EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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*

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

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.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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:**	**Experiment number**: MX2441
Beamline: CM01	**Date of experiment**: from: 31/08/2022 to: 02/09/2022	**Date of report**: 04/09/2023
Shifts: 6	**Local contact(s)**: Gregory EFFANTIN	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

We had 2 days on the Titan KRIOS to collect data on an RNA polymerase (RNAP) transcription termination complex trapped at a socalled intrinsic terminator. When RNAP encounters this intrinsic terminator, it undergoes a conformational change and enters a paused state before releasing the transcript. The RNA transcript forms a hairpin structure, which is critical for RNA release. Unlike previous attempts, we used a non-natural base in the DNA template to trap RNAP precisely at the termination site after it has transcribed a roughly 100 nucleotide long transcript. This approach has disadvantages and advantages. The disadvantage is a much larger sample heterogeneity (e.g. some RNAP molecules are not escaping from the promoter region and are thus not reaching the region of interest on the DNA). The advantage is that we are able to see functional states that are not observed when this type of complex is assembled directly (i.e. without prior transcription of the DNA template). As a consequence of the larger sample heterogeneity and the larger set of functional states, we require much larger datasets than usual. This allows us to classify the different functional states and still reach high-resolution.

We obtained time at the ESRF CM01 to collect one of our target complexes (*E. coli* RNAP trapped at a transcription terminator). We used UltrAuFoil grids, which were pre-screened on a Glacios microscope. We had thus a pretty good idea about the grid quality. Our local contact was Gregory EFFANTIN. We have worked with Gregory before and he helped us to collect some of our best datasets so far. This was also true for this data. Sanjay DEY, a postdoc from the lab, joined for data collection.

We collected a very large dataset of close to 15000 movies. We were able to identify several functional states and an example for a transcription termination complex is shown below.

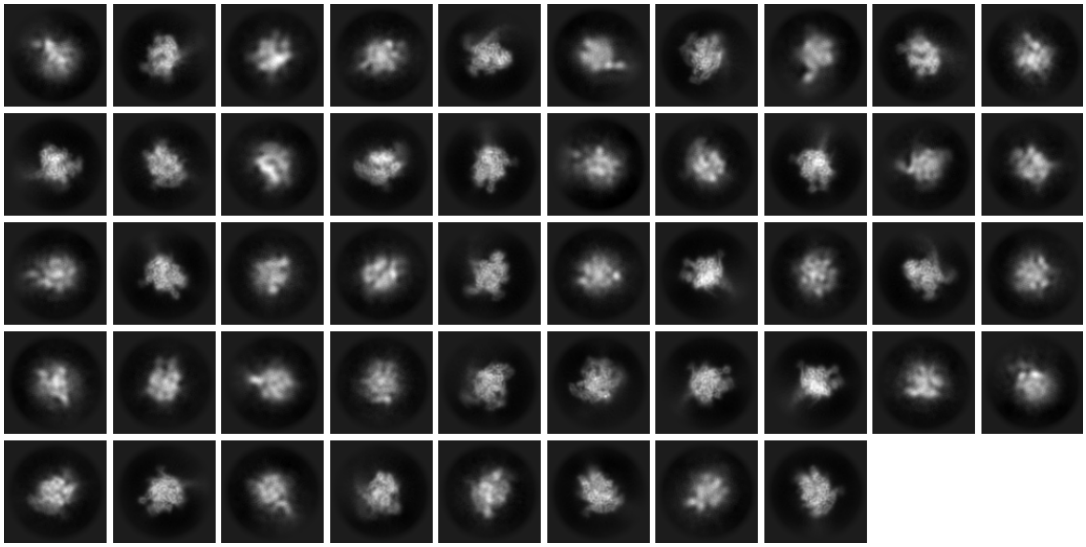

Figure 1: representative 2D classes for one of the functional states of a transcription termination complex.

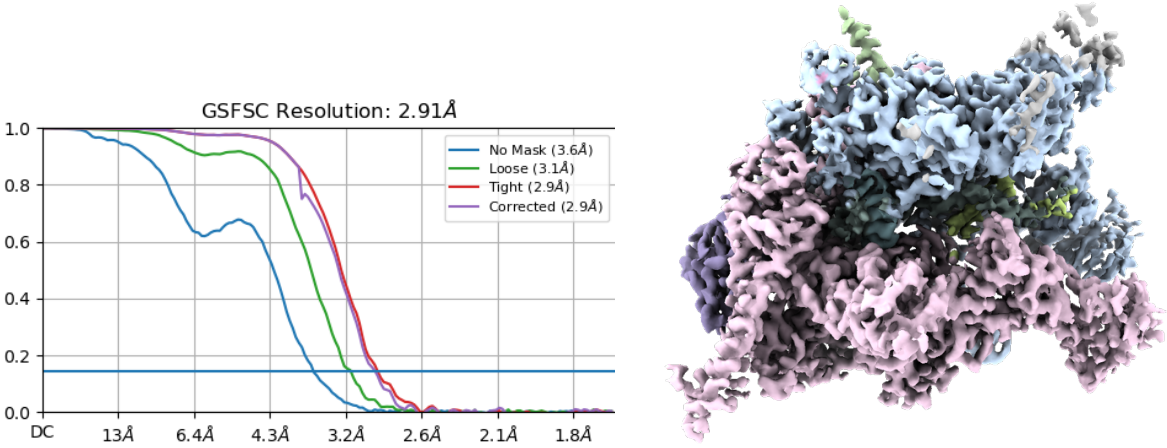

Figure 2: Nominal resolution estimate for one of the fucntional states, which represents a termination intermediate (left) and corresponding reconstruction colored by protein chains (right). We can see side chain conformations in the protein core and in ordered regions.