EUROPEAN SYNCHROTRON RADIATION FACILITY
INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF	**Experiment title:** Identifying the proteins involved in biomineralization through the structural analysis of mollusc shells grown in sub-optimal conditions using 3D Bragg ptychography	**Experiment number**: EV326
Beamline: ID13	**Date of experiment**: from: 4/07/18 to: 8/07/18	**Date of report**: 1/03/20
Shifts: 6	**Local contact(s)**: T. Gruenewald	*Received at ESRF:*

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Report:

The EV326 proposal was allocated beamtime together with the EV327 proposal. As it was not possible to perform a full experiment in only 6 shifts, we decided to use the whole allocated beamtime to perform the EV326 proposal. This choice was motivated by the advent of the whole biomineralization project.

This proposal aimed at investigating the mysterious mechanisms of biomineralization. To this end, we proposed to establish the 3D crystalline structures of calcareous mollusc shells raised in several sub-optimal (T°, pH) conditions. Those physico-chemical conditions were expected to affect the mineralization mechanisms and thereby modify the mollusc metabolism, which should adapt through production of biomineralizing organic molecules. Hence, the environmental conditions, which most perturb the biomineralizing processes, at the nanometer scale, should be pointed out. Proteomic and transcriptomic studies were planned on the perturbed molluscs, to identify the biomineralizing molecules. Our strategy relied on 3D Bragg ptychography (3DBP), in order to elucidate the crystalline structure at the most generic length scale for biomineralization. Indeed, within a large single-crystalline prismatic units, AFM shows the systematic presence of an organo-mineral granular structure, which underlines the generics of the biocrystallization process as well as the length-scale at which to focus.

For this experiment, a large series of samples were produced by our collaborators (IFREMER, French Polynesia) under 9 different physico-chemical conditions (T = 26, 29 and 32°C and pH = 7.4, 7.8, 8.2). The shells were mounted onto a metallic pin and previously characterized with polarized optical microscopy and coherent Raman. Depending on the raising conditions, It was clear that the shells present very different structural properties with respect to the reference shell (T = 29°C and pH = 8.2).
In order to align the samples in Bragg conditions and perform Bragg ptychography, we used the Eiger 4M detector on the trail. For nanodiffraction, the camera was placed at about 0.1m from the sample holder and located at about 2 m for the Bragg ptychography data acquisition.

In total, four samples were investigated with nanodiffraction and 3D Bragg ptychography. They were chosen among the most extreme raising conditions, as those conditions are expected to present the most stricking differences with the reference shell. As expected from the preliminary characterisation, the crystalline quality were degraded (large angular spreading) and a large angular range was needed to explore the full rocking

curve. It resulted in long measurement times and rocking curve of more than 600 angular steps. The analysis is on the way and some preliminary 3D reconstructions are presented in Figure 1.

Note that proteomics analysis has been done in the meantime and evidenced the possibility to extract observable signals regarding anomalies in the protein production, under sub-optimal conditions. On the contrary to our expectations, it was not possible to evidence the up- or down-regulations of one a few sets of systematic proteins, among the few 20-30 identified proteins. It likely indicates that the various proteins acts at different steps of the biomineralization cycle. In order to sort this out, a multi-scale structural analysis of a large number of shells has been initiated, combining chemical and crystalline structural sensitive methods.
Now, identifying the proteins at play at the most fundamental length scale of biomineralization (the granules), critically relies on our capability to investigated a large number of shells with Bragg ptychography. Given the progresses done on our side on the method (large field of view, sparse data set) and the increased brilliance of the ESRF-EBS, we now expect to be able to perform a full Bragg ptycho scan in about 1 hour.

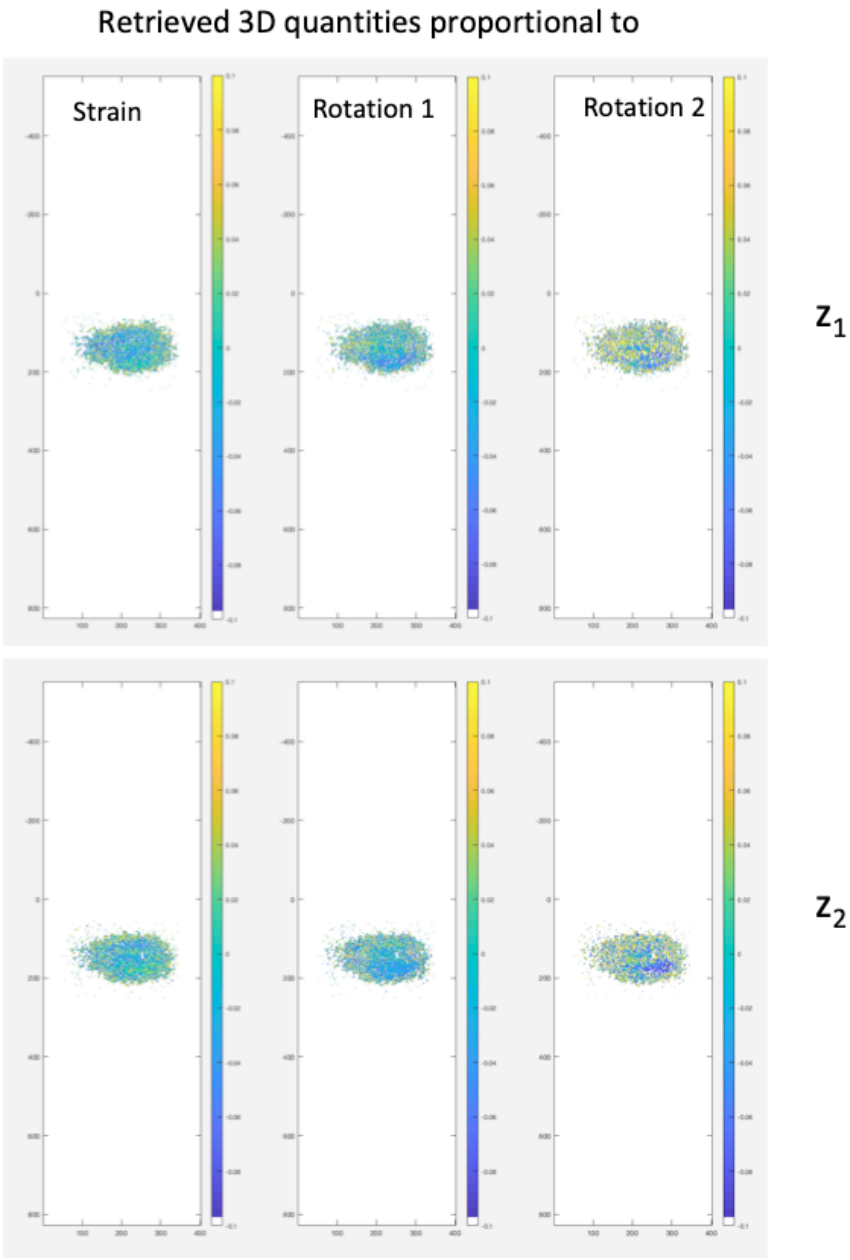

Figure 1: 3D Bragg ptychography recontruction on shell #88 (pH = 7.4 and T = 32°C). The cross sections along the (x,y) plane are shown for two different positions along z (a): z1 and (b) z2. The shown quantities are proportional to the strain, and the two lattice rotations around the y and x axis, respectively. Additional efforts are needed to reduce the noise in the reconstruction. However, some iso-strain and iso-oriented domains can already be distinguished.