EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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-2994
Beamline:	**Date of experiment**: from: 24 Sep 2010 to: 27 Sep 2010	**Date of report**: 1st Sep 2011
Shifts:	**Local contact(s)**: Dr T. Narayanan	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(1) Khan, S.*, Nan, R.*, Gor, J., Mulloy, B. & Perkins, S. J.* (UCL)

(2) Li, K.* & Perkins, S. J.* (UCL)

Report:

Publication: Khan, S., Nan, R., Gor, J., Mulloy, B. & Perkins, S. J. (2011). Multivalent binding of complement factor H to heparin provides new insights for function and disease. Submitted.

Abstract: Factor H (FH) with 20 short complement regulator (SCR) domains is a major serum regulator of complement, and is associated with diseases including age-related macular degeneration and atypical haemolytic uraemic syndrome. Heparin binding to FH at SCR-7 and SCR-20 is an analogue of FH binding to glycosaminoglycan-coated host cell surfaces. To elucidate the solution structures of heparin fragments dp6-dp36 bound to intact FH (where dp stands for degree of polymerisation), FH-heparin mixtures were studied by X-ray scattering and analytical ultracentrifugation. The X-ray radius of gyration R_G of FH slightly decreased from 8.4 to 7.3 nm for dp6 and dp12, showing that heparin blocked FH self-association. In the presence of dp18 to dp36, the R_G increased significantly from 10.4 to 11.7 nm, and the maximum lengths of FH increased from 35 nm to 40 nm, indicating the formation of compact oligomers. In contrast to the 12-15% of oligomers for free FH, ultracentrifugation showed that heparin induced up to 63% of structurally well-defined FH oligomers ranging from dimer to decamer. This corresponds to a moderate dissociation constant K_D of about 0.5 μM. Surface plasmon resonance of FH-dp32 mixtures gave a comparable K_D of 2.7 μM.

Molecular modelling of FH molecules cross-linked by heparin molecules explained the sedimentation coefficients of the oligomers without a need to invoke major conformational changes in FH. We obtain the first molecular view of the bivalent binding of FH to host cell surfaces at two independent sites, and this provides novel insights on complement-mediated immune disease.

Publication: Li, K. & Perkins, S. J. (2011) Complement C3u interacts independently with complement Factor H at two sites: implications for complement regulation. Submitted.

Abstract: In the alternative pathway of complement activation, factor H (FH) with 20 short complement regulator (SCR) domains regulates the major complement protein C3b. C3b shares high functional similarity with C3u (also known as $C3_{H20}$). Here, analytical ultracentrifugation and X-ray scattering were used to identify the stoichiometry and structure of the C3u-FH interaction. Size-distribution analyses showed that both 1:1 and 2:1 complexes of C3u-FH were observed in buffers with 137 mM and 50 mM NaCl. In 137 mM NaCl buffer, the 1:1 complex is predominant, and a dissociation constant K_D of 0.59 μM was determined. The maximum dimensions of the C3u-FH complexes were similar in a range of 30-33 nm, showing that FH did not become more compact in these. Starting from known structures for FH, C3u, FH-C3b and FH-C3d complexes, and the C3b dimer, a curve fit procedure for weakly-interacting macromolecules accounted for the FH-C3u scattering curves. For 137 mM NaCl buffer, unbound C3u and FH and a 1:1 C3u-FH complex were found, leading to a K_D value of 0.22 μM. In 50 mM NaCl buffer, the presence of 2:1 complexes was identified. The 1:1 and 2:1 C3u-FH complexes accounted for the observed sedimentation coefficients. Because no FH conformational changes were detected in the complexes, and two independent binding sites for C3u in FH were identified, our results clarify the bivalency of the C3u-FH interaction and show that the N-terminus and C-terminus of FH are functionally independent of each other.