Unveiling the reactive conformation of PEP Carboxylase with molecular dynamics

DESCAMPS Axel;[A] **Rodolphe Vuilleumier**[A] **CHERON Nicolas**[A]

A) UMR PASTEUR 8640 (CNRS-ENS-SU), 24 rue Lhomond, 75005 Paris.

nicolas.cheron @ens.psl.eu

The next time you drink a mojito on the terrace, you'll think of PEPC. Sugar cane and a number of other plants such as maize, sorghum and rye are plants with a photosynthesis mechanism called C4[1]. In these plants, CO2 fixation during the Calvin-Benson cycle is preceded by a CO2 capture step by the enzyme PEP Carboxylase (PEPC); this enzyme catalyses the reaction between bicarbonate (i.e. hydrated CO2) and the substrate PEP (phosphoenolpyruvate). C4 plants have a lower water requirement than other plants, which could prove to be crucial in the context of global warming and water shortages[2]. Our ultimate aim is to understand the growth of these plants by studying the reactivity of the enzyme responsible for carbon capture.
The active site of PEPC is located close to a mobile loop that isolates the active site from the solvent[1]. The opening/closing of this loop therefore plays a crucial role in the reactivity of the enzyme. The open structure of this loop was solved only in 2018 and no closed structure is currently known. In this project, we aimed at answering the question: « is it possible to predict a closed conformation that can be used to understand the catalytic activity of the enzyme? ». To do so, we developed a methodology based on:

1. the analysis of the potential energy of the psi and phi angles during short simulations out of equilibrium;
2. simulations with a modified force field based on results from the previous analysis, followed by enhanced sampling simulations;
3. clustering by a new methods and estimation of the most stable conformation by forcing the reopening of the loop.

This methodology was tested on enzymes for which the open and the closed conformations was known, and we observed a very good agreement between the experimental closed structure and the predicted one. Encouraged by these results, we tested the method on the PEPC to obtain as closed conformation

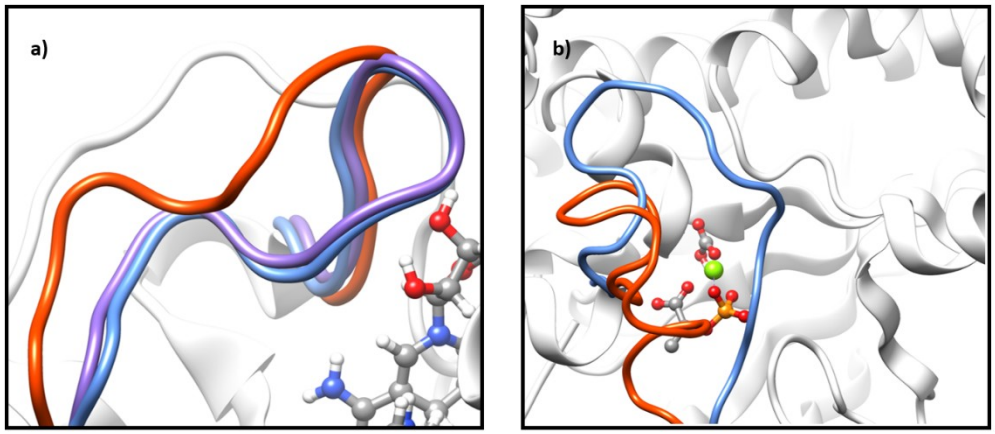

Figure 1. **a)** Superposition of the crystallographic structure of DHFR in orange (open) and Closed (purple) and the predicted (blue). **b)** Superposition of the crystallographic structure of PEPC in orange (open) and the predicited (blue).

Keywords: Molecular dynamic , PEPC ,Methodological developement

1 *S M. Darabi et S. Seddigh, « Structural, functional, and phylogenetic characterization of phosphoenolpyruvate carboxylase (PEPC) in C_4 and CAM plants », Caryologia, vol. 71, n° 3, p. 272-288, juill. 2018, doi: 10.1080/00087114.2018.1465762.*

2 *S. Lu, X. Bai, W. Li, et N. Wang, « Impacts of climate change on water resources and grain production », Technological Forecasting and Social Change, vol. 143, p. 76-84, juin 2019, doi: 10.1016/j.techfore.2019.01.015.* .