The Addition of Protease Inhibitors to Improve PBLP Purification From *E.coli* for Further Investigation

Plasmodium, the parasite that causes malaria, undergoes a complex life cycle within its host that includes multiple developmentally distinct stages. Previous studies have identified a *Plasmodium* BEM46-like protein (PBLP) that is expressed throughout the parasite life cycle, and deletion of which is detrimental to plasmodium replication at multiple stages. Despite PBLP's significance in the life cycle, its enzymatic function is unknown. In this study, we expressed and purified wild-type and catalytically-dead PBLP in order to test its activity against a variety of substrates to better understand its enzymatic function.We computationally docked a number of possible substrates onto predicted structures of both full length and truncated variants of PBLP. We found that ester-containing molecules had high affinity for PBLP, suggesting that PBLP may act as an esterase. Attempts to express truncated PBLP in vitro revealed that PBLP was susceptible to proteolysis, so we added phenolmethylsulfonyl fluoride (PMSF) and other protease inhibitors in a second expression and purification attempt. Expression of truncated PBLP significantly improved compared to the initial experiment, although the catalytically-dead strain remained unsuccessful. Substrate assays using p-nitrophenyl (PNP) acetate, butyrate, and octanoate were inconclusive in identifying PBLP's substrates. Future successful identification of PBLP's substrates and enzymatic activity may provide new insight in plasmodium biology, which may support the development of malaria treatment involving PBLP in future research.