Expression and purification of the novel Thioredoxin-like protein 1 of the malaria parasite *Plasmodium*

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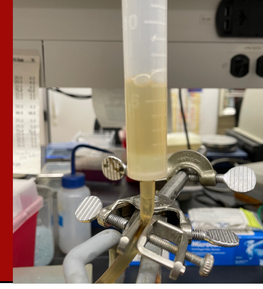

Abstract

As the protozoan parasite *Plasmodium* is transmitted from humans to mosquitoes, it changes its cell morphology between developmental stages as well as its response to changes in its environment. To accomplish this the parasite must reorganize its cytoskeleton. Microtubules in *Plasmodium*, although structurally highly similar to human microtubules, perform highly specialized functions, specifically in generating and maintaining the highly polarized banana shape of the ookinete.

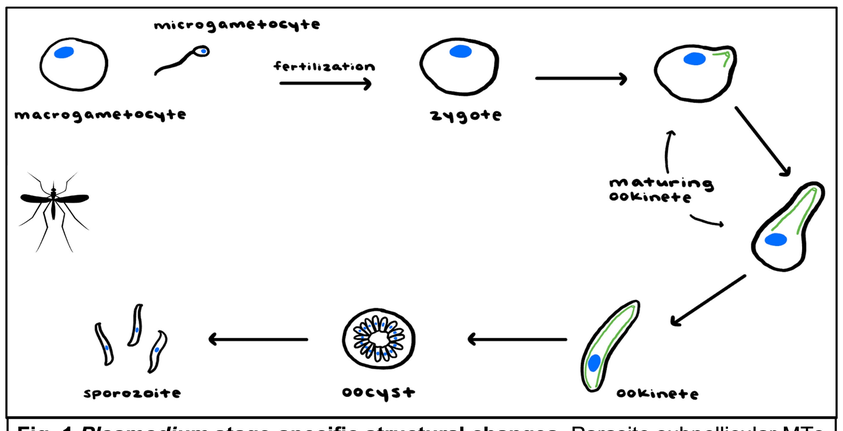

Fig. 1 *Plasmodium* stage-specific structural changes. Parasite subpellicular MTs (green) polymerize and depolymerize due to regulation of MAPS. MT elongation helps the parasite achieve the highly polarized shape of the ookinete.

Specialized functions of microtubules are facilitated by microtubule associated proteins (MAPs). Very little is known about MAPs in *Plasmodium*.

Background Information

• *pbtrxl-1* is a single copy gene that is organized into three exons. The gene spans a length of 1021 bp and is located on chromosome 8 of the *P. berghei* genome. Its open reading frame is comprised of 621 bp, which codes for a 206 AA protein with a calculated molecular weight of 23.4 kDa and an isoelectric point of 7.03.

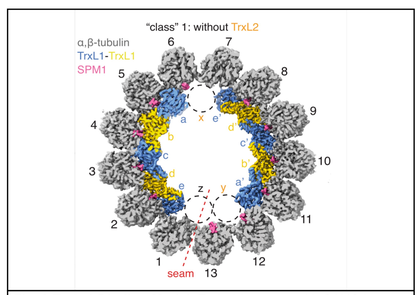

Fig. 2 TrxL-1 Binding Model. TrxL-1 interacts with SAXO1 (SPM1) inside of microtubules in *Toxoplasma* (Wang, 2021).

•Investigations into the TrxL-1 protein of the related parasite Toxoplasma revealed that TrxL-1 does not bind to microtubules but potentially interacts with microtubule-associated proteins (MAPs) TLAP2 and SAXO1 (Liu, 2013).

•Previous work in our lab indicates that TrxL-1 is highly upregulated during the ookinete stage in the mosquito.

•Using the CRISPR system, a TrxL-1KO parasite strain was generated, showing that the gene is not essential for parasite development in mice or mosquitoes. Initial analysis of the KO parasites did, however, detect a delay in parasite development during ookinete development and maturation. Misshapen ookinetes were present in the TrxL-1KO strain (Fig. 3).

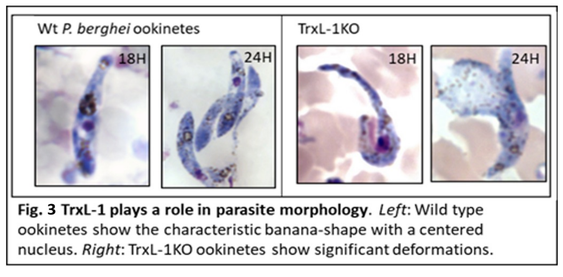

Fig. 3 TrxL-1 plays a role in parasite morphology. *Left*: Wild type ookinetes show the characteristic banana-shape with a centered nucleus. *Right*: TrxL-1KO ookinetes show significant deformations.

•We hypothesize that TrxL-1 may play a role in microtubule regulation in *Plasmodium* ookinetes.

References

Liu J, Wetzel L, Zhang Y, Nagayasu E, Ems-McClung S, Florens L, Hu K. Novel thioredoxin-like proteins are components of a protein complex coating the cortical microtubules *of Toxoplasma gondi*i. Eukaryot Cell. 2013 Dec;12(12):1588-99. doi: 10.1128/EC.00082-13. Epub 2013 Jul 19. PMID: 23873863; PMCID: PMC3889574.

Wang X, Fu Y, Beatty WL, Ma M, Brown A, Sibley LD, Zhang R. Cryo-EM structure of cortical microtubules from human parasite *Toxoplasma gondii* identifies their microtubule inner proteins. Nat Commun. 2021 May 24;12(1):3065. doi: 10.1038/s41467-021-23351-1.

Acknowledgements

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Methods

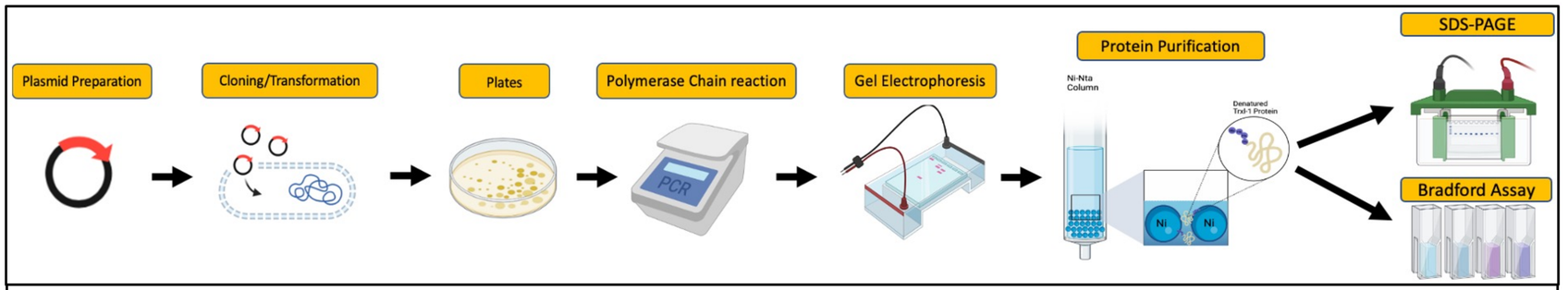

Fig. 4 Cloning and Purification Process. Flowchart indicates steps of expressing and purifying TrxL-1. **Plasmid Preparation)** Prepared plasmid from DH5α cells. **Transformation)** Transformed M15 cells with plasmid from plasmid preparation. **Plates)** Bacteria was plated with ampicillin and kanamycin to select for transformed bacteria. **PCR and Gel Electrophoresis)** Test to confirm presence of plasmid in M15 cells. **Protein purification)** Expression and isolation of TrxL-1. **SDS-PAGE)** Analytical confirmation of purified TrxL-1. **Bradford Assay)** Quantification of purified TrxL-1.

Results

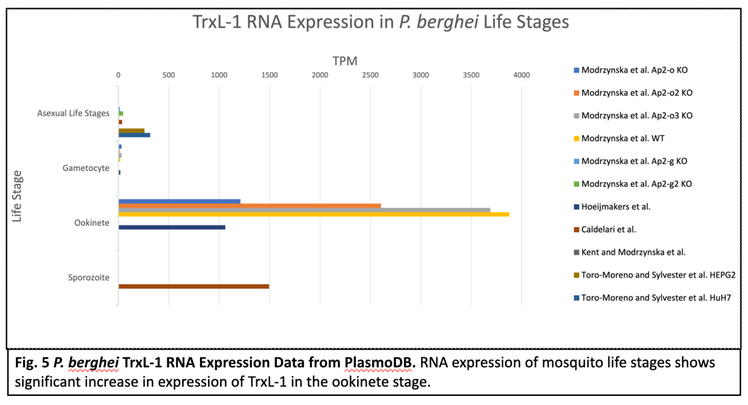

Fig. 5 *P. berghei* TrxL-1 RNA Expression Data from PlasmoDB. RNA expression of mosquito life stages shows significant increase in expression of TrxL-1 in the ookinete stage.

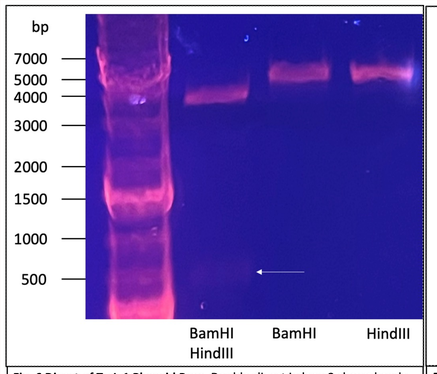

Fig. 6 Digest of TrxL-1 Plasmid Prep. Double-digest in lane 2 shows bands corresponding to pQE30 vector and TrxL-1 insert.

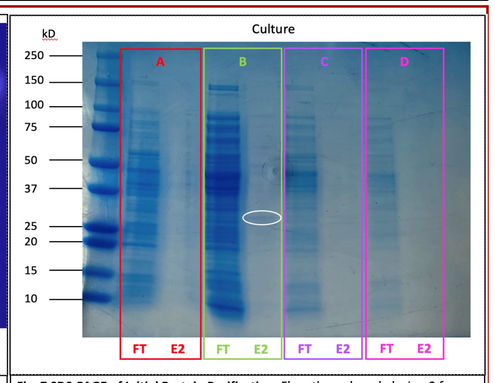

Fig. 7 SDS-PAGE of Initial Protein Purification. Flow-through and elusion 2 for Cultures A-D are shown. The band of interest around 27 kD is circled.

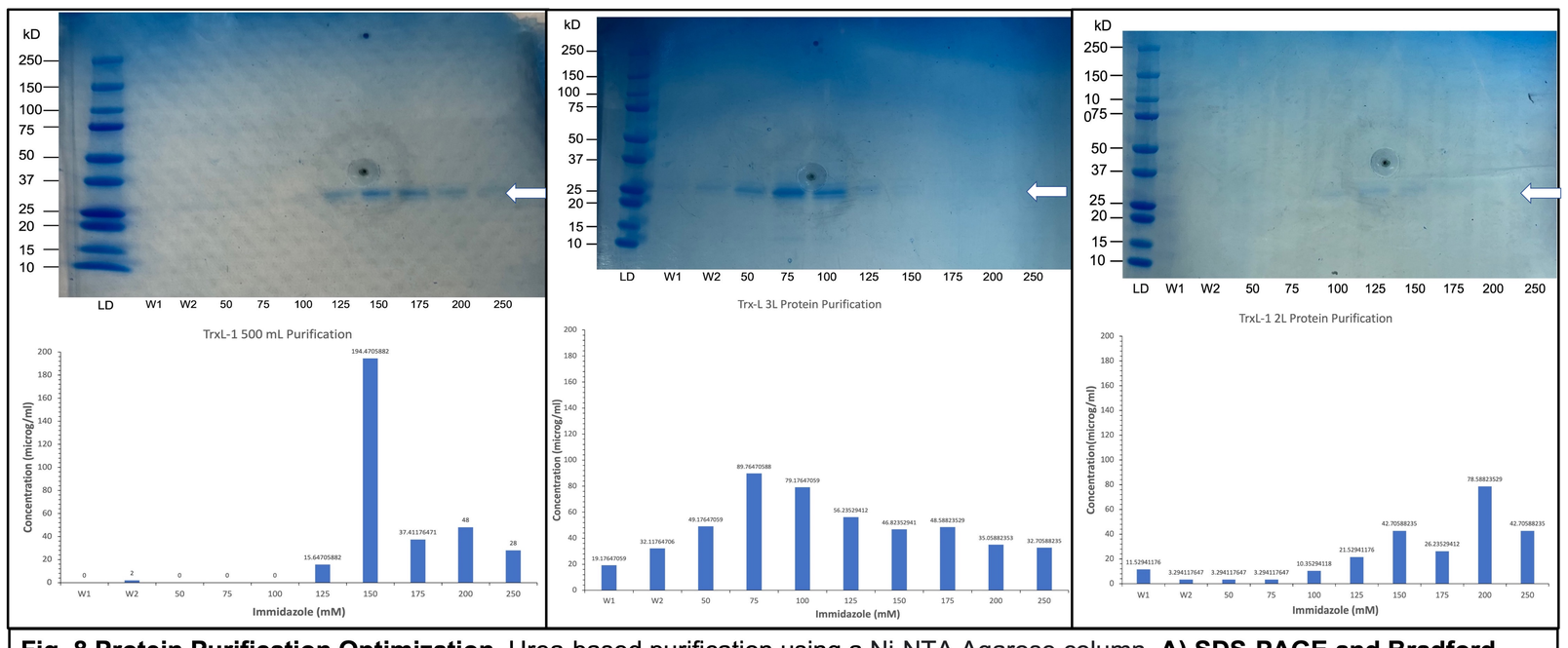

Fig. 8 Protein Purification Optimization. Urea-based purification using a Ni-NTA Agarose column. **A) SDS-PAGE and Bradford Assay for Purification A.** A 500 mL culture with a 0.5 mL column was used. Cells were incubated with PMSF, DNAase, and lysozyme for 1 hour. **B) SDS-PAGE and Bradford Assay for Purification B.** A 3 L culture with a 2 mL column was used. **C) SDS-PAGE and Bradford Assay for Purification C.** A 2 L culture with a 1 mL column was used. Sonication time was increased to 1-minute rounds.

Summary

- Data base analysis shows upregulation of TrxL-1 in the ookinete stage
- Cloning and transformation of pQE30 TrxL-1 into M15 expression cells
- Successful expression and purification of TrxL-1
- Optimization of TrxL-1 purification scheme

Ongoing

- •Send out purified TrxL-1 for antibody production
- •Perform qPCR to analyze expression of TrxL-1 in mosquito life stages
- •Visualize parasite development in wild type and KO parasites using x methods
- •Immunofluorescence assay to visualize microtubules of wild type and KO parasites