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:** Structural basis of class C metabotropic glutamate receptor 5 signal transduction	**Experiment number**: MX-2516
Beamline: CM01	**Date of experiment**: from: 2023/03/24 to: 2002/03/26	**Date of report**: 2023/11/01
Shifts: **6**	**Local contact(s)**: Gregory Effantin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report: CONFIDENTIAL

The goal of this experiment was to collect a full data set of $mGlu_5$ receptor bound to agonist and a positive allosteric modulator. Around 20000 movies were collected at 0.84Å/pix sampling. Images were processed using cryosparc. Very good 2D classes revealing intact molecules were obtained (**Fig1**) and 3D reconstruction was obtained and refined to an overall resolution of ~3.0Å. In short , we have obtain a map that allowed to build water molecules in the large extracellular domain of the receptor, agonist in the binding site and also to analyse the stabilising effect of PAM on receptor active state. As part of this data set, we also identified population of particles visually identical to $mGlu_5$ inactive state receptor (i.e., with the TM domain separated apart) and the number of particles was sufficient for medium-resolution EM reconstruction to ~4.1Å (**Fig. 1**). This structure reveals a closed conformation (c) of the VFTs, with agonist bound, but stabilised in an inactive (R) state, which we propose to be in an intermediate-active conformation, Rcc (**Fig. 1**). Alltogether with additional $mGlu_5$ structure representing several other PAM bound conformation of the receptor, this work will make an significant impact in the field as part of the publication entitle “Conformational diversity in class C GPCR positive allosteric modulation” that has be submitted for publication and also deposited to preprint server for biology, BioRxiv.

Figure 1. 2D classification of $mGlu_5$ receptor bound to agonist and positive allosteric modulator (A), and for intermediate active state Rcc (D). CryoEM maps coloured according to the local resolution plot of Full-lenght $mGlu_5$ as determined with Relion 4.0 are shown in two different views for agonist- and PAM bound state (B) and quisqualate-bound intermediate active state Rcc (E). Corresponding Fourier shell correlation (FSC) curves of two half-maps with mask (blue) and the map and model (red) are shown (C and F).