EUROPEAN SYNCHROTRON RADIATION FACILITY

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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**: LS2409
Beamline: ID13	**Date of experiment**: from: 11.05.2015 to: 15.05.2015 from: 27.06.2015 to: 29.06.2015	**Date of report**: 01.09.2015
Shifts: 24	**Local contact(s)**: Manfred Burghammer (email: burgham@esrf.fr) Martin Rosenthal	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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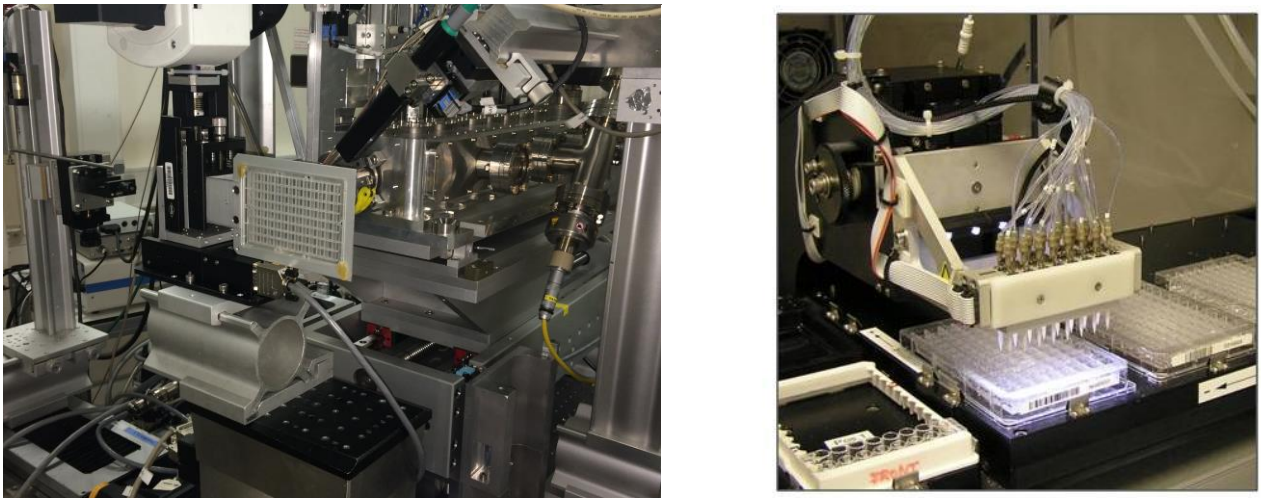
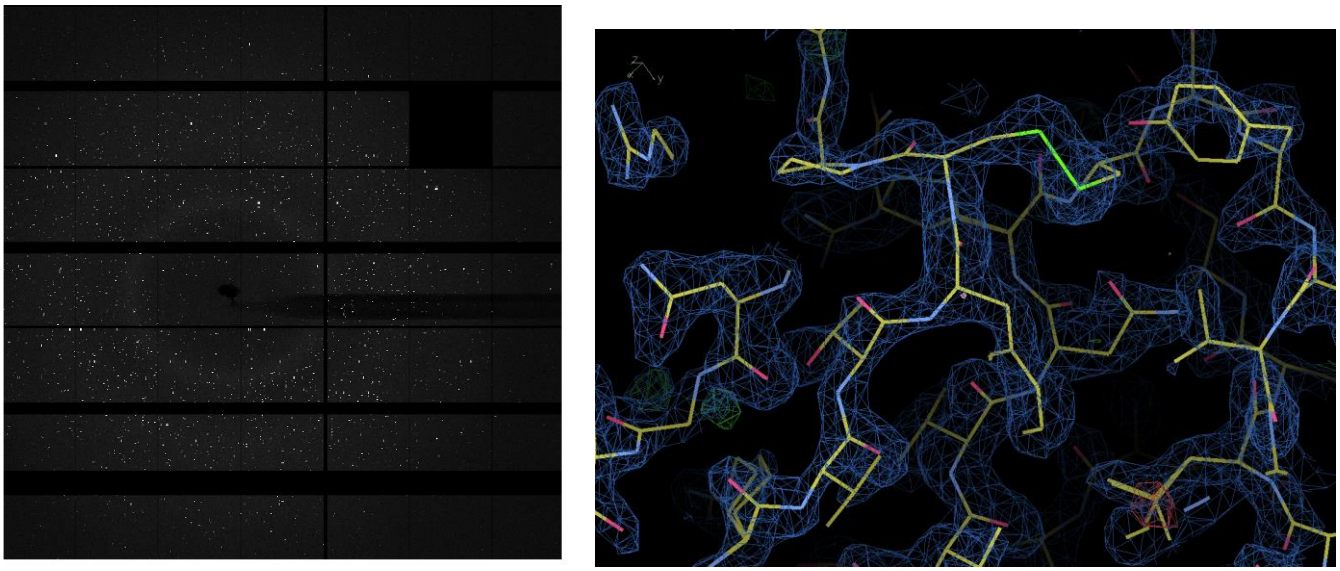
Report: The aim of the proposal was to develop serial diffraction methods, which are complementary to efforts currently taken at hard X-ray free electron laser (FEL) facilities. In previous proposals, we already succeeded with solid support crystal mounting techniques [1], LCP-injectors [2] and for now we decided to collect the data using special crystallization plates (Pic.1) that were created at HTX-lab in EMBL, Grenoble [3]. This system is based on a new crystallization plate that allows growing crystals on very thin films that can then be excised with a laser beam to recover the crystalline material. Due to their design, CrystalDirect plates allow to collect diffraction data in-situ with very low background. CrystalDirect systems offer a lot of advantages, like absence of the mechanical stress for the crystals (as no tools enter the crystallization drop), full automation of the crystal-harvesting process (Pic.1), availability to know in advance all positions of the crystals, systematic testing the large numbers of crystals.



Pic.1 Set up of the CrystalDirect plates at ID13 (left), Cartesian Robot (right)

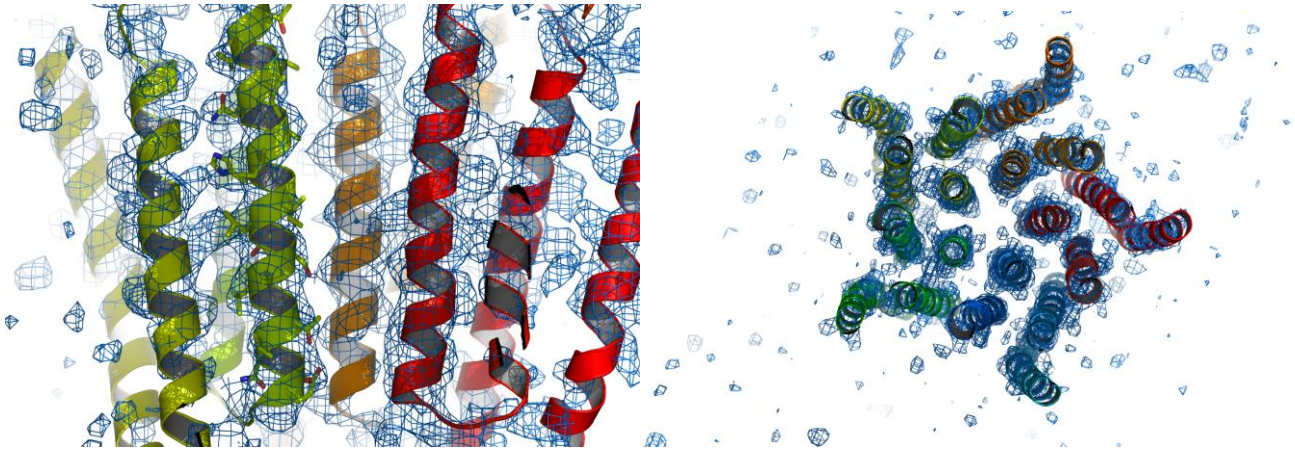
First test was performed with the model protein Thaumatin (Pic.1). The collection of the data was very fast due to the great equipment of the ID13 beamline, for instance, new ultra-fast EIGER 4M detector and the computational infrastructure that can process the large volume of data. We just needed several hours to collect 400 000 frames in room temperature, of which around 12000 were indexed. The structure was solved using Nanopeakcell [1] and CrystFEL[4] software, the best resolution was 1.9 A ° (Pic.2).



Pic.2 Maximum projection of the one of the dataset of the model protein Thaumatin (left), Refinement of the Thaumatin till 1.9 A°, using coot (right)

Structure determination of membrane proteins is one of the most critical steps to be mastered before achieving breakthroughs in life science and medicine (for now only 551 structures of the membrane proteins has been solved), it is also a notoriously difficult and time-consuming procedure. We performed first test with

CrystalDirect plates in room temperature with the membrane protein Glic, which structure has never been solved in room-temperature before. We collected around 1 400 000 frames, of which around 8000 were indexed. This conventionally collected data set has the best resolution of 3.9 A°. The current preliminary dataset is promising, although more frames would be needed, the maps show clearly the positions of the helices (unchanged compared to the structure at cryo temperature) and even some side chain positions (side view image).



Pic.3 Side view of the membrane protein Glic (left), top view (right).

With CristalDirect plates it is easy to understand from the beginning of the experiment which crystallization conditions are better to use and what size of the crystal will give the best diffraction. There is no special sample preparation, changing of the plates is absolutely not time consuming. In 96 wells it is possible to set up different concentration of the protein or even different crystallization conditions due to the full automation of the crystal-harvesting process. The film, which cover plates almost doesn't have any background and in future we are planning to test new films of different materials.

The next steps are to improve resolution for Glic (using improvement of the crystallization conditions) and to collect a dataset for the 5-HT3 receptor, which has a high scientific impact, because these receptors are ion channels that mediate fast neurotransmission and they are first-class therapeutic targets for a number of conditions (such as depression, neurodegenerative diseases, nausea, and irritable bowel syndrome).

References:

[1] N.Coquelle et all. Raster-scanning serial protein crystallography using micro- and nano-focused synchrotron beams Acta Crystallogr D 2015 May 1; 71(Pt 5): 1184–1196

[2] P.Nogly et all. Lipidic cubic phase serial millisecond crystallography using synchrotron radiation DOI: 10.1107/S2052252514026487

[3] Marquez, J. A., and Cipriani, F. (2014) CrystalDirect: a novel approach for automated crystal harvesting based on photoablation of thin films. Methods in molecular biology 1091, 197-203

[4] White, A., R. A. Kirian, Martin, A.V.,Aquila, A.et al.,. J. Appl. Cryst.(2012) 45, 335–341