EUROPEAN SYNCHROTRON RADIATION FACILITY

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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 RNA polymerase elongation ccomplexes bound to transcription factors**	**Experiment number**: MX-2216
Beamline: CM01	**Date of experiment**: from: 20/09/2019 to: 22/09/2019	**Date of report**: 07/10/2019
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 RNA polymerase (RNAP) elongation complex bound to a regulatory RNA in July 2019. We were scheduled in September 2019 (20/09 – 22/09). Our local contact was Gregory Effantin. I came with a postdoc from my team to the ESRF.
We brought Quantifoil R 2/2 as well as UltrAuFoil R 1.2/1.3 grids with different sample concentrations. We pre-screened the Quantifoil grids on a FEI Glacios microscope. However, something between screening and arrival of the grids at the ESRF went wrong because all of the pre-screened ones had serious ice contamination. Luckily the UltrAuFoil grids looked good. Thanks to the outstanding support by our local contact we were able to screen the all the gold grids on the first day, identify several good ones, and select enough squares and holes to collect data that gave us almost 5000 images over the course of three days of data collection.

We had collected on a related RNA polymerase elongation complex before but needed additional data in presence of a transcription factor. Although we had reached high resolution before, the regulatory RNA was always disordered. We had evidence from preliminary reconstructions that the transcription factor would help because it is known to interact and stabilize RNA. We brought in total eight grids frozen under different conditions and Gregory Effantin, our local contact, helped us screen them on the first day. After identifying a good one he selected all the holes for data acquisition and everything went very smooth. We started data collection mid afternoon on the first day. We are very grateful for the excellent support that we received.
We have now transferred the data and started processing. We were very pleased because we already got some very promising results. From just 80,000 particles, we were able to obtain a reconstruction at a nominal resolution of about 3.2Å – this is the highest resolution we have obtained so far for RNAP complexes. In addition, we identified a small population (10% of the particles or about 8,000), which adopts a new conformation not observed before for *E. coli* RNAP (Figure 1). This trip was also informative because we collected at a smaller pixe size (0.9Å/pixel instead of 1.1Å/pixel) and we used the UltrAuFoil gold grids instead of holey carbon grids. Both changes compared to previous data collections appear to be beneficial and we will from now on adjust our strategy.

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 and at the ESRF – as I said, this dataset from the ESRF appears to be the best so far and we are very excited about it. I would like to conclude by thanking the team behind the Titan KRIOS at the ESRF for their excellent work and support! I would also like to congratulate them because this instrument produces consistently very good datasets and I hope to be able and access it in the future.

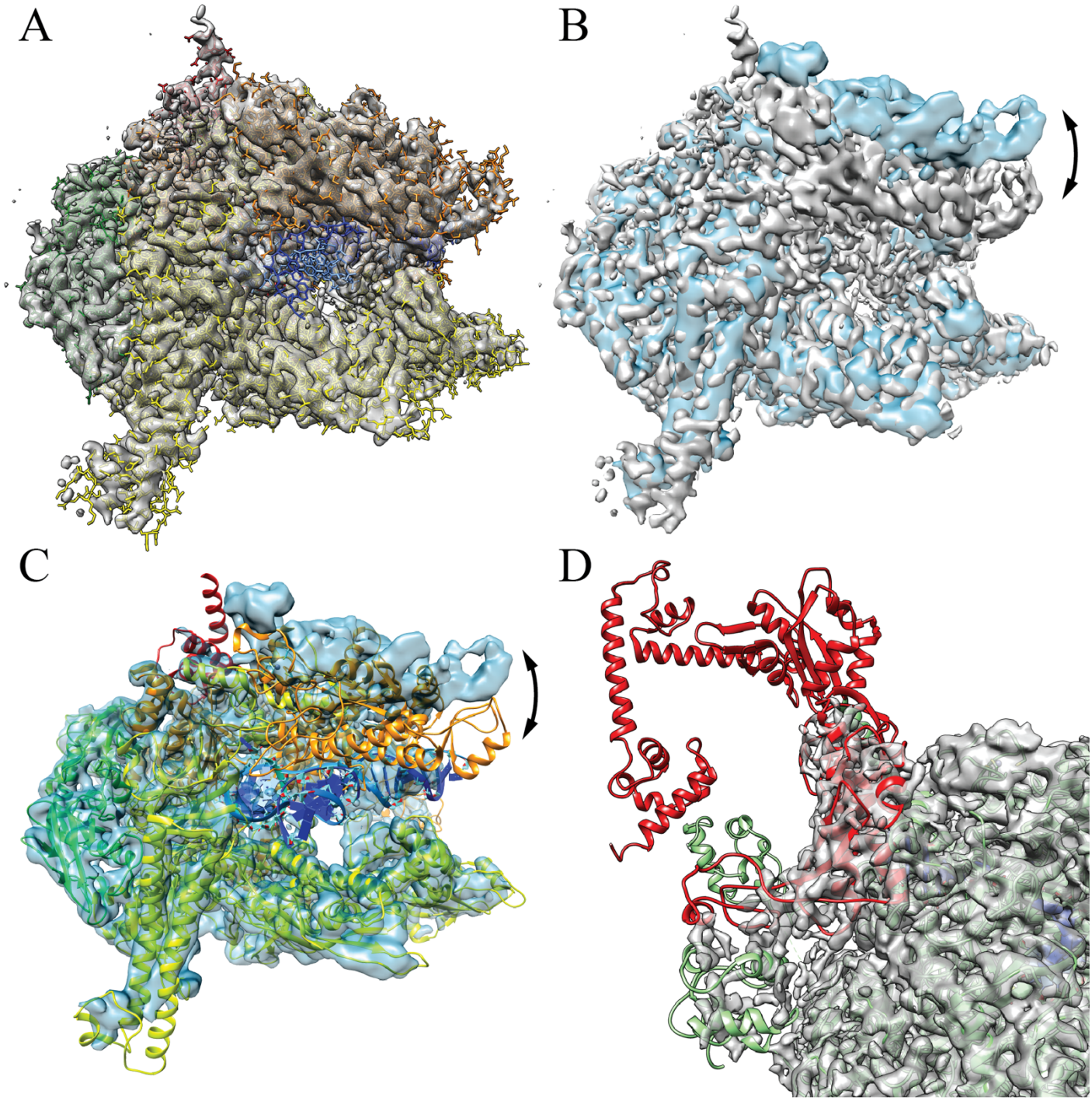

Figure 1.: Preliminary results of this dataset are as follows: 3D classification suggests the presence of two populations: The larger popularion (80,000 particles, 90% of the total particles) refines to 3.2Å nominal resolution (A). A second, rare population (8,000 particles, 10% of the total particles) refines to a nominal resolution of about 4Å. It is clear that the conformation of the rare state is different from the major state (B, arrow, compare blue vs. grey map). Likewise, a model that fits the major state, would require adjustments in a mobile RNAP domain to fit the map of the rare state (C, arrow). Density for the known ordered domain of the transcription factor is also visible (D, red). Although density for the non-coding RNA is visible (not-shown), it does not appear to adopt a defined state and we are still trying to improve the density by more extensive 3D classification.