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:** Molecular basis of the activity of factor B of complement	**Experiment number**: WT-40
Beamline: ID02	**Date of experiment**: 27 Feb 2003, 27 May 2003, 16 Dec 2003 (1 day each)	**Date of report**: 1st Sept 2005
Shifts: 9	**Local contact(s)**: Dr Stephanie Finet	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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

Hannan, J. P., Guthridge, J. M. & Holers, V. M. (University of Colorado Health Sciences Center, USA)

Report:

Solution structure of the complex between CR2 SCR 1-2 and C3d of human complement: an X-ray scattering and sedimentation modelling study. (2005) *J. Mol. Biol.* **346**, 859-873. Gilbert, H. E., Eaton, J. T., Hannan, J. P., Holers, V. M., and Perkins, S. J.

Abstract: Complement receptor type 2 (CR2, CD21) forms a tight complex with C3d, a fragment of C3, the major complement component. Previous crystal structures of the C3d-CR2 SCR 1-2 complex and free CR2 SCR 1-2 showed that the two SCR domains of CR2 form contact with each other in a closed V-shaped structure. SCR 1 and SCR 2 are connected by an unusually long eight-residue linker peptide. Medium resolution solution structures for CR2 SCR 1-2, C3d, and their complex were determined by X-ray scattering and analytical ultracentrifugation. CR2 SCR 1-2 is monomeric. For CR2 SCR 1-2, its radius of gyration R_G of 2.12 ± 0.05 nm, its maximum length of 10 nm and its sedimentation coefficient $s^o_{20,w}$ of 1.40 ± 0.03 S do not agree with those calculated from the crystal structures, and instead suggest an open structure. Computer modelling of the CR2 SCR1-2 solution structure was based on the structural randomization of the eight-residue linker peptide joining SCR 1 and SCR 2 to give 9,950 trial models. Comparisons with the X-ray scattering curve indicated that the most favoured arrangements for the two SCR domains corresponded to an open V-shaped structure with no contacts between the SCR domains. For C3d, X-ray scattering and sedimentation velocity experiments showed that it exists as a monomer-dimer equilibrium with a dissociation constant of 40 μM. The X-ray scattering curve for monomeric C3d gave an R_G value of 1.95 nm, and this together with its $s^o_{20,w}$ value of 3.17 S gave good agreement with the monomeric C3d crystal structure. Modelling of the C3d dimer gave good agreements with its scattering and ultracentrifugation parameters. For the complex, scattering and ultracentrifugation experiments showed that there was no dimerisation, indicating that the C3d dimerisation site was located close to the CR2 SCR 1-2 binding site. The R_G value of 2.44 ± 0.1 nm, its length of 9 nm and its $s^o_{20,w}$ value of 3.45 ± 0.01 S showed that its structure was not much

more elongated than that of C3d. Calculations with 9,950 models of CR2 SCR 1-2 bound to C3d through SCR 2 showed that SCR 1 formed an open V-shaped structure with SCR 2 and was capable of interacting with the surface of C3d. We conclude that the open V-shaped structures formed by CR2 SCR 1-2, both when free and when bound to C3d, are optimal for the formation of a tight two-domain interaction with its ligand C3d.

Extended linkers between CR2 SCR-1 and SCR-2 and the Fc fragment in CR2-Ig by X-ray and neutron scattering, analytical ultracentrifugation and constrained molecular modelling. (2005). Submitted for publication..Gilbert, H. E., Aslam, M., Guthridge, J. M., Holers, V. M. &. Perkins, S. J.

Abstract: Complement receptor 2 (CR2; CD21) is a membrane-bound regulator of complement activation, being comprised of 15 or 16 short complement repeat (SCR domains). A recombinant glycosylated human CR2 SCR 1-2 domain pair was engineered with the Fc fragment of a mouse IgG1 antibody to create a chimaera CR2-Ig containing the major ligand binding domains. Such a chimaera has therapeutic potential as a complement inhibitor or immune modulator. X-ray and neutron scattering and analytical ultracentrifugation identified its domain structure in solution, and provided a comparison with controversial folded-back crystal structures for deglycosylated CR2 SCR 1-2. The radius of gyration R_G of CR2-Ig was determined to be 5.39 ± 0.14 and 5.29 ± 0.01 nm by X-ray and neutron scattering respectively. The maximum dimension of CR2-Ig was determined to be 17 nm. The molecular weight of CR2-Ig ranged between 101,000 to 107,000 Da by neutron scattering and sedimentation equilibrium, in good agreement with the sequence-derived value of 106,600 Da. Sedimentation velocity gave a sedimentation coefficient of 4.49 ± 0.11 S. Stereochemically-complete models for CR2-Ig were constructed from crystal structures for the CR2 SCR 1-2 and mouse IgG1 Fc fragments. The two SCR domains and the Fc fragment were joined by randomised conformational peptides. The analysis of 35,000 possible CR2-Ig models showed that only those models in which the two SCR domains were arranged in an open V-shape in random orientations about the Fc fragment accounted for the scattering and sedimentation data. It was not possible to define one single conformational family of Fab-like fragment relative to the Fc fragment. This flexibility is attributed to the relatively long linker sequence and the absence of the antibody light chain in CR2-Ig. The modelling also confirmed that the structure of CR2 SCR 1-2 is more extended in solution than in its crystal structure.