Antimalarial Drug Discovery: Structural Insights

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Chapter 1

Introduction and Scope of the Thesis

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Malaria remains one of the deadliest diseases on earth. In the latest report, the World Health Organisation (WHO) estimated more than 200 million cases of malarial infection in 2016 worldwide, where 445 thousand have been fatal, despite enormous efforts in malaria control and elimination. A highly complex lifecycle, striking ability to develop drug resistance, as well as poor availability and misuse of current drugs in malaria-endemic countries make malaria eradication more and more challenging [1-3]. A constant supply of novel antimalarial drugs with orthogonal action modes is needed to eliminate or at least delay the emergence of global drug resistance of the malarial parasite [4]. This thesis is a compilation of academic publication discussing the methods used in antimalarial drug research. While the main attention is focused on the drug target validation in malarial parasite, we also report crystallographic data and preliminary characterization of several enzymes within essential pathways, previously reported or suggested to be promising drug targets. These data will provide additional basis for rational drug discovery against malaria.

In **Chapter 2,** we discuss the so-called "Harlow-Knapp" effect in antimalarial research, a trend previously described for human kinase research [5, 6]. Despite the pressing need for a constant supply of novel antimalarial drugs targeting diverse parasitic systems, the majority of efforts have rather been focused on optimization of the existing scaffolds and further investigation of validated and druggable pathways of the parasite. A summary of the knowledge available for these pathways with specific focus in pathway interplay is used to generate a "road map" for further antimalarial drug development and new target identification [7]. Furthermore, a promising approach for novel drug target validation applied to a highly complex organism, such as *Plasmodium falciparum*, is suggested.

Drug target validation in parasitic systems is a challenging and expensive process, especially in cases when the parasite has a complex lifecycle and multiple host organisms. In **Chapter 3,** we continue the discussion on current methods that have been used for antimalarial drug target vali-

dation [8]. Despite constant improvements, the genetic manipulation toolset used to validate drug targets in the majority of "standard" cases, remains insufficient in malaria. In addition to the overview of the current antimalarial toolset and associated "gaps", we discuss an alternative route for specific inhibition of the target enzymes. Extreme specificity of inter-oligomeric interactions provides an opportunity to exploit oligomerization as a tool in drug validation. We suggest a novel strategy of highly specific *in vivo* functional modulation of the selected target proteins for their validation, the Protein Interference Assay (PIA).
The PIA assay requires the knowledge of the quaternary structure of the target protein as well as detailed knowledge of the residues involved in these oligomeric interactions. The availability of spatial structure as well as basic biophysical characterization can provide such information. We will focus on X-ray protein crystallography as the dominant method of protein-structure determination. However, recent advances in such techniques as cryoEM, NMR spectroscopy and *in silico* structure modeling suggest, that these methods could soon reach the functionality of X-ray crystallography and will be used for routine measurements as often [9-11]. The perspectives of X-ray crystallography will be further discussed in **Chapter 8**.

In addition to the several examples of PIA-applications discussed in **Chapters 2**&**3**, in **Chapter 4,** we describe the crystal structure of malate dehydrogenase from *Plasmodium falciparum* (*Pf*MDH)[12]. We show how these data can be used in order to modulate the activity of the enzyme *in vitro* with high specificity, without recourse to questionable genetic manipulations or laborious and expensive inhibitor design. We report structure-based mutations designed to manipulate its quaternary structure, resulting in significantly altered activity of the enzyme. Furthermore, we show that the modified mutants can be incorporated into the native assembly *in vitro* and render the resulting chimeric enzyme inactive.

In **Chapter 5,** we report the crystal structure and preliminary characterization of the unliganded aspartate transcarbamoylase from *Plasmodium falciparum Pf*ATC, another potential target for antimalarial drug discov-

ery [13]. The reported structural and mutagenic data can further be used in PIA-validation of *Pf*ATC as a drug target. The trimeric nature, as well as the location of each of the three active sites at the oligomeric interfaces, make *Pf*ATC a good target for PIA, as introduction of one mutant copy into the native trimeric assembly would likely result in significant or full activity loss. Such experiments both *in vitro* and *in vivo* are ongoing (Bosch, Lunev, Batista *et al.*, in preparation).

In **Chapter 6,** we report identification of the lead-compound inhibiting *Pf*ATC as well as its preliminary biophysical characterization using the combination of (semi)high-throughput Differential Scanning Fluorimetry (DSF), X-ray crystallography and activity measurements [14]. 2,3-Napthalenediol was identified using DSF based on a significantly increased thermal stability of *Pf*ATC in its presence. Further assays, as well as crystal structure of the complex confirmed the binding of the compound. The location of the binding of 2,3-Napthalenediol suggests that it can be further developed into a specific *Pf*ATC inhibitor. In this chapter we also report preliminary attempts to generate and characterize a family of structurally-related compounds in order to provide additional data for further drug-development.

In **Chapter 7**, we report the expression, crystallization and X-ray data collection of pyridoxal kinase from *Plasmodium falciparum* (*Pf*PdxK) [15]. *Pf*PdxK is involved in the vitamin B6 metabolism and has been previously suggested as a promising drug target [16-20].

Chapter 8 is a summary of this thesis. Furthermore, we briefly discuss the future perspectives of antimalarial drug discovery and the methods used. Emergence and rapid evolution of novel research tools as well as further advances in high-throughput methods used in drug screening, crystallization, data collection, processing and analysis will likely significantly affect “classic” drug discovery. We believe that all available tools should be used in synergy in order to fight malaria and other devastating diseases.

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