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 with and without transcription factors 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 100msec. The reactants were allowed to interact for a defined time interval (100 msec to 300 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 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:

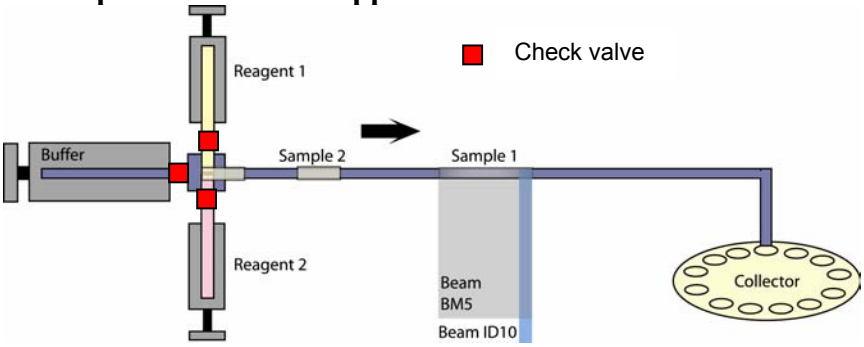

Figure 1: The modified stopped flow apparatus. Two separate motors drive the syringes. One motor facilitates mixing of the reagents (1) and (2) and the second one drives the mixed sample across the beam and then into the collection tube after defined reaction intervalls.

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 in order to improve the control of the flow system. Undesired cutting of the DNA and radiation stress of the machine parts were suppressed by providing a lead shield in front of the thermostated chamber and a lead channel behind the exposure capillary.

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 footprinting experiments is about 30% so that most of DNA molecules are cleaved not more than once. The optimal exposure time turned out to be 0.5 to 1.5 milliseconds, depending on the beam current and experimental conditions. We have found a linear relationship of exposure time and beam current. Having a beam size of 1.6mm H x 1.2mm the volume which can be exposed at once is 0.6 microliters, so it takes 20-60 msec to expose the whole 24 µl of the sample assuming a flow rate of 0.4µl/msec.

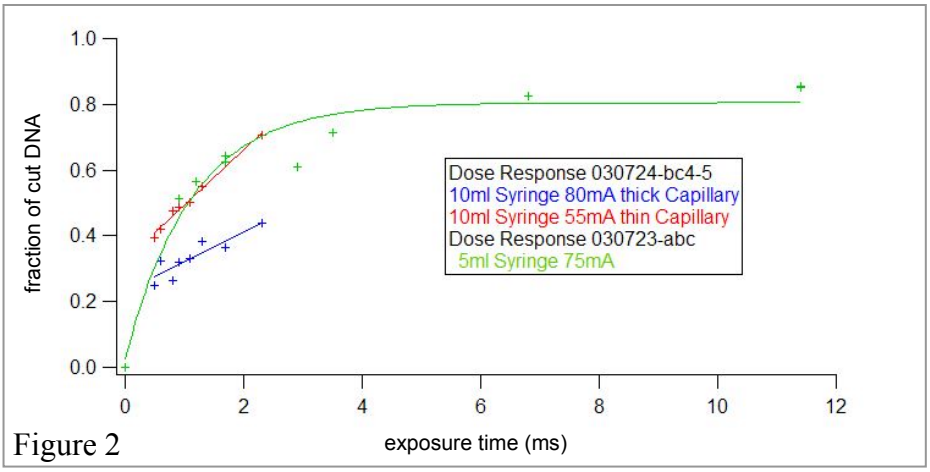

Figure 2

The maximum speed of the machine is presently limited due to non-linear-effects of the stepping motors. Also relaxation-effects of the teflon-tubings are significant at speeds above 1m/s (=mm/msec).

During our last beamtime in July we were able to carry out a dose response experiment at high current (hybrid mode ~180 mA). Our data indicate that the exposure time can be reduced, if a higher current is used. This would enable us to shorten the exposure time and reduce the dead time of the experiment. In order to make use of the shorter exposure time the stopped flow has to be adapted as well as the mechanical components. The first , with respect to the flow rates and the later one with respect to the tolereance to radiation stress. 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 without and with CAP. RNA polymerase was mixed with a DNA fragment of 170 base pairs containing the promoter, allowed to react between 93 msec and 300 sec, exposed to the x-ray beam for 1.5 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 using the same methods as described in the previous reports.

Figure 3A shows our result from RNA polymerase-DNA binding kinetics experiment without CAP, using the promoter A1 from phage T7. The abscissa shows the base positions on the promoter DNA (+1 indicates the starting point of transcription), the ordinate indicates the reaction time and the color shows the degree of protection according to the color code. The Fig 3a shows that binding of RNA polymerase and DNA is formed in at least two steps. The first intermediate formed within 400msec interacts upstream of the promoter with the bases at positions -60, -50, -40, -30, -18. The formation of the second intermediate is characterized by an extension of the protected region further downstream including the transcription start site (+1). Characteristic reaction rates can be obtained for both intermediates (Fig. 3b) by analyzing the kinetics

of individual base positions (Figure 3B), such as -32, -18 and +1, +13, respectively. In order to show the strength of the methode a different transcription system was analysed kinetically, namely the complex of the transcription factor CAP bound to the promoter lacUV5 and RNA polymerase.
Figure 3C, D show, that the binding characteristics are similar in both systems, but the binding of polymerase appear to be faster in the later case.

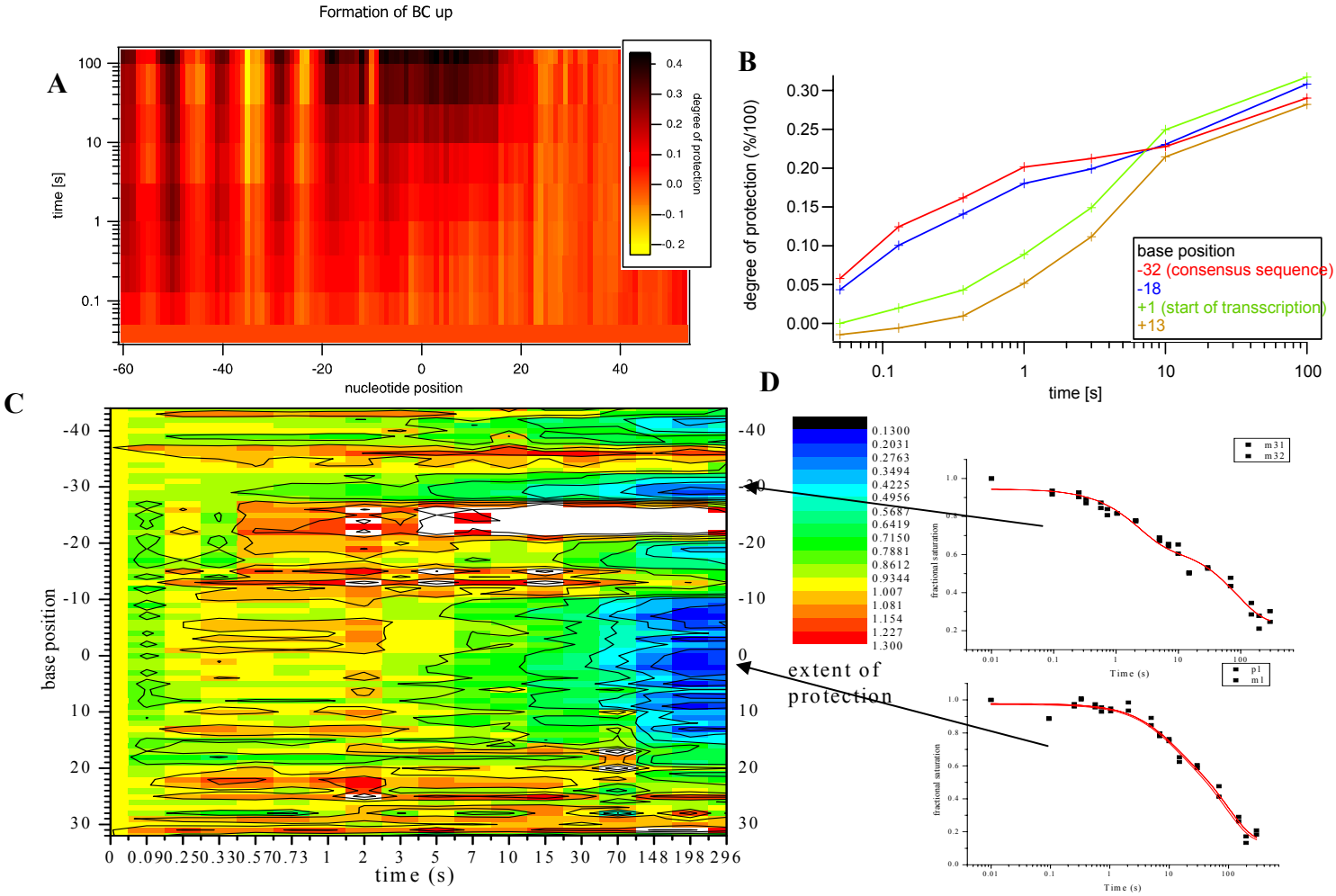

Figure 3

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. The advance to the previous experiments (Nov, Jan, Feb and Mar) is that we can obtain kinetics in a higher time resolution, reproducibility and precision. In order to increase the time resolution of our experiments to offset-times below 100msec we are planning to change the drive motors of the stopped flow and we are applying for funds to buy these motors. In a second enhancement, we want to adapt the machine to higher beam current. We are currently writing two articles for publication with these results.