Chapter

Protein-Protein Interactions in Malaria: Emerging Arena for Future Chemotherapeutics

Rahul Pasupureddy, Sriram Seshadri, Rajnikant Dixit and Kailash C. Pandey

Abstract

Malaria is one of the most deadly diseases infecting humans. Advances in elimination and vector control have reduced the global malaria burden in the past decade; however, the emerging threat of drug resistance and suboptimal vaccine efficacies threaten global eradication efforts. Unlocking novel drug and vaccine targets while simultaneously mitigating spread of resistant strains seems to be the need of the hour. Protein-protein interactions (PPIs), an integral part of host-pathogen cross-talk and parasite survival, have only recently emerged as promising drug targets. Large PPI networks (interactome) are being developed to better our understanding of various parasite biochemical pathways. In this chapter, we throw light on several newly characterized protein-protein interactions between the host (humans) and parasite (plasmodium) in key processes such as hemoglobin degradation, enzyme regulation, protein export, egress, invasion, and drug resistance and further discuss their viability for development as novel chemotherapeutic targets.

Keywords: malaria, proteases, drug resistance, protein-protein interactions, host-parasite interactions, interactome

1. Introduction

Malaria is one of the deadliest diseases to affect humans, with the latest WHO reports indicating ~445,000 deaths in 2017 alone [1]. More alarmingly, despite decades of advances in controlling the malaria epidemic, death rates caused by malaria seem to have plateaued in the past 3 years, indicating drug resistance and re-emergence. Drug resistance to the current frontline antimalarials have been confirmed by many recent studies and steadily observed over increasing geographic coverage [2]. Thus, it is of utmost importance to develop novel antimalarials, with different modes of action and distinct targets, if possible in conjunction, to check the onslaught of malaria. This chapter looks at such potential scope for antimalarial drug development: disruption of protein-protein interactions in the malaria parasite *Plasmodium*, crucial for survival and proliferation.

2. PPIs: the basics

Protein-protein interactions (PPIs) constitute the fundamental backbone required for occurrence of any biological event. They are defined as the residue level interactions between either the same protein (dimers, trimers, or other multimers) or diverse proteins (protein complexes). These basic interactions are necessary for a myriad of functions such as kinase signaling, receptor binding, proteolytic digestion, apoptosis regulation, and antigen-antibody interactions [3, 4]. Disruptions in the protein interaction networks (PINs) as a result of PPI inhibition have been shown to cause several diseases where either single or multiple biochemical pathways are affected [5]. Owing to their fundamental roles in almost every process imaginable, PPIs have emerged as attractive therapeutic targets in several diseases. Several forms of cancer were also shown to have dysregulated protein interaction networks (PINs) [6]. Similarly, PPI disruptions have been observed in several autoimmune as well as parasitic diseases. Small peptides that infiltrate cellular defenses and specifically bind to target structures are already in development. Taken together, targeting PPIs though challenging can provide a novel understanding of biochemical processes as well as uncover new ways to combat diseases like malaria.

PPIs can be generally categorized into several groups depending on their function or the type of interactions. They include internal (hot-spots) or external (surface), obligate (permanent) or non-obligate (transient), stabilizing or destabilizing, ability to induce conformational changes in either of the partner molecules, peptide-protein or peptide-peptide interactions, and contiguous or discontiguous epitope binding [7] (**Figure 1**).

Some types of PPIs such as membrane PPIs can be difficult to characterize. While dedicated techniques like the split-ubiquitin membrane yeast two-hybrid (MYTH) system were developed to specifically detect membrane protein interactions [8], these techniques are still considered time-consuming and labor-intensive. Such bottlenecks make it hard to generate a complete picture of the membrane interactome. Even for reliable bioinformatic models for detection of membrane

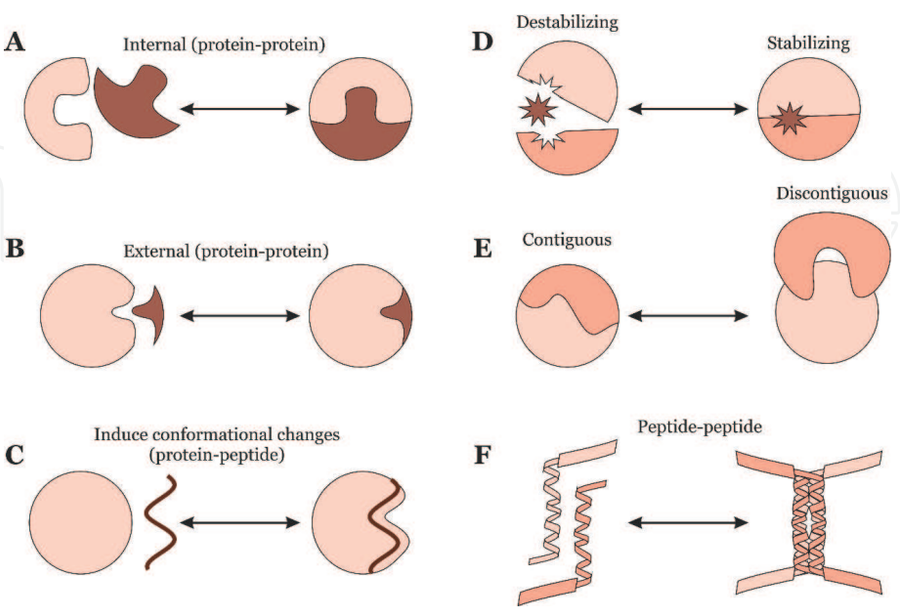

Figure 1.
Categorization of PPIs based on types of interactions. (A) Internal, where the site of interaction lies buried inside such as "hot-spots", (B) external, where proteins interact at the surface, (C) where a peptide could induce conformational changes upon binding, (D) interactions can be stabilized or dissociated based on the type of cofactor/compound binding, (E) whether the epitope binding is contiguous or not, and (F) if both interacting partners are peptides/small molecules.

PPIs to be developed, there need to be large sets of positive, false-positive, as well as negative data to accurately train such models, which are currently unavailable for membrane PPIs [9]. Thus, decoding membrane PPIs even through bioinformatic approaches remains challenging.

3. PPIs in malaria

Malaria traditionally has been treated using inhibitors which target the broad spectrum proteasome offering several advantages as compared to specific protein inhibitors. Specific inhibitors had comparatively low efficacy in vivo. Also, inhibitors targeting a specific protein/ligand could potentially inhibit parasite growth only in stages when the target proteins are expressed. Broad spectrum antimalarials, such as the current frontline drugs artemisinins (ARTs) and their combination therapies (ACTs), target and break down various cellular pathways including but not limited to hemozoin formation, DNA repair, and mitochondria machinery, which make them highly potent within short exposure times [10, 11]. However, exposure to various cellular targets leads to the rapid development of drug resistance. While resistance to chloroquine worldwide was observed after ~40 years of continued use, resistance to ARTs was achieved in a relatively short span of a decade, from its inception in late 1990s to the first reported resistance in 2008 [12]. While this rapid emergence resistance was partially attributed to suboptimal drug regimens and poor administrative practices, the same could be attributed to earlier drugs as well. Thus, compounds that are specific/flexible to the target protein are the need of the hour. This section deals with and summarizes current knowledge about crucial PPIs in various biochemical pathways of the malaria parasite *Plasmodium falciparum*.

3.1 Hemoglobin hydrolysis

Hemoglobin hydrolysis is one of the most targeted pathways for treatment as it is fundamental for parasite survival and involves numerous proteins [13]. Majority of earlier and currently used drugs disrupt multiple protein interactions. Several studies have been conducted recently that target individual PPIs and design inhibitors based on those interactions. Our lab has previously identified a "hot-spot" region in falcipains, the principal hemoglobinases of *P. falciparum* [14]. Falcipains contain a pro and a mature domain, with the pro domain bound to and blocking access to the active site in the mature domain. The interactions between these two domains, termed "hot-spot" interactions, dissociate under acidic conditions and are essential for hemoglobinase activity. This specific hot-spot was identified at the interface of pro and mature domains in falcipain-2 (FP2) and falcipain-3 (FP3). The study further demonstrated that synthetic compounds, NA01 and NA03, specifically bound to this hot-spot region and stabilized these interactions. Thus, even in the presence of an acidic environment (pH - 5.5), pro-mature domains remained intact, rendering them inactive [15].

Falcipains, owing to their crucial role in Hb degradation, are considered as attractive chemotherapeutic targets. Several inhibitors were designed based on the interactions of the FP2 and the active site inhibitor E64. Molecular dynamics (MD) simulations indicated that two sets of residues, namely, recruiter groups A (rgA) and B (rgB) (rgA (D170, Q171, C168, G169, A151, and G230); rgB (K76, N77, and N81)) of FP2 are primarily involved in the initial binding with E64 about 80% and 14% of the time, respectively, before finally proceeding to bind with the active site residues [16]. Efforts elsewhere have focused on selective inhibition of falcipains rather than indiscriminate inhibition including its human host cathepsin isoforms.

While the S1 and S3 subsite residues have been conserved across *Plasmodium* and human cysteine proteases, the P2 residue in the S2 pocket in falcipains contained an acidic amino acid (Asp234 in FP2 and Glu243 in FP3) as compared to neutral residues in cathepsins (Leu209 in Cat-K, Ala215 in Cat-L, and Phe211 in Cat-S). A class of peptidomimetic amino-nitrile compounds known to inhibit cysteine proteases was further engineered to exploit the P2-region charge differences and inhibit falcipains specifically [17, 18].

Falcipains also contain a domain at their C-terminal called the hemoglobin binding domain (Hb domain), a β-hairpin loop which protrudes away from the active site. Deletion of this 14 amino acid domain ablated the ability of falcipains to degrade Hb, thus indicating a necessary role of this domain in Hb capture prior to degradation at the active site [19]. Our lab recently published another study that identified crucial protease-substrate PPIs within this domain. A functionally conserved single amino acid position in both falcipains (Glu185 in FP2 and Asp194 in FP3) was found to be essential for Hb interactions, with activated falcipain mutants unable to degrade Hb even with accessibility to the active site. Molecular docking results indicated both the residues interacted with Hb-α as well as Hb-β subunits with interactions mediated primarily through this position (**Figure 2**). A specific inhibitor which could target this position could have potential applications in arresting the parasite hemoglobin degradome [20].

Hemoglobin degradation as a source for parasite growth was also shown to be dependent on the hemoglobin tetramer composition. Children (<5 years) have different Hb subunits (HbF, $\alpha_2\gamma_2$) as compared to adults (HbA, $\alpha_2\beta_2$), and malaria mortality rates have consistently indicated child mortality to be higher (61% of

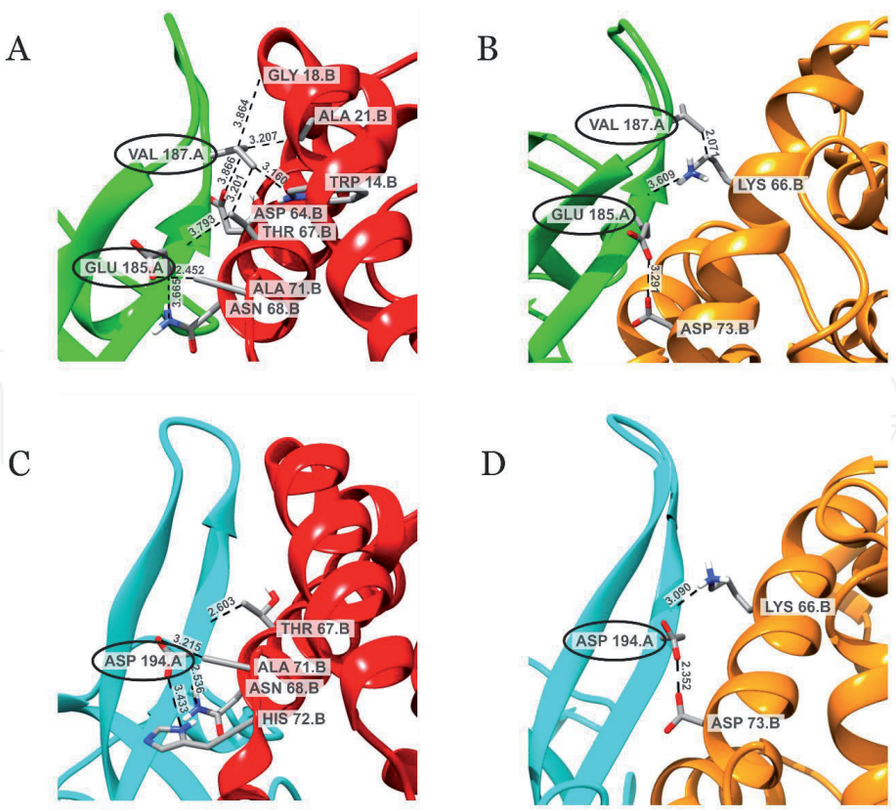

Figure 2.
Interactions of falcipains (FP2, FP3) with Hb (α, β) chains. A residue level interaction map of the interactions between Hb-binding motif of FP2 (green) with Hb α chain (a; red) and Hb β chain (B; orange) of hemoglobin. Similarly, a view of interactions between C-terminus Hb-binding motif of FP3 (blue) with Hb α chain (C; red) and Hb β chain (D; orange). Bond lengths of interactions have been indicated in angstrom units (Å). Adapted and modified from [20].

deaths in 2017) [20]. The essential amino acid isoleucine (I) was found to be a main differentiating factor as it is absent in both α and β chains but present in γ subunit and makes up to 99% of encoded proteins in *P. falciparum* proteome [21]. Thus, compounds that interfered with the isoleucine acquiring pathways, such as targeting the two *P. falciparum* isoleucyl tRNA synthetases (IRSs), have been described. The compound thiaisoleucine, where γ-methylene of isoleucine was substituted by a sulfur atom, was found to potently inhibit cytosolic IRS, while another compound, mupirocin, a known inhibitor of methicillin-resistant *S. aureus* (MRSA) IRS, inhibited the apicoplast-localized IRS [22]. Of the 36 putative aminoacyl tRNA synthetases present in *P. falciparum*, nearly 5 have been targeted with potent inhibitors, and more are being tested [23, 24].

3.2 Invasion/egress

Both invasion and egress are important events in the erythrocytic stage and are responsible for the malaria symptoms including chills and fever. The process of invasion requires a host of proteins to be secreted from its apical organelles including rhoptry bodies and micronemes, among others, and is precisely coordinated. The parasite initially aligns the merozoite apical region toward the host erythrocyte and forms a tight junction at the apex, progressing as the moving junction (MJ) pushes the parasite into the erythrocyte, with the erythrocyte surface forming a ring around the engorged parasite, which would later become the parasitophorous vacuolar membrane (PVM) [25].

While the process of moving junction (MJ) formation and important players involved in the process were well elucidated in *P. falciparum*, the other major causative agent of malaria, *P. vivax*, remains poorly understood, partially owing to difficulties in culturing *P. vivax* in vitro. Recombinant *P. vivax* rhoptry neck protein 2 (PvRON2), based on literature evidence of involvement of PfRON2 in MJ formation and invasion, showed that both rhoptry proteins PvRON2 and PvRON4 bound preferentially to CD71-labeled human reticulocytes rather than normocytes [26]. More importantly, the cysteine-rich C-terminal PvRON2 region strongly interacts with PvAMA1 domains II and III, similar to earlier reports of PfRON2-PfAMA1 interactions [27].

The *P. falciparum* genome encodes 10 aspartic proteases termed plasmepsins, a few of which including PMIX and PMX have remained functionally uncharacterized until recently. Conditional knockdown (KD) using TetR-aptamer regulators inserted at PMIX and PMX loci using CRISPR-Cas9 gene editing tools indicated a drastic decrease in parasite that could egress in PMIX-KD and role of PMX-KD in both invasion and egress. Further, PMX was found to be involved in the processing of a semi-proenzyme PfSUB1 to mature protein, a step crucial in both egress and invasion and also in the final processing step of a known egress protein, SERA5 [28]. Another study was simultaneously published that attempted to inhibit the activity of both PfPMIX and PfPMX. A hydroxyl-ethyl-amine (HEA) scaffold-based compound, 49c, was shown to potently inhibit both the proteases in vitro and in vivo at nanomolar concentrations and reiterated the role of these two proteases in egress and invasion [29]. MD simulations indicated that the flap tip and hinge regions present in both the plasmepsins were very well stabilized by the rigid structure of compound 49c, leading to higher-binding free energy as compared to control plasmepsin inhibitor pepstatin [30].

For a *Plasmodium* parasite to successfully invade different types of cells at different life stages, it must display multiple families of receptor molecules on its surface to perform various functions such as gliding and traversal between hepatocyte cells or other motile functions. To successfully egress from erythrocytes,

the parasite expresses a merozoite-thrombospondin-related anonymous protein (MTRAP) which interacts with the tetrameric glycolytic enzyme, fructose-1,6-bisphosphate aldolase, for powering the actomyosin motors required for movement [31]. However, continuous detachment and reattachment of these two enzymes are required for proper gliding motility. Therefore, compounds have been described that specifically stabilize MTRAP-aldolase interactions thereby rendering the parasite immotile. Of the 400 Medicines for Malaria Venture's (MMV) Malaria Box inhibitor library, a single compound (C4) was found to significantly inhibit hepatocyte invasion and led to abnormal gliding movements in a dose-dependent manner. A structure of C4 bound to the TRAP-aldolase complex could provide better insight into the key interactions of the inhibitor and help design more potent compounds [31].

3.3 Protein export

The protein export element (PEXEL) comprising of the conserved sequence RxLxE/Q/D is found in the N-terminus of ~300 proteins bound for export in the *Plasmodium* proteome. The aspartic protease plasmepsin V (PMV) was shown to be the sole protease involved in the cleavage of the PEXEL domain at the ER, specifically cleaving after the leucine residue (RxL$^{\downarrow}$xE/D/Q). The first, third, and fifth conserved positions were thoroughly probed for role in protease activity and showed that the first Arg and third Leu are essential for PMV recognition and cleavage, while the fifth E/Q/D position was shown not to be essential for cleavage but for trafficking out of PVM [32]. Owing to the importance of PMV, peptidomimetic compounds that resembled PEXEL were developed. While statin-based compounds were the traditional inhibitors of aspartic proteases, inhibitors based on HEA moiety were also shown to be strong inhibitors of aspartic proteases. A compound named LG20 consisted of PEXEL-like motif R-L-[L-HEA-A]-E-A, where the L-HEA-A mimics the aspartic protease transition state, while HEA motif is proteolytically uncleavable by PMV. Overall, the compound successfully inhibited PMV activity with concentrations in the picomolar range [33].

PfEMP1 is one the most studied exported protein as it is one of the exported in abundant quantities to the outer surface and thus is an attractive target along with others such as circumsporozoite protein (CSP). Immunoprecipitation (IP) and mass spectrometry studies of a GFP-tagged minimal section of PfEMP1 (PfEMP1B) identified novel targets in different cellular components including parasitophorous vacuole (PV), Maurer's clefts, the plasmodium translocon of exported proteins (PTEX) translocon, and a novel exported protein-interacting complex (EPIC). Several new interacting partners including parasitophorous vacuole protein-1 (PV1), PV2, and exported protein-3 (EXP3) have been identified, all of whom localized to the newly described EPIC [34]. Finally, a comprehensive pathway of PfEMP1 export has been suggested, where PfEMP1 is initially translocated to the ER and then trafficked to PTEX machinery and out of PVM with the aid of EPIC, where finally it is received by host erythrocytic chaperonin complex, the TCP-1 ring complex (TRiC), and transported to erythrocyte surface [34].

IP assays coupled with truncated-construct interaction assays have helped identify few prominent PMV-partner PPIs. The *P. falciparum* ortholog of signal peptidase complex 25 (PfSPC25) was found to interact with PMV as well as PfSec61 and PfSec62 translocon. Together, the PfSPC25-PfSec61-PfSec62 interactome along with PMV was found to regulate the entry of the effector cargo into the ER [35]. Apart from export, protein-protein interactions during autophagy, where different cellular components are engulfed and fused with vacuoles or lysozymes, can be considered as important PPI targets. The *P.*

falciparum autophagy-related protein 8 (PfAtg8) upon activation binds to PfAtg3 through a thioester bond, and this complex promotes the membrane assembly for the formation of autophagosomes. Solved crystal structure of the PfAtg3-PfAtg8 complex identified an additional region, called the A-loop, having very low sequence similarity to that of humans, thus providing a new avenue for drug development [36]. MD simulations revealed several crucial PPIs including H-bonds, van der Walls contacts, and other electrostatic interactions that are crucial for their interactions. Crucially, a peptidomimetic compound mimicking the PfAtg3 segment WLLP that interacts with PfAtg8 was identified to achieve maximum potency [37].

3.4 PPIs mediating drug resistance

The emerging threat of resistance to the current frontline drug artemisinin (ART), and its combination therapies (ACTs), is a cause of great concern. The mechanism of ART action, though generally agreed to generate free radical species which disrupt several essential pathways in the parasite, is highly debated. Immunoprecipitation studies with chemically tagged ART analogue (AP1) revealed that ART interacts with over a dozen proteins and is predominantly activated by free heme rather than free ferrous ions. *P. falciparum* Kelch 13 (PfK13) was identified as a key marker of ART resistance in several studies, most notably by Ariey et al., 2008 [38]. The studies have identified residues in the six Kelch propeller domains that are responsible for crucial protein-protein interactions, with the mutants failing to interact with its substrates *P. falciparum* phosphatidylinositol-3-phosphate (PfPI3P) [39]. PfPI3P has been shown to be involved in the unfolded protein response (UPR) pathway, thus helping the parasite cope with the free radical-induced stress (**Figure 3**). Localization studies indicated the PfK13 to co-localize with the *P. falciparum* erythrocyte membrane protein (PfEMP1) and majorly concentrated at various proteostasis system in the parasite including ER, cytoplasm, and the UPR. Specifically, PfK13 mutants were shown to elevate the PI3P-vesicle production and amplification, and their export throughout the parasite and into the infected erythrocyte [40].

Structural analysis and MD simulations of PfK13 indicated the presence of two evolutionarily highly conserved domains in the broad-complex, tramtrack, and bric à brac (BTB) domain and in the shallow binding pocket formed by the six propeller domain repeats. These domains displayed a different electrostatic surface potential unlike the rest of the bottom PfK13 face and are rich in highly conserved arginine and serine residues. While the BTB domain was shown to be involved in the recruitment of a scaffold protein Cullin, the propeller domain pocket binds to the substrate molecules for further ubiquitination. MD simulations showed that the validated PfK13 markers such as C580Y and R539T mutants induced a significant structural destabilization in the shallow pocket region as compared to wild type while maintaining the overall structural integrity. Specifically, the C580Y mutant disrupted a disulfide bridge (C532-C580) and additional H-bonds created with neighboring residues not present in wild type strains. In the case of R539T mutant, it was shown to have substantially less H-bond interactions along with a complete loss of a salt bridge interaction with E606 while also losing another inter-blade H-bond interaction [41]. Overall, these mutants lead to significantly diminished levels of functional protein which cause diminished substrate interaction, ultimately promoting the PfPI3P-mediated unfolded protein response pathway. Our own group, through Co-IP and mass spectrometric studies, has identified several novel proteins including Trx-like mero protein (PF3D7_1104400), pyridoxal kinase (PF3D7_0616000), trafficking protein particle complex subunit 3 (TRAPP), and

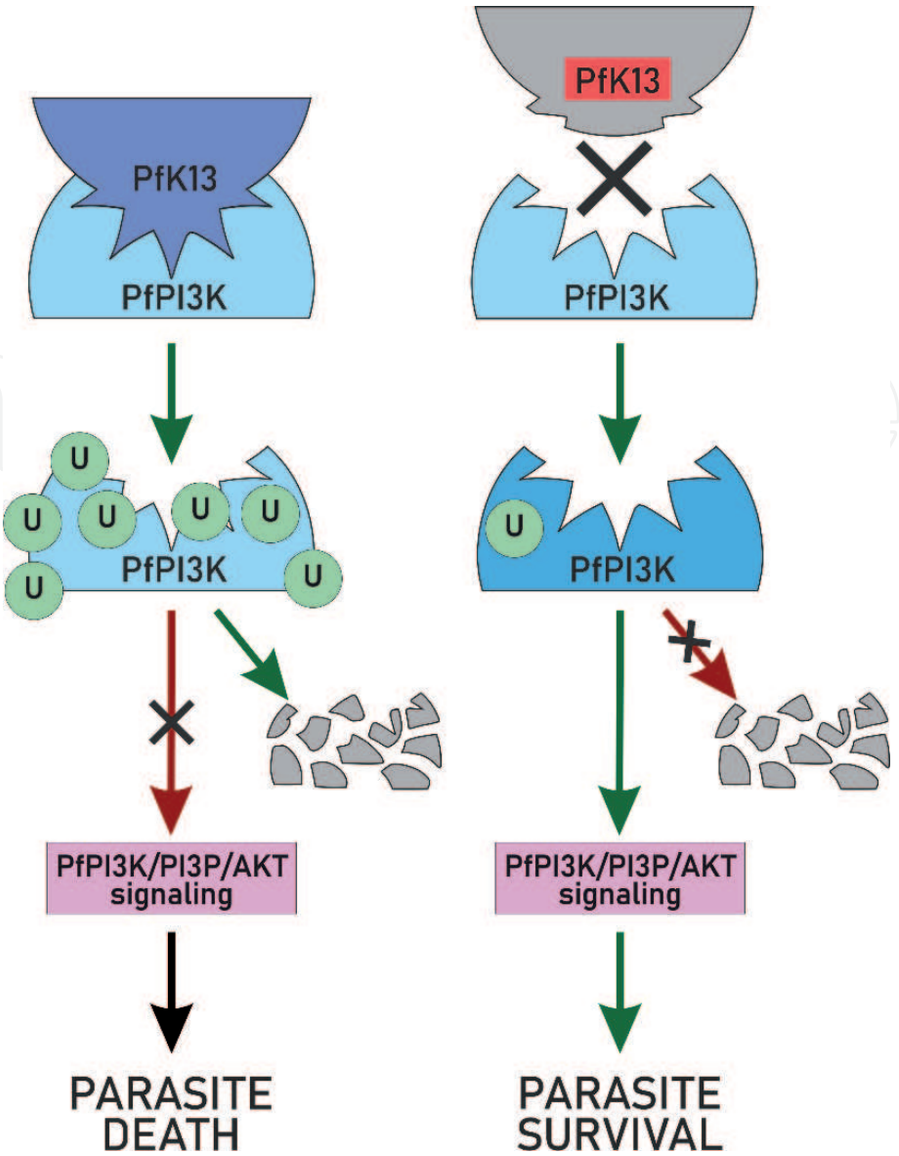

Figure 3.
Role of PfK13 in mediating ART resistance. In the presence of ART, wild PfK13 binds with PfPI3K, which results in PfPI3K ubiquitination and degradation, thus dysregulation of phosphoinositide signaling pathways ultimately leading to parasite death, while in ART-resistant strains, mutated PfK13 does not bind to PfPI3K initiating PI3P signaling thus promoting parasite survival. Adapted and modified from [39].

putative (PF3D7_0418500) that interact with PfK13 and could potentially have a role in stress-mediated response (Atul et al., 2019, unpublished).

4. PPI inhibition: peptidomimetics and design

While several strategies have been employed for PPI inhibition, bioinformatics-based drug design has been at the forefront to design specific PPI inhibitors. Structure-based drug design, where the solved structure of an enzyme in complex either with an inhibitor or a natural substrate was used to design inhibitors, was popularized in the 1990s. However, rational design for PPI inhibitors needs to overcome some common hurdles such as low proteolytic stability, analyzing extensive libraries of candidate molecules, and in some cases low ligand efficiency when compared to standard active site inhibitors. Thus, several strategies are employed in modern drug synthesis to overcome these problems. Short linear peptides tend to have lower conformational stability; thus cyclization of

the peptide is preferred which rigidifies the structure in an active configuration. Certain modifications in the backbone of the peptide such as backbone extension, side-chain shifting to nitrogen atoms (peptoids), and altering the stereochemistry can also be applied. Peptoids can easily fold into helices or other structures as they consist of repeated nitrogen-substituted glycine units that give an added advantage of mimicking the peptide structure and function. Stereochemistry of a compound can be changed by using D-peptides instead of L-peptides as they are more susceptible to proteolytic degradation and are one of the most common strategies to develop potent bioactive compounds. Another modification involves using β-peptides; peptides with amino group bound at β-carbon instead of α-carbon for each amino acid, often called as foldamers, could confer additional proteolytic stability both in vitro and in vivo. A class of oligopeptides (<80 residues in length) called as miniproteins can also be utilized as they have a rigid, well-defined three-dimensional structure. The 19 kDa fragment of merozoite surface protein 1 (MSP1), the fragment that is finally displayed on the merozoite surface after several processing steps, was fused along with a glycosylphosphatidylinositol (GPI) tag used to create a miniprotein, which was successfully targeted by antibodies specific to the miniprotein and inhibited erythrocyte invasion [42]. Substantial effort has been made to develop rational strategies in designing PPI inhibitors for target proteins that have no well-defined binding site (so-called "hot-spot") and, thus, have previously been considered undruggable (**Figure 4**).

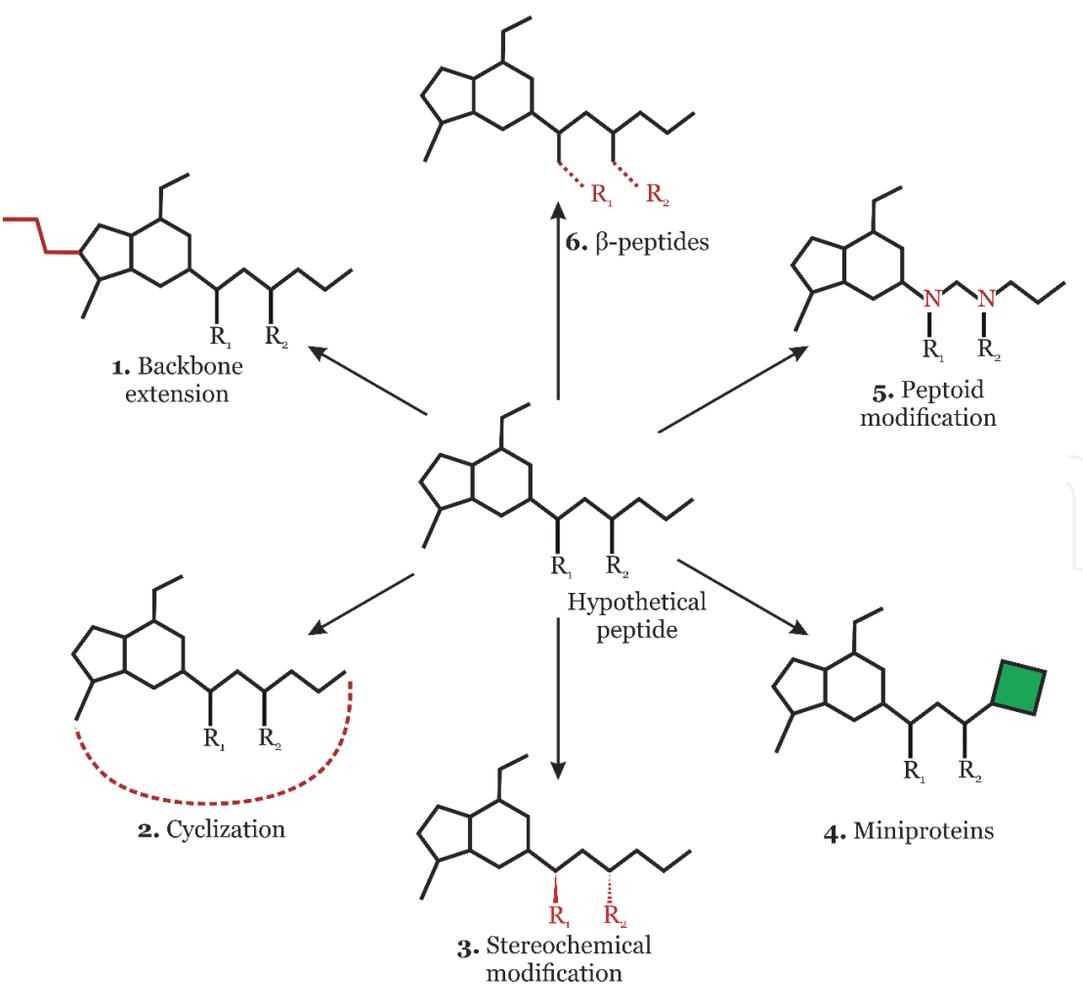

Figure 4.
Strategies for improving the potency of PPI inhibitors. Schematic showing the various ways peptides can be modified (highlighted in red) to achieve potency or improve bioavailability.

As described in the earlier section, Villa et al. designed and synthesized peptidomimetics belonging to 1,2,3-triazoles, specifically 1,4-disubstituted 1,2,3-triazoles. These compounds mimicked the contacts made by PfAtg3 template structure containing the residues W-L-L-P, as this template was shown to have the majority of interactions with PfAtg8. Of the four compounds synthesized, compound 2 (C2) exhibited prominent inhibition (IC_{50}–3.8 μM) in vitro, while C1 had better inhibitory effects in vivo [37].

Natural inhibitors of proteases are one of the best studies substrate groups in malaria as they are highly specific, stable, and reversible. *Plasmodium spp.* contain inhibitors of cysteine proteases (ICPs) and endogenous macromolecular inhibitors, which regulate the activity of cysteine proteases. Orthologs of ICPs are also observed in other apicomplexan groups such as *Trypanosoma cruzi*, whose ICP is called as chagasin [43]. Chagasin was shown to potently bind to the active site of both falcipains, FP2 and FP3. More importantly, three loop regions termed BC-, DE-, and FG loops

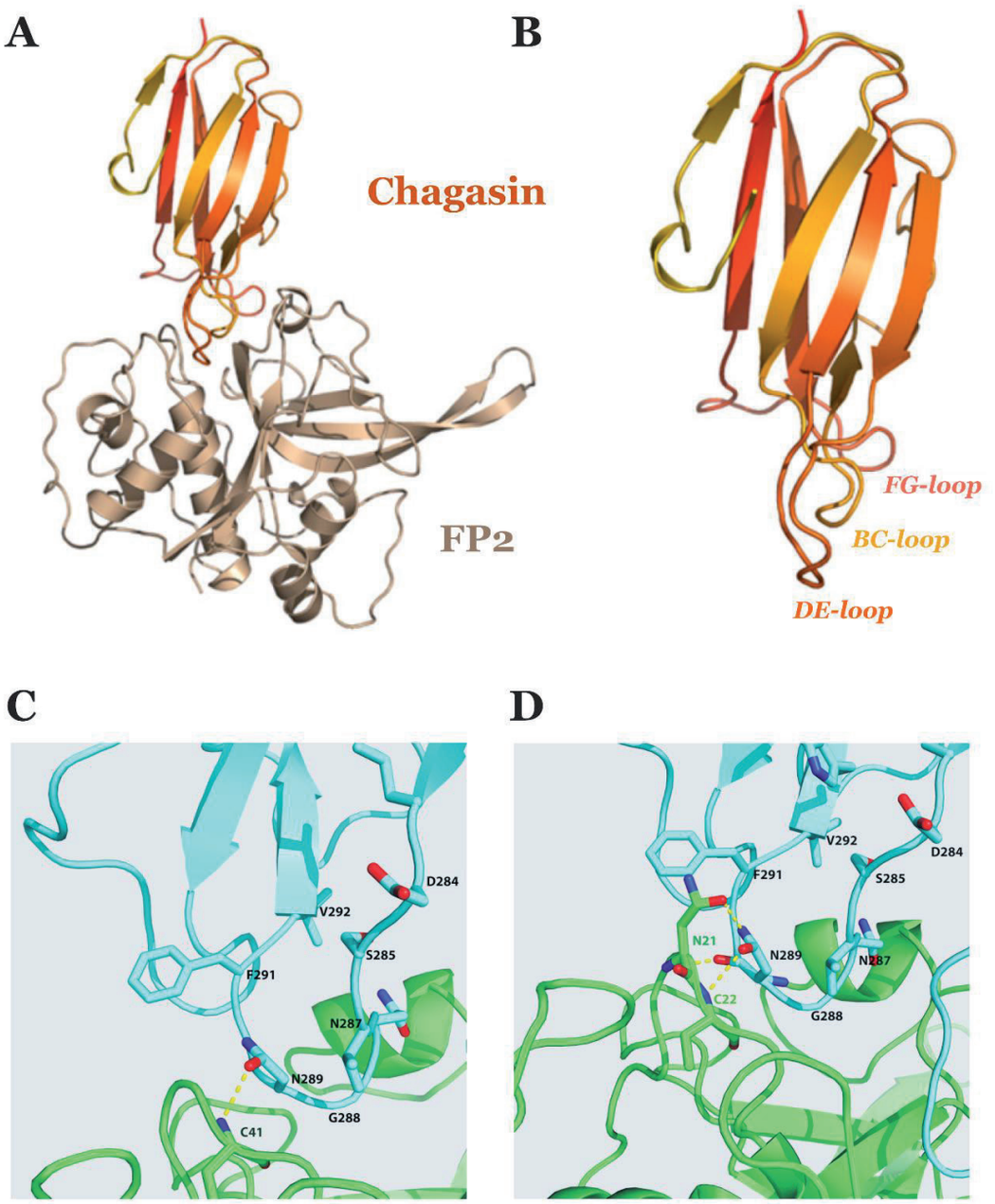

Figure 5.
Crucial interactions between falcipains and ICPs chagasin and falstatin. (A) Solved structure of FP2 and chagasin in that the ICP bound to the active site of falcipain and that (B) three loops BD, DE, and FG are involved in this interactions. (C, D) mutagenesis studies indicated that just the BC loop of falstatin was sufficient for inhibiting both FP2 (C) and FP3 (D), respectively. Adapted and modified from [44, 45].

were shown to be involved in active site inhibition (**Figure 5A,B**) [44]. However, further studies with the *P. falciparum* ICP, falstatin, indicated that unlike other ICPs, just the BC loop was sufficient for falcipain inhibition and that Asn289 of falstatin formed stabilizing hydrogen bonds and hydrophobic interactions (**Figure 5C,D**) [45].

5. Conclusion

Protein-protein interactions play roles of utmost importance in the growth and survival of any organism. Thus, focused targeting of such interactions specific to parasite can help produce robust and effective drugs. Recent research has indicated a renewed interest in targeting PPIs in the field of malaria. Various PPIs in pathways essential for parasite survival, erythrocyte invasion/egress, drug resistance, and others have been elucidated. These new classes of peptidomimetic compounds would form the future defense against an ever-increasing resistant parasite threat. Targeting PPIs offers several advantages over active site inhibition as 'hot-spots' are more flexible as compared to the active site and thus can be more selective in terms of drug interactions. In contrary to active site, the interactions at allosteric sites and exosites in an enzyme occur away from the active site; thus they tend to fall under less drug pressure and are less likely to develop resistance.

Acknowledgements

We thank NIMR, New Delhi, for providing basic infrastructure facilities. We also thank Indian Council of Medical Research for providing fellowship assistance to Mr. Rahul (45/16/2019-Bio/BMS).

Conflict of interest

The authors declare no conflict of interest.

Author details

Rahul Pasupureddy[1,2], Sriram Seshadri[2], Rajnikant Dixit[1] and Kailash C. Pandey[1*]

1 Host-Parasite Interaction Biology Group, ICMR-National Institute of Malaria Research, New Delhi, India

2 Institute of Science, Nirma University, Ahmedabad, Gujarat, India

*Address all correspondence to: pandey.kailash70@gmail.com; kailash.pandey@icmr.gov.in

References

[1] World Health Organization. World Malaria Report 2018; 2018. ISBN 9789241564403

[2] Tilley L, Straimer J, Gnädig NF, et al. Artemisinin action and resistance in *Plasmodium falciparum*. Trends in Parasitology. 2016;**32**:682-696

[3] Davies DR, Cohen GH. Interactions of protein antigens with antibodies. Proceedings of the National Academy of Sciences of the United States of America. 1996;**93**:7-12

[4] Calejo AI, Taskén K. Targeting protein-protein interactions in complexes organized by a kinase anchoring proteins. Frontiers in Pharmacology. 2015;**6**:1-13

[5] Rao A, Bulusu G, Srinivasan R, et al. Protein-protein interactions and disease. Protein Interact. 2012;**8**:143-154. ISBN: 978-953-51-0244-1

[6] Liu K-QQ, Liu Z-PP, Hao J-KK, et al. Identifying dysregulated pathways in cancers from pathway interaction networks. BMC Bioinformatics. 2012;**13**:126

[7] Scott DE, Bayly AR, Abell C, et al. Small molecules, big targets: Drug discovery faces the protein-protein interaction challenge. Nature Reviews. Drug Discovery. 2016;**15**:533-550

[8] Snider J, Kittanakom S, Curak J, et al. Split-ubiquitin based membrane yeast two-hybrid (MYTH) system: A powerful tool for identifying protein-protein interactions. Journal of Visualized Experiments. 2010;(36):e1698

[9] Zhang X, Francis BF. New perspectives in predicting membrane protein-protein interactions. New Achievements in Evolutionary Computation. 2010;**7**:157-172. ISBN: 978-953-307-053-7

[10] Muangphrom P, Seki H, Fukushima EO, et al. Artemisinin-based antimalarial research: Application of biotechnology to the production of artemisinin, its mode of action, and the mechanism of resistance of plasmodium parasites. Journal of Natural Medicines. 2016;**70**:318-334

[11] Pasupureddy R, Atul SS, et al. Current scenario and future strategies to fight artemisinin resistance. Parasitology Research. 2019;**118**:29-42

[12] Dondorp AM, Nosten F, Yi P, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. The New England Journal of Medicine. 2009;**361**:455-467

[13] Goldberg DE. Hemoglobin degradation. Malaria: Drugs, Disease and Post-genomic Biology. 2005;**295**:275-291

[14] Sundararaj S, Singh D, Saxena AK, et al. The ionic and hydrophobic interactions are required for the auto activation of cysteine proteases of *Plasmodium falciparum*. PLoS One. 2012;**7**:e47227

[15] Pant A, Kumar R, Wani NA, et al. Allosteric site inhibitor disrupting auto-processing of malarial cysteine proteases. Scientific Reports. 2018;**8**:e16193

[16] Salawu EO. In silico study reveals how E64 approaches, binds to, and inhibits falcipain-2 of *Plasmodium falciparum* that causes malaria in humans. Scientific Reports. 2018;**8**:1-13

[17] Bekono BD, Ntie-Kang F, Owono Owono LC, et al. Targeting cysteine proteases from *Plasmodium falciparum*: A general overview, rational drug design

and computational approaches for drug discovery. Current Drug Targets. 2018;**19**:501-526

[18] Nizi E, Sferrazza A, Fabbrini D, et al. Peptidomimetic nitrile inhibitors of malarial protease falcipain-2 with high selectivity against human cathepsins. Bioorganic & Medicinal Chemistry Letters. 2018;**28**:1540-1544

[19] Pandey KC, Wang SX, Sijwali PS, et al. The *Plasmodium falciparum* cysteine protease falcipain-2 captures its substrate, hemoglobin, via a unique motif. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:9138 LP-9139143

[20] Pasupureddy R, Verma S, Pant A, et al. Crucial residues in falcipains that mediate hemoglobin hydrolysis. Experimental Parasitology. 2019;**197**:43-50

[21] Awasthi V, Chattopadhyay D, Das J. Potential hemoglobin A/F role in clinical malaria. Bioinformation. 2017;**13**:269-273

[22] Istvan ES, Dharia NV, Bopp SE, et al. Validation of isoleucine utilization targets in *Plasmodium falciparum*. Proceedings of the National Academy of Sciences. 2011;**108**:1627-1632

[23] Manickam Y, Chaturvedi R, Babbar P, et al. Drug targeting of one or more aminoacyl-tRNA synthetase in the malaria parasite *Plasmodium falciparum*. Drug Discovery Today. 2018;**23**:1233-1240

[24] Saint-Léger A, Sinadinos C, Ribas de Pouplana L. The growing pipeline of natural aminoacyl-tRNA synthetase inhibitors for malaria treatment. Bioengineered. 2016;**7**:60-64

[25] Burns AL, Dans MG, Balbin JM, et al. Targeting malaria parasite invasion of red blood cells as an antimalarial strategy. FEMS Microbiology Reviews. 2019;**43**:223-238

[26] Bermúdez M, Arévalo-Pinzón G, Rubio L, et al. Receptor–ligand and parasite protein–protein interactions in *Plasmodium vivax*: Analysing rhoptry neck proteins 2 and 4. Cellular Microbiology. 2018;**20**:e12835

[27] Hossain ME, Dhawan S, Mohmmed A. The cysteine-rich regions of *Plasmodium falciparum* RON2 bind with host erythrocyte and AMA1 during merozoite invasion. Parasitology Research. 2012;**110**:1711-1721

[28] Nasamu AS, Glushakova S, Russo I, et al. Plasmepsins IX and X are essential and druggable mediators of malaria parasite egress and invasion. Science (80-). 2017;**358**:518-522

[29] Pino P, Mukherjee B, Klages N, et al. A multistage antimalarial targets the plasmepsins IX and X essential for invasion and egress. Science (80-). 2017;**358**:522-528

[30] Munsamy G, Agoni C, Soliman MES. A dual target of Plasmepsin IX and X: Unveiling the atomistic superiority of a core chemical scaffold in malaria therapy. Journal of Cellular Biochemistry. 2019;**120**:7876-7887

[31] Boucher LE, Hopp CS, Muthinja JM, et al. Discovery of plasmodium (M) TRAP-aldolase interaction stabilizers interfering with sporozoite motility and invasion. ACS Infectious Disease. 2018;**4**:620-634

[32] Chang HH, Falick AM, Carlton PM, et al. N-terminal processing of proteins exported by malaria parasites. Molecular and Biochemical Parasitology. 2008;**160**:107-115

[33] Gambini L, Rizzi L, Pedretti A, et al. Picomolar inhibition of plasmepsin V,

an essential malaria protease, achieved exploiting the prime region. PLoS One. 2015;**10**:e0142509

[34] Batinovic S, Mchugh E, Chisholm SA, et al. An exported protein-interacting complex involved in the trafficking of virulence determinants in plasmodium-infected erythrocytes. Nature Communications. 2017;**8**:1-14

[35] Marapana DS, Dagley LF, Sandow JJ, et al. Plasmepsin V cleaves malaria effector proteins in a distinct endoplasmic reticulum translocation interactome for export to the erythrocyte. Nature Microbiology. 2018;**3**:1010-1022

[36] Hain AUP, Weltzer RR, Hammond H, et al. Structural characterization and inhibition of the plasmodium Atg8-Atg3 interaction. Journal of Structural Biology. 2012;**180**:551-562

[37] Villa S, Legnani L, Colombo D, et al. Structure-based drug design, synthesis and biological assays of *P. falciparum* Atg3–Atg8 protein–protein interaction inhibitors. Journal of Computer-Aided Molecular Design. 2018;**32**:473-486

[38] Ariey F, Witkowski B, Amaratunga C, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014;**505**:50-55

[39] Mbengue A, Bhattacharjee S, Pandharkar T, et al. A molecular mechanism of artemisinin resistance in *Plasmodium falciparum* malaria. Nature. 2015;**520**:683

[40] Bhattacharjee S, Coppens I, Mbengue A, et al. Remodeling of the malaria parasite and host human red cell by vesicle amplification that induces artemisinin resistance. Blood. 2018;**131**:1234-1247

[41] Coppée R, Jeffares DC, Miteva MA, et al. Comparative structural and evolutionary analyses predict functional sites in the artemisinin resistance malaria protein K13. Scientific Reports. 2019;**9**:e10675

[42] Gilson PR, O'Donnell RA, Nebl T, et al. MSP119 miniproteins can serve as targets for invasion inhibitory antibodies in *Plasmodium falciparum* provided they contain the correct domains for cell surface trafficking. Molecular Microbiology. 2008;**68**:124-138

[43] Santos CC. Chagasin, the endogenous cysteine-protease inhibitor of *Trypanosoma cruzi*, modulates parasite differentiation and invasion of mammalian cells. Journal of Cell Science. 2005;**118**:901-915

[44] Wang SX, Pandey KC, Scharfstein J, et al. The structure of Chagasin in complex with a cysteine protease clarifies the binding mode and evolution of an inhibitor family. Structure. 2007;**15**:535-543

[45] Sundararaj S, Saxena AK, Sharma R, et al. Cross-talk between malarial cysteine proteases and falstatin: The BC loop as a hot-spot target. PLoS One. 2014;**9**:e93008