Elemental signatures in fossil and modern otoliths: Proxies for biomineralizing protein functionality and diagenetic alteration

The sensation of gravity and the proper functioning of the balance receptors in vertebrate animals are provided by specialized biomineral structures located in the inner ear, which are an integral part of the sensory system. In bony fishes they are called otoliths. These fascinating structures are composed of calcium carbonate layers interspersed with regions rich in organic materials. Besides calcium, otoliths also contain trace elements, which are often treated as archives of environmental changes, fish migration patterns, and even dietary habits. Much like other marine organisms that build calcium carbonate skeletons (e.g., corals, foraminifera), many geochemists assume that the elemental and isotopic composition of fish otoliths reflects chemistry of the surrounding seawater, with systematic deviations known as "*vital effects*." However, growing evidence suggests that these *vital effects*, sometimes strongly influenced by environmental changes, are more significant and less predictable than previously thought. In otoliths, the mechanisms by which trace elements are incorporated into the calcium carbonate structure remain unclear. While several theories exist, none sufficiently explains the role of the organic matrix, which is both rich and diverse in otoliths. Of particular interest are proteins with unique amino acid compositions, which dominate the organic matrix and may actively influence the incorporation of trace elements into the otolith structure.

This project aims to uncover the role of otolith proteins not only in forming calcium carbonate crystals in trace-element-enriched environments but also in actively incorporating trace elements into the otolith structure. To achieve this, our team will conduct biomimetic experiments using proteins that regulate otolith biomineralization in the presence of trace elements. These proteins, extracted from various sources, will be systematically analyzed to study the formation of crystalline structures, mesocrystals, and amorphous phases. A key focus will be on liquid-liquid phase separation (LLPS), a process where macromolecule solutions spontaneously separate into two distinct phases with different concentrations. Various physicochemical factors modulate LLPS, creating dense droplets that may serve as unique environments for biomineralization. *In vitro* biomineralization assays under different conditions will also explore how trace elements influence the biological activity of otolith biominerlization proteins and affect the microenvironment in which biominerals form.

A major mystery in biomineralization research is understanding how the organic matrix integrates into the mineral structure and the role trace elements play in this process. One hypothesis suggests that organic molecules attach to specific crystallographic planes of calcium carbonate through stereochemical recognition mechanisms, becoming embedded within the crystal lattice as the layers grow. Another proposes that organic components are simply trapped in voids and channels within the biomineral, without direct incorporation into the crystal network.

The complex mineral-organic nature of biominerals, including otoliths, makes them an excellent model for studying poorly understood diagenetic processes - those that alter the original structural and geochemical characteristics of biominerals. Diagenesis may occur *via*: (1) incorporation of elements from sediments into the biomineral structure, and/or (2) release of elements from decomposing organic matter, which are then integrated into the mineral lattice. Recent findings suggests that regions of biominerals rich in organic materials, behaving as nanocomposites, can act as selective ion-exchange „highways”. In these zones, calcium ions are replaced by others from the external environment - without altering the mineral's original structure. This means that an otolith, while remaining aragonite, could have its elemental composition significantly altered, challenging interpretations of past environmental conditions (e.g., paleotemperature). This surprising discovery will be rigorously tested in the project using novel diagenetic experiments on modern otoliths, exceptionally well-preserved fossil otoliths, and biomimetically formed crystals containing organic components.

The project will deepen our understanding of the key factors influencing otolith biomineralization in model organisms. It will introduce innovative experimental approaches and create new interdisciplinary perspectives, such as in an astrobiological context, enabling the identification of biogenic minerals from elemental "fingerprints" and therefore providing insights into the search for extraterrestrial life.