Malaria has remained a major threat to the public health and economic development especially in the tropical regions of the world. There are four plasmodium parasites that cause malaria: Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, and Plasmodium ovale. Of the four parasites, Plasmodium falciparum is the most lethal. Due to the lack of an effective malaria vaccine, P. falciparum parasites continue to threaten the global malaria control and elimination programs. Resistance of malarial parasites to some of the existing drugs calls for a need to identify new therapeutic targets. Integral membrane proteins, which are metal transporters, are increasingly becoming targets for drug development. These proteins are essential for metabolism of metal ions and are required for growth and replication of the Plasmodium parasites. Understanding the structure and function of these proteins will provide valuable information to aid in the eradication of this devastating disease. Copper is an essential nutrient that Plasmodium falciparum needs in order to colonize its host. Its cellular levels are carefully regulated by a copper efflux protein (PfCuP-ATPase) to reduce its toxicity.

PfCuP-ATPase is the largest copper transporter of the Pib P-type ATPase family made up of 2563 amino acid residues with a molecular weight of 298.8 kDa. The number of metal-binding domains in copper ATPases varies among organisms. In PfCuP-ATPase, the
number of metal-binding domains and how the copper ATPase functions is only partially understood. I have identified three N-terminal domains of \textit{PfcuP-ATPase} found within the first 564 amino acids. Surprisingly, only one domain contains the copper binding motif MXCXXC. There is a long linker of 320 amino acids between the first and the second domains, and the second domain is directly adjacent to domain three. I pursued biophysical studies to better understand the structural organization of these domains. Each domain was separately cloned using recombinant technology (over) and expressed in \textit{E. coli}. Domain three is the most soluble and relatively resistant to chemical denaturation with guanidine hydrochloride. In contrast, domain two is less stable. It retains only 50\% of its helical structure at 1.35 M GuHCl. Strikingly, domain one is resistant to thermal denaturation and it has a melting midpoint of 78\°C. Domains two and three, when heated, precipitate out of solution. However, when they are expressed together as a two-domain construct (\textit{PfcuP-MBD2-3}), thermal denaturation shows a sigmoidal curve with a melting midpoint of 47.9\°C. The chemical denaturation of \textit{PfcuP-MBD2-3} produced a double sigmoidal curve and the second sigmoid correlates to the denaturation of domain three. \textsuperscript{15}N-labelled samples were prepared and \textsuperscript{1}H-\textsuperscript{15}N HSQC NMR experiments were performed for four different constructs. Subsequently, a \textsuperscript{13}C,\textsuperscript{15}N sample of domain three was prepared and triple resonance NMR experiments were performed. NMR resonance assignments and the subsequent Rosetta modelling shows domain three to have a ferredoxin fold that is similar to the metal binding domains of the human Wilson disease protein.

Copper binding studies were performed under anaerobic conditions with Cu\textsuperscript{I}(CH\textsubscript{3}CN\textsubscript{4})\textsuperscript{+} as the copper source, and we obtained a \textit{K}_D of 1.35 \times 10^{-18} M. The copper ATPase of \textit{P. falciparum} diverges from that of the kingdom \textit{Animalia}. The presence of a two domain construct, like that of human Wilson protein metal-binding domains five and six (PDB ID: 2EW9), yet lacking a metal-binding motif in domain three is intriguing. This finding highlights the structural versatility and stability of the dual ferredoxin fold.