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: <u>https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do</u>

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 <u>each project</u> 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 : MX2367
Beamline:	Date of experiment:	Date of report:
CM01	from: 23/02/2022 to:	25/02/2022 15/07/2022
Shifts:	Local contact(s):	Received at ESRF:
9	Alessandro Grinzato	
Names and affiliations of applicants (* indicates experimentalists): Albert WEIXLBAUMER Vita VIDMAR		
Institute of IGBMC - 1 1, rue Lau BP 10142	nt of Integrated Structural Biology f Genetics and Molecular and Cellular Bio UMR 7104 - U 1258 rent Fries KIRCH CEDEX	ology
FRANCE		

Report:

We had 9 shifts on the Titan KRIOS to collect data on a functional RNA polymerase elongation complex bound to DNA Topoisomerase I. We were scheduled in February 2022 (23/02 - 25/05). Our local contact was Alessandro Grinzato.

We brought two UltrAuFoil gold grids, which were pre-screened on our Glacios microscope. The grids were shipped a week ahead of the scheduled experiment and stored at the ESRF by our local contact. Thanks to the outstanding support by our local contact we were able to select enough squares and holes and collect data that gave us more than 21.500 images over the course of the two days. We had collected on this RNA polymerase elongation complex before but needed additional data because the earlier reconstruction did not refine to high resolution and it was clear that the complex adopts multiple different conformational states. We brought two grids frozen and 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.

Update July 15th 2022:

The data has now been processed and refined by Vita Vidmar. We have several reconstructions: 1) RNAP in complex with DNA topoisomerase I at about 2.7A resolution; and 2) RNAP alone as a result of 3D classification at 2.5A resolution. The RNAP Topoisomerase I complex will play a critical role in a publication on the interplay of transcription and DNA topology. The RNAP alone, while not providing newbiological insights, is one of the highest resolution reconstruction of *E. coli* RNAP and we plan to use to provide a better refined model for the community.