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 antibodies important in biotechnology and disease	**Experiment number:** MX-1595
Beamline: BM29	**Date of experiment:** from: 25 Apr 2014 to: 26 Apr 2014	**Date of report:** 12/04/2015
Shifts: 3	**Local contact(s):** Dr Adam Round	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(1) Rayner, L. E., Hui, G. K., Gor, J., Heenan, R. K., Dalby, P. A. & Perkins S. J. (UCL)
(2) 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/Leicester).
(3) Wright, D. W. & Perkins, S. J. (UCL)

(1) Publication:

Rayner, L. E., Hui, G. K., Gor, J., Heenan, R. K., Dalby, P. A. & Perkins S. J. (2015). The solution structures of two human IgG1 antibodies show conformational stability and accommodate their C1q and FcγR ligands. *J. Biol. Chem.* 290, 8420-8438. Pubmed 25659433.

Abstract: The human IgG1 antibody subclass shows distinct properties compared to the IgG2, IgG3 and IgG4 subclasses, and is the most exploited subclass in therapeutic antibodies. It is the most abundant subclass, has a half-life as long as that of IgG2 and IgG4, binds the FcγR receptor, and activates complement. There is limited structural information on full-length human IgG1 because of the challenges of crystallisation. To rectify this, we have studied the solution structures of two human IgG1 6a and 19a monoclonal antibodies in different buffers at different temperatures. Analytical ultracentrifugation showed that both antibodies were predominantly monomeric, with sedimentation coefficients $s^0_{20,w}$ of 6.3 S - 6.4 S. Only a minor dimer peak was observed, and the amount was not dependent on buffer conditions. Solution scattering showed that the X-ray radius of gyration R_g increased with salt concentration, while the neutron R_g values remained unchanged with temperature. The X-ray and neutron distance distribution curves *P(r)* revealed two peaks, *M1* and *M2*, whose positions were unchanged in different buffers to indicate conformational stability. Constrained atomistic scattering modelling revealed predominantly asymmetric solution structures for both antibodies with extended hinge structures. Both structures were similar to the only known crystal structure of full-length human IgG1. The Fab conformations in both structures were suitably positioned to permit the Fc region to bind readily to its FcγR and C1q ligands without steric clashes, unlike human IgG4. Our molecular models for human IgG1 explain its immune activities, and we discuss its stability and function for therapeutic applications.

(2) Publication:

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 asymmetric solution structures of native and patient monomeric human IgA1 reveal new insights on IgA nephropathy. Submitted for publication.

Abstract: IgA nephropathy (IgAN) is a leading cause of chronic kidney disease in developed countries, in which immune complexes containing IgA1 antibody are deposited in the mesangium and result in glomerular damage. Native IgA1 contains an *O*-glycosylated 23-residue hinge region that joins its Fab and Fc regions. IgA1 in IgAN has a poorly galactosylated hinge region. Here, the solution structures of monomeric IgA1 from the plasma of a healthy subject and three patients with reduced or elevated *O*-linked galactose levels were determined. Analytical ultracentrifugation confirmed that all four IgA1 glycoproteins were monomeric with similar sedimentation coefficients $s^0_{20,w}$ of 6.2-6.4 S. X-ray scattering showed that the radius of gyration R_g significantly increased with IgA1 concentration, indicating self-association, but two peaks *M1* and *M2* seen in their distance distribution curves *P(r)* were unchanged with concentration. Neutron scattering in heavy water indicated a similar solution structure, however IgA1 was prone to aggregation. Atomistic modelling based on comparisons of 127,000 and 177,000 conformationally-randomised IgA1 structures with the experimental scattering curves extrapolated to zero concentration revealed similar extended Y-shaped asymmetric solution structures. The modelling suggested that the *N*-glycans at Asn263 were folded back against the Fc surface, while the C-terminal tailpiece conformations were undefined. The Fab and Fc regions of full-length IgA1 were positioned asymmetrically to allow ample space for the binding of two FcαR receptors to the Fc region. This study of monomeric IgA1 suggested that hinge *O*-galactosylation and IgAN were linked through the different exposure, flexibility and aggregation of the extended *O*-galactosylated hinge structures.

(3) Publication:

Wright, D. W. & Perkins, S. J. (2015). SCT: A suite of programs for comparing atomistic models to small angle scattering data. *J. Appl. Crystallographr*. In press.

Abstract: 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.

Examples are given based on our modelling of antibodies.