Nanoscale microstructure and texture patterns of bivalve nacre

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Biological hard tissues are hierarchical composites. EBSD is one of the best methods available for structural characterization of biological tissues, since it provides microstructure imaging and crystal orientation determination on several hierarchical levels. While the conventionally used 20 kV acceleration voltage yields EBSD with a spatial resolution in the micrometer range, high resolution, low kV (8 to 15) EBSD renders a 100-400 nm step resolution. This enables the investigation of nanostructures such as orientation patterns in the nacreous parts of biological skeletons.

We could map orientation patterns of calcite with high resolution, low kV (8 to 15) EBSD. This rendered measurements with 100-400 nm step resolution and enabled for the first time the investigation of biological nanostructures, especially orientation patterns in nacre and the nacreous parts of biological skeletons. The investigated specimens are the nacreous portions of the oyster *Crassostrea gigas* and of the bivalve *Mytilus edulis.* Further, we investigated the shells of *Elliptio crassidens*, *Cristaria plicatus* (composed entirely of nacreous aragonite) and the pearl of the freshwater mollusk *Hyriopsis cumingii*. The aragonite nanoplatelets are untwined single crystals that assemble to platelets. Stacks of almost equally oriented platelets form clusters with distinct orientations. Within a cluster the orientation goes across the platelets, while neighbouring platelets that belong to different clusters often form twin related orientations (rotation by 60 degrees around the c-axis). The normal to the platelets is the c-axis (setting: a=4.96 Å, b=7.97 Å, c=5.75 Å). The size of the correlated clusters and the abundance of twin relationships between adjacent platelets varies significantly between the investigated mollusk species.

For *Crassostrea gigas*, *Cristaria plicatus* and the pearl of *Hyriopsis cumingii* one crystal orientation dominates, such that the overall texture pattern has a 3D single crystal like appearance. For *Mytilus edulis* and *Elliptio elliptio* the three twin orientations are of similar abundance.

3D STXM tomography of Fe(II)-oxidizing bacteria

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In pH-neutral environments ferrous iron can be oxidized under anoxic or microoxic conditions by Fe(II)-oxidizing bacteria. Different microbial metabolisms of Fe(II) oxidation and biomineralization of these bacteria have been identified and characterized so far. Initial Fe(III) mineral precipitation in the periplasm and subsequently cell encrustation was observed with the mixotrophic, nitrate-reducing, Fe(II)-oxidizing *Acidovorax* sp. strain BoFeN1 isolated from anoxic littoral sediments from Lake Constance, Germany [1]. Iron mineral precipitation in vicinity to the cell was shown for the phototrophic, anaerobic Fe(II)-oxidizing *Rhodobacter* sp. strain SW2 [2]. Also, under microaerophilic conditions iron minerals can be deposited within extracellular polymeric structures such as twisted stalks or sheats which are extruded by microaerophilic *Gallionella* strains [3].

To further our understanding of the different mineralization patterns and mechanisms of Fe-biomineralization, we investigated BoFeN1, SW2 and an environmental biofilm containing twisted stalks (similar to those observed for *Gallionella* strains) from an abondoned silver mine in Germany. We conducted conventional soft X-ray scanning transmission microscopy (STXM) in combination with angle-scan tomography measurements allowing for a 3D reconstruction. The advantage of STXM is the combination of high spatial resolution (≈10 nm) with the possibility of identifying and quantifying cell components such as proteins and lipids, extracellular polymeric substances (EPS) and iron minerals. Therefore, we acquired image sequences across the C1s, O1s and Fe2p absorption edges for BoFeN1, SW2 and an environmental biofilm containing twisted stalks. Compositions maps of the macromoleculars components were obtained by linear combination fits of reference spectra of proteins, lipids, polysaccharides and iron minerals. The association of the Fe-phases with the organic components of the cell-mineral aggregates was then analyzed quantitatively in 3D by correlation analysis.

Our results confirmed that iron is precipitated within the periplasm of BoFeN1, in contrast to SW2 and the environmental biofilm where the iron precipiates are closely asscociated with organic expolymers.

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