ESRF	**Experiment title:** The ultrastructure of *Loxosceles* silk ribbons	**Experiment number**: **LS-3155**
Beamline: ID13	**Date of experiment**: from: 17 February 2023 to: 19 February 2023	**Date of report**: 28/2/2023
Shifts: 9	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Christian Riekel: ESRF
Karolina Peter: Univ. Natural Resources & App. Life Sc.- BOKU, Vienna, Austria
Sakr Charbel: Univ. Natural Resources & App. Life Sc.- BOKU, Vienna, Austria
Sarah Isabella Stadlmayer: Medical University of Vienna, Austria
Hidaji Aida Naghilouye: Medical University of Vienna, Austria
Hannes Schniepp (remote): William&Mary, Nano & Biomaterials lab, Williamsburg, Virginia, USA

Report:

The aim of the proposal is to collect diffraction patterns from single *Loxosceles* silk ribbons with a width of 6-8 microns and a thickness of about 50 nm in order to determine its hierarchical structural organization. Scanning SAXS/WAXS experiments were performed in transmission geometry at the nanofocus branch of the ID13 beamline using a ~70 nm (hxv) beam of about $2x10^{11}$ ph/s @ 13.0 keV.

Selected samples provided by the co-proposer Prof. H. Schniepp and his group are shown in Figure 1a-c. For the most challenging sample of single ribbons on a Si_3N_4 substrate, promising areas with "flat" ribbons (see Fig. 1c) documented at Schniepp' s lab, could be readily localized on-line using the ID13 beamline microscope.

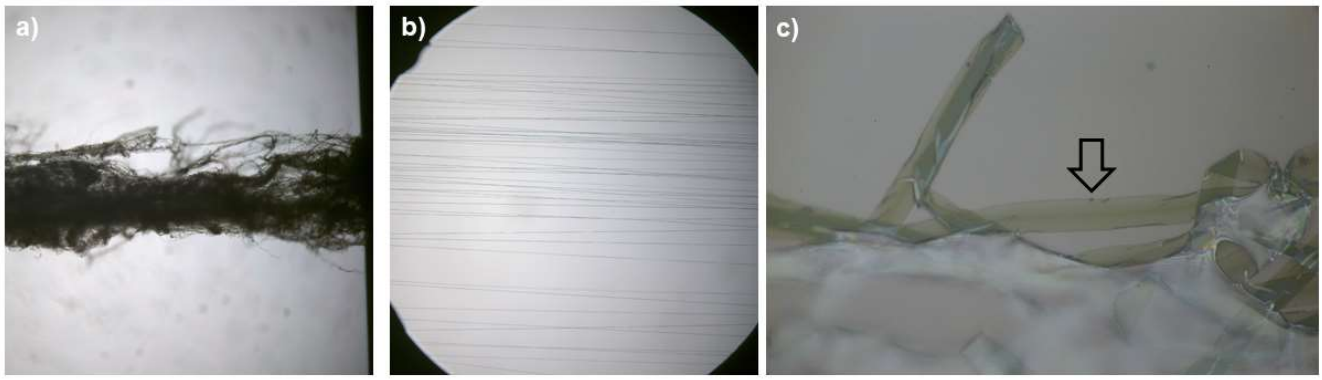

Figure 1 a) Raw *Loxosceles* web silk stretched on Si_3N_4 membrane. b) *Loxosceles* ribbon obtained forced silking of spider, wound several times around metal O-ring. c) Area of Si_3N_4 membrane with deposited *Loxosceles* ribbons. The arrow indicates a flat ribbon of about 8 μm diameter and 50 nm thickness.

Scattering patterns were recorded with -typically- 10-20 ms exposure to limit radiation damage. All samples shown in Figure 1a-c were probed for a detector-to-sample distance covering the WAXS-range. Single, flat ribbons were also probed at a larger detector distance, optimized for SAXS.

A short summary of the experiments performed is provided below. A detailed analysis of the recorded patterns is in progress.

(1) ***Raw bundle of Loxosceles silk collected from a web.***

The aim was obtaining a well-defined pattern for comparison with the patterns from single ribbons. WAXS patterns with fiber texture but with significant azimuthal spread were recorded at several positions of a raw bundle of ribbons from a web (Figure 1a.).

(2) ***Single ribbon extruded from Loxosceles spiders by forced silking, wound around metal O-ring.***

The aim was obtaining highly oriented SAXS/WAXS patterns by averaging multiple patterns from a single ribbon. Scanning SAXS/WAXS was performed with lateral resolution down to 50 nm normal to the ribbon's axis and down to 500 nm along the ribbons axis to limit radiation damage propagation. The ribbon could be imaged at the scanned areas by density projections based on the strongest equatorial peaks or SAXS. Preliminary analysis of a single scanned area scanned revealed the strongest (020)/(210) equatorial peaks of the poly(L-alanine) structure with a narrow angular spread.

(3) ***Single extruded ribbons deposited on Si_3N_4 substrates of 1-micron thickness.***

The aim was exploring in particular the hierarchical organization at the ribbons interface. Multiple ribbons were scanned with the same lateral resolution as for (2), revealing SAXS& WAXS density projections.