Electronic Supporting Information

Templated and self-limiting calcite formation directed by coccolith organic macromolecules

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Experimental Procedures

Isolation of coccolith-associated macromolecules

Isolation of the base plates and soluble macromolecules followed a procedure described previously.[9] Briefly, *Pleurochrysis carterae* CCMP645 was grown in artificial seawater medium. ~5×10^6 cells were harvested by centrifugation at 2.000 g and re-suspended in 1 M KNO_3 solution for 20 minutes. This treatment induced the release of the coccoliths from the surface of the cells. The cells and coccoliths were pelleted, and the supernatant was removed. The pellet was re-suspended in a 50% Percoll® (GE Healthcare, Germany) solution and centrifuged at 5.000 g, resulting in the separation of the heavy coccoliths from the lighter cells floating on top of the Percoll® cushion. The cellular material was decanted, and the Percoll® step was repeated once more. In order to remove residual Percoll® particles, the coccoliths were washed three times with an aqueous solution saturated with respect to $CaCO_3$.

For isolation of the organic material associated with coccoliths, the mineral was dissolved with 0.1 M EDTA. The organic material was desalted by three rounds of dialysis against MilliQ-H_2O using Spectra/Por® 7 50 kDa cutoff (Spectrum Laboratories, Inc.) When needed, the desalted material was separated into a fraction containing the base plates, and a fraction containing the soluble macromolecules, using centrifugation at 20.000 g for 15 minutes. All steps were carried out at room temperature.

In vitro crystallization experiments

The experiments were done using 200 µL of coccolith associated organic fraction containing ~10^7 base plates and ~50 µg × mL^{-1} soluble macromolecules. Base plate number was estimated based on the number of cells used (and ~30 coccoliths/cell), soluble macromolecules concentration was determined by lyophilizing and weighing the content extracted from a known number of cells grown in 1L culture. For experiments with varying ratios of base plates to soluble macromolecules, the two fractions were mixed in the needed volumes. $CaCl_2$ solution was added to a final concentration of 1 mM (or other concentrations when indicated). After 10 minutes (enough time in order to allow the macromolecular recognition reaction to complete) 20 µL of 100 mM Na_2CO_3 were added to the solution in 5 µL steps.

Sample analyses

For SEM imaging, 10 µL of sample was placed on Isopore™ membrane filters (Merck Millipore, Germany). Excess solution was blotted from the opposite side of the filter through the 100 nm pores. The filters were coated with 2-3 nm platinum and imaged with a Jeol JSM7500F.

For AFM imaging, the NanoWizard® 3 (JPK, Germany) was used in tapping mode. 10 µL of sample were added to 1 mL MilliQ-H_2O, the calcified base plates were pelleted by centrifugation and re-suspended in 5 µL MilliQ-H_2O. The sample was dried on a freshly cleaved mica surface.

For scanning transmission electron microscopy (STEM), 4 µL of calcified base plate sample was placed on a holey carbon coated TEM grid (Quantifoil, Germany) with 600

nm holes. The solution was blotted through the holes, and the base plates were trapped on the film. Dark-field imaging, bright-field imaging, and electron-energy loss spectroscopy (EELS) were performed on a Tecnai G2 F20 X-Twin microscope equipped with a field-emission electron source (operated at 200 kV), a Fishione high-angle annular dark-field (HAADF) detector at 330 mm camera length (diffraction contrast + Z contrast) for image acquisition in the STEM mode, a Gatan Tridiem imaging filter for acquisition of energy-filtered images and EEL spectra.

For EBSD measurements and the recording of transmission Kikuchi diffraction (TKD) analysis in SEM, the samples were prepared in an identical procedure to the TEM samples. TKD analysis was carried out using Aztec software and Oxford NORDLYS II detector mounted on FEI Quanta 200 FEG ESEM (operated at 20 keV in the high-vacuum mode). The TKD resolution is in the order of 10nm while step size was 60nm for mapping the crystal orientation within mineralized base plates. Further statistical analysis was performed using Oxford Instruments HKL Channel 5 software for pole figure and inverse pole figure presentations.

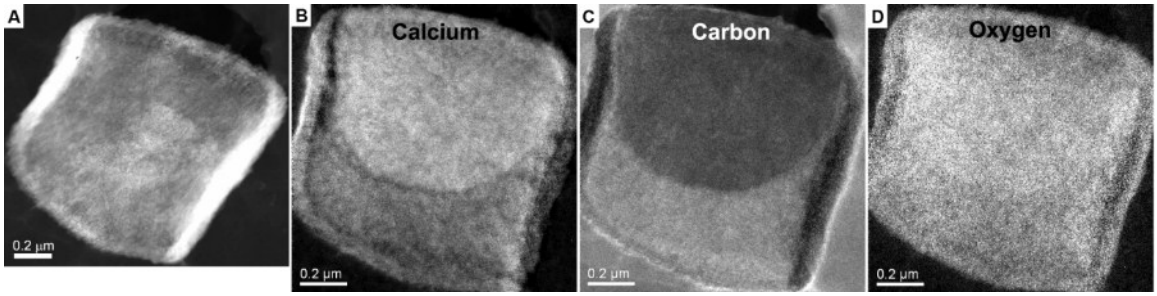

Figure S1. A) HAADF-STEM image of a calcified base plate. B-D) EELS maps of calcium, carbon, and oxygen, of the same field of view. Note the hole in the carbon support film that is prominent in the carbon map.

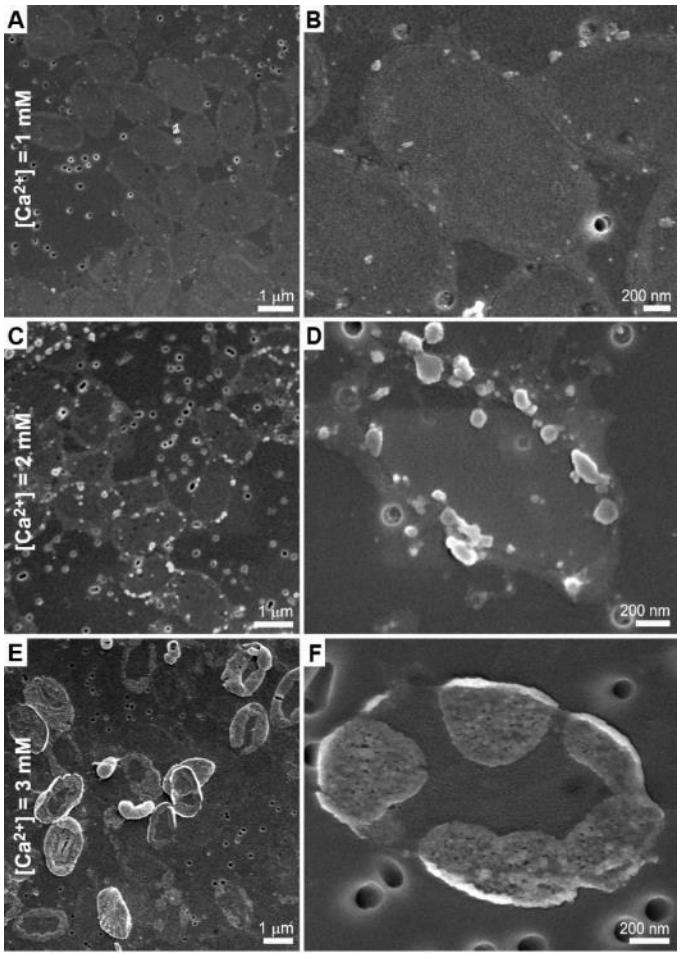

Figure S2. Base plate crystallization experiments at various [Ca], dried 10 minutes after the addition of carbonate. Both low-magnification images showing the overall appearance of the sample (A,C,E), and higher magnification of representative base plates (B,D,F) are shown. Panels B, D are the same as Fig 3. C,D in the main text.

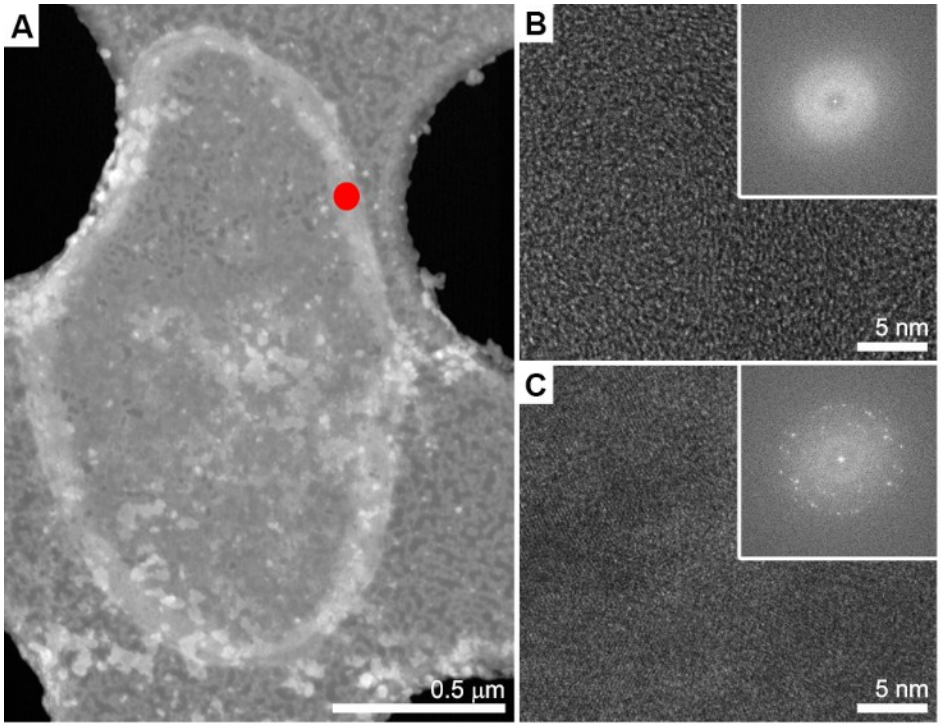

Figure S3. A) HAADF-STEM image of a base plate dried 10 minutes after the addition of carbonate to a crystallization solution containing 3 mM calcium ions. Carbonate addition induces a compositional change in the dense Ca-rich phase located at the base plate rim, even before mineral growth can be detected. B) High-resolution imaging of the dense phase on the base plate rim (indicated with red circle), showing amorphous pattern (inset shows diffraction pattern (FFT) of the image). C) After several minutes of irradiation, some crystalline calcitic domains appear in the same location, suggesting that the initial amorphous phase contained calcium and carbonate ions that crystallized under the influence of the electron beam.

Table S1. The results of crystallization experiment under various carbonate buffer conditions. The same amount of organic macromolecules and [Ca] = 1 mM were used in all experiments.

		Carbonate buffer concentration		
		5 mM	**10 mM**	**20 mM**
pH	**7**	No precipitation	No precipitation	No precipitation
	8	No precipitation	No precipitation	Calcified base plates + other precipitates after 24 h
	9	No precipitation	Rare formation of calcified base plates	Calcified base plates
	10	No precipitation	Calcified base plates after 10 h	Calcified base plates
	11	Calcified base plates after 20 h	Calcified base plates after 4 h	Calcified base plates + rhombohedral calcite

Table S2. The influence of raising [Ca] and changing the ratio between the base plates and the soluble macromolecules on the outcome of the crystallization experiments.

Tested parameter	Result
Calcium concentration	
1 mM	As described in Figures 1 and 4
2 mM	After 10 min, as in Figure 4. After 4 hours, mostly calcified base plates are found, with rare appearance of rhombohedral crystals.
3 mM	After 10 min, as in Figure 4. After 4 hours, most precipitate is in the form of rhombohedral crystals, some calcified base plates and some vaterite.
10 mM	Unspecific precipitation of various crystal morphologies.
[Base plates]:[Soluble macromolecules]	
1:3	No precipitation was observed after 4 hours.
1:1	Similar results to the experiments with the un-fractionated organic extract.
5:1	Calcified base plates with some distinct rhombohedral facets that are smaller than 100 nm.
10:1	Calcified base plate with rhombohedral facets up to micrometer long (Figure 3).
33:1	Only rhombohedral crystals were observed, not related to the base plates.
∞:1	Only rhombohedral crystals were observed, not related to the base plates.