EUROPEAN SYNCHROTRON RADIATION FACILITY

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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:** Structure of properdin in the complement system	**Experiment number**: WT-53
Beamline: ID02	**Date of experiment**: 18 Dec 2004, 18 May 2005 (1 day each)	**Date of report**: 1st Sept 2005
Shifts: 6	**Local contact(s)**: Dr Stephanie Finet	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Sun, Z.*, Eaton, J. T.*, & Perkins, S. J.* (UCL)

Reid, K. B. M. (Oxford University)

Hu, Y.* & Bouloux, P. M. G. (Royal Free Hospital, London)

Report:

The multidomain solution structures of the complement protein properdin dimer and trimer by X-ray scattering, analytical ultracentrifugation and constrained modeling. (2004) *J. Mol. Biol.* **343**, 1327-1343. Sun, Z., Reid, K. B. M. and. Perkins, S. J.

Abstract: Properdin regulates the alternative pathway of the complement system of immune defence by stabilising the C3 convertase complex. It contains six thrombospondin repeat type I (TSR-1 to TSR-6) domains and an N-terminal domain. Properdin exists as either a dimer, trimer or tetramer. In order to determine the solution structure of multiple TSR domains, the molecular structures of dimeric and trimeric properdin were studied by X-ray scattering and analytical ultracentrifugation. Guinier analyses showed that the dimer and trimer have radii of gyration R_G values of 7.5 nm and 10.3 nm respectively, and cross-sectional radii of gyration R_{XS} values of 1.3 nm and 1.5 nm respectively. Distance distribution functions showed that the maximum lengths of the dimer and trimer were 25 nm and 30 nm respectively. Analytical ultracentrifugation gave sedimentation coefficients of 5.1 S and 5.2 S for the dimer and trimer forms respectively. Homology models for the TSR domains were constructed using the crystal structure of the TSP-2 and TSP-3 domains in human thrombospondin as templates. Properdin could be represented by seven TSR domains, not six as previously believed, since the crystal structure determined for TSR-2 and TSP-3 showed that the N-terminal domain (TSR-0) could be represented by a truncated TSR domain with the same six conserved Cys residues found in TSR-1 to TSR-6. Automated constrained molecular modelling revealed the solution conformations of multiple TSR domains in properdin at medium resolution. The comparison of 3,125 systematically generated conformational models for the trimer with the X-ray data showed that good curve fits could be obtained by assuming that the linker between adjacent TSR domains possessed limited flexibility. Good trimer models correspond to partially collapsed triangular structures, and extended triangular shapes do not fit the data. The corresponding 3,125 models for the dimer revealed a similar

outcome in which a partially collapsed TSR structure gave good fits. The models account for the effect of mutations that cause properdin deficiencies, and suggest that the biologically active TSR-4, TSR-5 and TSR-6 domains are exposed for protein-protein interactions. The role of the other TSR domains in properdin may be to act as spacers to make TSR-4, TSR-5 and TSR-6 accessible for function.

Extended domain solution structure of the extracellular matrix protein anosmin-1 by X-ray scattering, analytical ultracentrifugation and constrained modeling. *J. Mol. Biol.* **350**, 553-570. (2005) Hu, Y., Sun, Z., Eaton, J. T., Bouloux, P. M. G. & Perkins, S. J.

Abstract: Kallmann's syndrome corresponds to a loss of sense of smell and hypogonadotrophic hypogonadism. Defects in anosmin-1 result in the X-linked inherited form of Kallmann's syndrome. Anosmin-1 is an extracellular matrix protein involved in cell adhesion and neurite outgrowth, and interacts with heparan sulphate, urokinase-like plasminogen activator and the fibroblast growth factor receptor. Anosmin-1 comprises an N-terminal cysteine rich (Cys-box) domain and a whey acidic protein like (WAP) domain, followed by four fibronectin type III (FnIII) domains. The solution structures of recombinant proteins containing the first three domains (PIWF1) and all six domains (PIWF4) were determined by X-ray scattering and analytical ultracentrifugation. Guinier analyses showed that PIWF1 and PIWF4 have different radii of gyration R_G values of 3.1 nm and 6.7 nm respectively, but similar cross-sectional radii of gyration R_{XS} values of 1.5 nm and 1.9 nm respectively. Distance distribution functions showed that the maximum lengths of PIWF1 and PIWF4 were 11 nm and 23 nm respectively. Analytical ultracentrifugation gave sedimentation coefficients of 2.52 S and 3.55 S for PIWF1 and PIWF4 respectively. The interpretation of the scattering data by constrained modelling requires homology models for all six domains in anosmin-1. While models were already available for the WAP and FnIII domains, that for the Cys-box domain was not. A homology model was derived from database searches which suggested this may resemble the cysteine-rich region of the insulin-like growth factor receptor. Automated constrained molecular modelling based on joining the individual anosmin-1 domains with structurally-randomised linkers resulted in 10,000 models for anosmin-1. A trial-and-error search showed that about 0.1% to 1.4% of these models gave satisfactory curve fits to the X-ray data. The best models showed that the three and six domains in PIWF1 and PIWF4 were extended. The inter-domain linkers in anosmin-1 could not all be extended at the same time, and there was evidence for inter-domain flexibility. Models with folded-back domain arrangements do not fit the data. These solution structures account for the known biological function of anosmin-1, in particular its ability to interact with its three macromolecular ligands.