## Publishable executive summary

### Summary of the project objectives

*Plasmodium falciparum* causes severe malaria and about 2 million human deaths annually. The main obstacle to combat the disease is increasing resistance of the parasites to existing drugs and the lack of a protective vaccine. Therefore, it is imperative that new suitable drug targets in the parasite`s metabolism are identified, assessed and validated. Vitamins are organic compounds required in small amounts to ensure normal metabolic functions. Since they are not synthesized by humans, they need to be supplied via nutrients in trace amounts. The absence of vitamin biosynthesis in humans suggests that specific targeting of these parasite pathways with inhibitors is feasible and thus vitamin biosynthesis of *Plasmodium* might offer excellent potential for the development of novel chemotherapeutics against malaria with specific toxicity towards the parasites without affecting the host`s metabolism. In the first instance we were focussing on vitamin $B_6$ biosynthesis, as this important nutrient is required as a co-factor for a wide variety of essential metabolic functions in protein and amino acid metabolism and also has been implicated in the defence against oxidative stress in other eukaryotes. Using reverse genetic approaches, the suitability of two of the vitamin $B_6$ synthesising enzymes Pdx1 and Pdx2 respectively, as drug targets should be validated. In addition, their precise biological functions and potential interactions with other cellular components should be analyzed. Further the biochemical, biophysical and structural features of both enzymes should be assessed in order to be able to rationally design specific inhibitors that interfere with the parasite's proteins activities and functions.

### Contractors involved

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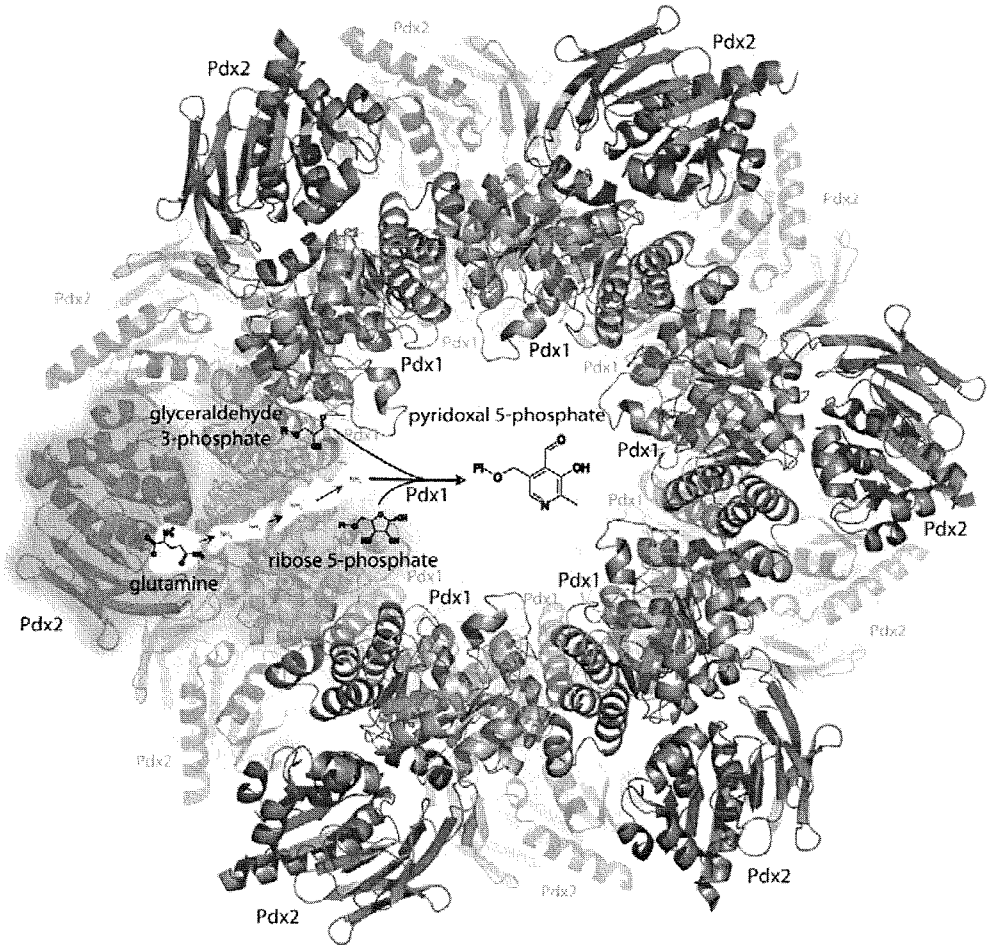
**Work performed and results achieved so far and main elements of the publishable results**

Vitamin $B_6$ is one of nature's most versatile cofactors. Most organisms synthesize vitamin $B_6$ via a recently discovered pathway employing the proteins Pdx1 and Pdx2. In course of this project it turned out that Pdx1 is the actually synthase of the complex synthesizing the biological active $B_6$ vitamer pyridoxal-5-phosphate (PLP) from $NH_3$, which is provided by the glutaminase partner Pdx2 and ordinary sugars.




Three-dimensional structure of the PLP synthase.

We have done an in depth characterization of the respective orthologs from the malaria parasite, *Plasmodium falciparum.* Expression profiling of Pdx1 and 2 revealed that parasite blood stages possess a functional vitamin $B_6$ *de novo* biosynthesis. Using reverse genetic approaches the suitability the vitamin $B_6$ synthesising enzymes Pdx1 and Pdx2 as drug targets was investigated. Gene knock-out studies in the mouse malaria model system revealed that the parasite possesses a functional vitamin $B_6$ uptake system, which can at least in part compensate for loss of function of de novo biosynthesis, suggesting that the latter is not essential for parasite survival and as a consequence not a suitable target for antimalarial drug development. The contribution of vitamin $B_6$ uptake and/or salvage versus vitamin $B_6$ de novo biosynthesis to the overall PLP homeostasis was addressed by vitamin $B_6$

depletion experiments and by gene knock-outs affecting either parasite`s de novo vitamin $B_6$ biosynthesis or vitamin $B_6$ uptake and/or salvage. Whereas the growth of intraerythrocytic parasites, which are the parasite forms causing the symptoms of malaria and as a consequence are the target of almost all antimalarials, was not affected by the gene knock-out abolishing the formation of the biological active $B_6$ vitamer pyridoxal-5-phosphate (PLP) upon uptake or salvage, the knock-out of the gene required for de novo vitamin $B_6$ biosynthesis resulted in a growth delay of these parasite forms. These findings suggest that PLP de novo biosynthesis is superior to vitamin $B_6$ uptake and/or salvage as the latter were not able to fully compensate for loss of PLP de novo biosynthesis and, in addition, demonstrate that vitamin $B_6$ de novo biosynthesis is not simply a back up system coming into action when required, but instead is the main supplier of the biological active $B_6$ vitamer PLP. Knock-outs of genes required for vitamin $B_6$ uptake and/or salvage or vitamin $B_6$ de novo biosynthesis severely affected the mosquito stages of the parasite life cycle resulting in dramatic reduction of parasite numbers from 90 – 98%. Parasites mosquito stages, in which formation of PLP upon uptake and/or salvage was abolished, were no longer able to establish infections in mice.
Although our studies clearly rule out vitamin $B_6$ de novo biosynthesis as a suitable target for antimalarial drug development, the current project had a tremendous impact on the biological, biochemical, biophysical and structural characterization of the vitamin $B_6$ synthesising enzyme complex and pushed on the knowledge and expertise on this topic significantly. In the following only two examples are given. Parallel to scientists from the United States, our consortium discovered the substrates of Pdx1, which were unknown at the beginning of the project. In addition, the first three dimensional structure of the Pdx1-Pdx2 complex being published arouse from the VITBIOMAL consortium.

**The plan of using and disseminating the knowledge**

Knowledge has been disseminated to the general public by press releases and the scientific community by publications, on national as well as international conferences by oral presentation and on poster as well as in higher education, e.g. in lectures and student courses.

**Project logo**:

**Reference of the project public website**: http://www.hyg.uni-heidelberg.de/vitbiomal/