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.

	Experiment title: The S. aureus aberrant large ribosomal subunit with bL27 N-terminal attached to catalytically inactive Prp cysteine protease.	**Experiment number**: mx-2006

Beamline: CM01	**Date of experiment**: from: 26.1.18 to: 29.1.18	**Date of report**: 13.3.18
Shifts: 9	**Local contact(s)**: HONS Michael	*Received at ESRF:*

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Report:

Ribosomal protein bL27 is a component of the large ribosomal subunit found only in eubacteria and in the ribosomes of mitochondria and chloroplasts. The bL27 N-terminus protrudes into the PTC and has been shown to play a critical role in substrate stabilization during protein synthesis [4]. The bL27 of *S. aureus* (and other Firmicutes) is encoded with an N-terminal extension (9 amino acids longer, compared to *E. coli* bL27 sequence) that is not present in most Gram-negative organisms, and is absent from mature ribosomes. This extension is not present in all Gram-positive bacteria. Pathogens of varying lineages with N-terminally extended bL27 include *Enterococcus fecaelis, Bacillus anthracis, Mycoplasma pneumoniae, Streptococcus pneumoniae, Sneathia aminii and Fusobacterium nucleatum*.

Ribosomal protein bL27 is encoded by rpmA gene, its deletion in *E. coli* led not only to a severe growth defect but also harmed the peptidyl transferase activity [2] probably due to incomplete assembly of the large ribosomal subunit, indicating a pivotal role for bL27 activity [3]. A specific cysteine protease (Prp) that performs post-translational cleavage of bL27 in a sequence specific manner has been identified [4] in bacteria containing the bL27 N-terminal extension. In S. aureus, both bL27 and Prp have been shown to be essential [4].

Our collaborators, Prof. Gail Christie (Virginia Commonwealth University School of Medicine, Virginia, USA) have demonstrated, in unpublished work, by complementation analysis that not only bL27 is essential, but its cleavage is essential for cell growth. Therefore, the action of Prp is a bona fide novel drug target. The decrease of growth in these strains indicates that the ribosomal particles they produce are probably not active, therefore should shed more light on the role of bL27 in assembly. bL27 is known to be one of the last proteins

that join the large subunit assembly process [5]. However, little is known about its final processing for its ribosome incorporation.

Prp provides a target for the development of antibiotics specific to *S. aureus* and other related Firmicute pathogens in which this specific bL27 processing occurs. The structure of Prp bound to its substrate is not known, and that structure is the most desirable one for structure based drug design. The existing crystal structure of *S. aureus* Prp (PDB ID: 4PEO) lacks the loop that includes the active site residues histidine 22 and cysteine 34. Structural modeling based on functional analysis of the Prp enzyme suggests rearrangement of the flexible loop upon binding of the correct peptide substrate is required for the active site to assume the proper conformation. [45].

Results:

S. aureus with full length bL27 and a plasmid with catalytically inactive Prp mutant C34A were grown (limited by 3-4 doubling times of the bacteria because they rely on existing healthy ribosomes to continue growth). The inactive Prp mutant C34A which binds to un-cleaved bL27 before or during assembly, was over expressed. This binding causes a build-up of pre-50S (aberrant) particles that are highly toxic to the cell and cannot form 70S [4]. Ribosome were purified using a sucrose gradient 10%-40% with high Mg^{2+}concentration, the "healthy" 50S paired with the 30S to form 70S, so the aberrant 50S with attached Prp will be separated.

Full length bL27 rProtein, with un-cleaved 9 amino acids at its' N-terminal, as well as Prp enzyme C34A mutant were detected by LC-MS/MS in the aberrant 50S particle sample.

The presence of full length bL27 rProtein, with un-cleaved 9 amino acids at its' N-terminal and Prp enzyme in the aberrant 50S particles peak was also examined by Western blot, using polyclonal antibodies . bL27 was detected in aberrant particles as a doublet that appears to contain large amounts of un-cleaved bL27 and only a small amount of the cleaved form. In addition, Prp enzyme was detected by immunoblot in the aberrant 50S particles sample.

Cryo-EM grids (Quantifoil 1.2/1.3) of the aberrant 50S sample were prepared from solutions of concentration of 1mg/ml. Data were collected on the Titan Krios FEI operating at 300 kV acceleration voltage at CM01 beamline, Grenoble, France and at a nominal underfocus of $\Delta z = (-0.6)- (-1.5)$ μm using K2 GATAN camera and automated data collection with EPU software. The camera was calibrated at nominal magnification of 130K× resulting in 1.067 Å pixel size at the specimen level. The camera was set up to collect 20 frames, total exposure time was was 8sec with a dose of 23 ē Å −2 (where ē specifies electrons).

Data processing was done using RELION 2.1 [6]. 3,234 movies (**Fig. 1**) were aligned by the whole image motion correction method. the contrast transfer function of every image was determined using CTFFIND4.1 in the RELION 2.1 workflow. Particle auto picking was done with a template (from manual particle picking) and about 500,000 particles were picked. Particles were extracted with a box size of 400 × 400 pixels. Owing to unexpected problems in 2D classification, so far we were not able to fully process the collected data Currently, we are making efforts to understand the source of the problem.

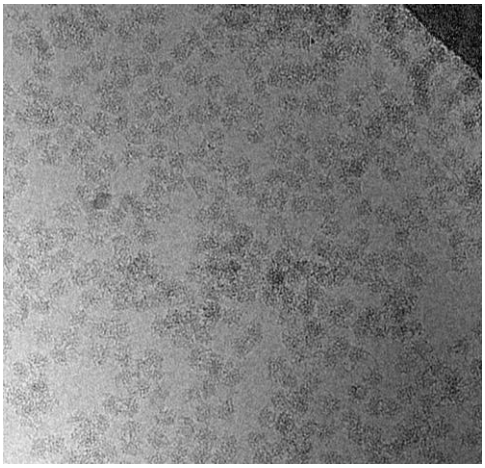

Fig. 1:cryo-EM micrograph of the aberrant 50S particle, was taken at CM01 beamline, Grenoble, France with K2 camera onTitan Krios FEI.

References:

1. Voorhees, R.M., et al., *Insights into substrate stabilization from snapshots of the peptidyl transferase center of the intact 70S ribosome.* Nature structural & molecular biology, 2009. 16(5): p. 528-533.
2. Maguire, B.A., et al., *A protein component at the heart of an RNA machine: the importance of protein L27 for the function of the bacterial ribosome.* Molecular cell, 2005. 20(3): p. 427-435.
3. Wower, I.K., J. Wower, and R.A. Zimmermann, *Ribosomal protein L27 participates in both 50 S subunit assembly and the peptidyl transferase reaction.* Journal of Biological Chemistry, 1998. 273(31): p. 19847-19852.
4. Wall, E.A., et al., *Specific N-terminal cleavage of ribosomal protein L27 in Staphylococcus aureus and related bacteria.* Molecular microbiology, 2015. 95(2): p. 258-269.
5. Chen, S.S. and J.R. Williamson, *Characterization of the ribosome biogenesis landscape in E. coli using quantitative mass spectrometry.* Journal of molecular biology, 2013. 425(4): p. 767-779.
6. S. H. W. Scheres. 2012. A Bayesian View on Cryo-EM Structure Determination. J Mol Biol. 415(2): 406-418.