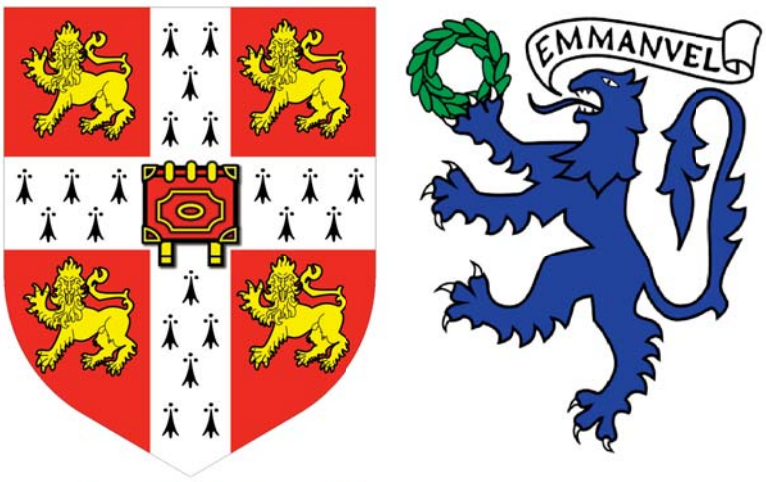
Identification and characterisation of host-pathogen protein-protein interactions in the blood stages of malaria



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For Julia, Nick and Tim

Declaration

I hereby declare that the contents of this thesis are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other, University. This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration, except where specifically indicated in the text. This thesis does not exceed the word limit set by the Faculty of Biology.

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Abstract

Malaria is a widespread and serious disease which affects billions of people. Protein-protein interactions occurring between host and *Plasmodium* parasites are critical to the pathogenesis of malaria and thus represent prime targets for greatly-needed novel therapeutics. Identifying these host-pathogen interactions is challenging, but recent advances in our understanding of parasite biology and in high-throughput biomolecular interaction detection methods have paved the way to a number of successes.

In this work I produced a library of recombinant *Plasmodium falciparum* proteins to screen for interactions with human receptors in a number of high-throughput assays. Using an established ELISA-based protein-protein interaction detection method, I identified an interaction between *P. falciparum* merozoite protein 7 (PfMSP7) and human P-selectin (SELP). I used surface plasmon resonance and flow cytometry approaches to validate this interaction and, by screening more widely across the MSP7 protein family, identified that SELP-binding is a conserved property of multiple MSP7s in at least three *Plasmodium* species. The evolutionary conservation indicates that SELP-MSP7 interactions might have an important function in malaria. Isolating the interacting regions of SELP and PfMSP7 to the secreted, flexible N-terminus of PfMSP7 and the known ligand-binding domains of SELP led to a hypothesis that PfMSP7 could prevent the leukocyte recruitment and activation properties of SELP. I used PfMSP7 to block the interactions between SELP and leukocyte ligands *in vitro*, providing support to this hypothesis. Further evidence will be required to determine whether *Plasmodium* MSP7 proteins and their interactions with SELP mediate an immunomodulatory mechanism in malaria, and whether the MSP7 proteins represent useful therapeutic targets.

I also developed a biochemical co-purification assay aiming to detect additional interactions between recombinant *P. falciparum* merozoite extracellular proteins and those present in human serum. This assay was successful in detecting previously-identified interactions but did not identify novel binding partners for 56 *P. falciparum* ligands. By expanding the screen or by decreasing its stringency this method could facilitate the identification of further receptors for *Plasmodium* ligands which could in turn, like the interaction between SELP and MSP7s, aid our understanding of how host and pathogen interact to cause disease.

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