	Experiment title:	Experiment number:
	GEOMETRIC FRUSTRATION CONTROLLED MORPHOGENESIS OF PROTEIN CRYSTALS	SC-5302
Beamline: ID16a	**Date of experiment:** from: 24 Nov 2022 to: 28 Nov 2022	**Date of report:**
Shifts: 12	**Local contact(s):** Dmitry Karpov	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Dr. Igor Zlotnikov, B CUBE – Center for Molecular Bioengineering, TU Dresden, Germany

*Abisheik John Victor Jothi, B CUBE – Center for Molecular Bioengineering, TU Dresden, Germany

Background:

Demospongiae, a class of sponges, synthesize mineralized siliceous skeletal elements, glass spicules, which provide the animals with structural support and mechanical strength. The spicules, made of hydrated amorphous silica, exhibit a fascinating diversity of the most intricate species-specific three-dimensional (3D) morphologies displaying high spatial regularity and symmetry.(*1*) Whether the spicules have a simple elongated needle-like shape (Fig. 1A) or branch to form a tetrapod-like or a star-like morphology (Figure 1B) or even in the case of spatially more complex morphologies, all branches of the spicules harbor a proteinaceous axial filament (inserts in Fig. 1A and 1B, Fig. 1C-1D). The filament, up to 2 µm in diameter, is predominantly composed of spatially ordered enzymatically active proteins, silicateins, which catalyse silica fabrication and act as a template for its deposition. Astonishingly, as it is evident from the shape of the filament, the silicateins inside assemble to form slender crystals throughout the entire spicule. Crystal habit of the filaments reflects the three-fold symmetry of the crystal. It is well accepted that axial filaments play an essential role in directing the process of silica biomineralization and thus governing the morphogenesis of sponge spicules.

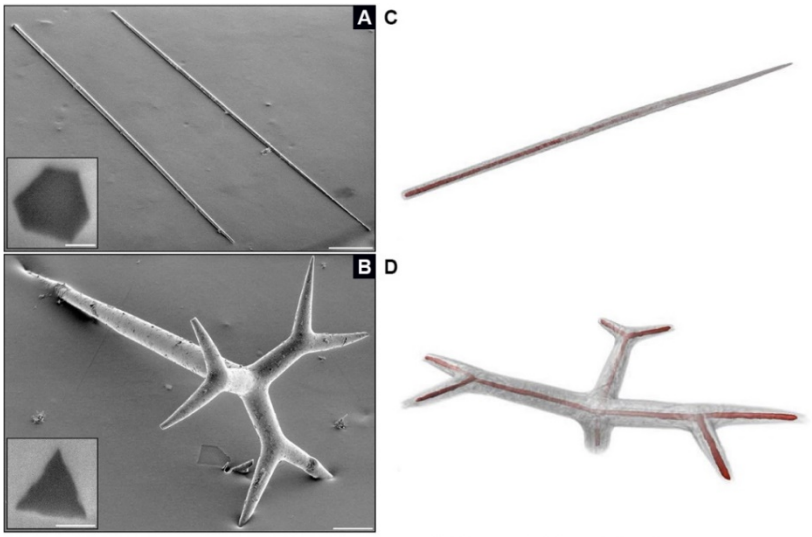

Figure 1: The morphology of spicules and their inner axial filaments. A) Spicule from the demosponge *T. aurantium*. Scale bar, 100 µm. Inset: Cross section of the spicule showing the axial filament.Scale bar, 1 µm. B) Spicule from the demosponge *S. ponderosus*. Scale bar, 100 µm. Inset: Cross sections of the main shaft of the spicule showing the axial filament. Scale bar, 1 µm. C) A 3D reconstruction of the spicule in (A) obtained using microtomography on beamline ID19 in ESRF showing the spicule and the internal axial filament in red. D) A 3D reconstruction of the spicule in (B) obtained using microtomography on beamline ID19 in ESRF showing the spicule and the internal axial filament in red.

Therefore, our recent work focused on the question: **Being a single crystalline entity, what governs the morphology of the axial filament?** Our recent work in ESRF and SLS enabled us to (*i*) determine the structure of silicatein and its crystal packing characteristics(*2*), (*ii*) determine that the high spatial symmetry of the spicules is achieved through branching of the protein crystal on very specific crystallographic planes(*1*) and (*iii*) provide new details on the mechanism of silica polymerization by the protein. However one main question remains: **How do the cells control the branching events of the protein crystal during assembly?**

Almost a century ago German mineralogist Ferdinand Bernauer discovered that a large

fraction of slender organic compounds could form twisted molecular crystals. Whenever the constituents of an assembly are required to distort in order to be joined together, their assembly is associated with geometric frustration. In the case the effect is cumulative, the frustration builds up and leads to a progressive twist of the crystal lattice and other elastic phenomena.(*3*)
We hypothesize that geometric frustration plays a significant role in the morphogenesis of the axial filament. Periodicity that is enforced during the assembly of the silicatein crystal should result in residual elastic strains that lead to a twist of the slender protein crystals and changes in its crystal habit.(*3–5*) The twist of the lattice in the axial filament of six different sponge species was already recently demonstrated by us on ID13. Further information on the changes in protein crystal habit during spicule morphogenesis together will theoretical calculations provided by Prof. Efrati(*3–5*) (Weizmann Institute of Science, Israel) will allow us to establish the role of the cumulative elastic strain in the branching of the protein crystal.

Experiments and Setup at ID16:
The holotomographic imaging method on the beamline ID16A-NI was used to study the evolution of the chrystal habbit of the eaxial filament. The exceptionally small beam and the resulting nanometer-sized resolution matched well the small diameter of the axial filament the shape of which needed to be resolved. We succesfully followed the morphological evolution of symmetrically branched spicules in three Demosponge species: *Stryphnus ponderous*, *Geodia cydonium* and *Tethia aurantium*. The imaging beamline ID16A made it possible to perform zoom-projected imaging that can be combined into phase-retrieved holotomographic reconstructions of small samples. The high flux, high resolution, dedicated software and our experience on ID16A provided unprecedented 3D reconstructions of the morphology of protein crystals produced by living organisms. A total of 24 spicules collected from the three organisms were investigated. „Low" resolution overview holotomography scans followed by a number of local-tomography using the higher resolutions of the experimental setup of 30 nm at 17 KeV and 0.3 s exposure time were performed. The local scans were used to image the crystal habit of the axial filament at different regions of the spicule. The holotomographic datasets were phase retrieved to quantify local structural variations in density and reconstructed to create 3D information on the protein crystal. Finally, the different scans were merged to provide comprehensive information of the shape of the axil filament during its formation.

Results
The information presented in Figure 2 demostrates our preliminary analysis of the obtained data, where we not only succesfully follow the shape of the protein crystal along its long axis, but also extract quantitative information on its relative shape changes during growth. Our further analysis of the filament using Bragg-CDI on ID01 will allow us to coralate between these morphological changes with the behvaiour of the protein lattice.

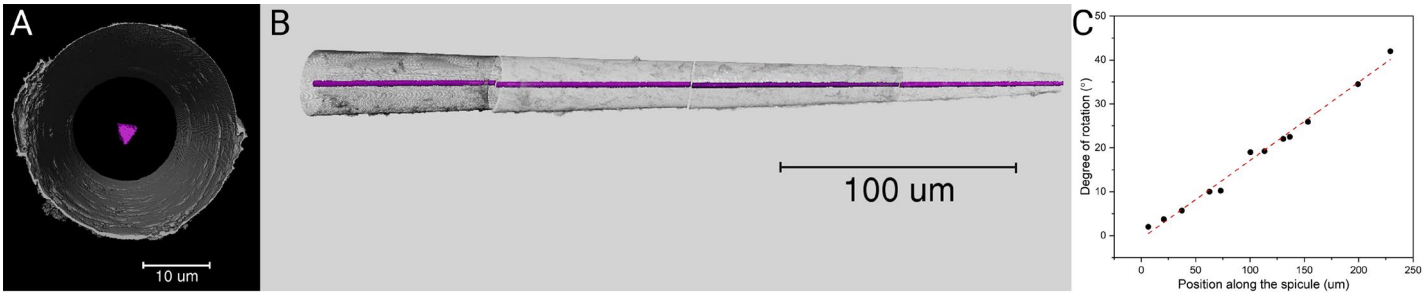

Figure 2: *(A) A cross section of the axial filament (purple) from the needle like spicule of sirrounded by amourphous silica the edge of which is marked in grey. (B) 3D reconstruction of the entire needle like spicule. (C) The magnitude of the rotation of the triangular crystal habit of the axial filament across the entir espicule showing a permanent twist of 0.17 degrees per micron.*

References

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