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Selective Ion Accumulation in Biomineralizing Marine Acantharia

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Separating chemically similar ions from complex mixtures presents a major technological challenge. While most living organisms show an indiscriminate transport of earth alkaline cations, the marine protists *Acantharea* build their entire endoskeleton from strontium sulfate ($SrSO_4$) [1]. A key question to be addressed is how Acantharia selectively sequester $SrSO_4$ mineral despite the very low levels of strontium compared to calcium ions in the oceans. Within the vesicle enclosing the $SrSO_4$ crystals, the local Sr^{2+} and SO_4^{2-} ion concentration has to exceed supersaturation to allow for $SrSO_4$ mineral deposition. We speculate that specific ion-channels or ion-binding proteins may be involved in selective ion uptake. Fundamental insights into the biological system will help us find engineered solutions for a broad range of chemical separation problems involving heavy metal ions in aqueous environments, as found in drinking water purification, nuclear waste management, or the chemical industry

Towards the goal of understanding the principles of ion selection in Acantharia, we mapped the elemental composition across the uncultured protist cell using scanning synchrotron X-ray fluorescence microscopy (Advanced Photon Source, Argonne National Laboratory) [2] [3], providing quantitative elemental information with a spatial resolution down to 80nm and a sensitivity down to parts per million [3] [4]. Synchrotron X-ray microfluorescence (XRF) mappings were obtained from freshly collected, chemically fixed Acantharian cells from Southern California (Figure 1a). Here, the cytoplasm contains Ca^{2+} and Sr^{2+} in a 26:1 ratio, suggesting that Sr^{2+} is enriched with respect to seawater with a 116:1 ratio [5] (Figure 1b,c). We conclude that after seawater uptake, a further ion-selective step must occur at the level of the periplasmatic membrane enclosing the spicules.

Acantharia cells harvested in Villefranche-sur-Mer (France) were high-pressure frozen, freeze-substituted, and resin-embedded. Synchrotron XRF elemental maps of ultramicrotome sections show a broad distribution of sulfur across the cellular tissue and the outer membrane (Figure 1d). Trace metals bound in metalloproteins help us identify ultrastructural elements, for instance cellular membranes, nuclei (Cu), or endosymbiotic microalgae (Zn). Colocalization of sulfur hotspots with calcium indicated an intracellular precipitation of $CaSO_4$ crystals. In addition, we used correlative X-ray absorption spectroscopy for analysis of sulfur speciation (Figure 1e) [6]. Synchrotron sulfur *K*-edge X-ray absorption near edge structure (μ-XANES) allows us to discern sulfur species in different oxidation states depending on the position of the absorption edge. Thereby, we confirm that these sulfur-rich regions (P2, P3) display a higher proportion of inorganic sulfate compared to sulfur-containing amino acids or sulfated polysaccharides (Figure 1f). The abundance of sulfate is known to favor the deposition of scarcely soluble sulfate minerals ("sulfate trap" mechanism), as previously observed in desmid green algae [7]. [9]

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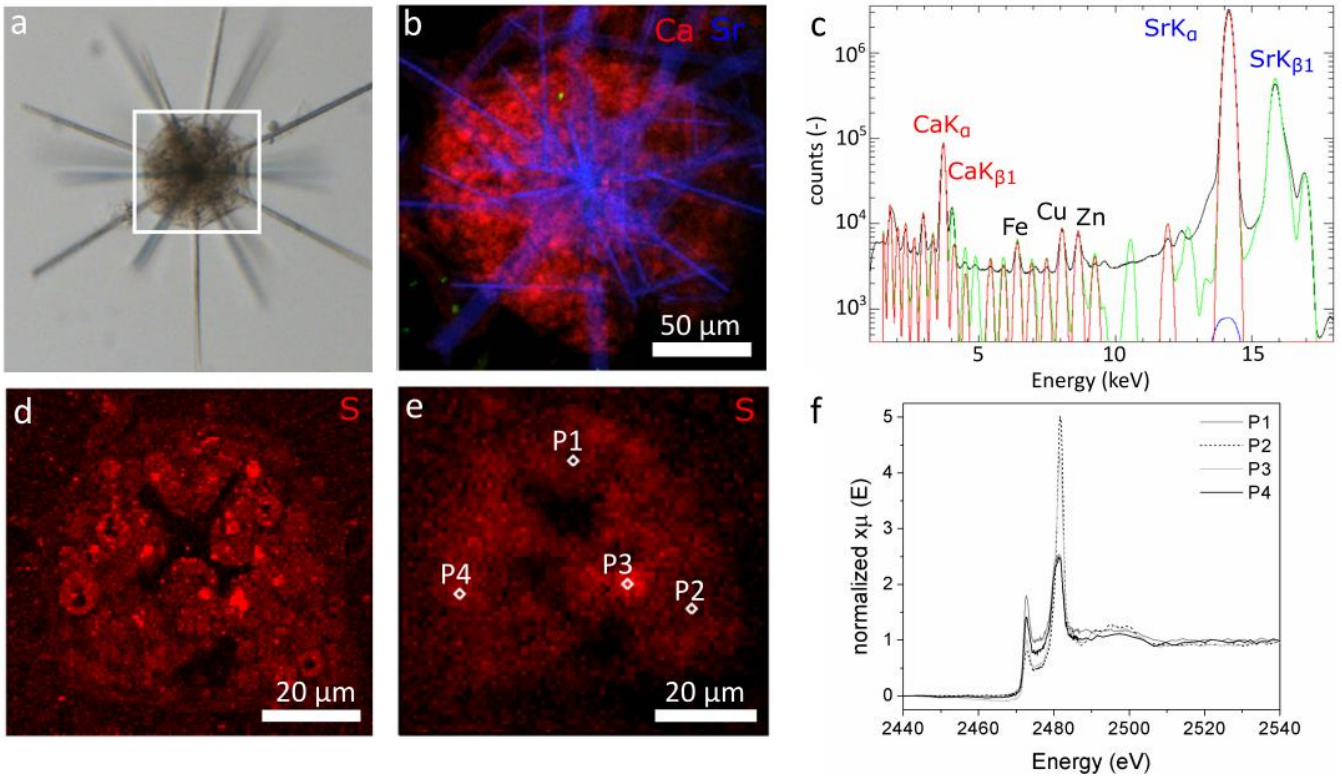

Figure 1. a) Light microscope image of whole, chemically fixed Acantharian cell collected in San Pedro, Southern California. b) Synchrotron X-ray fluorescence mapping of whole organism (2-ID-E, 17keV, 500nm pixel size). c) Average XRF spectrum, background and fitted peaks from µ-XRF mapping. d) Synchrotron X-ray fluorescence mapping of ultramicrotome section from high-pressure frozen, freeze-substituted Acantharian cell from Villefranche-sur-Mer, France (9-ID-B/Bionanopobe, 10keV, 500nm pixel size). e) Synchrotron X-ray fluorescence mapping at sector 13-ID-E (2.4keV, 1.5µm pixel size). f) Sulfur *K*-edge µ-XANES spectra from selected regions of interest.

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