EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Effect of hydration on the structure of spiders cuticular hair sensilla	**Experiment number**: SC 5329
Beamline: ID13	**Date of experiment**: from: 14.02.2023 to: 17.02.2023	**Date of report**: 17.05.2023
Shifts: 9	Local contact(s): MELNIKOV Alexey	*Received at ESRF:*

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Report:

The experiment SC 5329 was performed on the nanobranch of ID13 with a beam cross-section of (70x70) nm² at an energy of 13 keV with exposure times usually 50 ms per point. The goal of the experiment was for perform high resolution (at sub-micron scale) XRF/ XRD mapping of tactile hair sensilla of the spider *Cupiennius salei* to study the chitin-protein structure and composition of these mechanosensors. We were able to use again the fibre-tracking macro developed by the beam line scientist, during our previous experiment, that allowed us to acquire of multiple maps region along shaft of a hair sensillum in an automated fashion. This experiment was complementary to the measurements previously performed in experiment SC 5187, since in the recent experiment we focused on the role of water within the hydrated hair sensilla. Additionally, besides samples listed in the initial proposal we were able to study the sensilla in an actuated state in dry and hydrated conditions. Also, submicron resolution maps of hair shaft cross-sections were studied looking into the distribution of metal and halogen ions with XRF. Due to the good support of the beamline scientist and we were able to was to measure all planned samples and a few additional ones.

Results

The goal is to quantify chitin/protein ratio, the chitin fibre orientation and compare it between the different regions along the hair. For that, the tactile hair sensilla were dissected from the tarsus of *C. salei* keeping the hair socket intact, which anchors the sensillum in the cuticle. For that, the cuticle was manually cut into sections with a thickness of less than 50 μm but with a length of up to 1 mm. The samples were glued away from the region of interest (ROI) to silicon nitride (SiN) membranes (1 μm thickness) to provide a mechanical support without compromising signal/noise and increasing background signal. Two different approaches were used to hydrate the sample; in the first approach, the cavity of the SiN membrane was filled with deionized and filtered water, than vanillin was applied on the frame and closed with a second membrane. The membrane sandwich was than sealed with super glue or nail polish. This setup ensured the hydration of the sensilla, but had the drawback, that gas bubbles formed at during acquisition of high-resolution maps, which can move the sensilla during the measurement. To prevent this we also prepared membrane sandwiches in which we placed a hydrogel prepared form low melting agarose (1.5 % wt), to facilitate a humid environment for the sensillum, which indeed corresponds better to the natural

state. The risk of this sample setup is condensation at the membrane, however, we did not observe any interference during the measurements.

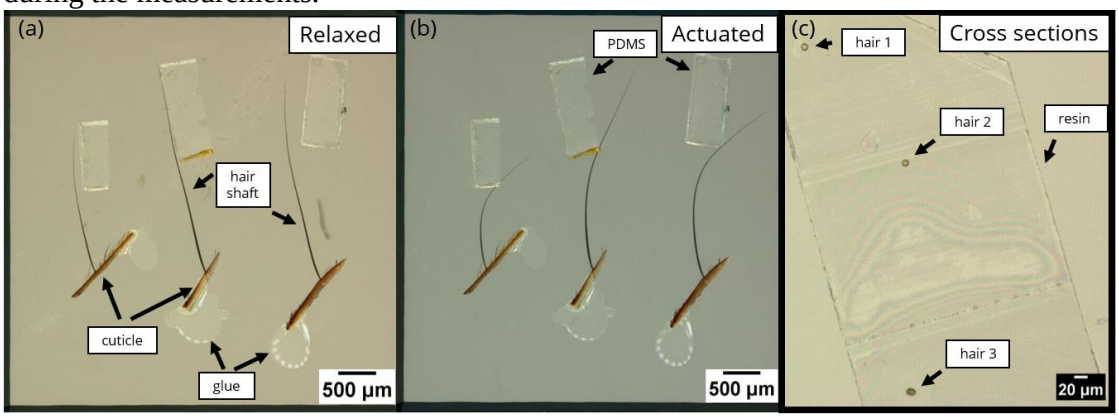

Figure 1: SiN membrane with glued tactile hair sensilla and PDMS blocks (a). Tactile hair are bend and fixed behind a PDMS block (b). Membrane with embedded tactile hair cross-sections (c).

As we were interested if the actuation on the sensilla had an impact on the fibre architecture samples with bend sensilla were prepared. For that the sensilla were glued in their relaxed state on the membrane then a small block of PDMS, which was cut out from a 100 µm thick PDMS film, as placed next to the sensillum and it was bend behind the block (Fig. 1b). These samples were also measured in under hydrated conditions. To understand the distribution of naturally occurring halogen and metal ions in the hair shaft, individual sensilla were embedded in EpoFix Resin and cut into 10 µm cross-sections, which also were placed on SiN membranes.

To detect the typical chitin-protein fibre diffractions we measured at a q range ranging from 0.1 to 40 nm^{-1}. Compared to the previous experiment, we increased the exposure time to 50 ms to improve the signal-to-noise ratio, considering the radiation damage. The XRF detector was optimized for each sample to obtain the best possible outcome. In Fig. 2 a&b 2D XRD plots of a dry and hydrated sensillum are shown, respectively. The signal was averaged over different areas of a single map and azimuthally integrated. The orange plot shows the respective background signal which will be subtracted and the individual chitin-(protein) peaks will be fitted. Fig. 2 c shows a SAXS intensity map (q range 0.2 -2 nm^{-1}) of an intact and dry hair. Based on the interfaces of the hollow cavity in the hair shaft, which are indicated by the yellow arrowheads, the cuticle facing and contact side of the hair were defined, respectively. The panel in Fig. 2d shows XRF maps of a hair cross-section for selected ions. It can be clearly seen that for example manganese is found in the periphery of the hair and the microtrichs while zinc is located at two regions in the shaft itself.

We aim to correlate the distribution of ions and the chitin-protein architecture of the tactile hair sensilla with their mechanical properties in future. Additionally, we performed high-resolution maps XRD/XRF of *C. salei* tibia section as well as course maps of the sections prepared from locust ovipositor.

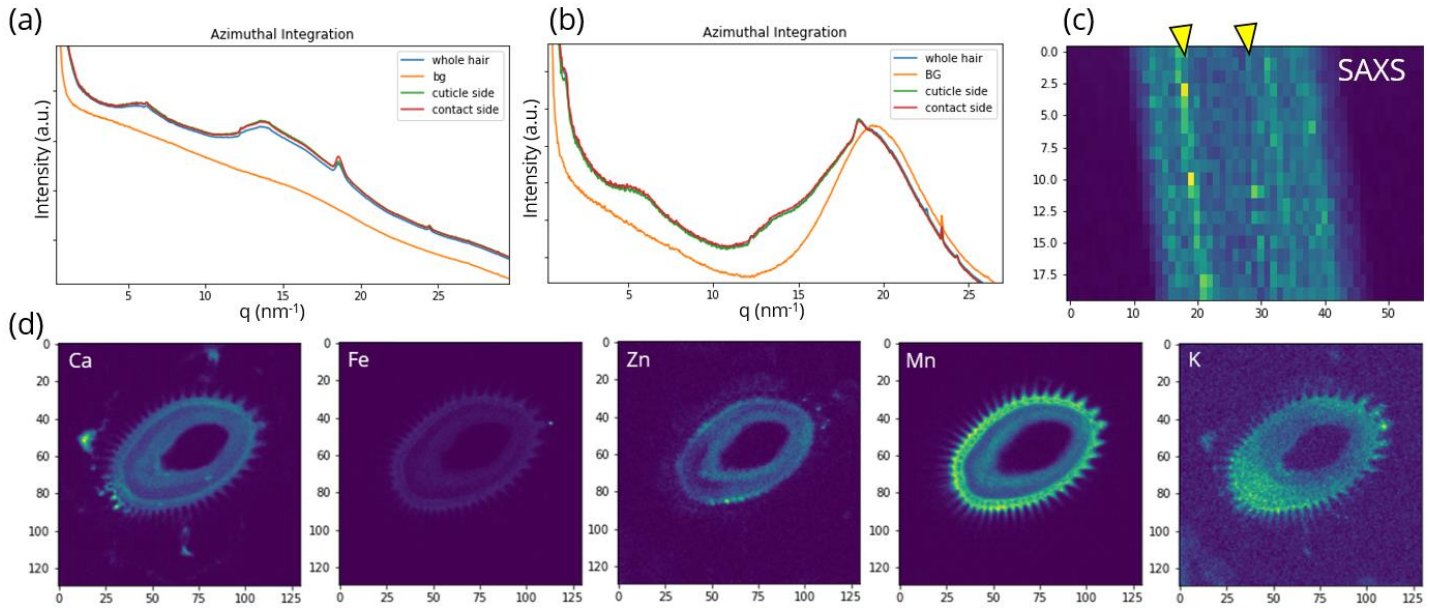

Figure 2: Integrated XRD signal plots in of dry (a) and hydrated (b) tactile hair sensilla. SAXS signal (q range 0.2-2 nm^{-1}) intensity map of a sensillum (c). High-resolution (200 nm step size) XRF maps of a tactile hair cross-section, showing the distribution of calcium, iron, zinc, manganese and potassium, respectively (d).