Plasmodium BEM46-like protein from the malaria parasite *Plasmodium Yoelii*: Computational models utilized to analyze alpha/beta-hydrolase characteristics and optimize expression protocols

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Introduction

- Malaria is a parasitic disease caused by the *Plasmodium* species, yoelli, and is responsible for approximately 240 million infections worldwide annually.
- Previous research discovered the enzyme PBLP (Plasmodium BEM46-like protein), exhibits characteristics of an Alpha/Beta hydrolase.
- The enzyme is conserved across all *Plasmodium* species and is expressed during all stages of the *Plasmodium yoelii* life cycle, which is localized to the parasitic membrane.
- Previous research by Dr. Carmona found that the deletion of PBLP impairs proliferation in infected red blood cells of hosts, leading to decreased infection levels (2015).
- These findings make PBLP a promising candidate for targeted drug development.

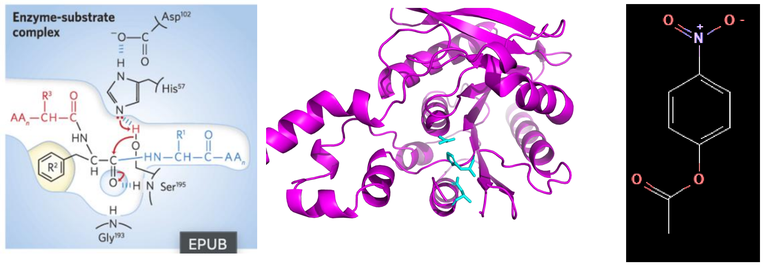
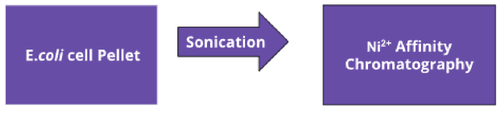


Figure.1A-C: Rendering of catalytic triad, Catalytic triad of Full length PBLP and Molecular structure of a PNP Acetate. A) Diagram shows covalent catalysis by SER. Chymotrypsin Catalytic Triad, serine protease acts on peptide(amide) bonds (Principles of Biochemistry, 2021). B) Catalytic triad of PBLP model from Alpha Fold3 predictive model (Abramson, J et al, 2024) . C) P-nitrophenyl Acetate, (Pubchem, NIH, 2024).

Specific Aims

- To refine expression protocols, and purification of two PBLP constructs that lack transmembrane domains- wild type (WT) and catalytically-dead mutant (S153N), using Plasmids containing the amino acid sequences for WT and S153N to transform competent BL21 *E.coli*.
- To extract PBLP and compare two lysis techniques, using sonication to break apart E.*coli* cells in the first stage and a lysozyme in the second stage.
- To determine the presence and concentration of PBLP identification using SDS-PAGE, which indicates the presence of protein at various molecular weights. PBLP weight being approximately 30 KDa.
- To produce protein for assays investigating enzymatic activity. PBLP-S153N has been modified to replace the catalytic serine with asparagine, which renders the catalytic triad ineffective.

Stage 1 Protocol:



Stage 2 Protocol:

Methods

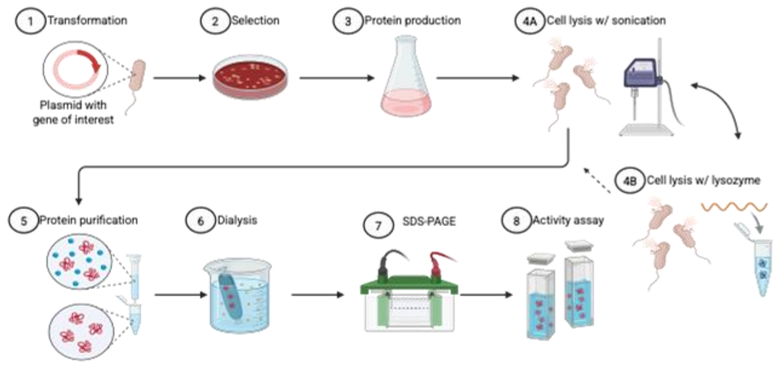


Figure.2: Methodologies for stage 1 and stage 2; showing Transformation, cell selection, protein induction, cell lysis, purification and analysis steps. A) Shows stage 1 methods with sonication technique for cell lysis. B) Shows stage 2 methods with addition of lysozyme for cell lysis in lieu of manual technique. Created in https://BioRender.com

Computational Results

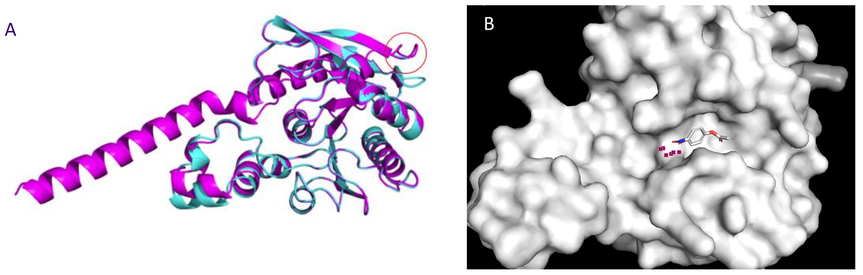


Figure. 3A & B: Computational predictive models from Alpha Fold 3 and Swissdock. A) Visual overlap of Alpha Fold 3 generated predicted models of full length and truncated PBLP proteins. Models generated from amino acid sequences, including AA sequence of transmembrane domain for full length and truncated without transmembrane sequence (Abramson, J et al, 2024). B) Truncated PBLP prediction model from SwissDock, using structure prediction from Alpha Fold 3, and the catalytic triad activity with P-nitrophenyl Acetate (Swissdock, 2024).

Experimental Results

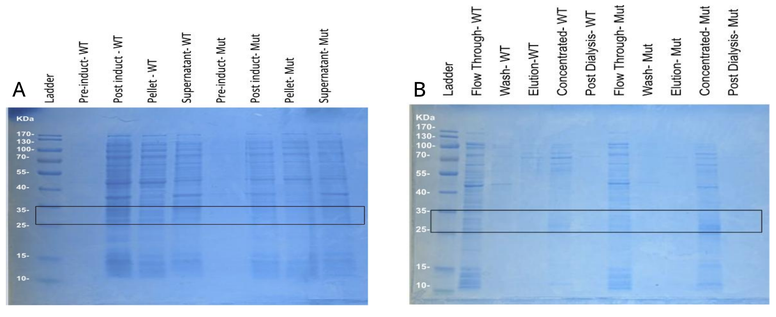
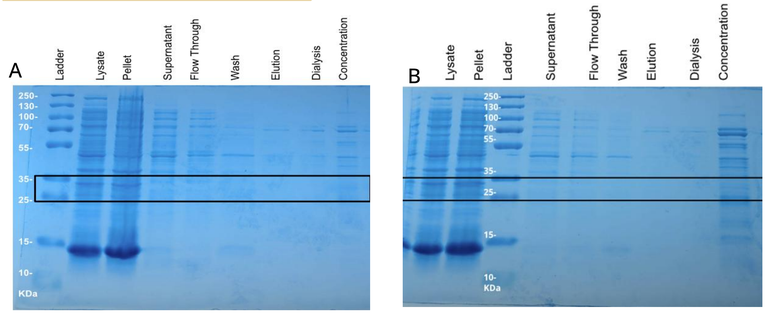
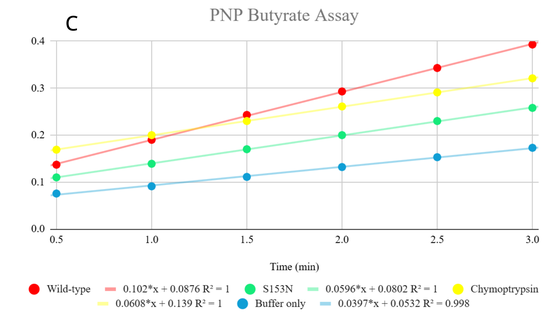
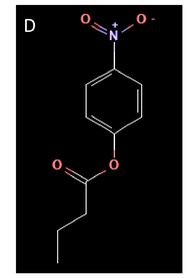


Figure. 4 A&B: SDS-Page results that highlight 30 KDa ranges for all samples during each step of protocol for Stage 1. A) Potential PBLP presence in all steps for WT and Mutant after preinduction stages. B) Potential PBLP presence in final concentration samples in comparison to previous samples from dialysis and Ni^2+ column chromatography

Experimental Results (continued)







E	FINAL PBLP CONCENTRATIONS:	
	Wild Type Stage 2:	1.01 * 10^ -5 M
	Wild Type Stage 1:	1.61*10^-5 M
	Mutant (S153N) Stage 2:	2.01 * 10^-5 M
	Mutant (S153N) Stage 1:	2.74*10^-5 M

Figure. 5 A-C: SDS-Page results that highlight 30 KDa ranges for all samples during each step of protocol for Stage 2, and results of enzymatic assay for Stage 2 PBLP samples. A) SDS-PAGE gel of stage 2 WT samples taken from various stages of purification. Possible faint bands representing PBLP WT at ~30 KDa in concentration step of protocol. B) SDS-PAGE gel of stage 2 Mutant- S153N; samples taken from various stages of purification. Possible faint bands representing PBLP MUT at ~30 KDa in concentration step of protocol. C) PNP assay results for stage 2 samples, with P-nitrophenyl butyrate as the substrate. Absorbance was higher with WT sample than catalytically dead mutant and chymotrypsin. D) PNP Butyrate molecular structure generated by pubchem and the NIH. E) Final PBLP concentrations after final centrifugation step. Values represent quantity of PBLP obtained in each stage; WT and Mutant.

Significance

- Alternative Stage. 2 E.coli cell lysis method was ineffective in comparison to Stage. 1 lysis method; sonication vs lysozyme, respectively.
- Wild Type PBLP forms were more enzymatically active with subtrats that contained shorter fatty acid tails such as P-nitrophenyl acetate and P-nitrophenyl butyrate, as it was predicted due to truncated hydrophobic pocket regions and transmembrane sections.
- Mutant forms were catalytically non-functional in enzymatic activity assays, as the serine was replaced with an asparagine amino acid.

References

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