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:** Movement of the RNA polymerase on the DNA template: a fast OH-radical footprinting study using short pulses of OH radicals produced by synchrotron radiation.	**Experiment number**: SC-1175/76
Beamline: BM5	**Date of experiment**: 16-05-03 to 21-05-03 20-07-03 to 26-07-03	**Date of report**: 23-07-2003
Shifts: BM5: 32	**Local contact(s)**: Dr. Gerhard GRUEBEL	*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.

Aim:

It is the aim of these groups to establish OH-radical X-ray footprinting on the beam line ID10A and use the method to follow the binding of RNA polymerase at the promoter DNA and translocation of RNA polymerase along the DNA during RNA synthesis.

Summary of the experimental approach:

RNA polymerase and DNA were mixed in a stopped flow device in the range of about 20msec. The reactants were allowed to interact for a defined time interval (20 msec to 200 sec). Subsequently, the polymerase-DNA complex was exposed to the x-ray beam for a defined period. During this period (about 1msec) the DNA was cleaved by OH-radicals generated by x-ray-induced radiolysis of water. Since the OH-radicals cleave predominantly those regions of DNA which are uncovered by the bound RNA polymerase, a "footprint" of the DNA-bound RNA polymerase is generated. Here we show for the first time how RNA polymerase binds to the cognate DNA, the promoter, by following the kinetics by "synchrotron footprinting".

Achievements:

Development of a new stopped flow device:

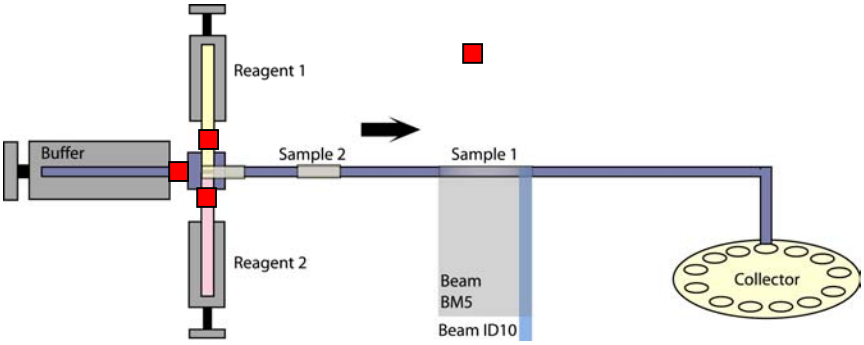


Figure 1: The modified stopped flow apparatus. Two separate motors drive the syringes. One motor drives the reagents (1) and (2) for mixing. After waiting for a defined reaction time, the second drive pushes the sample across the beam and then into the collection tube.

We have recently built a new stopped flow apparatus in order to make more efficient use of the beamtime available (Figure 1). The previous stopped flow apparatus required the user to enter the hutch after each time point was exposed. 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. This new machine has again been improved since our last use during the beamtimes at the end of February and March, check valves were added to avoid premixing of the reagents and other modifications were made to reduce sample dilution during long mixing times.

Figure 2 shows the results of an experiment where we varied the speed at which the sample is passed in front of the beam, thus changing the exposure time. As the speed increases the amount of DNA cleavage decreases. The amount of cleavage needed for these experiments is about 30% so that on average each molecule of DNA is cleaved only once. The optimal exposure time is 0.5 to 1.5 milliseconds, depending on the beam current and experimental conditions. We have found a linear correlation of exposure time to beam current. Since the beam size we are using is 1.6mm H x 1.2mm V, in this amount of time we expose one thirtieth of our sample.

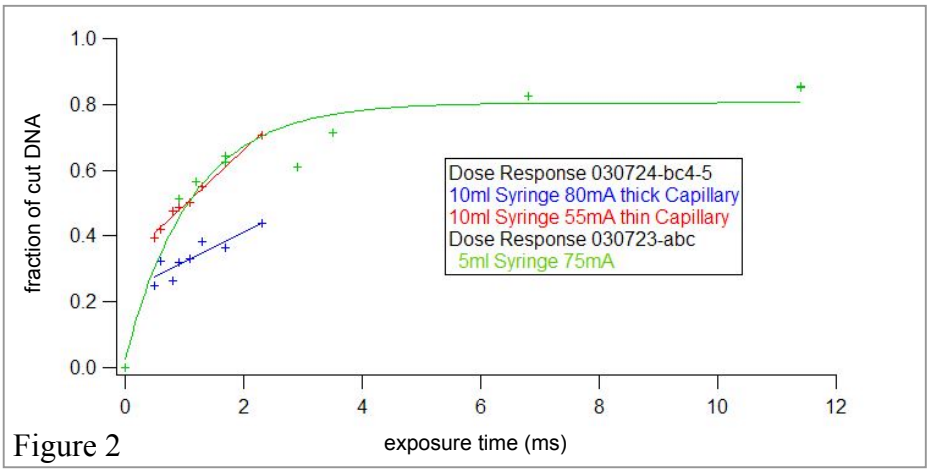


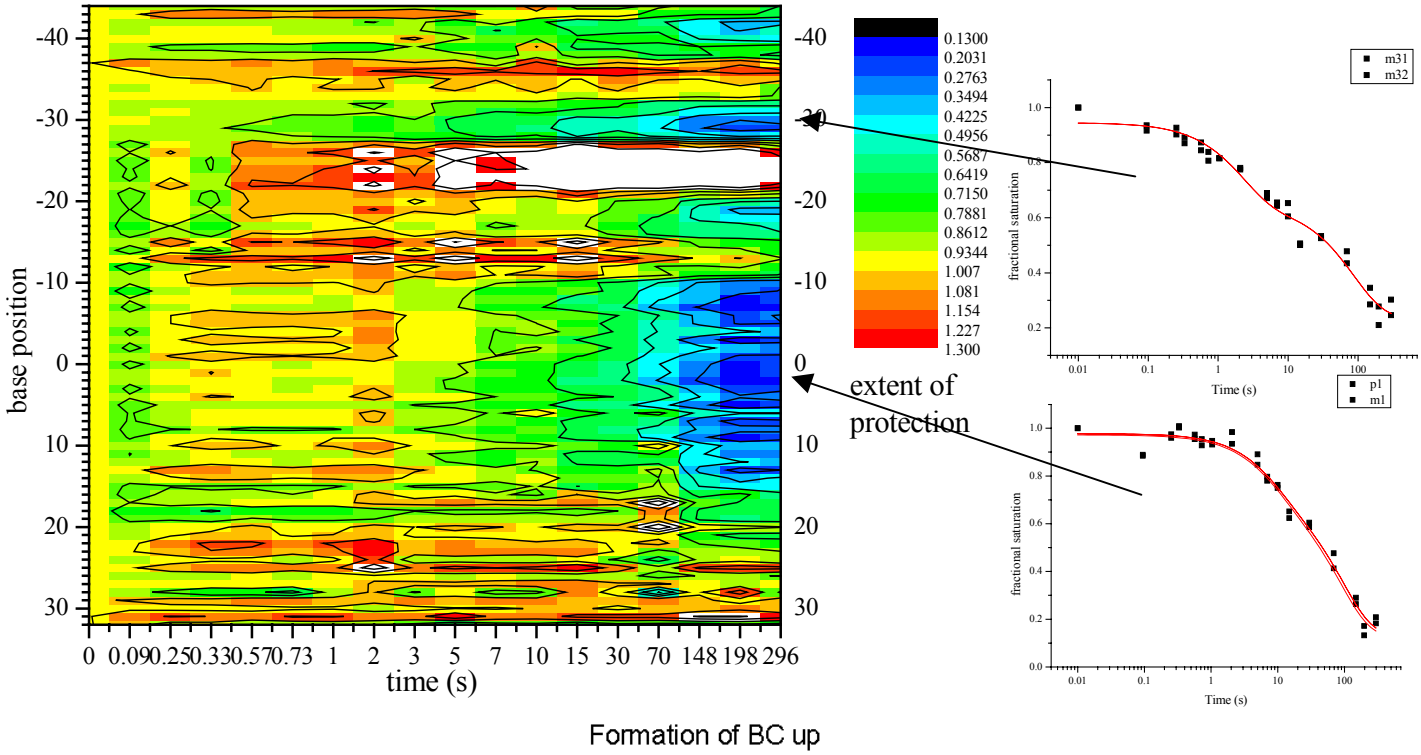
Figure 2

During our last beamtime in July we were able to carry out a dose response experiment at high current (hybrid mode ~180 mA). We thus determined that the cutting efficiency at lower current is still not saturated and that an increase in cutting is seen at the higher currents (from 1.3 to 0.5 ms). This shows that we can reduce the dead time of the experiment. The stopped flow will have to be adapted to decrease the mixing time of the reagents and increase the lifetime of the components' materials. To avoid unnecessary exposure of the quartz capillary we are now closing the beam during the longer mixing times.

Experiment (1): kinetics of binding of RNA polymerase to the promoter DNA. RNA polymerase was mixed with a DNA fragment of 110 base pairs containing the promoter, allowed to react between 93 msec and 300 sec, exposed to the x-ray beam for 1 msec and subsequently applied to a sequencing gel. The DNA fragments (obtained by OH-radical cleavage) were visualized by a fluorescence label at one end of the DNA (see figure in previous report).

Figure 3A shows a typical result from a RNA polymerase binding experiment. The regions of DNA becoming protected by the protein as a function of mixing time can be seen as a change in color (blue equals protection). A characteristic rate of formation of each contact can thus be measured (Figure 3B). A first intermediate is observed where the polymerase binds to the upstream part of the promoter. In a second intermediate the protection extends downstream as the polymerase contacts a larger area of DNA near the transcription initiation site. In this case there is an activator protein (CAP) prebound on the DNA which

guides the polymerase to the formation of an active complex. We have collected data for a mutant of this activator protein. Another experiment was carried out on a promoter called T7 A1 from phage DNA (Figure 3C). And a similar pattern of appearance of protection was observed.



Formation of BC up

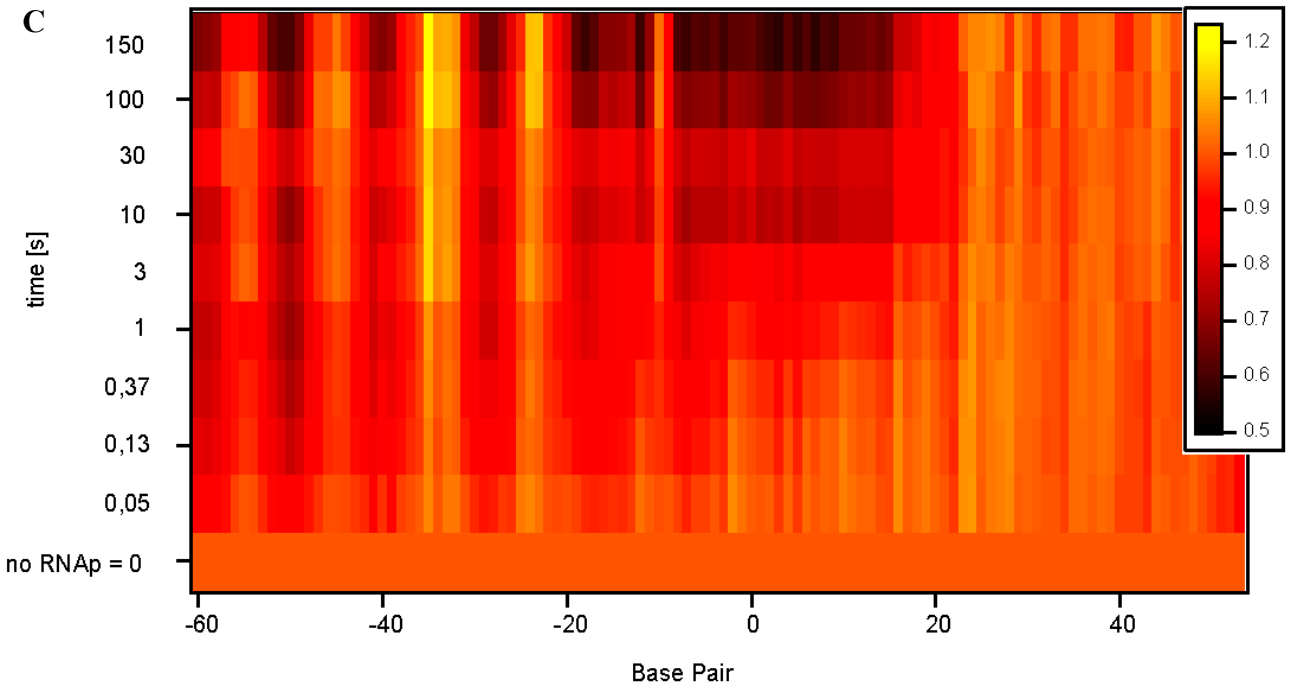


Figure 3

In conclusion: The data show 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. In our results we can observe the formation of a first intermediate within the dead time of the stopped-flow machine in which contacts are made between the protein and the DNA in the upstream region of the promoter where a highly conserved sequence is found. Subsequently the protein makes contacts with the rest of the promoter by wrapping of the DNA around the polymerase. In order to increase the time resolution of our experiment we are planning to change the drive motors of the stopped flow and we are applying for funds to buy these motors. We are currently writing two articles for publication with these results.