	Experiment title: Lattice rotations in the hybrid silica/protein crystals forming the skeletons of Demosponges	**Experiment number**: SC-4899
Beamline: ID13	**Date of experiment**: from: 27.10.2018 to: 30.10.2018	**Date of report**: 01.10.2020
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Background:

Nature is known to form a variety of complex silica-based architectures with exquisite structural control by the means of organic-template assisted biomineralization. For example, the skeletal structure of siliceous sponges consists of amorphous silica containing spicules. The spicules are built around a thin (usually less than 1 micron in diameter) inner axial filament consisting of enzymatically active protein, silicatein, that is responsible for the biomineralization of silica.[1] Recently, we have shown that, in the giant axial filament of the anchor spicule of the sponge *Monorhaphis chuni*, belonging to the sponge class Hexactinellida, the silicatein self-assembles into a perfectly ordered body-centered tetragonal structure.[2] Furthermore, using the Eshelby twist theory, we have demonstrated that this perfect protein crystal growth with the assistance of a screw dislocation situated at its core (the Barton-Cabrera-Franck mechanism).[3] According to this postulation, a screw dislocation located at the center of a slender crystal is expected to induce an elastic strain field that will lead to the twist of the lattice of that crystal. Indeed, our previous work demonstrated the existence of the twist in the filament of *Monorhaphis chuni*. The main objective of this beamtime was to investigate the crystalline nature of axial filaments in a variety of sponges from the class Demospongiae and, thus, generalizing this unique biomineralization scheme to all sponge species made of amorphous glass. Two specific goals were defined: (i) identifying the crystallographic properties of the axial filaments taken from the body of five different demosponge species (*Tethya aurantium, Stryphnus ponderosus, Rhizaxinella pyrifera, Geodia cydonium and Clathria prolifera*)[4] and (ii) registering an elastic Eshelby twist of the protein lattice that would suggest the presence of a screw dislocation at the core of the filament in all the studied species.

Experiments and Setup at ID13

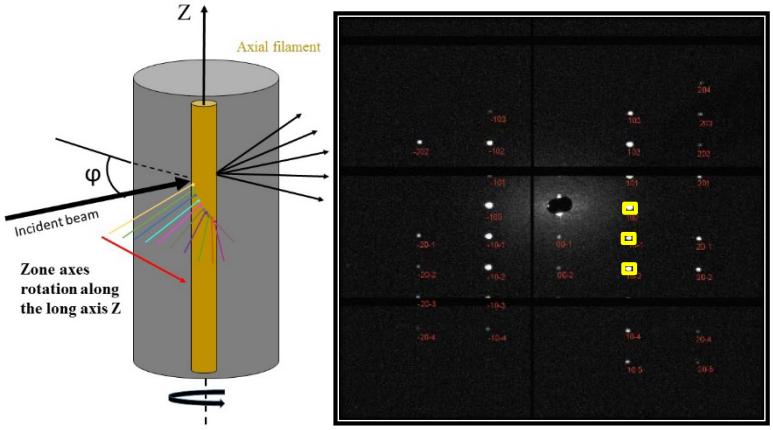
We used a monochromatic beam (13keV) and x-ray optics providing a sub-micrometer beam size. The covered q-range was approximately 0.2 to 15 nm^{-1}. Beamline ID13 at the ESRF perfectly met the high requirements (beamline optics, sample environment and stage, detector) for our experiment. Due to small dimensions of the axial filaments (micron range and below), we required a sub-micron focused beam. The scanning setup provided a step size in the range of the beam size (200 nm) and rotation ability that was necessary to follow the filament and the zone axis rotation along the filament. High-speed detectors together with a fast shutter were required in order to avoid significant radiation damage. We received 9 shifts, which included the time for samples

alignment, absorption scans to detect the location of the filaments inside the spicules and measurements at different positions along the filament in the different spicules.

Analysis and Results

The experiments were performed by mounting a needle-like spicule from all five investigated sponges with their long axis perpendicular to the incident beam. Then the center of each spicule was raster scanned using a mesh of 9 × 11 200 nm spots with a step size of 0.5 micron. This experiment was repeated 60 times by rotating the spicule around its long axis with an angular step of 0.1 degree and, thus, covering a range of 6 degrees (Fig. 1a). Therefore, a total of 5940 diffraction patterns per spicule were collected. The purpose of the measurement was twofold: (i) by creating a max projection using all of the diffraction patterns from a single spicule, the packing of the protein crystal in each species can be determined (Fig. 1b) and (ii) By following the max intensity of a single diffraction spot, as marked in Fig. 1b, we could determine the twist of the zone axis along each spicule (Fig. 1a). The results of these experiments are summarized in Fig. 1c.

First, astonishingly, the lattice parameters of the protein super-structure inside the axial filaments of all five different species were determined to be similar. In all, the protein crystal exhibits a hexagonal packing with similar lattice parameters. Second, all the species exhibited a lattice twist that was consistent by following a number of diffraction spots. As postulated, this twist suggests that the axial filaments comprising perfect protein crystals form using screw-dislocation assisted growth mode.



Demosponge	Lattice Parameters	Intensity maps of Lattice reflections	Angle of Lattice twist (Zone axis rotation)
Tethya aurantium	a = 5.82 ± 0.04283 nm	(100)	1.26306°/µm
	c = 11.82 ± 0.1055 nm	(10-2)	1.28338°/µm
		(10-1)	1.26306°/µm
Stryphnus ponderosus	a = 5.96 ± 0.0109 nm	(100)	2.35204°/µm
	c = 11.557 ± 0.008 nm	(101)	2.46515°/µm
		(102)	2.24514°/µm
Rhizaxinella pyrifera	a = 5.95 ± 0.009 nm	(100)	0.96195°/µm
	c= 11.46495+0.208 nm	(-10-1)	0.96835°/µm
		(10-1)	0.98436°/µm
Geodia cydonium	a = 5.98 ± 0.02 nm	(100)	1.39693°/µm
	c = 11.47 ± 0.019 nm	(-102)	1.31766 °/µm
		(-103)	1.34811°/µm
Clathria prolifera	a = 5.93 ± 0.0268 nm	(100)	0.64988°/µm
	c=11.516± 0.04836 nm		

Fig.1. (a) A schematic representation of the experimental set-up. (b) Max-projection of diffraction patterns obtained from the axial filament in the sponge *Tethya aurantium*. Yellow squares mark the diffraction spots that were used to follow the twist of the lattice. (c) Table summarizing the lattice parameters and the magnitude of lattice rotations from all five studied sponge species.

Impact

The current study provided unprecedented information on a unique biomineralization scheme in which the organism employs highly ordered protein structures to morph intrinsically disordered amorphous mineral phase – silica. Currently, a high resolution TEM tomography study is underway to directly visualize the presence of a screw dislocation in the lattices of the studied organisms. Finally, a master thesis was successfully written based on this work. The manuscript is currently in preparation.

References

1. Shimizu, K., Cha, J., Stucky, G. D. & Morse, D. E. Silicatein α: cathepsin L-like protein in sponge biosilica. *Proc. Natl. Acad. Sci.* **95**, 6234–6238 (1998).
2. Zlotnikov, I. *et al.* A perfectly periodic three-dimensional protein/silica mesoporous structure produced by an organism. *Adv. Mater.* **26**, 1682–1687 (2014).
3. Zlotnikov, I., Werner, P., Fratzl, P. & Zolotoyabko, E. Eshelby twist as a possible source of lattice rotation in a perfectly ordered protein/silica structure grown by a simple organism. *Small* **11**, 5636–5641 (2015).
4. Schoeppler, V. *et al.* Shaping highly regular glass architectures: A lesson from nature. *Sci. Adv.* **3**, eaao2047 (2017).