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**: MX2162
Beamline: CM01	**Date of experiment**: from: 22nd March 2019 to: 25th March 2019	**Date of report**:
Shifts: 9	**Local contact(s)**: Effantin Gregory	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Janesh Kumar*

Surbhi Dhingra*

***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 the dataset for ligand bound form of Kainate receptor using the Krios Cryo-Electron Microscope. With the help of the local contact (Dr? Effantin Gregory), we loaded 10 grids in the grid loader (prepared at National Centre for Biological Centre with Dr. Vinothkumar Kutti)and grids used in previous experiment at ESRF, MX-2120) containing ligand bound protein in different conditions. According to priority, we first tested the grids that were freshly prepared and found them with too much ice thickness and ice contamination, which could be due to shipping (it took 5 days for the dewar to reach the facility). We chose the grid #11 (same grid was used in MX-2120 for data collection) with slightly lesser ice thickness as compared to others and marked the holes in each square to be imaged. The following parameters were adjusted using grid hole of each square:

Magnification: 130,000; pixel size: 1.05Å; spot size: 5; dose rate: 7.43 e^-/p/s; total dose: 60.3e^-/Å^2; fractions (# frames): 40; exposure time: 9s; images per hole: 1; Tilt: -30°; amplitude contrast: 10%; defocus range (μm): -1.3 to -2.5; Energy filter was set to 20eV and the data was collected in super resolution counting mode. The data collection was monitored using the ExiMX interface; partial data processing (motion correction and CTF estimation) was done simultaneously as the data was collected. In addition, data collection statistics was performed by the system to measure the resolution distribution across movies, average motion per frame and astigmatism as shown in Figure 1. In total, 2121 movies were collected.

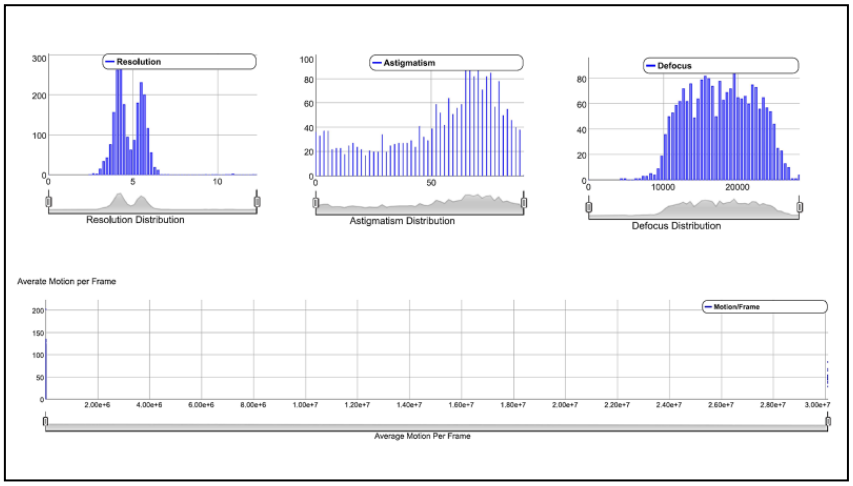

Figure1: Data collection statistics

Processed and raw data was transferred from the system to the harddisks using Rsync command line. Data processing was carried at home institute using CryoSPARC software version2.9. The motion corrected movies were imported in the software, CTF estimated and good micrographs were separated from the bad ones by checking individual micrographs. Template based picking was done from few micrographs, followed by particle extraction and 2D classification. Then, these particles were used as template to optimize the autopick and extract particles from all micrographs, and go for 2D classification. Initial processing showed that the data collection at this tilt angle (-30°) was not sufficient to obtain side views and we could observe only few titled views as well as a lot of drift even after motion correction. Furthermore, due to ice thickness of the micrographs we couldn't see much features in the later 2D classes. We could have obtained a better dataset if we were allowed to prepare fresh grids due to emergency conditions, we had the protein with us as well as the expert, but unfortunately, due to the rules and regulations of ESRF, we weren't able to do so. Hence, it would be necessary to optimize the tilt angle and collect better movies to obtain sufficient side views to build high resolution structure of kainate receptor. Figure 2 shows a glimpse of processing of movies.

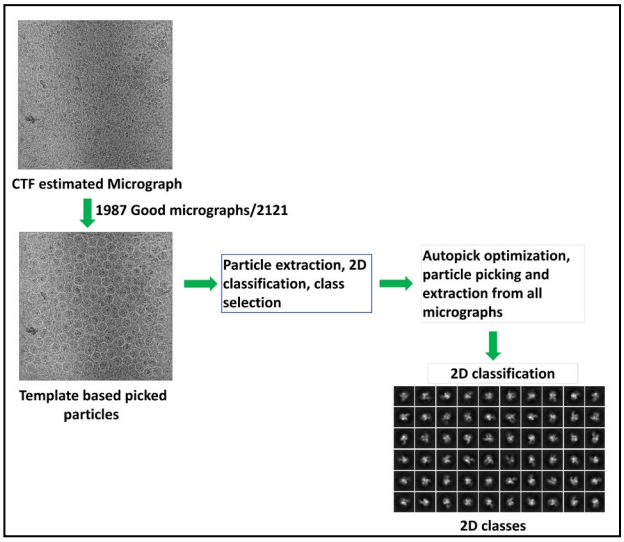

Figure2: Data processing of motion corrected and CTF estimated movies