEMP1 protein, displayed on the surface of the pRBC, is critical for this adhesion process and is one of the few parasite proteins that is directly exposed to immune effectors in the blood for more than a matter of seconds. *P. falciparum* has evolved a sophisticated mechanism to prevent PfEMP1 from recognition by the immune system. It only expresses one of a diverse range of approximately 60 PfEMP1-encoding *var* genes at any one time, such that antibodies raised against one PfEMP1 variant will be ineffective against parasites that display another[295]. Though their role in disease is less well-characterised, antigenic switching of this kind has also been observed in a number of pRBC surface proteins belonging to other multi-gene families. These include the *rif* family[178] and *stevor* family which has been recently implicated in RBC invasion and rosetting[238].

Plasmodium parasites are also thought to actively subvert the functioning of the immune system; this may be particularly important in protecting free merozoites from destruction when they are exposed between cycles of RBC lysis and invasion. There have been many reports that *Plasmodium* parasites can impair the maturation of antigen-presenting dendritic cells (DCs), possibly preventing them from stimulating parasite-killing activity in T-cells[197, 315, 340]. It has been suggested that the haemozoin, produced by the parasites' digestion of haemoglobin, can mediate the observed suppression of DC function[223]. A parasite-encoded homologue of human macrophage migration inhibitory factor (MIF) might also affect the function of antigen-presenting cells (APCs), stimulating them to produce cytokines that drive the premature maturation of $CD4^+$ T-cells such that their anti-parasite specificity is rapidly lost and does

not contribute to immunological memory[320].

Whilst there is increasing evidence that certain naturally-induced antibodies do protect hosts from infection (Section 1.1.5.1), there are a number of mechanisms by which parasites are thought to suppress the generation of an effective, long-lasting antibody response. Memory B-cell populations studied from people living in areas of high malaria transmission are considered under-responsive and potentially impaired in their ability to combat infection with *P. falciparum*[143]. Many merozoite antigens display a great deal of sequence diversity between isolates[18], indicating that they are under strong balancing selection exerted by the human immune system. Alongside the antigen-switching behaviour exhibited by *P. falciparum* proteins on the pRBC surface, this variability impairs the effectiveness of antibody repertoires generated during previous infections to neutralise new infecting strains of the parasite. The inability of the immune system to respond effectively to a malarial challenge, and the lack of long lasting immunological memory present obstacles to making an effective vaccine.

1.1.7 Current anti-malarial measures

1.1.7.1 Chemotherapeutic agents

A wide range of anti-malarial drugs has been developed and used both as treatments and as prophylaxis in immunologically naïve travellers to malaria zones. 4-Aminooquinolines, such as chloroquine, are safe and affordable drugs that have been effective against all species of *Plasmodium* known to affect humans. They form a complex with a waste product of haemoglobin digestion that is toxic to the parasite, and prevent its degradation to haemozoin[245]. Arylaminoalcohols (structural relatives of aminoquinolones) and artemisinin derivatives have been used for hundreds of years. There has been much speculation over the mechanism of action of these drugs; arylaminoalcohols (such as quinine) have been suggested, like aminoquinolones, to prevent the detoxification of haem degradation products. Artemisinin is thought to cause oxidative damage to the parasite but the precise targets are unclear[220]. More recently, drugs that target the parasite respiratory chain or biosynthetic pathways have been developed[356]. A number of antibiotics also have an antimalarial activity, acting on the prokaryote-derived apicoplast organelle (see Figure 1.2) upon which the parasite relies for fatty acid and isoprenoid biosynthesis[353]. However these antibiotics are not used as standalone treatment as they have a delayed-killing effect, dependent on the parasites' completion of an erythrocytic cycle[66]. In an effort to prevent the spread of drug resistance, many of these classes of drugs are used in combination with one another, and new anti-malaria compounds are being sought actively. Of note, nearly two million chemicals were recently screened for

anti-parasite activity, which has led to the identification of novel drug targets and many chemical starting-points for potential new drugs[104, 129].

1.1.7.2 Environmental management

There is currently no widely-available vaccine, so prevention of malaria in people living in endemic regions has relied solely on environmental management aimed at preventing infectious bites from occurring. Interventions that target mosquito vectors have been a major contributor to the eradication of the disease from many areas of the world, notably the USA where a relatively short campaign saw the eradication of malaria by the 1950s. This elimination process has involved the use of insecticides and the destruction of the stagnant-water habitats where mosquitoes breed. For the areas of the world where infected *Anopheles* vectors still persist, the WHO advocate that at-risk people sleep under an insecticide-treated bed net (ITN) and that indoor surfaces are regularly sprayed with insecticides (indoor residual spraying, IRS). They estimate that almost 60% of sub-Saharan African households have now been supplied with ITNs and that 135 million people (about 4% of the at-risk population) are protected by IRS[360]. In the small number of areas, often urban areas, where mosquito breeding sites are easily-identifiable, accessible and few, biological or chemical agents are sometimes used to eliminate developing larvae[247]. Innovative vector control strategies have been emerging in recent years. These include the generation of genetically-modified mosquitoes that are resistant to infection[204] or that produce predominantly male offspring, so as to drive a population crash[102]. Interventions of this kind rely on the large-scale generation and release of genetically-modified insects, which is currently a logistical challenge.

1.1.8 Threats to the elimination of malaria

1.1.8.1 Antimalarial drug resistance

Whilst prophylactic drug treatment for malaria is effective for travellers who visit malaria zones, the lifelong treatment of the billions at risk from malaria would not be practicable. The spread of drug-resistant parasites threatens the utility of these preventative and curative treatments. Chloroquine, once the first-line treatment for *P. falciparum* malaria, was considered to be a highly effective treatment until the emergence of resistant strains in the 1960s. Resistance is thought to have evolved in at least four independent incidences and has now spread such that chloroquine treatment failure rates are high in almost every country studied

to date[4], approaching 100% in a number of areas[246]. Similar emergences of drug-resistant parasite strains have led to an over-reliance on artemisinin-based therapies, but in recent years resistance to this drug has also been identified[225]. The use of combination therapies has been an important step in preventing the spread of antimalarial resistance, however multi-drug resistant strains are emerging, which is a serious concern. Adaptations in transporter proteins which allow the parasite to expel these drugs from their sites of action include mutations in the *pfcrt* gene and amplification of the *pfmdr1* gene, and have become particularly widespread[170, 246]. This does not necessarily mean that our repertoire of antimalarial drugs is now useless; just ten years after the withdrawal of chloroquine treatment in Malawi, chloroquine-sensitive parasites dramatically re-emerged such that treatment failure rates returned to negligible levels[188]. However the potential for chloroquine-resistant parasites to re-surface under the selective pressure of drug treatment is all too clear.

Many existing treatments, such as quinine and artemisinin, are derived from naturally occurring remedies discovered hundreds of years ago. We can no longer rely on chance discoveries; an in-depth knowledge of the molecular biology of the parasite will better inform the rational design of new drugs and the combinations of treatments that will best guarantee long-term treatment success.

1.1.8.2 Insecticide-resistant mosquitoes

Whilst the use of insecticides has been arguably the biggest contributor to a recent reduction in the global incidence of severe malaria, and is still effective in most areas, it is widely believed that the spread of resistant mosquitoes will threaten the continuation of this decline. Four main classes of insecticides are used against malaria, and mosquitoes resistant to each of them have been identified[11, 271]. Furthermore, almost all ITNs are treated with pyrethroids, against which there are the largest number of reported cases of resistant mosquitoes. It is estimated that if pyrethroids lose their efficacy over half of the benefit of current vector control programmes would be lost, resulting in over 100,000 additional deaths every year[249]. These concerns make the establishment of new preventative measures an urgent priority.

1.1.8.3 Challenges of vaccine development

Although antimalarial drugs and vector control have been highly effective in preventing malaria, the ideal for long-term prevention would be an effective and widely-available vaccine. More than 40 *P. falciparum* malaria vaccine formulations have reached the clinical trial stages of

[4]Chloroquine is still effective in some regions of Central America

development[300]. Two of the most-developed are based on sporozoite-stage antigens; although natural immunity is not thought to rely on targeting sporozoites, the induction of a neutralising anti-sporozoite response could result in sterile immunity, which would be the gold-standard of efficacy for a vaccine. RTS-S/AS01E is a vaccine based on the *P. falciparum* circumsporozoite protein (PfCSP). However, phase III clinical trials on this vaccine indicated a only partial efficacy, which declined to below 20% protection within four years[244]. 'Leaky' vaccines that do not provide sufficient protection have the potential to drive evolution of more virulent vaccine-resistant parasites[15], so an alternative is desperately needed. A whole-sporozoite vaccine, which may evoke a broader, more effective immune response against pre-erythrocytic parasites, is another initiative which has recently been tested. Whilst some early results are promising, there is scepticism about whether the production of such a vaccine (which involves the dissection of individual infected mosquitoes) would be scalable[86].

A growing number of blood-stage *P. falciparum* antigens are being developed as potential vaccines. These include a range of merozoite surface proteins such as PfMSP1, PfMSP3 and PfAMA1, but as yet none have shown high efficacy in field trials[82, 242, 308, 329]. Sequence diversity in blood-stage antigens may represent a major driving force behind low reported rates of protection mediated by vaccines of this kind. Immunisation against one variant of a particular antigen results in allele-specific immunity, such that individuals are only protected against parasites displaying that particular antigen variant. This means that vaccines using polymorphic blood-stage antigens will need to include a diverse range of protein variants, or focus on functionally-important conserved epitopes, to induce cross-strain immunity[80, 256]. Transmission-blocking vaccines that target the sexual stages of the parasite could be a useful tool in the global eradication of malaria. Pfs25, an ookinete surface antigen, is thought to be a suitable vaccine antigen[126]. However vaccines of this type require almost complete coverage to be effective, and voluntary uptake stands to be low since vaccination does not directly protect the recipient. Vaccines based on multiple antigens, perhaps from multiple life-cycle stages, may induce a broader, more effective immune response and help prevent the emergence of vaccine-escaping *Plasmodium* strains[200]. With emerging drug resistance and no certainty of a cost-effective vaccine in the immediate future, it will be important to develop new strategies for controlling the incidence and impact of malaria. The rational design of such interventions will demand a deeper understanding of parasite biology at the molecular level[125].

1.1.9 Research resources

The study of the molecular biology of the malaria parasite has been greatly aided by a growing number of resources and data sets. Perhaps the most influential development in recent decades has been the sequencing of *Plasmodium* genomes. Genomes of *P. falciparum*[107], *P. vivax*[49], *P. knowlesi*[260], the rodent parasite *P. yoelii*[48] and *P. reichenowi*[255]- a chimpanzee parasite very closely related to *P. falciparum* - are published. Draft assemblies have been produced for many isolates, including those from the rodent parasites *P. berghei* and *P. chabaudi*[132], the macaque pathogen *P. cynomolgi*[323] and the avian parasite *P. gallinaceum*. Genome sequencing is becoming more routine, such that thousands of *P. falciparum* genomes[203, 225] and a smaller number of *P. vivax* genomes[52, 140, 218, 235] have now been analysed, allowing deeper insights into the genetic variation that exists in nature. Annotated sequences are freely available and are constantly being improved[254, 343]. Complementing these genomes, transcriptome data has been generated for a number of different species at different stages of their lifecycles[32, 189, 253, 289, 355], and large proteome data sets are also published[98, 187, 270].

We are now able to culture *Plasmodium* parasites in isolated RBCs, which provides an *in vitro* system to study phenotypes of blood stage parasites. The first attempts to grow *P. falciparum*, *P. vivax* and *P. malariae* in human blood were made over a century ago[21], but it was not until the 1970s that the continuous growth of a *P. falciparum* strain could be achieved[335]. Since then *P. knowlesi* and a number of additional strains of *P. falciparum* have been successfully grown in culture[228], though we still lack the capacity to sustain the growth of *P. vivax* along with many *P. falciparum* field isolates outside of a host. *P. falciparum* cultures have been used extensively as a source of parasites for a range of studies into processes such as gene expression, protein localisation and RBC invasion[330]. Genetically manipulating these *Plasmodium* parasites, particularly by knocking-out genes-of interest, allows the functions of candidate genes to be determined. For instance the roles of hypothesised RBC invasion ligands have been investigated using cultured *P. falciparum* parasites that have been genetically altered to be deficient in PfEBA175, PfMSP7 and a range of other proteins[77, 78, 159]. Systematic approaches that can facilitate stable genetic modifications in *P. falciparum*, *P. vivax*, *P. knowlesi, P. berghei* and *P. yoelii* have been developed, making the study of candidate genes possible in *in vitro* and *in vivo* systems [50, 228, 230, 265, 371].

Appropriate animal models of malaria are required to study the course of infections *in vivo* and represent a valuable research resource. *Plasmodium* species that naturally infect African rats have been adapted to grow in laboratory mice and now represent the most common animal models of human malaria. Model systems using *P. chabaudi*, *P. berghei* or *P. yoelii* parasites

have been used with some success to study *P. falciparum* protein orthologues, such as the prospective immune evasion ligand PfPMIF[9] and PfTRSP, a putative hepatocyte invasion ligand[180]. However these systems are somewhat limited in their utility to study proteins that do not have orthologues in rodent malaria parasites, or to examine aspects of human malaria pathology that are not accurately replicated in rodent models. The *P. berghei* mouse model of cerebral malaria has been widely criticised, as the hallmark human symptom of pRBC sequestration does not appear to be replicated in brain microvasculature, though its pathological effects are perhaps substituted by leukocyte sequestration[43]. This makes the model inappropriate for the study of cytoadhesive interactions and anti-cytoadhesion therapies, and it also appears to have had limited power in predicting the efficacy of human vaccines; for instance antibodies against PbCSP protected mice from infection[269], yet immunogenic, human vaccines based on PfCSP have not shown the same success[120, 244]. To overcome some of these issues, adapted model systems are being developed, such as mice infected with *P. berghei* parasites expressing *P. falciparum* proteins[119] and chimeric mouse models that can carry human erythrocytes to support the growth and study of *P. falciparum*[154]. For studies that require the closest possible proxy for human infection, *Aotus* monkeys can be used as a host for *P. falciparum* and *P. vivax*[139]. However, there is widespread ethical objection to the use of non-human primates in research, such that their use is restricted to very specialised facilities and for experiments on only the most promising therapeutic targets.

To complement the data generated about *Plasmodium* parasites, there has also been a focus on the biology of the human host and mosquito vector. Tens of thousands of human genomes have been sequenced to date and these data are continually enhancing our understanding of the host factors underlying susceptibility to malaria (see 1.1.5.2), and provide an insight into how host and pathogen interact to cause disease.

1.2 Protein-protein interactions in malaria

1.2.1 Parasites interact directly with their human host

To establish disease, pathogens interact extensively with their hosts. Systematic screening approaches have identified a myriad of potential interactions between viral proteins and those of their hosts[45, 68]. In fact, one study suggests that all ten major proteins of human influenza viruses make interactions with multiple host factors[303]. With much larger genomes, the host-pathogen interactomes for bacterial and protozoal diseases are not as well characterised, but still over 60 such interactions are known in Salmonellosis alone[297]. *Plasmodium*'s com-

plex life cycle means that it interacts with a broad range of human and vector environments. *Plasmodium* parasites invade a range of different cell types at different points in the life cycle, and these processes are known to involve a series of protein-protein interactions (PPIs).

1.2.1.1 RBC invasion

RBC invasion by the merozoite is the most comprehensively studied process where host and *Plasmodium* cells interact. PPIs occurring at around a 20-30nm membrane-membrane distance, are thought to mediate the initial recognition between the cells. As the primary component of the merozoite surface, PfMSP1 has been implicated in mediating this initial interaction, perhaps with the abundant Band 3 protein on the erythrocyte surface[116]. A number of other inter-cell receptor-ligand pairs have been identified as part of the merozoite-RBC recognition process. Amongst these, *P. falciparum* erythrocyte-binding-like (EBL) family proteins PfEBA175, PfEBL1 and PfEBA140 are thought to bind RBC surface glycophorins A, B and C respectively[201, 207, 306]. A family of reticulocyte binding-like homologue (RH) proteins also have known RBC ligands, with PfRH4 and PfRH5 interacting with CR1 and BSG respectively[64, 328]. There are thought to be multiple pathways by which the the merozoite can enter the RBC, such that there is a substantial degree of redundancy in *P. falciparum*'s invasion ligands. This allows the parasite to infect hosts regardless of polymorphisms in their RBC receptors[78]. The interaction between PfRH5 and BSG appears to be particularly fundamental to the invasion process, as blocking this interaction with antibodies or recombinant proteins completely inhibits invasion in a wide range of *P. falciparum* isolates *in vitro*[64].

1.2.1.2 pRBC/host interactions

PfEMP1 has at least 20 hypothesised ligands which facilitate the sequestration of pRBCs away from innate immune destruction[285]. These include a range of endothelial ligands including CD36, Thrombospondin, ICAM-1, SELP and EPCR, with some variants able to bind placental CSA[17, 23, 24, 279, 302, 338]. Certain PfEMP1 variants are known to mediate rosetting phenotypes via their interactions with CR1[284], AB blood group antigens[47], heparan sulphate-like molecules[54] or possibly CD36[134], which has also been implicated as the host ligand responsible for platelet-mediated pRBC clumping[259]. A range of known PfEMP1 ligands are present on the surface of leukocytes, such that pRBCs may interact with immune effector cells via direct PPIs. pRBC binding to macrophage CD36 has been suggested to lead to phagocytosis and parasite clearance [210], however there is conflicting evidence that this pRBC/CD36 interaction subverts the functioning of DCs and causes the suppression of

immunological memory[340].

1.2.1.3 Sporozoite/host interactions

A number of sporozoite proteins are implicated in the parasites' migration from the skin to the liver, and in the invasion of hepatocytes. The major sporozoite surface protein, PfCSP, as well as PfTRAP are thought to be important hepatocyte invasion ligands, both suggested to bind to highly sulphated heparan sulphate proteoglycans (HSPGs) on the hepatocyte surface[100, 280]. PfTRSP-depleted parasites are unable to invade hepatocytes[180] and antibodies against ligands including PfSPATR, PfSTARP and PfEMP3 inhibit hepatocyte invasion [96, 128, 180]. Despite this range of potential invasion ligands, the identification of hepatocyte receptors has proved difficult. A recent model for cellular invasion by *Toxoplasma gondii*, another intracellular Apicomplexan parasite, hypothesises that the parasites insert their own invasion receptors into the host membrane[25]. This phenomenon is also thought to occur in *P. falciparum*-mediated RBC invasion where the interaction between PfAMA1 and RBC-targeted PfRON2 is thought to be critical[183]. If *Plasmodium* sporozoites also behave in this way, this may account for why host receptors for cell traversal and hepatocyte invasion have not been characterised.

1.2.1.4 Identification of novel interactions can provide important insights into parasite biology

Of over 5,000 potential protein-coding genes identified from the *P. falciparum* genome[107], we understand the functions of surprisingly few. To understand the intricacies of how *Plasmodium* parasites manipulate their hosts and gain access to a range of cell types, it will be invaluable to identify the interacting proteins of pathogen and host. The identification of these interactions will help us to understand the disease process at a molecular level and could help elucidate new targets for therapeutics. The most interesting proteins in these respects are perhaps those at the cell surface of, and secreted from, the parasite as they make direct contact with host cells and are directly exposed to host proteins and immune effectors. For example, by identifying receptor-ligands pairs in cell invasion, we could design safe, specific antibody or small-molecule inhibitors to target these processes and prevent the parasite from accessing the host cell. Blocking PPIs involved in cell invasion has shown promise for the treatment of viral infections[72, 298] and is being investigated as a therapeutic target for malaria[316, 354]. Similarly, specific drugs that could rapidly prevent the adhesive properties of pRBCs could be a valuable clinical tool[348]. Vaccines too could be based on antigens

that participate in pathological host-pathogen PPIs, inducing long-lasting antibody responses that can block these interactions. An understanding of the interactions that allow the parasite to evade the immune system will also contribute to the development of successful vaccines, advising their rational design to elicit the most effective immune response in the host.

1.2.2 Interaction discovery

1.2.2.1 Studies on candidate proteins

The majority of the known interactions involved in RBC invasion have been discovered following the observation that isolated *Plasmodium* ligands bind to the RBC surface. The identity of the receptors for each of these ligands has been inferred or narrowed-down based on the sensitivity of the interactions to enzymatic treatment of the RBC, or by the inability of ligands to bind RBCs naturally lacking specific receptors[5]. For example, the first of these RBC-binding ligands to be discovered, PfEBA175, could not bind to neuraminidase-treated RBCs, which implicated a sialylated protein as the receptor[46]. PfEBA175 was also unable to bind M^kM^k, Tn or En(a-) RBCs which do not express glycophorin A (GYPA) on their surface, thus leading to the identification of GYPA as the receptor[250, 307].
Similarly, the PfEBA175-related proteins PfEBL1 and PfEBA140 were shown to bind specifically to erythrocytes displaying glycophorins B and C respectively[196, 207]. Likewise a recombinant protein corresponding to a conserved sequence block from PfMSP1 bound to the RBC surface, but could not bind to the surface of RBCs from spherocytosis patients[138], which lack one of a number of cytoskeletal proteins including Band 3 and spectrin. A segment of the Band 3 protein, believed to contain the PfMSP1 binding domain was subsequently shown to block the interaction between PfMSP1 and the RBC surface[116]. PfRH4 was shown to interact with the erythroctyte surface in a neuraminidase-resistant but trypsin and chymotrypsin-sensitive manner. This restricted the potential receptor for PfRH4 to only a handful of proteins, including CR1, antibodies against which were shown to block the binding of PfRH4 to the erythrocyte surface[328]. Similarly, some of the known pRBC/endothelial interactions were initially identified following the observation that pRBCs could bind to a number of cell lines displaying human endothelial surface proteins[299, 339]. Anti-CD36 antibodies were able to prevent this adhesion, leading to CD36's eventual identification as a PfEMP1 ligand[17, 20]. Affinity purification methods have also been applied to the discovery of PPIs involved in malaria. This involves creating an isolatable binding reagent based on a protein-of-interest, incubating this reagent with the potential ligand and demonstrating that

[5]Since RBCs are anucleate it has not yet been possible to engineer cells lacking receptors of interest

the ligand co-purifies with the binding reagent. For instance, the earliest evidence that SELP might be an endothelial receptor for PfEMP1 came from an experiment where PfEMP1-coated beads were incubated with recombinant SELP. SELP could be detected on the surface of these beads, but not negative control beads, by Western blotting[302].

1.2.2.2 Screening approaches

The cell-binding approaches described in Section 1.2.2.1 have relied upon prior knowledge and hypothesis-driven rationale on which to select candidate *Plasmodium* or host proteins for study. The identification of the full repertoire of host-pathogen receptor-ligand interactions will require exhaustive, high-throughput, unbiased screening approaches. In recent years computational analyses have been used to infer novel host-pathogen PPIs occurring in malaria. New candidate interactions have been predicted based on both structural similarities with experimentally-determined interactions and correlation of the gene expression data for host and parasite[278, 362]. These approaches often generate a large number of candidate interactions, which require experimental validation, and refinement is still needed to reduce the false positive and false negative discovery rates.

The yeast-two-hybrid (Y2H) system has been systematically applied to discover nearly 3000 candidate *Plasmodium-Plasmodium* PPIs[181], and has also been used to screen for PPIs occurring between *Plasmodium* proteins and those expressed in the human liver and brain[345]. Whilst Y2H screening has provided a wealth of candidate interactions, and has been used in combination with computational approaches to help define the *Plasmodium-Plasmodium* PPI network[275], it is again limited by high rates of false positives and negatives. The cloning steps used to generate bait and prey libraries mean that only fragments of much larger proteins are screened and that a proportion of proteins will not be represented in the screening library. By expressing *Plasmodium* proteins in a heterologous system such as yeast, it is likely that a proportion of the protein fragments will be not be folded in their native conformation. Improvements in the efficiency of recombinant protein production have enabled high-throughput screening between libraries of full-length proteins, which has led to the identification of two more erythrocyte-merozoite PPIs (see 1.2.3).

Another set of approaches has screened *Plasmodium* proteins against cells that have been transfected to display human receptors-of-interest on their surface. An expresssion-cloning screening approach where COS cells were transfected with endothelial ligands was used to discover ICAM1 as another receptor for pRBCs[24]. More recently a much larger scale microarray-based reverse transfection system has been used to screen PfEMP1 against over 2500 potential human cell surface receptors, leading to the identification of EPCR as a pRBC

receptor[338].

1.2.2.3 *In vitro* validation and kinetic analysis of interactions

Many of the methods used to identify interactions (discussed in 1.2.2.1) are equally appropriate for use in generating evidence to validate the occurrence of an interaction predicted by computational or *in vitro* screening approaches. For example, for an interaction involving an isolated or recombinant RBC receptor, it would be useful to demonstrate that its binding partner could interact with the RBC surface in a manner dependent on the presence and availability of the RBC receptor. Conversely, for interactions discovered between a *Plasmodium* ligand and the RBC surface, it would be useful to validate the identity of the expected RBC receptor by demonstrating that the interaction can take place between isolated proteins. In either case, a routine test of interaction specificity is to demonstrate that the interaction can be blocked by an agent known to bind to either of the interacting proteins (for example, a monoclonal antibody or known ligand, as described in many of the above references[20, 64, 116, 302]). Affinity purification studies are another common approach used to validate interactions. Such experiments can be designed to validate interactions between recombinant proteins (for example between PfMSRP proteins and PfMSP1[215]), but it is preferable to design these experiments such that one binding partner can be used to co-purify, or 'pull down', its interacting protein from its native source, such as a parasite lysate or RBC membrane preparation, as has been demonstrated for the interaction between PfMSP1 and PfMSP7[160].

A number of methods that measure biophysical properties of binding proteins can be used to validate interactions. Specific binding interactions usually display saturable binding kinetics and thus these methods can be adapted to estimate interaction affinities. Surface plasmon resonance (SPR) is a widely used biophysical analysis method that can be used to determine kinetic parameters of interactions occurring between isolated proteins. The technology uses light directed at a metal-coated surface, which is reflected back onto a detector. Some of the light causes electrons to resonate at the chip surface (these electrons are surface plasmons), which results in a loss of intensity of the reflected light at a particular angle of detection. The surface plasmons travel parallel to the metal surface so are sensitive to the presence of other molecules along their path. Thus the binding of molecules to the surface of the chip can be quantified by measuring the shift in the angle where the dip in intensity of the reflected light can be detected. PfRH4/CR1 and PfRH5/BSG binding have been studied in this way, and as such have provided useful, quantitative information about the affinity and half-lives of these interactions[64, 328, 347].

1.2.3 Identifying new interactions: Approaches in the Wright Lab

Whilst hypothesis-driven studies on candidate proteins and a growing number of screening approaches have yielded a myriad of candidate PPIs for further investigation, the identification of additional host receptors for *Plasmodium* proteins has been impeded by technical difficulties. The first of these challenges is to produce sufficient quantities of correctly folded recombinant *Plasmodium* proteins, particularly since the genome of *Plasmodium falciparum* is predominated by A-T base pairs[107], which can be problematic for cloning and protein expression. The next hurdle to identifying host-pathogen PPIs is the low sensitivity of interaction detection methods; extracellular protein-protein interactions are often transient and of low-affinity and therefore not readily detected by classical biochemical purification techniques, which usually require stringent washing steps. In the laboratory where the work described in this thesis was carried out, we aim to overcome these challenges using a specialised protein expression system and a high-throughput interaction screening platform.

1.2.3.1 A mammalian expression system for *Plasmodium* proteins

By developing a high-throughput mammalian expression system based on the transient transfection of cells from the Human Embryonic Kidney line (HEK293 cells), we have been able to express a library of 62 merozoite cell surface and secreted proteins[65, 369]. This has acted as a valuable resource both to discover novel interactions and to use in *in vitro* assays to help determine their function. The details of this expression system are described in Section 2.1, but one of its key features is the use of codon-optimisation to overcome the A-T nucleotide bias that impairs routine molecular biology procedures. This process makes *Plasmodium* protein ectodomains much more amenable to expression in mammalian cells. Using this system, proteins can be pentamerised via the inclusion of a cartilage oligometric matrix protein (COMP) sequence in expression constructs[333]. These pentameric proteins have further increased our capacity to discover and study PPIs by increasing the avidity and stability of the interactions in which they participate (Figure 1.4B).

1.2.3.2 AVEXIS

Avidity-based extracellular interaction screening, or AVEXIS, is a technique developed in the Wright laboratory as a sensitive, high-throughput method for the discovery of protein-protein interactions[40]. Ectodomain regions of receptor proteins are expressed in HEK293 cells, such that protein libraries can be systematically screened against each other. One ectodomain library can be presented as an ordered array of 'bait' proteins immobilised on microtitre plates.

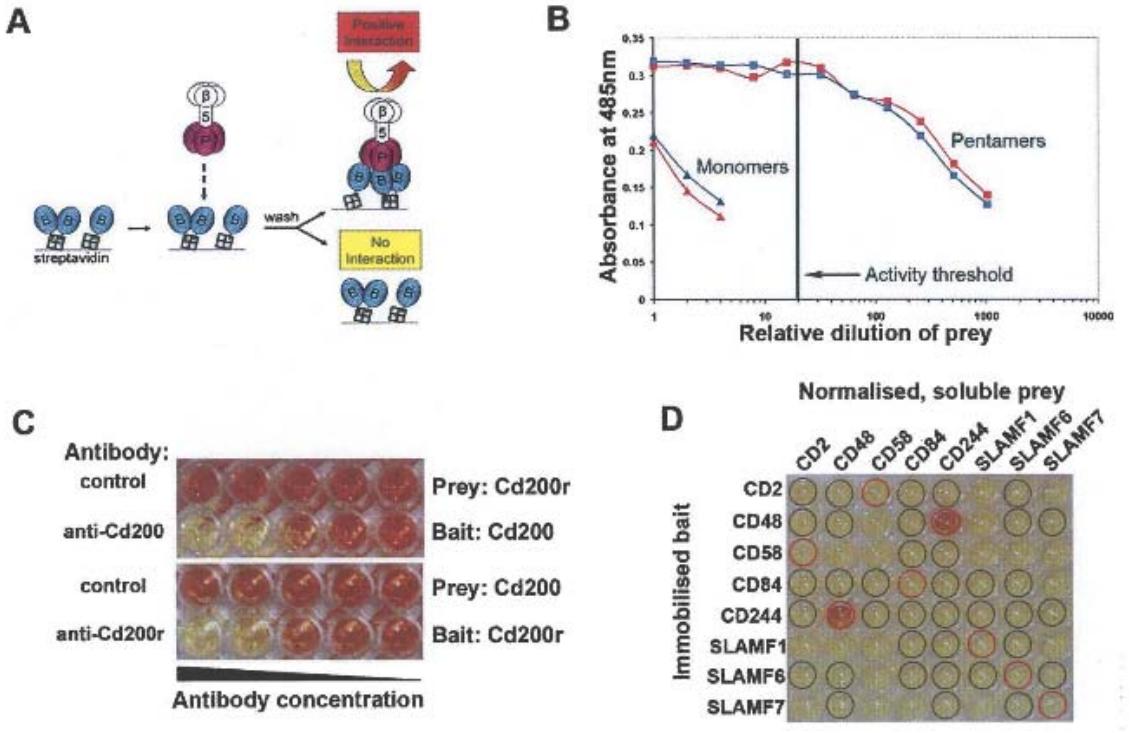

Figure 1.4: **AVEXIS as a highly sensitive interaction screening method**
Figure from *Large-scale screening for novel low-affinity extracellular protein interactions* by Bushell *et al.*, (2008)[40]. Reproduced with permission.
A. Diagramatic representation of the AVEXIS method. Biotinylated bait proteins (blue) are arrayed on the surface of a streptavidin-coated plate and incubated with pentameric prey proteins (red). Interacting prey proteins are captured by the immobilised baits and remain bound following a wash step. The β-lactamase activity of the prey hydrolyses nitrocefin, inducing a yellow-to-red colour change.
B. Pentamerisation of the prey proteins increases the avidity and stability of the Cd200/Cd200R interaction.
C. The specificity of Cd200/Cd200R interaction detection is shown, as an antibody against the bait protein can prevent the binding of the prey.
D. Example of an interaction screen: hits from the screen produce a red colour.

To detect interactions, these plates are probed with another library of pentamerised, enzyme-tagged ectodomain 'prey' proteins (Figure 1.4A). This approach has been used previously in this laboratory to identify receptor-ligand interactions involved in malaria. By screening a panel of merozoite proteins against a library of erythrocyte receptors, two novel interactions were identified and subsequently validated; SEMA7a was identified as a receptor for PfM-TRAP and BSG as a receptor for PfRH5[19, 64]. Both of these interactions are transient, with micromolar affinities calculated in SPR experiments; this demonstrates that AVEXIS is a

highly sensitive method, appropriate for the detection of low-affinity interactions.

1.3 Scope of this thesis

This thesis focuses on the use of existing methods and the development of novel methods for identifying host-pathogen protein interactions in malaria. In screening for interactions, *P. falciparum* merozoite surface protein 7 (PfMSP7) was identified as a potential ligand for human P-selectin (SELP) and the biochemistry of this interaction was characterised in detail. This work is summarised in three results chapters as follows:

Chapter 3: Identification and validation of an interaction between SELP and PfMSP7 This chapter describes the use of AVEXIS to test for novel interactions occurring between recombinant proteins from the *P. falciparum* merozoite and proteins from, but not restricted to, the human platelet. A novel interaction was identified between PfMSP7 and human SELP. These recombinant proteins were shown to be biochemically active and this interaction could also be observed in SPR experiments and in a flow cytometry-based assay developed as part of this project. Evidence is also presented that suggests that recombinant PfMSP7 oligomerises in solution, that this is a property of the protein's N-terminus, and that this oligomerisation might be important for its interaction with SELP.

Chapter 4: Biochemical investigations into the conservation and function of the interactions between *Plasmodium* MSP7s and SELP This chapter describes the biochemical and functional characterisation of the interactions between SELP, PfMSP7 and related proteins. AVEXIS was used to isolate the binding domains as the C-type lectin and/or EGF-like domains of SELP and the N-terminus of PfMSP7, and to screen more widely for interactions occurring between mammalian selectins and *Plasmodium* MSP7-family proteins. This revealed that SELP-binding is a conserved feature of multiple members of the *P. falciparum* MSP7 family. At least one *P. vivax* MSP7 protein also bound to SELP *in vitro* and the SELP/MSP7 interaction looked to be conserved in the *P. berghei* mouse model of infection. These data suggest that SELP-binding might be an important, previously unidentified, role for the PfMSP7 N-terminus and its numerous paralogues. The possibility that the PfMSP7/SELP interaction might play a role in RBC invasion was investigated and largely ruled-out. In *in vitro* binding experiments PfMSP7 could block the interaction that SELP makes with its known ligands, and can thus be implicated as having an immunomodulatory role.

Chapter 5: Development of a biochemical co-purification assay to detect interactions between *Plasmodium* merozoite proteins and human serum proteins This chapter describes the rational design of a biochemical co-purification procedure whereby recombinant *P. falciparum* merozoite proteins are immobilised on superparamagentic beads and used to isolate binding partners from normal human serum. The assay was optimised so as to balance maximal capture of interacting protein against minimal contamination with abundant non-specifically interacting serum proteins. The assay was shown to be very effective at detecting the high-affinity interaction between PfMSP3.4 and human IgM, which is an abundant serum component. The possibility that transient, low affinity interactions can also be detected by this approach was also demonstrated using the interaction between PfRH5 and BSG. A panel of over 50 merozoite proteins were screened for interactions with serum proteins using this method, though no novel interactions were identified.