TBIOMD 495

From Isolation to Function: Investigating a Novel Enzyme in *Plasmodium yoelii*

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Background:

- Malaria is a disease caused by parasites of the *Plasmodium* family.
- In *Plasmodium yoelii* a novel protein was discovered. (Groat-Carmona et al. 2015)
- The sequence of the protein matches the α/β-hydrolase, which is known to have a diverse array of functionalities.(Groat-Carmona et al. 2015)
- Can be inferred that the protein co-localizes within the parasitic plasma membrane (PPM). (Groat-Carmona et al. 2015)
- When knocked out, observed reduced infectivity in Malaria cases. (Groat-Carmona et al. 2015)

A.

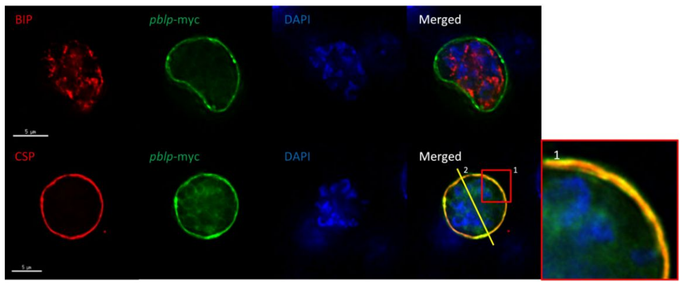
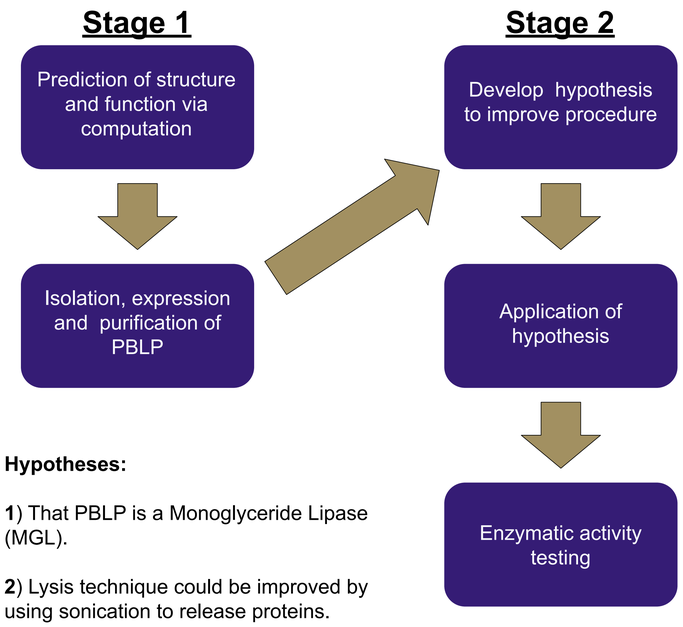


Figure A:
P. yoelii infected livers with PBLP-myc at 24 hours post inoculation. PBLP colocalizes with the circumsporozoite protein (CSP) which is associated with the parasitic plasma membrane. (Groat-Carmona et al. 2015)

Objectives:



Hypotheses:

1) That PBLP is a Monoglyceride Lipase (MGL).

2) Lysis technique could be improved by using sonication to release proteins.

Rationale: PBLP localizes to the parasitic plasma membrane (PPM) (Groat-Carmona et al. 2015). This supports the hypothesis of PBLP being a MGL due to its crucial role for membrane dynamics.

Computational Results:

C.

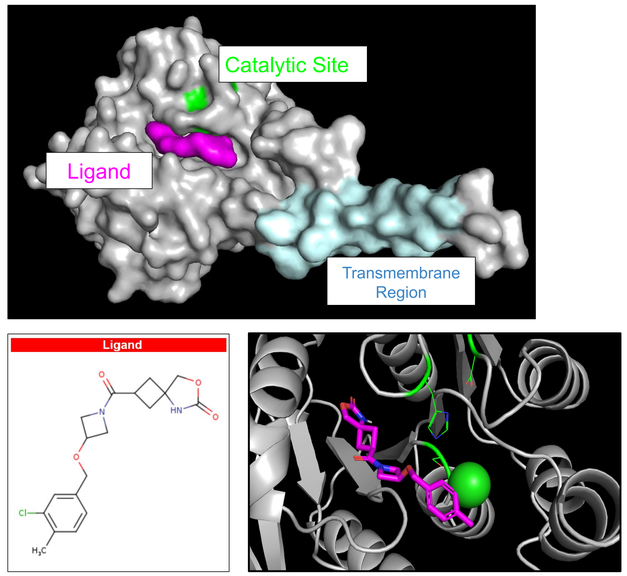


Figure C:
Computational results collected from various computational tools plugged into visualization via the program PyMol. Showcases how a potential ligand; a MGL inhibitor, can fit and interact within the active site.

Methods:

- *E.coli* transformation and expression of protein of interest.
- Ni^{2+} affinity chromatography.
  - Polyhistidine tags on PBLP have a strong affinity towards Nickel.
- Concentration
  - Post-Dialysis samples were concentrated using spin tubes.
  - Experimental 2x, Original Protocol 3.57x
- SDS-PAGE, to monitor concentration and successful isolation of PBLP.

D.

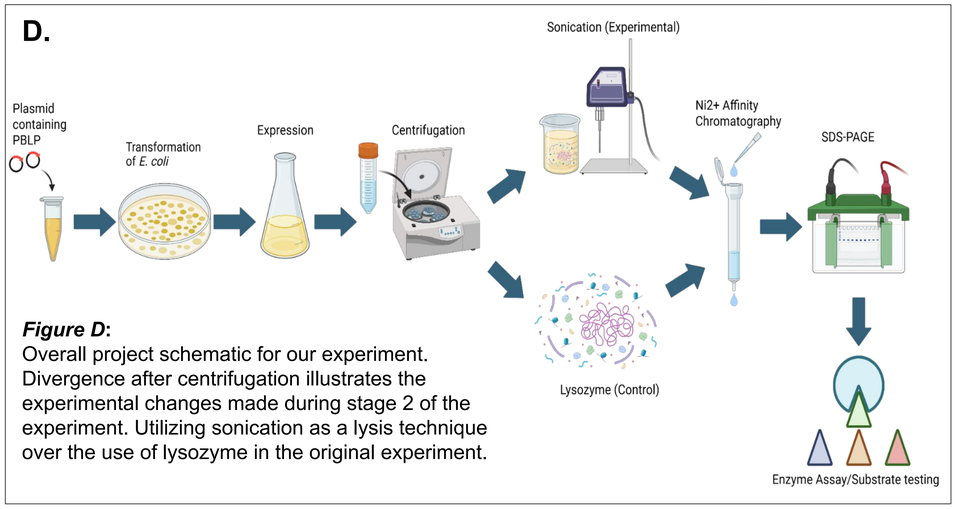


***Figure D*:**
Overall project schematic for our experiment. Divergence after centrifugation illustrates the experimental changes made during stage 2 of the experiment. Utilizing sonication as a lysis technique over the use of lysozyme in the original experiment.

Results:

E.

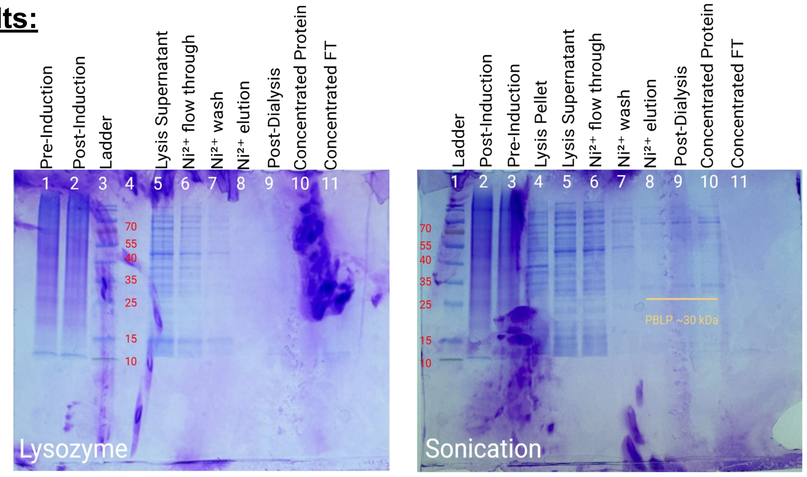
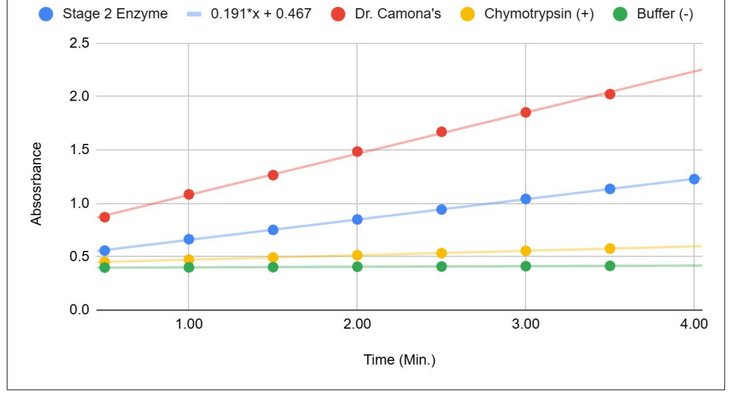


***Figure E*:** Comparison of the Lysozyme (Control) and Sonication gels. In wells 8–10 of the sonication gel, three bands are highlighted in yellow around the 30 kDa region, where the protein of interest, PBLP, is known to migrate. This suggests the presence of the protein in the experimental procedure gel.

Figure F:
Enzyme activity assay of multiple protein samples using p-nitrophenyl acetate substrate. Our PBLP product yielded 0.126 units of activity per 5000uL. Which supports the prediction that PBLP is a hydrolase.



Conclusion

- We were successful in the isolation of PBLP
- Sonication improved yield of PBLP via sonication lysis.
- Confirmed that PBLP is a hydrolase protein due to enzyme assay test.

Future Direction:

- Refinement of isolation protocol to improve yield.
- Testing of more substrates that relate to:
  - Lipid Esters, Amides, Thioesters.
- Testing Other substrates associated with α/β-hydrolase superfamily..

Acknowledgements:

I would like to thank Dr. Baughman for her guidance, knowledge, and enthusiasm passed onto us in regards to protein expression. I would also like to thank the rest of the research team for their collaboration and dedication to the project.

Reference:

- 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. Cell Microbiol. 17(12):1848–1867. doi:10.1111/cmi.12477.