N-myristoylation as a drug target in malaria: exploring the role of N-myristoyltransferase substrates in inhibitor mode of action

Anja C. Schlott[†,‡], Anthony A. Holder[†] and Edward W. Tate[‡*]

[†]Malaria Parasitology, The Francis Crick Institute, 1 Midland Rd, NW1 1AT, London, United Kingdom

[‡]Department of Chemistry, Imperial College London, Imperial College Rd, SW7 2AZ, London, United Kingdom

*E-mail: e.tate@imperial.ac.uk

Malaria continues to be a significant cause of death and morbidity worldwide and there is a need for new antimalarial drugs with novel targets. We have focused as a potential target for drug development on *N*-myristoyl transferase (NMT), an enzyme that acylates a wide range of substrate proteins. The NMT substrates in *Plasmodium falciparum* include some proteins that are common to processes in eukaryotes such as secretory transport and others that are unique to apicomplexan parasites. Myristoylation facilitates a protein interaction with membrane that may be strengthened by further lipidation, and inhibition of NMT results in incorrect protein localization and consequent disruption of function. The diverse roles of NMT substrates mean that NMT inhibition has a pleiotropic and severe impact on parasite development, growth and multiplication. To study the mode of action underlying NMT inhibition it is important to consider the function of proteins upstream and

downstream of NMT. In this work, we therefore present our current perspective on the different functions of known NMT substrates as well as comparing the inhibition of co-translational myristoylation to the inhibition of known targets upstream of NMT.

Keywords

N-myristoyl transferase, NMT, acyl transferase, myristoylation, palmitoylation, post-translational modification, protein lipidation, chemical proteomics

Malaria is caused by apicomplexan parasites of the genus *Plasmodium*, with an estimated 216 million clinical cases worldwide in 2016 causing around half a million deaths.[1] The spread of resistance to all front-line drugs (recently reviewed by Blasco et al.[2] as well as the low efficacy of the current vaccine[3], increases the need for new drugs with novel modes of action. Here we describe recent studies to define the substrates of *N*-myristoyl transferase (NMT) in the principle human parasite *Plasmodium falciparum* and the potential of this enzyme as a drug target for treatment of malaria.

_N_-myristoyltransferase as a drug target in _Plasmodium_

NMT catalyzes the transfer of the fatty acid myristate (C14) from myristoyl coenzyme A (myr-CoA) to the α-amino group of the N-terminal glycine residue of various protein substrates (Figure 1) forming an amide bond[4], and is a promising new drug target for malaria. Protein *N*-myristoylation is predominantly a co-translational process following removal of the initiator methionine.[4] Myristoylation promotes substrate localization to membrane and regulates protein complex assembly and protein stability although the exact role of myristoylation may be unclear.[5]

NMT is present in eukaryotes and was first discovered in *Saccharomyces cerevisiae*.[6] In contrast to all metazoa, which encode two NMT isoforms[7], *Plasmodium* spp. possess a single NMT enzyme, which was first reported in 2000 by the Holder lab.[8] NMT has been shown to be essential for survival of fungi and protozoa including the parasites *Leishmania major*, *Trypanosoma brucei* and *Trypanosoma cruzi*[9-13], recently review in Ritzefeld et al.[14] The enzyme was shown to be important in signaling pathways during development in mice[15] and C. elegans.[16] NMT is a cancer drug target and its inhibition is thought to lead to upregulation of proteins involved in endoplasmic reticulum (ER) stress and downregulation of cell cycle proteins.[17]

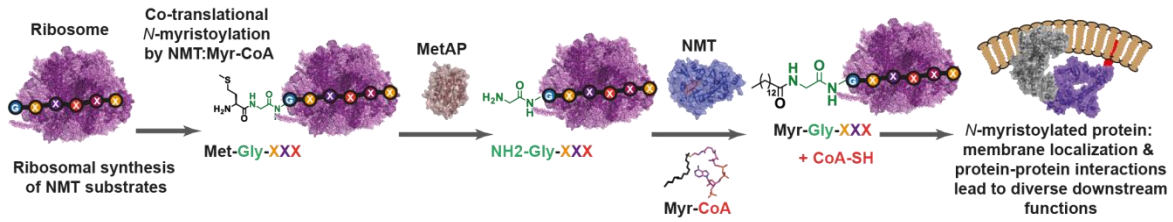


Figure 1: *N*- myristoylation is catalysed by the acyl transferase, *N*-myristoyl transferase (NMT), which attaches myristic acid to the N-terminal glycine of specific protein substrates. The modification is mostly co-translational

following removal of the initiator methionine by methionine aminopeptidase (MetAP). The reaction catalysed by NMT follows an ordered Bi-Bi double displacement mechanism with Myr-CoA binding inducing a conformational change in NMT to allow substrate engagement. This is followed by transfer of the myristic acid to the substrate by nucleophilic substitution. Myristoylation promotes substrate localization to membrane, protein-protein interactions and protein stability.

Metabolic labelling and tagging *via* click chemistry to study myristoylation

In silico analysis of the *P. falciparum* genome for potential NMT substrates indicated that up to 2% of the 5400 encoded proteins are NMT substrates.[5,18] Experimentally, just over 30 substrates have been identified in the schizont stage of parasite development in erythrocytes through a chemical proteomic approach using a myristic acid analogue (YnMyr) to label NMT substrates.[5] This metabolic tagging with click chemistry (MTCC) is used to attach the YnMyr alkyne group to an azide group on a capture reagent through copper-catalysed azide/alkyne cycloaddition (CuAAC).[19] The capture reagent may contain a biotin moiety, fluorophore or both for subsequent purification and protein identification by mass spectrometry (MS) or in-gel fluorescence of the labelled NMT substrates. The workflow for quantitative and comparative chemical proteomics, using a potent and selective NMT inhibitor (NMTi) as a tool to discriminate NMT substrates from non NMT-substrates, is outlined in Figure 2 and has been reviewed recently.[14]

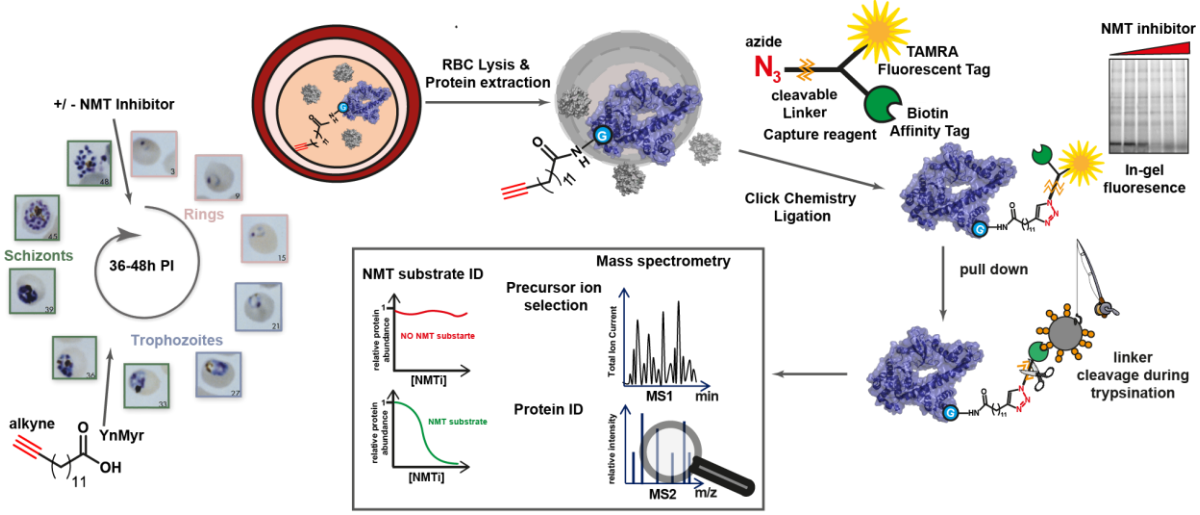


Figure 2: Synchronized parasite cultures are incubated with YnMyr, in the presence of the NMT inhibitor or DMSO carrier during the developmental stage of interest. Red blood cells (RBCs) containing parasites are lysed and proteins are extracted. The alkyne handle on YnMyr is ligated to a multifunctional capture reagent containing biotin and/ or TAMRA fluorophore through Cu(I)-catalyzed azide/alkyne cycloaddition (CuAAC). The fluorophore is used for in-gel visualization of the NMT substrates. The biotin allows NMT substrate enrichment on streptavidin coated beads. An additional optional cleavable linker allows the fluorophore and biotin handle to be removed after pull down and enables direct identification of the site of modification by MS/MS. After on-bead digestion with trypsin, NMT substrate protein peptide fragments are expected to decrease in samples with increasing NMTi concentration. Peptide fragments resulting from fragmentation of the reporter ions are submitted to MS2, generating individual spectra for each peptide.

An additional 28 proteins with N-terminal glycine are predicted to be myristoylated in the erythrocytic life cycle, of which nine belong to the PfEMP1 family. Fourteen of these proteins are synthesized earlier than 35 hours post invasion (h PI), defined as the duration after first invasion into newly infected red blood cell (RBC), and therefore would not be expected to be identified in the 40-45 h PI time window examined by Wright et al.[5]

Dual acylation of *N*-myristoylated proteins

Myristoylated proteins may also be *S*-acylated via thioesterification. By comparing the known myristoylated proteins with the *P. falciparum* palmitoyl proteome [20] that was acquired experimentally, 11 proteins were identified as likely dual acylated with a cysteine palmitoylation site either close to the N-terminus (most common, e.g. armadillo-domain containing rhoptry protein, ARO)[21] or at both the N- and C-termini (e.g. glideosome-associated protein 45, GAP45).[14] (Table 1) Cabrera et al., identified 18 proteins predicted to be dual acylated within the first 20 amino acids, while genome analysis suggests that there

are up to 25 proteins that can be both myristoylated and palmitoylated at the N-terminus. The Gibbs free energy released by interaction of the myristoyl group with membrane is thought to be insufficient to tightly anchor most substrates, creating the necessity for a second modification of the protein to complement myristoylation, such as a second acyl group near the N-terminus.[4] Experimental data making use of point mutations have shown that this second signal is necessary.[21-23] Therefore, palmitic acid (C16) is added as a reversible post-translational acylation to strengthen the interaction with membrane.

Table 1: List of NMT substrates likely to be dual acylated with at least one palmitoylation site

Protein ID	Protein name
PF3D7_1020900	ADP-ribosylation factor 1.1 (ARF1)
PF3D7_0414900	Armadillo-domain containing rhoptry protein (ARO)
PF3D7_0217500	Calcium-dependent protein kinase 1.1 (CDPK1)
PF3D7_1137300	CLPTM1 domain containing
PF3D7_1237700	Conserved protein unknown function
PF3D7_1222700	Glideosome-associated protein 45.1 (GAP45)
PF3D7_1460600	IMC protein 3.1 (ISP3)
PF3D7_1011000	IMC protein 1.1 (ISP1)
PF3D7_0810300	Protein phophatase (PPM5)
PF3D7_1310600	Ras-related protein Rab-5B, GTPase (Rab-5b)
PF3D7_0816200	Vacuolar protein-sorting protein (VPS2)

NMT substrates: where are they located and what is their function during parasite development in the erythrocyte?

NMT substrates are involved in a diverse range of biochemical and cellular pathways including: protein transport, secretion, ion channel regulation, protein homeostasis,[5] and formation of the inner membrane complex (IMC), a flattened vesicular structure lying just below the parasite plasma membrane (PPM)[24], as well as in parasite motility and development.[25] Their known biological functions range from hydrolase to kinase and phosphatase activities, and stabilizing the parasite motor complex that drives invasion (Figure 3). NMT substrates are localized to membranes including the PPM but also membranes of organelles such as the micronemes and rhoptries (Figure 3). In order to

understand the consequences of NMT inhibition and to find potential drug targets downstream of myristoylation, substrates identified to date are analyzed below for function, location and essentiality.

NMT substrates and their function at the PPM and IMC

The two most studied NMT substrates in the malaria parasite are calcium-dependent protein kinase 1 (CDPK1) and glideosome-associated protein 45 (GAP45). Both proteins have been shown to have N-terminal myristoylation and a nearby cysteine residue that can be modified by *S*-palmitoylation.[26,27] CDPK1 requires *N*-myristoylation for localization to the PPM[28], [29] (Figure 3), where it facilitates key functions of the asexual blood stage such as erythrocyte invasion by phosphorylating IMC components, including GAP45 and myosin A tail domain interacting protein (MTIP), and Raf kinase inhibitor protein (RKIP).[29,30] CDPK1 is likely non-essential in the asexual blood stage, at least in *P. berghei*[31-33], however, knockdown of CDPK1 in sexual stages resulted in developmental arrest and prevented parasite transmission to mosquitoes.[32] GAP45 is localized to both the PPM and the IMC (Figure 3)[23,34], where it is thought to be involved in recruiting and stabilizing the actomyosin motor complex, which drives merozoite invasion.[23], [35] Further investigation of GAP45 localization showed that the first 30 N-terminal amino acids of GAP45 are sufficient to localize a GFP fusion to the PPM, whereas a truncated GAP45 lacking the N-terminal 30 amino acids localizes to the IMC thanks to *S*-palmitoylation at the C-terminus[23]. This study used GAP45 variants expressed from an episomal plasmid, without deletion of the endogenous gene, and therefore it did not address the issue of whether myristoylation of GAP45 is essential for its function and therefore for the viability of the parasite.

Two additional NMT substrates called IMC sub-compartment proteins 1 and 3 (ISP1 and ISP3) have also been shown to localize at the IMC.[36] (Figure 3) Together with GAP45 they have important roles in regulating IMC formation by organizing the apical end of the cell through the establishment of polarity.

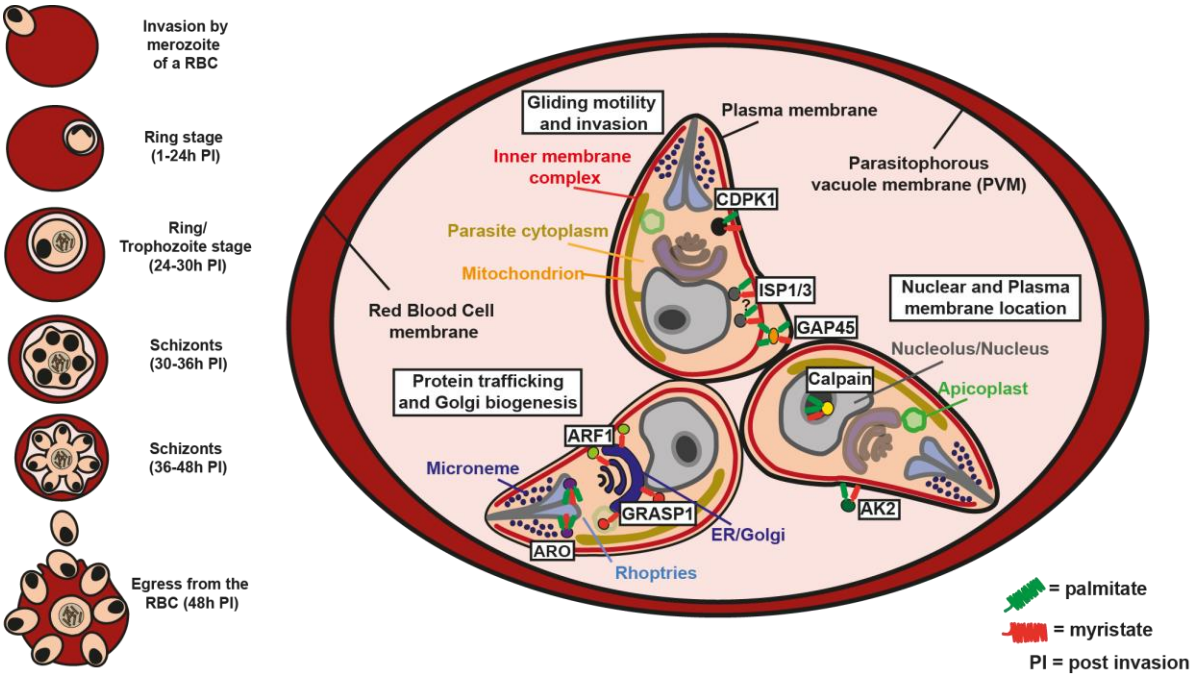


Figure 3: *Plasmodium falciparum* NMT substrates are involved in a variety of essential processes including host cell invasion, parasite gliding motility, protein trafficking and protein degradation. Inhibition of NMT leads to the mis-localization and mis-function of these substrates, causing catastrophic and irreversible failure to continue the life cycle.

NMT substrates involved in transport and secretory organelle localization

About 20% of the identified *N*-myristoylated proteins are involved in transport, making this the largest functional group (Figure 4). Transport includes the secretory pathway involved in exporting merozoite surface proteins including MSP1 to the PPM and soluble proteins to the parasitophorous vacuole (PV) towards the end of schizogony. Examples of NMT substrates involved in this process include ADP-ribosylation factor 1 (ARF1) and two ADP-ribosylation factor-like (ARL) proteins (PF3D7_0920500 and PF3D7_1034700) (Figure 3). All have putative

GTP hydrolase activity, and ARF1 was found to co-localize with a Golgi marker[37] and is amongst the most abundant *N*-myristoylated proteins identified by Wright *et al.*[5] In other organisms, ARF1 is involved in ER-Golgi vesicular trafficking, recruiting coat proteins to promote the assembly of a coating complex for cargo secretion by vesicular budding, and in determining cell structure by interacting with cytoskeletal factors; these functions are likely conserved in *Plasmodium spp*. due to ARF1's high homology across species.[38] The myristate of human ARF1 switches between a buried and an exposed state (the myristoyl-switch), allowing ARF1 to associate with and dissociate from membrane as part of its function.[4]

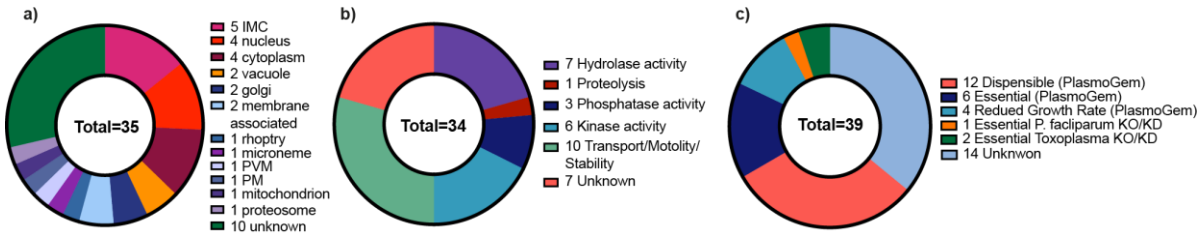


Figure 4: Analysis of known *N*-myristoylated proteins identified by Wright *et al*., a) Putative localization; b) Putative function. c) Putative essentiality for the erythrocyte life cycle. Assigned using PlasmoDB, GO Retriever, PlasmoGem, and literature searches. Some substrates received multiple assignments.

Some NMT substrates involved in transport are localized to the membrane of a secretory organelle such as the rhoptries or micronemes. ARO is localized to rhoptries at the apical end of merozoites[21] (Figure 3), and by analogy with its proposed role in *Toxoplasma gondii,* it is involved in rhoptry positioning to the apical end of the cell, where the rhoptries discharge their contents during host cell invasion.[39] An inducible *aro* gene knockout in *T. gondii* caused mis-localization and cytosolic dispersion of the rhoptries, resulting in severe defects in invasion but no effect on intracellular growth or egress from the host cell.[39] A recently characterized protein now named acylated pleckstrin-homology domain-containing protein

(APH) (PF3D7_0414600) was found to be myristoylated.[5] APH is homologous to a protein coded by a gene (TGME49_249970) found to be essential in a genome-wide CRISPR screen in *Toxoplasma.*[40] Myristoylation and palmitoylation of APH is thought to be involved in localizing the protein to the outer surface of micronemes, where it is involved in sensing phosphatidic acid (PA), inducing microneme secretion.[41]

Other known NMT substrates also have predicted transport functions, including Golgi re-assembly stacking protein 1 (GRASP1), Rab5b, a member of the Rab family of GTPases, vacuolar protein-sorting protein 2 (VPS2) (PF3D7_0816200), and vacuolar protein sorting-associated protein 46 (VPS46) (PF3D7_0906100). GRASP1 is highly conserved at the N-terminus across most species and is involved in maintaining Golgi integrity and positioning close to the nucleus due to its myristoylation motif.[42] The presence of two GRASP splicing variants has recently been reported leading to two different isoforms of which only GRASP1 is myristoylated whereas GRAPS2 lacks myristoylation and instead contains a hydrophobic N-terminal sequence interacting with the Golgi membrane; its location resembles the distribution of GRASP1 but its structure may result in a stronger binding to the membrane.[43] Rab5b is both myristoylated and palmitoylated[44], and has been shown to localize to the food vacuole and PPM.[34] It co-localizes with AK2 and both may be transported to the tubulovesicular network, extending from the PVM through a membrane-trafficking pathway that is still largely unknown.[45] In addition to being myristoylated and palmitoylated, the GTPase Rab5b may be prenylated on either of three internal cysteine residues[46], and these lipidations may be important for its localization to both the food vacuole and the parasitophorous vacuole.[47] Both VPS proteins were identified as myristoylated[5] and may play a role in vesicle tethering, by homology with VPS proteins in other organisms.[48] In

Plasmodium, vesicular trafficking involving VPS proteins may also prepare parasites for the host cell invasion process.[49]

NMT substrates with hydrolase activity

In addition to ARF1, the two ARF-like proteins and Rab5b, there are two additional NMT substrates with hydrolase activity, the alpha/beta hydrolases PF3D7_0805000 and Pf3D7_0403800, which have been identified as NMT substrates.[5] The 26S proteasome regulatory subunit 4 (PRT2) (PF3D7_1008400) was also identified as an NMT substrate through the identification of its modified N-terminal peptide by mass spectrometry. The mammalian homologue, PSMC1, is important for the function of the proteasome in platelet formation and brain development[50,51], and in *P. falciparum*, PRT2 is likely essential based on the result of the *P. berghei* gene knock out screen.[52] The proteasome is a known drug target in *Plasmodium.*[53,54]

NMT substrates and their involvement in signaling pathways

NMT substrates with a role in phosphorylation/dephosphorylation switches in signaling pathways include calcium-dependent protein kinase 4 (CDPK4), and CDPK1. CDPKs are absent from animals and have only been found in plants, ciliates, and apicomplexan parasites. Their exact roles are not fully understood, but they may regulate intracellular calcium levels and cell cycle progression through their stage-specific expression.[55] Both CDPK1 and CDPK4 are thought to be dispensable in the asexual life cycle but essential for sexual reproduction and mosquito transmission in *P. berghei*[56], where CDPK4 has a role in activating replication in haploid microgametocytes, assembling the mitotic spindle by phosphorylating microtubule-associated proteins, as well as completing cytokinesis by activating the motility of the male gamete.[57] For most of these functions myristoylation is

thought to be essential, providing an indication of its importance in sexual stages. A second smaller isoform of CDPK4 that is not myristoylated has been identified and is thought to be essential to complete late gametogenesis, while the longer myristoylated isoform may initiate the first round of DNA replication.[57] The myristoylated cAMP-dependent protein kinase A regulatory subunit (PKAr)[25,58] is the regulator of PKA, which is important in cell invasion during the asexual stages, in gametogenesis, as well as in merozoite formation inside hepatocytes, as reviewed in Wurtz *et al*., 2011.[59] PKAr was recently shown to be palmitoylated and predicted to be myristoylated in *Toxoplasma gondii.*[60] Because of its importance for many aspects of parasite biology, PKA represents an attractive drug target[58], although it may be difficult to obtain selectivity over the host enzyme. Two metal-dependent protein phosphatases (PPM) called PPM2 and PPM5 have also been identified as NMT substrates.[61] Their expression profile indicates their presence throughout the life cycle with elevated levels during schizogony and in activated gametocytes. Guttery *et al.* provided evidence for their essential function during sexual and sporogonic development of *P. berghei*, through gene disruption experiments.[61]

Two likely NMT substrates, calpain[62] and adenylate kinase 2 (AK2)[63] were not detected by Wright *et al*. in schizonts, probably due to their early expression in asexual blood stages.[5] Calpain has been localized to the nucleolus[62], and its successful knock down in *P. falciparum* showed it to be essential for cell cycle progression, with ring stage parasites unable to progress to S-phase.[64] A non-myristoylated Gly2 to Ala mutant (G2A) Calpain-YFP chimera expressed from an episomal plasmid accumulated in the cell cytoplasm, indicating the importance of *N*-myristoylation in targeting calpain to the nucleolus.[62] AK2 is expressed primarily in late stage gametocytes and ookinetes, and largely absent in asexual blood stage

parasites. However it was shown to be myristoylated in an artificial co-expression system of recombinant AK2 with NMT[63], and localized beyond the PPM to the PVM by co-localization with Exp1 by immunofluorescence imaging.[65] Combining live fluorescence imaging and biochemical approaches Thavayogarajah and co-workers found AK2 to be located at the outside of the PPM.[37] In AK2 most residues involved in AMP binding are conserved between the *Plasmodium* and human homologues.[63] Although AK2 was not found to be essential in the recent *P. berghei* screen[31] it is an interesting NMT substrate because its location suggests an alternative secretory pathway in *Plasmodium spp.* in which N-terminal myristoylation and adjacent palmitoylation are involved in translocating proteins across the PPM. [37]

NMT inhibition by small molecules

The knockdown of NMT activity using pharmacological NMT inhibitors (NMTi) is an important tool to investigate the phenotypic consequences of NMT inhibition without the need to generate a conditional genetic knockout of the essential and constitutively expressed single *nmt* gene in *P. falciparum.* In a tetracycline-induced knock-down of the *nmt* gene in *P. berghei*[66], parasites showed a severely impaired maturation resulting in abnormal morphology reminiscent of that observed by Wright *et al*. following NMTi treatment of *P. falciparum* parasites.[5]

Using the MTCC approach highlighted in Figure 2, several different NMT inhibitors (NMTis) have been evaluated in parasites to determine their TC_{50}, the concentration of NMTi at which 50% incorporation of YnMyr occurs, as visualized by decrease in fluorescence on a SDS-PAGE gel or by proteomic readouts[67] and to correlate it with the EC_{50} (i.e. parasiticidal activity *in vitro*). Inhibitors include previously reported Dundee University *T. brucei* NMTis[13,68], and

Imperial College *P. falciparum* NMTis developed from a benzothiophene scaffold antifungal series[69,70], as well as a new series of NMTis originating from two high-throughput screens (HTS) of 150,000 compounds.[71] Compounds from each series were found to inhibit YnMyr incorporation and parasite growth in a dose dependent manner, with a good correlation between the TC_{50} and EC_{50} showing direct engagement of compounds with NMT *in vitro.*[5,14] A recent review by Ritzefeld *et al*., highlights recent progress in the development of NMT inhibitors against *Plasmodium*, *Leishmania* and *Trypanosoma* parasites.[14]

Selective NMT inhibition for 45 hours leads to severe pleiotropic consequences affecting parasite development in the asexual blood stage, presumably due to the malfunction of multiple substrates and the downstream effects, and resulting in an irreversible failure of parasites to complete their life cycle. When myristoylation is inhibited from an early point in the erythrocyte cycle with a NMTi concentration four times greater than the EC_{50}, development is blocked at around 36 to 39 h PI, arresting the parasite at a stage with 4 to 7 nuclei compared to control parasites, which should normally contain up to 20 nuclei at 45 h PI.[5] NMTi-treated parasites fail to egress leading to a > 85% drop in parasitaemia. It has been hypothesized that these abnormal looking “pseudoschizonts” with 4 to 7 nuclei are arrested in development due to a failure to assemble the IMC during early schizogony, which is in turn due to the loss of essential IMC components, including NMT substrates such as GAP45, ISP1, and ISP3.[5] The expression of IMC markers (e.g. GAP45, MyoA, MTIP and ISP3) is greatly reduced in NMTi treated parasites, suggesting that parasite development stops before 38 h PI, a stage when GAP45 synthesis is normally complete.[5,23] It is also possible that the defect precedes the onset of the “pseudoschizont” phenotype and might be initiated by an earlier malfunction. Since treatment with a NMTi for 45 hours generates a severe phenotype, it is

important to ask what would be consequence of changing the duration or timing of the window of treatment. Such experiments may help in determining how NMT inhibition kills the parasite.

NMTi and protein homeostasis: a convergence of pathways

Even if an NMT inhibitor is added immediately after invasion there is a lag phase until parasite death is observed at around 36 to 38 h PI. A similar lag phase and arrested developmental stage is observed following parasite treatment with protein synthesis-inhibitors. It is interesting to note that total protein synthesis accelerates at around 18 to 24 h PI[72] and that myristoylation is a co-translational process, raising the possibility that NMTi and protein synthesis inhibition phenotypes may be connected through common essential downstream factors. However, Wright *et al*. showed that total protein synthesis is not directly affected by NMTis, as expected for a selective inhibitor of NMT, implying that the initial mechanism is distinct.[5] Many protein synthesis inhibitors stop parasite development in the transition from ring to trophozoite/schizont stage at the point where protein synthesis starts to increase. For example, Baragaña *et al.* showed that the translation elongation factor 2 (eEF2) inhibitor DDD107498 prevents trophozoite and schizont development, generating morphologically abnormal schizonts when treatment is commenced at ring stage.[73] Images from Giemsa-stained thin blood smears suggest that parasites arrest before DNA synthesis starts at around 24 h PI, and schizont formation was prevented, causing a 50% reduction in parasitaemia. It is possible that NMTi may in some way resemble inhibitors of a subset of protein synthesis, which would explain a similar phenotype. The parasite response to nutrient restriction, which in some ways phenocopies the effect of protein synthesis inhibition, is a universal evolutionary adaptation and may drive the process of differentiation

into gametocytes.[74] The efficacy of NMT inhibition in other stages of the life cycle has not been reported to date, but it will be interesting to see whether NMTi mimics the same broad spectrum of activity as DDD107498 throughout the life cycle.

In addition to steps of protein translation, other targets upstream of NMT include aminoacyl-tTNA synthases (reviewed recently by Khan[75]). Both a methionyl-tRNA and a lysyl-tRNA synthetase inhibitor prevent development of mature trophozoites and schizonts when treatment is started at ring stage.[76,77] Methionine aminopeptidase (MetAP) removes the initiator methionine preceding *N*-myristoylation, and is a possible upstream target since removal of the methionine is essential for myristoylation to occur[78]; the *P. falciparum* genome encodes four MetAP proteins of which MetAP1b is a target of the inhibitor XC11, which is 100-fold selective over the other two related PfMetAP1 enzymes. This 2-(2-pyridinyl)-pyrimidine inhibitor has an IC_{50} of 112nM and also prevents schizont formation when used to treat synchronized ring stage parasites.[78] Inhibition of NMT may also lead to an imbalance in favor of other N-terminal protein modifications (NPMs) such as *N*-α-acetylation (NAT) carried out by N-terminal acetyltransferase (NATs)[79], or N-terminal ubiquitination.[80] Usually these NPMs occur co-translationally through ribosome-associated protein biogenesis factors (RPBs) that interact with the ribosome and show a degree of competition in their binding.[81] For example, there is already some indication of competition between N-α-acetylation and *N*-myristoylation.[82]

In addition to the possible imbalance of different NPMs and protein mislocalization, the downstream consequences of NMT inhibition may also include protein misfolding, leading to protein degradation. In several types of cancer cell, NMT inhibition leads to cell death

through apoptosis during G1 phase, potentially as a result of ER stress.[17] It has been proposed that inhibition of myristoylation leads to an accumulation of mis-localized proteins that are degraded by the proteasome. Furthermore the *Plasmodium* 26S proteasome regulatory subunit 4 (PF3D7_1008400) is itself an NMT substrate[5] and malfunction of this subunit due to lack of myristoylation might reinforce the ER stress response.

Conclusions and Future Perspectives

Although many *Plasmodium* NMT substrates likely remain to be discovered, it is clear that several of those already identified experimentally have an essential role in the erythrocytic cycle of the malaria parasite. To determine the mode of action of NMT inhibition and to study the biology of its substrates, it is important to examine the function of proteins upstream and downstream of NMT, and the phenotype resulting from their ablation. Many known antimalarial agents kill the parasite by inhibiting targets involved directly or indirectly in protein synthesis, often in the last 24 to 48 hours of the erythrocytic cycle. This timing coincides with that of NMT inhibitors presumably because myristoylation is a co-translational process, and may affect a similar spectrum of essential proteins as protein synthesis inhibitors acting upstream of NMT. Studies to date have focused largely on asexual blood stages in *P. falciparum* and the mouse parasite, *P. berghei*. Future studies should address the impact of NMT inhibitors on other clinically important species such as *P. vivax,* and other stages important for therapy, including pre-erythrocytic stages in the liver (for prophylaxis and targeting dormancy in *P. vivax*) and sexual development in the host and vector (for transmission blocking). There is also significant scope for exploring mechanisms of resistance to NMTi, for example through induced resistance to one or more of the several NMTi chemotypes currently available or under development. Together, these experiments

will shed light on NMTi mode of action, reveal new parasite biology, and indicate the next steps for antimalarial NMTi development, for example by helping to identify suitable combinations with existing or novel drugs with complementary modes of action.

ABBREVIATIONS

APH, acylated pleckstrin-homology domain containing protein; ARO, armadillo-domain containing rhoptry protein; CDPK, calcium-dependent protein kinase; CuAAC, copper-catalysed azide/alkyne cycloaddition; ER, endoplasmic reticulum; GAP45, glideosome-associated protein 45; IMC, inner membrane complex; MS, mass spectrometry; MTCC, metabolic tagging with click chemistry; NMT, *N*-myristoyl transferase; NMTi, NMT inhibitor; PPM, parasite plasma membrane; PV, parasitophorous vacuole.

AUTHORS INFORMATION

ORCID

Edward Tate: 0000-0003-2213-5814

Anthony Holder: 0000-0002-8490-6058

ACKNOWLEDEMENTS

AS was a Francis Crick Institute/Imperial College London PhD student. Work in the Holder and Tate laboratories was supported by the Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001097), the UK Medical Research Council (FC001097), and the Wellcome Trust (FC001097).

References

1. World Health Organization. *World Malaria Report 2017*. *World Health Organization* (2017) (pp. 1–196). Geneva, Switzerland.
2. Blasco, B., Leroy, D., & Fidock, D. A. Antimalarial drug resistance: linking *Plasmodium falciparum* parasite biology to the clinic. *Nat Med.* (2017), *23*(8), 917–928. doi:10.1038/nm.4381
3. RTS, S. C. T. P., Agnandji, S. T., Lell, B., Fernandes, J. F., Abossolo, B. P., Methogo, B. G., et al. A Phase 3 Trial of RTS,S/AS01 Malaria Vaccine in African Infants. *N Engl J Med.* (2012), *367*(24), 2284–2295. doi:10.1056/NEJMoa1208394
4. Wright, M. H., Heal, W. P., Mann, D. J., & Tate, E. W. Protein myristoylation in health and disease. *J Chem Biol.* (2010), *3*(1), 19–35. doi:10.1007/s12154-009-0032-8
5. Wright, M. H., Clough, B., Rackham, M. D., Rangachari, K., Brannigan, J. A., Grainger, M., et al. Validation of N-myristoyltransferase as an antimalarial drug target using an integrated chemical biology approach. *Nat Chem.* (2014), *6*(2), 112–121. doi:10.1038/nchem.1830
6. Towler, D. A., Adams, S. P., Eubanks, S. R., Towery, D. S., Jackson-Machelski, E., Glaser, L., & Gordon, J. I. Purification and characterization of yeast myristoyl CoA:protein N-myristoyltransferase. *Proc Natl Acad Sci U S A.* (1987), *84*(9), 2708–2712.
7. Shrivastav, A., Varma, S., Lawman, Z., Yang, S. H., Ritchie, S. A., Bonham, K., et al. Requirement of N-myristoyltransferase 1 in the development of monocytic lineage. *J. Immunol.* (2008), *180*(2), 1019–1028. doi: 10.4049/jimmunol.180.2.1019
8. Gunaratne, R. S., Sajid, M., Ling, I. T., Tripathi, R., Pachebat, J. A., & Holder, A. A. Characterization of N-myristoyltransferase from *Plasmodium falciparum*. *Biochem J.* (2000), *348* (2), 459–463.
9. Wright, M. H., Paape, D., Storck, E. M., Serwa, R. A., Smith, D. F., & Tate, E. W. Global Analysis of Protein N-Myristoylation and Exploration of N-Myristoyltransferase as a Drug Target in the Neglected Human Pathogen Leishmania donovani. *Chem Biol.* (2015), *22*(3), 342–354. doi:10.1016/j.chembiol.2015.01.003
10. Wright, M. H., Paape, D., Price, H. P., Smith, D. F., & Tate, E. W. Global Profiling and Inhibition of Protein Lipidation in Vector and Host Stages of the Sleeping Sickness Parasite Trypanosoma brucei. *ACS Infect Dis.* (2016), *2*(6), 427–441. doi:10.1021/acsinfecdis.6b00034
11. Herrera, L. J., Brand, S., Santos, A., Nohara, L. L., Harrison, J., Norcross, N. R., et al. Validation of N-myristoyltransferase as Potential Chemotherapeutic Target in Mammal-Dwelling Stages of Trypanosoma cruzi. *PLoS Negl Trop Dis.* (2016), *10*(4), e0004540. doi:10.1371/journal.pntd.0004540
12. Brannigan, J. A., Smith, B. A., Yu, Z., Brzozowski, A. M., Hodgkinson, M. R., Maroof, A., et al. N-Myristoyltransferase from Leishmania donovani: Structural and Functional Characterisation of a Potential Drug Target for Visceral Leishmaniasis. *J Mol Biol.* (2010), *396*(4), 985–999. doi:10.1016/j.jmb.2009.12.032
13. Frearson, J. A., Brand, S., McElroy, S. P., Cleghorn, L. A. T., Smid, O., Stojanovski, L., et al. N-myristoyltransferase inhibitors as new leads to treat sleeping sickness. *Nature.* (2010), *464*(7289), 728–732. doi:10.1038/nature08893

14. Ritzefeld, M., Wright, M. H., & Tate, E. W. New developments in probing and targeting protein acylation in malaria, leishmaniasis and African sleeping sickness. *Parasitology.* (2017), *8*, 1–18. doi:10.1017/S0031182017000282
15. Yang, S. H., Shrivastav, A., Kosinski, C., Sharma, R. K., Chen, M.-H., Berthiaume, L. G., et al. N-myristoyltransferase 1 is essential in early mouse development. *J Biol Chem.* (2005), *280*(19), 18990–18995. doi:10.1074/jbc.M412917200
16. Tang, H., & Han, M. Fatty Acids Regulate Germline Sex Determination through ACS-4-Dependent Myristoylation. *Cell.* (2017), *169*(3), 457–469.e13. doi:10.1016/j.cell.2017.03.049
17. Thinon, E., Morales-Sanfrutos, J., Mann, D. J., & Tate, E. W. N-Myristoyltransferase Inhibition Induces ER-Stress, Cell Cycle Arrest, and Apoptosis in Cancer Cells. *ACS Chem Biol.* (2016), *11*(8), 2165–2176. doi:10.1021/acschembio.6b00371
18. Traverso, J. E. A., Giglione, C., & Meinnel, T. High-throughput profiling of N-myristoylation substrate specificity across species including pathogens. *Proteomics.* (2013), *13*(1), 25–36. doi:10.1002/pmic.201200375
19. Lang, K., & Chin, J. W. Bioorthogonal reactions for labeling proteins. *ACS Chem Biol.* (2014), *9*(1), 16–20. doi:10.1021/cb4009292
20. Jones, M. L., Collins, M. O., Goulding, D., Choudhary, J. S., & Rayner, J. C. Analysis of protein palmitoylation reveals a pervasive role in *Plasmodium development* and pathogenesis. *Cell Host Microbe.* (2012), *12*(2), 246–258. doi:10.1016/j.chom.2012.06.005
21. Cabrera, A., Herrmann, S., Warszta, D., Santos, J. M., John Peter, A. T., Kono, M., et al. Dissection of minimal sequence requirements for rhoptry membrane targeting in the malaria parasite. *Traffic.* (2012), *13*(10), 1335–1350. doi:10.1111/j.1600-0854.2012.01394.x
22. Wetzel, J., Herrmann, S., Swapna, L. S., Prusty, D., John Peter, A. T., Kono, M., et al. The role of palmitoylation for protein recruitment to the inner membrane complex of the malaria parasite. *J Biol Chem.* (2015), *290*(3), 1712–1728. doi:10.1074/jbc.M114.598094
23. Ridzuan, M. A. M., Moon, R. W., Knuepfer, E., Black, S., Holder, A., & Green, J. L. Subcellular Location, Phosphorylation and Assembly into the Motor Complex of GAP45 during *Plasmodium falciparum* Schizont Development. *PloS One.* (2012), *7*(3), e33845. doi:10.1371/journal.pone.0033845
24. Frénal, K., Dubremetz, J.-F., Lebrun, M., & Soldati-Favre, D. Gliding motility powers invasion and egress in Apicomplexa. *Nat Rev Microbiol.* (2017), *45*, 597. doi:10.1038/nrmicro.2017.86
25. Wright, M. H., Clough, B., Rackham, M. D., Rangachari, K., Brannigan, J. A., Grainger, M., et al. Validation of N-myristoyltransferase as an antimalarial drug target using an integrated chemical biology approach. *Nat Chem.* (2014), *6*(2), 112–121. doi:10.1038/nchem.1830
26. Möskes, C., Burghaus, P. A., Wernli, B., Sauder, U., Dürrenberger, M., & Kappes, B. Export of *Plasmodium falciparum* calcium-dependent protein kinase 1 to the parasitophorous vacuole is dependent on three N-terminal membrane anchor motifs. *Mol Microbiol.* (2004), *54*(3), 676–691. doi:10.1111/j.1365-2958.2004.04313.x
27. Rees-Channer, R. R., Martin, S. R., Green, J. L., Bowyer, P. W., Grainger, M., Molloy, J. E., & Holder, A. Dual acylation of the 45 kDa gliding-associated protein (GAP45) in

Plasmodium falciparum merozoites. *Mol Biochem Parasitol.* (2006), *149*(1), 113–116. doi:10.1016/j.molbiopara.2006.04.008

28. Bansal, A., Singh, S., More, K. R., Hans, D., Nangalia, K., Yogavel, M., et al. Characterization of *Plasmodium falciparum* calcium-dependent protein kinase 1 (PfCDPK1) and its role in microneme secretion during erythrocyte invasion. *J Biol Chem.* (2013), *288*(3), 1590–1602. doi:10.1074/jbc.M112.411934
29. Green, J. L., Rees-Channer, R. R., Howell, S. A., Martin, S. R., Knuepfer, E., Taylor, H. M., et al. The motor complex of *Plasmodium falciparum*: phosphorylation by a calcium-dependent protein kinase. *J Biol Chem.* (2008), *283*(45), 30980–30989. doi:10.1074/jbc.M803129200
30. Kugelstadt, D., Winter, D., Pl ckhahn, K., Lehmann, W. D., & Kappes, B. Raf kinase inhibitor protein affects activity of *Plasmodium falciparum* calcium-dependent protein kinase 1. *Mol Biochem Parasitol.* (2007), *151*(1), 111–117. doi:10.1016/j.molbiopara.2006.10.012
31. Schwach, F., Bushell, E., Gomes, A. R., Anar, B., Girling, G., Herd, C., et al. PlasmoGEM, a database supporting a community resource for large-scale experimental genetics in malaria parasites. *Nucleic Acids Res.* (2015), *43*, D1176–82. doi:10.1093/nar/gku1143
32. Sebastian, S., Brochet, M., Collins, M. O., Schwach, F., Jones, M. L., Goulding, D., et al. A *Plasmodium* Calcium-Dependent Protein Kinase Controls Zygote Development and Transmission by Translationally Activating Repressed mRNAs. *Cell Host Microbe.* (2012), *12*(1), 9–19. doi:10.1016/j.chom.2012.05.014
33. Jebiwott, S., Govindaswamy, K., Mbugua, A., & Bhanot, P. Plasmodium berghei calcium dependent protein kinase 1 is not required for host cell invasion. *PloS One.* (2013), *8*(11), e79171. doi:10.1371/journal.pone.0079171
34. Ezougou, C. N., Ben-Rached, F., Moss, D. K., Lin, J.-W., Black, S., Knuepfer, E., et al. *Plasmodium falciparum* Rab5B is an N-terminally myristoylated Rab GTPase that is targeted to the parasite's plasma and food vacuole membranes. *PloS One.* (2014), *9*(2), e87695. doi:10.1371/journal.pone.0087695
35. Baum, J., Richard, D., Healer, J., Rug, M., Krnajski, Z., Gilberger, T.-W., et al. A conserved molecular motor drives cell invasion and gliding motility across malaria life cycle stages and other apicomplexan parasites. *J Biol Chem.* (2006), *281*(8), 5197–5208. doi:10.1074/jbc.M509807200
36. Poulin, B., Patzewitz, E.-M., Brady, D., Silvie, O., Wright, M. H., Ferguson, D. J. P., et al. Unique apicomplexan IMC sub-compartment proteins are early markers for apical polarity in the malaria parasite. *Biol Open.* (2013), *2*(11), 1160–1170. doi:10.1242/bio.20136163
37. Thavayogarajah, T., Gangopadhyay, P., Rahlfs, S., Becker, K., Lingelbach, K., Przyborski, J. M., & Holder, A. Alternative protein secretion in the Malaria Parasite *Plasmodium falciparum*. *PloS One.* (2015), *10*(4), e0125191. doi:10.1371/journal.pone.0125191
38. Donaldson, J. G., & Jackson, C. L. ARF family G proteins and their regulators: roles in membrane transport, development and disease. *Nat Rev Mol Cell Biol.* (2011), *12*(6), 362–375. doi:10.1038/nrm3117
39. Mueller, C., Samoo, A., Hammoudi, P.-M., Klages, N., Kallio, J. P., Kursula, I., & Soldati-Favre, D. Structural and functional dissection of *Toxoplasma gondii* armadillo repeats only protein. *J Cell Sci.* (2016), *129*(5), 1031–1045. doi:10.1242/jcs.177386

40. Sidik, S. M., Huet, D., Ganesan, S. M., Huynh, M.-H., Wang, T., Nasamu, A. S., et al. A Genome-wide CRISPR Screen in Toxoplasma Identifies Essential Apicomplexan Genes. *Cell.* (2016), *166*(6), 1423–1435.e12. doi:10.1016/j.cell.2016.08.019
41. Bullen, H. E., Jia, Y., Yamaryo-Botté, Y., Bisio, H., Zhang, O., Jemelin, N. K., et al. Phosphatidic Acid-Mediated Signaling Regulates Microneme Secretion in Toxoplasma. *Cell Host Microbe.* (2016), *19*(3), 349–360. doi:10.1016/j.chom.2016.02.006
42. Struck, N. S., de Souza Dias, S., Langer, C., Marti, M., Pearce, J. A., Cowman, A. F., & Gilberger, T. W. Re-defining the Golgi complex in *Plasmodium falciparum* using the novel Golgi marker PfGRASP. *J Cell Sci.* (2005), *118*(Pt 23), 5603–5613. doi:10.1242/jcs.02673
43. Struck, N. S., Herrmann, S., Langer, C., Krueger, A., Foth, B. J., Engelberg, K., et al. *Plasmodium falciparum* possesses two GRASP proteins that are differentially targeted to the Golgi complex via a higher- and lower-eukaryote-like mechanism. *J Cell Sci.* (2008), *121*(Pt 13), 2123–2129. doi:10.1242/jcs.021154
44. Quevillon, E., Spielmann, T., Brahimi, K., Chattopadhyay, D., Yeramian, E., & Langsley, G. The *Plasmodium falciparum* family of Rab GTPases. *Gene.* (2003), *306*, 13–25. doi:10.1016/S0378-1119(03)00381-0
45. Ebine, K., Hirai, M., Sakaguchi, M., Yahata, K., Kaneko, O., & Saito-Nakano, Y. *Plasmodium* Rab5b is secreted to the cytoplasmic face of the tubovesicular network in infected red blood cells together with N-acylated adenylate kinase 2. *Malar J.* (2016), *15*(1), 323. doi:10.1186/s12936-016-1377-4
46. Gisselberg, J. E., Zhang, L., Elias, J. E., & Yeh, E. The Prenylated Proteome of *Plasmodium falciparum* Reveals Pathogen-specific Prenylation Activity and Drug Mechanism-of-action. *Mol Cell Proteomics.* (2017), *16*(4 suppl 1), S54–S64. doi:10.1074/mcp.M116.064550
47. Howe, R., Kelly, M., Jimah, J., Hodge, D., & Odom, A. R. Isoprenoid biosynthesis inhibition disrupts Rab5 localization and food vacuolar integrity in *Plasmodium falciparum*. *Eukaryot Cell.* (2013), *12*(2), 215–223. doi:10.1128/EC.00073-12
48. Cai, H., Reinisch, K., & Ferro-Novick, S. Coats, tethers, Rabs, and SNAREs work together to mediate the intracellular destination of a transport vesicle. *Dev Cell.* (2007), *12*(5), 671–682. doi:10.1016/j.devcel.2007.04.005
49. Tomavo, S., Slomianny, C., Meissner, M., & Carruthers, V. B. Protein trafficking through the endosomal system prepares intracellular parasites for a home invasion. *PLoS Pathog.* (2013), *9*(10), e1003629. doi:10.1371/journal.ppat.1003629
50. Rezvani, N., Elkharaz, J., Lawler, K., Mee, M., Mayer, R. J., & Bedford, L. Heterozygosity for the proteasomal Psmc1 ATPase is insufficient to cause neuropathology in mouse brain, but causes cell cycle defects in mouse embryonic fibroblasts. *Neurosci Lett.* (2012), *521*(2), 130–135. doi:10.1016/j.neulet.2012.05.070
51. Shi, D. S., Smith, M. C. P., Campbell, R. A., Zimmerman, P. W., Franks, Z. B., Kraemer, B. F., et al. Proteasome function is required for platelet production. *J Clin Invest.* (2014), *124*(9), 3757–3766. doi:10.1172/JCI75247
52. Bushell, E., Gomes, A. R., Sanderson, T., Anar, B., Girling, G., Herd, C., et al. Functional Profiling of a Plasmodium Genome Reveals an Abundance of Essential Genes. *Cell.* (2017), *170*(2), 260–272.e8. doi:10.1016/j.cell.2017.06.030
53. Li, H., Ponder, E. L., Verdoes, M., Asbjornsdottir, K. H., Deu, E., Edgington, L. E., et al. Validation of the Proteasome as a Therapeutic Target in Plasmodium Using an

Epoxyketone Inhibitor with Parasite-Specific Toxicity. *Chem Biol.* (2012), *19*(12), 1535–1545. doi:10.1016/j.chembiol.2012.09.019
54. Li, H., O'Donoghue, A. J., van der Linden, W. A., Xie, S. C., Yoo, E., Foe, I. T., et al. Structure and function based design of Plasmodium-selective proteasome inhibitors. *Nature.* (2016), *530*(7589), 233–236. doi:10.1038/nature16936
55. Holder, A., Mohd Ridzuan, M. A., & Green, J. L. Calcium dependent protein kinase 1 and calcium fluxes in the malaria parasite. *Microbes Infect.* (2012), *14*(10), 825–830. doi:10.1016/j.micinf.2012.04.006
56. Billker, O., Dechamps, S., Tewari, R., Wenig, G., Franke-Fayard, B., & Brinkmann, V. Calcium and a calcium-dependent protein kinase regulate gamete formation and mosquito transmission in a malaria parasite. *Cell.* (2004), *117*(4), 503–514. doi:10.1016/S0092-8674(04)00449-0
57. Fang, H., Klages, N., Baechler, B., Hillner, E., Yu, L., Pardo, M., et al. Multiple short windows of Calcium-Dependent Protein Kinase 4 activity coordinate distinct cell cycle events during Plasmodium gametogenesis. *Elife.* (2017), *6*, e26524. doi:10.7554/eLife.26524
58. Haste, N. M., Talabani, H., Doo, A., Merckx, A., Langsley, G., & Taylor, S. S. Exploring the *Plasmodium falciparum* cyclic-adenosine monophosphate (cAMP)-dependent protein kinase (PfPKA) as a therapeutic target. *Microbes Infect.* (2012), *14*(10), 838–850. doi:10.1016/j.micinf.2012.05.004
59. Wurtz, N., Chapus, C., Desplans, J., & Parzy, D. cAMP-dependent protein kinase from *Plasmodium falciparum*: an update. *Parasitology.* (2011), *138*(1), 1–25. doi:10.1017/S003118201000096X
60. Caballero, M. C., Alonso, A. M., Deng, B., Attias, M., de Souza, W., & Corvi, M. M. Identification of new palmitoylated proteins in Toxoplasma gondii. *Biochimica et biophysica acta.* (2016), *1864*(4), 400–408. doi:10.1016/j.bbapap.2016.01.010
61. Guttery, D. S., Poulin, B., Ramaprasad, A., Wall, R. J., Ferguson, D. J. P., Brady, D., et al. Genome-wide functional analysis of Plasmodium protein phosphatases reveals key regulators of parasite development and differentiation. *Cell Host Microbe.* (2014), *16*(1), 128–140. doi:10.1016/j.chom.2014.05.020
62. Russo, I., Oksman, A., & Goldberg, D. E. Fatty acid acylation regulates trafficking of the unusual *Plasmodium falciparum* calpain to the nucleolus. *Mol. Microbiol.* (2009), *72*(1), 229–245. doi:10.1111/j.1365-2958.2009.06639.x
63. Rahlfs, S., Koncarevic, S., Iozef, R., Mailu, B. M., Savvides, S. N., Schirmer, R. H., & Becker, K. Myristoylated adenylate kinase-2 of *Plasmodium falciparum* forms a heterodimer with myristoyltransferase. *Mol Biochem Parasitol.* (2009), *163*(2), 77–84. doi:10.1016/j.molbiopara.2008.09.008
64. Russo, I., Oksman, A., Vaupel, B., & Goldberg, D. E. A calpain unique to alveolates is essential in *Plasmodium falciparum* and its knockdown reveals an involvement in pre-S-phase development. *Proc Natl Acad Sci U S A.* (2009), *106*(5), 1554–1559. doi:10.1073/pnas.0806926106
65. Ma, J., Rahlfs, S., Jortzik, E., Schirmer, R. H., Przyborski, J. M., & Becker, K. Subcellular localization of adenylate kinases in *Plasmodium falciparum. FEBS Lett.* (2012), *586*(19), 3037–3043. doi:10.1016/j.febslet.2012.07.013
66. Pino, P., Sebastian, S., Kim, E. A., Bush, E., Brochet, M., Volkmann, K., et al. A Tetracycline-Repressible Transactivator System to Study Essential Genes in Malaria

Parasites. *Cell Host Microbe.* (2012), *12*(6), 824–834. doi:10.1016/j.chom.2012.10.016
67. Thinon, E., Serwa, R. A., Broncel, M., Brannigan, J. A., Brassat, U., Wright, M. H., et al. Global profiling of co- and post-translationally N-myristoylated proteomes in human cells. *Nat Commun.* (2014), *5*, 4919. doi:10.1038/ncomms5919
68. Brand, S., Cleghorn, L. A. T., McElroy, S. P., Robinson, D. A., Smith, V. C., Hallyburton, I., et al. Discovery of a novel class of orally active trypanocidal N-myristoyltransferase inhibitors. *J Med Chem.* (2012), *55*(1), 140–152. doi:10.1021/jm201091t
69. Rackham, M. D., Brannigan, J. A., Moss, D. K., Yu, Z., Wilkinson, A. J., Holder, A., et al. Discovery of novel and ligand-efficient inhibitors of Plasmodium falciparum and Plasmodium vivax N-myristoyltransferase. *J Med Chem.* (2013), *56*(1), 371–375. doi:10.1021/jm301474t
70. Rackham, M. D., Brannigan, J. A., Rangachari, K., Meister, S., Wilkinson, A. J., Holder, A., et al. Design and synthesis of high affinity inhibitors of *Plasmodium falciparum* and *Plasmodium vivax* N-myristoyltransferases directed by ligand efficiency dependent lipophilicity (LELP). *J Med Chem.* (2014), *57*(6), 2773–2788. doi:10.1021/jm500066b
71. Bell, A. S., Mills, J. E., Williams, G. P., Brannigan, J. A., Wilkinson, A. J., Parkinson, T., et al. Selective Inhibitors of Protozoan Protein N -myristoyltransferases as Starting Points for Tropical Disease Medicinal Chemistry Programs. *PLoS Negl Trop Dis.* (2012), *6*(4), e1625. doi:10.1371/journal.pntd.0001625
72. Holder, A. A., & Freeman, R. R. Biosynthesis and processing of a *Plasmodium falciparum* schizont antigen recognized by immune serum and a monoclonal antibody. *J Exp Med.* (1982), *156*(5), 1528–1538. doi:10.1084/jem.156.5.1528
73. Baragaña, B., Hallyburton, I., Lee, M. C. S., Norcross, N. R., Grimaldi, R., Otto, T. D., et al. A novel multiple-stage antimalarial agent that inhibits protein synthesis. *Nature.* (2015), *522*(7556), 315–320. doi:10.1038/nature14451
74. Ono, T., Ohnishi, Y., Nagamune, K., & Kano, M. Gametocytogenesis induction by Berenil in cultured *Plasmodium falciparum*. *Exp Parasitol.* (1993), *77*(1), 74–78. doi:10.1006/expr.1993.1062
75. Khan, S. Recent advances in the biology and drug targeting of malaria parasite aminoacyl-tRNA synthetases. *Malar J.* (2016), *15*(1), 203. doi:10.1186/s12936-016-1247-0
76. Hoepfner, D., McNamara, C. W., Lim, C. S., Studer, C., Riedl, R., Aust, T., et al. Selective and Specific Inhibition of the *Plasmodium falciparum* Lysyl-tRNA Synthetase by the Fungal Secondary Metabolite Cladosporin. *Cell Host Microbe.* (2012), *11*(6), 654–663. doi:10.1016/j.chom.2012.04.015
77. Hussain, T., Yogavel, M., & Sharma, A. Inhibition of protein synthesis and malaria parasite development by drug targeting of methionyl-tRNA synthetases. *Antimicrob Agents Chemother.* (2015), *59*(4), 1856–1867. doi:10.1128/AAC.02220-13
78. Chen, X., Chong, C. R., Shi, L., Yoshimoto, T., Sullivan, D. J., & Liu, J. O. Inhibitors of *Plasmodium falciparum* methionine aminopeptidase 1b possess antimalarial activity. *PNAS.* (2006), *103*(39), 14548–14553. doi:10.1073/pnas.0604101103
79. Starheim, K. K., Gevaert, K., & Arnesen, T. Protein N-terminal acetyltransferases: when the start matters. *Trends Biochem Sci.* (2012), *37*(4), 152–161. doi:10.1016/j.tibs.2012.02.003

80. Ciechanover, A., & Ben-Saadon, R. N-terminal ubiquitination: more protein substrates join in. *Trends Cell Biol.* (2004), *14*(3), 103–106. doi:10.1016/j.tcb.2004.01.004
81. Giglione, C., Fieulaine, S., & Meinnel, T. N-terminal protein modifications: Bringing back into play the ribosome. *Biochimie.* (2015), *114*, 134–146. doi:10.1016/j.biochi.2014.11.008
82. Utsumi, T., Sato, M., Nakano, K., Takemura, D., Iwata, H., & Ishisaka, R. Amino acid residue penultimate to the amino-terminal gly residue strongly affects two cotranslational protein modifications, N-myristoylation and N-acetylation. *J Biol Chem.* (2001), *276*(13), 10505–10513. doi:10.1074/jbc.M006134200

For Table of Contents Use Only

