Expression and Purification of *Drosophila* Phosducin-like Protein-3

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Abstract

Our lab has demonstrated that PhLP-3 is redox active. We already confirmed that PhLP-3 homologues are present in evolutionary model organisms and possess enzymatic activity. In collaboration with Dr. Mierisch's lab, I have been working on expressing and purifying the PhLP-3 protein in *Drosophila*. I hypothesize that DmPhLP-3 (also referred to as CG4511) plays a significant role in the development of germ line cells. I performed a spin purification of DmPhLP-3 to ensure that the protein was being expressed. After a successful spin purification, I performed a large-scale stepwise purification using a gravity column. I successfully cloned, expressed, and purified truncated version of *Drosophila* PhLP-3 (DmPhLP-3 ΔH) in the stepwise purification. Afterwards, I focused on calculating the concentrations of DmPhLP-3 ΔH.

Introduction

Phosducin-like proteins (PhLPs) are highly conserved in eukaryotes from protozoa to humans, yet their biological function is not well understood. The PhLP family of proteins has been divided into three subfamilies due to an increasing number of PhLPs that are being identified in diverse organisms. The PhLP-1 subfamily is related to canonical phosducins identified in retina rod photoreceptor cells where they play a role in G-protein signaling. The PhLP-2 and PhLP-3 subfamilies are believed to play roles as co-chaperones in protein folding by formation of a ternary complex with the *chaperonin-containing t-complex polypeptide 1* (CCT). PhLP-3 is classified as a thioredoxin-like protein (Tlp) or thioredoxin-domain containing (TXNDC) protein, that research suggests plays a role in the process of protein folding of the cytoskeletal proteins: actin and tubulin. The thioredoxin (Trx) domain, also referred to as the Trx fold, is a compact 3D structure consisting of a twisted beta sheet sandwiched by alpha helices. Some Trxs and Tlps demonstrate thiol-based redox activity facilitated by [-CXXC-] active site motif found within the Trx-domain. PhLPs possess a Trx-domain that expresses the same structural composition. Our lab has recently demonstrated that PhLPs are redox active even though they do not contain the discernible [-CXXC-] active site motif of classical thioredoxins. PhLP-3 exhibits thiol-based redox activity carried out by a single cysteine in their respective active sites. Our lab has recently demonstrated that PhLPs are redox active even though they do not contain the discernible [-CXXC-] active site motif of classical thioredoxins. PhLP-3 exhibits thiol-based redox activity carried out by a single cysteine in their respective active sites. We have already confirmed that PhLP-3 homologues of evolutionary model organisms, such as round worms (*Caenorhabditis elegans*), fruit flies (*Drosophila*), zebrafish (*Danio*) and humans (*Homo sapiens*), also possess enzymatic activity (Sweeney et al., unpublished). The putative active site seems to be highly conserved in various species.

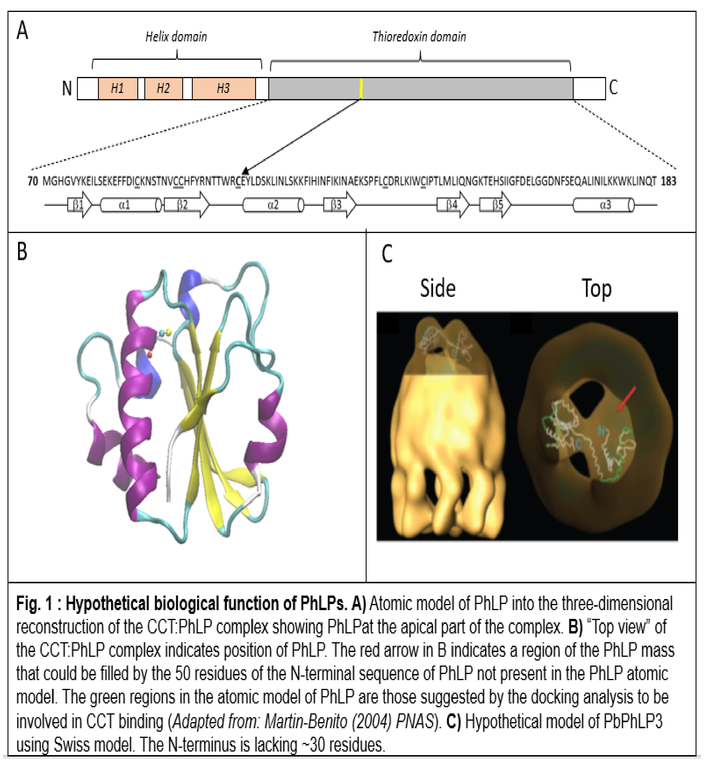
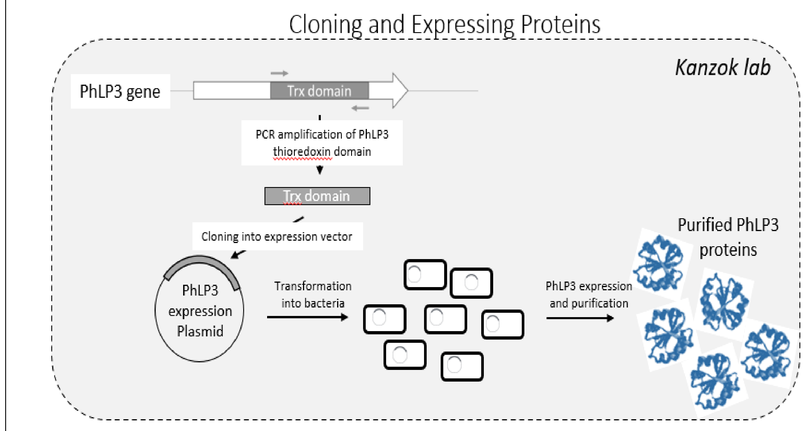


Fig. 1 : Hypothetical biological function of PhLPs. A) Atomic model of PhLP into the three-dimensional reconstruction of the CCT:PhLP complex showing PhLPat the apical part of the complex. **B)** "Top view" of the CCT:PhLP complex indicates position of PhLP. The red arrow in B indicates a region of the PhLP mass that could be filled by the 50 residues of the N-terminal sequence of PhLP not present in the PhLP atomic model. The green regions in the atomic model of PhLP are those suggested by the docking analysis to be involved in CCT binding (*Adapted from: Martin-Benito (2004) PNAS*). **C)** Hypothetical model of PbPhLP3 using Swiss model. The N-terminus is lacking ~30 residues.

Methods



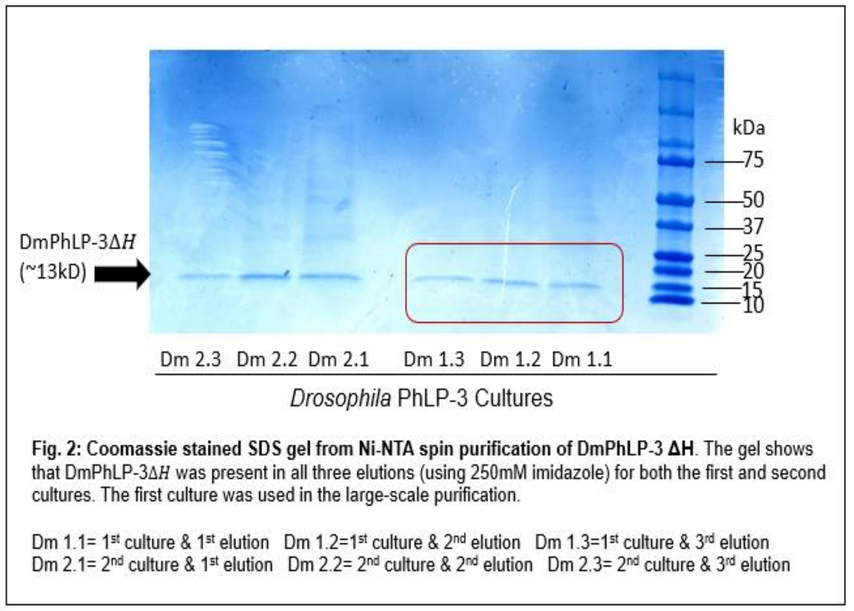
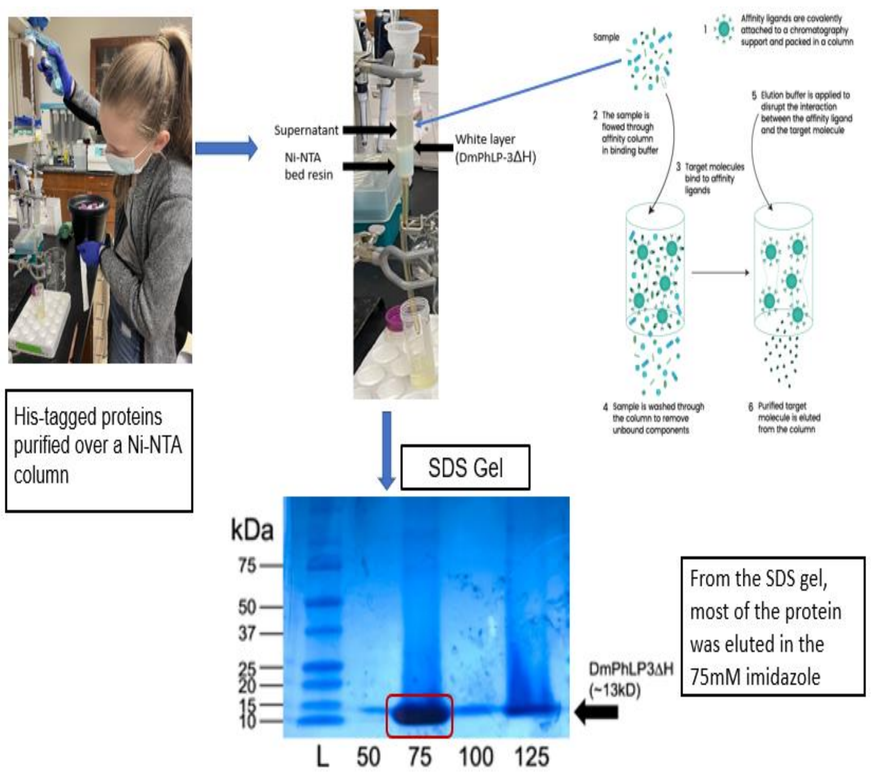


Fig. 2: Coomassie stained SDS gel from Ni-NTA spin purification of DmPhLP-3 ΔH. The gel shows that DmPhLP-3Δ*H* was present in all three elutions (using 250mM imidazole) for both the first and second cultures. The first culture was used in the large-scale purification.

Dm 1.1= 1st culture & 1st elution Dm 1.2=1st culture & 2nd elution Dm 1.3=1st culture & 3rd elution
Dm 2.1= 2nd culture & 1st elution Dm 2.2= 2nd culture & 2nd elution Dm 2.3= 2nd culture & 3rd elution



Results

- Successfully cloned, expressed, and purified the DmPhLP-3ΔH
- Calculated the concentrations of DmPhLP-3ΔH in the stepwise purification

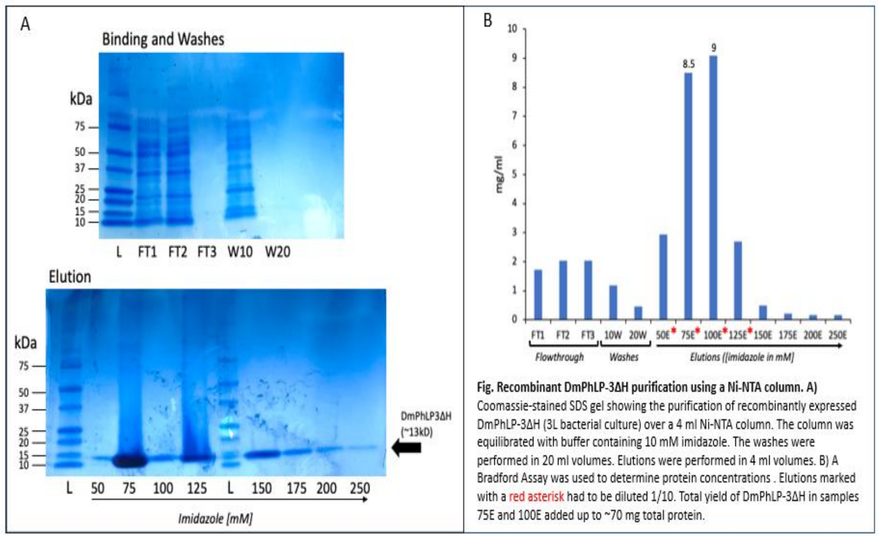


Fig. Recombinant DmPhLP-3ΔH purification using a Ni-NTA column. A) Coomassie-stained SDS gel showing the purification of recombinantly expressed DmPhLP-3ΔH (3L bacterial culture) over a 4 ml Ni-NTA column. The column was equilibrated with buffer containing 10 mM imidazole. The washes were performed in 20 ml volumes. Elutions were performed in 4 ml volumes. B) A Bradford Assay was used to determine protein concentrations . Elutions marked with a red asterisk had to be diluted 1/10. Total yield of DmPhLP-3ΔH in samples 75E and 100E added up to ~70 mg total protein.

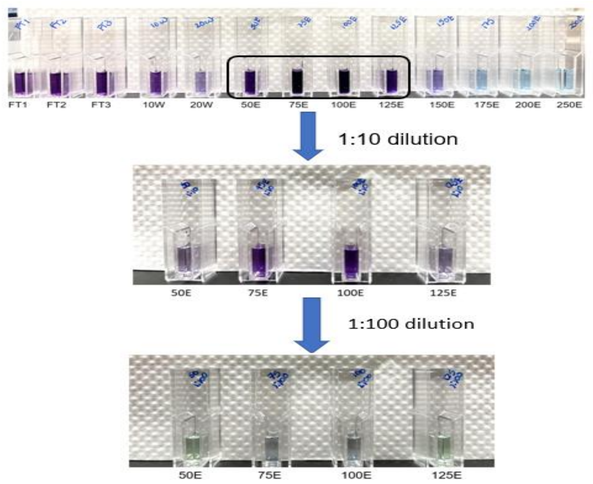
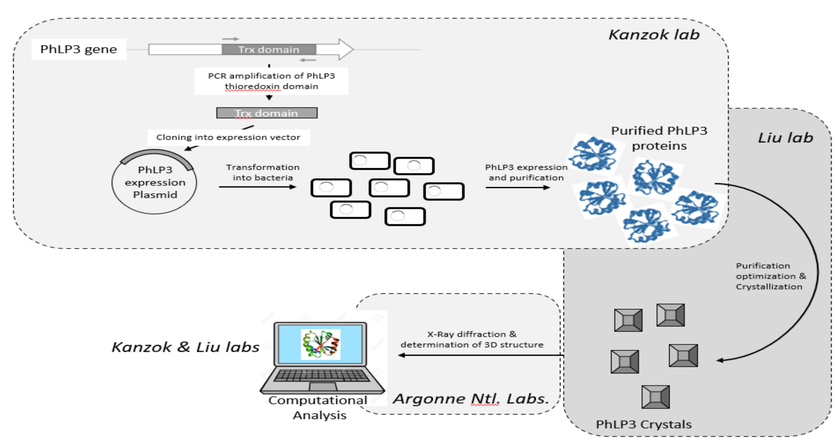


Fig. 4: Bradford Protein Assay Determination of DmPhLP-3 ΔH concentration. The darker the purple hue, the more protein present (B= blank, FT= flow through, W= wash, E= elution).

Next Steps

- Optimize buffer solution
- X-ray crystallography in Dr. Liu's lab
- DmPhLP-3 antibody production



Acknowledgments

I would like to thank LUROP for granting me the Mulcahy Scholarship and the opportunity to pursue research. I would also like to thank my lab members who showed me their support during my time in the lab. I would also like to thank Dr. Mierisch for allowing our labs to collaborate on this project.