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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-3482
Beamline: ID02	**Date of experiment**: from: 28 Nov 2012 to: 30 Nov 2012	**Date of report**: 1st Oct 2013
Shifts: 6	**Local contact(s)**: Dr Gudrun Lotze, Dr T. Narayanan	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(1) Nan, R.*, Tetchner, S.*, Rodriguez, E.*, Gor, J., Lengyel, I. & Perkins, S. J.* (UCL; UCL Inst. Of Ophthalmology)

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

(3) Rodriguez, E.*, Nan, R.*, Gor, J. & Perkins, S. J.* (UCL)

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

Report:

(1) Publication: Nan, R., Tetchner, S., Rodriguez, E., Pao, P.-J., Gor, J., Lengyel, I. & Perkins, S. J. (2013). Zinc-induced self-association of complement C3b and factor H: implications for inflammation and age-related macular degeneration. *J. Biol. Chem.* **288**, 19197-19210. Pubmed 23661701.

Abstract: The sub-retinal pigment epithelial deposits (sRPEds) that are a hallmark of age-related macular degeneration (AMD) contain both C3b and mM levels of zinc. C3 is the central protein of complement, while C3u is formed by the spontaneous hydrolysis of the thioester bridge in C3. During activation, C3 is cleaved to form active C3b, then C3b is inactivated by Factor I and Factor H to form the C3c and C3d fragments. The interaction of zinc with C3 was quantified using analytical ultracentrifugation and X-ray scattering. C3, C3u, and C3b associated strongly in >100 μM [Zn], while C3c and C3d showed weak association. With zinc, C3 forms soluble oligomers, while C3u and C3b precipitate. We conclude that the C3, C3u and C3b association with zinc depended on the relative positions of C3d and C3c in each protein. Computational predictions showed that putative weak zinc binding sites with different capacities exist in all five proteins, in agreement with experiment. Factor H forms large oligomers in >10 μM [Zn]. In distinction to C3b or Factor H alone, the solubility of the central C3b-Factor H complex was much reduced at 60 μM [Zn], and even more so at >100 μM [Zn]. The removal of the C3b-Factor H complex by zinc explains the reduced C3u/C3b inactivation rates by zinc. Zinc-induced precipitation may contribute to the initial development of sRPEds in the retina, as well as reducing the progression to advanced AMD in higher-risk patients.

(2) 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.* In press (publication date October 2013). 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.]

(3) Publication: Rodriguez, E., Nan, R., Gor, J. & Perkins, S. J. (2013) Extended and compact solution structures for complement C3b and C3u provide new insight into complement activation in the α_2-macroglobulin protein family In preparation.

Abstract: The formation of active C3b following the removal of C3a from its inactive precursor C3 is central to complement activation. C3u is produced by hydrolysis of the thioester in C3. Cleavage of C3b or C3u results in inactivated C3c and C3d, where C3d (TED domain) contains the thioester active site. Crystal structures for C3b in 50 mM NaCl buffer showed that C3c and C3d made contact with each other, while other crystal structures in the α_2-macroglobulin protein family show that the equivalent C3c and C3d regions are well separated. To reconcile these differences, we examined C3b, C3, C3u, C3c and C3d by ultracentrifugation and X-ray and neutron scattering. In 50 mM NaCl, C3b dimerised in a manner similar to C3 and C3u; we show these interactions arise from separate sites in C3c and C3d. The X-ray radius of gyration R_G values showed concentration dependences for C3, C3u, C3b and C3d, but not C3c. In 137 mM NaCl, no dimerization was seen. C3b was more extended in conformation than C3, but less extended than C3u. Scattering modelling showed that, in 50 mM NaCl, the TED domain of C3b and C3u made contact with the MG1 domain in C3c. For 137 mM NaCl, modelling fits showed that the TED and MG1 domains of C3b and C3u become separated by up to 2.4 nm. We conclude that the conformational variability of the TED domain in C3b and C3u in physiological buffer is significant for their functional roles.

(4) Publication: Rayner, L. E., Hui, G.-K., Gor, J., Dalby, P. A. & Perkins S. J. (2013) Conformational and Fc accessibility changes in the solution structures of human IgG4 by ultracentrifugation and X-ray and neutron scattering modeling. In preparation.

Abstract: Human IgG4 antibody shows distinct and therapeutically-useful properties compared to the IgG1, IgG2 and IgG3 antibody subclasses. 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. Here, we have studied the solution structure of wild-type IgG4(Ser) and a mutant IgG4(Pro) in different buffers and temperatures, where the proline substitution suppresses the formation of halfmers. By analytical ultracentrifugation, both IgG4(Ser) and IgG4(Pro) were principally monomeric with sedimentation coefficient $s^0_{20,w}$ values of 6.8 S and 6.6 S respectively. Monomer:dimer exchange was observed in heavy water buffer, being most pronounced at low temperature. By X-ray and neutron scattering, the X-ray radius of gyration R_G was unchanged with concentration in all buffers, while the neutron R_G values increased with concentration as the temperature decreased in heavy water buffers. The X-ray and neutron distance distribution curves *P(r)* revealed two peaks, *M1* and *M2*, whose positions shifted below 2 mg/ml to indicate concentration-dependent structures. In addition, dimer formation was seen at high concentration in heavy water buffer. Constrained scattering modelling revealed predominantly asymmetric solution structures for IgG4(Ser) with mostly extended hinge structures. The IgG4(Pro) solution structures showed similarly extended hinge structures. Both IgG4 structures showed Fab regions that were positioned sufficiently close to the Fc region to block its accessibility to C1q binding. Our new molecular models for IgG4 explain its inability to activate complement, and will facilitate further studies of its stability and function for therapeutic applications.