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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

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

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.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal 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:** **Using Rheo-SAXS/WAXS to understand silk spinning**	**Experiment number**: SC3210
Beamline: BM26B	**Date of experiment**: from: 28/02/2011 to: 06/03/2011	**Date of report**: 27/02/2012
Shifts: 6	**Local contact(s)**: Guiseppe Portale	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Background: Silks are protein polymers optimised for extrusion processed via controlled protein denaturation[1-3]. Stored in specialist organs glands as a concentrated gel, silk is transformed into a dry solid fibre as it flows through a hyperbolic spinning duct[4-6]. This transition is partially achieved through changes in chemical environment, but primarily through the application of a shear-stress field which triggers protein dehydration, denaturation and spontaneous aggregation into fibrils[2,7-9]. As a result, over the past decade rheological analysis has made significant contributions towards understanding this area[2,3,8,10-14]. Further insights were gained through the integration of rheological studies with measurements of structural properties and are the current focal point of our research. Our first step was to combine shearing with polarized infrared spectroscopy. This allowed us to monitor the conformational conversion (aggregation) process as well as molecular orientation[14]. Parallel Rheo-SANS measurements on LOQ (ISIS), on diluted silk feedstock gave tantalizing hints of structure development during shear, although being hampered by the limited *q*-range and temporal resolution. Hence the time and the tools were in the right place to submit to as Dubble can circumvent both limitations.

The aim of the experiment was to investigate *in situ* the shape, size and orientation of native silk proteins solutions under shear forces. By mimicking the shear induced processing on the x-ray beam line, we wanted to complement rheological and spectroscopic studies (Rheo-SANS and Rheo-IR) to expand our knowledge of silk spinning.

For **sample preparation**, Bombyx mori silkworms were cut open and the silk glands extracted and peeled before been washed of its sericin. All live samples were handled in the Biolab of ID17. A total of 119 worms were dissected. For the reconstituted silk fibroin (RSF) preparation, silkworm cocoons were degummmed and the fibres solubilised in a concentrated LiBr solution. The chaotropic agent was then removed by dialysis against water before was prepared using the standard protocol.[15]

The **Rheo-SAXS/WAXS** experiment conducted at Dubble BM26B employed a a modified CSS450 shearing cell (Linkam, Guildford, UK). With a gap of 700 μm, it was possible to shear the sample at constant rates, up to 100 s^{-1} (9.33 rad/s) at the x-ray beam spot. At least three shear rate repeats were performed at: 1, 5, 20, 30, 50 and 100 s^{-1} for a total of 85 runs. By positioning the SAXS detector 4.5 m away from the sample, we covered a *q* range from 0.003 to 0.18 $Å^{-1}$, working in 16 bunch mode.

For the **data analysis**, all scattering patterns were normalized by the intensity of the ionization chamber and the background from the empty cell subtracted. The scattering data was acquired using the newly implemented GDA software. For the data reduction of the generated nexus files, a modified MATLAB (Mathworks™) script written by Lian Apostol and Giuseppe Portal (BM26B-DUBBLE) was used to automate FIT2D (Copywrite 2005 Andy Hammersley /ESRF). The data was reduced to 200 radial bins from (0.003 to 0.18 $Å^{-1}$) and 18 azimuthal bins (20° per bin) before been save as ASCII files. The ASCII files were then imported, treated and plotted in Excel 2010 using a series of custom VBA macro (Microsoft©) that we wrote.

For the **results**, the data analysis of all the measurements is nearly completed, but still in progress. This experiment has proved very valuable in defining the conditions for testing successfully the native silk samples by x-ray scattering. For instance, we now know that a static sample can suffer radiation damage after only 0.4 seconds. Fortunately, it is possible to circumvent this effect by using a fast shutter and flowing in the sample cell at rate greater than 1 s^{-1}. We already knew from our previous rheology experiments that the shear induced conversion occurs only when silk is sheared above a critical shear rate given by the relaxation time of the material[9]. None of the samples sheared at rates lower than 20 s^{-1} saw its scattering changed. Figure 1 shows the scattering curve for native silk fibroin before and after 1 second of shear at a 100 s^{-1}rate. The high *q* region remained constant, suggesting that the smaller scales structures are not affected by shearing. However, the low *q* range intensity increases upon shearing. The scattering measurement can be correlated to an induced opacity in the sample (figure 1 inset) and is due to shear induced aggregation. The scattering pattern recorded (figure 2 inset) showed that the sheared sample oriented. The orientation distribution was quantified using the integration of the low *q* region as a function of the azimuthal angle (figure 2). A strong anisotropy was measured with an orientation distribution peaking perpendicular to the flow direction. It is then possible to conclude that the protein main chain is aligned predominantly along to flow. The orientation distribution can be described as a series of Legendre polynomial giving access to order parameter. Consequently, the fitting of the orientation distribution permits the calculation of the order parameters at each point in time to follow the orientation development (figure 3).

In **conclusion**, SC3210 was proven successful, and we have now overcome the obstacles that arose during the experiment (the limited number of acquisition frames and the development of analysis macros to analyse off site and exploitation of WAXS data). Not only has Dubble technically proven to be suitable to study our system, the data obtained has provided new insights into this area. Consequently, we are reapplying for beam time to complete this study, including temperature studies and reconstituted analogue comparisons.

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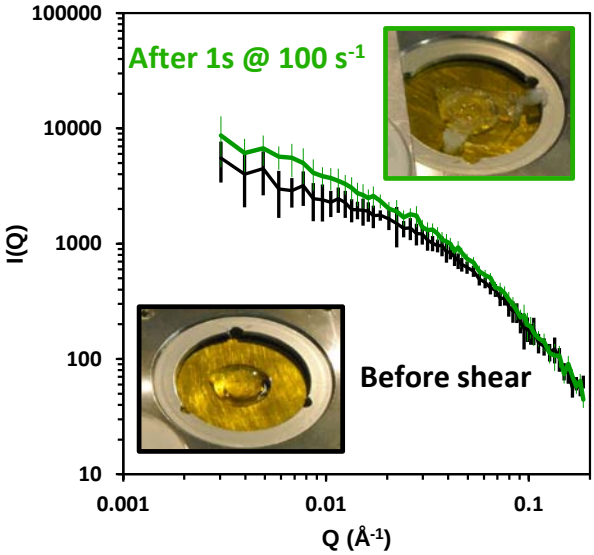

Figure 1

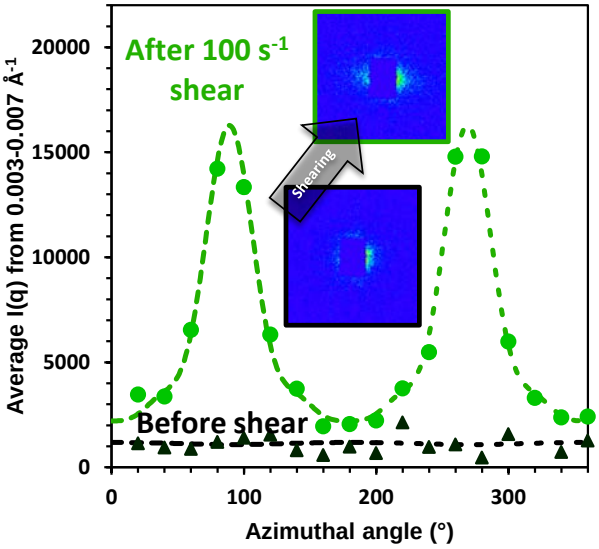

Figure 2

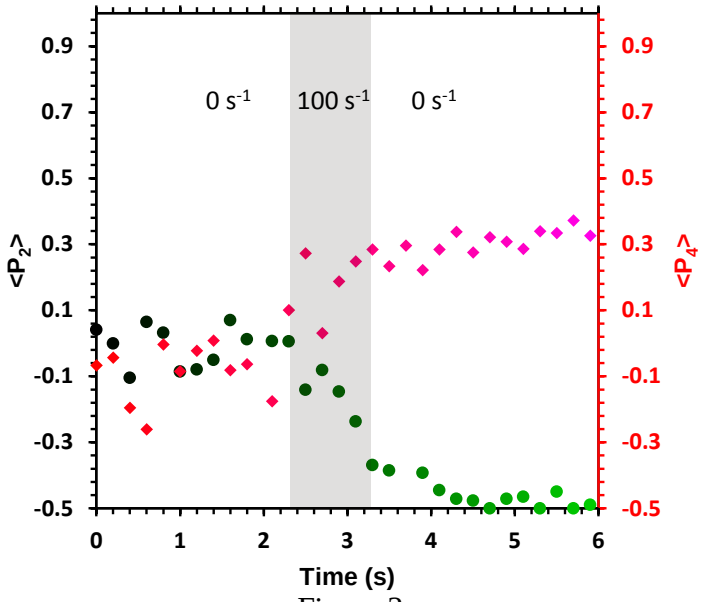

Figure 3