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-2893
Beamline:	**Date of experiment**: from: 11 Mar 2010 to: 14 Mar 2010	**Date of report**: 1st Mar 2011
Shifts:	**Local contact(s)**: Dr T. Narayanan	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Names and affiliations of applicants (* indicates experimentalists):

(1) Nan, R.*, Farabella, I., Schumacher, F., Miller, A.*, Gor, J., Martin, A.C.R., Jones, D.T., Lengyel, I. & Perkins, S. J.* (UCL)

(2) Khan, S.*, Rodriguez, E.*, Patel, R.*, Gor, J., Mulloy, B. & Perkins, S. J.* (UCL)

Report:

Publication: Nan, R., Farabella, I., Schumacher, F., Miller, A., Gor, J., Martin, A.C.R., Jones, D.T., Lengyel, I. & Perkins, S. J. (2011). Localisation of the major zinc-binding site of complement Factor H to the SCR-6/8 domains: possible implications for age-related macular degeneration. J. Mol. Biol. In press.

Abstract: The Tyr402His polymorphism of complement factor H (FH) with 20 short complement regulator (SCR) domains is associated with age-related macular degeneration (AMD). How FH contributes to disease pathology is not clear. Both FH and high concentrations of zinc are found in drusen deposits, the key feature of AMD. Heterozygous FH is inhibited by zinc which causes FH to aggregate. Here, zinc binding to homozygous FH was studied. By analytical ultracentrifugation, large amounts of oligomers were observed with both the native Tyr402 and the AMD-risk His402 homozygous allotypes of FH and both the recombinant SCR-6/8 allotypes with Tyr/His402. X-ray scattering also showed that both FH and SCR-6/8 allotypes strongly aggregated at > 10 μM zinc. The SCR-1/5 and SCR-16/20 fragments were less likely to bind zinc. These observations were supported by bioinformatics predictions. Starting from known zinc-binding sites in crystal structures, 202 putative partial surface zinc binding sites were predicted in FH, most of which were in SCR-6. Metal site prediction web servers also suggested that SCR-6 and other domains bind zinc. Predicted SCR-6/8 dimer structures showed that zinc binding sites could be formed at the protein-

protein interface that would lead to daisy-chained oligomers. It was concluded that zinc binds weakly to FH at multiple surface locations, most probably within the functionally-important SCR-6/8 domains, and this explains why zinc inhibits FH activity. Given the high pathophysiological levels of bioavailable zinc present in subretinal deposits, we discuss how zinc binding to FH may contribute to deposit formation and inflammation associated with AMD.

Publication: Khan, S., Rodriguez, E., Patel, R., Gor, J., Mulloy, B. & Perkins, S. J. (2011). The solution structure of heparan sulphate differs from that of heparin: implications for function. Submitted

Abstract: The highly sulphated polysaccharides heparin and heparan sulphate (HS) play key roles in the regulation of physiological and pathophysiological processes. Despite its importance, no molecular structures of free HS have been reported up to now. By combining analytical ultracentrifugation, small-angle X-ray scattering and constrained scattering modelling recently used for heparin, we have analysed the solution structures for eight purified HS fragments dp6 to dp18 and dp24 corresponding to the GlcA-GlcNAc domains of heparan sulphate. Unlike heparin, the sedimentation coefficient $s_{20,w}$ of HS dp6-dp24 showed a small rotor speed dependence, where $s_{20,w}$ values of 0.82 to 1.26 S (absorbance optics) and 1.05 to 1.34 S (interference optics) were determined. The corresponding X-ray scattering measurements of HS dp6-dp24 gave radii of gyration R_G values from 1.03 nm to 2.82 nm, cross-sectional radii of gyration R_{XS} values from 0.31 nm to 0.65 nm, and maximum lengths L from 3.0 nm to 10.0 nm. These data showed that HS has a longer and more bent structure than heparin. Constrained scattering modelling starting from 5,000-8000 conformationally-randomised HS structures gave best fit dp6-dp16 molecular structures that were longer and more bent than their equivalents in heparin. No fits were obtained for HS dp18 or dp24, indicating their higher flexibility. We conclude that HS displays an extended bent conformation that is significantly distinct from that for heparin. The difference is attributed to the different predominant monosaccharide sequence and reduced sulphation of HS, indicating that HS may interact differently with proteins compared to heparin.