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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:** The molecular structure of TTR amyloid fibrils	**Experiment number**: LS - 1976
Beamline:	**Date of experiment**: from: 05 September 2001 to: 06 September 2001 17 February 2002 17 February 2002	**Date of report**: 26 Sept 02
Shifts: 4	**Local contact(s)**: Dr. Christian RIEKEL Dr. Manfred ROESSLE	*Received at ESRF:*

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Report:

The aim of this experiment was to improve our knowledge on the molecular structure of amyloid fibrils in order to have a better insight on future terapeutic drugs.
Transthyretin (TTR) amyloid fibrils are the main component of the amyloid deposits occurring in Familial Amyloidotic Polyneuropathy patients. This is one of the twenty human proteins leading to protein aggregation disorders such as Alzheimer's and Creutzfeldt-Jakob diseases. The structural details concerning the association of the protein molecules in the fibrils are essential for a better understanding of the disease and consequently the design of new strategies for diagnosis and therapeutics.
New insights into the mechanism of fibril formation were obtained by X-ray diffraction analysis of FAP amyloid fibrils. It was shown that fibrils exhibit a cross β-sheet structure and two structural models were proposed: a continuous β-sheet helix or an association of units with a structure close to the TTR monomer. The two models are obviously different and neither of them refers to the packing interactions between the different subunits.

Amyloid fibrils were formed in vitro by acidification except for Leu55Pro TTR, the most aggressive variant described in the literature until now, that forms fibrils spontaneously when left for a few days at room temperature.

Fibrils formed in this way were aligned using two different methods:

- fibril solutions were drawn up into the siliconized glass capillary tubes which were then sealed at the top, placed into a 2-T magnet and allowed to dry at room temperature. As soon as a thin disk was formed, the capillary tubes were sealed with wax in order to retain some hydration of the sample. The X-ray diffraction patterns were only collected for the samples showing birefringence under cross-polarized light.
- a droplet of fibril suspension is placed between the ends of two glass capillaries, then the capillaries were drawn apart slowly and in small increments to facilitate the alignment of the fibrils as the droplet dries.

The X-ray diffraction data were collected using a 0.975 Å wavelength beam and a beam size of 5μm. Patterns were recorded on a MAR research imaging plate during exposure times of 10 to 60s. The background was recorded and subtracted to the diffraction pattern for elimination of the air scattering effects, using the software package FIT2. The sample to detector distance was calibrated with Ag-behenate.

The droplets that were stretched produce poorly oriented samples as X-ray diffraction patterns revealed essentially concentric circles. Better results were obtained with magnetic oriented samples. X-ray diffraction patterns of these samples revealed the characteristic cross-β pattern:
a sharp 4.85 Å reflection on the meridian and a diffuse equatorial 10.3 Å reflection. The meridional reflection indicates a structural repeat of 4.85 Å along the fibril axis usually attributed to the spacing of adjacent β strands oriented perpendicular to the fibril axis. The 10.3 Å equatorial reflection corresponds to the face-to-face distance of two β-sheets oriented parallel to the fibril axis.

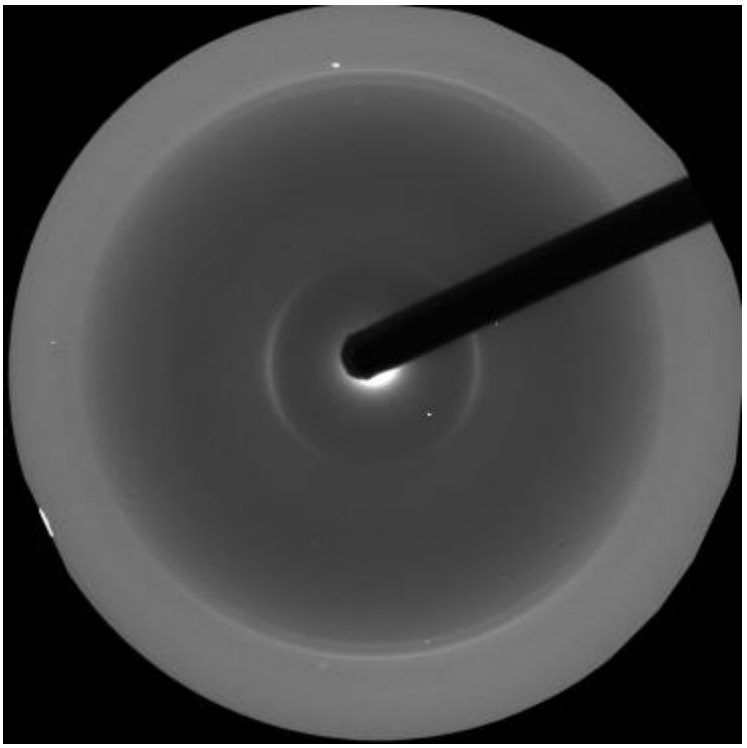

Figure 1. X-ray diffraction patterns from L55P-TTR amyloid. Specimen-to-film distance was 245mm and the exposure time 30s.

Lower intensity reflections were also observed. For example a 4.65 Å reflection on the meridian, of medium intensity. The 4.85 Å and 4.65 Å may represent sampling of a 4.75 Å reflection, corresponding to the backbone separation of the neighbouring main chains or may indicate the existence of a higher repeating unit along the fibril axis.
We are now interpreting the X-ray diffraction patterns and an article to be submited to J. Biol. Chem., also with data collected using other techniques, is in preparation.