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:** Structural kinetics of promoter recognition and transcription initiation by *E. coli* RNA polymerase by time-resolved X-ray footprinting. | **Experiment number**: SC-1062/63 |
|---|---|---|
| **Beamline**: BM5/ID10 | **Date of experiment**:BM5:29-10->31-10; 28-01->30-01-03<br>ID10: 6-11-02->11-11-02; 14-2-03->18-2-03 | **Date of report**: 28-02-2003 |
| Shifts:<br>BM5: 12<br>ID10: 27 | **Local contact(s)**:<br>ID10: Dr. Gerhard GRUEBEL BM5: Dr. Joanna HOSZOWSKA | *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 footprinting on the beam lines BM5 and ID10 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.

**Experimental approach in short:**

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:**

**1) Development of a new stopped flow device:**

Fig. 1 shows the device schematically.

Fig. 1

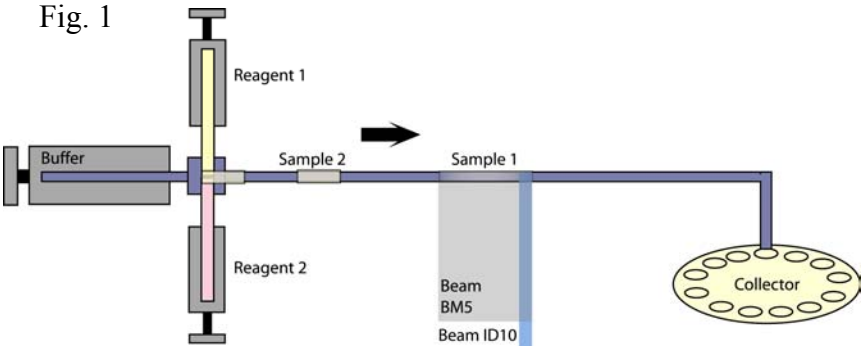



**Figure 1:** The new stopped flow apparatus. Two separate motors drive the syringes. One motor drives the reagents (1) and (2) for mixing. After waiting for a defined reaction time, the second drive pushes the sample across the beam and then into the collection tub

Two devices were built which were adapted to the requirements of BM5 and ID10 beam sizes. We have recently built a new stopped flow apparatus (Figure 1) in order to make more efficient use of the beamtime available. 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 already been improved since our first use during the beamtimes at the end of October (BM5) and beginning of November (ID10), for example it has been connected to a new sample collector; this new design reduces sample dilution during ejection from the machine into the tube.

**2) 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 93msec and 99sec, 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, as indicated in Fig. 2a. Fig.2a shows the scheme of the experiment. The arrows indicate OH-radical cleavage.

Fig. 2a

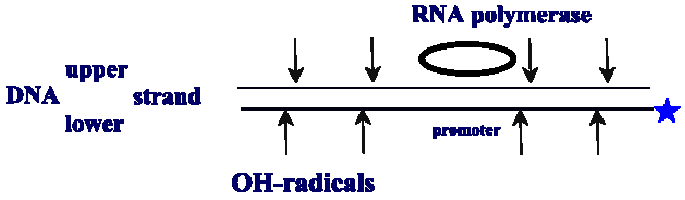
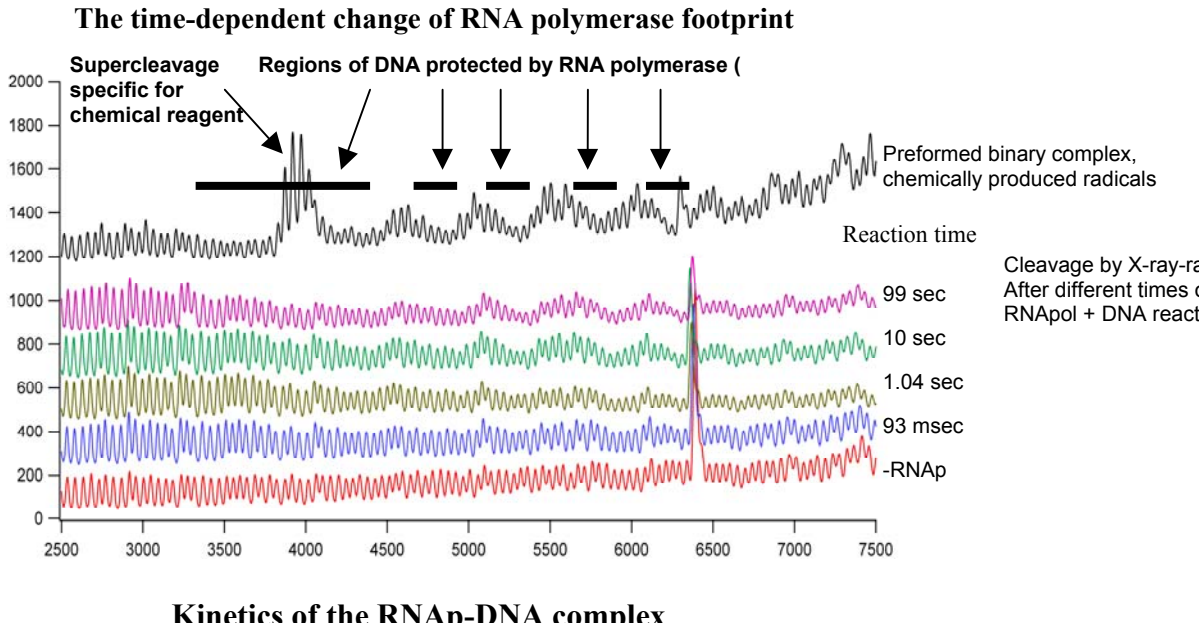



Fig. 2b shows the result. The lowest lane shows the fragment pattern of DNA without RNA polymerase and the lanes above the footprinting pattern after reaction time as indicated. The upper most line shows the footprint of the static RNA polymerase-DNA complex obtained by chemical OH-radical cleavage.

Fig. 2b



The lines in Fig. 2b show that the footprinting pattern develops with increasing reaction time (from bottom to top). However, the pattern is not yet fully developed, as the comparison with the upper most (static) footprint indicates. This indicates that the reaction is no yet finished. The reaction time has to be extended.
The patterns show an uneven distribution of the cleaved fragments. The small fragments are overrepresented This means that the exposure time was too long. For optimization the exposure time has to be shortened or the beam has to be attenuated. An alternative explanation is that the DNA is cut by scattered radiation before it reaches the exposure chamber. In the future we will add a shutter in order to block the beam between samples.

**In conclusion:** The data show that synchrotron OH-radical footprinting can be used to follow binding of RNA polymerase to the promoter DNA. However, the experimental conditions can be improved with respect to optimization of the exposure time and the reaction time.

**2) Experiment (2): translocation of RNA polymerase**

Fig. 3b shows real time translocation of RNA polymerase along the DNA using OH-radical footprinting for the first time.

**Experimental conditions:** The preformed RNA polymerase-DNA complex (binding complex (BC)) (lower lane in Fig. 3b) was preformed and mixed with the substrates, the nucleoside triphosphates, by means of the stopped flow apparatus. Addition of nucleotides allowed RNA synthesis and translocation of RNA polymerase. Fig. 3a shows schematically the process.

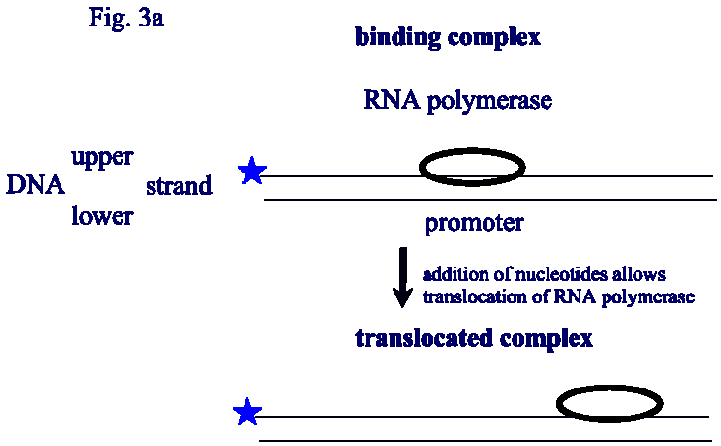



Fig. 3a: Transition from the binding to the translocated complex. (X) indicates the label.

Fig. 3b shows the results. The blue line corresponds to the footprint of the polymerase on the promoter, while the red line corresponds to the complex after the addition of nucleotides that has begun to move off the promoter and synthesize RNA. Below is the result of the data analysis showing that protection has been lost at the promoter and has appeared downstream. This kind of data analysis will allow us to follow the kinetics at each base on the DNA (each peak in the lines corresponds to one base).