

The Addition of Protease Inhibitors to Improve PBLP Purification From *E.coli* for Further Investigation

TBIOMD 495 | Rashad Muhammad, Kayla Libago, Hannah Baughman



Background

- **Plasmodium:** The genus of parasite responsible for causing malaria
- **Malaria:** A mosquito-borne illness responsible for thousands of deaths
- Plasmodium BEM46-like protein (**PBLP**) was discovered by Dr. Groat Carmona...
- PBLP was shown to be crucial for plasmodium development throughout all life stages.
- Although we're aware of PBLP's importance to the plasmodium life cycle...we do not know what it does!
- Previous attempts to purify PBLP in vitro have been widely unsuccessful...
- PBLP was able to be identified as a hydrolase...however we do not know what it hydrolyzes. Understanding how PBLP works may open doors to the development of malaria treatments in the future.

Road Map

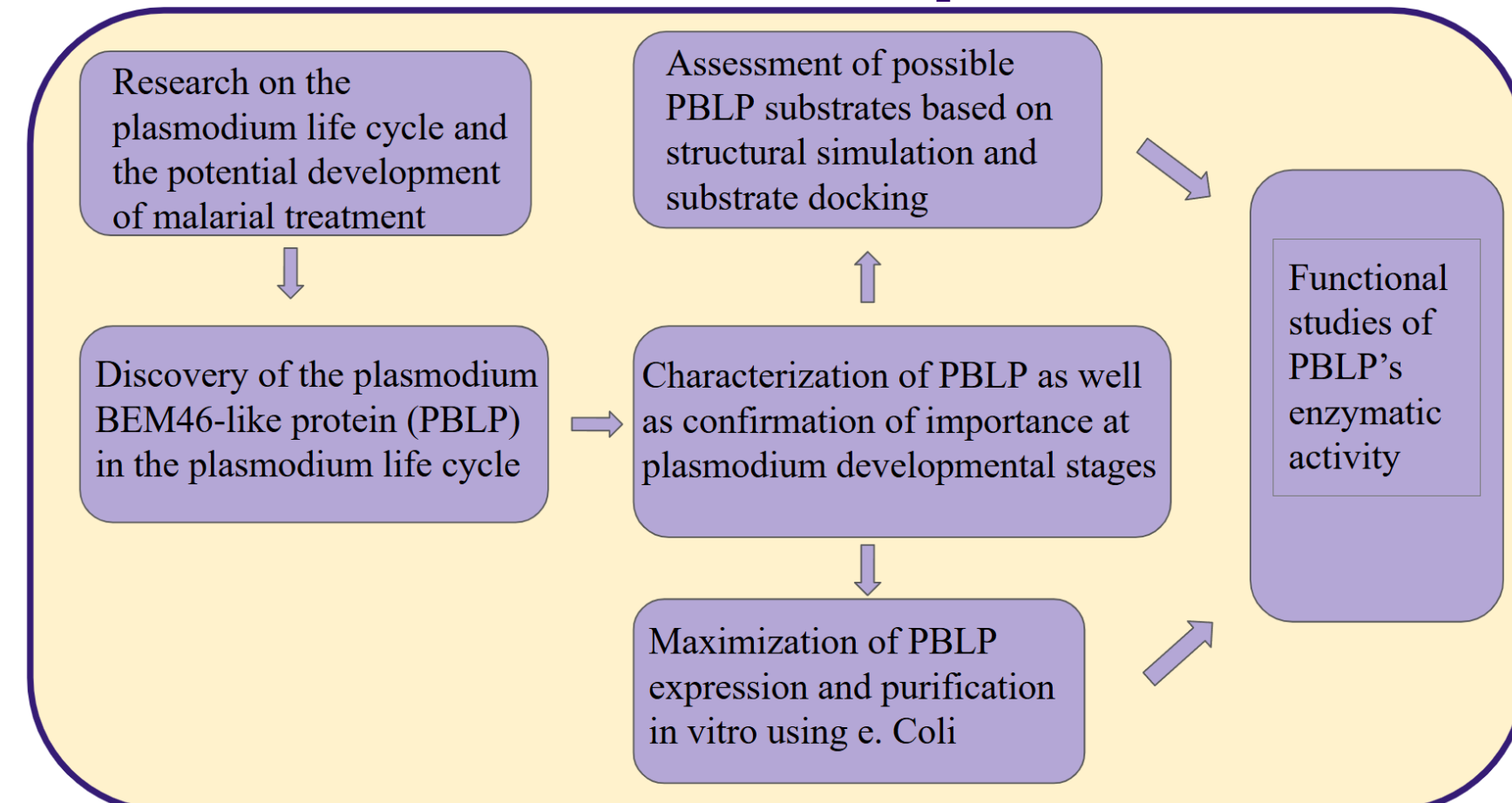


Figure 1. Roadmap from studies on the plasmodium life cycle, to the discovery and further assessing of the plasmodium BEM46-like protein (PBLP).

Research Objectives

- Computationally model the structure and substrate interactions of PBLP.
- Produce adequate amounts of truncated PBLP in vitro.
- Identify the substrate(s) that PBLP has affinity for to determine its function.

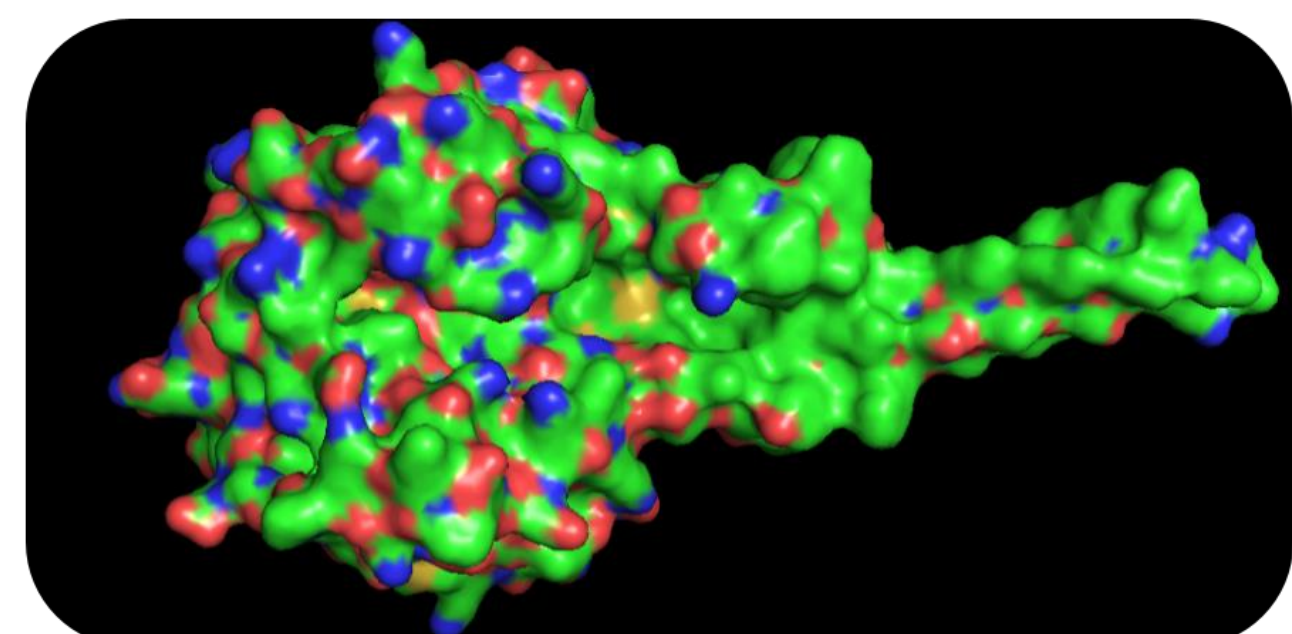


Figure 2. Full length PBLP structure estimated by AlphaFold and visualized with Pymol. The substrate binding site of the full-length structure is observed to be elongated horizontally.

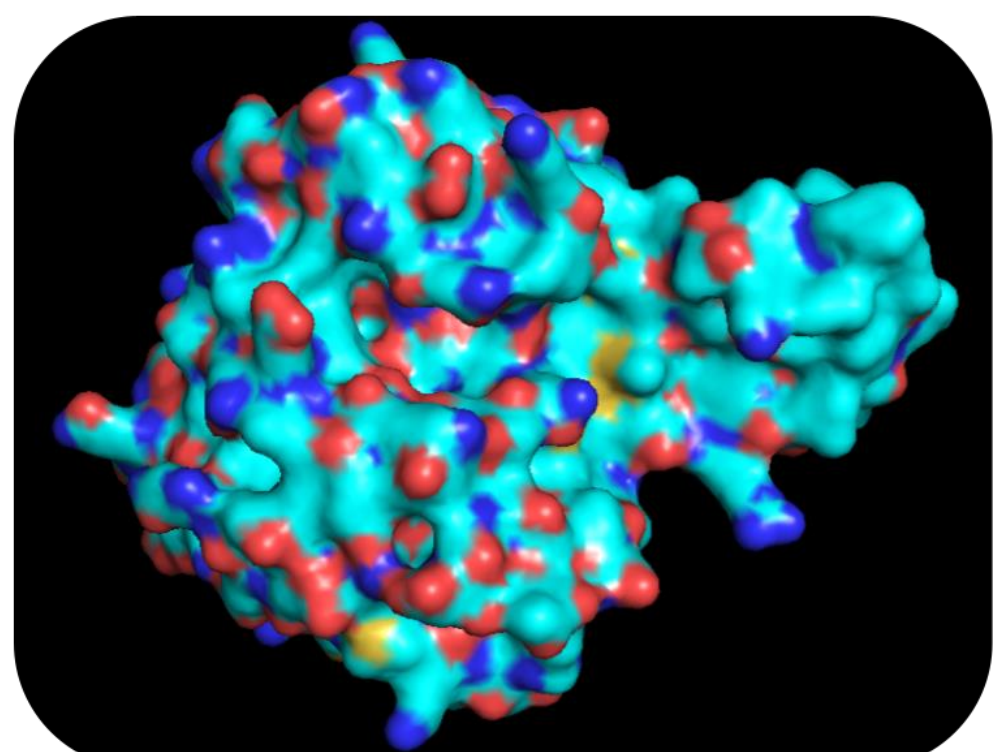


Figure 3. Truncated PBLP structure estimated by AlphaFold and visualized with Pymol. The truncated PBLP binding site is visibly clefted in comparison to the active site of the full-length structure.

Methods & Results Purification Flow Chart

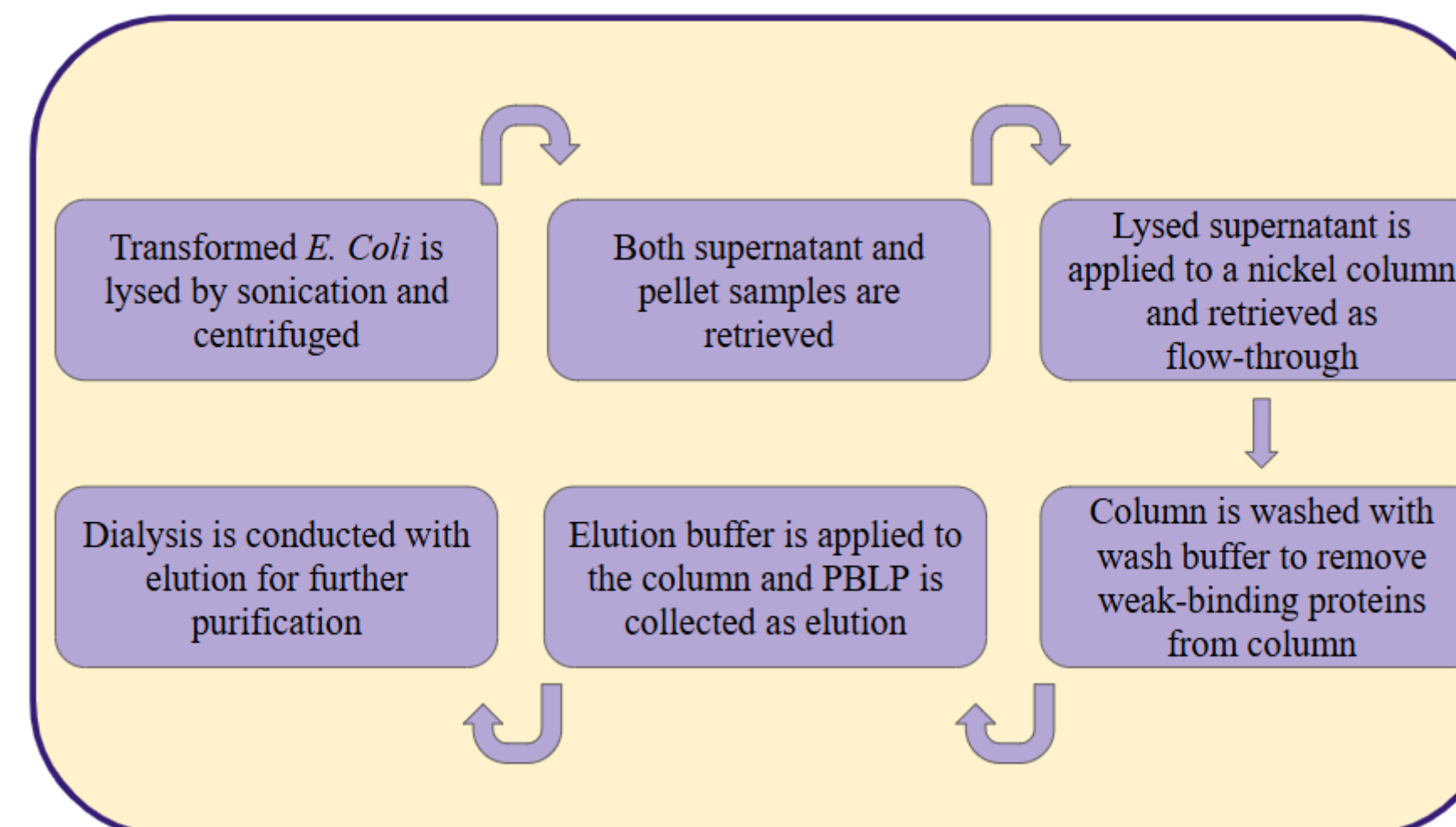


Figure 4. Flowchart of PBLP purification methods and SDS-PAGE sample retrieval

1st Attempt (W/o Protease Inhibitors)

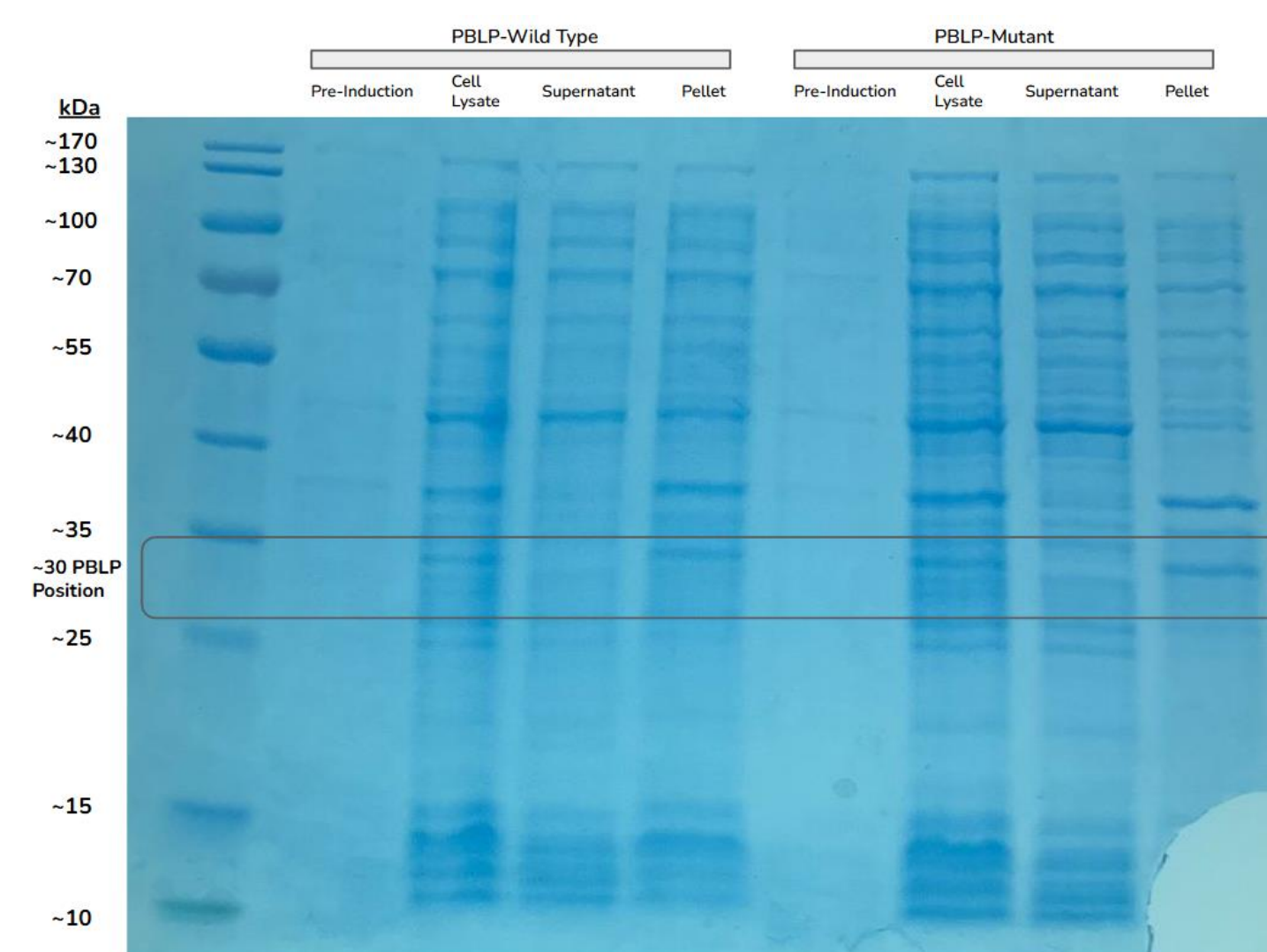


Figure 5. Stage 1 SDS-PAGE results of both wildtype and mutant PBLP samples collected pre-induction, after cell lysis, and in the pellet by-product of Nickel column chromatography. Minimally visible bands are observed in both cell lysate stages, and one band is visible in the pellet of the mutant. Bands of differing sizes were observed across both wild type and mutant PBLP samples, indicating the presence of non-targeted protein being amplified.

Protease inhibitors have been shown to improve protein purification by further protecting protein from degradation...could this be applied to PBLP?



Figure 6. Stage 1 SDS-PAGE results of both wildtype and mutant PBLP after attempting to amplify PBLP expression in *E. coli*. Samples were derived from various stages of the purification and amplification protocol. No visible PBLP bands were expressed across both wildtype and mutant PBLP across flow-through, wash, eluent, post-dialysis and concentrated stages of the procedure.

2nd Attempt (W/Protease Inhibitors)

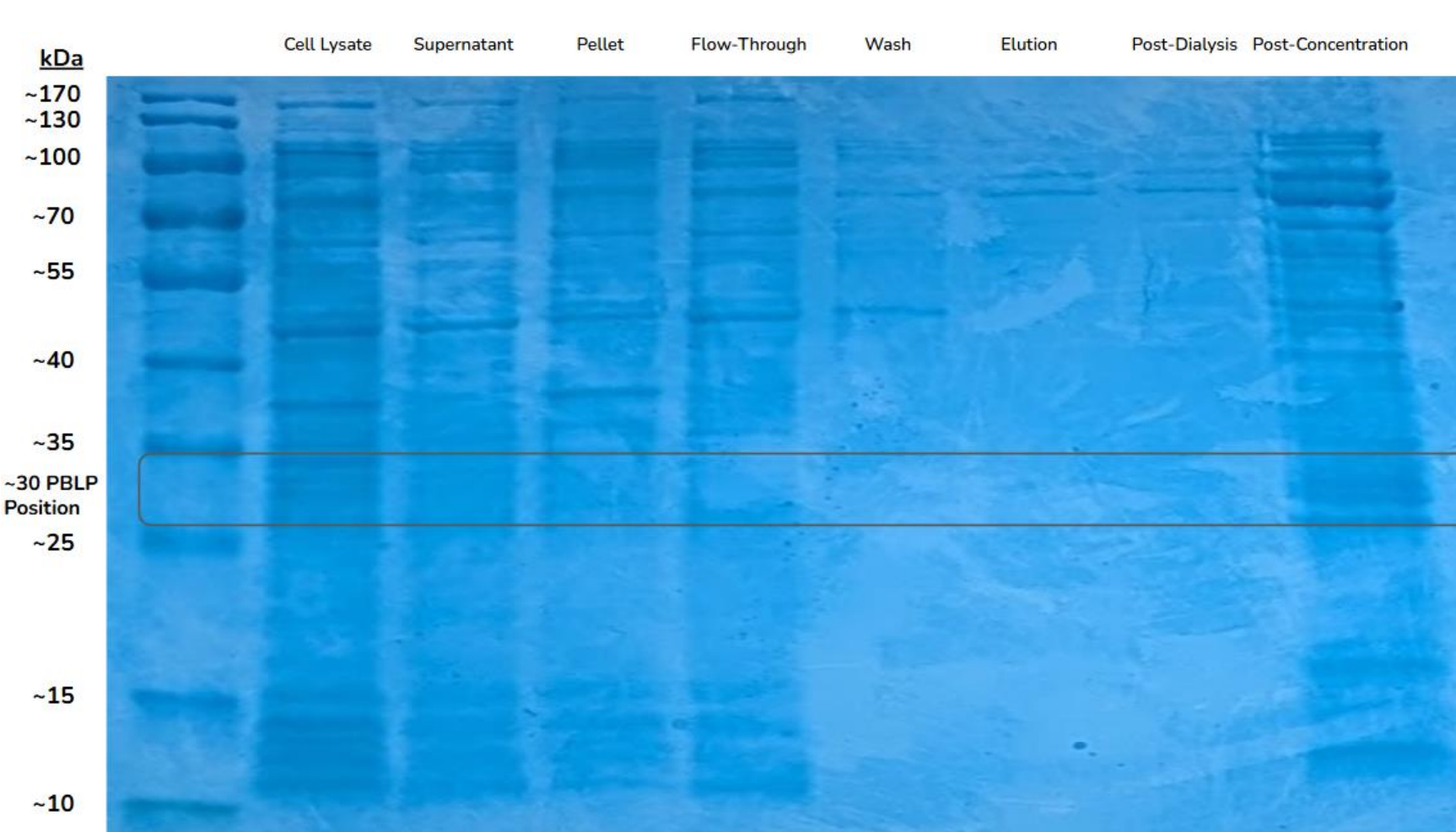


Figure 7. Stage 2 SDS-PAGE results of wildtype PBLP after amplification of expression in *E. coli*. Samples were derived from several stages of the purification and amplification protocol. Significantly visible bands were observed in both cell lysate and post-concentration lanes, with minor visibility at supernatant and flow-through lanes. No bands were observed from wash, elution and post-dialysis lanes. Presence of PBLP bands in the cell lysate lane may serve as indication that administered protease inhibitors and PMSF caused PBLP degradation during lysis. Visible bands across differing kDa values indicate presence of differing expressed proteins.

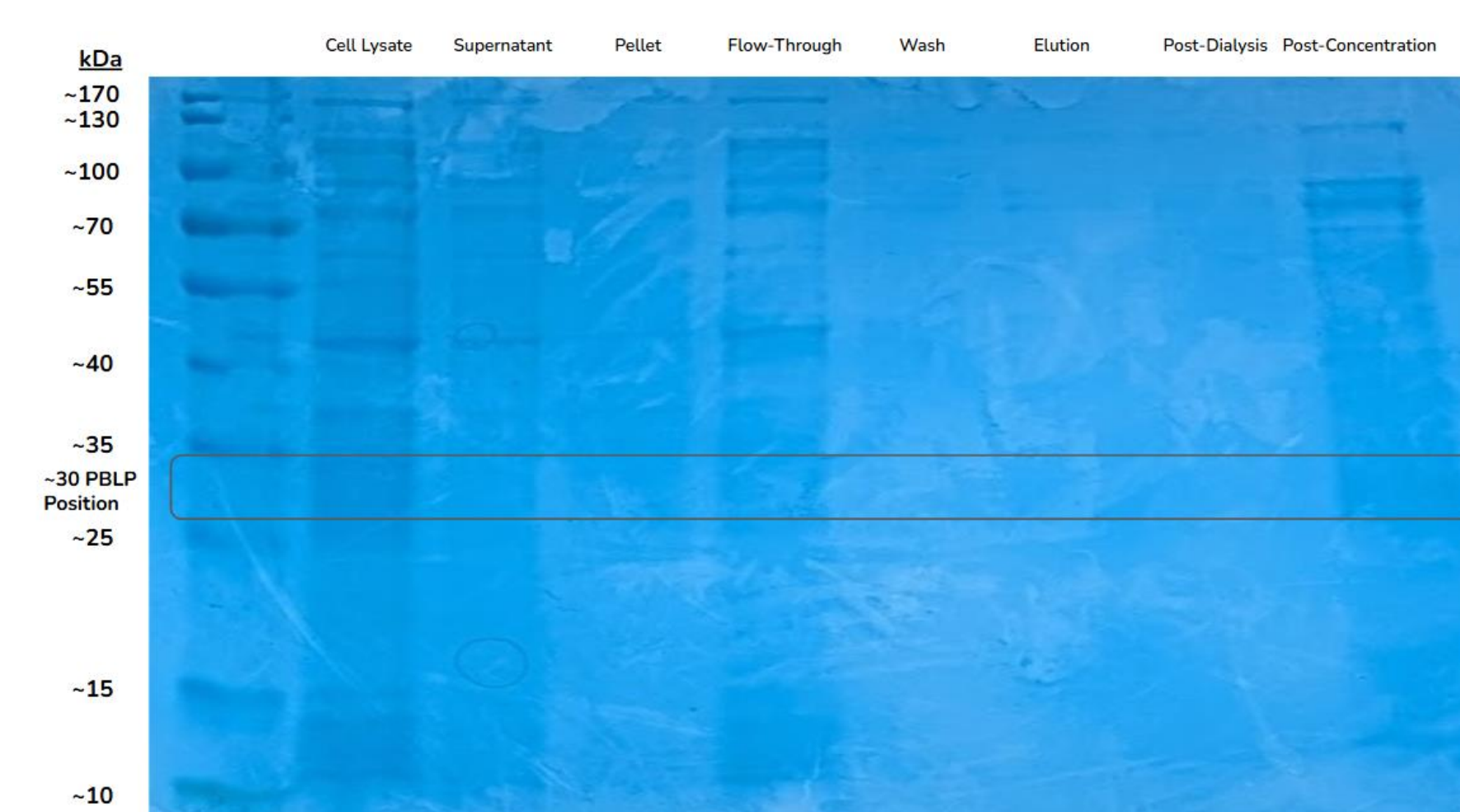


Figure 8. Stage 2 SDS-PAGE results of mutant PBLP post-amplification in *E. coli*. Minimally visible bands were observed in cell lysate, flow-through and post-concentration lanes. No bands were observed in pellet, wash, elution and post-dialysis lanes. Slightly visible bands reflect lower concentration of protein expression observed previously. Minimal bands observed across varying kDa's across cell lysate, flow-through and post-concentration lanes, indicating the expression of miscellaneous proteins.

Results (cont.)

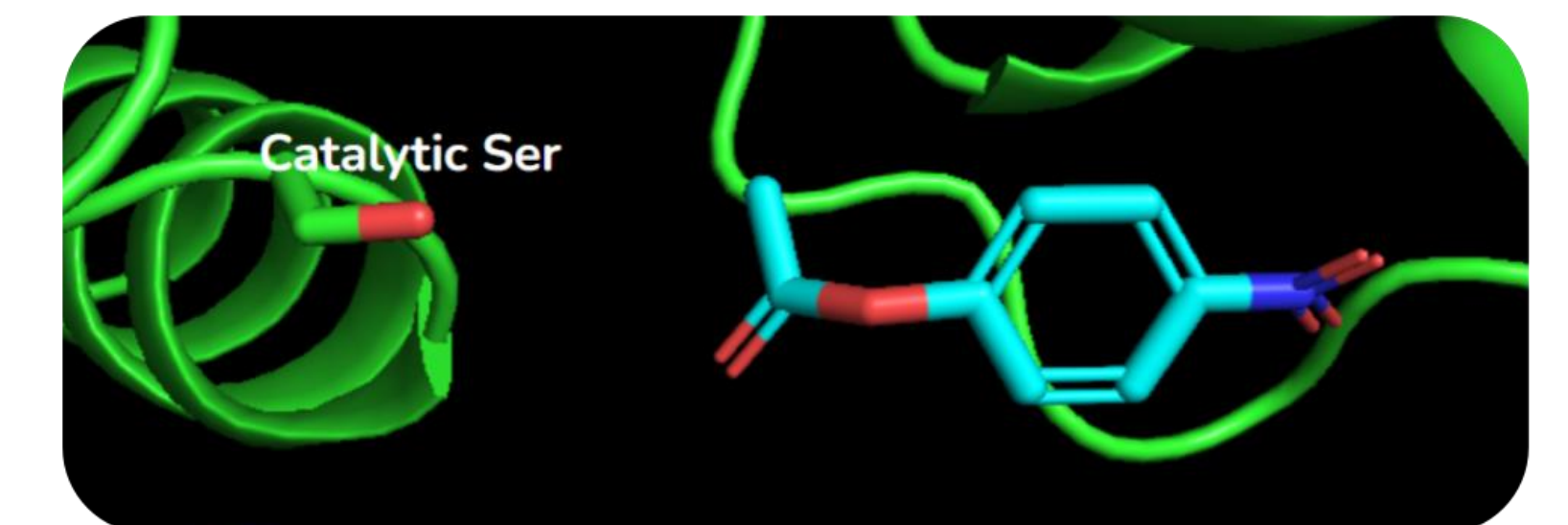


Figure 9. Docking result of p-nitrophenyl (PNP) acetate with the full length PBLP structure. Optimal proximity between the catalytic serine of full length PBLP and the carbonyl electrophile of PNP acetate was observed. Orientation of PNP acetate in the active site of full length PBLP was optimal.

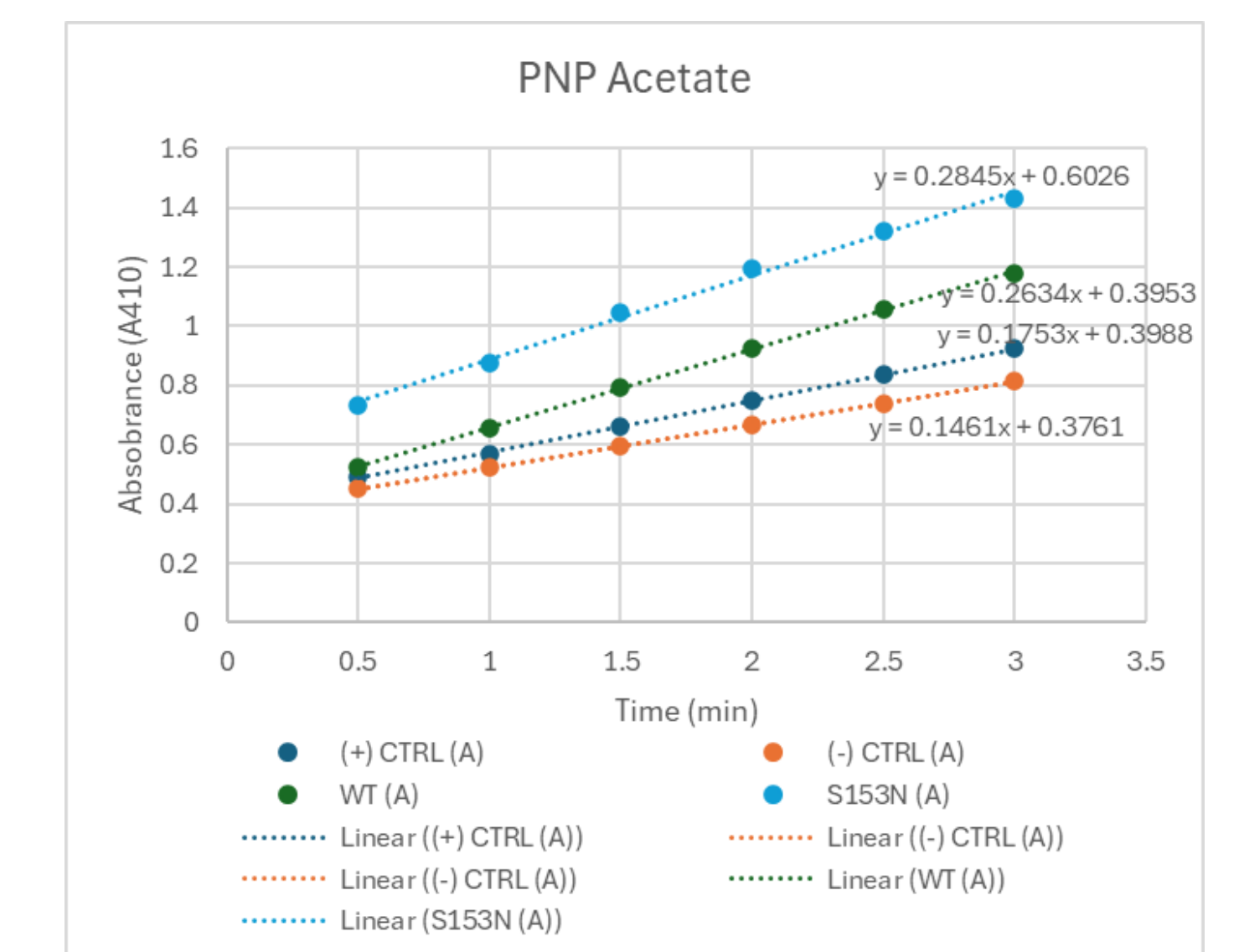


Figure 10. P-nitrophenyl assay (PNPA) results with truncated PBLP, the catalytically-dead mutant and both (+) and (-) control groups. The mutant had unexpectedly higher PNPA activity than both the truncated strain and (+) control. Observed results were inconsistent with expected findings, suggesting possible contamination and/or experimental error of the assays.

Conclusions & Future Directions

- Both attempts to produce PBLP and to identify PBLP's substrate were inconclusive.
- Addition of protease inhibitors showed to aid in protein expression in the WT PBLP but not the knockout strain.
- Additional refinement and investigation of the PBLP production methodology is needed, given the lack of viable success.
- Experimentation with alternate truncated strains.

Acknowledgements & References

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