Published in final edited form as:

Curr Bioinform. 2012 December 1; 7(4): . doi:10.2174/157489312803900965.

Comparative Genomics and Systems Biology of Malaria Parasites *Plasmodium*

Hong Cai^{1,#}, Zhan Zhou^{1,2,#}, Jianying Gu^{3,*}, and Yufeng Wang^{1,4,*}

¹Department of Biology, University of Texas at San Antonio, San Antonio, TX 78249, USA

²College of Life Sciences, Zhejiang University, Hangzhou 310058, P.R. China

³Department of Biology, College of Staten Island, City University of New York, Staten Island, NY 10314, USA

⁴South Texas Center for Emerging Infectious Diseases, University of Texas at San Antonio, San Antonio, TX 78249, USA

Abstract

Malaria is a serious infectious disease that causes over one million deaths yearly. It is caused by a group of protozoan parasites in the genus *Plasmodium*. No effective vaccine is currently available and the elevated levels of resistance to drugs in use underscore the pressing need for novel antimalarial targets. In this review, we survey omics centered developments in *Plasmodium* biology, which have set the stage for a quantum leap in our understanding of the fundamental processes of the parasite life cycle and mechanisms of drug resistance and immune evasion.

Keywords

Plasmodium; malaria; genomics; systems biology; transcriptomics; proteomics; interactome; drug; vaccine

Introduction

Malaria is a serious global infectious disease, responsible for over one million deaths yearly. It is caused by the protozoan parasites in the *Plasmodium* genus. Five species, *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* are human pathogens. The most severe form of malaria is caused by *P. falciparum*.

The life cycle of malaria parasites is complex, including multiple stages spanning the mosquito vector *Anopheles* and the human host. Infection begins when an infected female mosquito bites a host, injecting the parasites in the form of sporozoites into the bloodstream. The sporozoites enter the human liver in which they undergo asexual multiplication without any clinical symptoms. At the end of the liver stage, the parasites are released in the form of merozoites, which then invade red blood cells (RBCs, erythrocytes). During the RBC cycle, the parasites develop from small rings to metabolically active trophozoites and mature into schizonts, which later rupture and release merozoites. The RBC stage is responsible for the pathology associated with malaria, including recurring fever due to the lysis of the infected

* Address correspondence to these authors: Yufeng Wang at the University of Texas at San Antonio, Department of Biology, One UTSA Circle, San Antonio, TX 78249, USA; Tel: (210) 458-6492; Fax: (210) 458-5658; yufeng.wang@utsa.edu; Jianying Gu, jianying.gu@csi.cuny.edu.

#equally contributed

erythrocytes. Some merozoites develop into sexually differentiated gametocytes, which can develop into mature gametes when ingested by a mosquito. The gametes are fertilized to form zygotes which develop into ookinetes that penetrate the mosquito's midgut wall and form oocysts on the exterior surface. Inside the oocyst, thousands of active sporozoites develop and migrate to the mosquito's salivary glands, completing the life cycle.

Malaria is an old disease and it is striking back in recent years as parasites have developed increasing resistance to available antimalarial drugs. The completion of genome sequencing of various *Plasmodium* strains and the rapid development of transcriptomic, proteomic, metabolomic, and interactomic technologies have brought new hope to the development of more effective preventive and therapeutic strategies. This review is focused on recent advances in comparative genomics and systems biology of malaria parasites, which promise to make profound contributions to our understanding of pathogenesis, drug resistance, parasite biology and evolution.

Genomics of *Plasmodium*

The genome of *P. falciparum*, the most deadly human parasite, was completed and published in 2002 [1]. The partial sequence and contig information of *P. yoelii yoelii*, a rodent malaria parasite, were published at the same time [2]. Genome sequence data from nine *Plasmodium* strains are in various stages of completion and are available to the research community at PlasmoDB [3], a comprehensive genomic resource for malaria parasite research [1, 2, 4–6] (Table 1). With the advent of next-generation sequencing technology, the number of completed and ongoing sequencing projects is rapidly increasing. According to an updated version of the comprehensive sequencing proposal for *Plasmodium* (<http://www.genome.gov/Pages/Research/DER/PathogensandVectors/PlasmodiumWhitePaperV8.pdf>), as of March 21, 2011, US National Institute of Allergy and Infectious Diseases (NIAID) has approved the sequencing of 105 malaria parasite strains/isolates from a wide variety of geographical regions, including 75 *P. falciparum* isolates, nine isolates of *P. vivax*, the second most important human malaria parasite, representative simian malaria parasites (*P. reichenowi*, *P. cynomolgi*, *P. inui*, *P. coatneyi*, *P. fragile*), rodent parasites (*P. chabaudi*, *P. yoelii*, and *P. berghei*), avian parasite *P. relictum*, and a reptile parasite *P. mexicanum*.

The haploid *Plasmodium* genome is organized into 14 linear chromosomes, encoding about 5100–5800 open reading frames (ORFs) (Table 1). The genome size typically ranges from 16–26 Mb. The (A+T) content varies significantly among *Plasmodium* species: on average, 80.4% of the *P. falciparum* genome consists of A or T, and this proportion can be as high as 90% in introns and intergenic regions, while the *P. vivax* genome contains only 57.5% (A+T). The A+T richness presents multiple challenges in functional genomics of *P. falciparum*: low-complexity regions (LCRs) are dispersed all over the genome, which hampers accurate genome annotation, and over 60% of the ORFs were annotated as hypothetical proteins due to the lack of statistically significant sequence similarity to any known or characterized proteins; strong AT bias makes cloning in heterogeneous vector systems such as *E. coli* and yeast difficult [7]; it is difficult to design standard probes for quantifying mRNA expressions [8]; the vector for high throughput yeast two-hybrid (Y2H) assays for screening protein-protein interactions had to be modified to achieve a low false-positive detection rate [9]. Nevertheless, deciphering the parasite genomes has provided invaluable insights into various aspects of parasite biology, related to genetic diversity, parasite transmission, invasion, growth, development, response to drug administration and pathogenesis.

The core genome and lineage-specific features of the malaria parasites

Malaria parasites are rapidly evolving pathogens under constant endogenous and exogenous selection pressure from the host, the vector, and the environment. Phylogenetic analysis based on mitochondrial genome sequences reveals strong correlation between the parasites and their host ranges, suggesting a pathogen-host coevolution scenario (Fig. 1A) [10]. The avian and reptile parasites show close kinship, and rodent parasites are clustered into one single group. The human and nonhuman primate malaria parasites form two clades: *P. falciparum* and the chimpanzee parasite *P. reichenowi* are sister taxa, while divergent from other primate parasites. The origin of *P. falciparum* remains controversial: historically, it was thought to be derived from a bird/reptile parasite [11] and a big-bang mode of radiation; this hypothesis has recently been challenged by several independent lines of evidence showing that host switches occurred in multiple human and nonhuman primate parasites: *P. knowlesi*, whose natural host was thought to be macaques, indeed is a widely distributed human parasite [12], and similarly African apes have been shown to be natural hosts for *P. falciparum* [13–18]. Combining single genome amplification polymerase chain reaction (PCR) technique and sequence analyses of mitochondrial, apicoplast, and nuclear genes, Liu *et al.* (2010) suggested that *P. falciparum* originated from a single gorilla-to-human transmission [19]. Prugnolle *et al.* in a series of studies showed that *P. falciparum* can circulate in great apes and monkeys, and is not confined to gorillas [17, 18, 20]. Similarly, recent host transfers seem to have occurred among other human malaria parasites including *P. vivax* and *P. malariae* and monkey parasites *P. simium* and *P. brasilianum*. The host switching phenomena have been well defined and studied in viruses such as influenza A [21, 22]. The possibility of malaria parasites switching hosts could have profound impacts on the parasite evolution as new strains might be derived from host shift, representing new risks for human public health.

Consistent with the patterns revealed by phylogenetic analysis, genome alignment shows that the five genomes with completed sequences belong to three distinct groups (Fig. 1B).: Two rodent parasites, *P. chabaudi chabaudi* and *P. berghei*, are closely related to each other, with an average of 87% of nucleotide sequence similarity [5] and conserved chromosome synteny. *P. vivax* and *P. knowlesi* share conserved chromosome organization, while *P. falciparum* seems to have undergone significant chromosomal rearrangements.

Despite the diverse physiological and ecological niches of the malaria parasites, their genomes share common conserved features. Comparative genomic analysis of six genomes with complete annotations (*P. falciparum*, *P. vivax*, *P. chabaudi chabaudi*, *P. berghei*, *P. yoelii yoelii* and *P. knowlesi*) revealed that the core genome is comprised of 3,351 orthologous genes, accounting for 27–65% of the genomes [23]. In addition to the genes required for fundamental biology, the core genome contains components important for parasite-specific lifestyles. These include: (1) 15 orthologous clusters including various kinases and transcription factors involved in the cell cycle network; (2) At least one cluster that may participate in a G-protein coupled receptor (GPCR) signaling pathway. It was shown that signaling via human GPCR regulates parasite entry to the RBC and parasite infection is blocked by a GPCR inhibitor [24], suggesting that GPCR agonists can be potential drug targets; (3) 23 clusters related to parasite stress response; (4) six clusters relevant to pathogenesis or virulence, including the clusters encoding merozoite surface proteins (MSPs) [25], transmission-blocking target antigens [26], chloroquine resistance transporters and multidrug resistance proteins. These core components represent the minimum requirement for a successful life cycle that spans vertebrate hosts and mosquito vectors. Much of our current knowledge in parasite biology has been derived from studies conducted in non-human parasites, due to the restricted host specificity of human parasites. There are multiple advantages to using non-human parasites: the liver and mosquito stages

of the human parasites are difficult to study, while large scale *in vitro* cultivation and production of these stages for rodent parasites are well developed; it is feasible to study parasite-host interactions *in vivo* with rodent parasites as well as *in vivo* drug testing; gene knockouts in rodent hosts are useful to study protective immunity or immunopathology. Comparative genomic analysis confirms the conservation of the housekeeping genes, drug resistance genes, and surface antigens between the rodent and human parasites, suggesting that reasonable extrapolations from animal models can provide invaluable insights into the mechanisms of human malaria.

Comparative genomic analysis has also identified abundant lineage-specific expansions (LSEs), constituting about 5–9% of the genomes. The majority of the LSE families are hypothetical proteins with unknown functions. For example, 77% of the gene families that are expanded uniquely in *P. berghei* were hypothetical or putative proteins. Some of the LSEs encode surface antigens. In *P. falciparum*, the largest cluster is the RIFIN family with 165 paralogous members. Multiple gene duplications, followed by neo-functionalization and subfunctionalization, may give rise to the current distribution of the RIFIN family [27]; the antigenic variation observed in this enigmatic family suggests that it plays a role in cytoadherence and can serve as a vaccine target. The second largest LSE in *P. falciparum*, erythrocyte membrane protein 1 (PfEMP1), is also notorious for antigenic variation [28, 29]. Although antigenic variation is common in *Plasmodium* species as a mechanism for parasite evasion of the host immune response, different parasites may evolve lineage-specific surface antigens with tissue-specific activities. Vir genes, coding for variant antigens exposed on *P. vivax*-infected reticulocytes, are associated with spleen-specific cytoadherence in chronic infections [30, 31]. SICAVar-like antigens, the simian specific surface antigens, are present in *P. knowlesi*, and are implicated in parasite virulence [6].

Comparative genomics: searching for new drug and vaccine targets

Comparative genomics, in addition to its contribution to a better understanding of parasite evolution, offers a cost effective means to search for potential antimalarial targets *in silico*. Comparative analyses of evolutionarily related genes, gene products, structures, pathways, and networks have already lead to the discovery of potential drug targets, including (1) multiple enzymes such as 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase [32, 33], and apicoplast gyrase [34] that are essential for the replication, transcription, translation, and metabolism of the apicoplast. The apicoplast is an organelle that is required for parasite life cycle, as parasites are not viable when the metabolism and replication of apicoplast is inhibited [35, 36]. The proteins targeted to the apicoplast are considered to be attractive targets because this organelle is of prokaryotic origin and is distantly related to the human host; (2) kinases such as cyclin-dependent protein kinases (Pfmrk) [37], which may play an important role in DNA replication, transcription, and cell cycle regulation, and the plant-like calcium-dependent protein kinase (PfCDPK5) [38], which regulate the parasite exit from the RBCs; (3) transporters, which have demonstrated importance in drug resistance and nutrient acquisition from the host [39–44].

Data mining approaches combining traditional sequence similarity search, structural genomics, and machine learning techniques also identified a catalog of proteases that play versatile roles in parasite biology [45–47], including a series of aspartic proteases (plasmepsins) [48, 49], papain-type cysteine proteases (falcipains) [50, 51], and metallo aminopeptidases [52–54] that catalyze a cascade of reactions to digest host hemoglobins to liberate free amino acids for parasite nutrition, metacaspases that are potential mediators for programmed cell death-like pathways [55–57], a calpain that is crucial for parasite optimal growth and cell cycle progression [58], serine proteases (subtilases) that are essential for parasite invasion as well as egress from the erythrocytes [59–61], and several families of

proteases involved in heat shock response and stress response [62, 63]. In addition, three classes of proteases have been discovered in *P. falciparum* that mediate regulated intramembrane proteolysis (RIP), including an aspartic signal peptide peptidase (PfAPP) in the A22 presenilin family, two Site-2 metallo proteases (S2Ps) in the M50 family [64], and eight rhomboid serine proteases (PfROMs) in the S54 family [65–71]. RIP is a novel signaling mechanism, in which proteases process the transmembrane segments of the substrates, releasing cytosolic fragments that control the transcription of their effector proteins, thereby regulating cellular processes such as differentiation, metabolism, and protein turnovers [72]. Proteases are proven central players in RIP in bacterial, fungal, viral, and protozoan pathogens, and are emerging classes of therapeutic targets [73]. In *P. falciparum*, PfAPP has been implicated in merozoite invasion and parasite growth [69, 70]. Various PfROMs showed differential cleavage preference to an array of adhesins including the apical membrane antigen 1 (AMA1), the erythrocyte binding-like (EBL) family, the reticulocyte binding-like (RBL) family, and the thrombospondin-related anonymous protein (TRAP) family, suggesting that the parasite rhomboid proteases are involved in invasion [65]. The potentials of malarial proteases as drug targets have been evaluated via structural modeling, rational inhibitor design, and combinational compound screening [53, 74–81]. Many of these drug targets have no human homologs or have only a distant evolutionary relatedness to their counterparts in the humans; the drugs targeting to these candidate proteins therefore may have no or little side effects on the human system.

The search for potential drug targets in the superfamily of transcription factors, however, has been hindered by the remote homology between the transcriptional machinery in *P. falciparum* and that in other well-studied model organisms [82]. Combining hidden Markov model (HMM)-based domain search, secondary structural prediction, 3D structural homology modeling, phylogenetic analysis, and microarray transcriptomic data mining, Balaji *et al.* [83] discovered a novel family of transcriptional regulators, namely apicomplexan Ap2 (ApiAP2) family in *P. falciparum* and two other related apicomplexan parasites. This is the first and sole non-basal transcription factor (TF) family that has been identified in *Plasmodium*. ApiAP2 proteins contain the Apetala2 (AP2) domains that are commonly presented in plants, which are the key regulators for the ABC model of flower development [84]. It was postulated that the ApiAP2 proteins were acquired from the apicoplast progenitor or bacteria via transposition, followed by lineage-specific expansion [83]. Malaria parasites have 26–28 ApiAP2 members, with similar conserved domain architecture across different *Plasmodium* species. Unlike the plant ApiAP2 family which typically has one or two AP2 domains, four ApiAP2 proteins (PFF0670w, PF10_0075, PF11_0404, and PF13_0235) in *P. falciparum* have three AP2 domains. The roles of ApiAP2 proteins in parasite developmental control have been unraveled by functional genomic and transcriptomic analyses and gene knockout assays (See Painter *et al.* [85] for an excellent review). They have been implicated in transcriptional regulation throughout the life cycle, influencing parasite invasion, gametogenesis, oocyst development, sporozoite development, and gene silencing [86–91], opening a new line of targets for functional characterization.

Comparative genomics has been invaluable to malaria vaccine development in pinpointing potential antigens. Prior to the completion of genome sequencing, only about 30 antigens were being used as vaccine targets. Genome annotation predicted three large supergene families *var*, *rif*, and *stevor*, which code for PfEMP1, repetitive interspersed family (RIFIN) and sub-telomeric variable open reading frame (STEVAR), and a plethora of potential antigens that may contribute to parasite immune evasion [1]. For example, a series of transmission blocking vaccine (TBV) candidates are currently at various stage of experimental assessment and some of them are under pre-clinical trials, including Pfs25/Pvs25 [92], Pfs28/Pvs28 [93], Pfs48/45 [94], and Pfs230 [95, 96]. The TBVs target the

sexual or mosquito stage of the parasite and prevent the parasite transmission. New computational methods and wet-lab genome-wide screening have been used to identify novel vaccine candidates with distinct structure motifs [97–101]. Although high level of polymorphism and genetic diversity remain a major hurdle for malaria vaccine development [102], the availability of genome information provides a foundation for genome-wide scan and evaluation of vaccine targets.

The post-genomic era

The malaria research community is rapidly advancing upon the post-genomic era. High throughput technologies such as microarray DNA chip, mass-spectrometry, and yeast two-hybrid (Y2H) screening have enabled the rapid and large-scale characterization and profiling of the expression, regulation and interaction of the genes at the level of the transcriptome, proteome, metabolome, and interactome [103].

The application of microarray chips dates back to the beginning of millennium, even before the completion of the genome sequencing of *P. falciparum*. The first generation of microarray chips include shotgun genomic microarrays [104] and stage-specific cDNA microarrays [105]. Oligonucleotide arrays were custom designed and fabricated for *P. falciparum*, including an ORF-based array of long 70-mer oligonucleotides [106, 107] and short 25-mer oligonucleotide arrays [108, 109]. A groundbreaking work by Bozdech *et al.* [106] revealed, for the first time, that the transcription of genes in the blood stage in malaria parasite uses a “just-in-time” mechanism, i.e., genes are expressed only when they are required. At different stages in the RBC cycle, specific sets of genes are orchestrated to fulfill special needs for parasite development and infection. For example, the ring and early-trophozoite stage is characterized by the induction of genes important for transcription, translation, and fundamental metabolism such as glycolysis, while the schizont stage is characterized by the expression of genes associated with merozoite invasion and immune evasion. This study has also made an enormous impact on the bioinformatics community as it was used as a contest dataset in the 2004 Critical Assessment of Microarray Data Analysis (CAMDA) conference, which boosted the development of novel computational algorithms for microarray analysis. Many of these methods have been extended to systems-wide studies of other organisms or cellular systems. Another microarray study [109] that has significant impacts on malaria research focused on the temporal profiling of the entire life cycle of the parasite, spanning the blood stage, the gametocyte and the sporozoite stages. Many genes that were co-expressed seemed to be associated with correlated cellular processes such as cell cycle regulation, transport and trafficking, defense and virulence, cell-cell communication and signal transduction, suggesting the parasite uses a very efficient cellular machinery [106, 109]. The microarray chips for other *Plasmodium* species/strains, including *P. berghei* [5], *P. yoelii* [110, 111], *P. vivax* [112] have also been developed. Transcriptomic profiling is becoming one of the most powerful means of elucidating complex mechanisms that were previously impossible or difficult to study [113], including the mechanisms of cell cycle regulation [114], transcriptional control under various conditions [5, 115–120], gene silencing [121], genetic diversity, drug action and drug resistance [122–133], parasite invasion [134], pathogenesis and virulence [135–142], vector-parasite and host-parasite interactions [140, 143, 144], and sexual development [145]. The results have lead to the identification of novel drug and vaccine targets [47, 146–151]. ChIP (chromatin immunoprecipitation)-on-chip technology has been used to identify and characterize the cistrome, the whole complement of DNA-binding sites for target proteins, and to study the mechanisms of epigenetic modeling and programming [116, 119, 152–155]. RNA-Seq, which applies next-generation sequencing (NGS) technologies to the high-resolution sequencing of expressed RNA transcripts, has been successfully used in the human [156, 157], yeast [158, 159], and mouse systems [160]. The first RNA-Seq analysis in *P.*

falciparum was published in 2010. It made corrections to existing gene models, predicted rare temporal-specific alternative splicing events, identified novel transcripts and low abundance transcripts, and provided a blueprint of the transcriptomes at seven time points during the blood stage [89]. Although still in its infancy, RNA-Seq offers promises to achieve a fast, accurate, and comprehensive view of the parasite transcriptional and post-transcriptional regulation. Recently, Vignali *et al.* [161] design not-so-random (NSR) primers using a novel next-generation sequencing (NGS) technology, namely NSR-Seq [162], to profile the nonribosomal RNA transcripts in *P. falciparum*; they reported a signature profile of genes for parasites infecting children, demonstrating the value of NSR-Seq for the characterization of clinical samples.

Complementary to transcriptomic analysis, proteomics enables researchers to study the expression, function, and structure of proteins at a large scale. Typically, proteomic studies involve three steps: (1) sample isolation; (2) protein separation, for example, by two-dimensional (2-D) gel electrophoresis; (3) quantitative identification and analysis of the individual proteins by mass spectrometry or N-terminal sequencing. Systems-wide analyses of proteomes are possible due to the advent of high throughput technologies such as matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrometry, capillary liquid chromatography, followed by tandem mass spectrometry (LC-MS/MS), and Multidimensional Protein Identification Technology (MudPIT) which couples liquid chromatography and tandem mass spectrometry. The proteomes of the *P. falciparum* asexual trophozoites and schizonts, sexual stage gametocytes, and gametes were revealed by a high accuracy LC-MS/MS analysis [163], and the proteomes of the sporozoite, merozoite, trophozoite and gametocyte stages were elucidated using MudPIT [164]. Potential drug targets, such as proteases and transporters, as well as vaccine targets were found to be expressed at specific stages and subcellular locations in multiple *Plasmodium* strains [46, 47, 110, 165–169]. MudPIT also enabled the identification of surface antigens and epitopes expressed in the sporozoite and intrahepatic stages [170] and proteins on the surface of parasite-infected erythrocytes (PIESPs), which may play a role in pathogenesis and immunity [171]. LC-MS/MS identified several PfEMP1 variants in membrane protein-enriched *P. falciparum*-infected erythrocytes in laboratory isolates, field isolates and fresh placental parasite preparations, suggesting PfEMP1 may be associated with parasite adhesion in placental malaria [172]. It also identified glycosylphosphatidylinositol (GPI) - anchored membrane proteins which play diverse roles in parasite growth and invasion [173]. A quantitative proteomic method based on SILAC (stable isotope labeling by amino acids in cell culture) technology was developed to monitor protein expression and post-translationally modified variants in the blood stage and in the parasite responses to antimalarial drugs [174]. Proteomics has shed light on the mechanisms of parasite invasion and pathogenesis: MudPIT identified proteins that are localized to rhoptries, specialized secretory organelles, which are uniquely present within *Apicomplexa* parasites including *Plasmodium*, and are important for rupture, invasion, and RBC remodeling [175]. This technology also identified a group of membrane proteins in the Maurer's clefts (MCs), parasite-specific structures that are associated with parasite transport and secretion pathways [176]. Comparative LC-MS/MS proteomics between *P. falciparum* and *P. berghei* identified previously uncharacterized proteins that are essential for parasite growth and maturation in the mosquitoes [177]. The proteome of micronemes, *Apicomplexan*-specific secretory organelles that are important for gliding motility and host cell invasion, purified from the cultured ookinete, was recently published [178], revealing novel proteins that are related to vesicle trafficking and signaling functions for effective invasion. Proteomics offers a systematic way to characterize drug actions and drug resistance: 2-D gel electrophoresis and MALDI-TOF MS revealed the complex pharmacological effects of an antimalarial CoArtem, artemether and lumefantrine [179]; MudPIT analysis revealed expression changes in the trophozoites under chloroquine and artemisinin pressure [180]. Surface-enhanced

laser desorption/ionization (SELDI)- TOF-MS was developed to compare the global protein expression profiles between drug resistant and sensitive parasite strains, leading to the identification of molecular markers for drug susceptibility and action [181].

Metabolomics in malaria biology is aimed at the elucidation of the metabolism of *Plasmodium*. The approaches range from *in silico* inferences, as seen in PlasmoCyc, a pathway genome database (PGDB) for *P. falciparum* [182], the Malaria Parasite Metabolic Pathways (MPMP) database [183], and flux-balance analysis (FBA) [184], to wet-lab extraction and separation of metabolites and metabolic profiling using nuclear magnetic resonance (NMR) or MS (see [185] for a comprehensive review). The networks for lipid, polyamine, glycerol and carbon metabolism have been unveiled and novel enzymatic drug targets identified, enhancing our understanding of the biochemical environments in the host and vector, and of host-parasite/vector-parasite interactions [36, 186–189].

Interactomics provides a network view of the protein-protein interactions (PPI) and other interactions within a cell. Yeast two-hybrid (Y2H) screening identified 2,846 unique PPIs in *P. falciparum*, that may play roles in transcriptional regulation, chromatin modification, mRNA stability, parasite invasion [9]. Subsequent analysis [190] identified almost 3,000 interactions among about 1,300 (mostly ‘hypothetical’) proteins. They fell into 29 complexes, but the relatively small numbers of proteins with informative functional annotations impaired a detailed analysis of the interaction networks. Alternatively, the *P. falciparum* interactome was inferred by integrating functional genomic data in a Bayesian statistical framework [191]. Interactomic studies, which actually integrate the individual components of the malaria parasite into an overview of whole-organism interactions, represent a first step into the nascent field of systems biology.

Toward a systems level understanding of parasite biology

A growing body of evidence suggests that adaptive properties of malaria parasites, such as stress response, drug resistance, host entry, and pathogenesis, are complex traits involving associations among proteins, metabolites, DNAs, RNAs and other molecules in the cell. Systems biology approaches to the study of the malaria parasite began to appear shortly after the completion of the genome sequencing. Many of the studies cited above can be viewed as systems biology approaches in that a common goal is the synthesis of a high-level view of the malaria parasite. Isokpehi and Hide [151] used integrative data analysis to identify a set of proteins that showed stage-specific expression and discussed the possible importance of the gene products in parasite biology and as potential drug targets. Yeh *et al.* [182] searched for what they called “chokepoint” enzymes in *P. falciparum* by analyzing the interactions among proteins that had been mapped into metabolic pathways using the Pathway Tools software. Ralph *et al.* [36] used a comparative genomics approach to identify apicoplast-targeted proteins and were able to reconstruct apicoplast metabolic networks. Other studies, like the Y2H systems [9, 190], transcriptomic analysis ([88], for example) and computational deduction of the interactome [191] all attempt to place our knowledge of the gene (or protein) complement and its expression into a larger unified framework. These studies are significant milestones on the road to a true systems view of the parasite. Clearly, the field of systems biology is forging ahead, but just as clearly, computational methods for integrating data and inferring networks are needed [192–196]. It is worth emphasizing that the insights provided by omics approaches into the dynamics of biological networks will shed light on as yet unrecognized network interactions, thus providing a form of annotation feedback to the determination of system structure. A systems understanding of the *Plasmodium* parasites will result in a major step forward in the battle against malaria [197, 198].

Acknowledgments

This work is supported by NIH grants GM081068, SC1AI080579, and AI067543 to YW, and the PSC-CUNY Research Award PSCREG-39-497 to JG. YW is also supported by NIH grant RR013646. ZZ is supported by the government scholarship from China Scholarship Council. We thank the Computational Biology Initiative at UTSA for providing computational support. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences, National Institute of Allergy and Infectious Diseases, or the National Institutes of Health.

List of abbreviations

2-D	two-dimensional
AMA1	apical membrane antigen 1
CAMDA	Critical Assessment of Microarray Data Analysis
CDPK	calcium-dependent protein kinase
ChIP	chromatin immunoprecipitation
DOXP	1-deoxy-D-xylulose 5-phosphate
EBL	erythrocyte binding-like
FBA	flux-balance analysis
GPCR	G-protein coupled receptor
GPI	glycosylphosphatidylinositol
LCR	low-complexity region
LSE	lineage-specific expansion
LC-MS/MS	capillary liquid chromatography, followed by tandem mass spectrometry
MALDI-TOF	matrix-assisted laser desorption ionization-time-of-flight
MC	Maurer's cleft
MS	mass spectrometry
MPMP	Malaria Parasite Metabolic Pathways
MSP	merozoite surface protein
MudPIT	Multidimensional Protein Identification Technology
NGS	next-generation sequencing
NIAID	National Institute of Allergy and Infectious Diseases
NMR	Nuclear magnetic resonance
NSR	not-so-random
ORF	open reading frame
PCR	polymerase chain reaction
PfEMP1	<i>P. falciparum</i> erythrocyte membrane protein 1
PGDB	pathway genome database
PPI	protein-protein interaction
RBC	red blood cell
RBL	reticulocyte binding-like

Rifin	repetitive interspersed family
RIP	regulated intramembrane proteolysis
SELDI	Surface-enhanced laser desorption/ionization
SILAC	stable isotope labeling by amino acids in cell culture
Stevor	sub-telomeric variable open reading frame
TBV	transmission blocking vaccine
TRAP	thrombospondin-related anonymous protein
Y2H	yeast two-hybrid

References

1. Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, et al. Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature*. 2002; 419 (6906):498–511. [PubMed: 12368864]
2. Carlton JM, Angiuoli SV, Suh BB, Kooij TW, Pertea M, Silva JC, et al. Genome sequence and comparative analysis of the model rodent malaria parasite *Plasmodium yoelii yoelii*. *Nature*. 2002; 419 (6906):512–9. [PubMed: 12368865]
3. Aurrecoechea C, Brestelli J, Brunk BP, Dommer J, Fischer S, Gajria B, et al. PlasmoDB: a functional genomic database for malaria parasites. *Nucleic Acids Res*. 2009; 37 (Database issue):D539–43. [PubMed: 18957442]
4. Carlton JM, Adams JH, Silva JC, Bidwell SL, Lorenzi H, Caler E, et al. Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. *Nature*. 2008; 455 (7214):757–63. [PubMed: 18843361]
5. Hall N, Karras M, Raine JD, Carlton JM, Kooij TW, Berriman M, et al. A comprehensive survey of the *Plasmodium* life cycle by genomic, transcriptomic, and proteomic analyses. *Science*. 2005; 307 (5706):82–6. [PubMed: 15637271]
6. Pain A, Bohme U, Berry AE, Mungall K, Finn RD, Jackson AP, et al. The genome of the simian and human malaria parasite *Plasmodium knowlesi*. *Nature*. 2008; 455 (7214):799–803. [PubMed: 18843368]
7. LaCount DJ, Schoenfeld LW, Fields S. Selection of yeast strains with enhanced expression of *Plasmodium falciparum* proteins. *Mol Biochem Parasitol*. 2009; 163 (2):119–22. [PubMed: 19026694]
8. Bustamante LY, Crooke A, Martinez J, Diez A, Bautista JM. Dual-function stem molecular beacons to assess mRNA expression in AT-rich transcripts of *Plasmodium falciparum*. *Biotechniques*. 2004; 36(3):488–92. 94. [PubMed: 15038165]
9. LaCount DJ, Vignali M, Chettier R, Phansalkar A, Bell R, Hesselberth JR, et al. A protein interaction network of the malaria parasite *Plasmodium falciparum*. *Nature*. 2005; 438 (7064):103–7. [PubMed: 16267556]
10. Hayakawa T, Culleton R, Otani H, Horii T, Tanabe K. Big bang in the evolution of extant malaria parasites. *Mol Biol Evol*. 2008; 25 (10):2233–9. [PubMed: 18687771]
11. Rathore D, Wahl AM, Sullivan M, McCutchan TF. A phylogenetic comparison of gene trees constructed from plastid, mitochondrial and genomic DNA of *Plasmodium* species. *Mol Biochem Parasitol*. 2001; 114 (1):89–94. [PubMed: 11356517]
12. Cox-Singh J, Davis TM, Lee KS, Shamsul SS, Matusop A, Ratnam S, et al. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clin Infect Dis*. 2008; 46 (2):165–71. [PubMed: 18171245]
13. Duval L, Fourment M, Nerrienet E, Rousset D, Sadeuh SA, Goodman SM, et al. African apes as reservoirs of *Plasmodium falciparum* and the origin and diversification of the *Laverania* subgenus. *Proc Natl Acad Sci U S A*. 2010; 107 (23):10561–6. [PubMed: 20498054]

14. Holmes EC. Malaria: The gorilla connection. *Nature*. 2010; 467 (7314):404–5. [PubMed: 20864986]
15. Krief S, Escalante AA, Pacheco MA, Mugisha L, Andre C, Halbwax M, et al. On the diversity of malaria parasites in African apes and the origin of *Plasmodium falciparum* from Bonobos. *PLoS Pathog*. 2010; 6(2):e1000765, 1–12. [PubMed: 20169187]
16. Ollomo B, Durand P, Prugnolle F, Douzery E, Arnathau C, Nkoghe D, et al. A new malaria agent in African hominids. *PLoS Pathog*. 2009; 5(5):e1000446, 1–5. [PubMed: 19478877]
17. Prugnolle F, Durand P, Neel C, Ollomo B, Ayala FJ, Arnathau C, et al. African great apes are natural hosts of multiple related malaria species, including *Plasmodium falciparum*. *Proc Natl Acad Sci U S A*. 2010; 107 (4):1458–63. [PubMed: 20133889]
18. Prugnolle F, Durand P, Ollomo B, Duval L, Arieu F, Arnathau C, et al. A fresh look at the origin of *Plasmodium falciparum*, the most malignant malaria agent. *PLoS Pathog*. 2011; 7(2):e1001283, 1–8. [PubMed: 21383971]
19. Liu W, Li Y, Learn GH, Rudicell RS, Robertson JD, Keele BF, et al. Origin of the human malaria parasite *Plasmodium falciparum* in gorillas. *Nature*. 2010; 467 (7314):420–5. [PubMed: 20864995]
20. Prugnolle F, Ollomo B, Durand P, Yalcindag E, Arnathau C, Elguero E, et al. African monkeys are infected by *Plasmodium falciparum* nonhuman primate-specific strains. *Proc Natl Acad Sci U S A*. 2011; 108 (29):11948–53. [PubMed: 21730135]
21. Bandin I, Dopazo CP. Host range, host specificity and hypothesized host shift events among viruses of lower vertebrates. *Vet Res*. 2011; 42(1):67, 1–15. [PubMed: 21592358]
22. Webby R, Hoffmann E, Webster R. Molecular constraints to interspecies transmission of viral pathogens. *Nat Med*. 2004; 10 (12 Suppl):S77–81. [PubMed: 15577935]
23. Cai H, Gu J, Wang Y. Core genome components and lineage specific expansions in malaria parasites *plasmodium*. *BMC Genomics*. 2010; 11(Suppl 3):S13, 1–10. [PubMed: 21143780]
24. Harrison T, Samuel BU, Akompong T, Hamm H, Mohandas N, Lomasney JW, et al. Erythrocyte G protein-coupled receptor signaling in malarial infection. *Science*. 2003; 301 (5640):1734–6. [PubMed: 14500986]
25. Pachebat JA, Kadekoppala M, Grainger M, Dluzewski AR, Gunaratne RS, Scott-Finnigan TJ, et al. Extensive proteolytic processing of the malaria parasite merozoite surface protein 7 during biosynthesis and parasite release from erythrocytes. *Mol Biochem Parasitol*. 2007; 151 (1):59–69. [PubMed: 17097159]
26. Fanning SL, Czesny B, Sedegah M, Carucci DJ, van Gemert GJ, Eling W, et al. A glycosylphosphatidylinositol anchor signal sequence enhances the immunogenicity of a DNA vaccine encoding *Plasmodium falciparum* sexual-stage antigen, Pfs230. *Vaccine*. 2003; 21 (23): 3228–35. [PubMed: 12804852]
27. Joannin N, Abhiman S, Sonnhammer EL, Wahlgren M. Sub-grouping and sub-functionalization of the RIFIN multi-copy protein family. *BMC Genomics*. 2008; 9:19, 1–14. [PubMed: 18197962]
28. Hernandez-Rivas R, Hinterberg K, Scherf A. Compartmentalization of genes coding for immunodominant antigens to fragile chromosome ends leads to dispersed subtelomeric gene families and rapid gene evolution in *Plasmodium falciparum*. *Mol Biochem Parasitol*. 1996; 78 (1–2):137–48. [PubMed: 8813684]
29. Robinson BA, Welch TL, Smith JD. Widespread functional specialization of *Plasmodium falciparum* erythrocyte membrane protein 1 family members to bind CD36 analysed across a parasite genome. *Molecular Microbiology*. 2003; 47 (5):1265–78. [PubMed: 12603733]
30. del Portillo HA, Fernandez-Becerra C, Bowman S, Oliver K, Preuss M, Sanchez CP, et al. A superfamily of variant genes encoded in the subtelomeric region of *Plasmodium vivax*. *Nature*. 2001; 410 (6830):839–42. [PubMed: 11298455]
31. del Portillo HA, Lanzer M, Rodriguez-Malaga S, Zavala F, Fernandez-Becerra C. Variant genes and the spleen in *Plasmodium vivax* malaria. *Int J Parasitol*. 2004; 34 (13–14):1547–54. [PubMed: 15582531]
32. Jomaa H, Wiesner J, Sanderbrand S, Altincicek B, Weidemeyer C, Hintz M, et al. Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs. *Science*. 1999; 285 (5433):1573–6. [PubMed: 10477522]

33. Wiesner J, Jomaa H. Isoprenoid biosynthesis of the apicoplast as drug target. *Curr Drug Targets*. 2007; 8 (1):3–13. [PubMed: 17266527]
34. Dahl EL, Rosenthal PJ. Apicoplast translation, transcription and genome replication: targets for antimalarial antibiotics. *Trends Parasitol*. 2008; 24 (6):279–84. [PubMed: 18450512]
35. Ralph SA, Foth BJ, Hall N, McFadden GI. Evolutionary pressures on apicoplast transit peptides. *Mol Biol Evol*. 2004; 21 (12):2183–94. [PubMed: 15317876]
36. Ralph SA, van Dooren GG, Waller RF, Crawford MJ, Fraunholz MJ, Foth BJ, et al. Tropical infectious diseases: metabolic maps and functions of the *Plasmodium falciparum* apicoplast. *Nat Rev Microbiol*. 2004; 2 (3):203–16. [PubMed: 15083156]
37. Jana S, Paliwal J. Novel molecular targets for antimalarial chemotherapy. *Int J Antimicrob Agents*. 2007; 30 (1):4–10. [PubMed: 17339102]
38. Dvorin JD, Martyn DC, Patel SD, Grimley JS, Collins CR, Hopp CS, et al. A plant-like kinase in *Plasmodium falciparum* regulates parasite egress from erythrocytes. *Science*. 2010; 328 (5980): 910–2. [PubMed: 20466936]
39. Ecker A, Lakshmanan V, Sinnis P, Coppens I, Fidock DA. Evidence That Mutant PfCRT Facilitates the Transmission to Mosquitoes of Chloroquine-Treated *Plasmodium* Gametocytes. *J Infect Dis*. 2011; 203 (2):228–36. [PubMed: 21288823]
40. Joet T, Eckstein-Ludwig U, Morin C, Krishna S. Validation of the hexose transporter of *Plasmodium falciparum* as a novel drug target. *Proc Natl Acad Sci U S A*. 2003; 100 (13):7476–9. [PubMed: 12792024]
41. Johnson DJ, Fidock DA, Mungthin M, Lakshmanan V, Sidhu AB, Bray PG, et al. Evidence for a central role for PfCRT in conferring *Plasmodium falciparum* resistance to diverse antimalarial agents. *Mol Cell*. 2004; 15 (6):867–77. [PubMed: 15383277]
42. Kirk K, Howitt SM, Broer S, Saliba KJ, Downie MJ. Purine uptake in *Plasmodium*: transport versus metabolism. *Trends Parasitol*. 2009; 25 (6):246–9. [PubMed: 19423394]
43. Kirk K, Saliba KJ. Targeting nutrient uptake mechanisms in *Plasmodium*. *Curr Drug Targets*. 2007; 8 (1):75–88. [PubMed: 17266532]
44. Patel AP, Staines HM, Krishna S. New antimalarial targets: the example of glucose transport. *Travel Med Infect Dis*. 2008; 6 (1–2):58–66. [PubMed: 18342277]
45. Kuang R, Gu J, Cai H, Wang Y. Improved prediction of malaria degradomes by supervised learning with SVM and profile kernel. *Genetica*. 2009; 136 (1):189–209. [PubMed: 19057851]
46. Wang Y, Wu Y. Computer assisted searches for drug targets with emphasis on malarial proteases and their inhibitors. *Curr Drug Targets Infect Disord*. 2004; 4 (1):25–40. [PubMed: 15032632]
47. Wu Y, Wang X, Liu X, Wang Y. Data-mining approaches reveal hidden families of proteases in the genome of malaria parasite. *Genome Res*. 2003; 13 (4):601–16. [PubMed: 12671001]
48. Goldberg DE. Hemoglobin degradation. *Curr Top Microbiol Immunol*. 2005; 295:275–91. [PubMed: 16265895]
49. Rosenthal PJ. Hydrolysis of erythrocyte proteins by proteases of malaria parasites. *Curr Opin Hematol*. 2002; 9 (2):140–5. [PubMed: 11844998]
50. Rosenthal PJ. Cysteine proteases of malaria parasites. *Int J Parasitol*. 2004; 34 (13–14):1489–99. [PubMed: 15582526]
51. Rosenthal PJ, Sijwali PS, Singh A, Shenai BR. Cysteine proteases of malaria parasites: targets for chemotherapy. *Curr Pharm Des*. 2002; 8 (18):1659–72. [PubMed: 12132997]
52. Eggleston KK, Duffin KL, Goldberg DE. Identification and characterization of falcilysin, a metallopeptidase involved in hemoglobin catabolism within the malaria parasite *Plasmodium falciparum*. *J Biol Chem*. 1999; 274 (45):32411–7. [PubMed: 10542284]
53. McGowan S, Oellig CA, Birru WA, Caradoc-Davies TT, Stack CM, Lowther J, et al. Structure of the *Plasmodium falciparum* M17 aminopeptidase and significance for the design of drugs targeting the neutral exopeptidases. *Proc Natl Acad Sci U S A*. 2010; 107 (6):2449–54. [PubMed: 20133789]
54. Skinner-Adams TS, Stack CM, Trenholme KR, Brown CL, Grembecka J, Lowther J, et al. *Plasmodium falciparum* neutral aminopeptidases: new targets for anti-malarials. *Trends Biochem Sci*. 2010; 35 (1):53–61. [PubMed: 19796954]

55. Meslin B, Barnadas C, Boni V, Latour C, De Monbrison F, Kaiser K, et al. Features of apoptosis in *Plasmodium falciparum* erythrocytic stage through a putative role of PfMCA1 metacaspase-like protein. *J Infect Dis*. 2007; 195 (12):1852–9. [PubMed: 17492602]
56. Meslin B, Zalila H, Fasel N, Picot S, Bienvenu AL. Are protozoan metacaspases potential parasite killers? *Parasit Vectors*. 2011; 4:26, 1–7. [PubMed: 21356053]
57. Wang YF, Gu X. Functional divergence in the caspase gene family and altered functional constraints: Statistical analysis and prediction. *Genetics*. 2001; 158 (3):1311–20. [PubMed: 11454777]
58. Russo I, Oksman A, Vaupel B, Goldberg DE. A calpain unique to alveolates is essential in *Plasmodium falciparum* and its knockdown reveals an involvement in pre-S-phase development. *Proc Natl Acad Sci U S A*. 2009; 106 (5):1554–9. [PubMed: 19164769]
59. Blackman MJ. Proteases involved in erythrocyte invasion by the malaria parasite: function and potential as chemotherapeutic targets. *Curr Drug Targets*. 2000; 1 (1):59–83. [PubMed: 11475536]
60. Blackman MJ. Malarial proteases and host cell egress: an ‘emerging’ cascade. *Cell Microbiol*. 2008; 10 (10):1925–34. [PubMed: 18503638]
61. Blackman MJ, Fujioka H, Stafford WH, Sajid M, Clough B, Fleck SL, et al. A subtilisin-like protein in secretory organelles of *Plasmodium falciparum* merozoites. *J Biol Chem*. 1998; 273 (36):23398–409. [PubMed: 9722575]
62. El Bakkouri M, Pow A, Mulichak A, Cheung KL, Artz JD, Amani M, et al. The Clp chaperones and proteases of the human malaria parasite *Plasmodium falciparum*. *J Mol Biol*. 2010; 404 (3): 456–77. [PubMed: 20887733]
63. Rathore S, Sinha D, Asad M, Bottcher T, Afrin F, Chauhan VS, et al. A cyanobacterial serine protease of *Plasmodium falciparum* is targeted to the apicoplast and plays an important role in its growth and development. *Mol Microbiol*. 2010; 77 (4):873–90.
64. Kinch LN, Ginalski K, Grishin NV. Site-2 protease regulated intramembrane proteolysis: sequence homologs suggest an ancient signaling cascade. *Protein Sci*. 2006; 15 (1):84–93. [PubMed: 16322567]
65. Baker RP, Wijetilaka R, Urban S. Two *Plasmodium* rhomboid proteases preferentially cleave different adhesins implicated in all invasive stages of malaria. *PLoS Pathog*. 2006; 2(10):e113, 1–11. [PubMed: 17040128]
66. Brossier F, Jewett TJ, Sibley LD, Urban S. A spatially localized rhomboid protease cleaves cell surface adhesins essential for invasion by *Toxoplasma*. *Proc Natl Acad Sci U S A*. 2005; 102 (11): 4146–51. [PubMed: 15753289]
67. Dowse TJ, Pascall JC, Brown KD, Soldati D. Apicomplexan rhomboids have a potential role in microneme protein cleavage during host cell invasion. *Int J Parasitol*. 2005; 35 (7):747–56. [PubMed: 15913633]
68. Dowse TJ, Soldati D. Rhomboid-like proteins in Apicomplexa: phylogeny and nomenclature. *Trends Parasitol*. 2005; 21 (6):254–8. [PubMed: 15922242]
69. Li X, Chen H, Bahamontes-Rosa N, Kun JF, Traore B, Crompton PD, et al. *Plasmodium falciparum* signal peptide peptidase is a promising drug target against blood stage malaria. *Biochem Biophys Res Commun*. 2009; 380 (3):454–9. [PubMed: 19174148]
70. Li X, Chen H, Oh SS, Chishti AH. A Presenilin-like protease associated with *Plasmodium falciparum* micronemes is involved in erythrocyte invasion. *Mol Biochem Parasitol*. 2008; 158 (1): 22–31. [PubMed: 18160114]
71. O'Donnell RA, Hackett F, Howell SA, Treeck M, Struck N, Krnajska Z, et al. Intramembrane proteolysis mediates shedding of a key adhesin during erythrocyte invasion by the malaria parasite. *J Cell Biol*. 2006; 174 (7):1023–33. [PubMed: 17000879]
72. Brown MS, Ye J, Rawson RB, Goldstein JL. Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. *Cell*. 2000; 100 (4):391–8. [PubMed: 10693756]
73. Urban S. Making the cut: central roles of intramembrane proteolysis in pathogenic microorganisms. *Nat Rev Microbiol*. 2009; 7 (6):411–23. [PubMed: 19421188]
74. Carroll CD, Patel H, Johnson TO, Guo T, Orlowski M, He ZM, et al. Identification of potent inhibitors of *Plasmodium falciparum* plasmepsin II from an encoded statine combinatorial library. *Bioorg Med Chem Lett*. 1998; 8 (17):2315–20. [PubMed: 9873534]

75. Haque TS, Skillman AG, Lee CE, Habashita H, Gluzman IY, Ewing TJ, et al. Potent, low-molecular-weight non-peptide inhibitors of malarial aspartyl protease plasmepsin II. *J Med Chem.* 1999; 42 (8):1428–40. [PubMed: 10212129]
76. Kasam V, Zimmermann M, Maass A, Schwichtenberg H, Wolf A, Jacq N, et al. Design of new plasmepsin inhibitors: a virtual high throughput screening approach on the EGEE grid. *J Chem Inf Model.* 2007; 47 (5):1818–28. [PubMed: 17727268]
77. Li R, Chen X, Gong B, Selzer PM, Li Z, Davidson E, et al. Structure-based design of parasitic protease inhibitors. *Bioorg Med Chem.* 1996; 4 (9):1421–7. [PubMed: 8894100]
78. Liu P, Marzahn MR, Robbins AH, Gutierrez-de-Teran H, Rodriguez D, McClung SH, et al. Recombinant Plasmepsin 1 from the Human Malaria Parasite *Plasmodium falciparum*: Enzymatic Characterization, Active Site Inhibitor Design, and Structural Analysis. *Biochemistry.* 2009; 48 (19):4086–99. [PubMed: 19271776]
79. Scheidt KA, Roush WR, McKerrow JH, Selzer PM, Hansell E, Rosenthal PJ. Structure-based design, synthesis and evaluation of conformationally constrained cysteine protease inhibitors. *Bioorg Med Chem.* 1998; 6 (12):2477–94. [PubMed: 9925304]
80. Shah F, Mukherjee P, Desai P, Avery M. Computational Approaches for the Discovery of Cysteine Protease Inhibitors Against Malaria and SARS. *Current Computer-Aided Drug Design.* 2010; 6 (1):1–23. [PubMed: 20370692]
81. Shah F, Mukherjee P, Gut J, Legac J, Rosenthal PJ, Tekwani BL, et al. Identification of novel malarial cysteine protease inhibitors using structure-based virtual screening of a focused cysteine protease inhibitor library. *J Chem Inf Model.* 2011; 51 (4):852–64. [PubMed: 21428453]
82. Aravind L, Iyer LM, Wellemes TE, Miller LH. *Plasmodium* biology: Genomic gleanings. *Cell.* 2003; 115 (7):771–85. [PubMed: 14697197]
83. Balaji S, Babu MM, Iyer LM, Aravind L. Discovery of the principal specific transcription factors of Apicomplexa and their implication for the evolution of the AP2-integrase DNA binding domains. *Nucleic Acids Res.* 2005; 33 (13):3994–4006. [PubMed: 16040597]
84. Riechmann JL, Meyerowitz EM. The AP2/EREBP family of plant transcription factors. *Biol Chem.* 1998; 379 (6):633–46. [PubMed: 9687012]
85. Painter HJ, Campbell TL, Llinas M. The Apicomplexan AP2 family: integral factors regulating *Plasmodium* development. *Mol Biochem Parasitol.* 2011; 176 (1):1–7. [PubMed: 21126543]
86. Campbell TL, De Silva EK, Olszewski KL, Elemento O, Llinas M. Identification and genome-wide prediction of DNA binding specificities for the ApiAP2 family of regulators from the malaria parasite. *PLoS Pathog.* 2010; 6(10):e1001165, 1–15. [PubMed: 21060817]
87. Flueck C, Bartfai R, Niederwieser I, Witmer K, Alako BT, Moes S, et al. A major role for the *Plasmodium falciparum* ApiAP2 protein PfSIP2 in chromosome end biology. *PLoS Pathog.* 2010; 6(2):e1000784, 1–15. [PubMed: 20195509]
88. Llinas M, Bozdech Z, Wong ED, Adai AT, DeRisi JL. Comparative whole genome transcriptome analysis of three *Plasmodium falciparum* strains. *Nucleic Acids Res.* 2006; 34 (4):1166–73. [PubMed: 16493140]
89. Otto TD, Wilinski D, Assefa S, Keane TM, Sarry LR, Bohme U, et al. New insights into the blood-stage transcriptome of *Plasmodium falciparum* using RNA-Seq. *Mol Microbiol.* 2010; 76 (1):12–24. [PubMed: 20141604]
90. Yuda M, Iwanaga S, Shigenobu S, Kato T, Kaneko I. Transcription factor AP2-Sp and its target genes in malarial sporozoites. *Mol Microbiol.* 2010; 75 (4):854–63. [PubMed: 20025671]
91. Yuda M, Iwanaga S, Shigenobu S, Mair GR, Janse CJ, Waters AP, et al. Identification of a transcription factor in the mosquito-invasive stage of malaria parasites. *Mol Microbiol.* 2009; 71 (6):1402–14. [PubMed: 19220746]
92. Wu Y, Ellis RD, Shaffer D, Fontes E, Malkin EM, Mahanty S, et al. Phase 1 trial of malaria transmission blocking vaccine candidates Pfs25 and Pvs25 formulated with montanide ISA 51. *PLoS One.* 2008; 3(7):e2636, 1–9. [PubMed: 18612426]
93. Qian F, Aebig JA, Reiter K, Barnafo E, Zhang Y, Shimp RL Jr, et al. Enhanced antibody responses to *Plasmodium falciparum* Pfs28 induced in mice by conjugation to ExoProtein A of *Pseudomonas aeruginosa* with an improved procedure. *Microbes Infect.* 2009; 11 (3):408–12. [PubMed: 19146977]

94. Outchkourov NS, Roeffen W, Kaan A, Jansen J, Luty A, Schuiffel D, et al. Correctly folded Pfs48/45 protein of *Plasmodium falciparum* elicits malaria transmission-blocking immunity in mice. *Proc Natl Acad Sci U S A*. 2008; 105 (11):4301–5. [PubMed: 18332422]
95. Doi M, Tanabe K, Tachibana S, Hamai M, Tachibana M, Mita T, et al. Worldwide sequence conservation of transmission-blocking vaccine candidate Pvs230 in *Plasmodium vivax*. *Vaccine*. 2011; 29 (26):4308–15. [PubMed: 21514344]
96. Gerloff DL, Creasey A, Maslau S, Carter R. Structural models for the protein family characterized by gamete surface protein Pfs230 of *Plasmodium falciparum*. *Proc Natl Acad Sci U S A*. 2005; 102 (38):13598–603. [PubMed: 16155126]
97. Kulangara C, Kajava AV, Corradin G, Felger I. Sequence conservation in *Plasmodium falciparum* alpha-helical coiled coil domains proposed for vaccine development. *PLoS One*. 2009; 4(5):e5419, 1–11. [PubMed: 19492090]
98. Mu J, Awadalla P, Duan J, McGee KM, Keebler J, Seydel K, et al. Genome-wide variation and identification of vaccine targets in the *Plasmodium falciparum* genome. *Nat Genet*. 2007; 39 (1): 126–30. [PubMed: 17159981]
99. Regis DP, Dobano C, Quinones-Olson P, Liang X, Graber NL, Stefaniak ME, et al. Transcriptionally active PCR for antigen identification and vaccine development: in vitro genome-wide screening and in vivo immunogenicity. *Mol Biochem Parasitol*. 2008; 158 (1):32–45. [PubMed: 18164079]
100. Villard V, Agak GW, Frank G, Jafarshad A, Servis C, Nebie I, et al. Rapid identification of malaria vaccine candidates based on alpha-helical coiled coil protein motif. *PLoS One*. 2007; 2 (7):e645. [PubMed: 17653272]
101. Anders RF, Adda CG, Foley M, Norton RS. Recombinant protein vaccines against the asexual blood stages of *Plasmodium falciparum*. *Hum Vaccin*. 2010; 6 (1):39–53. [PubMed: 20061790]
102. Mu J, Seydel KB, Bates A, Su XZ. Recent Progress in Functional Genomic Research in *Plasmodium falciparum*. *Curr Genomics*. 2010; 11 (4):279–86. [PubMed: 21119892]
103. Winzeler EA. Applied systems biology and malaria. *Nat Rev Microbiol*. 2006; 4 (2):145–51. [PubMed: 16362033]
104. Hayward RE, DeRisi JL, Alfadhli S, Kaslow DC, Brown PO, Rathod PK. Shotgun DNA microarrays and stage-specific gene expression in *Plasmodium falciparum* malaria. *Mol Microbiol*. 2000; 35 (1):6–14. [PubMed: 10632873]
105. Mamoun CB, Gluzman IY, Hott C, MacMillan SK, Amarakone AS, Anderson DL, et al. Co-ordinated programme of gene expression during asexual intraerythrocytic development of the human malaria parasite *Plasmodium falciparum* revealed by microarray analysis. *Mol Microbiol*. 2001; 39 (1):26–36. [PubMed: 11123685]
106. Bozdech Z, Llinas M, Pulliam BL, Wong ED, Zhu J, DeRisi JL. The Transcriptome of the Intraerythrocytic Developmental Cycle of *Plasmodium falciparum*. *PLoS Biol*. 2003; 1(1):E5, 1–16. [PubMed: 12929205]
107. Bozdech Z, Zhu J, Joachimiak MP, Cohen FE, Pulliam B, DeRisi JL. Expression profiling of the schizont and trophozoite stages of *Plasmodium falciparum* with a long-oligonucleotide microarray. *Genome Biol*. 2003; 4(2):R9, 1–15. [PubMed: 12620119]
108. Le Roch KG, Zhou Y, Batalov S, Winzeler EA. Monitoring the chromosome 2 intraerythrocytic transcriptome of *Plasmodium falciparum* using oligonucleotide arrays. *Am J Trop Med Hyg*. 2002; 67 (3):233–43. [PubMed: 12408661]
109. Le Roch KG, Zhou Y, Blair PL, Grainger M, Moch JK, Haynes JD, et al. Discovery of gene function by expression profiling of the malaria parasite life cycle. *Science*. 2003; 301 (5639): 1503–8. [PubMed: 12893887]
110. Tarun AS, Peng X, Dumpit RF, Ogata Y, Silva-Rivera H, Camargo N, et al. A combined transcriptome and proteome survey of malaria parasite liver stages. *Proc Natl Acad Sci U S A*. 2008; 105 (1):305–10. [PubMed: 18172196]
111. Zhou Y, Ramachandran V, Kumar KA, Westenberger S, Refour P, Zhou B, et al. Evidence-based annotation of the malaria parasite's genome using comparative expression profiling. *PLoS One*. 2008; 3(2):e1570, 1–16. [PubMed: 18270564]

112. Bozdech Z, Mok S, Hu G, Imwong M, Jaidee A, Russell B, et al. The transcriptome of *Plasmodium vivax* reveals divergence and diversity of transcriptional regulation in malaria parasites. *Proc Natl Acad Sci U S A*. 2008; 105 (42):16290–5. [PubMed: 18852452]
113. Llinas M, DeRisi JL. Pernicious plans revealed: *Plasmodium falciparum* genome wide expression analysis. *Curr Opin Microbiol*. 2004; 7 (4):382–7. [PubMed: 15358256]
114. Gissot M, Refour P, Briquet S, Boschet C, Coupe S, Mazier D, et al. Transcriptome of 3D7 and its gametocyte-less derivative F12 *Plasmodium falciparum* clones during erythrocytic development using a gene-specific microarray assigned to gene regulation, cell cycle and transcription factors. *Gene*. 2004; 341:267–77. [PubMed: 15474309]
115. Fang J, Zhou H, Rathore D, Sullivan M, Su XZ, McCutchan TF. Ambient glucose concentration and gene expression in *Plasmodium falciparum*. *Mol Biochem Parasitol*. 2004; 133 (1):125–9. [PubMed: 14668019]
116. Cui L, Miao J, Furuya T, Li X, Su XZ, Cui L. PfGCN5-mediated histone H3 acetylation plays a key role in gene expression in *Plasmodium falciparum*. *Eukaryot Cell*. 2007; 6 (7):1219–27. [PubMed: 17449656]
117. Singh K, Agarwal A, Khan SI, Walker LA, Tekwani BL. Growth, drug susceptibility, and gene expression profiling of *Plasmodium falciparum* cultured in medium supplemented with human serum or lipid-rich bovine serum albumin [corrected]. *J Biomol Screen*. 2007; 12 (8):1109–14. [PubMed: 18087074]
118. Young JA, Johnson JR, Benner C, Yan SF, Chen K, Le Roch KG, et al. In silico discovery of transcription regulatory elements in *Plasmodium falciparum*. *BMC Genomics*. 2008; 9:70, 1–21. [PubMed: 18257930]
119. Cui L, Miao J, Furuya T, Fan Q, Li X, Rathod PK, et al. Histone acetyltransferase inhibitor anacardic acid causes changes in global gene expression during in vitro *Plasmodium falciparum* development. *Eukaryot Cell*. 2008; 7 (7):1200–10. [PubMed: 18487348]
120. Scholz M, Fraunholz MJ. A computational model of gene expression reveals early transcriptional events at the subtelomeric regions of the malaria parasite, *Plasmodium falciparum*. *Genome Biol*. 2008; 9(5):R88, 1–15. [PubMed: 18505551]
121. Ralph SA, Bischoff E, Mattei D, Sismeiro O, Dillies MA, Guigon G, et al. Transcriptome analysis of antigenic variation in *Plasmodium falciparum*--var silencing is not dependent on antisense RNA. *Genome Biol*. 2005; 6(11):R93, 1–12. [PubMed: 16277748]
122. Kidgell C, Volkman SK, Daily J, Borevitz JO, Plouffe D, Zhou Y, et al. A systematic map of genetic variation in *Plasmodium falciparum*. *PLoS Pathog*. 2006; 2(6):e57, 1–16. [PubMed: 16789840]
123. Eklund EH, Fidock DA. Advances in understanding the genetic basis of antimalarial drug resistance. *Curr Opin Microbiol*. 2007; 10 (4):363–70. [PubMed: 17709280]
124. Cramer A, Marfurt J, Mugittu K, Maire N, Regos A, Coppee JY, et al. Rapid microarray-based method for monitoring of all currently known single-nucleotide polymorphisms associated with parasite resistance to antimalaria drugs. *J Clin Microbiol*. 2007; 45 (11):3685–91. [PubMed: 17804664]
125. Zhang GQ, Guan YY, Sheng HH, Zheng B, Wu S, Xiao HS, et al. Multiplex PCR and oligonucleotide microarray for detection of single-nucleotide polymorphisms associated with *Plasmodium falciparum* drug resistance. *J Clin Microbiol*. 2008; 46 (7):2167–74. [PubMed: 18448699]
126. Jiang HY, Yi M, Mu JB, Zhang L, Ivens A, Klimczak LJ, et al. Detection of genome-wide polymorphisms in the AT-rich *Plasmodium falciparum* genome using a high-density microarray. *Bmc Genomics*. 2008; 9:398, 1–15. [PubMed: 18724869]
127. Ganesan K, Ponmee N, Jiang L, Fowble JW, White J, Kamchonwongpaisan S, et al. A genetically hard-wired metabolic transcriptome in *Plasmodium falciparum* fails to mount protective responses to lethal antifolates. *PLoS Pathog*. 2008; 4(11):e1000214, 1–13. [PubMed: 19023412]
128. Steenkeste N, Dillies MA, Khim N, Sismeiro O, Chy S, Lim P, et al. FlexiChip package: an universal microarray with a dedicated analysis software for high-throughput SNPs detection linked to anti-malarial drug resistance. *Malar J*. 2009; 8:229, 1–9. [PubMed: 19828052]

129. Tan JC, Patel JJ, Tan A, Blain JC, Albert TJ, Lobo NF, et al. Optimizing comparative genomic hybridization probes for genotyping and SNP detection in *Plasmodium falciparum*. *Genomics*. 2009; 93 (6):543–50. [PubMed: 19285129]
130. Dharia NV, Sidhu AB, Cassera MB, Westenberger SJ, Bopp SE, Eastman RT, et al. Use of high-density tiling microarrays to identify mutations globally and elucidate mechanisms of drug resistance in *Plasmodium falciparum*. *Genome Biol*. 2009; 10(2):R21, 1–13. [PubMed: 19216790]
131. Ibrahim ML, Steenkeste N, Khim N, Adam HH, Konate L, Coppee JY, et al. Field-based evidence of fast and global increase of *Plasmodium falciparum* drug-resistance by DNA-microarrays and PCR/RFLP in Niger. *Malar J*. 2009; 8:32, 1–9. [PubMed: 19236701]
132. Ballif M, Hii J, Marfurt J, Cramer A, Fafale A, Felger I, et al. Monitoring of malaria parasite resistance to chloroquine and sulphadoxine-pyrimethamine in the Solomon Islands by DNA microarray technology. *Malar J*. 2010; 9:270, 1–7. [PubMed: 20925934]
133. Nair S, Miller B, Barends M, Jaidee A, Patel J, Mayxay M, et al. Adaptive Copy Number Evolution in Malaria Parasites. *Plos Genetics*. 2008; 4(10):e1000243, 1–10. [PubMed: 18974876]
134. Gaur D, Furuya T, Mu J, Jiang LB, Su XZ, Miller LH. Upregulation of expression of the reticulocyte homology gene 4 in the *Plasmodium falciparum* clone Dd2 is associated with a switch in the erythrocyte invasion pathway. *Mol Biochem Parasitol*. 2006; 145 (2):205–15. [PubMed: 16289357]
135. Duraisingh MT, Voss TS, Marty AJ, Duffy MF, Good RT, Thompson JK, et al. Heterochromatin silencing and locus repositioning linked to regulation of virulence genes in *Plasmodium falciparum*. *Cell*. 2005; 121 (1):13–24. [PubMed: 15820675]
136. Carret CK, Horrocks P, Konfortov B, Winzeler E, Qureshi M, Newbold C, et al. Microarray-based comparative genomic analyses of the human malaria parasite *Plasmodium falciparum* using Affymetrix arrays. *Mol Biochem Parasitol*. 2005; 144 (2):177–86. [PubMed: 16174539]
137. Mok BW, Ribacke U, Winter G, Yip BH, Tan CS, Fernandez V, et al. Comparative transcriptomal analysis of isogenic *Plasmodium falciparum* clones of distinct antigenic and adhesive phenotypes. *Mol Biochem Parasitol*. 2007; 151 (2):184–92. [PubMed: 17196675]
138. Oakley MS, Kumar S, Anantharaman V, Zheng H, Mahajan B, Haynes JD, et al. Molecular factors and biochemical pathways induced by febrile temperature in intraerythrocytic *Plasmodium falciparum* parasites. *Infect Immun*. 2007; 75 (4):2012–25. [PubMed: 17283083]
139. Ribacke U, Mok BW, Wirta V, Normark J, Lundberg J, Kironde F, et al. Genome wide gene amplifications and deletions in *Plasmodium falciparum*. *Mol Biochem Parasitol*. 2007; 155 (1): 33–44. [PubMed: 17599553]
140. Tuikue Ndam N, Bischoff E, Proux C, Lavstsen T, Salanti A, Guitard J, et al. *Plasmodium falciparum* transcriptome analysis reveals pregnancy malaria associated gene expression. *PLoS One*. 2008; 3(3):e1855, 1–9. [PubMed: 18365010]
141. Siau A, Silvie O, Franetich JF, Yalaoui S, Marinach C, Hannoun L, et al. Temperature shift and host cell contact up-regulate sporozoite expression of *Plasmodium falciparum* genes involved in hepatocyte infection. *PLoS Pathog*. 2008; 4(8):e1000121, 1–13. [PubMed: 18688281]
142. Wurtz N, Desplans J, Parzy D. Phenotypic and transcriptomic analyses of *Plasmodium falciparum* protein kinase A catalytic subunit inhibition. *Parasitol Res*. 2009; 105 (6):1691–9. [PubMed: 19779742]
143. Xu X, Dong Y, Abraham EG, Kocan A, Srinivasan P, Ghosh AK, et al. Transcriptome analysis of *Anopheles stephensi*-*Plasmodium berghei* interactions. *Mol Biochem Parasitol*. 2005; 142 (1): 76–87. [PubMed: 15907562]
144. Lovegrove FE, Pena-Castillo L, Mohammad N, Liles WC, Hughes TR, Kain KC. Simultaneous host and parasite expression profiling identifies tissue-specific transcriptional programs associated with susceptibility or resistance to experimental cerebral malaria. *BMC Genomics*. 2006; 7:295, 1–17. [PubMed: 17118208]
145. Young JA, Fivelman QL, Blair PL, de la Vega P, Le Roch KG, Zhou Y, et al. The *Plasmodium falciparum* sexual development transcriptome: a microarray analysis using ontology-based pattern identification. *Mol Biochem Parasitol*. 2005; 143 (1):67–79. [PubMed: 16005087]

146. Daily JP, Le Roch KG, Sarr O, Ndiaye D, Lukens A, Zhou Y, et al. In vivo transcriptome of *Plasmodium falciparum* reveals overexpression of transcripts that encode surface proteins. *J Infect Dis*. 2005; 191 (7):1196–203. [PubMed: 15747257]
147. Claessens A, Ghumra A, Gupta AP, Mok S, Bozdech Z, Rowe JA. Design of a variant surface antigen-supplemented microarray chip for whole transcriptome analysis of multiple *Plasmodium falciparum* cytoadherent strains, and identification of strain-transcendent rif and stevor genes. *Malar J*. 2011; 10(1):180, 1–16. [PubMed: 21718533]
148. Tan JC, Miller BA, Tan A, Patel JJ, Cheeseman IH, Anderson TJ, et al. An optimized microarray platform for assaying genomic variation in *Plasmodium falciparum* field populations. *Genome Biol*. 2011; 12(4):R35, 1–10. [PubMed: 21477297]
149. Dharia NV, Bright AT, Westerberger SJ, Barnes SW, Batalov S, Kuhen K, et al. Whole-genome sequencing and microarray analysis of ex vivo *Plasmodium vivax* reveal selective pressure on putative drug resistance genes. *Proc Natl Acad Sci U S A*. 2010; 107 (46):20045–50. [PubMed: 21037109]
150. Dharia NV, Plouffe D, Bopp SE, Gonzalez-Paez GE, Lucas C, Salas C, et al. Genome scanning of Amazonian *Plasmodium falciparum* shows subtelomeric instability and clindamycin-resistant parasites. *Genome Res*. 2010; 20 (11):1534–44. [PubMed: 20829224]
151. Isokpehi RD, Hide WA. Integrative analysis of intraerythrocytic differentially expressed transcripts yields novel insights into the biology of *Plasmodium falciparum*. *Malar J*. 2003; 2(1): 38, 1–13. [PubMed: 14617379]
152. Westerberger SJ, Cui L, Dharia N, Winzeler E, Cui L. Genome-wide nucleosome mapping of *Plasmodium falciparum* reveals histone-rich coding and histone-poor intergenic regions and chromatin remodeling of core and subtelomeric genes. *BMC Genomics*. 2009; 10:610, 1–17. [PubMed: 20015349]
153. Broadbent KM, Park D, Wolf AR, Van Tyne D, Sims JS, Ribacke U, et al. A global transcriptional analysis of *Plasmodium falciparum* malaria reveals a novel family of telomere-associated lncRNAs. *Genome Biol*. 2011; 12(6):R56, 1–15. [PubMed: 21689454]
154. Lopez-Rubio JJ, Mancio-Silva L, Scherf A. Genome-wide analysis of heterochromatin associates clonally variant gene regulation with perinuclear repressive centers in malaria parasites. *Cell Host Microbe*. 2009; 5 (2):179–90. [PubMed: 19218088]
155. Lopez-Rubio JJ, Riviere L, Scherf A. Shared epigenetic mechanisms control virulence factors in protozoan parasites. *Curr Opin Microbiol*. 2007; 10 (6):560–8. [PubMed: 18024150]
156. Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nat Genet*. 2008; 40 (12):1413–5. [PubMed: 18978789]
157. Sultan M, Schulz MH, Richard H, Magen A, Klingenhoff A, Scherf M, et al. A global view of gene activity and alternative splicing by deep sequencing of the human transcriptome. *Science*. 2008; 321 (5891):956–60. [PubMed: 18599741]
158. Nagalakshmi U, Wang Z, Waern K, Shou C, Raha D, Gerstein M, et al. The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science*. 2008; 320 (5881):1344–9. [PubMed: 18451266]
159. Wilhelm BT, Marguerat S, Watt S, Schubert F, Wood V, Goodhead I, et al. Dynamic repertoire of a eukaryotic transcriptome surveyed at single-nucleotide resolution. *Nature*. 2008; 453 (7199): 1239–43. [PubMed: 18488015]
160. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat Methods*. 2008; 5 (7):621–8. [PubMed: 18516045]
161. Vignali M, Armour CD, Chen J, Morrison R, Castle JC, Biery MC, et al. NSR-seq transcriptional profiling enables identification of a gene signature of *Plasmodium falciparum* parasites infecting children. *J Clin Invest*. 2011; 121 (3):1119–29. [PubMed: 21317536]
162. Armour CD, Castle JC, Chen R, Babak T, Loerch P, Jackson S, et al. Digital transcriptome profiling using selective hexamer priming for cDNA synthesis. *Nat Methods*. 2009; 6 (9):647–9. [PubMed: 19668204]

163. Lasonder E, Ishihama Y, Andersen JS, Vermunt AMW, Pain A, Sauerwein RW, et al. Analysis of the *Plasmodium falciparum* proteome by high-accuracy mass spectrometry. *Nature*. 2002; 419 (6906):537–42. [PubMed: 12368870]
164. Florens L, Washburn MP, Raine JD, Anthony RM, Grainger M, Haynes JD, et al. A proteomic view of the *Plasmodium falciparum* life cycle. *Nature*. 2002; 419 (6906):520–6. [PubMed: 12368866]
165. Cooper RA, Carucci DJ. Proteomic approaches to studying drug targets and resistance in *Plasmodium*. *Curr Drug Targets Infect Disord*. 2004; 4 (1):41–51. [PubMed: 15032633]
166. Gelhaus C, Fritsch J, Krause E, Leippe M. Fractionation and identification of proteins by 2-DE and MS: towards a proteomic analysis of *Plasmodium falciparum*. *Proteomics*. 2005; 5 (16): 4213–22. [PubMed: 16196089]
167. Korir CC, Galinski MR. Proteomic studies of *Plasmodium knowlesi* SICA variant antigens demonstrate their relationship with *P. falciparum*. *EMPI Infect Genet Evol*. 2006; 6 (1):75–9.
168. Patra KP, Johnson JR, Cantin GT, Yates JR 3rd, Vinetz JM. Proteomic analysis of zygote and ookinete stages of the avian malaria parasite *Plasmodium gallinaceum* delineates the homologous proteomes of the lethal human malaria parasite *Plasmodium falciparum*. *Proteomics*. 2008; 8 (12):2492–9. [PubMed: 18563747]
169. Chen JH, Jung JW, Wang Y, Ha KS, Lu F, Lim CS, et al. Immunoproteomics profiling of blood stage *Plasmodium vivax* infection by high-throughput screening assays. *J Proteome Res*. 2010; 9 (12):6479–89. [PubMed: 20949973]
170. Doolan DL, Southwood S, Freilich DA, Sidney J, Graber NL, Shatney L, et al. Identification of *Plasmodium falciparum* antigens by antigenic analysis of genomic and proteomic data. *P Natl Acad Sci USA*. 2003; 100 (17):9952–7.
171. Florens L, Liu X, Wang YF, Yang SG, Schwartz O, Peglar M, et al. Proteomics approach reveals novel proteins on the surface of malaria-infected erythrocytes. *Molecular and Biochemical Parasitology*. 2004; 135 (1):1–11. [PubMed: 15287581]
172. Fried M, Wendler JP, Mutabingwa TK, Duffy PE. Mass spectrometric analysis of *Plasmodium falciparum* erythrocyte membrane protein-1 variants expressed by placental malaria parasites. *Proteomics*. 2004; 4 (4):1086–93. [PubMed: 15048989]
173. Gilson PR, Nebl T, Vukcevic D, Moritz RL, Sargeant T, Speed TP, et al. Identification and stoichiometry of glycosylphosphatidylinositol-anchored membrane proteins of the human malaria parasite *Plasmodium falciparum*. *Mol Cell Proteomics*. 2006; 5 (7):1286–99. [PubMed: 16603573]
174. Nirmalan N, Sims PF, Hyde JE. Quantitative proteomics of the human malaria parasite *Plasmodium falciparum* and its application to studies of development and inhibition. *Mol Microbiol*. 2004; 52 (4):1187–99. [PubMed: 15130134]
175. Sam-Yellowe TY, Florens L, Wang TM, Raine JD, Carucci DJ, Sinden R, et al. Proteome analysis of rhoptry-enriched fractions isolated from *Plasmodium merozoites*. *Journal of Proteome Research*. 2004; 3 (5):995–1001. [PubMed: 15473688]
176. Sam-Yellowe TY, Florens L, Johnson JR, Wang T, Drazba JA, Le Roch KG, et al. A *Plasmodium* gene family encoding Maurer's cleft membrane proteins: structural properties and expression profiling. *Genome Res*. 2004; 14 (6):1052–9. [PubMed: 15140830]
177. Lasonder E, Janse CJ, van Gemert GJ, Mair GR, Vermunt AM, Douradinha BG, et al. Proteomic profiling of *Plasmodium* sporozoite maturation identifies new proteins essential for parasite development and infectivity. *PLoS Pathog*. 2008; 4(10):e1000195, 1–18. [PubMed: 18974882]
178. Lal K, Prieto JH, Bromley E, Sanderson SJ, Yates JR 3rd, Wastling JM, et al. Characterisation of *Plasmodium* invasive organelles; an ookinete microneme proteome. *Proteomics*. 2009; 9 (5): 1142–51. [PubMed: 19206106]
179. Makanga M, Bray PG, Horrocks P, Ward SA. Towards a proteomic definition of CoArtem action in *Plasmodium falciparum* malaria. *Proteomics*. 2005; 5 (7):1849–58. [PubMed: 15832369]
180. Prieto JH, Koncarevic S, Park SK, Yates J 3rd, Becker K. Large-scale differential proteome analysis in *Plasmodium falciparum* under drug treatment. *PLoS One*. 2008; 3(12):e4098, 1–9. [PubMed: 19116658]

181. Koncarevic S, Bogumil R, Becker K. SELDI-TOF-MS analysis of chloroquine resistant and sensitive *Plasmodium falciparum* strains. *Proteomics*. 2007; 7 (5):711–21. [PubMed: 17295353]
182. Yeh I, Hanekamp T, Tsoka S, Karp PD, Altman RB. Computational analysis of *Plasmodium falciparum* metabolism: organizing genomic information to facilitate drug discovery. *Genome Res*. 2004; 14 (5):917–24. [PubMed: 15078855]
183. Ginsburg H. Progress in in silico functional genomics: the malaria Metabolic Pathways database. *Trends Parasitol*. 2006; 22 (6):238–40. [PubMed: 16707276]
184. Plata G, Hsiao TL, Olszewski KL, Llinas M, Vitkup D. Reconstruction and flux-balance analysis of the *Plasmodium falciparum* metabolic network. *Mol Syst Biol*. 2010; 6:408, 1–15. [PubMed: 20823846]
185. Lakshmanan V, Rhee KY, Daily JP. Metabolomics and malaria biology. *Mol Biochem Parasitol*. 2011; 175 (2):104–11. [PubMed: 20970461]
186. Clark K, Niemand J, Reeksting S, Smit S, van Brummelen AC, Williams M, et al. Functional consequences of perturbing polyamine metabolism in the malaria parasite, *Plasmodium falciparum*. *Amino Acids*. 2010; 38 (2):633–44. [PubMed: 19997948]
187. Lian LY, Al-Helal M, Roslaini AM, Fisher N, Bray PG, Ward SA, et al. Glycerol: an unexpected major metabolite of energy metabolism by the human malaria parasite. *Malar J*. 2009; 8:38, 1–4. [PubMed: 19267910]
188. Schwarzer E, Kuhn H, Valente E, Arese P. Malaria-parasitized erythrocytes and hemozoin nonenzymatically generate large amounts of hydroxy fatty acids that inhibit monocyte functions. *Blood*. 2003; 101 (2):722–8. [PubMed: 12393662]
189. Tonhosolo R, D'Alexandri FL, de Rosso VV, Gazarini ML, Matsumura MY, Peres VJ, et al. Carotenoid biosynthesis in intraerythrocytic stages of *Plasmodium falciparum*. *J Biol Chem*. 2009; 284 (15):9974–85. [PubMed: 19203994]
190. Suthram S, Sittler T, Ideker T. The *Plasmodium* protein network diverges from those of other eukaryotes. *Nature*. 2005; 438 (7064):108–12. [PubMed: 16267557]
191. Date SV, Stoeckert CJ Jr. Computational modeling of the *Plasmodium falciparum* interactome reveals protein function on a genome-wide scale. *Genome Res*. 2006; 16 (4):542–9. [PubMed: 16520460]
192. Mitrofanova A, Kleinberg S, Carlton J, Kasif S, Mishra B. Predicting malaria interactome classifications from time-course transcriptomic data along the intraerythrocytic developmental cycle. *Artif Intell Med*. 2010; 49 (3):167–76. [PubMed: 20580212]
193. Huang Y, Tienda-Luna IM, Wang Y. A Survey of Statistical Models for Reverse Engineering Gene Regulatory Networks. *IEEE Signal Process Mag*. 2009; 26 (1):76–97. [PubMed: 20046885]
194. Tienda-Luna IM, Yin Y, Carrion MC, Huang Y, Cai H, Sanchez M, et al. Inferring the skeleton cell cycle regulatory network of malaria parasite using comparative genomic and variational Bayesian approaches. *Genetica*. 2008; 132 (2):131–42. [PubMed: 17562188]
195. Brehelin L, Dufayard JF, Gascuel O. PlasmoDraft: a database of *Plasmodium falciparum* gene function predictions based on postgenomic data. *BMC Bioinformatics*. 2008; 9:440, 1–15. [PubMed: 18925948]
196. Pavithra SR, Kumar R, Tatu U. Systems analysis of chaperone networks in the malarial parasite *Plasmodium falciparum*. *PLoS Comput Biol*. 2007; 3 (9):1701–15. [PubMed: 17941702]
197. Dharia NV, Chatterjee A, Winzeler EA. Genomics and systems biology in malaria drug discovery. *Curr Opin Investig Drugs*. 2010; 11 (2):131–8.
198. Wuchty S, Adams JH, Ferdig MT. A comprehensive *Plasmodium falciparum* protein interaction map reveals a distinct architecture of a core interactome. *Proteomics*. 2009; 9 (7):1841–9. [PubMed: 19333996]
199. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*. 1987; 4 (4):406–25. [PubMed: 3447015]
200. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol Biol Evol*. 2011 Aug 18. [Epub ahead of print].
201. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One*. 2010; 5(6):e11147, 1–17. [PubMed: 20593022]

Fig. 1.

(A) A phylogenetic tree of *Plasmodium* parasites. The tree was inferred from partial mitochondrial genomes using the neighbor-joining method based on the nucleotide sequences with Kimura 2-parameter distance [199]. Evolutionary analyses were conducted in MEGA5 [200]. The option of complete deletion of gaps was used for tree construction. 1,000 bootstrap replicates were used to infer the reliability of branching points. Bootstrap values of >50% are presented. The *Leucocytozoon sabrazei* mitochondrial sequence was set as the outgroup. : *P. falciparum* and *P. reichenowi*; : rodent parasites; : other primate parasites; : avian and reptile parasites. The Maximum Parsimony and Maximum Likelihood methods give virtually the same topology (data not shown). (B) Genome alignment of five *Plasmodium* genomes, using MAUVE 2.3.1 [201]. The 14 chromosome regions are marked with arrows.

Table 1

The status of *Plasmodium* genome projects and the summary statistics.

Species and Strains	Genome Size(Mb)	# Chr	# ORF	AT%	Sequence Status	Ref
<i>P. berghei</i> ANKA	18.0	14	5864	76.3	Assembly	[5]
<i>P. chabaudi</i> chabaudi AS	16.9	14	5698	75.7	Assembly	[5]
<i>P. falciparum</i> 3D7	23.3	14	5403	80.6	Complete	[1]
<i>P. knowlesi</i> H	23.5	14	5188	62.5	Complete	[6]
<i>P. vivax</i> Sal-1	26.8	14	5433	57.7	Assembly	[4]
<i>P. yoelii</i> yoelii 17XNL	23.1	14	5878	77.4	Contigs and partial sequence published	[2]
<i>P. falciparum</i> IT	23.3	14	5446	73.2	Assembly	
<i>P. reichenowi</i>	25-27	14	N/A	N/A	Contigs	
<i>P. gallinaceum</i>	~25	14	N/A	N/A	Contigs	