Imaging intracellular calcium accumulation in coccolithophores using cryo-X-ray tomography

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Biogenic calcite precipitation in the oceans is one of the key drivers of the marine carbon cycle. Coccolithophore algae are dominant marine calcifiers that produce coccoliths, minute calcitic scales, inside specialized intracellular compartments. During coccolith formation some environmental parameters are recorded in the mineral composition as chemical proxies by a mechanism that differs from solution-mediated precipitation. Not much is known about the intracellular pathways that are responsible for the transport of the constituent ions from seawater to the growing coccolith. Elucidating this process is crucial in order to understand the influence of these algae on modern ocean systems and to extract paleoceanographic data from coccoliths in sediments.

We cultured the two model coccolithophore species *Emiliania huxleyi* and *Pleurochrysis carterae* in order to map intracellular calcium. Coccolith producing cells were rapidly frozen and preserved at cryogenic conditions in order to maintain their intracellular organization intact. We used synchrotron soft-X-ray tomography to acquire high-resolution images of single cells with a resolution of 30 nm. The reconstructed 3D cellular volume shows all major cellular organelles, as well as an immature coccolith enclosed in its intracellular vesicle. In addition, the cell also contained different intracellular compartments packed with highly absorbing material. Imaging the cells with X-ray energies around the Ca L-edge showed that some of these compartments, about one for each cell, are enriched with Ca. *In situ* spectroscopic analysis showed that this is a disordered Ca-rich phase.

These findings suggest that intermediate stages are part of the calcium accumulation process from the extracellular environment to the coccolith vesicle. These intermediate stages may involve the formation of amorphous precursor phases, and thus might be responsible for the ‘vital effects’ observed in the composition of biogenic minerals such as coccoliths.