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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-3381
Beamline: ID02	**Date of experiment:** from: 5 Jul 2012 to: 8 Jul 2012	**Date of report:** 4th Mar 2013
Shifts: 6	**Local contact(s):** Dr Gudrun Lotze, Dr T. Narayanan	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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

(2) Khan, S.*, Gor, J., Mulloy, B. & Perkins, S. J.* (UCL; NIBSC)

(3) Nan, R.*, Tetchner, S.*, Rodriguez, E.*, Gor, J., Lengyel, I. & Perkins, S. J.* (UCL; UCL Inst. Of Ophthalmology)

Report:

Publication: Rayner, L. E., Kadkhodayi-Kholghi, N., Heenan, R. K., Gor, J., Dalby, P. A. & Perkins S. J. (2013). The solution structure of rabbit IgG accounts for its interactions with the Fc receptor and complement C1q and its conformational stability. *J. Mol. Biol.* **425**, 506-523. Pubmed 23178865.

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 showed a principally monomeric species which is well resolved from small amounts of a dimeric species. 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. The maximum dimension increased with concentration because of dimer formation. Constrained scattering modelling reproducibly revealed very similar asymmetric solution structures for monomeric rabbit IgG in different buffers, in which the Fab-Fc and Fab-Fab pairs were separated by

maximally-extended hinge structures. The dimer was best modelled by two pairs of 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.

Publication: Khan, S., Gor, J., Mulloy, B. & Perkins, S. J. (2013). ***Corrigendum.*** Semi-rigid solution structures of heparin by constrained X-ray scattering modelling: new insight into heparin-protein complexes. *J. Mol. Biol.* In press.

Publication: Nan, R., Tetchner, S., Rodriguez, E., Gor, J., Lengyel, I. & Perkins, S. J. (2013). Zinc-induced self-association of complement C3b and factor H: implications for inflammation and age-related macular degeneration. In preparation/submission imminent.

Abstract: The sub-retinal pigment epithelial deposits (sRPEds) that are a hallmark of age-related macular degeneration (AMD) contain both C3b and mM levels of bioavailable zinc. C3 is the central protein of complement,while C3u is formed by the spontaneous hydrolysis of the thioester bridge in C3. During activation, C3 is cleaved to form active C3b, then C3b is inactivated by Factor I and Factor H to form the C3c and C3d fragments. The interaction of zinc with C3 was quantified using analytical ultracentrifugation and X-ray scattering. C3, C3u, and C3b associated strongly in >100 μM [Zn], while C3c and C3d showed weak association. With zinc, C3 forms soluble oligomers, while C3u and C3b precipitate. We conclude that the C3, C3u and C3b association with zinc depended on the relative positions of C3d and C3c in each protein. Predictions showed that putative weak zinc binding sites with different capacities exist in all five proteins, in agreement with experiment. Factor H forms large oligomers in >10 μM [Zn]. In distinction to C3b or Factor H alone, the solubility of the central C3b-Factor H complex was much reduced at 60 μM [Zn], and even more so at >100 μM [Zn]. The removal of the C3b-Factor H complex by zinc explains the reduced C3u/C3b inactivation rates by zinc. Zinc-induced precipitation may contribute to the initial development of sRPEds in the retina, and the reduced progression to advanced AMD in higher-risk patients.