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:** Structural Biology of an Enterobacterial Exopolysaccharide Secretion System	**Experiment number**: MX-2159
Beamline: CM01	**Date of experiment**: from: 18.01.2019 to: 21.01.2019	**Date of report**: 04.02.2019
Shifts: 9	**Local contact(s)**: KANDIAH Eaazhisai	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Bacterial cellulose, or nanocellulose, is an important matrix component in the biofilms of many free-living and clinically important bacterial species. *E. coli* and many other b- and g-proteobacteria share conserved multi- component *bcs* operons for cellulose secretion, where the encoded subunits are proposed to assemble in sophisticated secretory nanomachines. We recently reported a low-resolution structure of a multi-component Bcs macrocomplex encompassing most of the inner-membrane and cytosolic components (Krasteva *et al.*, *Nature Communications* 2017 (1)) and are currently aiming to obtain high-resolution cryo-EM data on the same sample. We collected a first dataset on the CM01 at the ESRF in January 2019. The purified Bcs macrocomplex was flash frozen on Quantifoil R 1.2/1.3 grids and the freezing conditions were optimized by initial screening on a Talos Arctica microscope at the IECB Bordeaux. The optimal freezing conditions were reproduced for data collection on the CM01 and a total of 4093 movies were collected in electron counting mode using an energy filter. We collected 3 movies per grid hole using a nominal magnification of 130 000 x, a pixel size of 1.05248 A^2, and a range of defocus values between 0.8 and 2.8 microns as set in EPU. The data was pre-processed using MotionCorr 2 and Gctf for motion correction and CTF estimation, respectively. Overall the data was of good quality, with an average resolution per micrograph at about 3.1A, going as high as 2.3A and with only a fraction of micrographs having an estimated resolution of about 4A. We obtained a preliminary structure reconstruction with an overall resolution of about 4A, pending particle polishing, movie and multi-body refinement. Nevertheless, the particles and resultant 3D reconstruction were characterized by significant gradient of estimated local resolution, with very limited resolution for the transmembrane and cytosolic domains of the macrocomplex (likely due to the presence of a detergent micelle, partial complex dissociation and/or conformational flexibility or variability). As we could only select between 30-50 good particles per frame, the total dataset was relatively small in particle count and 3D classification did not yield enriched enough classes to increase the local resolution for these regions. We will therefore aim to increase the overall particle count by collecting an additional dataset at the same microscope settings in order to be able to merge the data and reliably reclassify and refine the 3D structure reconstruction(s).