Master 2 Internship
Laboratory BIP
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Photosynthesis regulation: effect of supramolecular organisation on enzymatic regulation in the green microalgae *Chlamydomonas reinhardtii*.

The assimilation of CO_2 by photosynthetic organisms thanks to the Calvin Benson Bassham (CBB) cycle is largely responsible for global primary productivity of organic compounds and its efficient functioning requires it to be regulated to cope with varying environmental conditions. The molecular mechanisms responsible for the dark/light regulation of CBB cycle are well described, and include redox reactions, metabolites concentrations and pH transition in the chloroplast. Protein-protein interactions, involving an intrinsically disordered protein, CP12, and two enzymes, the phosphoribulokinase (PRK) and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), also regulate the CBB cycle [1,2]. To study the origin of this regulation, we have used the model green alga *Chlamydomonas reinhardtii* and describe the molecular transitions (redox potential, structural transition) of CP12 [3]. Nevertheless, in its physiological environment inside the chloroplast, the native CP12 protein is expected to encounter a highly complex physico-chemical environment (Figure). The effect of high molecular crowding and supramolecular organisation on enzymatic regulation remains enigmatic. The student recruited on this project will dedicate her/his internship on developing new methodologies to tackle this question.

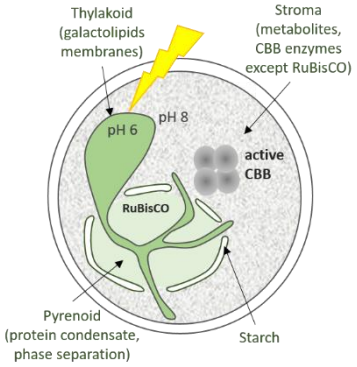

Figure: Schematic of a chloroplast.

Recombinant CP12 protein will be produced in *Escherichia coli* and purified in high amount (currently performed in the laboratory) and its structural properties will be investigated using NMR in complex environments such as Natural Deep Eutectic Solvent (NaDES)[1], liposomes[2], thylakoids and isolated chloroplasts from *C. reinhardtii* [3].

[1] Known NaDES will be prepared using amino acid, sugars and organic acids.

[2] Liposomes of the main lipids found in photosynthetic membranes, galactolipids, will be designed and characterised using well-established protocols in the group.

[3] Chloroplasts and thylakoid membranes will be purified using standard protocols.

Most experiments will be prepared *in vitro* and present a high feasibility. However, the most challenging part of this internship will be to incorporate CP12 within the algal chloroplast.

This internship will start in January/February 2023 and could be followed by a PhD thesis.

1. Gérard, C. et al. A Trajectory of Discovery: Metabolic Regulation by the Conditionally Disordered Chloroplast Protein, CP12. *Biomolecules* **2022**, *12*, 1047, doi:10.3390/biom12081047.
2. Gérard, C. et al. Reduction in Phosphoribulokinase Amount and Re-Routing Metabolism in *Chlamydomonas Reinhardtii* CP12 Mutants. *International Journal of Molecular Sciences* **2022**, *23*, 2710, doi:10.3390/ijms23052710.
3. Launay, H. et al. Flexibility of Oxidized and Reduced States of the Chloroplast Regulatory Protein CP12 in Isolation and in Cell Extracts. *Biomolecules* **2021**, *11*, 701, doi:10.3390/biom11050701.