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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: Structural Characterisation of alpha-synuclein fibrils and Oligomers** | **Experiment number**: 26-02-664 |
| **Beamline**: DUBBLE | **Date of experiment**: from: 18-11-2013 to: 21-11-2013 | **Date of report**: 19-12-2013 |
| **Shifts:** 6 | **Local contact(s)**: Giuseppe Portale | *Received at ESRF:* |


**Names and affiliations of applicants** (*indicates experimentalists):

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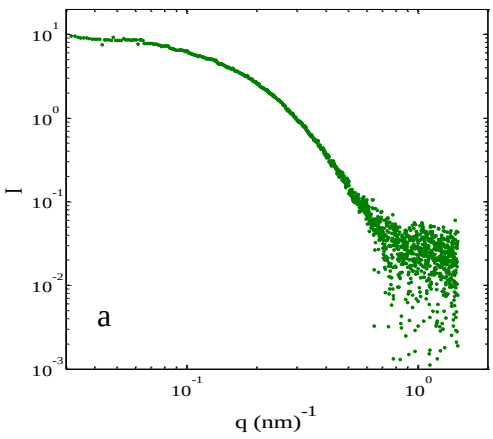
University of Twente, Nanobiophysics group.
Other experimenters: Slav Semerdzhiev and Anja Stefanovic
University of Twente, Nanobiophysics group.


## Report:

The neuronal protein α-synuclein is natively unfolded, but it can adapt a number of different conformational states depending on environmental conditions and addition of cofactors. This protein plays a critical role in the molecular mechanism of Parkinson's disease. The aim of this SAXS experiment was to investigate the structure of different α-synuclein oligomers and fibrils.

Our experiment had several sub-experiments. First of all, we are interested in determining the aggregation numbers, size and shapes of several oligomeric species of wild-type α-synuclein and α-synuclein mutants. In figure 1 the scattering curve alpha-synuclein WT oligomers (figure 1a) and its Guinier plot (figure 1b) are presented. From the Guinier plot we estimated a radius of gyration of ~8 nm. We will further try to determine the shape of these oligomers by fitting the curves and try to estimate the aggregation numbers. Kratky plots of the oligomer scattering curves will give us some information about the globularity of these structures.



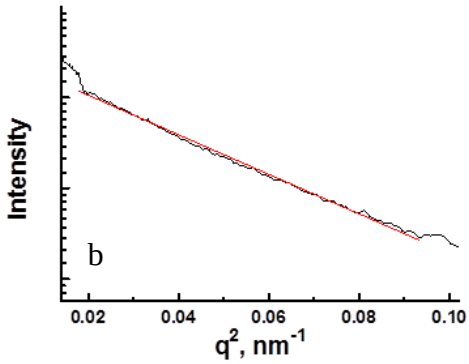




Figure 1a. Scattering curve of α-synuclein wild type oligomers and figure 1b. Guinier plot of figure 1a.

In another sub-experiment we studied the structure of α-synuclein/tubulin fibrils. In figure 2a the scattering curves of 100 μM αS with different concentrations of tubulin are presented. It can be seen that the scattering intensity decreases as function of the tubulin concentration. It is known that tubulin seeds the formation of the α-synuclein fibrils. Figure b and c are fluorescent microscopy images of 100 μM αS with 0.2 μM and 0.3 μM tubulin,

respectively. At the highest tubulin concentration the fibrils are much shorter. That the intensity is lower at higher tubulin concentrations could be due to the scattering particles being smaller.

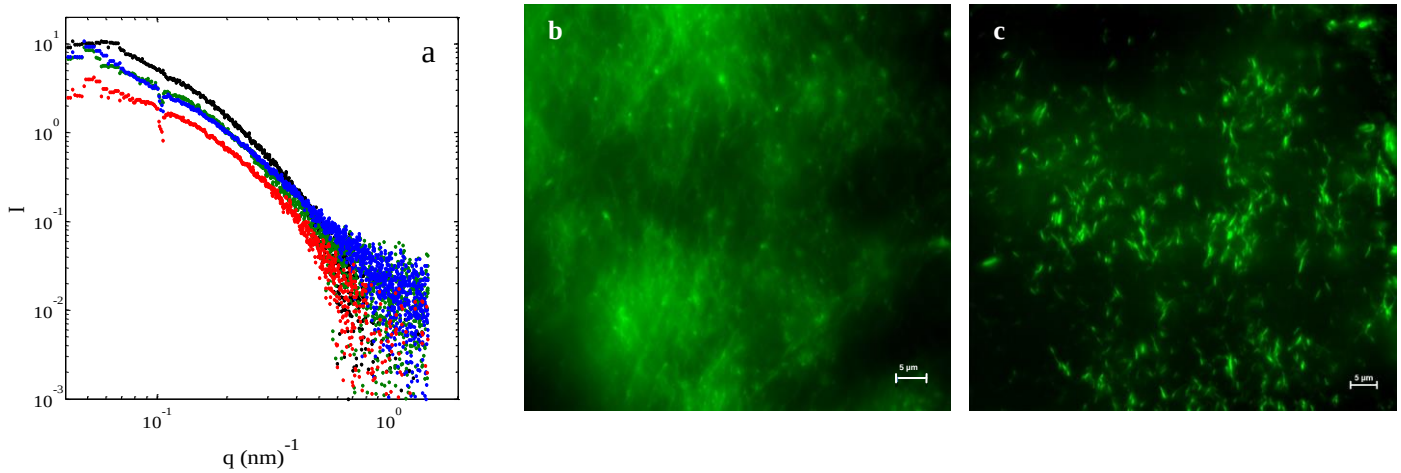



Figure 2a. scattering curves of ● 100 µM αS, ● 100 µM αS+ 0.1 µM tubulin, ● 100 µM αS+ 0.2 µM tubulin and ● 100 µM αS+ 0.4 µM tubulin in a 10 mM Tris buffer pH=7.4. Figure b and c, fluorescence microscopy images of 100 µM αS+ 0.2 µM and 100 µM αS+ 0.3 µM in a 25 mM phosphate buffer pH=7.3.

The interactions between the amyloid fibrils are strongly influenced by the ionic strength. To get some insight in the mechanism of these interactions we used different cations, keeping the anion constant. In figure 3 X-ray scattering curves of the monovalent salts NaCl and CsCl (figure 3a) and the divalent salts $CaCl_2$ and $BaCl_2$ (figure 3b) are presented.

For both the monovalent and divalent salts the scattering intensity of the gels with the heavier cation is higher. This indicates that the ions are present in the gels. The valence of the ions seems to be very important, since the scattering intensity of the gels containing $BaCl_2$ is much higher than the scattering intensity of the gels containing monovalent ions (figure 3b).

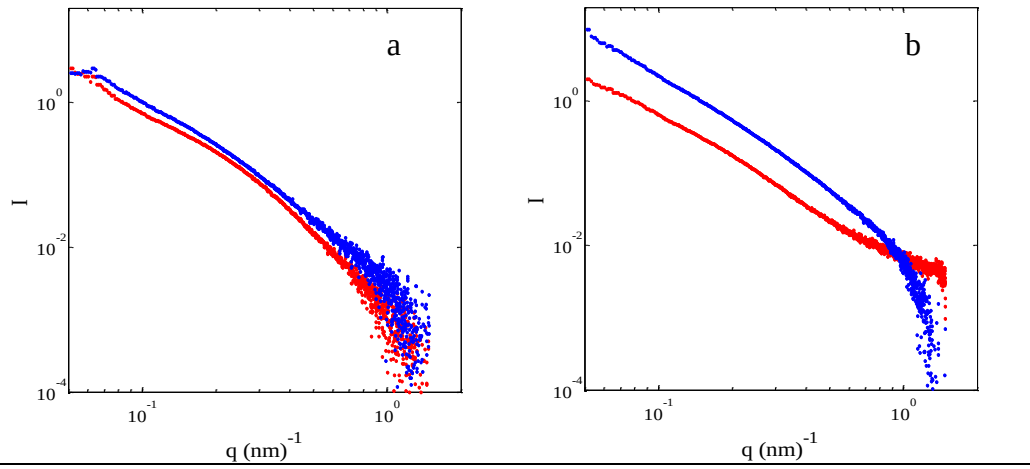



Figure 3a. Small Angle X-ray Scattering of 300 µM αS with ● 600 mM NaCl and ● 600 mM CsCl. Figure 3b. Small Angel X-ray Scattering of 300 µM αS with ● 200 mM $CaCl_2$ and ● 200 mM $BaCl_2$

Another experiment we have performed is measuring the Small Angle X-ray Scattering of 300 µM αS gels with different ages (1, 2, 3, 4 and 5 weeks) as function of the temperature, of which we measured the rheology at our own lab. Unfortunately we have not been able so far to reduce the data of this experiment, due to the very little support and poor manual of the data reduction software. Therefore we are unable to show any of these data at this stage.

**Conclusion**

So far the results of these scattering experiments have given valuable insight in determining the size of the oligomers, in the structural aspects of alpha-synuclein tubulin fibril formation and in the mechanism of gel formation at high ionic strength. We hope that, once we are able to reduce the temperature data, we have new insights in the structures that are forming during increasing and decreasing the temperature of alpha-synuclein gels that could help us with the interpretation of our rheology data.