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:** Molecular mechanisms underlying the prominent DNA double-strand breaks (DSBs) repair pathway, the NHEJ (non-homologous end joining)	**Experiment number**: MX2519
Beamline: CM01	**Date of experiment**: from: 20230612 to: 220230612	**Date of report**:
Shifts: 6	**Local contact(s)**: Grégory Effantin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):
Vincent MORIN, PhD student, and Paloma FERNANDEZ VARELA, Research Engineer

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Report:

Our previous request for rolling acces for cryo electron microscopy concerns a project to characterise the molecular mechanisms underlying the prominent DNA double-strand breaks (DSBs) repair pathway, the NHEJ (Non Homologous End Joining) in budding yeast *S. cerevisiae*. In this model system, we are trying to obtain a synapse complex consisting of Ku, Lif1-Dnl4 and Nej1 proteins *(Fig.1A)* along the lines of what was obtained in 2021 on the publications by Chen and Chaplin (DOI: 10.1038/s41586-021-03458-7 and DOI: 10.1016/j.molcel.2021.07.005). CryoEM tests were first carried out as part of the thesis project of Vincent MORIN, which produced encouraging initial volumes thank to the time requested at the ESRF (MX2519 session). During this session, 11,000 images were recorded then and processed by Vincent MORIN in our institute at the CEA Saclay. The results are quite satisfactory and have enabled us to characterise Ku on DNA in form of monomer or dimers, which could open up a field of study concerning the dynamics of this protein-DNA interaction. The resolutions obtained are 2.7 Å for Ku – DNA monomer *(Fig.1B)*, and lower for the dimers.

We also observed a Ku - Dnl4 complex with an acceptable resolutionof 4 Å *(Fig.1C)*. As the data set is heterogeneous *(Fig.1D)*, the aim is to continue this work by i) obtaining a homogeneous data set for Ku dimers, ii) improving the resolution for Ku - Dnl4 complex to be able to determine with certainty the precise interaction zones in the two proteins and iii) carrying out a session with the aim of obtaining the entire NHEJ synapse thanks to the biochemical improvements we have recently made using the grafix system.

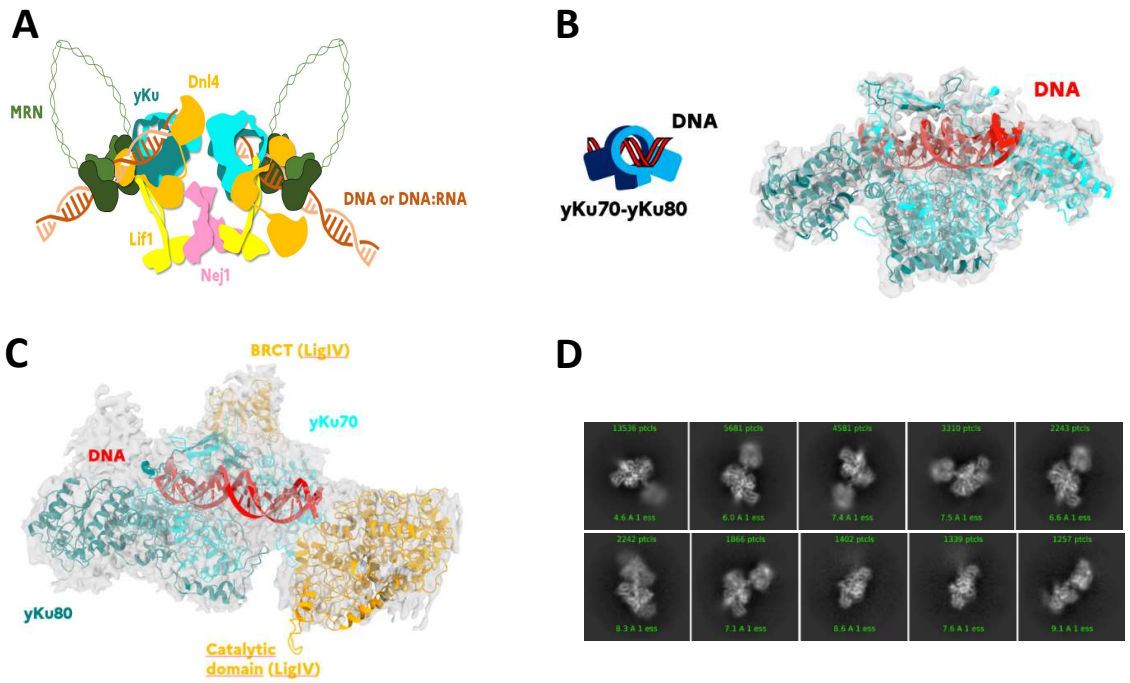

Figure 1. A) Schematic representation of a NHEJ synapse in S. cerevisiae *(role of MRN difficult to distinguish). B) Ku - DNA complex map at 2.7 Å resolution. C) Ku - DNA - Dnl4 complex map at 4 Å resolution. D) Example of 2D classes from the collected dataset*