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:** Complexs of S. aureus ribosome with new linezolid derivatives	**Experiment number**: mx-2183

Beamline: CM01	**Date of experiment**: from: 05.07.19 to: 08.07.19	**Date of report**: 20.1.20
Shifts: 9	**Local contact(s)**: Gregory Effantin	*Received at ESRF:*

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Report:

The rapid emergence and spread of multi-drug resistant bacteria, alongside the negligible activity of the major pharma companies, cause a world crisis. Hence, the current situation is frequently described as a "catastrophe". Consequently, in 2014 the World Health Organization (WHO) warned that the antibiotic resistance is leading to "post-antibiotic era", and declared it a substantial threat to human health. Even the World Bank estimated that up to 3.8% of the global economy will be lost by 2050 because of resistance to antibiotics.

The ribosomes, the multi-components (rRNA and rProteins) universal cellular particles that translate the genetic code into proteins are the target for ~40% of antibiotics in clinical use. During the last three decades the structure and function of the ribosomes have been the main objects of our scientific activities. Within this framework, we determined the structures of several bacterial ribosomes, and decifered how the currently used antibiotics paralyze them, as well as the molecular bases of the various resistance mecanisms. Among those we focused on the ribosome inhibition by Linezolid (**Fig. 1**), which is a synthetic antibiotic drug that belongs to the class of oxazolidinones. It was approved by the Food and Drug Administration (FDA) in April 2000 to treat Gram-positive pathogen infections. Being a synthetic drug, no preexisting resistance mechanisms were known, hence resistance to it was expected to emerge rather slowly. Despite these expectations, linezolid resistance was acquired by a specific 23S rRNA point mutation (G2576U) (E. coli rRNA numbering is used throughout) was aquiered by *Staphylococcus aureus* (SA) (Tsiodras, Gold et al. 2001) approximately a year after it was approved for treatment. Together with resistance mechanisms identified later, linezolid resistance reached the level of <1% of SA clinical isolates within 10 years (Endimiani, Blackford et al. 2011).

Recently, it was shown that new derivatives of linezolid (**Fig. 1**) that were synthesized by our collaborators (Yang, Chen et al. 2015) are useful inhibitors against SA strains. Moreover, superior *in vitro* inhibition of LYP-2 and YB-6 over LYP-1 and 1622 by 1-2 orders of magnitude was deteced. Consequently, we are studying the structures of the complexes of these compounds with the SA ribosome, aiming at the definition of the determinants for inhibition by these compounds as well as suggesting possible mechanisms for resistance and selectivity.

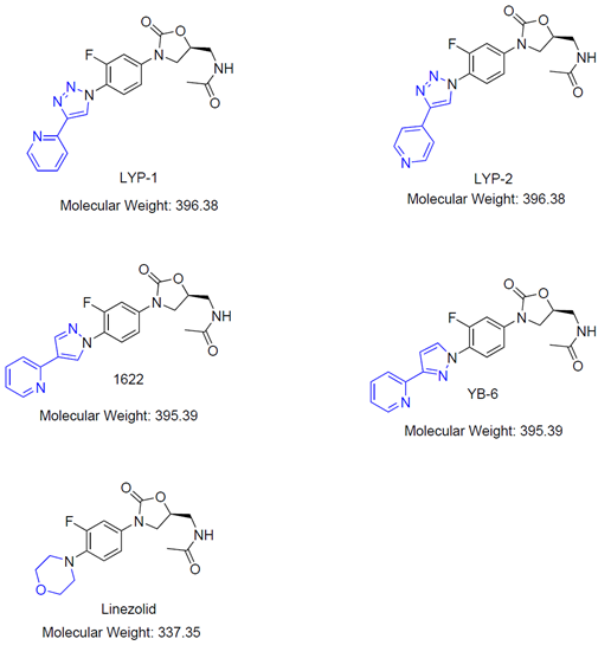


Fig. 1*: Chemical structure of linezolid derivatives that we plan to study in complex with SA ribosome. Linezolid structure is also shown for comparison purposes*

Results:

Cryo-EM grids (Quantifoil 2/2) of the SA 70S ribosome (SA70S) complex samples 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 $\Delta z = (-0.5)$- (-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 1.052 Å pixel size at the specimen level. The camera was set up to collect 40 frames, total exposure time was was 4 sec/movie with a dose of 20.3 $e^{-}Å^{2}$ collecting 5 movie/hole.

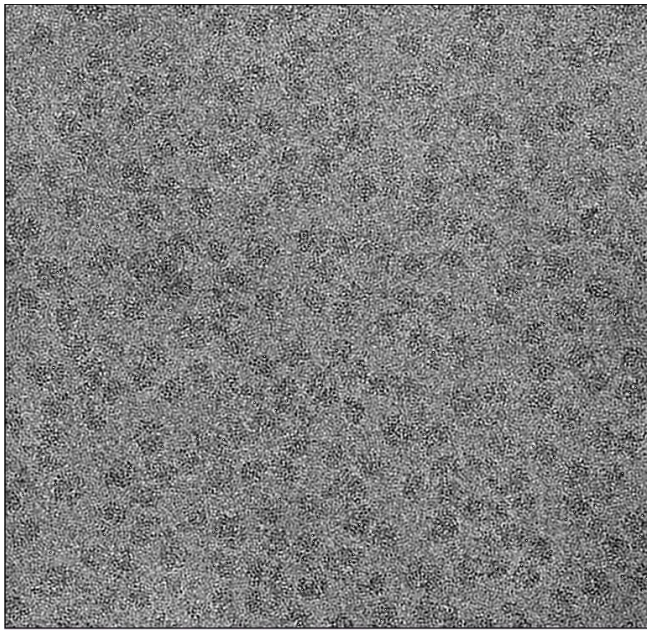


Fig. 2: *cryo-EM micrograph of the 70S complex with compound YB-6, was collected at CM01 beamline, Grenoble, France with K2 camera onTitan Krios FEI.*

All data processed using Relion 3 (Scheres, 2018). 6480 movies (**Fig. 2**) were aligned by the whole image motion correction method. The contrast transfer function of every image was determined using CTFFIND4.1 of the RELION 3 workflow. Particle auto picking was done with a template (from auto particle picking). About 1,230,958 particles were picked. Due to relion's limitations we had to reduce the number of particles

before 2D clasification, therfore we selected 575,600 particles with estimated resolution higher than 2.78 Å . 2D classification and selection of 45 (out of 380) classes yielded 510,675 particles (**Fig. 3**). Performing 3D classification resulted in 10 classes. Among them, 6 classes were selected for 3D image reconstruction (**Fig. 5**). 300,518 particles were used for 3D auto-refinement and the reconstructed map reached 3.00Å. After post-processing the final resolution was 2.69Å. A CTF refinment, followed by Bayesian polishing was preformed, and the second 3D reconstruction reached 2.82Å. After post-processing the final resolution is 2.51Å.
The high resolution of the resulting map enabled unambiguouse modeling of the nucleotides in the core of the ribosome. In addition, we could identify unmodeled electron density in the core of the ribosome, in close proximity to linezolid known binding site. that could fit our compaund (**Fig. 5**).

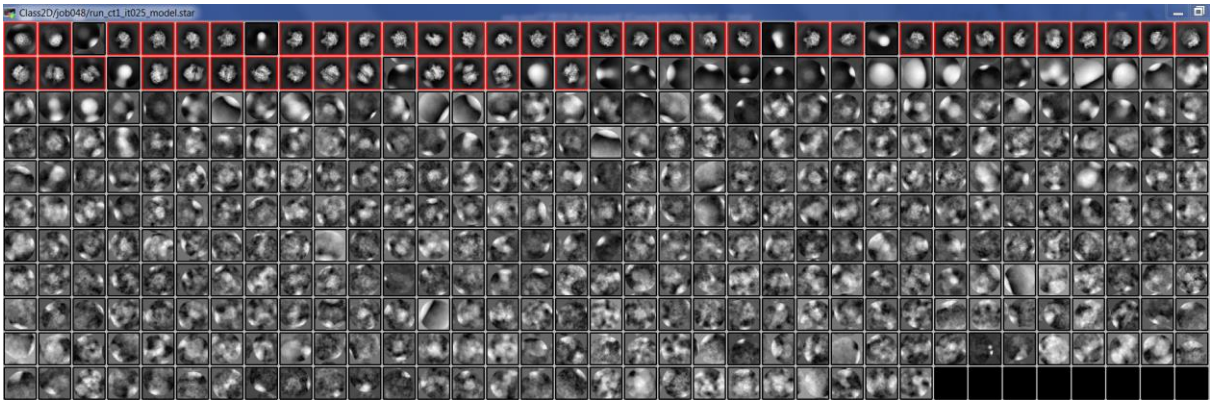
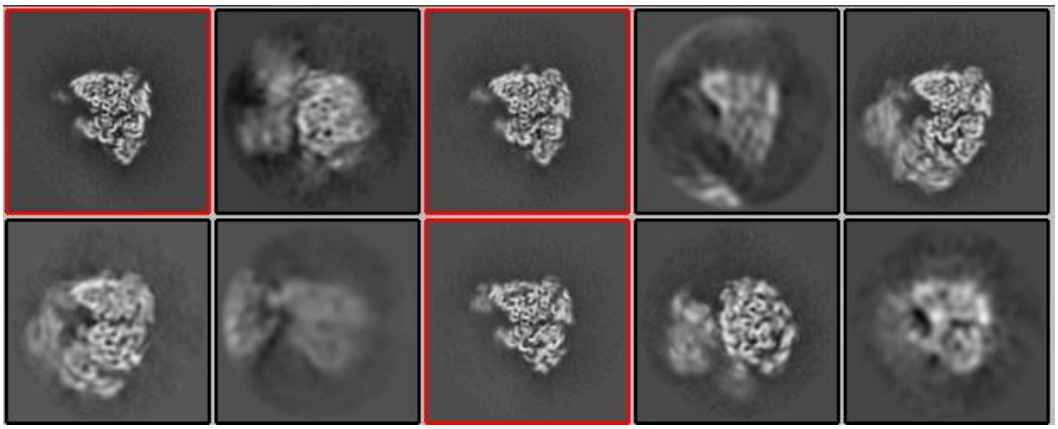
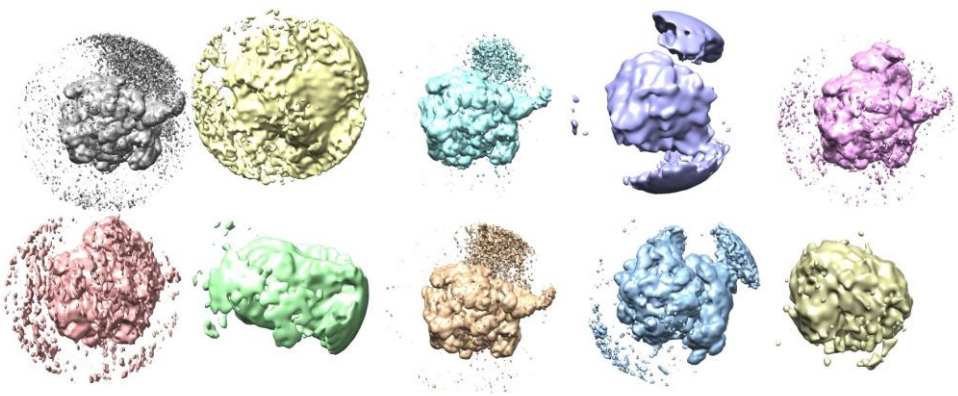


Figure 3: *380 classes of the 1st 2D classification of 510,675 particles (in red square are the 45 selected classes that were used for further analysis)*





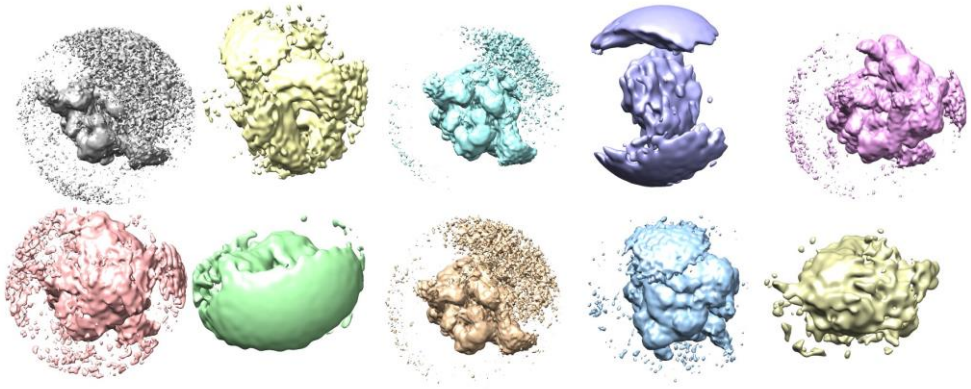


Figure 4: *A. 10 classes of the 3D classification of 510,675 particles. 3 classes were selected (squared in red) for further analysis. B-C. two views (180 deg apart) of the 10 3D classes, (Figure 4B-C were created using Chimera).*

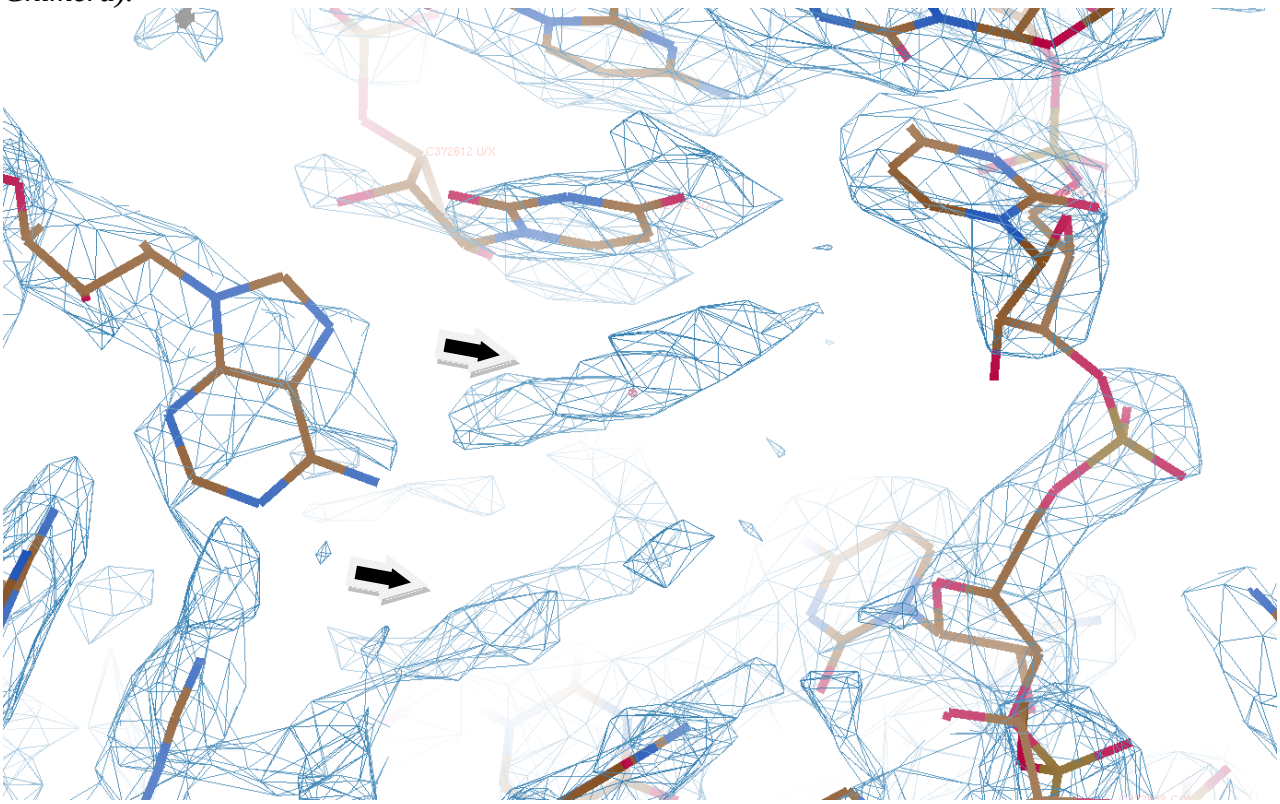


Figure 5: *The electron density map (blue) and a model of the nucleotides (gold) in the ribosome core, surrounding the expected binding site. The SA ribosome model was docked into the electron density and an extra electron density can be observed (pointed with black arrows). The extra density results from YB-6 that was bound to the ribosome. The two unmodeled electron densities blobs that were detacted, led to a speculatation that 2 molecules of YB-6 are bound (Figure 5 was created using Coot).*

References:

all in : http://www.weizmann.ac.il/sb/faculty_pages/Yonath/00Publications.html and

Endimiani, A., M. Blackford, et al. (2011). "Emergence of linezolid-resistant Staphylococcus aureus after prolonged treatment of cystic fibrosis patients in Cleveland, Ohio." Antimicrobial agents and chemotherapy **55**(4): 1684-1692.

Tsiodras, S., H. S. Gold, et al. (2001). "Linezolid resistance in a clinical isolate of *Staphylococcus aureus*." The Lancet **358**(9277): 207-208.

Yang, T., G. Chen, et al. (2015). "Discovery of a Teraryl Oxazolidinone Compound (S)-N-((3-(3-Fluoro-4-(4-(pyridin-2-yl)-1 H-pyrazol-1-yl) phenyl)-2-oxooxazolidin-5-yl) methyl) acetamide Phosphate as a Novel Antimicrobial Agent with Enhanced Safety Profile and Efficacies." Journal of medicinal chemistry **58**(16): 6389-6409.

S. H. W. Scheres. 2012. A Bayesian View on Cryo-EM Structure Determination. J Mol Biol. 415(2): 406-418.
Emsley, Paul, et al. "Features and development of Coot." Acta Crystallographica Section D: Biological Crystallography 66.4 (2010): 486-501.