

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 ccomplexes bound to transcription factors	Experiment number: MX-2026
Beamline: CM01	Date of experiment: from: 02/07/2018 to: 04/02/7018	Date of report: 14/08/2018
Shifts: 9	Local contact(s): Gregory Effantin	<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 RNA polymerase elongation complex in April 2018. We were scheduled in July 2018 (02/07 – 04/07). Our local contact was Gregory Effantin.

We brought several grids from two different batches, which were pre-screened on our Polara microscope. Thanks to the outstanding support by our local contact we were able to identify a good grids, select enough squares and holes and collect data that gave us about 4000 images.

We had collected on a related RNA polymerase elongation complex before at the ESRF (February 2018) but needed additional data because the earlier reconstruction did not resolve part of the RNA transcript we were interested. We brought several grids frozen under differetn 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 smooth.

We would not have been able to get the quality and size of the dataset without his support. Although we are still refining the structure, we have a reconstruction at about 3.3Å resolution (Figure 1). We have collected data from closely related complexes at Titan KRIOS microscopes equipped with K2 cameras at IGBMC, Strasbourg, Biozentrum Basel, Switzerland, EMBL - Heidelberg, Germany, and NeCEN, the Netherlands but the ESRF data gave us the highest resolution we have obtained so far. We could easily fit X-ray models of RNA polymerase, can model side chains in many parts of the structure and can see all ligands. Most importantly, we can see the RNA 3'-end, which is ordered but in a conformation not seen before. We are currently preparing the manuscript and are confident that this reconstruction will play a prominent role.

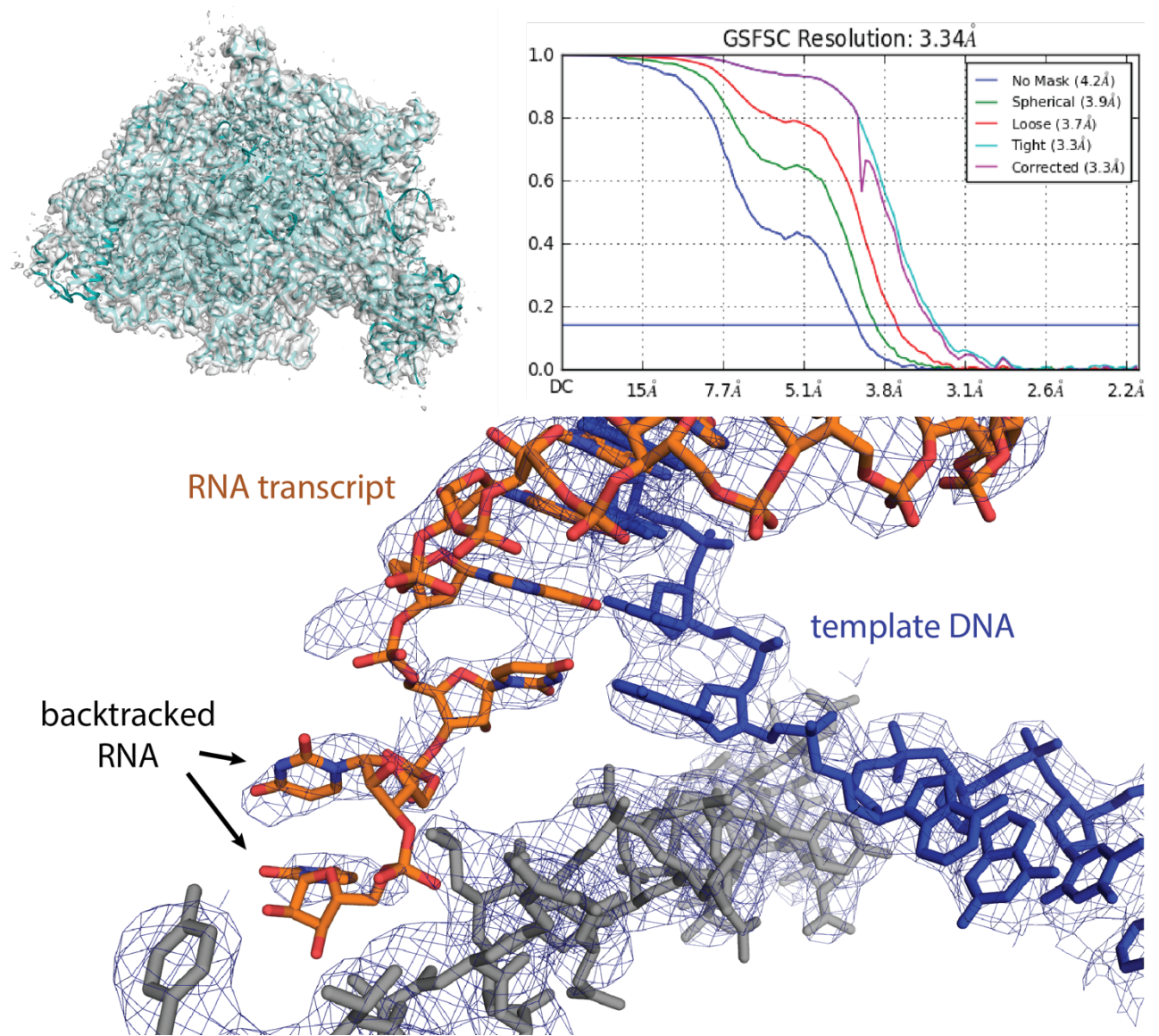


Figure 1.: A reconstruction of functional RNA polymerase complex resulting from data collected at ESRF. The data produced the highest resolution reconstruction that we have obtained so far (3.3 Å resolution, top). More importantly for the question we were addressing: the 3'-end of the RNA transcript is resolved in the active site and (bottom).