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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 RNA polymerase-promoter interactions and the mechanisms of transcriptional regulation.***	**Experiment number**: **SC-2093**
Beamline: ID10C	**Date of experiment**: from: 08-02-2007 8 am to: 12-02-2007 8 am	**Date of report**: 01-09-2007
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]. Our current aims include: 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 and of promoter mutants (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).

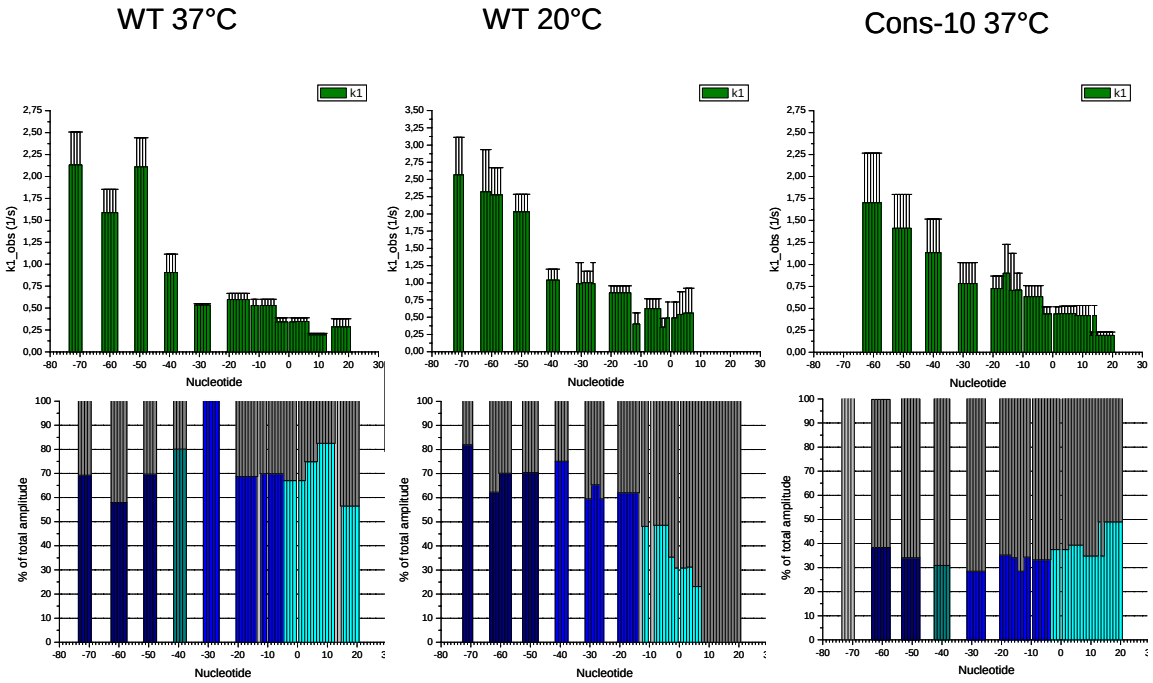
X-ray footprinting can be also be applied to the study of the solvent accessible surface of a protein [2]. The oxidation of amino acid side chains by the oxygen radicals produced during exposure to the X-rays can be detected by mass spectrometry of the protein’s peptides following protease digestion. Differences in the rate of oxidation, as measured by the amount of product as a function of X-ray dose, can be correlated with changes in solvent accessibility resulting from specific conformational changes or the formation of multi-

protein complexes. This approach can be especially useful for the study of large protein complexes that are difficult to crystallize or to measure the dynamics of conformational changes.

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 at the -10 region, necessary for the subsequent synthesis of RNA onto the template strand. This process is highly temperature dependent, thus we expected that the pathway of promoter recognition could be changed by lowering the temperature. Parallel experiments were carried out on a mutant of the promoter where the -10 sequence has been mutated to facilitate its binding and opening by the protein. The combination of these experiments will allow us to distinguish the effect that temperature on the DNA opening step from the effect on previous DNA-binding steps and conformational changes.

The figure below shows the result several combined experiments from the beamtimes in July 2006 and February 2007. We have obtained additional datasets during the latest beamtime in July 2007 that are currently being analyzed. In the plots in the first row each bar correponds to the rate at which each base is becoming protected from cleavage. The left side of the plot corresponds to the 'upstream' part of the promoter, while on the right side is the -10 and the 'downstream' region. At least two families of rates can be distinguished in the wild type promoter experiments, the upstream region becomes protected at faster rates, in addition the region from +8 to +20 only becomes protected during the last rate-limiting step at 20°C. On the other hand on the mutant promoter the decrease in the rates is more gradual. The second row of plots shows the amplitude of the fast phase whose rate is shown above. The second part of the signal appears at slower rate corresponding to the rate limiting step. Surprisingly, in the mutant promoter the fast phase has a smaller amplitude, even though this sequence is more easily recognized by the enzyme. It is possible that the the formation of these contacts earlier in the pathway might inhibit or slow down the formation of the final stable structure. We are cuurently writing an article based on these results, including the more recent ones obtained during the July beamtime.

We have also obtained encouraging preliminary results for the protein footprinting experiments. We have identified several modification sites on the surface of the protein, this will be useful in order to study the oligomerization process of the protein, DnaA, that is dependent on the bidning of ATP and DNA. We are currently optimizing the mass spectrometry protocol to obtain an increased pepetide coverage of the protein.



[1] Sclavi, B., Zaychikov, E., Rogozina, A., Walther, F., Buckle, M., Heumann, H., (2005) *PNAS USA*, **102**, 4706-4711.[2] Takamoto K, Chance MR. (2006) Radiolytic protein footprinting with mass spectrometry to probe the structure of macromolecular complexes. *Annu Rev Biophys Biomol Struct*.**35**:251-76.