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Análise do estado redox e seu efeito sobre a proliferação de *Plasmodium falciparum* em eritrócitos geneticamente diferentes

Tese apresentada ao programa de Pós-Graduação em Biologia da Relação Patógeno-Hospedeiro do Departamento de Parasitologia do Instituto de Ciências Biomédicas da Universidade de São Paulo para obtenção do Título de Doutor em Ciências.

Área de concentração: Biologia da Relação Patógeno-Hospedeiro

Orientador: Prof. Dr. Carsten Wrenger

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Analysis of the Redox Status and its Effect on the Proliferation of *Plasmodium falciparum* in Genetically Different Erythrocytes

Ph. D. Thesis presented to the Post-graduation program Biology of Host-Pathogen Interactions at the Institute of Biomedical Sciences of the University of São Paulo, in order to obtain the degree of Doctor in Sciences

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RESUMO

Meissner, KA. Análise do estado redox e seu efeito sobre a proliferação de Plasmodium falciparum em eritrócitos geneticamente diferentes. Tese (Doutorado em Parasitologia).

Malária, causada por parasitas *Plasmodium spp.*, ainda contribui com cerca de 400 mil mortes anuais sendo uma das mais vastas doenças de nosso tempo. *Plasmodium falciparum*, que causa a malária tropical, leva a forma mais severa da doença. Não obstante, há alguns grupos com resistência nativa conhecidas como, por exemplo, a siclemia ou enzimopatias como no caso da deficiência da glicose 6-fosfato desidrogenase. Apesar dos anos de pesquisa, até hoje os exatos mecanismos que conferem proteção, permanecem desconhecidos. Contudo, várias hipóteses, como o aumento da resposta imune inata ou a resposta melhorada contra os danos oxidativos dentro dos eritrócitos são discutidos. Este trabalho foca nos sistemas de defesa contra danos oxidativos em *Plasmodium falciparum* usando parasitas geneticamente modificados em células sanguíneas vermelhas anormais. O aumento de diferentes sistemas antioxidantes deveria fornecer um olhar aprofundado dos mecanismos de proteção destes eritrócitos modificados. Neste trabalho demonstramos a importância da Glutationa-S-Transferase para a sobrevivência do parasita em eritrócitos com a deficiência da glicose 6-fosfato desidrogenase. Isso leva a hipótese de que níveis aumentados de ROS nas células vermelhas geram uma alta quantidade de xenobióticos no parasita, resultando na morte da célula.

Palavras-chave: Malária. Glutathiona. Deficiência em G6PD. Estresse oxidativo.

ABSTRACT

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Malaria, caused by *Plasmodium* spp., remains with more than 400.000 deaths annually one of the vastest diseases of our time. *Plasmodium falciparum*, is the most dangerous species leading to severe malaria. Nevertheless, there are some native resistances known like sickle cell trait or enzymopathies such as glucose-6-phosphate dehydrogenase deficiency. However, the protection mechanism is still unknown. Hypotheses like a better innate immune response or the increased oxidative stress inside the altered erythrocytes are discussed. This work is focusing on the oxidative defence system of *P. falciparum* using transgenically modified parasites cultured in wild-type and abnormal red blood cells. Elevated expression levels of different anti-oxidative systems in *P. falciparum* should give a deeper insight of the protection mechanism of the altered erythrocytes. In this work, we show the importance of the plasmodial Glutathione-S-Transferase (*Pf*GST) for the proliferation of the malaria pathogen in erythrocytes with glucose-6-phosphate dehydrogenase deficiency. This leads to the hypothesis that the increased ROS level in these red blood cells generating a high amount of xenobiotics within the parasite which results in cell death.

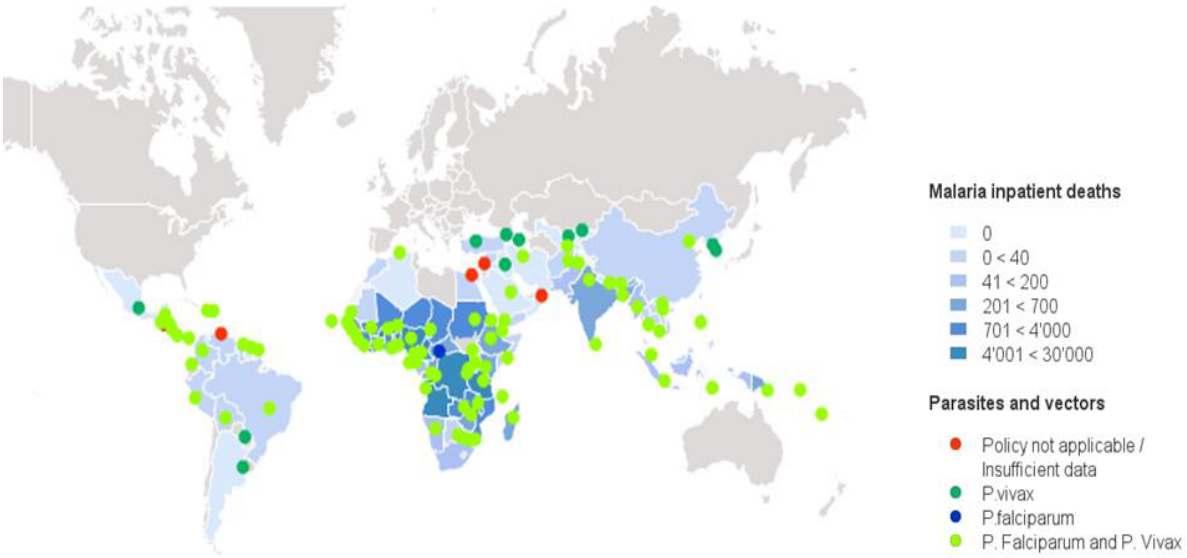
Keywords: Malaria. Glutathione. G6PD deficiency. Oxidative stress.

1 INTRODUCTION

1.1 History and Distribution of Malaria

Malaria is a life-threatening disease with nearly a quarter of the world's population living in areas of high risks. In 2015, 212 million malaria cases, leading to about half a million deaths, were recorded, most of them African children under the age of five years. With over 90% of all malaria cases and 92% of malaria deaths shows the Sub-Saharan Africa harbouring the highest global malaria burden. On the American continent, 18 countries are endemic for malaria and the majority of cases occur in one of the nine amazonic countries, which includes Brazil (1).

Figure 1 – World distribution and cases of death through malaria.



Spots are demonstrating the global distribution of *P. falciparum* and *P. vivax* infections correlating with the number of death in blue caused by malaria in 2016. Data were available at http://www.who.int/en/ 31.01.2017.

The history of malaria extends into antiquity with records from China in 2700 before Christ (BC), Greece and the Roman Empire. Already Hippocrates mentioned in the 5th century BC the correlation between marshes and the characteristic periodic fevers of disease (2). Suspecting that the reason for the disease was the miasmas rising from the swamps, the name malaria occurred, which is driven from the Italian mal'aria, bad air. However, the disease continued spreading around the Mediterranean Sea up to the 19th century including central Europe. Through the

systematic draining of swamps and the use of insecticide, malaria has been finally eradicated in the 1960s in Europe (3).

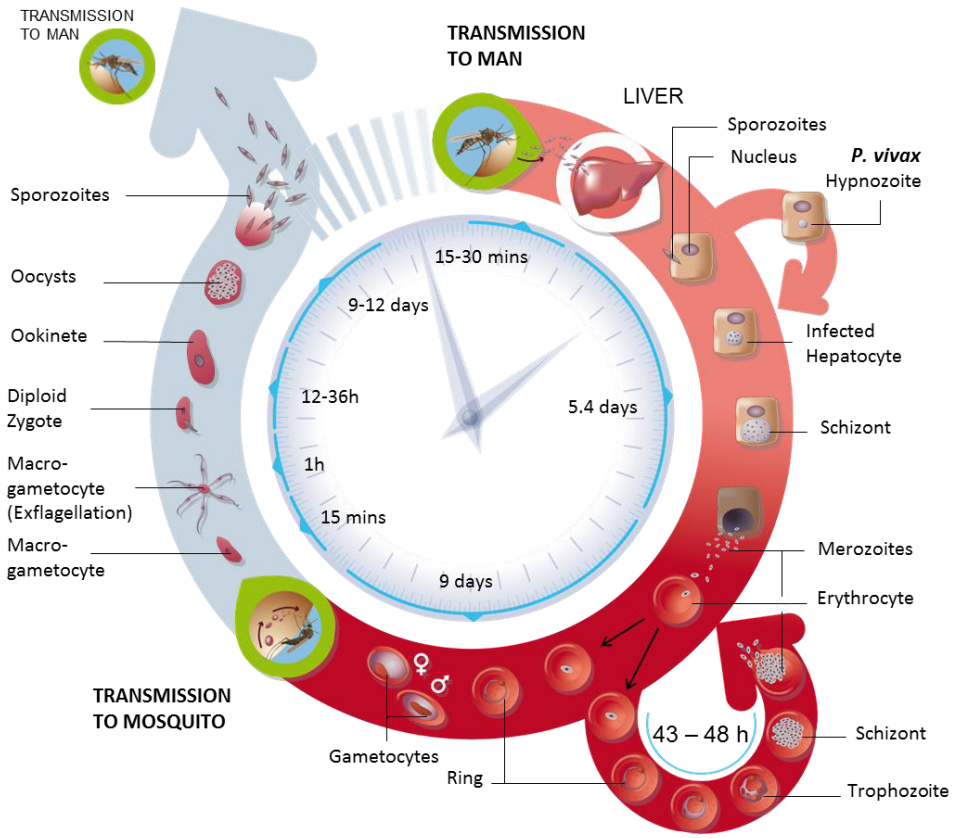
In 1880 Alphonse Laveran, a French army surgeon was the first to find the parasite in the blood of a patient suffering from malaria (2). Almost 20 years later Ronald Ross, a British medical doctor, introduced the anopheles mosquito as a possible vector for transmission of the malaria parasite (4). Additional 50 years were needed to find the missing link between after the infection of the parasite and its occurrence in the human blood. When in 1947, Henry Shortt and Cyril Garnham, were finally able to show a primary division of the parasite in liver cells (5). Subsequently, Krotoski and colleagues discovered that some *P. vivax* strains could remain in this liver stage for several months (6).

Up today there are more than 200 known species of the genus *Plasmodium (P.)*, but just five of them are causing human malaria, including *P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi* and the most virulent, *P. falciparum* (7). The genus *Plasmodium* belongs to the phylum Apicomplexa, which consist of a large group of unicellular eukaryotes sharing the same invasion machinery, the apical complex. Together with dinoflagellates and ciliates, they are forming the higher group of Alveolata (8).

All *Plasmodium* spp share a complex life cycle happening within an insect and a vertebrate host. The human malaria is transmitted via the female Anopheles mosquito, which injects sporozoites during the blood meal. After invading liver cells each sporozoite can mature into up to 40,000 merozoites, which will then be released into the blood stream via merosomes (9). However, *P. vivax* and *P. ovale* are able to form hypnozoites, a special form of sporozoites, which can remain in the liver for several months before proceeding to the blood stage. The released merozoites can infect red blood cells (RBCs), which start to remodel these cells in order to facilitate their proliferation and differentiation from ring to trophozoite and then into schizont. One of the reasons of the high virulence of *P. falciparum* is the export of *Pf*EMP1 (*P. falciparum* infected erythrocyte membrane protein 1) to the infected RBC (iRBC) surface. *Pf*EMP allows the iRBC to bind to the endothelium avoiding the clearance by the spleen and are leading to a disrupted blood flow which can cause cerebral or placental malaria when occurring in the brain or placenta (10). The asexual blood cycle ends with the haemolysis and release of new merozoite forms into the

bloodstream, resulting in both anaemia and periodic fevers characteristic of the disease. While most of the merozoites will reinfect other erythrocytes, some follow a different path differentiating into male and female gametocytes. These gametocytes will differentiate into gametes within the mid-gut of a female *Anopheles* mosquito after the next blood meal and the sexual proliferation can take place. After formation of the diploid zygote, the zygote differentiates to the ookinete and later oocysts and subsequently, new sporozoites are formed. The released sporozoites migrate to the mosquito's salivary gland, where they will be transmitted during the next blood meal of the mosquito (11).

Figure 2 – Life cycle of *Plasmodium* spp.



The life cycle of *Plasmodium* spp. is occurring in two hosts. After a blood meal of the Anopheles spec. mosquito, the parasite infects human hepatocytes and proliferates into merozoites. While an infection with *P. vivax* or *Plasmodium ovale* can lead to a sporozoite differentiation into hypnozoites in all other cases merozoites will directly infect RBC and replicate via schizogony. This assexually replication can be repeated several times. Other merozoites develop into male and female gametocytes that infect mosquitoes when taken up by the next blood meal. The sexual stages mature into the mosquito gut where they fuse and form an ookinete. The ookinete develops into the oocyst which releases new sporozoites that migrate to the insect's salivary glands Source: modified from (11).

Due to the increased prevention and vector control, the global malaria mortality rates have been reduced by 29% and even by 35% among children under 5 since 2010 (1). Nevertheless, continuous outbreaks of vector resistance associated with the spread of resistance among parasites to classical antimalarial treatments are the reason for the devastating effects of this disease. Therefore continuous discovery and development of novel antimalarials are needed to combat malaria (12,13).

1.2 Conquer malaria

The primary way to overcome malaria is the vector control via insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS) in areas of high risks. The most effective and affordable insecticide is 1,1,1-Trichloro-2,2'bis(p-chlorophenyl) ethane DDT, the first synthetic organic (14). It was intensively used to combat malaria in 1940s to 1970s before it was banned in 1972 because of its impact on the environment and human health (14). However after several years of debating 2006 WHO gave again a clean bill to use of DDT to combat malaria in Africa due of the great burden of malaria and the current expensive and ineffective vector control strategies (15,16).

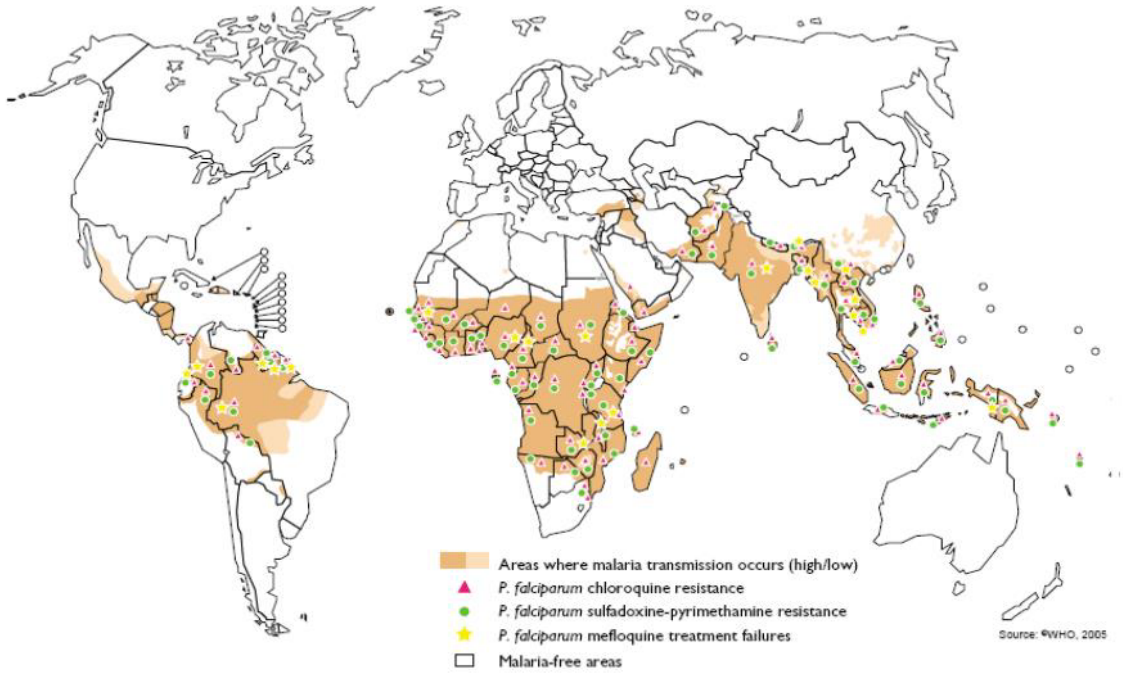
The alternative, long-lasting insecticidal nets (LLINs) are containing pyrethroids, which lead to a protection of up to 3 years and is highly recommended especially for young children and pregnant women in endemic areas. Between 2010 and 2015, the use of these ITNs increased in sub-Saharan Africa there by 80%. However, mosquito resistances to pyrethroids are already reported and in some areas, even all 4 classes of insecticides show already a decreased effect (1).

1.2.1 Drug treatment

One of the oldest known antimalarials is the quinine, an alkaloid derived from the bark of the cinchona tree. It was brought in the 17th century from Peru to Europe and was first isolated in the 19th century from French researchers Pierre Joseph Pelletier and Joseph Bienaimé Caventou. However, the first synthetic antimalarial drug, methylene blue, was introduced 1891 by Guttmann and Ehrlich. Methylene blue is a specific inhibitor of the glutathione reductase and interferes with the haemozoin

polymerisation (17). Because its uncommen side effects (green urine and blue sclera) it was discarded as antimalarial and replaced with chloroquine (CQ)(18). However because of its potential to reverse CQ resistance, today methylene blue is again considered as potential drug against *Plasmodium* spp. (18). In 1940 CQ took over as the new antimalarial drug of choice (19). Already in 1934, Hans Andersag discovered the quinine-related CQ (20), which was massively used worldwide. All 4-aminoquinolines, including quinine, CQ, mefloquine, amodiaquine and the quinoline-methanols, are supposed to interfere with the plasmodial heme detoxification inside the digestive vacuole (DV) killing thereby the parasite (21,22). Despite the advantages of CQ, such as high efficacy, low production costs and low toxicity, the need for new drugs raises because of the appearance of CQ-resistant *Plasmodium* strains in the late 1950s (2). The resistance is mediated through mutations in the *P. falciparum* chloroquine resistance transporter (*Pf*CRT) located on the DV membrane allowing the efflux of CQ (23,24).

Figure 3 – Occurrence of antimalarial drug resistance of *P. falciparum*.



The resistance of *P. falciparum* to the still in use antimalarial drugs, such as chloroquine, sulfadoxine-pyrimethamine and mefloquine are already widespread as seen in the global map from 2005 (25).

However, antifolates were discovered as an alternative, acting through the inhibition of the biosynthesis of tetrahydrofolate (the active form of folate, vitamin B9), solely

present in the parasite. Antibiotics like sulfadoxine (a sulfonamide antibiotic) inhibit the enzyme dihydropteroate synthetase, while pyrimethamine serves as an inhibitor of the dihydrofolate reductase and dihydropteroate synthase (26,27). To enhance the effect both drugs were used in combination to inhibit two different steps in the same biosynthesis. Nevertheless, in 1970 the first resistances were noted in Thailand and from there spread rapidly through Asia and to the African continent (Figure 3) (24,26).

Because of the fast spreading of resistances against all known antimalarials new drugs were urgently needed and in the 1970s isolated artemisinin from the Chinese herb qinghaosu seemed to be the solution. Artemisinin was effective against all multi-drug resistant parasites (28). There exist several artemisinin derivates, which all are reducing the blood parasitemia very rapidly. However, the drug half-life is very short which is the reason why is the drug only given in combination with other antimalarials, known as artemisinin combination therapy (ACTs)(29). The mode of action of artemisinin is still not cleared but it is suggested that artemisinin gets activated by iron which in turn inhibits *Pf*ATP6 (a calcium pump) which leads to parasites death (30). Recently was demonstrated that artesunate, an artemisinin derivative, is inhibiting a novel membrane bound GST (*Pf*GST2/*Pf*EXP1) (31). Untill today ACTs are the treatment of choice for uncomplicated malaria. Nevertheless, parasites with resistance to artemisinin were identified already in 5 countries of South East Asia (Cambodia, Laos, Myanmar, Thailand and Viet Nam). Fortunately, ACTs seem still successful when used in combination with other drugs. However, the search for new antimalarial drugs has to continue and novel drug targets have to be discovered before the spread of the ACT-resistances.

Another strategy for new antimalarials is to block the parasite transmission by targeting the liver or the sexual stages of the parasite. Till today there is just one drug family available attacking also hypnozoites. The 8-aminoquinolines like as primaquine is the only available drug to use for relapsing malaria caused by *P. vivax* or *P. ovale*. The mechanism of action is still unclear but probably involves cytochrome P450s and monoamine oxidase, as well as the formation of reactive intermediates (32). However, due to the risk of hemolytic anaemia, glucose-6-phosphate-deficient patients infected with malaria should not be treated by

primaquine, as well as a pregnant woman. Since the combat against the liver and sexual stage could lead to a clinically relevant reduction of malaria, new drugs for these stages are important. Tafenoquine could be one of these promising agents showing already several benefits over primaquine (32).

1.2.2 Vaccination

The use of vaccination against malaria would lead a big step forward to the eradication of this disease. However, the complex live cycle with their multistage and the genetic diversity of *Plasmodium* spp. hinder the approaches to develop a vaccine hence there is currently no effective vaccination available. However, in 2015 the first malaria vaccine RTS,S/AS01 against *P. falciparum* was accepted for pilot implementations in 3 countries in sub-Saharan Africa. Before, large clinical trials in 7 countries in Africa were completed with positive evaluation by the European Medicines Agency (1).

RTS,S/AS01 targets pre-erythrocytic stages of the *Plasmodium* infection. This might result in a reduction of liver stage schizonts releasing merozoites, which will induce the blood stage proliferation. Previous field trials showed already a partial protective effect, with efficiencies around 30-50% (33). This seems rather disappointing but this pilot project could open the way to an increased attention on the discovery of vaccines. However the vaccination program with RTS,S/AS01 is intended to start in 2018 (1).

1.2.3 Innate Resistances

Knowing the long lasting history of human malaria it is certainly no surprise that this disease had a selective pressure on the human genome evolution. This was first recognised and described in 1949 by J.B.S. Haldane. He found a correlation between malaria-endemic regions around the Mediterranean Sea and the local frequency of thalassemia which seemed to mediate a protective effect against human malaria (34,35). Consequently, Haldane formulated his hypothesis of “balanced polymorphisms”, where the enhance fitness against malaria acquired from a heterozygote carrier phenotype would prevail over the disadvantages of the

homozygote phenotype, which causes the genetic disease. This hypothesis is today known as the “malaria hypothesis” and was first confirmed in 1954 for the sickle cell trait (36). Additionally to the sickle cell trait and thalassemia, there are several other red blood cells mutations which provide a certain resistance to malaria, like Glucose 6-phosphate dehydrogenase (G6PD) deficiency, pyruvate kinase deficiency, the absence of Duffy antigens and other haemoglobin (Hb) mutations (HbC, HbE). The precise mechanisms of action are despite years of research still unknown. However, two major reasons are discussed in the literature. On the one hand, the proliferation of the parasite within altered erythrocytes could be impaired because of limited access to Hb or the increased oxidative stress. On the other hand, an enhanced immune response could diminish the appearance of differently formed RBCs.

1.2.3.1 Sickle cell disease

Sickle cell trait is the term of heterozygote sickle cell anaemia, which was first described by J.B. Herrick in 1910 (37). It is driven by a single point mutation in the β chain of the Hb gene, resulting in the exchange of glutamate at position 6 to valine. A Hb molecule with such a mutation is termed HbS. As a result, the deoxygenated HbS tetramer gained a hydrophobic motif which mediates the binding between a β1 of one HbS molecule to the β2 chain of another. This results in an aggregation of HbS molecules to long polymers which disrupt the erythrocytic shape and their flexibility generating cellular dehydration and oxidative stress (38). Patients having sickle cell disease suffer primarily from vaso-occlusions and hemolytic anaemia.

In 2013 about 3.2 million people were diagnosed with the sickle-cell disease and another 43 million with sickle-cell trait (39). The highest occurrence of this HbS mutation is in sub-Saharan Africa which involves around 80% of all sickle cell diseases in children (40). The heterozygote HbAS form consisting of one WT Hb gene (HbA) and one HbS gene show protection against malaria, respectively. This is demonstrated by a lower parasite density in infected HbS children compared to HbAA children as well as by a decrease in severe malaria and mortality of 50-90%. This occurrence has been analysed for more than 50 years (36,41,42) and the respective mode of action has been studied as well.

However, a precise mechanism has not been identified yet and all hypotheses relating to a protective role against malaria fall into three main categories. Early work suggested both, erythrocytes containing HbS are less supportive for *P. falciparum* proliferation under low oxygen tensions as well as a reduction of the parasite invasion event into HbS carrying erythrocytes under low oxygen levels (43,44). Further it has been observed that HbS cells deposit oxidized, denaturated haemoglobin at the inner site of the erythrocytic membrane (45), which occurs to a higher extent in HbS- than in HbA-red blood cells (RBC) and is even forced by the release of non-heme iron that also binds to the RBC membrane (46,47). Due to this denaturating, pro-oxidative environment, the intracellular proliferation of the malaria parasite might be attenuated (48). Secondly, an increased degree of phagocytosis of the respective infected erythrocytes could explain the low parasitemia in HbS carriers (49,50). Recently, data have been accumulated which suggest that HbS might be involved in pathophysiological consequences of *P. falciparum* by reducing the amount of proteins such as *Pf*EMP1 encoded by the var-gene family on the surface of the erythrocyte which leads to a higher level of sequestration (51,52). Indeed in a very recent study by Cyrklaff and colleagues (53), it has been implicated that HbS carrying erythrocytes influence the actin cytoskeleton and the Maurer's cleft formation and thereby impair the vesicle transport towards the erythrocytic surface. More recently, it has been suggested that HbS is mediating a higher tolerance of the host as shown by a non-reduction of the parasite quantity or virulence (54,55). Although these experiments were of some controversial nature as already outlined by (56), the focus was on how the parasite is proliferating in an elevated oxidative environment. Humans who are sickle cell carriers have higher levels of free, non-protein bound heme in the blood circulation (57), which is potentially toxic, due to its oxidative nature. It has been suggested that increased levels of human heme oxygenase 1 (HO-1) might detoxify free heme to CO, biliverdin and iron that binds subsequently to the protein ferritin H chain in HbS blood and thereby renders complicated (cerebral) malaria (54). However, it remains questionable whether the protective nature of the increased level of free heme in HbS carriers is related to a higher tolerance to an increased level of oxidative stressor-mediated by HO-1 or to a higher susceptibility of the parasite by a decreased parasitemia (36) within a pro-oxidative environment.

1.2.3.2 Thalassemia

Similar to the sickle cell trait, thalassemia is also hemoglobinopathies driven by the decrease in synthesis of α- or β-globin (α- and β-thalassemia). In 2013 about 208 million cases were noted with about 4.7 million severe forms of thalassemia and resulting in 25,000 deaths (39,58). It mostly occurs around the Mediterranean Sea, Middle Eastern, South Asian and sub-Saharan Africa correlating with the distribution of malaria infections. In some of this regions, α-thalassemia occurs even in up to 50% of the population (59). This is indicating the former propose of Haldane, that milder forms of thalassemia provide a certain protection against malaria (34).

The α-thalassemia disorder involves the genes HBA1 and HBA2 encoding for the Hb α-chains on chromosome 16. The severity of the disease depends on if one (-α/αα), two(--/αα; -α/-α), three (--/-α) or all four (--/--) genes are inactive either by deletion or point mutations. Because of the followed excess of β- or γ-chains in new-borns unstable HbH tetramers consisting of 4 beta chains are formed. The formation of γ-chains tetramers in a homozygote α^0 fetus often results in a soon death because of the high affinity to oxygen, which hinders the transport of oxygen to the tissues. Nevertheless, α-thalassemia shows a protective effect against *P. falciparum* infection around 40% (60–64). The mechanism of action is still unknown but it has been reported that the parasite density in blood is not different than for (αα/αα) children (60,61,63–65). This rejects the possibility of impaired growth or an enhanced removal of iRBC by the immune system as a possible mode of action (60,66). Currently, a discussion of a weakened cytoadherence as the reason for the protective effect has been initiated (59).

The decrease of active β-globin driven by gene mutation is the cause of β-thalassemia. Up today there are around 300 mutations described (67). The most common are HbE where the glutamic acid at position 26 is exchanged for a lysine. HbE carriers usually show no clinical effects (68,69), but provide an resistance advantage towards *P. vivax* infections, and to a lesser extent to *P. falciparum* (70–72).

1.2.3.3 Duffy antigen negative

The Duffy antigen chemokine receptor (DARC), also known as Fy glycoprotein (FY) is a glycoprotein receptor on the surface of red blood cells (73). The receptor serves for several chemokines and is important for the merozoite invasion into RBC of *P. vivax* and *Plasmodium knowlesi* (74,75). In 1950 the first antigen (Fya) of the DARC-family was discovered, followed several other resulting in a total of six: FyA, FyB, Fy3, Fy4, Fy5 and Fy6 (76,77). However, the mediated resistance is dependent mainly on two of these antigens, FyA and FyB. A single nucleotide substitution polymorphism (SNIP) (DARC 46 T → C) in the promoter region of the Duffy antigen gene results in the suppression of their expression. Duffy negative includes individuals which have this mutation on both alleles (78).

Similar to *P. falciparum, P. vivax* is distributed around tropical countries. However, in West and Central Africa, a low occurrence is noticed. In parallel up to 90% of the population of this area shows a lack of Duffy receptors (75,79). It is believed that the heavy burden of *P. vivax* forced the selection of a Duffy-negative population over the time, resulting in the elimination of *P. vivax* in this region (80).

Nevertheless, there is evidence that *P. vivax* is able to infect Duffy negative RBC as it was shown for populations in Western Kenya, the Brazilian Amazon region and Madagascar (81–83). Although the resistance is not protecting completely against *P. vivax* infection it remains a good example of innate resistance against malaria.

1.2.3.4 Pyruvate kinase deficiency

A recent example of the malaria hypothesis is the pyruvate kinase (PK) deficiency mediated resistance towards malaria (84). PK catalyses the last step of anaerobic glycolysis converting phosphoenolpyruvate (PEP) into pyruvate releasing ATP. Because of the absence of mitochondria in mature erythrocytes, this step is responsible for creating about 50% of the RBC total ATP production (85). Therefore PK deficiency leads to a decreased ATP concentration resulting in a shorter erythrocyte lifespan (85).

PK deficiency was first described in 1961 and is caused by mutations in the PK, liver and RBC (PKLR) gene on chromosome 1q21 (86,87).Today more than 200

mutations are known which result in clinical symptoms like nonspherocytic hemolytic anaemia in homozygous and compound heterozygotes patients (88,89). With one out of 20,000 persons who have PK deficiency this disease is the second most common enzymopathy after G6PD deficiency (90).

Recently a geographical co-distribution between malaria and PK deficiency was shown by Machado and colleagues demonstrating the highest prevalence in the Middle East and sub-Saharan Africa (84). The protective effect was already revealed for in vivo malaria infection in the murine models and under culture conditions using human PK-deficient blood (91). The mediated resistance and the co-distribution with human malaria suggest that it might be a selective pressure resulting in the development of PK deficiency variants (92,93).

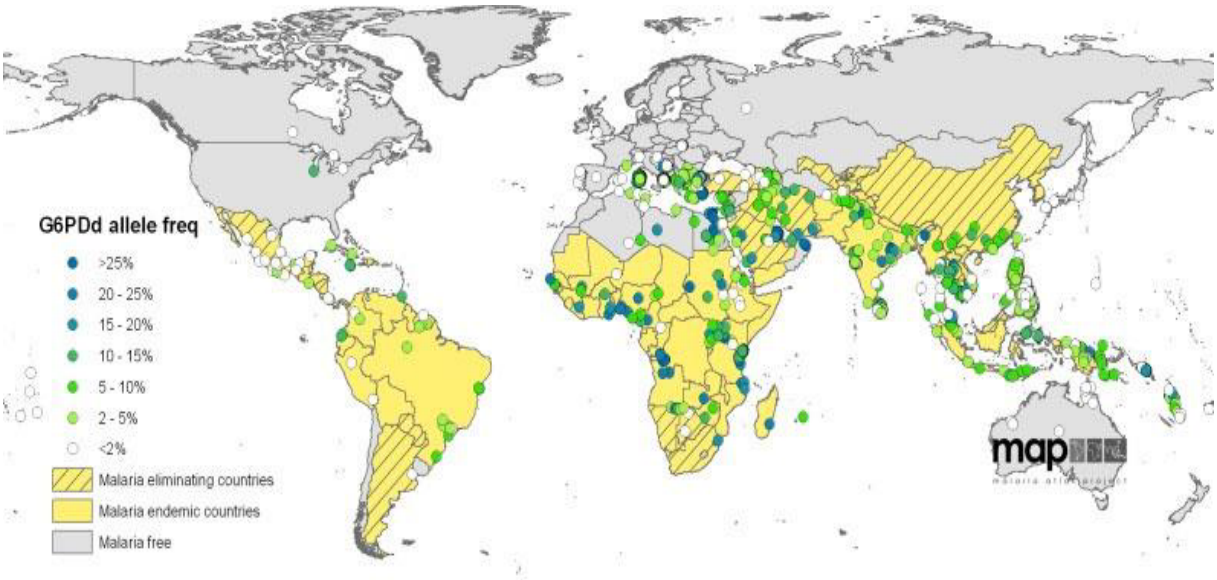
1.2.3.5 Glucose 6-phosphate dehydrogenase deficiency

The most common enzymopathy is a G6PD deficiency with more than 400 million cases worldwide (94). Although G6PD mutations are distributed all over the world the main prevalence is in Africa, southern Europe and Asia, as well as the Middle East and southern Pacific islands (Figure 4). This is remarkably similar to the world distribution of malaria leading to the idea of a G6PD deficiency mediated protective effect (80).

G6PD deficiency was first known as favism because of the pathological symptoms like hemolytic anaemia occurring after the consumption of fava beans (95). In 1956 G6PD deficiency was described for the first time, seeing low levels of G6PD in patients with hemolytic anaemia using the antimalarial drug primaquine. Subsequently, the correlation of favism and G6PD deficiency was published (96).
G6PD is catalysing the first step of the pentose phosphate pathway (PPP) providing the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). NADPH is the reducing agent in many enzymatic reactions and is playing a key role in protection against oxidative stress via the glutathione system. Especially in erythrocytes which lack mitochondria, the PPP is the only source of NADPH making the G6PD essential to counterbalance oxidative stress (97).

The G6PD deficiency is a X-linked, hereditary genetic disease with a gene location at the telomeric region of the long arm (98,99). Because of the X-linked pattern the disease occurs more in males who are either G6PD deficient or not. Females, however, have two alleles and can be heterozygote mosaics because of the X chromosome inactivation (lyonization) (100).

Figure 4 – World distribution of G6PD deficiency.



World prevalence of G6PD deficiency using coloured data points compared to the malaria distribution as background map (101).

G6PD is active as a tetramer or dimer with a $NADP^+$ molecule in each subunit. The quaternary structure is essential for the enzyme activity and therefore, it is no surprise that often G6PD deficiency is due to mutations interfering with the enzyme conformation (100). There are about 140 different mutations described which are all located in the enzyme coding sequence (102). Most of them are single base exchanges leading to various biochemical and clinical different phenotypes. However, mostly G6PD deficient individuals show no clinical symptoms when not exposed to oxidative stress triggers like drugs, infection or fava beans (103). In some severe cases, patients develop neonatal jaundice or acute hemolytic anaemia which could lead to permanent neurological damage or death (104).

As already suspected G6PD deficiency mediates resistance against malaria. It was already seen, that individuals with this condition have a protection up to 50% against

severe *P. falciparum* malaria (105,106). More recent studies show a protective effect also against *P. vivax* infection (107,108). In both infections, a lower parasite density was found. However no mechanism of action for G6PD deficiency mediates malaria resistance is known, there are several theories which are discussed, like an enhanced phagocytosis (109–111) or the impairment of parasite growth because of increased oxidative stress. It was already refuted that the reason is an increase of antibodies against *P. falciparum* merozoite surface protein 2 (MSP2). G6PD deficient individuals showed even a lower level of MSP2 IgG3 antibodies (112). Moreover it is still discussed if the protective effect is given for male hemizygote or just for female heterozygote individuals (106,113–117).

1.3 Oxidative stress

1.3.1 Formation of reactive oxygen species

Reactive oxygen species (ROS) is a term to describe by oxygen- (O_2) derivates driven free radicals, such as superoxide anion ($O_2^{\bullet -}$), alkoxyl radical (RO•), peroxyl radical (ROO•), and the highly toxic hydroxyl radical (HO•), as well as nonradicals like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl) and ozone (O_3). Reactive nitrogen species (RNS) are similar reactive molecules containing nitrogen, such as nitric oxide. They are by-products of the normal cell metabolism or driven by exogenous sources like drugs, xenobiotics or pollutants. ROS can be important signalling molecules for cell proliferation and differentiation (118,119) as well as harmful to the cell and tissues leading to apoptosis and cell death (120). Therefore the balance of ROS formation and detoxification is essential for the cellular homoeostasis and depends on pro- and antioxidant enzymatic reactions as well as antioxidant molecules.

One of the main sources of ROS production is the mitochondrion. Through the inner mitochondrial membrane, electrons are translocated via the electron transport chain (ETC) reducing in their final step O_2 to water and so finally producing adenosine triphosphate (ATP) via the ATP-synthase complex. Here about 1-2% of the electrons pass through and produce in the presence of metal ions (present in Complex I and

Complex III) ROS, such as $O_2^{\bullet-}$ and H_2O_2 via the Fenton and/or Haber-Weiss reactions leading to the dangerous HO• (121,122):

$$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\bullet$$

$$O_2^{\bullet-} + H_2O_2 \rightarrow HO\bullet + OH^- + O_2$$

Also the endoplasmic reticular (ER) can be responsible for the increase of ROS for e.g. through monooxygenases like cytochrome P450 (CYP) (123). CYPs are important for the detoxification of the ER and catalyse the oxygenation of an organic substrate by reducing O_2 to water.

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

When the reaction of O_2 and the organic substrate not tightly coupled, electron equivalents derived from NADPH can react directly with O_2 forming a CYP–oxygen complexes which will dissociate and form ROS as $O_2^{\bullet-}$ and H_2O_2 and HO• (123–125).

Furthermore, another significant source of cellular ROS are the peroxisomes, which are ubiquitous subcellular organelles. They are responsible for the β-oxidation of fatty acids, biosynthesis of ether phospholipids as well as the metabolism of ROS. Certain enzymes like the flavin oxidases catalyse the reaction of O_2 with organic substrates. In this oxidative reaction ROS can be generated. It is already described that peroxisomes are responsible for H_2O_2, $O_2\cdot^-$ and $^1O_2$ cellular production (126). Thus peroxisomes are present in almost all eukaryotic cells (127,128) some organisms suffered its evolutionary loss, such as several parasitic lineages including *Plasmodium* spp (129).

The last important source to mention are the NADPH oxidase (NOX) complexes which are the only enzymes, whose biological function is the production of ROS. The first NOX described was the phagocyte NOX2 (NOX2/gp91phox) which is highly expressed in granulocytes and monocyte-macrophages (reviewed in (119). Here ROS generation is used for the active killing of microorganisms. However, up today six more NOX homologous were found, which are localised in almost every tissue.

Interestingly, though, a defence function is just described for NOX2. The active generation of ROS suggests that this molecule has to have an important role for cell development and survival. Besides host defence, NOX driven ROS production has been associated with the posttranslational processing of proteins, cellular signalling, regulation of gene expression, and cell differentiation (119).

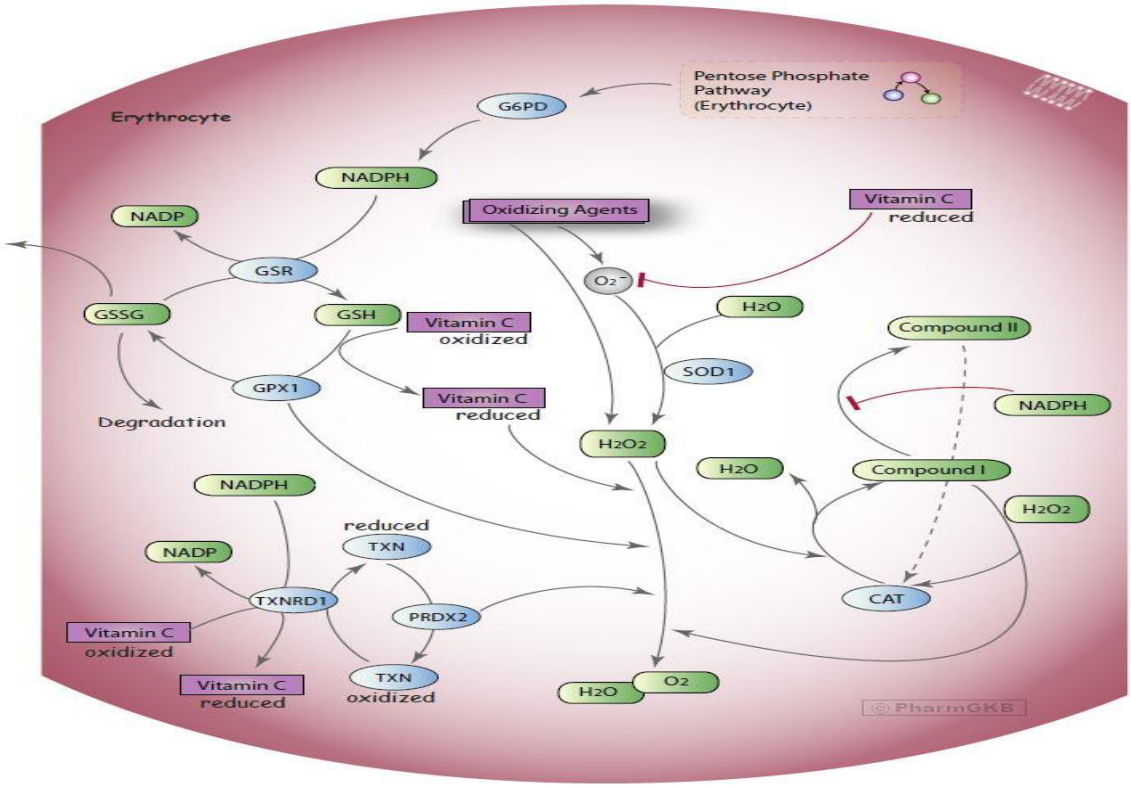
Despite the advantages of ROS generation, an increase of this molecules results in dangerous cell damage. In normal cells, intracellular levels of ROS are maintained in balance with intracellular biochemical antioxidants and when this balance is disrupted it can be called an oxidative stress situation. In this case, ROS reacts with several molecules like carbohydrates, proteins, lipids, or nucleic acids, resulting in consequences such as lipid peroxidation or unfolded protein response (UPR) in the ER. If the oxidative stress level is too high and too much damage occurs, a cell will undergo apoptosis or programmed cell death.

1.3.2 The oxidative defence system of erythrocytes

The primary biological function of erythrocytes is the transport of O_2 from the lungs to all blood tissues. Therefore the cytoplasm is rich of haemoglobin, which can bind O_2 via its iron-containing heme group. To use the whole cytosolic capacity RBC lacks mostly all organelles as well as the nucleus. Although RBCs are not exposed to ROS-driven by the above-mentioned organelles like mitochondrion, ER and peroxisomes RBCs are continuously exposed to both cellular and extracellular ROS (130). One of the main intracellular sources of ROS in RBS is the autoxidation of Hb under hypoxic conditions, leading to the formation of $O_2^{\bullet -}$ which will rapidly convert to H_2O_2. Because of the iron-containing heme group the generation of HO• via the Fenton and/or Haber-Weiss reactions is also possible. New approaches show that RBCs contain a ROS producing NOX and therefore enzymatically catalysing ROS. It is suspected that these RBC-NOX are involved in sickle cell disease, although RBC-NOX were also found in healthy erythrocytes (131). Xenobiotic floating in the blood stream is exogenous exposure of RBCs to ROS as well as free heme after haemolysis as well as free radicals released via neutrophils and macrophages into the plasma (132). However, experiments showed that as well as intracellular as well as extracellular generated ROS are rapidly neutralised by the RBC antioxidant

system (132). To do so RBCs have a comprehensive antioxidant system (Figure 5) involving both non-enzymatic antioxidants like glutathione and ascorbic acid and enzymatic antioxidants including superoxide dismutase, catalase (133) glutathione peroxidase (134) and PRDX-2 (135,136).

Figure 5 – Oxidative defence system of red blood cells.



The oxidative defence system of RBCs consisting of superoxide dismutase, catalase the glutathione and thioredoxin system as well as peroxiredoxin-2 and the essential vitamin C. Source: (137).

Via autoxidation or Fenton reactions resulted cytosolic $O_2^{\bullet-}$ is converted to H_2O_2 by superoxide dismutase. This can be either neutralized by the glutathione (GSH) system or via TXN both NADPH-dependent systems. GSH is a small tripeptide (γ-L-Glutamyl-L-cysteinylglycine) which serves as an important antioxidant due to its thiol group. On the other hand, TXN is a small redox protein which facilitates the reduction of other proteins. Both GSH and thioredoxin reductase (TXNR1) are able to reduce vitamin c (ascorbic acid) which subsequently neutralises $O_2^{\bullet-}$, H_2O_2 and oxygen free radicals (137).

GSH is also important together with peroxiredoxin 2 (PRDX2) to stabilise haemoglobin by preventing and reversing oxidation that causes disulphide cross-links between globin chains. The in this way resulting a change of haemoglobin structure is the potential reason for the formation of the typical G6PD deficient RBCs appearance the 'Heinz bodies'. Finally, RBCs contain a catalase (CAT) which is NADPH-independent and is able to catalyse H_2O_2 to H_2O and O_2.

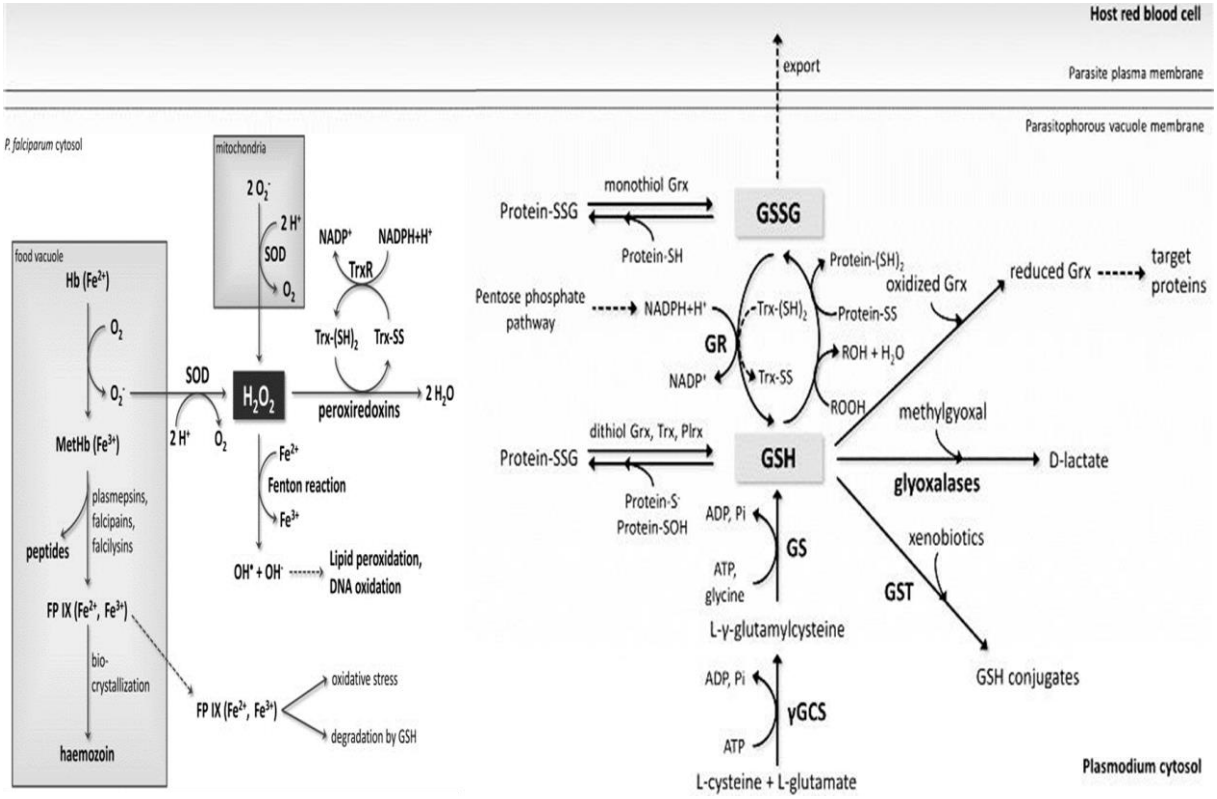
1.3.3 The oxidative defence system of P. falciparum

During the erytrocytic stage of *P. falciparum*'s life cycle the parasite has to adept himself to the oxidative environment of the host cell. Therefore he needs an effective oxidative defence system which protects him from ROS driven by the RBC, as well as from the host immune response and his own ROS products driven by the degradation of Hb (138). The parasite uptakes Hb from the RBCs cytosol and digests it inside the DV. The released highly reactive heme is than bound as a non-toxic criystal, the haemozoin. However, small amounts of free heme are released into the parasites cytosol where it reacts with O_2 to $O_2^{\bullet-}$ and H_2O_2 which can lead to oxidative damage and parasites death (139). Another main source of ROS in *P. falciparum* is the mitochondrion as already described above. Nevertheless *Plasmodium* spp. does not possess a peroxisome (129) or NOX complexes.

The adaptation to this oxidative milieu is critical to the parasites survival, therefore already in the early blood stages at least five different antioxidant proteins are expressed (140). However, Plasmodium lacks a catalase and glutathione peroxidase (141,142) and therefore it has established several other mechanisms to detoxify H_2O_2 and other ROS (Figure 6). Superoxide dismutases in the cytosol (*Pf*SOD1) and in the mitochondrion (*Pf*SOD2) catalyze the reaction from $O_2^{\bullet-}$ to H_2O_2. Cytosolic H_2O_2 either react with $O_2^{\bullet-}$ to the highly reactive HO• or can be neutralized by TRX dependent peroxidases, which are part of the main system to remain the redox homeostasis together with the GSH system (143). Both GSH and TRX antioxidant mechanisms are NADPH dependent and can be recycled by GSH or TRX reductases. Plasmodium is able to synthesis GSH *de novo* an important small molecule for the detoxification via *Pf*GST and glyoxalases. Plasmodium contains two *Pf*GSTs, one localised in the parasites cytosol detoxifying *e.g.* hemin and different

intracellular xenobiotics (144). *Pf*GST2 previously known as *Pf*EXP1 is localised in the parsitophorous vacuolar membrane and is contributing in the hemin/haematin detoxification (145). *Pf*GST2 was also shown to be a potential target of the antimalarial artesunate (31). GSH is also an important co-factor for the glyoxalases which is reacts with a toxic by-product of the glycolysis, methylglyoxal to D-lactate, which than can be secreted (146).

Figure 6 – Oxidative defence system of *P. falciparum*.



The oxidative defence system of *P. falciparum* is important to conter ROS driven from mitochondria and Hb digestion. Superoxide dismutase catalyze the reaction from $O_2^{\bullet-}$ to H_2O_2 which will be neutralized via the TRX system. The *de novo* synthesis of GSH is present in the parasite providing GSH as a co-factor for *Pf*GSTs and other detoxifying enzymes like glycolases. Source: modified from (147).

Interestingly *P. falciparum* contains the biosynthesis pathway for Vitamin B6, as well as the possibility for scavenging it from the blood plasma. Vitamin B6 is a co-factor in more than 100 enzymatic reactions and additionally is highly relevant to quench oxidative stress, due to its role in singlet oxygen ($^1O_2$) detoxification (148,149).

6 CONCLUSION

*Pf*GST is one of the most abundant protein in *P. falciparum* and highly important for parasites survival. Its main function is the detoxification of hydrophobic and endogenous/exogenous electrophilic compounds, eliminating cytotoxic hemin by coupling GSH and neutralization of H_2O_2. In this we work reinforce the importance of *Pf*GST showing its role in enhancing parasite's growth in G6PD deficient RBCs, which are known to mediate malaria resistance. Those cells are more susceptible to oxidative stress resulting in the formation of Heinz body, erythrocyte destruction and subsequent hemolytic anemia. Under normal conditions G6PD deficient erythrocytes can cope with low levels of ROS as indicated by no difference in ROS concentration, RBC appearance and leading to no clinical symptoms.

P. falciparum infected G6PD deficient RBCs show just a slight increase in intracellular ROS compared to WT blood but have a comparable growth behavior in the first days of culturing. However after long term culturing (more than 15 days) the proliferation of the pathogen is decreasing. This effect was reversed by overexpression of plasmodial *Pf*GST, leading to an equivalent growth in G6PD deficient blood as in WT blood. Moreover *Pf*GST overexpressing decreases the ROS occurrence in both blood types. This result leads to the proposal that after long term culturing, elevated exogenous ROS alters the redox homeostasis of G6PD deficient RBCs leading to lipid peroxidation and oxidation of DNA and proteins. Overexpression of the plasmodial *Pf*GST is protecting the parasite by detoxifing oxidised biomolecules via coupling GSH and exporting GS-X conjugates via MRP outside of the cell. Additionally *Pf*GST is able to neutralize the access of H_2O_2 and thereby increasing the fitness of *P. falciparum* inside G6PD deficient RBCs.

However there are several other enzymes which have been reported to be involved in the defence of oxidative stress including a recently identified membrane-bound *Pf*GSTF2/*Pf*EXP1. In order to verify whether *Pf*GST is the only enzyme to enable *P. falciparum* to proliferate normally in G6PD-deficient RBCs further analyses of other ROS detoxifying pathways are required.

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REFERENCES*

1. WHO. World Malaria Report 2016 [Internet]. 2016 [cited 2017 Mar 13]. Available from: http://apps.who.int/iris/bitstream/10665/252038/1/9789241511711-eng.pdf?ua=1

2. Cox FEG. History of human parasitology. Clin Microbiol Rev [Internet]. 2002 Oct [cited 2017 Feb 13];15(4):595–612. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12364371

3. Majori G. Short history of malaria and its eradication in Italy with short notes on the fight against the infection in the mediterranean basin. Mediterr J Hematol Infect Dis [Internet]. 2012 [cited 2017 Mar 13];4(1):e2012016. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22550561

4. Ross R. On some Peculiar Pigmented Cells Found in Two Mosquitos Fed on Malarial Blood. Br Med J [Internet]. 1897 Dec 18 [cited 2017 Mar 13];2(1929):1786–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20757493

5. Shortt HE, Garnham PCC. Pre-erythrocytic stage in mammalian malaria parasites. Nature [Internet]. 1948 Jan 24 [cited 2017 Mar 13];161(4082):126. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18900752

6. Krotoski WA, Collins WE, Bray RS, Garnham PC, Cogswell FB, Gwadz RW, et al. Demonstration of hypnozoites in sporozoite-transmitted Plasmodium vivax infection. Am J Trop Med Hyg [Internet]. 1982 Nov [cited 2017 Mar 13];31(6):1291–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6816080

7. Pain A, Böhme U, Berry AE, Mungall K, Finn RD, Jackson AP, et al. The genome of the simian and human malaria parasite Plasmodium knowlesi. Nature [Internet]. 2008 Oct 9 [cited 2017 Mar 13];455(7214):799–803. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18843368

8. Morrison DA. Evolution of the Apicomplexa: where are we now? Trends Parasitol [Internet]. 2009 Aug [cited 2017 Mar 13];25(8):375–82. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1471492209001433

9. Kishore U. Target pattern recognition in innate immunity [Internet]. Springer Science+Business Media; 2009 [cited 2017 Mar 13]. 202 p. Available from: https://books.google.com.br/books?id=p5Oay0xBypwC&pg=PA175&lpg=PA175&dq=40,000+merozoites,+which+will+then+be+released+into+the+blood+stream&source=bl&ots=nQNuwx7Oyh&sig=gy3wrCnA00BBiNAfep4Gq_a36zk&hl=de&sa=X&ved=0ahUKEwiDw_T2h9TSAhVCGpAKHSwVDWQQ6AEINDAF#v=onepage&q=40%2C000 merozoites%2C which will then be released into the blood stream&f=false

10. Biryukov S, Stoute JA. Complement activation in malaria: friend or foe? Trends Mol Med [Internet]. 2014 May [cited 2017 Mar 13];20(5):293–301. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1471491414000021

11. Delves M, Plouffe D, Scheurer C, Meister S, Wittlin S, Winzeler EA, et al. The Activities of Current Antimalarial Drugs on the Life Cycle Stages of

Plasmodium: A Comparative Study with Human and Rodent Parasites. Beeson JG, editor. PLoS Med [Internet]. 2012 Feb 21 [cited 2017 Mar 13];9(2):e1001169. Available from: http://dx.plos.org/10.1371/journal.pmed.1001169

12. Kronenberger T, Lindner J, Meissner KA, Zimbres FM, Coronado MA, Sauer FM, et al. Vitamin B6-dependent enzymes in the human malaria parasite plasmodium falciparum: A druggable target? Biomed Res Int. 2014;2014.

13. Lindner J, Meissner KA, Schettert I, Wrenger C. Trafficked proteins - Druggable in plasmodium falciparum ? Int J Cell Biol. 2013;

14. Beard J, Australian Rural Health Research Collaboration. DDT and human health. Sci Total Environ [Internet]. 2006 Feb 15 [cited 2017 Apr 9];355(1–3):78–89. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15894351

15. Raghavendra K, Barik TK, Reddy BPN, Sharma P, Dash AP. Malaria vector control: from past to future. Parasitol Res [Internet]. 2011 Apr 13 [cited 2017 Apr 9];108(4):757–79. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21229263

16. Sadasivaiah S, Tozan Y, Breman JG. Dichlorodiphenyltrichloroethane (DDT) for indoor residual spraying in Africa: how can it be used for malaria control? Am J Trop Med Hyg [Internet]. 2007 Dec [cited 2017 Apr 9];77(6 Suppl):249–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18165500

17. Schirmer RH, Coulibaly B, Stich A, Scheiwein M, Merkle H, Eubel J, et al. Methylene blue as an antimalarial agent. Redox Rep [Internet]. 2003 Oct 19 [cited 2017 Apr 9];8(5):272–5. Available from: http://www.tandfonline.com/doi/full/10.1179/135100003225002899

18. Suwanarusk R, Russell B, Ong A, Sriprawat K, Chu CS, Pyaephyo A, et al. Methylene blue inhibits the asexual development of vivax malaria parasites from a region of increasing chloroquine resistance. [cited 2017 Apr 9]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4267499/pdf/dku326.pdf

19. Greenwood D. The quinine connection. Journal of Antimicrobial Chemotherapy. 1992.

20. Solomon VR, Lee H. Chloroquine and its analogs: a new promise of an old drug for effective and safe cancer therapies. Eur J Pharmacol [Internet]. 2009 Dec 25 [cited 2017 Mar 13];625(1–3):220–33. Available from: http://linkinghub.elsevier.com/retrieve/pii/S001429990900870X

21. Roepe PD. Molecular and physiologic basis of quinoline drug resistance in *Plasmodium falciparum* malaria. Future Microbiol [Internet]. 2009 May [cited 2017 Mar 13];4(4):441–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19416013

22. Bray PG, Mungthin M, Hastings IM, Biagini GA, Saidu DK, Lakshmanan V, et al. PfCRT and the trans-vacuolar proton electrochemical gradient: regulating the access of chloroquine to ferriprotoporphyrin IX. Mol Microbiol [Internet].

2006 Oct [cited 2017 Mar 13];62(1):238–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16956382

23. Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, Ferdig MT, et al. Mutations in the P. falciparum digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Mol Cell [Internet]. 2000 Oct [cited 2017 Mar 13];6(4):861–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11090624

24. Ecker A, Lehane AM, Clain J, Fidock DA. PfCRT and its role in antimalarial drug resistance. Trends Parasitol [Internet]. 2012 Nov [cited 2017 Mar 13];28(11):504–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23020971

25. WHO. World malaria report 2005 [Internet]. World Health Organization; 2005 [cited 2017 Mar 19]. 294 p. Available from: http://www.who.int/malaria/publications/atoz/9241593199/en/

26. Sridaran S, McClintock SK, Syphard LM, Herman KM, Barnwell JW, Udhayakumar V. Anti-folate drug resistance in Africa: meta-analysis of reported dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) mutant genotype frequencies in African Plasmodium falciparum parasite populations. Malar J [Internet]. 2010 Aug 30 [cited 2017 Mar 13];9(1):247. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20799995

27. Gesase S, Gosling RD, Hashim R, Ord R, Naidoo I, Madebe R, et al. High Resistance of Plasmodium falciparum to Sulphadoxine/Pyrimethamine in Northern Tanzania and the Emergence of dhps Resistance Mutation at Codon 581. Gregson A, editor. PLoS One [Internet]. 2009 Feb 24 [cited 2017 Mar 13];4(2):e4569. Available from: http://dx.plos.org/10.1371/journal.pone.0004569

28. Mutabingwa TK. Artemisinin-based combination therapies (ACTs): Best hope for malaria treatment but inaccessible to the needy! Acta Trop [Internet]. 2005 Sep [cited 2017 Mar 13];95(3):305–15. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0001706X05001592

29. Nosten F, White NJ. Artemisinin-based combination treatment of falciparum malaria. Am J Trop Med Hyg [Internet]. 2007 Dec [cited 2017 Mar 13];77(6 Suppl):181–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18165491

30. Shandilya A, Chacko S, Jayaram B, Ghosh I, Haak J. A plausible mechanism for the antimalarial activity of artemisinin: A computational approach. Sci Rep [Internet]. 2013 Aug 29 [cited 2017 Mar 13];3:33–8. Available from: http://www.nature.com/articles/srep02513

31. Lisewski AM, Quiros JP, Ng CL, Adikesavan AK, Miura K, Putluri N, et al. Supergenomic network compression and the discovery of EXP1 as a glutathione transferase inhibited by artesunate. Cell [Internet]. 2014 Aug 14 [cited 2017 Apr 5];158(4):916–28. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0092867414009258

32. Campo B, Vandal O, Wesche DL, Burrows JN. Killing the hypnozoite – drug

discovery approaches to prevent relapse in *Plasmodium vivax*. Pathog Glob Health [Internet]. 2015 May 18 [cited 2017 Mar 13];109(3):107–22. Available from: http://www.tandfonline.com/doi/full/10.1179/2047773215Y.0000000013

33. Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Aide P, et al. Duration of protection with RTS,S/AS02A malaria vaccine in prevention of Plasmodium falciparum disease in Mozambican children: single-blind extended follow-up of a randomised controlled trial. Lancet [Internet]. 2005 Dec 10 [cited 2017 Mar 13];366(9502):2012–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16338450

34. Haldane JBS. The rate of mutation of human genes. Hereditas [Internet]. 1949 Jul 9 [cited 2017 Mar 13];35(S1):267–73. Available from: http://doi.wiley.com/10.1111/j.1601-5223.1949.tb03339.x

35. Hedrick PW. Resistance to malaria in humans: the impact of strong, recent selection. Malar J. 2012;11:1.

36. Allison AC. Protection afforded by sickle-cell trait against subtertian malareal infection. Br Med J [Internet]. 1954 Feb 6 [cited 2017 Mar 13];1(4857):290–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/13115700

37. Herrick JB. Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia. 1910. Yale J Biol Med [Internet]. 1910 [cited 2017 Mar 13];74(3):179–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11501714

38. Brittenham GM, Schechter AN, Noguchi CT. Hemoglobin S polymerization: primary determinant of the hemolytic and clinical severity of the sickling syndromes. Blood [Internet]. 1985 Jan [cited 2017 Feb 14];65(1):183–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3965046

39. Vos T, Barber RM, Bell B, Bertozzi-Villa A, Biryukov S, Bolliger I, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet [Internet]. 2015 Aug 22 [cited 2017 Mar 14];386(9995):743–800. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26063472

40. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. Lancet [Internet]. 2010 Dec 11 [cited 2017 Feb 14];376(9757):2018–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21131035

41. Beet EA. Sickle cell disease in the Balovale District of Northern Rhodesia. East Afr Med J [Internet]. 1946 Mar [cited 2017 Mar 13];23:75–86. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21027890

42. Allison AC. The distribution of the sickle-cell trait in East Africa and elsewhere, and its apparent relationship to the incidence of subtertian malaria. Trans R Soc Trop Med Hyg [Internet]. 1954 Jul [cited 2017 Mar 13];48(4):312–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/13187561

43. Friedman MJ. Erythrocytic mechanism of sickle cell resistance to malaria

(Plasmodium falciparum/hemoglobin S/in vitro culture/cyanate). 1978;75(4):1994–7.

44. Pasvol G, Weatherall DJ, Wilson RJ. Cellular mechanism for the protective effect of haemoglobin S against P. falciparum malaria. Nature [Internet]. 1978 Aug 17 [cited 2017 Mar 13];274(5672):701–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/353566

45. Browne P, Shalev O, Hebbel RP. The molecular pathobiology of cell membrane iron: the sickle red cell as a model. Free Radic Biol Med [Internet]. 1998 Apr [cited 2017 Mar 13];24(6):1040–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9607615

46. Sheng K, Shariff M, Hebbel RP. Comparative oxidation of hemoglobins A and S. Blood [Internet]. 1998 May 1 [cited 2017 Mar 13];91(9):3467–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9558406

47. Hebbel RP. Sickle hemoglobin instability: a mechanism for malarial protection. Redox Rep [Internet]. 2003 Oct 19 [cited 2017 Mar 13];8(5):238–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14962356

48. Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogerson S, Ginsburg H. Oxidative stress in malaria parasite-infected erythrocytes: Host-parasite interactions. International Journal for Parasitology. 2004.

49. Abu-Zeid YA, Abdulhadi NH, Hviid L, Theander TG, Saeed BO, Jepsen S, et al. Lymphoproliferative responses to Plasmodium falciparum antigens in children with and without the sickle cell trait. Scand J Immunol [Internet]. 1991 Aug [cited 2017 Mar 13];34(2):237–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1866602

50. López C, Saravia C, Gomez A, Hoebeke J, Patarroyo MA. Mechanisms of genetically-based resistance to malaria. Gene. 2010.

51. Cholera R, Brittain NJ, Gillrie MR, Lopera-Mesa TM, Diakité SAS, Arie T, et al. Impaired cytoadherence of Plasmodium falciparum-infected erythrocytes containing sickle hemoglobin. Proc Natl Acad Sci U S A [Internet]. 2008 Jan 22 [cited 2017 Mar 13];105(3):991–6. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0711401105

52. Fairhurst RM, Baruch DI, Brittain NJ, Ostera GR, Wallach JS, Hoang HL, et al. Abnormal display of PfEMP-1 on erythrocytes carrying haemoglobin C may protect against malaria. Nature [Internet]. 2005 Jun 23 [cited 2017 Mar 13];435(7045):1117–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15973412

53. Cyrklaff M, Sanchez CP, Kilian N, Bisseye C, Simpore J, Frischknecht F, et al. Hemoglobins S and C Interfere with Actin Remodeling in Plasmodium falciparum-Infected Erythrocytes. Science (80-) [Internet]. 2011 Dec 2 [cited 2017 Mar 13];334(6060):1283–6. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.1213775

54. Ferreira A, Marguti I, Bechmann I, Jeney V, Chora Â, Palha NR, et al. Sickle

Hemoglobin Confers Tolerance to Plasmodium Infection. Cell [Internet]. 2011 Apr [cited 2017 Mar 13];145(3):398–409. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0092867411003849

55. Haque A, Engwerda CR. An antioxidant link between sickle cell disease and severe malaria. Cell [Internet]. 2011 Apr 29 [cited 2017 Mar 13];145(3):335–6. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0092867411004211

56. Rosenthal PJ. Lessons from Sickle Cell Disease in the Treatment and Control of Malaria. N Engl J Med [Internet]. 2011 Jun 30 [cited 2017 Mar 13];364(26):2549–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21714653

57. Muller-Eberhard U, Javid J, Liem HH, Hanstein A, Hanna M. Plasma concentrations of hemopexin, haptoglobin and heme in patients with various hemolytic diseases. Blood [Internet]. 1968 Nov [cited 2017 Mar 13];32(5):811–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/5687939

58. Naghavi M, Wang H, Lozano R, Davis A, Liang X, Zhou M, et al. Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet [Internet]. 2015 Jan 10 [cited 2017 Mar 14];385(9963):117–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25530442

59. Krause MA, Diakite SAS, Lopera-Mesa TM, Amaratunga C, Arie T, Traore K, et al. α-Thalassemia Impairs the Cytoadherence of Plasmodium falciparum-Infected Erythrocytes. Spielmann T, editor. PLoS One [Internet]. 2012 May 18 [cited 2017 Feb 14];7(5):e37214. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22623996

60. Williams TN, Wambua S, Uyoga S, Macharia A, Mwacharo JK, Newton CRJC, et al. Both heterozygous and homozygous + thalassemias protect against severe and fatal Plasmodium falciparum malaria on the coast of Kenya. Blood [Internet]. 2005 Mar 15 [cited 2017 Feb 14];106(1):368–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15769889

61. Mockenhaupt FP, Ehrhardt S, Gellert S, Otchwemah RN, Dietz E, Anemana SD, et al. +-thalassemia protects African children from severe malaria. Blood [Internet]. 2004 Oct 1 [cited 2017 Feb 14];104(7):2003–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15198952

62. Wambua S, Mwangi TW, Kortok M, Uyoga SM, Macharia AW, Mwacharo JK, et al. The Effect of α +-Thalassaemia on the Incidence of Malaria and Other Diseases in Children Living on the Coast of Kenya. Pasvol G, editor. PLoS Med [Internet]. 2006 Apr 18 [cited 2017 Feb 14];3(5):e158. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16605300

63. May J, Evans JA, Timmann C, Ehmen C, Busch W, Thye T, et al. Hemoglobin variants and disease manifestations in severe falciparum malaria. JAMA [Internet]. 2007 May 23 [cited 2017 Mar 13];297(20):2220–6. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.297.20.2220

64. Allen SJ, O'Donnell A, Alexander ND, Alpers MP, Peto TE, Clegg JB, et al. alpha+-Thalassemia protects children against disease caused by other infections as well as malaria. Proc Natl Acad Sci U S A [Internet]. 1997 Dec 23 [cited 2017 Mar 13];94(26):14736–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9405682

65. Veenemans J, Andang'o PEA, Mbugi E V, Kraaijenhagen RJ, Mwaniki DL, Mockenhaupt FP, et al. Alpha+ -thalassemia protects against anemia associated with asymptomatic malaria: evidence from community-based surveys in Tanzania and Kenya. J Infect Dis [Internet]. 2008 Aug 1 [cited 2017 Feb 14];198(3):401–8. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1086/589884

66. Fowkes FJI, Allen SJ, Allen A, Alpers MP, Weatherall DJ, Day KP. Increased Microerythrocyte Count in Homozygous α+-Thalassaemia Contributes to Protection against Severe Malarial Anaemia. Pasvol G, editor. PLoS Med [Internet]. 2008 Mar 18 [cited 2017 Feb 14];5(3):e56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18351796

67. Thein SL. The molecular basis of β-thalassemia. Cold Spring Harb Perspect Med [Internet]. 2013 May 1 [cited 2017 Mar 13];3(5):a011700. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23637309

68. Flint J, Harding RM, Boyce AJ, Clegg JB. The population genetics of the haemoglobinopathies. Baillieres Clin Haematol [Internet]. 1993 Mar [cited 2017 Feb 14];6(1):215–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8353314

69. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ [Internet]. 2001 [cited 2017 Feb 14];79(8):704–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11545326

70. Ray RN, Chatterjea JB, Chaudhuri RN. Observations on the resistance in hb e thalassaemia disease to induced infection with Plasmodium vivax. Bull World Health Organ [Internet]. 1964 [cited 2017 Feb 14];30:51–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14122440

71. Kuesap J, Chaijaroenkul W, Rungsihirunrat K, Pongjantharasatien K, Na-Bangchang K. Coexistence of Malaria and Thalassemia in Malaria Endemic Areas of Thailand. Korean J Parasitol [Internet]. 2015 Jun [cited 2017 Feb 14];53(3):265–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26174819

72. Hutagalung R, Wilairatana P, Looareesuwan S, Brittenham GM, Gordeuk VR. Influence of Hemoglobin E Trait on the Antimalarial Effect of Artemisinin Derivatives. J Infect Dis [Internet]. 2000 Apr [cited 2017 Mar 13];181(4):1513–6. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1086/315373

73. Moore S, Woodrow CF, McClelland DB. Isolation of membrane components associated with human red cell antigens Rh(D), (c), (E) and Fy. Nature

[Internet]. 1982 Feb 11 [cited 2017 Mar 14];295(5849):529–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6799838

74. Miller LH, Mason SJ, Dvorak JA, McGinniss MH, Rothman IK. Erythrocyte receptors for (Plasmodium knowlesi) malaria: Duffy blood group determinants. Science [Internet]. 1975 Aug 15 [cited 2017 Mar 14];189(4202):561–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1145213

75. Miller LH, Mason SJ, Clyde DF, McGinniss MH. The Resistance Factor to *Plasmodium vivax* in Blacks. N Engl J Med [Internet]. 1976 Aug 5 [cited 2017 Mar 14];295(6):302–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/778616

76. Chown B, Lewis M, Kaita H. The duffy blood group system in caucasians: evidence for a new allele. Am J Hum Genet [Internet]. 1965 Sep [cited 2017 Mar 14];17:384–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14334737

77. Cutbush M, Mollison PL, Parkin DM. A New Human Blood Group. Nature [Internet]. 1950 Feb 4 [cited 2017 Mar 14];165(4188):188–9. Available from: http://www.nature.com/doifinder/10.1038/165188b0

78. Zimmerman PA, Woolley I, Masinde GL, Miller SM, McNamara DT, Hazlett F, et al. Emergence of FY*A(null) in a Plasmodium vivax-endemic region of Papua New Guinea. Proc Natl Acad Sci U S A [Internet]. 1999 Nov 23 [cited 2017 Mar 14];96(24):13973–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10570183

79. Schmid P, Ravenell KR, Sheldon SL, Flegel WA. DARC alleles and Duffy phenotypes in African Americans. Transfusion [Internet]. 2012 Jun [cited 2017 Mar 14];52(6):1260–7. Available from: http://doi.wiley.com/10.1111/j.1537-2995.2011.03431.x

80. Carter R, Mendis KN. Evolutionary and historical aspects of the burden of malaria. Clinical Microbiology Reviews. 2002.

81. Menard D, Barnadas C, Bouchier C, Henry-Halldin C, Gray LR, Ratsimbasoa A, et al. Plasmodium vivax clinical malaria is commonly observed in Duffy-negative Malagasy people. Proc Natl Acad Sci [Internet]. 2010 Mar 30 [cited 2017 Mar 13];107(13):5967–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20231434

82. Ryan JR, Stoute JA, Amon J, Dunton RF, Mtalib R, Koros J, et al. Evidence for transmission of Plasmodium vivax among a duffy antigen negative population in Western Kenya. Am J Trop Med Hyg [Internet]. 2006 Oct [cited 2017 Mar 13];75(4):575–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17038676

83. Cavasini CE, de Mattos LC, Couto Á, Couto V, Gollino Y, Moretti LJ, et al. Duffy blood group gene polymorphisms among malaria vivax patients in four areas of the Brazilian Amazon region. Malar J [Internet]. 2007 Dec 19 [cited 2017 Mar 13];6(1):167. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18093292

84. Machado P, Manco L, Gomes C, Mendes C, Fernandes N, Salomé G, et al. Pyruvate kinase deficiency in sub-Saharan Africa: identification of a highly frequent missense mutation (G829A;Glu277Lys) and association with malaria. PLoS One [Internet]. 2012 [cited 2017 Mar 13];7(10):e47071. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23082140

85. Grace RF, Zanella A, Neufeld EJ, Morton DH, Eber S, Yaish H, et al. Erythrocyte pyruvate kinase deficiency: 2015 status report. Am J Hematol [Internet]. 2015 Sep [cited 2017 Mar 14];90(9):825–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26087744

86. Valentine WN, Tanaka KR, Miwa S. A specific erythrocyte glycolytic enzyme defect (pyruvate kinase) in three subjects with congenital non-spherocytic hemolytic anemia. Trans Assoc Am Physicians [Internet]. 1961 [cited 2017 Mar 14];74:100–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/13924348

87. Tanaka KR, Valentine WN, Miwa S. Pyruvate kinase (PK) deficiency hereditary nonspherocytic hemolytic anemia. Blood [Internet]. 1962 Mar [cited 2017 Mar 14];19:267–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/13919474

88. Warang P, Kedar P, Ghosh K, Colah R. Molecular and clinical heterogeneity in pyruvate kinase deficiency in India. Blood Cells, Mol Dis [Internet]. 2013 Oct [cited 2017 Mar 14];51(3):133–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23770304

89. Pissard S, Max-Audit I, Skopinski L, Vasson A, Vivien P, Bimet C, et al. Pyruvate kinase deficiency in France: a 3-year study reveals 27 new mutations. Br J Haematol [Internet]. 2006 Jun [cited 2017 Mar 14];133(6):683–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16704447

90. Ayi K, Min-Oo G, Serghides L, Crockett M, Kirby-Allen M, Quirt I, et al. Pyruvate Kinase Deficiency and Malaria. N Engl J Med [Internet]. 2008 Apr 24 [cited 2017 Mar 14];358(17):1805–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18420493

91. Min-Oo G, Fortin A, Tam M-F, Nantel A, Stevenson MM, Gros P. Pyruvate kinase deficiency in mice protects against malaria. Nat Genet [Internet]. 2003 Dec 2 [cited 2017 Mar 14];35(4):357–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14595440

92. Machado P, Pereira R, Rocha AM, Manco L, Fernandes N, Miranda J, et al. Malaria: looking for selection signatures in the human PKLR gene region. Br J Haematol [Internet]. 2010 Feb 8 [cited 2017 Mar 14];149(5):775–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20377593

93. Alves J, Machado P, Silva J, Gonçalves N, Ribeiro L, Faustino P, et al. Analysis of malaria associated genetic traits in Cabo Verde, a melting pot of European and sub Saharan settlers. Blood Cells, Mol Dis [Internet]. 2010 Jan 15 [cited 2017 Mar 14];44(1):62–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19837619

94. Peters AL, Noorden CJF Van. Glucose-6-phosphate Dehydrogenase Deficiency and Malaria: Cytochemical Detection of Heterozygous G6PD

Deficiency in Women. J Histochem Cytochem [Internet]. 2009 Nov [cited 2017 Mar 14];57(11):1003–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19546473

95. Mehta AB. Glucose-6-phosphate dehydrogenase deficiency. Postgr Med J [Internet]. 1994 [cited 2017 Mar 14];70:871–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2398001/pdf/postmedj00048-0016.pdf

96. Alving AS, Carson PE, Flanagan CL, Ickes CE. Enzymatic deficiency in primaquine-sensitive erythrocytes. Science [Internet]. 1956 Sep 14 [cited 2017 Mar 14];124(3220):484–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/13360274

97. Patra KC, Hay N. The pentose phosphate pathway and cancer. Trends Biochem Sci [Internet]. 2014 Aug [cited 2017 Mar 14];39(8):347–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25037503

98. Szabo P, Purrello M, Rocchi M, Archidiacono N, Alhadeff B, Filippi G, et al. Cytological mapping of the human glucose-6-phosphate dehydrogenase gene distal to the fragile-X site suggests a high rate of meiotic recombination across this site. Proc Natl Acad Sci U S A [Internet]. 1984 Dec [cited 2017 Mar 16];81(24):7855–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6595664

99. Trask BJ, Massa H, Kenwrick S, Gitschier J. Mapping of human chromosome Xq28 by two-color fluorescence in situ hybridization of DNA sequences to interphase cell nuclei. Am J Hum Genet [Internet]. 1991 Jan [cited 2017 Mar 16];48(1):1–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1985451

100. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet (London, England) [Internet]. 2008 Jan 5 [cited 2017 Mar 16];371(9606):64–74. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0140673608600732

101. Howes RE, Piel FB, Patil AP, Nyangiri OA, Gething PW, Dewi M, et al. G6PD Deficiency Prevalence and Estimates of Affected Populations in Malaria Endemic Countries: A Geostatistical Model-Based Map. von Seidlein L, editor. PLoS Med [Internet]. 2012 Nov 13 [cited 2017 Mar 19];9(11):e1001339. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23152723

102. Vulliamy T, Luzzatto L, Hirono A, Beutler E. Hematologically important mutations: glucose-6-phosphate dehydrogenase. Blood Cells Mol Dis [Internet]. 1997 Aug [cited 2017 Mar 16];23(2):302–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9410474

103. Arese P, Gallo V, Pantaleo A, Turrini F. Life and Death of Glucose-6-Phosphate Dehydrogenase (G6PD) Deficient Erythrocytes - Role of Redox Stress and Band 3 Modifications. Transfus Med Hemother [Internet]. 2012 Oct [cited 2017 Mar 16];39(5):328–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23801924

104. Elyassi AR, Rowshan HH. Perioperative management of the glucose-6-

phosphate dehydrogenase deficient patient: a review of literature. Anesth Prog [Internet]. 2009 [cited 2017 Mar 16];56(3):86–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19769422

105. Gilles HM, Fletcher KA, Hendrickse RG, Lindner R, Reddy S, Allan N. Glucose-6-phosphate-dehydrogenase deficiency, sickling, and malaria in African children in South Western Nigeria. Lancet (London, England) [Internet]. 1967 Jan 21 [cited 2017 Mar 16];1(7482):138–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4163314

106. Ruwende C, Khoo SC, Snow RW, Yates SN, Kwiatkowski D, Gupta S, et al. Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. Nature [Internet]. 1995 Jul 20 [cited 2017 Mar 16];376(6537):246–9. Available from: http://www.nature.com/doifinder/10.1038/376246a0

107. Leslie T, Briceño M, Mayan I, Mohammed N, Klinkenberg E, Sibley CH, et al. The impact of phenotypic and genotypic G6PD deficiency on risk of plasmodium vivax infection: a case-control study amongst Afghan refugees in Pakistan. Rogerson SJ, editor. PLoS Med [Internet]. 2010 May 25 [cited 2017 Mar 16];7(5):e1000283. Available from: http://dx.plos.org/10.1371/journal.pmed.1000283

108. Louicharoen C, Patin E, Paul R, Nuchprayoon I, Witoonpanich B, Peerapittayamongkol C, et al. Positively selected G6PD-Mahidol mutation reduces Plasmodium vivax density in Southeast Asians. Science [Internet]. 2009 Dec 11 [cited 2017 Mar 16];326(5959):1546–9. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.1178849

109. Ayi K, Turrini F, Piga A, Arese P. Enhanced phagocytosis of ring-parasitized mutant erythrocytes: a common mechanism that may explain protection against falciparum malaria in sickle trait and beta-thalassemia trait. Blood [Internet]. 2004 Nov 15 [cited 2017 Mar 16];104(10):3364–71. Available from: http://www.bloodjournal.org/cgi/doi/10.1182/blood-2003-11-3820

110. Arese P, Turrini F, Schwarzer E. Band 3/complement-mediated recognition and removal of normally senescent and pathological human erythrocytes. Cell Physiol Biochem [Internet]. 2005 Nov 15 [cited 2017 Mar 16];16(4–6):133–46. Available from: http://www.karger.com/?doi=10.1159/000089839

111. Cappadoro M, Giribaldi G, O'Brien E, Turrini F, Mannu F, Ulliers D, et al. Early phagocytosis of glucose-6-phosphate dehydrogenase (G6PD)-deficient erythrocytes parasitized by Plasmodium falciparum may explain malaria protection in G6PD deficiency. Blood [Internet]. 1998 Oct 1 [cited 2017 Mar 16];92(7):2527–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9746794

112. Courtin D, Milet J, Bertin G, Vafa M, Sarr JB, Watier L, et al. G6PD A-variant influences the antibody responses to Plasmodium falciparum MSP2. Infect Genet Evol [Internet]. 2011 Aug [cited 2017 Mar 16];11(6):1287–92. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1567134811001225

113. Luzzatto L. G6PD deficiency: a polymorphism balanced by heterozygote advantage against malaria. Lancet Haematol [Internet]. 2015 Oct [cited 2017 Mar 16];2(10):e400–1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26686037

114. Uyoga S, Ndila CM, Macharia AW, Nyutu G, Shah S, Peshu N, et al. Glucose-6-phosphate dehydrogenase deficiency and the risk of malaria and other diseases in children in Kenya: a case-control and a cohort study. Lancet Haematol [Internet]. 2015 Oct [cited 2017 Mar 16];2(10):e437–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26686045

115. Bienzle U, Ayeni O, Lucas AO, Luzzatto L. Glucose-6-phosphate dehydrogenase and malaria. Greater resistance of females heterozygous for enzyme deficiency and of males with non-deficient variant. Lancet (London, England) [Internet]. 1972 Jan 15 [cited 2017 Mar 16];1(7742):107–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4108978

116. Guindo A, Fairhurst RM, Doumbo OK, Wellems TE, Diallo DA. X-Linked G6PD Deficiency Protects Hemizygous Males but Not Heterozygous Females against Severe Malaria. Krishna S, editor. PLoS Med [Internet]. 2007 Mar 13 [cited 2017 Mar 16];4(3):e66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17355169

117. Sirugo G, Predazzi IM, Bartlett J, Tacconelli A, Walther M, Williams SM. G6PD A- deficiency and severe malaria in The Gambia: heterozygote advantage and possible homozygote disadvantage. Am J Trop Med Hyg [Internet]. 2014 May [cited 2017 Mar 16];90(5):856–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24615128

118. Rhee SG. Redox signaling: hydrogen peroxide as intracellular messenger. Exp Mol Med [Internet]. 1999 Jun 30 [cited 2017 Apr 5];31(2):53–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10410302

119. Bedard K, Krause K-H. The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology. Physiol Rev [Internet]. 2007 Jan 1 [cited 2017 Apr 5];87(1):245–313. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17237347

120. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med [Internet]. 2010 Mar 15 [cited 2017 Apr 5];48(6):749–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20045723

121. Reiter RJ, Melchiorri D, Sewerynek E, Poeggeler B, Barlow-Walden L, Chuang J, et al. A review of the evidence supporting melatonin's role as an antioxidant. J Pineal Res [Internet]. 1995 Jan [cited 2017 Apr 5];18(1):1–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7776173

122. Sadrzadeh SM, Graf E, Panter SS, Hallaway PE, Eaton JW. Hemoglobin. A biologic fenton reagent. J Biol Chem [Internet]. 1984 Dec 10 [cited 2017 Apr 5];259(23):14354–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6094553

123. Hrycay EG, Bandiera SM. Involvement of Cytochrome P450 in Reactive Oxygen Species Formation and Cancer. In: Advances in pharmacology (San Diego, Calif) [Internet]. 2015 [cited 2017 Apr 4]. p. 35–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26233903

124. Hrycay EG, Bandiera SM. The monooxygenase, peroxidase, and peroxygenase properties of cytochrome P450. Arch Biochem Biophys [Internet]. 2012 Jun 15 [cited 2017 Apr 5];522(2):71–89. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0003986112000057

125. Lewis DF V. Molecular modeling of human cytochrome P450–substrate interactions. Drug Metab Rev [Internet]. 2002 Jan 15 [cited 2017 Apr 5];34(1–2):55–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11996012

126. del Río LA, López-Huertas E. ROS Generation in Peroxisomes and its Role in Cell Signaling. Plant Cell Physiol [Internet]. 2016 Apr 14 [cited 2017 Apr 5];57(7):pcw076. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27081099

127. del Rio LA. Peroxisomes. Preface. Subcell Biochem [Internet]. 2013 [cited 2017 Apr 5];69:vii–x. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25039096

128. Foyer CH. Plant Peroxisomes: Biochemistry, Cell biology and Biotechnological Applications: Edited by Alison Baker, Ian A. Graham, Kluwer Academic Publishers, Dordrecht, The Netherlands, 2002. 505 pp., ISBN 1402005873, €180. Plant Sci [Internet]. 2003 Nov [cited 2017 Apr 5];165(5):1171–2. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0168945203003248

129. Žárský V, Tachezy J. Evolutionary loss of peroxisomes--not limited to parasites. Biol Direct [Internet]. 2015 Dec 23 [cited 2017 Apr 5];10:74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26700421

130. Kumerova A, Lece A, Skesters A, Silova A, Petuhovs V. Anaemia and antioxidant defence of the red blood cells. Mater Med Pol [Internet]. [cited 2017 Apr 5];30(1–2):12–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10214469

131. George A, Pushkaran S, Konstantinidis DG, Koochaki S, Malik P, Mohandas N, et al. Erythrocyte NADPH oxidase activity modulated by Rac GTPases, PKC, and plasma cytokines contributes to oxidative stress in sickle cell disease. Blood [Internet]. 2013 Mar 14 [cited 2017 Apr 5];121(11):2099–107. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23349388

132. Mohanty JG, Nagababu E, Rifkind JM. Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. Front Physiol [Internet]. 2014 [cited 2017 Apr 5];5:84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24616707

133. Gonzales R, Auclair C, Voisin E, Gautero H, Dhermy D, Boivin P. Superoxide dismutase, catalase, and glutathione peroxidase in red blood cells from patients with malignant diseases. Cancer Res [Internet]. 1984 Sep [cited 2017 Apr 5];44(9):4137–9. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/6589047

134. Nagababu E, Chrest FJ, Rifkind JM. Hydrogen-peroxide-induced heme degradation in red blood cells: the protective roles of catalase and glutathione peroxidase. Biochim Biophys Acta [Internet]. 2003 Mar 17 [cited 2017 Apr 5];1620(1–3):211–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12595091

135. Lee T-H, Kim S-U, Yu S-L, Kim SH, Park DS, Moon H-B, et al. Peroxiredoxin II is essential for sustaining life span of erythrocytes in mice. Blood [Internet]. 2003 Feb 27 [cited 2017 Apr 5];101(>12):5033–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12586629

136. Nagababu E, Mohanty JG, Friedman JS, Rifkind JM. Role of peroxiredoxin-2 in protecting RBCs from hydrogen peroxide-induced oxidative stress. Free Radic Res [Internet]. 2013 Mar 9 [cited 2017 Apr 5];47(3):164–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23215741

137. McDonagh EM, Bautista JM, Youngster I, Altman RB, Klein TE. PharmGKB summary: methylene blue pathway. Pharmacogenet Genomics [Internet]. 2013 Sep [cited 2017 Apr 9];23(9):498–508. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23913015

138. Francis SE, Sullivan DJ, Goldberg and DE. Hemoglobin metabolism in the malaria parasite Plasmodium falciparum. Annu Rev Microbiol [Internet]. 1997 Oct [cited 2017 Apr 5];51(1):97–123. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9343345

139. Egan TJ, Combrinck JM, Egan J, Hearne GR, Marques HM, Ntenteni S, et al. Fate of haem iron in the malaria parasite Plasmodium falciparum. Biochem J [Internet]. 2002 Jul 15 [cited 2017 Apr 5];365(Pt 2):343–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12033986

140. Foth BJ, Zhang N, Chaal BK, Sze SK, Preiser PR, Bozdech Z. Quantitative Time-course Profiling of Parasite and Host Cell Proteins in the Human Malaria Parasite Plasmodium falciparum. Mol Cell Proteomics [Internet]. 2011 Aug 1 [cited 2017 Apr 5];10(8):M110.006411-M110.006411. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21558492

141. Sztajer H, Gamain B, Aumann K-D, Slomianny C, Becker K, Brigelius-Flohe R, et al. The Putative Glutathione Peroxidase Gene of Plasmodium falciparum Codes for a Thioredoxin Peroxidase. J Biol Chem [Internet]. 2001 Mar 9 [cited 2017 Apr 5];276(10):7397–403. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11087748

142. Clarebout G, Slomianny C, Delcourt P, Leu B, Masset A, Camus D, et al. Status of Plasmodium falciparum towards catalase. Br J Haematol [Internet]. 1998 Oct [cited 2017 Apr 5];103(1):52–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9792289

143. Müller S, Gilberger TW, Krnajski Z, Lüersen K, Meierjohann S, Walter RD. Thioredoxin and glutathione system of malaria parasite Plasmodium falciparum. Protoplasma [Internet]. 2001 [cited 2017 Apr 5];217(1–3):43–9.

Available from: http://www.ncbi.nlm.nih.gov/pubmed/11732337

144. Wrenger C, Schettert I, Liebau E. Oxidative stress in human infectious diseases – present and current knowledge about its druggability.

145. Müller S. Role and Regulation of Glutathione Metabolism in Plasmodium falciparum. Molecules [Internet]. 2015 Jun 8 [cited 2017 Mar 14];20(6):10511–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26060916

146. Urscher M, Alisch R, Deponte M. The glyoxalase system of malaria parasites—Implications for cell biology and general glyoxalase research. Semin Cell Dev Biol [Internet]. 2011 May [cited 2017 Apr 5];22(3):262–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21310259

147. Jortzik E, Becker K. Thioredoxin and glutathione systems in Plasmodium falciparum. Int J Med Microbiol [Internet]. 2012 Oct [cited 2017 Apr 5];302(4–5):187–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22939033

148. Knöckel J, Müller IB, Butzloff S, Bergmann B, Walter RD, Wrenger C. The antioxidative effect of de novo generated vitamin B6 in Plasmodium falciparum validated by protein interference. Biochem J [Internet]. 2012 Apr 15 [cited 2017 Mar 14];443(2):397–405. Available from: http://biochemj.org/lookup/doi/10.1042/BJ20111542

149. Butzloff S, Groves MR, Wrenger C, Müller IB. Cytometric quantification of singlet oxygen in the human malaria parasite Plasmodium falciparum. Cytom Part A [Internet]. 2012 Aug [cited 2017 Apr 5];81A(8):698–703. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22736452

150. Ménard R. Malaria : methods and protocols. Humana Press; 2013. 626 p.

151. Sambrook J, Russell DW (David W. Molecular cloning : a laboratory manual. Cold Spring Harbor Laboratory Press; 2001.

152. Crabb BS, Rug M, Gilberger T-W, Thompson JK, Triglia T, Maier AG, et al. Transfection of the Human Malaria Parasite <I>Plasmodium falciparum<I> In: Parasite Genomics Protocols [Internet]. New Jersey: Humana Press; 2004 [cited 2017 Mar 14]. p. 263–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15153633

153. Wrenger C, Müller S. The human malaria parasite Plasmodium falciparum has distinct organelle-specific lipoylation pathways. Mol Microbiol. 2004;

154. Müller IB, Knöckel J, Eschbach M-L, Bergmann B, Walter RD, Wrenger C. Secretion of an acid phosphatase provides a possible mechanism to acquire host nutrients by *Plasmodium falciparum*. Cell Microbiol [Internet]. 2010 May 1 [cited 2017 Mar 14];12(5):677–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20070315

155. Trager W, Jensen JB. In vitro cultivation of malaria parasites - Cultivation of erythrocytic stages*. Bull World Health Organ [Internet]. 1977 [cited 2017 Mar 14];55(3):363–5. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2366733/pdf/bullwho00446-

0223.pdf

156. Das Gupta R, Krause-Ihle T, Bergmann B, Müller IB, Khomutov AR, Müller S, et al. 3-Aminooxy-1-aminopropane and derivatives have an antiproliferative effect on cultured Plasmodium falciparum by decreasing intracellular polyamine concentrations. Antimicrob Agents Chemother [Internet]. 2005 Jul [cited 2017 Mar 14];49(7):2857–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15980361

157. Wu Y, Sifri CD, Lei HH, Su XZ, Wellems TE. Transfection of Plasmodium falciparum within human red blood cells. Proc Natl Acad Sci U S A [Internet]. 1995 Feb 14 [cited 2017 Mar 14];92(4):973–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7862676

158. Romano RL, Liria CW, Machini MT, Colepicolo P, Zambotti-Villela L. Cadmium decreases the levels of glutathione and enhances the phytochelatin concentration in the marine dinoflagellate Lingulodinium polyedrum. J Appl Phycol [Internet]. 2016 Aug 20 [cited 2017 Mar 16];1–10. Available from: http://link.springer.com/10.1007/s10811-016-0927-z

159. Hiller N, Fritz-Wolf K, Deponte M, Wende W, Zimmermann H, Becker K. Plasmodium falciparum glutathione S-transferase--structural and mechanistic studies on ligand binding and enzyme inhibition. Protein Sci [Internet]. 2006 Feb [cited 2017 Mar 14];15(2):281–9. Available from: http://doi.wiley.com/10.1110/ps.051891106

160. Müller IB, Knöckel J, Groves MR, Jordanova R, Ealick SE, Walter RD, et al. The Assembly of the Plasmodial PLP Synthase Complex Follows a Defined Course. Song H, editor. PLoS One [Internet]. 2008 Mar 19 [cited 2017 Mar 16];3(3):e1815. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18350152

161. Ertl P, Rohde B, Selzer P. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J Med Chem. 2000 Oct;43(20):3714–7.

162. Brenk R, Schipani A, James D, Krasowski A, Gilbert IH, Frearson J, et al. Lessons learnt from assembling screening libraries for drug discovery for neglected diseases. ChemMedChem. 2008 Mar;3(3):435–44.

163. Hawkins PCD, Skillman AG, Warren GL, Ellingson BA, Stahl MT. Conformer generation with OMEGA: algorithm and validation using high quality structures from the Protein Databank and Cambridge Structural Database. J Chem Inf Model. 2010 Apr;50(4):572–84.

164. Hawkins PCD, Skillman AG, Nicholls A. Comparison of shape-matching and docking as virtual screening tools. J Med Chem. 2007 Jan;50(1):74–82.

165. Tobergte D, Curtis S. Molecular Operating Environment (MOE). J Chem Inf Model. 2013;53(9):1689–99.

166. Smilkstein M, Sriwilaijaroen N, Kelly JX, Wilairat P, Riscoe M. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. Antimicrob Agents Chemother [Internet]. 2004 May [cited 2017

Mar 14];48(5):1803–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15105138

167. Croom E. Metabolism of Xenobiotics of Human Environments. In: Progress in molecular biology and translational science [Internet]. 2012 [cited 2017 Mar 14]. p. 31–88. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22974737

168. Saha D, Tamrakar A. www.asianpharmaonline.org Xenobiotics, Oxidative Stress, Free Radicals Vs. Antioxidants: Dance Of Death to Heaven's Life. Asian J Res Pharm Sci. 2011;1(2):36–8.

169. Perbandt M, Eberle R, Fischer-Riepe L, Cang H, Liebau E, Betzel C. High resolution structures of Plasmodium falciparum GST complexes provide novel insights into the dimer–tetramer transition and a novel ligand-binding site. J Struct Biol [Internet]. 2015 Sep [cited 2017 Mar 14];191(3):365–75. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1047847715300125

170. Meissner KA, Lunev S, Wang Y-Z, Linzke M, Batista FA, Wrenger C, et al. Drug target validation methods in malaria - Protein interference assay (PIA) as a tool for highly specific drug target validation. Curr Drug Targets. 2016;17(16).

171. Atamna H, Ginsburg H. Origin of reactive oxygen species in erythrocytes infected with Plasmodium falciparum. Mol Biochem Parasitol [Internet]. 1993 Oct [cited 2017 Apr 6];61(2):231–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8264727

172. Omodeo-Salè F, Motti A, Basilico N, Parapini S, Olliaro P, Taramelli D. Accelerated senescence of human erythrocytes cultured with Plasmodium falciparum. [cited 2017 Apr 7]; Available from: http://www.bloodjournal.org/content/bloodjournal/102/2/705.full.pdf?sso-checked=true

173. Gazzinelli RT, Kalantari P, Fitzgerald KA, Golenbock DT. Innate sensing of malaria parasites. Nat Rev Immunol [Internet]. 2014 Oct 17 [cited 2017 Apr 9];14(11):744–57. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25324127

174. Pandey A V, Bisht H, Babbarwal VK, Srivastava J, Pandey KC, Chauhan VS. Mechanism of malarial haem detoxification inhibition by chloroquine. Biochem J [Internet]. 2001 Apr 15 [cited 2017 Apr 7];355(Pt 2):333–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11284719

175. Bolchoz LJC, Gelasco AK, Jollow DJ, McMillan DC. Primaquine-induced hemolytic anemia: formation of free radicals in rat erythrocytes exposed to 6-methoxy-8-hydroxylaminoquinoline. J Pharmacol Exp Ther [Internet]. 2002 Dec 1 [cited 2017 Apr 7];303(3):1121–9. Available from: http://jpet.aspetjournals.org/cgi/doi/10.1124/jpet.102.041459

176. Haynes RK, Krishna S. Artemisinins: activities and actions. Microbes Infect [Internet]. 2004 Nov [cited 2017 Apr 7];6(14):1339–46. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1286457904002734

177. Roth EF, Raventos-Suarez C, Rinaldi A, Nagel RL, Nagel RL. Glucose-6-

phosphate dehydrogenase deficiency inhibits in vitro growth of Plasmodium falciparum. Proc Natl Acad Sci U S A [Internet]. 1983 Jan [cited 2017 Apr 6];80(1):298–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6337374

178. Liebau E, Bergmann B, Campbell AM, Teesdale-Spittle P, Brophy PM, Lüersen K, et al. The glutathione S-transferase from Plasmodium falciparum. Mol Biochem Parasitol [Internet]. [cited 2017 Apr 7];124(1–2):85–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12387854

179. Hubatsch I, Ridderström M, Mannervik B. Human glutathione transferase A4-4: an alpha class enzyme with high catalytic efficiency in the conjugation of 4-hydroxynonenal and other genotoxic products of lipid peroxidation. Biochem J [Internet]. 1998 Feb 15 [cited 2017 Apr 7];175–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9461507

180. Danielson UH, Esterbauer H, Mannervik B. Structure-activity relationships of 4-hydroxyalkenals in the conjugation catalysed by mammalian glutathione transferases. Biochem J [Internet]. 1987 Nov 1 [cited 2017 Apr 7];247(3):707–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3426557

181. Srivastava P, Puri SK, Kamboj KK, Pandey VC. Glutathione-S-transferase activity in malarial parasites. Trop Med Int Health [Internet]. 1999 Apr [cited 2017 Apr 7];4(4):251–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10320651

182. Harwaldt P, Rahlfs S, Becker K. Glutathione S-Transferase of the Malarial Parasite Plasmodium falciparum: Characterization of a Potential Drug Target. Biol Chem [Internet]. 2002 Jan 15 [cited 2017 Apr 7];383(5):821–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12108547

183. Fritz-Wolf K, Becker A, Rahlfs S, Harwaldt P, Schirmer RH, Kabsch W, et al. X-ray structure of glutathione S-transferase from the malarial parasite Plasmodium falciparum. Proc Natl Acad Sci [Internet]. 2003 Nov 25 [cited 2017 Apr 7];100(24):13821–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14623980

184. Deponte M, Becker K. Glutathione S-transferase from Malarial Parasites: Structural and Functional Aspects. In: Methods in enzymology [Internet]. 2005 [cited 2017 Apr 7]. p. 241–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16399390

185. Ahmad R, Srivastava AK, Tripathi RP, Batra S, Walter RD. Synthesis and biological evaluation of potential modulators of malarial glutathione-S-transferase(s). J Enzyme Inhib Med Chem [Internet]. 2007 Jan 4 [cited 2017 Apr 7];22(3):327–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17674815

186. Sturm N, Hu Y, Zimmermann H, Fritz-Wolf K, Wittlin S, Rahlfs S, et al. Compounds structurally related to ellagic acid show improved antiplasmodial activity. Antimicrob Agents Chemother [Internet]. 2009 Feb 1 [cited 2017 Apr 7];53(2):622–30. Available from: http://aac.asm.org/cgi/doi/10.1128/AAC.00544-08

187. Beutler E, Duparc S, G6PD Deficiency Working Group. Glucose-6-phosphate dehydrogenase deficiency and antimalarial drug development. Am J Trop Med Hyg [Internet]. 2007 Oct [cited 2017 Apr 9];77(4):779–89. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17978087

188. Fletcher KA, Barton PF, Kelly JA. Studies on the mechanisms of oxidation in the erythrocyte by metabolites of primaquine. Biochem Pharmacol [Internet]. 1988 Jul 1 [cited 2017 Apr 9];37(13):2683–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2839199