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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 using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now 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:** Oligomerization process of mutants and a peptide from the ovine prion protein	**Experiment number:** SC-2637
Beamline: ID02	**Date of experiment:** from: May 7th to: May 8th	**Date of report:** 15-08-2009
Shifts: 6	**Local contact(s):** Shirley Callow	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr Cécile Dreiss * King's College London, Pharmaceutical Science Division, London, United Kingdom

Miss Nesrine CHAKROUN * King's College London, Pharmaceutical Science Division, London, United Kingdom

Dr Human REZAEI * INRA, Equipe Biologie Physico-chimique des Prions, Jouy-en-Josas, France

Dr Marc MALFOIS - Diamond Light Source, Non-crystalline diffraction beam line**,** Didcot, United Kingdom

Report:

Our work focuses on the prion protein (PrP), linked to Creutzfeldt-Jacob and bovine spongiform encephalopathy (BSE). We have studied the molecular mechanisms and kinetics of ovine PrP (OvPrP) oligomerization over the past few years [1-4] using a combination of biophysical techniques. These studies have shown that OvPrP partially unfolds into 3 intermediate states, which aggregate independently into 3 distinct oligomers O1, O2 (very instable) and O3. The same pattern of oligomerization is obtained with the C-terminal globular domain of the PrP, namely ΔOvPrP. Furthermore we have identified *a minimal region* (H2H3) [5] involved in the PrP structural conversion and oligomerization leading to the same pattern of oligomerization as the entire PrP.

In the present study, SAXS has been used to determine the low resolution structure of each of the monomeric species (ΔOvPrP and H2H3) and the different oligomers obtained from their oligomerization.

The following figures show selected data obtained for the monomeric species ΔOvPrP and H2H3 and oligomers from H2H3 domain using Svergun's ATSAS suite programs (GNOM, DAMMIN) [6].

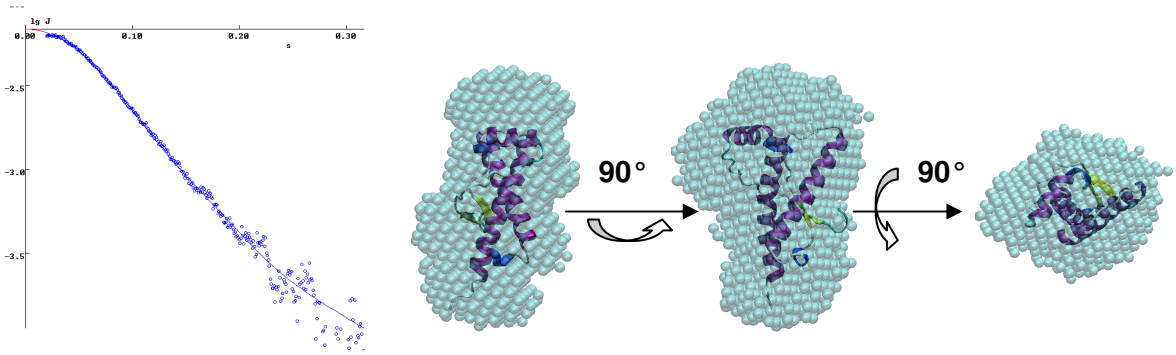

Fig1. *Scattering intensity I(q) from SAXS experiments for ΔOvPrP monomer at 269μM and low-resolution shape compared to the crystal structure of the ΔOvPrP domain (PDB 1UW3). Fitting the data using GNOM enabled the determination of a Radius of gyration Rg=1.96nm. DAMMIN was used for the sturcture determination by averaging 10 independent runs.*

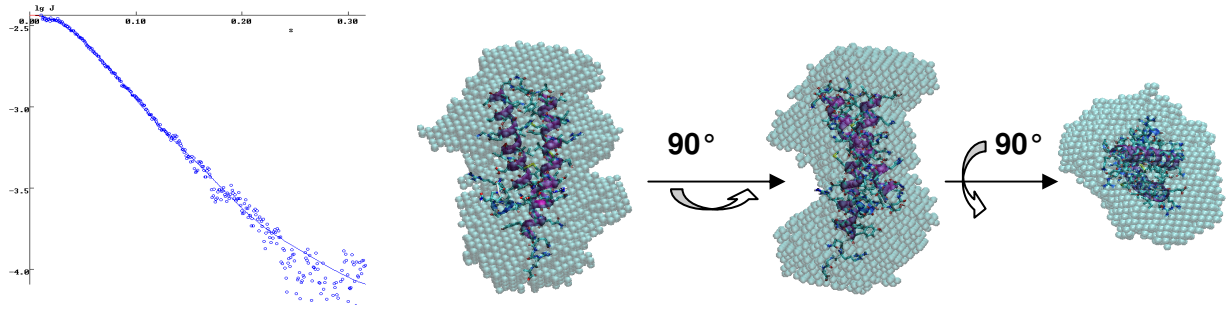

Fig2. *Scattering intensity I(q) from SAXS experiments for H2H3 monomer at 187 µM and low-resolution shape compared to the crystal structure of the H2H3 domain (from PDB 1UW3). Fitting the data using GNOM enabled the determination of a Radius of gyration Rg=2.03nm. DAMMIN was used for the sturcture determination by averaging 10 independent runs.*

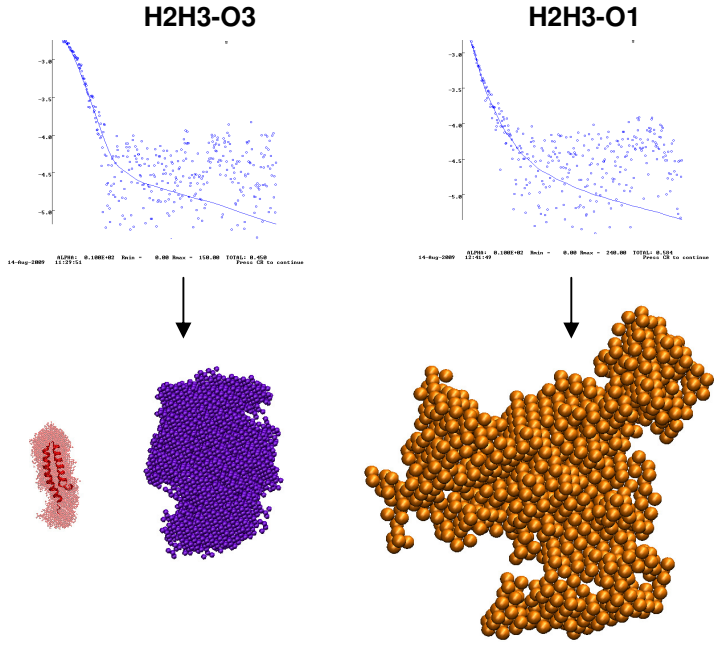

Fig3. *Scattering intensity I(q) from SAXS experiments for H2H3 oligomers O3 and O1 and low-resolution shapes Fitting the data using GNOM enabled the determination of a Radius of gyration Rg= 45.04 Å for H2H3-O3 oligomer and 85 Å for H2H3-O1 oligomer. DAMMIN was used for the sturcture determination by averaging 10 independent runs, H2H3 monomer (red) is shown as reference.*

SAXS data for the each oligomer have successfully been collected and we are still in the process of analysing the data. These data will help to construct for the first time an architectural model of monomer assembly into the oligomers. These preliminary results have been presented at the European Biophysical Societies Association conference in Genoa [6] (invited talk and poster – award for best poster) and will be presented at the XIV International Conference on Small-Angle Scattering 2009 (poster).

We have also carried out oligomerization experiments of selected mutants, for which the oligomerization pathway is selected and leads to the formation of only one oligomer. Preliminary data clearly show the progress of the oligomerization over time, but the set-up available was not fully adequate and will be optimized in future experiments, using a home-made thermostated 'kinetic cell'.

1. Eghiaian, F., et al., *Diversity in prion protein oligomerization pathways results from domain expansion as revealed by hydrogen/deuterium exchange and disulfide linkage.* Proc Natl Acad Sci U S A, 2007. **104**(18): p. 7414-9.
2. Rezaei, H., et al., *Amyloidogenic unfolding intermediates differentiates sheep prion protein variants.* J Mol Biol, 2002. **322**(4): p. 799-814.
3. Rezaei, H., et al., *Sequential generation of two structurally distinct ovine prion protein soluble oligomers displaying different biochemical reactivities.* J Mol Biol, 2005. **347**(3): p. 665-79.
4. Rezaei, H., et al., *High yield purification and physico-chemical properties of full-length recombinant allelic variants of sheep prion protein linked to scrapie susceptibility.* Eur J Biochem, 2000. **267**(10): p. 2833-9.
5. Chakroun, N., et al., The oligomerization properties of PrP are restricted to the H2H3 domain. PNAS, submitted
6. http://www.embl-hamburg.de/ExternalInfo/Research/Sax/software.html
7. Eur Biophys J (2009) 38 (Suppl 1) S102, S204
8. SAS conference, Oxford, September 2009