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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-1801
Beamline: BM29	**Date of experiment**: from: 22 Nov 2015 to: 23 Nov 2015	**Date of report**: 02/08/2016
Shifts: 3	**Local contact(s)**: Dr Petra Pernot (email: rejma@esrf.fr)	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):
(1) 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)
(2) S. J. Perkins* et al. (UCL)
(3) Fung, K.-W.*, Wright, D. W., Gor, J., Swann, M. & Perkins, S. J.* (UCL/Farfield)
(4) Walker, K. T.*, Nan, R.*, Wright, D. W., Gor, J., Bishop, A. C., Makhatadze, G. I., Brodsky, B. & Perkins, S. J.* (UCL/Tufts-USA/Troy-USA)

(1) Abstract: 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. (2016) Zinc-induced oligomerisation of zinc α2 glycoprotein reveals multiple fatty acid binding sites. *Biochem. J*. **473**, 43-54.

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. (247 words)

(2) Abstract: Perkins, S. J., Wright, D. W., Zhang, H., Brookes, E. H., Chen, J., Irving, T. C., Krueger, S., Barlow, D. J., Edler, K. J., Scott, D. J., Terrill, N. J., King, S. M., Butler, P. D. & Curtis, J. E. (2016) Atomistic modelling of scattering data in the Collaborative Computational Project for Small Angle Scattering (CCP-SAS). *J. Appl. Cryst.* Manuscript returned for acceptance.

In solution small-angle scattering, the major global investment in X-ray and neutron sources has resulted in huge advances in the throughput and accuracy of experimental measurements. Analytical model fitting, and more recently non-atomistic real-space and *ab-initio* modelling approaches, have been crucial to exploiting the data produced and answering important questions about a broad range of complex materials. The capabilities and sophistication of today's computer resources and simulation technologies now provide a unique opportunity to model scattering data at the atomistic level; this extends the range of questions that can be addressed and provide deeper insights into the physics and chemistry of the systems studied. Realizing this potential however, requires integrating the experimental data with a new generation of modelling

software. To achieve this, the CCP-SAS collaboration (http://www.ccpsas.org/) is developing open-source, high-throughput, and user-friendly software for the atomistic and coarse-grained molecular modelling of scattering data. Its implementation involves a modular approach in which GenApp provides the deployment infrastructure for running applications on both standard and high-performance computing hardware, and SASSIE-Web provides an online workflow framework into which a variety of modules can be plugged to prepare structures, carry out simulations, calculate theoretical scattering data, and compare results to experimental data. The breadth of CCP-SAS is illustrated by case studies: (i) inter-domain flexibility in two- to six-domain proteins as exemplified by HIV-1 Gag, MASP and ubiquitin; (ii) the hinge conformation in human IgG2 and IgA1 antibodies; (iii) the complex formed between a hexameric protein Hfq and mRNA; and (iv) synthetic 'bottlebrush' polymers.

(3) Abstract: Fung, K.-W., Wright, D. W., Gor, J., Swann, M. J. & Perkins, S. J. (2016) Domain structure of human complement C4b extends with increasing ionic strength: implications for its regulatory mechanism. Submitted for publication.

During the activation of complement C4 to C4b, the exposure of its thioester domain (TED) is crucial for the attachment of C4b to activator surfaces. In the C4b crystal structure, TED formed an Arg^{104}-Glu^{1032} salt bridge with its neighbouring macroglobulin (MG1) domain. Here, we examined the domain structure and oligomerisation of C4b. Dual polarisation interferometry of C4b immobilised at a sensor surface showed that the maximum thickness of C4b increased by 0.7 nm with increase in ionic strength from 50 mM to 137 mM NaCl. Analytical ultracentrifugation showed that the sedimentation coefficient $s_{20,w}$ of monomeric C4b of 8.41 S in 50 mM NaCl buffer decreased to 7.98 S in 137 mM NaCl buffer, indicating that C4b became more extended. Small angle X-ray scattering reported similar R_G values of 4.89-4.90 nm for C4b in 137-250 mM NaCl. Atomistic scattering modelling of the C4b conformation showed that TED and the MG1 domain were separated by 4.7 nm in 137-250 mM NaCl, this being greater than that of 4.0 nm in the C4b crystal structure. Our data reveal that in low ionic strengths, both at surfaces and in solution, C4b forms compact TED-MG1 structures stabilised by a salt bridge interaction. In solution, physiologically-relevant ionic strengths lead to the separation of TED and the MG1 domain, meaning this is less able to bind to its complement regulators. Because C4b exhibits salt-dependent conformational changes similar to those seen for complement C3b, this confirms the functional importance of this movement for both proteins.

(4) Abstract: Walker, K. T., Nan, R., Wright, D. W., Gor, J., Bishop, A. C., Makhatadze, G. I., Brodsky, B. & Perkins, S. J. (2016) Non-linearity of the collagen triple-helix in solution and implication for collagen function. Submitted for publication.

Collagen adopts a characteristic supercoiled triple helical conformation which requires a repeating $(Xaa\text{-}Yaa\text{-}Gly)_n$ sequence. Here, an experimental study is reported on the flexibility of varying lengths of collagen triple-helical peptides, composed of the most stable Pro-Hyp-Gly units. In addition, one unblocked peptide, $(POG)_{10unblocked}$, was compared with the blocked $(POG)_{10}$ to study the significance of end effects. Complementary analytical ultracentrifugation and small angle X-ray scattering data, coupled with molecular dynamics simulations, indicated that the longer triple-helical peptides are less well explained by a linear structure. Atomistic models were obtained of the best fitting peptide structures, selected out of a very large ensemble of possible structures obtained by molecular dynamics simulations. A small amount of non-linearity was observed in these best fit triple-helical structures, with an approximation of the degree of bending estimated as 4-17º. The studies described here lay the basis for the further study on collagen peptides of varying sequence and stability in order to clarify the role of molecular rigidity in collagen function and disease.