Expression, Purification, and Hydrolase Characterization of PBLP, a Protein from the Malaria Parasite *Plasmodium yoelii*

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Background

- In 2023 alone, globally, 597,000 malaria-related deaths and 263 million new cases of malaria were reported.[2]
- *Plasmodium* BEM46-like protein (PBLP) sequence orthologs are evolutionarily conserved across all *Plasmodium* parasite species. Yet, the protein's role in progressing infection has not been definitively identified.[1]
- PBLP shares structural and sequence homology with members of the α/β-hydrolase superfamily.[1]

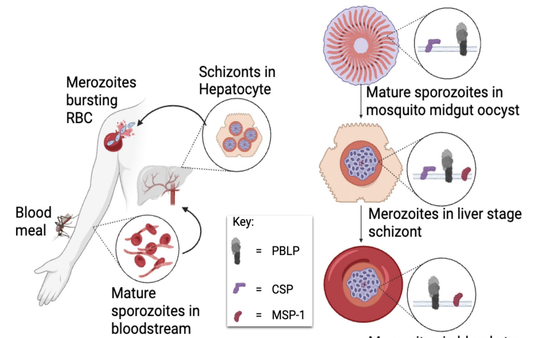
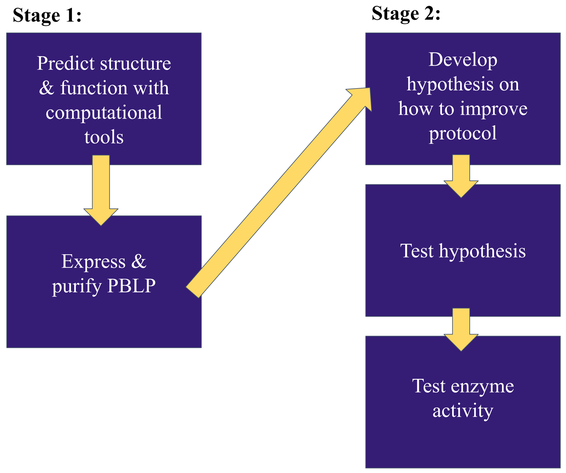


Fig 1. The *Plasmodium* parasite completes its asexual life cycle in mammalian hosts, and PBLP co-localizes with a selection of parasitic plasma membrane (PPM) proteins at various life stages of *P. yoelii*.

Research Objectives



Hypothesis (two-fold):

1. PBLP functions as a lipid esterase that hydrolyzes ester bonds of PPM lipids.
2. Adding dithiothreitol (DTT) as a reducing agent to isolation, column, and dialysis buffers will result in a greater yield of soluble PBLP.

Rationale: PBLP's inferred membrane-associated involvement in *Plasmodium* life cycle advancement and evolutionary conservation makes it a high-priority protein to functionally investigate, as it may be a beneficial target to incorporate in combination therapies.

Wet Lab Methods

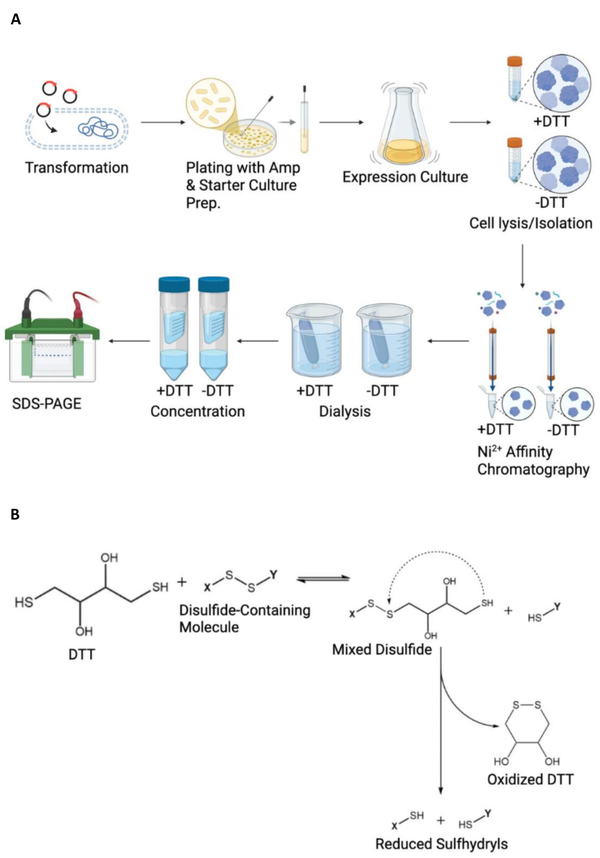
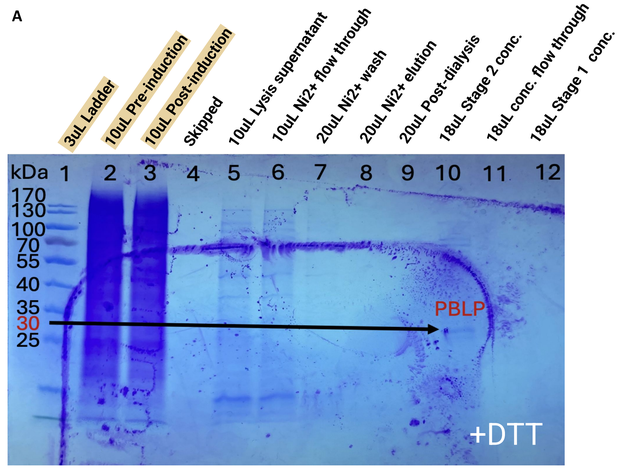


Fig 2. PBLP expression, isolation, and purification. A. Stepwise Stage 2 laboratory experiment with alterations to the original protocol: the addition of DTT to isolation, column, and dialysis buffers. Schematic created using BioRender illustration software. B. Mechanism of DTT acting as a reducing agent, adapted from Iris Biotech (2020).

Experimental Results



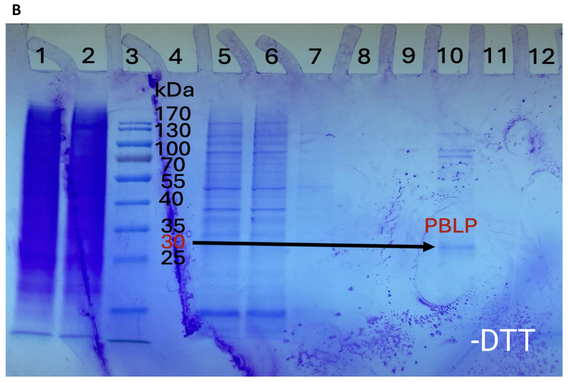


Fig 3. SDS-PAGE gel results reveal purified PBLP protein present in the Stage 2 concentrate samples for both -DTT control (B) and +DTT experimental (A) conditions. The presence of other bands not a 30 kDa in lanes 10 indicates only a partial purification of PBLP, and the absence of bands in lanes 8 and 9 indicates that protein concentrations were too low for detection prior to sample concentration.

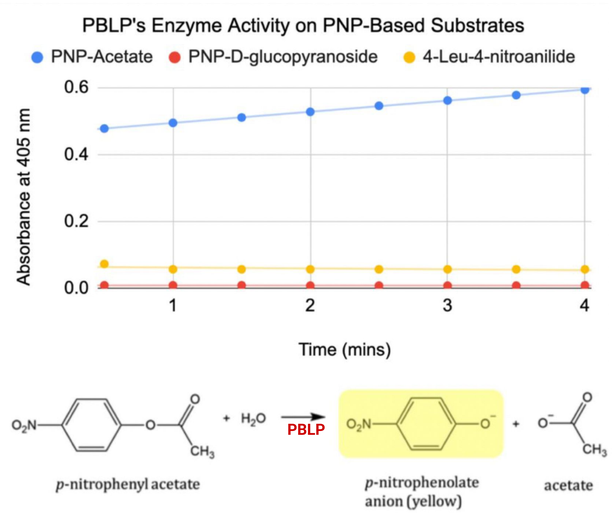
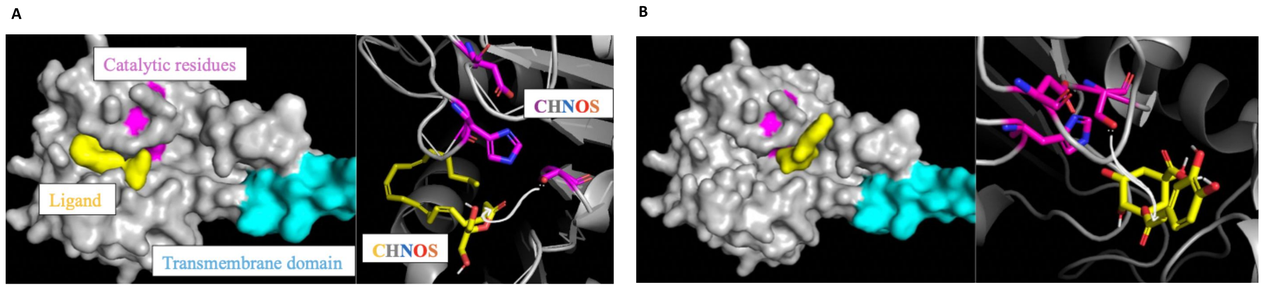
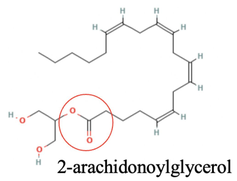


Fig 5. PBLP hydrolyzes the ester bond of PNP-Acetate to produce PNP and acetate products. Absorbance at 405 nm measured the amount of chromogenic product (PNP) produced by the enzymatic hydrolysis of each substrate.

Computational Results





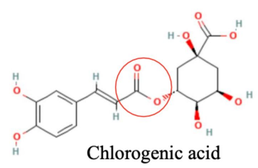


Fig 4. Putative membrane-associated ligands of PBLP display favorable conformations at the active site that position ester carbonyl groups proximal to the serine nucleophile of the Ser-His-Asp catalytic triad. A. 2-arachidonoylglycerol as the substrate. B. chlorogenic acid as the substrate. Images are PyMOL-generated visualizations using the AlphaFold3 prediction of the full-length structure of PBLP. Positions of 2-AG and chlorogenic acid were predicted using SwissDock protein docking.

Discussion/Conclusions

Did DTT improve the yield of soluble PBLP?

- PBLP was successfully expressed, isolated, and partially purified from the *E. coli* cells under both +DTT and -DTT conditions.
- The concentrate bands were similar intensities for both the +/-DTT conditions, however the -DTT prep was more concentrated. Therefore, we know that the addition of DTT facilitated a greater recovery of soluble and natively folded PBLP.
  - ➔ +DTT: 15.8-fold
  - ➔ -DTT: 45-fold
  - ➔ Stage 1: 2.8-fold

What do we know about PBLP activity now?

- We have experimentally confirmed that PBLP has hydrolase activity.
- Given the structure of PNP Acetate, we know that PBLP cleaved an ester bond to produce PNP product.

Next Steps

- Additional assays with PPM-associated substrates in *Plasmodium* would help determine PBLP's significance to the parasite.
- Consideration of pharmaceutical development involving PBLP as an antimalarial target. Continue PBLP gene knockout investigations.[1]

Acknowledgements & References

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