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.

<table>
<tr><td>ESRF</td><td>Experiment title: CryoEM structure Determination of the Vault Particle Cap Region</td><td>Experiment number:
MX-2092</td></tr>
<tr><td>Beamline:</td><td>Date of experiment:
from: 22/06/2018 to: 25/06/2018</td><td rowspan="2">Date of report:
10/01/19
Received at ESRF:</td></tr>
<tr><td>Shifts:</td><td>Local contact(s): Michael Hons</td></tr>
<tr><td colspan="3">Names and affiliations of applicants (* indicates experimentalists):
Prof. Nuria Verdaguer (main proposer). Dr Pablo Guerra *. Miss Maria Gonzalez*.
Molecular Biology Institute of Barcelona. IBMB-CSIC. Parc Cientific de Barcelona. Josep Samitier 1 – 5. 08028 BARCELONA. SPAIN</td></tr>
</table>

Report:

- **Overview:**

We acquired movies of recombinant vaults from *Mus musculus* in gold grids using the FEI Titan Krios electron microscope in counting mode with EPU at x130000 (pixel size of 1.067 Å/pixel). We used an electron dose of 6.4 electrons/pixel/second and each movie contains 30 frames recorded in 6 seconds. The whole dataset has 5039 movies.

- **Quality of data:**

The movies collected present a high heterogenity in the particle distribution, showing regions completely crowded of particles and regions with just very few particles. Also the particle itself presents structural heterogenity due to the presence of half- and full- particles or opening intermediates in the same area. These facts, joined to the big size of the particle (700 Å), limit the number of particles available per movie (FIGURE 1).

The quality of the images is considerably high, reaching the 2.8 Å resolution in the CTF estimation (Figure 1).

- **Status and progress of evaluation**

A total of 128657 particles were automatically picked with 700 x 700 box size in pixel and extracted with a pixel size of 1.07 Å. All particles were subjected to two-dimensional and three-dimensional classification using RELION 2.1. 17924 particles were selected and refined applying C1 symmetry. To enhance signal, the mask is generated from cryoEM data to focus the refinement in the vault region. Following "gold standard" refinement protocol, the current reconstructed map has been obtained to 8.4 Å of resolution. At this resolution we are not able to determine the structure of each monomer in the cap region and we can not apply any symmetry.

At this point we decide to make the localized three-dimensional reconstruction of the cap region of the vault, using the method described by Ilca et al., 2015. After determining the particle orientations, the cap regions were extracted and treated as single particles. The number of particles were duplicated and subjected to re-alignment and refinement but the reconstruction obtained did not improve the quality of the map.

In one of the first 3D classifications, we obtained one class comprised 7539 half-vault particles picked and extracted using relion. All these particles where subjected to two dimensional (Figure 2) and three dimensional classification and 7515 particles were selected and refined using C1 symmetry. Following "gold standard" refinement protocol, the current reconstructed map (Figure 3) has been obtained to 7.9 Å of resolution.

- **Results**

Unfortunately we have not been able to obtain a map of the half vault with sufficient quality to discern the readjustments of symmetry in the cap region. We have tried all the different methods that are available in the processing of the data, but it has not improved the quality of the maps in this region. However, we have obtained for the first time the structure of individual half vaults, showing surprising characteristics: the circular shape of the waist present in the whole particle is broken as a consequence of half vault diassembly (Figure 3).

Now we have written a draft including this structure among other experiments, that are in concordance with a new suggested vault-aperture mechanism. This article will be submitted in the next months. But if we will be able to obtain more particles to calculate this model we could arise to higher resolution, obtaining more details about the structural changes produce in the aperture of the particle. Taking into account the CTF estimation of our movies, we have information until 3.4 Å of resolution.

We are working in a mutant of the Major vault protein that generates , mostly, half-vaults in solution. We consider that just collecting another dataset like this one, but with this new mutant vaults, we will have enough particles to obtain a reconstraction near atomic resolution.

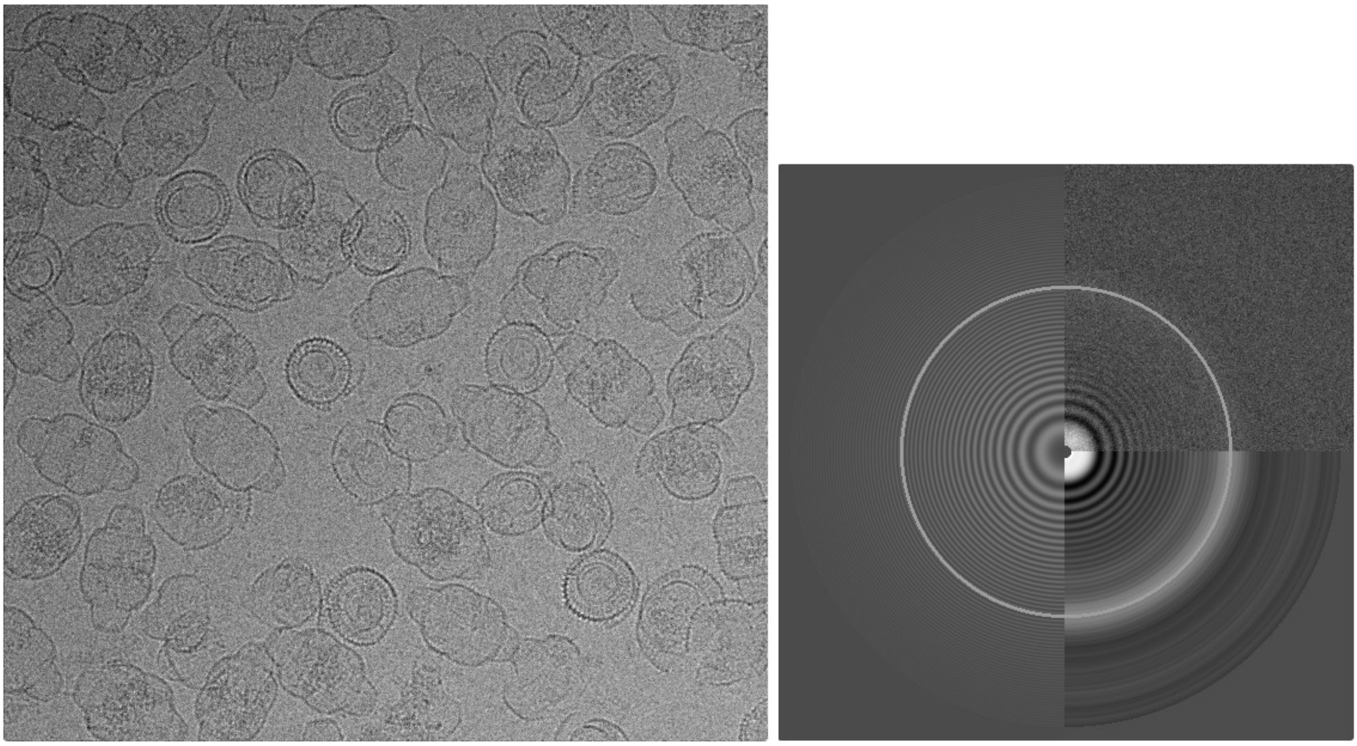

FIGURE 1. Cryo-micrographs and Fourier transforms of *D. discoideum* vaults acquired with a FEI Titan Kryos microscope equipped with a K2. Thon rings can reach at close to 2.4 Å^{-1}.

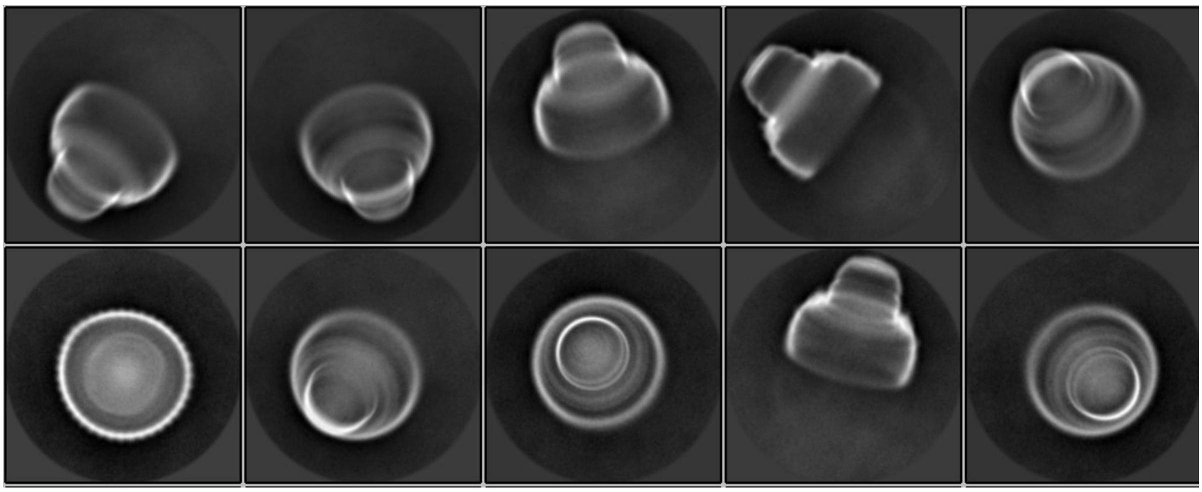

FIGURE 2. 2D classes of *D .discoideum* vaults obtained using the software Relion 2.1(Scheres, 2012).

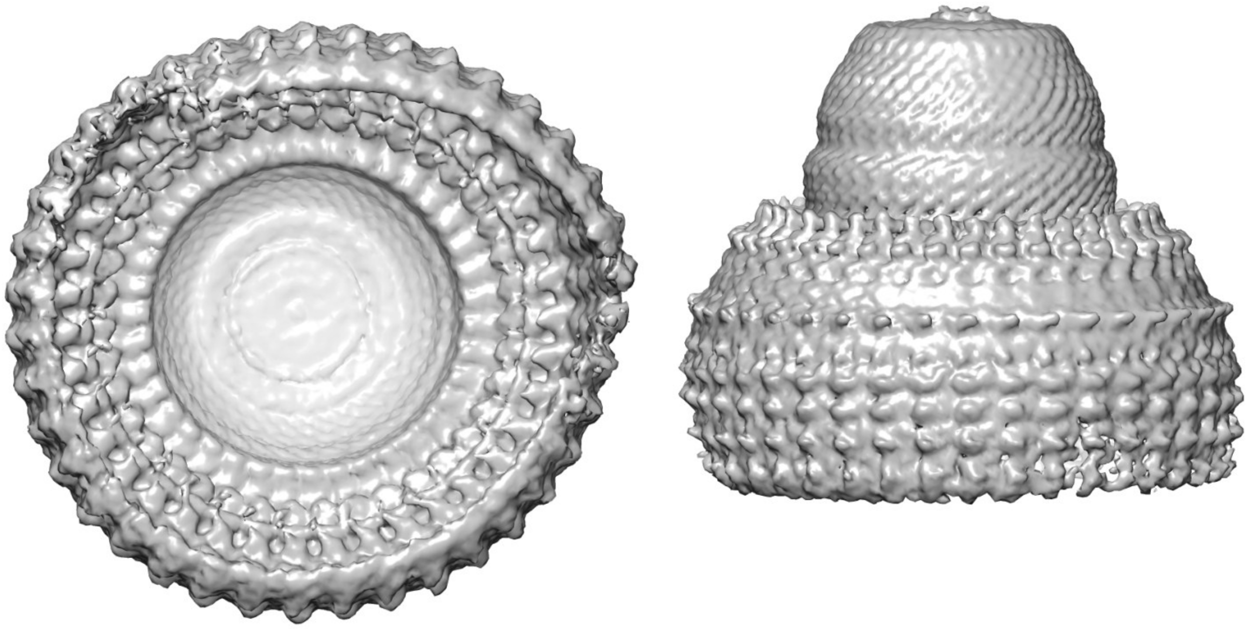

FIGURE 3. Lateral (left) and top (right) views of a 3D reconstruction of the *D. discoideum* vaults at 3.9 Å of resolution. They are in D13 symmetry

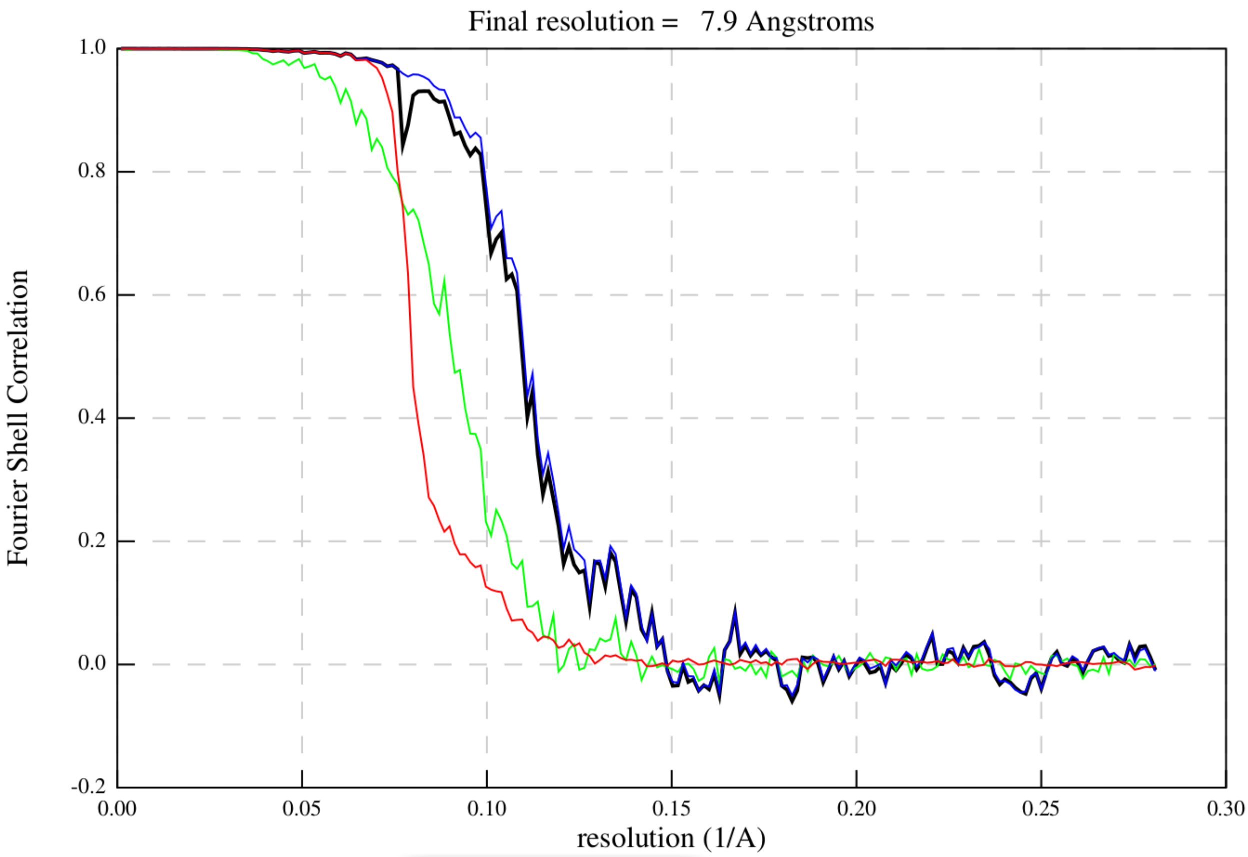

FIGURE 4. Fourier shell correlation correlation corrected (black line), unmasked maps(green), masked maps (blue) and phase randomised maps (red) showing that the resolution (FSC 0.143) is 3.9 Å.