EUROPEAN SYNCHROTRON RADIATION FACILITY

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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: X-ray footprinting of both DNA and proteins for the study of transcription regulation and the immune response.**	**Experiment number**: **SC-2919**
Beamline: ID10C	**Date of experiment**: from: 19 May 2010 to: 23 May 2010	**Date of report**: 31-08-2010
Shifts: 12	**Local contact(s)**: Federico Zontone	*Received at ESRF:*

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Report:

During the last beamtime, in May 2010, we completed the work on protein footprinting of the complement protein complex C3b-H of the innate immune response and we began a new project on the transcription regulation of sigma54 RNA polymerase (RNAP) in collaboration with Martin Buck and Nicolas Joly at Imperial College in London. In addition, two other sets of experiments tested the feasibility of possible applications of this technique: the first project concerns the mechanism of inhibition of RNA polymerase by the antibiotic lipiarmycin, in collaboration with Kostantin Brodolin from the Université de Montpellier 1, UMR 5236, CPBS; the second collaboration is focused on the probing of the structure of the RNA found within a viral capsid, in collaboration with Peter Stockley, at the Atsbury Centre for Structural Molecular Biology at the University of Leeds.

Summary of the experimental approach: The hydroxyl radicals produced from the radiolysis of water during irradiation of a DNA or protein sample with an X-ray beam can be used to probe the solvent accessible surface of biological macromolecules[1]. The main advantage of using an X-ray beam from a synchrotron light source is the high flux of photons allowing for microsecond exposure times and thus permitting a high time resolution of the experiment. The abstraction of a proton from the backbone sugar of polynucletides DNA or RNA by the hydroxyl radical results in the cleavage of the chain that can be detected and quantitated subsequently in the laboratory. Only those sites on the polynucleotide that are accessible to the solvent, and thus not specifically bound by the protein, will be cut. In case of protein footprinting instead, the oxidation products on the amino acid side chains are detected by mass spectrometry of the peptides obtained by cleaving the sample with a proteolytic enzyme following after x-ray exposure[2]. A specifically modified stopped flow apparatus is used in order to control the exposure time in the microsecond timescale and to mix the samples in the millisecond to minute timescale.

Project 1. The latest results from the protein footprinting project are part of the PhD thesis of Maxime Le Mignon, in the LAMBE laboratory in Evry. Maxime has recently successfully defended his PhD and we are now in the process of preparing a manuscript to publish the results of this work. The laboratory of Dr. Daniel has been studying the formation of the C3b-H complex involved in the regulatory cascade of the innate

immune system leading to discrimination between microbes and host. While the structure of the C3b protein is known, factor H is a highly flexible array of 20 homologous short consensus repeats (SCR) domains. The large size (187 kDa for C3b and 154 kDa for factor H) and flexibility of the partners in this complex has created a significant challenge in the determination of its structure. A new data acquisition and analysis protocol was developed in order to resolve and identify by nano-LC MS/MS mass spectrometry the large number of peptides produced from the digestion of this macromolecular complex, and especially to identify and quantify the amino acids in these peptides that become modified by the hydroxyl radicals. This new approach resulted in 80% of sequence coverage, a significant improvement from the previous protocols. Figure 1A shows the combined results obtained from hydroxyl radical footprinting using both synchrotron radiation and a gamma source (which however requires much longer exposure times). These results, together with data obtained by crosslinking and chemical modification experiments has permitted the construction of a high resolution model of the C3b-H complex (Figure 1B).

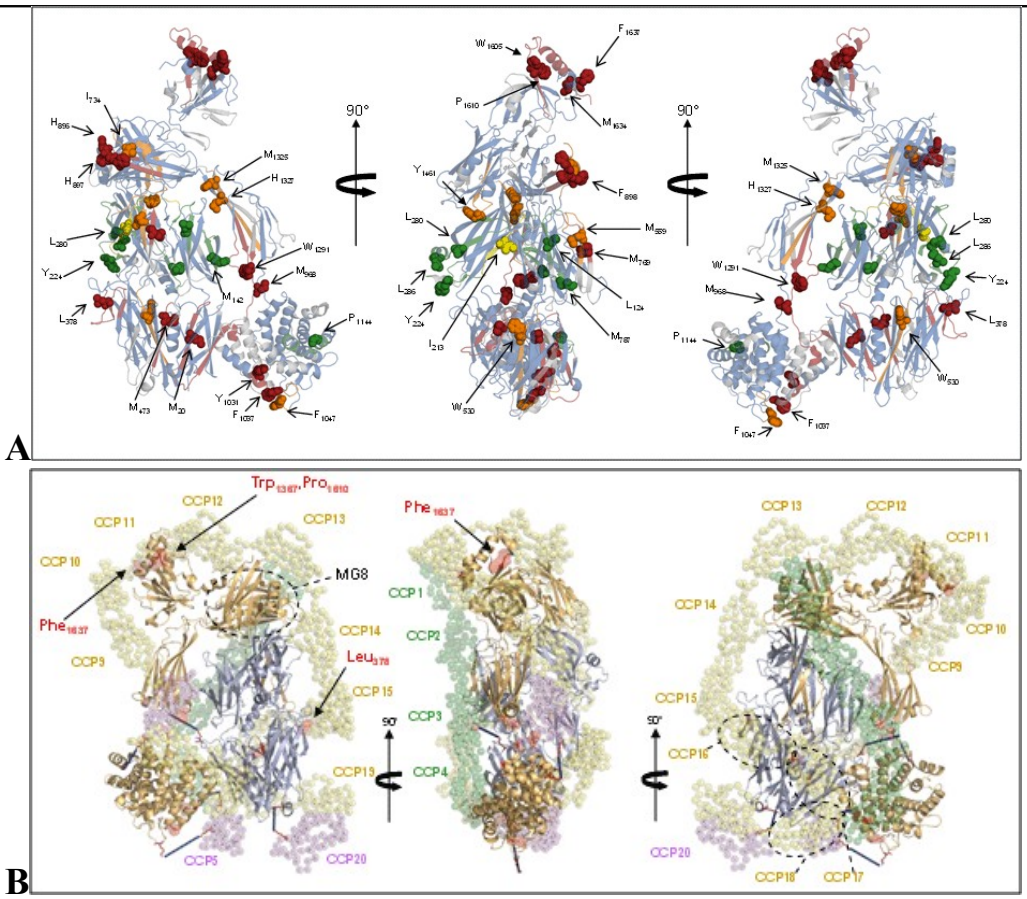

Figure 1. Differential modification of the C3b protein by hydroxyl radicals in complex with factor H. A. Hydroxyl radical footprinting reveals the surface amino acids that change in reactivity upon formation of the C3b-H complex. Decreased reactivity (red), increased reactivity (yellow) and no change (green). **B.** These results, combined with data from other biochemical approaches, have provided the first high resolution model of this key regulatory complex in the innate immune response cascade (C3b in ribbon and H in spheres).

Project 2. During this beamtime we have also obtained some very encouraging preliminary results on the new project on the interaction of sigma54 RNA polymerase and the promoter DNA, which is the region upstream of a gene where transcription initiation takes place. The subtle changes in the protection pattern observed in the presence of the transcription activator PspF reflect the unusual mode of interaction of this oligomeric protein with RNAP resulting in both proteins interacting with the same region of DNA but on opposite sides of the double helix. We are still in the process of analyzing these results and we are looking forward to continuing this work during the beamtime in October and in the future. This project is a logical evolution from our previous work on the caracterization of the formation of a transcription initiation complex by sigma70 RNAP that we obtained by this same experimental approach [3, 4].

[1].B. Sclavi, S. Woodson, M. Sullivan, M. Chance, M. Brenowitz, Methods Enzymol. 295, 379 (1998). [2].K. Takamoto, M. R. Chance, Annu.Rev.Biophys.Biomol.Struct. 35, 251 (2006). [3] B. Sclavi et al., Proc Natl Acad Sci U S A 102, 4706 (2005). [4] Rogozina, A., Zaychikov, E., Buckle, M., Heumann, H., Sclavi, B. (2009) *Nucleic Acids Research*, **37**(16) 5390–5404.