EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** Mechanisms underlying kainate receptor functions	**Experiment number**: MX2241
Beamline: CM01	**Date of experiment**: from: 13th March 2020 to: 16th March 2020	**Date of report**:
Shifts: 9	**Local contact(s)**: Gregory Effantin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Janesh Kumar*

Anshul Assaiya*

***National Centre for Cell Science, S. P. Pune University, Pune-411007, India**

Report:

For the allocated beamtime at CM01 beamline, we were able to collect a large dataset (8969 movies) for ligand bound form of mutant GluK3 receptor using the Krios Cryo-Electron Microscope. With the help of the local contact (Gregory Effantin), we loaded 8 grids in the grid loader containing ligand bound protein. First we screened the prepared grids and selected the best grids with good ice thickness and particle distribution. We chose grid no.6 and marked good squares with intermittent ice thickness imaged. The following parameters were adjusted using grid hole of each square:

The grids were loaded on Titan Krios accelerated at 300keV and a total of 8969 multi-frame movies (40 frames/movie) were collected using a K2 detector with a physical pixel size of 1.052 in counting mode with a total dose of 43.72 e/Å^2. Movies were motion-corrected using UCSF Motioncorr2 (Zheng et al., 2017). The contrast transfer function of the aligned micrographs was estimated using CTFFIND4, and particles were manually picked from a few micrographs in cryoSPARCv2 (Punjani et al., 2017; Rohou and Grigorieff, 2015). The picked particles were extracted and subjected to 2D classification. Class averages with good features were selected and used to train Topaz (Bepler et al., 2019) for automated particle picking in cryoSPARCv3.1. 151525 particles were picked initially; however, after several 2D and 3D classification rounds, 56089 particles were used for final 3D reconstruction. The manuscript from this work is under preparation.