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Diagnosis and therapy of malaria under the conditions of a developing country - the example of Burkina Faso.

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ACT	Arteminsinin-based combination therapy
AE	Accidents and Emergency
AIDS	Acquired Immune Deficieny Syndrome
AQ	Amodiaquine
AS	Artesunate
BRENDA	Braunschweig Enzyme Database
CD	Chlorproguanil-Dapsone
CHR	Centre Hospitalier Régional
CHU	Centre Hospitalier Universitaire
CHUP	Centre Hospitalier Universitaire Pédiatrique
CM	Centre Médical
CMA	Centre Médical avec Antenne chirurgicale
CQ	Chloroquine
CSPS	Centre de Santé et de Promotion Sociale
DALY	Disability-adjusted life years
DDT	Dichloro-diphenyl-trichlorethane

DNA	Deoxyribonucleic acid
DHA	Dihydroartemisinin
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthetase
DIC	Disseminated intravascular coagulation
EUR	Euro
FCFA	Franc CFA
G6PD	Glucose 6-Phosphate Dehydrogenase
GDP	Gross Domestic Product
GFATM	Global Fund to fight AIDS, Tuberculosis, and Malaria
GMP	Good Manufacturing Practice
GNI	Gross National Income
GPI	Glycosylphosphatidylinositol
GR	Glutathione Reductase
GSH	Glutathione
GSSG	Glutathione Disulfide
HDI	Human Development Index
HIV	Human Immunodeficiency Virus
ICAM-1	Intra-Cellular Adhesion Molecule 1
iNOS	Inducible Nitric Oxide Synthetase
IPT	Intermittent preventive treatment
IRS	Indoor residual spraying

ITN	Insecticide-treated bed-nets
KEGG	Kyoto Encyclopedia of Genes and Genomes
LDH	Lactate Dehydrogenase
LPS	Lipopolysacharide
MB	Methylene Blue
mdr	Multi drug resistant
MQ	Mefloquine
mRNA	Messenger-Ribonucleic acid
NGO	Non-governmental organization
PAMPs	Pathogen-Associated Molecular Patterns
PARP	Poly-ADP-Ribose-Polymerase
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank
Pfam	Protein Families Database of Alignments and Hidden Markov Models
PfATP6	*P. falciparum* ATPase 6
PfCRT	*P. falciparum* Chloroquine-Resistance Transporter
PfEMP-1	*P. falciparum* Erythrocyte Membrane Protein 1
PfHRP II	*P. falciparum* Histidine-Rich Protein II
PfPgh1	*P. falciparum* P-glycoprotein homologue protein 1
PfHRP-2	*P. falciparum* Histidine-Rich Protein 2
Pfmdr1	*P. falciparum* multi-drug resistance gene 1

pLDH	Parasite Lactate Dehydrogenase
PPP	Purchasing power parity
RBC	Red blood cell
RBM	Roll Back Malaria
ROS	Reactive oxygen species
RT	Reverse Transcriptase
SERCA	Sarco-/Endoplasmic Reticulum Calcium ATPase
SMART	Simple Modular Architecture Research Tool
SP	Sulfadoxine - Pyrimethamine
SSA	Sub-Saharan Africa
TNF	Tumor Necrosis Factor
Tpx	Thiol peroxidase
UN	United Nations
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNDP	United Nations Development Programme
WHO	World Health Organization

Chapter 1

Introduction

Ourefata was two years old when he was brought to the emergency department of the university pediatric hospital Charles de Gaulle in Ouagadougou, Burkina Faso.
For two days now, the boy had been suffering from high fever, in spite of Paracetamol that his mother had given him for the fever. Early that morning, he had had a convulsion. Seeking help, his mother had brought him to the hospital. When they arrived, the child was comatous. He was febrile, breathing heavily, his skin was yellowish and pale.
In his blood, Plasmodium falciparum - the causative organism of malaria tropica - was found abundantly.
The child was given Quinine and i.v. - fluid with glucose to balance his low blood sugar. He was wrapped into wet cloth and recieved Aspirine to lower the temperature. As he was suffering from respiratory distress, oxygen was administered by a nasal cannula. He did not improve.
Ourefata died that very day in the evening, one of estimated 1 - 3 Million victims of malaria that year.

Worldwide, malaria alone is estimated to account for 5% of global disease burden. When narrowed down to children below 14 years of age, this number mounts up to 10% [WHO, 2006b] - as can be seen in figure 1.1.
World Health Organization (WHO) appraises the total number of malarial cases to 300 - 500 Million and death toll to 1 - 3 Million per year. Of those, more than 80% occur in children under five years of age in Sub-Saharan Africa (SSA) [WHO, 2005b].

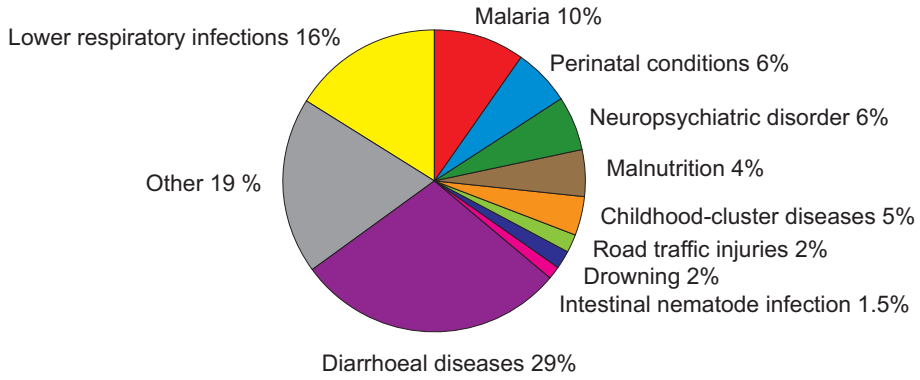

Figure 1.1: Global disease burden among children 0 - 14 years, measured in DALYs.
Data: World Health Report 2006 [15]

Reasons for this accumulation are multiple, ranging from distinct features of epidemiology, ecology and biology of both malaria parasites and vectors, to immunology of the exposed population.
Another important aspect of disease burden has only been focused on recently: the socio-economic background is in many ways mutually correlated with peculiarities of the disease, its impact on health care, economy and demographic development. Whenever discussing strategies to fight malaria, these interdependencies have to be adressed.
Decades back, during colonial hegemony throughout the endemic regions of malaria, financial aspects were scarcely relevant, fighting malaria used to be a matter of safeguarding trade relations as well as political and military power. The end of colonialism throughout the world also regularly meant a downfall of health care. After ongoing inner quarrels, urbanization, corruption and economic crisis took the better of the post-independency elation, far too often social networks were - if ever present before - neglected and left to decay.
Nowadays, situation has worsend most where limited resources prevent an effective and area-wide health system. Malaria has thus more and more become a disease of poverty [Sachs and Malaney, 2002] and poses immense burdens for population and economic prospects equally.

When in the 1950s thanks to new therapies and vector control measures malaria burden started to drop world-wide, SSA was the only area not to profit from this progress. Most

international concerted efforts to eradicate the disease were discouraged by fear of political, infrastructural, and financial drawbacks in SSA. Consequently, malaria incidence in SSA escalated since the 1970s (see figure 3.13), causing further decline of already crippled health systems.
Strategies to fight, control, or eradicate malaria include vector control, case management, and prophylaxis. These strategies need to be properly adjusted to the situation on-site: A successful treatment strategy for example has to take into account drug resistance profiles, but also economic feasibility, availablility and sustainable supply of required medication. Also, correct case management is vital for disease control.

In this thesis, therory and practice of malaria control in Burkina Faso, a developping country situated in Western Africa, are weighed against each other. Burkina Faso is hereby used as a representative example for a country in SSA with high endemicity for malaria. I will summarize and analyse the state of malaria control policy and its practical implementation in Burkina Faso, pointing out shortfalls in diagnostic as well as in treatment approaches, and specifically focus on MB as an attractive treatment alternative.
To achieve this, a portray of Burkina Faso's health system with its specific socioeconomic problems in regard to malaria is given, using data obtained from the ministry of health of Burkina Faso, and my own impressions during a 2005 visit to Burkina Faso.
Then, for a detailed view on the clinical challenges malaria is putting on this country, I describe the everyday clinical practice of malaria treatment in Burkina Faso. I will sketch typical features of malaria in pediatric patients I assessed in Burkina Faso, performing a retrospective study of case files from the university pediatric hospital in Burkina Faso's capital, Ouagadougou. Special interest is payed to clinical presentation and pre-treatment as important reasons for diagnostic and therapeutic shortcomings in everyday routine as compared to an optimal scheme proposed by WHO standards. This part is currently prepared for publication.
Current treatment policy strongly advocates Arteminsinin-based combination therapy (ACT) for malaria control. I will first sketch therapeutic principles and properties of these antimalarial drugs based on literature review. Special interest is laid on known targets, resistance features, and pharmacological properties. Artemisinin resistance is highlighted as a serious threat for current therapeutic strategies using homology mod-

elling of the putative target, PfATP6. One major drawback of ACTs is their price, which is highlighted using data on price ranges that were surveyed on-site through different channels (wholesalers, retailers, ministry of health data). Economic aspects of possible therapeutic strategies tend to be neglected by pharmacological studies.

In this context the use of Methylene Blue (MB) as a most interesting candidate for treatment of uncomplicated malaria is discussed. MB, the first synthetic drug ever used against malaria [Guttmann and Ehrlich, 1891], disappeared after other anti-malarial drugs (e.g. CQ) were introduced to the market in the 1960s. It was rediscovered some years ago [Schirmer et al., 2003], and currently MB is being tested in phase IIb trials in Burkina Faso [Coulibaly et al., 2009]. Previous studies could show that under the treatment with MB, no serious adverse advents occurred [Zoungrana et al., 2008]. MB treatment is well received in the local population [Schirmer et al., 2003].

The possible use of a MB-based combination strategy as alternative first-line therapy of uncomplicated malaria in Burkina Faso is best embraced by the acronym "bonaria" drug: "bon" in the Latin word stem stands for safe and effective, "a" for "affordable", "r" for "registered", and "ia" for "internationally available". A comprehensive approach combining bioinformatical analyses, literature studies, and cost-effectiveness analyses is chosen to illuminate the "bonaria" properties of MB: Bioinformatical analyses provide exciting tools to investigate new drug strategies or combination therapies for malaria. MB is a subversive substrate and specific inhibitor of *P. falciparum* Glutathione Reductase (GR). By pathway modelling some key aspects of a MB-based strategy are considered: resistance potential against standard drugs, model predictions, and measurements of specific additive pathways, and anti-resistance effects of MB. These data were also part of a recent publication [Zirkel et al., 2012].

A cost-effectiveness study shows the favourable properties of MB-based combination therapy compared to established drug combinations currently used in Burkina Faso.

Malaria control is feasible, but there are many obstacles still to overcome. Here, I use the example of Burkina Faso to illustrate how and why malaria control efforts often fail to reach those most in need of it. MB combination therapy is one potential solution for better achievement under these circumstances and for the first time comprehensively discussed in this thesis.

Chapter 2

Materials and Methods

In order to display a holistic approach to the current situation of disease burden and control measures, different investigation procedures were chosen:

2.1 Public Health Data

An extensive literature search of scientific databases (PubMed, GoogleScholar), WHO, UN, and World Bank data collections, as well as of online representations of the Ministry of Health of Burkina Faso was performed, and I interviewed employees and co-workers of the Ministry of Health in Burkina Faso. Especially Dr. Arsène Ouedraogo, director of pharmaceutical provisioning at the Mininstry of Health of Burkina Faso, was very helpful in establishing contacts and providing information.

2.2 Clinical Data

2.2.1 Setting and time frame

During a three months' elective stay at the Accidents and Emergency (AE) department of the Centre Hospitalier Universitaire Pédiatrique (CHUP) "Charles de Gaulle" in the capital of Burkina Faso, Ouagadougou, I was able to directly observe and participate in

the daily routine handling of malaria patients. Also, during another two weeks, I visited the hospitals in Yako and Nouna, two smaller towns in Burkina Faso, as well as community health centres in respectively near those towns.
In order to objectify these observations, I conducted a retrospective study evaluating the clinical records of patients admitted to the AE department of CHUP "Charles de Gaulle" with the consent of the hospital's authorities. Data were collected during the middle of the rainy season, from 31st of August, 2005 to 27th of September, 2005, which is the peak season of malaria transmission in Burkina Faso.

2.2.2 Ethical considerations, mode of data collection

During a three months' elective period, I participated in and observed the everyday clinical routine of patient care in different health care levels in Burkina Faso. The elective period was part of my medical training and was covered by the medical faculty of the university of Ouagadougou. Consent for this elective as well as my visits to other facilities of patient care in Burkina Faso (Hospital of Yako, Hospital of Nouna, Community Health Centres of Nouna and Yako) was each sought and given by the head of these institutions, in verbal and written form.
The photographs displayed in this thesis - unless otherwise identified - are taken during these visits. Where patients appear on these photographs, the informed consent of parents was sought prior to photographing the child.

In order to display and evaluate the disease burden as well as difficulties of diagnosis and treatment of malaria in practice, I was allowed to conduct a retrospective study, using the records of all children admitted to the AE department of the CHUP "Charles de Gaulle" during one month in the middle of the rainy season. With the consent of the hospital's authorities, I retrospectively collected data from the hospital's patient files and processed them anonymously. All data used in the following were exclusively taken and filed for therapeutic purposes and according to the clinical routine procedures of the hospital. I did not interfere in any diagnostic or therapeutic decisions, no additional assessment or treatment was performed on behalf of this study. Only after treatment had finished, these data were obtained from the hospital's patient files and anonymously

processed with the written permission from the clinic director as well as from the director of medical affairs (these include medical ethics, all operating procedures, auditing) of the pediatric university hospital "Charles de Gaulle" in Ouagadougou.

From 31st of August, 2005 until 27th of September, 2005 the number of all children recorded in the documentation of the AE department was taken down. All children aged zero to five years and being treated for malaria during their sojourn in the hospital were enclosed in the study. For those patients, their clinical data were retrieved from the hospital's records, anonymized and noted according to the standardized form that can be seen in figure 2.1, as far as possible. Children with confirmed other causes of illness, such as positive lumbar puncture for bacterial meningitis, were excluded.

No additional data or laboratory tests or any other tests were assessed or performed on behalf of this study. All given data were exclusively taken and noted for therapeutic purpose following the common clinical routine procedures of the hospital. Consequently, this resulted in a high number of cases that were lacking parts of clinical information: In a number of cases not all clinical findings were completely retrievable. Also, some laboratory examinations were not performed or noted. Reasons for these gaps predominantly were lacking documentation. Also, some examinations were not considered medically useful. As parents had to pay several laboratory exams separately, some were not performed as parents may not have been able to afford them and presumptive treatment was instead administered.

2.2.3 Statistical analyses

Basic descriptive summary statistics are reported and agreement was tested with Pearson χ^2. Differences in diagnostic characteristics (sensitivity and specificity) between severe malaria definitions were studied with logistic regression. The data was processed using SPSS 16, Excel2007, and R.

2.3 Available chemotherapy in Burkina Faso

Using literature studies (PubMed) as well as database searches (BioMed Central, Drugbank), I compiled a list of all currently available antimalarial drugs. A drug wholesaler in Ouagadougou, the capital of Burkina Faso, was interrogated and pointed out which of these drugs were actually available in Burkina Faso at which price. There are eight drug wholesalers in Burkina Faso: D.P.B.F. (Distribution Pharmaceutique de Burkina Faso), CO.PHA.DIS. (Coopérative Pharmaceutique de Distribution), LABOREX - Burkina, PHARMA-PLUS, FASO GALIEN, C.A.ME.G. (Centrale d'Achat des Médicaments Génériques), I.S.D.A. (Infusia Santé Diffusion Afrique), and CO.PHAR.ME.G. (Comptoir Pharmaceutique de Médicaments Génériques). The interrogation was conducted at D.P.B.F. in August, 2008, with the aid of Dr. Salam Sawadogo.

Prices were converted from Franc CFA (FCFA) to Euro (EUR) and then computed as price per treatment for comparison. Dosage and length of treatment were adapted from WHO standards. In case no WHO standards were available, suggestions of the manufacturer were cited. Treatment data always refer to children under five years of age. The calculation of price per treatment refers to a child of 10 kg, which was the mean weight of children treated in the monitored hospital. As this thesis concentrates on treatment of uncomplicated malaria, only application forms feasible at home were included (oral route or rectal suppositories).

2.4 Bioinformatical analyses

Bioinformatical approaches are useful tools to endorse and/ or to direct experimental findings aimed to achieve malaria control. As an example to display some of these methods, MB impact on Plasmodium and effects of drug resistance and drug combination were modelled. Also, to show how 3D protein modelling can help explaining and identifying (possible) resistance mechanisms, artemisinin resistance is simulated with such a 3D model of its putative target structure.

2.4.1 Homology modelling

Three-dimensional protein structures provide valuable insights into protein function, allowing an effective design of experiments, such as studies of resistance-related mutations or the structure based design of specific inhibitors useful as drugs. Despite new methods in the field of experimental structure solution, no structural information is available for the vast majority of protein sequences: Taken together, the UniProtKB/Swiss-Prot and TrEMBL databases [Wu et al., 2006, Jain et al., 2009] hold about 850,000 sequence entries, whilst only 20,000 experimental protein structures are deposited in the Protein Data Bank [Berman et al., 2003] (see Appendix A). This knowledge gap is currently best bridged by comparative or homology modeling, using software tools like Swiss-Model [Schwede et al., 2003]:

Model building requires at least one experimentally solved 3D-structure that has a significant amino acid sequence similarity to the target sequence and can thus be used as template. Homology modelling is performed by software tools like Swiss-Prot [Schwede et al., 2003] (`http://swissmodel.expasy.org/`) and comprises the following four steps:

1. Template selection: To select templates for a given protein (respectively its amino acid structure), the sequences of the template structure library are searched. If these templates cover distinct regions of the target sequence, and if at least one modeling template is available that has a sequence identity of more than 25% with the submitted target sequence, the automated modeling procedure will start.

2. Structural target-template alignment is then generated after removing incompatible templates by calculating a local pair-wise alignment of the target sequence to the identified template structures.

3. Model building: The backbone atom positions of the template structure are averaged to generate the core of the model. Templates with significantly deviating atom positions are again excluded. The template coordinates cannot be used to model regions of insertions or deletions in the target-template alignment. To generate the non-aligned parts of the protein model, and to reconstruct side chains, constraint

space programming is used: an ensemble of possible/ compatible structures is constructed from libraries; a scoring scheme, which accounts for force field energy, steric hindrance and unfavorable neighboring effects as well as favorable interactions like hydrogen bond or disulfide bridge formation is applied to select the most likely conformation. Deviations in the protein structure geometry, which have been introduced by the modeling algorithm when joining rigid fragments are regularized in the last modeling step by steepest descent energy minimization using the GROMOS96 force field [van Gunsteren et al., 1996].

4. Evaluation: Several tools are provided by Swiss-Model to allow the user to evaluate the reliability of the model. Inaccurate target-template alignments are the most frequent source of errors in models. This is especially true when the sequence similarity between the target and the template sequence drops below 40% [Schwede et al., 2003].

2.4.2 Modelling drug resistance

Resistance development can be visualized e.g. by homology modelling as presented above. Generally, structural modelling allows to a certain extent prediction of resistance development provided some mutations are known [Becker, 2011]. Here, as an example the proposed (main) drug target of artemisinins, *P. falciparum* ATPase 6 (PfATP6), is modelled with the active binding site for artemisinins. Point mutations in accordance with recent data [Mu et al., 2010] were included then to create resistance by an ill-fitting active centre.

2.4.3 Metabolic network modelling

Network-based pathway analysis helps comprehending the molecular mechanisms of a particular organism, correlating genomic and proteomic data with molecular physiology. To assess the properties that emerge from these networks, convex analysis is used. The latter is a branch of mathematics that enables the analysis of inequalities and systems of linear equations, to generate a convex set of vectors that can be used to characterize

a biochemical network [Papin et al., 2004]. The precondition for such a mathematical model is the balancing of all internal metabolites in a steady state flux, hence the term "flux balance analysis". Several bioinformatical tools have been developped in order to facilitate these network analyses, using computational linear programming algorithms [Orth et al., 2010]. Different approaches exist to analyse such networks, which, though closely related, exhibit quite distinct features. Two of these, elementary modes [Schuster et al., 2000] and extreme pathways [Schilling et al., 2000], are discussed further here to describe the effects of a multi-target drug like MB.

Mathematical representation of metabolism:

Biological networks can be represented by a stoichiometric matrix S of size n^*m. The columns and rows of this matrix correspond to the reactions (n) in the network and the involved metabolites (m), respectively. The entries in each column are the stoichiometric coefficients of the m metabolites participating in a reaction, a negative coefficient indicates a metabolite consumed, and a positive coefficient a metabolite that is produced. A stoichiometric coefficient of zero is used for every metabolite that does not participate in a particular reaction. The flux through all of the associated reactions in a network is represented by the vector v. At steady state, i.e. the state in which no internal metabolite accumulates or is depleted over time, mass balance in the network can be represented by the flux-balance equation:

$$Sv = 0 \tag{2.1}$$

The set of all possible solutions to equation 2.1 can be described by a set of basis vectors. Using convex analysis, a set of chemically valid (i.e. thermodynamically feasible) basis vectors is generated to describe the solutions to equation 2.1, by applying inequality constraints on the flux values of the irreversible reactions:

$$v_i \geq 0 \tag{2.2}$$

where v_i is the flux through reaction i.

A set of valid solutions to equation 2.1 subject to the constraints in equation 2.2 can be described as a high-dimensional cone that is located in a space where each axis corresponds to a reaction flux [Papin et al., 2004, Orth et al., 2010].

Elementary modes Elementary modes provide mathematical tools to define and comprehensively describe all biochemical routes that are both stoichiometrically and thermodynamically feasible within a given organism [Schuster et al., 2000, Dandekar and Sauerborn, 2002]. As shown above, all metabolic pathways are degraded into a unique set of elementary modes for a given network. Each elementary mode consists of the minimum number of reactions that it needs to exist as a functional unit ("genetic independence"). If any reaction in an elementary mode were removed, the whole elementary mode could not operate as a functional unit ("non-composability") [Schuster et al., 2000, Dandekar and Sauerborn, 2002]. Thereby, elementary modes form a set comprising all routes through a metabolic network [Papin et al., 2004].

Extreme Pathways The extreme pathways are the systemically independent subset of elementary modes that is, no extreme pathway can be represented as a non-negative linear combination of any other extreme pathways [Papin et al., 2004]: Satisfying the requirement of systemic independence can result in fewer extreme pathways than elementary modes: For example, linear combinations of the extreme pathways might satisfy the genetic independence requirement of the elementary modes. Therefore, the extreme pathways are not a set of all genetically independent routes through a metabolic network; rather, they are the edges of the high-dimensional convex solution space of a biochemical network, and as such are the convex basis vectors [Schilling et al., 2000, Papin et al., 2004].

2.4.4 Modelling combination therapies

Specific bioinformatical strategies are available to create drug combinations and tools to calculate combination effects of different drugs. Software tools like metatool, YANA [Schwarz et al., 2005], or YANAsquare [Schwarz et al., 2007] simulate the effect of drug combinations on metabolism based on different algorithms for flux balance analysis as discussed above, for instance. Topological analysis of a pathway network as calculated by elementary modes identifies readily which key enzymes have to be hit to block many routes in the network. Given target knowledge, the drop out of different pathways after

giving a combination of drugs is easily calculated [Schwarz et al., 2007, Becker, 2011]. Here, the use of extreme pathway calculation was used to evaluate the strain-specific metabolic networks of Plasmodium in regard to MB's mode of action and to model the effect of combination therapy involving MB.

Extreme Pathway Calculation

Calculation of all possible metabolic pathways: Extreme pathway calculation is used to evaluate the strain-specific metabolic network around known drug targets in all contained pathways. A stoichiometric matrix ($S : n^*m$) is prepared; the columns and rows correspond to the biochemical enzymes in the network (n) and the involved internal (produced or consumed within the network) metabolites (m), respectively. All of these internal metabolites have to be balanced. This is achieved by combining different enzymes so that in the end no internal metabolite accumulates or is depleted over time (see equation 2.1).

Calculation of pathway activities: Relying on a convex basis computation, a null-space matrix v capable of fulfilling the equation 2.1 is calculated. The quality of the match to experimentally measured gene expression values can be calculated as follows: observed gene expression is assumed to be proportional to enzyme expression and taken as a direct estimate of the observed enzyme activity E_{obs} according to gene expression data. In our case, gene expression data were obtained from Katja Becker and Stefan Rahlfs from the Interdisciplinary Research Center of Justus Liebig University, Giessen.
This is of course an over-simplification since there are many additional factors and mechanisms regulating enzyme activities. Furthermore, this assumes that without gene expression data all core pathways are used equally (flux value = 1). The changes, particularly stronger/ higher expression, are assumed to be proportional to the change in gene expression. However, these combined errors are greatly reduced by considering the complete network and the many available constraints according to the PCR-measured, significantly higher or lower gene expression data and by including the available time-resolved data from transcriptome databases (e.g. PlasmoDB).

First, each enzyme activity E_{pred} is calculated summing over the predicted activity A of each extreme pathway A_i containing this specific enzyme in its extreme pathway:

$$E_{pred} = \Sigma A_i \tag{2.3}$$

Next, for the complete system of enzymes with significant gene expression changes, the squared deviation between predicted enzyme activity E_{pred} and observed enzyme activity E_{obs}, as estimated according to gene expression data as mentioned above, is minimized:

$$min(\Sigma(E_{pred} - E_{obs})^2) \tag{2.4}$$

This least-squares error minimization task can be achieved via different strategies. For best results in this minimization, we first used the genetic algorithm in YANAsquare and then a steepest descent routine written in R [R Development Core Team, 2006].

2.4.5 Preparing the redox pathway model and the influence of MB

The bioinformatics analysis on the redox network effects by MB or other antimalarial drugs alone or in combination and compared in wild type and resistant mutations, was obtained step by step, refining the analysis results by including more experimental data (our own or public) in each step. The workflow is depicted in 2.2, the bioinformatical background of these steps has been described above. This part of my thesis was prepared in close collaboration with Alexander Cecil and Janina Zirkel and published [Zirkel et al., 2012].

First, genome annotation uses sequence analysis of the available data on the DNA sequence to identify all strain-specific enzymes and compared them to standard enzyme and pathway data from KEGG database. Central metabolic network with redox enzymes is set up accordingly, and all available pathways are calculated, solving the stochiometric matrix by elementary mode calculation. Model refinement by fitting the gene expression data to the measured enzyme activities achieves to describe how pathways and metabolic flow change under different conditions (infected / uninfected and over time). For resistance modeling, the enzyme activities for resistant/ sensitive strains are altered according to

antibiotic sensitivity using further gene expression data as constraints. Finally, predictions on drug combination effects by means of enzyme activity comparisons between sensitive and resistant strains are compared with available public clinical data.

Modeling of all pathways

Pathways of the central and redox metabolic networks: In order to set up the central metabolism of *P. falciparum* 3D7 with a focus on redox metabolism for building a network model, we used the database Kyoto Encyclopedia of Genes and Genomes (KEGG) [Kanehisa et al., 2008] and complemented the enzyme data given by it by using sequence analysis, expert knowledge, and literature [Michal, 1999]. The software package YANAsquare [Liang et al., 2011] provides a database import tool, the KEGG Browser. This tool was used to import pathways of the central metabolism of *P. falciparum* from the KEGG database [Kanehisa et al., 2008]. In order to achieve maximum model accuracy, proteins escaping previous annotation by KEGG were added to the preliminary pathway setup via sequence analysis and by incorporating data from PlasmoDB [Aurrecoechea et al., 2009]. The stoichiometric validity of the enzymatic reactions considered was checked according to literature [Michal, 1999], and the stoichiometric matrix of all enzyme reactions in the network was established (see table F.1). Then the set of all possible pathways that cannot be dissected any further - the elementary modes - was calculated. These now showed all metabolic pathways accessible for the system. At this stage, no transcription data or experimental mRNA expression data were incorporated.

Fluxes: In order to model all pathways of the system, the convex basis vectors [Papin et al., 2002, Larhlimi and Bockmayr, 2009] of the flux distribution were calculated applying YANA [Liang et al., 2011]. After calculating the set of all potential pathways, it was important to determine actual fluxes for different pathways in the model. For this we used expression data from PlasmoDB [Aurrecoechea et al., 2009] and the malaria transcriptome database [Bozdech et al., 2003] regarding the MB-free situation. The latter database contains a relative mRNA abundance for every hour of the intraerythrocytic cycle of parasite development based on a 70-mer oligonucleotide microarray. Next, enzyme

expression values and relative changes in the MB treatment situation were estimated according to RT-PCR results obtained from Katja Becker and Stefan Rahlfs, University of Giessen (Table 2.1)[1]

Table 2.1: Gene expression differences under the influence of MB: Results of RT-PCRmeasurements ofgene expression changes in cell culture. The gene expression changes were measured with 18SRNA.

Enzyme	Relative mRNA expression after 9h	Relative mRNA expression after 12h	Relative mRNA expression after 18h
Thioredoxin reductase	0.55 (± 0.002)	0.74 (± 0.003)	0.61 (± 0.002)
Glutathione reductase	0.73 (± 0.003)	1.49 (± 0.006)	1.2 (± 0.005)
Glyoxalase I	not determined	0.51 (± 0.002)	0.48 (± 0.002)
Glyoxalase II	0.9 (± 0.004)	0.36 (± 0.002)	0.38 (± 0.002)
Lactate dahydrogenase	0.7 (± 0.003)	0.93 (± 0.004)	1.93 (± 0.008)
1-Cys-peroxiredoxin	0.59 (± 0.002)	0.79 (± 0.003)	1.27 (± 0.005)
Glutaredoxin	0.88 (± 0.004)	0.63 (± 0.003)	0.7 (± 0.003)
Glutamate-cysteine-ligase	0.61 (± 0.002)	0.55 (± 0.002)	0.54 (± 0.002)
Thioredoxin-dependent peroxidase 1	0.45 (± 0.002)	0.49 (± 0.002)	0.39 (± 0.002)
Glutaredoxin-like protein 1	0.51 (± 0.002)	0.76 (± 0.003)	0.66 (± 0.003)

These enzyme activities were first-order (ignoring allosteric regulation etc.) approximated by applying YANA and R according to the best fit for the distribution of the flux modes for the given expression data. Enzymes catalyzing more than one reaction were considered with each reaction modeled independently in order to obtain a more accurate calculation for enzyme activity and convex base mode calculations.

[1]Gene expression studies in cell culture by RT-PCR, comparing gene expression changes after exposure to MB in correlation to a standard transcript indicated possible drug targets. As the standard, we employed 18SRNA, which was found to be very stable over the incubation period [Zirkel et al., 2012].

Modeling resistance mutations with and without MB: In order to calculate the effects of resistance against antimalarial drugs, we investigated different scenarios. The following enzymes were considered drug targets (in sensitive strains, see figures 4.19, 4.18, and 4.20: *P. falciparum* Chloroquine-Resistance Transporter (PfCRT) and *P. falciparum* multi-drug resistance gene 1 (Pfmdr1) for Chloroquine (CQ) resistance, and Dihydrofolate reductase (DHFR) and Dihydropteroate synthetase (DHPS) for Sulfadoxine - Pyrimethamine (SP) resistance (see Appendix B).

The activity of the drug-targeted enzymes in a sensitive strain under drug influence was defined to be zero but stayed normal for the resistant strains. All remaining enzyme activities were estimated according to the measured PCR data as well as the malaria transciptome database. Calculated fluxes were then fitted according to all these enzyme activity values. In this manner we created two clusters of four scenarios each (see Table 2.2; details for the calculated convex base pathway activities for all scenarios are given in tables F.2 to F.9). For more detail on the workflow of these calculations, please refer to [Schwarz et al., 2005, 2007].

Table 2.2: The different drug treatment scenarios

Scenario	Enzyme activities according to drug resistance phenotype			
	no. 1	no. 2	no. 3	no. 4
1: without MB	Wild type strain, no resistance	CQ-resistant strain: administration of sulfadoxine	Sulfadoxine-resistant strain: administration of CQ	Strain resistant to CQ and sulfadoxine: administration of CQ and sulfadoxine

Continuation of Table 2.2

Scenario	Enzyme activities accordingto drugresistance phenotype			
	no. 1	no. 2	no. 3	no. 4
2: with MB added	Wild type strain, MB administered	CQ-resistant strain: administration of sulfadoxine and MB	Sulfadoxine-resistant strain: administration of CQ and MB	Strain resistant to CQ and sulfadoxine: administration of CQ, sulfadoxine, and MB

Modeling *Plasmodium* resistance mutations and drug combinations

For detailed analysis of the resistance effects and drug combinations, we first simulated CQ-resistant *Plasmodium* parasites (PfCRT); in the CQ-resistant strain, hemozoin is formed by the oxidation of Fe^{2+} to Fe^{3+}, and Fe^{3+} to protoporphyrin IX even in the presence of CQ. Furthermore, the inhibition of DHFR and DHPS in a wild type strain was compared to plasmodial parasites resistant to such inhibition. In order to model the effects of these drugs on a sensitive strain *in silico*, we set the activities of drug-targeted enzymes to zero in order to simulate a best case scenario of complete sensitivity to the drug. For all enzymes that were not drug targets, we approximated their expression strengths, if available, by using the gene expression data [Bozdech et al., 2003]. Applying YANAsquare we fitted the complete network fluxes for three resistance scenarios (see below) according to the data from the gene expression database or PlasmoDB [Aurrecoechea et al., 2009, Bozdech et al., 2003]. Missing data were then calculated according to the total network fluxes, taking into account full or no activity for sensitive or resistant drug targets. Furthermore, for the scenarios with MB, the relative enzyme changes noted in the section above were taken into account as a first-order estimate for MB action.

numéro			
date, heures			
sexe			
age			
poids (kg)			
Histoire médicale (dernières 24 h):			
fièvre	oui	non	
convulsions	oui	non	
vomissement	oui	non	
diarrhée	oui	non	
manger/boire	bien	mal	rien
Traitement avant la consultation:			
referé de:			
médicaments:	spécification:		
antipaludiques			
anti-inflammatoires			
antibiotiques			
médicaments traditionels			
Examen			
asthénie	oui	non	
Conjonctives	pâle	moyen. colorée	normale
Tongue	pâle	moyen. colorée	normale
Palmes	pâle	moyen. colorée	normale
sclères (jaunisse)	oui	Non	
Symptômes			
Splénomégalie:	oui	Non	
Hépatomégalie:	oui	Non	
respiration:	battement d'aile du nez	tirages intercostales	normale
coma score (Blantyre)	réponse verbale	réponse motorique	mouvement des yeux
pouls			
fréquence de respiration			
température (rectal)			
Laboratoire:			
goutte épaisse	positive	negative	
Trophozoites : espèce			
Trophozoites : nombre			
hémoglobin tot.			
glycémie			
Sortie:			
amélioré	à la démande	décedé	

Figure 2.1: Standardized form for data collection from patient files

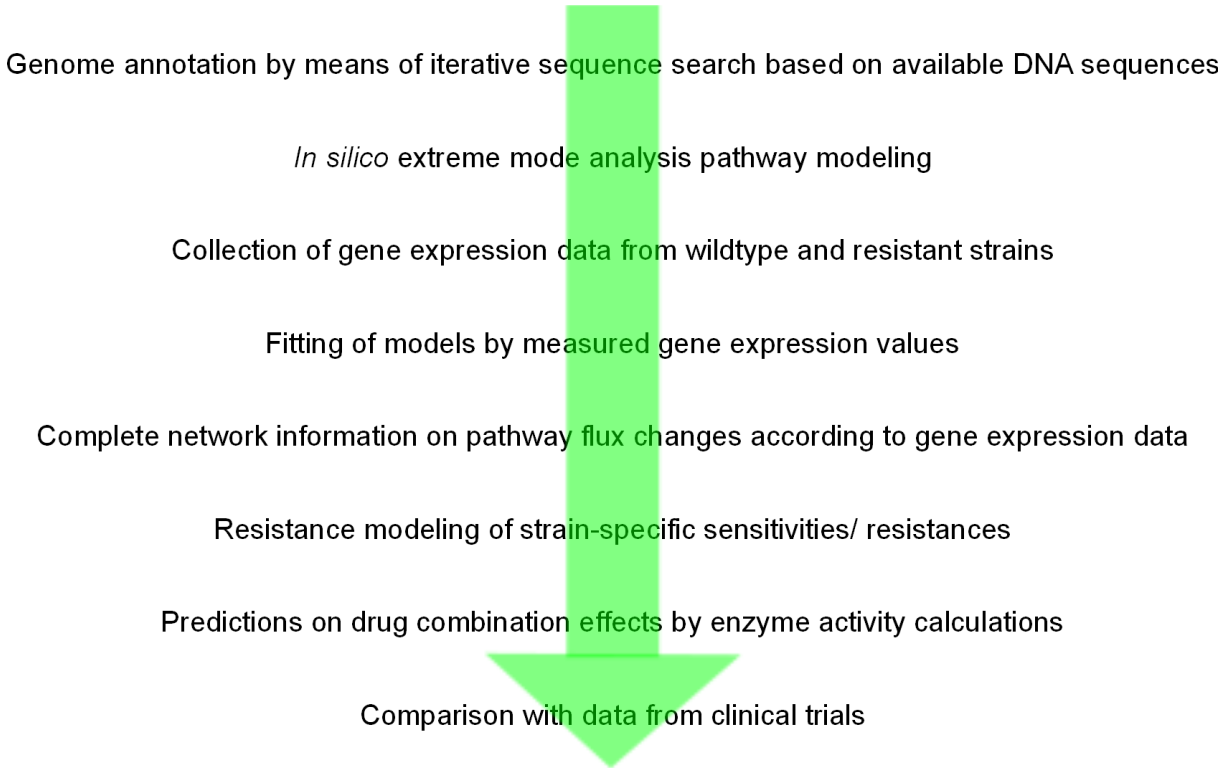

Figure 2.2: Workflow of redox pathway modeling

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Chapter 3

Theoretical foundations: aspects of malarial disease

3.1 Transmission and infectious cycle

3.1.1 Plasmodium spp.: the causative organism of malaria

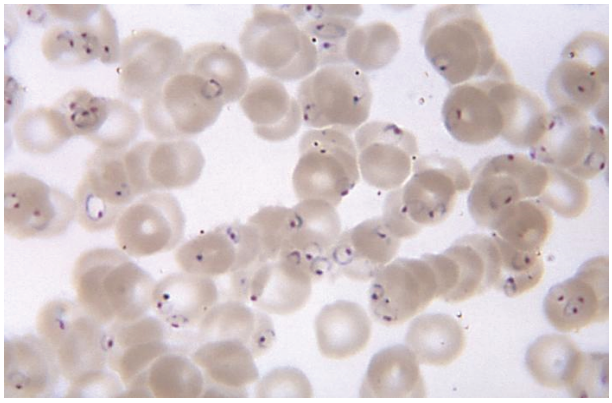

Figure 3.1: *P. falciparum* on blood smear micrograph: note the RBCs that contain multiple parasites, which is more common to *P. falciparum* than other Plasmodium spp. Photograph: Greene [6]

Malaria in humans is caused by five species of apicomplexan protozoa of the genus *Plasmodium*: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. The first three of the enumerated species are exclusively found in humans. That is, the schizogony

form, which is the causative organism of the disease malaria. The sporogony form is found in insect vectors, where sexual development occurs.

P. vivax, *P. ovale* and *P. malariae* are the causative agents of comparatively benign forms of malaria, Malaria tertiana (for *P. vivax* and *P. ovale*) and Malaria quartana (*P. malariae*). Only recently discovered *P. knowlesi* is responsible for malarial cases in South-East-Asia that bear features of falciparum malaria [Singh et al., 2004]. By far most of the severe cases of malarial disease are though attributable to *P.falciparum*, the causative agent of the potentially deadly malaria tropica [Warrell and Gilles, 2002]. Throughout SSA, more than 75 % of all malaria cases are due to *P.falciparum* [WHO, 2008c], this thesis will therefore focus on *P.falciparum* only.

3.1.2 Anopheles spp.: the vector for Plasmodium spp.

Figure 3.2: Anopheles gambiae. Photograph: Karim [7]

Anopheles is the only genus of mosquitos that is able to transmit human malaria. They are distributed worldwide, but are most common in tropical regions.

Mosquitos usually feed on plant juices - only when reproducing, female anophelines feed on blood to provide proteins and amino acids required for the maturation of eggs. Female *Anopheles* are predominantly attracted to mammals by a range of different chemical and physical stimuli. Once attracted - usually by night - they bite a minor incision into the host's skin, then enter the hole with their proboscis to search a capillary to start their blood meal on. During this process, mosquito saliva is pumped into the wound.

Blood is digested and within 2 - 3 days a batch of eggs is laid into free water. Passing

through aquatic developmental stages (eggs, larvae, pupae), after 7 to 20 days (under tropical conditions), the adult mosquito will emerge.

Temperature plays an important role in generation duration, which is why under colder climate the cycle is usually more extended in time.

Predominant in african countries are *Anopheles funestus*, *arabiensis* and *gambiae* (the two latter both belonging to the *Anopheles gambiae* complex). All of these express a high tendency to feed on humans only, they prefer rather late nightly hours for feeding and are most regularly found inside houses. Also, especially *Anopheles gambiae* is known for a very aggressive biting behaviour, feeding repeatedly from different people during one night and thus enhance the probability of spreading malaria immensely [Warrell and Gilles, 2002]. In figure 3.2 a picture of Anopheles gambiae can bee seen.

3.1.3 Infectious cycle

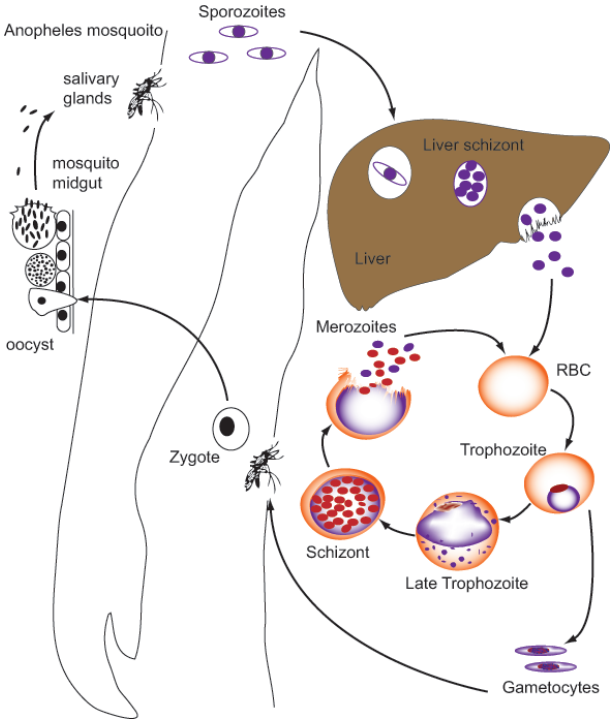

Figure 3.3: Simplified life-cycle of *P. falciparum*

When *Anopheles* bites a malaria-infected host, it will take up RBCs infected with gametocytes, the gamete-forming cells that perist in the peripheral blood of the host.

Within the gut of the mosquito, these gametocytes are triggered to form gametes. Consequently, male and female gametes will fuse to form the zygote, which again transforms into the ookinete. The ookinete then invades the mosquito's mid-gut wall where it differentiates into the oocyst. The nucleus undergoes repeated endomitosis whilst the oocyst increases considerably in size, resulting in a large, polyploid cell. When big enough, up to 8000 sporozoites are formed from the oocyst and emerge from the mid-gut to infect the salivary glands of the mosquito. There, after a whole duration of approximately ten days and one last maturation step, the sporozoites are ready to infect their next host.

Each time an infected mosquito bites, approximately 20 sporozoites are released into the victim. One usually is enough to cause an infection. After inoculation, the sporozoite is quick to invade hepatocytes, where the host cell form a parasitophorus vacuole that seperates the parasite from the host cell cytoplasm.
Thereafter, the sporozoite starts vegetative growth, often referred to as primary tissue schizogony. During this phase, the tissue schizont is feeding from its host cell. The schizont is forming some 30,000 merozoites. The host cell finally ruptures and gives way for the merozoites to enter the blood stream, where they within minutes invade RBCs. There, it resides inside a parasitophorus vacuole again and redifferentiates into the feeding trophozoite. When seen under the light microscope, trophozoites have a ring-like appearance with the nucleus at one side of the disc-shaped parasite (see figure 3.1). The cytoplasm of the RBC, mainly consisting of hemogloboin, is phagocytosed and digested. The unique problems arising from this specific diet will be discussed later on. Similar to primary tissue schizogony, again the nucleus will undergo repeated divisions, forming several merozoites that will enlarge until the erythrocytic hull bursts and releases toxic residual debris and merozoites into the blood stream. The newly released merozoites infect fresh erythrocytes. Whereas the majority of merozoites entering an RBC will re-enter the described cycle, a small fraction will develop into gametocytes, which then again can be taken up by mosquitos [Warrell and Gilles, 2002]. A simplified version of the infectious cycle can be seen in figure 3.3.

3.2 Characteristics of a life inside the RBC: Redox metabolism of *P. falciparum*

The unique lifestyle of malarial parasites inside the RBC consitutes an excellent shelter from the host's immune system. On the downside, it entails an enormous Achilles heel for the parasite: oxidative stress.

3.2.1 Hemoglobin digestion

When, as delineated above, the trophozoite lingers inside the RBC, it starts feeding from its host cell's cytoplasm, mainly consisting of hemoglobin. While the globin part is easily hydrolysed into its building blocks, ferriprotoporphyrin IX, more conveniently referred to as heme [Cohen et al., 1964, Aikawa, 1972, Chou et al., 1980], is set free. Heme is highly toxic, due to the high redox potential of its central component, iron [Orjih et al., 1981]. Also, heme accumulates in phospholipid barriers, destabilizing them and thus interfering with the maintenance of ionic balance in the cytosol and the food vacuole [Ginsburg et al., 1998, Zhang et al., 1999]. Different ways by which the parasite disposes of toxic heme are known to date:

Approximately 30% of heme is polymerised into insoluble crystals of hemozoin pigment [Ginsburg et al., 1998], which can be seen with the light microscope as brown crystalline malaria pigment. Heme has been shown by Slater et al. to be dimerized by an iron-carboxylate bond into insoluble β-hematin which consequently crystallizes into hemozoin [Slater et al., 1991, Pagola et al., 2000, Sullivan, 2002]. A "heme polymerase"-activity is needed to form β-hematin in vivo, but so far no single enzyme promoting this chemical step has been found in Plasmodium. Instead, a histidine rich protein as well as lipids (especially linoleates) derived from the food vacuole's membrane have been shown to promote β-hematin formation in living parasites [Dorn et al., 1995, Sullivan, 2002, Fitch, 2004].

Up to $70 - 80\%$ of freed heme escapes polymerization. Another way of detoxifying heme may be by a Glutathione (GSH)-mediated process of destruction [Atamna and

Ginsburg, 1995, Ginsburg et al., 1998, Zhang et al., 1999]: GSH is a tripeptide that occupies a central position in the maintenance of redox equilibrium of cells. It will be discussed in further detail later on in this chapter.

Free heme can also dissolve into, and translocate across, phospholipid membranes and in this way reach the cytosol of the parasite or that of the host cell, where further mechanisms exist for its detoxification. Eventually heme even exits the RBC and enters the bloodstream, where it binds to serum albumin or to hemopexin provided by the host [Ginsburg et al., 1998].

3.2.2 Oxidative stress and redox metabolism

The relationship between the redox status of malarial parasites and that of their host is complex, alterations in redox equilibrium contributing both to disease pathology and manifestation as well as to its abatement.

Its own metabolism induces much of this oxidative stress in *P. falciparum*: the degradation of host hemoglobin generates redox-active by-products abundantly, due to the high metabolic rate of the rapidly growing and multiplying trophozoite. These, like free heme and Reactive oxygen species (ROS), are highly toxic to the parasite as they heighten oxidative stress immensely and threaten the redox equilibrium crucial for the survival of any cell. The major neutralisation pathways for heme have been discussed in detail above, most importantly these include heme polymerisation [Slater and Cerami, 1992] and GSH-mediated heme degradation (by either parasite or host cell) [Atamna and Ginsburg, 1995, Ginsburg et al., 1998]. However, if even a small amount of free heme escapes these detoxification mechanisms, by its high redox potential it could cause severe damage to nucleic acids, proteins, lipids, and membranes, finally inhibiting parasite enzymes and lyse parasitised RBCs [Loria et al., 1999, Becker et al., 2004].

Apart from this metabolically derived oxidative stress, the host's immune response adds to the oxidative burden of the parasitised RBC by phagocytosis mechanisms and the production of nitric oxide and oxygen radicals [Becker et al., 2004]. As the latter also contribute to malarial disease, these mechanisms will be discussed in more detail elsewhere (see section 3.3.3).

Oxidative stress is thus an important clinical and pathological factor and obviously holds promise to be an effective therapeutic principle in malarial disease, as nature's own experiments suggest: inherited RBC defects that heighten oxidative stress in the host's RBC (such as Glucose 6-Phosphate Dehydrogenase (G6PD)-deficiency and sickle cell disease) prevail especially in countries where malaria is or was highly endemic, as they provide partial protection against this disease [Beutler et al., 1955, Eckman and Eaton, 1979, Atamna and Ginsburg, 1995].

To tackle the pro-oxidative environment in which the parasite has to survive, *P. falciparum* maintains a complex and tightly interweaved redox system that is designed to keep intracellular levels of ROS low and consists of a number of enzymatic and non-enzymatic antioxidants. Whilst a good review on these systems is given in [Mueller, 2004], here, I will only discuss those of special interest for the mode of action of MB:

The GSH - system:

GSH is a tripeptide that represents one of the major thiol redox buffer in almost all aerobic cells [Sies, 1989]. For a more precise review of the role of GSH in *P. falciparum* I recommend reference [Becker et al., 2003b]. The GSH - system comprises GSH itself, NADPH, GR, GSH-S-transferase, and glutaredoxin.
As can be seen in figure 3.4, GSH serves as an electron donor and is oxidised to Glutathione Disulfide (GSSG), whilst the latter is reduced back to GSH by the enzyme GR, using NADPH as an electron donor. To restore NADPH stocks of the cell, the resulting $NADP^+$ is reduced back to NADPH by G6PD and glucose-6-phosphogluconate dehydrogenase, which are key enzymes of the hexose monophophate shunt (also referred to as pentose phosphate pathway). The latter is a metabolic pathway using glucose-6-phosphate as substrate to generate energy equivalents [Becker et al., 2004].

The high ratio of [GSH]/[GSSG] necessary for an adequate antioxidant defence (10-100:1) is maintained through the concerted action of GR [Färber et al., 1998, Mueller, 2004] and the hexose monophosphate shunt, and an extensive de novo synthesis of GSH [Becker et al., 2004, Atamna and Ginsburg, 1997, Ayi et al., 1998, Luersen et al., 2000]. At the same time, the parasitised RBC looses its ability to synthesise GSH de novo because

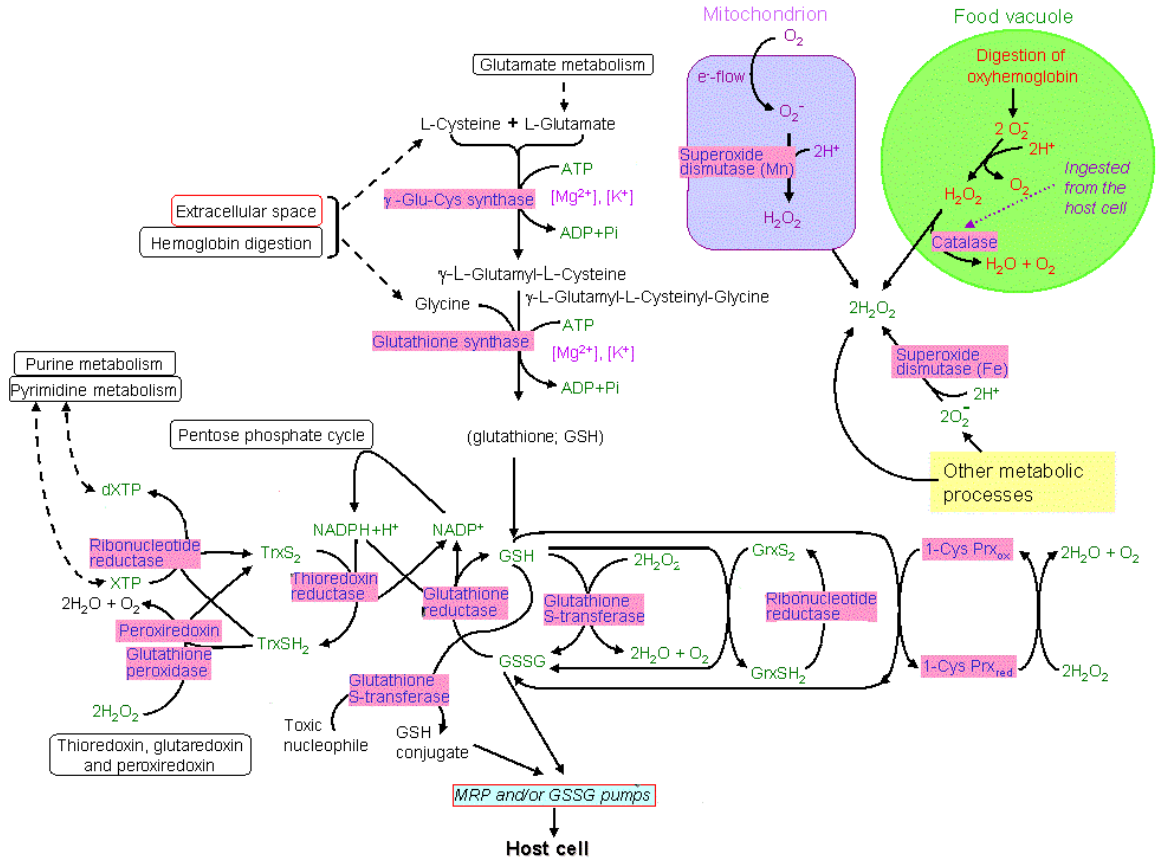

Figure 3.4: Redox metabolism in *P. falciparum*: details see text.
Illustration: Ginsburg [5]

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the intermediate γ-glutamyl-cysteine leaks out of the cell [Atamna and Ginsburg, 1997]. To compensate for this loss, GSSG is actively transported from the parasite into the host cell compartment where it is reduced to GSH by the resident GR and the highly up-regulated hexose monophosphate shunt. Thus, parasite and host cell cooperate in sustaining high GSH levels, while a net efflux of GSH is countervailed mainly by an extensive de novo synthesis from the parasite [Atamna and Ginsburg, 1997, Ayi et al., 1998, Luersen et al., 2000, Becker et al., 2004].

Apart from its central role as redox buffer, GSH also non-enzymatically degrades free heme [Atamna and Ginsburg, 1995], and is a cofactor for several redox-active enzymes such as GSH-S-transferase [Harwaldt et al., 2002] and glutaredoxin [Rahlfs et al., 2001]. The latter catalyse a variety of reactions, a number of which are still hypothetical:

P.falciparum possesses one GSH-S-transferase [Harwaldt et al., 2002], that may serve as hydroperoxide peroxidase (as GSH peroxidases are absent from the parasite's genome) [Becker et al., 2004, Mueller, 2004]. Also, it might stabilise sequestration of free heme [Harwaldt et al., 2002]. Glutaredoxin belongs to the thioredoxin superfamily, its typical "CPYC" redox active site [Rahlfs et al., 2001] confers interaction with proteins containing sulphydryl groups that need to be either oxidized or reduced. *P. falciparum* possesses one classic glutaredoxin and another glutaredoxin-like protein, named P. falciparum 1-Cys-glutaredoxin-like protein-1 [Rahlfs et al., 2001, 2002], the role of the latter remains to be elucidated.
(Classic) Plasmodium glutaredoxin was shown to protect cells against oxidative damage [Luikenhuis et al., 1998], catalyse ribonucleotide reductase and thus DNA synthesis [Holmgren, 2000], and they are associated with transcriptional control [Prieto-Alamo et al., 2000]. It also acts as an efficient reductant for plasmoredoxin, another member of the thioredoxin superfamily that is discussed in the following paragraph [Rahlfs et al., 2001, 2002, Becker et al., 2003a].

The thioredoxin system:

The proteins belonging to the thioredoxin superfamily are characterised by a specific active site "CxxC" motif and the so-called thioredoxin fold [Holmgren, 2000, Rahlfs et al., 2002]. They are redox messengers that interact with a variety of redox-active proteins and metabolites and are depicted in figure 3.4. The proteins comprising the thioredoxin redox system of *P. falciparum* are thioredoxin reductase, thioredoxin and thioredoxin-dependent peroxidases.

Thioredoxin itself is a small (approximately 12 kDa) protein with a typical "CGPC" active site motif [Kanzok et al., 2000, Krnajski et al., 2001]. When oxidised, the cysteine (C) residues form a disulphide and thereby reduce peroxidases resp. peroxiredoxins [Krnajski et al., 2001, Rahlfs et al., 2001], ribonucleotide reductase [Rahlfs et al., 2003] and GSSG [Kanzok et al., 2000]. The disulphide form is reduced back by NADPH-dependent thioredoxin reductase [Williams et al., 2000], which is an interesting drug target: *P.falciparum* thioredoxin reductase differs from its human analogue and knock-

out experiments of the gene coding for *P. falciparum* thioredoxin reductase had lethal effects on the parasite [Krnajski et al., 2002].

Thioredoxin-dependent peroxidases and peroxiredoxins are a ubiquitous group of peroxidases that is structurally distinguished into three classes determined by the number of active-site cysteines that exert their reductive activity [Wood et al., 2003]. *P. falciparum* possesses three genes encoding genuine peroxiredoxins, and a GSH peroxidase-like protein that preferably uses thioredoxin as a reducing cofactor [Rahlfs et al., 2001, Sztajer et al., 2001, Becker et al., 2004]. Peroxiredoxins efficiently reduce hydrogen peroxide and probably represent one of the major peroxide detoxifying proteins of the malarial parasite, especially as it lacks classic peroxidases such as catalase and GSH-dependent peroxidases [Krnajski et al., 2001, Sztajer et al., 2001, Kanzok et al., 2002, Rahlfs et al., 2002, Becker et al., 2003b,a, Mueller, 2004, Buchholz et al., 2008a]. Other functions of the peroxiredoxins remain uncertain and still need to be investigated further.
It remains to be stressed that all members of the thioredoxin superfamily have overlapping functions in *P. falciparum*, whilst thioredoxin reductase - as the only enzyme that restores the important co-factor thioredoxin - is essential for the survival of intraerythrocytic *P. falciparum* [Krnajski et al., 2002].

Plasmoredoxin

Plasmoredoxin was identified in the genome of *P. falciparum* by Becker et al., homology models showing sequence similarities with thioredoxin genes [Becker et al., 2003a]: The deduced amino acid sequence shares typical structural and functional characteristics with redox active proteins of the thioredoxin superfamily and contains the active site "WCKYC". Again, cysteine residues are arranged to form a disulphide when reduced. The purified gene product is reduced by GSH, but much faster by dithiols like thioredoxin and glutaredoxin. As demonstrated by Western blotting, the protein is expressed in blood-stage forms of malarial parasites. Potential roles of plasmoredoxin can be inferred from its functional similarities with other proteins of the thioredoxin superfamily. In vitro-experiments showed reduced plasmoredoxin to be active as reductive agent and to reduce GSSG. However, reduction of hydrogen peroxide and hydroxyethyl disulfide by

plasmoredoxin was negligible. Plasmoredoxin also provided electrons for ribonucleotide reductase, the enzyme catalyzing the first step of DNA synthesis [Becker et al., 2003a]. The plasmoredoxin gene is highly conserved and found exclusively in malarial parasites, which makes it an interesting candidate for drug design. Knock-out experiments revealed a non-vital role for plasmoredoxin, though [Buchholz et al., 2008a].

3.3 Malarial disease: clinical presentation and pathophysiology

3.3.1 Clinical presentation of uncomplicated malaria

When parasites enter their human host's blood stream, they immediately invade the liver where they undergo a pre-erythrocytic maturation step inside hepatocytes. During this part of the cycle, no symptoms nor functional impairment can be detected. The hepatocytic stage only leads to minimal histopathologic changes as only few hepatocytes are affected. Once the parasite reaches the erythrocytic cycle, pathological processes provoking malaria begin:

Fever is the most common symptom of malaria. This, as well as the other characteristic - but by far not specific - flu-like symptoms as headache and prostration, most probably arise from cytokines released from macrophages at the time of schizont rupture. Gastrointestinal symptoms, notably nausea, vomiting, diarrhea and abdominal pain are also commonly seen in *P. falciparum* malaria. The spleen playing a major role in parasite clearance enlarges considerably, showing both hyperplasia of red and white pulp and massive congestion of splenic sinuses by parsitized RBCs. Splenomegaly may contribute to thrombocytopenia, another common finding in *P. falciparum* malaria. The extent of thrombocytopenia shows some correlation with severity of illness, that may culminate in Disseminated intravascular coagulation (DIC). Hepatomegaly is also commonly seen in malaria, mainly reflected by sequestered blood cells, sinusoidal enlargement, and Kupffer cell hyperplasia. Hepatocytes usually show only mild changes with mitochondrial swelling and some storage abnormalities, as well as loss of microvilli of the bile canaliculi, which has

been suggested as the basis for hepatic dysfunction and cholestasis that may complicate malarial disease. [Warrell and Gilles, 2002, WHO, 2000]

Determinants of clinical presentation

In 1 - 2% the infection will turn into life-threatening disease, severe malaria [Snow et al., 1999]. Only recently the remarkable differences in clinical presentation of severe disease in different parts of the world have been adressed: for example, in Southeast Asia, a region of rather low transmission, severe malaria tends to affect all age groups and regularly presents as multi-organ-failure [White, 1987], whereas in Sub-Saharan-Africa - where high and stable transmission occurs - the disease mainly affects children under five and regularly presents as impaired consciousness, severe anemia and / or respiratory distress [Marsh et al., 1995]. Obviously, several aspects have to be taken into account to explain the clinical outcome of malarial disease that are summarized in figure 3.5:

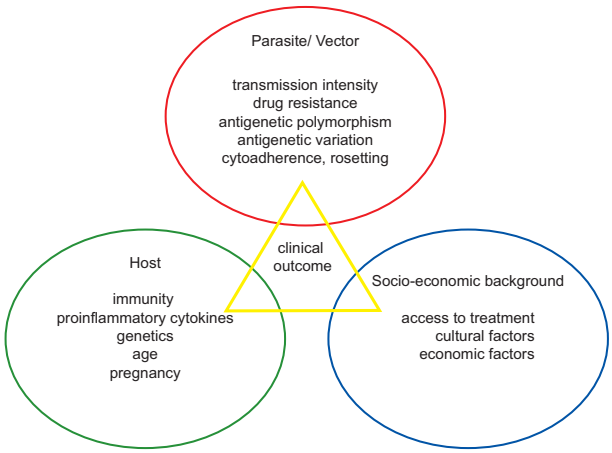

Figure 3.5: Clinical outcome of malarial infections is depending on many different factors, such as specifities of parasite, host and socio-economic background. Adapted from Miller et al. [10]

Semi-immunity Distinct features of host's immunology have been recognised for their role in modifying the symptoms of the disease: In holoendemic regions, patients exposed to repeated infections develop semi-immunity against the parasite, not clearing the infection, but minimalising the experienced symptoms [Bottius et al., 1996]. Semi-immunity is lost

when patients travel to other regions, facing different parasite strains, or when moving out of a holoendemic region for too long, as regular "training" intervals are needed to form semi-immunity. Also, when the immunity status of the patient is altered, e.g. in pregnancy [Gilles et al., 1969], old age [Dondorp et al., 2008b], or when acquiring HIV / AIDS [Wabwire-Mangen et al., 1989, Whitworth et al., 2000], susceptibility to *P. falciparum* is increased, which explains the higher risk malaria poses for these population groups.

In holoendemic regions of SSA, children under five years of age are those most at risk from malaria [Snow et al., 1999]. However, infants rarely acquire the disease: until about six moths of age, children remain remarkably resistant to malarial disease [Doolan et al., 2009]. This protection has long been thought to be associated with the presence of acquired maternal IgG-antibodies. This though plausible explanation was enfeebled by clinical studies showing antibody levels at birth not to be associated with resistance to malaria infection [Riley et al., 2000]. Alternatively, the protection of infants have been suggested to be associated with parasite growth-inhibiting factors such as lactoferrin and secretory IgA found in breast milk and in maternal and infant sera [Kassim et al., 2000]. Also, high percentage of fetal hemoglobin [Pasvol et al., 1976] acts as passive protection against *P. falciparum* infections. Beginning at around three to four months of age, infants become susceptible to severe disease, as the protection starts to vanish. The risk of severe disease increases with age and peaks in children two to four years old - depending on frequency of transmission. After this peak, from about five years of age, the frequency of clinical disease begins to diminish and the risk of mortality sharply decreases, when semi-immunity establishes [Doolan et al., 2009].

Genetic polymorphism Host genetic polymorphisms also alter the susceptibility to Plasmodia: sickle cell trait and G6PD deficiency are well known to protect patients from malaria, allowing these traits to spread widely e.g. in African populations [Beutler et al., 1955, Eckman and Eaton, 1979]. In West Africa, *P. vivax* has essentially disappeared, as this parasite relies on Duffy blood group antigen to invade RBCs, and the population in West Africa is largely Duffy blood group negative [Miller et al., 1976, 2002]. Several other genetic polymorphisms, mainly concerning RBC constitution and metabolism, as well as immune mechanisms, have been identified to have an impact on malaria mortality and

morbidity. To cover all of these would go beyond the scope of this thesis. A comprehesive review on this topic is given in reference [Verra et al., 2009].

3.3.2 Clinical presentation of severe malaria

Severe malaria identifies those patients at increased risk of dying. As this definition is rather difficult to assess for, WHO established a definition based on clinical signs and diagnostic patterns.

A patient presenting with one or more of the signs given in table 3.3.2 is suffering from "severe malaria" [WHO, 2000]. As my findings suggest (see section 4.2), this definition is not appliable in most holo-endemic settings: A number of included indicators are not routinely feasible (e.g. X-Ray, blood gas analysis, serum creatinine levels, or urine output measurements) in most affected develloping countries due to lack of financial, apparative, and personnel resources. While being sensitive, this definition is bound to over-estimate malarial cases due to the often non-decisive symptoms included (see section 4.2).

In a prospective study of 1844 children in a Kenyan District Hospital, Marsh et al [Marsh et al., 1995] showed that life-threatening malaria in African children is well predictable by the presence of three major clinical symptoms: cerebral malaria, respiratory distress and severe anemia (see figure 3.6). This rather simple approach is far better adapted, as it relies on clinical signs only (for hemoglobin measurements, see 3.4). Still, specificity of a case definition for severe malaria was shown to vary significantly: Another Kenyan study showed that among children under two years of age who presented with symptoms compatible with severe malaria, the malaria-attributable fraction of these cases was only 85%. This increased up to 95% when children with identified co-morbidities like meningitis, lower respiratory tract infection, bacteraemia, and gastroenteritis with severe dehydration were excluded, and a threshold of 2500 parasites/μL was applied. Other influencing factors established in this study were the intensity of transmission, and the markers of severity that were included [Bejon et al., 2007].

Table 3.1: Criteria for severe malaria as defined by WHO [WHO, 2000]

criteria for severe malaria	**defined as**
Prostration	Inability to sit, in young children who are not able to sit the inability to feed
Impaired consciousness	Inability to localize a painful stimulus (Blantyre Coma Score ≤ 4 [Marsh et al., 1995])
Respiratory distress	Any of the following: deep breathing, nasal flaring, intercostal indrawing [Marsh et al., 1995]
Repeated generalized convulsions	≥ 3 convulsions in 24 h
Circulatory collapse	Systolic blood pressure ≤ 50 mmHg (for age 1-5 years)
Pulmonary edema	Radiologic evidence of pulmonary edema
Abnormal bleeding	Spontaneous bleeding from gums, nose, gastrointestinal tract and/ or laboratory evidence of DIC
Jaundice	Visible jaundice (scleral icterus)
Hemoglobinuria	Macroscopic hemoglobinuria
Severe anemia	Hb ≤ 5 g/dl
Hypoglycemia	Whole blood glucose ≤ 40 mg/dl
Acidosis	Arterial pH ≤ 7.25 or plasma $HCO_3^- \leq 15$ mmol/l
Hyperlactataemia	Plasma lactate ≥ 5 mmol/l
Hyperparasitaemia	Parasite count $\geq 250{,}000$ parasites/µl (for non-immune patients)
Renal failure	In children: urine output ≤ 12 ml/kg/24h and/or serum creatinine ≥ 3 mg/dl

Cerebral malaria is one of the most common presentations of life-threatening malaria. It is characterised by altered consciousness in the presence of detectable parasitemia (and the absence of other obvious causes such as hypoglycemia or meningitis). Children often

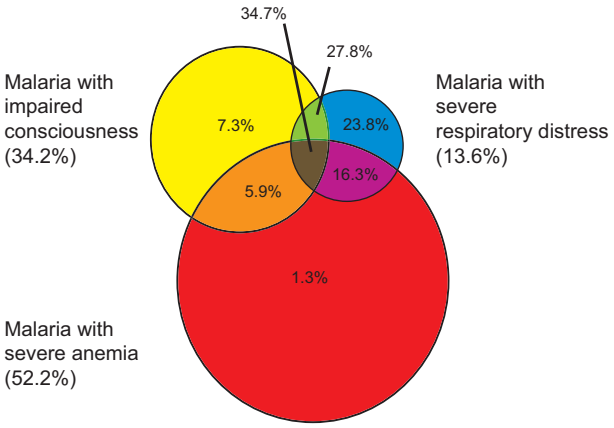

Figure 3.6: Prevalence, overlap and mortality for major clinical subgroups of severe malaria. Percentage in parentheses give prevalence of the given symptom, whereas within the circles, mortality is given as percentage of affected children. Adapted from Marsh et al. [9].

present with convulsions and/ or impaired consciousness. Coma develops quickly, is often preceded by (repeated) convulsions, and is commonly attended with abnormal posturing, such as opisthotonus (see figure 3.7), neurologic abnormalies (e.g. of pupillary reflexes) and altered breathing patterns. Examination of cerebrospinal fluid and blood glucose is important in these cases to exclude other pathologies that may mimic cerebral malaria and may be easily, respectively differently, treated. [Warrell and Gilles, 2002]

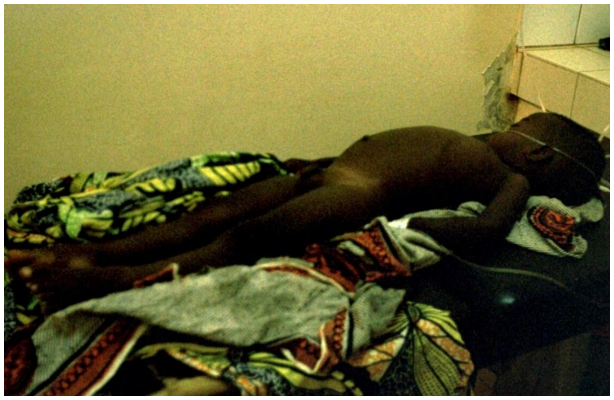

Figure 3.7: Opisthotonus in a comatose child suffering from malaria in Burkina Faso; the cerebrospinal fluid cell count was normal. Permission to publish was given.

Prognosis of cerebral malaria differs significantly for adults and children: case fatality rates range from 15 to 30%, but while more than 97% of surviving adults do not show

any detectable neurological residue, up to 12% of children are discharged with neurologic sequelae of some kind [Brewster et al., 1990].

Respiratory distress - characterised by deep breathing, intercostal recession and nasal flaring - is the chief clinical presentation of metabolic acidosis [English et al., 1996a] and has been shown to be a major predictor of a fatal outcome in malarial disease [Taylor et al., 1993, Marsh et al., 1995, English et al., 1996a, Maitland and Marsh, 2004].

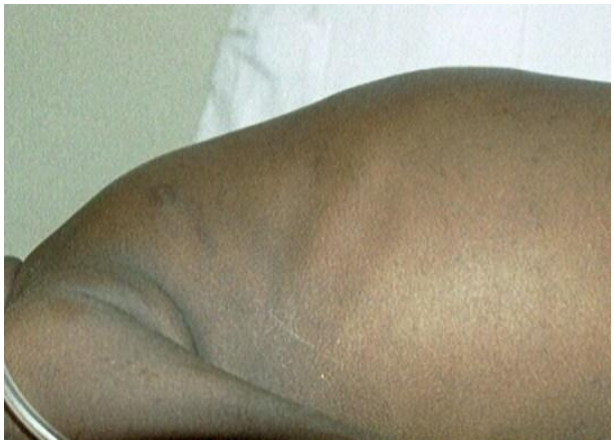

Figure 3.8: Respiratory distress in a child suffering from malaria in Burkina Faso; note the intercostal indrawings. Permission to publish was given.

Serum lactate may be high reflecting acidosis, but serum base excess was shown by English et al. to be best correlated with a fatal outcome [English et al., 1997]. Acidosis is strongly correlated with hypoglycemia [Taylor et al., 1993], another important risk factor of severe illness. Also, severe anemia may be present. Resuscitation with rapid transfusion of blood in those severely anemic or crystalloids, if total hemoglobin is above 5 g/dl, results in quick resolution of acidosis in the majority of cases [English et al., 1996b]. Respiratory distress in a febrile child may also hint towards pneumonia, which is why in settings where no imaging can be obtained, parallel treatment of both malaria and bacterial pneumonia may be reasonable [WHO, 2000].

Severe anemia is the most common single complication of severe malaria in children under five years of age. It is defined as a hemoglobin level below 5 g/dl. Useful clinical signs for anemia are nail-bed, palmar or conjunctival pallor, as well as prostration and

respiratory distress; the latter two may be absent in a child chronically anemic due to habituation effects.

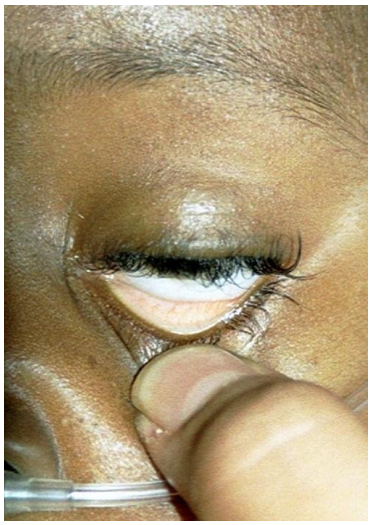

Figure 3.9: Anemia in a child suffering from malaria in Burkina Faso; conjunctival pallor is a useful clinical sign to detect anemia, hemoglobin level in this child was 3.8 g/dl. Permission to publish was given.

In malaria endemic areas of SSA, incidence rates of malaria-related anemia among children rates from 30 to 90% [WHO, 2000, Premji et al., 1995], indicating the huge morbidity burden exerted from malaria. Blood transfusion is a life-saving intervention for the severely anemic, however, the availability and safety of blood for transfusion is highly variable throughout the endemic regions of Africa. Notably, Human Immunodeficiency Virus (HIV) poses an important risk factor in many areas, therefore, blood should not be transfused unless the immediate risk of death from anemia is judged to exceed the risks of transfusion [Fleming, 1997, Menendez et al., 2000].

3.3.3 Pathophysiology of severe malaria

Pathophysiology of severe malaria is yet only scarcely understood and matter of intensive research. For years, severe malaria was pictured as essentially two major syndromes: (a) severe anemia and (b) cerebral malaria. Only in recent years has it been recognized that severe malaria is a complex multi-system, multi-organ disorder with many similarities to sepsis sydrome, although still there are many secrets to unveil.

Sequestration and cytoadherence Post-mortem studies have repeatedly demonstrated sequestration of parasitised RBCs within the capillary bed of many tissues, being greatest in the vasculature of the brain [MacPherson et al., 1985, Oo et al., 1987]. By sequestering within small capillaries, the parasite successfully compasses the passage through the spleen, where parasitised RBCs are usually filtered and destroyed [Stich et al., 2000].

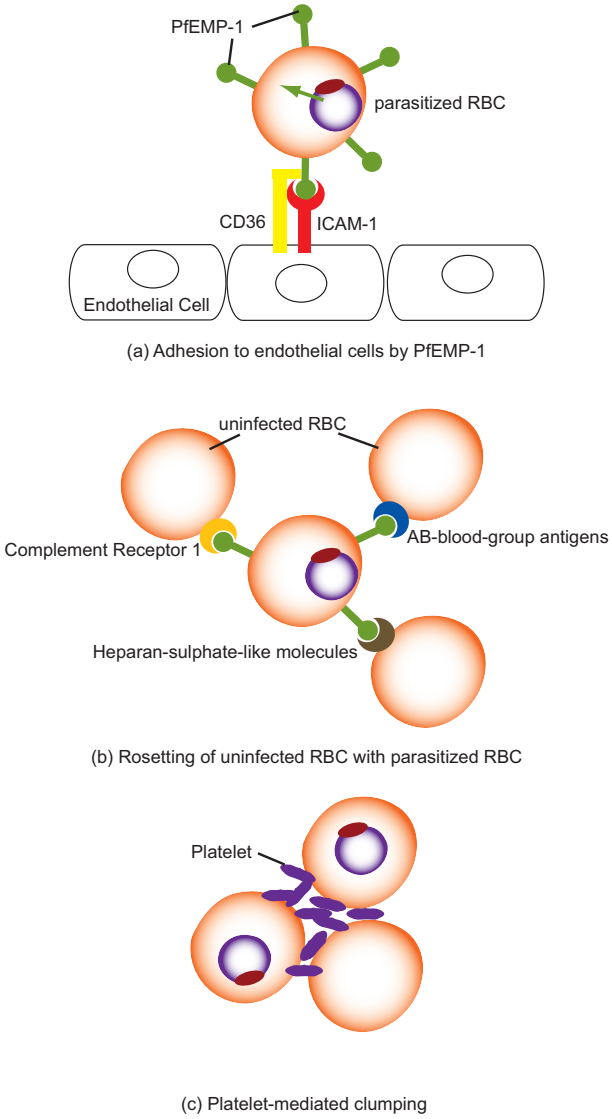

Figure 3.10: (a) Endothelial adherence of parasitised RBC by PfEMP-1,
(b) rosetting of non-infected and parasitised RBC, and
(c) platelet-mediated clumping.

Adhesion to the endothelium is mediated by *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP-1), a protein coded by the highly variable *var* - gene - cluster in *P.*

falciparum [Baruch et al., 1995, Smith et al., 1995, Su et al., 1995]. PfEMP-1 is expressed during the second half of the intra-erythrocytic growth cycle. It appears on the surface of parasitised RBCs, concentrating in so-called knobs, visible structures on the membrane of affected erythrocytes [Udeinya et al., 1981, Roberts et al., 1985, Howard and Gilladoga, 1989]. There, PfEMP-1 binds to several endothelial receptors such as Intra-Cellular Adhesion Molecule 1 (ICAM-1) (also referred to as CD54) and CD36 [Berendt et al., 1989, McCormick et al., 1997] and consequently sequesters parasitised RBCs in capillaries by a rolling mechanism similar to the adhesion of leucocytes during an inflammatory reaction [Howard and Gilladoga, 1989, Nash et al., 1992]. Endothelial cells in different tissues do express different and variable amounts of receptors; to successfully adhere to these cells, the parasite can bind to several receptors. By similar means, rosetting of infected with uninfected erythrocytes [David et al., 1988, Udomsangpetch et al., 1989] and platelet-mediated clumping occurs [Pain et al., 2001], as can be seen in figure 3.10.

However, it has proven rather difficult to ellucidate how adhesion progresses to pathology. Several mechanisms have been proposed, including impaired endothelial cell function by the adhesion of infected RBCs, obstructed blood flow [Dondorp et al., 2004b, 2008a] and consequent acidosis [Planche and Krishna, 2006] as well as production of damaging pro-inflammatory mediators as a result of adherence [Schofield, 2007].

Inflammatory mediators have more and more been recognized for their central role in malaria pathology, and the striking resemblance of severe malaria with septic reactions has led to the concept of similar pathomechanisms in these diseases. An excellent review on current understanding of these interactions, briefly displayed in the following and figure 3.11, is given in reference [Clark and Cowden, 2003].

It is important to realise that systemic inflammatory response is a highly regulated event. Provoked by toxins (e.g. LPS of gram-negative bacteria) that are commonly summarised as Pathogen-Associated Molecular Patterns (PAMPs), host cells (lymphocytes, macrophages, endothelial cells etc.) release pro-inflammatory soluble protein molecules that induce an intricate network of other molecules that activate and mediate cellular defenses [Bone, 1991]. In case of falciparum malaria, this "malaria toxin" is now widely

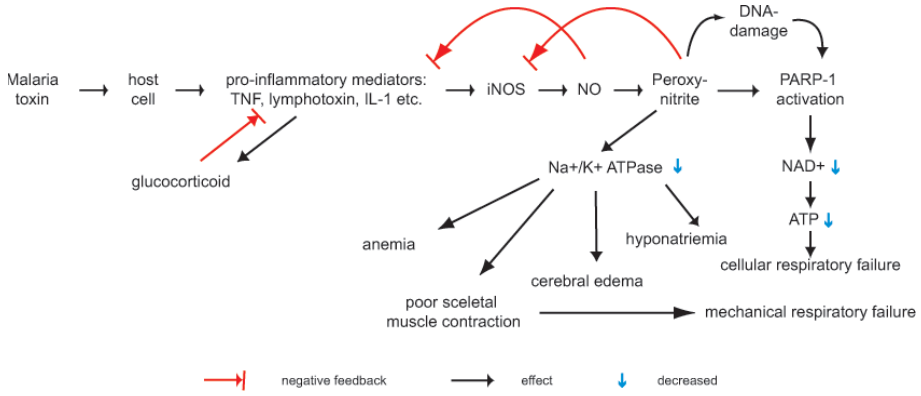

Figure 3.11: Role of inflammatory cytokines in malaria pathogenesis. Illustration adapted from Clark and Cowden [3]

thought to be a GPI-toxin derived from malarial parasites [Schofield and Hackett, 1993]. These pathways have to be carefully balanced by anti-inflammatory mediators and pathways, though: should they be up-regulated excessively, they begin to alter host physiology and cause illness. Fatal pathology may result if the provoking toxin is potent, abundant, or both [Bone, 1991, Clark and Cowden, 2003].

One of the first and therefore best studied pro-inflammatory mediators in acute illness is TNFα: it was found in malaria patients in direct proportion to the severity of illness [Grau et al., 1989, Kwiatkowski et al., 1990], and its central role in inflammatory reactions not only to bacterial endotoxins, but also in auto-immune diseases and tumor cell defense has been described *in extenso* elsewhere. Other pro-inflammatory mediators, such as lymphotoxin and IL-2 are less well studied, but probably contribute no less to disease pathogenesis [Clark and Cowden, 2003]. Nitric oxide (NO) seems to play a central role in downstream processes, as the above mentioned inflammatory mediators, as well as tissue hypoxia induce NO production via Inducible Nitric Oxide Synthetase (iNOS) [Kilbourn and Belloni, 1990, Rockett et al., 1991, 1992]. NO itself is involved in many different networks that are yet not fully understood. Among others, it regulates blood flow [Kilbourn et al., 1990], influences cell membrane potentials by inhibiting ATPase-driven Na^+/K^+ pump [Guzman et al., 1995], increases the expression of glucose transporter GLUT-1 [Balon and Nadler, 1996], and is suspected to impair mitochondrial respiration, thus blocking ATP-synthesis [Castro et al., 1994, Cleeter et al., 1994, Hibbs et al., 1988, Stuehr and Nathan, 1989]. Its derivative peroxinitrite is thought to play a major role here,

but it also leads to further energy depletion: Poly-ADP-Ribose-Polymerase (PARP), a nuclear enzyme selectively activated by DNA strand breaks, catalyses the successive transfer of ADP-ribose units from its subtrate NAD^+ to a variety of substrates. Peroxinitrite has been described to over-activate PARP, thus leading to NAD^+ and consequent ATP-depletion [Khan et al., 2002, Szabo, 1996]. PARP also interferes in gene expression, as it induces a central transcription factor, NFκ, that again enables the expression of several genes essential to pro-inflammatory pathways, including synthesis of Tumor Necrosis Factor (TNF), various interleukines, ICAM-1 and iNOS [Hauschildt et al., 1992, Le Page et al., 1998]. Figure 3.11 summarizes the above described reactions. It has to be stressed that - though intriguing, as many features of severe malaria may be explained thus - these connections are to date mainly hypothetical and have not been shown *in vivo* to apply for severe malaria in full.

3.4 Diagnosis of malaria

3.4.1 Clinical diagnosis

Malaria is usually and best confirmed by microscopic evaluation of a stained thick and/or thin blood film. Microscopy enables the identification of parasites at a species level, it is quantitative and has a remarkable sensitivity that so far has not been out-performed by various modern techniques like antigen detection methods, Polymerase Chain Reaction (PCR) and quantitative buffy coat technique [Warrell and Gilles, 2002].

In situations in which laboratory facilities are not available - as in much SSA - a presumptive diagnosis may need to be made on clinical features alone, a fact that led to the development of clinical algorithms that are adapted to local needs [Redd et al., 1996]. However, although clinical algorithms might be easy to apply for minimally trained health care workers and have been argued thus to be cost-effective, there still is a significant risk of over-diagnosis and over-treatment in holoendemic areas [Font et al., 2001], whilst in areas with only seasonal transmission these algorithms have been shown to fatally underestimate the number of malarial cases [Luxemburger et al., 1998]. Studies in Malawi even suggested that microscopy-based diagnoses may actually be more cost-effective than

presumptive treatment [Jonkman et al., 1995]. Still, one has to keep in mind that in *falciparum* malaria, based on the principle of cytoadherence illustrated above, microscopy can sometimes be false-negative when only once examined [Delley et al., 2000]. Thus, treatment should never depend on microscopy alone and should not be delayed in a case where severe disease is clinically suspected.

3.4.2 Blood examination for malaria parasites

Microscopy

The thick film (or drop, as it sometimes is alternatively termed) provides the sensitivity to detect even low parasitemia, whereas the thin film provides the specificity and species determination.

The thick film is not fixed prior to staining, so that the RBCs are lysed, allowing parasites to be seen in a much larger volume of blood. A drop of blood (preferably from capillary blood withdrawal) is applied on a clean glass slide, the blood is evenly spread with the corner of another slide to make a circle with moderate thickness that will just allow one to read through it. After drying, the slide is stained with Giemsa stain for 20 - 30 minutes, rinsed and after drying evaluated under the microscope.

For a thin film, a small drop of blood is deposited on a glass slide. Then, it is spread evenly by the means of another glass slide, so that an unbroken layer of single blood cells with a "tongue" not touching the edge of the slide is generated. The thin film has to be fixed with methyl alcohol and then stained identical to the thick film.

The thick film method is, in practised hands, by far the best for general clinical use. Standard practise requires that 100 to 200 microscopic fields have to be examined to establish the diagnosis. In case of a negative slide, repeated blood films must be taken every four hours and examined, as in *P. falciparum* infections the parasites may be sequestered in capillaries in 50% of the erythrocytic cycle and therefore are not always present in the peripheral blood [Warrell and Gilles, 2002].

Rapid tests

Rapid tests for malaria have become available at a wide range in recent years. So far, none of those were able to out-perform microscopy [Ashley et al., 2009, Zikusooka et al., 2008]. However, microscopy requires expertise and equipment, both of which are often not procurable in endemic settings, where public health structures are poorly or non-functioning. Rapid tests may fill the diagnostic gap in these settings, as they can be utilised by minimally skilled personell at a reasonable price (cost per test approximately US$ 1,00) [Proux et al., 2001]. Rapid tests detect parasitic antigenes. So far, methods based on immunochromatographic detection of *P. falciparum* Histidine-Rich Protein 2 (PfHRP-2) and the Parasite Lactate Dehydrogenase (pLDH) have been commercialized:

ParaSight-F® (produced by Becton Dickinson) was the first rapid malaria antigen test to be developed. It detects PfHRP-2, a molecule present in the parasite throughout the erythrocytic cycle of *P. falciparum*. A monoclonal antibody to PfHRP-2 is bound to a nitrocellulose/ glass-fibre dipstick, forming a line across the dipstick, that, when in contact with lysed infected blood, will bind the specific antigen of *P. falciparum*. The positivity of the test is then visualized by a polyclonal anti-PfHRP-2 antibody labelled with a coloured marker, which produces a visible line on the dipstick [Warrell and Gilles, 2002, Shiff et al., 1993].

By similar means, OptiMal® (produced by Flow Inc.) detects pLDH, that is produced by all human malaria parasite species during intra-erythrocytic growth. By detecting anti-genetic differences between pLDH isoforms, this test also differentiates between malaria species [Warrell and Gilles, 2002, Piper et al., 1999].

3.4.3 Further laboratory tests

Other laboratory tests may need to be performed to detect signs of severe disease: Hemoglobin levels to detect (severe) anemia are generally measured photometrically, for settings in which no laboratory is accessible quickly enough, WHO has developped a colour scale that is easy and cheap to apply [Lewis et al., 1998].

To detect hypoglycemia, which is essential in a comatose patient, blood glucose measurements are needed. Based on photometric measurements, there is a variety of different apparatuses, especially a number of portable ones that are easy to handle, but unfortunately rather expensive due to the cost of test strips.

Blood gas measurements may be needed to objectify the presence and extent of acidosis, but again, these apparatuses are expensive in acquisition and attendance.

Interestingly, to the best of my knowledge, only little research has been done on cost-effective and easy-to-apply alternatives to measurements of blood glucose and - gases, although hypoglycemia and acidosis are major risk factors of malaria mortality and in the majority of cases relatively well treatable. Especially as allocation of resources to diagnostic laboratory testing is often not a priority for resource-limited health care systems, this might be a field worth exploring, as insufficient laboratory diagnostic testing leads to unnecessary expenditures due to over-treatment and compromises patient care [Petti et al., 2006].

3.5 Strategies of malaria control

3.5.1 Economic impact of malaria

Where malaria prevails, economic prospects are bleak. As illustrated in figure 3.12, poverty on a global scale is concentrated in the tropical and subtropical zones, the same geographical boundaries that most closely frame malaria transmission. Analyses of Sachs and colleagues [Gallup and Sachs, 2001, Sachs and Malaney, 2002] were able to show that economic growth in countries in which a high proportion of the population lived in regions of *P. falciparum* malaria transmission was constricted substantially due to malaria, even after controlling for the other standard growth determinants used in macroeconomic analyses. According to their studies malarious countries lost an average growth rate of 1.9% per-capita Gross Domestic Product (GDP) per year in between 1965 and 1990 [Sachs and Malaney, 2002].

The extent of malaria burden as well as the accuracy of the correlation of poverty and

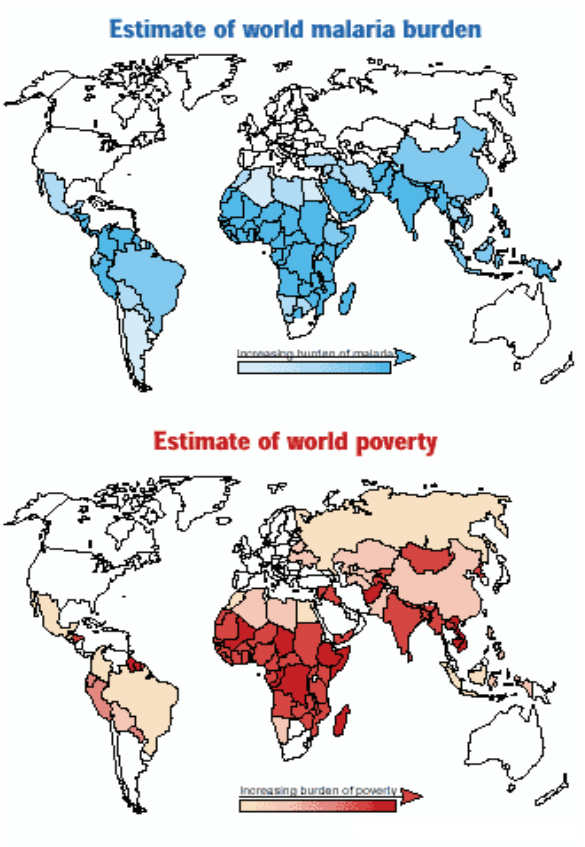

Figure 3.12: Poverty and malaria burden worldwide.
Figure from Roll Back Malaria campaign [11].

malaria strongly suggest their vice versa interdependencies to express a causality rather than a coincidence.

Prevention, diagnosis, and treatment of the disease imply high private and public medical costs, as will be discussed in more detail later. A dimension even more important in terms of economy is foregone income, which generally is estimated by calculating the value of lost workdays as a result of malaria, based on estimated wages/ income. In the case of mortality, foregone income is estimated by calculating the capitalized value of future lifetime earnings that would have been earned by those who died prematurely as a result of the disease, based on projected incomes for different age groups, basic longevity data and age-specific mortality rates [Sachs and Malaney, 2002]. Studies estimated the average foregone income to approximately 1% of GDP in malarious countries.

Nonetheless it is argued by many economists that these accounts still underestimate

the effects of malaria on longterm economic growth and development as malaria imposes economic costs well beyond direct medical costs and foregone incomes. Easiest to comprehend, though difficult to assess, are macroeconomic costs that arise from the negative impact of endemic malaria on human resources, trade, foreign investments, and tourism: Residents of sites of holo-endemic malaria transmission generally develop semi-immunity, diminishing morbidity and mortality of this disease [Bottius et al., 1996]. Semi-immunity is quickly lost in the absence of regular re-infection, such as during a period of education or employment away from the malarious region, which especially affects highly skilled personnel. Also, antigenic diversity limits the effect of semi-immunity geographically, hindering human mobility even within malarious regions as movements of this kind increase risk of death or disease for the concerned. By limiting such movement, malaria implies economic cost because human mobility permits labour to move to regions where it is most productive [Sachs and Malaney, 2002].

Risk of malaria infection also depresses economic incentives to expand markets into malarious regions, and foreign investors are likely to be discouraged by fear of disease-related loss of productivity for both their native and expatriate employees. Industries such as tourism are particularly hard hit by malaria transmission, as tourists understandably shun the risk of acquiring a potential life-threatening disease. In a globalized economy in which international trade and finance is critical for economic development, these adverse effects on foreign trade and investment are likely to be of tremendous macroeconomic importance [Sachs and Malaney, 2002].

While it seems impossible to grasp the human suffering resulting from one to three million deaths caused annually by the disease, the economic value of resources invested in infants who do not survive into adulthood can be significant, too. Estimates of the percentage of productive time parents spend in child-rearing show that substantial cost arise from high child mortality rates: For the example of Ivory Coast (a neighbouring country of Burkina Faso), sociologist David Reher calculated productive time "lost" by parents for a child that does not survive to its fifth birthday to avarage 1516 hours per child [Reher, 1995].

Historical evidence has shown that high infant and child mortality rates are linked

closely to high fertility rates [Rosenzweig and Schultz, 1983, Yamada, 1985, Galloway et al., 1998]. The disease will thus lead to a high fertility rate in regions of intense malaria transmission [Sachs and Malaney, 2002]. Especially among poor families a high reproductive rate might result in reduced investments on education per child. Also, parents may choose to invest only in those of their children who are most likely to amortize the cost of education. This entails especially discrimination against girls, as in a high-fertility environment women are likely to spend a considerable portion of their working years involved in child-rearing activities rather than in the labour force [Das Gupta, 1987]. A low level of eductaion among women is again a risk factor for child mortality and thus another vicious circle begins. Malaria impairs education by more direct means as well: In Kenya it was found that primary school students miss 11% of school days per year because of malaria, and secondary school students miss 4.3% of school days [Leighton and Foster, 1993]. An even more severe consequence arises from the impact of malaria on cognitive development and learning abilities [Holding and Snow, 2001]: a considerable amount of children show neurologic residues after an episode of cerebral malaria [Brewster et al., 1990]; parasitemia, chronic anemia and poor nutritional status due to recurrent episodes of malaria can impair brain function and development [Al Serouri et al., 2000, Shiff et al., 1996]. Also, malaria impacts fetal development and is related to low birthweight, which again is a risk factor for neurosensory, cognitive and behavioural development of children [Gilles et al., 1969, McCormick et al., 1992].

In an era where "human capital constitutes a key factor in economic growth" [Sachs and Malaney, 2002], the impact of malaria on economic growth rates through the mechanism of depressing the rate of human capital accumulation is likely to be considerable and especially harms development prospects of countries lacking natural resources such as Burkina Faso.

3.5.2 Malaria eradication efforts

Efforts to eradicate malaria have been numerous throughout the centuries: First attempts were started by Roman settlers who drained the swamplands and thus established malaria control by diminuishing the breeding grounds of the vector mosquitos. As knowledge and

methods improved, the measures grew more and more effective. The invention of the pesticide DDT and the highly effective antimalarial drug CQ in the mid-1930s consequently launched a world-wide eradication programme by the WHO in 1955 [Mayo and Brady, 1955]. Although the programme was abandonned due to chronic financial problems in 1972 [Bruce-Chwatt, 1984, Najera, 1989], in the temperate zones of the world it was largely successful and until 1978 led to malaria eradication in 37 countries (of which 27 are in Europe or the Americas) [Roll Back Malaria campaign, 2008]. In tropical regions, results were rather discouraging though:

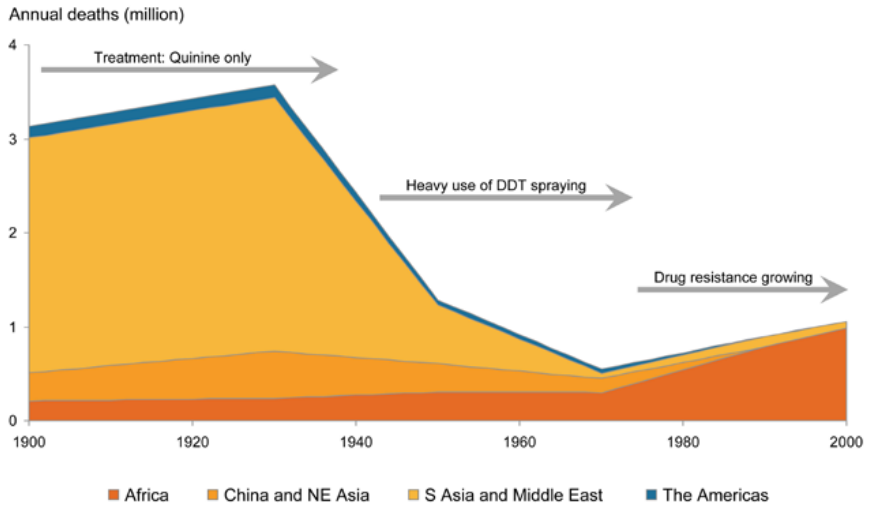

Figure 3.13: Evolution of malaria mortality world-wide and subdivided in regions. Figure from Roll Back Malaria campaign [12]

In SSA eradication efforts were considered infeasible from the start, and "malaria control" was instead targeted. As can be seen in figure 3.13, even these efforts were futile, and SSA practically did not profit from the world-wide decrease in malaria mortality achieved until the 1970s [Carter and Mendis, 2002, WHO, 2008a]. During the 1980s, following the increase in parasite and vector resistance to anti-malarial drugs and insecticides, and largely ignored by national and international health policy, malaria began to rise again [Carter and Mendis, 2002]. The most dramatic increase in malaria mortality and morbidity was seen in SSA, where next to the globally emerging resistance problem the weakening of malaria control programs, rapid decentralization, deteriorating primary health services, and the development of humanitarian crisis situations fuelled the spreading of the disease.

As a result of rapidly growing malarial cases at the verge of the 21st century, international efforts finally re-focussed on malaria, and of WHO's "Global Malaria Control Strategy", adopted in 1992, the Roll Back Malaria (RBM) partnership was mounted in 1998 to coordinate global efforts in combating malaria [Trigg and Kondrachine, 1998, WHO, 2008a]. For African countries, the Decalaration of Abuja signed by most African leaders on April 25th, 2000, translates the goals of the RBM Partnership into tangible political action. Today, the RBM partnership comprises hundreds of partners, including malaria endemic countries, their donor countries, the Global Fund to fight AIDS, Tuberculosis, and Malaria (GFATM), the private sector, NGOs, and research institutions. Central to the actions of RBM are the UN Millennium Development Goals, which call for halting and reversing the incidence of malaria by 2015.

To achieve this ambitious aim, a number of promising control strategies were launched, such as Insecticide-treated bed-nets (ITN), Indoor residual spraying (IRS), and Intermittent preventive treatment (IPT) for pregnant women. Also, strategies to improve malaria treatment and diagnostics are part of RBM's global malaria action plan, such as ACTs and rapid diagnostic tests, and the strengthening of local health systems [WHO, 2009, Roll Back Malaria campaign, 2008]. The adoption of these strategies into regional health policies and treatment protocols is mainly endorsed with financial funding from the GFATM and other sponsors.

With these measures impressive results were accomplished: Approximately half of the malaria endemic countries have been able to reduce malaria cases and deaths by 50% or more between 2000 and 2010. Still, SSA profits least from these advances, and reduction of malaria morbidity was most pronounced in countries with low transmission rates [WHO, 2009]. Most African countries have - partly or completely - integrated the recommended actions into their official malaria treatment and control strategies, as compliance with the global malaria action plan secures access to aid funds. Burkina Faso did so in 2005/06. Nevertheless, a distressing discrepancy often exists between these written strategic plans and the reality.
A good example is given by the "boom and bust"-story of Artemisinine: After strong WHO recommendations, backed by the GFATM announcement to shift financial sup-

port to countries using ACT treatment for malaria, and the groundbreaking agreement of drug firm Novartis to supply the only currently available fixed dose ACT (artemether-lumefantrine, Coartem®) at cost price, within only a few months in 2004 nearly 20 high-endemic countries changed their national treatment policies to ACT.

As demand soared, drug supplies were suddenly depleted and the producers of Artemisinins were unable to keep up. Prices for raw artemisinin rocketed, and many countries were not or insufficiently supplied with the needed drug [Cyranoski, 2004]. The consequent boom in Artemisinin production led to excess supply in 2007, which in turn caused prices to drop significantly and farmers to plant more profitable crops instead. Recent analyses show that for its long agricultural production time (12 - 14 months), floods and droughts in suitable cultivation areas, as well as unstable market conditions, a renewed shortage of Artemisinin supply is likely to occur [Kindermans et al., 2007, Van Noorden, 2010, WHO, 2008c].

Obviously, the main cause for scarce implementation is the lack of sufficient funding. The global malaria action plan accounted the cost to achieve RBM's goals to add up to 6.9 Billion US-$ in 2010 [Roll Back Malaria campaign, 2008]. Of those, despite great fundraising efforts, only 1.5 Billion US-$ were financed in 2010 [WHO, 2010].

3.5.3 The "Plan strategique"

Burkina Faso's National Malaria Control Programme is a Ministry of Health unit designed to coordinate all national activities towards malaria control. Since 1991 it issues the regularly updated "plan strategique nationale de lutte contre le paludisme", which frames all measures taken to attain the goals foreseen in the Declaration of Abuja and also draws a balance of the achievements and shortfalls. Thus, establishment of national treatment protocols falls within the remit of the National Malaria Control Programme, as well as the supply with antimalarial drugs and ITNs, initiation and surveillance of vector control measures, health education, and statistical surveillance. Governmental health institutions are under direct supervision of the Programme unit, for as long as malaria control is involved. Also, malaria research is coordinated. Four research centres in the country are engaged in malaria research: The Centre National de Recherche et de Formation sur le

Paludisme and the Institut de Recherche en Sciences de la Santé in Ouagadougou, the Centre Muraz in Bobo Dioulasso, and the Centre de Recherche en Santé de Nouna in the small town Nouna. Fundraising and the distribution of those funds is part of the National Malaria Control Programme's responsibility, for which it works together with private, bi- and multilateral partners of Burkina Faso, and Non-governmental organization (NGO)s. Obviously, with so many different players involved and given the fact that the team of the Malaria Control Programme includes no more than ten staff members [Kouyate et al., 2007], cooperation and coordination tends to be rather poor in reality [Ministry of Health of Burkina Faso, 2006a]. Still, a 25% reduction of malarial cases was achieved in 2000-2005 [Ministry of Health of Burkina Faso, 2006a].

When in 2005 CQ resistance in parts of Burkina Faso went up to 63.3%, national treatment protocol was changed to ACTs as first line treatment (Artemether - Lumefantrine or Artesunate - Amodiaquine), according to RBM programmes. Unfortunately, this decision was taken despite mention that ACT at that time was not available through governmental drug channels [Ministry of Health of Burkina Faso, 2006b]. Until only recently, CQ was still used in more than 90% of home-based malaria treatments in Burkina Faso [Tipke et al., 2009]. Notably, the registration approval for CQ was revoked in 2009 [A. Ouedraogo, personal communication] as a response to this study. The most recent changes in malaria control strategies in Burkina Faso will be further discussed later on.

Chapter 4

Results

The aim of this thesis is to compare theory and practice of malaria control in Burkina Faso, thereby focussing on antimalarial therapy. MB is displayed as a possible alternative first-line treatment for uncomplicated malaria and thus a possibility to safeguard ACTs as "reserve antimalarials", as the current way of dealing with malaria burden threatens to fuel resistance development to this last line of defense against malaria.
The distinct problems of malaria control in Burkina Faso are discussed under special consideration of socio-economic aspects. Malaria poses a high burden on the country's health system. Most of this burden arises from cost for medication and inpatient care. The actual presentation and handling of malarial disease on-site is shown from a retrospective evaluation of patient files from a hospital in Burkina Faso. I show that and why malaria therapy is sub-optimal. Therapeutic options for malaria available and used in Burkina Faso are reviewed under special consideration of their cost. Price considerations affect treatment significantly, but tend to be cast aside in most studies. Special attention is drawn to the risk of resistance devellopment against artemisinins. MB is highlighted as most interesting drug candidate for a combination therapy strategy for Burkina Faso. MB targets the redox metabolism of *P. falciparum*, which is analyzed in detail using bioinformatic tools. Also, possible drug combination partners are discussed applying bioinformatic methods like elementary mode analyses and extreme pathway modelling. Economic aspects of the use of MB combinations as a "bonaria" (good drug against malaria) drug for uncomplicated malaria are shown by cost effectiveness analysis.

4.1 Aspects of malaria control in Burkina Faso

4.1.1 An introduction to the country

Geography and Climate

Burkina Faso is located in Western Africa, landlocked between Mali, Niger, Benin, Togo, Ghana, and Ivory Coast. In terms of vegetation and climate, it can be divided into two areas: The northern part belongs to the Sahel zone, a dry savannah that forms the transition from Sahara desert to the tropical zone and has only very little rain in the rainy season from May to October. The southern part is characterized by a more humid climate, less prone to droughts, with partly arable grassy savannah and few forests.

Figure 4.1: Political map of Burkina Faso

map: CIA [2]

History and Politics

Upper Volta, as Burkina Faso was formerly known, achieved independence from France in 1960. During the 1970s and 1980s, repeated military coups shook the country until President Blaise Compaoré came to power in a 1987 military coup and held the first multiparty elections in the early 1990s. Compaoré has won every election since then and currently still holds the post of President in a semi-dictatorial administration.

Population

The 2006 held population census counted 13.6 Million inhabitants, of which approximately 46% are under the age of 15. The annual growth rate of its population is 3%, the fertility rate is 5.8 children born per woman [UNICEF, 2011, CIA, 2010]. In 2010, the urban population was estimated 26% of the whole population, and of those most are concentrated in the country's two major cities, the capital Ouagadougou (appr. 1.3 Million), and Bobo-Dioulasso (appr. 500,000). Population density also follows the above indicated geographical zones with a high population density in the middle (surrounding Ouagadougou) and southern regions. In the northern part only few, often noamdic tribes live mainly on breeding livestock (see figure 4.2).

Burkina Faso has over 60 ethno-linguistic groups, the largest of which is Mossi with over 40%. 50% of the Burkinabe are Muslim, approximately 40% follow animistic indigenous beliefs, and 10% are Christian (mainly Roman Catholic). In everyday life, religious beliefs are handeled with great tolerance [U.S. Department of State, 2007].

Economy

Burkina Faso's limited natural resources result in poor economic prospect for the majority of its citizens: The GDP was US$ 8.6 billion (PPP) in 2010. For comparison: Germany's GDP was estimated to be US$ 3.3 trillion (PPP) in the same year [CIA, 2010] (see table 4.1). Many Burkinabe depend on employment as seasonal farm workers in neighbouring countries, namely Ivory Coast and Ghana. Recent unrest in these areas,

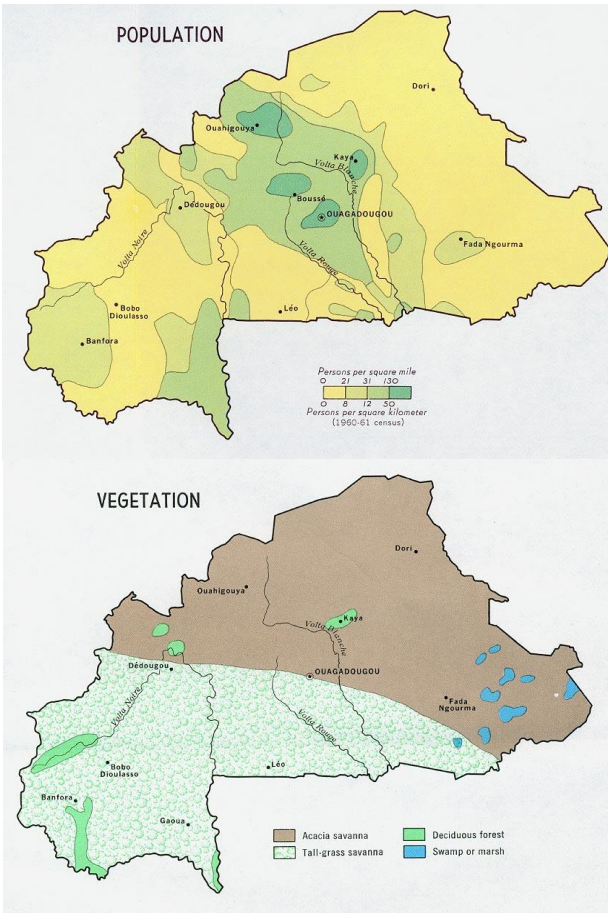

Figure 4.2: Burkina Faso - population density and vegetation map: University of Texas, Austin [13]

especially in the wake of the 2002-2007 civil war in Ivory Coast, hinders employment and is posing a high burden on the people of Burkina Faso [CIA, 2010]. About 90% of the population is engaged in subsistence agriculture, which is vulnerable to frequently occuring droughts. Cotton crops and gold exploration form the basis of Burkina Faso's few exports. It won't surprise in this context that Burkina Faso is one of the world's poorest countries: The Gross National Income (GNI) per capita is US$ 510, 56.5% of Burkina Faso's population live below the poverty line [The World Bank] (see table 4.1). Table 4.1 summarizes important socio-economic markers for Burkina Faso in comparison with an industrialized, highly developed country such as Germany. Data are taken from references [The World Bank], [WHO], and [Ministry of Health of Burkina Faso, 2008a]. Definitions of these factors are given in Appendix D.

Table 4.1: Comparison of substantial economic and demographic markers: Burkina Faso versus Germany

	Burkina Faso	Germany
GDP	8.6 billion US$	3,306 billion US$
GNI	8.0 billion US$	3,476 billion US$
GNI per capita	510 US$	42,450 US$
HDI	0.305 (rank 161)	0.885 (rank 10)
Absolute poverty	56.5 %	none
Fertility rate	5.8	1.4
Crude birth rate	47/1,000 people	8/1,000 people
Life expectancy at birth	53 years	80 years
Under-5-mortality rate	166/1,000 life births	4/1,000 life births
Infant mortality rate	91/1,000 births	4/1,000 births
Health expenditure per capita and year	38$	4,629$
Births attended by skilled health staff	54%	100%
Population with access to improved water source	76% (rural: 72% urban: 95%)	100%
Population with access to sanitation facilities	11% (rural: 6% urban: 33%)	100%
Malnutrition prevalence in children	10%	1.1%
Adult literacy rate	29%	99%
Mean years of schooling	1.3 years	12.2 years
Primary school enrollment, net	32.8%	88.1%

4.1.2 The health system of Burkina Faso

Health care architecture

The health system of Burkina Faso is based on three levels of care: primary, secondary and tertiary: see figure 4.3

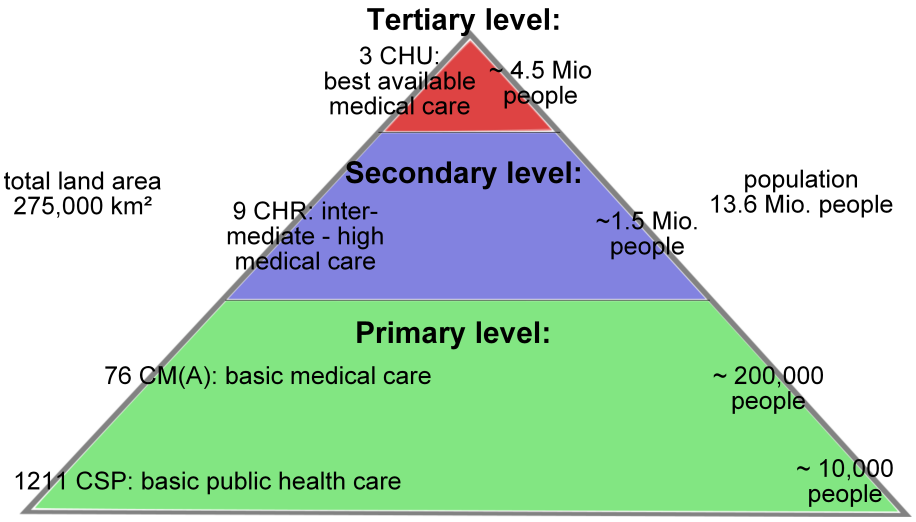

Figure 4.3: The health system of Burkina Faso - a pyramidical architecture. On the left side number of executive units is indicated, the right side shows the (theoretical) number of people within the operating range (rounded). Data as of [Ministry of Health of Burkina Faso, 2008a]

The primary level is represented by the "district sanitaire", in which 1211 health care centres (Centre de Santé et de Promotion Sociale (CSPS)) are primarily assigned with health advocacy. These should usually employ one nurse, one assistant midwive and one unskilled helper. Within the responsibility of a CSPS lie public health functions such as surveillance of pregnacy, uncomplicated deliveries, surveillance of child growth and development, and vaccinations. They only supply very basic health care, such as small wound treatment, or handing out of medications for e.g. uncomplicated malaria. If further health care is needed, they transfer to one of 76 medical centres (33 Centre Médical (CM) and 43 Centre Médical avec Antenne chirurgicale (CMA)). These are covering the basic medical needs of the population of one "district sanitaire". The district hospitals are mainly run by nurses with supervision of usually two physicians [Kouyate et al., 2007].

A range of basic diagnostic means should be provided (laboratory, X-Ray, ultrasound) and all regularly occuring diseases should be treatable here. The CMA also offer small surgery (e.g. appendectomy, cesarian sections).

The secondary level is composed of nine regional hospital centres (Centre Hospitalier Régional (CHR)), which are reference units for the medical centres, and are run by physicians. More complicated cases can be treated and hospitalised here.

The tertiary level is representing the best and most specialized medical care within the country [Ministry of Health of Burkina Faso, 2008a]. Three university hospitals (Centre Hospitalier Universitaire (CHU)) are covering the total country area of approximately 275,000 km^2. The university hospitals are located in the capital Ouagadougou (CHU "Yalgado Ouedraogo", CHUP "Charles de Gaulle") and in the second largest town, Bobo Dioulasso in the South-West of the country (CHU "Sanou Sourou").

Beside the public sector represented by the above given institutions there is a rapidly growing private sector consisting of a considerable number of charitable health care centres that are mainly financed by religious and - to lesser extent - non-religious aid organisations from abroad, and a small number of private consultants who most of the time are employed in one of the country's hospitals and health care centres, sustaining their income by offering private medical practice. The 2008 census of the private medical sector counted approximately 1000 medical institutions in the whole country - ranging from non-profit public health care units to private cabinets. Notably, 95% of these centres are located in the southern part of the country, and most of these center in and nearby the two major towns, Ouagadougou and Bobo-Dioulasso [Ministry of Health of Burkina Faso, 2008b, 2010].

Also, traditional medicine plays a major role in health care, which in Burkina Faso is even aknowledged by law (article 143 of law no. 23/94/AMD). There is no official recognition for the qualification as traditional health practitioner, but a formal training programme in traditional medicine exists in Burkina Faso. According to WHO statistics, more than 80% of the population in Burkina Faso use traditional medicine [WHO, 2001b].

Health care cost

The total health expenditure of 220 billion FCFA (335 million EUR) is 6.8% of Burkina Faso's GDP. The per-capita health expenditure derived from this amount is 17,000 FCFA (25 EUR) per year.

Burkina Faso's health system is subsidised by the state, but patients have to pay fees for each consultation and usually seperately for all medical services, such as injections, pharmaceutical products, hospitalisation etc.. Indeed, as can be seen in table 4.2, over one third of the total health expenditure of Burkina Faso is payed for by private households. The rest of the expenses is approximately equally split between government expenditure and external funds, such as the GFATM, bi- or multilateral agreements, or NGOs. A national social security system or private health insurance are practically non-existent: Burkina Faso's "Caisse nationale de securité sociale" only covers a rough 10% of the population and pays pensions to their insured, but does not foresee a health insurance. Next to this, a small number of both profit and non-profit private health insurances exists, but their penetration in the population is only marginal [Ministry of Health of Burkina Faso, 2010]. Thus, the population bears a huge part of health care cost, to the disadvantage of those most threatend by disease.

Focussing on the amounts spent, the biggest single part of health expenditures, 29.5%, is directed towards pharmaceutical products only. This is even more obvious when cost for malaria is singled out: 15.2 billion FCFA (23.2 million EUR), i.e. 63.5% of all health expenditures for malaria and 6.9% of all health expenditures in Burkina Faso were spent for pharmaceutical malaria treatment in 2007 [Ministry of Health of Burkina Faso, 2010] (see table 4.3).

Table 4.2: Health expenditure in Burkina Faso: Actual cost and percentage of total health care expenditure. Data are as given from WHO's National Health Accounts [WHO] and Statistics provided by Burkina Faso's Ministry of Health [Ministry of Health of Burkina Faso, 2010] for 2007. For definitions of the different matters see Appendix D

	cost in FCFA	% of total health exp.
Total health expenditure	220 x 10^9	100%
Capital sources: who pays for health?		
Public/ Government health expenditure	68.7 x 10^9	31.2%
Private health expenditure	86.8 x 10^9	39.5%
Out-of pocket health expenditure	84.2 x 10^9	38.3%
External sources	65.5 x 10^9	29.3%
Bilateral or multilateral cooperations	38.0 x 10^9	17.2%
UN fonds or non-state organisations	26.4 x 10^9	12.0%
Expenditures: what is the money spent on?		
Curative health care	57.8 x 10^9	26.3%
Inpatient	43.6 x 10^9	19.8%
Outpatient (excl. prescribed medical products)	14.2 x 10^9	6.4%
Medical products	65.4 x 10^9	29.7%
Pharmaceuticals	64.8 x 10^9	29.5%
Other devices, e.g. glasses, wheelchairs, ITNs	0.6 x 10^9	0.2%
Public health programmes	44.1 x 10^9	20%
Administration	19.5 x 10^9	8.9%
Miscellaneous, e.g. research, training of personnel, etc.	33.2 x 10^9	15.1%

Table 4.3: Health expenditure for malaria for malaria treatment, control and prophylaxis in Burkina Faso. Data are derived from [Ministry of Health of Burkina Faso, 2010].

	cost in FCFA	% of total health exp.	% of exp. for malaria
Total cost of malaria	23.9 x 10^9	10.8%	100%
Capital sources for malaria			
Private expenditure for malaria	15.2 x 10^9	6.9%	63.6%
Public expenditure for malaria	6.0 x 10^9	2.7%	25.1%
External expenditure for malaria	2.7 x 10^9	1.2%	11.3%
Expenditures for malaria			
Medical products	15.5 x 10^9	7.0%	64.8%
ITNs	0.3 x 10^9	1.3%	1.4%
pharmaceuticals	15.2 x 10^9	6.9%	63.5%
Medical research	0.7 x 10^9	0.3%	3.0%
Administration	0.1 x 10^9	0.04%	0.4%
Public health measures for malaria control	0.1 x 10^9	0.04%	0.4%
Curative care for malaria patients	7.5 x 10^9	3.4%	31.4%
Outpatients	2.1 x 10^9	0.9%	8.7%
Inpatients	5.4 x 10^9	2.4%	22.5%

Main health care problems

Poverty and Health: The correlations of health and economic status are common sense: Poverty creates ill-health because it forces people to live in environments that make them sick, without decent shelter and nutrition, access to medical care, clean water or adequate sanitation. In the world today, poverty can be regarded as the number one cause of ill-health [WHO, 1995]. Among others, the poor have higher than average child mortality [WHO and OECD, 2003], a relation that is shown most vividly when evaluating the world-wide distribution of child mortality and wealth combined (see Figure 4.4). A

poor country such as Burkina Faso has a nearly 50-fold higher under-5-mortality rate (16.6%) compared to a rich country such as Germany (0.4%).

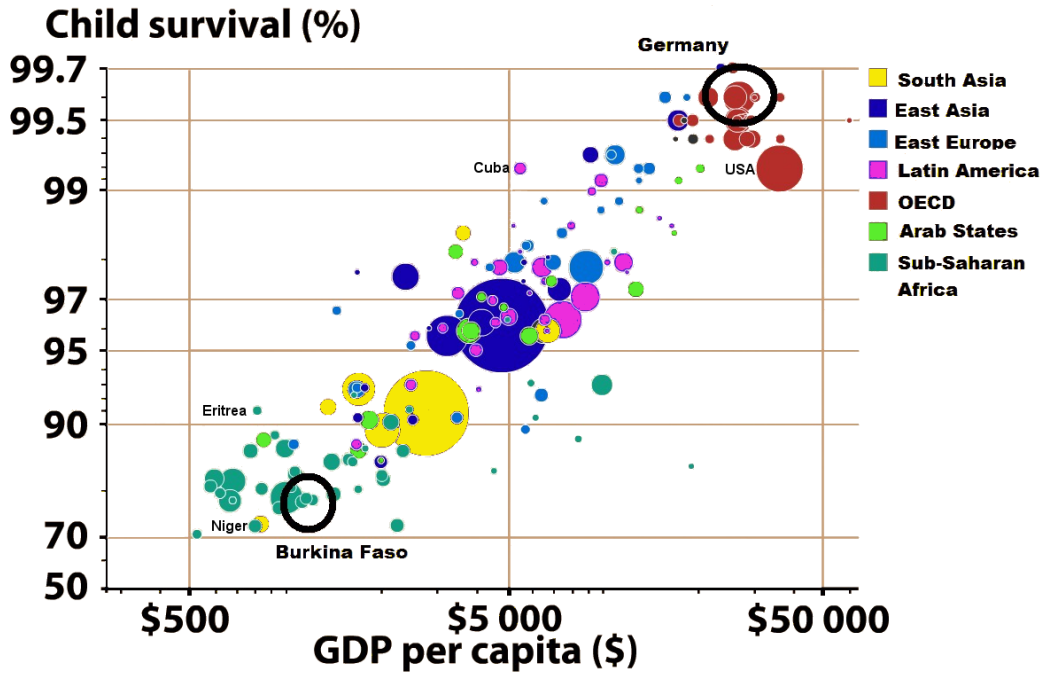

Figure 4.4: Child survival up to five years of age dependent on GDP per capita: each bubble represents one country; colour of the bubbles indicates region, size population.
figure: free material from www.gapminder.org, slightly altered. Data are as of 2003, derived from UNDP statistics. Gapminder.org [4]

Despite this striking inequality, it took quite a while until poverty and health were finally appearing on the international agenda, in political as in scientific terms [Feinstein, 1993, WHO and OECD, 2003]. Also, whilst documentation and quantification of health inequalities in dependence to socio-economic factors are very much advanced, the explanation for - and the right measures to counteract - them is far more difficult to achieve [Feinstein, 1993, WHO, 1995]. Another look at Figure 4.4 e.g. reveals most interesting exceptions from the general rule "Wealth equals Health": Niger for example is approximately at the same economic level as Eritrea, but Eritrea has a much higher child survival rate, whereas Cuba has the same child survival rate as the USA while being substantially poorer than the latter.

Today, health is a central issue in development assessment: The HDI, a measure for

standard of living, is computed from three dimensions: health, education, and financial wealth. Burkina Faso is rated 0.305 and is therefore ranked as low as 161 of 169 countries displayed on the HDI [UNDP, 2010].

Education: is another socioeconomic factor that is highly interweaved with both economy and health. Australian demographer John Caldwell was the first to show, in his seminal paper on Nigeria, the important influence of mother's education on child survival even after control for other factors of enhanced socioeconomic status [Caldwell, 1979]: he showed that children of un-educated women had a significant higher risk of dying than those of educated mothers, and that this risk diminishes coherently with educational status of the mother. Caldwell suggested several pathways whereby mother's education might enhance child survival, most notably a shift from fatalistic acceptance of health outcomes towards implementation of simple health knowledge, and a shift in the familial power structures, permitting the educated woman to exert greater control over health choices for her children [Caldwell, 1979, Hobcraft, 1993]. Other studies showed strong education effects on postneonatal risk, undernutrition in childhood, and non-use of health services [Hobcraft et al., 1984, Mensch et al., 1985, Bicego and Boerma, 1993].

As can be seen in Table 4.1, only 29% of Burkina Faso's population is literate. School enrollment is generally low in this country (32.8%) and most children only visit school for a few months (mean schooling time 1.3 years) [The World Bank]. Burkina Faso's school system is modeled after the french education system. Primary education starts at age seven and is bound to last for six years. Primary school is completed with an exam, the passing of which is required for secondary education. In 2009, according to UNESCO statistics, 78% of all children were enrolled to primary education, whilst only 43% completed their primary education [The World Bank]. Secondary school is divided into two main parts: the junior high school level (four years) followed by the senior high school level (three years). Secondary school enrollment is only 20% in Burkina Faso [The World Bank]. A junior high school diploma entitles the student for entrance in vocational schools for further professional training (usually two to three years), as e.g. nurses, midwives, primary schoolmasters, administration clerks etc.. The senior high school study program closes with the baccalaureate exam, upon which the student can

enroll in a university. Tertiary education enrollment only sums up to 3% of the Burkinabe population [The World Bank], which results in a huge lack of highly trained personnel as e.g. medical doctors.

This low level of education and especially literacy has to be met in any strategy to improve health and health care among the poor. Basic knowledge e.g. on disease handling and risk factors, but also treatment instructions such as how and when to take medications have to be taught in adequate ways. Figures 4.5 and 4.6 show posters that are used in a health care post in Yako, a small town appr. 100 kilometres north-west of Ouagadougou that I visited for some days during my stay in Burkina Faso.

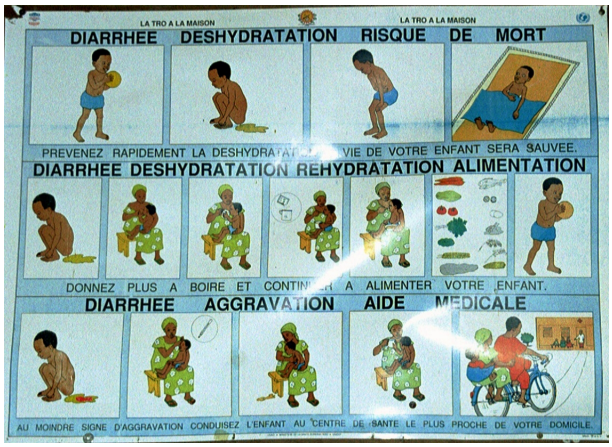

Figure 4.5: Health education in Burkina Faso: because of the high illiteracy rate, health care workers employ drawn pictures to explain mothers what to do and what risk signs to look out for in their sick children.
Picture taken with kind allowance of Soeur Martine, health care worker

In Burkina Faso, as noted above, approximately 60 different languages exist. The unifying language, French, is taught at primary level, but when education is only scarce, people are often not or only insufficiently able to communicate with doctors or other health staff. This impedes health education even further and also complicates patient care.

Malnutrition is what usually springs to mind whenever thinking about poverty. Indeed, it is the first mentioned factor to define poverty in the World Development Report

Figure 4.6: Health education in Burkina Faso: to explain treatment regimes to people lacking basic education, drawn pictures are employed.
Picture taken with kind allowance of Soeur Martine, health care worker

for 1980: "Poverty is a condition of life so characterized by malnutrition, illiteracy and disease as to be beneath any reasonable definition of human decency" [The World Bank, 1980].

Malnutrition is a mortality risk on its own already (4% of global disease burden is due to malnutrition [WHO, 2006b], see Figure 1.1), but the burden related to indirect effects of malnutrition is an order of magnitude higher: Underweight children are more vulnerable to almost all infectious diseases and have a lower prognosis for full recovery, mortality risk of underweight children from diseases such as malaria, measles, or diarrhoea being as high as 52% [Caulfield et al., 2004].

In Burkina Faso, a total of approximately 250,000 (10%) of children are undernourished, and 32,000 children (1.4%) are severly malnourished [Ministry of Health of Burkina Faso, 2008a].

Water and sanitation Access to proper drinking water and sanitation are main aspects of health in any country. Many diseases are caused by the ingestion of pathogens in unsafe drinking-water, in contaminated food, or from unclean hands. Inadequate sanitation and insufficient hygiene promote the transmission of these pathogens. Diarrhoea alone

accounts for 29% of global disease burden in children [WHO, 2006b] (see figure 1.1). Other communicable diseases closely linked to unsafe drinking-water, poor santiation and hygiene include intestinal nematode infections, schistosomiasis, lymphatic filariasis and Trachoma. Associated diseases and mortality risks enhance this burden even further: An estimated 50% of malnutrition in children is associated with repeated diarrhoea or intestinal nematode infections. Also, malaria can be fought by improving water supply and sanitation: by eliminating stagnant water bodies, modifying the contours of reservoirs, introducing drainage or improving the management of irrigation schemes vector habitats and thus disease transmission can be reduced in many places [WHO, 2008b, Pruss et al., 2002].

While a lot of progress has been achieved to supply Burkina Faso's population with improved water sources (76%), the vast majority (89%) does not have access to proper sanitation facilities.

Infrastructure Infrastructure is weak in Burkina Faso. In a country of approximately 275,000 km^2, a total of 92,495 km roadways exists, but of those only 3,857 km are paved [CIA, 2010]. Journeys from one point to another are thus very difficult, especially when not living in one of the bigger towns. For long-distance journeys most Burkinabe rely on public transportation by one of the few bus lines unfrequently criss-crossing the country. For short distances, the major transportaion means is by foot, bike or "moto" - small scooters which are often seen in the streets loaded with whole families and households. Cars are still not very common, and most cars seen in the streets are taxis of some kind.

These drawbacks of infrastructure handicap not only, but in a quite special way, Burkina Faso's health care system: As can be seen in figure 4.3, a CSPS in Burkina Faso has to provision approximately 10,000 people in theory. The theoretic radius within which these people live around a CSPS is thus estimated to 7.60 km. As for varying population density this can only be a rough estimate. According to statistics of the Ministry of health of Burkina Faso, 60% of its population live in a radius of 10 km or less from any medical institution [Ministry of Health of Burkina Faso, 2008a]. This may sound not much, but when an ill child has to be transported in a country where most roads are unpaved, not

Figure 4.7: Public transport in Burkina Faso - a public bus connecting the small town Yako to Ouagadougou is unloaded

frequented by public transport, and people rely on such basic means of transport as a bicycle or a donkey-drawn cart, if at all - then 10 km equals an impediment that will in most cases result in a waiting attitude as long as the child is not critically ill. In a study of treatment seeking behaviour in a rural area of Burkina Faso, Mueller et al. stated that the frequency of medical consultations were highly associated with distance to the health centre or hospital, as well as with a more severe illness presentation [Mueller et al., 2003a]. Distance, as well as low social status, result in lower access to medical services: whilst among the urban population, 70% of the population have access to medical services, in rural areas as little as 29% of the population have such access [Ministry of Health of Burkina Faso, 2008a].

Personnel Shortage of trained personnel is one of the major problems Burkina Faso's health system has to cope with: In 2007, Burkina Faso had 441 medical doctors, 38 surgeons and dentists, 58 pharmacists, 604 trained midwives, and 7741 registered nurses [Ministry of Health of Burkina Faso, 2008a]. For comparison: The German town Munich has 1.3 million inhabitants - a tenth of Burkina Faso's population. The Bavarian Association of Statutory Health Insurance Physicians reports Munich to have appr. 4,000 established medical doctors - which does not count those doctors working in one of Munich's 46 hospitals. Formal health service especially in the rural parts of Burkina Faso is thus mainly performed by nurses, midwives, and mainly unskilled helpers. Only from the regional level on, hospitals have specialist units and are run by physicians [Kouy-

ate et al., 2007]. In order to ensure a minimum level of care, the Ministry of Health of Burkina Faso framed minimum personnel standards for the maintenance of a basic health care institution: a CSPS is to employ at least one nurse, one assistant midwive, and one unskilled helper. In reality, between 40 and 10% of all CSPS - depending on region - do not meet even this basic standard [Ministry of Health of Burkina Faso, 2008a]. This problem is especially pronounced in the rural regions as they are less attractive to highly skilled personnell due to low infrastructure and life quality: The cities of Ouagadougou and Bobo-Dioulasso together concentrate 54% of all medical doctors, 57% of all midwives, 59% of all pharmacists, and 33% of all nurses working in Burkina Faso [Ministry of Health of Burkina Faso, 2006a]!

Malaria burden in Burkina Faso

As can be seen in table 4.1, life expectancy is 53 years in Burkina Faso, as opposed to 80 years in Germany. Most of this discrepancy may be explained by Burkina Faso's high under-5-mortality rate of 166 per 1,000 live births (compare: Germany 4/1,000) and an infant mortality rate of 91/1,000 births. Children are thus carrying the highest burden of disease in Burkina Faso. In this age group, infectious diseases are the main health threats [WHO, 2006b] (see figure 1.1). Acute respiratory infections, diarrhea, bacterial meningitis and malaria together were responsible for 64% of all medical consultations in 2007 [Ministry of Health of Burkina Faso, 2008a].

Table 4.4: Main causes of medical consultations in Burkina Faso, 2007.

cause		number (rounded)
all consultations		7,000,000
all infectious diseases		5,500,000
	malaria	3,000,000
	HIV/AIDS	3,500
	tuberculosis	2,000
trauma/ accidents		400,000
others		1,100,000

Malaria alone accounts for 41% of all medical consultations; 57% of all hospitalisations and 32% of all death cases are attributable to malaria in Burkina Faso [Ministry of Health of Burkina Faso, 2008a]. In the under five age group, this is even more pronounced, with malaria being the reason for 48.4% of consultations, 54% of hospitalisations and 76.5% of all deaths [Ministry of Health of Burkina Faso, 2010]. Malaria - as well as most of the other communicable diseases (respiratory tract infections, meningitis) is seasonal in Burkina Faso. Most cases are seen in the rainy season from May to October, where humidity and warmth allows perfect breeding conditions for the malaria vector, *Anopheles*.

Table 4.5: Main causes of hospitalisation and deaths for children under the age of five years in Burkina Faso, 2007

disease	hospitalisations	deaths
anemia	9,550	656 (letality 6.8%)
acute diarrhea	4,035	113 (letality 2.8%)
acute respiratory tract infection	14,894	346 (letality 2.3%)
malaria	66,498	3,355 (letality 5.0%)
malnutrition	3,535	280 (letality 7.9%)
meningitis	6,088	493 (letality 8.1%)

Table 4.5 shows the main causes of hospitalisation and, of those, death in children under five in Burkina Faso in 2007. It is obvious that malaria easily surpasses all other illnesses in this compilation, even without taking into account that anemia in malaria-endemic countries is to a considerable amount caused and/or worsened by malaria. Overall, malarial cases (not only the hospitalised ones) sum up to more than 1.3 million in children under five per year in Burkina Faso. Of those, approximately 10% are classified "severe malaria" and would therefore have to be treated in hospitals, i.e. in this age group we are facing approximately 150,000 patients per year to be treated in hospitals. As can be seen in table 4.5, merely half of these are actually treated under sufficient medical care [Ministry of Health of Burkina Faso, 2008a].

4.2 Clinical presentation of malaria in a tertiary hospital in Burkina Faso

During a three months' elective at the CHUP "Charles de Gaulle" in Ouagadougou, the capital of Burkina Faso, I was able to get a detailed impression of what malaria in the context of a developing country presents like. To objectify these impressions I was permitted by the hospital's authorities to perform a retrospective analysis of patient files. Every patient aged 0 to 5 years being admitted to the hospital under the presumptive diagnosis of malaria within one month observation period was included in this study, to form a representative pattern of the situation in this tertiary hospital.
Also, by visiting different district hospitals and dispensaries, I had the chance to experience first-hand the structure of Burkina Faso's health system, and thus have an insight to a working health system under the conditions of a developing country:
In Yako, a small town two hours east of Ouagadougou, I visited a small CSPS and a CMA for three days, whilst in Nouna, for another ten days I was able to observe the daily routine of a regional hospital, another CSPS, and in the Nouna Research Centre.

4.2.1 Medical conditions at CHUP "Charles de Gaulle"

Setting

The CHUP "Charles de Gaulle" disposes of 126 beds. It has seven clinical departments: Nourissons (for infants), Grands Enfants (for older children), Maladies infectieuses (infectious diseases), Chirurgie (surgery), Urgences (AE), Réanimation (intensive care) and Consultations externes (outpatients). A fully equipped laboratory, a medical imaging department and a pharmacy are also part of the hospital.

Most acute patients are first admitted to the AE - department, where first-line treatment is administered until patients are stable enough to be sent home or, if needed, referred to the other departments of the hospital. The main causes for referral to the AE department were acute infectious diseases, mainly acute respiratory infections, malaria, acute gastroenteritis and meningitis, apart from accidents that were directly taken care

of by the surgical department. Usually, there was one doctor in charge, in most cases during the day one intern student was assigned to the AE department additionally, plus three to five nurses. There were no additional workers (e.g. secretaries).

Admission to the AE department had to be paid by the patients' parents and was 11,000 FCFA (approximately 17 EUR): Medications such as i.v. fluids, quinine, antibiotics (whichever was in stock, usually ampicilline, gentamicine and ceftriaxone), anti-convulsants (diazepam and phenobarbital), anti-pyretics (acetylsalicylic acid) and diuretics (furosemide) were comprised in this "admission package". All used medications were generic. Laboratory exams were included as well: thick blood film examination, hemoglobin and leucocyte count, glycemia and electrolytes (sodium, potassium) were usually ordered. Every exam or medication outside this "admission package" had to be paid for and organised seperately. In case the admission fee could not be afforded, the hospital's social service investigated the family's indigence. By which means decisions to grant aid or not were taken, I could not descry. Also, I was not able to get detailed information on the magnitude of these cases.

Data were collected during one month in the middle of the rainy season. Whilst malaria is holoendemic in Burkina Faso, the incidence of malaria is especially pronounced in this season, as rainwater is collecting nearly everywhere to form perfect breeding places for Anopheles mosquitos.

Figure 4.8: Main street in Nouna after a heavy rainfall: a perfect breeding ground for mosquitos

4.2.2 Issues

The main objective of the retrospective study presented here was to get a well-founded impression of malaria burden in SSA, and to objectify my impressions from every-day routine in the AE department of a tertiary hospital. Primarily, a descriptive approach was chosen to

- a. define, which clinical signs were triggering parents to seek medical advice
- b. analyse the referral and pre-treatment patterns of patients presented at a tertiary hospital in Burkina Faso
- c. ways to enhance the identification of patients at immediate risk and
- d. point out reasons for which presumptive treatment is necessary even in the best outfitted hospital in Burkina Faso.

Especially during the rainy season malaria is the main cause of hospitalisation in Burkina Faso, but its clinical signs are often non-specific (see chapter 3). Other frequent infectious diseases have to be seperated from severe malaria: Bacterial meningitis may present very similar to cerebral malaria, with high fever, impaired consciousness, and convulsions being most common signs. Respiratory infections such as pneumonia or bronchiolitis are associated with high fever and respiratory distress - also important clinical signs for severe malaria. Malnutrition and intestinal parasitoses can present with hypoglycemia and anaemia, diarrhea is a most common sign of acute gastroenteritis - but all of these may be present in malaria, too. Considering these facts, malaria seems bound to be over-diagnosed in a number of cases ("all is malaria"). To determine whether this actually is the case was one of the aims for this study. At the same time, a focus is layed on clinical signs that define severe malaria, and possible means to optimise the differential diagnosis with simultaneous consideration of limited resources as present in Burkina Faso.

On the other hand most studies in developping countries such as Burkina Faso suggest that treatment deferral is one of the main causes of preventable death in children under the age of five [Mueller et al., 2003a]. Reasons for this have been pointed out earlier. It

seems that for effective reduction of malaria mortality, early treatment is vital, and even WHO recommendations explicitly state that "treatment should not depend on fulfilling [laboratory] criteria" [WHO, 2000]. This principle competes with the above suspected over-treatment (which, given the epidemiological features of malaria in a holo-endemic setting, could trigger drug resistance development). The happy medium is yet to be found. Being a tertiary hospital and the last referral hospital in Burkina Faso, it seems likely that only advanced cases are admitted here, and most children have recieved treatment before. The patterns of pre-treatment, previous medical consultations, and referral were especially focussed on in the presented study. Aspects of these data are currently prepared for publication.

4.2.3 Results

Patients

Table 4.6: Patients

	no.
All children admitted to the hospital	542
Treated for (suspected) Malaria	343
Noted cases	289
Missing cases	54

During the observation period, 542 patients up to 5 years of age were seen in the AE department. The main diagnosis upon discharge was decisive for inclusion in this study: of all patients, 343 (63.3%) were admitted and consequently treated for malaria. The other 199 (36.7%) were treated for other illnesses, mainly acute gastroenteritis, acute respiratory infections, bacterial meningitis, less frequently HIV/AIDS, severe malnutrition, acute asthma and other non-infectious conditions. As the files of 54 children could not be retrieved at all, 289 children were included in the observation study. Of these, 179 files lacked several information:

- 44 children were not recorded when leaving the hospital, thus their "end point" could not be defined.
- 52 children had one or more laboratory exams not performed (thick blood film examination lacked in 21 cases, Hemoglobin counts in 10 cases and blood sugar was not measured in 47 cases). Reasons for lacking results were:
    1. Medical reasons: especially hemoglobin and blood sugar-measurements were not always considered necessary when children did not clinically appear anemic or hypoglycemic. Thus the treating physician did sometimes not order these. In some cases, patients had recent thick drop results from outward referral hospitals, thus the examination was not repeated.
    2. Financial reasons: When neither the family nor social service payed for the hospitalisation of the patient, laboratory exams were often not performed. Especially tests that had to be paid seperately were not performed when parents could not afford to pay for it.
    3. Lacking documentation: Some tests were not properly documented and could not be retrieved.
- In 137 cases anamnestic information and/ or parts of clinical examinations were not properly documented.

Unfortunately it was not possible to seperate the cases in which information were lacking because of incomplete documentation from the ones that were sketchy because of medical or financial reasons. In order not to exclude too many socially weak patients and thus bias the findings, the following analysis is on behalf of all 289 recorded patients. This results in differing total numbers, as those patients with insufficient data for a specific question were not counted. In the following tables "total" thus indicates the number of patients with all corresponding data available. If no total number is given, prevalence refers to all 289 patients.

Gender distribution 55% male patients and 45% female patients were presented at the hospital. A slight tendency towards preference of boys can be seen under consideration

of the overall gender distribution in Burkina Faso ($\chi^2 = 2.683$, $df = 1$, $p = 0.101$).

Table 4.7: Gender distribution

gender	prevalence	
	no.	*%*
Female	130	45
Male	159	55
Total	289	100

Table 4.8: Gender distribution in Burkina Faso: National Institute of Statistics and Demography of Burkina Faso, Estimation for 2005

gender	prevalence	
	no.	*%*
Female	1,259,330	50,2
Male	1,251,348	49,8
Total	2,510,678	100

Age distribution Patients were included until the age of 5 years (60 months). The youngest patients were one month of age, nearly 75% of all patients seen were aged 2 months to 2 years (see figure 4.9).

121 infants (Aged one month up to one year) (41.9%) and 167 toddlers (1 year to 5 years old) (57.8%) were treated for malaria, whilst only 1 neonate (aged 1 month) was included in the observations.

Growth status Upon admission, the weight of the children was taken. Figure 4.10 displays the age-adjusted growth status of the children within the WHO percentiles of weight-for-age distribution. According to WHO percentiles, 42% of all children (girls: 54 of 129, boys: 66 of 158) were underweight for their age (3rd percentile and below).

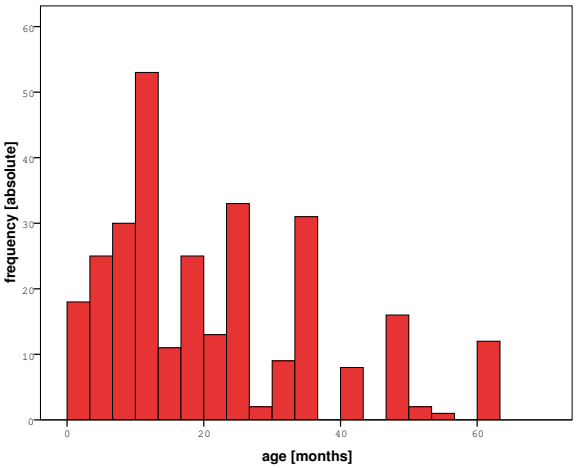

Figure 4.9: Age distribution

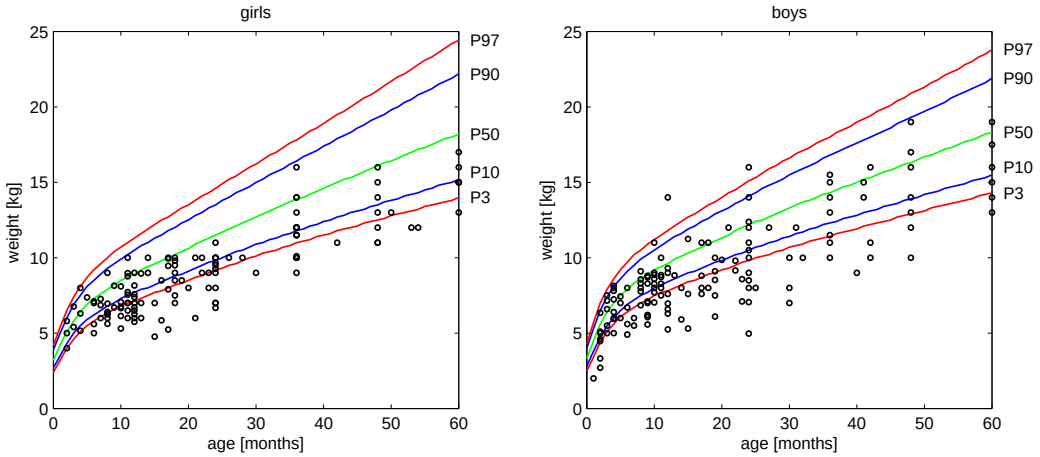

Figure 4.10: Weight-for-age of observed children, percentiles from WHO [16]

Clinical assessment at CHUP "Charles de Gaulle"

On admission, routinely all children were examined and history was taken from the accompanying person. Quite regularly, translation by one of the nurses or other accompanying persons was needed as parents were only speaking their local language. The hospital's official language was French.

Sex, age and weight of the child were taken. Parents were routinely asked whether the child had been assessed in an outward hospital before (during the recent episode of disease), and were referred to the hospital, or wheter they came directly from home. Parents were interviewed for observed signs of illness. Questions usually asked were if the

child felt hot, if convulsions, vomiting or diarrhea occurred and if the child was able to feed. Furthermore, parents were asked for any given treatment during the recent episode of disease. Common antimalarial drugs such as CQ, Amodiaquine (AQ), SP, Quinine, and Artemether® were assessed as well as antipyretic and antibiotic drugs and traditional medication. On admission, pulse and breathing rate were counted and temperature was measured rectally, in older children who refused acceptance the axillary temperature was noted plus a standard deviation of 0.5°C added. Clinically observed prostration, signs of anemia such as pallor of conjunctivae, tongue and palms, hepato- and splenomegaly and signs of respiratory distress such as nasal flaring and thoracic indrawings were noted. Children were judged according to the Blantyre coma score for eye movements, verbal and motor response to guarantee comparability of the scores. At discharge, the clinical outcome was noted.

Routine diagnostic procedures In terms of diagnostics, as mentioned above, the CHUP "Charles de Gaulle" is very well equipped: the laboratory equipment was provided by the french partner hospital of CHUP "Charles de Gaulle" and attended regularly according to hospital standards. Laboratory personnell consisted of well-trained pharmacists and technicians. The hospital was also equipped with X-Ray. Unfortunately, three out of the four weeks observation period, the X-ray machine was broken down. Children who were considered of needing an X-ray (of the chest, most regularly) had to be sent to town, where parents had to pay seperately for the X-Ray. Ultrasound was also available.

Despite the various diagnostic possibilities, few of these were regularly used at the AE department: A venous blood sample was generally taken from every child admitted; routinely, a quantitative examination of a blood film for malarial parasites and to determine hemoglobin concentration and white blood-cell count was performed. Blood sugar and electrolytes were analyzed whenever the treating physician did suspect a metabolic disorder in a child. Stool samples were microbiologically tested in cases where acute gastroenteritis was suggested (there was a local cholera epidemic during the time), lumbar puncture was done when menigitis had to be ruled out as a differential diagnosis. Additional measurements such as plasma creatinine, liver enzymes and blood gas analysis were feasible, as well as imaging studies. Those were only infrequently performed, when

clinically indicated, as most of these examinations had to be paid for seperately. Instead, presumptive treatment was regularly applied.

Anamnestic symptoms

In order to establish which clinical signs were noticed by parents and prompt them to seek medical advice, the anamnestic givings were screened for clinical signs of (possible) severe malaria: fever, convulsions, vomiting, diarrhea, inappetence, and prostration. Whilst only one patient (0.3%) according to anamnesis presented no such clinical sign (and one did not have any anamnesis documented), in 287 patients (99.7%) any of the above mentioned signs were observed by parents during the current episode of disease. Of those, fever was clearly the most prominent clinical sign (277 patients, 96%).

Fever alone was recorded in only three cases, indicating that most parents were seeking medical help only when other factors supervened. Febrile episodes were most frequently accompanied by inappetence (196/264; 74.2%), prostration (118/263; 44.9%), vomiting (106/287; 37.0%), and convulsions (69/288; 24.0%).

Table 4.9: Anamnesis of all cases

Assessed symptoms	total	prevalence	
		no.	*%*
fever	288	277	96.2
convulsion(s)	288	70	24.3
vomiting	287	112	39.0
diarrhea	288	74	25.7
inappetence	264	205	77.7
observed prostration	264	81	30.7

Pretreatment

Parents either were referred to the hospital from another health institution (secondary hospitals, primary health units, private practitioners), or did present to the hospital directly from home. Being a reference health unit, 203 of 275 (73.8%) patients were referred, but still 72 (26.2%) used the hospital as "primary health centre".

Table 4.10: Way of transfer for all cases

Way of transfer	prevalence	
	no.	*%*
Referred from another health institution	203	73.8
Came directly from home	72	26.2
Valid: Total	275	100
Missing	14	
Total	289	

161 of 239 (67.4%) of patients have been treated with antimalarials previous to their admittance to the hospital. Of those, 59.9% (91 of 152) were tested negative for trophozoites, 3.9% (6 of 152) had less than 1000 trophozoites per μl, and in 36.2% (55 of 152), malaria could still be confirmed after having recieved antimalarial treatment. There was no significant difference for the results of the thick drop examination dependent on previous antimalarial treatment ($\chi^2 = 0.387$, $df = 1$, $p = 0.534$). Interestingly, only 13 of 239 questioned parents (5.4%) stated having given traditional remedies to their children, while in other studies (conducted in rural areas of Burkina Faso), 13 - 18% of febrile episodes were treated with traditional medication by parents [Mueller et al., 2003a, Tipke et al., 2009].

Table 4.11: Premedication in all cases

Premedication	total	prevalence	
		no.	*%*
Antimalarial treatment	239	161	67.4
Specification:			
CQ	239	32	13.4
AQ	239	69	28.9
Quinine	239	82	34.3
Coartem ®	239	3	1.3
Antipyretic medication	239	129	54.0
Antibiotic treatment	239	71	29.7
Traditional Medication	239	13	5.4
Others	239	128	53.6

Table 4.12: Premedication in all cases with confirmed Malaria

Premedication	total	prevalence	
		no.	*%*
Antimalarial treatment	77	55	71.4
Specification:			
CQ	77	13	16.9
AQ	77	13	16.9
Quinine	77	34	44.2
Coartem®	77	1	1.3
Antipyretic medication	77	42	54.5
Antibiotic treatment	77	17	22.1
Traditional Medication	77	4	5.2
Others	77	41	53.2

Table 4.13: Premedication dependent on previous consultation for all cases

Antimalarial treatment				Way of transfer	
CQ	AQ	Quinine	Coartem®	home	health inst.
-	-	-	-	22	54
+	-	-	-	4	19
-	+	-	-	19	24
-	-	+	-	8	50
-	-	-	+	2	0
+	+	-	-	1	2
+	-	+	-	0	4
-	+	+	-	2	14
Total				58	167

54 of 167 (32.3%) patients referred from another health institution did not receive any antimalarial treatment; for those with no previous medical consultation 58 patients 22 (37.9%) recieved no antimalarial therapy. Whilst pretreatment in general was not dependent on previous consultation of another health institution ($\chi^2 = 0.603$, $df = 1$, $p = 0.438$), the sort of pretreatment differed significantly ($\chi^2 = 12.927$, $df = 3$, $p = 0.005$). Of 113 (67.7%) who did recieve antimalarials from a previously consulted health institution most (50, 44.2%) had recieved Quinine, 24 (21.2%) AQ, and 19 (16.8%) CQ. For those having recieved home treatment (36, 62.1%), 19 (52.8%) recieved AQ, 8 (22.2%) Quinine, and only 4 (11.1%) CQ. Combination treatment, as called for by WHO since 2001, was not administered: only 23 patients had recieved more than one drug (20 referred, 3 from home), and these were no combination treatments, but follow-ups after initial treatment failure: 18 of those referred had been given CQ (4) resp. AQ (14) as home treatment before recieving Quinine from the referring health institution. Despite high failure rates, CQ was quite commonly used. Contrary to the expectation there was no significant difference in the use of CQ between medical personnel and lay people ($\chi^2 = 1.502$, $df = 1$, $p = 0.220$).

Table 4.14: Premedication dependent on previous consultation for all cases with confirmed Malaria

Antimalarial treatment				Way of transfer	
CQ	AQ	Quinine	Coartem®	home	health inst.
-	-	-	-	3	19
+	-	-	-	2	9
-	+	-	-	2	5
-	-	+	-	2	25
-	-	-	+	1	0
+	+	-	-	0	0
+	-	+	-	0	1
-	+	+	-	1	2
Total				11	61

Antimalarial treatment did not differ significantly depending on severity of disease: of 133 patients with severe malaria following Marsh's definition, 92 had recieved pretreatment, as did 67 of 104 classified as uncomplicated malaria ($\chi^2 = 0.596$, $df = 1$, $p = 0.440$) (for the WHO definition of severe malaria those results were comparable: $\chi^2 = 0.468$, $df = 1$, $p = 0.494$).

Treatment at CHUP "Charles de Gaulle"

All children included in the presented study were treated for malaria at the hospital. Outpatients were not seen in the AE department.

Antimalarial therapy always consisted of i.v. administration of Quinine. In some cases where bacterial infection - most commonly pneumonia or meningitis - as a differential diagnosis to malaria could not immediately be ruled out, an empirical antibiotic therapy was administered in addition to the antimalarial therapy. Those patients in which a confirmed other illness ruled out malaria during the course of their stay at the hospital were excluded from this study.

For (suspected) malaria, treatment consisted of intravenously administered Quinine, usually in 5% glucose solution (= 5 g glucose per 100ml solution) 10 ml/kg and electrolytes added (NaCl 3 g/l, KCl 1.5 g/l, Ca 1 g/l). After an initial loading dose of 20 mg/kg Quinimax® was given over four hours, a maintenance dose of 10 mg/kg was administered every twelve hours for seven days. Treatment was changed to oral Quinine or AQ if the patient was discharged before the treatment course was complete.

Additional interventions were ordered as necessary:

When fever was $\geq$ 38.5°C, antipyretic measures were taken. Primarily, acetylsalicylic acid (10 mg/kg/6h) was given, in very young children paracetamol (10 mg/kg/6h) was given. If fever didn't drop sufficiently, children were enveloped in clothes soaked with cold water.

Acute convulsions were treated by diazepam 0.5 mg/kg rectally; if convulsions didn't stop within 30 minutes, treatment was repeated to a maximum dose of 3 mg/kg/24h.

If anemia was present, but hemoglobin levels $\geq$ 5 g/dl, infusion of Glucose solution as noted above was raised to 25-30 ml/kg/d. In case of severe anemia, whole blood transfusion was ordered. Usually, 20 ml/kg body weight blood was transfused over four hours. When no adept donor blood was in stock, parents and other relatives of the child were tested and donated for the child.

Dehydrated children were rehydrated with Ringer Lactate according to the degree of dehydration.

Hypoglycemia was treated by adjusting the amount of glucose in the infusion: 2-3 ml/kg of 30% respectively 1 ml/kg of 50% glucose solution was adminstered.

In case of respiratory insufficiency, oxygen could be administered. There were no possibilities to intubate children at the AE department, if intubation was needed, the child had to be transfered to the intensive care unit, which was scarcely feasible. Comatose children were usually oxygenated by the placement of oxygen nasal cannulae. Nasogastric tubes and urinary catheters theoretically should have been placed in these cases, but were not available during the observation time. Accompanying persons were taught to check

on the patient constantly and to alert doctors in case of clinical deterioration. No means of continuos monitoring of vital parameters was available.

In some cases where bacterial infection - most commonly pneumonia or meningitis - as a differential diagnosis to malaria could not immediately be ruled out, an empirical antibiotic therapy was administered additional to the antimalarial therapy. For suspected meningitis, a lumbar puncture was performed, but treatment was administered before laboratory findings were back. The treatment course consisted of Ampicilline 200 mg/kg/d, in children up to one year of age Gentamicin 3-5 mg/kg/d was additionally given. For pneumonia, normal treatment consisted of Ampicilline (100-200 mg/kg/d), in severe cases a 3rd generation Cephalosporine (eventually plus gentamicine) was given.

Confirmed malaria

Confirmed malaria was defined by the findings of the thick blood film. If Trophozoites were $1000/\mu l$ or above the child was considered as presenting with confirmed malarial disease for the presented study.

Table 4.15: Confirmed Malaria

	prevalence	
	no.	*%*
No malarial parasites in thick blood film	158	59.4
Trophozoites in thick blood film, $\leq 1000/\mu l$	10	3.8
Trophozoites $\geqq 1000/\mu l$ - Confirmed Malaria	98	36.8
Valid: Total	266	100
Missing	23	
Total	289	

Of 266 children probed for parasitemia, 108 (40.6%) were positive for *P. falciparum*. 10 patients failed to fulfill minimal criteria for "confirmed" malaria, which was set to 1000 trophozoites in periphereal blood. For 98 patients (36.8%) malaria was thus considered

being confirmed. All analyses for malarial cases refer to the latter.

Differential diagnosis

As stated above, all children with suspected malaria were treated before confirmation of the diagnosis. Negative microscopy did not result in discontinuation of antimalarial treatment, unless another differential diagnosis was confirmed (e.g. a positiv lumbar puncture for bacterial meningitis). The latter were excluded from this study. Also, thick drop examinations were not repeatedly taken in case of a negative result. Instead, presumtive antimalarial (eventually plus antibiotic) treatment was administered.

Only 116 of 274 (42.3%) patients were exclusively treated for malaria. Most common differential diagnoses were (lower) respiratory tract infections and meningitis with 80 (29.2%) respectively 29 (10.6%) patients, who were treated with antimalarials and antibiotics.

Table 4.16: Differential diagnosis for all cases

Diagnosis	prevalence	
	no.	*%*
Malaria	116	42.3
Malaria and/ or meningitis	29	10.6
Malaria and/ or respiratory infection	80	29.2
Malaria and/ or gastrointestinal infection	33	12.0
Malaria and/ or typhoid fever	2	0.7
Malaria and/ or skin infection	2	0.7
Malaria and/ or hepatitis	7	2.6
Malaria and/ or malnutrition	2	0.7
Malaria and/ or HIV/AIDS	2	0.7
Malaria and/ or endocarditis	1	0.4
Valid: Total	274	100
Missing	15	
Total	289	

When only considering those with confirmed malaria, these numbers did not "improve", as still 39.6% of these were treated dually for malaria and respiratory tract infections (24.5%) and meningitis (15.4%) respectively.

Table 4.17: Differential diagnosis for all cases with confirmed malaria

Diagnosis	prevalence	
	no.	*%*
Malaria	41	45.1
Malaria and/ or meningitis	14	15.4
Malaria and/ or respiratory infection	22	24.2
Malaria and/ or gastrointestinal infection	10	11.0
Malaria and/ or skin infection	1	1.1
Malaria and/ or hepatitis	3	3.3
Valid: Total	91	100
Missing	7	
Total	98	

Severe malaria

Severe malaria identifies those patients at increased risk of dying. As reviewed in chapter 3 in order to translate this definition to a clinically applicable sorting algorithm, there are different approaches. Here, the defining symptoms set by WHO (see table 3.3.2) and a more basic definition used by Kenian malariologist Kevin Marsh and co-workers [Marsh et al., 1995] (see figure 3.6) are used and compared. For the WHO definition, not all defining symptoms and findings could be included, as

1. some information were lacking due to patchy documentation
2. clinical signs did not occur (e.g. hemoglobinuria)
3. certain measurements were not feasible and/ or not routinely performed in AE (e.g. blood pressure measurements, thoracic X-Ray, blood gas analysis, lactate and

creatinine level measurements, urine output).

Table 4.18: Symptoms of severe disease in all cases

Symptoms	total	prevalence	
		no.	*%*
impaired consciousness	285	64	22.5
respiratory distress	280	83	29.4
severe anemia	279	76	27.2
prostration	263	123	46.8
jaundice	98	9	9.2
hypoglycemia	242	49	20.2
hyperparasitemia	266	7	2.6
cerebral malaria (impaired consciousness and/ or convulsions)	285	97	34.0
Severe malaria (WHO)	266	246	92.5
Severe malaria (Marsh)	285	159	55.8

Of all children up to five years of age presented at the hospital, 246 of 266 showed any clinical sign for severe malaria as defined by WHO. As clinical signs for (severe) malaria are ambiguous, and not in all cases differential diagnoses could be excluded (e.g. lumbar puncture was not performed to exclude meningitis, X-ray was not available and pneumonia could not be ruled out), the following analyses refer to only those patients with microscopically confirmed malaria:

Of all cases with confirmed malaria 93.5% presented with clinical signs of severe disease, according to WHO definition. In contrast, applying the definition as described by [Marsh et al., 1995] (from hereon referred to as "Marsh definition"), only 63.9% are identified as presenting with severe malaria. Of those distinguished "severe malaria" (WHO resp. Marsh definition), 38.7% (87 of 225) and 42.8% (62 of 145) were actually malaria positive in microscopy examinations.

Table 4.19: Symptoms of severe disease in all cases with confirmed Malaria

Symptoms	total	prevalence	
		no.	*%*
impaired consciousness	97	31	32.0
respiratory distress	97	27	27.8
severe anemia	98	30	30.6
prostration	92	35	38.0
jaundice	98	9	9.2
hypoglycemia	88	21	23.9
hyperparasitemia	98	7	7.1
cerebral malaria (impaired consciousness and/ or convulsions)	97	42	43.3
Severe malaria (WHO)	93	87	93.5
Severe malaria (Marsh)	97	62	63.9

Although identifying different amounts of patients, the outcome was identical: both definitions for severe malaria rightfully diagnosed 83.3% (5 of 6) patients with confirmed malaria who died. The latter showed signs for cerebral malaria (one showed respiratory distress additionally) (3 of 5, 60%) and severe anemia (one with, one without respoiratory distress) (2 of 5, 40%). One patient (16.7%) was not identified by either one of these definitions, and was considered to suffer from uncomplicated malaria by both applied algorithms.

While sensitivity is high for both, WHO and Marsh-definition, specificity is higher for the latter: when looking at mortality as endpoint, while sensitivity is 0.83 for both algorithms, in terms of specificity, WHO only attains 0.06 compared to 0.34 for Marsh's algorithm (see tables 4.20 and 4.21).

Table 4.20: Sensitivity and specificity of WHO's definition for severe malaria predicting mortality

Severe malaria	mortality		
	died	survived	Σ
severe malaria	5	72	77
no severe malaria	1	5	6
Σ	6	77	

Table 4.21: Sensitivity and specificity of Marsh's definition for severe malaria predicting mortality

Severe malaria	mortality		
	died	survived	Σ
severe malaria	5	53	58
no severe malaria	1	5	28
Σ	6	80	

When focussing on the defining symptoms, the reasons for this difference is quite obvious: While the definition construct according to Marsh et al. does only take into account two clinical and one laboratory signs (impaired consciousness, respiratory distress, and severe anaemia), WHO considers more details. Of those, in the study group "prostration" (123 of 263) and "hypogylcemia" (49 of 242) quite clearly were the preponderating symptoms and likely the ones causing the observed difference.

Outcome

219 of 245 children (89.4%) were discharged after successful treatment, 18 (7.3%) died, and 8 (3.3%) left the hospital against medical advice. Of 18 dead patients, 6 were probed positive for malaria, 10 had no trophozoites in their thick drop. In two patients, laboratory exams were not performed, as they died shortly after arrival. Of the 10 suspected, but

unconfirmed malarial cases, 9 were identified as "severe malaria" by WHO and 8 by Marsh's definition. 15 of the 18 patients who died were considered as presenting with severe malaria by both applied definitions, of the remaining 3, two were classified as uncomplicated by both definitions; and only one patient was considered a severe case by WHO definition, but not Marsh's definition. As mentioned earlier, all patients included in this study were treated as malarial cases, disrespectful of the result of the thick drop. The occurence of fatal cases was equal in both groups, those with confirmed and those with unconfirmed malaria ($\chi^2 = 0.000$, $df = 1$, $p = 0.989$).

Table 4.22: Outcome and Confirmed Malaria

	survived	died
Confirmed Malaria	81	6
Unconfirmed Malaria	136	10

4.2.4 Summary of results

Patients

Malaria burden is huge in holo-endemic countries such as Burkina Faso. As shown here, during one month in the middle of the rainy (and thus also malaria-) season, the AE department was faced with 542 patients up to five years. 343 (63.3%) of those were treated for malaria.

55% male patients and 45% female patients were presented at the hospital. A slight tendency towards preference of boys can be seen under consideration of the overall gender distribution in Burkina Faso ($\chi^2 = 2.683$, $df = 1$, $p = 0.101$). This may prompt the impression that families take more expenses for male children than for girls. Burkina Faso's society indeed is paternalistic, and although household studies did not find health expenditures biased by sex [Sauerborn et al., 1996], Plan International, a NGO highly active in Burkina Faso, admonishes commonly occurring gender discrimination for female children [Plan Burkina Faso, 2011]. The observed effect is too small for a definite

conclusion, though.

Another observed effect deserves more attention: when applying WHO percentiles, 42% of all children were underweight for their age (3rd percentile and below), compared to an international normative standard of physiological child growth [WHO, 2008]. Burkina Faso's official statistics only speak of 10% undernourished children [Ministry of Health of Burkina Faso, 2008a]. This points to a by far underestimated deficit of growth - and thus hindered potential for development of Bukinabe children against their global peer. The vice-versa causal relationship of malaria and malnutrition have been discussed before (see section 4.1); it does not seem far fetched then to assume at least part of this grwoth deficit is attributable to malaria.

Patient history

In order to establish which clinical signs were noticed by parents and prompt them to seek medical advice, the anamnestic givings were screened for clinical signs of (possible) severe malaria: fever, convulsions, vomiting, diarrhea, inappetence and prostration. In nearly all (99.7%) patients any of these signs were observed by parents, with fever naturally being the most prominent clinical sign (96%). Still, fever alone was recorded in only three cases, indicating that most parents were seeking medical help only when other factors supervened. Febrile episodes were most frequently accompanied by inappetence (74.2%), prostration (44.9%), vomiting (37.0%), and convulsions (24.0%).

Being a reference health unit, most (73.8%) children were referred to the hospital from another health institution (secondary hospitals, primary health units, private practitioners), but still 26.2% used the hospital as "primary health centre". This of course is attributable to the urban surrounding of the hospital, as those parents living nearby will not search a peripherical health centre first. Approximately two thirds of patients had been treated with antimalarials previous to their admittance to the hospital (overall: 67.4%; home treatment: 62.1%; referred from another health institution: 67.7%). Interestingly, there was no significant difference for the results of the thick drop examination dependent on previous antimalarial treatment ($\chi^2 = 0.387$, $df = 1$, $p = 0.534$). This shoulnd't be misinterpreted as common treatment failure, although this might be

relevant for some of the cases, as a high proportion of children did not recieve optimal treatment (see below). From the records, no discrimination was possible as for how long treatment had been given, nor whether given treatment was properly administered. To establish which cases are real treatment failures due to resistance, and in which cases original treatment simply wasn't adept for the severity of the disease, more detailed information on the duration and mode of drug administration would be needed. Whilst pretreatment in general was not dependent on previous consultation of another health institution ($\chi^2 = 0.603$, $df = 1$, $p = 0.438$), the sort of pretreatment differed significantly ($\chi^2 = 12.927$, $df = 3$, $p = 0.005$):

Of those having recieved treatment from a health practicioner before, most (44.2%) had recieved Quinine, 21.2% AQ, and 16.8% CQ. For those having recieved home treatment, 52.8% recieved AQ, 22.2% Quinine, and only 11.1% CQ.

Combination treatment, as called for by WHO since 2001, was not administered: only 23 patients had recieved more than one drug (20 referred, 3 from home), and these were no combination treatments, but follow-ups after initial treatment failure: most had been given AQ resp. CQ as home treatment before recieving Quinine from the referring health institution.

Despite high failure rates, CQ was quite commonly used. Contrary to the expectation there was no significant difference in the use of CQ between medical personnel and lay people ($\chi^2 = 1.502$, $df = 1$, $p = 0.220$). This clearly shows that treatment protocols as suggested by WHO are not satisfactorily implemented on-site.

Antimalarial treatment was not influenced by severity of disease: of those patients with severe malaria following Marsh's definition, 69.2% had recieved pretreatment, as did 64.4% of those classified as uncomplicated malaria ($\chi^2 = 0.596$, $df = 1$, $p = 0.440$) (for the WHO definition of severe malaria those results were comparable: $\chi^2 = 0.468$, $df = 1$, $p = 0.494$).

Interestingly, only 5.4% stated having given traditional remedies to their children, while in other studies (conducted in rural areas of Burkina Faso), 13 - 18% of febrile episodes were treated with traditional medication by parents [Mueller et al., 2003a, Tipke et al., 2009]. The urban setting of Ouagadougou might play an important role in this

finding, as people in urban surroundings tend to be better educated and traditional beliefs to be rejected [Ofovwe et al., 2002].

Diagnosis and treatment of malaria

Unfortunately, the clinical signs of malaria are often non-specific (see chapter 3). Other frequent infectious diseases have to be seperated from severe malaria: Bacterial meningitis may present very similar to cerebral malaria, with high fever, impaired consciousness and convulsions being most common signs. Respiratory infections such as pneumonia or bronchiolitis are associated with high fever and respiratory distress - also important clinical signs for severe malaria. Malnutrition and intestinal parasitoses can present with hypoglycemia and anaemia, diarrhea is a most common sign of acute gastroenteritis - but all of these may be present in malaria, too.

Identifying those patients most at risk is the most important demand on medical personnel under these circumstances. Different algorithms have been proposed for this task. While all of the mentioned 343 patients were hospitalised and treated according to national treatment standard for severe malaria, this seems to be over-estimated, and thus a cost-intensive over-treatment (in terms of unnecessary hositalisation): applying WHO standards, 93.5% of those with confirmed malaria were correctly identified as severe malaria. When applying an algorithm proposed by [Marsh et al., 1995], much less (63.9%) would have been identified - leaving a high number of supposedly over-treated cases. Although identifying different amounts of patients, the outcome was identical: both definitions for severe malaria rightfully diagnosed 83.3% patients with confirmed malaria who died. One patient (16.7%) was not identified by either one of these definitions, and was considered to suffer from uncomplicated malaria by both applied algorithms. The striking difference of the two groups prompts the impression that while sensitivity is high for both, WHO's and Marsh's definition, specificity is higher for the latter. Indeed, when looking at mortality as endpoint, while sensitivity is 0.83 for both algorithms, in terms of specificity, WHO only attains 0.06 compared to 0.34 for Marsh's algorithm (see tables 4.20 and 4.21). When focussing on the defining symptoms, the reasons for this difference is quite obvious: While the definition construct according to Marsh et al. does only take

into account two clinical and one laboratory signs (impaired consciousness, respiratory distress, and severe anaemia), WHO considers more details, most of which are ambiguous for themselves.

Whilst all algorithms discussed above stress the importance of laboratory testing to compensate for the poor specificity of the clinical diagnosis, in reality this is often not properly performed. In CHUP "Charles de Gaulle", thick drop testings were only performed once, on arrival of the patient. Antimalarial treatment was administered irrespective of the test results. Only 36.8% of all patients were tested positive for parasites. As discussed in chapter 3, especially venous blood samples (as performed in AE) may be negative despite present parasitemia [Delley et al., 2000]. For this reason, it is required to perform repeated examinations when malaria is suspected and the first test is negative. In reality, as treatment in severe cases should not be delayed, this is not feasible. On the other hand in a semi-immune population parasitemia might be present, but the patient might not suffer from malaria [Bottius et al., 1996]. Also, not in all cases differential diagnoses could be excluded (e.g. lumbar puncture was not performed to exclude meningitis, X-ray was not available and pneumonia could not be ruled out). As shown, in 39.6% of all confirmed malarial cases an alternative diagnosis was still considered and also treated. This harbours an enormous potential for over-treatment with both antimalarials and antibiotics, as most likely the double treatment was unnecessary in a majority of the cases. To the best of my knowledge, this so far has not been systematically studied, but as socio-economic costs may be huge, further studies are needed to confirm this suspicion and to quantify its impact.

Considering these facts, malaria seems bound to be over-diagnosed in a number of cases ("all is malaria"). On the other hand most studies in developping countries such as Burkina Faso suggest that treatment deferral is one of the main causes of preventable death in children under the age of five [Mueller et al., 2003a]. It seems that for effective reduction of malaria mortality, early treatment is vital, and even WHO recommendations explicitly state that "treatment should not depend on fulfilling [laboratory] criteria" [WHO, 2000]. This principle competes with the suspected over-treatment (which, given the epidemiological features of malaria in a holo-endemic setting, could trigger drug resistance development).

The bottom line

Malaria is one of the most dangerous diseases in Burkina Faso. Its clinical signs unfortunately are far from decisive and can in most cases also be interpreted as manifestations of other (also frequent) infectious diseases. To overlook any of the contemplable differential diagnoses could be fatal. Quick and reliable means to establish the right diagnosis is thus crucial. The lack of these (often basic) diagnostic means - even in the best-outfitted hospital of Burkina Faso - results in cost-intensive over-/ double-treatment.

Also, the definition of severe disease poses a problem not to underestimate: to identify those patients most at risk with high sensitivity, but also high specificity, is still unsatisfactorily met. WHO definition, though very adept for medical conditions e.g. in Germany, is not properly appliable in Burkina Faso. Even in the best-outfitted hospital, out of 15 defining symptoms only 10 were properly to elevate. Sensitivity of this definition was high (83%), but with poor specificity (0.6%). The definition suggested by [Marsh et al., 1995] offers the same sensitivity, but a far better (though still far from impressive) specificity of 34%.

Case management is highly affected by the shocking dimensions of disease burden: during one month, 343 out of 542 patients (only those under 5 years of age!) were presenting with (suspected) malaria to be hospitalized. To attend to all these patients, there usually were one doctor, one intern student, and three to five nurses present. The resulting workload on each of these is huge, as it includes admission, ongoing patient care - many of them critically ill, and discharge of patients. It seems understandable that under these circumstances diagnostic steps like repeating of blood examinations etc. is not easily feasible.

Also due to the sheer mass of patients, co-morbidities like malnutrition tend to be overlooked: when applying WHO standards, 42% of all children treated for malaria were underweight. Still, only 0.7% of all children were actively treated for malnutrion alongside with antimalarial treatment. This poses a great risk for children to slip "under the radar" and present with repeated episodes of severe disease, associated with a considerably higher mortality risk [Caulfield et al., 2004]. (These results are consistent with findings of similar studies in Burkina Faso [Sawadogo et al., personal communication].) Measures to better

identify these patients and to take proper care of them, feasible under realistic conditions as presented here, are desperately needed.

Since 2001, WHO treatment protocols foresee the discontinuation of CQ-monotherapy and the implementation of (artemisinin-containing) combination therapy. From pretreatment data of patients included in this study, Artemisinins were virtually absent, as was combination treatment. Pretreatment data suggest that observance of these guidelines is poor and knowledge on resistance rates need improvement: even patients referred from other health professionals had recieved CQ in 16.8% of the cases. With resistance levels of $\geq 20\%$, this drug is virtually useless in Burkina Faso [Tinto et al., 2001, 2002], a fact that should be well known among health professionals.

Another "result" not explicity mentioned above should not go unnoticed here: As shown above, 343 patients were treated for severe malaria during the observation period. Of those, only 110 files were complete, without missing details on laboratory exams, or sketchy anamnesis or examination findings. Proper documentation is in many cases not done. From my personal experience several reasons are to hold responsible for this, but mainly here again - as so many of the above mentionned shortcomings - are due to the overwhelming mass of patients facing a severely under-staffed medical team.

4.3 Therapy of malaria in Burkina Faso: Availability and price considerations

4.3.1 Antimalarial therapy

Various compounds have been identified as antimalarials. For a long time though, Chloroquine (CQ) was by far the most widely used antimalarial drug, it was highly effective, cheap and well tolerated [Warrell and Gilles, 2002]. First reports on CQ resistance in East Africa emerged in the late 1970s [Campbell et al., 1979], from where it continually spread throughout the whole continent [Menon et al., 1987]. Nowadays, CQ has been rendered virtually useless in most endemic settings due to high resistance rates that were fuelled by its often inconsidered mass-use [Payne, 1988, Draper et al., 1988, Foster, 1995, Wernsdorfer and Payne, 1991]. Sulfadoxine - Pyrimethamine (SP) was widely implemented as second-line treatment when CQ was failing, but resistance was quickly spreading against this drug, too [Draper et al., 1988, Basco and Ringwald, 1999, Ogutu et al., 2000]. Mefloquine (MQ) was then used against multi-resistant strains of *P. falciparum*, but here again resistance emerged quickly [Draper et al., 1988, Brasseur et al., 1990]. Figure 4.11 shows the current resistance situation worldwide which nowadays has to be faced.

Facing this situation, WHO as well as most experts on the field favour combination therapy for the treatment of Malaria: This concept has already proven effective against various infectious diseases like leprosy, tuberculosis and HIV/AIDS as well as in cancer chemotherapy [Nosten and Brasseur, 2002].

Rationale of combination therapies

To prevent the further development of resistance and to better exploit the potential of existing individual drugs, combination therapy is a promising way of improving antimalarial treatment.

The rationale for the use of combination therapy is based on the concept that drug resistance arises from random mutations of the parasite's genome which inevitably will be selected if they protect the parasite from harmful drug effects. If two drugs with different

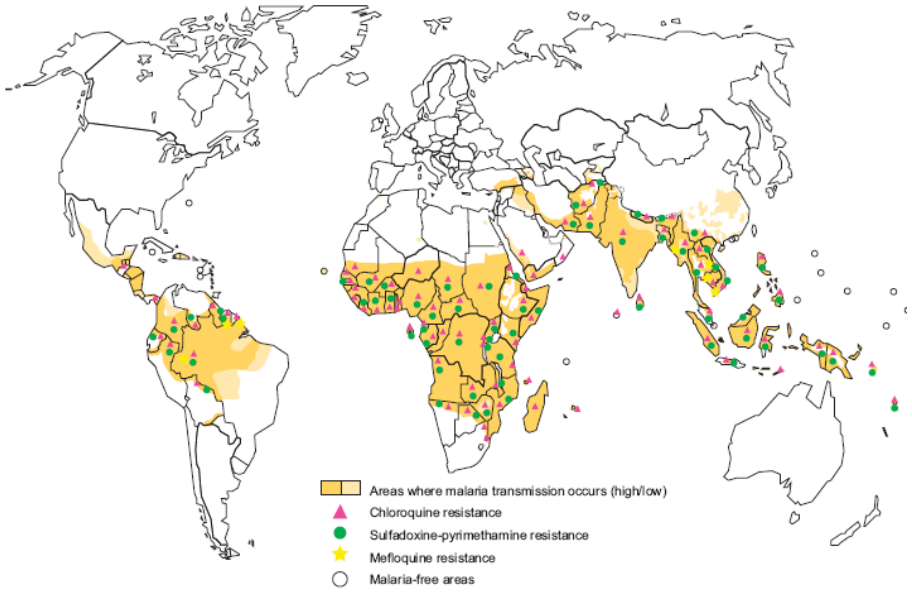

Figure 4.11: Drug resistance of *P. falciparum*, World 2004.
Illustration: WHO [14]

mechanisms of action are combined, probability of a double mutation that will knock out susceptibility to both drugs simultaneously is the product of the individual chances for each drug administered [White and Olliaro, 1996, Nosten and Brasseur, 2002, Bell and Winstanley, 2004]. It has been shown that combination therapy can extend the life-time of existing antimalarial drugs if properly used: In 1994, after ten years of first-line use, MQ resistance in north-western Thailand was as high as 50%, when local treatment was changed to MQ plus Artesunate (AS)-combination. Six years later, in 2000, treatment efficacy of the MQ-AS combination remained over 95%. At the same time, sensitivity to MQ increased in this region, whilst in other areas of Thailand - where MQ monotherapy was continued - sensitivity for MQ decreased even further [Brockman et al., 2000]. Nonetheless, a combination ideally shouldn't include drugs that are already constrained by high resistance rates, as not to provoke resistance against the remaining, efficient combination partner: "combination therapy cannot extend the useful therapeutic life of a failing drug" [Warrell and Gilles, 2002]. This also implies that combined drugs should have compatible pharmacokinetic and pharmacodynamic properties [White and Olliaro, 1996]: An important principle of any combination therapy is the mutual protection of two

drugs on each other, therefore the parasite should never be exposed to one drug alone. This means that half-lifes of combined drugs should be matched in order not to expose parasites to low concentrations of either drug on its own [Bloland et al., 2000, Bell and Winstanley, 2004]. In reality, this often is not the case as the example of AS-MQ shows. Partner drugs may also have synergistic or additive effects on the parasite, thus increasing or accelerating the therapeutic effect. To clear parasites from the blood quickly and sustainable is one major challenge of a successfull drug regimen. The example of the combination of AS and MQ, a regimen now widely used in South-East Asia, may demonstrate this in detail: AS has a very high parasite reduction ratio; with a three day drug regimen parasitic load is reduced some 10^8-fold, although due to its short half-life, AS is not able to clear all remaining parasites within this time frame - as monotherapy it has to be administered over seven days. MQ on the contrary is a slow acting drug with a comparably long half-life, meaning the drug remains active for longer. When combined, AS reduces the parasite load quickly, leaving less parasites for MQ to tackle when its concentration is still relatively high. This reduces the probablity of resistance selection, as resistance is usually acquired gradually, meaning that parasites first become less sensitive to low concentrations of antimalarial drugs before they are fully resistant [White, 1997]. This concept worked very well in Thailand (s.a.), where transmission is generally low: studies estimated the number of infections per person to 0.4 per year [Price et al., 1996]. In most of SSA the number of infective bites is much higher, although there is a high variety depending on climatic differences. For Burkina Faso, 100 infective bites per person and year have been estimated [Kelly-Hope and McKenzie, 2009]. In this context, where it is much more likely that a given malaria patient will be re-exposed to malaria shortly after the treatment and at a time when blood drug levels may still be in a range exerting selective pressure for resistance, mismatched half lives become far more influential, stressing the need for pharmacokinetic and -dynamic matched combinations in SSA [Bloland et al., 2000].

Apart from delaying or even preventing resistance development, combination therapy often allows the dose of the individual drugs to be reduced and therefore diminish adverse effects and/ or to shorten the therapeutic course, which could beneficially influence therapy adherence. For example, standard treatment regimen of Artemisinin monotherapy

has to be administered for at least seven days, whereas in combination e.g. with lumefantrine (CoArtem® or Riamet®), treatment lasts only for three days. On the other hand, this also means that combinations of drugs which express adverse pharmacological interaction or additional toxicity cannot be considered as combination partners [Warrell and Gilles, 2002, Nosten and Brasseur, 2002, Bell and Winstanley, 2004].

Here, another important factor comes into play: drug adherence is a crucial point in any drug strategy. Self-treatment is very common in developing countries and the help of a health care professional is often requested only when self-treatment is failing [McCombie, 1996]. In Burkina Faso, a study conducted among 1640 febrile patients with malaria showed that 69% were self-treated [Mueller et al., 2003a]. This harbours extreme risk of improper drug use and especially by underdosage or discontinuation of treatment after initial improvement this leads to further resistance development [Foster, 1995]: of children treated at home in Kenya and Togo, only 12 and 30% respectively recieved adequate dosage of CQ [Deming et al., 1989, Ruebush et al., 1995]. In these holo-endemic settings, even sub-optimal drugs and -dosages are often sufficient to reduce parasites and relieve symptoms, as semi-immunity prevails in the affected population. Thus, treatment will be discontinued in order to save the drugs for the next episode and resistance is once again fuelled [McCombie, 1996, Bloland et al., 2000]. Education of parents [Kidane and Morrow, 2000], drug-retailers [Marsh et al., 2004], as well as health-care workers [Delacollette et al., 1996] may enhance the proper use of antimalarials and should be an essential point in any eradication effort. Also, regimens have to be easy to understand and to comply with: Combination therapy can be co-administered, which does not require a special formulation but may be more complex to explain to patients and difficult to comply with, especially when a high percentage of patients or parents are illiterate as in Burkina Faso. Also, it harbours the risk of continued monotherapy. Treatment with a fixed combination seems thus to be less error prone and may provide a better adherence rate [Bloland et al., 2000, Nosten and Brasseur, 2002, Bell and Winstanley, 2004].

Combination therapy obviously augments treatment cost. This fact has to be properly adressed before implementation of any new drug under the conditions of a poor-resource country such as Burkina Faso. A new combination strategy, as effective and well-tolerated

as it may be, will inevitably fail to be utilized if costs double per treatment course - not mentioning the 20-fold increase that is implied when changing first-line treatment protocols from CQ to ACT in SSA. In a community-based study in Burkina Faso, home treatment of malaria consisted of CQ in 93%, despite the recent policy change to ACT as first-line treatment of uncomplicated malaria in Burkina Faso [Tipke et al., 2009].

Another problem arising more and more in developing countries should be adressed here: Counterfeit antimalarial drugs have been repeatedly found, first in South-East Asia [Dondorp et al., 2004a], and increasingly in African countries, too: a survey conducted in Cameroon found insufficient or inactive ingredients in 38% of preparations labeled CQ, 78% of those labeled quinine, and 12% of tablets labeled as an antifolate agent [Basco, 2004]. In Kenya and Congo, fake or substandardly dosed Artemisinins were found in up to two thirds of all tested products [Atemnkeng et al., 2007]. The trade in counterfeit drugs undoubtedly results in many deaths, but it is lucrative and carries little risk of imprisonment in regions where the trade in pharmaceuticals is not rigorously regulated [Newton et al., 2002]. Substandard pharmaceuticals may not only result from criminal activity, but also from improper storage, as was shown in surveys conducted in Nigeria and Tanzania [Taylor et al., 2001, Minzi et al., 2003].

Properties of current antimalarial agents

Table 4.23 summarises important pharmacological properties of classic antimalarial agents. More detail on those is given in Appendix B.

Table 4.23: Pharmacological properties of classic antimalarial agents

drug	Mechanism of action	Mechanism of resistance	half-life time
CQ/AQ	Heme detoxification	efflux via PfCRT[1]	1 - 2 months
Quinine	Heme detoxification	efflux via Pfmdr1	10 - 12 hours
Antifolates (SP)	Pyrimidine synthesis	DHFR and DHPS mutation	4 - 8 days

[1] while sharing the same resistance mechanism [Tinto et al., 2008], AQ was shown to maintain efficacy against CQ-resistant strains of *Plasmodium [Olliaro et al., 1996]*

4.3.2 Artemisinins

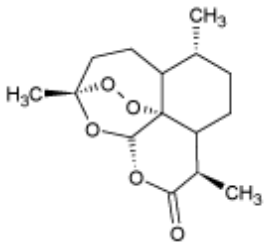

Figure 4.12: Structure of Artemisinine

The sweet wormwood plant "Qinghao" (*Artemisia annua*), was used centuries ago as an antipyretic in China. Earliest proof is found in a medical handbook dating from the Jin dynasty (265 - 420) [Cui and Su, 2009]. In the late 1960s, the Chinese government launched a huge coordinated effort in order to discover antimalarial principles in various medicinal herbs, including Qinghao. "Qinghaosu", later to be called Artemisinin, was finally isolated from the plant in the early 1970s by Chinese scientists [Qinghaosu Antimalarial Coordinating Group, 1979]. Artemisinin is a sesquiterpene lactone peroxide, a chemical structure hitherto unknown in biology (see figure 4.12) that carries an endoperoxide which is essential to the mechanism of action of this drug. Although Artemisinins are by far the most rapidly acting antimalarials ever developed, killing malarial parasites already in nanomolar concentrations, it took nearly 15 years until the parasitocidal potential of these drugs was finally recognised on a broad scale. Today, Artemisinins form a central part of WHO strategies to "Roll Back Malaria" [Woodrow et al., 2005, Krishna et al., 2008]. However, supply shortage [Cyranoski, 2004], its high price, and recent evidence for resistance against Artemisinins [Krishna et al., 2006] have severely pruned enthusiastic hopes thrust upon these drugs.

Pharmacology of Artemisinins

Mode of action: Artemisins are active against all blood stages of *P. falciparum* [ter Kuile et al., 1993], including gametocytes [Kumar and Zheng, 1990], and maintain activity against otherwise multidrug-resistant parasites.

The mechanisms of antimalarial action of Artemisinins are yet not well understood and

subject of considerable debate. Several mechanisms have been proposed, but so far there is no decisive evidence pointing towards the absolute validity of any of these, nor has a multi-target mechanism yet been proven. The following will briefly sketch these theories, for more elaborate reviews, I recommend references [Haynes and Krishna, 2004], [Golenser et al., 2006], [Cui and Su, 2009], and [O'Neill et al., 2010].

The endoperoxide bridge is essential to the antimalarial action of Artemisinins, as replacement of one of the peroxidic oxygens with e.g. a carbon produces a molecule ineffective against *P. falciparum* [Krishna et al., 2004, Haynes and Krishna, 2004]. It has been suggested that Artemisinins are activated by ring opening of this peroxide to generate free radicals, a theory supported by experimental findings which show that free radical scavengers antagonise the antimalarial activity of Artemisinins whilst other free radical generators promote them [Krungkrai and Yuthavong, 1987]. Two different models of radical formation were established, both of which are mediated by Fe^{2+}, either in ferrous heme or exogenous free iron. The selective toxicity of Artemisinins could be explained thus, as *P. falciparum* accumulates ferrous heme and free ferrous ions when digesting hemoglobin of its host cell in its food vacuole. Once formed, the Artemisinin-derived free radicals are theorized to damage the parasite. It was soon agreed that free radicals would kill via a rather indiscriminate process, a view that is hard to integrate with the exceptionally high, fast, and specific activity of Artemisinins [Haynes et al., 2004, Woodrow et al., 2005]. More specific intracellular targets were then sought, and various possible points of action have been suggested since:

Heme alkylation is thought to block polymerisation of this highly toxic compound of parasitic hemoglobin digestion. This theory is supported by *in vitro* experiments showing heme-Artemisinin adducts to form [Meshnick et al., 1991], and consequently to bind to *P. falciparum* Histidine-Rich Protein II (PfHRP II), a putative heme polymerase [Kannan et al., 2002]. Artemisinins have also been shown to inhibit the falcipains, a papain family cysteine protease that aid hemoglobin degradation [Pandey et al., 1999].

This theory, though widely supported for decades, has not remained undisputed: So far, inhibition of heme polymerization was not shown *in vivo* [Meshnick, 1996, Haynes et al., 2003]. It has also been argued that the effects of Artemisinin on early ring stages,

where heme is not yet present [Skinner et al., 1996], nor their considerable effect on other parasites as *Babesia* [Jones-Brando et al., 2006] and *Toxoplasma* [Kumar et al., 2003], which do not produce heme at all [Krishna et al., 2008, Golenser et al., 2006] can be adequately explained thus. Several studies identified possible other drug targets, e.g. endoperoxide-dependent parasite membrane damage [Hartwig et al., 2009, del Pilar Crespo et al., 2008] or inhibition of the mitochondriac respiratory chain [Krungkrai et al., 1999, Li et al., 2005]; but although protein alkylation as a central mechanism of action is well established, a single molecular target is yet to be identified which has a direct role in cell death.

In a localization experiment with radio-labeled Artemisinins it was shown that the drug mainly accumulates in the membrane-bound protein fraction of Plasmodium [Asawamahasakda et al., 1994] - which would contradict the hypothetized effect on heme degradation taking place primarily in the food vacuole.

An alternative mechanism of action for Artemisinins has been proposed by Krishna and colleagues, based on inhibition of the malarial parasite's Sarco-/Endoplasmic Reticulum Calcium ATPase (SERCA), an ATP-driven Ca^{2+}-transporter [Eckstein-Ludwig et al., 2003]. SERCAs are present in a wide range of organisms and act to reduce cytosolic free calcium concentrations by actively concentrating Ca^{2+} into membrane-bound stores. This activity is critical to cellular survival because it allows intracellular signalling by large increases in free Ca^{2+} to recur, by rapidly restoring concentrations [Haynes and Krishna, 2004]. In an transfection experiment with *Xenopus laevis* oocytes Artemisinins inhibited the SERCA of malarial parasites, PfATP6, with particular specificity [Eckstein-Ludwig et al., 2003]. Further evidence supporting this theory is the effect of Artemisinin on the *Toxoplasma gondii* SERCA homolog, where Artemisinin perturbed calcium homeostasis [Nagamune et al., 2007].

Construction of the three-dimensional protein model of PfATP6 and subsequent docking simulation showed that Artemisinins bind to PfATP6 via hydrophobic interactions, exposing the peroxide bonds outside of the binding pocket and thus allowing cleavage of the peroxide bridge by Fe^{2+}, which may ultimately inhibit PfATP6 function and lead to parasite death [Jung et al., 2005].

The finding that a single amino acid mutation in PfATP6 modulates the parasite's sen-

sitivity to Artemisinins in vitro [Uhlemann et al., 2005] further supported the proposed mechanism of action.
Although intriguing, to this date the mode of action of Artemisinins remains to be clarified, and the SERCA-hypothesis could not yet be verified nor rebuted.

Mechanisms of resistance: So far, no Artemisinin resistance has been found in field isolates of *P. falciparum*. In laboratory models though, resistance has been successfully selected [Afonso et al., 2006], proving the potential of the parasites to become resistant to this last resort of malaria control. Also, alarming evidence for decreased efficacy of Arteminsin-containing combinations has emerged recently from the Thai-Cambodian border, a region where multi-resistant parasite strains have emerged earlier [Wongsrichanalai and Meshnick, 2008, Noedl et al., 2008, Dondorp et al., 2009]. Resistance mechanisms are yet unclear.
In 2002 - 2003, decreased in vitro sensitivity of P. falciparum to artemether was reported in French Guiana and was associated with the S769N mutation in the *P. falciparum* SERCA-gene [Jambou et al., 2005]. Homology models of PfATP6 against its SERCA orthologues in man, mouse, and Anopheles suggest that few point mutations at domain boundaries of PfATP6 would be enough to create resistance: see figure 4.13 [Becker, 2011]. The minor differences in structure regarding PfATP6 are selectively targeted by Artemisinins (e.g. position 263, suggested to be involved in Artemisinin binding to PfATP6, or in position 769, proposed to be related to decreased sensitivity to Artemether *in vitro* [Jambou et al., 2005]) show that resistance could involve only minor structural changes which in this case can investigated by homology modelling [Becker, 2011]. This result is also backed by genome-wide analyses [Mu et al., 2010].

Pharmacokinetics: Artemisinin itself is a highly crystalline compound that does not dissolve in oil or water and thus can only be given by the enteral route. It is the parent compound for semisynthetic derivatives that have been chemically modified to produce Artesunate (AS), Artemether, Arteether, Dihydroartemisinin (DHA), and artelinic acid. These compounds have variously been formulated for oral, rectal, and parenteral administration.

Once absorbed, the Artemisinin derivatives are converted primarily to DHA and then to inactive metabolites via hepatic cytochrome P-450 and other enzyme systems. DHA is itself a potent antimalarial with an elimination half life of about 45 minutes. The extent of conversion to DHA differs between derivatives, and time to maximum DHA concentration is typically one to two hours. Artemisinin, as well as Artemether and Arteether contribute to antimalarial activity themselves and are metabolised more slowly, whilst AS is hydrolysed within minutes to DHA, its antimalarial activity largely mediated by this metabolite. DHA is mostly (90%) bound to plasma proteins. The absolute bioavailability of antimalarial activity differ from 30 - 60%, depending on derivative, age, grade of illness and administration route [Woodrow et al., 2005, Roll Back Malaria campaign, 2001].

Toxicity, adverse effects: Artemether and Arteether were neurotoxic in animal models at doses higher than those used in the treatment of malaria in humans [Brewer et al., 1994]. So far, no notable side effects have been reported in patients treated with Artemisinins [Roll Back Malaria campaign, 2001].

Artemisinin-based combination therapies

Monotherapy with Artemisinin derivatives requires a seven day regimen and even then is hampered in its efficacy by a high recrudescence rate. Artemether - Lumefantrine, later to be produced by Novartis under the names of Co-Artem® and Riamet®, was the first commercially available Artemisinin-containing combination therapy. In 2001, WHO adopted ACTs as its primary drug strategy in the fight against malaria [WHO, 2001a]. Next to Artemether-Lumefantrine one other fixed dose ACT is currently available on the market in Burkina Faso: DHA-Piperaquine. Piperaquine has a very long elimination half-life (8-18 days), which leaves a long "unprotected" interval for Piperaquine in the combination with DHA with its very short half-life time [Davis et al., 2005].

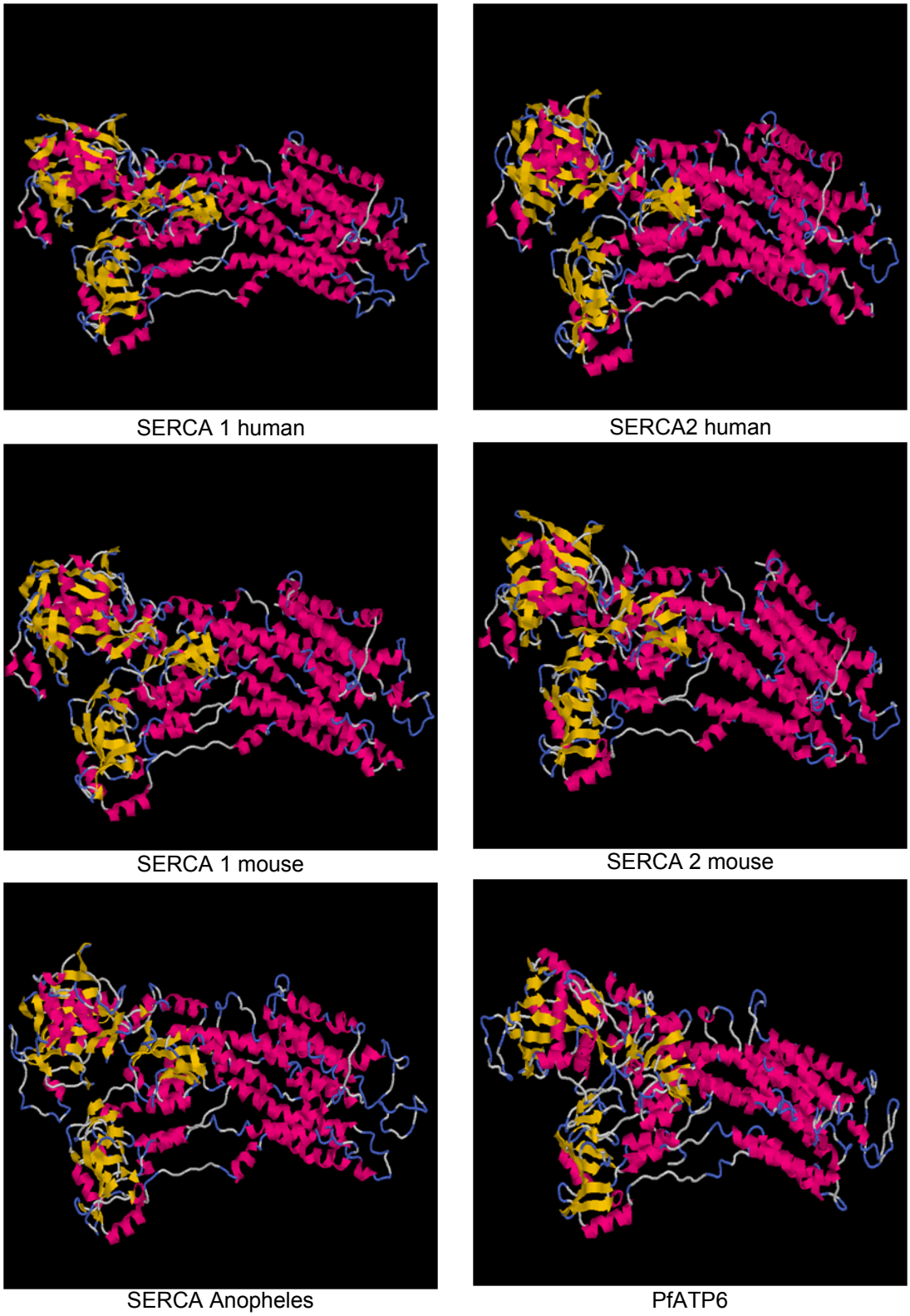

Figure 4.13: Homology modelling of SERCA orthologues, SERCA of man (top), mouse (middle), Anopheles and Plasmodium's PfATP6 (bottom) helices: red, strands: brown, loops: blue
Reproduced with allowance from Becker [1].

4.3.3 Antimalarial agents available in Burkina Faso

By questioning one of Burkina Faso's drug wholesalers, data on currently available antimalarial treatment were collected. Table 4.24 gives the results. The complete price list, including details on manufacturers, package size, and wholesales prices can be found in Appendix F.3.

In 2001, WHO adopted ACTs as its primary drug strategy in the fight against malaria [WHO, 2001a]. Of 106 endemic countries, 56 had adopoted this new strategy in their health policies to the year 2006 [WHO, 2010, Roll Back Malaria campaign, 2006]. In Burkina Faso, national treatment policy designates Artemether-Lumefantrine (alternatively AS-AQ) as first-line treatment for uncomplicated malaria since 2005. Unfortunately, the common saying "paper doesn't blush" applies to this context only too well: as will be displayed in more detail later on, the large majority of patients seen in Burkina Faso had still recieved CQ, AQ, or SP as first-line treatment despite drug resistance. Given that these treatments were available at treatment cost of a few Cents each, this won't surprise.

Table 4.24: Price per treatment of commercially available antimalarials in Burkina Faso. The table gives detailed data on the presented price considerations. Data on treatment are as suggested by WHO. In case no WHO standards were available, suggestions of the manufacturer were cited. Treatment data always refer to children under 5 years of age. The calculation of price per treatment refer to a child of 10 kg. Only application forms feasible at home were included (oral route or rectal suppositories).

drug	recommended total treatment dose	price-range/ treatment [EUR]
	"Classic" antimalarials	
CQ	25 mg/kg (over 3 days)	0.15 - 0.63
AQ	25-35 mg/kg (over 3 days)	0.24 - 1.34

Continuation of Table 4.24

drug	recommended total treatment dose	price-range/ treatment [EUR]
SP	25 mg/kg S + 1.25 mg/kg P (single dose)	0.10 - 0.28
Quinine	168 mg/kg (3 x 8 mg/d for 7 d)	1.42 - 3.96
Halofantrine	24 mg/kg (3 doses of 8 mg every 6 h)	0.92 - 1.54
	Artemisinins	
Artemether	16 mg/kg (over 7 d; loading dose 4 mg/kg followed by 2 mg/kg/d)	1.49 - 4.87
AS	s.a.	0.53 - 2.65
DHA	s.a.	1.63 - 4.84
	ACTs	
AS + MQ	only children $\geq$ 15 kg: 1 blister of 6 tablets = 1 therapy	4.72
AS + SP	total course (12 kg - 20 kg): 1.5 tbl. over 24 h	1.34
AS + AQ	AS 12 mg/kg + AQ 30 mg/kg (AS 4 mg/kg/d + AQ 10 mg/kg/d; given for 3 d)	1.33 - 3.33
Artemether + Lumefantrin	children 10 to $\leq$ 15 kg: 2 x 1 tbl./ d for 3 d (= 6 tbl.)	1.65 - 4.19
DHA + Piperaquine	only children $\geq$ 6 yrs (6-11 yrs: 4 tbl.; 11-16 yrs: 6 tbl.; adults: 8 tbl.; given over 3 days)	3.77

Table 4.25: Balance of antimalarials sold: results from a representative ministry of health survey in Burkina Faso in 2009. Data: personal communication of Dr. Arsène Ouedraogo, Chef de service coordination et statistique des approvisionnements pharmaceutiques, ministry of health of Burkina Faso.

drug	units sold	price per unit [EUR]	total price [EUR]
Amodiaquine			
AQ syrup (50 mg/5 ml), 60 ml	27,041	0.41	11,208.35
AQ-hydrochloride tbl. 200 mg	1,290,610	0.01	15,248.33
Sulfadoxine-Pyrimethamine			
SP (500 + 25 mg) tbl.	2,586,648	0.03	74,095.13
Quinine			
Quinine resorcine (200mg/ 2ml), 2ml inj.	1,803,345	0.12	220,072.47
Quinine resorcine (400mg/ 4ml), 4ml inj.	2,600,777	0.17	437,681.72
Quinine sulfate tbl. 300 mg	22,098,030	0.05	1,089,142.80
Artesunate + Amodiaquine			
Fixed combination AS + AQ (100 + 270 mg), blister of 6 tbl., adults	478,047	0.72	343,983.93
AS + AQ (100 + 270 mg), blister of 3 tbl., adolescents	217,533	0.48	105,457.59
AS + AQ (50 + 135 mg), blister of 3 caps., children	1,530,263	0.32	489,903.94
AS + AQ (25 + 67.5 mg), blister of 3 tbl., infants 2-11 months	1,304,013	0.26	339,941.03

In 2008/09, an official policy change in Burkina Faso's antimalarial treatment strategy was performed by the ministry of health. Following the observance that despite high

failure rates and despite treatment guidelines recommending otherwise, monotherapy especially with CQ was still continued widely, CQ was banned from the market. Also, artemisinins as non-fixed combinations were revoked to comply to a WHO call. AS - AQ was backed as national first line treatment protocol for uncomplicated malaria (together with artemether-lumefantrine, which in that year was not available for Burkina Faso as for shortage problems).

Table 4.25 shows the 2009 balance of antimalarials sold in Burkina Faso via the ministry of health - supervised drug wholesalers. Prices represent wholesale prices, not the market prices given in Table 4.24. For comparison of wholesales prices, please refer to Appendix F.3. It should be noted that only about 10% (3,529,856/33,936,307 units) of the sold drugs were ACTs, although thanks to subsidarisation programmes the price for these combinations was reduced to merely a tenth of the price given in 2007. Clearly, the cheaper medications are more often bought: 77% (25,975,288/33,936,307) of the sold antimalarials were AQ, SP, and Quinine tablets, which cost less than a tenth of the price for ACTs. It seems thus that - under the given circumstances - price influences therapy significantly.

4.4 Methylene Blue as antimalarial - a solution for Burkina Faso?

The use of the histologic and bacteriologic stain MB as an antimalarial agent was first reported by Paul Ehrlich (1854-1915) and Paul Guttmann (1834-1893). In 1891 they published two case studies in the weekly journal "Berliner Klinische Wochenschrift" [Guttmann and Ehrlich, 1891]: Malaria was then twice cured by the administration of 0.5-0.7 g/d of MB p.o. over 14 days. Alleviation of symptoms was achieved within 1-3 days, by which time blood smears were cleared from Plasmodia, too. There were no severe adverse effects noted, apart from intensive, but reversible, blue staining of urine and faeces due to MB. In one case spastic dysuria occurred but was easily handled by the co-administration of ground nutmeg.

Whilst MB became a structural prototype for various other drugs, as an antimalarial on its own it was quickly forgotten when in the 1960s it was replaced by its derivatives, the 4-aminoquinolines [Schirmer et al., 2003].

Today, MB is still in use for various clinical indications: Most relevant is the treatment of methemoglobinemia [Mansouri and Lurie, 1993, Coleman and Coleman, 1996] - which, among other etiologies, may be a complication of malarial infection [Anstey et al., 1996]. Also, MB is employed for prevention and treatment of ifosfamide-induced neurotoxicity in cancer chemotherapy [Pelgrims et al., 2000] as well as for supportive treatment of anaphylactic reactions [Evora and Simon, 2007, Oliveira Neto et al., 2003]. It has even been reported to have a beneficial effect on sepsis patients [Donati et al., 2002], and research on the use of MB in Alzheimer's disease is promising [Gura, 2008, Wischik et al., 1996]. Furthermore due to its antiseptic properties it is utilized in topical antiseptic lotions and to inactivate virus pathogens in fresh frozen plasma [Wieding et al., 1993]. The intense blue colour of MB solutions also makes it an ideal candidate for visualisation purposes, e.g. to trace sentinel lymph nodes in breast surgery [Simmons et al., 2003].

As an antimalarial, MB was rediscovered when resistance rates of *P. falciparum* against common antimalarial drugs such as CQ were eskalating and the search for alternative drugs was launched. MB is a well-known coumpound already approved for therapeutic

purposes in many countries and produced at low cost. This fact, combined with its high antimalarial potency, make this drug a most interesting candidate to re-investigate as an antimalarial for low-income countries such as Burkina Faso [Mandi et al., 2005]. In the following, an extensive *in silico* analysis refines the view on the multi-hit strategy evidenced by MB on plasmodial redox pathways. Second, suitable combination partners for MB in an antimalarial drug combination are discussed by bioinformatical simulations of combination effects, using the example of CQ and SP. The bioinformatical data shown here are part of a recent publication [Zirkel et al., 2012] where I participated as one of the equally contributing first authors. Last, economical and practical aspects of the introduction of MB combinations for pediatric malaria in Burkina Faso are comprehensively discussed for the first time in this thesis.

4.4.1 Analyzing thiol-dependent redox networks in the presence of Methylene Blue and other anti-malarial agents with RT-PCR-supported *in silico* modeling

Chemical properties and pharmacology of Methylene Blue

MB (CAS No. 61-73-4) is a heterocyclic aromatic chemical compound soluble in water and alcohol and was first synthesized as a dye by German chemist Heinrich Caro in 1876. The dye soon proved to be useable in a variety of ways. Among others, its property to specifically stain histologic features made it part of widely used staining techniques, such as Giemsa's stain [Giemsa, 1904].

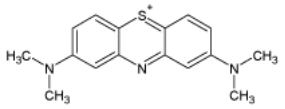

Figure 4.14: Chemical structure of Methylene Blue

Synthesis: The first preparation of MB was performed by Heinrich Caro in 1876, for which German dye fabricant BASF recieved a patent in 1877. MB is prepared from its precursor dimethylaniline in three steps: first, dimethylaniline is treated with

sodium nitrite in hydrochloric acid solution to form nitroso-dimethylaniline. The latter is then reduced by hydrogen sulphide (H_2S), giving p-amino-dimethylaniline. Alternatively, metals can be used as reducing agents. The third step comprises the oxidation of p-aminodimethylaniline, and the introduction of a thiosulphonic acid group into the molecule. Oxidation is achieved with ferric chloride in the presence of hydrogen sulphide, and by adding thiosulphuric acid to the reaction the thiosulphonic acid group is introduced and consequently oxidised to close the ring to form MB [Caro, 1878].

The basic chemical steps are still used for MB synthesis today, though in some ways refined. One major drawback of MB used as pharmaceutical are marginal impurities with heavy metals, originating from the synthesis [Pascual et al., 2011]. MB is though approved by the U.S. Food and Drug Administration for treatment of methemoglobinemia. Oral treatment (Urolene Blue) was used in clinical trials in Burkina Faso without heavy metal related toxicity being observed [Meissner et al., 2006]. Also, the Hazardous Substances Data Bank (HSDB) of U.S.-National Library of Science does not mention any heavy metal induced toxicity (`http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@rn+@rel+61-73-4`, last checked 29.3.2012). Generic MB is produced by several dozens of manufacturers worldwide. French pharmaceutical enterprise Provepharm recently developed a new synthesis pathway minimising the heavy metal impurities [Pascual et al., 2011] (International Patent No. PCT/FR/2007/001193). Their product Proveblue® got certified by the European Medinines Agency.

Pharmacologic properties: A number of studies in animal models as well as in healthy volunteers and malaria patients has shown MB to be savely administered and well absorbed both intravenously and orally. After i.v. and p.o. administration its terminal half-life ranges between 4 and 6 hours. Whole blood levels as well as the area under the concentration-time curve (AUC) of MB were generally lower for oral than for i.v. administration, although absorption rates after oral administration have been shown to be excellent in humans. This effect most probably arises from variable concentrations reached in different organs depending on the mode of administration. Excretion is mainly through the urine, in reduced as well as in unchanged form [DiSanto and Wagner, 1972b,c,a, Peter et al., 2000, Burhenne et al., 2008, Walter-Sack et al., 2009].

In cell culture studies it has been shown that MB possesses a high level of antimalarial activity, as well as a high selectivity for *P. falciparum*. No cross resistance with CQ nor MQ was observed in these tests [Vennerstrom et al., 1995, Atamna et al., 1996].

MB accumulates inside the RBC [Sass et al., 1967]. In *P. falciparum*-infected RBCs accumulation is even higher than in non-infected cells. This finding may be explained by the fact that in acidic cellular compartments such as the parasite food vacuole (pH 5.0-5.4) MB is reduced to Leuco-MB, which in turn is ionized and thus turned into a relatively membrane impermeant form [Vennerstrom et al., 1995] (see figure 4.15).

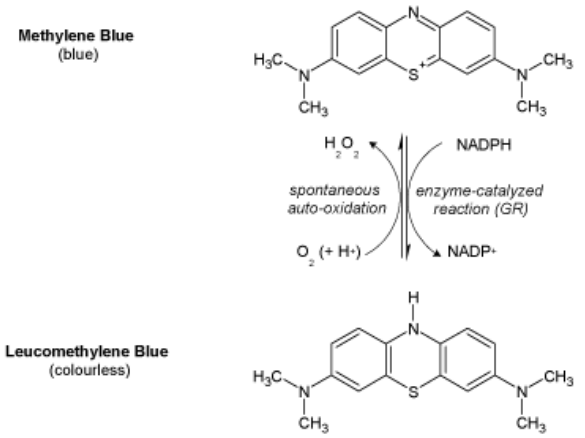

Figure 4.15: MB as a redox-cycling substrate of GR

Also, the strongly increased hexose monophosphate shunt activity in parasitized RBCs facilitates significantly higher levels of reductive uptake of MB [Atamna et al., 1994] (see section 3.2). Inside the parasitized RBC Leuco-MB is spontaneously oxidized back into MB, which was shown to activate the hexose monophosphate shunt even further [Deslauriers et al., 1987]. Combined with experimental findings that MB inhibits the polymerization of heme into hemozoin [Atamna et al., 1996, Deharo et al., 2002] (in analogy to its chemical relatives, the 4-aminoquinolines), and selectively inhibits GR in *P. falciparum* [Färber et al., 1998], it seems that its mode of action is primarily based on interferences with the parasite's fragile redox equilibrium (see section 3.2):

Mode of action of MB

MB specifically inhibits Plasmodium GR [Färber et al., 1996, 1998, Sarma et al., 2003, Becker et al., 2004]. GR is though unlikely to be the primary molecular target for MB, as the IC_{50} of parasite growth inhibition by MB is far below the K_i for GR inhibition by MB [Meierjohann et al., 2002, Mueller, 2004]. This suggests that the compound has additional effects that lead to parasite death [Sarma et al., 2003], like the inhibition of hemozoin formation [Atamna et al., 1996]. Some of the putative targets like two-methemoglobin and β-hematin are metabolites that are not controllable by the genome of the parasite, which makes resistance development against MB unlikely [Atamna et al., 1996, Schirmer et al., 2003, Vennerstrom et al., 1995]. Indeed, resistance to MB seems to be rather difficult to evolve: in experiments chronically exposing *P. berghei* (rodent malaria) to MB, only a very modest drug resistance to MB could be induced [Thurston, 1953].
MB also has a gametocytocidal effect [Coulibaly et al., 2009], an effect of high relevance to cut the infectious cycle of *P. falciparum*.

It was suggested that MB is a subversive substrate of GR and other antioxidant disulfide reductases such as thioredoxin reductase [Krauth-Siegel et al., 2005, Buchholz et al., 2008b]. The term subversive substrate indicates that this compound changes the physiological function of the enzyme to the opposite, in this context meaning turning the antioxidant disulfide reductases into pro-oxidant enzymes. Thus, due to the action of MB the reducing milieu that the enzymes are meant to protect is disturbed by those very enzymes. The mechanism of MB is that of a redox-cycling agent (see figure 4.15). In each cycle, MB is reduced by NADPH or NADH in an enzyme-dependent manner. The resulting leuco-MB undergoes rapid auto-oxidation, with the products being MB and H_2O_2. In balance, each catalytic cycle leads to the loss of NAD(P)H and O_2, while H_2O_2 is produced. Furthermore, NAD(P)H and O_2, which are needed for the pathogen's metabolism, are consumed in the pathological reaction cycles, and the [NADPH]/[$NADP^+$] ratio is likely to be affected. GSSG, the physiological substrate of GR, is expected to be more slowly reduced in succession, which leads to toxic effects of GSSG. In addition, there is less GSH available in the parasite as a substrate of GSH-S-transferase for the detoxification

of heme and other lipophilic compounds [Becker et al., 2003b].

Our own data back the assumption that the high antimalarial potency of MB derives from pleiotropic effects on different plasmodial redox pathways. This part of my thesis was prepared in close collaboration with Alexander Cecil and Janina Zirkel and published [Zirkel et al., 2012]. While Mrs. Zirkel's work extends to an area-wide implementation of MB in a malaria eradication strategy, the scope of this thesis is rather focussed on the potential of MB to serve as a regular compound used for the treatment of uncomplicated malaria.

In silico redox pathway analysis under the influence of MB

Details on the preparation of the redox network is given in chapter 2.

After preparing a basic network of P. falciparum redox metabolism in YANAsquare (figure 4.16 gives a graphic impression of these elementary modes), the MB-induced enzyme changes in this redox network were modeled and further analyzed, taking into account PCR data: mRNA activities for key redox enzymes were measured. A bioinformatical flux model calculated all fluxes as well as their changes in the whole network. Figure 4.17 summarizes the result obtained; thicker arrows represent strong pathway fluxes (Appendix F.1, Table F.1 lists all involved enzymes). YANAsquare estimated for this an overall flux distribution according to overall expression data from PlasmoDB [Aurrecoechea et al., 2009] and the malaria transcriptome database [Bozdech et al., 2003]. Changes were calculated using the gene expression changes of the measured key enzymes under different conditions as constraints. Though an individual gene expression change is only a rough and indirect estimate of the actual enzyme activity (as factors such as translation, protein turnover and enzyme activation are not captured), usage of all measured values as constraints helps to reduce the overall fitting error (remaining error: only few percent, see [Liang et al., 2011, Cecil et al., 2011]).

Without MB, 25 hours after invasion into the human RBC, when the developmental state of *P. falciparum* is the rapidly growing trophozoite, we found comparatively strong pathway fluxes (defined as relative changes ≥ 0.15) in the following modes: protein protection (modes 2, 3, 6, 7, 11, and 35), generation of keto sugars (modes 18 and 23), and

lactate production (mode 26). We conclude that the parasite spends its main activity on these metabolic processes and that these pathways play an inportant role for *P. falciparum* survival and growth inside the host cell. The model shown in figure 4.16 shows the steady state fluxes as present in the undisturbed parasite.

In the experiments conducted, the administration of MB slows down all enzymes participating in these processes. GR, after an initial down regulation at 9 hours, showed an up-regulation to 149% after 12 hours, which declines slightly after 18 hours (Table 2.1). Also after initial down regulation, 1-Cys-peroxiredoxin and Lactate Dehydrogenase (LDH) showed an up regulation of mRNA expression over the initial value after 18 hours under the chosen MB concentrations (15 nM)[2]. The real-time PCR results were furthermore compared to the mRNA expression data of the DeRisi Database 25 hours after parasite invasion into the erythrocyte. In order to analyze the effects of MB on the redox metabolism, we performed extreme pathway analyses and flux calculations with R. Under the influence of MB after 12 hours, the protein protection modes 3, 6, 7, 11, and 22 are reduced by more than 4%. The strongest reductions (more than 10%) are calculated for protein protection modes 3, 6, 7, and 11, using thioredoxin and/ or glutaredoxin. Ribose phosphate production for nucleotide metabolism is reduced by more than 17% in the most affected mode. The flux calculated for lactate generation is also reduced by 4%, whereas the generation of keto sugars remains nearly stable with minor fluctuations in the different modes (see table F.12). These results show that the parasite is subjected to more oxidative stress, as the protein protection modes are more affected. Furthermore, ribose phosphate production is reduced under these stress conditions. Consequently these effects are parasiticidal if lethal concentrations are used.

According to the calculated fluxes, the gene expression data for MB action on the parasite led to reduced protection against oxidative stress at multiple places in the network. Regarding the enzyme activity calculated under the influence of MB, we can show that enzymes participating in redox protection are less active and that the parasite is exposed to oxidative stress. For optimal activity (parasiticidal effect) a higher concentration ($>$ 20 nM) and/ or longer incubation times ($>$ 18 hours) are required.

[2]NB: these are non-lethal concentrations. This approach was used to include long-term effects of MB, including parasite recovery, into the model.

MB and MB-based combination therapies in *in silico* analysis and clinical trials

The workflow of the modeling of *Plasmodium* resistance mutations and drug combinations is described in chapter 2. Results were obtained for the following scenarios of drug combinations and different sensitivity or resistance of *Plasmodium*:

Scenario 1: SP-resistant strain. When CQ is acting alone on a SP-resistant strain in comparison to a SP sensitive strain, the enzyme activity of glutaredoxin reaction 6, GSH-peroxidase-like Thiol peroxidase (Tpx), thioredoxin, and thioredoxin reductase is lowered by at least 10%. GR increases its activity by 15%, which could be part of a compensatory process. In the case of thioredoxin there is a reduction of more than 30%, and for thioredoxin reductase of more than 70% in enzyme activity. For the latter two enzymes, the effect is very similar to that caused by the administration of MB alone. However, when additional MB is added to the CQ treatment, we could note several strong effects in some key redox enzymes; for example, thioredoxin reductase is completely inhibited by the MB/CQ combination. Thioredoxin is also influenced and the direction of the reaction changes, while increasing two-fold. This was also calculated for glutaredoxin reaction 6, although in this case activity decreases. Those effects could be a result of a complete loss in activity of thioredoxin reductase. The GSH-peroxidase-like Tpx reduces its activity by more than 50%, whereas CQ alone reduces activity only by 13%. Thioredoxin 2 has a 73% loss in activity under MB/CQ combination treatment. When inhibited, no hemozoin can be formed, and Fe^{2+} accumulates in the cell. The *in silico* effects are modeled to be the same as *in vitro* and *in vivo*. This results in an accumulation of Fe^{2+} after the administration of CQ and the inhibition of the enzyme ferrochelatase. The modeling shows that redox pathways, especially the enzymes involved in protein protection against oxidative stress, are very active. Under the influence of CQ, thioredoxin reductase activity is inhibited, and mode 22 for protein protection is consequently less active. Glutaredoxin 6, GSH-peroxidase-like Tpx, and thioredoxins 1 and 2 also have a loss in activity when CQ is added. The ferrochelatase only actively detoxifies Fe^{2+} when no compound is added. GR gets more active when CQ is added [see figure 4.18).

Scenario 2: CQ-resistant strain. When sulfadoxine is added to a CQ-resistant strain, we observe an increase in activity in glutaredoxin reactions 2, 3, 4, and 5, in thioredoxin-dependent peroxidase by 15-18%, and 7% for glutaredoxin reaction 6 and for GSH-peroxidase-like Tpx. Thioredoxin reductase shows an increase of 56%. A decrease in enzyme activity is calculated for thioredoxin reaction 2, GR, ferrochelatase, and thioredoxin 1 by up to 10%. When MB is added, we calculated a strong enhancement of these effects for glutaredoxin reactions 2, 3, 4, and 5, thioredoxin-dependent peroxidase, GR, and thioredoxin 1 and 2. The activity of glutaredoxin reactions 2, 3, 4, and 5 and thioredoxin-dependent peroxidase is increased by nearly 45% (note that glutaredoxin reaction 4 changes its direction). GR shows a decrease in activity by 18%, as does thioredoxin 2. The activity of thioredoxin 1 decreases by 77% (with sulfadoxine alone only 10%). In this calculation thioredoxin reductase now shows an 8% decrease in activity, whereas it was more active under the influence of sulfadoxine alone (56%). GSH-peroxidase-like Tpx shows similar effects: with sulfadoxine there was a 7% increase in activity; with sulfadoxine/MB there was a 47% decrease (see figure 4.19).

Scenario 3: multi drug-resistant strain. When sulfadoxine and CQ are added to the Multi drug resistant (mdr) strain, an increase in activity of glutaredoxin reactions 2, 3, and 5 by 11% and GR by 14% is calculated. A decrease in activity by 17% is shown for glutaredoxin reaction 6, GSH-peroxidase-like Tpx, and ferrochelatase. There is a decrease by 9% for thioredoxin reaction 2, whereas thioredoxin reaction 1 is decreased by 61% and thioredoxin reductase by 80%. MB added to the sulfadoxine/ CQ combination causes severe changes in nearly all key redox enzymes. Thioredoxin reductase is completely reduced. Thioredoxin dependent peroxidase and glutaredoxin reaction 4 increase their activity by 18%, whereas the ferrochelatase is reduced by 13%. In the case of glutaredoxin reactions 2, 3, 5, and 6, GSH-peroxidase-like Tpx, and thioredoxin reactions 1 and 2, the activity is reduced and the direction of reaction changes. This indicates a more severe effect on the parasite than the combination treatment alone (see figure 4.20). As shown by metabolic flux modeling,the sulfadoxine-resistant strain shows an increased activity in glutaredoxin reactions 2, 3, 4, 5, and 6, in GSH peroxidase-like Tpx, thioredoxin-dependent peroxidase, and especially in thioredoxin reductase.

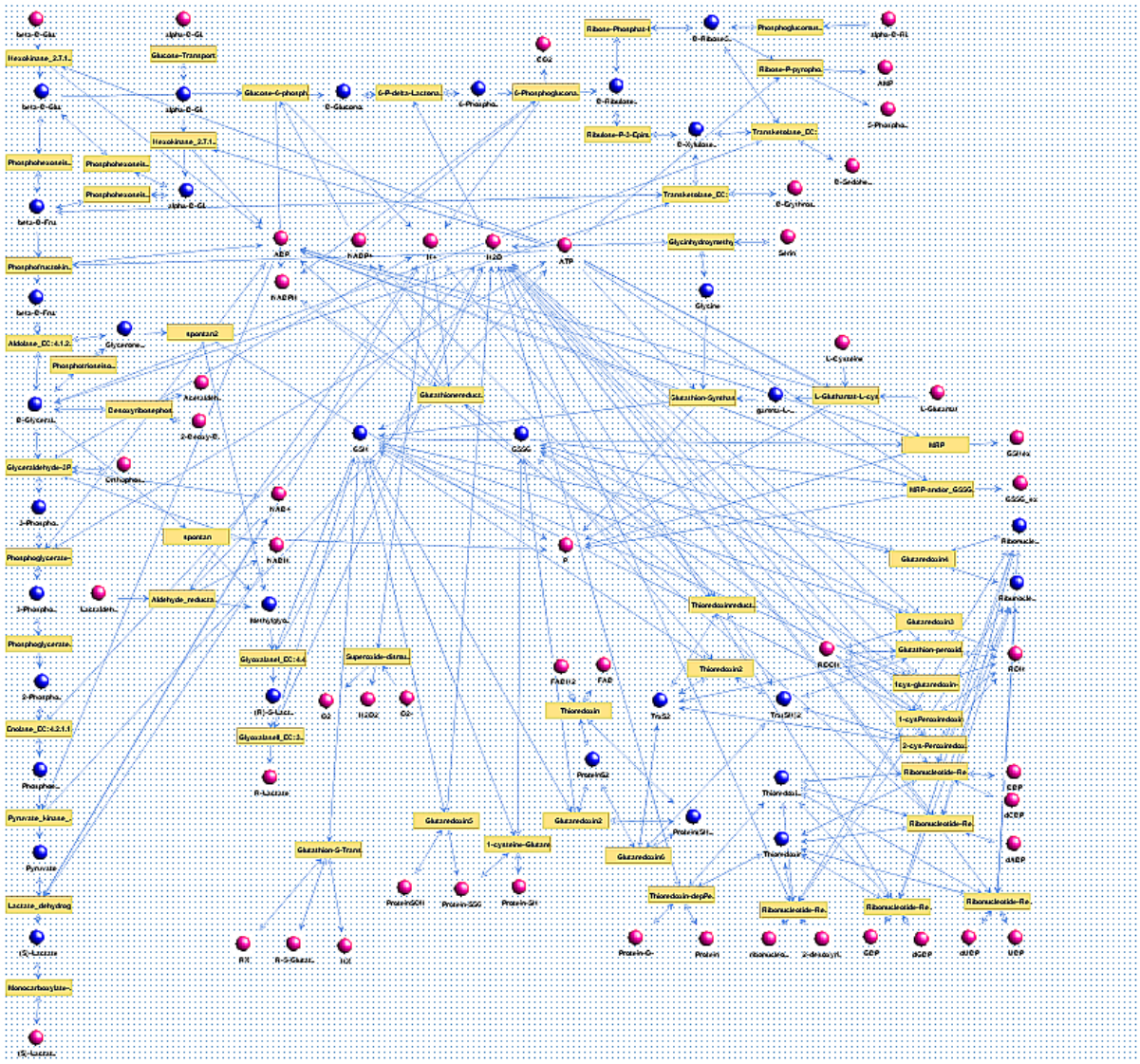

Figure 4.16: Pathways of plasmodial redox metabolism: Brown boxes refer to enzymes, a metabolite (names in black) is either substrate or product of a metabolic reaction. External metabolites (pink balls) are either taken up from the environment (substrate) or constitute the end-product of an enzymatic reaction. These are the sources or drains of the metabolic fluxes modeled and hence need not to be balanced. Internal metabolite (blue balls) concentration has to satisfy the steady state condition of the model as it is produced to cover the need of reactions using it in turn again as a substrate. Each of these internal metabolites has to be balanced by the enzymatic reactions of the metabolic network. All pathways are shown as equally active (blue arrows indicate fluxes) as this calculation indicates only which pathways could be used at all. For further details, see text.
Figure as published in Zirkel et al. [17]

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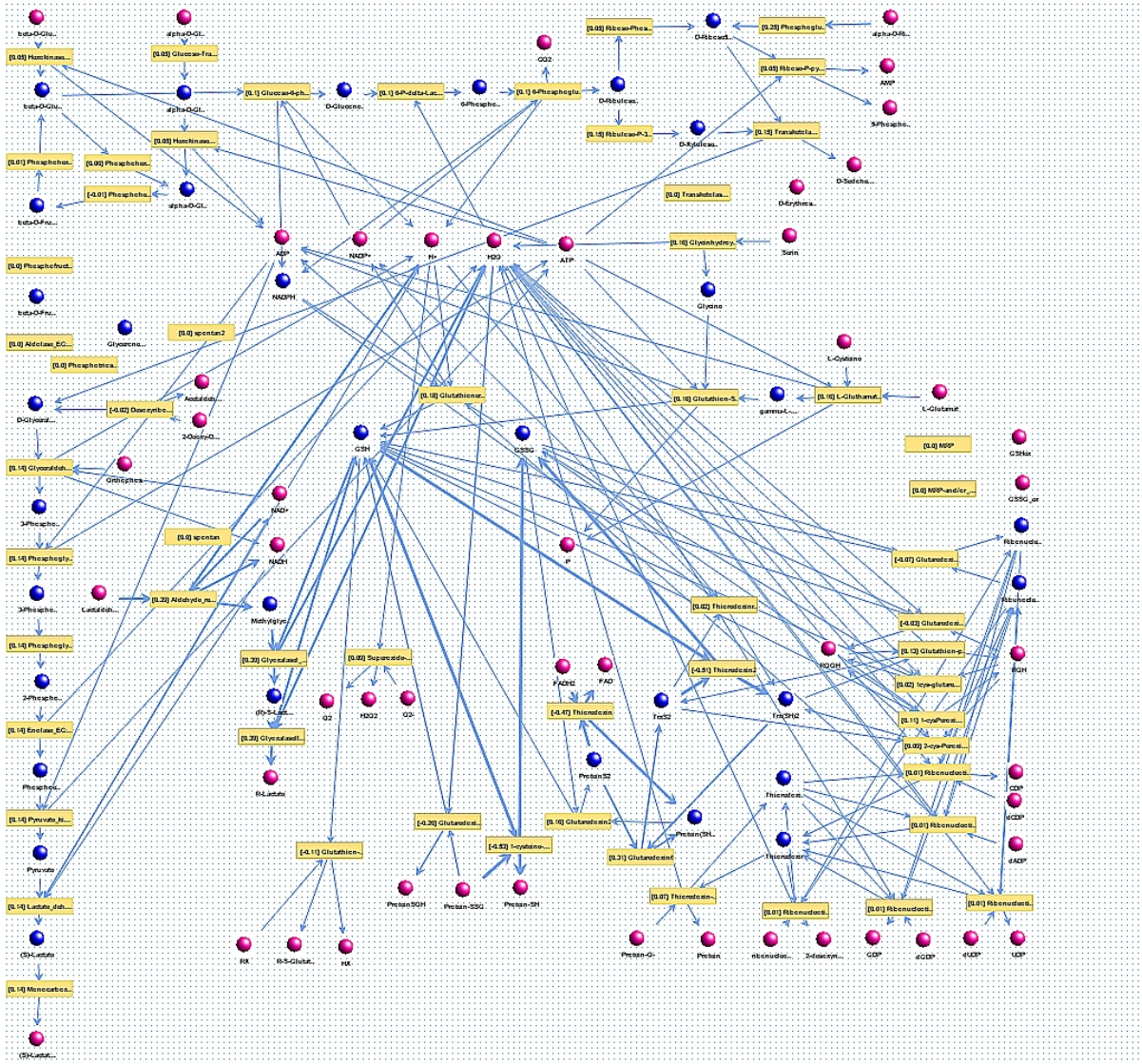

Figure 4.17: Changes in the *P. falciparum* redox flux network after MB drug action: Results incorporating the transcription data and experimental mRNA expression data nine hours after incubation with MB are shown. For colour codes see figure 4.16. Thicker arrows represent a stronger pathway flux. With MB there is a shift in redox protection, including GR. Fluxes change according to the utilization of the different pathways in the actual experiment and as a result of MB drug action (thinner and thicker arrows).
Figure as published in Zirkel et al. [17]

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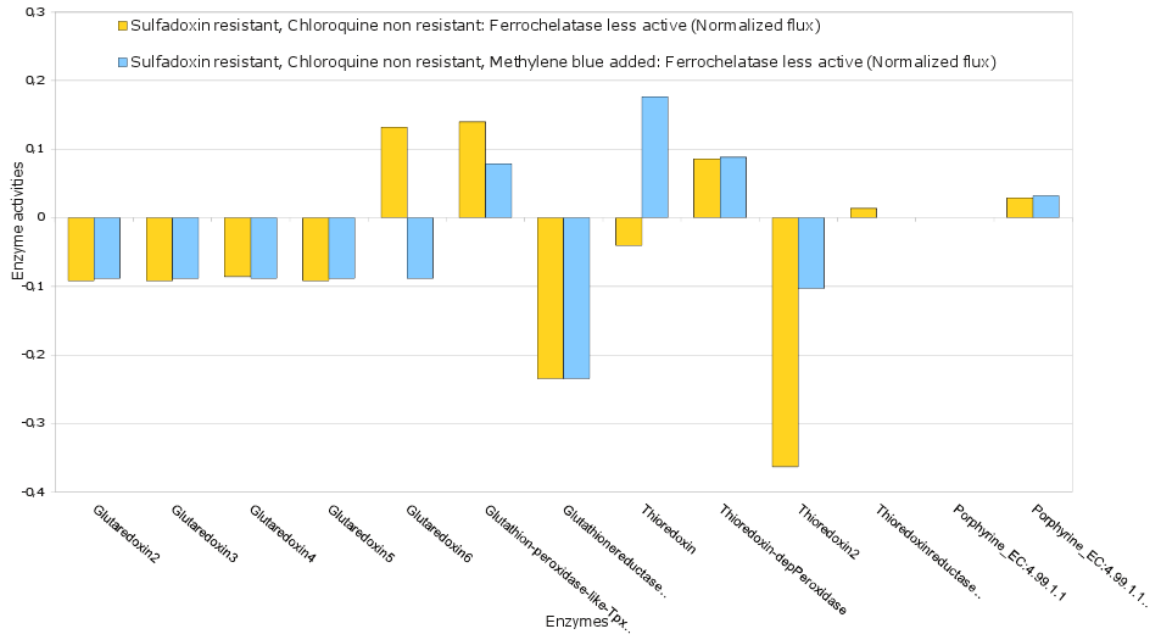

Figure 4.18: Changes in key redox enzymes - SP-resistant strain. Shown are the effects of CQ on key redox enzymes in a SP-resistant strain (yellow bar). Also shown are the effects of a combination of CQ and MB (blue bars), as well as the effects of MB on a non-resistant strain (greyish blue bar). As a baseline, the enzyme activities of a non-resistant strain without any added compounds were taken.
Figure as published in Zirkel et al. [17]

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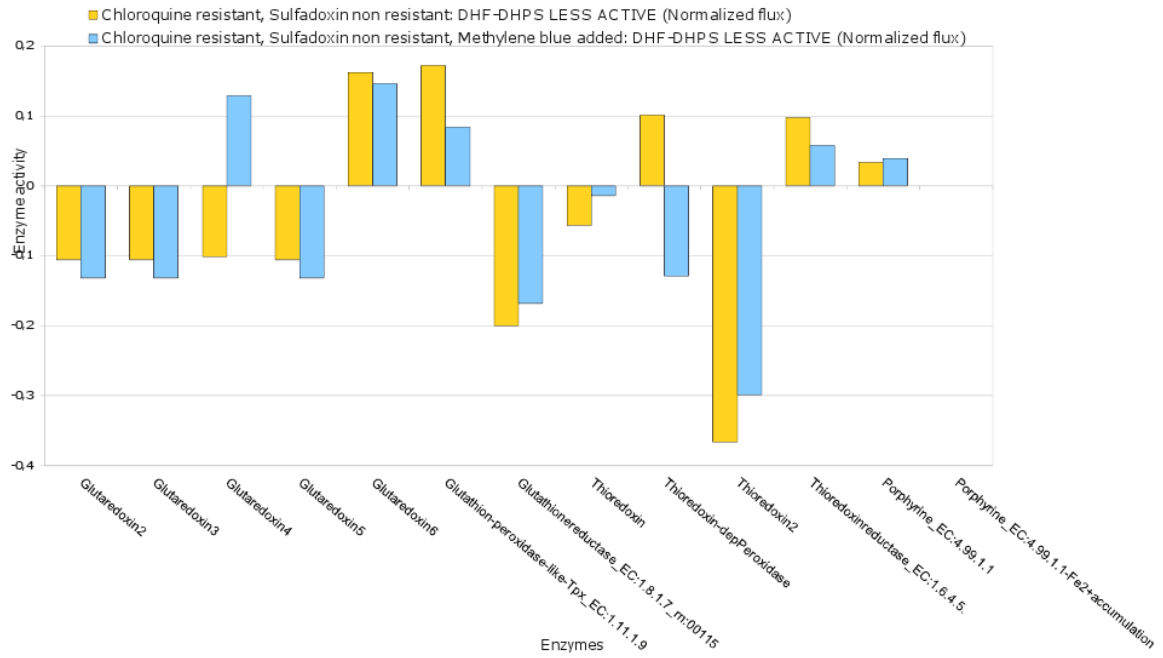

Figure 4.19: Changes in key redox enzymes - CQ-resistant strain. Shown are the effects of sulfadoxine on key redox enzymes in a CQ-resistant strain (yellow bar). Also shown are the effects of a combination of sulfadoxine and MB (blue bars), as well as the effects of MB on a non-resistant strain (greyish blue bar). As a baseline, the enzyme activities of a non-resistant strain without any added compounds were taken.

Figure as published in Zirkel et al. [17]

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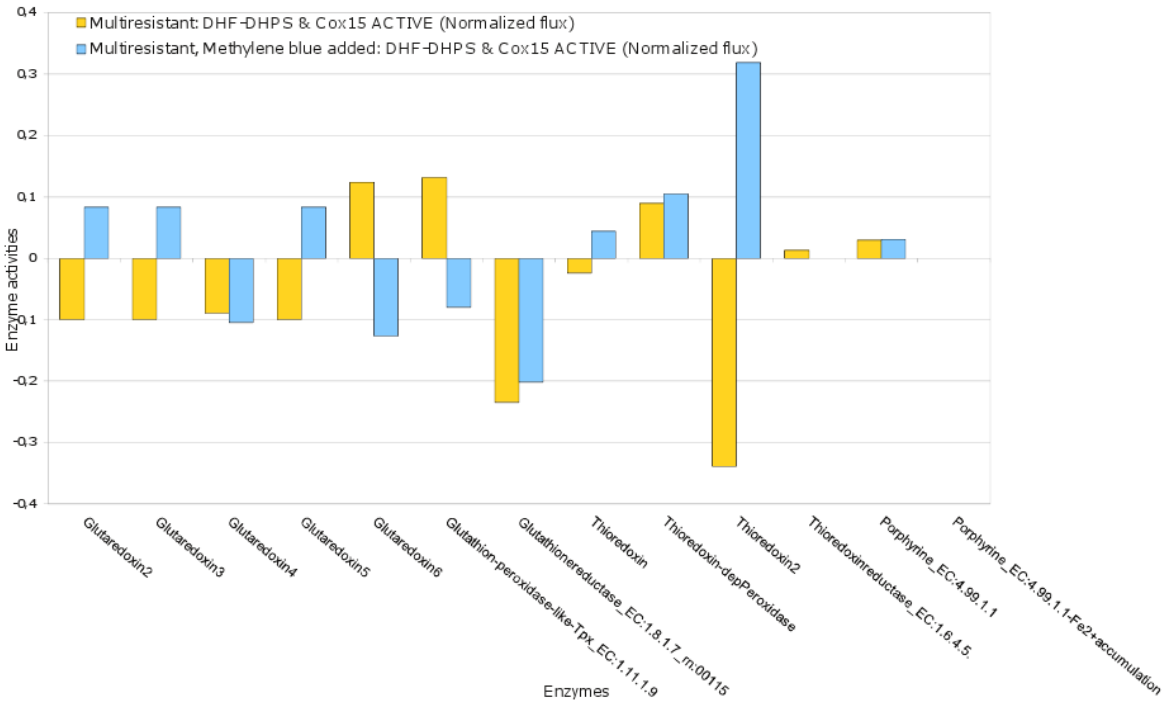

Figure 4.20: Changes in key redox enzymes - mdr strain. Shown are the effects of CQ and sulfadoxine on key redox enzymes in a mdr strain (yellow bar). Also shown are the effects of a combination of CQ, sulfadoxine, and MB (blue bars), as well as the effects of MB on a non-resistant strain (greyish blue bar). As a baseline, the enzyme activities of a non-resistant strain without any added compounds were taken. Figure as published in Zirkel et al. [17]

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Clinical trials involving MB:

The first trials re-establishing MB as an antimalarial were based on the observation that the antimalarial effects of CQ is enhanced by low GSH levels [Dubois et al., 1995, Meierjohann et al., 2002, Schirmer et al., 2003]. On the assumption to undermine parasite CQ resistance by the combination with MB, this combination was tested in clinical trials in Nouna, Burkina Faso [Rengelshausen et al., 2004, Mandi et al., 2005, Meissner et al., 2005]. No severe adverse effects occurred in these trial, even in G6PD deficient individuals MB was savely administered. One property of MB turned out to be an annoyance though: its bitter flavour could impair compliance, especially in children. Gut et al [Gut et al., 2008] suggested to solve this problem by binding MB to cation exchange materials as pharmaceutical carriers in order to mask the undesirable properties. A sweet granulate or syrup can thus be fabricated and was used with good compliance in henceforth conducted studies. Coulibaly et al. were able to show that MB-based therapies are accepted by the communities in spite of another side effect: the blue discoloration of urine resulting from MB intake were not considered problematic for drug adherence. On the contrary, blue (washable) spots in clothes or diapers indicated patient compliance to caregivers and health workers [Coulibaly et al., 2009]. The blue color of the urine can also be used as an indicator that the MB-containing drug combination has not been faked.

The combination effect of MB and CQ that was hoped for was not found in the above cited clinical trials, though: parasite clearance after CQ-MB combination did not significantly differ from CQ monotherapy [Meissner et al., 2005, 2006]. In vitro, MB was found be antagonistic with CQ, as well as with other quinoline antimalarials like AQ. With MQ and quinine, MB showed additive effects, whilst synergistic effects of MB with artemisinin, AS, and artemether for *P. falciparum* and other parasite strains were demontrated [Akoachere et al., 2005]. Following these observations, a trial comprising 180 children aged 6 - 10 years with uncomplicated falciparum malaria was conducted in Nouna, Burkina Faso [Zoungrana et al., 2008]: Tested combinations were the standard regimen, AQ plus AS, against MB with either AS or AQ. Most interestingly, whilst parasite clearance time was the shortest with MB - AS, this combination was clearly inferior to AQ plus AS and AQ plus MB in follow-up as opposed to the in-vitro findings:

By day 28, the parasitological clearance rate was lowest for MB - AS (62%), intermediate for the standard treatment AQ - AS (82%), and highest for MB - AQ (95%). However, treatment duration and -dosage are not yet to be considered optimised. Especially as clinical trials testing MB monotherapy suggest MB to act slowly, and needed to be given for at least 7 days to be efficacious in the treatment of falciparum malaria on follow-up [Bountogo et al., 2010]. The combination with a fast-acting antimalarial could turn out to be beneficial thus.

4.4.2 Methylene Blue as antimalarial: Implementation in Burkina Faso

Economic aspects of MB implementation

The acronym "bonaria" epitomises the concept of an "ethical" drug [Schirmer et al., 2003, Becker, 2011]: "bon" in the Latin word stem means safe and effective, "a" affordable for patients who need it, "r" already registered for other medical indications, and "ia" internationally available.
Although costing millions of lives, only 1% of all newly approved drugs between 1975 and 1999 were registered for diseases that like malaria prevail in developping countries, evidence of a huge innovation gap for these "unprofitable" diseases. The pharmaceutical industry argues that research and development is too costly and risky to invest in low-return neglected diseases [Trouiller et al., 2002]. To bridge this gap, ethical drugs that are necessary to prevent and cure disease, but unlikely to generate profits are designed following the "bonaria" principle [Becker, 2011]. MB does not only fulfill these criteria, but is also an example for the reconcilability of ethical and economic value:

The pleiotropic impact on Plasmodium, and the deep interference with the parasite's redox metabolism - an achilles heel resulting from its unique hideaway inside RBCs - make MB a most effective antimalarial drug, as has been shown in the preceeding section. Cross resistance with other drugs does not occur and quick resistance development seems unlikely, given the way of action including not only many targets, but also pathways that cannot be easlily bypassed and are of vital importance to the parasite.

MB is also active against the gametocytes of *P. falciparum*. This property brings about the chance to interrupt the transmission cycle from patients to mosquitoes [Buchholz et al., 2008b, Coulibaly et al., 2009], and make it a most interesting candidate for broad scale use.

Pharmacokinetics and bioavailability of MB given orally have been investigated in several studies [Akoachere et al., 2005, Bountogo et al., 2010, Walter-Sack et al., 2009]. These showed MB to be safely administered even to individuals with G6PD deficiency. No severe adverse effects were noted in clinical studies, and acceptance of MB was high in the local population.

One major drawback is the bad (bitter) taste of MB in aqueous solution, but the development of a taste-masked formulation (a sweet granulate) of MB takles this problem fulfilling these criteria have recently been developed [Gut et al., 2008, Schirmer et al., 2011].

Recently, MB has been criticised for synthesis-related impurities, heavy metals[3] being the major contaminants [Pascual et al., 2011]. Although heavy-metal related toxcitity was not observed in clinical studies in Burkina Faso [Mandi et al., 2005, Meissner et al., 2005, 2006, Akoachere et al., 2005, Zoungrana et al., 2008], and preparations of MB used in these studies are conform to requirements of the US- and European Pharmacopoeial Convention, this should be cautiously monitored in long-term studies to avoid accumulation effects.

Proveblue is a MB preparation obtained by a new synthetic pathway with a heavy-metal-free process involving pharmaceutical-grade reagents (patent no. PCT/FR/2007/001193) [Pascual et al., 2011, Dormoi et al., 2012]. As this new synthesis got patented, its product cost are substantially higher - although it seems doubtable that this is only due to higher production cost (see table 4.26).

The current regimen used for antimalarial therapy in studies is 12 mg/kg body weight MB administered p.o. twice daily for 3 days, i.e. in total 72 mg/kg [Zoungrana et al., 2008]. Observances during these studies hint towards a good efficacy and tolerability of a once-daily regimen of 15 mg/kg body weight (Olaf Mueller, personal communication). This regimen would enourmously simplify drug intake - and reduce total drug amount to

[3] Aluminium, Iron, Copper, Tin, Chromium, Manganese, Molybdenum, and Cadmium

45 mg/kg body weight - with consequent appealing effects on drug adherence and cost.

In Burkina Faso, as well as in most other countries, MB is approved for the treatment of methemoglobinemia. Approval as an antimalarial seems within reach given the existing studies.
Theoretically, MB could be produced at cost comparable to those of CQ, as synthesis is uncomplicated and basic products are easily available. As a raw product with $\geq 95\%$ purity, MB is available from many providers of chemical and pharmaceutical products worldwide, of which table 4.26 only gives a small selection[4].

Table 4.26: Cost of MB: Raw material prices from different manufacturers worldwide.
A standard treatment regimen as currently used in clinical studies consists of 15 mg/kg/d for 3 days = 45 mg/kg. Applying a medium weight of 10 kg, total treatment dose is 450 mg.

Manufacturer	Price per kg	Price per treatment
MubyChem, India	170.00 EUR	0.07 EUR
Sigma Aldrich, USA	262.50 EUR	0.12 EUR
Provepharm, France	400,000 EUR	180 EUR

Since 2007, however, obtaining MB from pharmaceutical companies has become more and more difficult. Curiously coinciding with the practical implementation of MB in Alzheimer's therapy, the price of GMP-validated MB underwent a steep increase [Schirmer et al., 2011] (see table 4.26). As no shortage of the raw materials of MB synthesis occurred in the meantime, this can only be explained by ongoing market-rearrangements.
In order to alleviate the price pressure evolving from possible uses of MB for Alzheimer's - with consequent economic value as this disease prevails most in high-income industrialised countries - a production (partly) detached from the free market could be an interesting

[4]prices were taken from a online search at manufacturer's homepages and a search of an international pharmacy (EuroMed, Fuerth, Germany) conducted in April, 2012

solution. This already proved effective for Artemether-Lumefantrine (Coartem®), the fixed-dose ACT produced by the swiss pharmaceutical giant Novartis. In 2001 Novartis and WHO launched a public-private partnership providing Coartem® at cost-price to the poor. Between 2001 and 2011, more than 400 million Coartem® treatments were thus delivered to WHO. Another example is given by Highly Active Anti-Retroviral Therapy (HAART): In 1996, this combination therapy became available, and within only four years, death rates for HIV/AIDS patients had dropped by 84% in developped countries [Porter et al., 2003]. At a cost of US$10,000-15,000 per person and year, these drugs were beyond the reach for the majority of people infected with HIV in developping countries; only 2% of people living with HIV/AIDS in these regions had access to HAART [WHO, 2002] at the beginning of the new millennium. At that time, an Indian pharmaceutical company started to produce generic antiretrovirals, reducing the price for HAART to approximately 300 US$ per person and year. The thus triggered price war, together with concerted pressure from NGOs and governments of developping countries highly affected by the HIV/AIDS pandemic finally forced the large pharmaceutical companies to lower the price of their antiretroviral drugs [Avert, 2011, MSF, 2001].

As the synthesis of MB is long beyond patent protection, a non- or low-profit production of MB, respectively a MB-based combination therapy financed by means of e.g. the GFATM seems possible.

MB synthesis is in many respects similar to that of CQ, thus prices should be comparable. India's National Pharmaceutical Pricing Authority, a 1995 established organization of the Government of India, fixes regularly updated prices of controlled bulk drugs to enforce reasonable drug prices for its citizens. Price settings of this authority are highly regulated to assure fair pricing for manufacturer and consumer alike [5], and should thus display a realistic price range for bulk drugs such as antimalarials. The 2008 update (NPPA Order Sl. No. 43) set the price of CQ to 861 Indian Rupees (12.45 EUR) per kg.

Taking CQ as example, MB could be produced at cost not exceeding 15 EUR per kg.

[5] Data on production prices are collected by standardised questionnaires, cost-audit reports, and verification by plant visits, if required. These are then processed and adapted to actual technical parameters and personnell standards (salaries and wages) (for more detailed information, see `http://www.nppaindia.nic.in/index1.html`, last checked May 16th, 2012)

Of course, portioning, packaging, and transportation cost will add to this bulk price. But even assuming the active pharmaceutical ingredient only to account for 10% of the final package price, MB treatment cost would be approximately 7 EURCents[6].

MB is not a quick-acting drug. It should therefore only be used for uncomplicated malaria, and in combination with a suitable partner drug. The ideal combination partner should thus be quick acting, to counterbalance MBs slowly occuring parasitocidal effect. Also, half lives of the partner drug should be matched, and additive - ideally synergistic - effects with MB are preferable.

AQ-MB is a combination that expresses many of these desirable effects: in *in-vitro* studies this combination showed additive results [Dormoi et al., 2012], *in vivo* synergistic effects were even noted [Zoungrana et al., 2008]. The two drugs have comparable half-live times of their active ingredients, and AQ is a standard antimalarial drug in Burkina Faso. Moreover, it is cheap (0.24 EUR per treatment). A fixed-dose treatment of MB and AQ would definitely be worth exploring for its use as an area-wide treatment strategy for Burkina Faso.

[6]This refers to a child of 10 kg, treated with 15 mg/kg once daily for 3 days.

Chapter 5

Discussion

The fight against malaria is a major task of global health policy today. Malaria not only threatens millions of lives, it also is a major setback for human development in affected areas. Poor countries such as Burkina Faso suffer most from malaria burden. However, they are also those least equipped to tackle this burden. WHO has termed a variety of measures to fight and control malaria, which in many studies have proven good efficacy [WHO, 2008a, Nosten and Brasseur, 2002, Bloland et al., 2000, Brockman et al., 2000]. It is thus surprising how little attention is drawn to their practical implementation under "real life"-conditions in current scientific analyses.

Here, I show that malaria treatment in Burkina Faso is not in accordance with theoretical constructs of antimalarial therapy. Reasons for these shortcomings are various, as discussed in this thesis, but can ultimately be reduced to a tremendous imbalance of patients versus health personnel and the unspecific presentation of malarial disease combined with lacking diagnostic means.

First of all the overwhelming number of patients severely overcharge health structures: 1.3 million malaria cases in children under five years of age occur annually in Burkina Faso, of which approximately 10% (150,000) are classified as severe malaria. During the monitored four weeks of my retrospective study presented in this thesis, the AE department was faced with 542 patients under five years of age to be hospitalized. 343 (63.3%) of those were presenting under the suspected diagnosis of malaria and treated as

such.

One factor influencing these numbers surely is the undecisive nature of clinical signs for malaria, which can in many cases be interpreted as manifestations of other, also frequently occuring infectious diseases like bacterial meningitis or pneumonia.
As discussed in chapter 3, especially venous blood samples (as performed in AE) may be negative despite present parasitemia [Delley et al., 2000]. Also, antimalarial pretreatment can result in false-negative microscopy. For this reason, it is required to perform repeated examinations when malaria is suspected, should the first test be negative.
On the other hand most studies in developing countries such as Burkina Faso suggest that treatment deferral is one of the main causes of preventable death in children under the age of five [Mueller et al., 2003a]. It seems that for effective reduction of malaria mortality, early treatment is vital, and even WHO recommendations explicitly state that "treatment should not depend on fulfilling [laboratory] criteria" [WHO, 2000].
Another aspect is complicating the presentation of malaria: In a semi-immune population parasitemia can be present but the patient might not suffer from malaria [Bottius et al., 1996]. To overlook any of the contemplable differential diagnoses to malaria, like meningitis or pneumonia, could be fatal. Again, due to lack of diagnostic means, not in all cases differential diagnoses could be excluded (e.g. lumbar puncture was not performed to exclude meningitis, X-ray was not available and pneumonia could not be ruled out). As shown, in nearly 40% of all confirmed malarial cases an alternative diagnosis was still considered and also treated. This harbours an enormous potential for over-treatment with both antimalarials and antibiotics, as most likely the double treatment was unnecessary in a majority of the cases. Quick and reliable means to establish the right diagnosis is thus crucial. The lack of these (often basic) diagnostic means - results in cost-intensive over-/ double-treatment.

Also, the definition of severe malaria poses a problem not to underestimate: to identify those patients most at risk - and thus to be hospitalised - with high sensitivity, but also high specificity, is still unsatisfactorily met. WHO definition, though very adept for medical conditions e.g. in Germany, is not properly appliable in Burkina Faso. Even in the best-outfitted hospital, out of 15 defining symptoms only 10 were properly to

elevate. Sensitivity of this definition was high (83%), but with poor specificity (0.6%). The definition suggested by [Marsh et al., 1995] offers the same sensitivity, but a far better (though still far from impressive) specificity of 34%. Contrary, it relies only on three signs that are easy to assess for: impaired consciousness, respiratory distress, and severe anaemia. Thus, cost-intensive hospitalisation could be avoided if diagnostic means were better adapted and/ or exploited.

To achieve this, though, health care units need to be better equipped with personnel. The above mentioned shortcomings noted in my study did not result from ignorance or slothfulness of the medical personnel, but in most cases were due to the overwhelming mass of patients facing a severely under-staffed medical team:
To attend to all these patients, there usually were one doctor, one intern student, and three to four nurses present. The resulting workload on each of these is huge, as it includes admission, ongoing patient care - many of them critically ill, and discharge of patients. It seems understandable that under these circumstances diagnostic steps like repeating of blood examinations etc. is not easily feasible.
Medical conditions are even worse in peripherical health care units of Burkina Faso: In 2007, Burkina Faso had 441 medical doctors, 38 surgeons and dentists, 58 pharmacists, 604 trained midwives, and 7741 registered nurses [Ministry of Health of Burkina Faso, 2008a]. Formal health service especially in the rural parts of Burkina is thus mainly performed by nurses, midwives, and mainly unskilled helpers. Only from the regional level on, hospitals have specialist units and are run by physicians (see Figure 4.3) [Kouyate et al., 2007]. In order to ensure a minimum level of care, the Ministry of Health of Burkina Faso framed minimum personnel standards for the maintenance of a basic health care institution: a CSPS is to employ at least one nurse, one assitant midwive, and one unskilled helper. In reality, between 40 and 10% of all CSPS - depending on region - do not meet even this basic standard [Ministry of Health of Burkina Faso, 2008a]. This problem is especially pronounced in the rural regions as they are less attractive to highly skilled personnel due to low infrastructure and life quality: The cities of Ouagadougou and Bobo-Dioulasso together concentrate 54% of all medical doctors, 57% of all midwives, 59% of all pharmacists, and 33% of all nurses working in Burkina Faso [Ministry of Health of Burkina Faso, 2006a].

This carries about a blatant unmet medical need in the rural regions of Burkina Faso. Although binding 22.5% of the expenses for malaria in Burkina Faso, merely half of the patients suffering from severe malaria according to statistics are actually treated under sufficient medical care (see table 4.5).
As can be seen in figure 4.3, a CSPS in Burkina Faso has to provision approximately 10,000 people in theory. The theoretic radius within which these people live around a CSPS is thus estimated to 7.60 km. As for varying population density this can only be a rough estimate. According to statistics of the Ministry of health of Burkina Faso, 60% of its population live in a radius of 10 km or less from any medical institution [Ministry of Health of Burkina Faso, 2008a]. This may sound not much, but when an ill child has to be transported in a country where most roads are unpaved, not frequented by public transport and people rely on such basic means of transport as a bicycle or a donkey-drawn cart, if at all - then 10 km equals an impediment that will in most cases result in a waiting attitude as long as the child is not critically ill. In a study of treatment seeking behaviour in a rural area of Burkina Faso, Mueller et al. stated that the frequency of medical consultations were highly associated with distance to the health centre or hospital, as well as with a more severe illness presentation [Mueller et al., 2003a]: whilst among the urban population, 70% of the population have access to medical services, in rural areas as little as 29% of the population have such access [Ministry of Health of Burkina Faso, 2008a].

Poor access to medical care of course compromises treatment seeking behaviour, but early antimalarial treatment is delayed for other reasons as well: The low level of education and especially literacy as present in Burkina Faso brings about that basic knowledge e.g. on disease handling and risk factors are often scarce. Paired with the low level of medical care especially in the rural parts of Burkina Faso, a high number of malarial episodes is bound to be left un- or insufficiently treated.
Language barriers are augmenting this issue: In Burkina Faso approximately 60 different languages exist. The unifying language, French, is taught at primary level, but when education is only scarce, people are often not or only insufficiently able to communicate with doctors or other health staff, as I experienced first hand. Even simple treatment instructions such as how and when to take medications were sometimes difficult to communicate,

not to mention the difficulties in taking the medical history of a patient.

As these considerations suggest, the fight against malaria in Burkina Faso has to struggle with two seemingly opposed problems: On one side, a serious over-estimation - and cost-intensive over-treatment - of malarial cases in hospitals badly equipped for proper diagnostic courses. On the other side, malaria presentation is likely to be aggravated due to treatment deferral that results from low education levels, bad infrastructure and a poor level of medical care especially in the rural parts of the country.

Since 2001, WHO treatment protocols foresee the discontinuation of CQ-monotherapy and the implementation of (artemisinin-containing) combination therapy. Artemisinins were virtually absent from pretreatment data of patients included in this study, as was combination treatment. Since then, only little progress was made, as only 10% of all sold antimalarial treatments in 2009 were ACTs (see Table 4.25).
This clearly shows that treatment protocols are not satisfactorily implemented on-site, and knowledge on resistance rates need improvement: even patients referred from other health professionals had recieved CQ in 16.8% of the cases. With resistance levels of $\geq$ 20%, this drug is virtually useless in Burkina Faso [Tinto et al., 2001, 2002], a fact that should be well known among health professionals.

Antimalarial drugs in Burkina Faso account for 63.5% of all medical cost for malaria (see Table 4.3). Economic aspects are of vital importance for any treatment strategy: Nearly two thirds of all medical cost are borne by patients, who will shun expensive drugs if these cannot be afforded. Also, available funds are bound by high treatment cost and cannot be used elsewhere.
CQ for years was the drug of choice for its antimalarial potency and good tolerability, paired with with an unbeatable low price. In Burkina Faso, CQ treatment only cost 15 EUR Cents for the end-consumer. This might explain why despite high resistance rates and after official treatment policy change towards ACTs, CQ was continuously used for treatment of febrile illness. In 2009, CQ was consequently banned from the Burkinabe market. During the time of my study 13.4% of all patients had recieved CQ.
At that time, AQ had already replaced CQ as primary treatment for uncomplicated malaria: 52.8% of all patients having recieved home treatment in my study had been

treated with this drug. Its price of 24 Cents per treatment for the end consumer - though considerably more expensive than CQ - is still quite attractive. It is currently recommended in combination with AS as first line treatment for uncomplicated malaria [Ministry of Health of Burkina Faso, 2006a]. By extensive RBM-financed subsidy programmes cost of this treatment have been reduced from 1.33 EUR and up to 0.26 (infants) - 0.72 (adults) EUR per treatment (wholesales bulk price).
AQ-resistance in Burkina Faso is approximately 8% [Mandi et al., 2008], which is acceptable - though far from ideal. A combination strategy ideally should not include a failing drug, for this could imply factual monotherapy with the remaining effective compound. AQ yet has an agreeable action profile, but continued mass-use as monotherapy could accelerate resistance selection, as the underlying mechanisms of AQ and CQ resistance are identic [Tinto et al., 2008]. Thus, AQ monotherapy should be banned from the market to prolong its useful life in combination treatment.

Quinine is the drug of choice for severe malaria and should in these cases be administered intravenously according to national treatment protocol in Burkina Faso [Ministry of Health of Burkina Faso, 2006a]. Disrespectful of this fact, it was often used in Burkina Faso as oral treatment, even in home treatment: 34.3% of all patients had recieved Quinine before presenting at the hospital, and 22.2% of those treated at home had taken this drug. Still, it is the drug most frequently sold in Burkina Faso after the removal of CQ: 65% of all sold antimalarials in 2009 were Quinine tablets. The oral route is not sensible in a case of severe malaria, thus it seems likely that most of these medications were used for the treatment of uncomplicated malaria. This seems a dangerous trend, as Quinine therapy carries a substantial potential for serious side effects. Also, the long duration of therapy (7 days minimum) prompts the risk of non-adherence respectively discontinuation of treatment after initial amelioration of symptoms (especially in a semi-immune population). Currently, quinine remains highly effective against African *P. falciparum* strains, but as the example of South-East Asia has shown, extensive use has before triggered quinine resistance [Wongsrichanalai et al., 2002, Woodrow and Krishna, 2006]. Thus, this drug should be reserved for treatment of severe malaria.

Artemisinin emerged on the global stage in the 1990s, and was the light at the end of

the tunnel in a time where resistance rates rocketed and caused a bleak outlook on malaria control. Since, ACTs took the world by storm. They are the fastest acting antimalarials ever known, do not express any severe adverse effects, and are effective even against multi-drug resistant parasites. Unfortunately, as in all head-over-heels love stories, there are a few catches to be taken into the account:

The unacceptable high price of Artemisinins is by far the greatest of these. The artemisinin raw material is derived from the chinese wormwood plant *Artemisia annua*. Although a very undemanding plant to grow, the yield of artemisinin fluctuates considerably, depending e.g. on climate, altitude, and soil - i.e. suitable agricultural terms are needed for its commercial cultivation. Production is currently centered in East and South-East Asia (China, Vietnam) with a few upcoming cultivation areas in East Africa. Under optimal conditions, production ranges from 6 to 14 kg of artemisinin raw material per hectare [Kindermans et al., 2007]. Even on these terms, unpredictable events like droughts or floods ruining whole crops and thus threatening artemisinin supplies uncontrollably, become more likely under the ongoing climatic changes. Within the foreseeable future, cultivation as a bottleneck of production will remain to dictate a minimum price level for artemisinins. Current projections estimate that cost could be sustainably held at US$ 250-300 (EUR 195 - 235) per kg, provided appropriate mechanisms to reduce market fluctuations are taken [Kindermans et al., 2007]. This price is unlikely to be able to compete with all-synthetic drugs like CQ and MB on the long run.

In Burkina Faso, national treatment policy designates Artemether - Lumefantrine (alternatively AS - AQ) as first-line treatment for uncomplicated malaria since 2005. Unfortunately, the common saying "paper doesn't blush" applies to this context only too well: in the presented study, the large majority of patients in Burkina Faso still recieved monotherapy treatment, many even CQ despite drug resistance. Even after the ban of CQ in 2008/09, ACTs are still only used in 10% of the cases as data presented here show (see table 4.25). Despite agressive subsidaries this combination treatment still costs five to ten times more than the next cheaper available alternative. When as in Burkina Faso these cost mostly have to be tackled by the patient alone this price is still too high for a majority of patients.

Another important factor to be taken into account is resistance development. So far,

no Artemisinin resistance has been found in field isolates of *P. falciparum*. In laboratory models though, resistance has been successfully selected [Jambou et al., 2005, Afonso et al., 2006], proving the potential of the parasites to become resistant to this last resort of current Malaria control.

The *in silico* data presented in section 4.3 (Figure 4.13) suggest that few point mutations at domain boundaries of PfATP6 would be enough to create resistance: Minor differences in structure regarding PfATP6 compared to its orthologues of e.g. man are selectively targeted by artemisinins, showing that resistance could involve only few structural changes and could thus be easier to develop than previously thought. These results of course strongly rely on the absoluteness of the PfATP6-target as underlying mechanism of action of artemisinins, and only suggest one possible resistance mechanism. Still, even the possibility of such an easy way of resistance development needs to be addressed, given the fact that currently artemisinins form the last line of defence against malaria.

Considering a possible resistance mechanism that is as easy to develop as presented in this thesis, the best possible protection of artemisinin efficacy should be the ultimate goal of any treatment strategy. It seems thus odd that WHO so uncompromisingly focusses on ACTs alone: Combination treatment is an important principle to improve therapeutic effect, e.g. by exploiting synergistic effects or shortening treatment courses. It also protects the single components of a drug combination against resistance and thus extends the useful life of a drug.

On the other hand, the principle of combination therapy is not necessarily bound to artemisinins. It could be beneficial to concentrate also on alternative drug combinations in order to conserve artemisinins comparable to reserve antibiotics.

However, for current WHO policy so strongly advocates ACTs and aid grants are bound unequivocal to this policy, chances for such a solution are scarce. In 2005, after Burkina Faso's appropriation request for the purchase of ACTs was rejected by the GFATM, the authorities asked the World Bank to use a portion of an existing US$12 million loan from the Global Strategy and Booster Program to purchase SP and AQ as an interim solution. This combination had been tested with good results in Burkina Faso [Zongo et al., 2005] before. However, the request was rejected with the argument that WHO recommends only ACTs [Kouyate et al., 2007].

This example becomes even more delicate considering that in 2005 ACTs were practically not available on the market for Burkina Faso: In 2004, after strong WHO recommendations and ensuing GFATM funding, and the groundbreaking agreement of drug firm Novartis to supply the only currently available fixed dose ACT (artemether-lumefantrine, Coartem®) at cost price, within only a few months in 2004 nearly 20 high-endemic countries changed their national treatment policies to ACT. As demand soared, drug supplies were suddenly depleted and the producers of Artemisinins were unable to keep up. Prices for Artemisinin rocketed from US$ 350 to over 1000, and many countries like Burkina Faso were not or insufficiently supplied with the needed drug [Cyranoski, 2004].

MB combinations could serve as an interesting alternative to ACTs - but also MB could be an interesting candidate to investigate further for combination therapy with artemisinins:
The modeling data as presented in this thesis, obtained in close collaboration with Janina Zirkel, Alexander Cecil, and Katja Becker's group at the Interdisciplinary Research Center of Giessen University, show that there is a multi-hit strategy evidenced by the MB effect. While Mrs. Zirkel's work extends to an area-wide implementation of MB in a malaria eradication strategy, the scope of this thesis is rather focussed on the potential of MB to serve as a regular compound used for the treatment of uncomplicated malaria. The pleiotropic impact of MB on Plasmodium, and the deep interference with the parasite's redox metabolism - an achilles heel resulting from its unique hideaway inside RBCs - make this a most effective antimalarial drug. In the PCR-backed modeling data presented here, GR protection is down-regulated, and only some recovery occurs. This compensatory up-regulation of GR observed is though likely to be counterproductive for the parasite, since MB is a subversive substrate turning the anti-oxidative GR into a pro-oxidative enzyme. Furthermore, the pathways of various protein protection modes, detoxification of 2-oxoaldehydes, generation and conversion of keto sugars, the lower part of glycolysis, and GSH synthesis are inhibited. The presented estimates are of course only semi-quantitative statements. As the presented study targeted only to underline the capabilities of MB, this was considered appropriate. The limits of estimates based on gene expression have been discussed previously [Cecil et al., 2011]; in the presented model, multiple constraints lower the residual fitting error to PCR data for most fluxes to only a small percentage.

A sub-lethal concentration of MB[1] was used on purpose, in order to monitor subsequent changes in gene expression that include parasite recovery.
The bioinformatical analysis presented here clearly shows the trophozoitocidal effects of MB via multiple direct hits on different enzymes, as well as via further redox pathway changes and impairment.
The gametocytocidal effects of MB reported elsewhere [Coulibaly et al., 2009] involve very similar pathways, and can be modeled with the same approach. This gametocytocidal property brings about the chance to interrupt the transmission cycle from patients to mosquitoes [Buchholz et al., 2008b, Coulibaly et al., 2009], especially in broad scale use.

Metabolic modelling of different drug resistance mechanisms as performed here point out that MB, due to its broad redox network pathway effects, can be well combined with different standard drugs used in malaria therapy. The different simulations detailed here show that different resistant strains become sensitive again when tackled by a combination therapy with MB. MB action is not impaired by the other drug resistance mechanisms investigated in the different scenarios. Even the Multi drug resistant situation is not enough to successfully inhibit the action of MB on several different redox and metabolic pathways, as also shown in in-vitro studies [Pascual et al., 2011]. The presented data show that MB is a good drug for a combined attack on several pathways, in particular those involved in redox protection. It is thus a good candidate for drug combinations, as also suggested by previous work on the combination of MB with CQ, AQ, or in combination with artemisinins [Zoungrana et al., 2008, Coulibaly et al., 2009, Dormoi et al., 2012].
Quick resistance development seems unlikely, given the way of action including not only many targets, but also pathways that cannot be easlily bypassed and are of vital importance to the parasite: Additional gene copies in these redox pathways are rare, and they only partially affect the pleiotropic action of MB. Furthermore, unbalanced duplications would even lead to additional redox stress (e.g. as known for trisomia 21 where an additional copy of peroxidase is not balanced by a sufficient catalase).
The models shown here are not only capable of performing those calculations for the different strains of *P. falciparum* 3D7. The process of preparing metabolic network models

[1] 15 nM for 18 hours corresponding to $5xIC_50$ determined after 72 hours

is highly modular: the enzymes comprising the metabolic pathways of *P. falciparum* 3D7 differ only slightly in other strains of Plasmodium. The pipeline described in this work is easily and quickly transferable to other malaria strains for which the genome sequence is known, respectively, to examine other drugs provided their primary target is known. Thus, the work displayed here can help to complement or even direct further studies on antimalarial treatment and new combination therapies.

MB has been studied for its safety, tolerability, and pharmacokinetics on-site in Burkina Faso with excellent results, even in G6PD-deficient individuals it is safe to administer without sever adverse effects arising [Akoachere et al., 2005, Bountogo et al., 2010, Walter-Sack et al., 2009, Mandi et al., 2005, Meissner et al., 2005, Zoungrana et al., 2008]. The often reported side effect of reversible blueish or greenish discolorations of urine and faeces was well tolerated if properly explained beforehand, and could even be used as indicator for drug adherence and/ or to unmask counterfeit drugs [Schirmer et al., 2011].

In Burkina Faso MB is approved for the treatment of methemoglobinemia. Approval as an antimalarial seems within reach given the existing studies. Theoretically, MB could be produced at cost comparable to those of CQ, as synthesis is uncomplicated and basic products are easily available.

MB thus brings about all properties of a "bonaria" drug. This acronym epitomises the concept of an "ethical" drug [Schirmer et al., 2003, Becker, 2011]: "bon" in the Latin word stem means safe and effective, "a" affordable for patients who need it, "r" already registered for other medical indications, and "ia" internationally available.

Although costing millions of lives, only 1% of all newly approved drugs between 1975 and 1999 were registered for diseases that like malaria prevail in developping countries, evidence of a huge innovation gap for these "unprofitable" diseases. The pharmaceutical industry argues that research and development is too costly and risky to invest in low-return neglected diseases [Trouiller et al., 2002]. To bridge this gap, ethical drugs that are necessary to prevent and cure disease, but unlikely to generate profits are designed following the "bonaria" principle [Becker, 2011]. MB does not only fulfill these criteria, but is also an example for the reconcilability of ethical and economic value:

As a raw product with $\geq$ 95% purity, MB is available from many providers of chemical and pharmaceutical products worldwide, currently at prices around 170 EUR per

kg. Since 2007, however, curiously coinciding with the practical implementation of MB in Alzheimer's therapy, the price of GMP-validated MB has gone up by a factor of 100 [Schirmer et al., 2011]. This steep increase can only be explained by ongoing market-rearrangements.

In a scenario of MB production independently from market speculations, e.g. a generic production in India, and/ or within a public-private partnership backed by WHO and GFATM, this price could sustainably be reduced to approximately EUR 15 per kg, assuming comparable synthesis cost for CQ and MB.

Of course, portioning, packaging, and transportation cost will add to this bulk price. But even assuming the active pharmaceutical ingredient only to account for 10% of the final package price, MB treatment cost would be approximately 7 EUR Cents[2].

Compared to a projected minimum price of 195 EUR per kg artemisinin active pharmaceutical ingredient, and applying the same considerations for portioning, packaging and transport as given above for MB, treatment cost add up to 23 EUR Cents[3] - thrice the projected cost of MB.

The perfect partner drug for a MB-based combination strategy remains to be identified and should be subject to further studies.

MB is not a quick-acting drug, a treatment duration of 7 days is needed for sustained parasite clearance [Bountogo et al., 2010]. It should therefore only be used for uncomplicated malaria, and in combination with a suitable partner drug. The ideal combination partner should thus be quick acting, to counterbalance MBs slowly occuring parasitocidal effect. Also, half lives of the partner drug should be matched (MB: 4-6 hours), and additive - ideally synergistic - effects with MB are preferable. Our pathway model as presented here can endorse time- and resource-consuming experimental studies to identify promising partner drugs.

MB-AQ is a combination that expresses many of these desirable effects: *in-vitro* studies showed this combination to express additive results [Dormoi et al., 2012], *in vivo* synergistic effects were even noted [Zoungrana et al., 2008]. In follow-up studies, a three day regimen of MB-AQ achieved 95% parasitological clearance rate at day 28, far better than

[2]This refers to a child of 10 kg, treated with 15 mg/kg once daily for 3 days.

[3]This refers to a child of 10 kg, treated with 4 mg/kg once daily for 3 days.

AS-AQ.

AQ is a standard antimalarial drug in Burkina Faso, and it is relatively cheap. A fixed-dose treatment of MB and AQ could be put on the market at a price of about 10 EUR Cents and would definitely be worth exploring for its use as an area-wide treatment strategy for Burkina Faso.

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Zusammenfassung

Malaria ist eine Krankheit, die uns vor große Herausforderungen stellt. Insbesondere die weltweit verbreiteten Resistenzen, die viele Therapieoptionen nutzlos werden lassen, haben den Kampf gegen die Malaria in den letzten Jahrzehnten deutlich verkompliziert. Schätzungen gehen davon aus, dass Malaria jährlich 1 bis 3 Millionen Todesopfer fordert. Mortalität und Morbidität der Erkrankung konzentrieren sich dabei in besonderer Weise auf Kinder unter fünf Jahren in Afrika südlich der Sahara.
In der hier vorgestellten Doktorarbeit analysiere ich den aktuellen Stand der Malaria-Kontrolle in Burkina Faso und zeige beispielhaft auf, warum diese Krankheit eine derart große Bürde für die Volksgesundheit darstellt und wo Ansatzpunkte zur Verbesserung der Kontrollmaßnahmen zu sehen sind, mit einem besonderen Fokus auf Diagnostik und Therapieoptionen. Dabei wird MB als Therapieoption genauer beleuchtet.

Um die besonderen Gegebenheiten eines Landes wie Burkina Faso - welches hier als repräsentatives Beispiel für einen Staat mit hoher Endemizität für Malaria herangezogen wird - aufzuzeigen, wird ein Porträt des Landes und seines Gesundheitssystems insbesondere unter Sozio-Ökonomischen Gesichtspunkten gezeichnet. Burkina Faso ist ein sehr armes Land, über die Hälfte seiner Bevölkerung lebt unterhalb der Armutsgrenze. Die Kosten von Malaria sind für diese Menschen gigantisch, und insbesondere die Kosten von Medikamenten wiegen schwer.
Eine retrospektive Studie aus Fallakten des Universitäts-Kinderkrankenhauses in Burkina Fasos Hauptstadt Ouagadougou zeigt vor allem, dass allein die Fallzahlen überwältigend sind, und vor allem die spezifische Diagnose der schweren Verlaufsform der Malaria ist unter den vorherrschenden Bedingungen eine Mammutaufgabe. Die Behandlungsvorschriften wie von der WHO vorgegeben werden weder vom Gesundheitssystem noch von der

Therapie zu Hause erfüllt, wie in den präsentierten Daten für die Vorbehandlung zeigen. Die zur Verfügung stehenden Malaria-wirksamen Therapeutika sind leider dank Resistenzentwicklung - oft durch unbedachten Masseneinsatz verursacht - sehr begrenzt. Artemisinine sind momentan das einzige Mittel gegen welches noch keine Resistenzen im Feld nachgewiesen wurden. Mittels Homologie-Modellierung zeige ich auf wie einfach eine solche Resistenzentwicklung jedoch denkbar wäre. Artemisinine sollten daher durch sehr gezielten Einsatz als "letzter Trumpf" möglichst lange vor Resistenzentwicklung geschützt werden, ähnlich wie Reserveantibiotika gegen Multi-resistente Keime.
MB ist ein hervorragender Kandidat für eine Kombinationsbehandlung gegen Malaria und eventuell eine Option, Artemisinine länger zu "schonen". Hier wird dieses Medikament mit bioinformatischen Mitteln genauer in seinen Wirkmechanismen beleuchtet und in Kombination mit anderen Medikamenten getestet mittels einer experimentell gestützten bioinformatischen Pathway-Modellierung. Durch diese Netzwerk-Analyse wurden verschiedene Angriffspunkte von MB auf das Redox-Netzwerk der Malariaerreger identifiziert.
Daraufhin wurden CQ und SP-Resistenzen *in silico* simuliert. Weitere Analysen zeigten dabei, dass MB synergisitische Wirkungen mit anderen Therapeutika gegen Malaria aufzeigt, wenn sie zielgerichtet eingesetzt werden. Finanziell gesehen hat MB Potenzial, ein zweites CQ zu werden, und somit endlich wieder die Kosten der Behandlung für Menschen die in Armut leben erschwinglich zu machen.

Malaria Kontrolle ist erreichbar, aber suboptimale Diagnosestellung und Behandlung behindern das Erreichen dieses Zieles. Hierfür muss eine angepasste, dezentrale und hochgradig standardisierte Primärbehandlung unkomplizierter Malaria implementiert werden und für eine bessere Verfügbarkeit dieser gesorgt werden. Leider leidet die Finanzierung der Kampagnen gegen Malaria an chronischer Unterversorgung. Um den maximalen Nutzen aus den vorhandenen Mitteln ziehen zu können ist eine günstigere medikamentöse Therapie ein entscheidender Beitrag, zumal Medikamente den größten Einzelbetrag im Kampf gegen Malaria verbrauchen.

Summary

Malaria is a challenging infection with increasing and wide-spread treatment failure risk due to resistance. With a estimated death toll of 1-3 Million per year, most cases of Malaria affect children under the age of five years in Sub-Saharan Africa. In this thesis, I analyse the current status of malaria control (focussing on diagnosis and therapy) in Burkina Faso to show how this disease burdens public health in endemic countries and to identify possible approaches to improvement. MB is discussed as a therapeutic option under these circumstances.

Burkina Faso is used as a representative example for a country in Sub-Saharan Africa with high endemicity for malaria and is here portrayed, its health system characterised and discussed under socioeconomic aspects.
More than half of this country's population live in absolute poverty. The burden that malaria, especially treatment cost, poses on these people cannot be under-estimated.
A retrospective study of case files from the university pediatric hospital in Burkina Faso's capital, Ouagadougou, shows that the case load is huge, and especially the specific diagnosis of severe malaria is difficult to apply in the hospital's daily routine. Treatment policy as proposed by WHO is not satisfactorily implemented neither in home treatment nor in health services, as data for pretreatment clearly show.
In the face of growing resistance in malaria parasites, pharmacological combination therapies are important. Artemisinins currently are the last resort of malaria therapy. As I show with homology models, even this golden bullet is not beyond resistance development. Inconsidered mass use has rendered other drugs virtually useless before. Artemisinins should thus be protected similar to reserve antibiotics against multi-resistant bacteria.
There is accumulating evidence that MB is an effective drug against malaria. Here the

biological effects of both MB alone and in combination therapy is explored via modeling and experimental data. Several different lines of MB attack on Plasmodium redox defense were identified by analysis of the network effects. Next, CQ resistance based on Pfmdr1 and PfCRT transporters as well as SP resistance were modeled *in silico*. Further modeling shows that MB has a favorable synergism on antimalarial network effects with these commonly used antimalarial drugs, given their correct application.
Also from the economic point of view MB shows great potential: in terms of production price, it can be compared to CQ, which could help to diminuish the costs of malaria treatment to affordable ranges for those most affected and struk by poverty.

Malaria control is feasible, but suboptimal diagnosis and treatment are often hindering the achievment of this goal. In order to achieve malaria control, more effort has to be made to implement better adjusted and available primary treatment strategies for uncomplicated malaria that are highly standardised. Unfortunately, campaigns against malaria are chronically underfinanced. In order to maximize the effect of available funds, a cheap treatment option is most important, especially as pharmaceuticals represent the biggest single matter of expense in the fight against malaria.

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"Leider läßt sich eine wahrhafte Dankbarkeit mit Worten nicht ausdrücken."

Johann Wolfgang von Goethe, 1749-1832

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Lebenslauf

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Appendix A

Databases and bioinformatical tools

A.1 General databases

Different genome-, enzyme-, and pathway databases exist and are used for bioinformatical analyses as described in chapter 2. The most important ones for the performed analyses are listed in the following:

GenBank (`http://www.ncbi.nlm.nih.gov/genbank/`) is part of the USA-based National Center for Biotechnology Information (NCBI). GenBank is a comprehensive database that contains publicly available nucleotide sequences for more than 300,000 organisms. GenBank searches are performed with the NCBI Entrez retrieval system, which integrates data from the major DNA and protein sequence databases along with taxonomy, genome, mapping, protein structure and domain information, and the biomedical journal literature via PubMed. The search tool "BLAST" (Basic Local Alignment Search Tool) provides sequence similarity searches of GenBank and other sequence databases [Benson et al., 2009].

UniProtKB/Swiss-Prot (`http://www.uniprot.org/`) provides a central resource on protein sequences and functional annotation. The UniProt Knowledgebase (UniProtKB) is a "joint venture" of the Swiss Institute of Bioinformatics (SIB), the European Bioinformatics Institute (EBI) and the Protein Information Resource (PIR) at Georgetown

University. Two different, non-redundant protein sequence data sets are available: the manually annotated UniProtKB/Swiss-Prot section, which relies on experimental results and scientific conclusions, and the automatically annotated UniProtKB/TrEMBL section using protein coding sections from EMBL and automatically translating them into amino acid sequences [Wu et al., 2006, Jain et al., 2009].

Protein Families Database of Alignments and Hidden Markov Models (Pfam) (`http://pfam.sanger.ac.uk/`) is a database of protein families. Proteins are generally composed of one or more functional regions, commonly termed domains. Different combinations of domains give rise to the diverse range of proteins found in nature. The identification of domains that occur within proteins can therefore provide insights into their function. There are two components to Pfam: Pfam-A and Pfam-B. Pfam-A entries are high quality, manually curated families. Pfam-B entires are automatically generated. Although of lower quality, Pfam-B families can be useful for identifying functionally conserved regions when no Pfam-A entries are found [Finn et al., 2010].

Simple Modular Architecture Research Tool (SMART) (`http://smart.embl.de/`) is another online tool for the identification and annotation of protein domains. It provides a platform for the exploration and comparative study of domain architectures in both proteins and genes. SMART contains manually curated models for well over 700 protein domains [Letunic et al., 2009].

Protein Data Bank (PDB) (`http://www.rcsb.org/pdb/`) is a repository for the coordinates and related information for protein structures as well as nucleic acids and large macromolecular complexes that have been determined using X-ray crystallography, NMR and electron microscopy techniques. Some thousand of theses structures are recorded here, and by homology modelling, even the structure of unknown proteins can be predicted [Berman et al., 2003].

Expasy (`http://www.expasy.org/`) is a bioinformatics resource portal dedicated to proteomics and operated by the Swiss Institute of Bioinformatics. It provides access to a

variety of databases and analytical tools for specific tasks relevant to proteomics, similarity searches, pattern and profile searches, post-translational modification prediction, topology prediction, primary, secondary and tertiary structure analysis and sequence alignment of enzymes [Gasteiger et al., 2003].

Braunschweig Enzyme Database (BRENDA) is curated at the technical university of Braunschweig (`http://www.brenda-enzymes.org/`). It contains biochemical and molecular information on all classified enzymes as well as software tools for querying the database and calculating molecular properties. The database covers information on classification and nomenclature, reaction and specificity, functional parameters, occurrence, enzyme structure and stability, mutants and enzyme engineering, preparation and isolation, the application of enzymes, and ligand-related data. The data in BRENDA are manually curated from more than 79,000 primary literature references [Chang et al., 2009].

KEGG (`http://www.kegg.jp/`) is a database of biological systems that integrates genomic, chemical and systemic functional information. KEGG provides a reference knowledge base for linking genomes to coherent biochemical operations within cells and whole organisms, and also contains data on approved drugs and data on genetic and/ or metabolic fundamentals of diseases [Kanehisa et al., 2008].

A.2 Plasmodium-specific databases

Apart from these general databases,a number of Plasmodium-specific databases exist, too:

PlasmoDB (`http://PlasmoDB.org`) is a functional genomic database for Plasmodium spp. only. PlasmoDB belongs to a family of genomic resources that are housed under the EuPathDB Bioinformatics Resource Center umbrella. The latest release, PlasmoDB 8.2, contains annotated genomes, evidence of transcription, proteomics evidence, protein function evidence, population biology and evolution of *P. falciparum* (strain 3D7), *P. vivax*, *P. yoelii*, *P. berghei*, *P. chabaudi*, and *P. knowlesi* [Aurrecoechea et al., 2009].

Malaria Transkriptome Database DeRisi hosted by the university of California, San Francisco (`http://malaria.ucsf.edu/comparison/index.php`), comprises experimentally obtained data of intraerythrocytic developmental cycle transcriptome of the HB3 strain of *P. falciparum* [Bozdech et al., 2003]. These transcriptome data are also allocated in the PlasmoDB Database (see above).

Malaria Parasite Metabolic Pathways (`http://sites.huji.ac.il/malaria/`), curated by Hagai Ginsburg of the Hebrew University of Jerusalem, Israel, displays information on enzyme pathways by amalgamating genomic, transcriptomic, and proteomic data obtained from literature and other databases into construction of metabolic pathways [Ginsburg, 2006].

Appendix B

Pharmacology of "classic" antimalarial drugs

B.1 Chloroquine

Though not completely understood yet, obviously CQ is a multi-target drug that interferes deeply with the parasite's metabolism. This might explain its remarkable long efficacy despite inconsidered mass-use. CQ is still the drug most frequently used worldwide, despite the fact that CQ resistant strains of *P. falciparum* are emerging everywhere and that resistance rates are constantly growing. CQ resistance in Burkina Faso today is exceeding 20% [Tinto et al., 2001, 2002], whilst a remarkable difference of resistance rates in urban vs. rural settings can be observed [Mueller et al., 2003b, Meissner et al., 2008], with urban settings expressing higher resistance rates. However, such resistance rates interdict further use of CQ in Burkina Faso as of now.

CQ is a diprotic weak base with the relative proportions of the neutral, mono-protonated (CQH^+), and di-protonated (CQH_2^{2+}) species varying with pH. Its structure is displayed in figure B.1.

Mode of action: CQ is a 4-aminoquinoline drug with blood schizonto- and gametocytocidal effects on *P. falciparum* that only acts against those parasite stages actively

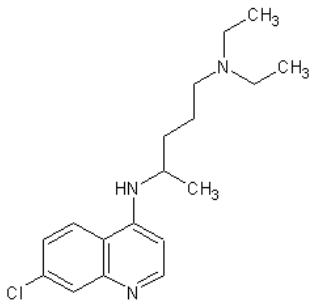

Figure B.1: Structure of Chloroquine

degrading hemoglobin. It accumulates in the parasite's acidic food vacuole, which leads to typical morphologic changes in the parasite, such as food vacuole swelling and pigment clumping [Yayon et al., 1984, Krogstad et al., 1985]. Accumulation occurs due to the chemical characteristics of CQ: the neutral molecule enters the parasite and its internal compartments via simple diffusion. When the base enters the acidic environment of the parasite's acidic food vacuole (pH = 5), the equilibrium is shifted toward the CQH_2^{2+} species, which is unable to diffuse across the membrane and becomes trapped, thereby accumulating to high concentrations within this compartment [Martin et al., 2009].

In the food vacuole, aminoquinoline-containing antimalarials such as CQ were repeatedly shown to bind to heme [Cohen et al., 1964, Aikawa, 1972, Chou et al., 1980]. CQ's efficacy is thought to lie in its ability to interrupt heme detoxification in the parasite [Chou et al., 1980, Dorn et al., 1998]: By binding to heme, CQ is thought to disrupt heme detoxification [Chou et al., 1980, Egan et al., 1994, Slater and Cerami, 1992, Dorn et al., 1995, Sullivan et al., 1996, Egan et al., 1999, Pagola et al., 2000, Fitch, 2004]. Also, Ginsburg et al. were able to show that heme degradation by GSH is inhibited when CQ or AQ are present. By this mechanism another way of detoxifying hemeis blocked, which may enhance the toxic effect of heme even further [Ginsburg et al., 1998]. Other theories proposed for mechanism of action of CQ include increased vacuolar pH, by which supposedly hemoglobin degradation is halted [Homewood et al., 1972, Krogstad et al., 1985].

Also, a couple of parasitic enzymes could be inhibited by CQ, such as vacuolar phospholipase [Zidovetzki et al., 1993, 1994], hemoglobin proteases [Gluzman et al., 1994], and inhibition of hydrogen peroxide degradation of heme [Loria et al., 1999].

Though not completely understood yet, obviously CQ is a multi-target drug that interferes deeply with the parasite's metabolism. This might explain its remarkable long efficacy despite inconsidered mass-use. However, today CQ is rendered virtually useless in most settings against *P. falciparum* due to resistance mechanisms that will be reviewed in the following paragraph.

Mechanisms of resistance: Mechanisms of resistance to CQ started to be unveiled when it was discovered that CQ-resistant *P. falciparum* accumulates significantly less CQ in its acidic food vacuole than susceptible parasites [Fitch, 1969, Verdier et al., 1985]. Krogstad et al. then found resistant parasites to release CQ from the food vacuole 40 to 50 times more rapidly than susceptible ones, although their initial rates of CQ accumulation were the same [Krogstad et al., 1987]. Also, Verapamil and other calcium channel blockers slowed the release and increased the accumulation of CQ by resistant (but not susceptible) *P. falciparum* [Martin et al., 1987, Krogstad et al., 1987]. Another consistent finding in CQ resistant parasites was a slightly lower vacuolar pH than in CQ sensitive parasites, indicating (CQ-dependent and Verapamil-sensitive) H^+ efflux from the vacuole [Martiney et al., 1995, Ursos et al., 2000, Lehane et al., 2008], that was theorized to interfere with CQ accumulation.

PfCRT on chromosome 7 of *P. falciparum* was identified by genetic analyses [Wellems et al., 1991] to be associated with antimalarial drug resistance, and following research localized PfCRT to the digestive vacuole membrane where it was supposed to work as a transporter involved in drug flux and/or pH regulation [Fidock et al., 2000, Wellems and Plowe, 2001]. Many polymorphisms of PfCRT have been identified, but the substitution of threonine for lysine in codon 76 (Thr76) was repeatedly shown to associate absolutely with resistance against CQ. In a field study in Mali, Thr76-PfCRT was found in all parasite samples from patients with clinical CQ resistance [Djimde et al., 2001]. Just recently, Martin et al. were able to show that mutant PfCRT actually transports CQ: a positive charge from lysine at the substrate-binding-site of wild-type PfCRT usually prevents CQ in its protonated form from interacting with the transporter. When this positive charge is lost through mutation that substitutes lysine with threonine, the substrate specificity of Thr76-PfCRT is altered and allows the transport of the protonated drug. Thus, CQ

can be transported out of the vacuole (following the concentration gradient), and away from its site of action. Verapamil inhibits the transport of CQ via Thr76-PfCRT, as do a range of quinolines including quinine and AQ [Martin et al., 2009].

Pfmdr1, located on chromosome 5 of *P. falciparum*, was another gene discovered in the course of identifiying the underlying cause of CQ resistance. Whilst its significance in resistance against MQ is largely undisputed nowadays [Woodrow and Krishna, 2006], the role of Pfmdr1 in CQ-resistant malaria has been challenged [Reed et al., 2000]. The product of this gene is *P. falciparum* P-glycoprotein homologue protein 1 (PfPgh1) and is located in the digestive vacuole membrane. PfPgh1 is an analog to the glycoproteins overexpressed in cancer cells where they function as pumps expelling cytotoxic drugs (ATP-binding cassette transporters). Another analogy that led to the identification of Pfmdr1 as possible efflux-pump is the Verapamil-induced inhibition of multidrug resistance phenotype in cancer cells [Tsuruo et al., 1981]. Pfmdr1 was subsequently found in *P. falciparum* [Foote et al., 1989], an association between Pfmdr1 single nucleotide polymorphisms and CQ resistance was noted [Foote et al., 1990], and subsequently tested in a large number of field studies that produced conflicting results [Woodrow and Krishna, 2006]. Although CQ transport is altered in the cells with modified PfPgh1 [Reed et al., 2000], the associations between isolated mutations of Pfmdr1 and CQ remain uncertain. Many studies that report on PfCRT-polymorhism note that, although the Thr76 mutation may be essential for the resistant phenotype, it is also present to a lesser degree in CQ-sensitive strains, suggesting that several genes may be involved. A significant association between both mutated Pfmdr1 and PfCRT has been noted in Africa [Babiker et al., 2001, Adagu and Warhurst, 2001] and provides support for a convergence of polymorphisms necessary for a resistance phenotype. The slow development of CQ resistance may signal such a multifactorial mechanism [Wellems and Plowe, 2001, Wongsrichanalai et al., 2002, Woodrow and Krishna, 2006].

It though seems that CQ resistance is not a favourable mutation for *P. falciparum*: When selective pressure by continued use of CQ is lifted, as done in Malawi, where this drug was banned from the market in 1993, CQ sensitivity re-established within seven years: The prevalence of the chloroquine-resistant PfCRT genotype decreased from 85% in 1992 to 13% in 2000 [Kublin et al., 2003]. This poses an exciting possibility of restoring the

extraordinary efficacy of CQ by re-introducing it as part of a combination therapy after a decade of consequent banning.

Pharmacokinetics: Ingested CQ is rapidly absorbed, with peak plasma concentrations reached within two hours following oral or rectal application [White, 1985]. Intramuscular or subcutaneous injection is feasible though rarely used as it has to be given fractionated in order to prevent rapid absorption and resulting hypotension [White et al., 1988]. CQ is extensively bound to tissues, in plasma, 55% is bound to proteins. About half of the absorbed drug is cleared unchanged by the kidney, the rest is biotransformed in the liver. Therapeutic blood concentrations persist for 6-10 days after a single dose, but terminal elimination half-life is one to two months [Warrell and Gilles, 2002].

Toxicity, adverse effects: CQ is generally well tolerated in therapeutic dosages. When plasma-concentrations are too high or raising too quickly (e.g. after intravenous injection), severe adverse effects as systolic hypotension, ECG abnormalties and associated clinical signs may occur [Warrell and Gilles, 2002]. Especially in dark-skinned people, pruritus is a tiresome problem that may arise from the high affinity of CQ to melanocytes [Bell and Winstanley, 2004].

B.2 Amodiaquine

Mode of action: AQ is a 4-aminoquinoline like CQ and has a very similar mode of action.

Mechanisms of resistance: Studies suggested that CQ resistant parasite strains may remain sensitive against AQ [Olliaro et al., 1996]. In Burkina Faso, AQ was tested for efficacy and safety in 117 children. After correction for re-infection, the response rate to AQ was 92% by day 14 [Mandi et al., 2008]. Meanwhile, AQ was shown to share the same mechanism of resistance as CQ in principle [Tinto et al., 2008], and is therefore of only limited use as an alternative to CQ - at least in monotherapy.

Pharmacokinetics: After uptake, it is rapidly and extensively converted to a pharmacologically active metabolite, desethyl-AQ. Data on the terminal elimination half-life of desethyl-AQ are insufficient, it has been detected in the urine several months after administration, indicating that it may be similar to CQ in this respect, too [WHO, 2006a].

Toxicity, adverse effects: The use of AQ has been discouraged after fatal adverse drug reactions were reported in the mid-1980s, when travellers using AQ as a prophylaxis developed agranulocytosis and liver damage [Hatton et al., 1986, Neftel et al., 1986]. However, in therapeutic rather than prophylactic treatment, AQ was shown to induce only slightly more adverse events (10.7% for AQ vs. 8.8% for CQ), and none of these were considered serious [Olliaro et al., 1996]. It is more palatable, though, and produces less pruritus than CQ, which may support its acceptance especially in children. In Burkina Faso, AQ was tested for efficacy and safety in 117 children. No serious adverse events occurred in this study [Mandi et al., 2008].

B.3 Quinine

Quinine is an alkaloid derived from the bark of the Cinchona tree that originated from South America. There, it had been used by Inca Indians of Peru and Bolivia long before the Jesuits brought the Cinchona to Europe in the seventeenth century. Since then, it had been used therapeutically to cure febrile "agues" - notably cases of Malaria that then were still endemic in many parts of Europe. Quinine today is widely used as first line treatment for severe malaria. Due to its considerable side effects, long duration of therapy and the risk of resistance selection, its use for uncomplicated malaria should be restricted to cases where no other therapeutic option is available.

Mode of action and mechanisms of resistance: Quinine is widely thought to act by interfering with parasite heme metabolism, similar to its derivatives, the 4-aminoquinolines. The drug is usually effective against *P. falciparum* resistant to CQ as well as antifolate

combinations. Clinical resistance that seems to be related to Pfmdr1 polymorphisms occurs occasionally in South-East Asia, where it has been extensively used for malaria therapy [Wongsrichanalai et al., 2002, Woodrow and Krishna, 2006]. Strains of *P. falciparum* from Africa are generally highly sensitive to quinine [Roll Back Malaria campaign, 2001].

Pharmacokinetics: Quinine is an quinoline alkaloid base that is prepared as a salt, containing various amounts of Quinine hydrochloride, dihydrochloride, sulfate, bisulfate and gluconate. Quinine is largely absorbed when taken orally, the drug is distributed throughout body fluids being highly protein bound. Quinine is extensively metabolized in the liver and is subsequently excreted in the urine, mainly as hydroxylated metabolites. Its elimination half-life is rather short with ten to twelve hours [Warrell and Gilles, 2002, Roll Back Malaria campaign, 2001].

Toxicity, adverse effects: Cinchonism, a symptom complex characterized by tinnitus, hearing impairment, and sometimes vertigo or dizziness, occurs in a high proportion of treated patients at common therapeutic dosages. The symptoms are usually reversible and generally develop on the second or third day of treatment. Dose-related cardiovascular, gastrointestinal and central nervous system effects may arise following excessive infusion or from accumulation following oral administration. Severe hypotension may develop if the drug is injected too rapidly. Hypoglycemia may be caused by quinine since the drug stimulates secretion of insulin from pancreatic β-cells [Roll Back Malaria campaign, 2001]. Blackwater fever used to be a well-known and dreaded complication of malaria in long-term european expatriates who took quinine on irregular basis as intermittent prophylaxis and/ or treatment for malaria. Hemolytic crisis with massive hemoglobinuria and consecutive renal failure in these patients likely is caused by quinine-triggered immune hemolysis [Tran et al., 1996] - which is why the use of quinine as a prophylactic regimen was discouraged since the 1950s.

B.4 Mefloquine

MQ (Lariam®) is a quinoline closely related to quinine. It has been widely used in South-East Asia after CQ and SP failure, but owing to its long elimination half-life of 15 to 40 days and consequent long-lived subtherapeutic concentrations in the blood, resistance was quick to develop in South-East Asia and is to be expected especially in areas of high transmission. MQ resistance seems to be related to mutations in Pfmdr1, that acts as efflux pump for the drug [Price et al., 2004]. *P. falciparum* resistance to MQ is accompanied by cross-resistance to Quinine and Halofantrine. In contrast, laboratory studies have shown some increase in the sensitivity of MQ-resistant isolates to CQ [Basco and Le Bras, 1992, Nateghpour et al., 1993]. As of now, only sporadic reports of drug failure and in vitro evidence of reduced sensitivity for MQ have been reported from Africa, as this drug is only rarely used there, due to its relatively high price and its problematic profile of adverse effects. The main problem relates to the drug's potential for inducing neuropsychiatric adverse reactions. There have also been concerns about the other pronounced adverse effects, such as dizziness, vomiting and cardiotoxicity [Roll Back Malaria campaign, 2001, Warrell and Gilles, 2002].

B.5 Other quinoline drugs

B.5.1 Halofantrine

Halofantrine is a phenanthrene methanol active against *P. falciparum* infections that are resistant to CQ and to antifolate combinations. *In vitro* studies indicated cross-resistance between MQ and halofantrine. Halofantrine resistance is easy to produce in laboratory models and is accompanied by increased susceptibility to CQ and decreased susceptibility to MQ and quinine [Nateghpour et al., 1993, Basco and Le Bras, 1992].
The elimination half life of Halofantrine varies with the individual but is generally 24-48 hours for the parent drug and twice as long for the biologically active desbutyl metabolite. The functional elimination half-life is therefore four to five days. The major route of elimination is the faeces.

The most important adverse effect is a marked cardiotoxicity with an increase of the ECG QT-interval and consequent risk for cardiac arrhythmia [Warrell and Gilles, 2002]. It is therefore not recommended in malaria control programmes.

B.5.2 Primaquine

Primaquine is an 8-aminoquinoline highly active against the gametocytes of all malaria species found in humans and against hypnozoites of the relapsing malarial parasites, *P. vivax* and *P. ovale*. It is the drug of choice for follow-up treatment of these relapsing malaria forms. For the treatment of *falciparum* malaria it has no practical relevance.

B.5.3 Piperaquine

Piperaquine is a bisquinoline antimalarial drug that was first synthesised in the 1960s, and used extensively in China and Indochina for the next 20 years. With the development of piperaquine-resistant strains of *P. falciparum* and the emergence of the Artemisinin derivatives, its use declined during the 1980s. However, during the next decade, piperaquine was rediscovered by Chinese scientists as suitable for combination with an Artemisinin derivative [Davis et al., 2005].

Bisquinolines are related to CQ and other quinoline drugs and probably share their mode of action whilst resistance development seems to be hindered by steric inhibition of efflux pumps by bulky side chains. Indeed, Piperaquine was shown to be highly active against CQ resistant and -sensitive strains of *P. falciparum* alike [Basco and Ringwald, 2003] and is well tolerated [Batty et al., 2008].

The drug is quickly absorbed, highly lipid-soluble and has a large volume of distribution, its elimination half-life is rather long (8-18 days) [Liu et al., 2007]. Clearance is markedly higher in children than in adults [Hung et al., 2004].

B.5.4 Lumefantrine

Lumefantrine is an aryl amino alcohol similar to quinine, MQ and halofantrine. It is absorbed with a lag time of 2 hours, followed by peak plasma concentrations 8 hours after oral administration. Elimination hlaf-life is 1-6 days [Warrell and Gilles, 2002]. Lumefantrine is only available as component of the ACT Co-Artem®/ Riamet®.

B.6 Antifolate drugs

Mode of action of antifolates: Because Plasmodium in humans can capture and use the host's purines but not its pyrimidines, the parasites must synthesize the latter [Le Bras and Durand, 2003]. This fact may be therapeutically exploited by drugs that block pyrimidine-sythesis: the anti-folates Pyrimethamine, Sulfadoxine, Proguanil and Dapsone are utilised in this way. Tetrahydrofolic acid (respectively its anion, tetrahydrofolate) is an important co-factor in the synthesis of purines and pyrimidines, that again are building blocks of DNA. Figure B.2 shows the central steps of pyrimidine metabolism pathways and the drug targets for antifolate drugs.

Sulfadoxine and Dapsone both act as competetive inhibitors of the active site of DHPS, which promotes the formation of the folate precursor dihydropteroate, that again is the precursor of dihydrofolate. Synergistic to this, Pyrimethamine and Proguanil as well as its derivative Chlorproguanil tackle DHFR, an enzyme needed in the synthesis of tetrahydrofolate from dihydrofolate [Watkins et al., 1985].

To exploit the synergistic effect, these drugs are given in various combinations:

- SP (Fansidar®) is a combination of two antifolate drugs: Sulfadoxine and Pyrimethamine. The proportion in a fixed combination is 20 part Sulfadoxine to 1 part Pyrimethamine.

  SP, which has potent efficacy against CQ-resistant *P. falciparum*, became the standard second-line therapy against CQ-resistant malaria. However, resistance to SP was recognized at the Thai-Cambodian border in the 1960s, and failures occurred in

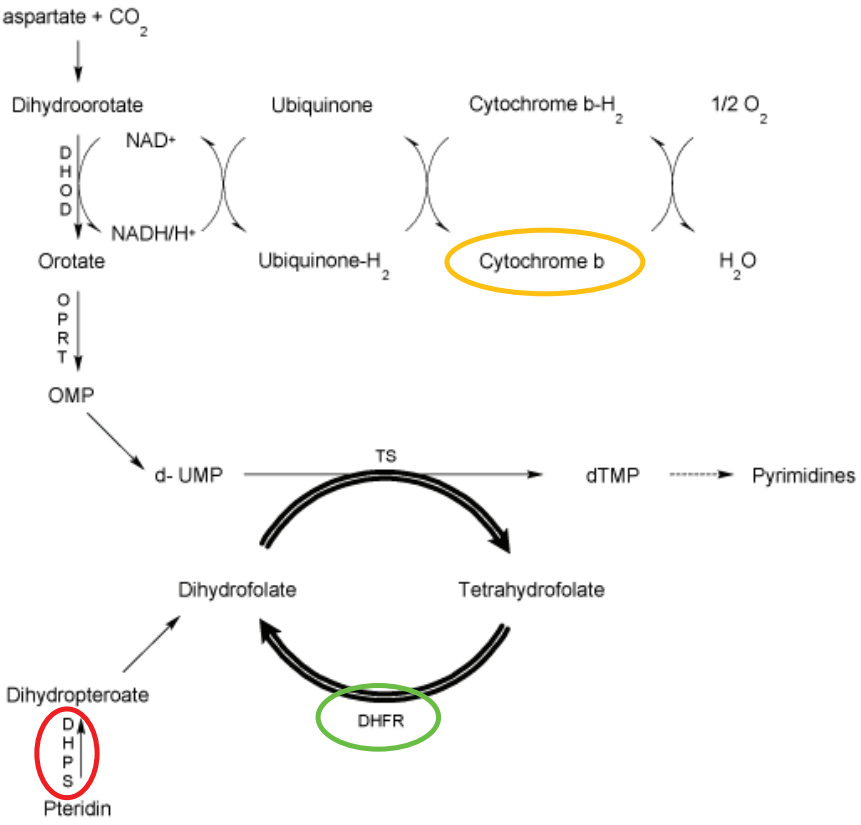

Figure B.2: A highly simplified illustration of metabolic pathways of pyrimidine synthesis and targets of antifolate drugs: DHPS circled in red, DHFR circled in green; in orange, the target of atovaquone is indicated (cytochrome b). Illustration: adapted from Le Bras and Durand [8]

refugee camps in Thailand in the 1970s [Wongsrichanalai et al., 2002]. Resistance rates in Burkina Faso were found to range between 10 and 20% [Tinto et al., 2002, 2007].

SP is commercially available in Burkina Faso as tablets containing 500 mg Sulfadoxine + 25 mg Pyrimethamine, it is also available as an injectable for i.m. injection.

Both components of SP show long elimination half-lives of approximately 4 to 8 days [Winstanley, 2000]. The slow elimination favours development of resistance to SP [Watkins and Mosobo, 1993]. There is no cross-resistance with other antimalarial agents such as 4-aminoquinolines, quinine, halofantrine or Artemisinin derivatives. SP is administered as a single-dose regimen either *per os* or as i.m.-injection. The single-dose scheme obviously heightens compliance. Pyrimethamine is extensively

metabolized whereas only a small proportion of sulfadoxine is metabolized. Excretion of both components is mainly through the urine [Roll Back Malaria campaign, 2001].

SP is generally well tolerated when used at the recommended therapeutic doses. Serious adverse events may arise under a prophylactic regimen due to hypersensitivity to the sulfonamide component that involve the skin and mucous membranes, including life-threatening toxic epidermal necrolysis (Stevens-Johnson Syndrome). For this reason, the combination is no longer recommended for prophylactic use [Roll Back Malaria campaign, 2001].

- Proguanil-Atovaquone (Malarone®) is a synergistic drug combination of the antifolate Proguanil and Atovaquone, a drug blocking cytochrome b, a central electron donor in the mitochondriac respiratory chain. Atovaquone-Proguanil is not licensed in most of Sub-Saharan Africa, due to its high price (EUR 40 to 50 per treatment). It is often used as prophylaxis or stand-by therapeutic in travelers from industrialised countries.

- Chlorproguanil-Dapsone (CD) (LapDap®) is a synergistic antifolate combination of Chlorproguanil with Dapsone that was developed by a public-private partnership as a low-cost treatment for uncomplicated falciparum malaria [Lang and Greenwood, 2003]. Its major benefit was thought to be the fact that the half-lives of its components are considerably shorter than SP and well-matched [Winstanley, 2001, Sulo et al., 2002]. However, in clinical trials LapDap® - although effective in line with expectations - showed a significant rate of adverse effects, namely an alarming reduction in hemoglobin concentration observed in patients with G6PD-deficiency [Wootton et al., 2008]. G6PD-deficiency is a hereditary enzyme disorder which is estimated to affect 10-25% of the population in sub-Saharan Africa. The G6PD enzyme is important for the normal functioning of red blood cells and deficiency of the enzyme in certain individuals can only be detected using a blood test which is often not a practical option in Africa. On the basis of these data, further development of CD was terminated and the product was recalled in countries where it had already been licensed [GSK press release, 2008].

Mechanisms of resistance to antifolates: The resistance of *P. falciparum* to Pyrimethamine is due to simple point mutations in the gene encoding for DHFR [Peterson et al., 1988, Basco et al., 1995, Plowe et al., 1996], which reduce the drug's affinity without affecting the enzyme's operation on its natural substrate. Similar mutations in the genes encoding for DHPS govern resistance to Sulfadoxine [Brooks et al., 1994]. It seems that mutations in the DHPS-gene (Glu540, Gly437) occur only after the DHFR mutations manifest. Also, multiple mutations in DHFR diminish the efficacy of the enzyme on dihydrofolate which suggests that additional mutations are unfavourable to the parasites in the absence of drug pressure [Sirawaraporn et al., 1997]. When the parasite has become virtually insensitive to Pyrimethamine due to serial triple mutations (Asn108, Ile51 and Arg59) in the DHFR-gene, Chlorproguanil is still active [Plowe et al., 1997, Kublin et al., 2002]. *P. falciparum* becomes insensitive to Chlorproguanil only when a fourth mutation of the DHFR-gene, Leu164, has occurred. This pattern of DHFR mutations is widely present in South America and Southeast Asia, but only infrequently found in Africa yet [WHO, 2005a]. However, cross-resistance has been demonstrated between Chlorproguanil and Pyrimethamine, and the potential use of CD to treat strains resistant to SP seems thus severely limited [Kublin et al., 2002].

Appendix C

Currency in Burkina Faso

Burkina Faso's currency is the West African CFA Franc (FCFA, ISO currency code XOF). This currency was created in 1945 and today is still the common currency for all former French colonies in West Africa. As for its history, the FCFA has a fixed exchange rate to the EUR: 100 FCFA = 0.00152449 EUR; or 1 EUR = 655.957 FCFA

In this thesis, when prices were calculated in EUR, the above given exchange rate was used for FCFA prices and the amount rounded.

Appendix D

Economic Indicators used for Global Health characteristics

To characterise such imprecise terms as "wealth", "developmental status" and "public health", a number of economic and demographic indices are often used. As these often do not come in handy definitions, the most important ones - that are also used in this thesis - will be explained in the following. Given definitions are as given by references [The World Bank, UNDP, 2010, UNICEF, 2011, WHO].

D.1 Economic markers

Gini coefficient The Gini coefficient measures the extent to which the distribution of income (or consumption) among individuals or households within a country deviates from a perfectly equal distribution. To calculate it, the Lorenz curve is used, a cumulative distribution function of the empirical probability distribution of wealth. A value of 0 represents perfect equality, a value of 100 perfect inequality. In reality, figures range between 23 (Sweden) and 70 (Namibia) [CIA, 2010, UNDP, 2010].

Gross national income Gross National Income or Product (GNP) is a measure of income from all "nationals" - firms and households - of a country, whether they are

resident or not. GNI and GDP are often used homologous, which isn't exactly correct: The difference is that while GDP defines its scope according to location (i.e. product produced within a country's borders), GNI defines its scope according to ownership and summarises all products produced by enterprises owned by a country's citizens. In other words: GNI = GDP + income from nationals based abroad − profits generated by foreign firms or individuals operating in the country and then remitted back to their origin country.

Gross domestic product GDP summarises the market value of all final goods and services produced within a country in a given period. Being an aggregate figure, it does not account for the size of a nation. Therefore, GDP is often stated as GDP per capita in which total GDP is divided by the resident population in a given period to get a measure of the wealth of its citizens.

Human development index The HDI is a statistic value that was established to rank countries according to their developmental status and is a measure for a country's standard of living. It combines three dimensions: Life expectancy at birth, mean years of schooling and expected years of schooling and GNI per capita (Purchasing power parity (PPP) US$). The HDI is annually determined by the United Nationa Development Programme and published in its Human Development Report.

Poverty line Poverty is usually defined in relation to the median income of a country. WHO defines the national poverty line as 50% of the median income of a given country, the country itself may use other standards. The poverty line should represent the minimum level of income deemed necessary to achieve an adequate standard of living within that country. In contrast, "absolute poverty" is internationally defined. The most commonly used definition is that of "less than a dollar per day"; the most recent calculation by the World bank in 2008 defined the limit at $ 1.25 a day.

Purchasing power parity Economic values such as GDP/GNI do not take into account differences in relative costs of goods between (and within) countries. PPP is a concept that rules out different exchange rates of national currencies and makes them

internationally comparable. In the figurative sense, PPP is the price in terms of one currency that a certain basket of goods will achieve in a given country. The best-known purchasing power adjustment is the "international dollar", in other sources, the US dollar (resp. its purchasing power in a given year) is used.

D.2 Demographic markers

Birth rate The crude birth rate indicates the number of live births occurring during the year, per 1,000 population estimated at midyear. Subtracting the crude death rate from the crude birth rate provides the rate of natural increase, which is equal to the rate of population change in the absence of migration.

Fertility rate The total fertility rate gives the average number of children that would be born alive to a woman during her lifetime if she were to bear children at each age in accordance with the prevailing age-specific fertility rates.

Growth rate Annual population growth rate for year t is the exponential rate of growth of midyear population from year t-1 to t, expressed as a percentage.

Life expectancy at birth Life expectancy gives the number of years a newborn infant would live if prevailing patterns of mortality at the time of its birth were to stay the same throughout its life.

D.3 Health indicators

Risk factors

Access to improved sanitation facilities Access to improved sanitation facilities refers to the percentage of the population with at least adequate access to excreta disposal

facilities that can effectively prevent human, animal, and insect contact with excreta. Improved facilities range from simple but protected pit latrines to flush toilets with a sewerage connection. To be effective, facilities must be correctly constructed and properly maintained.

Access to improved water source Access to an improved water source refers to the percentage of the population with reasonable access to an adequate amount of water from an improved source, such as a household connection, public standpipe, borehole, protected well or spring, and rainwater collection. Unimproved sources include vendors, tanker trucks, and unprotected wells and springs. Reasonable access is defined as the availability of at least 20 liters a person a day from a source within one kilometer of the dwelling.

Adult literacy rate Adult literacy rate is the percentage of people ages 15 and above who can, with understanding, read and write a short, simple statement on their everyday life.

Births attended by skilled health staff Births attended by skilled health staff are the percentage of deliveries attended by personnel trained to give the necessary supervision, care, and advice to women during pregnancy, labor, and the postpartum period; to conduct deliveries on their own; and to care for newborns.

Malnutrition in children, prevalence of Prevalence of child malnutrition is the percentage of children under age 5 whose weight for age is more than two standard deviations below the median for the international reference population ages 0-59 months.

Primary school enrolment ratio Net enrollment ratio is the ratio of children of official school age based on the International Standard Classification of Education 1997 who are enrolled in school to the population of the corresponding official school age. Primary education provides children with basic reading, writing, and mathematics skills

along with an elementary understanding of such subjects as history, geography, natural science, social science, art, and music.

Mortality rate Mortality rate is a measure of the number of deaths in some population, scaled to the size of that population, per unit time. Mortality rate is typically expressed in units of deaths per 1000 individuals per year.

Specific mortality rates

Infant mortality rate The probability of dying between birth and age one, per thousand births is given as infant mortality rate.

Under-5-mortality rate The under-5-mortality rate gives the probability of number of children dying between birth and age five, per 1,000 life births and year.

Maternal mortality ratio Maternal mortality ratio is the number of women who die during pregnancy and childbirth (until 42 days after giving birth), per 100,000 live births.

Health expenditure

Disability adjusted life years The DALY is a measure of overall disease burden, expressed as the number of years lost due to ill-health, disability or early death. One DALY is equal to one year of healthy life lost. The DALY is becoming increasingly common in the field of public and global health assessments. It combines mortality and morbidity into a common metric and allows to quantify health liabilities due to specific diseases.

(National) health expenditure Health expenditure comprises all expenditures during a given time period (usually a year) directed to restore, enhance and preserve the health of the population (as a whole as well as of individuals) of a country. In terms of national health accounts they identify the source of money spent on health care:

- Total health expenditure is the sum of all health expenditures. It covers public and private health care: the provision of health services (preventive and curative), family planning activities, nutrition activities, and emergency aid designated for health. It does not include provision of water and sanitation.

- Private health expenditure The sum of outlays for health by private entities. It includes direct household (out-of-pocket) spending, private insurance, charitable donations, and direct service payments by private corporations.

- Public health expenditure The sum of recurrent and capital spending from government budgets, external borrowings and grants (including donations from international agencies and NGOs), and health insurance funds used for health care. Public plus private health expenditure result in total health expenditure.

- Governmental health expenditure: The sum of outlays for health maintenance, restoration or enhancement paid for in cash or supplied in kind by government entities, such as the Ministry of Health, other ministries, parastatal organisations, social security agencies (without double-counting the government transfers to social security and to extra-budgetary funds). Includes transfer payments to households to offset medical care costs and extra-budgetary funds to finance health services and goods. The revenue base of these entities may comprise multiple sources, including external funds. In this thesis, public equals governmental health expenditure, as the Ministry of Health of Burkina Faso does not differentiate the origin of their health expenses, and social security agencies are practically non-existent [Ministry of Health of Burkina Faso, 2010].

- Household health expenditure Household or out of pocket health expenditure is any direct outlay by households, including gratuities and in-kind payments, to health practitioners and suppliers of pharmaceuticals, therapeutic appliances, and other

goods and services whose primary intent is to contribute to the restoration or enhancement of the health status of individuals or population groups. It is a part of private health expenditure.

- External funds' health expenditures are the sum of all non-national payments directed toward health care. These are e.g. payments from the World Fund to the government for malaria control programmes or donations from charitable organisations handing out ITNs to families. As for their nature, they are usually included into private resp. governmental health expenditures and thus are not to be added to the latter when computing total helath expenditure.

Health care cost These identify all emerging costs from health care. In terms of national health accounts, they identify where the money goes to respectively what it is spent on specifically.

- Curative health care cost comprise all expenses for stationary and ambulatory health care, i.e. cost for all diagnostic and therapeutic interventions, including those for traditional medicine.

- Public health care cost summarizez all health care cost directed to public health interventions. Public health focuses on prevention of disease by education (e.g. promotion of hand washing, family planning, or breastfeeding), specific intervention strategies (e.g. vaccinations, distribution of ITNs etc.).

Appendix E

Blantyre Coma Scale

The widely used Glasgow coma scale was modified by Molyneux et al. in 1989 to be applicable to young children [Molyneux et al., 1989]. Total score ranges from 0 to 5, "2" being defined as "unrousable coma".

Table E.1: Blantyre Coma Scale

	Score
Best motor response	
Localizes painful stimulus	2
Withdraws limb from pain	1
Non-Specific or absent response	0
Verbal response	
Appropriate cry	2
Moan or inappropriate cry	1
None	0
Eye movements	
Directed (e.g., follows mother's face)	1
Not directed	0

Appendix F

Supplementary material

F.1 Bioinformatical data

Full list of enzymes of YANAsquare modes: table F.1

Extreme modes calculated by YANAsquare models: tables F.2 - F.5

Extreme modes with added MB calculated by YANAsquare models: tables F.6 - F.9

Changes of pathway activities in CQ/ sulfadoxine-resistant strains: tables F.10 - F.12

Table F.1: List of enzymes used to build metabolic web for *P. falciparum*

Enzyme name	reversible?	Reaction equation
1-cysPeroxiredoxin	true	$2GSH + ROOH = GSSG + H_2O + ROH$
1-cysteine-Glutaredoxin	true	$GSH + Protein - SSG = GSSG + Protein - SH$
1-cys-glutaredoxin-like-protein1 (EC:1.11.1.15)	true	$2GSH + ROOH = GSSG + H_2O + ROH$
2-cys-Peroxiredoxin (EC:1.11.1.15_r2)	true	$ROOH + Trx(SH)_2 = H_2O + ROH + TrxS_2$
6-P-delta-Lactonase (EC:3.1.1.31)	true	$D - Glucono - 15 - lactone6 - phosphate + H_2O = 6 - Phospho - D - gluconate$
6-Phosphogluconatedehydrogenase (rn:R01528)	false	$6 - Phospho - D - gluconate + NADP^+ = CO_2 + D - Ribulose - 5 - phosphate + H^+ + NADPH$
Aldehyde-reductase (EC:1.1.1.21)	false	$Lactaldehyde + NAD^+ = H^+ + Methylglyoxal + NADH$
Aldolase (EC:4.1.2.13_rn:R01070)	true	$\beta - D - Fructose16 - bisphosphate = D - Glyceraldehyde3 - phosphate + Glyceronephosphate$
Deoxyribosephosphataldolase (EC:4.1.2.4_rn:R01066)	true	$2 - Deoxy - D - ribose5 - phosphate = Acetaldehyde + D - Glyceraldehyde3 - phosphate$
DHF-reductase (EC:1.5.1.3_01x_rn:R00936)	false	$NAD^+ + Tetrahydrofolate = Dihydrofolate + H^+ + NADH$
DHF-reductase (EC:1.5.1.3_01x_rn:R00937)	false	$Folate + H^+ + NADH = NAD^+ + Tetrahydrofolate$
DHF-reductase (EC:1.5.1.3_01x_rn:R00939)	false	$Dihydrofolate + H^+ + NADPH = NADP^+ + Tetrahydrofolate$
DHF-reductase (EC:1.5.1.3_01x_rn:R00940)	false	$Folate + H^+ + NADPH = NADP^+ + Tetrahydrofolate$
DHF-reductase (EC:1.5.1.3_01x_rn:R02235)	false	$Folate + H^+ + NADH = Dihydrofolate + NAD^+$
DHF-reductase (EC:1.5.1.3_01x_rn:R02236)	false	$Folate + H^+ + NADPH = Dihydrofolate + NADP^+$
DHPS (EC:2.5.1.15_rn:R03066)	false	$2 - Amino - 4 - hydroxy - 6 - hydroxymethyl - 78 - dihydropteridine + ATP = AMP + Dihydropteroate$
DHPS (EC:2.5.1.15_rn:R03067)	false	$2 - Amino - 78 - dihydro - 4 - hydroxy - 6 - (diphosphooxymethyl)pteridine + 4 - Aminobenzoate = Dihydropteroate + Diphosphate$
Dihydrofolate-synthetase (EC:6.3.2.12_rn:R02237)	false	$ATP + Dihydropteroate + L - Glutamate = ADP + Dihydrofolate + Orthophosphate$
Dihydroneopterin-aldolase (EC:4.1.2.25_rn:n/a)	false	$Dihydroneopterine + H_2O = 2 - Amino - 4 - hydroxy - 6 - hydroxymethyl - 78 - dihydropteridine + Orthophosphate$
Enolase (EC:4.2.1.11_rn:R00658)	true	$2 - Phospho - D - glycerate = H_2O + Phosphoenolpyruvate$

Continuation of Table F.1

Enzyme name	reversible?	Reaction equation
Glucose-6-phosphate1-dehydrogenase (EC:1.1.1.49_rn:R02736)	false	$NADP^+ + \beta - D - Glucose6 - phosphate = D - Glucono - 15 - lactone6 - phosphate + H^+ + NADPH$
Glucose-Transporter	true	$\alpha - D - Glucose_e x = \alpha - D - Glucose$
Glutaredoxin2	true	$2GSH + ProteinS_2 = GSSG + Protein(SH)_2$
Glutaredoxin3	true	$2GSH + ROOH = GSSG + H_2O + ROH$
Glutaredoxin4	true	$2GSH + Ribonucleotide - reductase - ox = GSSG + Ribunocleotide - reductase - red$
Glutaredoxin5	true	$GSH + ProteinSOH = H_2O + Protein - SSG$
Glutaredoxin6	true	$ProteinS_2 + Trx(SH)_2 = Protein(SH)_2 + TrxS_2$
Glutathione-peroxidase-like-Tpx (EC:1.11.1.9)	true	$2ROOH + Trx(SH)_2 = 2H_2O + ROH + TrxS_2$
Glutathione-S-Transferase2 (EC:2.5.1.18)	true	$GSH + RX = HX + R - S - Glutathione$
Glutathione-Synthase (EC:6.3.2.3)	false	$ATP + Glycine + gamma - L - Glutamyl - L - Cysteine = ADP + GSH + P$
Glutathione reductase (EC:1.8.1.7_rn:00115)	true	$GSSG + H^+ + NADPH = 2GSH + NADP^+$
Glyceraldehyde-3P-dehydrogenase (EC:1.2.1.12_rn:R01061)	true	$D - Glyceraldehyde3 - phosphate + NAD^+ + Orthophosphate = 3 - Phospho - D - glyceroylphosphate + H^+ + NADH$
Glycinhydroymethyl-transferase	true	$L - Serine + Tetrahydrofolate = 510 - Methylenetetrahydrofolate + Glycine + H_2O$
GlyoxalaseI (EC:4.4.1.5)	false	$GSH + Methylglyoxal = (R) - S - Lactoylglutathione + H_2O$
GlyoxalaseII (EC:3.1.2.6)	false	$(R) - S - Lactoylglutathione + H_2O = GSH + R - Lactate$
GTP-Hydrolase (EC:3.5.4.16_01x-rn:R00428)	false	$GTP + H_2O = Formamidopyrimidinenucleosidetriphosphate$
GTP-Hydrolase (EC:3.5.4.16_01x-rn:R04639)	false	$25 - Diamino - 6 - (5 - triphosphoryl - 34 - trihydroxy - 2 - oxopentyl) - amino - 4 - oxopyrimidine + H_2O = 2 - Amino - 4 - hydroxy - 6 - (erythro - 123 - trihydroxypropyl)dihydropteridinetriphosphate$
GTP-Hydrolase (EC:3.5.4.16_01x-rn:R05046)	false	$Formamidopyrimidinenucleosidetriphosphate + H_2O = 25 - Diaminopyrimidinenucleosidetriphosphate + Formate$
GTP-Hydrolase (EC:3.5.4.16_01x-rn:R05048)	false	$25 - Diaminopyrimidinenucleosidetriphosphate = 25 - Diamino - 6 - (5 - triphosphoryl - 34 - trihydroxy - 2 - oxopentyl) - amino - 4 - oxopyrimidine$
Hexokinase (EC:2.7.1.1_rn:R01600)	false	$ATP + \beta - D - Glucose = ADP + \beta - D - Glucose6 - phosphate$
Hexokinase (EC:2.7.1.1_rn:R01786)	false	$ATP + \alpha - D - Glucose = ADP + \alpha - D - Glucose6 - phosphate$

Continuation of Table F.1

Enzyme name	reversible?	Reaction equation
L-Glutamate-L-cysteine-Synthase (EC:6.3.2.2)	false	$ATP + L-Cysteine + L-Glutamate = ADP + P + \gamma - L-Glutamyl-L-Cysteine$
Lactate Dehydrogenase (EC:1.1.1.27_rn:R00703)	true	$H^+ + NADH + Pyruvate = (S)-Lactate + NAD^+$
Monocarboxylate-Transporter	true	$(S)-Lactate = (S)-Lactate_ex$
MRP	false	$ATP + GSH = ADP + GSHex + P$
MRP- and/or GSSG-Pumps	false	$ATP + GSSG = ADP + GSSG_ex + P$
One-carbon-pool (rn:R00945)	true	$L-Serine + Tetrahydrofolate = 510-Methylenetetrahydrofolate + Glycine + H_2O$
One-carbon-pool (rn:R02101)	false	$510-Methylenetetrahydrofolate + dUMP = Dihydrofolate + dTMP$
One-carbon-pool (rn:R03940)	false	$10-Formyltetrahydrofolate + L-Methionyl-tRNA = N-Formylmethionyl-tRNA + Tetrahydrofolate$
One-carbon-pool (rn:R04125)	false	$S-Aminomethyldihydrolipoylprotein + Tetrahydrofolate = 510-Methylenetetrahydrofolate + Dihydrolipoylprotein + NH_3$
Phosphofructokinase (EC:2.7.1.11_rn:R04779)	false	$ATP + \beta-D-Fructose6-phosphate = ADP + \beta-D-Fructose16-bisphosphate$
Phosphoglucomustase (EC:5.4.2.2_rn:R01057)	true	$alpha-D-Ribose1-phosphate = D-Ribose5-phosphate$
Phosphoglycerate-kinase (EC:2.7.2.3_rn:R01512)	true	$3-Phospho-D-glyceroylphosphate + ADP = 3-Phospho-D-glycerate + ATP$
Phosphoglycerate-mutase (EC:5.4.2.1_rn:R01518)	true	$3-Phospho-D-glycerate = 2-Phospho-D-glycerate$
Phosphohexoseisomerase (EC:5.3.1.9_rn:R02739)	true	$\alpha-D-Glucose6-phosphate = \beta-D-Glucose6-phosphate$
Phosphohexoseisomerase (EC:5.3.1.9_rn:R02740)	true	$\alpha-D-Glucose6-phosphate = \beta-D-Fructose6-phosphate$
Phosphohexoseisomerase (EC:5.3.1.9_rn:R03321)	true	$\beta-D-Glucose6-phosphate = \beta-D-Fructose6-phosphate$
Phosphotrioseisomerase (EC:5.3.1.1_rn:R01015)	true	$D-Glyceraldehyde3-phosphate = Glyceronephosphate$
Porphyrine (EC:1.3.3.3)	false	$CoproporphyrinogenIII + O_2 = 2CO_2 + 2H_2O + Protoporpyhrinogen$
Porphyrine (EC:1.3.3.4)	false	$3O_2 + 2Protoporpyhrinogen = 6H_2O + 2Protoporphyrin$
Porphyrine (EC:2.3.1.37)	false	$Glycine + Succinyl-CoA = 5-Aminolevulinate + CO_2 + CoA$
Porphyrine (EC:2.5.1.-)	false	$Heme = HemeO$
Porphyrine (EC:2.5.1.61)	false	$H_2O + 4Porphobilinogen = Hydroxymethylbilane + 4NH_3$
Porphyrine (EC:4.1.1.37)	false	$UroporphyrinogenI = 4CO_2 + CoproporphyrinogenI$
Porphyrine (EC:4.1.1.37_2)	false	$UroporphyrinogenIII = 4CO_2 + CoproporphyrinogenIII$
Porphyrine (EC:4.2.1.24)	false	$5-Aminolevulinate = 2H_2O + Porphobilinogen$

Continuation of Table F.1

Enzyme name	reversible?	Reaction equation
Porphyrine (EC:4.2.1.75)	false	$Hydroxymethylbilane = H_2O + UroporphyrinogenIII$
Porphyrine (EC:4.4.1.17)	false	$Apocytochromec + Heme = Cytochromec$
Porphyrine (EC:4.99.1.1)	false	$Fe^{2+} + Protoporphyrin = 2H^+ + Heme$
Porphyrine (EC:COX15)	false	$HemeO = HemeA$
Porphyrine, spontaneous	false	$Hydroxymethylbilane = UroporphyrinogenI$
Pyruvate-kinase (EC:2.7.1.40_rn:R00200)	false	$ADP + Phosphoenolpyruvate = ATP + Pyruvate$
Ribonucleotide-Reductase (EC:1.17.4.1_R04294)	true	$2 - deoxyribonucleoside - diphosphate + H_2O + Ribonucleotide - reductase - ox + Thioredoxindisulfide = Ribunocleotide - reductase - red + Thioredoxin + ribonucleoside - disphosphate$
Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017)	true	$H_2O + Ribonucleotide - reductase - ox + Thioredoxindisulfide + dADP = ADP + Ribunocleotide - reductase - red + Thioredoxin$
Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02018)	true	$H_2O + Ribonucleotide - reductase - ox + Thioredoxindisulfide + dUDP = Ribunocleotide - reductase - red + Thioredoxin + UDP$
Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02019)	true	$H_2O + Ribonucleotide - reductase - ox + Thioredoxindisulfide + dGDP = GDP + Ribunocleotide - reductase - red + Thioredoxin$
Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02024)	true	$H_2O + Ribonucleotide - reductase - ox + Thioredoxindisulfide + dCDP = CDP + Ribunocleotide - reductase - red + Thioredoxin$
Ribose-P-pyrophosphokinase (EC:2.7.6.1_rn:R01049)	false	$ATP + D - Ribose5 - phosphate = 5 - Phospho - alpha - D - ribose1 - diphosphate + AMP$
Ribose-Phosphate-Isomerase (EC:5.3.1.6_rn:R01056)	true	$D - Ribose5 - phosphate = D - Ribulose5 - phosphate$
Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529)	true	$D - Ribulose5 - phosphate = D - Xylulose5 - phosphate$
spontaneous	false	$D - Glyceraldehyde3 - phosphate = Methylglyoxal + P$
spontaneous2	false	$Glyceronephosphate = Methylglyoxal + P$
Superoxide-dismutase (EC:1.15.1.1)	false	$2H^+ + 2O^{2-} = H_2O_2 + O_2$
Thioredoxin	true	$FADH_2 + ProteinS_2 = FAD + Protein(SH)_2$
Thioredoxin-depPeroxidase	true	$Protein - O - + Thioredoxin = H_2O + Protein + Thioredoxindisulfide$

Continuation of Table F.1

Enzyme name	reversible?	Reaction equation
Thioredoxin2	true	$GSSG + Trx(SH)_2 = 2GSH + TrxS_2$
Thioredoxinreductase (EC:1.6.4.5)	false	$H^+ + NADPH + TrxS_2 = NADP^+ + Trx(SH)_2$
Transketolase (EC:2.2.1.1_rn:R01641)	true	$D-Ribose5-phosphate + D-Xylulose5-phosphate =$ $D-Glyceraldehyde3-phosphate + D-Sedoheptulose7-phosphate$
Transketolase (EC:2.2.1.1_rn:R01830)	true	$D-Glyceraldehyde3-phosphate + \beta-D-Fructose6-phosphate =$ $D-Erythrose4-phosphate + D-Xylulose5-phosphate$
Triphosphate lyase (EC:4.2.3.12_rn:R04286)	false	$2-Amino-4-hydroxy-6-(erythro-123-trihydroxypropyl)dihydropteridinetriphosphate + 2H_2O =$ $6-Pyruvoyltetrahydropterin + Triphosphate$
Triphosphate-phosphohydrolase (EC:3.1.3.1_rn:R04620)	false	$2-Amino-4-hydroxy-6-(erythro-123-trihydroxypropyl)dihydropteridinetriphosphate + 3H_2O =$ $Dihydroneopterine + 3Orthophosphate$

Table F.2: Extreme modes of *P. falciparum* 3D7 for a wildtype strain (no resistances, no compounds added)

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
1	0,03550214	2	true	2	-1 Glycinhydroymethyl-Transferase; 1 One-carbon-pool (rn:R00945)
2	0,18927750	3	true	3	1 2-cys-Peroxiredoxin (EC:1.11.1.15_r2); -1 Glutaredoxin6; 1 Thioredoxin
3	0,24827750	3	true	3	-1 Glutaredoxin6; 1 GSH-Peroxidase-like-Tpx (EC:1.11.1.9); 1 Thioredoxin
4	0,12242836	3	true	3	-1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R03321)
5	0,05332433	2	true	2	1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R04294); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017)
6	0,21577177	4	true	4	-1 1cys-Glutaredoxin-like-protein1 (EC:1.11.1.15); 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
7	0,35187177	4	true	4	-1 1-cys-Peroxiredoxin; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
8	0,13847177	4	true	4	-1 Glutaredoxin3; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
9	0,05332433	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02024)
10	0,13847177	5	true	5	-1 Glutaredoxin2; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin; -1 Thioredoxin-depPeroxidase
11	0,57607120	6	true	6	-1 Glutaredoxin4; 1 Glutaredoxin6; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; -1 Thioredoxin2
12	0,05332433	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02018)
13	0,05332433	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02019)

Continuation of Table F.2

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
14	0,02767480	5	true	5	-1 1-Cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 GSH-S-Transferase2 (EC:2.5.1.18); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
15	0,13847177	5	true	5	-1 1-Cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 Glutaredoxin5; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
16	-0,14189903	6	true	5	1 Desoxyribose-P-Aldolase (EC:4.1.2.4_rn:R01066); -2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -1 Transketolase (EC:2.2.1.1_rn:R01641)
17	0,14112955	5	false	5	1 1-Cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
18	0,17966192	12	false	11	1 Enolase (EC:4.2.1.11_rn:R00658); 1 Glyceraldehyde-3P-Dehydrogenase (EC:1.2.1.12_rn:R01061); 1 Lactate-Dehydrogenase (EC:1.1.1.27_rn:R00703); 1 Monocarboxylate-Transporter; 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphoglycerate-Kinase (EC:2.7.2.3_rn:R01512); 1 Phosphoglycerate-Mutase (EC:5.4.2.1_rn:R01518); 1 Pyruvatekinase (EC:2.7.1.40_rn:R00200); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 Transketolase (EC:2.2.1.1_rn:R01641)
19	0,11928531	6	false	6	1 Hexokinase (EC:2.7.1.1_rn:R01600); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)
20	0,10219637	2	false	2	1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Pyrophosphokinase (EC:2.7.6.1_rn:R01049)
21	0,14100070	1	false	1	1 Superoxide-Dismutase (EC:1.15.1.1)
22	0,09630498	7	false	7	-1 Glutaredoxin4; 1 Glutaredoxin6; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; 1 Thioredoxin-Reductase (EC:1.6.4.5.)

Continuation of Table F.2

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
23	0,15464255	18	false	13	1 6-P-δ-Lactonase (EC:3.1.1.31); 1 6-Phosphogluconate-Dehydrogenase (rn:R01528); 1 Glucose-6-P-1-Dehydrogenase (EC:1.1.1.49_rn:R02736); 2 Glutaredoxin4; 2 GSH-Rreductase (EC:1.8.1.7_rn:00115); -2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -2 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Thioredoxin-depPeroxidase; -1 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
24	0,10150761	16	false	8	1 Aldolase (EC:4.1.2.13_rn:R01070); 1 Phosphofructokinase (EC:2.7.1.11_rn:R04779); -5 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphotriose-Isomerase (EC:5.3.1.1_rn:R01015); -2 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -3 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
25	0,00000000	8	false	7	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous; 1 Transketolase (EC:2.2.1.1_rn:R01641)
26	0,19287431	3	false	3	1 Aldehyde-Reductase (EC:1.1.1.21); 1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6)
27	0,14112955	6	false	5	2 1-Cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP- and/or GSSG-Pumps; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
28	0,00000000	6	false	6	1 DHFR (EC:1.5.1.3_01x_rn:R00936); 1 DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
29	0,07247485	6	false	6	1 Glucose-Transporter; 1 Hexokinase (EC:2.7.1.1_rn:R01786); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)

Continuation of Table F.2

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
30	0,00000000	9	false	8	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphotriose-Isomerase (EC:5.3.1.1_rn:R01015); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous2; 1 Transketolase (EC:2.2.1.1_rn:R01641)
31	0,00000000	39	false	12	(4 DHFR (EC:1.5.1.3_01x_rn:R00939); -4 Glutaredoxin4; -4 GSH-Reductase (EC:1.8.1.7_rn:00115); 4 Glycinhydroxymethyl-Transferase; 4 One-carbon-pool (rn:R02101); 4 Porphyrine (EC:2.3.1.37); 1 Porphyrine (EC:2.5.1.61); 1 Porphyrine (EC:4.1.1.37); 4 Porphyrine (EC:4.2.1.24); 1 Porphyrine (EC:spontaneous); 4 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 4 Thioredoxin-depPeroxidase
32	0,00000000	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1-Fe^2+-accumulation); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
33	0,02733251	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
34	0,02895586	7	false	7	-1 1-Cysteine-Glutaredoxin; 1 DHFR (EC:1.5.1.3_01x_rn:R00939); 1 GSH-Synthase (EC:6.3.2.3); -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Glycinhydroxymethyl-Transferase; 1 L-Glutamate-L-Cysteine-Synthase (EC:6.3.2.2); 1 One-carbon-pool (rn:R02101)
35	0,28335771	7	false	7	1DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 One-carbon-pool (rn:R02101); 1 One-carbon-pool (rn:R04125); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase

Table F.3: Extreme modes of *P. falciparum* 3D7 for less active DHF and DHPS (CQ-resistant, sulfadoxine added).

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
1	0.004165815	2	true	2	-1 Glycinhydroxymethyl-Transferase; 1 One-carbon-pool (rn:R00945
2	0.180262233	3	true	3	1 2-cys-Peroxiredoxin (EC:1.11.1.15_r2); -1 Glutaredoxin6; 1 Thioredoxin
3	0.239262169	3	true	3	-1 Glutaredoxin6; 1 GSH-peroxidase-like-Tpx (EC:1.11.1.9); 1 Thioredoxin
4	0.103040009	3	true	3	-1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R03321)
5	0.054126352	2	true	2	1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R04294); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017)
6	0.224549750	4	true	4	-1 1cys-glutaredoxin-like-protein1 (EC:1.11.1.15); 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
7	0.360649601	4	true	4	-1 1-cysPeroxiredoxin; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
8	0.147249834	4	true	4	1 Glutaredoxin3; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
9	0.054126352	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02024)
10	0.147249834	5	true	5	-1 Glutaredoxin2; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin; -1 Thioredoxin-depPeroxidase
11	0.509545893	6	true	6	-1 Glutaredoxin4; 1 Glutaredoxin6; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; -1 Thioredoxin2
12	0.054126352	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02018)
13	0.054126352	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02019)

Continuation of Table F.3

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
14	-0.115070754	5	true	5	-1 1-Cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 GSH-S-Transferase2 (EC:2.5.1.18); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
15	0.147249834	5	true	5	-1 1-Cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 Glutaredoxin5; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
16	-0.078911521	6	true	5	1 Deoxyribose-P-Aldolase (EC:4.1.2.4_rn:R01066); -2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -1 Transketolase (EC:2.2.1.1_rn:R01641)
17	0.132262225	5	false	5	1 1-Cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
18	0.170683240	12	false	11	1 Enolase (EC:4.2.1.11_rn:R00658); 1 Glyceraldehyde-3P-Dehydrogenase (EC:1.2.1.12_rn:R01061); 1 Lactate-Dehydrogenase (EC:1.1.1.27_rn:R00703); 1 Monocarboxylate-Transporter; 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphoglycerate-Kinase (EC:2.7.2.3_rn:R01512); 1 Phosphoglycerate-Mutase (EC:5.4.2.1_rn:R01518); 1 Pyruvatekinase (EC:2.7.1.40_rn:R00200); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 Transketolase (EC:2.2.1.1_rn:R01641)
19	0.096690900	6	false	6	1 Hexokinase (EC:2.7.1.1_rn:R01600); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)
20	0.122356648	2	false	2	1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Pyrophosphokinase (EC:2.7.6.1_rn:R01049)
21	0.141054461	1	false	1	1 Superoxide-dismutase (EC:1.15.1.1)
22	0.135861652	7	false	7	-1 Glutaredoxin4; 1 Glutaredoxin6; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; 1 Thioredoxinreductase (EC:1.6.4.5.)

Continuation of Table F.3

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
23	0.100397666	18	false	13	1 6-P-δ-Lactonase (EC:3.1.1.31); 1 6-Phosphogluconate-Dehydrogenase (rn:R01528); 1 Glucose-6-P-1-Dehydrogenase (EC:1.1.1.49_rn:R02736); 2 Glutaredoxin4; 2 GSH-Reductase (EC:1.8.1.7_rn:00115); -2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -2 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Thioredoxin-depPeroxidase; -1 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
24	0.144504901	16	false	8	1 Aldolase (EC:4.1.2.13_rn:R01070); 1 Phosphofructokinase (EC:2.7.1.11_rn:R04779); -5 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphotriose-Isomerase (EC:5.3.1.1_rn:R01015); -2 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -3 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
25	0.000000000	8	false	7	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous; 1 Transketolase (EC:2.2.1.1_rn:R01641)
26	0.192916676	3	false	3	1 Aldehyde-reductase (EC:1.1.1.21); 1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6)
27	0.132262225	6	false	5	2 1-Cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP- and/or GSSG-Pumps; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
28	0.000000000	6	false	6	1 DHFR (EC:1.5.1.3_01x_rn:R00936); 1 DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
29	0.067293057	6	false	6	1 Glucose-Transporter; 1 Hexokinase (EC:2.7.1.1_rn:R01786); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)

Continuation of Table F.3

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
30	0.000000000	9	false	8	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphotrioseisomerase (EC:5.3.1.1_rn:R01015); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous2; 1 Transketolase (EC:2.2.1.1_rn:R01641)
31	0.000000000	39	false	12	4 DHFR (EC:1.5.1.3_01x_rn:R00939); -4 Glutaredoxin4; -4 GSH-Reductase (EC:1.8.1.7_rn:00115); 4 Glycinhydroxymethyl-Transferase; 4 One-carbon-pool (rn:R02101); 4 Porphyrine (EC:2.3.1.37); 1 Porphyrine (EC:2.5.1.61); 1 Porphyrine (EC:4.1.1.37); 4 Porphyrine (EC:4.2.1.24); 1 Porphyrine (EC:spontaneous); 4 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 4 Thioredoxin-depPeroxidase
32	0.000000000	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1-Fe^2+-accumulation); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
33	0.023118816	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
34	0.000000000	7	false	7	-1 1-Cysteine-Glutaredoxin ; 1 DHFR (EC:1.5.1.3_01x_rn:R00939); 1 GSH-Synthase (EC:6.3.2.3); -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Glycinhydroxymethyl-Transferase; 1 L-Glutamate-L-Cysteine-Synthase (EC:6.3.2.2); 1 One-carbon-pool (rn:R02101)
35	0.158425136	7	false	7	1 DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 One-carbon-pool (rn:R02101); 1 One-carbon-pool (rn:R04125); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase

Table F.4: Extreme modes of *P. falciparum* 3D7 for less active ferrochelatase (sulfadoxine-resistant, CQ added).

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
1	0.01107674	2	true	2	-1 Glycinhydroxymethyl-Transferase; 1 One-carbon-pool (rn:R00945)
2	0.17461941	3	true	3	1 2-cys-Peroxiredoxin (EC:1.11.1.15_r2); -1 Glutaredoxin6; 1 Thioredoxin
3	0.23361941	3	true	3	-1 Glutaredoxin6; 1 GSH-peroxidase-like-Tpx (EC:1.11.1.9); 1 Thioredoxin
4	0.07739945	3	true	3	-1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R03321)
5	0.05463234	2	true	2	1 Ribonucleotide-Reductase (EC:1.17.4.1_R04294); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017)
6	0.23049611	4	true	4	-1 1cys-glutaredoxin-like-protein1 (EC:1.11.1.15); 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
7	0.36659613	4	true	4	-1 1-cysPeroxiredoxin; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
8	0.15319611	4	true	4	-1 Glutaredoxin3; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
9	0.05463234	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02024)
10	0.15319611	5	true	5	-1 Glutaredoxin2; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin; -1 Thioredoxin-depPeroxidase
11	0.60540687	6	true	6	-1 Glutaredoxin4; 1 Glutaredoxin6; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; -1 Thioredoxin2
12	0.05463234	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02018)
13	0.05463234	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02019)
14	0.04239609	5	true	5	-1 1-cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 GSH-S-Transferase2 (EC:2.5.1.18); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase

Continuation of Table F.4

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
15	0.15319611	5	true	5	-1 1-cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 Glutaredoxin5; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
16	-0.14807101	6	true	5	1 Deoxyribose-P-Aldolase (EC:4.1.2.4_rn:R01066); -2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -1 Transketolase (EC:2.2.1.1_rn:R01641)
17	0.12632956	5	false	5	1 1-cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
18	0.18053624	12	false	11	1 Enolase (EC:4.2.1.11_rn:R00658); 1 Glyceraldehyde-3P-Dehydrogenase (EC:1.2.1.12_rn:R01061); 1 Lactate-Dehydrogenase (EC:1.1.1.27_rn:R00703); 1 Monocarboxylate-Transporter; 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphoglycerate-Kinase (EC:2.7.2.3_rn:R01512); 1 Phosphoglycerate-Mutase (EC:5.4.2.1_rn:R01518); 1 Pyruvate-Kinase (EC:2.7.1.40_rn:R00200); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 Transketolase (EC:2.2.1.1_rn:R01641)
19	0.09472815	6	false	6	1 Hexokinase (EC:2.7.1.1_rn:R01600); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)
20	0.11093138	2	false	2	1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Pyrophosphokinase (EC:2.7.6.1_rn:R01049)
21	0.14099469	1	false	1	1 Superoxide-dismutase (EC:1.15.1.1)
22	0.02300644	7	false	7	-1 Glutaredoxin4; 1 Glutaredoxin6; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; 1 Thioredoxin-Reductase (EC:1.6.4.5.)

Continuation of Table F.4

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
23	0.20981061	18	false	13	1 6-P-δ-Lactonase (EC:3.1.1.31); 1 6-Phosphogluconate-Dehydrogenase (rn:R01528); 1 Glucose-6-P-1-Dehydrogenase (EC:1.1.1.49_rn:R02736); 2 Glutaredoxin4; 2 GSH-Reductase (EC:1.8.1.7_rn:00115); -2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -2 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Thioredoxin-depPeroxidase; -1 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
24	0.09053873	16	false	8	1 Aldolase (EC:4.1.2.13_rn:R01070); 1 Phosphofructokinase (EC:2.7.1.11_rn:R04779); -5 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphotrioseisomerase (EC:5.3.1.1_rn:R01015); -2 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -3 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
25	0.00000000	8	false	7	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous; 1 Transketolase (EC:2.2.1.1_rn:R01641)
26	0.19306453	3	false	3	1 Aldehyde-Reductase (EC:1.1.1.21); 1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6)
27	0.12632956	6	false	5	2 1-cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP- and/or GSSG-Pumps; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
28	0.38889148	6	false	6	1 DHFR (EC:1.5.1.3_01x_rn:R00936); 1 DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
29	0.12087227	6	false	6	1 Glucose-Transporter; 1 Hexokinase (EC:2.7.1.1_rn:R01786); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)

Continuation of Table F.4

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
30	0.00000000	9	false	8	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphotrioseisomerase (EC:5.3.1.1_rn:R01015); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous2; 1 Transketolase (EC:2.2.1.1_rn:R01641)
31	0.00000000	39	false	12	4 DHFR (EC:1.5.1.3_01x_rn:R00939); -4 Glutaredoxin4; -4 GSH-Reductase (EC:1.8.1.7_rn:00115); 4 Glycinhydroxymethyl-Transferase; 4 One-carbon-pool (rn:R02101); 4 Porphyrine (EC:2.3.1.37); 1 Porphyrine (EC:2.5.1.61); 1 Porphyrine (EC:4.1.1.37); 4 Porphyrine (EC:4.2.1.24); 1 Porphyrine (EC:spontaneous); 4 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 4 Thioredoxin-depPeroxidase
32	0.02399060	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1-Fe^2+-accumulation); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
33	0.00000000	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
34	0.00681835	7	false	7	-1 1-cysteine-Glutaredoxin; 1 DHFR (EC:1.5.1.3_01x_rn:R00939); 1 GSH-Synthase (EC:6.3.2.3); -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Glycinhydroxymethyl-Transferase; 1 L-Glutamate-L-Cysteine-Synthase (EC:6.3.2.2); 1 One-carbon-pool (rn:R02101)
35	0.20008914	7	false	7	1 DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 One-carbon-pool (rn:R02101); 1 One-carbon-pool (rn:R04125); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase

Table F.5: Extreme modes of *P. falciparum* 3D7 for multiresistant strains (resistant to sulfadoxine and CQ; CQ and sulfadoxine added).

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
1	0.013981991	2	true	2	-1 Glycinhydroxymethyl-Transferase; 1 One-carbon-pool (rn:R00945)
2	0.161130841	3	true	3	1 2-cys-Peroxiredoxin (EC:1.11.1.15_r2); 1 Glutaredoxin6; 1 Thioredoxin
3	0.220130969	3	true	3	1 Glutaredoxin6; 1 GSH-peroxidase-like-Tpx (EC:1.11.1.9); 1 Thioredoxin
4	-0.108441632	3	true	3	1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R03321)
5	0.055877240	2	true	2	1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R04294); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017)
6	0.243857043	4	true	4	1 1cys-Glutaredoxin-like-protein1 (EC:1.11.1.15); 1 Glutaredoxin4; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
7	0.379957338	4	true	4	1 1-cysPeroxiredoxin; 1 Glutaredoxin4; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
8	0.166556876	4	true	4	1 Glutaredoxin3; 1 Glutaredoxin4; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
9	0.055877240	2	true	2	1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02024)
10	0.166556876	5	true	5	1 Glutaredoxin2; 1 Glutaredoxin4; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin; 1 Thioredoxin-depPeroxidase
11	0.566519587	6	true	6	1 Glutaredoxin4; 1 Glutaredoxin6; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin; 1 Thioredoxin-depPeroxidase; 1 Thioredoxin2
12	0.055877240	2	true	2	1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02018)
13	0.055877240	2	true	2	1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02019)

Continuation of Table F.5

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
14	-0.095919650	5	true	5	1 1-cysteine-Glutaredoxin; 1 Glutaredoxin4; 1 GSH-S-Transferase2 (EC:2.5.1.18); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
15	0.166556876	5	true	5	1 1-cysteine-Glutaredoxin; 1 Glutaredoxin4; 1 Glutaredoxin5; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
16	-0.148983275	6	true	5	1 Deoxyribosephosphataldolase (EC:4.1.2.4_rn:R01066); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 Transketolase (EC:2.2.1.1_rn:R01641)
17	0.113048484	5	false	5	1 1-cysteine-Glutaredoxin; 1 Glutaredoxin4; 1 MRP; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
18	0.180699041	12	false	11	1 Enolase (EC:4.2.1.11_rn:R00658); 1 Glyceraldehyde-3P-Dehydrogenase (EC:1.2.1.12_rn:R01061); 1 Lactate-Dehydrogenase (EC:1.1.1.27_rn:R00703); 1 Monocarboxylate-Transporter; 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphoglycerate-Kinase (EC:2.7.2.3_rn:R01512); 1 Phosphoglycerate-Mutase (EC:5.4.2.1_rn:R01518); 1 Pyruvate-Kinase (EC:2.7.1.40_rn:R00200); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 Transketolase (EC:2.2.1.1_rn:R01641)
19	0.142813939	6	false	6	1 Hexokinase (EC:2.7.1.1_rn:R01600); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)
20	0.109719497	2	false	2	1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Pyrophosphokinase (EC:2.7.6.1_rn:R01049)
21	0.140891848	1	false	1	1 Superoxide-dismutase (EC:1.15.1.1)
22	0.021482206	7	false	7	1 Glutaredoxin4; 1 Glutaredoxin6; 1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin; 1 Thioredoxin-depPeroxidase; 1 Thioredoxinreductase (EC:1.6.4.5.)

Continuation of Table F.5

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
23	0.204131870	18	false	13	1 6-P-delta-Lactonase (EC:3.1.1.31); 1 6-Phosphogluconated-Dehydrogenase (rn:R01528); 1 Glucose-6-P-1-Dehydrogenase (EC:1.1.1.49_rn:R02736); 2 Glutaredoxin4; 2 GSH-Reductase (EC:1.8.1.7_rn:00115); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 2 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 2 Thioredoxin-depPeroxidase; 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)
24	0.093981883	16	false	8	1 Aldolase (EC:4.1.2.13_rn:R01070); 1 Phosphofructokinase (EC:2.7.1.11_rn:R04779); 5 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphotriose-Isomerase (EC:5.3.1.1_rn:R01015); 2 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 2 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 3 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)
25	0.000000000	8	false	7	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous; 1 Transketolase (EC:2.2.1.1_rn:R01641)
26	0.192968332	3	false	3	1 Aldehyde-Reductase (EC:1.1.1.21); 1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6)
27	0.113048484	6	false	5	2 1-cysteine-Glutaredoxin; 1 Glutaredoxin4; 1 MRP- and/or GSSG-Pumps; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
28	0.381630913	6	false	6	1 DHFR (EC:1.5.1.3_01x_rn:R00936); 1 DHFR (EC:1.5.1.3_01x_rn:R00939); 1 Glutaredoxin4; 1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
29	0.078878107	6	false	6	1 Glucose-Transporter; 1 Hexokinase (EC:2.7.1.1_rn:R01786); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)

Continuation of Table F.5

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
30	0.000000000	9	false	8	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphotrioseisomerase (EC:5.3.1.1_rn:R01015); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous2; 1 Transketolase (EC:2.2.1.1_rn:R01641)
31	0.000000000	39	false	12	4 DHFR (EC:1.5.1.3_01x_rn:R00939); 4 Glutaredoxin4; 4 GSH-Reductase (EC:1.8.1.7_rn:00115); 4 Glycinhydroxymethyl-Transferase; 4 One-carbon-pool (rn:R02101); 4 Porphyrine (EC:2.3.1.37); 1 Porphyrine (EC:2.5.1.61); 1 Porphyrine (EC:4.1.1.37); 4 Porphyrine (EC:4.2.1.24); 1 Porphyrine (EC:spontaneous); 4 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 4 Thioredoxin-depPeroxidase
32	0.000000000	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); 8 Glutaredoxin4; 8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1-Fe^2+-accumulation); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
33	0.024497192	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); 8 Glutaredoxin4; 8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
34	0.008718887	7	false	7	1 1-cysteine-Glutaredoxin; 1 DHFR (EC:1.5.1.3_01x_rn:R00939); 1 GSH-Synthase (EC:6.3.2.3); 1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Glycinhydroxymethyl-Transferase; 1 L-Glutamate-L-cysteine-Synthase (EC:6.3.2.2); 1 One-carbon-pool (rn:R02101)
35	0.193498730	7	false	7	1 DHFR (EC:1.5.1.3_01x_rn:R00939); 1 Glutaredoxin4; 1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 One-carbon-pool (rn:R02101); 1 One-carbon-pool (rn:R04125); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase

Table F.6: Extreme modes of *P. falciparum* 3D7 for a wildtype strain (no resistances, MB added).

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
1	0,03415607	2	true	2	-1 Glycinhydroxymethyl-Transferase; 1 One-carbon-pool (rn:R00945)
2	0,16843896	3	true	3	1 2-cys-Peroxiredoxin (EC:1.11.1.15_r2); -1 Glutaredoxin6; 1 Thioredoxin
3	0,11743894	3	true	3	-1 Glutaredoxin6; 1 GSH-peroxidase-like-Tpx (EC:1.11.1.9); 1 Thioredoxin
4	0,15070773	3	true	3	-1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R03321)
5	0,05314473	2	true	2	1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R04294); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017)
6	0,10707029	4	true	4	-1 1cys-Glutaredoxin-like-Protein1 (EC:1.11.1.15); 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
7	0,22619036	4	true	4	-1 1-cysPeroxiredoxin; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
8	0,13669031	4	true	4	-1 Glutaredoxin3; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
9	0,05314473	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02024)
10	0,13669031	5	true	5	-1 Glutaredoxin2; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin; -1 Thioredoxin-depPeroxidase
11	0,45818687	6	true	6	-1 Glutaredoxin4; 1 Glutaredoxin6; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; -1 Thioredoxin2
12	0,05314473	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02018)
13	0,05314473	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02019)

Continuation of Table F.6

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
14	0,04829026	5	true	5	-1 1-Cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 GSH-S-Transferase2(EC:2.5.1.18); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
15	0,13669031	5	true	5	-1 1-Cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 Glutaredoxin5; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
16	0,03022726	6	true	5	1 Deoxyribose-P-Aldolase (EC:4.1.2.4_rn:R01066); -2 Phosphogluco-Mutase (EC:5.4.2.2_rn:R01057); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -1 Transketolase (EC:2.2.1.1_rn:R01641)
17	0,12055349	5	false	5	1 1-Cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
18	0,17932449	12	false	11	1 Enolase (EC:4.2.1.11_rn:R00658); 1 Glyceraldehyde-3P-Dehydrogenase (EC:1.2.1.12_rn:R01061); 1 Lactate-Dehyrogenase (EC:1.1.1.27_rn:R00703); 1 Monocarboxylate-Transporter; 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphoglycerate-Kinase (EC:2.7.2.3_rn:R01512); 1 Phosphoglycerate-Mutase (EC:5.4.2.1_rn:R01518); 1 Pyruvate-Kinase (EC:2.7.1.40_rn:R00200); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 Transketolase (EC:2.2.1.1_rn:R01641)
19	0,08158773	6	false	6	1 Hexokinase (EC:2.7.1.1_rn:R01600); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)
20	0,08830794	2	false	2	1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-pyrophosphokinase (EC:2.7.6.1_rn:R01049)
21	0,14097235	1	false	1	1 Superoxide-dismutase (EC:1.15.1.1)
22	0,01934514	7	false	7	-1 Glutaredoxin4; 1 Glutaredoxin6; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; 1 Thioredoxin-Reductase (EC:1.6.4.5.)

Continuation of Table F.6

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
23	0,14896761	18	false	13	1 6-P-delta-Lactonase (EC:3.1.1.31); 1 6-Phosphogluconate-Dehydrogenase (rn:R01528); 1 Glucose-6-P1-dehydrogenase (EC:1.1.1.49_rn:R02736); 2 Glutaredoxin4; 2 GSH-Reductase (EC:1.8.1.7_rn:00115); -2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -2 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Thioredoxin-depPeroxidase; -1 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
24	0,08502037	16	false	8	1 Aldolase (EC:4.1.2.13_rn:R01070); 1 Phosphofructokinase (EC:2.7.1.11_rn:R04779); -5 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphotrioseisomerase (EC:5.3.1.1_rn:R01015); -2 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -3 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
25	0,04974439	8	false	7	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous; 1 Transketolase (EC:2.2.1.1_rn:R01641)
26	0,15315625	3	false	3	1 Aldehyde-Reductase (EC:1.1.1.21); 1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6)
27	0,12055349	6	false	5	2 1-Cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP- and/or GSSG-Pumps; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
28	0,00000000	6	false	6	1 DHFR (EC:1.5.1.3_01x_rn:R00936); 1 DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
29	0,05172020	6	false	6	1 Glucose-Transporter; 1 Hexokinase (EC:2.7.1.1_rn:R01786); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)

Continuation of Table F.6

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
30	0,00000000	9	false	8	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphotriose-Isomerase (EC:5.3.1.1_rn:R01015); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous2; 1 Transketolase (EC:2.2.1.1_rn:R01641)
31	0,00000000	39	false	12	4 DHFR (EC:1.5.1.3_01x_rn:R00939); -4 Glutaredoxin4; -4 GSH-Reductase (EC:1.8.1.7_rn:00115); 4 Glycinhydroxymethyl-Transferase; 4 One-carbon-pool (rn:R02101); 4 Porphyrine (EC:2.3.1.37); 1 Porphyrine (EC:2.5.1.61); 1 Porphyrine (EC:4.1.1.37); 4 Porphyrine (EC:4.2.1.24); 1 Porphyrine (EC:spontaneous); 4 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 4 Thioredoxin-depPeroxidase
32	0,00000000	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1-Fe2+accumulation); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
33	0,02822463	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
34	0,01911563	7	false	7	-1 1-Cysteine-Glutaredoxin; 1 DHFR (EC:1.5.1.3_01x_rn:R00939); 1 GSH-Synthase (EC:6.3.2.3); -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Glycinhydroxymethyl-Transferase; 1 L-Glutamate-L-Cysteine-Synthase (EC:6.3.2.2); 1 One-carbon-pool (rn:R02101)
35	0,28090636	7	false	7	1 DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 One-carbon-pool (rn:R02101); 1 One-carbon-pool (rn:R04125); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase

Table F.7: Extreme modes of *P. falciparum* 3D7 for less active DHF and DHPS and added MB (CQ resistant; sulfadoxine and MB added).

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
1	0.003705426	2	true	2	-1 Glycinhydroxymethyl-Transferase; 1 One-carbon-pool (rn:R00945)
2	0.149361218	3	true	3	1 2-cys-Peroxiredoxin (EC:1.11.1.15_r2); -1 Glutaredoxin6; 1 Thioredoxin
3	0.098361138	3	true	3	-1 Glutaredoxin6; 1 GSH-Peroxidase-like-Tpx (EC:1.11.1.9); 1 Thioredoxin
4	0.104657833	3	true	3	-1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R03321)
5	-0.056415281	2	true	2	1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R04294); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017)
6	0.126174031	4	true	4	-1 1cys-Glutaredoxin-like-Protein1 (EC:1.11.1.15); 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
7	0.245294218	4	true	4	-1 1-cysPeroxiredoxin; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
8	0.155794078	4	true	4	-1 Glutaredoxin3; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
9	-0.056415281	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02024)
10	0.155794078	5	true	5	-1 Glutaredoxin2; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin; -1 Thioredoxin-depPeroxidase
11	0.352528658	6	true	6	-1 Glutaredoxin4; 1 Glutaredoxin6; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; -1 Thioredoxin2
12	-0.056415281	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02018)
13	-0.056415281	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02019)

Continuation of Table F.7

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
14	0.067393939	5	true	5	-1 1-cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 GSH-S-Transferase2 (EC:2.5.1.18); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
15	0.155794078	5	true	5	-1 1-Cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 Glutaredoxin5; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
16	-0.082763049	6	true	5	1 Deoxyribosephosphataldolase (EC:4.1.2.4_rn:R01066); -2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -1 Transketolase (EC:2.2.1.1_rn:R01641)
17	0.101381174	5	false	5	1 1-Cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
18	0.162657571	12	false	11	1 Enolase (EC:4.2.1.11_rn:R00658); 1 Glyceraldehyde-3P-Dehydrogenase (EC:1.2.1.12_rn:R01061); 1 Lactate-Dehydrogenase (EC:1.1.1.27_rn:R00703); 1 Monocarboxylate-Transporter; 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphoglycerate-Kinase (EC:2.7.2.3_rn:R01512); 1 Phosphoglycerate-Mutase (EC:5.4.2.1_rn:R01518); 1 Pyruvate-Kinase (EC:2.7.1.40_rn:R00200); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 Transketolase (EC:2.2.1.1_rn:R01641)
19	0.098216298	6	false	6	1 Hexokinase (EC:2.7.1.1_rn:R01600); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)
20	0.123514449	2	false	2	1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Pyrophosphokinase (EC:2.7.6.1_rn:R01049)
21	0.140921560	1	false	1	1 Superoxide-Dismutase (EC:1.15.1.1)
22	0.067778062	7	false	7	-1 Glutaredoxin4; 1 Glutaredoxin6; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; 1 Thioredoxin-Reductase (EC:1.6.4.5.)

Continuation of Table F.7

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
23	0.104497504	18	false	13	1 6-P-δ-Lactonase (EC:3.1.1.31); 1 6-Phosphogluconate-Dehydrogenase (rn:R01528); 1 Glucose-6-P-1-Dehydrogenase (EC:1.1.1.49_rn:R02736); 2 Glutaredoxin4; 2 GSH-Reductase (EC:1.8.1.7_rn:00115); -2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -2 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Thioredoxin-depPeroxidase; -1 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
24	0.142020949	16	false	8	1 Aldolase (EC:4.1.2.13_rn:R01070); 1 Phosphofructokinase (EC:2.7.1.11_rn:R04779); -5 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphotriose-Isomerase (EC:5.3.1.1_rn:R01015); -2 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -3 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
25	0.000000000	8	false	7	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous; 1 Transketolase (EC:2.2.1.1_rn:R01641)
26	0.186286383	3	false	3	1 Aldehyde-Reductase (EC:1.1.1.21); 1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6)
27	0.101381174	6	false	5	2 1-Cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP- and/or GSSG-Pumps; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
28	0.000000000	6	false	6	1 DHFR (EC:1.5.1.3_01x_rn:R00936); 1 DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
29	0.067597773	6	false	6	1 Glucose-Transporter; 1 Hexokinase (EC:2.7.1.1_rn:R01786); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)

Continuation of Table F.7

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
30	0.000000000	9	false	8	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphotrioseisomerase (EC:5.3.1.1_rn:R01015); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous2; 1 Transketolase (EC:2.2.1.1_rn:R01641)
31	0.000000000	39	false	12	4 DHFR (EC:1.5.1.3_01x_rn:R00939); -4 Glutaredoxin4; -4 GSH-Reductase (EC:1.8.1.7_rn:00115); 4 Glycinhydroxymethyl-Transferase; 4 One-carbon-pool (rn:R02101); 4 Porphyrine (EC:2.3.1.37); 1 Porphyrine (EC:2.5.1.61); 1 Porphyrine (EC:4.1.1.37); 4 Porphyrine (EC:4.2.1.24); 1 Porphyrine (EC:spontan); 4 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 4 Thioredoxin-depPeroxidase
32	0.000000000	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1-Fe^2+-accumulation); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
33	0.023006859	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxylmethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
34	0.000000000	7	false	7	-1 1-Cysteine-Glutaredoxin; 1 DHFR (EC:1.5.1.3_01x_rn:R00939); 1 GSH-Synthase (EC:6.3.2.3); -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Glycinhydroxylmethyl-Transferase; 1 L-Glutamate-L-Cysteine-Synthase (EC:6.3.2.2); 1 One-carbon-pool (rn:R02101)
35	0.155981439	7	false	7	1 DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 One-carbon-pool (rn:R02101); 1 One-carbon-pool (rn:R04125); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase

Table F.8: Extreme modes of *P. falciparum* 3D7 for less active ferrochelatase and added MB (sulfadoxine-resistant; CQ and MB added.

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
1	0.007981358	2	true	2	-1 Glycinhydroxymethyl-Transferase; 1 One-carbon-pool (rn:R00945)
2	0.171036125	3	true	3	1 2-cys-Peroxiredoxin (EC:1.11.1.15_r2); -1 Glutaredoxin6; 1 Thioredoxin
3	0.120036123	3	true	3	-1 Glutaredoxin6; 1 GSH-peroxidase-like-Tpx (EC:1.11.1.9); 1 Thioredoxin
4	0.160669895	3	true	3	-1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R03321)
5	0.052926801	2	true	2	1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R04294); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017)
6	0.104625852	4	true	4	-1 1cys-glutaredoxin-like-protein1 (EC:1.11.1.15); 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
7	0.223745857	4	true	4	-1 1-cysPeroxiredoxin; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
8	0.134245853	4	true	4	-1 Glutaredoxin3; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
9	0.052926801	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02024)
10	0.134245853	5	true	5	-1 Glutaredoxin2; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin; -1 Thioredoxin-depPeroxidase
11	0.156853481	6	true	6	-1 Glutaredoxin4; 1 Glutaredoxin6; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; -1 Thioredoxin2
12	0.052926801	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02018)
13	0.052926801	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02019)

Continuation of Table F.8

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
14	-0.105908604	5	true	5	-1 1-Cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 GSH-S-Transferase2 (EC:2.5.1.18); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
15	0.134245853	5	true	5	-1 1-Cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 Glutaredoxin5; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
16	-0.140226218	6	true	5	1 Deoxyribosephosphataldolase (EC:4.1.2.4_rn:R01066); -2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -1 Transketolase (EC:2.2.1.1_rn:R01641)
17	0.123108687	5	false	5	1 1-Cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
18	0.170898526	12	false	11	1 Enolase (EC:4.2.1.11_rn:R00658); 1 Glyceraldehyde-3P-Dehydrogenase (EC:1.2.1.12_rn:R01061); 1 Lactate-Dehydrogenase (EC:1.1.1.27_rn:R00703); 1 Monocarboxylate-Transporter; 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphoglycerate-Kinase (EC:2.7.2.3_rn:R01512); 1 Phosphoglycerate-Mutase (EC:5.4.2.1_rn:R01518); 1 Pyruvate-Kinase (EC:2.7.1.40_rn:R00200); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 Transketolase (EC:2.2.1.1_rn:R01641)
19	0.117972578	6	false	6	1 Hexokinase (EC:2.7.1.1_rn:R01600); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)
20	0.086218620	2	false	2	1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Pyrophosphokinase (EC:2.7.6.1_rn:R01049)
21	0.140997978	1	false	1	1 Superoxide-Dismutase (EC:1.15.1.1)
22	0.000000000	7	false	7	-1 Glutaredoxin4; 1 Glutaredoxin6; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; 1 Thioredoxinreductase (EC:1.6.4.5.)

Continuation of Table F.8

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
23	0.206731012	18	false	13	1 6-P-δ-Lactonase (EC:3.1.1.31); 1 6-Phosphogluconate-Dehydrogenase (rn:R01528); 1 Glucose-6-P-1-Dehydrogenase (EC:1.1.1.49_rn:R02736); 2 Glutaredoxin4; 2 GSH-Reductase (EC:1.8.1.7_rn:00115); -2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -2 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Thioredoxin-depPeroxidase; -1 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
24	0.115753221	16	false	8	1 Aldolase (EC:4.1.2.13_rn:R01070); 1 Phosphofructokinase (EC:2.7.1.11_rn:R04779); -5 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphotriose-Isomerase (EC:5.3.1.1_rn:R01015); -2 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -3 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
25	0.000000000	8	false	7	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous; 1 Transketolase (EC:2.2.1.1_rn:R01641)
26	0.186355577	3	false	3	1 Aldehyde-Reductase (EC:1.1.1.21); 1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6)
27	0.123108687	6	false	5	2 1-Cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP- and/or GSSG-Pumps; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
28	0.379371542	6	false	6	1 DHFR (EC:1.5.1.3_01x_rn:R00936); 1 DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
29	0.064238597	6	false	6	1 Glucose-Transporter; 1 Hexokinase (EC:2.7.1.1_rn:R01786); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)

Continuation of Table F.8

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
30	0.000000000	9	false	8	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphotrioseisomerase (EC:5.3.1.1_rn:R01015); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous2; 1 Transketolase (EC:2.2.1.1_rn:R01641)
31	0.000000000	39	false	12	4 DHFR (EC:1.5.1.3_01x_rn:R00939); -4 Glutaredoxin4; -4 GSH-Reductase (EC:1.8.1.7_rn:00115); 4 Glycinhydroxymethyl-Transferase; 4 One-carbon-pool (rn:R02101); 4 Porphyrine (EC:2.3.1.37); 1 Porphyrine (EC:2.5.1.61); 1 Porphyrine (EC:4.1.1.37); 4 Porphyrine (EC:4.2.1.24); 1 Porphyrine (EC:spontan); 4 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 4 Thioredoxin-depPeroxidase
32	0.024073026	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1-Fe^2+-accumulation); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
33	0.000000000	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
34	0.000000000	7	false	7	-1 1-Cysteine-Glutaredoxin; 1 DHFR (EC:1.5.1.3_01x_rn:R00939); 1 GSH-Synthase (EC:6.3.2.3); -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Glycinhydroxymethyl-Transferase; 1 L-Glutamate-L-Cysteine-Synthase (EC:6.3.2.2); 1 One-carbon-pool (rn:R02101)
35	0.198307345	7	false	7	1 DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 One-carbon-pool (rn:R02101); 1 One-carbon-pool (rn:R04125); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase

Table F.9: Extreme modes of *P. falciparum* 3D7 for multiresistant strains and added MB (CQ and sulfadoxine resistant; CQ, sulfadoxine and MB added).

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
1	0.01441165	2	true	2	-1 Glycinhydroxymethyl-Transferase; 1 One-carbon-pool (rn:R00945)
2	-0.17549682	3	true	3	1 2-cys-Peroxiredoxin (EC:1.11.1.15_r2); -1 Glutaredoxin6; 1 Thioredoxin
3	-0.12454966	3	true	3	-1 Glutaredoxin6; 1 GSH-peroxidase-like-Tpx (EC:1.11.1.9); 1 Thioredoxin
4	-0.06292834	3	true	3	-1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R03321)
5	0.05879846	2	true	2	1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R04294); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017)
6	-0.10010707	4	true	4	-1 1cys-glutaredoxin-like-protein1 (EC:1.11.1.15); 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
7	0.28805035	4	true	4	-1 1-cysPeroxiredoxin; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
8	-0.12969505	4	true	4	-1 Glutaredoxin3; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
9	0.05879846	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02024)
10	-0.12969505	5	true	5	-1 Glutaredoxin2; 1 Glutaredoxin4; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin; -1 Thioredoxin-depPeroxidase
11	-0.49878081	6	true	6	-1 Glutaredoxin4; 1 Glutaredoxin6; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; -1 Thioredoxin2
12	0.05879846	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02018)
13	0.05879846	2	true	2	-1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02019)

Continuation of Table F.9

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
14	-0.04138897	5	true	5	-1 1-Cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 GSH-S-Transferase2 (EC:2.5.1.18); -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
15	-0.12969505	5	true	5	-1 1-Cysteine-Glutaredoxin; 1 Glutaredoxin4; -1 Glutaredoxin5; -1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin-depPeroxidase
16	0.09069177	6	true	5	1 Deoxyribosephosphataldolase (EC:4.1.2.4_rn:R01066); -2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -1 Transketolase (EC:2.2.1.1_rn:R01641)
17	0.05863309	5	false	5	1 1-Cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
18	0.17072246	12	false	11	1 Enolase (EC:4.2.1.11_rn:R00658); 1 Glyceraldehyde-3P-Dehydrogenase (EC:1.2.1.12_rn:R01061); 1 Lactate-Dehydrogenase (EC:1.1.1.27_rn:R00703); 1 Monocarboxylate-Transporter; 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphoglycerate-Kinase (EC:2.7.2.3_rn:R01512); 1 Phosphoglycerate-Mutase (EC:5.4.2.1_rn:R01518); 1 Pyruvate-Kinase (EC:2.7.1.40_rn:R00200); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 Transketolase (EC:2.2.1.1_rn:R01641)
19	0.10431479	6	false	6	1 Hexokinase (EC:2.7.1.1_rn:R01600); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)
20	0.08565630	2	false	2	1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Pyrophosphokinase (EC:2.7.6.1_rn:R01049)
21	0.14102903	1	false	1	1 Superoxide-Dismutase (EC:1.15.1.1)
22	0.00000000	7	false	7	-1 Glutaredoxin4; 1 Glutaredoxin6; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Thioredoxin; 1 Thioredoxin-depPeroxidase; 1 Thioredoxinreductase (EC:1.6.4.5.)

Continuation of Table F.9

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
23	0.22160177	18	false	13	1 6-P-δ-Lactonase (EC:3.1.1.31); 1 6-Phosphogluconate-Dehydrogenase (rn:R01528); 1 Glucose-6-P1-Dehydrogenase (EC:1.1.1.49_rn:R02736); 2 Glutaredoxin4; 2 GSH-Reductase (EC:1.8.1.7_rn:00115); -2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02739); -1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); -2 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); -1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Thioredoxin-depPeroxidase; -1 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
24	0.05909384	16	false	8	1 Aldolase (EC:4.1.2.13_rn:R01070); 1 Phosphofructokinase (EC:2.7.1.11_rn:R04779); -5 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); -1 Phosphotriose-Isomerase (EC:5.3.1.1_rn:R01015); -2 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); -2 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); -3 Transketolase (EC:2.2.1.1_rn:R01641); -1 Transketolase (EC:2.2.1.1_rn:R01830)
25	0.00000000	8	false	7	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous; 1 Transketolase (EC:2.2.1.1_rn:R01641)
26	0.10850367	3	false	3	1 Aldehyde-Reductase (EC:1.1.1.21); 1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6)
27	0.05863309	6	false	5	2 1-Cysteine-Glutaredoxin; -1 Glutaredoxin4; 1 MRP- and/or GSSG-Pumps; 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
28	0.36747103	6	false	6	1 DHFR (EC:1.5.1.3_01x_rn:R00936); 1 DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase
29	0.05430869	6	false	6	1 Glucose-Transporter; 1 Hexokinase (EC:2.7.1.1_rn:R01786); 1 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphohexose-Isomerase (EC:5.3.1.9_rn:R02740); 1 Transketolase (EC:2.2.1.1_rn:R01641); 1 Transketolase (EC:2.2.1.1_rn:R01830)

Continuation of Table F.9

Extr. Mode no.	activity	flux sum	rever-sible?	path-length	reactions
30	0.11664678	9	false	8	1 GlyoxalaseI (EC:4.4.1.5); 1 GlyoxalaseII (EC:3.1.2.6); 2 Phosphoglucomutase (EC:5.4.2.2_rn:R01057); 1 Phosphotrioseisomerase (EC:5.3.1.1_rn:R01015); 1 Ribose-P-Isomerase (EC:5.3.1.6_rn:R01056); 1 Ribulose-P-3-Epimerase (EC:5.1.3.1_rn:R01529); 1 spontaneous2; 1 Transketolase (EC:2.2.1.1_rn:R01641)
31	0.00000000	39	false	12	4 DHFR (EC:1.5.1.3_01x_rn:R00939); -4 Glutaredoxin4; -4 GSH-Reductase (EC:1.8.1.7_rn:00115); 4 Glycinhydroxymethyl-Transferase; 4 One-carbon-pool (rn:R02101); 4 Porphyrine (EC:2.3.1.37); 1 Porphyrine (EC:2.5.1.61); 1 Porphyrine (EC:4.1.1.37); 4 Porphyrine (EC:4.2.1.24); 1 Porphyrine (EC:spontaneous); 4 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 4 Thioredoxin-depPeroxidase
32	0.00000000	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1-Fe^2+-accumulation); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
33	0.02408068	83	false	15	8 DHFR (EC:1.5.1.3_01x_rn:R00939); -8 Glutaredoxin4; -8 GSH-Reductase (EC:1.8.1.7_rn:00115); 8 Glycinhydroxymethyl-Transferase; 8 One-carbon-pool (rn:R02101); 2 Porphyrine (EC:1.3.3.3); 1 Porphyrine (EC:1.3.3.4); 8 Porphyrine (EC:2.3.1.37); 2 Porphyrine (EC:2.5.1.61); 2 Porphyrine (EC:4.1.1.37_2); 8 Porphyrine (EC:4.2.1.24); 2 Porphyrine (EC:4.2.1.75); 2 Porphyrine (EC:4.99.1.1); 8 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 8 Thioredoxin-depPeroxidase
34	0.01260773	7	false	7	-1 1-Cysteine-Glutaredoxin; 1 DHFR (EC:1.5.1.3_01x_rn:R00939); 1 GSH-Synthase (EC:6.3.2.3); -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 Glycinhydroxymethyl-Transferase; 1 L-Glutamate-L-Cysteine-Synthase (EC:6.3.2.2); 1 One-carbon-pool (rn:R02101)
35	0.18607812	7	false	7	1 DHFR (EC:1.5.1.3_01x_rn:R00939); -1 Glutaredoxin4; -1 GSH-Reductase (EC:1.8.1.7_rn:00115); 1 One-carbon-pool (rn:R02101); 1 One-carbon-pool (rn:R04125); 1 Ribonucleotide-Reductase (EC:1.17.4.1_rn:R02017); 1 Thioredoxin-depPeroxidase

Table F.10: Comparison of pathway activities in CQ/ sulfadoxine resistant strains.

Extreme Mode Number	Activity of wildtype, no compounds added	Activity of CQ resistant strain	Activity of Sulfadoxine-resistant strain
1	0,03550214	0,00416582	0,01107674
2	0,18927750	0,18026223	0,17461941
3	0,24827750	0,23926217	0,23361941
4	0,12242836	0,10304001	0,07739945
5	0,05332433	0,05412635	0,05463234
6	0,21577177	0,22454975	0,23049611
7	0,35187177	0,36064960	0,36659613
8	0,13847177	0,14724983	0,15319611
9	0,05332433	0,05412635	0,05463234
10	0,13847177	0,14724983	0,15319611
11	0,57607120	0,50954589	0,60540687
12	0,05332433	0,05412635	0,05463234
13	0,05332433	0,05412635	0,05463234
14	0,02767480	-0,11507075	0,04239609
15	0,13847177	0,14724983	0,15319611
16	-0,14189903	-0,07891152	-0,14807101
17	0,14112955	0,13226223	0,12632956
18	0,17966192	0,17068324	0,18053624
19	0,11928531	0,09669090	0,09472815
20	0,10219637	0,12235665	0,11093138
21	0,14100070	0,14105446	0,14099469
22	0,09630498	0,13586165	0,02300644
23	0,15464255	0,10039767	0,20981061
24	0,10150761	0,14450490	0,09053873
25	0,00000000	0,00000000	0,00000000
26	0,19287431	0,19291668	0,19306453
27	0,14112955	0,13226223	0,12632956
28	0,00000000	0,00000000	0,38889148
29	0,07247485	0,06729306	0,12087227
30	0,00000000	0,00000000	0,00000000
31	0,00000000	0,00000000	0,00000000
32	0,00000000	0,00000000	0,02399060
33	0,02733251	0,02311882	0,00000000
34	0,02895586	0,00000000	0,00681835
35	0,28335771	0,15842514	0,20008914

Table F.11: Comparison of pathway activities in CQ/ sulfadoxine resistant strains, MB added

Extreme Mode Number	Activity of wildtype, MB added	Activity of CQ resistant strain; MB added	Activity of Sulfadoxine-resistant strain, MB added
1	0,03415607	0,00370543	0,00798136
2	0,16843896	0,14936122	0,17103613
3	0,11743894	0,09836114	0,12003612
4	0,15070773	0,10465783	0,16066990
5	0,05314473	-0,05641528	0,05292680
6	0,10707029	0,12617403	0,10462585
7	0,22619036	0,24529422	0,22374586
8	0,13669031	0,15579408	0,13424585
9	0,05314473	-0,05641528	0,05292680
10	0,13669031	0,15579408	0,13424585
11	0,45818687	0,35252866	0,15685348
12	0,05314473	-0,05641528	0,05292680
13	0,05314473	-0,05641528	0,05292680
14	0,04829026	0,06739394	-0,10590860
15	0,13669031	0,15579408	0,13424585
16	0,03022726	-0,08276305	-0,14022622
17	0,12055349	0,10138117	0,12310869
18	0,17932449	0,16265757	0,17089853
19	0,08158773	0,09821630	0,11797258
20	0,08830794	0,12351445	0,08621862
21	0,14097235	0,14092156	0,14099798
22	0,01934514	0,06777806	0,00000000
23	0,14896761	0,10449750	0,20673101
24	0,08502037	0,14202095	0,11575322
25	0,04974439	0,00000000	0,00000000
26	0,15315625	0,18628638	0,18635558
27	0,12055349	0,10138117	0,12310869
28	0,00000000	0,00000000	0,37937154
29	0,05172020	0,06759777	0,06423860
30	0,00000000	0,00000000	0,00000000
31	0,00000000	0,00000000	0,00000000
32	0,00000000	0,00000000	0,02407303
33	0,02822463	0,02300686	0,00000000
34	0,01911563	0,00000000	0,00000000
35	0,28090636	0,15598144	0,19830735

Table F.12: Comparison of the wildtype strains with and without added MB

Extreme Mode Number	Activity of wildtype, MB added	Activity of wildtype, no compounds added	Change enzyme flux [%]: no compounds vs. MB added
1	0,03415607	0,03550214	-0,13 Folate synthesis
2	0,16843896	0,18927750	-2,08 Protein Protection
3	0,11743894	0,24827750	-13,08 Protein Protection
4	0,15070773	0,12242836	2,83 Conversion of keto sugars
5	0,05314473	0,05332433	-0,02 Deoxyribunucleotide production
6	0,10707029	0,21577177	-10,87 Protein Protection
7	0,22619036	0,35187177	-12,57 Protein Protection
8	0,13669031	0,13847177	-0,18 Protein Protection
9	0,05314473	0,05332433	-0,02 Deoxyribunucleotide production
10	0,13669031	0,13847177	-0,18 Protein Protection
11	0,45818687	0,57607120	-11,79 Protein Protection
12	0,05314473	0,05332433	-0,02 Deoxyribunucleotide production
13	0,05314473	0,05332433	-0,02 Deoxyribunucleotide production
14	0,04829026	0,02767480	2,06 Protein Protection
15	0,13669031	0,13847177	-0,18 Protein Protection
16	0,03022726	-0,14189903	-17,21 Ribose phosphate for nucleotide metabolism
17	0,12055349	0,14112955	-2,06 Protein Protection
18	0,17932449	0,17966192	-0,03 Generation of keto sugars
19	0,08158773	0,11928531	-3,77 Ribose phosphate for nucleotide metabolism
20	0,08830794	0,10219637	-1,39 Ribose phosphate for nucleotide metabolism
21	0,14097235	0,14100070	0,00 Superoxide protection
22	0,01934514	0,09630498	-7,70 Protein Protection
23	0,14896761	0,15464255	-0,57 Generation of keto sugars
24	0,08502037	0,10150761	-1,65 Generation of keto sugars
25	0,04974439	0,00000000	4,97 Generation of keto sugars
26	0,15315625	0,19287431	-3,97 Lactacte production
27	0,12055349	0,14112955	-2,06 Protein Protection
28	0,00000000	0,00000000	0,00 PP using glutathione reductase
29	0,05172020	0,07247485	-2,08 Generation of keto sugars
30	0,00000000	0,00000000	0,00 Generation of keto sugars
31	0,00000000	0,00000000	0,00 Heme protection
32	0,00000000	0,00000000	0,00 Heme protection
33	0,02822463	0,02733251	0,09 Heme protection
34	0,01911563	0,02895586	-0,98 Glutathione production
35	0,28090636	0,28335771	-0,25 Protein Protection

F.2 Supplementary clinical data

Anamnestic symptoms :

Table F.13: Anamnesis of all cases with confirmed malaria

Assessed symptoms	Patients with admission data available	prevalence	
		no.	*%*
fever	98	95	96.9
convulsion(s)	98	30	30.6
vomiting	98	35	35.7
diarrhea	98	31	31.6
inappetence	92	69	75.0
observed prostration	92	26	28.3

Pretreatment and referral :

Table F.14: Way of transfer for all cases with confirmed Malaria

Way of transfer	prevalence	
	no.	*%*
Referred from another health institution	79	84.9
Came directly from home	14	15.1
Valid: Total	93	100
Missing	5	
Total	98	

Table F.15: Antimalarial pretreatment dependent on referral: prevalence of pre-administered antimalarial treatment for home-treatment and referred patients

	health institution	home
recieved antimalarials	113	36
no pretreatment	54	22

Table F.16: Specification of antimalarial pretreatment dependent on referral: prevalence of pre-administered antimalarial treatment for home-treatment and referred patients

	recieved antimalarials	no pretreatment
recieved CQ	19	4
recieved AQ	24	19
recieved Quinine	50	8
no pretreatment	54	22

Table F.17: Pretreatment with CQ dependent on referral: prevalence of pre-administered antimalarial treatment with CQ for home-treatment and referred patients

	health institution	home
recieved CQ	25	5
no CQ given	142	53

Table F.18: Confirmed malaria dependend from antimalarial pretreatment

	Confirmed Malaria	Unconfirmed Malaria
recieved antimalarials	55	97
no pretreatment	22	47

Table F.19: Confirmed malaria, defined as severe case following WHO definition, and antimalarial pretreatment

		pretreatment given	
severe malaria	malaria confirmed	yes	no
yes	yes	51	18
yes	no	77	41
no	yes	2	3
no	no	10	1

Table F.20: Confirmed malaria, defined as severe case following Marsh's definition, and antimalarial pretreatment

		pretreatment given	
severe malaria	malaria confirmed	yes	no
yes	yes	37	13
yes	no	48	22
no	yes	18	9
no	no	47	25

Clinical examination :

Table F.21: Assessed symptoms at arrival for all cases

Assessed symptoms	Patients with admission data available	prevalence	
		no.	*%*
fever	284	172	60.6
hyperpyrexia	284	9	3.2
prostration	263	123	46.8
impaired consciousness	285	64	22.5
coma	285	40	14.0
clinical signs of anemia	287	216	75.3
clinical signs of severe anemia	287	105	36.6
jaundice	287	20	7.0
hepatomegaly	279	88	30.4
splenomegaly	279	42	15.1
Respiratory distress	280	83	29.6
Tachypnea	203	133	65.5

Table F.22: Assessed symptoms at arrival, only cases with confirmed malaria

Assessed symptoms	Patients with admission data available	prevalence	
		no.	*%*
fever	96	57	59.4
hyperpyrexia	96	4	4.2
prostration	92	35	38.0
impaired consciousness	97	31	32.0
coma	97	20	20.6

Continuation of Table F.22

Assessed symptoms	Patients with admission data available	prevalence	
		no.	*%*
clinical signs of anemia	98	86	87.8
clinical signs of severe anemia	98	48	49.0
jaundice	98	9	9.2
hepatomegaly	97	40	41.2
splenomegaly	97	17	17.5
Respiratory distress	97	27	27.8
Tachypnea	76	53	69.7

Laboratory results :

Table F.23: Laboratory findings for all cases

Laboratory findings	Patients with admission data available	prevalence	
		no.	*%*
hemoglobin: severe anemia	279	76	27.2
hemoglobin: moderate anemia	279	100	35.8
hypoglycemia	242	49	20.2

Table F.24: Laboratory findings for all cases with confirmed Malaria

Laboratory findings	Patients with admission data available	prevalence	
		no.	*%*
hemoglobin: severe anemia	98	30	30.6
hemoglobin: moderate anemia	98	49	50.0
hypoglycemia	88	21	23.9

Outcome :

Table F.25: Outcome for all cases

Outcome	prevalence	
	no.	*%*
Ameliorated	219	89.4

Continuation of Table F.25

Outcome	prevalence	
	no.	*%*
Left against medical advice	8	3.3
Died	18	7.3
Valid: Total	245	100
Missing	44	
Total	289	

Table F.26: Outcome for all cases with confirmed Malaria

Outcome	prevalence	
	no.	*%*
Ameliorated	79	90.8
Left against medical advice	2	2.3
Died	6	6.9
Valid: Total	87	100
Missing	11	
Total	98	

F.3 Supplementary data on medication prices

CQ :

Table F.27: Price table: Chloroquine

label	amount	producer	wholesale price	retail sales price
Nivaquine tablets 100mg	B/20	Rhone Poulenc (France)	FCFA 958 (EUR 1,46)	FCFA 1265 (EUR 1,93)
Nivaquine syrup 0,5% (50mg/ 5ml)	150 ml	Rhone Poulenc (France)	FCFA 1377 (EUR 2,10)	FCFA 1818 (EUR 2,77)
Resochine tablets 250mg	B/30	Bayer (Germany)	FCFA 2179 (EUR 3,32)	FCFA 2876 (EUR 4,38)
Dulciquine 300 mg tablets	B/6	KC-Pharma (Syria)	FCFA 733 (EUR 1,12)	FCFA 968 (EUR 1,48)
Dulciquine syrup (50mg/ 5ml)	60 ml	KC-Pharma (Syria)	FCFA 752 (EUR 1,15)	FCFA 993 (EUR 1,51)

AQ :

Table F.28: Price table: Amodiaquine

label	amount	producer	wholesale price	retail sales price
Flavoquine 200mg tablets	B/16	Hoechst Marion Roussel (Sanofi Aventis Group) (France)	FCFA 1259 (EUR 1,92)	FCFA 1662 (EUR 2,53)
Flavoquine syrup 50mg/5ml	60ml	Hoechst Marion Roussel (Sanofi Aventis Group) (France)	FCFA 1597 (EUR 2,43)	FCFA 2108 (EUR 3,21)
Camoquin syrup 50mg/5ml	60ml	Park Devis Senegal (Pfizer Group) (Senegal)	FCFA 1438 (EUR 2,19)	FCFA 1898 (EUR 2,89)
Camoquin tablets 200mg	B/9	Park Devis Senegal (Pfizer Group) (Senegal)	FCFA 1014 (EUR 1,55)	FCFA 1338 (EUR 2,04)

Quinine :

Table F.29: Price table: Quinine

label	amount	producer	wholesale price	retail sales price
Quinine- & Quinidine Gluconate & Cinchonine- & Cinchonidine Chlorhydrate				
Quinimax 125 tablets	B/18	Sanofi Synthelabo (France)	FCFA 2635 (EUR 4,02)	FCFA 3478 (EUR 5,30)
Quinimax 500 tablets	B/9	Sanofi Synthelabo (France)	FCFA 4138 (EUR 6,31)	FCFA 5462 (EUR 8,33)
Quinimax 125 injectable ampoules	B/3	Sanofi Synthelabo (France)	FCFA 1021 (EUR 1,56)	FCFA 1348 (EUR 2,06)
Quinimax 250 injectable ampoules	B/3	Sanofi Synthelabo (France)	FCFA 1408 (EUR 2,15)	FCFA 1859 (EUR 2,83)
Quinimax 500 injectable ampoules	B/3	Sanofi Synthelabo (France)	FCFA 2467 (EUR 3,76)	FCFA 3256 (EUR 4,96)
Quinine- & Quinidine- & Cinchonine- & Cinchonidine Chlorhydrate				
Malarix 250 tablets	B/12	Exphar (Belgium)	FCFA 1879 (EUR 2,86)	FCFA 2480 (EUR 3,78)
Malarix 250 injectable ampoules	B/10	Exphar (Belgium)	FCFA 1831 (EUR 2,79)	FCFA 2417 (EUR 3,68)
Quinine- & Quinidine- & Cinchonine- & Cinchonidine Bichlorhydrate & Resorcine				
Paluject injectable ampoules 400mg	B/6	Aventis (France)	FCFA 1549 (EUR 2,36)	FCFA 2045 (EUR 3,12)
Quinine Chlorhydrate				
Surquina 250 tablets	B/18	Innotech Int. (France)	FCFA 1894 (EUR 2,89)	FCFA 2500 (EUR 3,81)

Continuation of Table F.29

label	amount	producer	wholesale price	retail sales price
Surquina 490 injectable ampoules	B/3	Innotech Int. (France)	FCFA 1894 (EUR 2,89)	FCFA 2504 (EUR 3,82)
Surquina 245 injectable ampoules	B/3	Innotech Int. (France)	FCFA 1324 (EUR 2,02)	FCFA 1748 (EUR 2,66)
		Quinine Formiate		
Arsiquinoforme 250 tablets	B/15	Sanofi Synthelabo (France)	FCFA 2029 (EUR 3,09)	FCFA 2678 (EUR 4,08)

Halofantrine :

Table F.30: Price table: Halofantrine

label	amount	producer	wholesale price	retail sales price
Halfan 250 tablets	B/6	GlaxoSmithKline (France)	FCFA 2864 (EUR 4,37)	FCFA 3780 (EUR 5,76)
Halfan 100mg / 5ml syrup	45ml	GlaxoSmithKline (France)	FCFA 2864 (EUR 4,37)	FCFA 3780 (EUR 5,76)

SP :

Table F.31: Price table: Sulfadoxine-Pyrimethamine

label	amount	producer	wholesale price	retail sales price
Fansidar 500mg/25mg tablets	B/3	Roche (France)	FCFA 826 (EUR 1,26)	FCFA 1090 (EUR 1,66)
Fansidar 500mg/25mg injectable ampoules	B/2	Roche (France)	FCFA 1924 (EUR 2,93)	FCFA 2540 (EUR 3,87)
Maloxine 500mg/25mg injectable ampoules	B/2	Exphar (Belgium)	FCFA 1074 (EUR 1,64)	FCFA 1418 (EUR 2,16)
Madar 500 mg/25 mg tablets	B/3	Caplin-Point (India)	FCFA 308 (EUR 0,47)	FCFA 407 (EUR 0,62)
Maloxine 500mg/25mg tablets	B/12	Exphar (Belgium)	FCFA 1879 (EUR 2,86)	FCFA 2480 (EUR 3,78)
Combimal inj 2 ml injectable ampoules	B/3	Ajanta-Pharma (India)	FCFA 1155 (EUR 1,76)	FCFA 1525 (EUR 2,32)

Artemisinins :

Table F.32: Price table: Artemisinins

label	amount	producer	wholesale price	retail sales price
		Artemether		
Paluther 80mg injectable ampoules	B/8	Rhone Poulenc (France)	FCFA 10309 (EUR 15,72)	FCFA 13608 (EUR 20,75)
Artésiane 80mg injectable ampoules	B/5	Dafra Pharma (Belgium)	FCFA 4583 (EUR 6,99)	FCFA 6050 (EUR 9,22)
Artésiane 40mg suppositories	B/6	Dafra Pharma (Belgium)	FCFA 2348 (EUR 3,58)	FCFA 3099 (EUR 4,72)
Artésiane 300mg/100ml syrup		Dafra Pharma (Belgium)	FCFA 3005 (EUR 4,58)	FCFA 3967 (EUR 6,05)
G-vither 300mg/100ml syrup	100ml	GVS LBS (Guyane)	FCFA 2573 (EUR 3,92)	FCFA 3396 (EUR 5,18)
G-vither 80mg injectable ampoules	B/1	GVS LBS (Guyane)	FCFA 6876 (EUR 10,48)	FCFA 9076 (EUR 13,84)
Artenam 100mg injectable ampoules	B/7	Ebewe Pharma (Australia)	FCFA 6058 (EUR 9,24)	FCFA 7997 (EUR 12,19)
Amether -Denk syrup 180mg	60ml	Denk-pharma (Germany)	FCFA 2723 (EUR 4,15)	FCFA 3594 (EUR 5,48)
Artenam 50mg tablets	B/14	Ebewe Pharma (Australia)	FCFA 3230 (EUR 4,92)	FCFA 4264 (EUR 6,50)
Artésiane suppositories 160 mg	B/6	Dafra Pharma (Belgium)	FCFA 5011 (EUR 7,64)	FCFA 6615 (EUR 10,08)
		Artesunate		
Arsumax 50mg tablets	B/12	Sanofi Synthelabo (France)	FCFA 2536 (EUR 3,87)	FCFA 3348 (EUR 5,10)
Arinate 100mg tablets	B/6	Dafra Pharma (Belgium)	FCFA 2330 (EUR 3,55)	FCFA 3076 (EUR 4,69)
Arinate 50mg tablets	B/6	Dafra Pharma (Belgium)	FCFA 1973 (EUR 3,01)	FCFA 2604 (EUR 3,97)
Plasmotrim 200mg tablets	B/6	Mepha (Switzerland)	FCFA 3475 (EUR 5,30)	FCFA 4585 (EUR 6,99)
Plasmotrim 50mg tablets	B/12	Mepha (Switzerland)	FCFA 2443 (EUR 3,72)	FCFA 3225 (EUR 4,92)
Plasmotrim 50mg suppositories	B/6	Mepha (Switzerland)	FCFA 1976 (EUR 3,01)	FCFA 2608 (EUR 3,98)
Plasmotrim 200mg suppositories	B/6	Mepha (Switzerland)	FCFA 3475 (EUR 5,30)	FCFA 4585 (EUR 6,99)
Arthesis 50mg tablets	B/12	NYD (Switzerland)	FCFA 2000 (EUR 3,05)	FCFA 2640 (EUR 4,02)
Arsunate-Denk tablets 200 mg plus	B/6	Denk pharma (Germany)	FCFA 2911 (EUR 4,44)	FCFA 3843 (EUR 5,86)

Continuation of Table F.32

label	amount	producer	wholesale price	retail sales price
Arsunate-Denk tablets 200 mg jun	B/6	Denk pharma (Germany)	FCFA 1973 (EUR 3,01)	FCFA 2604 (EUR 3,97)
		Dihydroartemisinine		
Cotecxin 60mg tablets	B/8	Beijing Cotec Pharmaceuticals (China)	FCFA 2010 (EUR 3,06)	FCFA 2653 (EUR 4,04)
Cotexcin syrup 160mg/ 80ml	80ml	Beijing Cotec Pharmaceuticals (China)	FCFA 1934 (EUR 2,95)	FCFA 2553 (EUR 3,89)
Artemax 60mg tablets	B/8	G.A.P (Greece)	FCFA 2507 (EUR 3,82)	FCFA 3309 (EUR 5,04)
Alexin 60mg tablets	B/8	GVS LBS (Guyane)	FCFA 2433 (EUR 3,71)	FCFA 3212 (EUR 4,90)
Alexin 160mg/ 80ml syrup	100ml	GVS LBS (Guyane)	FCFA 3005 (EUR 4,58)	FCFA 3967 (EUR 6,05)

ACTs :

Table F.33: Price table: ACTs

label	amount	producer	wholesale price	retail sales price
		Artesunate + Mefloquine		
Artequin 600mg/750mg tablets	B/6	Mepha (Switzerland)	FCFA 3100 (EUR 4,73)	FCFA 4092 (EUR 6,24)
Artequin 300mg/375mg tablets	B/6	Mepha (Switzerland)	FCFA 2348 (EUR 3,58)	FCFA 3099 (EUR 4,72)
		Artesunate + Sulfamethoxypyrazine + Pyrimethamine		
Coarinate 200mg/500mg/25mg tablets	B/3	Dafra Pharma (Belgium)	FCFA 3078 (EUR 4,69)	FCFA 4063 (EUR 6,19)
Coarinate enfant 100mg/250mg/12,5mg tablets	B/3	Dafra Pharma (Belgium)	FCFA 2005 (EUR 3,06)	FCFA 2647 (EUR 4,04)
		Artesunate + Amodiaquine		
Arsucam 50mg/200mg tablets	B/3	Sanofi Synthelabo (France)	FCFA 2066 (EUR 3,15)	FCFA 2727 (EUR 4,16)
Arsucam 50mg/200mg tablets	B/6	Sanofi Synthelabo (France)	FCFA 2320 (EUR 3,54)	FCFA 3062 (EUR 4,67)
Arsucam 50mg/200mg tablets	B/12	Sanofi Synthelabo (France)	FCFA 3306 (EUR 5,04)	FCFA 4364 (EUR 6,65)
		Artemether + Lumefantrine		
Coartem 20mg/120mg tablets		Novartis (France)	FCFA 3288 (EUR 5,01)	FCFA 4340 (EUR 6,62)
Coartesiane paediatric suspension		Dafra Pharma (Belgium)	FCFA 3222 (EUR 4,91)	FCFA 4253 (EUR 6,48)

Continuation of Table F.33

label	amount	producer	wholesale price	retail sales price
		Dihydroartemisinine + Piperaquine		
Duo-Cotecxin tablets	B/8	Beijing Cotec Pharmaceuticals (China)	FCFA 3747 (EUR 5,71)	FCFA 4946 (EUR 7,54)