Plasmodium BEM46-like Protein (PBLP) from the Malaria Parasite *Plasmodium yoelii*: From Computational Prediction to *in vitro* Hydrolase Activity

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Introduction

- Malaria causes >260 million cases and ~600,000 deaths per year (WHO, 2023).
- Drug resistance to current antimalarials is increasing, creating a need for new therapeutic targets (WHO, 2023).
- PBLP is conserved across Plasmodium species and shows structural similarity to α/β-hydrolase enzymes, including a predicted Ser–His–Asp catalytic triad (Groat-Carmona et al. 2015).
- PBLP knockout parasites display reduced liver- and blood-stage development (Groat-Carmona et al. 2015).

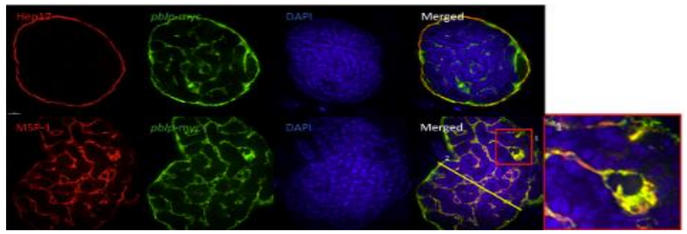

Figure 1. Localization of PBLP in Plasmodium yoelii developmental stages.
Immunofluorescence images of an exo-erythrocytic schizont from infected mouse liver tissue at ~45 hpi. PBLP-myc is shown in green, the parasitophorous vacuole membrane marker Hep17 is shown in red, and nuclei are stained with DAPI (blue). In the merged image, PBLP localizes along the parasite plasma membrane and outlines cytomere structures, supporting a role in merozoite formation.
Adapted from Groat-Carmona et al., 2015.

Research Objectives

- To characterize PBLP by identifying likely substrates and optimizing protein purification conditions
- Stage 1 focused on in silico prediction of structure & substrate candidates and initial expression of recombinant PBLP
- Stage 2 focuses on improving PBLP solubility using reducing conditions and preparing protein for activity assays

Research Question: **Can we determine whether PBLP functions as a lipid esterase, and can DTT improve recombinant protein solubility enough to enable future enzymatic testing?**

Computational Results

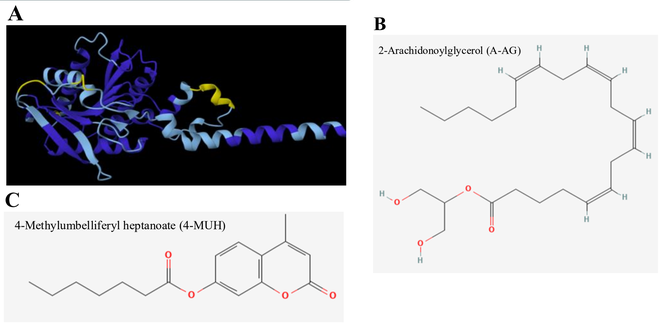

Figure 2. Computational prediction of PBLP structure and candidate substrates.
(A) AlphaFold3 predicted structure of full-length PBLP, including the predicted transmembrane helix (not present in the recombinant construct used experimentally)
(B) Structure of 2-arachidonoylglycerol (2-AG).
(C) Structure of 4-methylumbelliferyl heptanoate (4-MUH).
Both ligands were selected based on predicted compatibility with the hydrolase-like binding pocket.

Ligands	Docking Score (kcal/mol)
2-AG	-5.84
4-MUH	-5.39

Table 1. Docking scores for predicted PBLP substrate candidates.

Experimental Methods

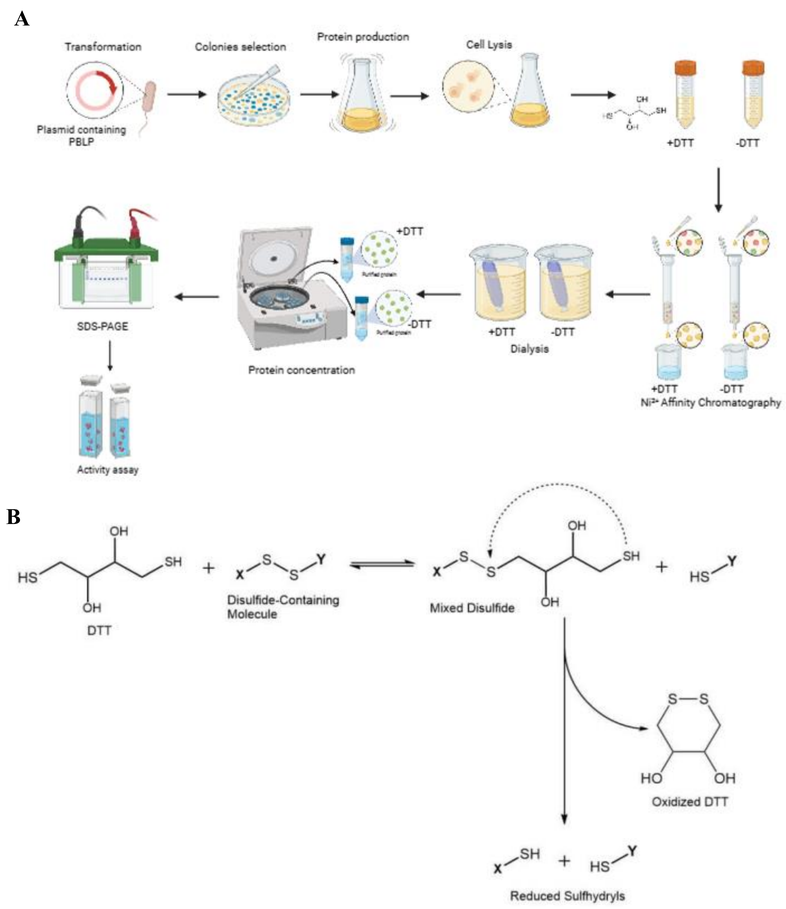

Figure 3. Experimental workflow and DTT reduction mechanism.
(A) Schematic overview of Stage 2 wet lab workflow. Truncated His-tagged PBLP was expressed in E. coli, followed by parallel purification under reducing (+DTT) and non-reducing (–DTT) conditions. Steps included cell lysis, Ni^{2+} affinity chromatography, dialysis, concentration, SDS-PAGE, and substrate activity assay.
(B) Chemical mechanism of DTT-mediated disulfide bond reduction. DTT first forms a mixed disulfide with the target, then undergoes intramolecular cyclization to yield oxidized DTT and two reduced sulfhydryls.
Figure adapted from BroadPharm, "Protocol for S–S Bond Reduction by DTT," Version 2.0 (2022).

Experimental Results

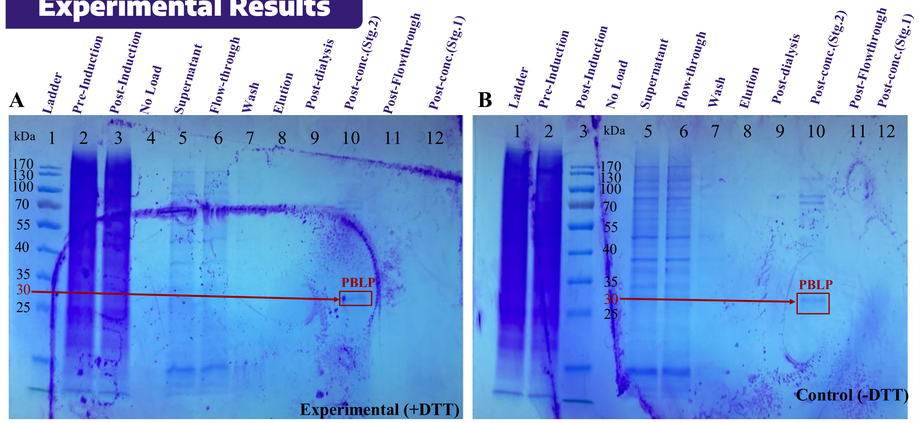

Fig. 3. SDS-PAGE of purified PBLP under reducing and non-reducing conditions.
(A) +DTT: A distinct ~30 kDa PBLP band appears after concentration, indicating improved solubility under reducing conditions.
(B) -DTT: The ~30 kDa band is present but weaker, and additional bands suggest partial purification.

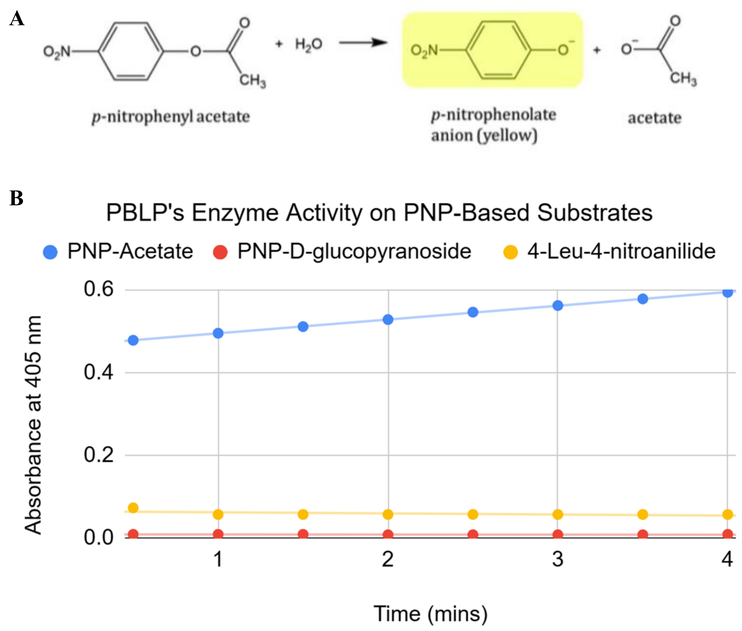

Figure 4. Enzyme assay schematic and activity results for PNP-based substrates.
(A) Hydrolysis reaction of p-nitrophenyl acetate. Cleavage of the ester bond releases p-nitrophenolate, which produces a yellow color measurable at 405 nm.
(B) PBLP activity on p-nitrophenyl (PNP) substrates over time. A steady increase in absorbance is observed only for PNP-acetate, while PNP-D-glucopyranoside and 4-Leu-4-nitroanilide show minimal signal, indicating limited or no turnover.

Discussion

- Docking suggests PBLP may act on lipid-like ester molecules.
- PBLP showed activity toward PNP-acetate, which is a small, non-specific model substrate that many hydrolases can cleave. This reaction does not reveal much about PBLP's true substrate preference, and the lack of activity on the other PNP-based substrates suggests they are unlikely to be biological targets.
- +DTT conditions produced more soluble PBLP, supporting the idea that reducing agents help stabilize the protein.
- Partial purification likely limited the enzyme assay results.
- More purified protein and testing lipid-based substrates will be needed to determine PBLP's true function.

Future Directions

- Further purify PBLP to reduce contaminating proteins.
- Optimize expression to increase protein yield.
- Re-run enzyme assays with more concentrated PBLP.
- Test additional lipid-like substrates based on docking.
- Use more sensitive assays (fluorescent or LC-MS) to detect activity.

Acknowledgements

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References

- 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.
- World Health Organization (WHO). 2023. World malaria threats map [Internet]. Geneva (CH): WHO; [cited 2025 May 6]. Available from: https://apps.who.int/malaria/maps/threats