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:** CryoEM Study of Trypsin Modulating Oostatic Factor Binding to E. coli Ribosomes	**Experiment number**: MX-2301
Beamline: CM01	**Date of experiment**: from: 20 Jul 2020 to: 22 Jul 2020	**Date of report**:
Shifts: 6	**Local contact(s)**: Michael Hons	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Anastasia Lilina*
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Report:

Allocated beamtime was used as followed: 2 hours for preparing grid atlases, 3 hours for grid screening and selecting the best grid, the remaining time was used for data collection. As a result, we were able to collect a dataset of 9830 movies for E.coli ribosome.

In total 6 grids with frozen sample were loaded into the microscope. However only grids #3 and #10 were showing good ice quality and decent particle distribution. In total 10 grid squares were selected on grid#10. Examples of a good grid square and a grid hole with particles are shown in figure 1A and 1B. The data were collected with the following parameters: magnification 165k, pixel size 0.827 Å/pixel, number of frames per movie 40, dose per movie 45.0 $e^-/Å^2$. The overall statistics for resolution and astigmatism distribution is shown in figure 1C.

Processed data were copied from ESRF server using rsync command and raw data were copied on hard drive and shipped to Leuven. During the download, we were giving priority to the pre-processed images that we could use directly for CTF estimation and particle picking. Data processing was carried at KU Leuven using CryoSparc v 2.15.

Initial processing was done using first 1700 micrographs and 190k particles were picked using blob picker. After partical inspection and several rounds of 2D classifcation, we had 93k particles suitable for 3D reconstruction. Initial map resolution was 3.0 Å. Afterwards, preliminary 3D reconstruction was used to generate 2D templates for optimized particle picking. Template picker was applied to 2556 micrographs with estimated CTF-fit resolution better than 3.5Å. In total 500k particles were picked followed by particle extraction and 2D

classification. Selected 2D classes for 3D reconstruction are shown in fig. 2A. 3D reconstruction revealed 2 classs with resolution 2.85Å and 3.01Å (fig. 2B and 2C) .

The binding site of the trypsin modulating oostatic factor is still being refined but the data look very promising and suitable for future publication.

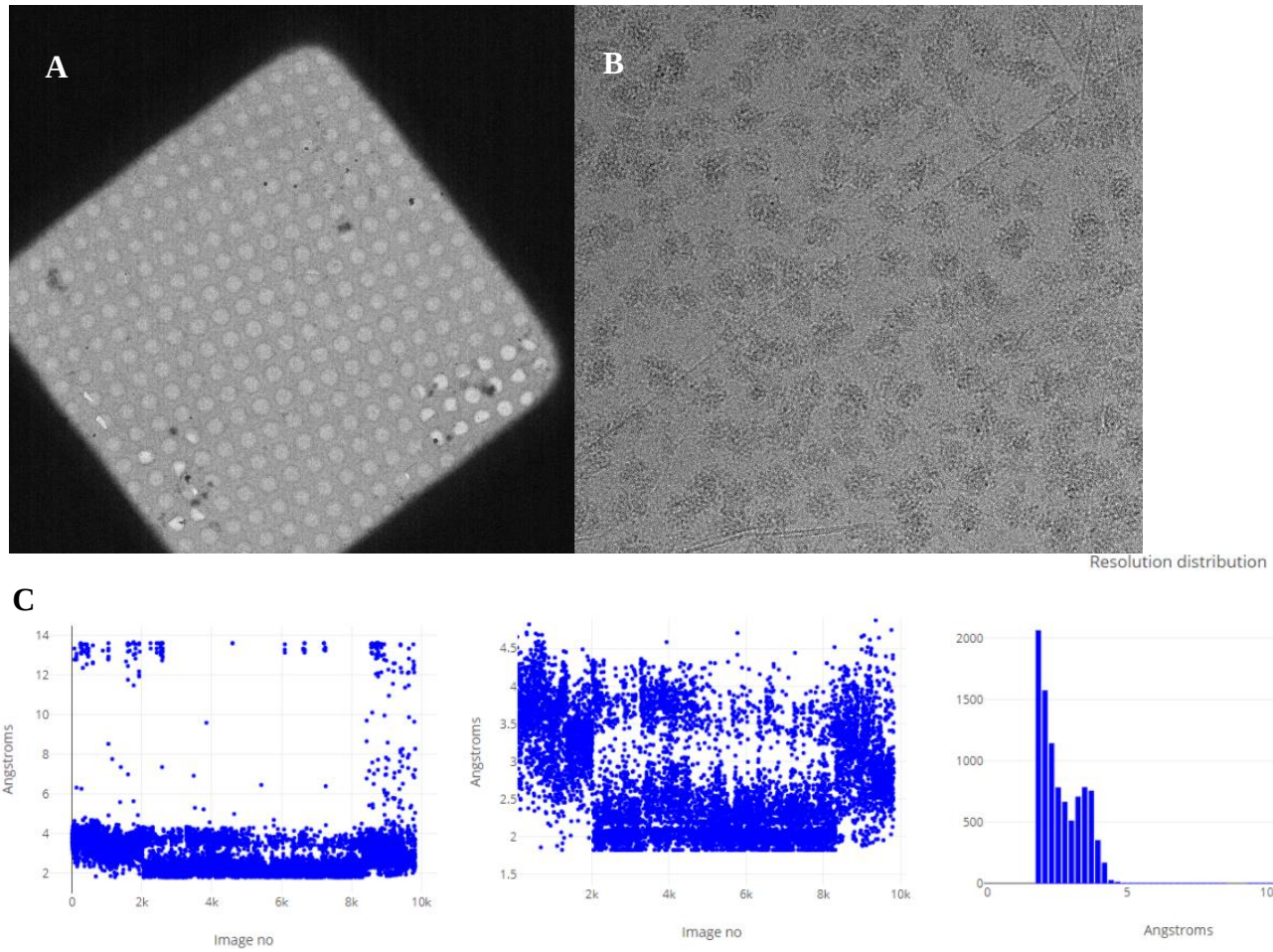

Figure 1. A. Example of selected squares for data collection. B. Carbon hole with graphene oxide containing vitrified ribosomes C. Data collection statistics.

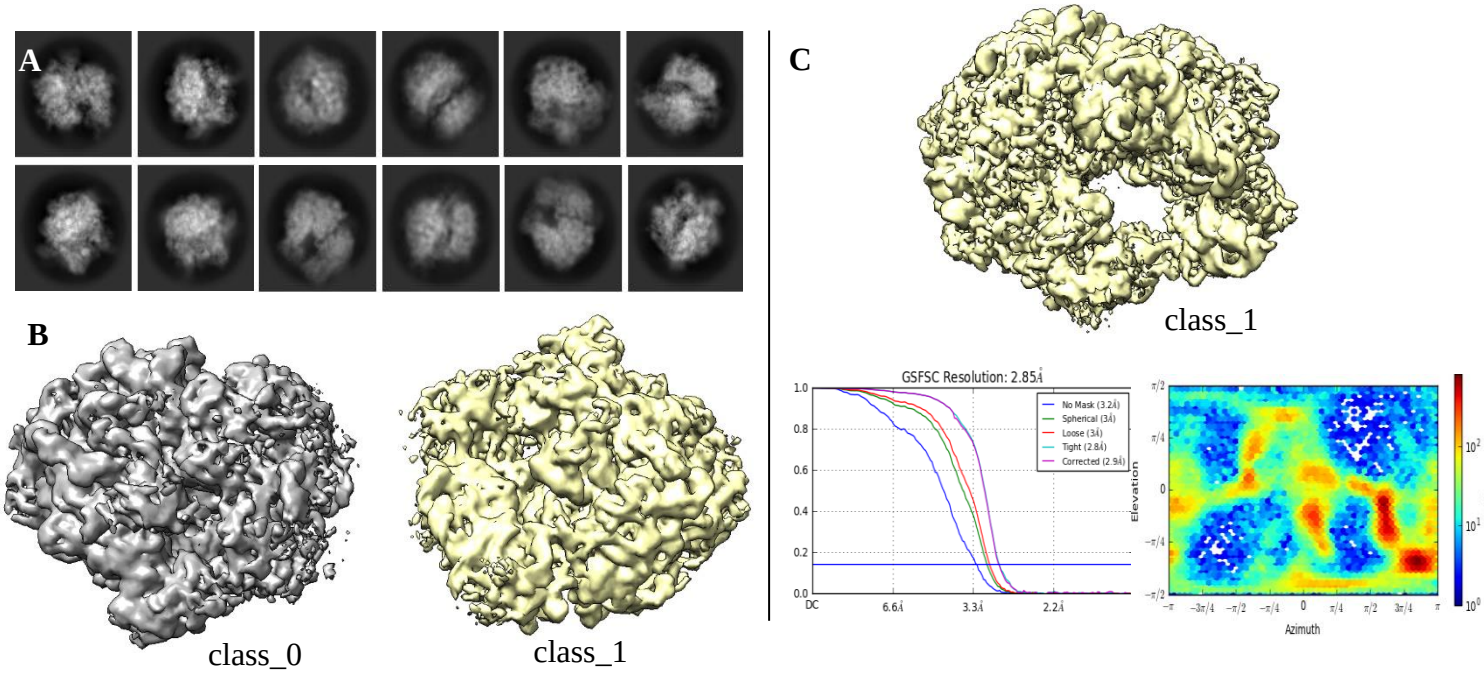

Figure 2. A. Selected 2D classes for ab-initio reconstruction. B. 3D classes obtained after ab-initio reconstruction and refinement. C. Homegeneous refinement of one of the classes obtained during ab-initio reconstruction.