	Experiment title: **Dislocation driven biomineral morphogenesis**	**Experiment number**: LS-3051
Beamline: ID01	**Date of experiment**: from: 12 Apr 2022 to: 16 Apr 2022	**Date of report**: 16.09.2022
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Report:

Background:

Biologically formed minerals have the morphology and crystallographic properties that are significantly different from their abiotically formed counterparts. Specifically, a number of species form highly **branched single-crystalline** endoskeletal building units at ambient conditions, which is far beyond the ability of current human technology. Understanding how living organism orchestrate the growth of these structures will not only provide unprecedented knowledge in the field of biological materials and a number of domains in earth sciences, but will also provide us with explicit tools for designing and generating these architectures synthetically. Our previous work, performed at the ESRF, suggests that defects play an important role in biogenic crystal growth and hence, biomineralized tissue morphogenesis.[1,2] In the proposed experiment, we tested the hypothesis that organisms employ screw dislocations to direct the growth of extremely complex but symmetric singe-crystalline entities. Here, Bragg-CDI (BCDI) experiments were employed to identify and localize these dislocations in the crystalline endoskeleton of a number of species, with the goal to establish dislocation-driven crystal growth as key mechanism in controlled morphogenesis of intricate extracellular biological materials.

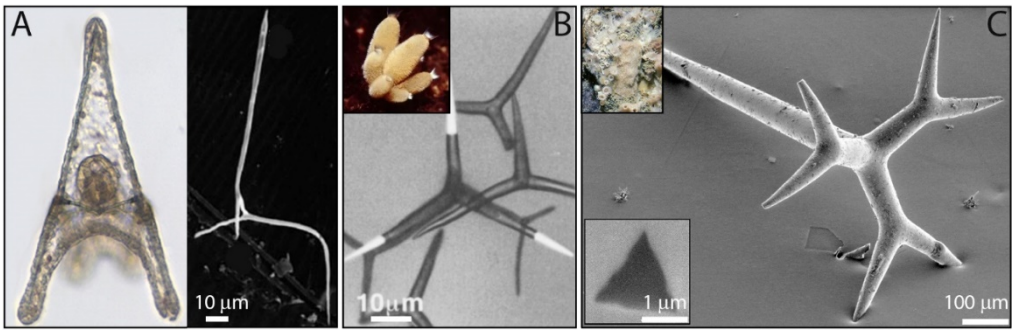

Figure 1: (A) sea urchin larval spicule in *P. lividus*. Left: the entire larvae of the animal 200 µm in height. Right: single-crystalline calcite spicule. (B) Calcitic spicules from *Sycon sp.*. Insert: The entire organism is 1 cm in size. (C) Amorphous glass spicule from the demosponge *S. ponderosus*. Upper Inset: Part of the organism. Bottom Inset: FIB-ed cross section of the main shaft of the spicule showing the crystalline axial filament laying along the spicule.

The most studied branched systems are:

(1) Calcitic sea urchin larval spicules, such as *Paracentrotus lividus*. It contains a branched calcitic structure that despite its branched form exhibits single crystalline properties (Fig. 1A). This organism is a significant model system to study mineral transport in a biological setting.[3]

(2) Spicules from the calcareous sponges, such as *Sycon raphanus*. The body of the sponge is reinforced by numerous highly regular branched calcitic spicules. Branching occurs in specific crystallographic orientations resulting in the high symmetry of the spicules (Fig. 1B).[4]

(3) Siliceous spicules from glass sponges, such as *Stryphnus ponderosus*. Similarly to calcitic sponges, the body of these animals is reinforced by highly regular building units. However, here they are made of amorphous glass the deposition of which is templated by a protein crystal (Fig. 1C, axial filament). A series of our previous publications demonstrated that (i) the crystallographic branching of this protein crystal is responsible for the morphogenesis and the high symmetry of the spicules (ID16a, ID13 and ID19)[1] and (ii) these protein crystals grow with the assistance of a screw dislocation located at the center of the filament resulting in an Eshelby twist of the entire crystal (ID13).[5]

In all three examples presented above, our understanding of how the animals control the crystallographic branching of these crystalline assemblies is limited. However, based on our work with glass spicules, we postulate that the branching and growth of these inorganic and organic crystals is assisted by screw dislocations that direct the highly controlled morphogenesis of these structures. In light of the success of Bragg-CDI in imaging such dislocations in similar synthetic calcite crystals,[6] we proposed to employ this method to describe the existence of screw dislocation in the above three model systems.

Experiment:

BCDI at ID01 was employed to visualize the dislocation-induced strain fields in the studied species in 3D. Five 1x1x1 μm^3 cubes were lifted out of central parts of different branches from the three studied structures, 15 samples in total) using the FIB-SEM method. The size of the samples in all cases was less than 1 micron. In the case of the calcific samples, EBSD pre-characterization was used to determine sample orientation in space. In the case of glass spicules, the samples were mounted in such a wat that the incident beam was aligned parallel to the basal plane of the hexagonal structure of the protein lattice.[7]

To our disappointment, despite our numerous attempts we were unable to obtain a useful diffraction signal from the calcitic samples. This is extremely surprising as EBSD measurements provided clear patterns. However, in the case of the protein crystal, we were able to obtain a diffraction signal useful for further reconstruction. Please see the Results section.

Analysis and Results

Instead of using FIB-ed samples that provided no diffraction, signal entire spicules were measured. Due to the large lattice parameters of the hexagonal superstructure (a ~ 6 nm , c ~ 11 nm), all the diffraction spots were immediately visible with the detector positioned along the incident beam (Fig. 2A). This fact significantly shortened the time required for searching a reflection from a single crystal. Due to the elongated nature of the crystal, the reconstructions was performed in 2D only. To follow the evolution of lattice properties along the filaments, the experiments were repeated every 10 micron along the filament and thus, generating a pseudo 3D information. Although these scans demonstrated our ability to successfully measure and analyze the data obtained from these samples (Fig. 2B-2C), additional beam time will be required to complete the data using the newly developed measurement approach.

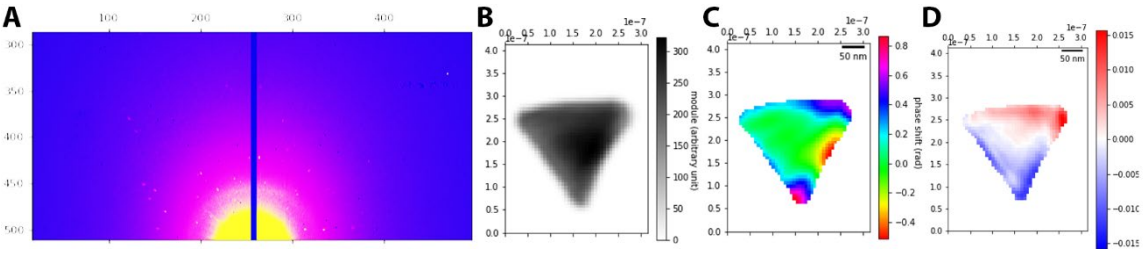

Fig. 2: (**A**) A diffraction pattern obtained from the filament from *G. cydonium* needle-like spicule with the detector positioned along the incident beam. Multiple reflections are clearly visible. 2D slices showing the reconstructed (**B**) amplitude and (**C**) phase across the filament. (**D**) 2D slice of one independent component of the strain tensor showing regions of compressive (negative) and tensile (positive) strain obtained from the retrieved phase. The triangular shape of the filament is reconstructed.

Impact

Our data is the first ever mapping of lattice distortions in protein crystals provided by BCDI. Provided additional beam time, we expect to obtain full crystallographic data on the axial filament in a number of species, including tensorial data on the evolution of strain across forming protein crystals and further information on local lattice properties, defects and crystallographic twists. This will not only provide the first experimental evidence to geometric frustration in protein crystals, but will also demonstrate the role of spontaneous processes in biological tissue morphogenesis.

Bibliography

[1] Schoeppler, V. *et al. Sci. Adv.* 3, eaao2047 (2017). **[2]** Beliaev, M. *et al.*, *Nat. Phys.* 17, 410–415 (2021). **[3]** Winter, M. R. *et al.*, *PLoS Comput. Biol.* 17, 1–28 (2021). **[4]** Rossi, A. L. *et al., J. Struct. Biol.* 196, 164–172 (2016). **[5]** Zlotnikov, I. *et al.*, *Small* 11, (2015). **[6]** Ihli, J. *et al., Nat. Commun.* 7, 17–19 (2016). **[7]** Görlich, S. *et al., Proc. Natl. Acad. Sci.* 117, 31088–31093 (2020).