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:** Design of species specific next generation novel antibiotics: resistance, selectivity and ecological issues	**Experiment number**: MX-2003
Beamline: CM01	**Date of experiment**: from: 23.1.18 to: 26.1.18	**Date of report**: 13/3/18
Shifts: 9	**Local contact(s)**: KANDIAH Eaazhisai	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Ada Yonath, The department of structural Biology, The Weizmann institute of science, Israel
Anat Bashan, The department of structural Biology, The Weizmann institute of science, Israel
*Yehuda Halfon , The department of structural Biology, The Weizmann institute of science, Israel
*Zohar Eyal, The department of structural Biology, The Weizmann institute of science, Israel
Ella Zimmerman, The department of structural Biology, The Weizmann institute of science, Israel
Giuseppe Cimicata, The department of structural Biology, The Weizmann institute of science, Israel
Donna Matzov, The department of structural Biology, The Weizmann institute of science, Israel
Elinor Breiner Goldstein **,** The department of structural Biology, The Weizmann institute of science, Israel

Report:

The rapid emergence and spread of multi drug resistance alongside the negligible activity of major pharma companies in the design of new antibiotics cause a world crisis. Hence, the current situation is frequently described as a "catastrophe" Hence, 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 ~4% of the global economy will be lost by 2050 because of resistance to antibiotics.

The treatments of infections with the available arsenal of clinically used antibiotics have been badly affected by the appearance of multidrug- resistant strains. For example, at present many infections are caused by highly resistant bacterial strains of Staphylococcus aureus. These Gram-positive, versatile and potentially aggressive strains are among the most worrisome pathogenic bacteria. In this study we attempt to decipher the structural basis for *S. aureus* mutant in the large subunit protein uL22 that is resistant to erythromycin (SA70SL22). uL22 resides on the wall of the polypeptide exit tunnel but the erythromycin binding site is more than 12 Å away from it.

During our MX-2003 session at CM01, 3,452 micrographs were collected with the following settings: each micrograph is composed of 40 frames where each was collected for 0.2s which leads to an 8s movie with a total dose of 40 $e^-/Å^2$ per movie. From these movies 529,786 particles were selected for 2D classification. Then 145,897 particles were selected for 3D classification and 124,731 particles were selected for 3D refinement. After post processing we obtained a 3.58Å reconstructed map of the SA70SL22 (**Fig. 1**).

After fitting as a starting model that of the SA70S (PDBID: 5TCU) into the cryo-EM reconstructed map it can be seen (**Fig. 2**) that the large subunit, 50S part, led to a quite nice and crispy map that covers most of the initial model while the small subunit, 30S part, is less resolved. Consequently, the map is of lower resolution and parts of the particle are absent from the map. We often observe this phenomena in cryo-EM reconstrutctions of empty ribosomes (as the current sample). Zooming into the deletion site in rProtein uL22 of the starting model, some density is missing (**Fig. 3**). Therefore there are slim chances for modeling the deletion mutant uL22 rProtein in our current reconstructed map. At this point we plan to try and include more

particles in the calculations. We might be able to obtain a better map if we pick more particles after 2D classification, when using only the large subunit initial model, ignoring the small subunit part, and applying focus refinement on it. However, the dificulties in tracing the loop of interest may be the result of the disorder caused by the deletion, and this is the reason for the problematic resistant phynotype. Consequently, more data treatment is neededin order to determine if more data collection is needed.

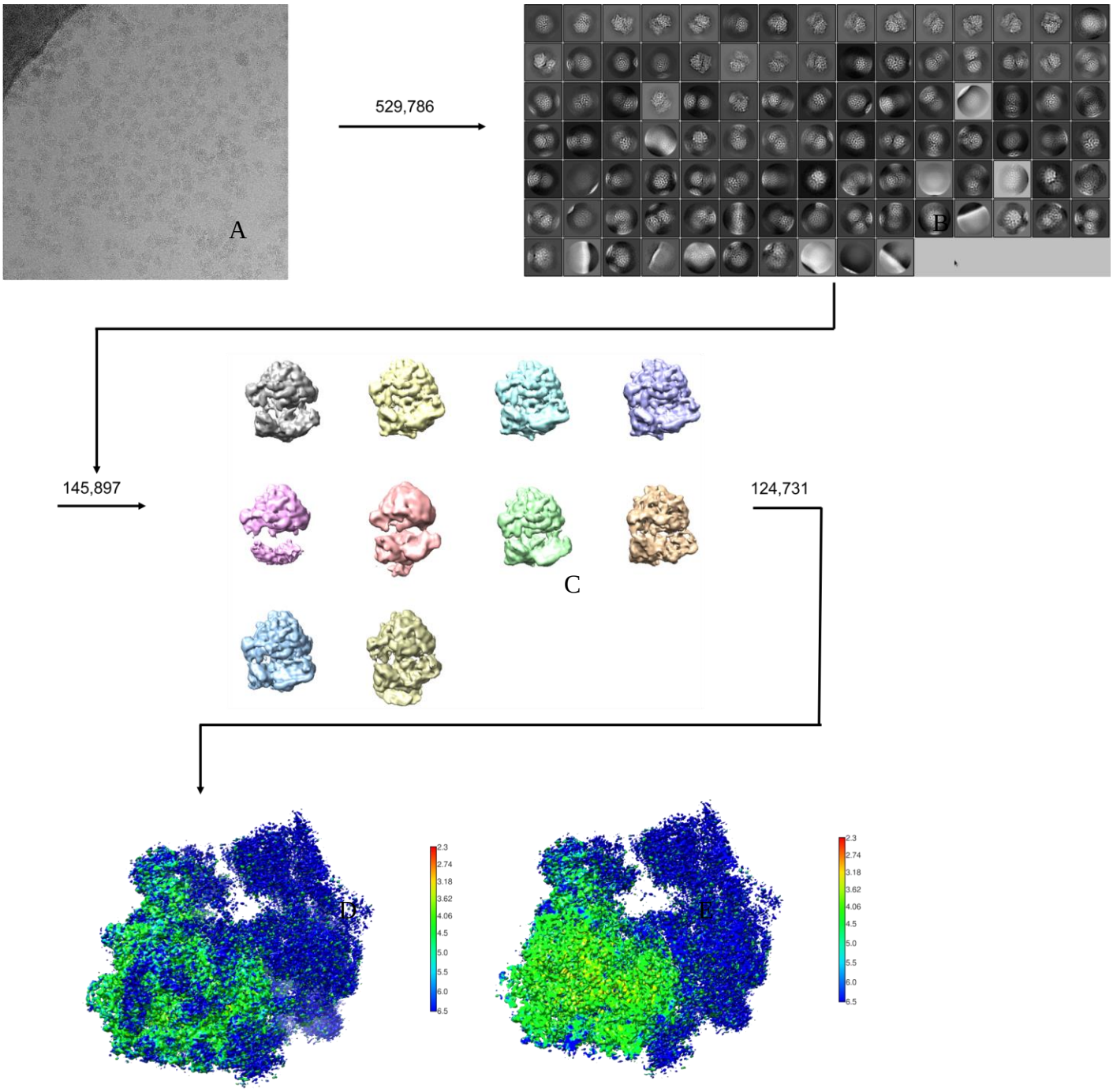

Fig. 1: The cryo-EM image processing flow chart for the project. (A) An image from a motion corrected movie; (B) A 2D classification of the particles selected from the movies; (C) 3D classification of the selected particles from the 2D classification; (D) An image of the map resolution distribution, calculated using ResMap and displayed via chimera (E) An image of the 70s ribosome and its cross section showing the resolution distribution through the particle.

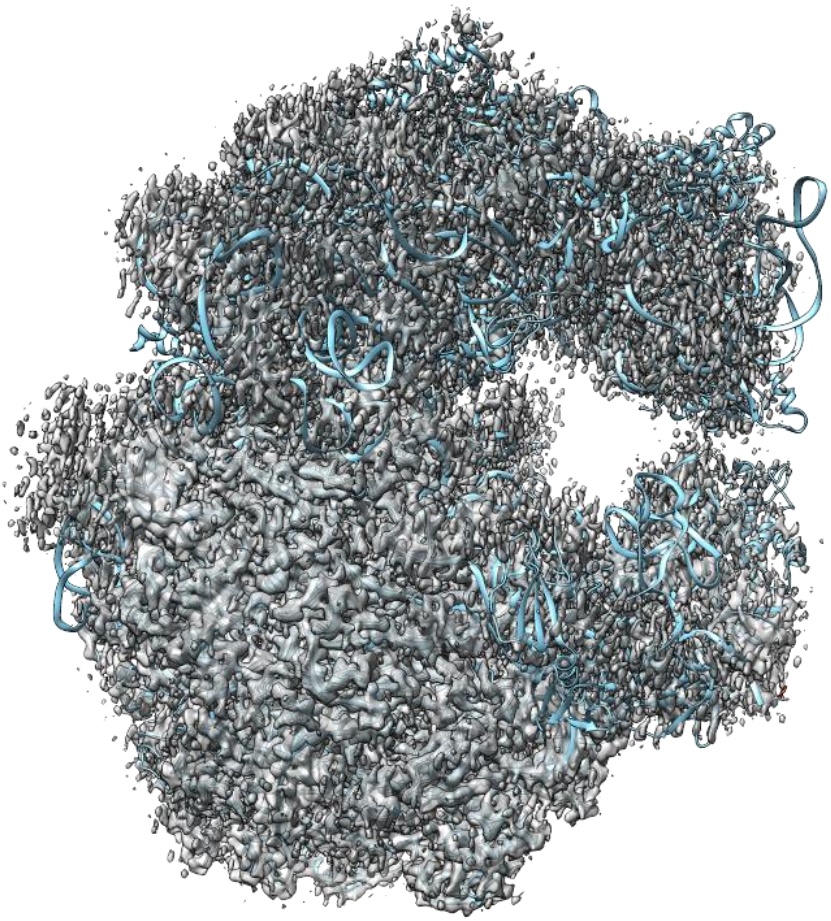

Fig. 2: Fitting the model in the EM map.

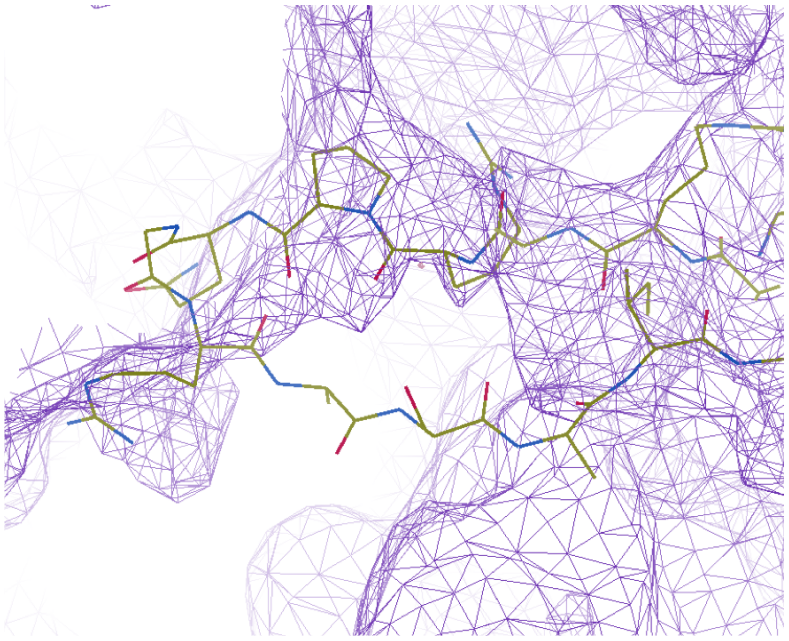

Fig. 3: The uL22 mutation site in the cryoEM reconstructed map. The density for the entire loop is missing from the map.