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:** Lead optimisation and structure-guided design of a new chemical series of ethionamide booster for tuberculosis combination therapy	**Experiment number**: MX1866
Beamline: ID30A-1	**Date of experiment**: from: 21-10-2016 to: 22-10-2016	**Date of report**:
Shifts: 3	**Local contact(s)**: Matthew Bowler	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

René Wintjens, Université Libre de Bruxelles, Belgium

Alexandre Wohlkönig, Vrij Universiteit Brussels, Belgium

Alain Baulard, Pasteur Institute of Lille, France

Report: In this first run, 50 crystals were tested and about 40 data were collected. Most crystals (29) were part of the main project (structure-guided design of ethionamide booster), while 21 crystals were of three side-projects (more details described below).

ESRF	**Experiment title:** Lead optimisation and structure-guided design of a new chemical series of ethionamide booster for tuberculosis combination therapy	**Experiment number**: MX1866
Beamline: ID30A-1	**Date of experiment**: from: 13-12-2016 to: 15-12-2016	**Date of report**:
Shifts: 3	**Local contact(s)**: Matthew Bowler	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

René Wintjens, Université Libre de Bruxelles, Belgium

Alexandre Wohlkönig, Vrij Universiteit Brussels, Belgium

Alain Baulard, Pasteur Institute of Lille, France

Report: From the 50 crystals sent, 36 complete data were acquired (details here below).

ESRF	**Experiment title:** Lead optimisation and structure-guided design of a new chemical series of ethionamide booster for tuberculosis combination therapy	**Experiment number**:
Beamline: ID30-A	**Date of experiment**: from: 05-03-2017 to: 07-03-2017	**Date of report**:
Shifts: 3	**Local contact(s)**: Matthew Bowler	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

René Wintjens, Université Libre de Bruxelles, Belgium

Alexandre Wohlkönig, Vrij Universiteit Brussels, Belgium

Alain Baulard, Pasteur Institute of Lille, France

Report: 27 complete data were collected (details below).

General report:

Objectives:

Samples from four different projects were investigated during the four MX1866 runs.

● **1.** Structure-based drug design of EthR2 chemical modulators (main project): Recently, we have discovered that inhibition of the transcriptional repressor EthR2 leads to the awakening of a new ethionamide bio-activation pathway and to the reverting of the acquired and innate ethionamide-resistance. We are currently developing EthR2-inhibitory compounds to drive medicinal chemistry towards new EthR2 modulators for using in combination therapy against tuberculosis.

● **2.** Structural investigation of the interaction between *Mycobacterium tuberculosis* MabA (FabG1) and a series of drug candidates: we have been trying to get crystal structures of MabA protein in complex with several new inhibitors. This protein represents a target of choice to a drug discovery program against tuberculosis.

● **3.** Crystal structure of lipY, a putative drug target of *Mycobacterium tuberculosis*: solving the structure of M. tuberculosis lipY should pave the way for starting a structure-based drug design program.

● **4.** Molecular mechanisms of the yeast Mep2 ammonium transport: optimal production conditions have been developed to obtain the Mep2 membrane protein in fully-active form. We would like to solve the crystal structure of an active form, that should provide important insights into the transport mechanism.

Results and the conclusions of the study:

● **1.** EthR2: the two first runs (21-10-2016 and 14-12-2016) yielded few exploitable results. All the resolved EthR2 crystal structures showed only weak occupancy for ligands. Note that co-crystals were obtained by soaking. In the third run (05-03-2017) we tested several EthR2 crystals obtained by different soaking procedures using the same ligand, thereby allowing to determine the optimal condition of soaking. Data collected confirmed the effectiveness of our soaking procedure and after structure determination by molecular replacement and the initial refinement steps, we are sure to have at least 5 high-resolution EthR2 structures in complex with 5 different chemical modulators (resolution ranged from 1.81 to 2.10 Å). In parallel, we have developed a procedure to obtain by co-crystallization EthR2 in complex with SMARt-420, the first ligand studied and published. This structure was resolved at 2.00 Å. In summary, the 6 EthR2 complex structures resolved here will be presented and described in details in two future publications, with coordinates and structure factors deposited to the protein databank.

● **2.** MabA: about 35 crystals were tested and 25 data were collected. More than 20 structures were solved with resolution ranging from 1.63 Å to 2.95 Å. However, no ligand, substrate or co-factor was present in obtained structures. Thus, despite all the efforts (co-crystallization, soaking, screening in presence of ligand, temperature, guanidinium chloride, etc), it seems evident therefore that MabA protein is reluctant to form crystals containing any ligand, such as substrate, co-factor or synthetic inhibitor.

● **3.** lipY: 12 crystals were tested and 4 data were acquired. We failed to solve the structure by molecular replacement. We tried I-SAD (one data) and Hg-SAD (one data) phasing, but without any success, probably due to the fixed wavelength of ID30-A (12.8 keV).

● **4.** Mep2: 39 different crystals were tried. Most of them gave no diffraction. Only one incomplete data on run of 05-03-2017 could be processed correctly, giving by molecular replacement a structure solved at 4.65 Å. Currently, we are fine screening the initial crystallographic condition to improve Mep2 crystal quality.