



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 via the User Portal:

<https://www.esrf.fr/misapps/SMISWebClient/protected/welcome.do>

Reports supporting requests for additional beam time

Reports can 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.



Experiment title: Structural studies on RNA polymerase elongation complexes bound to transcription factors

Experiment number:
MX-2026

Beamline: CM01	Date of experiment: from: 12/02/2018 to: 16/02/2018	Date of report: 17/04/2018
Shifts: 9	Local contact(s): Eaazhisai Kandiah	<i>Received at ESRF:</i>

Names and affiliations of applicants (* indicates experimentalists):

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FRANCE

Report:

We have applied for 9 shifts on the Titan KRIOS to collect data on a functional RNA polymerase elongation complex in November 2017. We were scheduled in February 2018 (12/2 – 16/2). Our local contact was Eaazhisai Kandiah.

We brought several grids from two different batches, which were pre-screened on our Polara microscope. Unfortunately, we cannot recover the grids from the Polara to use them on the Titan. In this sense, it is difficult for us to know what the actual grids will look like on the Titan. I am explaining this because we ran into some problems: all the grids we checked at ESRF had a lot of cracked carbon and we had a hard time finding intact squares (without cracks). Thanks to the great support by our local contact we collected data on two different grids and ended up with a bit over 2600 images.

We had some minor problems related to the K2 camera: at one point, part of the recorded images contained a completely black area - luckily we monitored data collection remotely and noticed the problem relatively soon (the setup to monitor progress of the data collection is extremely useful). I am not sure but it looked like as if part of the camera temporarily failed. Our local contact was able to fix the problem by restarting the camera and computer remotely and we did not lose too much time. On the second day there was a software problem, where EPU was not able to map the holes (at the magnification required to image the grid squares). We started collection anyway because it seemed EPU was still able to find the holes (at the higher magnification required to locate the individual holes). However, our local contact noticed that still many holes were skipped. She was kind enough to select more squares and holes in our absence and continue data collection for us!

I would like to emphasise that we would not have been able to get the quality and size of the dataset without the support from Eaazhisai Kandiah. Although we are still refining the structure, we have a reconstruction at about 4Å resolution (Figure 1). We could fit X-ray models of RNA polymerase and can see all ligands. One problem we face is that the RNA 3'-end seems disordered and/or degraded.

We have since performed additional biochemical experiments and ordered new and chemically modified RNA oligonucleotides that give greater chemical stability and result in a more homogenous sample (according to our transcription assays). We would like to collect data on this complex and hence I would like to apply for more time on the Titan KRIOS at ESRF.

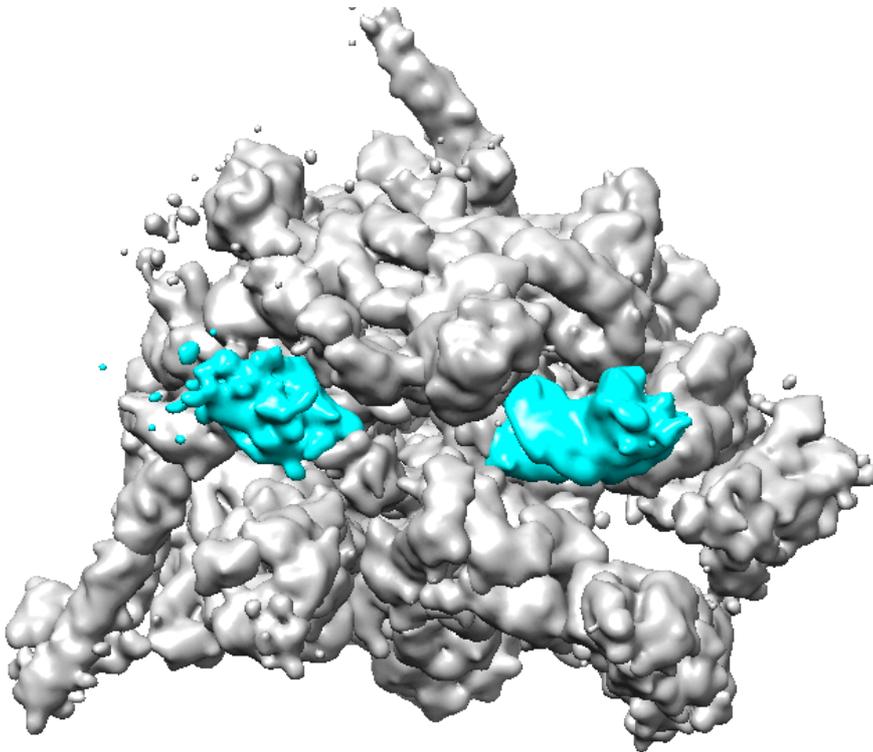


Figure 1.: A reconstruction of functional RNA polymerase complex resulting from data collected at ESRF. Extra density can be seen for ligands (e.g. upstream and downstream DNA duplex shown in cyan). Unfortunately, the reconstruction did not resolve the 3'-end of the RNA transcript in the active site (not shown).