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 via the User Portal:

https://wwws.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.

ESRF	**Experiment title: Structural studies on the coupling of transcription and translation**	**Experiment number**: MX-2207
Beamline: CM01	**Date of experiment**: from: 03/06/2019 to: 05/06/2019	**Date of report**: 05/09/2019
Shifts: 9	**Local contact(s)**: Eaazhisai KANDIAH	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Albert WEIXLBAUMER, PhD

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Report:

We have applied for 9 shifts on the Titan KRIOS to collect data on a functional complex of RNA polymerase bound to a transcription factor called GreA. GreA is involved in transcriptional proof reading and cleaves the RNA 3'-end to remove erroneous bases. The goal is to obtain reconstructions of RNA polymerase after it made a misincorporation in the nascent RNA transcript. We want to do this for all 4 possible misincorporation events (i.e. rATP, rUTP, rGTP, and rCTP). Our local contact was Eaazhisai KANDIAH and I traveled to the ESRF with a complex containing a misincorporated rATP.
I brought several grids from a batch, which was pre-screened locally on a Glacios microscope. Even though the atlases for several grids looked good, we noticed problems when taking a closer look at the holes. We thus had to manually search through many squares and holes to identify suitable ones. This was tedious but our local contact was supportive and helped to select enough holes to collect a large dataset.

We had collected on RNA polymerase (RNAP) elongation complexes before at the ESRF (February 2018, July 2018, and November 2018) and in each case obtained high-quality data. We have recently published a paper in *Mol. Cell* that contains reconstructions obtained from ESRF data. This is to say that we were extremely pleased with te quality of the data we have received so far and were looking forward to the results from this trip. Ayesha, a PhD student in my team, had spent some time on optimizing complex formation and grid-freezing protocols. I felt bad that the grids did not look better despite our efforts to screen them beforehand. The lesson learned for me is that it is not sufficient to collect atlases but we will also need to check a few squares and holes across the grid.

In the end, thanks to the support we got, we collected in total about 4420 micrographs and have picked more than 450.000 particles.
After 2D classification and clean-up, Ayesha obtained an initial 3D reconstructions and was able to classify the particles further. At the moment she is able to reach a nominal resolution of about 3.9Å (Figure 1). She can see all the ligands (DNA, RNA, GreA) and also sees that the active site is in a new and unusual conformation,

which had not been seen before. We have collected a dataset on the complex that misincorporated rUTP and see the same active site conformation. This is encouraging and very exciting. We now need to do reconstructions on complexes with rCTP and rGTP misincorporations. We hope to resolve some of the controversies in the field and maybe we will also be able to provide a structural explanation for the widely observed sequence dependent differences in the RNA cleavage rates.
I have submitted a new proposal to the ESRF and am looking forward to receiving feedback on it.

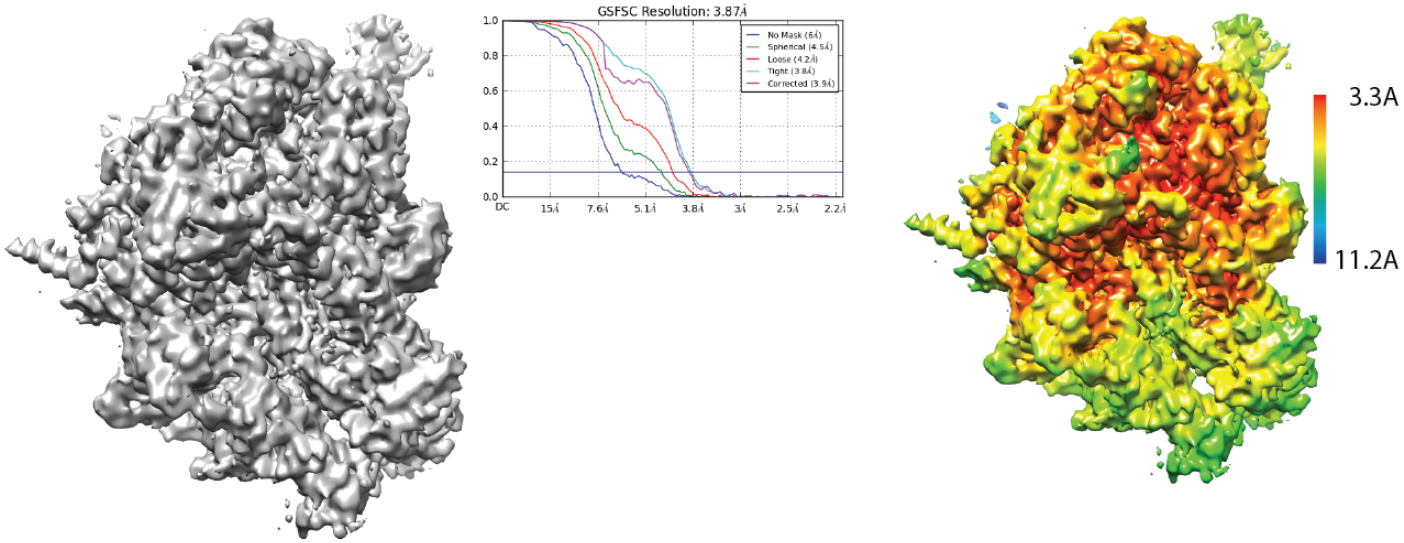

Figure 1: Preliminary reconstructions
After initial rounds of refinement in CryoSPARC, we obtained a reconstruction that refined to a nominal resolution of about 3.9Å. We can see density for the bound transcription factor, and nucleic acid ligands (not shown). In addition, we can see that the active site is in a new and unusual conformation. We are confident this will allow us to resolve some of the controversies in the field with respect to the role of elements such as the RNA polymerase trigger loop and its role in the cleavage reaction.