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The Oligomeric Protein Interference Assay Method for Validation of Antimalarial Targets

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**rijksuniversiteit
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Actions

The work described in this thesis was carried out at the Structural Biology Unit, Department of Drug Design (Groningen Research Institute of Pharmacy at the University of Groningen, The Netherlands) and Unit for Drug Discovery (Department of Parasitology, Institute of Biomedical Sciences at the University of São Paulo, Brazil) and was financially supported by CNPq, Science Without Borders fellowship (project number 2013/17577-9), CAPES/Nuffic MALAR-ASP (053/14) network and Marie Skłodowska-Curie grant Agreement No. 675555, Accelerated Early stage drug discovery (AEGIS).

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The Oligomeric Protein Interference Assay Method for Validation of Antimalarial Targets

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Rector Magnificus Prof. C. Wijmenga
and in accordance with
the decision by the College of Deans.

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University of São Paulo
on the authority of the
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Double PhD degree

This thesis will be defended in public on
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*"Research is to see what everybody else has seen,
and to think what nobody else has thought"*

Albert Szent-Györgyi

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GENERAL INTRODUCTION AND SCOPE OF THE THESIS

MALARIA remains a devastating global parasitic disease. There were an estimated 219 million cases and 435,000 related deaths in 2017[1, 2]. From the six *Plasmodium* species capable of causing human malaria; *Plasmodium falciparum* and *Plasmodium vivax* pose the greatest global health threat. The first is prevalent in Africa, disproportionately accounting for most malaria cases and deaths globally, whereas the second is a temperate zone infecting parasite[3, 4]. Currently, the only vaccine against malaria approved for is the RTS, SA/AS01[5]. Although this approach has the potential to reduce the number of cases by 25% and 50% in infants and young children respectively, this rate is still precarious in a global scenario[6, 7]. Moreover, *Plasmodium* is known to develop strategies in evading the immune system when challenged with vaccines over time[8].

Artemisinin-based combination therapies (ACTs) are currently the most effective frontline therapies against *P. falciparum*[1, 3, 4, 9]. These became the potent solution to the previous regime of resistance to drugs such as sulfadoxine/pyrimethamine and chloroquine in the mid-1900's[10, 11]. Unfortunately, drug-resistance to artemisinin and its derivatives has emerged in Southeast Asia[12–16]. This highlights the need for constant basic research into the life cycle and metabolism of *Plasmodium* in order to identify areas of parasite's vulnerability. Once new targets are identified and validated, they can be used to generate new therapeutics that uniquely target the malaria parasite.

Validation is a crucial process in the drug discovery pipeline[17, 18]. Drugs mostly target and inhibit specific components of a biological process to produce a therapeutic effect[19]. Hence, the right toolset that would allow the specific and optimal inhibition *in vivo* is indispensable[18, 20]. The blood stage proliferation of the malaria parasite in humans is critically dependent on specific metabolites, and their survivability is believed to be tightly dependent on availability and metabolism of carbon nutrients[21, 22]. Although many key enzymes in carbon metabolism and related pathways could represent potential drug targets, the target validation process is far from simple, partly owing to the complex nature of the parasite's life cycle[18, 20]. This complexity has led to limited tools to properly validate antimalarial targets *in vivo*[4]. Current attempts with the genetic

approaches, the small molecules, and siRNA tools among many others as antimalarial validation probes have shown significant limitations. These constraints call for new innovative, highly specific validation tools[18, 20].

In **Chapter 1**, we discuss the currently employed antimalarial target validation tools. Despite the recent developments in the field, there remain pitfalls for each existing methodology. In this context, we introduce the oligomeric Protein Interference Assay (PIA) as a valuable addition to the current antimalarial validation toolbox.

The PIA principle applied *in vitro* is the subject of **Chapter 2**. The initial mutagenesis experiments based on structural analysis of the plasmodial enzymes aspartate aminotransferase (*PfAspAT*) and malate dehydrogenase (*PfMDH*) are described here. The ability of the mutants to form a complex with their wild-type counterparts, consequently impairing their function, is evaluated and discussed in this chapter.

Following up the *in vitro* experiments, **Chapter 3** describes the first application of PIA *in vivo*. Aiming to provide proof-of-concept of the effect of oligomeric interface distortion, *P. falciparum* parasites were transfected with constructs encoding for mutant AspAT and MDH enzymes and had their growth profiles monitored.

The initial application of PIA to two components of the plasmodial aspartate metabolism encouraged us to apply this technique to an enzyme that connects this pathway to another essential pathway within *P. falciparum*. Aspartate transcarbamoylase (*PfATC*) catalyses the condensation of aspartate and carbamoyl phosphate to form N-carbamoyl-L-aspartate and inorganic phosphate, the second step of the pyrimidine biosynthetic pathway. The crystallographic structure of this enzyme, as well as initial mutagenesis experiments supporting its possible validation by PIA, are reported in **Chapter 4**.

In **Chapter 5**, we describe the evaluation of *PfATC* essentiality by PIA. Aiming to evaluate the effect of inactivating mutations in the structure and folding of this enzyme, crystallographic and static light scattering data analysis are reported. The growth of parasites transfected with mutant *PfATC* alone and in combination with *PfAspAT* was monitored. The effect of the expression of these mutants in parasites' proliferation is disclosed in this chapter.

The findings reported in this thesis are summarized in **Chapter 6**. This chapter also contains the future perspectives for the use of PIA as a novel target validation technique in the field of infectious diseases.

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