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:** Solution structures of the SCR domains in complement factor H and related proteins	**Experiment number**: SC-3652
Beamline: BM29	**Date of experiment**: from: 12 July 2013 to: 14 July 2013	**Date of report**: 25th Apr 2014
Shifts: 6	**Local contact(s)**: Dr Martha Brennich, Dr Petra Pernot	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(1) Khan, S.*, Fung, K. W., Rodriguez, E., Patel, R., Gor, J., Mulloy, B. & Perkins, S. J.* (UCL; NIBSC)

(2) Perkins, S. J.*, Fung, K.-W.* & Khan, S.* (UCL)

(3) Rayner, L.*, Hui, G.-K.*, Gor, J., Heenan, R. K., Dalby, P. A. & Perkins S. J.* (UCL)

(4) Hui, G.-K.*, Rayner, L.*, Gor, J., Heenan, R. K., Dalby, P. A. & Perkins S. J.* (UCL)

Report:

(1) Publication: Khan, S., Fung, K. W., Rodriguez, E., Patel, R., Gor, J., Mulloy, B. & Perkins, S. J. (2013). The solution structure of heparan sulphate differs from that of heparin: implications for function. *J. Biol. Chem.* **288**, 27737-27751. Pubmed 23921391.

Abstract: The highly sulfated polysaccharides heparin and heparan sulfate (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 dp24 corresponding to the predominantly unsulfated GlcA-GlcNAc domains of heparan sulfate. Unlike heparin, the sedimentation coefficient $s_{20,w}$ of HS dp6-dp24 showed a small rotor speed dependence, where similar $s_{20,w}$ 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 5,000-12,000 conformationally-randomised HS structures gave best fit dp6-dp24 molecular structures that were longer and more bent than their equivalents in heparin. Alternative fits were obtained for HS dp18 and dp24, indicating their higher bending and flexibility. We conclude that HS displays bent conformations that are 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. [**Note:** Following publication of our original 2011 study, we regrettably discovered an error in the anomeric configuration of our heparan sulfate structural models. This present 2013 study supersedes the 2011 study which has been withdrawn.]

(2) Publication: Perkins, S. J., Fung, K.-W. & Khan, S. (2014). Molecular interactions between complement factor H and its heparin and heparan sulphate ligands. *Front. Immunol.* **5**, 126, 1-14. Pubmed 24744754.

Abstract: Complement factor H (CFH) is the major regulator of the central complement protein C3b in the alternative pathway of complement activation. A molecular view of the CFH interaction with native heparan sulfate (HS) is central for understanding the mechanism of how surface-bound CFH interacts with C3b bound to host cell surfaces. HS is composed of sulfated heparin-like S-regions that alternate with desulfated NA-regions. Solution structural studies of heparin (equivalent to the S-regions) and desulfated HS (the NA-regions) by scattering and ultracentrifugation showed that each structure was mostly extended and partially bent, but with greater bending and flexibility in the NA-regions compared to the S-regions. Their solution structures have been deposited in the Protein Data Bank. The largest HS oligosaccharides showed more bent and flexible structures than those for heparin. A folded-back domain structure for the solution structure of the 20 domains in CFH was determined likewise. CFH binds to the S-regions but less so to the NA-regions of HS. The bivalent interaction of CFH-heparin was observed by ultracentrifugation, and binding studies of CFH fragments with heparin-coated sensor chips. In common with other CFH interactions with its physiological and pathophysiological ligands, the CFH-heparin and CFH-C3b interactions have moderate micromolar dissociation constants K_D, meaning that these complexes do not fully form *in vivo*. The combination of the solution structures and binding studies indicated a two-site interaction model of CFH with heparin at cell surfaces. By this, the bivalent binding of CFH to a cell surface is co-operative. Defective interactions at either of the two independent CFH-heparin sites reduce the CFH interaction with surface-bound C3b and lead to immune disorders.

(3) Publication: Rayner, L. E., Hui, G. K., Gor, J., Heenan, R. K., Dalby, P. A. & Perkins S. J. (2014) **The Fab conformations in the solution structure of human IgG4 restrict access to its Fc region: implications for functional activity**. Submitted for publication.

Abstract: Human IgG4 antibody shows therapeutically-useful properties compared to the IgG1, IgG2 and IgG3 subclasses. Thus IgG4 does not activate complement, and shows conformational variability. These properties are attributable to its hinge region, which is the shortest of the four IgG subclasses. Using high throughput scattering methods, we have studied the solution structure of wild-type IgG4(Ser222) and a hinge mutant IgG4(Pro222) in different buffers and temperatures, where the proline substitution suppresses the formation of half-antibody. Analytical ultracentrifugation showed that both IgG4 allotypes were principally monomeric with sedimentation coefficients $s^0_{20,w}$ of 6.6-6.8 S. A monomer-dimer equilibrium was observed in heavy water buffer at low temperature. Scattering showed that the X-ray radius of gyration R_G was unchanged with concentration in 50-250 mM NaCl buffers, while the neutron R_G values showed a concentration-dependent increase as the temperature decreased in heavy water buffers. The distance distribution curves *P(r)* revealed two peaks, *M1* and *M2* that shifted below 2 mg/ml to indicate concentration-dependent IgG4 structures, in addition to IgG4 dimer formation at high concentration in heavy water. Constrained X-ray and neutron scattering modelling revealed asymmetric solution structures for IgG4(Ser222) with extended hinge structures. The IgG4(Pro222) structure was similar. Both IgG4 structures showed that their Fab regions were positioned close enough to the Fc region to restrict C1q binding. Our new molecular models for IgG4 explain its inability to activate complement, and clarifies aspects of its stability and function for therapeutic applications.

(4) Publication: Hui, G. K., Rayner, L. E., Gor, J., Heenan, R. K., Dalby, P. A. & Perkins S. J. (2014) **The Fab conformations in the solution structure of human IgG1 permit access to its Fc region: implications for functional activity**. Manuscript in publication.

Abstract: The corresponding studies for human IgG1 have been performed to show that its solution structure permits complement activation to occur. All the data processing and modelling are completed, and this work is being written up for publication.