Functional characterization of *Plasmodium* kinase regulatory networks

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Malaria is caused by parasitic protozoa of the genus *Plasmodium* that are responsible for almost 250 million infections annually, leading to nearly a million deaths. The parasite's progression through its complex life cycle in the vertebrate hosts and in the *Anopheles* vector mosquito are tightly controlled by signalling pathways involving diverse second messengers and protein kinases. Global gene knock out screens in different *Plasmodium* species have identified critical functions for parasite protein kinases in regulating key events in the parasite transmission cycle[1,2]. From these and other studies cyclic guanosine monophosphate (cGMP) emerges as an important second messenger that controls different events in the life cycle through PKG, a cGMP dependent protein kinase[3]. We now believe that one function of PKG is to control cellular calcium levels, which in turn regulate a panel of unusual calcium dependent protein kinases (CDPKs) with a plant-like domain architecture, that act as more stage specific effector kinases to elicit appropriate cellular responses in the different life cycle stages[4,5]. Genetic studies in *P. falciparum* of humans, and in *P. berghei*, a parasite infecting rodents, have provided evidence that parasite egress from infected erythrocytes signals through CDPK5[6], that gametocyte activation requires CDPK4[7], that translational activation of mRNAs is mediated by CDPK1[8] and that gliding of the motile zygote in the mosquito midgut is mediated by CDPK3[9].

In vivo substrates of *Plasmodium* kinases have so far remained elusive and our lack of knowledge of specific downstream effector pathways is a major roadblock to a deeper understanding of malaria pathogenesis and transmission. In this project we propose to tackle this problem by combining a powerful chemical genetic approach with quantitative phosphoproteomics and computational analysis[10]. We have already generated a panel of genetically modified *P. berghei* lines, which express either wild type PKG, CDPK1 or CDPK4, or a mutated version of each kinase that are rendered resistant to selective inhibitors. Using some of these strains we have developed a mass-spectrometry protocol to measure the changes in phosphosite abundances after genetic or chemical inhibition using a quantitative phosphoproteomics approach.

The ESPOD fellow will use *P. berghei* kinase mutants and knock-out lines to study the regulatory networks and functions of selected protein kinases such as PKG, CDPK1 and CDPK4. First, the fellow will measure the relative changes in phosphosite abundance after kinase disruption. Briefly, phosphosites from the different samples will be enriched in metal affinity columns. Following high resolution liquid chromatography samples will be analyzed by tandem mass spectrometry on an orbitrap instrument. Each sample will be analyzed by label free quantitation based on synthetic isotope labelled spike peptides that will be used to quantify and normalise the data for comparative analysis. These experiments will yield a quantitative proteome and phosphoproteome profile after kinase disruption. Down-regulated phosphosites are expected to be highly enriched in kinase targets but will also contain sites that are indirectly regulated by downstream effectors. To address this issue the fellow will develop kinase-target predictors. The phosphoprotemics data will first be used to build a kinase recognition position-specific model. Given that kinase-substrate recognition *in-vivo* depends on multiple factors (e.g. co-localization, co-expression, interactions with scaffold proteins) the fellow will also build a functional interaction network for *P. berghei* using standard machine learning approaches to integrate different features from publicly available data (e.g. expression data, interactions from orthologous protein pairs, domain-domain interaction propensity). The kinase specificity models will be combined with the

functional interaction network and the quantitative phosphoproteomics to derive a high confidence kinase network[11]. In addition the *P. berghei* phosphoproteome will be studied in detail to rank phosphosites according to their functional relevance[10]. The fellow will obtain structural models of *P. berghei*'s phosphoproteins and their predicted interaction networks (using MODELLER and Interactome3D) and phosphosite conservation information using the previously published *P. falciparum* and *T. gondii* phosphoproteomes[12]. Having established a high confidence kinase regulatory network that will elucidate the biological role of the targeted kinases, the fellow will then be able to return to the wet lab to test key hypotheses experimentally. This project will generate highly relevant new insights into malaria parasite biology, while at the same time advancing the experimental and computational methodology required to study kinase signaling in general. The methods developed in the course of this project will be useful to very wide research community.

For this project we will bring together expertise from different research groups and facilities at the WTSI and EBI. In the Sanger Institute Malaria Programme Oliver Billker's laboratory has generated genetically modified parasite lines for this project and shares with Julian Rayner's team a keen interest in studying how *Plasmodium* development and transmission is regulated through post-translational protein modifications (PTMs). Both will host and advise the fellow for the parasite cell biology component of the project. Jyoti Choudhary leads a state-of-the-art mass spectrometry facility at WTSI, where she has already established methods to identify >2000 *Plasmodium* proteins and >10k phosphosites. Pedro Beltrao at EBI has a strong track record in predicting regulatory protein networks from global datasets of PTMs and will guide the computational aspects of the project.

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