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-2265
Beamline: ID02	**Date of experiment**: 29-30 Jan 2008 (2 days)	**Date of report**: 29th Feb 2008
Shifts: 6	**Local contact(s)**: Dr Stephanie Finet	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(1) Okemefuna, A. I. *, Gilbert, H. E. *, & Perkins, S. J. * (UCL) Griggs, K. M., Ormsby, R. J. & Gordon, D. L. (Flinders University, Australia)

(2) Nan, R. *, Gor, J. & Perkins, S. J. * (UCL)

(3) Furtado, P. B. *, Huang, C. Y. *, Ihyembe, D. *& Perkins, S. J. * (UCL) Hammond, R. A. & Marsh, H. C. (Avant Immunotherapeutics, USA)

Report:

Publication: Okemefuna, A. I., Gilbert, H. E., Griggs, K. M., Ormsby, R. J., Gordon, D. L. & Perkins, S. J. (2008). The regulatory SCR-1/5 and cell-surface-binding SCR-16/20 fragments of Factor H reveal partially folded-back solution structures and different self-associative properties. J. Mol. Biol. **375**, 80-101.

Abstract: Factor H (FH) is a plasma glycoprotein that plays a central role in regulation in the alternative pathway of complement. It is composed of 20 short complement regulator (SCR) domains. The SCR-1/5 fragment is required for decay acceleration and cofactor activity, while the SCR-16/20 fragment possesses binding sites for complement C3d and heparin. X-ray scattering and analytical ultracentrifugation showed that SCR-1/5 was monomeric while SCR-16/20 formed dimers. The Guinier radius of gyration R_G of 4.3 nm for SCR-1/5 and those of 4.7 nm and about 7.8 nm for monomeric and dimeric SCR-16/20 respectively showed that their structures are partially folded back and bent. The distance distribution function *P(r)* showed that SCR-1/5 has a maximum dimension of 15 nm while monomeric and dimeric SCR-16/20 are 17 nm and about 27 nm long respectively. The sedimentation coefficient of 2.4 S for SCR-1/5 showed no concentration dependence, while that for SCR-16/20 was 2.8 S for the monomer and 3.9 S for the dimer. Sedimentation equilibrium data showed that SCR-1/5 is monomeric while SCR-16/20 exhibited a weak monomer-dimer equilibrium with a dissociation constant of 16 μM. The constrained scattering and sedimentation modelling of SCR-1/5 and SCR-16/20 showed that partially folded-back and bent flexible SCR arrangements fitted both data sets better than extended linear arrangements, and that the dimer was best modelled in the SCR-16/20 model by an end-to-end association of two SCR-20 domains. The SCR-1/5 and SCR-16/20 models were conformationally similar to the previously-determined partially folded-back structure for intact wild-type FH, hence suggesting a part-explanation of the intact FH structure. Comparison of the SCR-16/20 model with the crystal structure of C3b clarified reasons for the distribution of mutations leading to atypical haemolytic uraemic syndrome.

Publication: Nan, R., Gor, J. & Perkins, S. J. (2008). Implications of the progressive self-association of wild-type human Factor H for complement regulation and disease. J. Mol. Biol. **375**, 891-900.

Abstract: Factor H (FH) is a major regulator of complement alternative pathway activation. It is composed of 20 short complement regulator (SCR) domains, and is genetically associated as a risk factor for age-related macular degeneration. Previous studies on FH suggested this existed in monomeric or dimeric forms. Improved X-ray scattering and analytical ultracentrifugation methodology for wild-type FH permitted a clarification of these oligomeric properties. Data at lower concentrations revealed a dependence of the X-ray R_G values on concentration that corresponded to the weak self-association of FH. Global sedimentation equilibrium fits indicated that a monomer-dimer equilibrium best described the data up to 1.3 mg/ml with a fitted dissociation constant K_D of 28 μM, and that higher oligomers formed at increased concentrations. The K_D showed that about 85% to 95% of serum FH will be monomeric in the absence of other factors. Size-distribution analyses in sedimentation velocity experiments showed that monomeric FH was the major species but that as many as six different oligomeric forms co-existed with this. The data were explained in terms of two weak dimerisation sites recently identified in the SCR-6/8 and SCR-16/20 fragments of FH with similar K_D values. These observations indicate a mechanism for the progressive self-association of FH, and may be relevant for complement regulation and the formation of drusen deposits that are associated with age-related macular degeneration.

Publication: Furtado, P. B., Huang, C. Y., Ihyembe, D., Hammond, R. A., Marsh, H. C. & Perkins, S. J. (2008). The partly-folded back solution structure arrangement of the 30 SCR domains in human complement receptor type 1 (CR1) permits access to its C3b and C4b ligands. J. Mol. Biol. **375**, 102-118.

Abstract: Human complement receptor type 1 (CR1, CD35) is a Type I membrane-bound glycoprotein that belongs to the regulators of complement activity (RCA) family. The extracellular component of CR1 is comprised of 30 short complement regulator (SCR) domains, whereas complement receptor type 2 (CR2) has 15 SCR domains and factor H (FH) has 20 SCR domains. The domain arrangement of a soluble form of CR1 (sCR1) was studied by X-ray scattering and analytical ultracentrifugation. The radius of gyration R_G of sCR1 of 13.4 ± 1.1 nm is not much greater than those for CR2 and FH, and its R_G/R_0 anisotropy ratio is 3.76, compared to ratios of 3.67 for FH and 4.1 for CR2. Unlike CR2, but similar to FH, two cross-sectional R_G ranges were identified that gave R_{XS} values of 4.7 ± 0.2 nm and 1.2 ± 0.7 nm respectively, showing that the SCR domains adopt a range of conformations including folded-back ones. The distance distribution function *P(r)* showed that the most commonly occurring distance in sCR1 is at 11.5 nm. Its maximum length of 55 nm is less than double those for CR2 or FH, even though sCR1 has twice the number of SCR domains compared to CR2 Sedimentation equilibrium experiments gave a mean molecular weight of 235 kDa for sCR1. This is consistent with the value of 245 kDa calculated from its composition including 14 N-linked oligosaccharide sites, and confirmed that sCR1 is a monomer in solution. Sedimentation velocity experiments gave a sedimentation coefficient of 5.8 S. From this, the frictional ratio (f/f_0) of sCR1 was calculated to be 2.29, which is greater than those of 1.96 for CR2 and 1.77 for FH. The constrained scattering modelling of the sCR1 solution structure starting from homologous SCR domain structures generated 5000 trial conformationally-randomised models, 43 of which gave good scattering fits to show that sCR1 has a partly folded-back structure. We conclude that the inter-SCR linkers show structural features in common with those in FH, but differ from those in CR2, and the SCR arrangement in CR1 will permit C3b or C4b to access all three ligand sites.