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Plasmodial Hsp40s: New Avenues for Antimalarial Drug Discovery

Running Title: Plasmodial Hsp40s in Malaria Intervention

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ABSTRACT

Malaria, an infectious disease caused by *Plasmodium* spp, is one of the world's most dangerous diseases, accounting for more than half a million deaths yearly. The long years of co-habitation between the parasite and its hosts (human and mosquito), is a testimony to the parasite's ability to escape the immune system and develop drug resistance mechanisms. Currently, an important search area for improved pharmacotherapy are molecular chaperones of the heat shock protein family, abundant in *Plasmodium falciparum* and contributing to its continuous survival and development. Thus far, small molecule inhibitor studies on *P. falciparum* Hsp70s and Hsp90s have indicated that they are promising antimalarial targets. However, not much attention has been given to Hsp40s as potential antimalarial drug targets. Hsp40s are known to function as chaperones by preventing protein aggregation, and as co-chaperones, by regulating the chaperone activities of Hsp70s to ensure proper protein folding. There are only a limited number of reviews on Hsp40s as drug targets, and the few reviews on plasmodial Hsp40s tend to focus largely on the intra-erythrocytic stage of the parasite life cycle. Therefore, this review will summarize what is known about Hsp40s throughout the malaria parasite life cycle, and critically evaluate their potential to serve as new avenues for antimalarial drug discovery.

1. INTRODUCTION

1.1. The burden of malaria and available intervention approaches

Malaria, though preventable and curable, remains a life-threatening disease, negatively impacting on the health and economy of millions of people, and accounting for the high rate of child and maternal mortality, mostly in sub-Sahara Africa [1,2]. Five species of *Plasmodium* are known to cause malaria infection in humans [3,4], of which *P. falciparum* is one of the most abundant and clinically important species, *and* remains the causative agent of the most virulent, severe and dangerous form of human malaria [5]. The World Health Organization '2015 World Malaria Report', which showed that 88% of the estimated 214 million new cases of malaria, and alarming 90% of 438,000 estimated deaths from malaria occurring in Africa is a serious call for concern [6]. However, while the actual malaria mortality burden may be higher, considering such factors as lack of sanitation, malnutrition, lack of or reduced access to medications, poverty and the location of many of the poor countries affected by malaria in the tropical zones [7], the inclusion of malaria as an essential components of the World Health Organization (WHO) Sustainable Development Goal (SDG) number 3 [8–10], is a step in the right direction.

Preventive measures aimed at controlling malaria is a critical step towards the eradication of the disease. They are broadly divided into two – infection control and vector control. Infection control involves the use of chemoprophylaxis to prevent the development of malaria following an occasional mosquito bite [11-14]. On the other hand, the main focus of vector control is to prevent the transmission of the parasite from mosquitoes to human and involves the use of residual spraying of insecticides, the use of insect repellent cream or spray, sleeping under insecticide impregnated bed nets (ITNs), proper sanitation and personal and general hygiene [15,16]. However, to ensure long lasting prevention, both approaches (infection control and vector control) should be combined. Meanwhile, notwithstanding the use of these preventive measures [15], chemotherapy remains an indispensable approach in malarial prevention and treatment. In fact, consistent with WHO “Roll Back Malaria” initiative and current sustainable development goal [8–10,17], and in addition to chemoprophylaxis, an important intervention strategy in curbing maternal and child death includes prevention from mosquitoes and prompt malaria treatment [18]. However, the effective use of chemotherapy and ITNs has been hampered respectively by the parasite’s ability to develop drug resistance [19,20], and the mosquito’s ability to resist pyrethroids, the active principle of the ITNs [21,22], making a case for the constant search for new, affordable and sustainable pharmacotherapy.

1.2. Human and mosquito cycle of infection and the involvement of chaperones

Humans and female *Anopheles* mosquitoes are two important hosts needed for the complete reproductive life cycle of malaria parasites (Figure 1).

Figure 1: The life cycle of *Plasmodium falciparum*.
The parasite life cycle traverses two hosts (human and mosquito) with each stage involving complex cellular and molecular modifications. Sporozoite-infected saliva is deposited into the human host during a blood meal by female *Anopheles* mosquitoes and the sporozoites make their way to the liver, develop over time into merozoites (that are released into blood stream to invade erythrocytes). The clinical symptoms of the disease are associated with the invasion of the erythrocytes by the parasite, its growth, division inside the host cell and the cyclic cell lysis and reinvasion of new erythrocytes. Some of the merozoites will eventually develop into gametocytes (male and female) which can be taken in by mosquitoes following another blood meal.

The stages of the development of the parasite include sporozoites (infective stage), merozoites (erythrocytes invading stage), trophozoites (multiplying form in erythrocytes), and gametocytes (sexual stages), as well as intermediate changes that

take place following the fusion of microgametes and macrogametes, namely, zygotes, ookinete and oocysts (Figure 1). In mosquitoes, the sporogony or sexual phase of the parasite life cycle leads to the formation of numerous infective forms of the parasites. This phase involves fusion of male and female gametes to form a zygote [23,24], which subsequently develop into ookinete, and oocyst [25]. Sporozoites are produced from oocysts by cellular division, released into the mosquito's body cavity when the oocysts burst and then invade the mosquito salivary glands, ready to be transmitted to the human hosts at any available opportunity for a blood meal [23,24,26-28]. Following a mosquito's blood meal, the successful colonization of the liver by the sporozoites initiates the parasite reproductive life cycle in humans, followed subsequently by erythrocyte invasion which accounts for the clinical manifestation of the disease [29-34]. The development of the sporozoites takes place in the hepatocytes, within the parasitophorous vacuoles (PV), forming schizonts, containing thousands of merozoites [35-37], and stored in vesicles called merosomes [38,39]. The speedy invasion of erythrocytes by merozoites begins the intra-erythrocytic stage of parasite development [33], known to occur with precise cyclic accuracy and characterized by repeated cycles of growth, division inside the host cell, cell lysis, release of hundreds of merozoites and reinvasion of new erythrocytes. The quick disappearance of merozoites from circulation into the erythrocytes, its successful attachment, invasion and establishment in the erythrocytes, and the subsequent PV formation within the erythrocytes, not only protect the parasite, but also creates a conducive environment for intra-erythrocytic development to take place [5,33,39-42]. Finally, the human phase of the parasite life cycle ends with the development of merozoites to gametocytes (macro- and micro-gametes), waiting to infest another mosquito.

Therefore, the malaria parasite life cycle is a complex process involving specialized and unique stages of development in humans as well as female *Anopheles* mosquitoes (Figure 1). These stages are unique in shape, structure and in specialized protein profiles, including surface proteins and exportomes that play critical roles in the survival and development of the parasite by ensuring successful invasion and protection against the host immune response [5,43-48]. It should be noted that the continuous changes in surface proteins as well as metabolic pathways during these stages, not only help the parasites to survive the host immune response, but also create challenges for drug and vaccine development [43]. One of the major families of proteins found to be exported during the intra-erythrocytic stage, are the molecular chaperones of the heat shock protein (Hsp) family [42,44,49-52]. The most extensively characterized Hsp families that function as molecular chaperones are heat shock protein 70 (Hsp70), heat shock protein 90 (Hsp90) and the subject of this review, the heat shock protein 40 (Hsp40) protein family [53- 55].

2. PLASMODIAL HSP40 CHAPERONE / CO-CHAPERONE

The correct and proper folding of a given polypeptide into its native three-dimensional conformation is an essential factor for the successful production of a functional protein. While all the steric information for protein folding is encoded in the primary amino acid sequence of a protein, under the highly concentrated and complex cellular environment where this occurs, proper folding of many polypeptides would not be possible without the assistance of molecular chaperones [53,54,56]. Molecular chaperones can be said to be housekeepers, organizers, stabilizers and facilitators as they ensure proper protein folding for functional activities, timely translocation of proteins across intracellular membranes or timely degradation of proteins misfolded beyond repair [53,54]. The structure and function of molecular chaperones in health and disease states in general [57] and in malaria pathogenesis [58] have been extensively reviewed. The Hsp40 family is one of the most diverse families of molecular chaperones. Like all Hsp40 proteins, plasmodial Hsp40 proteins are defined by the presence of a ~70 amino acids domain, called the J domain, originally identified in the *E. coli* Hsp40, DnaJ, and characterized by an invariant

tripeptide HPD (His-Pro-Asp) motif [59,60] (Figure 2). They are known to function alone as a chaperone or as a co-chaperone by regulating Hsp70, thereby facilitating proper folding, translocation, or degradation of substrate polypeptides [61–74]. The classification of Hsp40s into type I to IV has been done on the basis of four canonical domains, namely, a J-domain, having a conserved tripepetide HPD motif [75]; a Gly/Phe-rich region (GF-domain) [76,77]; a cysteine-rich zinc binding domain [78]; and a C-terminal domain [79,80]. All the domains are present in type I, while type II lack only the zinc binding domain. Moreover, type III have just the J–domain, while type IV possess a J-like domain with a non-conserved corrupted HPD motif [75,81] (Figure 2). In *P. falciparum*, at least 49 Hsp40s consisting of 2 type Is, 9 type IIs, 25 type IIIs and 13 type IVs, with a high degree of conservation, have been identified [55]. Also, *P. falciparum* is known to possess the highest number (*n = 19*) of exported Hsp40 proteins among *Plasmodium* species, consisting of 3 type IIs, 5 type IIIs and 11 type IVs [44,55].

Figure 2: Schematic representation of the domains present in Hsp40
Schematic representation of Hsp40 type I to IV, showing the helix I to IV and the conserved HPD motif (corrupted in the case of type IV) of the J-domain. Through the J-domain, Hsp40 can interact with and stimulate the ATPase activity of Hsp70. Other domains in Hsp40 are involved in regulation and stabilization of Hsp70-substrate binding (GF region), stabilization of the Hsp40 tertiary structure (Zn-binding domain) and capture of protein substrates and dimerisation (C-terminal domain). GF region = Gly/Phe-rich region (GF-domain), Zn-binding domain = cysteine-rich zinc binding domain.

3. PLASMODIAL HSP40s AT DIFFERENT STAGES OF THE PARASITE LIFE CYCLE

Note, from this section forward, when first mentioned, plasmodial proteins are identified using common nomenclature, followed in brackets by their current I.Ds / previous I.Ds as contained in PlasmoDB [117].

3.1. The intermediate stage: gametocytes

The early stage of sexual differentiation during gametocytogenesis is marked by profound export of putative proteins called *P. falciparum* gametocyte exported proteins (PfGEXPs) such as Pfg14.744 (PF3D7_1477300 / PF14_0744), PfRex3 (PF3D7_0936300 / PFI1755C), PfGEXP5 (PF3D7_0936600 / PFI1770W) and PfGEXP10 (PF3D7_0113900 / MAL1P4.12, PFA0670C) [82,83]. Microarray analysis identified 11 genes that were up-regulated in sexually committed *P. falciparum* parasites [84], of which Pfs16, a sexual stage-specific protein precursor (PF3D7_0406200 / MAL4P1.61, PFD0310w) and Pfg27/25, a gamete antigen 27/25 (G27/25) (PRCDC_1301100 / PRD_1302100), were then considered as the first molecular markers of gametocytes, due to their expression within 24 hours of parasite development [85]. But PfGEXP5 (PF3D7_0936600 / PFI1770W) may now be considered as the first molecular marker based on its high expression during the early stages of sexual development and its active export into the cytosol of infected erythrocytes [83]. PfGEXP5 now represents a useful post-invasion sexual stage marker which could be used for the identification of sexually committed ring stage parasites and detection of malaria transmission reservoirs in infected individuals [83]. This supports a specific role for exported proteins in mediating the process of gametocytogenesis.

The repertoire of proteins known to be exported during the gametocyte stages of parasite development has been expanded to include PfGECO, a type IV Hsp40 protein (PF3D7_1253000 / PFL2550w, 2277.T00508, PF12_0010) [86]. Some important features of this protein include expression at all gametocyte stages I to IV, export into the cytosol of infected erythrocytes, lack of over-expression upon heat shock, and successful knock out of its gene with no compensatory expression of the other type IV Hsp40 genes or any negative impacts on the targeting of other exported gametocyte proteins [86]. These findings suggest that the protein may be involved in, but not essential for gametocytogenesis. In fact, the gene encoding PfGECO was found to be one of 16 whose mutagenesis putatively distrupted gametocytogenesis; however, the lack of rescue

by expression of the wild type gene again put into question its requirement for gametocytogenesis [87]. Nevertheless, the export of PfGECO into the infected erythrocytes cytosol along with other related proteins does suggest that it may be involved in trafficking of other exported proteins [33,88]. The expression of PfGECO in early stages (I and II) of gametocytogenesis was reported to be similar to previous microarray expression data [89]. Also, knobs are not produced and the essential cytoadherence and virulence factor *P. falciparum* erythrocyte membrane protein-1 (PfEMP1) (PF3D7_0100100 / MAL1P4.01, PFA0005W) is virtually absent from the surface of the stage I gametocyte-infected erythrocytes [90,91]. These reports further support the fundamental functional role of protein export and red blood cell remodelling in gametocyte differentiation. Therefore, epigenetic factors and specific transcriptional regulators involved in gametocyte commitment and development may help provide additional information regarding the process of cellular differentiation [92].

3.2. Development in mosquitoes

The schizogony phase of parasite development takes place in female *Anopheles* mosquitoes, following the ingestion of gametocytes infected blood. In humans, every progenitor of a single erythrocytic schizont has the capacity to develop into a gametocyte (male or female) and it has been reported that the expression of *P. falciparum*, Nima-related kinase-4 (Pfnek-4), a member of protein kinases superfamily of proteins (PF3D7_0719200 / MAL7P1.100), might be important in identifying the sexually committed gametocytes [93,94]. There are important features that differentiate male from female gametocytes [95,96]. For instance, while, the male gametocytes have plasma membrane and nucleus with a much reduced network of ribosome and endoplasmic reticulum [95,96], the female gametocytes possess apicoplast, mitochondria and endoplasmic reticulum that are well developed and important for rapid development into zygotes [97]. The drastic change from the human to mosquito environment also makes it essential for the gametocytes to undergo rapid molecular and cellular changes while entering into the sporogonic cycle. Among the known changes that take place during gametogenesis are temperature and pH changes and the timely release of xanthurenic acid by the mosquito to trigger male gamete exflagellation [98,99]. *P. falciparum* cGMP-dependent protein kinase (PF3D7_1436600 / PF14_0346) [100] is known to be essential for this xanthurenic acid–mediated male gamete exflagellation [100]. Also, essential to fertilization are two proteins belonging to 6-cysteine repeat protein family, namely, P48/45, a male-gamete-specific protein (PFIT_1345900 / PFIT_PF13_0247), which is uniquely expressed by male gametes, and P230 (PF3D7_0209000 / PF02_0086, PFB0405w) [101–104].

In *P. falciparum*, some other genes have also been implicated in ookinetes formation, including CDPK4, a *Plasmodium* calcium-dependent kinase 4 (PF3D7_0717500 / PF07_0072) [95], MAPK-2, a mitogen-activated protein kinase-2 (PF3D7_1113900 / PF11_0147) [105,106], and NIMA, (never in mitosis/*Aspergillus*)-related protein kinase (Nek-4) [107], as well as CDPK3, a micronemal protein (PF3D7_0310100 / MAL3P3.17, PFC0420w) [108] and circumsporozoite- and thrombospondin-related anonymous protein (TRAP)-related protein (CTRP) (PF3D7_0315200 / PFC0640w), that are also essential for the ookinete motility in the mosquito gut [109]. Finally, the ookinetes need to be transformed into oocysts, and oocysts into sporozoites, an infective form, which subsequently invade the mosquito salivary gland. Secreted ookinete adhesive protein (SOAP) (PF3D7_1404300 / PF14_0040) [110] and CTRP [111] are involved in the transformation of ookinetes into oocysts. On the other hand, LAPs (lectin adhesive-like proteins) (PF3D7_1446200 / PF14_0439) play a critical role in transformation of oocysts into sporozoites [112], while thrombospondin-related anonymous protein (TRAP), a micronemal protein (PF3D7_1335900 / PF13_0201) has been shown to aid sporozoite invasion of the mosquito salivary gland and its subsequent continuous sequence of stick-and-slip motility in the human host [32,113–115].

While plasmodial Hsp40s are not among the major proteins involved in the process of schizogony, there are certain members involved in early stages of this process. For instance, a type IV Hsp40 (PF3D7_1143200 / PF11_0443) was found to

be expressed during schizgony and could not be knocked out. This Hsp40 contains two trans-membrane (TM) domains, is present in the endoplasmic reticulum (ER) of early schizonts and then subsequently found to accumulate in rhoptries at the apex of merozoites [116]. Furthermore, the data on the transcriptomes of 7 sexual and asexual life stages of *P. falciparum* as contained in PlasmoDB [117] suggests that up to 41 plasmodial Hsp40s could be expressed during the mosquito stage of the parasite life cycle, especially, the ookinete (Figure 3). This apparent high number of Hsp40s warrants further investigation, to both validate their expression at the mosquito stage and to determine the significance of their role in parasite development and pathogenesis.

Figure 3: Transcriptomes of plasmodial Hsp40s at selected stages of the malaria parasite life cycle.
The expression pattern is shown for each gene ranging from lowest (light colour) to highest (dark colour). Data used were obtained from PlasmoDB [117] and presented in Supplementary Table 1. Data used were generated using one full cycle of synchronized *Plasmodium falciparum* 3D7 parasite cultures, from where samples were harvested for ring, early trophozoite, late trophozoite, and schizont stages, respectively at 8, 19, 30, and 42 h post infection. Gametocytes were produced from asexual cultures harvested at day 8 (stage II gametocytes) and day 15 (stage V gametocytes). Ookinetes were harvested from 30 mosquito midguts 24 h after a *P. falciparum* infected blood meal. Samples were sequenced using Illumina methods. The values represent transcript levels of fragments per kilobase of exon model per million mapped reads (RPKM) for Uniquely and Non-Uniquely mapped sequences. Non-Unique sequences are presented to indicate the maximum expression potential of the respective gene. Classification by type is shown as I, II, III and IV, and as exported (EP), resident (RE), essential (Y), and nonessential (N) proteins. Essentialities were obtained by WHO-TDR [198] prediction and/or sourced from the literature [45,49,50,61,86,153,157,172,174,176,178,179,182–184,199]. R is ring, ET is early trophozoite, LT is late trophozoite, S is schizont, G-II is gametocyte II, G-V is gametocyte V, and O is ookinete.

3.3. The intermediate stage: sporozoites

The transversal of sporozoites from where they are deposited below the skin to the liver is essential for the establishment, and continuous survival and development of the parasite in the human host. Implicated in the ability of sporozoites to transverse the host cells prior to hepatocyte infection, include proteins secreted by the micronemes, namely, sporozoite protein essential for cell traversal 1 (SPECT1) (PF3D7_1342500 / MAL13P1.212) and SPECT2 (PFIT_0407300 / PFIT_PFD0430c) [118,119], as well as TRAP-like Protein [120], a sporozoite secreted phospholipase [121], cell traversal protein for ookinete and sporozoite [122] and circumsporozoite protein (CSP) (PF3D7_0304600 / MAL3P2.11, PFC0210c) [123]. While having no effect on gliding, the absence of *SPECT1* or *SPECT2* in mutant sporozoites has been reported to prevent sporozoite migration through host cells [118].

Gene expression analysis and mass spectrometry data revealed that about 28 Hsp40s have potential to be expressed at the sporozoite stage of the parasite life cycle [43,84,124–128]. Apart from 17 that were predicted to be expressed at all stages of parasite life cycle, gene expression analysis showed the potential expression of two type III (PF3D7_1126300 and PF3D7_1307200) and one type IV (PF3D7_1039100) Hsp40s [124–127]. On the other hand, mass spectrometry data revealed an additional five type III (PF3D7_0220100, PF3D7_0806500, PF3D7_1002800, PF3D7_1328800 and PF3D7_0920100) and three type IV (PF3D7_1039100, PF3D7_1102200 and PF3D7_0201700) Hsp40s were expressed at the sporozoite stage [43,127,128]. While, from the available data [129], none of these are currently shown to be important for sporozoite development and invasion, the expression of a polymorphic array of PfEMP1 (25 PfEMP1 isoforms) [43] and demonstration of PfHsp70-1 (PF3D7_0818900 / PF08_0054) [130] at the sporozoite stage, suggest potential functional interaction with these Hsp40s, which may form part of the sporozoite survival mechanism. Therefore functional characterization of these sporozoite-stage Hsp40s may help to pave the way for their potential usefulness as targets of drug and vaccine development.

3.4. Development in humans

3.4.1. Liver stage: hypnozoites

The entering of sporozoites into the liver signals another important phase in the reproductive cycle of the parasite. To begin liver stage development, sporozoites have to migrate through several hepatocytes and finally form a PV to settle in [131]. Also, to prevent the deleterious effects of the phagocytic action of Kuppfer cells, a receptor mediated-mechanism involving the specific interaction of CSP and liver-specific low-density lipoprotein receptor-related protein-1 (LRP-1), is employed to suppress the immune action of the Kuppfer cells [132]. Other parasite molecules that have been shown to be involved in sporozoite invasion of the hepatocytes, formation of the PV membrane and growth and development, include two 6-cyteine proteins, namely, P36 (PF3D7_0404400 / MAL4P1.42, PFD0210c) and P52/P36p (PF3D7_0404500 / MAL4P1.43, PFD0215c) [119], and microneme proteins such as sporozoite surface protein-2 (SSP2), or TRAP and their homologue, circumsporozoite- and TRAP-related protein, as well as CSP [133,134], sporozoite low complexity asparagine-rich protein (SAP1) [135] and sporozoite and liver stage asparagine-rich protein (SLARP) (PF3D7_1147000 / PF11_0480) [21]. Also, the expression of a bud emergence BEM46-like protein (PF3D7_0818600 / MAL8P1.66) has been identified during the liver stage of the *Plasmodium* life cycle [136], although its exact role in liver stage development needs further elucidation. Although data from gene expression and mass spectrometry studies [43,84,124–128] have indicated the expression of plasmodial Hsp40s at all stages of parasite development, there is a paucity of information on liver stage plasmodial Hsp40s and their functional characterization. The identification and functional characterization of plasmodial Hsp40s at the liver stage therefore represents a wide-open and exciting avenue for further investigation.

3.4.2. Erythrocytic invasion

Erythrocyte invasion marks an essential step towards the successful establishment of clinical features of infection through a series of steps involving merozoite binding and reorientation, followed by deformation of the erythrocyte, specific interaction and junction formation, and finally entrance of the parasite into the erythrocytes. Among the parasite proteins that have been documented to play critical roles at different steps in ensuring successful invasion include merozoite surface protein-1 (MSP-1) (PF3D7_0930300 / PFI1475w), known to be involved in merozoite initial binding [137], and apical membrane antigen-1 (AMA-1) (PF3D7_1133400 / PF11_0344), said to play a role in the initial contact, reorientation and erythrocyte deformation [138]. Also of importance to parasite invasion and establishment within host cells are rhoptry neck proteins, namely, RON2 (PF3D7_1452000 / PF14_0495), RON4 (PF3D7_1116000 / PF11_0168) and RON5 (PF3D7_0817700 / MAL8P1.73), through their interactions with AMA-1 at the point of invasion to form the much needed AMA-1-RON complex [139–141]. This complex also encourages the establishment of the parasite within the PV, and when prevented, the invasion of merozoites is inhibited [140,142]. Furthermore, EBA-175 (PF3D7_0731500 / MAL7P1.176, PF07_0128), a 175 kDa erythrocyte binding antigen, duffy-binding protein (DBP) (PF3D7_1035700 / PF10_0348) and the reticulocyte binding-like proteins (PF3D7_1371600 / PF13_0115) aid the parasites in their bid to recognize and bind effectively to different erythrocyte receptors [143,144]. To our knowledge, no plasmodial Hsp40 has been found to play an essential and direct role in promoting parasite invasion of erythrocytes. However, there have been extensive studies on the potential role in erythrocyte invasion of a type IV Hsp40, the ring-infected erythrocyte surface antigen (RESA; PF3D7_0102200 / PFA0110w). RESA is exported to the erythrocyte cell membrane, where it binds and stabilizes spectrin resulting in protection of the infected erythrocyte from mechanical and thermal degradation, and increased resistance to further parasite invasion (127, 145). By preventing secondary erythrocyte invasion, RESA is proposed to provide a selective advantage on the parasite by enhancing parasite survival (145).

3.4.3. Intra-erythrocytic development

The intra-erythrocytic phase of parasite development is responsible for the clinical manifestation of the disease. It is characterized by host cell remodelling or modification, which generates conditions for the parasite to develop, survive and establish itself in an environment devoid of the necessary cellular machinery for protein trafficking [40,42,47,50]. This remodelling process, which includes cytoadherence and knob formation, provides the parasite the much needed enabling environment for effective metabolism and protection from destruction by the spleen [146–148]. PfEMP1 is associated with this phase of parasite development, and its translocation to the erythrocyte surface is known to be dependent in part on *P. falciparum* erythrocyte membrane protein-3 (PfEMP3) (PF3D7_0201900 / PF02_0019, PFB0095C) [149] and PfHsp70-x (PF3D7_0831700 / MAL7P1.228) [150]. The type IV Hsp40 *P. falciparum* erythrocyte membrane protein-2 (PfEMP2) (also called mature parasite-infected erythrocyte surface antigen, MESA; PF3D7_0500800 / PFE0040c) and KAHRP (knob-associated histidine rich protein - PF3D7_0202000 / PF02_0020, PFB0100C) are also reported to be associated with knobs [151], with KAHRP distruption leading to a loss of ability to cytoadhere [146,152,153]. In addition, skeleton binding protein 1 (SBP1) (PF3D7_0501300 / MAL5P1.14, PFE0065W) [154] and the membrane associated histidine-rich protein 1 (MAHRP1) (PF3D7_1370300 / MAL13P1.413) [155], are proposed to be required for cytoadherence [156–158].

During the intra-erythrocytic phase, a high percentage of chaperones / co-chaperones of the heat shock protein family are produced, used and / or exported (44,55). The exported parasite proteins, also known as the exportome, (Figure 4A), are routed through the ER to the Golgi apparatus, carried away by vesicular transport and subsequently released into the lumen of the PV [46,48,159]. From the PV membrane, proteins are transported across into the host erythrocytes, a process known to be mediated by a pentameric motif called *Plasmodium* export element (PEXEL) or host targeting signal (HT) [49,52,152] or through other alternative export pathways, as may be applicable to several PEXEL-negative exported proteins (PNEPs) [148]. Consistent with transcriptome profiling (Figure 3), proteome analyses of the parasite life cycle revealed that a total of 2,415 proteins are highly expressed, with 1,049 in sporozoites, 839 in merozoites, 1,036 in trophozoites and 1,147 in gametocytes [31,160]. Also, a study that used a long-oligonucleotide microarray, reported an expression profile of the schizont and trophozoite stages of *P. falciparum*, including a number of Hsps that were enriched in trophozoites [125]. These Hsps include PfHsp70-1, PfHsp86 (PF3D7_0708400 / PF07_0029), and the Hsp40 homologues, PfEMP2 and RESA [125].

Figure 4: Export of parasite proteins into the cytosol of infected erythrocytes.
The parasite proteins meant for export are processed in the endoplasmic reticulum (ER) and the Golgi for onward movement by vesicular transport into the parasitophorous vacuole (PV). The proteins to be exported are probably maintained in an unfolded state before they can be translocated by the *Plasmodium* translocon of exported proteins (PTEX) complex across the parasitophorous vacuole membrane (PVM) into the cytosol of the infected red blood cell (RBC). Both PEXEL-containing (PEPs) and PEXEL-negative export proteins (PNEPs) are possibly exported via the same mechanism [48]. The zoomed out portion (B) shows the components of the PTEX translocon and how proteins are maintained in an unfolded state during export by plasmodial exported chaperones. Furthermore, plasmodial exported chaperones and host chaperones capture the exported protein in the erythrocyte cytosol to facilitate its folding. EC is erythrocyte cytosol, PV is parasitophorous vacoule and PC is parasite cytosol. The diagram was adapted from [46].

Furthermore, important to protein translocation into the cytosol of *P. falciparum* infected erythrocytes are members of the *Plasmodium* translocon of exported proteins (PTEX) [161] (Figure 4B) and the ring-exported protein 1 and 2 (REX1, PF3D7_0935900 / PFI1735C; and REX2, PF3D7_0936000 / PFI1740C) [36,148,162]. The PTEX proteins are restricted within the *Plasmodium* genus, localised to the parasitophorous vacuole membrane (PVM), required for blood stage growth and bind specifically to their exported proteins [161]. Among the identified proteins that were shown to be bona fide members of the PTEX family are PTEX150 (PF3D7_1436300 / PF14_0344, Pf112), Hsp101 (PF3D7_1116800 / PF11_0175), EXP2

(PF3D7_1471100 / PF14_0678), PTEX88 (PF3D7_1105600 / PF11_0067) and TRX2 (PF3D7_1345100 / MAL13P1.225) [161,163–167] (Figure 4). Similar to an earlier report of the inability to generate gene knockouts of *P. falciparum* PTEX150 [161], all the five members of PTEX, except TRX2, could not be deleted in *Plasmodium berghei* and were also expressed in early gametocytes, mosquito and liver stages [165]. This suggested that in addition to being essential for protein trafficking (to and from infected erythrocytes) [161,163–165], these proteins were essential for the intra-erythrocytic development and inter-hosts survival of the parasite. The reported substantial reduction in proteins trafficking (PEXEL and PEXEL-negative exported proteins), including PfEMP1, and the strong negative effects on the capacity of the parasite to complete the erythrocytic cycle in parasite cell lines devoid of essential (Hsp101 or PTEX150), and non-essential (TRX2) PTEX components, further confirmed the importance of PTEX to parasite survival and development [163]. Moreover, for efficient protein export, the integrity of the PTEX complex must be maintained. It appears that the maintenance of such integrity depends largely on Hsp101, a ClpB-like AAA+ ATPase component of PTEX [166]. This is because the ablation of its function caused an almost total blockage of protein export, resulting in accumulation of proteins meant for export within the vacuole, a condition that disrupted the association of Hsp101 with other PTEX components [166]. Thus, Hsp101 can be regarded as the power house of the PTEX complex. Furthermore, we propose that the PTEX translocon, especially Hsp101, works closely with plasmodial exported chaperones and host chaperones to ensure that exported proteins are competent to be translocated, and that they are properly folded once they reach their final destination in the erythrocyte cytosol (Figure 4B).

At least two typical type II Hsp40 proteins, namely, PFA0660w (PF3D7_0113700 / MAL1P4.10), and PFE0055c (PF3D7_0501100.1 / MAL5P1.12) are exported together with PfHsp70-x into mobile membrane structures within the cytosol of infected erythrocytes called J-dots [150,187]. The J-dots, whose defining characteristic was the presence of J-domain containing Hsp40s, appear to be distinct from the Maurer's clefts as they are highly mobile and share none of the Maurer's clefts antigenic determinants [187]. Also, the difference in intra-erythrocytic velocity when compared to an earlier described highly mobile 'vesicle like structures' in the *P. falciparum*-infected erythrocyte, constitute another distinctive feature of J-dots [187,188]. New evidence has shown that the substrate binding domains of the J-dot-resident Hsp40 proteins is necessary and sufficient for J-dot targeting, which only occurred in *P. falciparum* infected human erythrocytes, supporting the idea of J-dots being *P. falciparum*-specific [189]. Furthermore, biochemical evidence has recently been provided for a specific functional interaction between PFA0660w and PfHsp70-x [61]. The inability to produce viable parasites in the absence of PFA0660w, indicates that it might be essential for the intra-erythrocytic development and survival of the parasite [45]. Therefore, given the recent successful modulation of basal and co-chaperone stimulated activity of PfHsp70-x [179], the screening and identification of small molecule modulators of the PfHsp70-x / PFA0660w system will enable its functional significance and potential as a drug targets to be probed.

Among the parasite-resident *P. falciparum* Hsp40s whose activities have been investigated are Pfj1 (PFD0462w / PF3D7_0409400) and Pfj4 (PFL0565w / PF3D7_1211400) (type I and type II Hsp40s, respectively). They were first identified by Watanabe [168] and their J-domains were subsequently shown to be functional in a prokaryotic assay system [169]. Although Pfj1 was predicted to be localized to mitochondria [168], it has been reported to be localized to the apicoplast and potentially involved in DNA replication [170]. Furthermore, using *in vitro* assays Pfj1 was shown to be capable of functionally interacting with cytosolic PfHsp70-1, a situation that may be unlikely *in vivo* [171,172]. On the other hand, the co-localization, co-immunoprecipitation and co-fractionation of Pfj4 with PfHsp70-1 support the idea of direct or indirect functional and specific interactions between them [173]. It has been proposed that important information necessary for subtrate specificity and functional interactions of Pfj4 may be contained in the G/F-rich and C-terminal regions [173]. This, coupled with the report of the increase in expression of Pfj4 upon heat shock [173], could be an indication of distinct roles for Pfj1 and

Pfj4. Pfj4 may therefore play a critical role in cytoprotection, and by extension, parasite intra-erythrocytic survival and development in association with PfHsp70-1.

Furthermore, PfHsp40, a type I Hsp40 protein (PF14_0359 / PF3D7_1437900), is localized to the parasite cytosol and expressed in all the stages of the intra-erythrocytic phase of parasite development in a similar pattern as PfHsp70-1, for which it showed potential functional interaction [124,172]. PfHsp40 stimulated the basal ATP hydrolytic rates of PfHsp70-1 and human Hsp70 and enhanced the protein aggregation suppression activity of PfHsp70-1 [172].

4. PLASMODIAL HSP40 CO-CHAPERONES AS DRUG TARGETS

The identification and potential pharmacological uses of small molecules that specifically interact with and modulate the basal and Hsp40 co-chaperone stimulated activities of Hsp70 is attracting attention as a potential approach in drug development [174–179]. The reported groups of small molecule inhibitors of Hsp70 include adenosine analogs (ATP mimics), spergualins, pyrimidinones, fatty acids (acyl benzamide, sulfogalactosyl ceramide, sulfogalactoglycerolipid), peptides, malonganenone (malonganenones A, B and C), and naphthaquinones (lapachol and bromo-β-lapachona) [176,178–183] (Figure 5 and Supplementary Figure 1). Of these classes of compounds, some members of spergualins, pyrimidinones, malonganenone and naphthaquinones have been shown to modulate plasmodial Hsp70 function and to possess antimalarial activity [176,178,179,182,183]. The biological activities of these antimalarial small molecules and their representative structures are presented in Table 1 and Figure 5, respectively. It is worth noting that the concentrations needed to achieve a reasonable modulation of plasmodial Hsp70 activity were significantly higher than the concentrations required for *in vitro* growth inhibition of *P. falciparum* [176,178,179,182,183]. Insight into the biological fate of these small molecules following their uptake by the parasite, such as the functional activities of their metabolites, and the possibility of modulating different biological pathways or processes, may help to explain this discrepancy. Nevertheless, the identification of small molecule compounds (such as pyrimidinone-amides, 15-deoxyspergualin, lapachol, bromo-β-lapachona and malonganenones A, B and C) (Figure 5) capable of specifically modulating the basal ATPase activity, Hsp40-stimulated ATPase activity or aggregation suppression activity of plasmodial Hsp70s [174,176,178,179,182–184], opens up the possibility of developing sustainable inhibitors of these chaperones.

Although members of each family of the identified small molecules share similar basic structures, the presence of different substituted side chains and functional groups may explain the difference in their inhibitory activities. For instance, the nine pyrimidinone-amide compounds which showed significant inhibition of *P. falciparum* growth and PfHsp70-1 ATPase activity, all shared a similar ester pyrimidine core at position C-4 with eight of them having alkyl groups at position N-1 [176]. However, there was a distinct morpholine moiety on DMT3024 and MAL3-39 and tetrasubstituted pyrrole side chain on MAL2-215 and MAL2-213 that might explain the differences in their inhibitory activities (Figure 5). Also, the inhibitory mechanisms of action of pyrimidinones may be connected with the presence of hydrophobic groups and the addition of steric bulk on its structure [185].

Furthermore, the potential of identifying small molecule inhibitors having selective inhibitory activities on chaperone / co-chaperone systems has been demonstrated [174,179,182]. In a recent study, lapachol, bromo-β-lapachona and malonganenones A, B and C were assessed for their modulatory effects on the steady-state basal and Hsp40-stimulated ATPase activities of PfHsp70-1, PfHsp70-x and human Hsp70 [179]. Bromo-β-lapachona and malonganenones A showed selective effects on the PfHsp70-1/Hsj1a and PfHsp70-1/PfHsp40 systems. While bromo-β-lapachona produced enhanced

Table 1. Biological activities of small molecules on *P. falciparum* parasite growth and Hsp40/Hsp70 function

COMPOUNDS		BIOLOGICAL ACTIVITIES OF THE COMPOUNDS			REFERENCES
Classes	**Compounds**	***P. falciparum* Growth *in vitro***	**Hsp70 function**	**Hsp40 – Hsp70 interaction**	
Pyrimidinones	DMT3024 DMT2264 MAL2-29 MAL2-39 MAL2-61 MAL2-213 MAL2-215 MAL3-39 JAB75	These compounds inhibited the growth of *P. falciparum in vitro* with IC_{50} values ranging from 0.03 - 1.6 µM. Showed no cytotoxicity to human cell lines	At 300 µM, some of these compounds showed inhibition of PfHsp70-1 steady state ATPase activity, especially DMT2264 (26%) and MAL3-39 (28%). At 100 µM each compound stimulated the PfHsp70-1 single turnover ATP hydrolysis rate. However, no change in ATPase activities were observed when used at the concentration that inhibited *P. falciparum* growth *in vitro*	DMT2264 and MAL3-39 inhibited the Hdj2-stimulated ATPase activity of human Hsp70 under steady-state conditions, whilst the PfHsp40-stimulated ATPase activity of PfHsp70-1 was only inhibited by DMT2264 under single-turnover conditions	[172,176]
Malonganenone	Malonganenone A Malonganenone B Malonganenone C	Apart from malonganenone B, *in vitro* parasite growth was inhibited with IC_{50} values of less than 20 µM. Showed no signs of cytotoxicity to human cell lines	All compounds showed similar and dose dependent inhibition of PfHsp70-1 aggregation suppression activity and nearly completely at 300 µM. However, only 300 µm malonganenone A produced about 20% inhibition of the aggregation suppression activity of PfHsp70-x. At 100 µM, malonganenone A enhanced the ATPase activity of PfHsp70-1 by ~15% but not PfHsp70-x	They all showed significant inhibition of Hsj1a-stimulated ATPase activity of PfHsp70-x, but to a lesser extent than lapachol and bromo-β-lapachona. Also, malonganenone A showed a selective inhibitory effect on the PfHsp70-1/PfHsp40 system	[178,179]
Naphthaquinones	Lapachol	*In vitro* parasite growth was inhibited with IC_{50} values of less than 20 µM. Showed varying degrees of cytotoxicity to human cell lines	Selective and concentration dependent inhibition of protein aggregation suppression activity of PfHsp70-1. At 100 µm, it inhibited PfHsp70-x basal ATPase activity by ~40% but not PfHsp70-1	Showed high (~50%) significant inhibition of Hsj1a-stimulated ATPase activity of PfHsp70-x	[178,179]

	Bromo-β-lapachona	*In vitro* parasite growth was inhibited with IC_{50} values of less than 20 µM. Showed varying degrees of cytotoxicity to human cell lines	Similar inhibition of protein aggregation suppression activity to the class of malonganenones (A, B, C) but does not produce complete suppression at 300 µM. However, it did inhibit aggregation suppression activity of PfHsp70-x at 100 µM (about 25%) and 300 µM (about 75%). At 100 µm, it inhibited PfHsp70-x basal ATPase activity by ~80% but not PfHsp70-1	Showed high (~90%) significant inhibition of Hsj1a-stimulated ATPase activity of PfHsp70-x.	[178,179]
Spergualins	15-deoxyspergualin	*In vitro* parasite growth was inhibited with IC_{50} values of 148 nM. As a tumoricidal agent, it is possibly as toxic on human cell lines	Known to cause Hsp70 sequestration and interfere with trafficking of nucleus-encoded proteins to the apicoplast with an IC_{50} of 443 µM, about 1000-fold increase above *in vitro* growth inhibition IC_{50}. It appears specific to PfHsp70 with C-terminal EEVD motif, as it selectively enhances their ATPase activity. But produces no effect on their chaperone activities.		[182,183]

Hsj1a-stimulated activity of PfHsp70-1, malonganenones A had no effect. On the other hand, although both showed inhibitory effects on PfHsp40-stimulated activity of PfHsp70-1, malonganenones A had a stronger inhibitory effect. Interestingly, while the basal and PfHsp40-stimulated ATPase activity of PfHsp70-1, as well as the Hsj1a-stimulated ATPase activity of PfHsp70-x were significantly altered by malonganenones A, the compound had no effect on the basal and Hsj1a-stimulated ATPase activity of human Hsp70 (HSPA1A) [179]. Thus, it could be argued that the varying effects of the small molecules on the activities of Hsp40/Hsp70 systems may be a function of the different Hsp40 interacting partners. The identification of malonganenone A is evidence that it is possible to develop chaperone / co-chaperone-based parasite-specific multi-targeted small molecule inhibitors. The development of antimalarial lead compounds from malonganenone A will require stabilization of its structure by modification of certain reactive centres (e.g. the α,β-unsaturated ketones, making it susceptible to nucleophilic attack resulting in unfavourable irreversible inhibition of Hsp70 by covalent interaction; see Figure 5) [179,186].

Figure 5: Selected antimalarial small molecule inhibitors of plasmodial Hsp70s.
All the small molecules have been tested on PfHsp70-1, while naphthaquinones and malonganenones were also tested on PfHsp70-x. They showed varying degrees of inhibition of parasite growth, basal and /or Hsp40-stimulated Hsp70 ATPase activities, and/or Hsp70 protein aggregation suppression activities. Sourced and adapted from [176,178].

5. SEQUENCE AND STRUCTURAL SIMILARITIES OF PLASMODIAL HSP40s

Several reports have been presented on the sequence and structural similarities among plasmodial Hsp40s and between them and other apicomplexa and humans [81,127,150,172-199]. A careful comparison between the Hsp40 proteins of *P. falciparum* and those of other apicomplexa and humans showed that most of the proteins, especially the exported proteins, are unique to *P. falciparum* [81,127]. Furthermore, while a few members of the types I, II and III *P. falciparum* Hsp40s have homologs among other apicomplexa and in humans, it is interesting to note that the type IV Hsp40 proteins with J-like domains are all unique to *P. falciparum* [81,127]. Unlike ubiquitous Hsp70s and Hsp90s with high levels of sequence and structural similarities and conservation within and across genomes [127,200-202], the expanded nature and uniqueness of many of the *P. falciparum* Hsp40s suggests that they are important for successful invasion and remodelling of human erythrocytes, and therefore potentially useful as avenues for new antimalarial drug discovery [81,127,199].

6. IMPLICATIONS FOR MALARIA PREVENTION AND DRUG DEVELOPMENT

Malaria infection remains a global disaster and although reduction in the rate of mortality and morbidity is being achieved [8,9], sustainable approaches to stemming the tide of malaria infection remains a global concern. This is largely due to environmental issues such as poverty, illiteracy and civil unrest or war, compounded by an increasing rate of resistance, not only to available drugs, but also to known active ingredients of insecticidal agents such as pyrethroids, organochlorines, organophosphates and carbamates [190,191]. Therefore, exploring new avenues such as the potential of plasmodial Hsp40 co-chaperones as an integral component of parasite survival and development, may help to develop new and much needed sustainable approaches towards malaria treatment.

Plasmodial parasites produce an array of proteins which help to aid its survival at different stages of its development. As noted in the preceding sections, studies on the functional activities of the majority of the currently known plasmodial Hsp40s have focused on the survival of the parasite within the unusual environment of the red blood cells. While gene expression studies, supported by the proteomics studies, have revealed up-regulated and high levels of expression of plasmodial chaperones, including Hsp40s, at the intra-erythrocytic stage of the parasite development [43,125,160], their expression is not limited to this stage [192]. As such, to take advantage of the potential of these proteins as new avenues for

drug discovery, their essentiality and druggability need to be ascertained. We have presented the available data on essentiality of plasmodial Hsp40s (Figure 3). Essentiality is here described as inability to provide a viable parasite when any particular gene was knocked out and/or if viable following knock out, inability of the resulting parasite to maintain essential functions or processes needed for its continuous survival and development. Druggability on the other hand is the possibility of targeting an essential protein, in this case using small molecules, with a view to modulating its functional activity, either alone or as interacting partners of druggable chaperones, and consequently disrupt parasite survival. In this regard, and with special focus on the intra-erythrocytic stages, the potential of Hsp40 co-chaperones as drug targets, with a view to developing specific inhibitors has been presented [177]. Like this review, we also noted that within the limit of information available to us, there are no known direct inhibitors of plasmodial Hsp40s. This is possibly due to paucity of information on the druggability of these proteins. While no plasmodial Hsp40 proteins have been directly targeted, their essentiality to the continuous survival and development of the parasite, viz-a-viz their functions alone or their functional interactions with and modulations of partner druggable Hsp70 proteins could constitute attractive avenues for drug discovery [174,176,178,179,182–184]. Thus, we have presented here updated information on small molecule inhibitors known to possess activities against the co-chaperone activities of plasmodial Hsp40s, especially with Hsp70s. Also, the selectivity of the identified small molecules appears to be directly connected with functional interaction of the different Hsp40 interacting partners. However, the abilities of these small molecules to disrupt the functional interactions of plasmodial Hsp40s with their counterpart Hsp70s [174,175,193], should serve as impetus for the design and development of Hsp40-specific small molecule inhibitors. In addition, it should be noted that for sustainable therapeutic intervention, focus on sexual development with a view to blocking malarial transmission should be intensified [194]. Thus, the available evidence in support of the small molecules points to a promising future of potential development of a new chaperone / co-chaperone-based antimalarial pharmacotherapy.

Plasmodial Hsp40s can also find usefulness in the development of new, effective and sustainable vaccines. Currently, large scale research efforts and funding are being expended in a bid to develop a vaccine against the malaria parasite [103,195,196]. Found in the forefront are: pre-erythrocytic vaccines which target sporozoites or hepatic stages or both; secondly, a blood stage vaccine which was aimed at preventing the erythrocyte invasion and adherence ability of parasitized RBCs; and thirdly, a transmission blocking vaccine in which parasite gametocytes proteins as well as proteins at sporogonic, or mosquito stages of the parasite are being explored as vaccine candidates [191,197]. While blood stage vaccines lack the capacity to prevent infection, they nonetheless have found usefulness in suppressing clinical symptoms of malaria. On the other hand, pre-erythrocytic vaccines use irradiated sporozoites to induce immune responses aimed at preventing erythrocytic invasion. And transmission blocking vaccines are designed to block the transmission of parasite from human to mosquitoes and/or mosquitoes to human, thereby preventing malaria from being transferred from one human host to the other [191]. In fact, a WHO release on vaccine development considered a transmission blocking vaccine as an ideal public good [198]. However, like other form of vaccines, a transmission blocking vaccine has challenges such as regulatory bottle necks [197] and an inability to prevent existing infections, recrudescence or the establishment of new infections. Thus, this type of vaccine has raised many concerns as to the benefits or otherwise of a vaccine that would not confer immediate protection on the recipients. Also, the multi-stage developmental life cycle of the malaria parasite with its attendant continuous changes in surface proteins raised an additional challenge to vaccine development. Therefore, redirecting research attention to molecular chaperones, such as plasmodial Hsp40s that are essential for parasite survival, exhibit multi-developmental stage expression profiles and are induced in response to stressors, may provide new avenues for drug discovery and vaccine development.

7. CONCLUSION

We have highlighted the potential of plasmodial Hsp40s as targets in the search for new and sustainable malaria pharmacotherapy. They could be successfully targeted as chaperones by interfering with their ability to suppress protein aggregation, and / or as co-chaperones by disrupting their ability to modulate Hsp70 functions. The successful targeting of plasmodial Hsp70s and Hsp90s with small molecule inhibitors, has shown us the door to exploring the potential of plasmodial Hsp40s as veritable new antimalarial drug targets. However, further detailed characterization of plasmodial Hsp40s and their functional interactions with partner chaperones, as well systematic structure-activity studies of small molecule modulators of these proteins, will provide the key to unlocking this exciting door to new discoveries.

8. CONFLICT OF INTEREST

The authors declare that there are no conflict of interest.

9. SUPPORTIVE/SUPPLEMENTARY MATERIALS

Supplementary Figure 1: Selected representatives of other small molecule inhibitors that modulate Hsp70s. These small molecules have been tested on various Hsp70s, but not plasmodial Hsp70s.. They have the potential, like those in Figure 5, to be effective against *Plasmodium falciparum*. Sourced and adapted from [180].

Supplementary Table 1: Raw Data for Uniquely and Non-uniquely mapped sequences represented in Figure 3.

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FIGURES

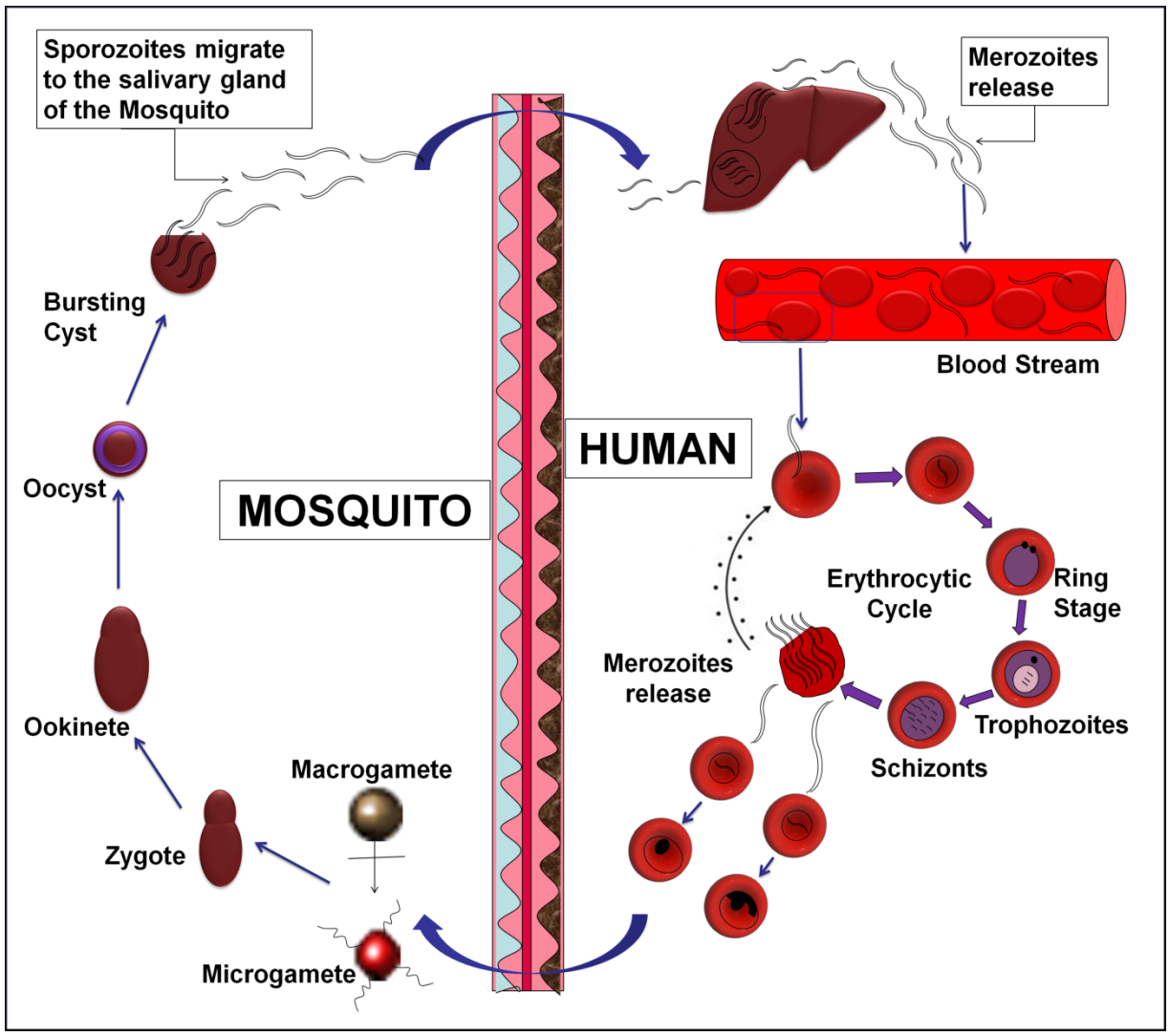
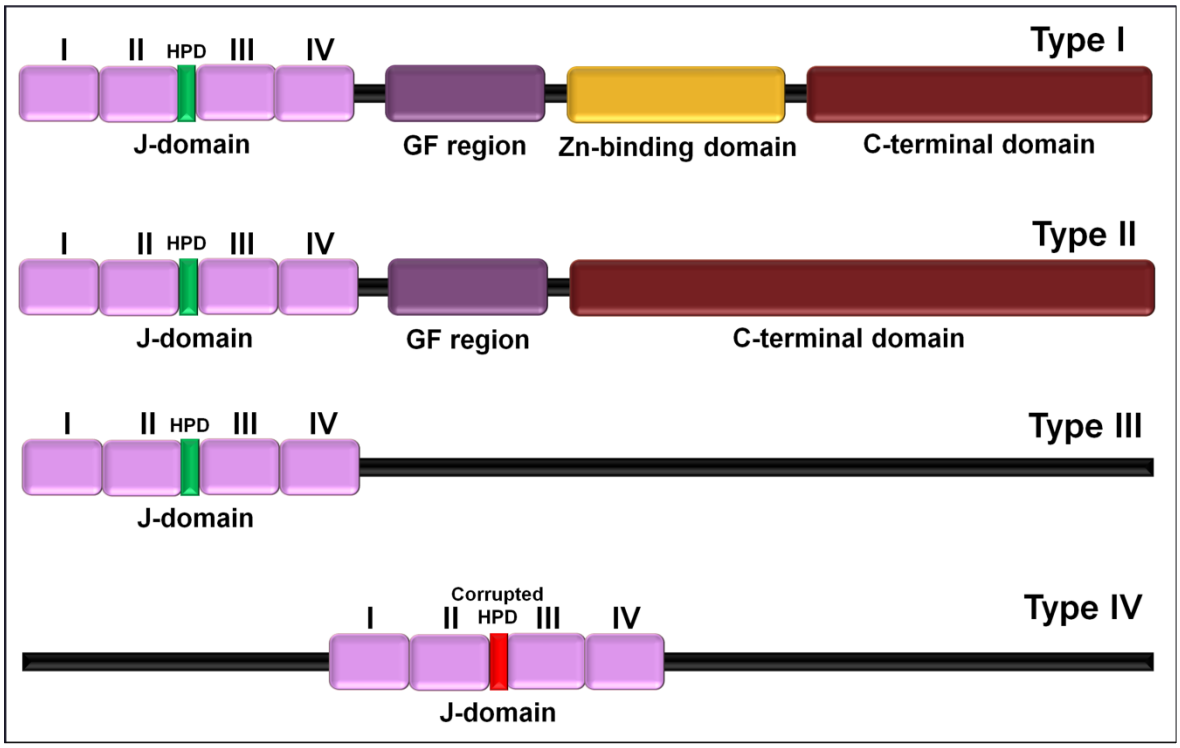


Figure 1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III
IV
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GF region
Zn-binding domain
C-terminal domain
Type II
I
II
HPD
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IV
J-domain
GF region
C-terminal domain
Type III
I
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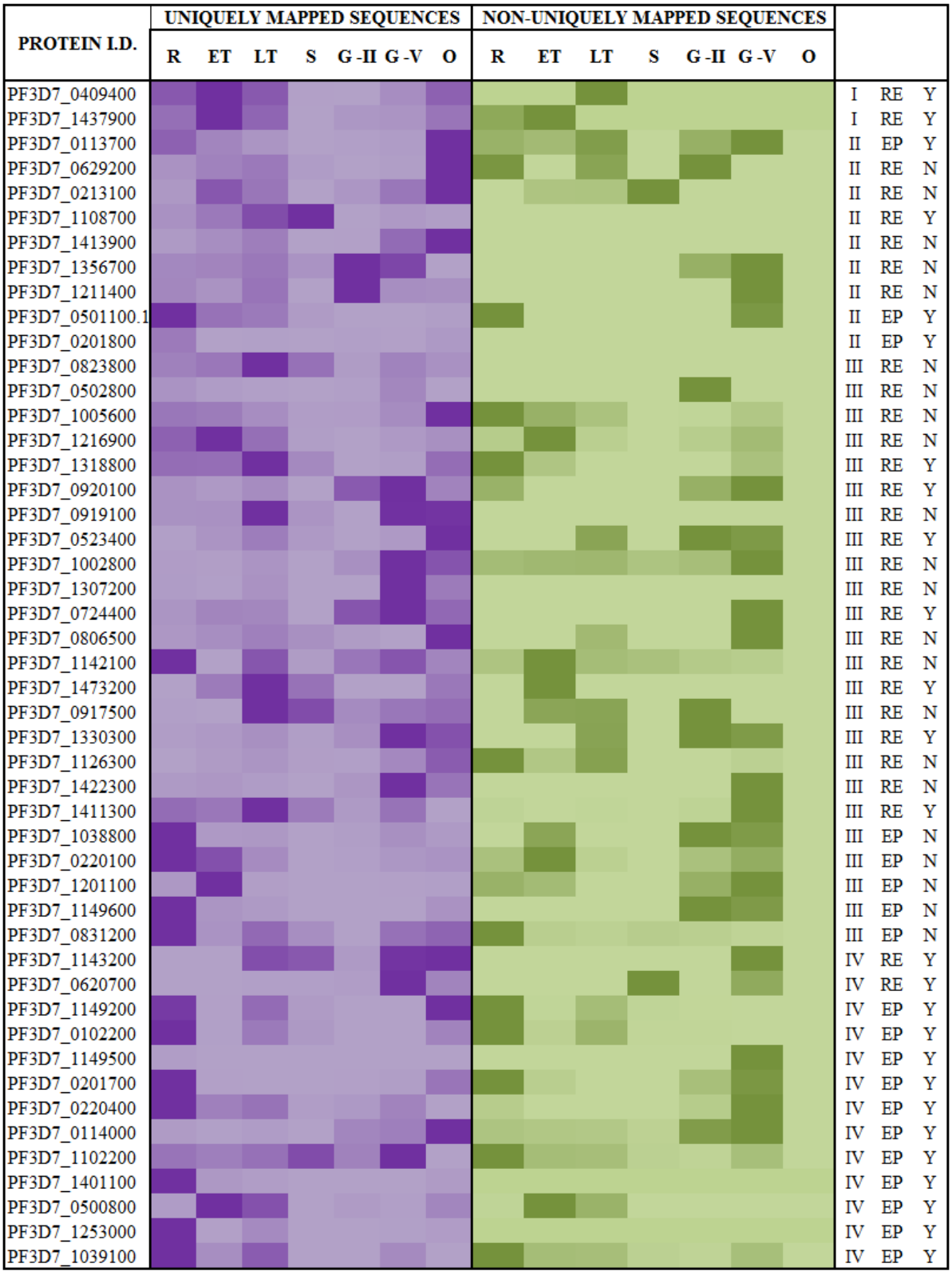
HPD
III
IV
J-domain
Type IV
Corrupted
I
II
HPD
III
IV
J-domain

Figure 2


PROTEIN I.D.
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NON-UNIQUELY MAPPED SEQUENCES
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R ET LT S G-II G-V O
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PF3D7_1437900 I RE Y
PF3D7_0113700 II EP Y
PF3D7_0629200 II RE N
PF3D7_0213100 II RE N
PF3D7_1108700 II RE Y
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PF3D7_1356700 II RE N
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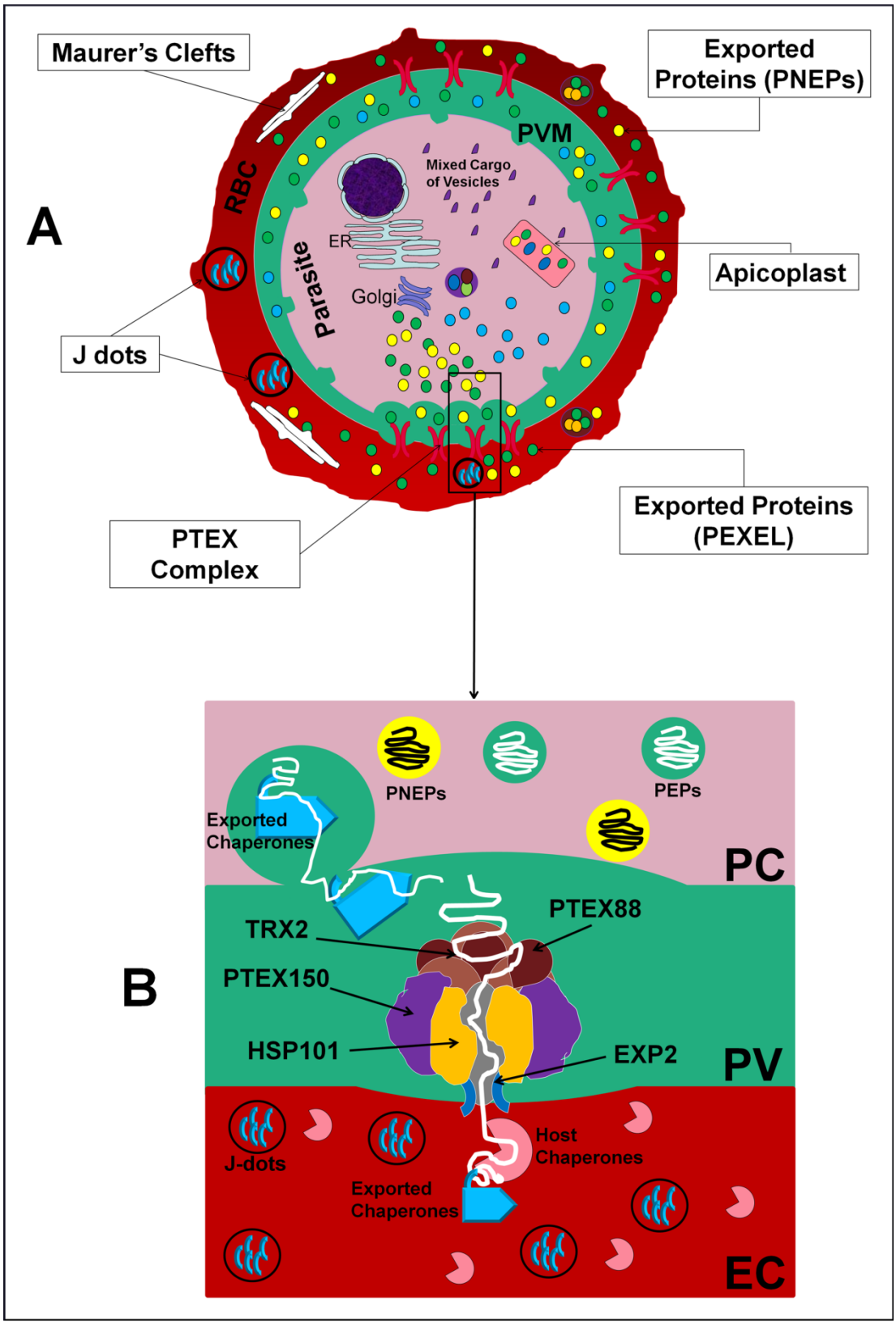
Figure 3


A
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Exported Proteins (PNEPs)
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Mixed Cargo of Vesicles
RBC
ER
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Golgi
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J dots
PTEX Complex
Exported Proteins (PEXEL)
B
PNEPs
PEPs
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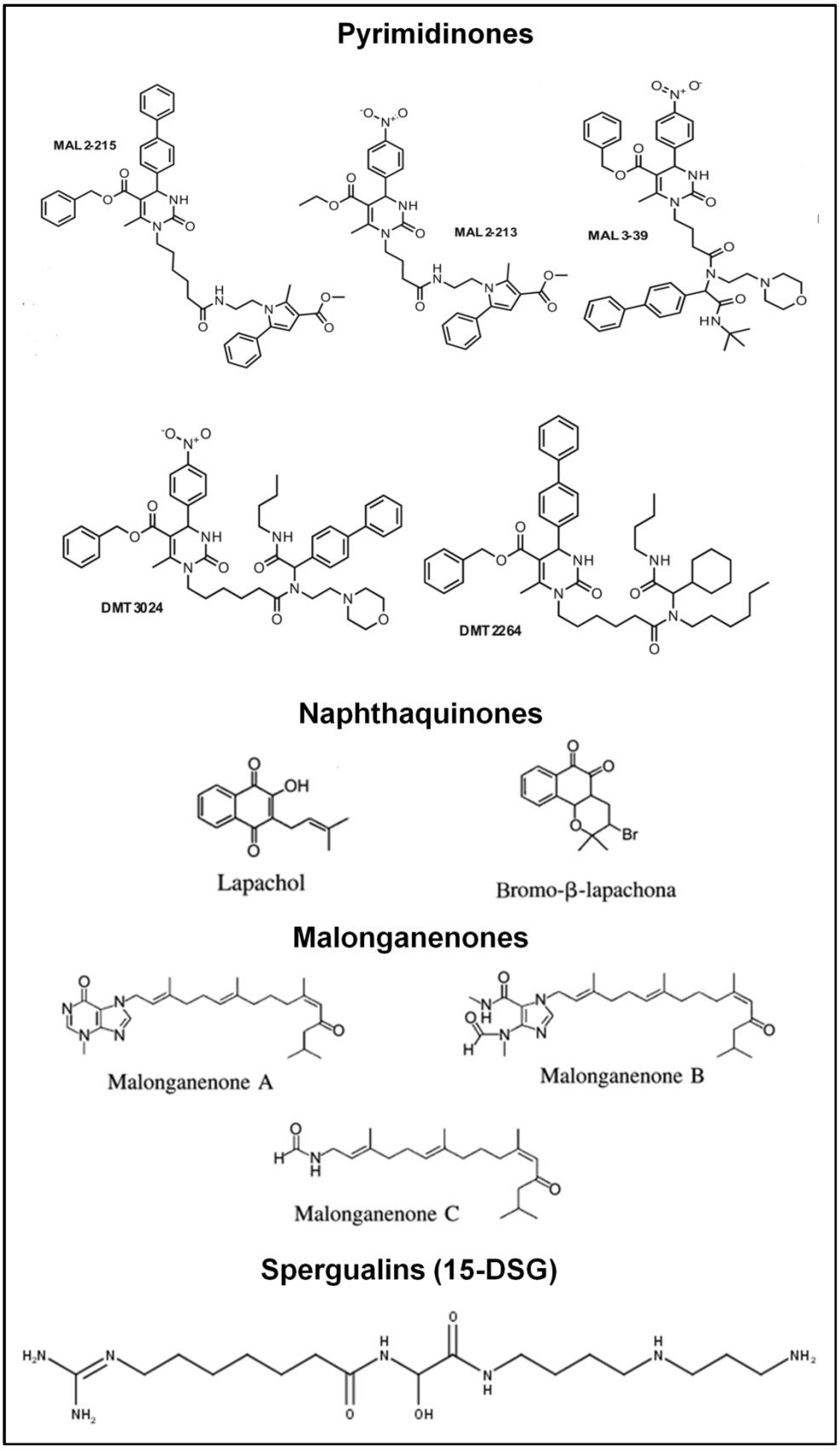
EC

Figure 4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DMT 3024
DMT 2264
Naphthaquinones
Lapachol
Bromo-β-lapachona
Malonganenones
Malonganenone A
Malonganenone B
Malonganenone C
Spergualins (15-DSG)

Figure 5