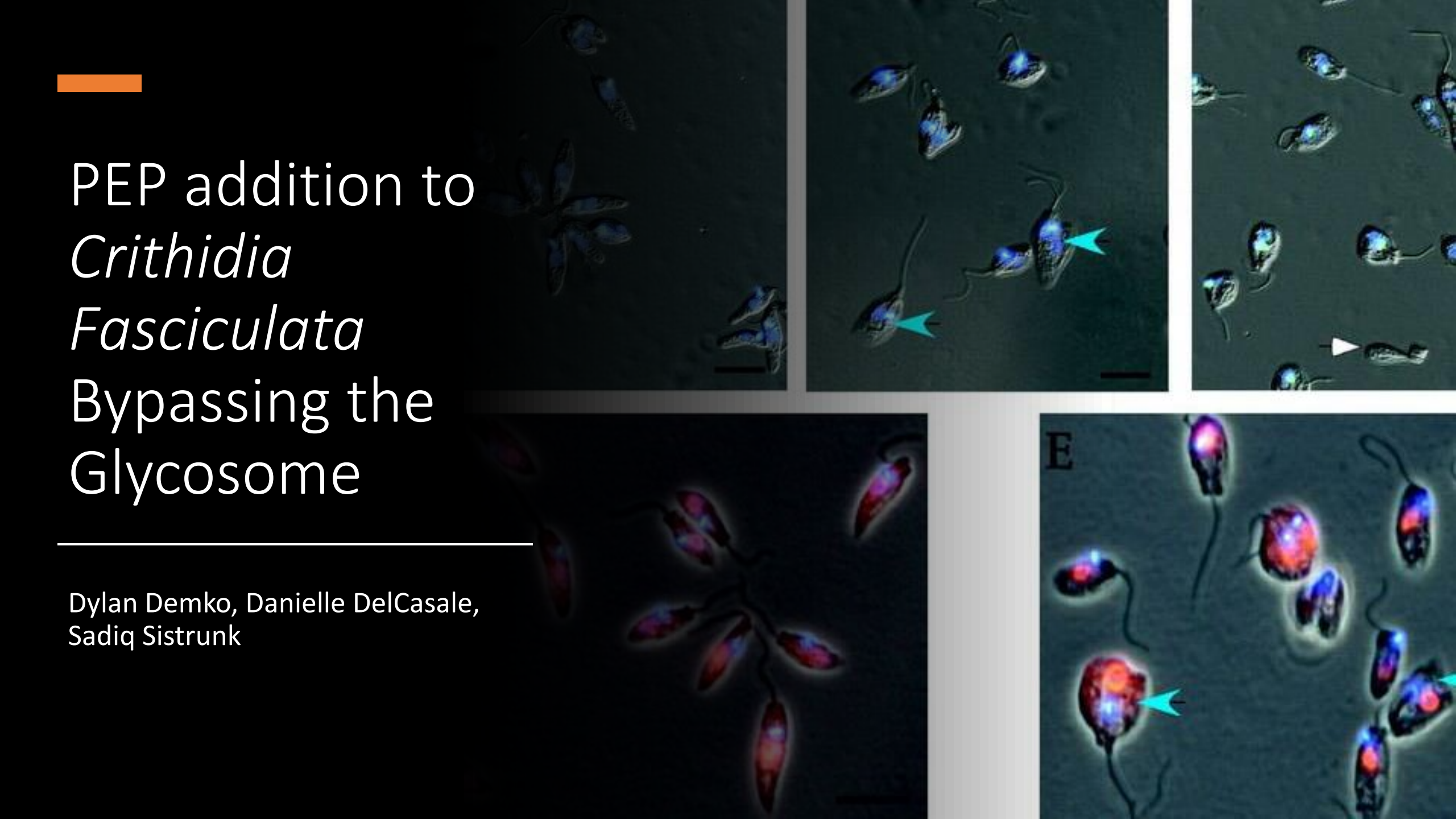
PEP addition to *Crithidia Fasciculata* Bypassing the Glycosome

Dylan Demko, Danielle DelCasale, Sadiq Sistrunk



Glycosomal/Glycolysis Pathways

- The glycosome compartmentalizes glycolysis (metabolic breakdown of glucose)
  - Steps consisting of glucose to 3-phosphoglycerate
- Addition of molecules at the end of the pathway may have a profound effect
  - Factors such as re-entry into the glycosome could play a role
  - PEP is what will be used as well as pyruvate

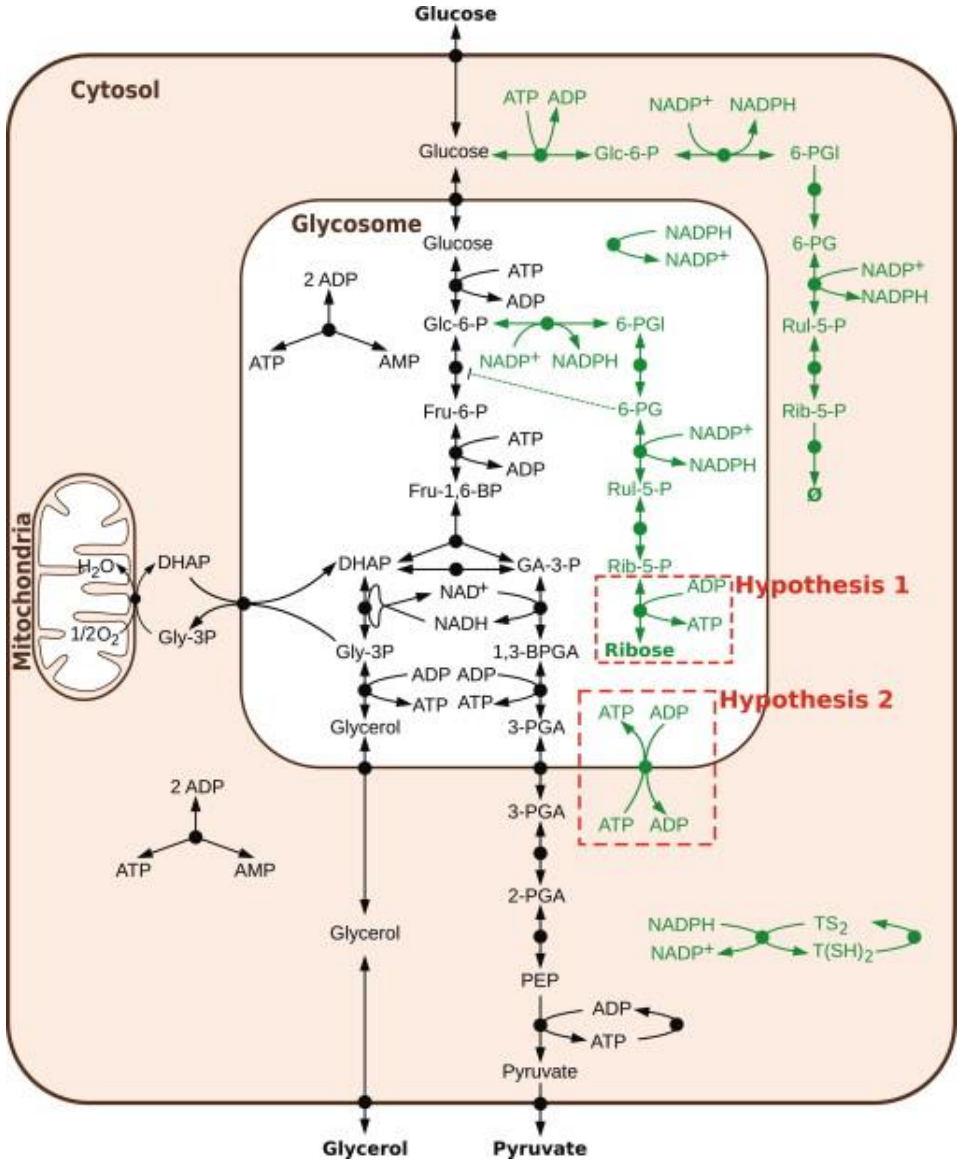
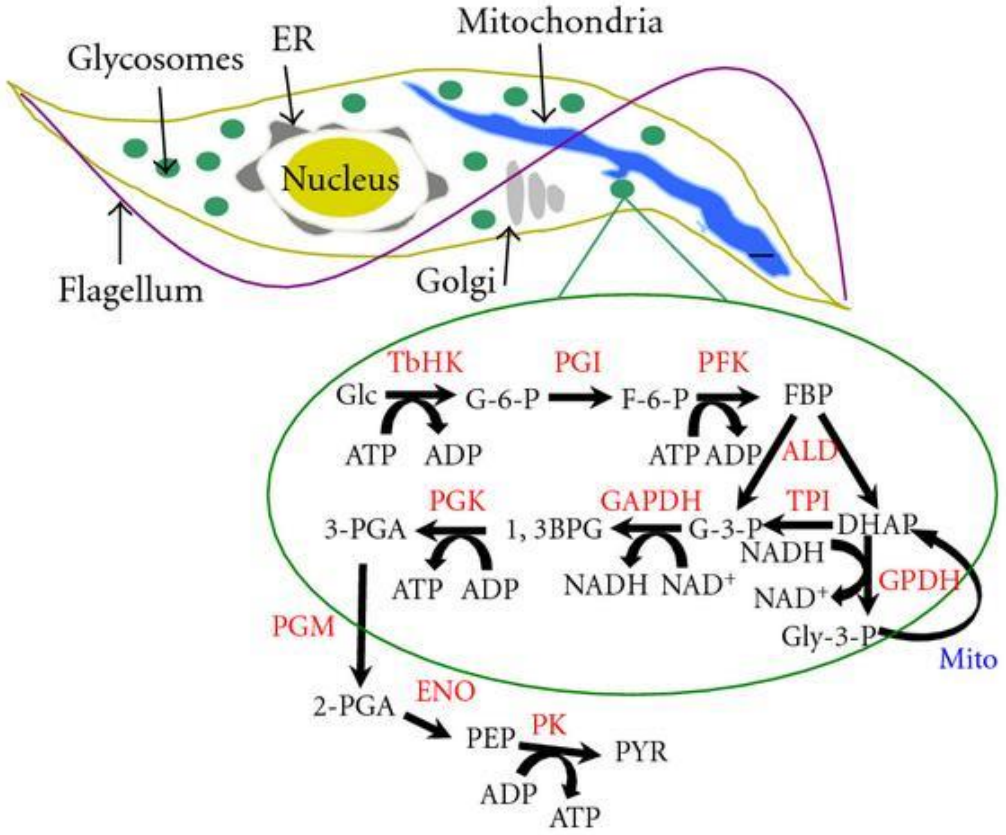


Figure showing how the glycosome bypasses typical glycolysis. Note: Figure is from a research article and area in green isn't of interest.

Hypothesis

- The reactions involving PEP will likely proceed forward
  - Due to the very negative Δ G
  - Excess of PEP pushing the reaction forward
  - May be influenced by re-entry into the glycosome
- Half of the payoff phase of glycolysis doesn't happen
  - *Crithidia fasciculata* depends on glycolysis for its energy
  - Energy consumption phase won't occur as well
- Hypothesis: *Crithidia fasciculata* depend on the glycosome for glycolysis, the addition of PEP would bypass this leaving the parasite energy deprived
- Alternative Hypothesis: The addition of PEP still leaves some ATP to be synthesized. This smaller amount of ATP is enough to sustain the parasites



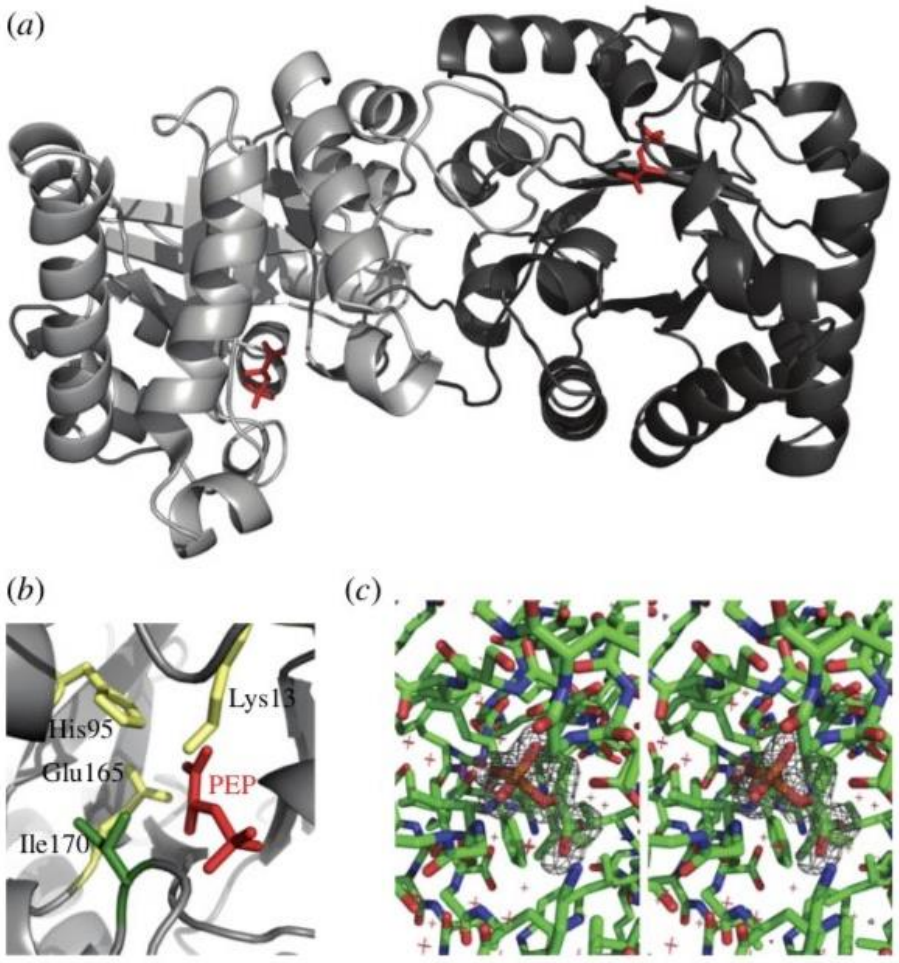


Figure 1. Co-crystal structure of TPI with bound PEP. (*a*) Schematic of the TPI–PEP crystallographic structure. PEP locates in the active centre of both subunits in the asymmetric TPI dimer. (*b*) The catalytic pocket of TPI bound to PEP. Catalytic residues are highlighted in yellow, PEP in red, isoleucine 170 in green. (*c*) Stereoscopic illustration of the PEP binding site environment including a difference map in which PEP has been removed from the model and was refined against the experimental data for five cycles. The map has been contoured at 4 s.d. and reveals positive density for the missing ligand.

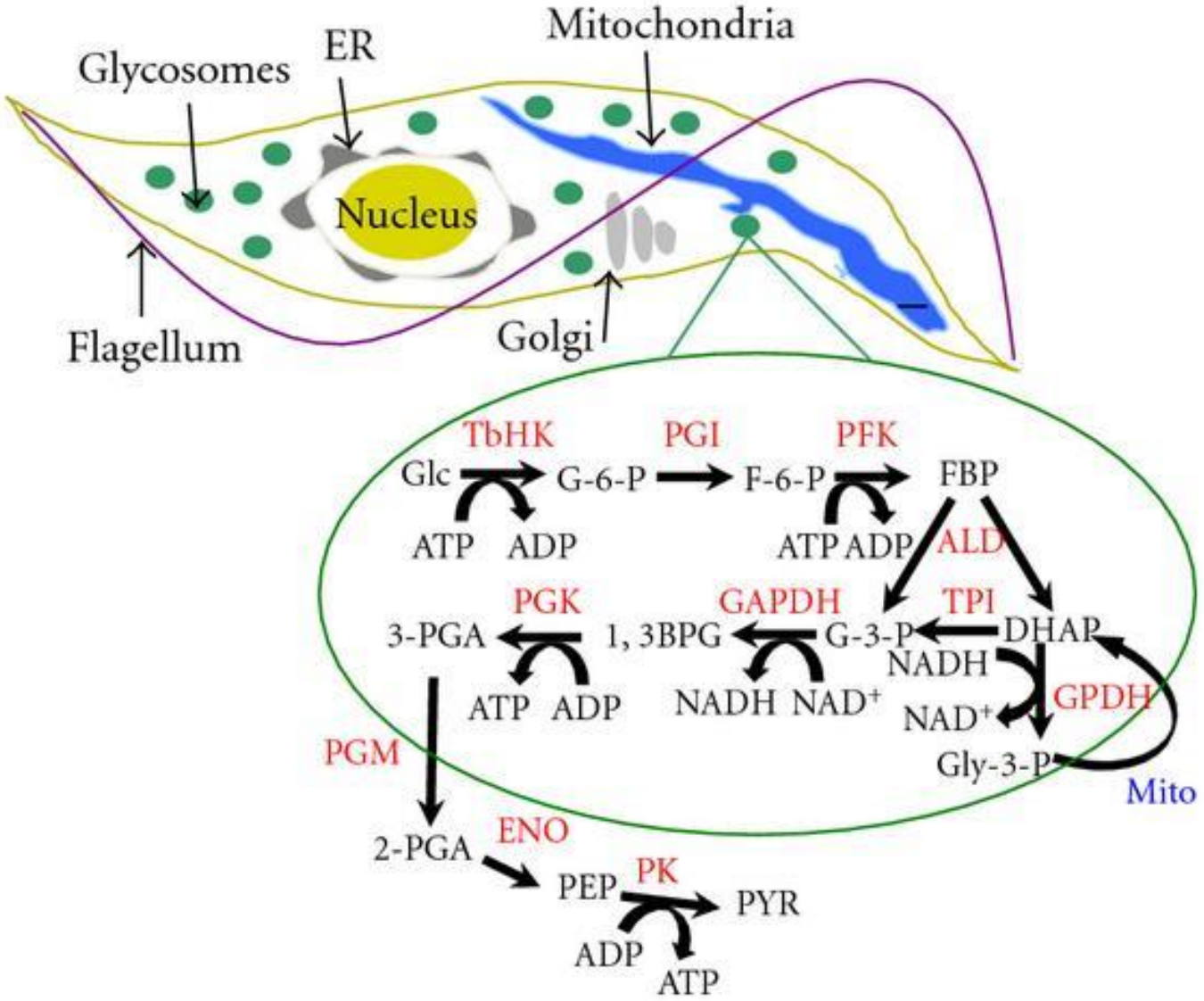
https://royalsocietypublishing.org/doi/pdf/10.1098/rsob.130232

Methodology: Glucose (GO) Assay

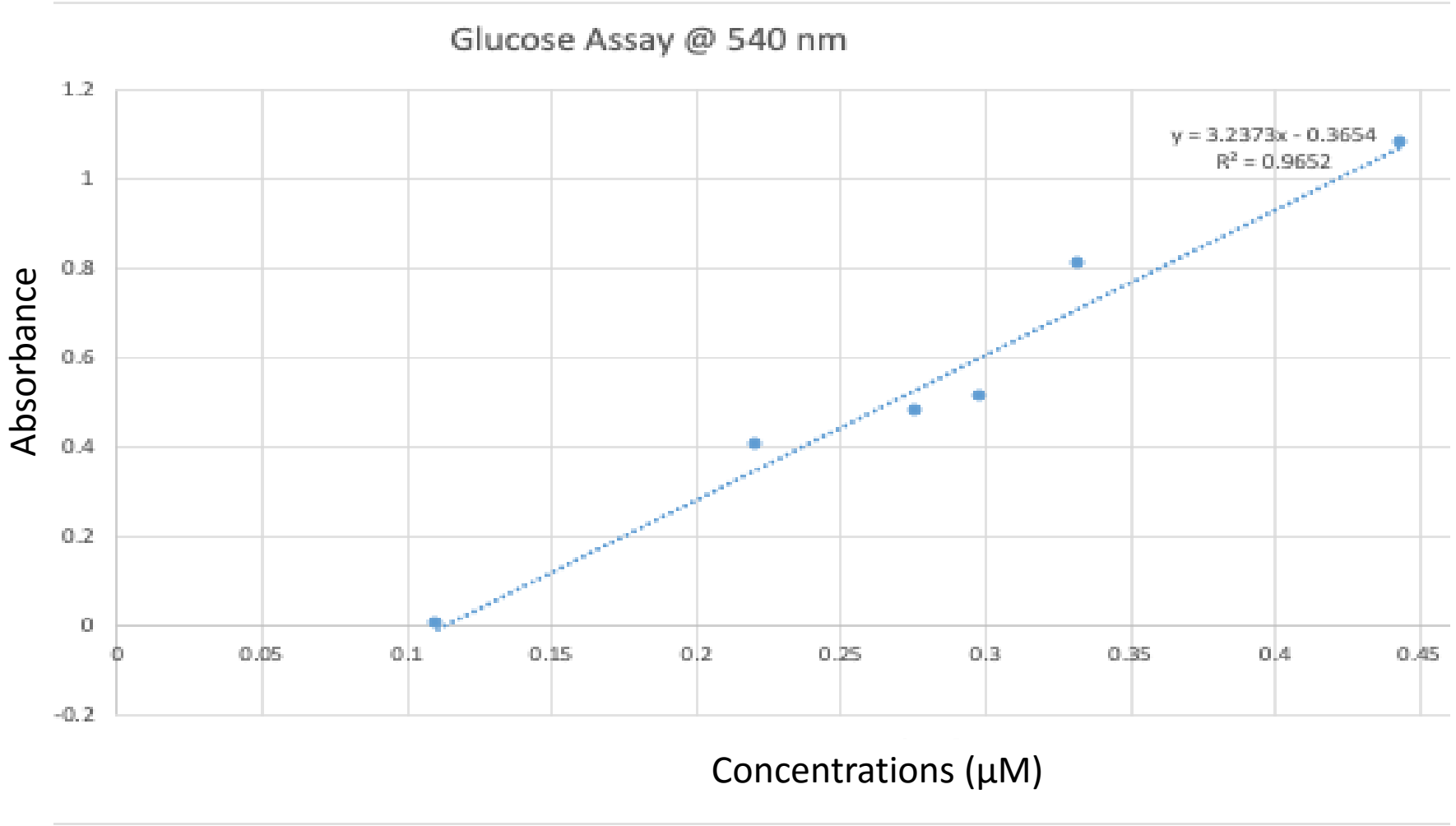
- A GO assay will be performed to measure the concentration after the addition of PEP
  - Prediction: PEP functions as a competitive inhibitor and will decrease glucose concentration with an increase in PEP
- Growth curves showing the concentration and presence of PEP after bypassing the glycosome

Protocol

- Control: *Crithidia fasciculata* cells with no PEP or Pyruvate added
- Experimental Variable: PEP, Pyruvate, and glucose are added to *Crithidia fasciculata* in increments
- Concentration of PEP or others that will be used: 1 µM, 25 µM and 50 µM
- Perform GO Assay to determine glucose activity after the addition of PEP and pyruvate
- Perform growth curves to see how the varying additions would affect the conditions of the parasitic cells

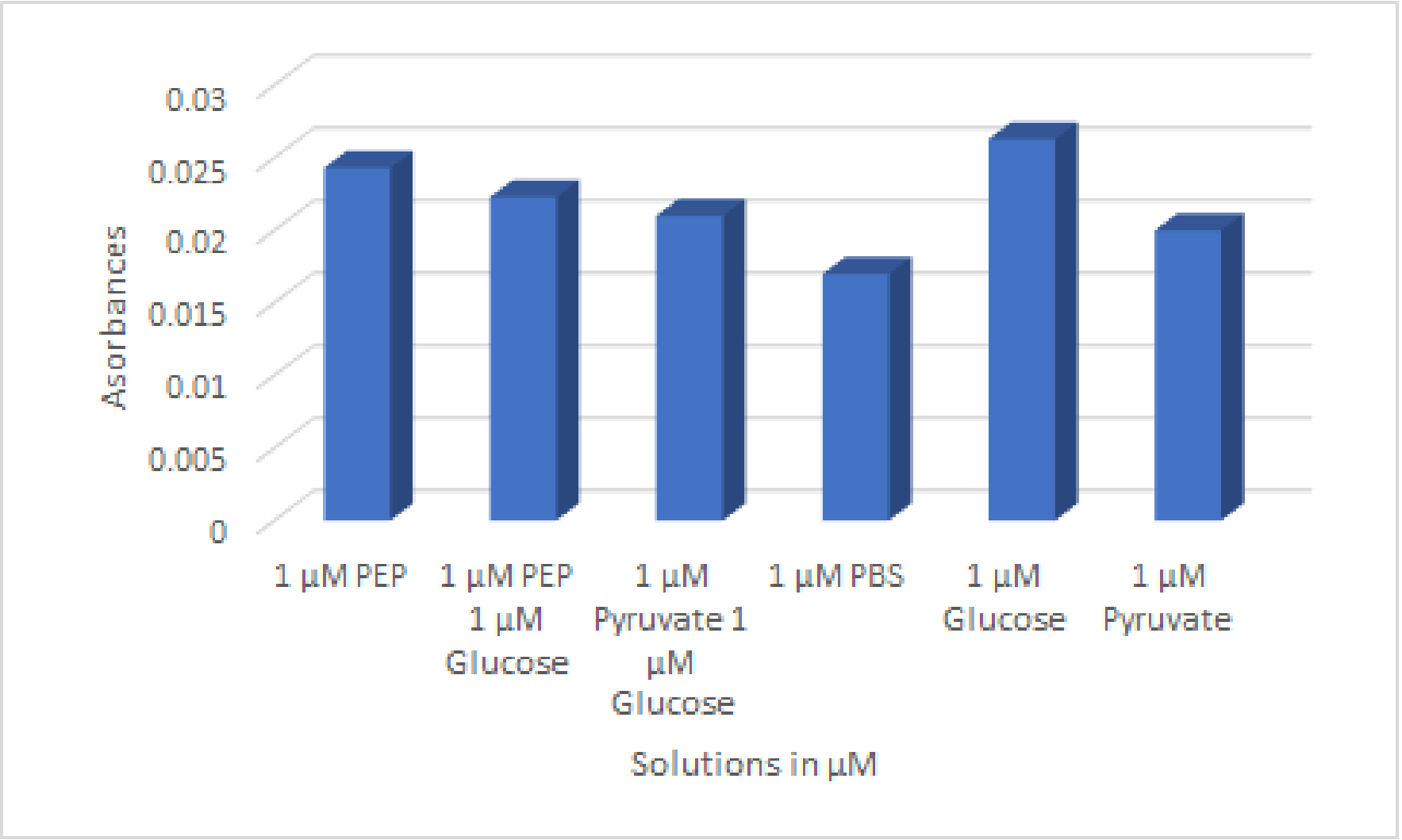


Data From GO Assay



- Performed 2/25/20
- The curve was obtained at 540 nm over 180 minutes at 37 °C

Experimental Data



- Performed 2/25/20 from 4:00 pm to 9:00 am

Conclusion/Future Directions

- Further results are delayed due to the pandemic.
- Further assays are to be performed, with PEP, and PYR additions.
- Growth curves are to be taken with samples in order to see effect of addition on the conditions of cells.
- Ultimately results are inconclusive until further testing can be done.

Literature Cited

Dimaio, John, et al. “The Single Mitochondrion of the Kinetoplastid Parasite Crithidia Fasciculata Is a Dynamic Network.” *Plos One*, vol. 13, no. 12, 2018, doi:10.1371/journal.pone.0202711.

Heiden, M. G. Vander, et al. “Evidence for an Alternative Glycolytic Pathway in Rapidly Proliferating Cells.” *Science*, vol. 329, no. 5998, 2010, pp. 1492–1499., doi:10.1126/science.1188015.