EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** Translocation of RNA-polymerase on the DNA-template: A fast OH-radical footprinting study using short pulses of OH-radicals produced by synchrotron radiation. Structural kinetics of transcription activation by the CAP protein.	**Experiment number**: SC-1294/95
Beamline: ID10A	**Date of experiment**: 24-09-2003 to 30-09-2003 and 03-12-2003 to 09-12-2003	**Date of report**: 01-04-2004
Shifts: 36	**Local contact(s)**: Dr. Federico ZONTONE	*Received at ESRF:*

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Report: This is the common report of two groups, namely CNRS/Ecole Normale Supérieure de Cachan (France) and the Max-Planck-Institute of Biochemistry (Germany). Both groups closely collaborate on the analysis of the transcription process using different physical chemical methods.

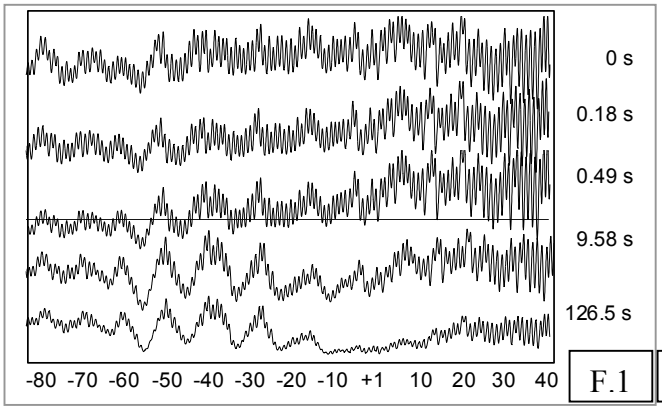
Aims: The first aim is to establish OH-radical X-ray footprinting at the beamline ID10A for the study of the kinetics of macromolecular interactions. The second aim is to use this method to study the mechanism of binding of RNA polymerase to promoter DNA, its translocation along the DNA during RNA synthesis and the role of transcriptional regulators during these key cellular processes.

Summary of the experimental approach: RNA polymerase and DNA are mixed in a stopped flow device in the range of about 20milliseconds. The reactants are allowed to interact for a defined time interval (20 msec to 200 sec). Subsequently, the polymerase-DNA complex are exposed to the x-ray beam for a defined period by being flowed across the X-ray beam in a quartz capillary. During this period (about 1msec) the DNA is cleaved by OH-radicals generated by x-ray-induced radiolysis of water. Since the OH-radicals cleave those backbone sugars of DNA which are solvent exposed, not protected by an interaction with the bound RNA polymerase, a "footprint" of the DNA-bound RNA polymerase is generated. The DNA fragments resulting from this cleavage reaction are separated by electrophoresis through a polyacrylamide gel and their relative amounts quantitated by detection of the fluorescence of a label present at their 5'end (alexa647).

Achievements: Our results show that we are able to carry out time-resolved x-ray footprinting at the beamline ID10A. Using this technique we have been able to identify several intermediates in the pathway of promoter recognition by RNA polymerase. In order to make more efficient use of the beamtime available we have built a new stopped flow apparatus. We can now collect up to 10 time points in a row without having to enter the hutch, and without having to open and close the beam shutter each time. The previous stopped flow apparatus required the user to enter the hutch after each time point was exposed. This new machine has again been improved since our last use during the beamtimes, check valves had already been added to avoid premixing of the reagents and other modifications were made to reduce sample dilution during long mixing times. However during our latest beamtime we have had problems with the reproducibility of the binding reactions data due to changes in the extent of mixing of the reagents. Some of these problems only become apparent after the data analysis process is near completion and therefore were not detected while we were working at the beamline. We believe that we have found the cause of these problems and we are developing

calibration control methods that will allow us to detect and avoid these kind of problems sooner. In addition we have now completed a protocol for data analysis that results in a decrease in the amount of time required in the future for this phase of the project. The results of this analysis are shown in the next section and are now being submitted for publication. In order to increase the reproducibility of the experiments and the efficacy of mixing of the reagents we will install on the stopped flow new syringe-drive motors with a smaller step size, increased speed, and a shorter pause time. These will be tested during future beamtimes.

Identification of short lived intermediates in real time during the binding of RNA polymerase to promoter DNA. RNA polymerase was mixed with a DNA fragment of 110 base pairs containing the T7A1 promoter, allowed to react between 93 msec and 300 sec, exposed to the x-ray beam for 1 millisecond and subsequently applied to a sequencing gel. Figure 1A shows the output from a gel, the results from a RNA polymerase binding experiment. Each peak corresponds to a DNA fragment of a different size, the difference between the peaks being only one nucleotide. The nucleotides are numbered with respect to the transcription start site (+1). The intensity of the peaks is determined by fitting these profiles to a set of Lorenzian curves. The change in peak intensity as a function of incubation time of the protein with the DNA is shown in figure 2. Some of the kinetics curves are multiphasic and the amplitude of the different phases is dependent on the position of the nucleotide in the promoter (fifure 3). Analysis of these results allows us to propose a mechanism for the recognition of this promoter by RNA polymerase (figure 4). A first set of intermediates is observed where the polymerase binds to the upstream part of the promoter (-55 to –22). In a second set of interm



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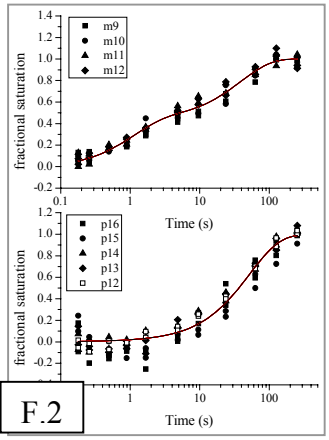

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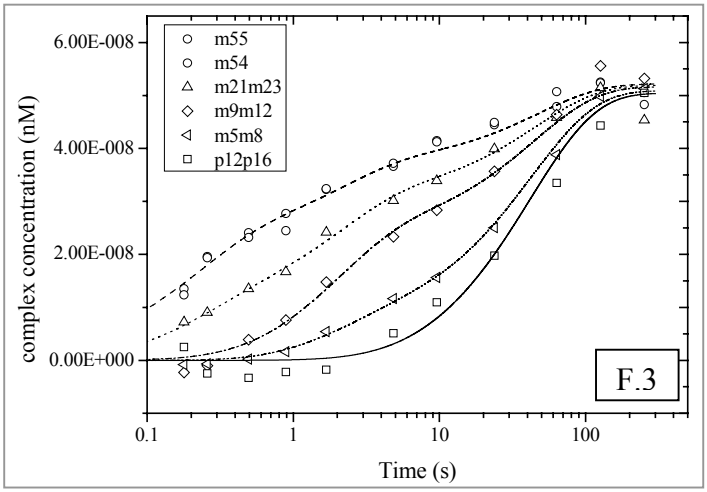

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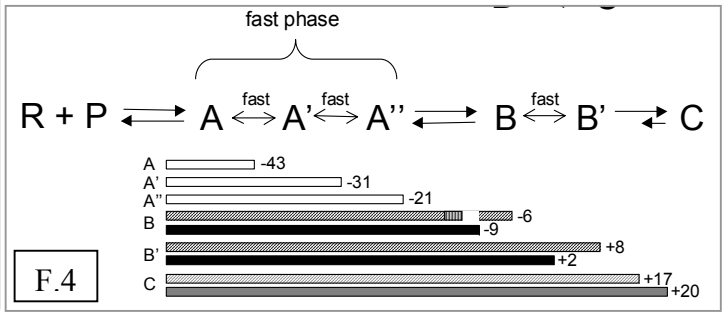

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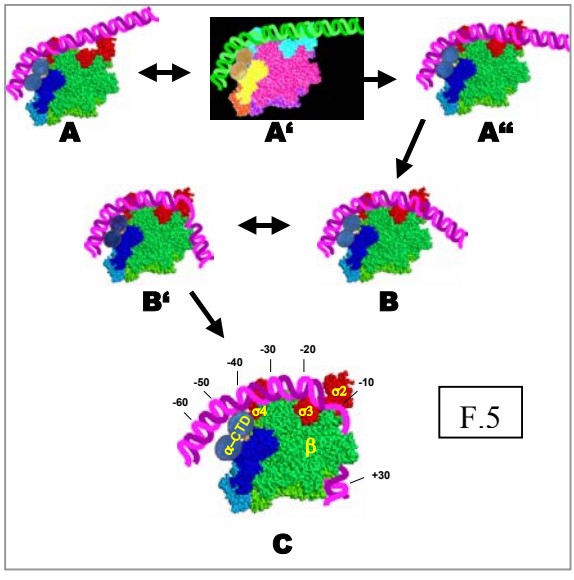

F.5

ediates the protection extends downstream as the polymerase begins to separate the two strands of the DNA. The final isomerization results in a protection down to nucleotide +20 as the double helix is completely enveloped by the protein domains. **Conclusions:** Our data shows that synchrotron OH-radical footprinting can be used to follow binding of RNA polymerase to the promoter DNA and to characterize the structure and the rate of formation of the different intermediates. Using the available structure of prokaryotic RNA polymerase holoenzyme we can propose a model for the sequence of structural isomerizations taking place during promoter binding (figure 5). In order to increase the time resolution of our experiment we are planning to change the drive motors of the stopped flow. We are currently submitting an article with these results and preparing a second on the mechanism of promoter escape upon transcription initiation.