Experimental report

21/10/2022

Proposal: 9-13-1036 **Council:** 4/2021

Title: Multimodal tools to resolve assembly mechanisms in biological processes: Silks as a model

Research area: Biology

This proposal is a new proposal

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Samples: Silk proteins

Instrument	Requested days	Allocated days	From	To
D22	3	3	17/09/2021	20/09/2021

Abstract:

Due to the inherent chemical and structural complexities of biological processes, the simultaneous combination of several techniques is necessary to gain the desired information about the mechanism and molecular basis for fibril/fibre assembly.
It is, therefore, our aim, using our promising NUrF (Neutron small-angle scattering ¿ UV-visible - Fluorescence) platform, to uniquely extract the rate laws and resolve the intermediates involved in the formation of silk protein fibrils. A correct description of the rate laws and individual intermediates will provide new insights in molecular regulation, transient interactions and solvation involved in the supramolecular assembly of fibre forming proteins.

Experimental report for proposal: 9-13-1036

Multimodal tools to resolve assembly mechanisms in biological processes: Silks as a model

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Summary
Due to biological processes' inherent chemical and structural complexities, the simultaneous combination of several techniques is necessary to gain the desired information about the mechanism and molecular basis for fibril/fibre assembly.
We implemented the NUrF (Neutron small-angle scattering – UV-visible - Fluorescence) platform to uniquely extract the rate laws and resolve the intermediates involved in forming silk protein fibrils.

Results

The setup

Figures 1 and 2 illustrate the NUrF setup at D22 with a single cuvette for kinetic measurements.

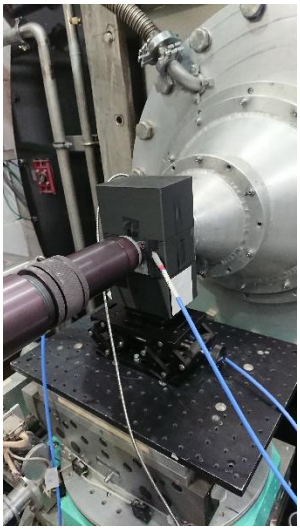

Figure 1. The NUrF setup fully closed.

Figure 2. NUrF setup from above without the top cover. The cuvette is visible in the centre and around the fibre optics and the neutron beam nozzle.

From a previous beamtime (9-13-947), we had integrated most of the NUrF into Nomad. The fluorescence system was as unreliable as in experiment 9-13-947. The reasons are still unclear.

The data

we used the NUrF sample environment to characterize the progressive acid-induced assembly of silk protein to resolve the mechanism of pH assembly and the physicochemical nature of the crosslinks.
The most intriguing questions about fibrillar silk gels are (i) what happens to the polymer chains by crosslinking and (ii) the network's fine structure.

Materials: Due to covid19 restrictions, we could not access live silkworms to extract native silk proteins. We instead used technical-grade silk. Briefly, silk fibres are dissolved in 9M

LiBr at 70deg C for 30 min. The dissolved silk is dialyzed for 3 days against a 10mM sodium phosphate buffer (NaP) solution. The dialyzed silk is recovered, centrifuged and stored, ready to use at 4deg C. Before any neutron experiment, we exchanged the aqueous buffer for a deuterated buffer using a desalting column.

The silk in 20mM NaP in D2O was diluted to the selected concentration (5, 10, 20 or 40 mg/ml), mixed with the acidifier and loaded in a 2mm cuvette. The kinetic of fibrillation was followed by taking measurements every 5 minutes.

Figure 3 illustrates the time course of the SANS for a 10mg/ml silk solution. There is a correlation peak appearing and disappearing during the kinetics.

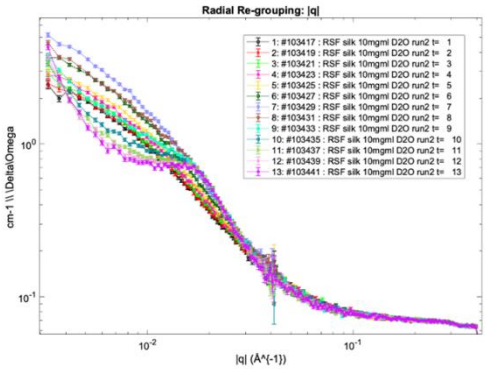

Figure 3. SANS time course of the silk fibrillation after acid induction.

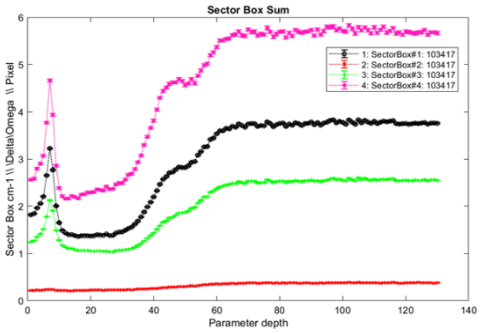

Figure 4. Radial sector averaging of the 2D SANS data. In red, the high q; in green and dark middle q and pink, the low q regions. The x-axis is equivalent to time.

A better visualization is by plotting the radial sector averaging of the 2D SANS data (Figure 4). 4 regions are represented, high q (red), middle q (green and dark) and low q (pink).

The equivalent time course using sector averaging suggests several steps in the processes. Unfortunately, the UV-visible spectra saturated early (high scattering) and the fluorescence had communication issues. Those issues meant no insights from the chemistry of the kinetics.

Looking at the effect of silk protein concentration. We found that the initial peak increase in intensity as the silk concentration increases. 5 and 10 mg/ml were very similar for the time profile. In contrast, 40mg/ml showed fewer features.

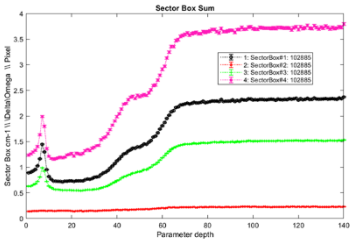

Figure 5. Radial sector averaging for 5mg/ml silk.

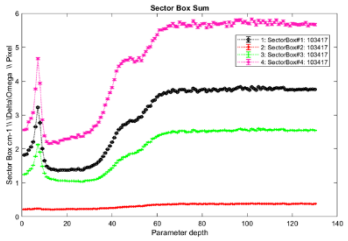

Figure 6. Radial sector averaging for 10 mg/ml silk.

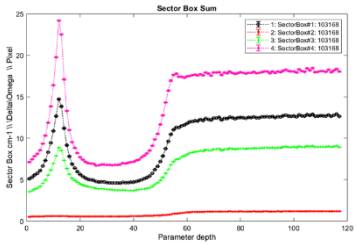

Figure 7. Radial sector averaging for 40 mg/ml silk.

Conclusion

We have demonstrated the possibility of fully exchanging silk protein's aqueous buffer into a buffered deuterated solution.
According to the SANS, the kinetics of acid-induced fibrillation in silk proceeded via multiple steps.
Difficulties with the UV-visible saturation and the fluorescence communication issues precluded any insights from the chemistry of the kinetics. For example, we used an extrinsic fluorescence dye (thioflavin T) to monitor the intermolecular crosslinks.