Chapter One

Introduction
Malaria is one of the most dreadful parasitic diseases ever to affect mankind. Nearly half of the world’s population is at risk to contract malaria, which is endemic in 99 countries. Malaria is a vector borne infectious disease, the causative agent of which is the apicomplexan protozoan parasite *Plasmodium*. Of the four species of *Plasmodium* that are able to infect humans (*P. ovale*, *P. vivax*, *P. malariae* and *P. falciparum*), *Plasmodium falciparum* is responsible for the severest form of this disease. Although preventable and treatable, the disease remains one of the leading causes of morbidity and mortality with approximately 216 million cases and 665,000 fatalities worldwide (WHO World Malaria Report 2011). Disease control has been primarily hindered by poverty, poor sanitation, weak health systems, drug and insecticide resistance and climate change. High risk groups include people indigenous to or visiting endemic areas with children and pregnant women being especially vulnerable. Symptoms of the disease include fever, shivering, body pain and nausea. Severe cases are characterized by anemia and organ failure leading to coma and death.

The life cycle of *Plasmodium falciparum* alternates between the human and mosquito host. The parasite propagates initially in human hepatocytes followed by infection of erythrocytes. The process of erythrocyte invasion is crucial as it facilitates entry in to the host cells, which if prevented can block subsequent growth of the parasite. Components of the motor complex called the glideosome that facilitate this process have been characterized in tachyzoites of *Toxoplasma* (Gaskins et al., 2004; Meissner et al., 2002), sporozoites of *Plasmodium* (Bergman et al., 2003) and more recently in the merozoites of *P. falciparum* (Baum et al., 2006; Green et al., 2006; Jones et al., 2006). Four proteins make up the complete motor complex, which are the glideosome associated proteins 50 and 45 (*PfGAP50* and *PfGAP45*), the myosin light chain homologue (*PfMTIP*) and a class XIV myosin (*PfMyoA*). Although correct targeting and assembly of the individual components into the functional complex is essential for invasion, the regulatory mechanisms that control it have not been deciphered yet.

The sequencing of the *Plasmodium* genome (Gardner et al., 2002) and other evidence suggests that several putative signaling proteins may be conserved in the malarial
parasite (Kappes et. al., 1999; Doerig, 1997; Srinivasan et. al., 2004; Aravind et. al., 2003; Ward et. al., 2004). Bioinformatic analysis has identified several putative protein kinases that are present in the Plasmodium genome. However, the biochemical characteristics, regulation and function of only a few of these are known (Ward et. al., 2004). Second messengers like cyclic nucleotides, phosphoinositides, calcium etc., are major driving forces responsible for transmitting signaling events in eukaryotic cells. Calcium is a ubiquitous second messenger that plays a vital role in all eukaryotic cells including Plasmodium (Garcia, 1999; Billker et. al., 2009; Nagamune et. al., 2008). Optimal levels of intracellular and extracellular calcium are essential for proper development of the parasite. Calcium mediated signaling events have been implicated in processes such as invasion (Wasserman and Chaparo, 1996), migration (Ishino et. al., 2006), gametogenesis (Billker et. al., 2004) and circadian rhythms (Hotta et. al., 2004). Phosphoinositides are potent secondary messengers that function in signaling and trafficking pathways by targeting proteins that are capable of binding to them via their phosphoinositide binding modules to specific cellular locations. Membrane targeting by phosphoinositides is dependent on their structure and relative abundance in cells. The binding domains themselves are diverse in structure as well as in their interactions with their targets. Some of the well characterized domains are the PH domain, the PX domain and the FYVE domain. PH domains are 100-150 amino acid modules that do not share a very high degree of sequence homology but possess a very similar 3-dimensional structure (Lemmon, 2003). The molecular networks that regulate parasite signalling pathways and contribute to important processes like erythrocyte invasion are not well deciphered. The work reported in this thesis is an attempt to contribute towards the understanding of parasite signal transduction pathways.

PfPKB is a Protein Kinase B like enzyme in Plasmodium falciparum. It shares a significant degree of similarity in its kinase domain with mammalian PKB although there are striking differences between both the proteins (Kumar et. al., 2004). The enzyme has been shown to play an important role in the invasion of erythrocytes by merozoites (Kumar et. al., 2004; Vaid et. al., 2008). PfCDPK1 is a calcium dependent protein kinase which has also been implicated in this process (Green et. al., 2008). The following studies were carried out to understand how these kinases may contribute to the process of host erythrocyte invasion by the parasite.
The work reported here addresses the following objectives:

I. Elucidation of the role of PfPKB in erythrocyte invasion by merozoites and identification of its targets.

II. Regulation of PfGAP45 by PfPKB and PfCDPK1.

III. Crosstalk between PfPKB and PfCDPK1.

IV. Identification and characterization of PH domains in *Plasmodium falciparum*. 