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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 RNA polymerase-promoter interactions and the mechanisms of transcriptional regulation.***	**Experiment number**: **SC-1984**
Beamline: ID10C	**Date of experiment**: from: 13-07-2006 8 am to: 17-07-2006 8 am	**Date of report**: 01-09-2006
Shifts: 8	**Local contact(s)**: Federico Zontone	*Received at ESRF:*

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Report: This is the common report of two groups located at the 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: Our first aim was to establish time-resolved OH-radical-mediated X-ray footprinting at beamline ID10C for the study of the kinetics of macromolecular interactions, this has been successfully accomplished, although we are continuing to improve our experimental setup in order to increase time resolution and reproducibility. The next aim was to use this method to study the mechanism of binding of RNA polymerase to promoter DNA, this has also been successfully accomplished and resulted in a publication in PNAS[1]. Since our last experiments at ESRF we have designed a new stopped flow apparatus that was built by BioLogic (Grenoble). During the beamtime of July 2006 we have carried out tests on this machine and we have used it for three main aims: 1.) the development of time-resolved hydroxyl radical (OH) footprinting on closed circular DNA; 2.) the use of time-resolved OH footprinting for the study of solvent accessible surfaces of multi-protein complexes; 3.) the study of the structural kinetics of RNA polymerase-DNA interactions as a function of temperature (a continuation of the work published in 2005). **Summary of the experimental approach:** RNA polymerase and DNA are mixed in a stopped flow device in the range of about 20 milliseconds. 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 1 msec) 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: By using time-resolved x-ray footprinting to probe changes in the solvent accessible sites on DNA we have been able to identify several intermediates in the pathway of promoter recognition by RNA polymerase. The kinetic analysis of these results showed that several intermediates can quickly equilibrate

within the longer times required for the rate limiting step to take place[1]. We next set out to study the effect of temperature on this process. An important step during promoter recognition by RNA polymerase is the melting of the double helix, necessary for the subsequent synthesis of RNA onto the template strand. This process is highly temperature dependent, thus we expected that the mechanism of promoter recognition could be changed by lowering the temperature. Our preliminary results show first of all that the new stopped flow apparatus is working correctly, the percentage of sample that is mixed has increased resulting in an improved signal to noise ratio (Figure 2). As a result the quality of the kinetic data is higher (Figure1) allowing for an accurate measurement of the rate of change of solvent accessibility at a resolution of a single base on the DNA (in the past we often had to « bin » the data for several bases, decreasing the structural resolution of this technique).

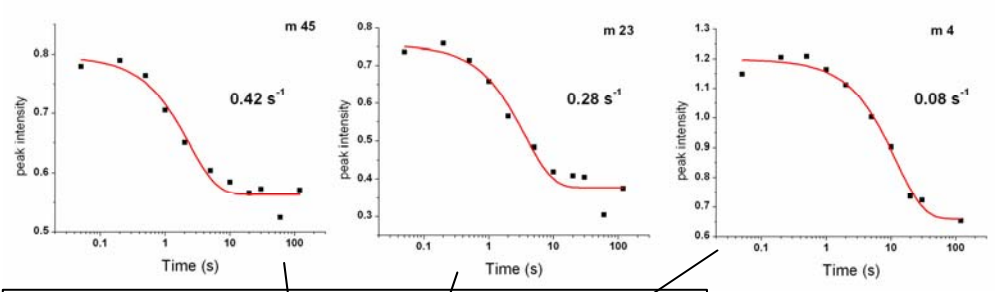

Figure 1. Plots of the decrease in solvent accessibility of individual bases as a funtction of time after mixing with RNAP

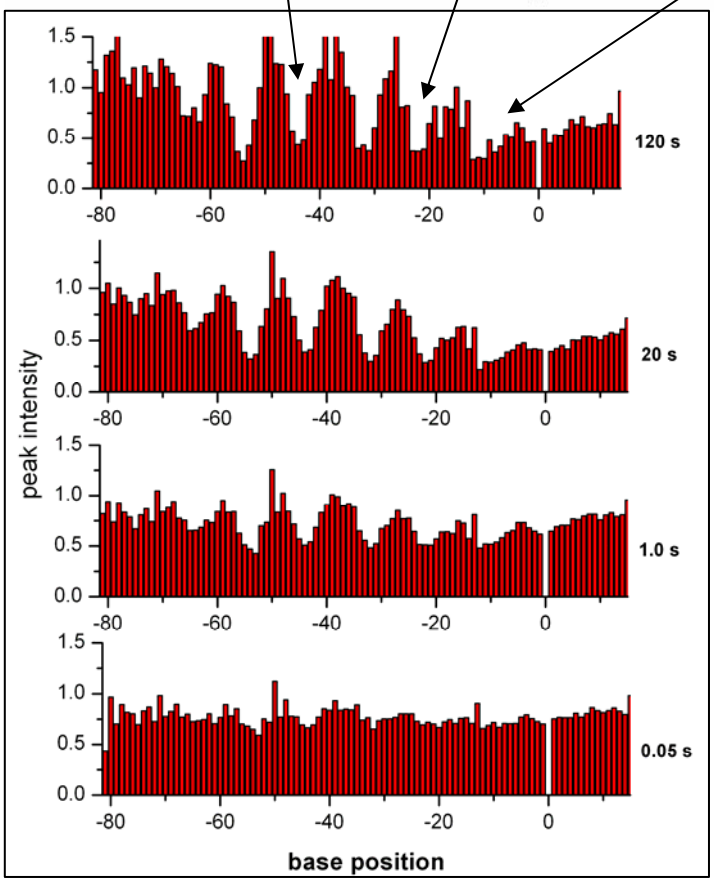

Figure 2. The height of each bar in the graph corresponds to the solvent accessibility of one base on the DNA. As the mixing time with the protein increases (bottom to top) the solvent accessibility of the bases contacted by the protein decreases, allowing for a detailed map of the interaction surface and its evolution through time.

These preliminary results allow us to distinguish at least three different sets of rates, corresponding to the formation of different structural intermediates in the process of promoter recognition. Comparison of these preliminary results obtained at 22°C with those obtained at 37°C shows that at 22°C the appearance of each structure is associated with a simple single exponential curve, instead of the double exponential observed at the higher tempearature. The rates of interconversion between the different intermediates are now slower, within the same order of magnitude as the rate-limiting step.

In our laboratory we have been studying the role of DNA topology in transcription regulation. In order to do this we use closed circular DNA that can be isolated with different degrees of supercoiling. The experiments described above were carried out with DNA fragments containing a fluorescent label at one end, in order to use circularized DNA for the same kind of experiments we need to set up an alternative method to quantitate the cleavage products. We decided to detect the cleavage products by the technique of primer extension. By this method an end-labeled oligonucleotide is hybridized to a specific site on the plasmid and then it is extended by a DNA polymerase up to a break in the DNA strand caused by the hydroxyl radicals. Preliminary results of these experiments show that we can obtain a cleavage pattern similar to the one obtained with end-labeled DNA. This will allow us to study a whole set of promoters whose activity is strictly dependent on the DNA being in a supercoiled state.

[1] Sclavi, B., Zaychikov, E., Rogozina, A., Walther, F., Buckle, M., Heumann, H., (2005) *PNAS USA*, **102**, 4706-4711.