Improving Purification and Investigating Substrate Specificity of Plasmodium BEM46-Like Protein (PBLP)

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Background

- Malaria is a major global health challenge caused by Plasmodium parasites. [1]
- PBLP is expressed by Plasmodium yoelii and is important for parasite development and infection. [1]
- PBLP knockout affects parasite maturation and infection rate. [1]
- However, PBLP's biological function and native substrate remain unknown. [1]
- Understanding PBLP activity may provide insight into parasite biology and potential intervention strategies.[1]

PBLP Computational Docking

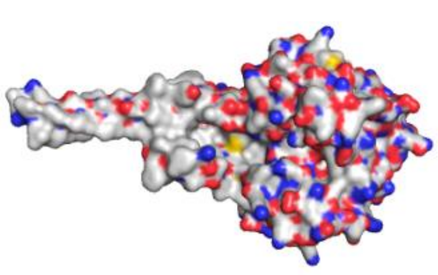


Figure 1: PBLP Full-Length computational result displaying the binding site generated through Alphafold.

Objective

- Improve PBLP expression and purification from E. coli.
- Investigate PBLP substrate specificity using computational predictions and enzyme assays.
- Determine whether substrate size influences PBLP activity.

Methods

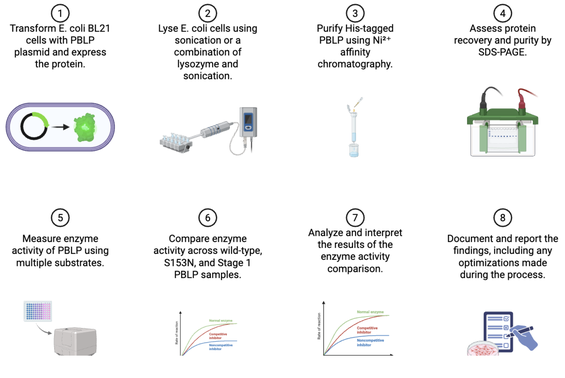


Figure 2: Flowchart of Methods used during project generated through BioRender.

Protein Purification Results

- Stage 1: WT PBLP and the S153N mutant were expressed and purified.
- Stage 2: The purification protocol was modified to improve soluble PBLP recovery.
- S153N is a catalytically inactive PBLP mutant.
- Stage 1 purification produced low PBLP recovery, prompting modification of the lysis protocol.
- The modified protocol produced a larger sample volume but lower protein concentration and total protein.
- SDS-PAGE showed PBLP primarily in the lysate and pellet, with minimal recovery during purification.
- Results suggest PBLP insolubility is a major factor limiting protein recovery.

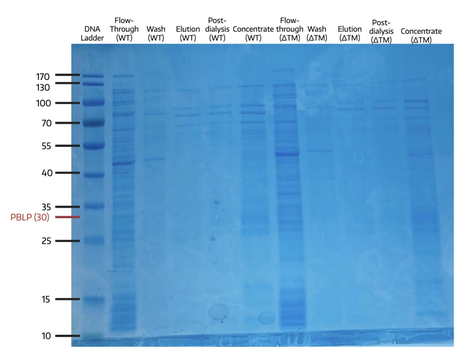


Figure 3: Stage 1 Wild Type and Mutant PBLP samples after purification by nickel affinity chromatography and dialysis show a faint band at ~30 kDa in the flow-through which implies possible presence of PBLP. Reduced or faint bands in the wash, elution, and post-dialysis fractions indicate a very low yield of PBLP during purification; a faint band at ~30 kDa is again noticed in the concentrated sample, indicating limited PBLP recovery in both strains. These results are consistent across both the Wild Type and Mutant PBLP protein.

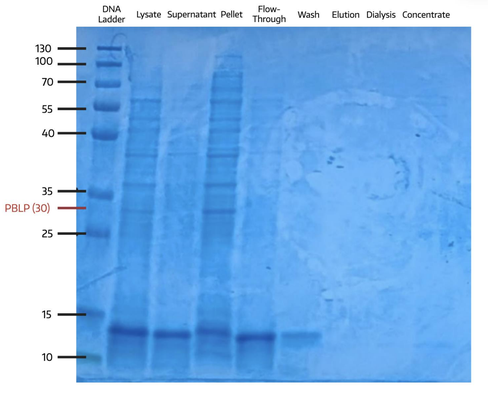


Figure 4A: New Sonication Method (Stage 2) Mutant PBLP expressed in *E. coli* before purification in stage 2 shows a faint band at ~30 kDa in the lysate and a thicker, slightly darker band in the pellet, indicating the majority of PBLP ends up insoluble but is present at higher levels compared to previous methods.

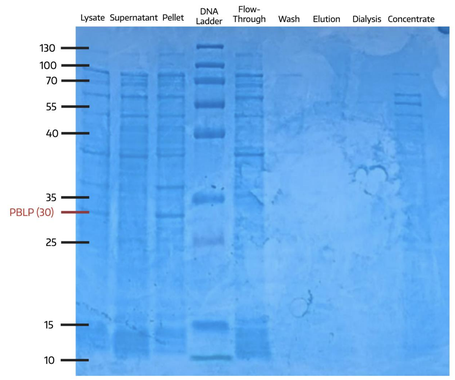
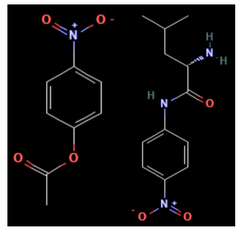


Figure 4B: Old Sonication Method (Stage 2) Mutant PBLP expressed in *E. coli* before purification in stage 2 shows a faint band at ~30 kDa in the lysate and a slightly darker band in the pellet; overall, both bands are lighter than those seen with the new sonication method, suggesting lower PBLP recovery.

Figure 5 + 6: PBLP Full-Length in teracting with P-Nitrophenyl Acetate (on top) and 4-Leucine 4-Nitroanilide (on bottom) generated through Swissdock. Image 1 ligand binds directly on Serine in the active site implying strong interaction and most promising docking result for full-length PBLP. Image 2 ligand binds directly on Serine in the active site implying strong interaction and most promising docking result for full-length PBLP. Below are chemical structure of P-Nitrophenyl Acetate (left) and 4-Leucine 4-Nitroanilide (right)



Enzyme Activity Results

- Candidate substrates were selected based on computational docking and structural similarity.
- p-Nitrophenyl acetate and 4-leucine-4-nitroanilide share aromatic and polar features.
- p-Nitrophenyl benzoate was included as a larger, structurally similar substrate.
- It showed the highest catalytic activity among the substrates tested.
- Activity generally decreased with increasing substrate size.

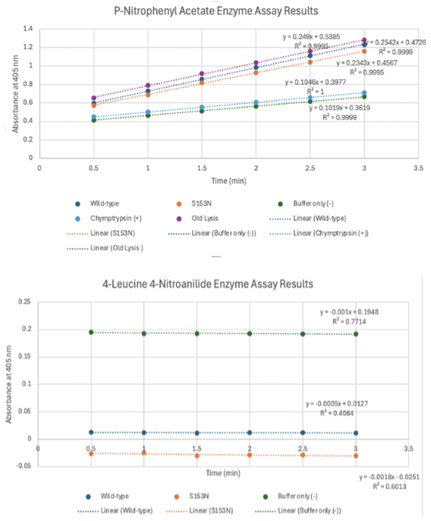


Figure 7: Enzyme Activity of PBLP Using p-Nitrophenyl Acetate (on top) and 4-Leucine-4-Nitroanilide (on bottom). PBLP activity was tested using Stage 1 WT, Stage 1 S153N mutant, and Stage 2 WT with the old lysis protocol. Buffer (–) and chymotrypsin (+) were included as controls for the p-nitrophenyl acetate assay. p-Nitrophenyl acetate showed activity in all PBLP samples, with Stage 2 old lysis showing the highest activity, while 4-leucine-4-nitroanilide showed minimal or no measurable activity.

Conclusion

- Results did not support the hypothesis that larger substrates would bind more effectively to PBLP.
- PBLP may instead favor smaller substrates.
- pNPA is a promising substrate for further investigation but is not confirmed as the native substrate.
- Improved protein solubility and additional substrate testing are needed to clarify PBLP function.

Future Directions

- Improve PBLP solubility and purification yield.
- Test additional small, structurally similar substrates.
- Optimize assays for substrates that precipitate.
- Further investigate PBLP activity toward p-nitrophenyl substrates.
- Identify PBLP's potential native substrate.

Acknowledgements

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- Jennifer Nguyen- Project Partner

References

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