M2 internship position

Post-translational modifications of photosynthetic enzymes

Context: Global population growth demands the creation of new resources or the improvement of existing ones. Since photosynthesis is used for many of our resources (food, clothing, building materials, biofuels…)[1], it is logical to aim at enhancing it, which starts by fully understanding this machinery. The Calvin-Benson cycle is the process that allows to capture CO_2 and to integrate it in the organism. One enzyme adds CO_2 to a substrate, and 10 enzymes are then used to regenerate the substrate and close the catalytic cycle. Our colleagues at Sorbonne University have applied X-ray crystallography to determine the structures of all these 11 enzymes from *Chlamydomonas reinhardtii*. During this journey, a striking observation was made: **when some native enzymes extracted from chloroplasts were purified, they were found to elute (with size-exclusion chromatography) at high molecular masses, whereas their purified recombinant counterparts eluted at masses corresponding to monomers**[2]. This means that the organism is capable of modifying enzymes in such a way that dimers or tetramers become the most stable structures[3].

Objectives: We surmise that the entry into a supramolecular assembly is conditioned by post-translational modifications (PTM), such as the phosphorylations of serines and threonines or the reversible oxidations of cysteines. Several PTM may also add up to alter the conformation of structural motives into local disorder. Thus, conformational switching could trap enzymes into states of increased affinities for partners, building up assemblies to potentiate catalysis. Putative sites of PTM have already been identified, and **our goals in this project are (1) to rank these sites to identify the most probable ones, (2) to investigate changes of structures after PTM.**

Project: We will perform molecular dynamics (MD) simulations to compute the relative stability of an enzyme phosphorylated at different sites. We will focus on two enzymes from the Calvin-Benson cycle (phosphoglycerate kinase and sedoheptulose biphosphatase) for which 5 and 7 sites were respectively identified. Using enhanced sampling simulations, we will identify new conformations and compute the melting temperature for each system, which will then be used to compare their stabilities[4]. Should this approach fail, we will rely on alchemical transformations to compare the stabilities of different systems and rank the sites. Once new structures are identified, we will perform classical MD simulations to compare the flexibility and dynamics of mobile loops.

Outcomes: Ranking the putative sites of PTM is of prime importance for our experimentalist colleagues, since it will guide them on the choice of mutants that could disturb the formation of multimers. These results will help understanding deeply the structural biology of condensates, and in particular the determinants that guide the formation of complexes and in the end modulate the catalytic potential of enzymes. Our ultimate goal is to reconstitute a functional Calvin-Benson cycle *in vitro*, and then engineer new-to-nature CO_2 capture systems using synthetic biology[5].

Environment: The project will take place in the CPCV laboratory (ENS-PSL, SU, CNRS), hosted by the Ecole Normale Supérieure in the Latin Quarter of Paris. The theoretical chemistry group of CPCV is multidisciplinary in terms of methods and applications, and is widely recognized for its activities. The group welcomes 15 to 20 interns, PhD students, and post-doctoral fellows.

Contact: Interested candidates must contact Nicolas Chéron (nicolas.cheron@ens.psl.eu) as soon as possible to get more information. Applicants must send a CV, a motivation letter, and if possible the name of references.

Extension: Extension of this project into a PhD thesis is possible for motivated applicants (funding already secured through the ANR project CALVINASSEMBLY), upon mutual agreement of the candidate and the supervisor.

Profile: Master in chemistry, biochemistry or biophysics. Knowledge of simulations would be an asset, as well as bash scripting and python analysis.

Keywords: molecular dynamics, enhanced sampling, enzymes, photosynthesis, CO_2.

Numerical tools: Gromacs software, python/bash scripts.

References:

[1] M. P. Johnson, *Essays Biochem.*, Vol. *60*, pp. 255–273, **2016**.

[2] T. Le Moigne, 'Structures et interactions des enzymes du cycle de Calvin-Benson-Bassham', These de doctorat, université Paris-Saclay, 2022.

[3] B. Gontero, G. Mulliert, et al., *Eur. J. Biochem.*, Vol. *217*, pp. 1075–1082, **1993**.

[4] G. Stirnemann and F. Sterpone, *J. Chem. Theory Comput.*, Vol. *11*, pp. 5573–5577, **2015**.

[5] S. Luo, C. Diehl, et al., *Nat. Catal.*, Vol. *6*, pp. 1228–1240, **2023**.