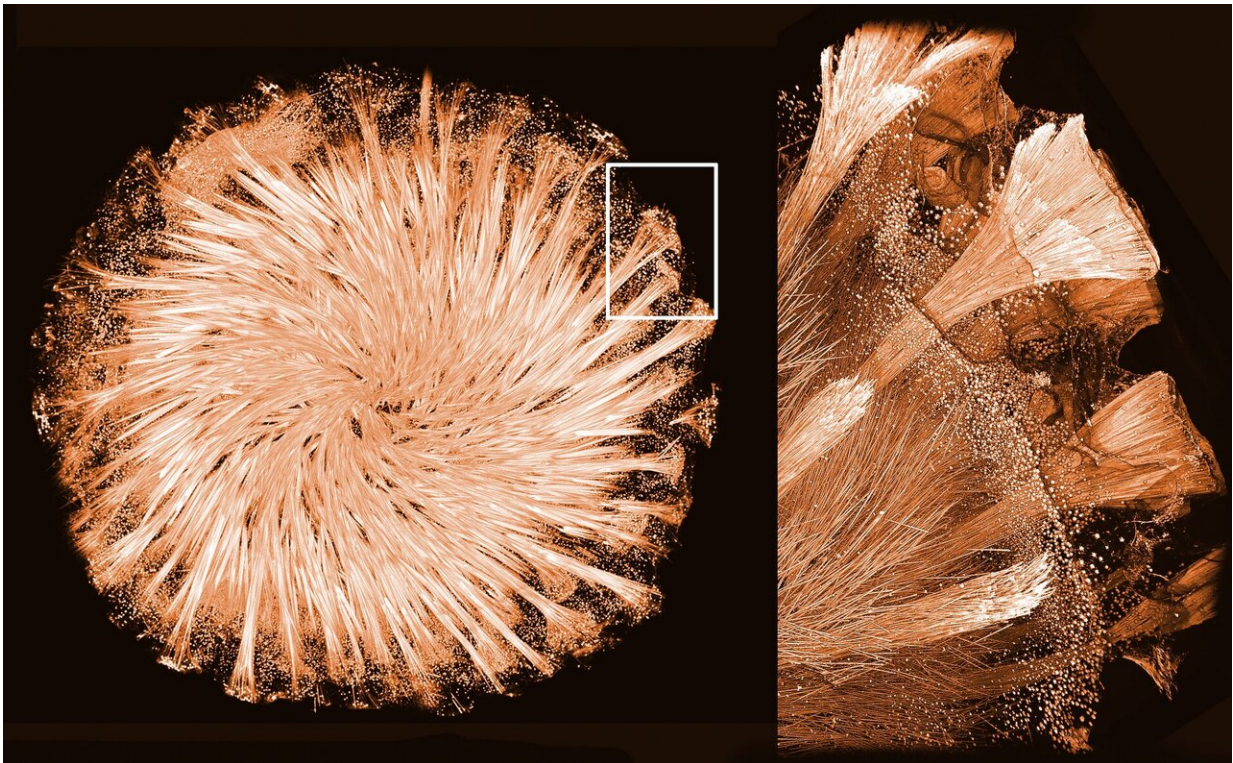


Scientists determine the structure of glass-shaping protein in sponges

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Glass sponge. Credit: Ronald Seidel/Igor Zlotnikov

Sponges are some of the oldest animals on Earth. They live in a wide range of waters, from lakes to deep oceans. Remarkably, the skeleton of some sponges is built out of a network of highly symmetrical glass structures. These glass scaffolds have intrigued researchers for a long

time. How do sponges manipulate disordered glass into the skeletal elements which are so regular? Researchers from B CUBE—Center for Molecular Bioengineering at TU Dresden together with the teams from the Center for Advancing Electronics Dresden (cfaed) and the Swiss Light Source at the Paul Scherrer Institute in Switzerland are the first to determine the three dimensional (3-D) structure of a protein responsible for glass formation in sponges. They explain how the earliest and, in fact, the only known natural protein-mineral crystal is formed. The results were published in the journal *PNAS*.

Glass sponges—as the name suggests—have a glass-based skeleton composed of a network of glass needles, hooks, stars, and spheres. To achieve such a unique architecture they have to manipulate the shape of disordered glass to form highly regular and symmetrical elements. Thin crystalline fibers made of a protein, known as silicatein, are present in channels inside of these glass elements. It is known that silicatein crystals are responsible for glass synthesis in sponges and for shaping the glass skeleton. However, until now efforts to determine the 3-D structure of this protein, describe how it assembles into crystals, and how those form the glass skeleton were unsuccessful. Mainly, because nobody was able to reproduce these crystals in the lab.

A team of researchers led by Dr. Igor Zlotnikov from the B CUBE—Center for Molecular Bioengineering at TU Dresden tried an unusual approach. Instead of producing silicatein in the lab and trying to obtain lab-grown crystals to study the structure, the researchers decided to take the glass needles from a sponge skeleton and analyze the tiny crystals that already exist inside.

The Zlotnikov group together with researchers from the Dresden Center for Nanoanalysis (DCN) at the Center for Advancing Electronics Dresden (cfaed) used high-resolution transmission electron microscopy (HRTEM) to take a closer look at silicatein crystals packed inside the

glass needles. "We have observed an exceptionally ordered and at the same time complex structure. Analyzing the sample we have seen that it is a mixture of an organic and inorganic matter. Meaning that both proteins and glass form a hybrid superstructure that somehow shapes the skeleton of [sponges](#)," explains Dr. Zlotnikov.

A traditional way of obtaining a 3-D structure of a protein is to expose its crystal to a beam of X-rays. Each protein crystal scatters the X-rays in a different way providing a unique snapshot of its internal arrangement. By rotating the crystal and collecting such snapshots from many angles, the researchers can use computational methods to determine the 3-D protein structure. Such an approach is widely used and is the basis of modern structural biology. It works well for crystals of at least 10 microns in size. However, the Zlotnikov group wanted to analyze silicatein crystals which were about 10 times smaller. When exposed to X-rays they were almost immediately damaged, making it impossible to collect a complete data set of snapshots from multiple angles.

With support from the team at PSI's Swiss Light Source (SLS), the researchers used a new emerging method known as serial crystallography. "You combine diffraction images from many crystals," says Filip Leonarski, beamline scientists at PSI, who was involved in the study. "With the traditional method you shoot a movie. With the new method you get many snapshot which you combine afterwards to decipher the structure." Each snapshot is taken at a different part of the tiny crystal or even from a different crystal.

In total, the researchers collected more than 3500 individual X-ray diffraction snapshots from 90 glass needles at completely random orientations. Using state-of-the-art [computational methods](#) they were able to find order within the chaos and assemble the data to determine the first complete 3-D structure of silicatein.

"Before this study, the structure of silicatein was hypothesized based on its similarity to other proteins," says Dr. Zlotnikov. Using the newly obtained 3-D [structure](#) of silicatein, the researchers were able to understand its assembly and function inside the glass skeleton of the sponge. They built a computational model of the superstructure within the glass needle and explained the initial complex images of the protein-glass superstructures obtained with the HRTEM.

"We provided detailed information on the existence of a functional 3-D protein-[glass](#) superstructure in a living organism. In fact, what we describe is the first known naturally occurring hybrid mineral-[protein](#) crystalline assembly," concludes Dr. Zlotnikov.

More information: Stefan Görlich et al. Natural hybrid silica/protein superstructure at atomic resolution, *Proceedings of the National Academy of Sciences* (2020). [DOI: 10.1073/pnas.2019140117](https://doi.org/10.1073/pnas.2019140117)

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