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POSTER #5

BIOMINERALIZATION IN DINOFLAGELLATES

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Beautiful examples of biomaterials occur in unicellular, eukaryotic microalgae. Among them dinoflagellates constitute one of the largest groups. Dinoflagellates are the second most abundant calcareous phytoplankton group and contribute substantially to the oceanic carbonate flux. Many species are associated with harmful algal blooms. For this reason there is a strong ecological interest in learning more about their complex life cycle. Surprisingly, dinoflagellates - one of the major constituents of phytoplankton - have remained largely unexplored.

Many dinoflagellate species produce (mineralized) resting stages (cysts) as a dormant, zygotic stage of their life cycle.[1] These cysts exhibit organic walls or calcareous and sometimes siliceous walls. Recently, fossilized calcite-cysts were shown to have extremely interesting, interwoven fibre-like structures.[2] These structures so far observed only in fossil cysts raise questions concerning biomineral formation in living dinoflagellate cysts.

The focus of previous studies has been the structural characterization of cysts and the en-/excystment process. At the moment, neither the detailed structure/function relations, nor the formation and stabilization mechanism of the mineral phase are known. For this reason, the primary goal is to obtain a detailed understanding of the biomineralization processes present in dinoflagellates during their complex life cycle.

Using the analytical methods Cryo-SEM/EDS, Cryo-FIB-SEM, (Cryo-)fluorescence, FT-IR, (Cryo-)micro-Raman spectroscopy and flow cytometry the biomineral formation process in several dinoflagellate species was studied to understand the cellular mechanisms, particularly for calcium carbonate in the cyst stage and organic crystals in the vegetative state.

Calcification in Leonella granifera

Cryo-SEM images of freeze-fractured cells of *L. granifera* (Fig. 1a) show vacuoles of different sizes on the cell periphery. These vacuoles are rich in potassium and phosphate (Cryo-EDS) and include small particles of unknown composition. After decalcification with EDTA the cells start to recalcify and these vacuoles start to form a ring surrounding the cell. To analyze if these vacuoles are part of the biomineralization process the cells were stained with Calcein-AM, a dye sensitive to calcium ions. Staining with Calcein-AM revealed enrichment in intracellular compartments and could be used to follow the shell formation process. In *L. granifera* strong evidence for a calcification mechanism proposed by Inouye and Pienaar[3] was found. Golgi-derived vesicles filled with particles are supposed to be part of the biomineralization process. Correlative approaches (Cryo-SEM, -Fluorescence and -Raman) are planned for a more detailed understanding of the shell formation process.

Guanine crystals in Calciodinellum operosum aff.

Cryo-SEM sections of vegetative cells of *C. operosum aff.* revealed vesicles filled with crystalline material in close association with the chloroplasts of the cell (Fig. 1b). The crystals exhibit no significant backscattering signal and are nitrogen-rich (Cryo-EDS). Crystalline anhydrous guanine could be identified. Because of the proximity of the crystal-filled vesicles and the chloroplasts we propose that in this non-

bioluminescent dinoflagellate these vesicles might act as light-scattering organelle enhancing photosynthesis efficiency.

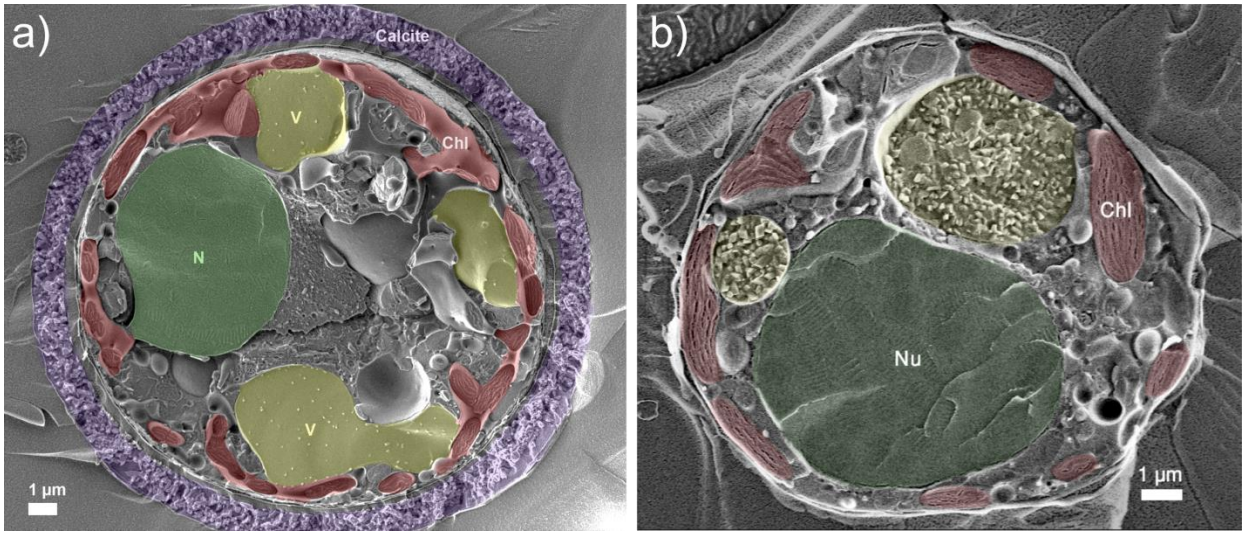

Figure 1. a) Cryo-SEM image of freeze-fractured calcarous cyst of *L. granifera.* b) Cryo-SEM section of a single cell of *C. operosum aff.* showing crystalline guanine deposits.

References:

1. a) F. J. R. Taylor, The Biology of dinoflagellates. Blackwell, Oxford, 1987; b) D. L. Spector, Dinoflagellates, Academic Press 1984.
2. J. E. Wendler, P. Bown, Nat. Commun. 2013, 4.
3. I. Inouye, R. N. Pienaar, S. African. J. Bot. 1983, 2, 63–75.