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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.

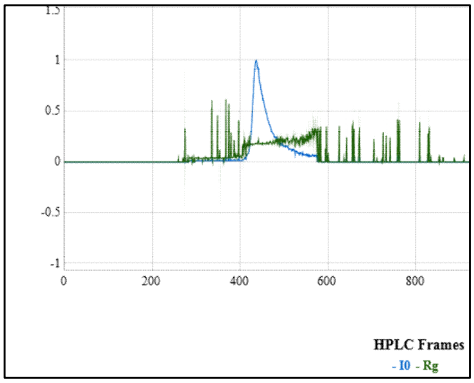
ESRF	**Experiment title:** Solution structures of complexes of complement factor H with C3, CRP and heparin	**Experiment number**: MX-1594
Beamline: BM29	**Date of experiment**: from: 24th Apr 2014 to: 25th Apr 2014	**Date of report**: 28th July 2014
Shifts: 3	**Local contact(s)**: Dr Adam Round	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(1) Dunne, O.*, Nan, R.,* Pao, P.J.* & Perkins, S. J.* (UCL)

Report:

Crystal structures of the crucial complex formed between complement C3d and Factor H SCR-19/20 revealed either a 1:1 or a 2:1 stoichiometry. To identify which stoichiometry was correct, online HPLC data collection was carried out on their mixtures in order to obtain SAXS data on their complex alone without either monomeric proteins present. First the individual proteins were measured using the online HPLC, then the mixture was studied. A 2.4 ml S-200 increase HPLC column was used.



SAXS data from SCR-19/20 alone from the online HPLC was consistent with previous SAXS measurements using the regular sample changer on BM29. The protein was eluted from the HPLC as a single peak (Figure 1). The radius of gyration R_G value was 2.50 ± 0.07 nm with a maximum dimension D_{max} of 8.7 nm (Figure 2)

Figure 1. SCR-19/20 I(0) profile from the online HPLC at 13 mg/ml. Blue is the I(0) value; green is the R_G value.

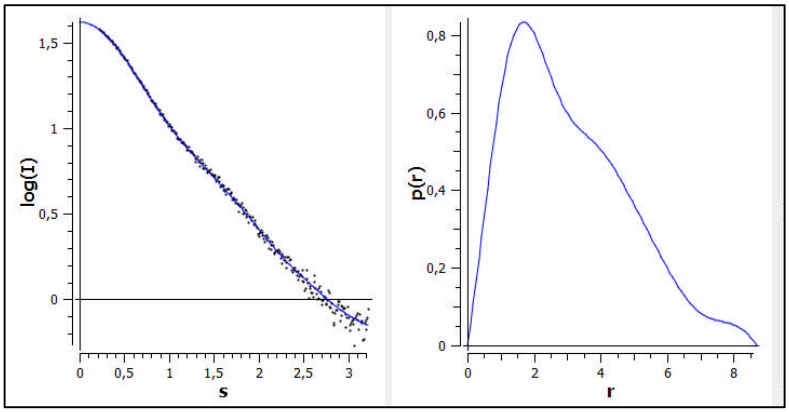
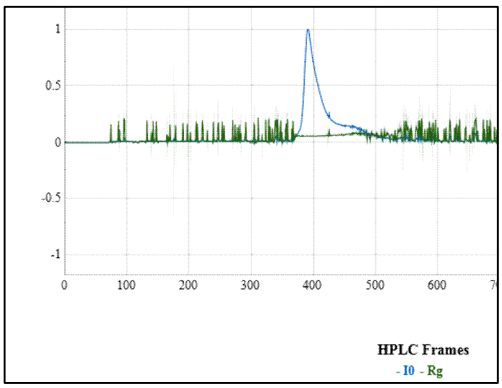


Figure 2. The SCR-19/20 *I(Q)* and *P(r)* curves, showing a D_{max} value of 8.7 nm.



C3d alone was eluted as a single peak (Figure 3). The Guinier and P(r) analyses were in agreement with previous SAXS measurements. The R_G value was 2.20 ± 0.06 nm and the D_{max} value was calculated to be 7.5 nm (Figure 4.)

Figure 3. C3d I(0) profile from the online HPLC at 8 mg/ml. Blue is the I(0) value; green is the R_G value.

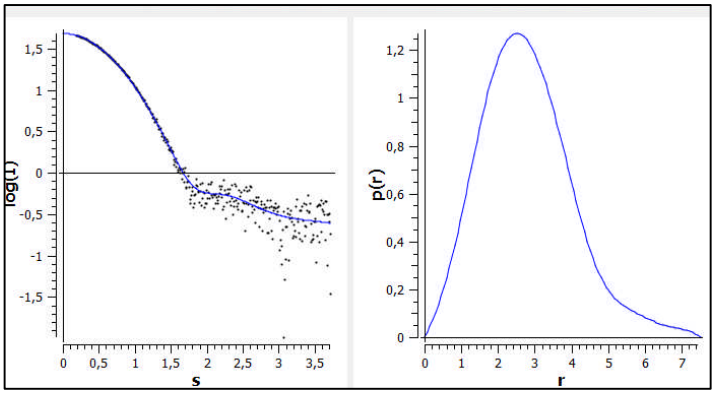


Figure 4. The C3d *I(Q)* and *P(r)* curves.

A **1:2 molar ratio** of the SCR-19/20 and C3d proteins was injected on the HPLC column. The resulting elution profile consisted of three resolved peaks (blue; Figure 5). Guinier and P(r) analyses were carried out on each of the three individual peaks.

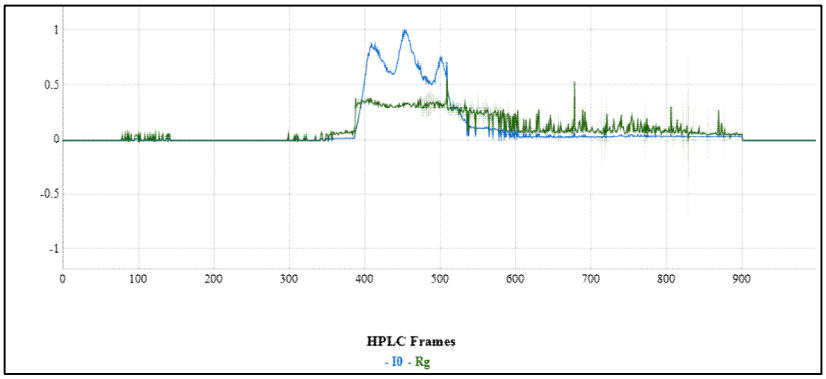


Figure 5. The C3d and SCR-19/20 mixture was measured using the online HPLC in a ~2:1 molar ratio (8.3 mg/ml C3d and 1.7 mg/ml SCR-19/20). Each of the three peaks gave similar R_G values (~3 nm) and similar D_{max} values (~10.4 nm). These values were similar to what had been observed when the mixture (complex and two monomers) were measured using the regular sample changer. Estimated mass calculations from the Porod volume indicated that, from left to right, the first peak corresponded to a mass of ~35-50 kDa, the second peak has a mass of ~40-60 kDa and the third peak has a mass of ~20-30 kDa. These values were comparable with the known molecular masses of C3d and SCR-19/20.

Conclusion

The elution profiles for the two separate proteins and their mixture successfully showed the expected single peaks for the free proteins and the expected three peaks for the mixture that corresponds to the free proteins and their complex. The observation of three peaks is attributable to the weak binding of C3d and SCR-19/20, meaning that peaks for the free and bound proteins will be present. What is not yet clear is whether a 1:1 or a 2:1 stoichiometry has been observed. This will only be resolved using a full concentration series and range of stoichiometries. We plan to repeat this experiment using the online HPLC on BM29, but will also collect data on the mixtures using the regular sample changer on BM29. Atomistic scattering modelling will be used to analyse the observed curves as the sum of the individual proteins and their two complexes, using the already known scattering curves for the individual proteins from crystallography.