EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

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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:** Solution structures of the SCR domains in complement factor H and related proteins	**Experiment number**: SC-3255
Beamline:	**Date of experiment**: from: 14 Sep 2011 to: 16 Sep 2011	**Date of report**: 1st Sep 2012
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; NIBSC)

(2) Rayner, L. E.*, Kadkhodayi-Kholghi, N.*, Gor, J., Dalby, P. A. & Perkins S. J.* (UCL)

Report:

Publication: Khan, S., Nan, R., Gor, J., Mulloy, B. & Perkins, S. J. (2012). Bivalent and co-operative binding of complement Factor H to heparan sulphate and heparin. *Biochem. J.* **444**, 417-428. Pubmed 22471560. http://dx.doi.org/10.1042/BJ20120183

Abstract: Factor H (FH) with 20 short complement regulator (SCR) domains is a major serum regulator of complement, and genetic defects in this are associated with inflammatory diseases. Heparan sulphate is a cell surface glycosaminoglycan composed of sulphated *S*-domains and unsulphated *NA*-domains. To elucidate the molecular mechanism of binding of FH to glycosaminoglycans, we performed ultracentrifugation, X-ray scattering and surface plasmon resonance with FH and glycosaminoglycan fragments. Ultracentrifugation showed that FH formed up to 63% of well-defined oligomers with purified heparin fragments (equivalent to *S*-domains), and indicated a dissociation constant K_D of about 0.5 μM. FH structures that are bivalently cross-linked at SCR-7 and SCR-20 with heparin explained the sedimentation coefficients of the FH-heparin oligomers. The X-ray radius of gyration R_G of FH in the presence of heparin fragments 18 to 36 monosaccharide units long increased significantly from 10.4 to 11.7 nm, and the maximum lengths of FH increased from 35 nm to 40 nm, confirming that large compact oligomers had formed. Surface plasmon

resonance of immobilised heparin with full-length FH gave K_D values of 1-3 μM, and similar but weaker K_D values of 4-20 μM for the SCR-6/8 and SCR-16/20 fragments, confirming co-operativity between the two binding sites. The use of minimally-sulphated heparan sulphate fragments that correspond largely to *NA*-domains showed much weaker binding, proving the importance of *S*-domains for this interaction. This bivalent and co-operative model of FH binding to heparan sulphate provided novel insights on the immune function of FH at host cell surfaces.

Publication: Rayner, L. E., Kadkhodayi-Kholghi, N., Gor, J., Dalby, P. A. & Perkins S. J. (2012). The solution structure of rabbit IgG accounts for its interactions with the Fc receptor and complement C1q and its conformational stability. Submitted.

Abstract: Solution structures for antibodies are critical to understand function and therapeutic applications. The stability of the solution structure of rabbit IgG in different buffers and temperatures was determined by analytical ultracentrifugation and X-ray and neutron scattering. Rabbit IgG is principally monomeric with a sedimentation coefficient $s_{20,w}$ of 6.6 S, and its dimer sedimented at 9.3 S. The proportion of dimer increased with increased concentration, decreased temperature and heavy water from 8% to 25% in all buffers except for high salt (250 mM NaCl). The Guinier X-ray radius of gyration R_G likewise increased with concentration in 137 mM NaCl buffer, but was unchanged in 250 mM NaCl buffer. The Guinier neutron R_G values increased as the temperature decreased. The X-ray and neutron distance distribution curves *P(r)* revealed two peaks, M1 and M2 whose positions did not change with concentration to indicate unchanged structures in all these conditions, while the maximum dimension increased with concentration because of dimer formation. Constrained scattering modelling revealed reproducible asymmetric solution structures for monomeric rabbit IgG, in which the Fab-Fc and Fab-Fab pairs were separated by maximally-extended hinge structures. These structures reflect the lack of conformational change detected in different buffers. The dimer was best modelled by two Fab regions forming tip-to-tip contacts. The intact rabbit IgG structures explained the ability of its two ligands, the Fc receptor and complement C1q, to bind to the top of its Fc region which is fully accessible and unhindered by the Fab regions.