A successful purification scheme of Plasmodium Bem46-Like Protein and a demonstration of its hydrolase activity

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Introduction

- PBLP is a Plasmodium BEM46-like protein from the malaria parasite Plasmodium yoelii (Groat-Carmona et al. 2015)
- BEM46-like proteins are in the highly conserved alpha/beta hydrolase superfamily (Groat-Carmona et al. 2015)
- BEM46-like proteins play an important role in cell growth and division and are understudied (Kollath-Leib et al. 2014)

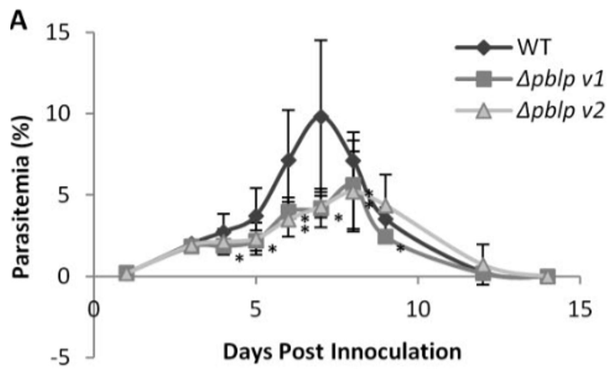


Figure 1. This figure shows the decreasing infectivity upon deletion of the PBLP gene within the malaria parasite compared to wild type. Adapted from: Groat-Carmona et al. 2015.

Computational Results

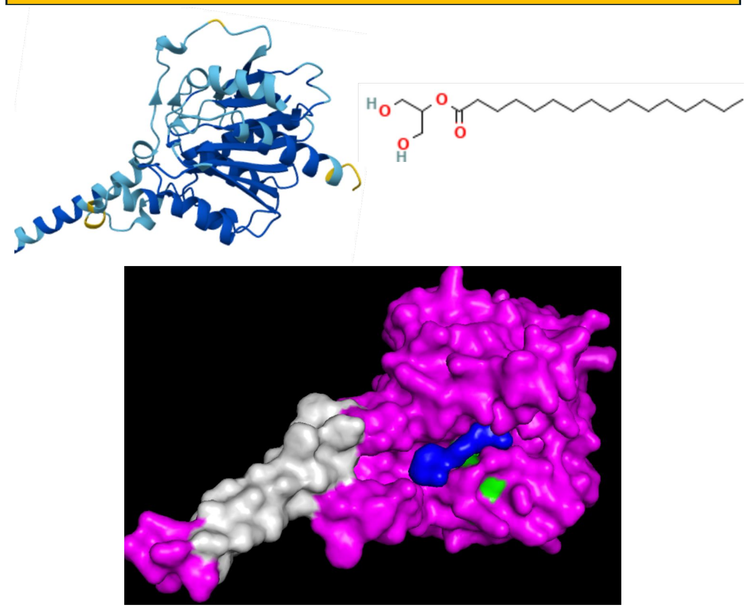


Figure 2. This figure shows promising predicted interactions within the Alphafold3 predicted structure of the PBLP protein and a potential ligand 2-Palmitoylglycerol identified as a potential substrate based on Dali and Blast homology searches. The ligand is nestled within the hydrophobic part of the active site with a nice fit and interactions with the catalytic serine and aspartic acid residues.

Stage 2 Methods

- Protein was lost in the pellet during stage 1, resulting in no usable protein
- Stage 2 tested an alternative procedure for release of the protein from the periplasmic space

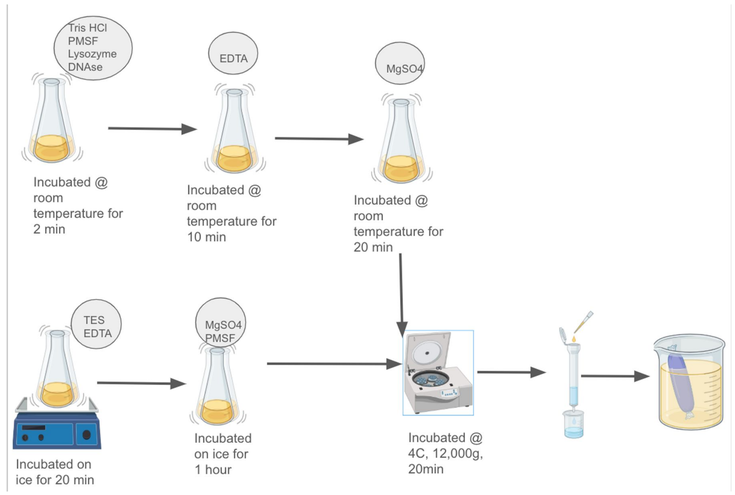


Figure 3. Schematic of the 2 different purification procedures followed. The E. coli was transfected with the PAG0023 plasmid that contained the gene for the PBLP protein and an ampicillin resistance gene. It was then cultured and expressed in a expression culture and incubated at 19 degrees Celsius overnight. Dr. Baughman then split up the expression culture into 2 500mL cultures for the lysis step which was different between the 2 purification procedures.

Stage 2 Results

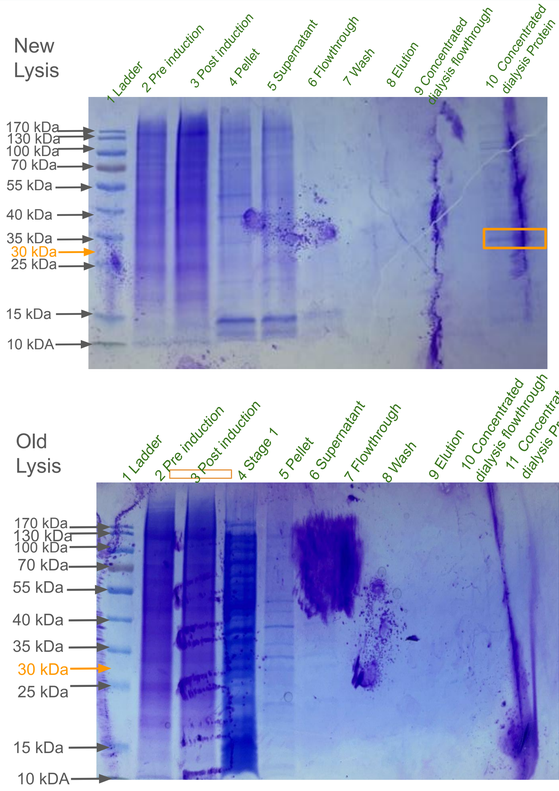
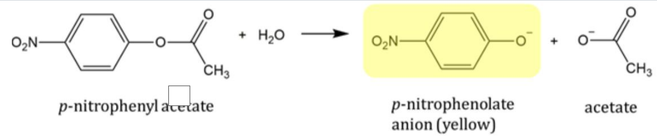


Figure 4. This figure shows a successful purification of the PBLP protein from E. coli with a moderate band at 30kDA in the concentrated post dialysis protein sample in gel to the left which represents the new lysis procedure.

Enzyme Activity Assay Results



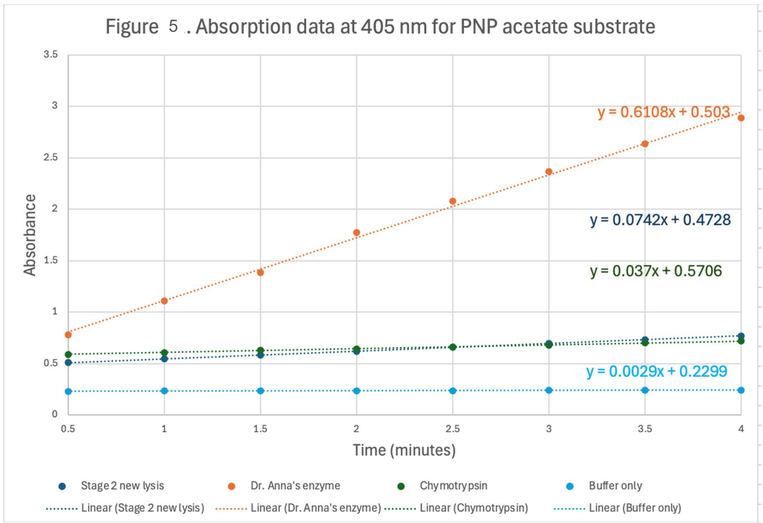


Figure 5. This figure shows PBLP has hydrolase activity with PNP acetate.

Conclusions and Future Directions

- We developed purification scheme that liberates PBLP from the periplasmic space of E. coli using osmotic shock
- Demonstrated the previously predicted hydrolase activity of PBLP through a p-nitrophenol-based enzyme assay
- Potential as an antimalarial drug target if further testing can determine its natural substrate
- Testing out more lipid based substrates
- Testing out 2-arachidonoylglycerol and it's analog's like arachidonoyl-1-thio-glycerol

References

1.Groat-Carmona AM, Kain H, Brownell J, Douglass AN, Aly ASI, Kappe SHI. 2015. A Plasmodium α/β-hydrolase modulates the development of invasive stages: An α/β-hydrolase modulates Plasmodium development of invasive stages. Cellular microbiology. 17(12):1848–1867. doi:10.1111/cmi.12477.

2. Kollath-Leiß K, Bönniger C, Sardar P, Kempken F. 2014. BEM46 shows Eisosomal Localization and Association with Tryptophan-Dervied Auxin Pathway in Neurospora crassa. Eukaryotic Cell. 13(8): 1051-1063.https://doi.org/10.1128/ec.00061-14