STUDYING THE ASSEMBLY OF CARBOXYSOME SHELLS OF CYANOBACTERIA

The Carboxysome is a bacterial micro-compartments (BMC) subtype that confines specialized enzymatic activities necessary for inorganic carbon (CO_2) fixation in cyanobacteria [1]. The shells of these complex macromolecular assemblies are composed exclusively of proteins. This envelope is assumed to play roles in protecting encapsulated enzymes or regulating the transit of molecules in and out of the compartment. Although often integrating 5 to 10 different protomers, all shell components can be classified as belonging to two structural families[2]. To the first family (Pfam 00936) belong the most abundant shell bricks, which associate as **h**examers (BMC-**H** protein) and the pseudohexameric BMC-T proteins, which arrange as trimers of two tandem BMC-H domains. Members of the second family (Pfam 03319) assemble as pentamers (BMC-P).

Despite impressive recent advances in the structural characterization of BMC shells [3], our understanding of how assembly is controlled *in vivo* or of the mechanisms of shell adaptation to environmental changes is still limited. This stage intends to contribute to the comprehension of such processes. In relation to that, several indirect evidences concur to indicate that, instead of being integrated within the shells, some BMC-T could play auxiliary functions (e.g. as shell-specialized chaperonins). Therefore, one of our goals will be to investigate the role played by the BMC-T of β-cyanobacteria, with a special emphasis on the badly characterized CcmO protein.

The stage will start with the construction of a plasmid containing the genes of the main carboxysome Ccm operon of β-cyanobacteria. Applying a deconstruction strategy, we will seek for the simplest constructs that still ensure reconstitution of shell particles in *E. coli* [4]. Preliminarily, shell assembly will be monitored using a high-throughput fluorescence technology based on the *in vivo* reconstitution of a split-GFP. The correct formation of shells will be next confirmed by transmission electron microscopy (EM) and cryo-EM, either by direct visualisation of cells or by the analysis of purified assemblies. Besides facilitating the selection of constructions/conditions causing increased particle yields (e.g. with changes of the BMC-H/BMC-P expression level ratio), the fluorescence screening will be exploited to investigate the participation of CcmO (or other BMC-T) to the assembly process.

The generated information will permit to better understand factors governing the complex process of shell assembly, thus impacting future efforts to engineer novel bio-inspired nano-reactors.

The stage would take place at TBI (INSA-Toulouse; Molecular and Metabolic Engineering, G. Truan)

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