# Chapter 5: Concluding discussion

The malaria burden falls heavily on sub-Saharan Africa, where more than 90% of the recorded deaths occur due to this parasitic infection and 5% of children die from severe malaria before the age of five (Phillips, 2001). Globally, malaria presents a threat to 40% of the world's population and is responsible for 2.7 million deaths per year, the majority of which are as a result of cerebral malaria caused by *P. falciparum* (Korenromp *et al.*, 2005). The burden is continuously intensified by the development of parasite resistance to all classes of currently used antimalarials including chloroquine, mefloquine, sulfadoxine-pyrimethamine and the most recent compound, artemisinin (Jambou *et al.*, 2005; Bathurst and Hentschel, 2006). New drug targets are thus urgently needed as a means for possible chemotherapeutic intervention, which are more effectively identified once the parasite physiology and host-parasite relationships are better understood. The presence or absence of specific essential pathway enzymes and differences in the structures between host and parasite enzymes can subsequently be identified and investigated in possible malaria disease prevention strategies (Assaraf *et al.*, 1984).

Urgent advances in vaccine development and drug discovery to curb the malaria burden can be assisted with the production of pure recombinant malarial proteins. The isolation of *P. falciparum* proteins using *E. coli* as an expression system is complicated by the parasite's codon bias and the presence of unique inserts and LCRs (Gardner *et al.*, 2002). Vedadi *et al*. performed a large-scale protein expression study in an attempt to obtain as many as possible crystal structures from seven different Apicomplexan genomes. The group managed to purify 304 proteins for which 97 crystal structures could be successfully determined. They showed that a codon-enhanced commercial *E. coli* strain can be used as an effective expression system for the recombinant protein purification and crystallisation of Apicomplexan proteins (Vedadi *et al.*, 2006). Their results supported the conclusions made by Mehlin *et al*. in which a weak association exists between A+T content and protein expression (Mehlin *et al.*, 2006). Once the proteins are isolated to homogeneity, structural biology studies can be performed when protein crystallisation seems dismal. These studies are important in the identification of novel drugs that specifically inhibit parasite enzymes without having to experimentally test thousands of chemicals. The chemical library can also be narrowed down by applying "Lipinski's rule of 5" as a general guideline. These rules estimate the solubility and permeability of chemical compounds, which deem them viable or not as potential drug candidates (Lipinski *et al.*, 2001).

Protein structural knowledge together with bioinformatic tools enhance drug discovery on several levels. The structure firstly gives an indication of the druggability of the protein with special focus on binding regions and molecular functions that may be exploited during inhibitor design (Hopkins and Groom, 2002). Secondly, it contributes to the design of a chemical molecule with optimised binding interactions to the target. And finally, the 3-dimensional structure of a proposed target can be used to virtually screen thousands of ligands for an ideal candidate that will exert the most direct effect while being the least toxic and cheapest to synthesise (Blundell *et al.*, 2005). Another important use of protein crystal structures are the insights they provide into antimalarial drug resistance; for example, the crystal structures of the wild type and mutant *P. falciparum* DHFR/TS proteins revealed molecular mechanisms used by the enzyme to lower the drug binding capacity of pyrimethamine and proguanil and at the same time provided templates for the design of new drugs to overcome drug resistance (Yuvaniyama *et al.*, 2003).

Homology models of all the polyamine pathway enzymes in *P. falciparum* have been determined while only PfSpdSyn has been crystallised (Dufe *et al.*, 2007). These structures provide important information on the active site residues involved in substrate, cofactor and inhibitor binding as well as regions involved in the dimerisation of the proteins (Birkholtz *et al.*, 2003; Wells *et al.*, 2006; Burger *et al.*, 2007).

The widespread prevalence of polyamines goes hand in hand with their roles in a multitude of functions in diverse processes ranging from cell proliferation and differentiation to cell death (Wallace *et al.*, 2003). DFMO in combination with bis(benzyl)polyamine analogues cures *P. berghei*-infected mice (Bitonti *et al.*, 1989), which provides strong support for combination chemotherapy targeting the polyamine pathway in rapidly proliferating parasites to control malaria infections. The levels of the polyamines are most pronounced during trophozoite and schizont stages when macromolecular synthesis is taking place (Assaraf *et al.*, 1984; Das Gupta *et al.*, 2005), which signify the importance of polyamines in rapid growth and parasite survival. ODC and AdoMetDC are the rate limiting enzymes in the polyamine pathway and are usually individually regulated on transcriptional, translational and post-translational levels. In *P. falciparum,* both these enzymes are uniquely encoded within a single open reading frame (PfAdoMetDC/ODC) (Müller *et al.*, 2000). The two decarboxylase activities of the bifunctional protein function independently (Wrenger *et al.*, 2001) but inter- and intradomain interactions occur, which are necessary for the stabilisation of the active bifunctional protein (Birkholtz *et al.*, 2004). The arrangement of both these proteins in a single complex provides an important difference between the host and parasite polyamine biosynthesis pathway enzymes that can be exploited during drug design to simultaneously inhibit both these enzymes.

In addition to the bifunctional arrangement of PfAdoMetDC/ODC, the ability of PfSpdSyn to synthesise both spermidine and spermine due to the absence of a gene encoding SpmSyn, (Haider *et al.*, 2005) provides further support in targeting the polyamine biosynthesis pathway of *P. falciparum*. Combination drugs targeting the bifunctional complex as well as PfSpdSyn would severely affect the intracellular polyamine pools. Polyamine-specific transporters in *P. falciparum* have not been identified and are also an important aspect to consider when designing novel inhibitors against polyamine biosynthesis enzymes. Inhibitor and product replenishment studies, however, suggested the presence of a putrescine transporter (Assaraf *et al.*, 1984; Das Gupta *et al.*, 2005) but the existence of a spermidine-specific transporter remains uncertain (Haider *et al.*, 2005). An ideal inhibitor must deplete intracellular polyamines by its combined action on the target enzyme/s and polyamine transporter/s. The treatment of polyamine biosynthesis enzymes with different inhibitors showed that these enzymes are essential for the growth of the parasites (Assaraf *et al.*, 1987; Das Gupta *et al.*, 2005; Haider *et al.*, 2005). In addition, the inability to successfully create null mutants of the polyamine biosynthesis pathway enzymes (Dr I.B. Müller, personal communication) reinforces the essentiality of polyamines in the survival of the parasites and suggests their value as potential drug targets.

In order for a parasite protein to be effective as a drug target, significant differences should exist between the parasite and host proteins for the exclusive inhibition of the parasite enzyme. The *P. falciparum* polyamine biosynthesis enzymes have been characterised on genetic, molecular and structural levels. Exceptional parasite-specific properties have been identified that may be exploited during lead drug development. Previous studies have shown that unique parasite-specific inserts in PfAdoMetDC and PfODC are involved in the activities of the respective and adjacent domains (Birkholtz *et al.*, 2004). These areas present innovative opportunities for designing drugs that target regions distant from the active sites.

The roles of the parasite-specific inserts in the PfAdoMetDC domain were investigated as previous studies showed that the presence of these seemingly unstructured areas are important in the complex formation and activities of the PfAdoMetDC/ODC protein (Birkholtz *et al.*, 2004). A logical way to determine the roles of these inserts is to simply remove them from the parental template and to subsequently assess the effects of the mutated proteins on protein activity. A decrease in activity would indicate that the inserts are important in the functioning of the proteins, may it be via direct involvement with the active site, protein-protein interactions forming the complex or by interacting with regulatory proteins. A literature study identified several PCR-based methods that can be employed for the deletion of large areas from genes. A comparative study was thus performed in order to determine which method is the most effective in deleting the largest A3 parasite-specific insert (>400 bp) in

the PfAdoMetDC domain from a large construct (~7400 bp). Application of the QCM and ExSite™ methods in deleting the insert did not produce PCR products while the inverse PCR and overlapping primer methods produced PCR products of the required size. A fifth method based on the inverse PCR method was additionally tested. The primers of the RE-mediated inverse PCR method contain unique RE sites at the 5'-ends of both the forward and reverse inverted tail-to-tail primers. PCR with these primers amplify the entire construct except for the desired region (insert) between the 5'-ends of the two primers. Subsequent PCR product digestion with the unique RE increases the number of deletion products by eliminating any primary parental product still containing the insert. But most importantly, RE digestion creates products with sticky-ends that improve the ligation efficiency during the circularisation of the mutant products into plasmids. The application of this deletion mutagenesis method also resulted in a PCR product of the correct size indicating that the A3 insert was possibly removed. RE mapping and nucleotide sequencing showed that the overlapping primer method was 40% efficient while the RE-mediated inverse PCR method resulted in 80% mutagenesis efficiency.

The RE-mediated inverse PCR method was also tested on a second *P. falciparum* protein; the method successfully removed a parasite-specific insert (>600 bp) form *PfPdxK* (~4600 bp) from all the clones tested, which indicates that the efficacy of the method is better on smaller templates. However, this is not dependent on the particular gene sequence. A highly efficient method was therefore created and tested which can be used to successfully remove large DNA regions (>100 bp) from abnormally large A+T rich *P. falciparum* genes (Williams *et al.*, 2007). The method proved to be invaluable in deciphering the involvement of the parasite-specific inserts in the structure and activity of PfAdoMetDC/ODC.

The smaller A1 and A2 inserts were removed with the overlapping primer method and were, together with the previously created A3 insert-deleted mutant, investigated in a structure-activity relationship study. The newly created deletion mutants were recombinantly expressed with C-terminal *Strep*-tags and isolated with *Strep*-Tactin affinity chromatography. Similar levels of recombinant proteins were isolated and subsequently visualised with SDS-PAGE. Gel electrophoresis showed that various contaminating proteins co-eluted with the desired ~160 kDa protein. The presence of three contaminating proteins (~60 kDa, ~70 kDa and 112 kDa) were identified in a previous study as being *E. coli* chaperones (HSP60 and HSP70), produced to aid the poor expression of the recombinant protein, and heterologously derived N-terminal PfAdoMetDC/ODC truncations sized ~70 kDa and ~112 kDa formed due to the presence of false internal translation initiation sites (Niemand, 2007). In addition to these three bands, the recombinant expression of the A2 and A3 insert-deleted proteins produced smaller proteins, which were located directly underneath the ~160 kDa bands. MS

analysis identified these as being PfAdoMetDC/ODC but the manner in which they were produced remains uncertain.

The affects that the inserts have on the activities of both the enzymes in the bifunctional complex were subsequently determined in an attempt to show that these inserts are evolutionary preserved as a result of the specific roles that they play in the bifunctional protein complex. The deletion of the sequence and structurally conserved A1 insert halved the PfAdoMetDC activity while the PfODC activity was depleted by 95%. This insert was predicted to have a similar role to that of the O1 insert due to their high Plasmodial conservation and lack of LCRs. The similarity and combined functional roles of these two inserts in the activities of both the domains in the bifunctional complex can therefore be exploited in the design of a single inhibitory compound capable of binding to both these areas. Such a compound would result in the reduction of PfODC activity by the inhibition of PfAdoMetDC activity (to ~50% of wild type activity) and, in turn, result in the reduction of PfAdoMetDC activity (to ~23% of the wild type activity) by the inhibition of PfODC activity.

The A2 and A3 deletion mutants severely affected both PfAdoMetDC and PfODC domain activities, which could possibly be due to the disruption of protein-protein interactions between these inserts and the remainder of the complex upon their removal. Based on these deletion mutagenesis studies, it thus seems that the two domains of the bifunctional protein are allosterically regulated via the presence of parasite-specific inserts to maintain optimum levels of polyamines. Large conformational changes upon deletion of these latter inserts could also be responsible for the depletion of the bifunctional activity. However, the roles of the various contaminant proteins are unknown and cannot be discounted at the current time.

Following the severe consequences of insert deletion on protein activity, the A2 and A3 inserts were analysed for the presence of secondary structures as these are often involved in protein-protein interactions across homodimeric interfaces (Guharoy and Chakrabarti, 2007). Popular secondary structure prediction programmes as well as HCA plots were used to analyse the inserts. It is expected that the large bifunctional PfAdoMetDC/ODC protein complex would contain a number of protein-protein interaction sites to stabilise the entire heterotetrameric protein complex and it was thus predicted that these inserts might be involved in additional interactions that are absent in the mammalian counterparts. Secondary structures are also involved in the formation of super-secondary structures that often characterise the function of a particular fold such as leucine zippers and β-barrels (Sansom and Kerr, 1995; Gromiha and Parry, 2004). Super-secondary structures of PfAdoMetDC/ODC include the PfAdoMetDC domain αββα-fold, the N-terminal α/β TIM and C-terminal modified Greek-key β-barrels of PfODC (Birkholtz *et al.*, 2003; Wells *et al.*, 2006).

Predicting secondary structures from amino acid sequences are also useful in the absence of a PfAdoMetDC/ODC crystal structure in which the location and structure of the parasite-specific inserts would be shown. Application of the prediction programmes and HCA identified two *Plasmodia* conserved structures that were further investigated, namely an α-helix in insert A2 and a β-sheet in insert A3. The helix was disrupted with an Ile to Pro point mutation while the sheet in insert A3 was deleted with the RE-mediated inverse PCR method. Once again, similar recombinant expression levels of the α-helix disrupted and wild type proteins were obtained while SDS-PAGE analysis showed the presence of several co-eluting contaminating proteins, which were higher with the mutated protein sample. These were attributed to possible degradation products formed as a result of the recombinant expression of the mutant sample and an increase in chaperone proteins rather than the formation of misfolded protein upon the introduction of the Pro helix-breaker residue.

Subsequent activity assays revealed some interesting results; only 15% PfAdoMetDC activity and 45% of the wild type PfODC remained upon the disruption of the conserved helix within insert A2. The effects of the mutation were thus communicated to the neighbouring PfODC domain, which indicates that this structure might be involved in interactions across the entire protein complex. These results are analogous to those when the conserved α-helix in insert O1 was disrupted in a similar manner. It thus seems plausible that secondary structures within the unique parasite-specific inserts are involved in the conformation of the bifunctional complex and the possibility that they are involved in protein-protein interactions for the dimerisation of the multimeric complex needs to be investigated.

Previous studies have also shown that the PfODC domain is more refractory to change and is thus largely affected once mutations are introduced within this domain and in the hinge region (Birkholtz *et al.*, 2004; Roux, 2006). Most importantly, the C-terminal PfODC domain is highly dependent on the presence of the N-terminal PfAdoMetDC domain and the hinge region connecting these two domains while PfAdoMetDC can function independently (Krause *et al.*, 2000; Wrenger *et al.*, 2001). These properties are mirrored in the malarial DHFR/TS bifunctional protein where the C-terminal TS domain is highly dependent on its interactions with the neighbouring N-terminal DHFR domain (Shallom *et al.*, 1999). It was previously thought that initialisation of the heterotetrameric complex takes place from the PfODC domain, but the results of these studies indicate that the parasite-specific inserts within the PfAdoMetDC domain are also responsible for the activities and/or stability of both the domains. These unique inserts can thus be investigated as possible drug targets for the disruption of the entire PfAdoMetDC/ODC complex activity.

Based on the results obtained, which showed an apparent functional role of the structurally conserved A1 insert and the importance of the conserved α-helix in insert A2 in the activities of both domains possibly via protein-protein interactions, the structurally distinct and conserved helix-containing O1 insert was further investigated. Previous studies showed that this insert is very important for both decarboxylase activities and that its deletion prevents dimerisation of the PfODC monomers and influences the association of PfODC with the adjacent PfAdoMetDC domain to form the bifunctional complex (Birkholtz *et al.*, 2004). The respective point mutations disrupting the helix (identified with secondary structure prediction programmes) and immobilisation of the insert resulted in severe activity depletions of both the decarboxylase domains, which demonstrated the essential roles of these delineated areas within O1 (Roux, 2006). The flexible Gly residues were shown with molecular dynamics to impart mobility on the insert, implying that this insert might be acting as an active site gatekeeper due to its close proximity to the active site as revealed with homology modelling (Birkholtz *et al.*, 2003; Roux, 2006). Alternatively, the mobile loop was thought to position the helix in such a way that it can interact with protein areas to establish the active dimeric proteins. If this is true then the O1 insert represents a valuable drug target, as dimerisation will be prevented leading to the inactivation of the entire protein and a subsequent loss in the biosynthesis of polyamines.

The O1 helix-disrupted and immobile mutations with the bifunctional gene as template were created in a previous study (Roux, 2006). The same mutations were also introduced in the monofunctional PfODC template. These two different monofunctional and bifunctional constructs were subsequently used in a study to determine whether these delineated areas are responsible for protein-protein interactions and dimerisation of the proteins. The different constructs were recombinantly expressed and purified with *Strep*-Tactin affinity chromatography. The recombinant proteins were applied to a size-exclusion column, which was calibrated with four standard proteins and the protein sizes were determined with FPLC. The ability of the respective mutated proteins to dimerise could thus be determined based on the elution volumes of the proteins and a linear regression equation. The sizes were confirmed with Western dot blot immunodetection with a *Strep*-tag II antibody. The wild type bifunctional and monofunctional proteins eluted in fractions corresponding to the sizes of the bifunctional heterodimeric and tetrameric proteins while the monofunctional proteins eluted as both monomeric and dimeric proteins. Both oligomeric states were eluted due to the rapid exchange of the ODC subunits between its monomeric and dimeric forms (Coleman *et al.*, 1994; Osterman *et al.*, 1994). The elution profiles of the immobile Gly mutant proteins were identical to that of the wild type protein, which means that even though the O1 insert loop is no longer mobile it is still takes part in protein-protein interactions for the dimerisation of the PfODC domain. The Gly mutant protein is therefore still capable of forming the bifunctional

complex but the ability of the substrate to stabilise the active site loop and induce an active site conformation may be affected (Dufe *et al.*, 2007), which explains the depletion in activity upon the creation of this immobile loop.

The abilities of the helix-disrupted proteins to form heterotetrameric PfAdoMetDC/ODC and dimeric PfODC proteins were lastly tested. The monofunctional PfODC protein only eluted as a monomeric protein while the bifunctional PfAdoMetDC/ODC protein exclusively eluted in a fraction corresponding to the size of the heterodimeric protein. This means that the O1 helix could no longer partake in protein-protein interactions and thus represents the exact delineated interacting area necessary for the dimerisation of the domains. Western blots confirmed these results. These studies once again reinforce the potential in targeting this insert with an inhibitory compound, which would prevent the complex formation of PfAdoMetDC/ODC leading to the simultaneous inactivation of both the enzymatic domains. Next, a proof-of-principle study was performed to test whether synthetic peptides targeting the O1 insert could affect bifunctional activity.

Experimental evidence proving the involvement of the O1 insert in the dimerisation of the PfODC and PfAdoMetDC/ODC proteins and activity of both the domains prompted the design of mechanistically novel, non-active site based peptides that could target and bind to this area to disrupt protein activity. Similar studies have been performed on another malarial protein, which showed that a peptide targeting the protein dimerisation interface resulted in a 50% loss in enzyme activity (Singh *et al.*, 2001). Three different peptides were designed: 1) NY-39 which is complementary to the entire 39 amino acid O1 insert; 2) LK-21 which is identical to only the α-helix within this insert; and 3) LE-21 which is similar to LK-21 but opposite in charge. The full-length PfAdoMetDC/ODC proteins were incubated at three different molar quantities with these different peptides and subsequently subjected to activity assays to determine the effects of the peptides on protein activity.

A 2 hr incubation at 22°C of the LE-21 peptide at a 1000x molar peptide excess depleted 40% of the PfODC activity while PfAdoMetDC activity increased by 66%. This peptide possibly forms salt bridges with the O1 helix in the target protein, thereby preventing it from interacting with its natural binding partner resulting in the inhibition of PfODC activity. The increase in PfAdoMetDC activity could be due to the alleviation of interdomain regulation or stabilisation in the absence of the adjacent domain. The exact interacting areas of the peptides on the full-length protein are, however, unknown and might explain why some activity still remained. The structures of the peptides in solution are also important and might mediate their ability to bind to the target site. A control peptide, which is not complementary to the target protein, should be tested to see if not just any peptide is capable of interacting

with PfAdoMetDC/ODC. The NY-39 peptide, which is identical to the O1 insert, would also compete with PfODC binding to its complementary site and caused an increase in both the PfAdoMetDC and PfODC activities. Finally, these inhibitory experiments should also be performed at physiological temperature, which was initially avoided due to the inhibitory effect the temperature had on the positive wild type control. The true effect of these ~2.3 kDa peptides on the polyamine levels can in future be tested *in vitro*. However, even if the peptides were to be taken up by the parasites via endocytosis, they would most probably end up in endosomes or the food vacuole, and not in the cytoplasm where PfAdoMetDC/ODC is located. These peptides, however, merely serve as a starting point for future studies aimed at using these peptides as scaffolds for the synthesis of chemical compounds that can be more effectively employed as therapeutic inhibitors.

In the last four years, studies performed on the bifunctional PfAdoMetDC/ODC complex by the introduction of various mutations and the use of synthetic peptides targeting the unique parasite-specific inserts have brought us closer to understanding the exact functions of these areas. Possible mechanisms have also been revealed that explain how polyamines levels are maintained by the tight regulation of the PfAdoMetDC and PfODC protein activities, which is possibly made more efficient as a result of their arrangement in a single bifunctional complex.

Table 5.1 summarises all the studies that have been performed involving the PfODC domain and how these affected protein activity and/or dimerisation. Several obvious trends can be seen in Table 5.1. Any alterations involving the conserved O1 insert prevented the dimerisation of the PfODC domain and therefore severely affected bifunctional activity. Bifunctional heterotetrameric complex formation only requires the presence of the two domains while dimerisation of PfODC depends on the hinge region. The flexible Gly residues of the O1 insert are important for the activities of both of the domains while a mobile insert is not required for the dimerisation of the PfODC domain or the complex formation of the heterotetrameric PfAdoMetDC/ODC complex. The effects of the insert O1 synthetic peptides on complex formation are still outstanding and would reveal their possible involvement in the disruption of protein-protein complexes.

An allosteric-type regulation of the domains in the bifunctional PfAdoMetDC/ODC domain is thus suggested since the activity of PfAdoMetDC becomes stabilised in the absence of the PfODC domain while the latter is inactive in the absence of the PfAdoMetDC domain (Krause *et al.*, 2000; Wrenger *et al.*, 2001). It is also possible that the opening and closure of the active site gate-keeping loop upon the binding and release of ornithine and putrescine,

respectively, is communicated to the PfAdoMetDC domain made possible by the interdomain interactions in the bifunctional complex

**Table 5.1: Summary of the effects of O1 insert mutations on protein activity and dimerisation as well as its targeting with peptide inhibitors**

| Mutation/treatment | %AdoMetDC activity | %ODC activity | Dimerisation | Reference |
|---|---|---|---|---|
| PfODC - hinge | NA | 3 | ✗ | Birkholtz *et al*., 2004 |
| PfODC + hinge | NA | 6 | ✓ | Birkholtz *et al*., 2004 |
| PfODCΔO1 | NA | 9 | ✗ | Birkholtz *et al*., 2004 |
| PfODCpO1a | NA | - | ✗ | Current study |
| PfODCpG2 | NA | - | ✓ | Current study |
| A/O - hinge | 67 | 50 | ✓ | Birkholtz *et al*., 2004 |
| A/OΔO1 | 23 | 6 | ✗ | Birkholtz *et al*., 2004 |
| A/OpO1a | 6 | 0 | ✗ | Roux, 2006 and current study |
| A/OpG2 | 33 | 3 | ✓ | Roux, 2006 and current study |
| O1 peptide + A/O | ↑ | ↑ | ? | Current study |
| Helix peptide + A/O | ↑ | No change | ? | Current study |
| Anti-helix peptide + A/O | ↑ | ↓ | ? | Current study |

Mutations that were performed: PfODC + hinge, monofunctional PfODC including 144 residues of the hinge region; PfODC - hinge, monofunctional PfODC without the hinge region; A/O - hinge, removal of residues 573-752 in the hinge region; ΔO1, deletion of the entire 39-residue O1 insert; pO1a, disruption of the α-helix within the O1 insert; pG2, mutagenesis of the flanking Gly residues in the O1 insert. The overall effects of the peptides after 2 hr incubation periods with the wild type bifunctional A/O protein at 22°C on protein activity are shown as either increasing (green) or decreasing (red) arrows. The abilities of the mutant monofunctional and bifunctional proteins to dimerise into dimeric PfODC or heterotetrameric PfAdoMetDC/ODC protein complexes are indicated with ticks or crosses. The activities were normalised as a percentage of the wild type PfAdoMetDC/ODC activity. "?" Shows that the results are outstanding. NA: non-applicable when the activity assays were performed with monofunctional PfODC as template. "-": When the activity was negligently low.

In conclusion, this study focussed on the parasite-specific inserts in the PfAdoMetDC domain and showed that these as well as the secondary structures within them are essential for the activities of both domains. The involvement of a PfODC parasite-specific insert in protein-protein interactions and dimerisation of the protein was subsequently evaluated. A conserved α-helix within this insert was shown to mediate the dimerisation of the PfODC domain and was targeted with a synthetic peptide, which depleted almost half of this protein's activity. This study has thus proven the importance of these inserts in the activity and structure formation of PfAdoMetDC/ODC. Finally, the design and application of a single inhibitory peptide that can recognize and bind to all the A1- and O1-type of parasite-specific inserts in *P. falciparum* proteins would lead to a global inhibition of proteins and would represent the ultimate drug to cure malarial infections.