EUROPEAN SYNCHROTRON RADIATION FACILITY

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.

	Experiment title: Structural kinetics of RNA polymerase elongation complexes and transcriptional regulation	**Experiment number**: SC-2220
Beamline: ID10	**Date of experiment**: from: 11-07-07 to: 15-07-07	**Date of report**: 02-03-2008
Shifts: 12	**Local contact(s)**: Federico Zontone	*Received at ESRF:*

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Report:

This is a report for an ongoing set of experiments using time-resolved X-ray footprinting to study the the dynamics of biological macromolecular interactions. We have set up this technique at the ID10 beamline of ESRF and we have been using it to study the structural kinetics of the process of promoter recognition by Escherichia coli RNA polymerase (RNAP). This work resulted in a first publication in 2005 in PNAS. In that work we had described the dynamics and the structure of the intermediates in the pathway to the transcriptionally active complex on the T7A1 promoter. We described, for the first time, the structural rearrangements taking place in the process of promoter recognition and open complex formation in addition we described the dynamics of the equilibria formed between the different intermediates in the pathway. We have since continued this work and have improved the experimental setup.

Aims: Having described this process on the wild type promoter at 37 degrees C we then decided to study a mutant promoter where one of the two key sequences recognized by the enzyme has been changed to its consensus form, in addition we also studied the pathway at a decreased temperature, 20 degrees C, where DNA strand separation becomes energetically unfavorable.

Another aim for the beamtime in july was to continue the development of the application of this technique to the study of the structure and dynamics of multiprotein complexes, more specifically the C1 complex of innate immunity response.

Summary of the experimental approach. The hydroxyl radicals produced from the radiolysis of water during irradiation with an X-ray beam can be used to probe the solvent accessible surface of biological macromolecules. The main advantage of using a sychrotron light source for the X-ray beam is the high intensity of photons resulting in 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 oxydation products on the amino acid side chains are detected by mass spectrometry of the peptides obtained by cutting the sample with the

tripsin enzyme after x-ray exposure. A specially 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.

Achievements. Our recent results on the sequence and temperature dependence of promoter-RNAP interactions, mainly obtained during the beamtimes in february and july 2007, are now part of a manuscipt that will be submitted in the next couple of months and are an integral part of the PhD thesis of Anastasia Rogozina.

Structural analysis of human complement protease C1r in the C1 complex using synchrotron footprinting. In collaboration with the laboratory of … in Evry we have begun the development of protein footprinting experiments. The analysis of the samples from the july beamtime by mass spectrometry has resulted in a preliminary map of the changes in solvent accessibility due to the formation of the C1 complex involved in the innate immunity response. (Figure 1 and 2). This large and flexible complex of the C1q hexamer with the C1r protease is very difficult to christallize therefore this is the first structural data on the protein interfaces involved in the formation of the complex.

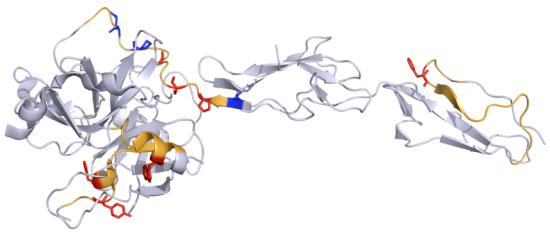
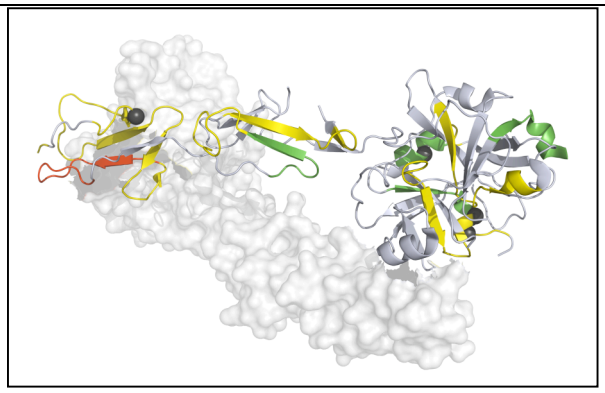


Figure 1. Structure of the zymogen catalytic domain of complement protease (CCP1, CCP2 and SP domains). Oxidized residues identified by NanoLC-MSMS experiments in the absence of C1q are shown in red; blue residues were not directly identified as modified by MSMS but proposed to be oxidized by MASCOT.

A.



B.

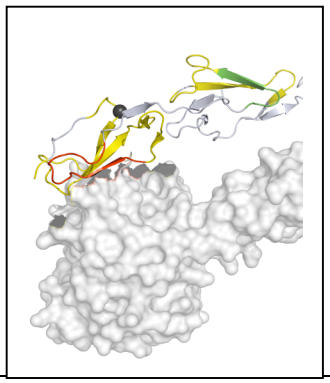


Figure 2. Surface accessibility modification of human C1r protease (modules SP-CCP2-CCP1) upon C1 complex formation.
(A) Crystal structure of the homodimeric dimeric form of CCP1-CCP2-SP C1r catalytic domain.
(B) Close view of the modified CCP1 region of C1r (peptide S352-R366, in red) displaying both protection and unprotection to oxidation upon C1 formation.
(C). Crystal structure of modules SP-CCP1 and CCP2 of C1r showing the modified and unprotected peptides in yellow (increase of accessibility in the complex), the unmodified peptides in green (no change of solvent accessibility upon complex formation) and the modified peptides with or without protection in red (2 conformations of the same peptide in solution). The undetected peptides are show in white. Key oxidized residues are labelled and shown as black balls.

C.