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

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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 complexes of complement factor H with C3, CRP, heparin and zinc	**Experiment number**: MX-1524
Beamline: BM29	**Date of experiment**: from: 29th Nov 2013 to: 30th Nov 2013	**Date of report**: 28th July 2014
Shifts: 3	**Local contact(s)**: Dr Adam Round	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(1) Rodriguez, E.*, Nan, R.,* Li, K., Gor, J. & Perkins, S. J.* (UCL)

(1) Li, K.* & Perkins, S. J.* (UCL)

Report:

Publication: A revised mechanism for the activation of complement C3 to C3b: a molecular explanation of a disease-related polymorphism

Elizabeth Rodriguez, Ruodan Nan, Keying Li, Jayesh Gor and Stephen J. Perkins. Manuscript to be submitted.

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). Analytical ultracentrifugation and X-ray and neutron scattering studies was 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 where its TED and MG1 domains were connected through the E1032-R102 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 E1032-R102 salt-bridge was determined by binding studies of C3d(E1032) and C3d(A1032) to immobilised C3c using surface plasmon resonance. The A1032 mutant did not bind while the E1032 form did. The high conformational variability of TED in C3b in physiological buffer showed that C3b is more reactive than previously thought. Because the E1032-R102 salt-bridge is

essential for the regulatory breakdown of C3b by Factor H, the functional difference between the major C3S (R102) and disease-associated C3F (G102) allotypes of C3b was experimentally verified.

Publication: Interaction of C3u at two sites on complement Factor H: a revised mechanism for complement regulation

Keying Li and Stephen J. Perkins. Manuscript submitted.

Abstract: In the alternative pathway of complement activation, factor H (FH) is the most important regulator of active C3b and a hydrolysed form termed C3u, also known as $C3_{H20}$. It had been long assumed that these form a 1:1 complex. Here, we show that the C3u-FH interaction is bivalent, with independently functional N-terminal and C-terminal regions. By analytical ultracentrifugation, size-distribution analyses resolved separate peaks for 1:1 and 2:1 complexes of C3u-FH in 137 mM and 50 mM NaCl buffers. In 137 mM NaCl, the 1:1 complex was predominant with a dissociation constant K_D of 0.59 μM. X-ray scattering analyses showed that the maximum dimension of the C3u-FH complexes was 30-33 nm, showing that FH did not form a more compact structure in the complex. Molecular modelling based on known structures for FH, C3u, and the FH-C3b and FH-C3d complexes accounted for the experimental sedimentation coefficients of the complexes. The models also accounted for the C3u-FH scattering curves. The scattering fit for 137 mM NaCl revealed unbound C3u and FH and a 1:1 C3u-FH complex, leading to a K_D value of 0.22 μM. The scattering fit for 50 mM NaCl revealed 2:1 complexes. Because two independent sites for C3u binding to FH were identified, together with no conformational change in FH, our results suggest a revised mechanism for FH regulatory control in which the N-terminal C3u/C3b degradation and C-terminal FH binding to host cell surfaces occur independently at opposite ends of FH.