Proposed BCHM 421/422 Project Outline – Project #2

Supervisor: Prof. William Plaxton (plaxton@queensu.ca; lab website: http://post.queensu.ca/~plaxton/)

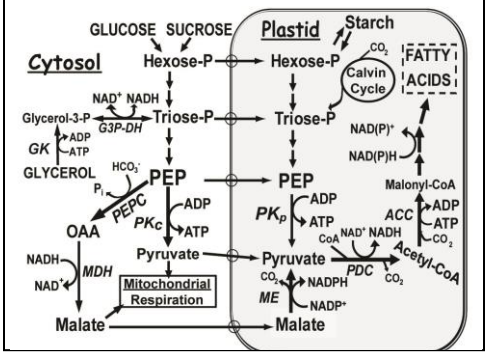
Title: ***Purification and characterization of chloroplast pyruvate kinase from Chlorella vulgaris: a metabolic 'bottleneck' to fatty acid and bio-oil synthesis in green algae***

Key words: green algae, triacylglycerides (bio-oil), allosteric enzyme, metabolic control, global warming

Project Goals:

Purify via FPLC and characterize physical and kinetic/regulatory properties the chloroplast isozyme of pyruvate kinase (**PKp**) from mixotrophic cultures of the model bio-oil accumulating green microalgae *Chlorella vulgaris,* cultivated with glycerol as a secondary carbon source. PKp is a tightly regulated allosteric enzyme of the glycolytic pathway that plays a pivotal role in controlling the production of both ATP and C-skeletons (i.e. pyruvate) needed for plastidic fatty acid and hence triacylglyceride (TAG) synthesis in both vascular plants and green algae (Fig. 1). It is well established that PKp represents a major 'metabolic bottleneck' - that owing to various poorly defined post-translational controls limits the flux of carbohydrates (derived from photosynthetic Calvin cycle, or exogenous C-sources such as glycerol) into fatty acids and hence TAG.

Fig 1. Alternative metabolic routes for production of fatty acids in vascular plants and green algae.



Note: This project is under auspices of an NSERC Strategic Projects grant entitled: "*Bio-oil Recovery & CO_2 Recycling by Waste Stream Enhanced Microalgal Growth & Low Energy CO_2-Related Extraction*" (PI: Pascale Champagne, Queen's Civil Engineering, w/ 5 co-applicants)

Experimental Approaches:

1. Soluble protein extraction from *C. vulgaris* using a French Press.

2. Continuous assays of enzyme (i.e. PK) activity, and protein concentration determination using a microplate spectrophotometer.

3. Enzyme (PKp) purification using a GE Healthcare Fast Protein Liquid Chromatography (FPLC) system (available in Plaxton lab)

4. SDS gel electrophoresis to assess purity of final PKp preparation and to determine its subunit size, coupled with analytical gel filtration FPLC to determine its native molecular mass (and thus quaternary structure).

5. Submit purified PKp to our expert mass spec collaborator (Yi-Min She) for LC MS/MS analysis (i.e., to pinpoint encoding gene, as well as any PTMs such as phosphorylation).

6. Time permitting = PKp kinetic characterization (pH activity profile, PEP and ADP saturation kinetics, screen and characterize allosteric effectors = activatory or inhibitory metabolites. Also, examine kinetic impact of any PTMs (detected in step #5) on PKp function.

Note: A research stipend may be available for an enthusiastic BCHM student who is keen to work full time with in our lab over the upcoming summer, prior to initiating a BCHM421 project in the fall.