EUROPEAN SYNCHROTRON RADIATION FACILITY

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Experiment Report Form

	Experiment title: Temperature- and alcohol-induced conformational and morphological changes in silk proteins.	**Experiment number**: MX1160
Beamline: ID14-3	**Date of experiment**: from: 11.12.2010 to: 12.12.2010	**Date of report**:
Shifts: 3	**Local contact(s)**: Petra Pernot	*Received at ESRF:*

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Report:

Summary: Silk protein conformational changes in different solvents were studied using SANS/SAXS and Circular Dichroism (CD) to gain insights in silks folding and assembly processes. The experiments were carried out on the same day at the instruments ILL-D11 (8-03-660) and ID14-3 (MX1160) as part of the PSB joint proposal initiative. We found that ID14-3 could be optimised to measure the highly shear sensitive silk proteins and that radiation damage could be avoided by continuously flowing the sample. Direct comparison of the sans and saxs data on concentration series, pH and urea perturbation showed a consistency and most of all great complementarity (saxs helps with high q background resolution). We fell, however, short in achieving the experimental aims. The temperature and alcohol perturbation were not implemented. We nevertheless were able to measure a range of silks from spiders and insects.

Materials and methods: Bombyx mori silkworm and Nephila edulis spider silk were dissected a day prior to the experiment. The silk proteins were extracted and immediately transferred to milliQ water or buffered solution. The solutions were left to homogenise overnight. Sample preparations were conducted at the ILL softmatter lab.

Specifically, glands from silkworm *Bombyx mori* were gently peeled to remove the epithelium and washed to remove sericin. The obtained silk fibroin was dissolved in milliq water and D2O overnight at 4 degree C.

H2O and a D2O stock solution were then combined by mass (maintaining the protein concentration) to prepare 0, 20, 60, 80 and 100% D2O silk fibroin solutions. Concentration series of the D2O as well as the H2O stock solutions were measured. Denaturation of the protein was induced chemically by altering the pH (basic and acidic) and using urea. All protein concentrations of the sample solutions were calculated from the dryweight of the stock solutions.
Aliquots of the samples were measured by circular dichroism spectroscopy to determine their secondary structures content.

Results:
The standard setup at ID14-3 was used with the capillary flow system (Sample-detector distance 2.43m, wavelength 0.931Å). We checked for radiation damage collecting series of short timeframes and check for any changes in intensity/shape of the scattering curve. We found that no radiation damage occurred within 3 seconds exposure time in a static system and at the highest used concentration (12mg/ml). A different cleaning protocol for the capillary system was implemented since the standard cleaning agent did not remove the aggregated silk protein from the capillary walls. We found that flushing a 12M urea solution through the system was sufficient to remove all residual protein.

Using the flow system we found that flow rates of 0.5 µl/sec showed no difference compared to a static frame scattering of our protein. It is important to note that in flow condition silk protein will fibrillate, therefore great care was taken to find the optimal condition to minimise both radiation damage and prevent flow induced fibrillation.

The data reduction was performed using the automated system provided at the beamline. During the measurement we encountered some variation in overall intensity in some samples and water runs (all frames of the run). The origin of the variation is yet to be determined.

H_2O-D_2O contrast study:
Comparing the effect of H2O and as solvent we found that in SAXS no significant changes in the shape of the scattering curve occurred (figure 1a). We found, however, a pronounced difference between H_2O and D_2O by SANS (figure 1b). Further investigation of the samples by CD confirmed that the secondary structure content in D2O was comparable to the one in H2O.

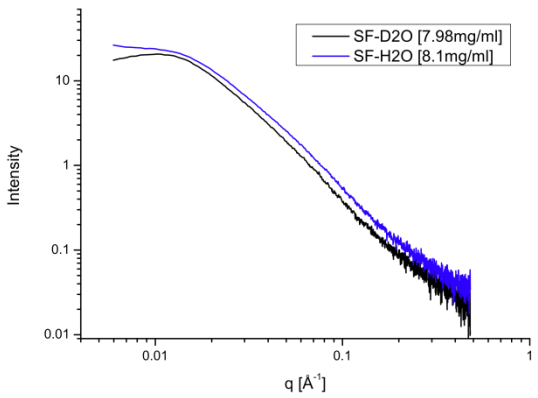

Figure 1(a): SAXS of Native Silk Fibroin run in 100%D2O (black) and 100%H2O (blue).

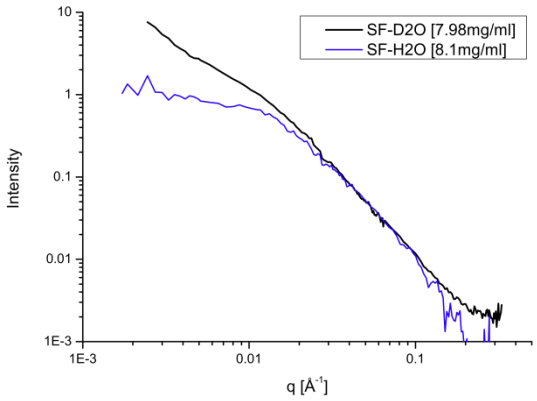

Figure 1(b): SANS for the same samples as in the SAXS show a clear difference.

The only difference in the SAXS of silk protein in H2O and D2O is the structure factor present in the D2O sample in figure 1a. A concentration series of silk protein in D2O did show a similar trend (measured only in SAXS). This interesting effect will be analyzed more in detail (Analysis in progress).

Temperature and alcohol induced gelation: Given the loading limitation and difficulties with temperature ramp it wasn't possible to systemically investigate the role of temperature and alcohol induced conformational changes. Therefore we studied conformational changes induced by pH and urea and followed up the interesting contrast difference we observed. The analysis of the data is in progress.

pH effects (Analysis in progress):
Direct change of the pH to acidic or alkaline condition showed strong changes in SANS. We expect to see strong changes by SAXS, but the capillary loading was a limitation. We found that the rapid gelation of the silk protein under pH change was not compatible with the loading mechanism at ID14-3. Alternatives are being sought.

Urea induced unfolding (in progress):
Urea denaturation is one of the key methodology in protein folding to understand the nature of the native and unfolded states as well as determining the thermodynamics of the denaturation process. For this experiment, silk proteins from the silkworm silk were dissolved in 12M urea and progressively diluted by an equivalent silk protein solution with no urea. This approach allows to reduce the urea concentration and keep the protein concentration constant. We found that urea denaturation of silk proteins was measurable by saxs and that the fully denatured protein adopts an extended rod conformation. Detailed data analysis is in progress to determine the size and shape of the different intermediates.

Multiple silk proteins (in progress):
A simple but revealing experiment consist of the comparison of multiple silk proteins with known functions. We preliminary were able to measure 4 out of 7 of a spider silk proteins, namely: Major ampullate (dragline and radial threads), Minor ampullate (auxilliary spiral thread), flagelliform (sticky spiral thread) and cylindiform (egg case silk). Each of these silks have a well determined function but also a well determinded secondary structure (CD and FTIR) in solution. We were able for the first time to measure the silks associated SAXS in solution. This result although preliminary suggests that the different silks measured have a different overall shape in solution.