Localization of biomineral proteins by Atomic Force Microscopy (AFM) with functionalized tips

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Biominerals are organo-mineral structures produced by living systems. In the metazoan world, they contribute, since the Cambrian, to the adaptation of organisms to different environments by fulfilling a variety of functions that go along with adapted morphologies. One of the aims of biomineralization is to understand how organisms "sculpt" these complex morphologies at nano scale: we know that occluded organic matrix (OM) proteins influence the lattice parameters of biominerals (1) and that many of them strongly interact with the mineral phase as shown by *in vitro* assays, but their exact roles are still to be known. The aim of my PhD is to understand the complex relationships between the organic and mineral phases. In the present study, I discuss the case of the Mediterranean fan mussel *Pinna nobilis*.

Like all bivalves, *Pinna nobilis*, the Mediterranean fan mussel, envelops its soft body with a highly ordered shell composed of two mineralized layers: the internal nacreous one is made of aragonite, while the outer one is made of long calcitic prisms of apparent monocrystalline texture, that grow perpendicularly to the surface of the shell. The prismatic layer comprises an assemblage of insoluble periprismatic matrix and very acidic "intracrystalline" proteins and sugars, together with pigments. The objectives of our study are:

1) To characterize novel shell proteins: We have analyzed the intra and inter-prismatic OM by gel electrophoresis, ELISA test and western blot. A novel prism-associated protein - provisionally named CSP3 - was identified owing to a cDNA library and its full sequence was further confirmed by transcriptomics and proteomics.
2) To localize the novel shell protein in the biomineral. To this end, a polyclonal antibody was elicited against two CSP3 peptides, and tested for its specificity before being purified from the serum. To know the distribution of CSP3 in the prisms, we have functionalized AFM tips with the purified anti-CSP3 antibody via covalent binding. Treated prism surfaces were subsequently scanned with the functionalized tip and adhesion curves/maps were obtained. Interestingly, we have identified for CSP3 a remarkable protein pattern (double layer) at the interface between the periprismatic sheath and mineral, which significance is discussed here.

Conclusion: this work represents the very first attempt to map a protein in a biomineral, owing to functionalized tip-AFM. We assert that this novel approach will be extremely useful in geosciences for mapping at molecular level specific macromolecules in biogenic or sedimentary carbonates.

Keywords: Biomineral, calcium carbonate, mollusc, shell, organic matrix, AFM

1. Zolotoyabko E (2017) Anisotropic Lattice Distortions in Biogenic Minerals Originated from Strong Atomic Interactions at Organic/Inorganic Interfaces. 4(1):1600189.