Developing an Experimental Protocol for Purification of a Malaria Parasite *Plasmodium Yoelii* Hydrolase Protein

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Background

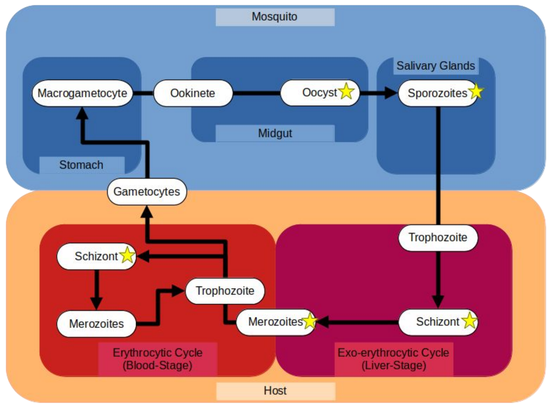


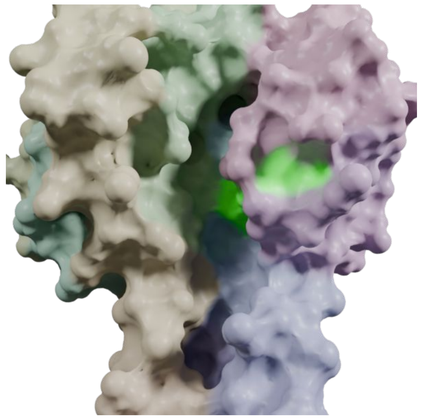
Figure 1. Flow chart of the life cycle of *Plasmodium* parasites. Stars indicate stages in which deletion of PBLP impacts parasite development.

Malaria is a deadly parasitic disease caused by *Plasmodium* parasites transmitted between mosquitoes and mammalian hosts (WHO 2024). Plasmodium Bud Emergence 46-Like Protein (PBLP) is a membrane-associated enzyme with unknown substrate discovered in *Plasmodium yoelii* which has significant importance in development of parasite invasive stages (Groat-Carmona et al. 2015). Understanding the function of this protein may provide useful insight in developing treatments for Malaria.

Objectives

- Investigate the 3D structure of PBLP, identify similar proteins, and find potential substrates for enzyme affinity testing (Stage 1).
- Develop an experimental protocol for purification of PBLP in lab conditions (Stage 1) and investigate variables which affect protein yield (Stage 2)
- Test enzyme affinity using purified protein samples (Stage 2).

Computer Modelling



Computer analysis was performed on the amino acid (AA) sequence of PBLP obtained by Groat-Carmona et al. using multiple databases:

- AlphaFold3 was used to estimate the 3D structure of the protein
- CLEAN search predicted EC number 3.1.2.22, indicating possible esterase activity
- Swissdock simulated substrate affinity using AlphaFold3 structure, indicated high affinity for phosphatidylserine, palmitic and anacardic acid.

Figure 2. Space-filling visualization of the active site of PBLP. The catalytic triad is highlighted in green. Figure generated in Blender 4.4 using Molecular Nodes toolkit.

Swissdock substrate affinities	
Name	**Affinity (kCal)**
Cytosine	-4.221
Glycine	-4.302
Palmitic Acid	-4.828
Phosphatidylserine	-5.304
Anacardic Acid	-5.920

Figure 3. Predicted substrate affinities. Lower values indicate more stable binding between the active site of PBLP and the substrate.

Experimental Methods

We performed two stages of protein purification, starting from scratch for stage 2.

- Modified PBLP gene excluding transmembrane domain was inserted into pAG0023 plasmid, transformed into *E. coli* cultures (pUC19 used as control). After transformation, bacteria were transferred to culture flasks to incubate

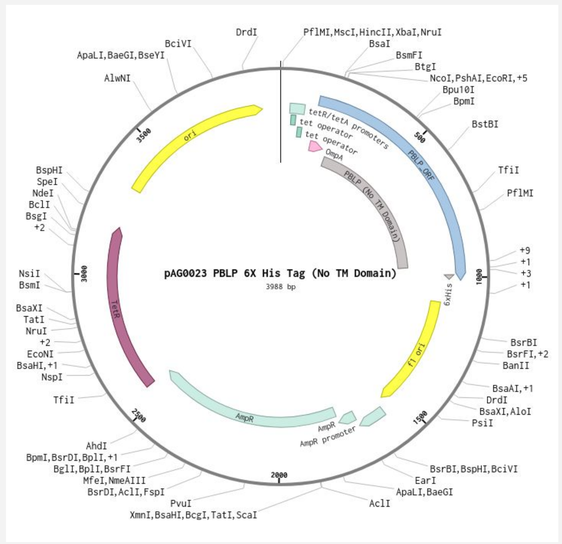


Figure 4. pAG0023 plasmid vector used in transformation. Contains modified His-tagged PBLP gene, Ampicillin/Tetracycline resistance genes and tet operator to allow selective induction of PBLP. Figure generated in Benchling.

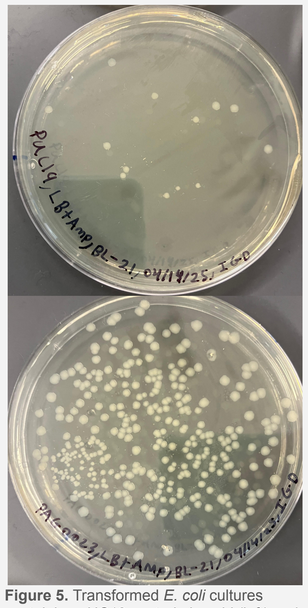


Figure 5. Transformed *E. coli* cultures containing pUC19 control plasmid (left) and experimental pAG0023 plasmid with PBLP gene (right).

- Expression of PBLP was induced by adding tetracycline, incubated at 18°C (Stage 1), 22°C and room temperature (Stage 2)
- Protein was purified from supernatant of lysed cells via Ni^{2+} column chromatography. Samples were collected before & during purification and run through SDS-PAGE to assess protein yield.

Results & Discussion - Protein Purification

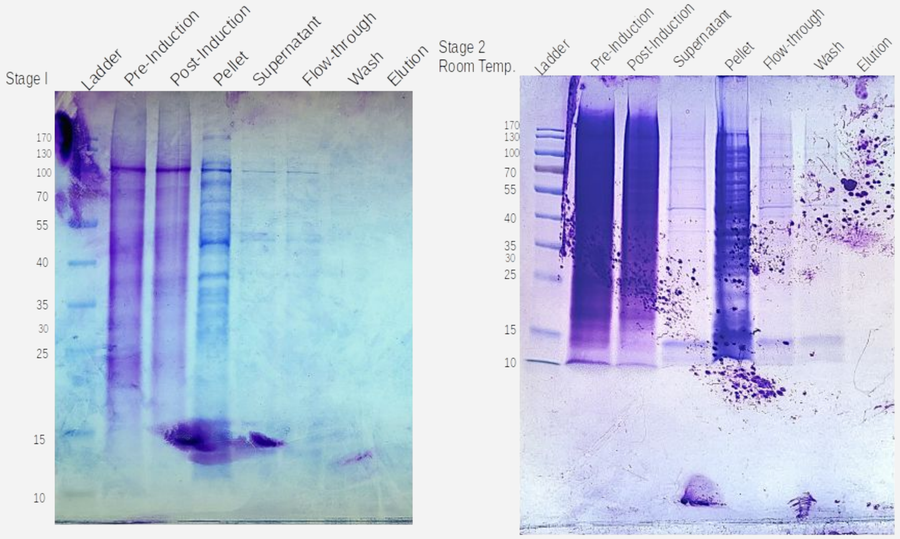


Figure 6. SDS-PAGE results from Stage 1 and 2 of product collected throughout the purification process. Ladder indicates protein mass in kilodaltons. PBLP has a molecular weight of 30 kDa.

- Expected size of PBLP is 30 kDa
- After purification, bands are too faint to interpret – PBLP cannot be observed in stage 1 or 2
- Results on effectiveness of incubation at different temperatures are inconclusive.

Results & Discussion - Enzyme Activity Assays

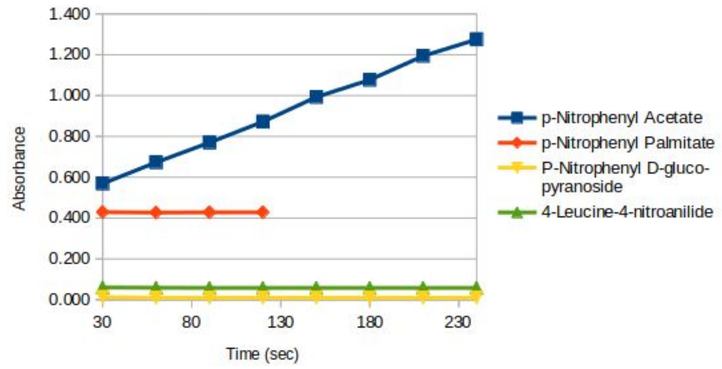


Figure 7. Enzyme assay results. Increasing absorbance over time indicates enzyme activity. Measurements of palmitate solution absorbance were stopped prematurely due to precipitation of the substrate.

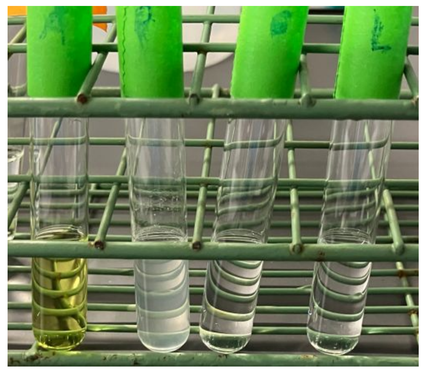


Figure 8. Enzyme activity assay results. From left to right: *p*-nitrophenyl acetate, *p*-nitrophenyl palmitate, *p*-nitrophenyl D-gluco-pyranoside, 4-Leucine-4-nitroanilide.

- Compounds with *p*-nitrophenyl groups produce yellow byproduct when ester bonds are cleaved, indicating enzyme activity
- PNP Acetate is a substrate for most hydrolases due to its small size
- Hydrolysis of PNP acetate confirms that PBLP has hydrolase activity

Conclusions & Further Research

- PBLP was successfully purified and exhibits some form of esterase activity, though due to the low specificity of PNP Acetate, a specific biological substrate is not clear
- Different conditions can be tested during amplification and expression to increase yield
- More substrates can be tested to gather data on the specificity of this enzyme.

Acknowledgements

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References

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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. Cellular microbiology. 17(12):1848–1867. doi:10.1111/cmi.12477.