

Proteomics in malaria research

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Genome sequence and proteome analysis of many parasitic organisms have provided new hope for the identification of new vaccine/drug targets and their corresponding inhibitors or drugs. Among these, the most significant progress has been made with the malaria parasite.

Malaria is the most prevalent tropical parasitic disease killing at least a million people annually. Genome sequences of different species of *Plasmodium* and its insect as well as vertebrate hosts have propelled the growth of integrated omics approaches in different spheres of malaria research, including understanding of the host-pathogen interactions, disease etiology and pathogenic mechanism, characterisation of stage-specific parasite proteome as well as post-translational modifications and elucidation of mechanisms of antimalarial drug action.

Proteomics is an effective tool for the identification of next-generation biomarkers and potential drug/vaccine targets. Nonetheless, this emerging field is also fraught with various challenges, such as *in vivo* proteomic profiling of human malaria parasites, expression and purification of proteins in large quantities, difficulties in targeting low-abundance target analytes within complex biological samples, and analysis and interpretation of huge multi-omics datasets.

Research leads

Plasmodium genomes encode about 5,300 proteins, more than half of which are hypothetical proteins since they do not show sufficient similarity with proteins from other organisms¹. In the last decade, a series of proteomics studies have tried to understand the expression of *Plasmodium* proteins at different parasite stages^{2,3} and also illustrated post-translational modifications that many of these proteins undergo^{4,5,6,7,8}.

These modifications have been crucial for protein functions such as haemoglobin degradation, host invasion and merozoite egress⁹. Proteome analyses have further led to the identification of a library of cell surface and secreted proteins that probably are responsible for host cell invasion and immune modulations¹⁰, organelle specific proteins and drug sensitive proteins¹¹. A

significant number of proteomics studies have also been performed to understand the development, pathogenesis and drug resistance in apicomplexan parasites¹²⁻¹⁹. Additionally, chemical proteomics approaches have provided new chemistries to develop new anti-malarials²⁰. A number of novel *Plasmodium* secretory proteins at asexual blood stages have been identified alongside a haemoglobin degradation-hemozoin formation complex^{21,22}.

Proteome analysis studies have led to the identification of potential new targets such as haemoglobin degradation enzymes²², enzymes/proteins of purine salvage pathway and of protein and polyamine metabolism²³, proteins associated with parasite specific trafficking/transport pathways^{24,25}, GPI anchored proteins²⁶, proteins associated with proteasome machinery and proteins linked with spread of drug resistance²⁷. These global proteomic studies have provided researchers enough arsenal to develop novel anti-parasitic strategies both for new drugs and vaccine development.

Signaling studies

Researchers have also noted the presence of several putative effectors of cell signaling in the parasite genome²⁸.

The post-genome era saw a flurry of activity in the use of reverse genetics to understand the function of enzymes like protein kinases and phosphatases^{4,29,30,31} – major modulators of protein phosphorylation. While these and other efforts revealed that signaling may regulate most stages of parasite development, the underlying mechanisms remained unclear and the signaling map of the parasite remains ambiguous.

Efforts to understand the role of second messengers like calcium and phosphoinositides (PIPs) in the parasite have resulted in explaining novel signaling and trafficking pathways. For instance, researchers have shown that phospholipase C-mediated calcium release may regulate protein kinases like PfCDPKs and PfPKB, which in turn may regulate key processes like host erythrocyte invasion and sexual differentiation³².

Using mass spectrometry, several regulatory phosphorylation sites on PfCDPK1³³ and its substrates like PfGAP45³⁴ have been identified. Indian teams have also identified several novel substrates for *Plasmodium* kinases. Some of these substrates have been validated by performing *in vitro* kinase assays with recombinant substrate proteins and LC-MS/MS analysis.

A combination of traditional approaches with the omics approach will help understand the mechanism through which signaling pathways regulate the development of malaria parasite.

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Identifying diagnostic and prognostic markers

Most of the attention on infectious diseases of the developing world has focused on the development of rapid diagnostic tests and novel therapeutics to ensure timely treatment and improved survival rates. Existing diagnostic tests are either too expensive or time consuming or difficult to implement in developing countries due to the lack of resources and expertise. The inability to predict disease severity is also a major challenge to effective clinical management and prevention of long-term malaria complications. These limitations have spurred the search for better diagnostic and prognostic markers in malaria that can be easily measured in body fluids.

For over a decade now, several attempts to discover novel biomarkers in human bio-fluids such as serum, plasma and urine have been made by various research groups. Such studies have involved the use of proteomic technologies to profile host responses to infectious diseases^{35, 36, 37, 38, 39}. The high-throughput proteomic technology platforms not only investigate the systemic alterations of protein expression in response to diseases but also enable visualisation of the underlying interconnecting protein networks and signaling pathways, facilitating the discovery of unique markers of infection⁴⁰. Proteomic technologies have also been used to discover biomarkers that demonstrate the presence of the infecting organisms⁴¹. One of the first attempts to unravel the proteome of the malaria parasite, *Plasmodium vivax* from clinical samples provided new leads towards

the identification of diagnostic markers, novel therapeutic targets and an enhanced understanding of malaria pathogenesis⁴².

Efforts to decipher host responses to malaria infection have revealed a panel of proteins with a distinct pattern of differential abundance that can discriminate malaria patients from healthy subjects and patients with other infectious diseases⁴³.

Similarly, analysis of serum proteome of dengue and leptospirosis patients has led to the identification of unique protein signatures and molecular targets^{44, 45, 46}. A comparative serum proteomic analysis of severe and non-severe malaria in search of prognostic markers using quantitative proteomics has highlighted the presence of muscular, cytoskeletal and anti-oxidant proteins in patient sera revealing extensive oxidative stress and cellular damage in severe malaria. These findings are currently being validated in a larger cohort of patients using immunoassays.

The application of proteomic technologies has shown promising leads. However, early disease detection, measurement of therapeutic efficacy, prediction of disease severity and tailored patient therapy are still some distance away.

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