ESRF	**Experiment title:** Analysis of Periodic 3D Protein/Silica Nanostructures Produced by Siliceous Sponges	**Experiment number**: **SC-4203**
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Report:

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. Recently, we have shown that, in the giant axial filament of the anchor spicule of the sponge *Monorhaphis chuni* (*M. chuni*, Fig. 1a), the silicatein self-assembles into a perfectly ordered body-centered tetragonal structure (*1*). The objective of this beamtime was to investigate the crystalline nature of axial filaments in a variety of spicules from two classes of siliceous sponges, thus, shedding light on a fundamentally novel biomineralization concept involving the growth of highly ordered mesoscale structures in the presence of proteins and putting these data in an evolutionary perspective.

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

We analyzed three types of spicules with increasing levels of spatial complexity taken from three different sponges. First were the needle-shaped megascleres from the sponge *Tethya aurantium* (Pallas, 1766) known as strongyloxea. The second type of spicules that we analyzed were megascleres from the sponge *Stryphnus ponderosus* (Bowerbank, 1866) called dichotriaenes. Finally, the third type were microscleres, called sterrasters, from *Geodia cydonium* (Linnaeus, 1767). To study the crystalline properties of the axial filament in these spicules, we performed an x-ray diffraction analysis using the small-angle x-ray scattering (SAXS) setup on ID13. Exceptionally sharp diffraction patterns collected from *T. aurantium* (Fig. 1A) and the main shaft of the megasclere in *S. ponderosus* (Fig. 1B) confirmed the highly regular single-crystalline nature of the axial filament in the spicules of these organisms. In both cases, by solving the diffraction patterns we found that the proteins are packed in a hexagonal crystal structure with lattice parameters of a = 5.95 ± 0.01 nm and c = 11.89 ± 0.01 nm in the case of *T. aurantium* and a = 5.96 ± 0.01 nm and c = 11.45 ± 0.04 nm in the case of *S. ponderosus*. Because of structural complexity of the sterrasters from the demosponge *G. cydonium*, it was not possible to perform diffraction experiments on a single axial filament. Therefore, we used the x-ray setup to accumulate a diffraction signal while raster-scanning an entire mature spicule. Because the axial filaments in the sterraster point radially in all directions, we obtained a ring-like diffraction pattern characteristic of a polycrystalline material (Fig. 1C). Similar to the axial filaments in *T. aurantium* and *S. ponderosus*, the proteins in *G. cydonium* are packed in a hexagonal crystal structure with lattice parameters of a = 5.93 ± 0.01 nm and c = 11.97 ± 0.05 nm. This data allowed us to solve an outstanding problem of silica sponge morphogenesis and to develop a model for branching principles of glass spicules in marine sponge(*2*).

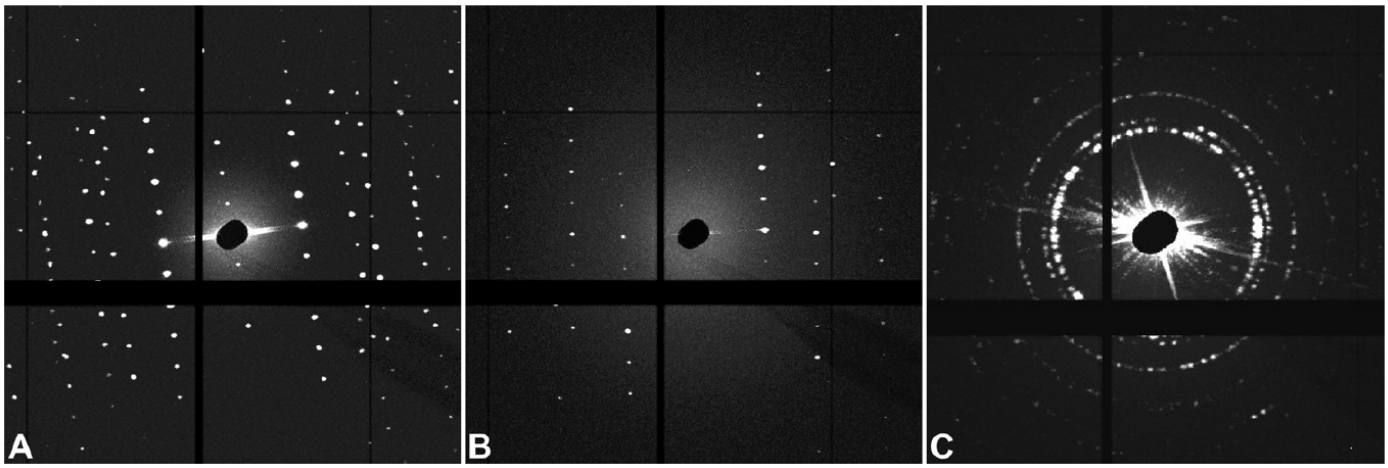

Fig. 1. X-ray diffraction analysis of protein crystals comprising the axial filaments of demosponge spicules. (A) X-ray diffraction pattern acquired from the strongyloxea from the demosponge *T. aurantium*. The pattern was obtained by accumulating the diffraction data while rotating the spicule around its long axis within an angular interval of 70°, the rotation axis being perpendicular to the incident beam. (B) X-ray diffraction pattern acquired from the main shaft of the dichotriaene from the demosponge *S. ponderosus*. The pattern as obtained by accumulating the diffraction data while rotating the spicule around the long axis o within an angular interval of 70°, the rotation axis being perpendicular to the incident beam. (C) X-ray diffraction pattern acquired from a mature sterraster from the demosponge *G. cydonium*. The pattern was obtained by raster-scanning the entire spicule with the incident beam perpendicular to the rastering plane.

1. I. Zlotnikov *et al.*, *Adv. Mater.* **26**, 1682–1687 (2014).
2. V. Schoeppler *et al.*, *Sci. Adv.*, **3** (10), eaao2047 (2017).