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-2136
Beamline: CM01	**Date of experiment**: from: 07/12/2018 to: 10/12/2018	**Date of report**: 28/02/2019
Shifts: 9	**Local contact(s)**: Gregory Effantin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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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 tethered to the 70S ribosomethrough the nascent mRNA in August 2018. We were scheduled in December 2018 (07/12 – 10/12). Our local contact was Gregory Effantin. Two users from my team (Maria Takacs, a research technician, and Michael Webster, a postdoc) traveled to the ESRF.
We brought several grids from a batch, which was pre-screened locally on our Polara microscope. Thanks to the outstanding support by our local contact, Maria and Michael were able to identify a good grid very quickly, select enough squares and holes and collect data that gave us about 5200 images. This was the fourth time we came to the ESRF but we have never started data collection as early as this time (around 3pm in the afternoon).

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 submitted a paper, which is curently under revision at *Mol. Cell* and 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. Maria and Michael had already spent some time on optimizing complex formation and grid-freezing protocols. We brought several grids frozen under different conditions and Gregory Effantin, our local contact, helped us screen those grids and select a suitable one for data collection. He selected all the holes for data acquisition and everything went extremely smoothly.

We collected in total 5220 micrographs and have picked more than 600.000 particles. We were very excited because initial CTF estimations suggested that 80% of the micrographs contained information to a spatial resolution corresponding to 2.3Å or better and only a very small proportion of micrographs were worse than 4.5Å.
I would like to stress that this complex is very challenging because we have compositional and conformational heterogeneity, which we cannot control biochemically. Processing and refining is therefore a time consuming task and we are far from being finished. After 2D classification and clean-up, Michael started to process the

data using downscaled particles corresponding to a pixel-size of 4.4Å. He obtained initial 3D reconstructions and was able to classify the particles based on whether or not density for RNAP was present. The reconstructions refined to the theoretical limit of 8.8Å. Michael now uses the classified and re-extracted particles (1x binned, 2.2Å pixel size) and refined them to 4.4Å resolution (again, the theoretical limit). He is able to control for conformational heterogeneity using novel software tools (Relion 3) and his results are already much better than anything that has been published before. We are confident that this dataset will be an important step forward in our efforts to obtain a high-resolution reconstruction of a transcribing/translating RNAP-70S ribosome complex.

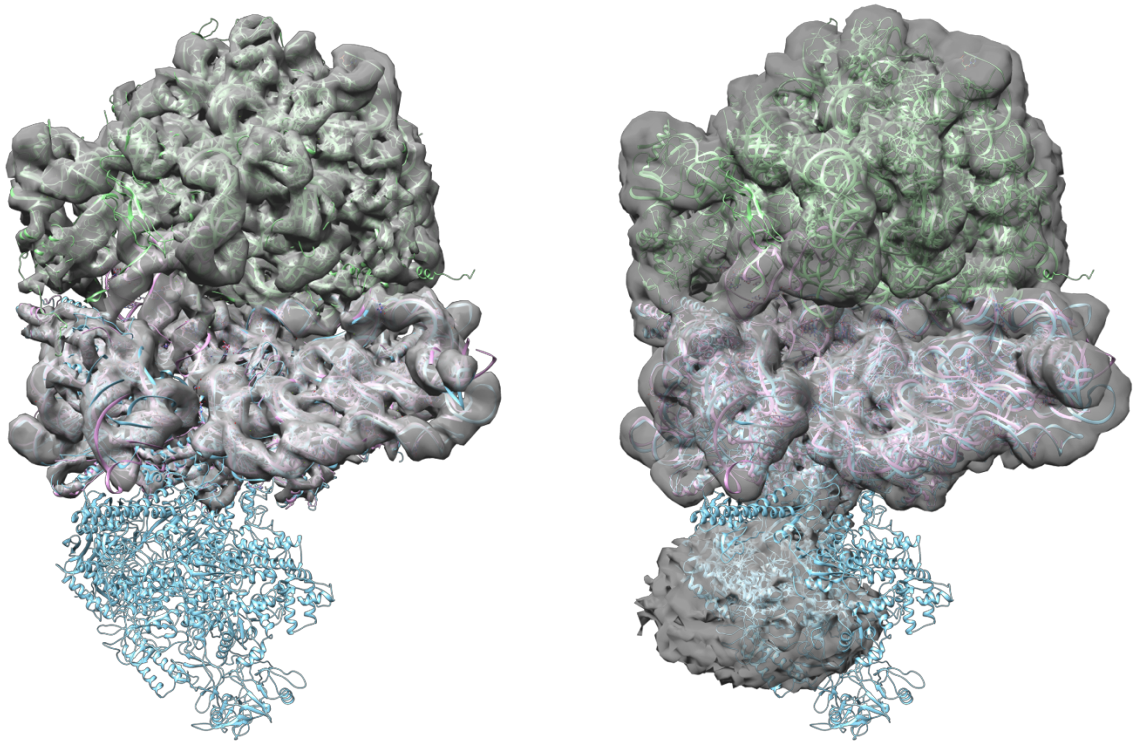

Figure 1: Preliminary reconstructions
Using downscaled particles, we have obtained reconstructions that refine to the theoretical resolution limit (left). At lower contour level (right), density for an RNAP tethered to the ribosome becomes visible. Focused refinement as well as multi-body refinement allowed us to confirm that this density is indeed RNAP, which is bound to the ribosome but rotates relative to the small ribosomal subunit.

UPDATE 02/28/2018

I would like to update the previous report because we now have a better understanding of the quality of the data. The density for the ribosome refines to below 3Å resolution (see Figure below) and after particle substraction and refinment of the density corresponding to the tethered RNAP, we get to below 7Å for it (see Figure below).

We have now started to use multi-body refinment as implemented in Relion ver. 3.0 and can model the movements of RNAP relative to the ribosome. This also allowed us to adjust our strategy and we believe we now know how to obtain even better samples, reach higher resolution, and resolve important controversies in the field with respect to the role of additional protein factors. However, for this we will need additional data and will apply for microscope time at the ESRF.

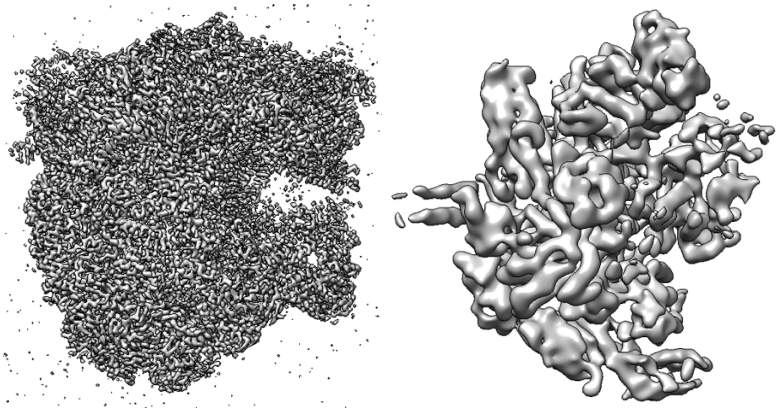

Figure 2: Improved reconstruction
We have now improved our reconstruction and used multi-body refinment to estimate the rotational movement of RNAP relative to the ribosome. This gave us a rconstruction of te ribosome around 3Å (left), which confirmed the presence of all ligands as expected. It also confirmed the presence of RNAP tethered to the ribosome – the density for RNAP refined to about 6.5Å (right). Because we know the orientation of RNAP relative to the ribosome and because we can estimate the rotational freedom, it already allows us to resolve important controversies in the field.