EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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-1460
Beamline: BM29	**Date of experiment:** from: 30 November 2012 to: 1 December 2012	**Date of report:** 28th July 2014
Shifts: 3	**Local contact(s):** Dr Chloe Zubieta, Dr Petra Pernot	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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

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

Report:

(1) 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**. J. Biol. Chem. **289**, 20740-20756. Pubmed 24876381. doi:10.1074/jbc.M114.572404

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 forms 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.

(2) Publication: Hui, G. K., Rayner, L. E., Gor, J., Heenan, R. K., Dalby, P. A. & Perkins S. J. (2014) **The solution structures of two human IgG1 molecules show conformational stability and accommodate its C1q and FcγR ligands: a study by ultracentrifugation and X-ray and neutron scattering modelling**. Manuscript in preparation.

Abstract: Human IgG1 antibody shows distinct and therapeutically-useful properties compared to the IgG2, IgG3 and IgG4 antibody subclasses. IgG1 is the most well studied and exploited subclass for the development of therapeutic antibodies due to numerous characteristics. It is the most abundant subclass, it has the longest plasma half-life and multifunctional as it can bind every type of human FcγR and can activate the complement cascade. Although it is the most-well understood antibody, only limited structural information is available for this due to the hinge flexibility between its Fab and Fc regions. Here, we have studied the solution structure of two different full-sized human IgG1 molecules IgG1 6a and IgG1 19a in different buffers and temperatures. By analytical ultracentrifugation, both IgG1 6a and IgG1 19a were principally monomeric with sedimentation coefficient $s^0_{20,w}$ values of 6.4 S and 6.3 S respectively. IgG1 dimer formation is minor and does not dependent on the buffer conditions. By solution scattering, the X-ray radius of gyration R_G values increased with salt concentration in different buffers, while the neutron R_G values remained unchanged with increase in temperature. The X-ray and neutron distance distribution curves *P(r)* revealed two peaks, *M1* and *M2*, that reflect the separation between the Fab and Fc regions. Since their positions remained unchanged, this indicated that the IgG1 structure is stable in different buffers. Constrained scattering modelling revealed predominantly asymmetric solution structures for both IgG1 6a and IgG1 19a with mostly extended hinge structures. Both IgG1 structures showed T- and Y-shaped conformations with the Fab regions suitably positioned to allow enough space for the Fc region to bind to its ligands such as C1q and FcγR without steric clashes. This outcoem is distinct from that for human IgG4. These IgG1 models validate the crystal structure of intact human IgG1. Our new molecular models for IgG1 explain its attributes to activate complement, and will facilitate further studies of its stability and function for therapeutic applications.

This project is completed. We expect that this will be shortly submitted for publication..