EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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.

<table>
<tr><td>ESRF</td><td>Experiment title:
Resolving the structure and composition of spiders cuticular hair sensilla</td><td>Experiment number:
SC 5197</td></tr>
<tr><td>Beamline:
ID13</td><td>Date of experiment:
from: 31.05.2022 to: 02.06.2022</td><td rowspan="2">Date of report:
02.09.2022
Received at ESRF:</td></tr>
<tr><td>Shifts:
9</td><td>Local contact(s): BURGHAMMER Manfred</td></tr>
</table>

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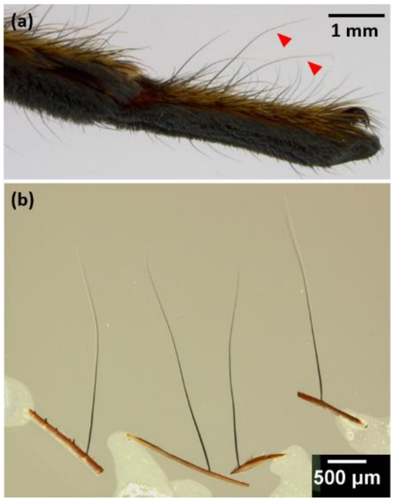
Report:

The experiment SC 5197 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 40 ms per point. The goal of the experiment was to perform high-real-space resolution (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. To study structure along the curved hair shaft the beam line scientist developed a fibre-tracking macro, which allowed us to acquire the data in a semi-automated manner. Starting from the socket of the sensilla, maps were acquired moving along the hair shaft with a defined spacing. Also, we are very grateful for the additional measurement time, which together with the efficient scanning procedure enabled us to measure further sample environment for our follow up experiment SC 5329.

<u>Results</u>

To study the chitin-protein architecture of the tactile hair sensilla individual tactile hair from the tarsus of *C. salei* were dissected with the cuticle embedding the socket of the sensillum. To assure reproducibility only the two longest tactile hairs, which can be found on every tarsus of a sub-adult and adult *C. salei*, were used (red arrowheads Fig. 1a). The cuticle sections were cut manually to 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 membranes (1 µm thickness) to provide a mechanical support without compromising signal/noise and increasing background signal (Fig. 1b).



To detect the typical chitin-protein fibre diffractions we measured at a q range ranging from 0.1 to 40 nm^{-1}. We determined the optimal exposure time and step size, which enabled us to obtain a good signal-to-noise ratio while reducing the radiation damage of the sample. Herein, the challenge was the decreasing diameter of the hair towards the tip, however, with 40 ms the diffraction pattern still showed signal of the chitin-protein fibres (Fig. 2 f,g). Fig 2 a-c depicts representative WAXS intensity maps of different region of the hair, the hairs socket, shaft and tip, respectively. The high-resolution maps of the WAXS intensity of the regions of the socket and hair shaft reveal details as the membrane suspending the hair or the hollow region within the shaft and its asymmetric structure. While the signal-to-noise ratio becomes worse towards the tip, averaging is done to improve the signal quality. 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 diffraction patterns are integrated and background subtracted. Then the resulting profiles will be exported for peak fitting in a customized tool developed in our group.

Besides, we detected the XRF, which due to the small beam size, provide us with high resolutions maps of different naturally occurring halogen and metal ions. We observed diverse distribution of elements, while for instance manganese was distributed homogenously (Fig. 2d) zinc was found predominantly at the convex side of the tactile hair shaft. Also, at the base of the sensillum we found that the socket and the membrane suspending the tactile hair were richer in zinc compared to the surrounding tissue. However, as only intact tactile hairs were studied the distribution across the hair shaft, as variation from the centre to the periphery could not be seen.

Due to the generous extension of the experiment time, we were able to measure additional samples besides the ones initially planned. With regard to the follow up experiment SC 5329 we tested high-resolution SAX/WAXS mapping of fully hydrated hair sensilla as well as *C. salei*. of pad long sections, tibia long section, diatom biofilms and weevil scales.

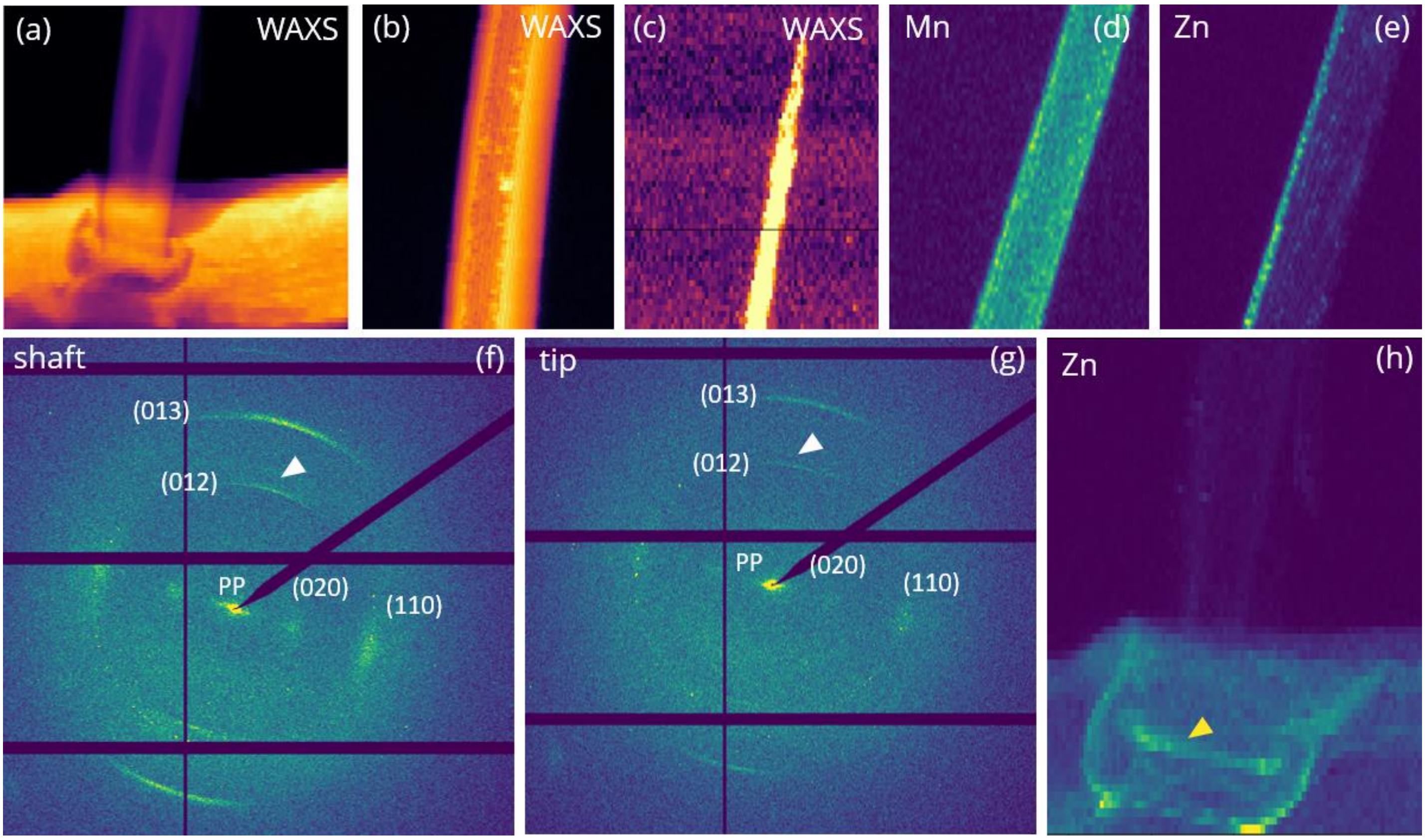


Figure 2: WAXS (021) signal intensity maps (0.5 *1 µm step size) of three regions along the hair, from base to tip (a-c). Diffraction patterns obtained at base (f) and tip (g). The white arrowheads indicate the protein peak, PP is the packing peak of chitin-protein fibres. XRF signal for manganese (d) and zinc at the hair shaft (e) and zinc at the socket (h), yellow arrowhead indicates the hair suspending membrane.