Expression and Purification of Glycosomal Proteins from *Trypanosoma brucei*

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BACKGROUND

- *Trypanosoma brucei* is kinetoplastid parasite
- Causes the disease "African Sleeping Sickness"
- Endemic to multiple regions of Africa
- Have specialized peroxisomes, called glycosomes that hold glycolytic enzymes
- Proteins called PEX 13 and PEX 14 make docking complex on glycosomes
- Two subtypes of 13- 13.1 and 13.2, both have YG rich region on N-terminal and 13.1 has SH3 rich region on C-terminal
- PEX 5 and PEX 7 transport proteins to docking complex

EXPERIMENT

Goals:

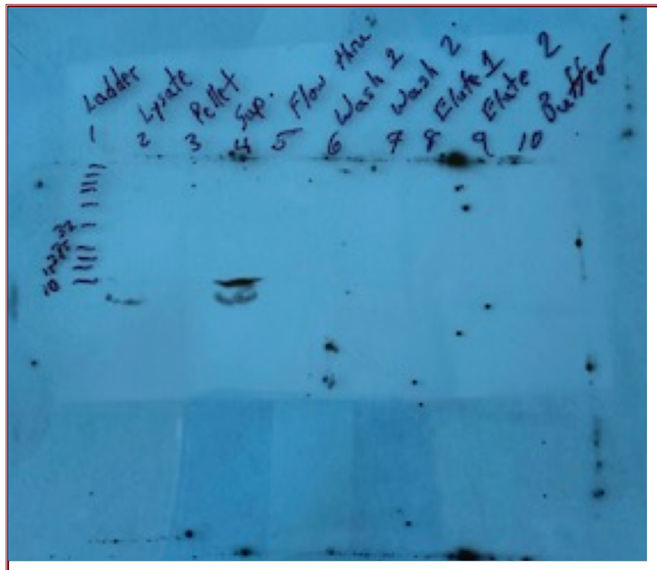
1. Gain expression of proteins 13.1 YG, 13.2 YG, and 13.1 SH3
2. Purify these proteins to allow for in vitro binding assays

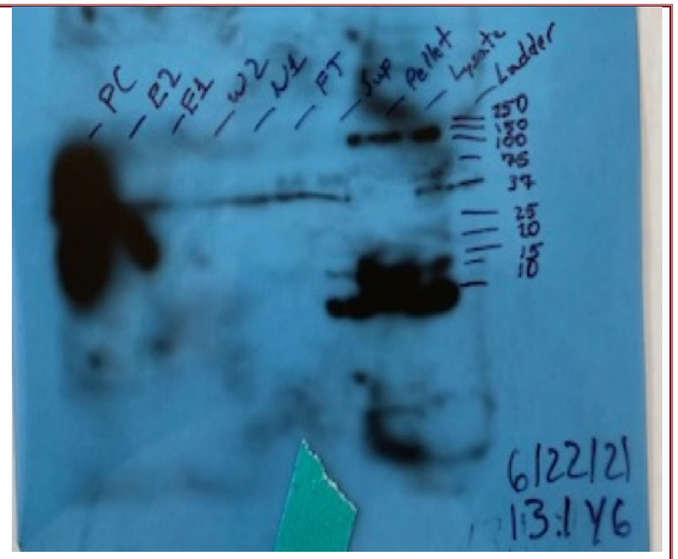
Methods:

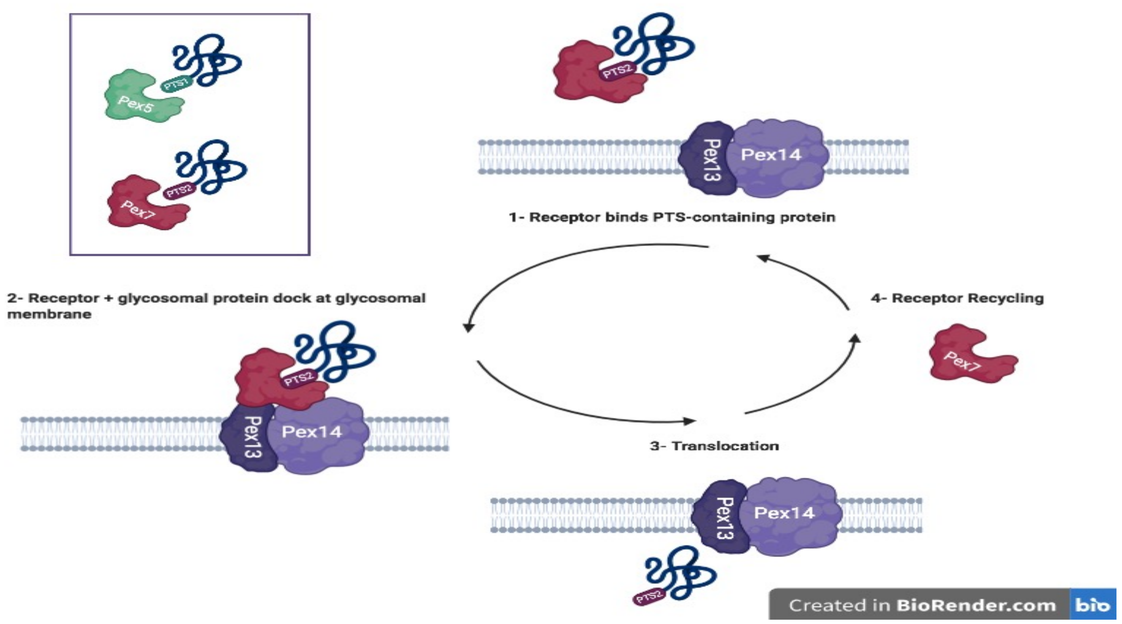
- Culture M15 *E. coli* cells with PQE30 plasmids
- Induce them to produce our target proteins
- Lyse the cells, wash them, and elute them with urea buffers
- SDS-Page analysis
- Western-blots and Coomassies

RESULTS

- Thought initially we were successful in purifying 13.1 YG
- Upon further trials, had inconclusive results with purifying 13.1 YG along with 13.2 YG and 13.1 SH3
- Found protein in 13.1 YG in the lysate, pellet, and supernatant, but not in any of the washes or eluates as we wanted to

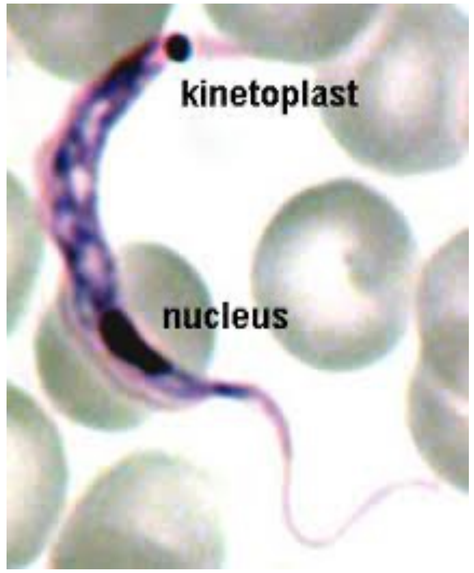






CONCLUSIONS/NEXT STEPS

- Inconclusive results so far
- Possible issue with cell line of *E. coli* (M15) we were using
- Transform in BL21 *E. coli* cells
- Glycerol stocks of bacteria with our plasmid DNA after successful transformation was completed
- Purify with that line of cells and then binding assays following that



Funding:

- National Institue of Health
- Clemson University and EPIC department