# Structural insight into the complex regulation of the Calvin–Benson cycle

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Photosynthetic CO2 fixation is a fundamental source of food, fuels and chemicals for human society. In the vast majority of photosynthetic organisms, carbon fixation is operated by the Calvin-Benson-Bassman (CBB) cycle [1]. CBB cycle is a pathway consisting of 13 reactions catalysed by 11 enzymes that are differentially regulated to coordinate the entire photosynthetic process in a constantly changing light environment.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoribulokinase (PRK) are two enzymes of the CBB cycle sharing peculiar features. Both enzymes use the products of light reactions for catalysis (NADPH for GAPDH, ATP for PRK), are regulated by thioredoxins and in the dark or under low photosynthetic conditions form inactive supramolecular complex with the regulatory scaffold protein CP12, mainly under the control of thioredoxins and pyridine nucleotides. In the light, complex dissociation allows GAPDH and PRK reactivation.

In land plants, the GAPDH/CP12/PRK complex coexists with an autoassembling heterotetrameric AB-GAPDH that is analogously regulated. Using small-angle X-ray scattering coupled with size-exclusion chromatography (SEC-SAXS) and cryo-electron microscopy (cryo-EM), we identified several oligomeric forms of AB-GAPDH [(A2B2)n-GAPDH oligomers with n=1, 2, 4, and 5] coexisting in a dynamic equilibrium [2]. Due to the sample's high compositional and conformational heterogeneity, we were able to resolve the structures of AB-GAPDH oligomers at resolution of approximately 3 Å for the two hexameric conformations of the most abundant oligomer A8B8.

The regulation of PRK rely on a pair of cysteines located in the active site, but not directly involved in catalysis. The crystal structure of PRK in reduced and oxidized form has elucidated the inhibition mechanism of this enzyme [3,4]. The structural comparison between eukaryotic and cyanobacterial PRKs, shows that the last lacks a loop of eighteen amino acids (named clamp loop) between the two regulatory cysteines, which has been proposed to participate in TRX binding [3]. Moreover, in order to investigate the PRK ligand and substrate binding mode, the crystal structures of CrPRK in complex with ATP and with ADP/Ru5P or AMP-PCP/Ru5P have been described.

With the recently described 3D structures of PRK, A4-GAPDH/CP12/PRK complex and AB-GAPDH oligomers the structural proteome of this ubiquitous regulatory system has been completed. This outcome opens a new avenue for understanding the regulatory potential of photosynthetic carbon fixation by laying the foundation for its knowledge-based manipulation.

#### [1] Michelet, L., Zaffagnini, M., Morisse, S., Sparla, F., Pérez-Pérez, M.E., Francia, F., Danon, A., Marchand, C.H., Fermani, S., Trost, P., & Lemaire, S.D. (2013) *Front. Plant Sci*, **4**, 470.

[2] Marotta, R., Del Giudice, A., Gurrieri, L., Fanti, S., Swuec, P., Galantini, L., Falini, G., Trost, P., Fermani, S. & Sparla, F. (2022) *Acta Crystallogr. D Struct. Biol*, **78**, 1399.

[3] Gurrieri, L., Del Giudice, A., Demitri, N., Falini, G., Pavel, N. V., Zaffagnini, M., Polentarutti, M., Crozet, P., Marchand, C. H., Henri, J. , Trost, P., Lemaire, S. D., Sparla, F. & Fermani, S. (2019) *Proc. Natl. Acad. Sci USA*, **116**, 8048.

[4] Wilson, R. H., Hayer-Hartl, M. & Bracher. A. (2019) *Acta Crystallogr.* F**75**, 278.