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

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:** Structural Studies on $GABA_A$ Receptors	**Experiment number**: MX1782
Beamline: ID23-2 ID23-1 ID30B	**Date of experiment**: from: 03/16 to: 06/16	**Date of report**:
Shifts:	**Local contact(s)**:	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Duncan Laverty*

Trevor Smart

Report:

ID23-2 5April 2016

The objective for this trip was to assess the diffraction limit for crystals of a eukaryotic ion channel following crystal growth optimization experiments. Previous diffraction experiments of crystal hits from intial sparse matrix screens had revealed diffraction extending beyond 4 angstrom, yet exhibiting susceptibility to radiation damage (preventing collection of complete datasets). Subsequent crystal optimization screens had yielded reproducible crystallization conditions, with many crystals of favorable morphology cryo-protected for this experimental session.

During the experimental session approximately 50 crystals were screened, with datasets collected for those exhibting strongest diffraction. Approximately 15 single crystal data sets ranging from 3.3-4 angstrom were collected. Autoprocessing of data at the beamline revealed a high degree of completeness for the collected data. The work carried out during this beamtime subsequently enabled us to determine, for the first time, the structure of a chimeric eukaryotic ion channel. This had formed a long-term goal of our work, and represents a significant breakthrough within the fields of ion channel structure and, more broadly, neurobiology. This work is currently in final stages of preparation for publication.

Additionally, invaluable information obtained during this session regarding the reproducibity of crystal diffraction has assisted subsequent efforts to obtain ligand-bound forms for this ion channel.

ID23-1 09May2016

During this session, diffraction data was collected for approximately 10 crystals of a eukaryotic ion channel. Reproducible diffraction (extending to ~3 angstrom) of 'optimized' crystals had been confimred in previous experimental sessions. Following structure determination of the 'apo' form of the ion channel (using diffraction data collected on beamline ID23-2), the objective for this session was to collect data for ligand bound forms of the ion channel in order to determine the molecular determinants of binding for allosteric modulators.

Efforts to collect data during this session were hampered by beamline issues affecting both on-site and remote data collection. We were however able to collect low resoltuion data sets for crystals of a ligand-bound form as well as for a different crystal form. This data has assisted in enabling us to determine, for the first time, the structure of an ion channel in complex with a specific endogenous positive allosteric modulator. In combination with data from previous experimental sessions, this work forms part of a manuscipt in the final stages of preparation for publication.

ID30B 11June 2016

During this session, diffraction data was collected for approximately 10 crystals of a eukaryotic ion channel in complex with a novel brominated-compound. Previous efforts to co-crystallize and determine the structure of an ion channel in complex with a specific inhibitory modulatory compound had been hampered by weak signal for bound ligand, preventing unambiguous positioning of ligand in electron density maps. A brominated-derivative was synthezised, and data collected at the absorption edge for bromine to assist in ligand identification and positioning in density maps. Crystals were screened for diffraction and data sets collected for those exhibting strongest diffraction. Despite the collection of data sets of good completeness, efforts to identify bound ligand remain ambiguous (potentially due to the presence of impurities in the chemically synthesized brominated-derivative compound).