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:** High-throughput screening campaign of inhibitors targeting the *Mycobacterium tuberculosis* repressor EthR2	**Experiment number**: MX1960
Beamline: ID30A-1	**Date of experiment**: from: 10-26-2017 to: 10-27-2017	**Date of report**: 01-18-2018
Shifts: 1	**Local contact(s)**: Didier Nurizzo	*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:** High-throughput screening campaign of inhibitors targeting the *Mycobacterium tuberculosis* repressor EthR2	**Experiment number**: MX1960
Beamline: ID30A-1	**Date of experiment**: from: 11-26-2017 to: 11-27-2017	**Date of report**: 01-18-2018
Shifts: 1	**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: Fifty crystals were tested and about 40 data were collected. Most crystals were part of the main project (structure-guided design of ethionamide booster), while other ones were of three side projects (more details described below).

General report:

Objectives:

Samples from four different projects were investigated during the two series of measurements (the two MX1960 shifts).

● **1.** High-throughput screening campaign of inhibitor targeting the Mycobacterium tuberculoisi repressor EthR2.

We have previously showed that manipulating the transcriptome of *Mycobacterium tuberculosis* (*Mtb*) using small molecules as modulators of the repressor EthR has the potential to increase the therapeutic index of the second line drug ethionamide (ETH). Recently, we have expanded this concept and discovered that inhibition of a second transcriptional repressor, EthR2, leads to the awakening of a new ETH bio-activation pathway and to the reverting of the acquired and innate ETH-resistance. We are currently developing EthR2-inhibitory compounds. The aim of the two MASSIF-1 shifts is to collect a large number of datasets of EthR2/inhibitor to drive medicinal chemistry towards new EthR2 modulators for a combination therapy against *Mtb*.

● **2.** Structural investigation of the interaction between *Mycobacterium tuberculosis* MabA (FabG1) and a series of drug candidates.

We are 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.** Structural investigation of a *Mycobacterium tuberculosis* target protein not yet structurally studies (starting project).

We have over-expressed and purified an essential *M. tuberculosis* protein not yet studied by X-ray or NMR, in order to develop a structure-based design program targeting this protein.

● **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 experiments:

● **1.** EthR2.

More than 50 structures in complex with different inhibitors were solved and refined at high resolution ranged from 1.3 to 2.5 Å. As a result, the bound ligands were perfectly well defined in the electron densities, allowing reliable modeling and robust analysis of the protein-ligand interactions. Clearly, the objectives of the experiment were fully achieved. The obtained structures will be described in at least four papers, that are being written (expected publication in 2018).

● **2.** MabA.

Twelve crystals were tested and only four diffraction data were collected. However, the crystal structures were solved and refined at only low resolution, and even more critical, we

failed again to obtain structures containing ligands. In conclusion, after all our attempts, we decided to stop trying to determine the MabA structure in complex with small compounds or ligands.

● **3.** Starting project.

Eigth crystals obtained from our initial crystallographic screens have been tested durng the second shift MX1960. Only two crystals gave diffraction but the inferred unit cell parameters indicated that these were crystals of small compounds. Further crystallographic screenings are currently in progress.

● **4.** Mep2.

Nine crystals were tested and only 3 diffraction data was acquired. Unfortunately, the solved structures turned out to be the structures of a yeast contaninant protein. We are continuing to focus our efforts on improving Mep2 protein production and purification.