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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 complexes of complement proteins	**Experiment number**: MX-1661
Beamline: BM29	**Date of experiment**: from: 7 Sep 2014 to: 8 Sep 2014	**Date of report**: 12/04/2015
Shifts: 3	**Local contact(s)**: Dr Petra Pernot	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(1) Rodriguez, E.*, Nan, R.*, Li., K., Gor, J. & Perkins, S. J.* (UCL)
(2) E. Rodriguez*, I. Iqbal, V. Suen, A. Miles, J. Gor, P. Adamson, D. L. Gordon and S. J. Perkins* (UCL/Flinders)
(3) O. Dunne*, R. Nan*, J. Gor, P. Adamson, D. L. Gordon, M. Moulin, V. T. Forsyth and S. J. Perkins* (UCL/Flinders/ILL)
(4) R. Nan*, C. M. Furze, D. W. Wright, J. Gor, R. Wallis, and S. J. Perkins* (UCL/Leicester)

(1) Publication: Rodriguez, E., Nan, R., Li., K., Gor, J. & Perkins, S. J. (2015). A revised mechanism for the activation of complement C3 to C3b: a molecular explanation of a disease-associated polymorphism. *J. Biol. Chem.* **290**, 2334-2350. Pubmed 25488663

Abstract: The solution structure of complement C3b is crucial for the understanding of complement activation and regulation. C3b is generated by the removal of C3a from C3. Hydrolysis of the C3 thioester produces C3u, an analogue of C3b. C3b cleavage results in C3c and C3d (TED). To resolve functional questions in relation to C3b and C3u, analytical ultracentrifugation and X-ray and neutron scattering studies were used with C3, C3b, C3u, C3c and C3d, using the wild-type allotype with R102. In 50 mM NaCl buffer, atomistic scattering modelling showed that both C3b and C3u adopted a compact structure, similar to the C3b crystal structure in which its TED and MG1 domains (MG: macroglobulin) were connected through the R102-E1032 salt-bridge. In physiological 137 mM NaCl, scattering modelling showed that C3b and C3u were both extended in structure with the TED and MG1 domains now separated by up to 6 nm. The importance of the R102-E1032 salt-bridge was determined using surface plasmon resonance to monitor the binding of wild-type C3d(E1032) and mutant C3d(A1032) to immobilised C3c. The mutant did not bind while the wild-type form did. The high conformational variability of TED in C3b in physiological buffer showed that C3b is more reactive than previously thought. Because the R102-E1032 salt-bridge is essential for the C3b-Factor H complex during the regulatory control of C3b, the known clinical associations of the major C3S (R102) and disease-linked C3F (G102) allotypes of C3b were experimentally explained for the first time.

(2) Abstract: Dual interaction of C3d with the C-terminus of complement factor H establishes a two-binding-site mechanism for complement regulation. E. Rodriguez, I. Iqbal, V. Suen, A. Miles, J. Gor, P. Adamson, D. L. Gordon and S. J. Perkins

Unravelling how factor H protects host cell surfaces from excessive complement activation is crucial for understanding inflammatory diseases. The C3d thioester region of C3b binds to the C-terminal short complement regulator SCR-19/20 domains of factor H. This interaction remains disputed, where crystallography showed either a 2:1 or a 1:1 stoichiometry. To resolve this controversy, we performed conventional and fluorescent-detected analytical ultracentrifugation (AUC). First, we showed from AUC that SCR-19/20 was monomeric in all buffers tested. Next, we showed that SCR-19/20 and C3d mixtures gave three peaks that corresponded to each of the SCR-19/20 alone, C3d and the 1:1 complex together, and the 2:1 complex. To show that the 1:1 complex had formed, we used fluorescent-labelled SCR-19/20 and three peaks

were again observed. Because previous surface plasmon resonance (SPR) by others did not reveal 2:1 complex formation, we repeated SPR experiments using both steady state and overlay methods. Two-state binding curves were observed in which one interaction was salt dependent, with K_D values of 1.3 μM and 4.7 μM in 50 mM NaCl and 137 mM NaCl respectively. Two-state binding curves were also observed by microscale thermophoresis. In confirmation of this, the crystal structures showed that the C3d site on SCR-19 is dominated by hydrogen bonding and hydrophobic contacts, while that on SCR-20 is dominated by ionic interactions. We conclude that C3d binding to SCR-19/20 is a two-state multimeric interaction. The presence of abundant C3d deposits at a host cell surface will augment factor H binding, thus down-regulating excess complement activation.

(3) Abstract: A dimerization site at SCR-17/18 in Factor H may explain its disease-causing mutations and a new mechanism for regulatory control O. Dunne, R. Nan, J. Gor, P. Adamson, D. L. Gordon, M. Moulin, V. T. Forsyth and S. J. Perkins

Complement Factor H (CFH) regulates the alternative pathway of complement through the interaction of its C-terminal SCR-19 and SCR-20 domains with surface bound C3b. CFH forms oligomers, and the SCR-16/20 domains contain one of at least two self-association sites in CFH. The rare disease atypical haemolytic uraemic syndrome (aHUS) is linked with up to 113 CFH mutations, of which up to 50 are found in the five domains of SCR-16/20. Because mutations in SCR-16/18 occur in a region of previously unknown functionality, we have analysed the dimerization of SCR-16/20 in detail. We expressed SCR-16/20, SCR-19/20, SCR-18/20, SCR-17/18, SCR-16/18, SCR-17 and SCR-18 in *Pichia*, some with His-tags and others without. Size-exclusion chromatography showed qualitatively that dimer formation occurred in many of these products. Quantitative *c(s)* size distribution analyses by analytical ultracentrifugation showed that dimer formation was often detected. Thus SCR-19/20 was monomeric, SCR-18/20 was partially dimeric, SCR-16/20, SCR-16/18, SCR-17/18 showed higher proportions of dimer, and SCR-17 and SCR-18 were mostly dimeric. These *c(s)* analyses localised the SCR-16/20 dimerization site to be at SCR-17 and SCR-18. These results are being corroborated using surface plasmon resonance and X-ray solution scattering. The dimerization site in the SCR-17/18 domains may explain why aHUS mutations are seen in SCR-17/18, in turn indicating that dimerization is functionally important. We propose that the self-association of CFH at SCR-17 and SCR-18 enables higher concentrations of CFH to be achieved at host cell surfaces in order to protect these better during high levels of inflammation.

(4) Abstract: The flexible solution structures of mannose-binding lectin-associated serine proteases-1 and -2 provide novel insight on lectin pathway activation R. Nan, C. M. Furze, D. W. Wright, J. Gor, R. Wallis, and S. J. Perkins

The lectin pathway of complement is activated by the recognition of carbohydrate patterns on pathogen cell surfaces by complexes comprising a recognition component (mannose-binding lectin (MBL), serum ficolins or collectin-K1) and an associated protease (MASP-1 or -2). MASP-1 activates MASP-2 and MASP-2 activates downstream components C4 and C4b-bound C2 to initiate the downstream reaction cascade. In order to clarify the molecular basis of lectin pathway activation, we determined two crystal structures for the N-terminal CUB1-EGF-CUB2 domains of rat MASP-1 and MASP-2 bound with Ca^{2+}, together with their solution structures from X-ray scattering and atomistic modelling. The solution structure for the CUB1-EGF-CUB2 dimer showed that the two CUB2 domains in MASP1 were tilted (upwards?), unlike its crystal structure, whereas those in MASP2 remain extended as seen in its crystal structure. We also determined the solution structures of MASP-1 and MASP-2 in their zymogen and activated forms by X-ray scattering. Crystal structures of the CUB1-EGF-CUB2 and SCR1-SCR2-SP subunits were used for atomistic scattering modelling. Both the zymogen and activated forms of MASP-1 and MASP-2 showed similar dimeric solution structures with lengths of 30 nm. Both structures were more compact than anticipated from homology models. The best-fit solution structures of the MASP-1 and MASP-2 dimers showed significant domain flexibility in the CUB2-SCR1-SCR2-SP region. In conclusion, our novel identification of flexible MASP domains structures changes our understanding of the way in which the MASP proteases activate the lectin pathway through their binding to MBL as a template.