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:** Kinetics of E.coli RNA polymerase promoter recognition and escape by time-resolved X-ray footprinting	**Experiment number**: LS-1564
Beamline: ID9	**Date of experiment**: from: 22 July 2000 to: 26 July 2000	**Date of report**: 31 August 2000
Shifts: 12	**Local contact(s)**: Michael Wulff	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The preliminary results from the first run at ESRF were very encouraging. The most important goal was accomplished: we determined that it is feasible to carry out time-resolved X-ray footprinting experiments at ESRF.

X-ray footprinting is a relatively new methodology which was developed at NSLS[1]. It is based on the production of hydroxyl radicals by the radiolysis of water caused by the X-rays. These radicals are used to cleave a polynucleotide (either DNA or RNA) at its solvent-accessible sites. If a protein covers the DNA for example it will protect the DNA from cleavage only at those sites that are in contact with the protein. The advantage of using a synchrotron X-ray beam to produce the hydroxyl radicals is that they can be produced fast enough (a few milliseconds) to carry out time-resolved experiments to study the formation of protein-nucleic acids complexes.

The time required to carry out this reaction at ID9 was about 2-3 ms. At NSLS the exposure time required is more in the order of 20 ms. We have used both hybrid mode and 16 bunch mode (the latter in the presence of both insertion devices) and had similar results, thanks to the helpful staff at the beamline, who also set up an easy way for us to align our sample. The only limiting factor right now for increased time-resolution is the size of the beam (1 x 3 mm), which allows us to expose only one tenth of the sample at a time. However under these conditions we were able to carry out some preliminary time-resolved experiments by placing a stopped-flow apparatus in front of the beam. This stopped-flow controls both the mixing time and exposure time of the sample.

[1] Sclavi, B., Woodson, S. A., Sullivan, M., Chance, M. R. and Brenowitz, M. (1998). Following the Folding of RNA with Time-resolved Synchrotron X-Ray Footprinting: *Methods in Enzymology*, **295**, 379-402.

In our laboratory we are studying the mechanism by which *E. coli* RNA polymerase recognizes the promoter on the DNA. It is known that this process involves several steps, including a large conformational change, as the protein changes its role from one in which it recognizes a specific DNA sequence and places the active site in the correct position, requiring tight contacts with the DNA, to that of a polymerase which has to be able to quickly slide on the double helix as it carries out the chemical reaction necessary to copy the DNA sequence into a new RNA molecule.

We are currently using a set of time-resolved techniques that allow us to follow different aspects of the conformational changes that take place during this mechanism.

The results from the time-resolved X-ray footprinting experiments measuring the rate of formation of the complex (Figure 1), are in good agreement with the data we had previously collected in the laboratory using different methods. The advantage of this synchrotron-based technique is that it can give us data of a much higher resolution than the other techniques we have available. X-ray footprinting will allow us to determine exactly where the protein is contacting the DNA at what time, instead of just measuring the rates of global changes. The next step therefore is to increase the quality of our samples, which will allow us to obtain a detailed picture of the different contacts made by the protein (*E. coli* RNA polymerase) on the DNA in the process of recognition of the promoter and the subsequent changes in conformation that take place during the formation of an active complex.

In the future this technique can be applied to the studied of the formation of multi-component systems on the DNA, where several proteins regulate the transcriptional activity of a given gene. X-ray footprinting has already been successfully applied to the study of the folding of a large RNA molecule[2]. In addition it can be used to look at the changes in solvent accessibility of the surface of a protein to directly study its changes in conformation and its interaction with other molecules[3]. Another future application of this technique is *in vivo* footprinting, which will take advantage of the fact that the radicals are produced directly from the water surrounding the macromolecules to be studied and there is no need to add any additional components for the cleavage reaction to take place.

This work was carried out in collaboration with the members of Hermann Heumann's laboratory from the Max-Planck Institut fuer Biochimie, Marinsried.

[2] Sclavi, B., Sullivan, M., Chance, M.R., Brenowitz, M., Woodson, S.A. (1998) RNA Folding at Millisecond Intervals by Synchrotron Hydroxyl Radical Footprinting. *Science*, **279**, 1940-1943.

[3] Maleknia SD, Brenowitz M, Chance MR. (1999) Millisecond radiolytic modification of peptides by synchrotron X-rays identified by mass spectrometry. *Anal Chem.* **71**(18):3965-73.

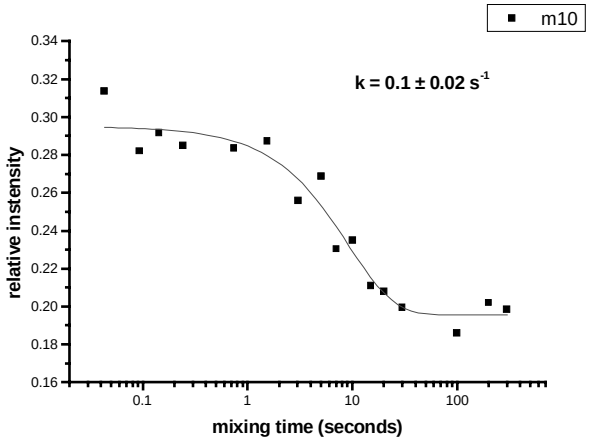

Figure 1: Time dependence of formation of the footprint of *E. coli* RNA polymerase on the lac UV5 promoter determined by time-resolved X-ray footprinting.