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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 Cystatin Amyloid Fibrils.**	**Experiment number**: MD281
Beamline: ID26	**Date of experiment**: from: 16/05/07 to: 22/05/07	**Date of report**: 29/08/07
Shifts: 17	**Local contact(s)**: Dr. Tsu-Chien Weng	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Using Sulfur K-edge XANES we were able to quantify disulfide crosslinks formed within amyloid fibrils of H.Cystatin B for 5 different double cysteine mutants (S7C/S45C, Q17C/A40C, Q46C/N52C, T51C/Q71C and Q71C/L80C. See Fig. 1). The pairs of cysteine residues were engineered at loci where native contacts are formed in the 3-dimensional crystal structure of H.Cystatin B. In order to determine the extent to which these contacts are preserved within H.Cystatin B amyloid, fibrils were grown under reducing conditions and then oxidised by exposure to air (labelled 'degassed'), diamide or peroxide prior to measurement of Sulfur K-edge XANES spectra.

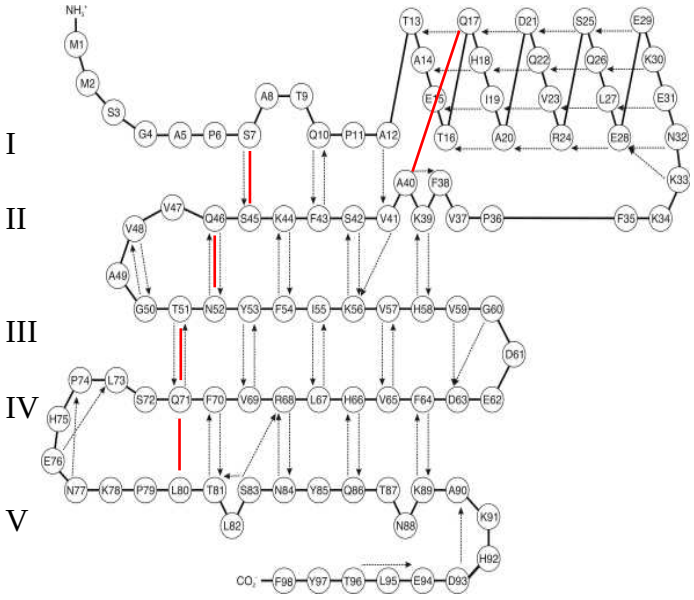
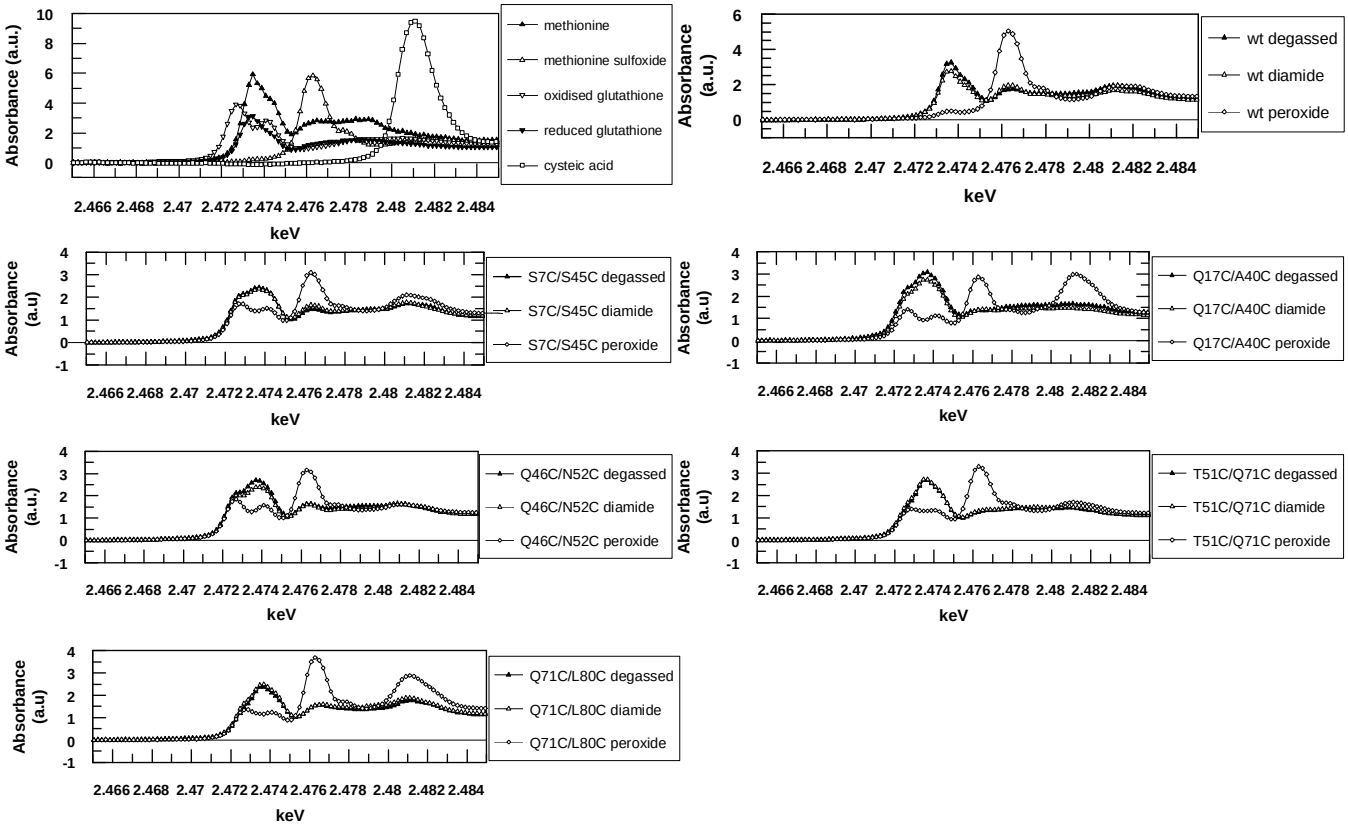


Fig. 1 Topology diagram of H.Cystatin B monomer showing locations of double cysteine mutants (joined in red); S7C/S45C, Q17C/A40C, Q46C/N52C, T51C/Q71C, Q71C/L80C. Strands 1-5 are labelled by Roman numerals.

Results

All Sulfur K-edge XANES spectra were calibrated to thiosulfate at



Reference compounds and controls (Wild-type spectra): Reference spectra were collected for a number of compounds relating to the various oxidation states of sulfur expected in our protein samples; methionine, methionine sulfoxide, reduced glutathione, oxidised glutathione and cysteic acid. Wild-type H Cystatin B contains no cysteine but has two methionines. Methionine is oxidised to methionine sulfoxide by peroxide. Exposure to air or diamide has no effect. The wild-type spectra were used to fit the data in linear combination with reference spectra for reduced/oxidised glutathione and cysteic acid.

S7C/S45C: This mutant was designed to probe whether or not native contacts between strands 1 and 2 are maintained within the fibril structure. Upon fitting the spectra we found that all of the cysteine present had been oxidised to cystine. This result implies that the native contact between strands 1 and 2 is preserved within the fibril structure.

Q17C/A40C: This mutant was designed to probe whether native contacts between the α-helix and strand 2 are maintained within the fibril structure. Upon fitting the data, we found that 70% of the cysteine was oxidised to cystine, whilst 30% remained reduced.

Q46C/N52C: This mutant was designed to probe whether native contacts between strand 2 and 3 are preserved within the fibril structure. This is particularly important as the protein domain swaps across this interface to form dimers, a process which has been suggested to be important in fibrilisation. We found that all of the cysteine had been oxidised to cystine, implying that the contact is preserved within the fibril structure.

T51C/Q71C: Upon fitting the spectra obtained for this mutant we found that all the cysteine present had been oxidised to cystine, imlying that the contact between strands 3 and 4 is preserved within the fibril structure.

Q71C/L80C: We found that 75% of the cysteine present had been oxidised to cystine whilst 25% remained reduced.