Laboratory: Isabel Abreu's Laboratory (PRPlants) at ITQB-NOVA. At PRPlants we are interested in how protein function is regulated and how it impacts the cell metabolism. We study important cellular processes, using plants as models, such as growth regulation, photosynthesis and abiotic stress response.

Title:

Quantification of protein levels of PEPC protein kinase

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Project description:

The enzyme phosphoenolpyruvate carboxilase (PEPC) catalyzes carbon fixation, as bicarbonate, in a pyruvate molecule to produce oxaloacetate. The enzyme is activated by the phosphorylation of a serine residue at the N-terminal of the protein. This is the best well known regulatory process involved in the control of PEPC activity. The kinase that phosphorylates PEPC, the PEPC protein kinase, is known but it is very low abundant, making its quantification, so far, impossible. Thus PEPC protein kinase levels are always inferred from the levels of the transcripts resulting from its gene. But, it is well know that transcript and protein levels usually do not correlate, due to the intricate regulation of post-transcriptional processes and protein transduction.

In this project, we aim to develop a mass-spectrometry (MS) method that will allow the quantification of PEPC protein kinase, for the first time, and to characterize PEPC protein kinase variation with maize photoperiod and its dependence on light, since PEPC phosphorylation only occurs in the first light hours of the day.

We will use maize leaves to prepare protein extracts. We will do tissue and cellular fractionation, since c4-maize photosynthetic PEPC is only present in the chloroplasts of mesophyll cells, to enrich protein extracts in PEPC protein kinase and will test for the presence of this protein by MS. Samples will also be collected in several time points during the 24h day/night regime, to determine protein variation. In parallel, we will clone PEPC protein kinase gene in a bacterial expression vector, and produce recombinant protein in *E. coli*, by affinity purification. The recombinant protein will be used in multiple-reaction monitoring (MRM) to set the basis for PEPC protein kinase quantification.

In the end the student will have done, cloning, cell transformation, recombinant protein production and purification, SDS-PAGE, tissue and cellular fractionations, maize protein extracts, and MS. Other common techniques done at the lab may also be used, when needed.