

## CaBioMinDB, a database for the proteins of CaCO<sub>3</sub> mineralized tissues: further application to the shell proteins of the freshwater mussel *Unio pictorum*

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Biom mineralization refers to the process by which living cells and organisms produce mineralized structures. It is a widespread phenomenon in numerous bacterial, archeal or eucaryotic phyla, where the most common biominerals are the phosphate and carbonate salts of calcium. Biomineralizations share a remarkable property: they are organo-mineral assemblages, where the dominant mineral is closely associated with a minor organic matrix. This later represents a mixture of proteins, glycoproteins and polysaccharides, which are secreted by the calcifying tissues during skeletogenesis and sealed within the skeleton during its growth. The organic matrix is essential for mineral formation and proteins are the key components of this process. Biomineral-associated protein sequences show particular characteristics: they possess repetitive sequence domains, consensus repeats and a repetitive occurrence of specific amino acids, such as Asp or Ala. Structurally, these proteins show some tendency to unfolded conformations and they do not exhibit strong homology to documented tertiary folds in protein databases (Evans, 2008). This can be related to their flexibility to interact with both mineral interfaces and organic-matrix assembly. Protein-mineral interactions are still in an early stage of comprehension and elucidation of this process relies on a large-scale analysis of the numerous proteins sequences.

This ongoing research project is focused on the proteins associated to CaCO<sub>3</sub> biominerals in metazoans. The construction of a relational database, CaBioMinDB, targeted on these proteins, is currently in progress. The aim of this biological database is to provide an automated selection and analysis of the calcifying protein sequences in order to characterize new patterns, motifs and domains, and establish new functional relations. To this end, the first approach consists of a semi-automated compilation of the reference proteins in a generalist protein database (UniprotKB). By this method it was possible to collect approximately all the proteins directly or putatively involved in calcium carbonate calcification and most significantly, bring together all the domains, motifs and functions described until now in this process. The second part consists in constructing the database by integrating different proteins analysis tools. This program is currently under validation, by employing the reference proteins which were previously collected.

Along with the database project, the identification of full-length transcripts encoding two main proteins from the nacreous matrix of the bivalve *Unio pictorum* (Marie *et al.*, 2007) is performed by cDNA screening with specific primers. Further characterization of the screening products is achieved by classical molecular biology and biochemical techniques such as PCR, SDS-PAGE, molecular cloning, Western-blotting and immunostaining. The analyzed sequences are integrated into CaBioMinDB, in order to obtain their relation to the other CaCO<sub>3</sub>-associated proteins.

### References:

Evans, J. S., 2008. « Tuning in » to mollusk shell nacre – and prismatic – associated protein terminal sequences. Implications for biomineralization and the construction of high performance inorganic-organic composites. Chem. Rev., 108 : 4455-4462.

Marie, B., Luquet, G., Pais De Barros, J. P., Guichard, N., Morel, S., Alcaraz, G., Bollache, L. and Marin, F., 2007. The shell matrix of the freshwater mussel *Unio pictorum* (Paleoheterodonta, Unionoida). Involvement of acidic polysaccharides from glycoproteins in nacre mineralization. FEBS Journal, 274 : 2933-2945.