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-2408
Beamline: ID02	**Date of experiment**: 10-12 Sep 2008 (2 days)	**Date of report**: 1st Mar 2009
Shifts: 6	**Local contact(s)**: Dr Anuj Shukla	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(1) Nan, R. *, Gor, J., Lengyel, I. & Perkins, S. J. * (UCL)

(2) Li, K. *, Okemefuna, A. I. *, Gor, J., Hannan, J. P., Asokan, R., Holers, V. M. & Perkins, S. J. * (UCL; Denver, Colorado, USA)

(3) Bonner, A. *, Almogren, A., Furtado, P. B. *, Kerr, M. A. & Perkins, S. J. * (UCL; Riyadh, Saudi Arabia; Leeds University

Report:

Publication: Nan, R., Gor, J., Lengyel, I. & Perkins, S. J. (2008). Uncontrolled zinc- and copper-induced oligomerisation of the human complement regulator Factor H and its possible implications for function and disease. J. Mol. Biol. **384**, 1341-1352.

Abstract: Polymorphisms in factor H (FH), a major regulator of complement activation, and the accumulation of high zinc concentrations in the outer retina are each associated with age-related macular degeneration (AMD). FH is inhibited by zinc, which causes FH to aggregate. To investigate this, zinc-induced FH self-association was quantitatively studied by X-ray scattering and analytical ultracentrifugation to demonstrate uncontrolled FH oligomerisation in conditions corresponding to physiological levels of FH and pathological levels of zinc in the outer retina. By scattering, FH at 2.8 – 7.0 μM was unaffected until [Zn] increased to 20 μM, whereupon the radius of gyration R_G values increased from 9 nm to 15 nm at [Zn] = 200 μM. The maximum dimension of FH increased from 32 nm to 50 nm, indicating that compact oligomers had formed. By ultracentrifugation, size-distribution analyses showed that monomeric FH at 5.57 S was the major species at [Zn] up to 60 μM. At [Zn] above 60 μM, a series of large oligomers were formed, ranging up to 100 S in size. Oligomerisation was reversed by EDTA. Structurally distinct large oligomers were observed for Cu, while Ni, Cd and Fe showed low amounts of oligomers, and Mg and Ca showed no changes. Fluid-phase assays showed reduced FH activities that correlated with increased oligomer formation. The results were attributed to different degrees of stabilisation of weak self-dimerisation sites in FH by transition metals. The relevance of metal-induced FH oligomer formation to complement regulation and AMD is discussed.

Publication: Li, K., Okemefuna, A. I., Gor, J., Hannan, J. P., Asokan, R., Holers, V. M. & Perkins, S. J. (2008) Solution structure of the complex formed between human complement C3d and full length complement receptor Type 2. J. Mol. Biol. **384**, 137-150.

Abstract: Complement receptor type 2 (CR2, CD21) is a cell surface protein that links the innate and adaptive immune response during the activation of B cells through its binding to C3d, a cleavage fragment of the major complement component C3. The extracellular portion of CR2 comprises 15 or 16 short complement regulator (SCR) domains in a partially folded-back but flexible structure. Here, the effect of C3d binding to CR2 was determined by analytical ultracentrifugation and X-ray scattering. The sedimentation coefficient of unbound CR2 is 4.03 S in 50 mM NaCl. Because this agrees well with a value of 3.93 S in 137 mM NaCl, the overall CR2 structure is unaffected by change in ionic strength. Unbound C3d exists in monomer-dimer and monomer-trimer equilibria in 50 mM NaCl, but as a monomer only in 137 mM NaCl. In c(s) size-distribution analyses, an equimolar mixture of the CR2-C3d complex in 50 mM NaCl revealed a single peak shifted to 4.52 S when compared to unbound CR2 at 4.03 S to show that the complex had formed. The CR2-C3d complex in 137 mM NaCl showed two peaks at 2.52 S and 4.07 S to show that this had dissociated. Solution structural models for the CR2 SCR-1/2 complex with C3d and CR2 SCR-1/15 were superimposed. These gave an average sedimentation coefficient of 4.57 S for the complex, in good agreement with the observed value of 4.52 S. It is concluded that CR2 does not detectably change conformation when C3d is bound to it. Consistent with previous analyses, its C3d complex is not formed in physiological salt conditions. The implications of these solution results for its immune role are discussed. To our knowledge, this is the first solution structural study of a large multidomain SCR protein CR2 bound to its physiological ligand C3d.

Publication: Bonner, A., Almogren, A., Furtado, P. B., Kerr, M. A. & Perkins, S. J. (2009). Location of secretory component on the Fc edge of dimeric IgA1 reveals insight into the role of secretory IgA1 in mucosal immunity. Mucosal Immunology (Nature Publishing Group), **2**, 74-84.

Abstract: Secretory IgA (SIgA) is the most prevalent antibody in the human body and a first line of defence in mucosal immunity. We located secretory component (SC) relative to dimeric IgA1 (dIgA1) within the SIgA1 structure using the constrained modelling of solution scattering and analytical ultracentrifugation data. The extended solution structure of dIgA1 is largely preserved within SIgA1. From conformational searches of SC locations, the best-fit SC models within SIgA1 show that SC is extended along the outermost convex edge of the Fc dimer in dIgA1. The topology of our SIgA1 structure reveals that it is able to bind to one FcαRI receptor molecule. SC binding to the Fc dimer confers protection to SIgA1 by the masking of proteolytically-susceptible surface sites from bacterial proteases in the harsh environment of the mucosa. The models support a “zipper-like” unfolding of SC upon dIgA1 in the formation and transportation of SIgA1 into the mucosa.