Poznar Monika

Biomineralization is a process of crystal formation under biologic control. An interesting example of biominerals are otoliths - gravity and sounds sensors of fishes. Similarly to other biologically formed minerals e.g. bone, teeth and shells, otoliths have highly hierarchical structure and precisely designed size, shape, polymorph and mechanical properties. Otolith consists of inorganic calcium carbonate and small fraction of organic matrix. Although organic matrix represents only about 5% of dry weight, it plays a crucial role in control of crystal nucleation and growth. Proteins involved in mineralization are usually acidic, calcium ions-binding, intrinsically disordered proteins (IDP). Until now it is not known why plenty of different IDP proteins are involved in this process.

Otolith matrix macromolecule-64 (OMM-64) is protein found in otoliths of rainbow trout. Along with otolin -1 and heparan sulfate glycosaminoglycans, OMM-64 is a part of high molecular weight aggregates (HMWAs), which control size, shape and polymorph of formed crystals. To better understand biomineralization process, role of individual HMWA's components and their synergistic effects, need to be investigated, however separation of HMWA's components from natural sources is extremely difficult. In this study efficient procedure of OMM-64 expression and purification in *E.coli* was developed. Detailed structural characteristic revealed that OMM-64 is a member of IDP family, among which variety of conformational states was described. OMM-64 is an extended native coil, however in some conditions like high temperature, low pH or presence of TFE, may be partially folded. In the presence of counter ions, being endolymph components, OMM-64 does not fold but collapse. The strongest effect was observed for calcium ions, which are natural ligand of OMM-64. The role of OMM-64 was proposed. It may serve as calcium ions storage molecules, which bind ca. 61 ions per OMM-64 molecule with low affinity (K_d = 0,93 mM). It was shown that OMM-64 being a part of $CaCO_3$ crystals control size, shape and stiffens of the crystals surface. Moreover it promotes crystals nucleation. In the presence of Mg^{2+} ions, protein partially protects aragonite formation, what may be crucial for polymorph selection. OMM-64 as intrinsically disordered, even inside the crystals, may serve as a template for precipitating inorganic phase. This work is a part of broadest studies, where role of IDP and individual otoliths and otoconia components is investigated to understand biomineralization mechanisms and role of proteins in design of extraordinary shapes and properties of crystals.