	Experiment title: Structural investigations of chitin fibers in adhesive hairs using nanodiffraction	**Experiment number**: SC-3736
Beamline: ID13	**Date of experiment**: from: 10.11.2013 to: 12.11.2013	**Date of report**: 03.03.2014
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Report:

We investigate hairy attachment systems of spiders, insects, and geckos, which enable these animals to walk upside down on plants, walls, on rough and smooth surfaces, and support a multiple of the body weight without the use of glue. These outstanding biological structures comprise of pads including hundreds to thousands of specially designed hairs that are made of composite materials consisting of proteins and reinforcing chitin or keratin fibres. The goal is to gain an in depth understanding of the working principle of the attachment and detachment processes of single hairs to a surface, which is likely based on the structurally determined material properties of the contact elements at the tips of the hairs. The accompanying changes of the shape of the contact elements and their self-orientation in parallel with a surface lead to the establishment of van der Waals forces between the hair tips and the substrate crucial for attachment.

The experiments were performed at the nanobranch of beamline ID13 with a beamsize of 100 x 100 nm and energy of 14.9 keV. Overall, two unattached and one attached tip of single adhesive hairs of the spider *Cupiennius salei* were scanned in the WAXS&SAXS configuration in fine detail with a resolution of 250 nm. Several attachment hairs on a

ladybug beetle´s (*Coccinella septempunctata*) attachment pad in a 21 x 42 µm window were scanned with a resolution of 300 nm. In the sample of the attached spider hair one spot was exposed 411 times for 2 seconds to check for the inorganic character of crystals found in a few spots during the scans of the 21 x 21 µm mesh of the hair tip. This inorganic matter, which did not show radiation damage, maybe were debris particles picked up during the preparation. Additionally, several line scans of the hair shaft were made at some distance from the hair tips in order to check for differences in fibre orientation.

The WAXS and SAXS signals achieved are of excellent intensity and beside the reconstruction of the hair outlines allow the proper analysis of single contact elements (figure 1 a, b). Taking the dimensions of the structures with a width of about 1 µm and a thickness of less than 100 nm into account, this result is breathtaking. The diffraction images show the WAXS signals aligned with the proposed fibre orientation and the WAXS signals of chitin perpendicular to them. Interestingly, the XRD signals from the hair shaft 50 µm away from the tip differ. They do not show aligned fibre orientation like in the tip regions, but rather amorphous rings. This finding points to a different structural embedding of the chitin fibres in the protein matrix, maybe similar to the plywood structure known from the skeletal cuticle of the spider[1]. From the samples of free standing whole beetle legs WAXS and SAXS signals allowing the reconstruction of single hairs could be recorded as well. In the chosen window several hairs of the attachment pad arranged roughly in parallel can be identified (figure 1 c, d).

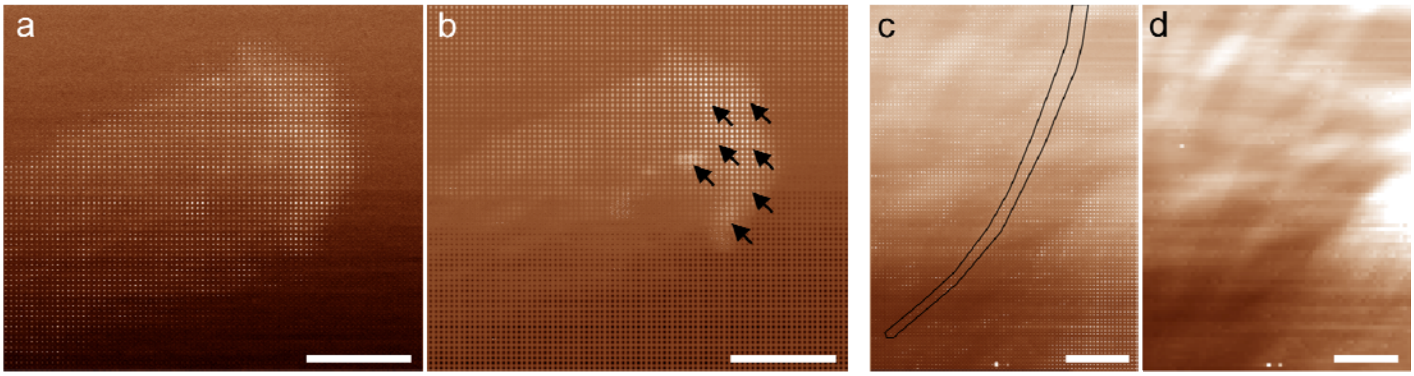

Figure 1 a Composite image of SAXS and **b** WAXS signals from the tip of a single spider adhesive hair, presumably in contact with the Si_3N_4 membrane. The arrows point to intense signals, where single contact elements are likely in contact with the substrate. **c** Composite image of SAXS signals from hairs of the tarsal attachment pad of the beetle *Coccinella septempunctata*. The outline of a single hair is drawn for clarification. **d** WAXS signal intensity map of the same detail as in **c**. Scale bars 5 µm.

The further thorough analysis of the data will yield detailed pictures of the distribution and alignment of the chitin fibres in biological hairy adhesive systems and the consequential adaptation of these structures to their specific working conditions. These results can help to improve artificial attachment devices. In the planned continuation of the experiment we focus on the changes of fibre orientation during the attachment process. For this purpose single hairs will be attached to the silicon nitride substrate *in situ* and different steps of the attachment process recorded.

References:

[1] Barth, F. G. 1973 Laminated composite material in biology. Microfiber reinforcement of an arthropod cuticle. Z. Zellforsch. 144, 409-433.