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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:** Solution structures of the SCR domains in complement factor H and related proteins	**Experiment number**: SC-3124
Beamline:	**Date of experiment**: from: 4 Mar 2011 to: 7 Mar 2011	**Date of report**: 1st Mar 2012
Shifts:	**Local contact(s)**: Dr T. Narayanan	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(1) Miller, A.*, Phillips, A.*, Gor, J., Wallis, R.* & Perkins, S. J.* (UCL; Leicester)

(2) Li, K.* Gor, J., Holers, V. M., Storek, M. J. & & Perkins, S. J.* (UCL; Denver; Alexion)

Report:

Publication: Miller, A., Phillips, A., Gor, J., Wallis, R. & Perkins, S. J. (2012). The near-planar solution structures of mannose-binding lectin oligomers provide insight on the activation of the lectin pathway of complement. *J. Biol. Chem.* **287**, 3930-3945. Pubmed 22167201

Abstract: The complement system is a fundamental component of innate immunity that orchestrates complex immunological and inflammatory processes. Complement comprises over 30 proteins that eliminate invading microorganisms while maintaining host cell integrity. Protein-carbohydrate interactions play critical roles in both the activation and regulation of complement. Mannose-binding lectin (MBL) activates the lectin pathway of complement via the recognition of sugar arrays on pathogenic surfaces. In order to determine the solution structure of MBL, synchrotron X-ray scattering and analytical ultracentrifugation experiments showed that the carbohydrate-recognition domains in the MBL dimer, trimer and tetramer are positioned close to each other in near-planar fan-like structures. These data were subjected to constrained modelling fits. A bent structure for the MBL monomer was identified starting from two crystal structures for its carbohydrate-recognition domain and its triple helical region. The MBL monomer structure was used to identify 10-12 near-planar solution structures for each of the MBL dimer, trimer and tetramer starting from

900-6,859 randomised structures for each. These near-planar fan-like solution structures joined at an N-terminal hub clarified how the carbohydrate-recognition domain of MBL binds to pathogenic surfaces. They also provided insight on how MBL presents a structural template for the binding and auto-activation of the MBL-associated serine proteases to initiate the lectin pathway of complement activation.

Publication: Li, K., Gor, J., Holers, V. M., Storek, M. J. & Perkins, S. J. (2012). Solution structure of TT30, a novel therapeutic agent for complement-mediated diseases, provides insight on its joint binding to complement C3b and C3d. *J. Mol. Biol.* In press.

Abstract: A novel therapeutic reagent TT30 was designed to be effective in diseases of the alternative pathway of complement such as paroxysmal nocturnal haemoglobinuria (PNH) and other diseases. TT30 is constructed from the first four short complement regulator (SCR) domains of complement receptor type 2 (CR2) that bind to complement C3d, followed by the first five SCR domains of complement factor H (CFH) that bind to complement C3b. In order to assess how TT30 binds to C3d and C3b, we determined the TT30 solution structure by a combination of analytical ultracentrifugation, X-ray scattering and constrained modelling. The sedimentation coefficients and radius of gyration of TT30 were unaffected by citrate or phosphate buffer saline buffers, and indicate an elongated monomeric structure with a sedimentation coefficient of 3.1 S and a radius of gyration R_G of 6.9 nm. Molecular modelling starting from 3,000 randomised TT30 conformations showed that high quality X-ray curve fits were obtained with extended SCR arrangements, showing that TT30 has a limited degree of inter-SCR flexibility in its solution structure. The best-fit TT30 structural models are readily merged with the crystal structure of C3b to show that the four CR2 domains extend freely into solution when the five CFH domains are bound within C3b. We re-evaluated the solution structure of the CR2-C3d complex that confirmed its recent crystal structure. This recent CR2-C3d crystal structure showed that TT30 is able to interact readily with C3d ligands in many orientations when TT30 is bound to C3b.