Published in final edited form as:

Int J Comput Biol Drug Des. 2014 ; 7(4): 369–383. doi:10.1504/IJCBDD.2014.066554.

Exploring systems affected by the heat shock response in *Plasmodium falciparum* via protein association networks

Timothy G. Lilburn,

Department of Bacteriology American Type Culture Collection Manassas, VA 20110, USA
tlilburn@atcc.org

Hong Cai,

Department of Biology South Texas Center for Emerging Infectious Diseases University of Texas
at San Antonio San Antonio, TX 78249, USA hong.cai@utsa.edu

Jianying Gu,

Department of Biology College of Staten Island City University of New York Staten Island, NY
10314, USA jianying.gu@csi.cuny.edu

Zhan Zhou, and

Department of Biology South Texas Center for Emerging Infectious Diseases University of Texas
at San Antonio San Antonio, TX 78249, USA zhouzhan1985@gmail.com

Yufeng Wang*

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

Abstract

The heat shock response is a general mechanism by which organisms deal with physical insults such as sudden changes in temperature, osmotic and oxidative stresses, and exposure to toxic substances. *Plasmodium falciparum* is exposed to drastic temperature changes as a part of its life cycle and maintains an extensive repertoire of heat shock response-related proteins. As these proteins serve to maintain the parasite in the face of anti-malarial drugs as well, better understanding of the heat shock-related systems in the malaria parasite will lead to therapeutic approaches that frustrate these systems, leading to more effective use of anti-malarials. Here we use protein association networks to broaden our understanding of the systems impacted by and/or implicated in the heat shock response.

Keywords

malaria; heat shock; *Plasmodium*; systems biology; network

*Corresponding author yufeng.wang@utsa.edu.

1. Introduction

Malaria is one of the most severe human infectious diseases. It is present in over 109 countries, predominantly in developing countries, affecting hundreds of millions of people yearly. Over three billion people live in endemic regions where malaria is a constant threat. The primary victims are children under five, pregnant women and travelers that are not immune to malaria. It was estimated by the World Health Organization (WHO) that a child will die from malaria about every minute.

The causative agents of malaria are a group of protozoan parasites from the Genus *Plasmodium*. Among five species that infect human, *P. falciparum* is the most deadly species. A major reason of the continuous morbidity and mortality rates of malaria is the fast evolution of parasites and the development of resistance to multiple antimalarial drugs. It remains largely unclear how parasites adapt to the host environment.

In this study, we report a systems level study of the heat shock response network, a major host stress response system in *P. falciparum*. This network was chosen for the following reasons: (1) The malaria parasite experiences frequent heat shock stress during its life cycle. A harsh heat shock occurs to the parasite when it is transmitted from a mosquito (25°C) to a human (37°C). Moreover, it also needs to adapt to the periodic fevers in the human host caused by the infection. Thermosensitive proteins are the central players in heat shock response, and have long been considered as promising drug and vaccine targets due to their important roles in stress response, signal transduction, cell cycle regulation, parasite invasion, and pathogenesis. (2) Heat shock proteins have been implicated in the rapid evolution of some pathogens that can be a response to stresses such as drug treatment (Cowen and Lindquist, 2005); (3) It is reasonable to hypothesize that the key components in the heat shock response can be identified through a comparative genomic approach. Previously, a catalog of heat shock proteins that are conserved in various human parasites was reported (Acharya et al., 2007), providing a foundation for our study. (4) A systems biology approach is feasible to identify key network components for new drug and vaccine development. A chaperone interaction network was derived from Yeast 2-hybrid (Y2H) protein-protein interaction assays (Pavithra et al., 2007). We propose that this network can be enhanced with new components and interactions that can be discovered by integrating disparate type of omics data that are currently available, including genome sequences from *P. falciparum* (Gardner et al., 2002) and its sibling species (Carlton et al., 2008, Carlton et al., 2002, Pain et al., 2008, Tachibana et al., 2012), transcriptomic profiles across developmental stages (Bozdech et al., 2003, Bozdech et al., 2008, Dharia et al., 2009, Le Roch et al., 2003, Tan et al., 2011, Tarun et al., 2008, Otto et al., 2010), temporal and spatial proteomic characterization (Florens et al., 2004, Florens et al., 2002, Lasonder et al., 2002, Lasonder et al., 2008), metabolomic characterization (Ginsburg, 2006, Ginsburg and Tilley, 2011, Lakshmanan et al., 2011, Plata et al., 2010), and protein-protein interactions (LaCount et al., 2005, Suthram et al., 2005). A better understanding of the parasite heat shock chaperone network will allow us to identify targets based on their key role in cellular networks.

2. Materials and methods

2.1 Search for heat shock proteins

Heat shock proteins were searched using two complementary approaches: (1) All-vs-all mutual BLAST searches were performed between *P. falciparum* proteins and known heat shock proteins in other species. Two proteins were considered putative orthologs if they were reciprocal best hits with E-value $<10^{-5}$ in a global alignment and if the difference in their lengths was less than 20% of the overall size of the protein. (2) query the *P. falciparum* genome for Gene Ontology categories related to heat shock response: heat shock protein binding (GO:0031072), response to heat (GO:0009408), and response to unfolded protein (GO:0006986). Conserved domains/motifs in *P. falciparum* sequences were identified by searching the InterPro database (Rosenthal, 2011). Multiple alignments of each protein were obtained by the program ClustalX (Larkin et al., 2007) and T-coffee (Di Tommaso et al., 2011), followed by manual inspection and editing. For each heat shock protein family, phylogenetic trees were inferred by the neighbor-joining, maximum-likelihood, and maximum parsimony methods, using MEGA6 (Tamura et al., 2013).

2.2 Network data and analysis

The complete set of protein-protein associations for *P. falciparum* was extracted from the STRING database (Franceschini et al., 2013). Each association between a pair of proteins has a confidence score (S) ranging from 0.15 to 0.999 that was inferred by Bayesian integration of the evidence supporting the association, such as sequence similarity, KEGG pathway assignment, chromosome synteny, phylogenetic relationship, and literature co-occurrence. Cytoscape (Smoot et al., 2010) package was used to visualize the protein-protein associations which were converted to undirected weighted graphs, where there was a single edge between any pair of proteins and the S value was used as the weight. The RNA-Seq transcriptomic profiles of gene expression during the red blood cell (RBC) stage were integrated in network graphs (Otto et al., 2010). Network properties were inferred using NetworkAnalyzer (Assenov et al., 2008). The default values were used for all the plugins. The set of proteins in the network that were directly associated with the heat shock proteins were screened using BiNGO (Maere et al., 2005) to determine if any categories of proteins, as identified by their Gene Ontology terms, were over-represented. The hypergeometric test was used with the Benjamini and Hochberg false discovery rate correction. A significance level of 0.05 was selected.

3. Results and discussion

3.1 Network analysis predicted that 552 proteins are associated with heat shock response and chaperone interactions in malaria parasite

Our sequence similarity and ontology-based searches predicted a list of 155 heat shock proteins, which constitute about 2.8% of the entire genome. This list was expanded from a list of 95 proteins that were previously reported (Acharya et al., 2007, Pavithra et al., 2007). They are key components in heat shock network and can be divided into protein families, including heat shock proteins (HSPs) of various molecular sizes such as Hsp90, Hsp60, Hsp 70, and Hsp 40, small Hsps, co-chaperones, and chaperonins.

One hundred out of the 155 predicted heat shock proteins are associated with 452 other proteins with high confidence score (>0.7) (Table 1). The 552 nodes are linked to each other via 5,844 edges; there are 6 connected components implying that the network is highly interconnected. The network centralization parameter is 0.094, indicating that the network is decentralized. The degree of connectivity of these 97 proteins ranges from 73 to one and the degree distribution offers a good fit to a power law distribution. This, as well as the high network heterogeneity coefficient (0.893), indicates that most nodes are connected to a few others and a few nodes are connected to many others.

The biological processes that are over-represented in this network include, not surprisingly, response to heat shock, but they also include previously under-characterized processes such as gene silencing, protein import, signal transduction, cytoskeleton organization, RNA processing, regulation of transcription, proteolysis, DNA replication, DNA recombination, chromatin remodeling, and vesicle-mediated transport.

3.2 Predicted protein set is implicated in important cellular processes

3.2.1 Hsp protein families—One of the most important and best-studied protein families in the heat shock network is the Hsp90 family. *P. falciparum* contains four Hsp90 orthologs: pfHsp90 (accession number PF3D7_0708400), Grp94 (PF3D7_1222300), TRAP1a (PF3D7_1443900), and TRAP1b (PF3D7_1118200). Their degrees of connectivity range from 18 to 3, and they are interconnected with each other (Figure 1).

PfHsp90 (PF3D7_0708400) is connected to 18 other proteins, among which eight are heat shock proteins (Figure 1). Integration of RNA-Seq data showed that PfHsp90 was co-expressed with at least two Hsp70 proteins, one Hsp60 proteins, two Hsp110 proteins, and co-chaperonin p23, suggesting that Hsp90 is regulated in a temporal-specific manner and is a member of the Hsp complex. In addition to classical Hsps, PfHsp90 is associated with proteins involved in: (1) DNA synthesis. PfHsp90 is associated with bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS), an attractive antimalarial target that is crucial for the synthesis of nucleotide precursor (Yuthavong et al., 2005, Gahtori et al., 2012). (2) Translation. PfHsp90 is associated with high confidence score with several large and small ribosomal proteins. (3) Signal transduction. The associations between pfHsp90 and calmodulin (PF3D7_1434200), and a putative serine/threonine kinase (PF3D7_0107600) suggest that it may play a role in signal transduction and posttranslational modification. (4) Hemoglobin digestion. Notably, a histo-aspartic protease (HAP, also known as plasmepsin III, PF3D7_1408100), a member of a protease-mediated cascade for parasite nutrition and development (Blackman, 2008), is involved in the heat shock network.

Other Hsp protein families may play versatile roles in parasite biology. As shown in the Hsp70 network (Figure 2), four Hsp70 proteins (PF3D7_1134000, PF3D7_0818900, PF3D7_0917900, and PF3D7_0831700) have connectivity that range from 36 to 9. The proteins associated are localized in different cellular compartments including cytosol, membrane, mitochondrion, apicoplast, and food vacuole. They are involved in (1) transcriptional regulation. A basic transcription factor and several zinc-finger proteins may coordinate response to heat shock. (2) Translation involving multiple ribosomal proteins and a peptide chain release factor. (3) Transport. Putative transporters, a translocase, and

secretory proteins are associated with *P. falciparum* Hsps. The very highly expressed knob-associated histidine-rich protein (PF3D7_0202000) associated with Hsp70 (PF3D7_0818900), as seen in Figure 2, is thought to work with Hsp70 on the red blood cell cytosolic side of the membrane during the operation of the PTEX translocon, helping the exported protein cross the membrane. (4) Hemoglobin digestion involving a cysteine peptidase falcipain-2, a promising antimalarial target (Goldberg, 2013, Rosenthal, 2011); (5) metabolisms catalyzed by various enzymes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), pyrroline carboxylate reductase, and haloacid dehalogenase-like hydrolase.

3.2.2 Surface antigens—Network analysis revealed previously unknown protein-protein associations. The catalog of heat shock proteins includes a couple of surface antigens, which indicate the potential interplay between heat shock response and parasite invasion. A mature parasite-infected erythrocyte surface antigen (MESA, PF3D7_0500800) was predicted to be in the heat shock network. MESA is an adhesin also known as pfEMP2. It belongs to the erythrocyte membrane protein family and is implicated in the invasion of red blood cells (RBCs). MESA is an attractive target for antimalaria design as *in vivo* assays showed that MESA and RBC protein 4.1R form an interaction that is required for trafficking and parasite survival (Black et al., 2008). Network analysis predicted that MESA, being an antigen itself, had three interaction partners with high confidence score (>0.7), including three other surface antigens: (1) antigen 332, which is a DBL-like protein (Pf332), (2) ring-infected erythrocyte surface antigen (RESA), and (3) a truncated pseudogene in the erythrocyte membrane protein 1 (PfEMP1) family (VAR1CSA). This suggests that MESA may not be only involved in parasite survival, but also implicated in antigenic variation. Because MESA and its associations remain under-characterized, we also examined the 33 partners with medium confidence score (>0.4). Yeast two-hybrid assays confirmed 31 associations. A role for MESA in protein trafficking is supported by its direct physical interaction with skeleton-binding protein 1 (SBP1), dynamin-like protein (DYN1), dynein-related AAA-type ATPase, and an unknown *Plasmodium* exported protein. Although its exact function remains unknown, MESA may also be involved in RNA splicing, protein turnover, signal transduction, gametocytogenesis, and chromatin remodelling.

The heat shock related protein set contains two ring-infected erythrocyte surface antigens (PF3D7_1149200 and PF3D7_0102200), which were expressed at significantly high levels at hour 0 of the intraerythrocyte RNA-Seq experiments of Otto et al (Otto et al., 2010). This correlates well with their putative roles in RBC invasion. These two proteins and the 50 proteins they interact with form a network that is less heterogenous and with a relatively high network centrality coefficient, indicating that they tend to interact more with each other than with proteins outside their group. Enrichment analysis shows that membrane-associated proteins are over-represented in this network; many of the proteins are of unknown function, but several point to a role in erythrocyte invasion, such as the apical membrane antigen (PF3D7_1133400), and the erythrocyte binding antigen-175 (PF3D7_0731500). The presence of six additional surface antigens in the RESA protein family highlights the interaction between parasite cytoadherence and response to heat stress.

3.2.3 Heat shock proteases—Heat shock response network is a prime example of how the parasites effectively utilize the available genetic resources to develop responses to environmental challenges and other adaptive phenotypes. In addition to chaperones and chaperonins, eight proteases are active mediators of the heat shock response networks (Figure 3). A major player in this subnetwork is PfHslV (PF3D7_1230400, also known as ClpQ). This ATP-dependent protease is of prokaryotic origin, and does not have homolog in the human host; therefore its inhibitor may have little side effect, a desirable feature as a promising drug target. It has three association partners, including ATP-dependent protease ATPase subunit ClpY (ClpY), a putative mitochondrial GrpE protein homolog, (MGE1), and another putative ATP-dependent protease (PF3D7_1414900). This protease-mediated stress response network also includes two other heat shock response proteases, an organelle processing peptidase, a leucyl aminopeptidase, and aminopeptidase P.

In summary, using a combined comparative genomic and network approaches, we showed that the malaria parasite possesses a rich repertoire of heat shock proteins that constitute the heat shock response network. This network is an adaptive system for the parasite to coordinate a wide variety of cellular processes from stress response, signal transduction, metabolic regulation, parasite invasion, to pathogenesis. The central components in this network may serve as novel drug and vaccine targets.

Acknowledgement

We thank PlasmoDB for providing access to malaria omic data. This work is supported by NIH grants GM100806, GM081068 and AI080579 to YW. This work received computational support from Computational System Biology Core, funded by the National Institute on Minority Health and Health Disparities (G12MD007591) from the National Institutes of Health.

Biographical notes

Timothy G. Lilburn received his Ph.D. in Microbiology from University of British Columbia, Canada. His research interests include evolution of biological systems and linking genotype to the virulence phenotype.

Hong Cai received his Ph.D. in Electrical Engineering from University of Texas at San Antonio (UTSA). Currently he is a research assistant professor at UTSA.

Jianying Gu currently is an Assistant Professor at City University of New York. She received her Ph.D. in Bioinformatics and Computational Biology from Iowa State University.

Zhan Zhou received his Ph.D. in Biochemistry and Molecular Biology from Zhejiang University, China. He was a visiting student at UTSA. Currently he is a postdoc at Fudan University, China.

Yufeng Wang currently is an Associate Professor at University of Texas at San Antonio. She received her Ph.D. in Bioinformatics and Computational Biology from Iowa State University. Her research is focused on systems biology and molecular evolution of microbial pathogens.

REFERENCES

- ACHARYA P, KUMAR R, TATU U. Chaperoning a cellular upheaval in malaria: Heat shock proteins in *Plasmodium falciparum*. *Molecular and Biochemical Parasitology*. 2007; 153:85–94. [PubMed: 17307260]
- ASSENOV Y, RAMIREZ F, SCHELHORN SE, LENGAUER T, ALBRECHT M. Computing topological parameters of biological networks. *Bioinformatics*. 2008; 24:282–4. [PubMed: 18006545]
- BLACK CG, PROELLOKS NI, KATS LM, COOKE BM, MOHANDAS N, COPPEL RL. In vivo studies support the role of trafficking and cytoskeletal-binding motifs in the interaction of MESA with the membrane skeleton of *Plasmodium falciparum*-infected red blood cells. *Mol Biochem Parasitol*. 2008; 160:143–7. [PubMed: 18482775]
- BLACKMAN MJ. Malarial proteases and host cell egress: an ‘emerging’ cascade. *Cell Microbiol*. 2008; 10:1925–34. [PubMed: 18503638]
- 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:E5. [PubMed: 12929205]
- BOZDECH Z, MOK S, HU G, IMWONG M, JAIDEE A, RUSSELL B, GINSBURG H, NOSTEN F, DAY NP, WHITE NJ, CARLTON JM, PREISER PR. The transcriptome of *Plasmodium vivax* reveals divergence and diversity of transcriptional regulation in malaria parasites. *Proc Natl Acad Sci U S A*. 2008; 105:16290–5. [PubMed: 18852452]
- CARLTON JM, ADAMS JH, SILVA JC, BIDWELL SL, LORENZI H, CALER E, CRABTREE J, ANGIUOLI SV, MERINO EF, AMEDEO P, CHENG Q, COULSON RM, CRABB BS, DEL PORTILLO HA, ESSIE K, FELDBLYUM TV, FERNANDEZ-BECERRA C, GILSON PR, GUEYE AH, GUO X, KANG’A S, KOOIJ TW, KORSINCZKY M, MEYER EV, NENE V, PAULSEN I, WHITE O, RALPH SA, REN Q, SARGEANT TJ, SALZBERG SL, STOECKERT CJ, SULLIVAN SA, YAMAMOTO MM, HOFFMAN SL, WORTMAN JR, GARDNER MJ, GALINSKI MR, BARNWELL JW, FRASER-LIGGETT CM. Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. *Nature*. 2008; 455:757–63. [PubMed: 18843361]
- CARLTON JM, ANGIUOLI SV, SUH BB, KOOIJ TW, PERTEA M, SILVA JC, ERMOLAEVA MD, ALLEN JE, SELENGUT JD, KOO HL, PETERSON JD, POP M, KOSACK DS, SHUMWAY MF, BIDWELL SL, SHALLOM SJ, VAN AKEN SE, RIEDMULLER SB, FELDBLYUM TV, CHO JK, QUACKENBUSH J, SEDEGAH M, SHOAIBI A, CUMMINGS LM, FLORENS L, YATES JR, RAINE JD, SINDEN RE, HARRIS MA, CUNNINGHAM DA, PREISER PR, BERGMAN LW, VAIDYA AB, VAN LIN LH, JANSE CJ, WATERS AP, SMITH HO, WHITE OR, SALZBERG SL, VENTER JC, FRASER CM, HOFFMAN SL, GARDNER MJ, CARUCCI DJ. Genome sequence and comparative analysis of the model rodent malaria parasite *Plasmodium yoelii yoelii*. *Nature*. 2002; 419:512–519. [PubMed: 12368865]
- COWEN LE, LINDQUIST S. Hsp90 potentiates the rapid evolution of new traits: drug resistance in diverse fungi. *Science*. 2005; 309:2185–9. [PubMed: 16195452]
- DHARIA NV, SIDHU AB, CASSERA MB, WESTENBERGER SJ, BOPP SE, EASTMAN RT, PLOUFFE D, BATALOV S, PARK DJ, VOLKMAN SK, WIRTH DF, ZHOU Y, FIDOCK DA, WINZELER EA. Use of high-density tiling microarrays to identify mutations globally and elucidate mechanisms of drug resistance in *Plasmodium falciparum*. *Genome Biol*. 2009; 10:R21. [PubMed: 19216790]
- DI TOMMASO P, MORETTI S, XENARIOS I, OROBITG M, MONTANYOLA A, CHANG JM, TALY JF, NOTREDAME C. T-Coffee: a web server for the multiple sequence alignment of protein and RNA sequences using structural information and homology extension. *Nucleic Acids Res*. 2011; 39:W13–7. [PubMed: 21558174]
- FLORENS L, LIU X, WANG YF, YANG SG, SCHWARTZ O, PEGLAR M, CARUCCI DJ, YATES JR, WU YM. Proteomics approach reveals novel proteins on the surface of malaria-infected erythrocytes. *Molecular and Biochemical Parasitology*. 2004; 135:1–11. [PubMed: 15287581]
- FLORENS L, WASHBURN MP, RAINE JD, ANTHONY RM, GRAINGER M, HAYNES JD, MOCH JK, MUSTER N, SACCI JB, TABB DL, WITNEY AA, WOLTERS D, WU YM,

- GARDNER MJ, HOLDER AA, SINDEN RE, YATES JR, CARUCCI DJ. A proteomic view of the *Plasmodium falciparum* life cycle. *Nature*. 2002; 419:520–526. [PubMed: 12368866]
- FRANCESCHINI A, SZKLARCZYK D, FRANKILD S, KUHN M, SIMONOVIC M, ROTH A, LIN J, MINGUEZ P, BORK P, VON MERING C, JENSEN LJ. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res*. 2013; 41:D808–15. [PubMed: 23203871]
- GAHTORI P, GHOSH SK, PARIDA P, PRAKASH A, GOGOI K, BHAT HR, SINGH UP. Antimalarial evaluation and docking studies of hybrid phenylthiazolyl-1,3,5-triazine derivatives: a novel and potential antifolate lead for Pf-DHFR-TS inhibition. *Exp Parasitol*. 2012; 130:292–9. [PubMed: 22233734]
- GARDNER MJ, HALL N, FUNG E, WHITE O, BERRIMAN M, HYMAN RW, CARLTON JM, PAIN A, NELSON KE, BOWMAN S, PAULSEN IT, JAMES K, EISEN JA, RUTHERFORD K, SALZBERG SL, CRAIG A, KYES S, CHAN MS, NENE V, SHALLOM SJ, SUH B, PETERSON J, ANGIUOLI S, PERTEA M, ALLEN J, SELENGUT J, HAFT D, MATHER MW, VAIDYA AB, MARTIN DMA, FAIRLAMB AH, FRAUNHOLZ MJ, ROOS DS, RALPH SA, MCFADDEN GI, CUMMINGS LM, SUBRAMANIAN GM, MUNGALL C, VENTER JC, CARUCCI DJ, HOFFMAN SL, NEWBOLD C, DAVIS RW, FRASER CM, BARRELL B. Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature*. 2002; 419:498–511. [PubMed: 12368864]
- GINSBURG H. Progress in in silico functional genomics: the malaria Metabolic Pathways database. *Trends Parasitol*. 2006; 22:238–40. [PubMed: 16707276]
- GINSBURG H, TILLEY L. *Plasmodium falciparum* metabolic pathways (MPMP) project upgraded with a database of subcellular locations of gene products. *Trends Parasitol*. 2011
- GOLDBERG DE. Complex nature of malaria parasite hemoglobin degradation [corrected]. *Proc Natl Acad Sci U S A*. 2013; 110:5283–4. [PubMed: 23513214]
- LACOUNT DJ, VIGNALI M, CHETTIER R, PHANSALKAR A, BELL R, HESSELBERTH JR, SCHOENFELD LW, OTA I, SAHASRABUDHE S, KURSCHNER C, FIELDS S, HUGHES RE. A protein interaction network of the malaria parasite *Plasmodium falciparum*. *Nature*. 2005; 438:103–7. [PubMed: 16267556]
- LAKSHMANAN V, RHEE KY, DAILY JP. Metabolomics and malaria biology. *Mol Biochem Parasitol*. 2011; 175:104–11. [PubMed: 20970461]
- LARKIN MA, BLACKSHIELDS G, BROWN NP, CHENNA R, MCGETTIGAN PA, MCWILLIAM H, VALENTIN F, WALLACE IM, WILM A, LOPEZ R, THOMPSON JD, GIBSON TJ, HIGGINS DG. Clustal W and Clustal X version 2.0. *Bioinformatics*. 2007; 23:2947–8. [PubMed: 17846036]
- LASONDER E, ISHIHAMA Y, ANDERSEN JS, VERMUNT AMW, PAIN A, SAUERWEIN RW, ELING WMC, HALL N, WATERS AP, STUNNENBERG HG, MANN M. Analysis of the *Plasmodium falciparum* proteome by high-accuracy mass spectrometry. *Nature*. 2002; 419:537–542. [PubMed: 12368870]
- LASONDER E, JANSE CJ, VAN GEMERT GJ, MAIR GR, VERMUNT AM, DOURADINHA BG, VAN NOORT V, HUYNEN MA, LUTY AJ, KROEZE H, KHAN SM, SAUERWEIN RW, WATERS AP, MANN M, STUNNENBERG HG. Proteomic profiling of *Plasmodium* sporozoite maturation identifies new proteins essential for parasite development and infectivity. *PLoS Pathog*. 2008; 4:e1000195. [PubMed: 18974882]
- LE ROCH KG, ZHOU Y, BLAIR PL, GRAINGER M, MOCH JK, HAYNES JD, DE LA VEGA P, HOLDER AA, BATALOV S, CARUCCI DJ, WINZELER EA. Discovery of gene function by expression profiling of the malaria parasite life cycle. *Science*. 2003; 301:1503–8. [PubMed: 12893887]
- MAERE S, HEYMANS K, KUIPER M. BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics*. 2005; 21:3448–9. [PubMed: 15972284]
- OTTO TD, WILINSKI D, ASSEFA S, KEANE TM, SARRY LR, BOHME U, LEMIEUX J, BARRELL B, PAIN A, BERRIMAN M, NEWBOLD C, LLINAS M. New insights into the blood-stage transcriptome of *Plasmodium falciparum* using RNA-Seq. *Mol Microbiol*. 2010; 76:12–24. [PubMed: 20141604]

- PAIN A, BOHME U, BERRY AE, MUNGALL K, FINN RD, JACKSON AP, MOURIER T, MISTRY J, PASINI EM, ASLETT MA, BALASUBRAMANIAM S, BORGWARDT K, BROOKS K, CARRET C, CARVER TJ, CHEREVACH I, CHILLINGWORTH T, CLARK TG, GALINSKI MR, HALL N, HARPER D, HARRIS D, HAUSER H, IVENS A, JANSSEN CS, KEANE T, LARKE N, LAPP S, MARTI M, MOULE S, MEYER IM, ORMOND D, PETERS N, SANDERS M, SANDERS S, SARGEANT TJ, SIMMONDS M, SMITH F, SQUARES R, THURSTON S, TIVEY AR, WALKER D, WHITE B, ZUIDERWIJK E, CHURCHER C, QUAIL MA, COWMAN AF, TURNER CM, RAJANDREAM MA, KOCKEN CH, THOMAS AW, NEWBOLD CI, BARRELL BG, BERRIMAN M. The genome of the simian and human malaria parasite *Plasmodium knowlesi*. *Nature*. 2008; 455:799–803. [PubMed: 18843368]
- PAVITHRA SR, KUMAR R, TATU U. Systems analysis of chaperone networks in the malarial parasite *Plasmodium falciparum*. *PLoS Comput Biol*. 2007; 3:1701–15. [PubMed: 17941702]
- 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. [PubMed: 20823846]
- ROSENTHAL PJ. Falcipains and other cysteine proteases of malaria parasites. *Adv Exp Med Biol*. 2011; 712:30–48. [PubMed: 21660657]
- SMOOT ME, ONO K, RUSCHEINSKI J, WANG PL, IDEKER T. Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics*. 2010; 27:431–2. [PubMed: 21149340]
- SUTHRAM S, SITTILER T, IDEKER T. The *Plasmodium* protein network diverges from those of other eukaryotes. *Nature*. 2005; 438:108–12. [PubMed: 16267557]
- TACHIBANA S, SULLIVAN SA, KAWAI S, NAKAMURA S, KIM HR, GOTO N, ARISUE N, PALACPAC NM, HONMA H, YAGI M, TOUGAN T, KATAKAI Y, KANEKO O, MITA T, KITA K, YASUTOMI Y, SUTTON PL, SHAKHBATYAN R, HORII T, YASUNAGA T, BARNWELL JW, ESCALANTE AA, CARLTON JM, TANABE K. *Plasmodium cynomolgi* genome sequences provide insight into *Plasmodium vivax* and the monkey malaria clade. *Nat Genet*. 2012; 44:1051–5. [PubMed: 22863735]
- TAMURA K, STECHER G, PETERSON D, FILIPSKI A, KUMAR S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol*. 2013; 30:2725–9. [PubMed: 24132122]
- TAN JC, MILLER BA, TAN A, PATEL JJ, CHEESEMAN IH, ANDERSON TJ, MANSKE M, MASLEN G, KWIATKOWSKI DP, FERDIG MT. An optimized microarray platform for assaying genomic variation in *Plasmodium falciparum* field populations. *Genome Biol*. 2011; 12:R35. [PubMed: 21477297]
- TARUN AS, PENG X, DUMPIT RF, OGATA Y, SILVARIVERA H, CAMARGO N, DALY TM, BERGMAN LW, KAPPE SH. A combined transcriptome and proteome survey of malaria parasite liver stages. *Proc Natl Acad Sci U S A*. 2008; 105:305–10. [PubMed: 18172196]
- YUTHAVONG Y, YUVANIYAMA J, CHITNUMSUB P, VANICHTANANKUL J, CHUSACULTANACHAI S, TARNCHOMPOO B, VILAIVAN T, KAMCHONWONGPAISAN S. Malarial (*Plasmodium falciparum*) dihydrofolate reductase-thymidylate synthase: structural basis for antifolate resistance and development of effective inhibitors. *Parasitology*. 2005; 130:249–59. [PubMed: 15796007]

Figure 1.

The proteins associated with Hsp90 subnetwork. Round nodes represent heat shock related proteins. The four Hsp90 orthologs are pfHSP90 (PF3D7_0708400), Grp94 (PF3D7_1222300), TRAP1a (PF3D7_1443900), and TRAP1b (PF3D7_1118200) and are highlighted with white labels. Color bar corresponds to the gene expression level (transcript levels of reads per kilobase of exon model per million mapped reads) at the 8-hour time point in the intraerythrocyte cycle (Otto et al., 2010).

Figure 2.

The proteins associated with Hsp70 subnetwork. The four Hsp70 orthologs (PF3D7_1134000, PF3D7_0818900, PF3D7_0917900, and PF3D7_0831700) are labelled with white backgrounds. Color bar corresponds to the gene expression level (transcript levels of reads per kilobase of exon model per million mapped reads) at the 8-hour time point in the intraerythrocyte cycle (Otto et al., 2010). The white node is a knob-associated histidine-rich protein that is highly expressed at this stage. There is no expression data for the black node.

Figure 3.

The proteins associated with heat shock proteases. Heat shock proteases are diamond-shaped nodes, other heat shock-related proteins are represented by round nodes. Color bar corresponds to the gene expression level (transcript levels of reads per kilobase of exon model per million mapped reads) at the 8-hour time point in the intraerythrocyte cycle (Otto et al., 2010).

Table 1Heat shock proteins in *P. falciparum* with protein-protein associations.

ID	Degree	Annotation
PF3D7_1143200	73	DnaJ protein, putative
PF3D7_0102200	45	ring-infected erythrocyte surface antigen (RESA)
PF3D7_0306800	43	T-complex protein beta subunit, putative
PF3D7_0708800	42	heat shock protein 110 (HSP110c)
PF3D7_0308200	41	TCP-1/cpn60 chaperonin family, putative
PF3D7_1134000	36	heat shock protein 70 (Hsp70-3)
PF3D7_1453700	35	co-chaperone p23 (P23)
PF3D7_1224500	34	histone chaperone ASF1, putative (ASF1)
PF3D7_0501100.2	31	heat shock protein 40, type II (HSP40)
PF3D7_1229500	31	T-complex protein 1, gamma subunit, putative
PF3D7_0322000	27	peptidyl-prolyl cis-trans isomerase (CYP19A)
PF3D7_1344200	26	heat shock protein 110, putative (HSP110)
PF3D7_1149600	26	DnaJ protein, putative
PF3D7_1132200	25	TCP-1/cpn60 chaperonin family, putative
PF3D7_0608700	25	chaperone, putative
PF3D7_1149200	24	ring-infected erythrocyte surface antigen
PF3D7_0220100	22	DnaJ protein, putative
PF3D7_1434300	21	Hsp70/Hsp90 organizing protein (HOP)
PF3D7_0320300	21	T-complex protein 1 epsilon subunit, putative
PF3D7_1357800	21	TCP-1/cpn60 chaperonin family, putative
PF3D7_1215300	20	10 kDa chaperonin (CPN10)
PF3D7_1310800	20	conserved Plasmodium protein, unknown function
PF3D7_0818900	19	heat shock protein 70 (HSP70)
PF3D7_0708400	18	heat shock protein 90 (HSP90)
PF3D7_0113700	18	heat shock protein 40, type II (HSP40)
PF3D7_1232100	18	60 kDa chaperonin (CPN60)
PF3D7_1124700	17	GrpE protein homolog,

ID	Degree	Annotation
		mitochondrial, putative (MGE1)
PF3D7_1437900	16	HSP40, subfamily A, putative
PF3D7_1116800	16	heat shock protein 101 (HSP101)
PF3D7_0917900	16	heat shock protein 70 (HSP70-2)
PF3D7_0213100	16	heat shock protein 40, putative
PF3D7_0324000	16	RESA-like protein
PF3D7_0214000	16	T-complex protein 1, putative
PF3D7_1416900	15	prefoldin subunit 2, putative
PF3D7_1216900	14	DNA-binding chaperone, putative
PF3D7_1318800	14	secretory complex protein 63 (SEC63)
PF3D7_1101800	13	RESA-like protein
PF3D7_0512000	12	prefoldin subunit, putative
PF3D7_0718500	12	prefoldin subunit 3, putative
PF3D7_0904500	12	prefoldin subunit, putative
PF3D7_1128100	12	cochaperone prefoldin complex subunit, putative
PF3D7_1443900	11	heat shock protein 90, putative (HSP90)
PF3D7_0308500	11	activator of Hsp90 ATPase, putative
PF3D7_1118200	10	heat shock protein 90, putative
PF3D7_1015600	10	heat shock protein 60 (HSP60)
PF3D7_0101400	10	RESA-like protein, pseudogene
PF3D7_0831700	9	heat shock protein 70 (HSP70-x)
PF3D7_1038800	8	RESA-like protein with PHIST and DnaJ domains
PF3D7_1333000	7	20 kDa chaperonin (CPN20)
PF3D7_0708600	6	heat shock protein 86 family protein
PF3D7_1039600	6	RESA-like protein, pseudogene
PF3D7_1423200	6	peptidyl-prolyl cis-trans isomerase (CYP52)
PF3D7_0201800	5	knob associated heat shock protein 40 (KAHsp40)
PF3D7_0724400	5	mitochondrial import

ID	Degree	Annotation
		inner membrane translocase subunit tim14, putative (PAM18)
PF3D7_0816600	5	ClpB protein, putative (ClpB1)
PF3D7_1239700	5	cell division protein FtsH, putative
PF3D7_1304500	4	small heat shock protein, putative
PF3D7_0629200	4	DnaJ protein, putative
PF3D7_1406600	4	ATP-dependent Clp protease, putative (ClpC)
PF3D7_1355500	4	serine/threonine protein phosphatase (PP5)
PF3D7_0424600	4	Plasmodium exported protein (PHISTb), unknown function
PF3D7_0907400	4	ATP-dependent protease ATPase subunit ClpY (ClpY)
PF3D7_0114000	4	RESA-like protein with DnaJ domain, putative (GEXP06)
PF3D7_0409400	3	heat shock protein 40 (DnaJ)
PF3D7_1211400	3	heat shock protein DNAJ homologue Pfj4 (Pfj4)
PF3D7_0831000	3	Plasmodium exported protein (PHISTb), unknown function (GEXP09)
PF3D7_0500800	3	mature parasite-infected erythrocyte surface antigen, erythrocyte membrane protein 2 (MESA)
PF3D7_1247400	3	FK506-binding protein (FKBP)-type peptidyl-prolyl isomerase (FKBP35)
PF3D7_1201100	3	RESA-like protein with PHIST and DnaJ domains
PF3D7_1410000	3	tetratricopeptide repeat family protein, putative
PF3D7_1102200	3	DnaJ protein, putative
PF3D7_1222300	3	endoplasmic, putative (GRP94)
PF3D7_1230400	3	ATP-dependent protease subunit ClpQ (ClpQ)
PF3D7_1139500.2	3	AAA family ATPase, putative
PF3D7_0220400	3	DnaJ protein, putative, pseudogene
PF3D7_0907300	3	conserved Plasmodium protein, unknown function

ID	Degree	Annotation
PF3D7_0528700	3	peptidyl-prolyl cis-trans isomerase (CYP23)
PF3D7_0307400	3	ATP-dependent Clp protease proteolytic subunit (ClpP)
PF3D7_1414900	3	ATP-dependent protease, putative
PF3D7_1406400	3	PPR repeat protein (PPR)
PF3D7_1320900	2	RNA-binding protein, putative
PF3D7_1108700	2	heat shock protein DnaJ homologue Pfj2
PF3D7_1401100	2	DnaJ protein, putative
PF3D7_0631000	2	Tetratricopeptide repeat protein, putative
PF3D7_1119600	2	peptidase, putative
PF3D7_0306200	1	activator of Hsp90 ATPase (AHA1)
PF3D7_1413900	1	DnaJ protein, putative
PF3D7_1215200	1	peptidyl-prolyl cis-trans isomerase (CYP32)
PF3D7_0527500	1	Hsc70-interacting protein (HIP)
PF3D7_0601600	1	Tetratricopeptide repeat protein, putative
PF3D7_1002800	1	DnaJ protein, putative
PF3D7_1307200	1	DnaJ protein, putative
PF3D7_0201700	1	DnaJ protein, putative
PF3D7_1115600	1	peptidyl-prolyl cis-trans isomerase (CYP19B)
PF3D7_1005600	1	DnaJ protein, putative
PF3D7_0708500	1	heat shock protein 86 family protein
PF3D7_0919100	1	DnaJ protein, putative
PF3D7_1120900	1	heat shock factor binding protein 1, putative
PF3D7_1039100	1	DnaJ protein, putative, pseudogene
PF3D7_1436800	1	ATP-dependent Clp protease proteolytic subunit (ClpR)