Biochemical investigations of *Plasmodium falciparum* erythrocyte invasion pathways using recombinant merozoite surface proteins produced in a mammalian expression system

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Declaration

This dissertation 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 dissertation does not exceed the word limit set by the Biology Degree Committee.

Summary

The invasion of erythrocytes by *Plasmodium falciparum* merozoites is mediated by low-affinity, extracellular interactions between surface proteins on the host and parasite cells. Identification of these interactions *in vitro* is technically challenging; not only are *P. falciparum* proteins hard to express recombinantly in heterologous systems, but detecting their transient interactions with erythrocyte receptors is difficult using traditional biochemical methods. Two significant advances in this area of research have recently been made in our laboratory. Firstly, a strategy has been optimised for the production of soluble full-length ectodomains of *P. falciparum* merozoite surface proteins, in a mammalian expression system. Secondly, an ELISA-based method, AVEXIS (avidity-based extracellular interaction screening) has been developed which enables low-affinity interactions between proteins to be identified in a high-throughput manner. The AVEXIS assay is, however, limited for use with soluble proteins and as such cannot be used for the analysis of multi-pass membrane proteins. In my PhD, I developed a flow-cytometry based, high-throughput assay for identifying low-affinity interactions between recombinant *P. falciparum* proteins and receptors displayed on the surface of cells. I used this assay in combination with other biochemical and biophysical methods such as AVEXIS and surface plasmon resonance to investigate selected aspects/interactions of erythrocyte invasion in three projects.

P. falciparum EBA175 is the known ligand of the erythrocyte receptor Glycophorin A, which carries the antigenic determinants of the human MN blood group system. In my first project, I showed that the mammalian-expressed, full-length ectodomain of EBA175 (*Pf*EBA175 FL) is functionally similar to native EBA175 isolated from parasite cultures and that it binds MN Glycophorin A with a slightly higher affinity than the MM form. I also found that *Pf*EBA175

RII, a truncated derivative containing only the extracellular region known to be essential for interacting with erythrocytes, binds to native Glycophorin A with a one order of magnitude lower affinity than *Pf*EBA175 FL, suggesting some role played by regions outside of RII to facilitate binding.

The closest relatives of *P. falciparum* are found amongst the *Laverania* family of great ape parasites, which appear to be strictly host-specific in their natural environments. In my second project, I investigated the potential contributions of EBA175-Glycophorin A and another parasite ligand-host receptor interaction, RH5-Basigin (BSG), towards the determination of host-specificity in *Laverania.* I only observed a relatively small difference in the affinities for native human Glycophorin A, between *Pf*EBA175 and orthologues of EBA175 from two chimpanzee parasites *P. reichenowi* and *P. billcollinsi*, which suggests that this interaction may not play a significant role in the determination of host-specificity in *Laverania*. On the other hand, I saw clear host-selectivity in the recognition of BSG orthologues from human, chimpanzee and gorilla, by *P. falciparum* RH5 and identified residues that confer this specificity by generating and testing site-directed mutants of the BSG orthologues.

Although multi-pass receptors are fairly abundant on the erythrocyte surface, no such receptor has yet been conclusively identified to play a role in erythrocyte invasion by *P. falciparum*. To potentially identify such a novel interaction, I screened a library of mammalian-expressed *P. falciparum* proteins against a panel of recombinant erythrocyte multi-pass receptors, in my third project. I also tested the library of merozoite proteins for binding to human erythrocytes and to a large panel of synthetic carbohydrates. A number of putative interactions were identified in the screens and are awaiting characterisation.

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