

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 the coupling of transcription and translation	Experiment number: MX-2180
Beamline: CM01	Date of experiment: from: 15/07/2019 to: 17/07/2019	Date of report: 05/09/2019
Shifts: 9	Local contact(s): Michael HONS	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): Albert WEIXLBAUMER, PhD Department of Integrated Structural Biology Institute of Genetics and Molecular and Cellular Biology IGBMC - UMR 7104 - U 1258 1, rue Laurent Fries BP 10142 67404 ILLKIRCH CEDEX FRANCE		

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 ribosome through the nascent mRNA in February 2019. We were scheduled for a mail-in experiment in July 2019 (15/07 – 17/07). Our local contact was Michael Hons. We shipped our grids in a dry shipper to the ESRF, where they were stored until the allocated time slot.

We sent several grids from a batch, which was pre-screened locally on a Glacios microscope and sent images of the grid atlases for various grids to our local contact. Thanks to the outstanding support by him, the data collection was going very well and we obtained a large dataset that members of my team were able to download shortly after the collection had finished.

We had collected similar complexes before at the ESRF (February 2018, July 2018, November 2018, December 2018, June 2019) and in each case obtained high-quality data. We have recently submitted a paper, which is now accepted at *Mol. Cell* and contains reconstructions obtained from ESRF data. This is to say that we were extremely pleased with the quality of the data we have received so far and were looking forward to the results from this trip. Maria and Michael, two people in my team working on the project, had already spent some time on optimizing complex formation and grid-freezing protocols. Based on our pre-screening and the atlases we collected, Michael Hons selected all the holes for data acquisition and everything went extremely smoothly.

We collected in total 5220 micrographs and have picked more than 385.000 particles.

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. However, after 2D classification and clean-up, Michael started to process the data using downsampled 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.

He is able to control for conformational heterogeneity using novel software tools (Relion 3). At the moment it refines to about 3.6Å resolution but we're confident to reach higher resolution. Importantly, we see density for a ligand, whose presence and location was controversial. We are confident this dataset is the last, missing one in a series of reconstructions that we had to do. We are now in the process of refining it further and have also started with model building and coordinate refinements.