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:** Development of Serial Crystallography with hard X-ray nano-beams	**Experiment number**: LS2384
Beamline: ID13	**Date of experiment**: from: 05.12.2014 to: 07.12.2014	**Date of report**:
Shifts: 6	**Local contact(s)**: Manfred Burghammer (email: burgham@esrf.fr)	*Received at ESRF:*

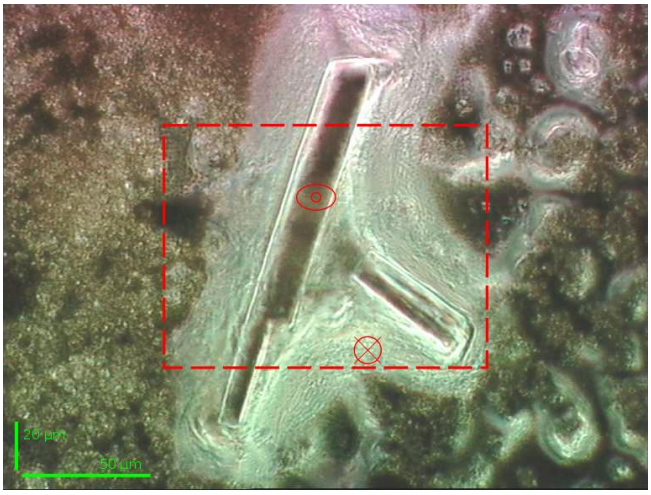
Names and affiliations of applicants (* indicates experimentalists):

A. Shilova[1],
[1] European Synchrotron Radiation Facility, Grenoble, France

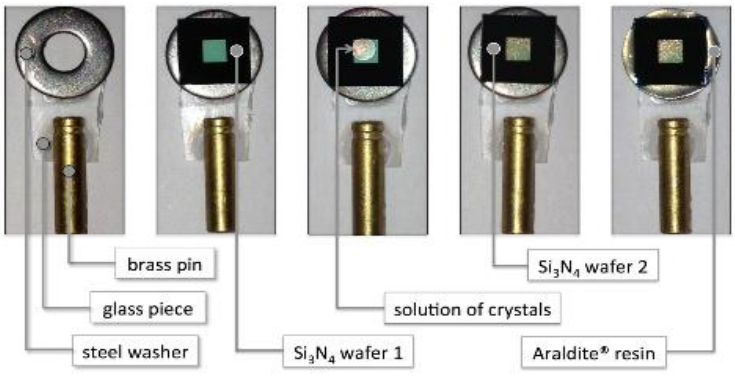
A. Woznicka, H. Nury, Ravaud Stephanie, E. Pebay-Peyroula[2]
[2] Institut de la Biologie Structurale Grenoble IBS, France.

Report:

Samples: Lysozyme micro-crystals were used several months in advance before the allocated shifts to optimize the setup and verify the method, i.e. to calibrate exposure and radiation damage. The main part of the experiment was focused on microcrystal ensembles of wild-type 5-HT3 receptor [1], which was grown at the IBS (Institut de Biologie Structurale, Grenoble, France). The size of the crystals were in the interval from 30 to 250 µm (Pic.1).



Pic.1 Crystals of 5-HT3 receptor membrane protein

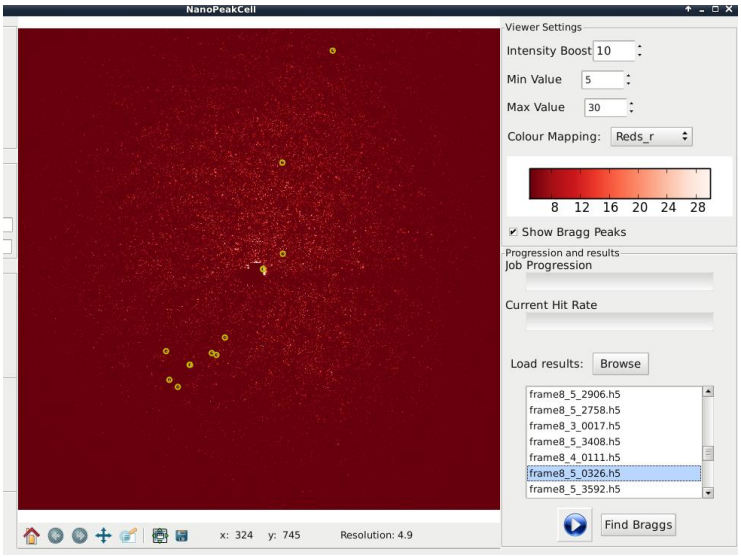


Pic.2 Sample preparation

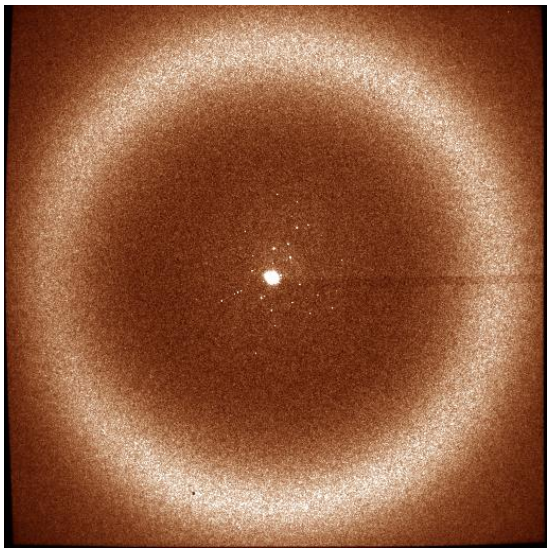
Method: 5HT3 receptor membrane protein was purified according to following steps: Detergent→Strep column→Trypsin digestion→PNGase digestion→SEC column. Protein

was concentrated up to 7-8 mg/ml, mixed with antibody and Cymal6 (for crystallization trials). Crystallization plates were set up by using vapour diffusion method. Samples were dispersed on silicon-nitride membranes with solid state support (Pic.2). These membranes supposed to be mounted into humidity controlled cells as windows, but we decided for the first attempt to do an experiment without humidity control chamber, due to the conditions. Experiment has been done at microfocus branch of ID13 with $2*10^{11}$ photons/sec micro-beam of 1 µm. Diffraction patterns was recorded with a 2-D detector (Frelon 4M 2kx2k detector).

Data analysis and final resolution: Data analysis were processed by using Crystfell[2] and Nanopeakcell[3]**.** We collected more than 100 000 images, of which less than 1000 were classified as hits using the Nanopeakcell program, giving an average hit rate of 0.8% (Pic.3). This conventionally collected data set has the best resolution of 4.9 A°. Nevertheless, these data was not enough to solve the structure of the receptor for this time (Pic.4).



Pic.3 Data analysis with Nanopeakcell.



Pic.4 Diffraction picture of 5-HT3 receptor.

However, we have 12 allocated shifts for the next round for this project and we are planning to use a humidity control chamber to improve the scattering contrast and to reduce the background. In addition, new EIGER 4M detector will give us new opportunity to improve resolution of the diffraction reflections and to increase speed of the data collection.

References:

[1] X-ray structure of the mouse serotonin 5-HT3 receptor Cherici Hassaine et all., Nature 512, 276-281 (2014)

[2] White, T. A. *et al.* CrystFEL: a software suite for snapshot serial crystallography. *J Appl. Crystallogr.* 45, 335–341 (2012).

[3] Coquelle N. et all Raster-scanning protein crystallography using micro and nano-focused synchrotron beams