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 complexes of complement proteins and antibodies	**Experiment number**: MX-1759
Beamline: BM29	**Date of experiment**: from: 21 Jun 2015 to: 22 Jun 2015	**Date of report**: 10/10/2015
Shifts: 3	**Local contact(s)**: Dr Adam Round	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):
(1) Hui, G. K.*, Wright, D. W., Vennard, O. L.*, Rayner, L. E.*, Pang, M.*, Yeo, S. C.*, Gor, J., Molyneux, K., Barratt, J. & Perkins, S. J.* (UCL)
(2) Zahid, H., Miah, L., Lau, A. M., Brochard, L., Hati, D., Bui, T. T. T., Drake, A. F., Gor, J., Perkins, S. J.* and McDermott, L. C.* (UCL/King's London)
(3) Nan, R.*, Furze, C. M., Wright, D. W., Gor, J., Wallis, R. & Perkins, S. J. * (UCL/Leicester)
(4) D. W. Wright, and S. J. Perkins* (UCL)

(1) Abstract: Hui, G. K., Wright, D. W., Vennard, O. L., Rayner, L. E., Pang, M., Yeo, S. C., Gor, J., Molyneux, K., Barratt, J. & Perkins, S. J. (2015). The solution structures of native and patient monomeric human IgA1 reveal asymmetric extended structures: implications for function and IgAN disease. *Biochem. J.* **471**, 255-265.

Native IgA1, for which no crystal structure is known, contains an *O*-galactosylated 23-residue hinge region that joins its Fab and Fc regions. IgA nephropathy (IgAN) is a leading cause of chronic kidney disease in developed countries. Because IgA1 in IgAN often has a poorly *O*-galactosylated hinge region, the solution structures of monomeric IgA1 from a healthy subject and three IgAN patients with four different *O*-galactosylation levels were studied. Analytical ultracentrifugation showed that all four IgA1 samples were monomeric with similar sedimentation coefficients, $s^0_{20,w}$. X-ray scattering showed that the radius of gyration, R_g, slightly increased with IgA1 concentration, indicating self-association, although their distance distribution curves, *P(r)*, were unchanged with concentration. Neutron scattering indicated similar R_g values and *P(r)* curves, although IgA1 showed a propensity to aggregate in heavy water buffer. A new atomistic modelling procedure based on comparisons of 177,000 conformationally-randomised IgA1 structures with the individual experimental scattering curves revealed similar extended Y-shaped solution structures for all four differentially-glycosylated IgA1 molecules. The final models indicated that the *N*-glycans at Asn263 were folded back against the Fc surface, the C-terminal tailpiece conformations were undefined, and hinge *O*-galactosylation had little effect on the solution structure. The solution structures for full-length IgA1 showed extended hinges, and the Fab and Fc regions were positioned asymmetrically to provide ample space for the functionally-important binding of two FcαR receptors to its Fc region. While no link between *O*-galactosylation and the IgA1 solution structure was detected, an increase in IgA1 aggregation with reduced *O*-galactosylation may relate to IgAN.

(2) Abstract: Henna Zahid, Layeque Miah, Andy M. Lau, Lea Brochard, Debolina Hati, Tam T. T. Bui, Alex F. Drake, Jayesh Gor, Stephen J. Perkins and Lindsay C. McDermott. (2015) Zinc-induced oligomerisation of zinc α2 glycoprotein reveals multiple fatty acid binding sites *Biochem. J.* Manuscript returned for acceptance.

Zinc α2 glycoprotein (ZAG) is an adipokine with a class I major histocompatibility complex protein fold and is associated with obesity and diabetes. Although its intrinsic ligand remains unknown, ZAG binds the dansylated C_{11} fatty acid, DAUDA, in the groove between the α1 and α2 domains. The surface of ZAG has about 15 weak zinc binding sites deemed responsible for precipitation from human plasma. Here the functional significance of these metal sites was investigated. Analytical ultracentrifugation and circular dichroism showed that zinc, but not other divalent metals, cause ZAG to oligomerise in solution. Thus ZAG

dimers and trimers were observed in the presence of 1 mM and 2 mM zinc. Molecular modelling of X-ray scattering curves and sedimentation coefficients indicated a progressive stacking of ZAG monomers, suggesting the ZAG groove may be occluded in these. Using fluorescence-detected sedimentation velocity, these ZAG-zinc oligomers were again observed in the presence of the fluorescent boron dipyrromethene fatty acid C_{16}-BODIPY. Fluorescence spectroscopy confirmed that ZAG binds C_{16}-BODIPY. ZAG binding to C_{16}-BODIPY, but not to DAUDA, was reduced by increased zinc concentrations. We conclude that the lipid binding groove in ZAG contains at least two distinct fatty acid binding sites for DAUDA and C_{16}-BODIPY, similar to the multiple lipid binding seen in the structurally-related immune protein Cd1c. In addition, because high concentrations of zinc occur in the pancreas, the perturbation of these multiple lipid binding sites by zinc may be significant in Type 2 diabetes where dysregulation of ZAG and zinc homeostasis occurs.

(3) Abstract: Nan, R., Furze, C. M., Wright, D. W., Gor, J., Wallis, R. & Perkins, S. J. (2015) The flexible solution structures of mannose-binding lectin-associated serine proteases-1 and -2 provide novel insight on lectin pathway activation. Manuscript to be submitted imminently (October 2015).

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, serum ficolins, collectin-LK or collectin-K1) and an associated protease (MASP-1 or MASP-2). MASP-1 activates MASP-2, and MASP-2 activates the downstream components C4 and C4b-bound C2. In order to clarify the molecular mechanism of lectin pathway activation, new crystal structures for the N-terminal CUB1-EGF-CUB2 domains of rat MASP-1 and MASP-2 bound with three Ca^{2+} ions were determined, together with their solution structures from X-ray scattering, analytical ultracentrifugation and atomistic modelling. The solution structure for the CUB1-EGF-CUB2 dimer suggested that the two CUB2 domains in MASP-1 and MASP-2 were tilted by as much as 90°, compared to the crystal structures, indicating that the EGF-CUB2 junction is a hinge allowing considerable flexibility. We also determined the solution structures of full-length MASP-1 and MASP-2 dimers in their zymogen and activated forms by X-ray scattering and ultracentrifugation, followed by atomistic scattering modelling based on crystal structures for the CUB1-EGF-CUB2 and SCR1-SCR2-SP fragments. Both forms of full-length MASP-1 and MASP-2 showed similar dimeric solution structures with lengths of 30 nm. These full-length structures were notably more bent than anticipated from crystal structures. We conclude that the best-fit solution structures of the MASP-1 and MASP-2 dimers also showed significant domain flexibility in the CUB2-SCR1-SCR2-SP region. This experimental identification of flexible MASP domain structures changes our understanding of how the MASP proteases bind to mannose-binding lectin to activate the lectin pathway of complement and indicates that both intra- and intercomplex activation mechanisms may be possible.

(4) Abstract: Wright, D. W. & Perkins, S. J. (2015). SCT: A suite of programs for comparing atomistic models to small angle scattering data. *J. Appl. Cryst.* **48**, 953-961.

Small angle X-ray and neutron scattering techniques characterize proteins in solution and complement high-resolution structural studies. They are of particular utility when large proteins cannot be crystallized or when the structure is altered by solution conditions. Atomistic models of the averaged structure can be generated through constrained modelling, a technique in which known domain or subunit structures are combined with linker models to produce candidate global conformations. By randomizing the configuration adopted by the different elements of the model, thousands of candidate structures are produced. Next, theoretical scattering curves are generated for each model for trial-and-error fits to the experimental data. From this, a small family of best-fit models is identified. In order to facilitate both the computation of theoretical scattering curves from atomistic models and their comparison to experiment, the SCT suite of tools were developed. SCT also includes programs which provide sequence-based estimates of protein volume (either incorporating hydration or not), and add a hydration layer to models for X-ray scattering modelling. The original SCT software, written in Fortran, resulted in the first atomistic scattering structures to be deposited in the Protein Data Bank, and 77 structures for antibodies, complement proteins and anionic oligosaccharides) were determined between 1998 and 2014. For the first time, this software is publicly available, alongside an easier-to-use reimplementation of the same algorithms in Python. Both versions of SCT have been released as open source software under the Apache 2 license, and available for download from https://github.com/dww100/sct.