EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Formβ

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:** CryoEM structure Determination of the Vault Particle from *Dictyostelium discoideum*	**Experiment number**: MX-2132
Beamline:	**Date of experiment**: from: 11/03/2019 to: 14/03/2019	**Date of report**: 30/07/19
Shifts:	**Local contact(s)**: Gregory Effantin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr Nuria Verdaguer (main proposer). Dr Pablo Guerra *. Miss Maria Gonzalez*.

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Report:

- **Overview:**

We acquired movies of recombinant vaults from *Dictoystelium discoideum* in gold grids using the FEI Titan Krios electron microscope in counting mode with EPU at x130000 (pixel size of 1.05 Å/pixel). We used an electron dose of 7.2 electrons/pixel/second and each movie contains 30 frames recorded in 6 seconds. The whole dataset has 2525 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 2.9 Å resolution in the CTF estimation (FIGURE 1).

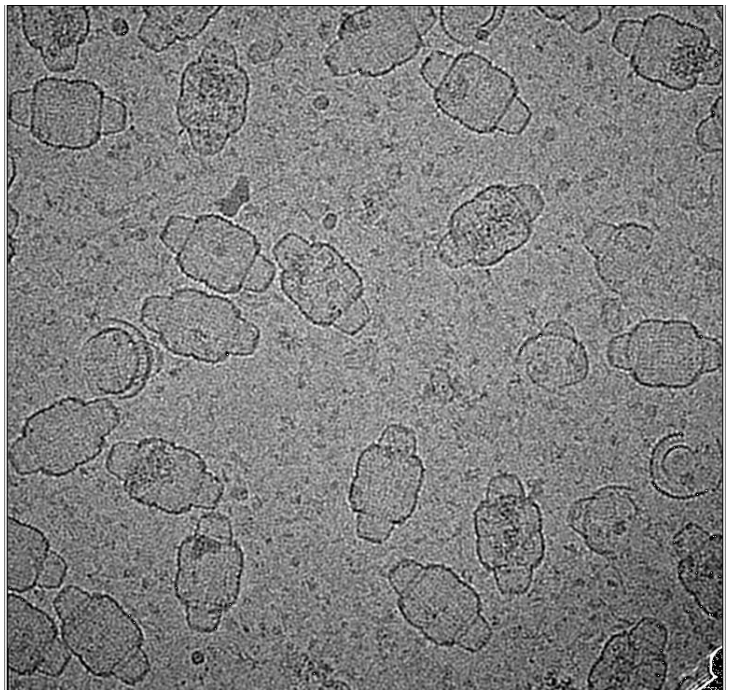
- **Status and progress of evaluation**

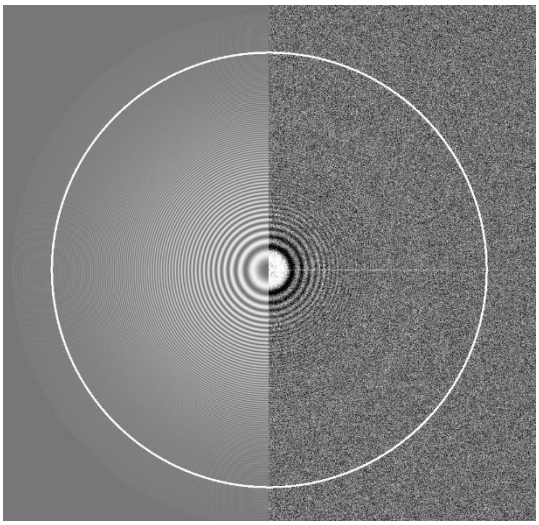
A total of 103546 particles were automatically picked with 700 x 700 box size in pixel and extracted with a pixel size of 1.05 Å. All particles were subjected for RELION 3.0 to two-dimensional (FIGURE 2) and three-dimensional classification. 12150 particles were selected, joined to the 18032 particles selected in the first acquisition of this project (MX-2018) and refined together using C3 symmetry (the lowest symmetry present in the particle). 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 (FIGURE 3) has been obtained to 3.85 Å of resolution.

- **Results**

Dictyostelium discoideum contains two MVP isoforms: MVPα and MVPβ and both proteins are required for the structural integrity of the vault particle. Our main goal was to decipher howe these two iosoforms are organised to conform the vault particle of Dictyostelium. We considered two possible scenarios: the D39 dihedral symmetry is conserved and each halve of the particle is composed of 39 copies of MVPα and MVPβ respectively or vaults from D. discoideum present a unique architecture with both isoforms intercalating in each vault half and thus exhibiting a new rotational symmetry.
As mentioned above, the processing of the data we have collected has resulted in a 3.85 Å map where we were able to trace the main chain of the MVP monomers and many of the bulky side chains . Unfortunately, we were not able to distinguish between the two MVP monomers, α and β, forming this particle. Now we are carrying out several software approximations using masks with the objective to solve this problem.

Additionally, some experiments have been planned with the objective to be able to distinguish the two monomers. We are going to fuse a peptide that is recognised by a commercial nanobody to one of the monomers. The complex nanobody-particle let us identify the position of these monmers in the assembly of the vault. Once we will have this information, we will be able to solve the structure.





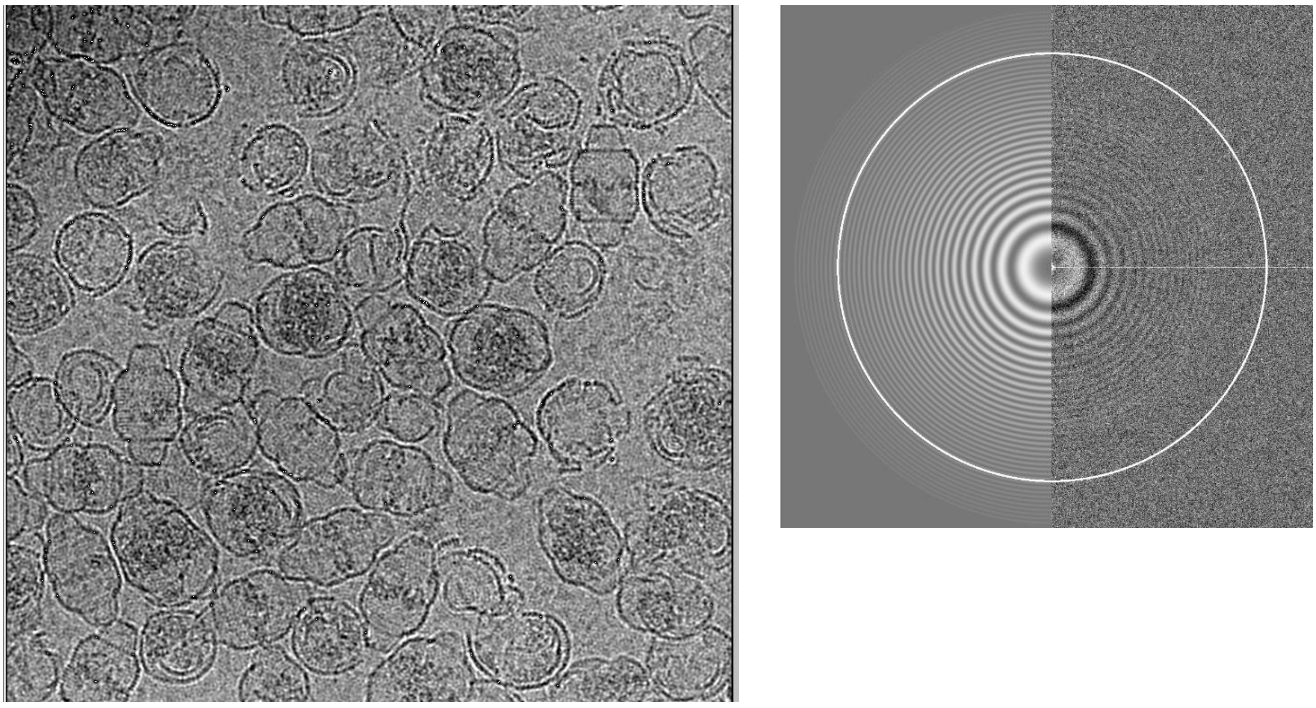


FIGURE 1. Cryo-micrographs and Fourier transforms of *D. discoideum* vaults acquired with a FEI Titan Kryos microscope equipped with a K2. Thon rings can reach at close to 2.9 $Å^{-1}$

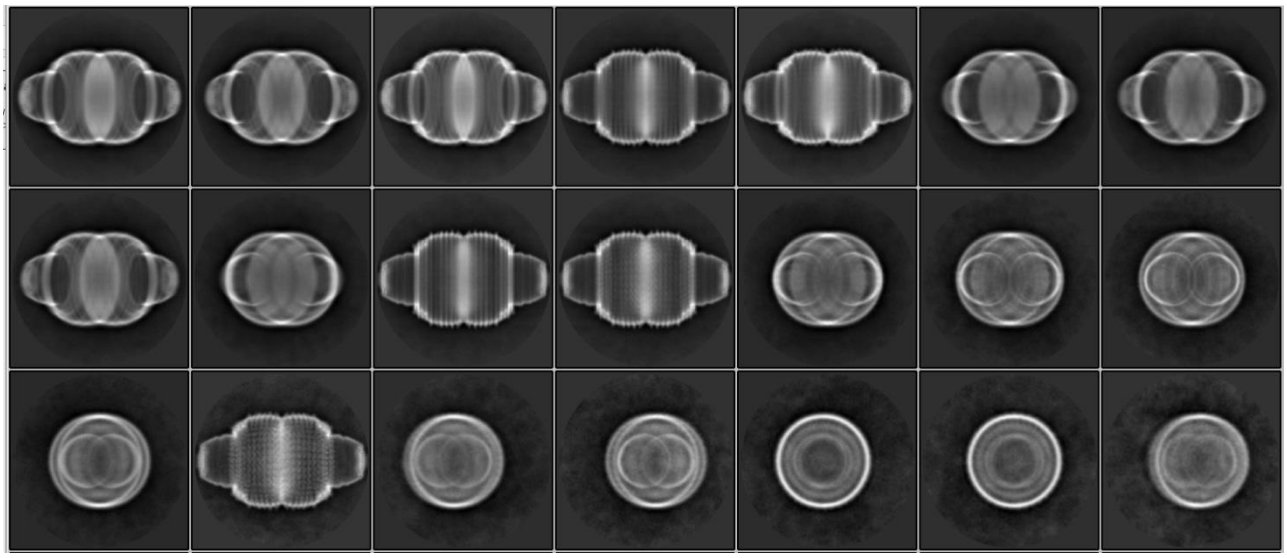


FIGURE 2. 2D classes of *D .discoideum* vaults obtained using the software Relion 3.0 (Scheres, 2012).

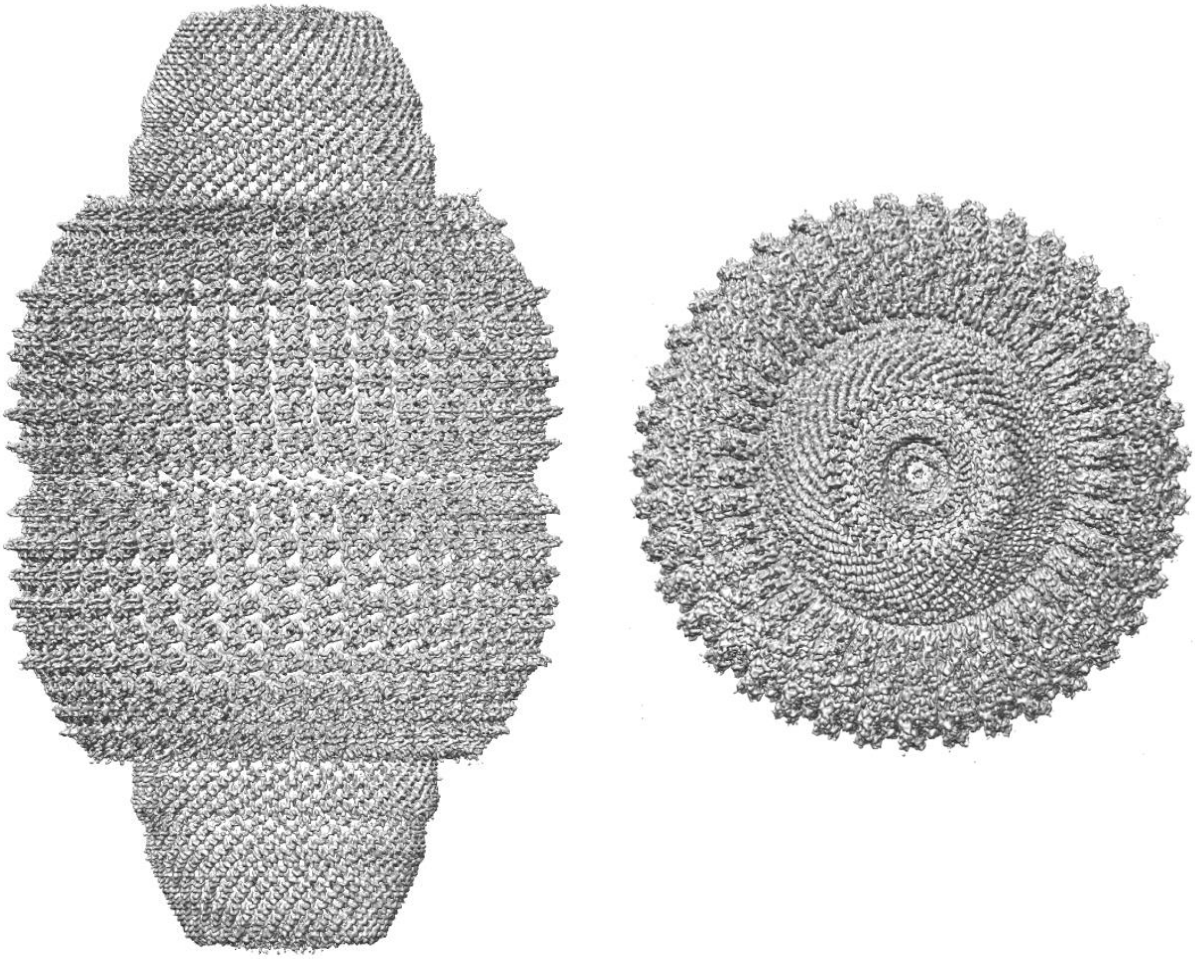


FIGURE 3. Lateral (left) and top (right) views of a 3D reconstruction of the *D. discoideum* vaults at 3.9 Å of resolution. They are in C3 symmetry

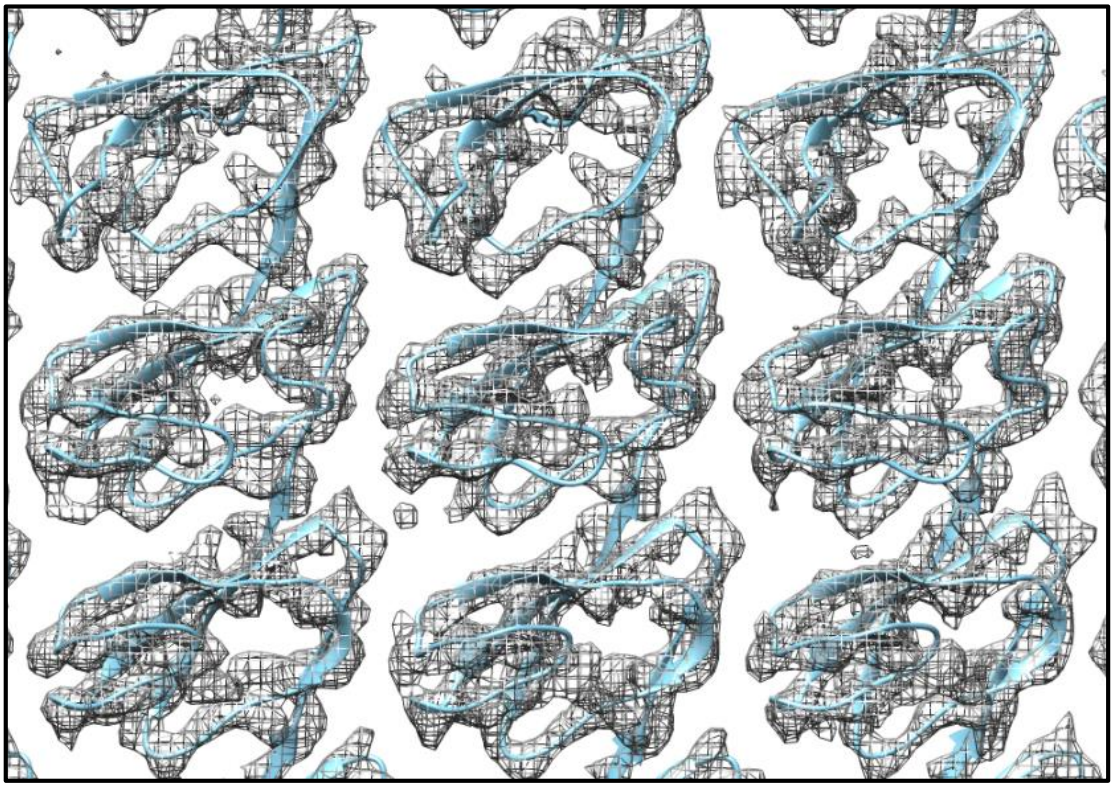


FIGURE 4. Magnified view at R3 to R7 domain of density map with the crystal structure of one MVP fitted in the density (PDBid: 4HL8).

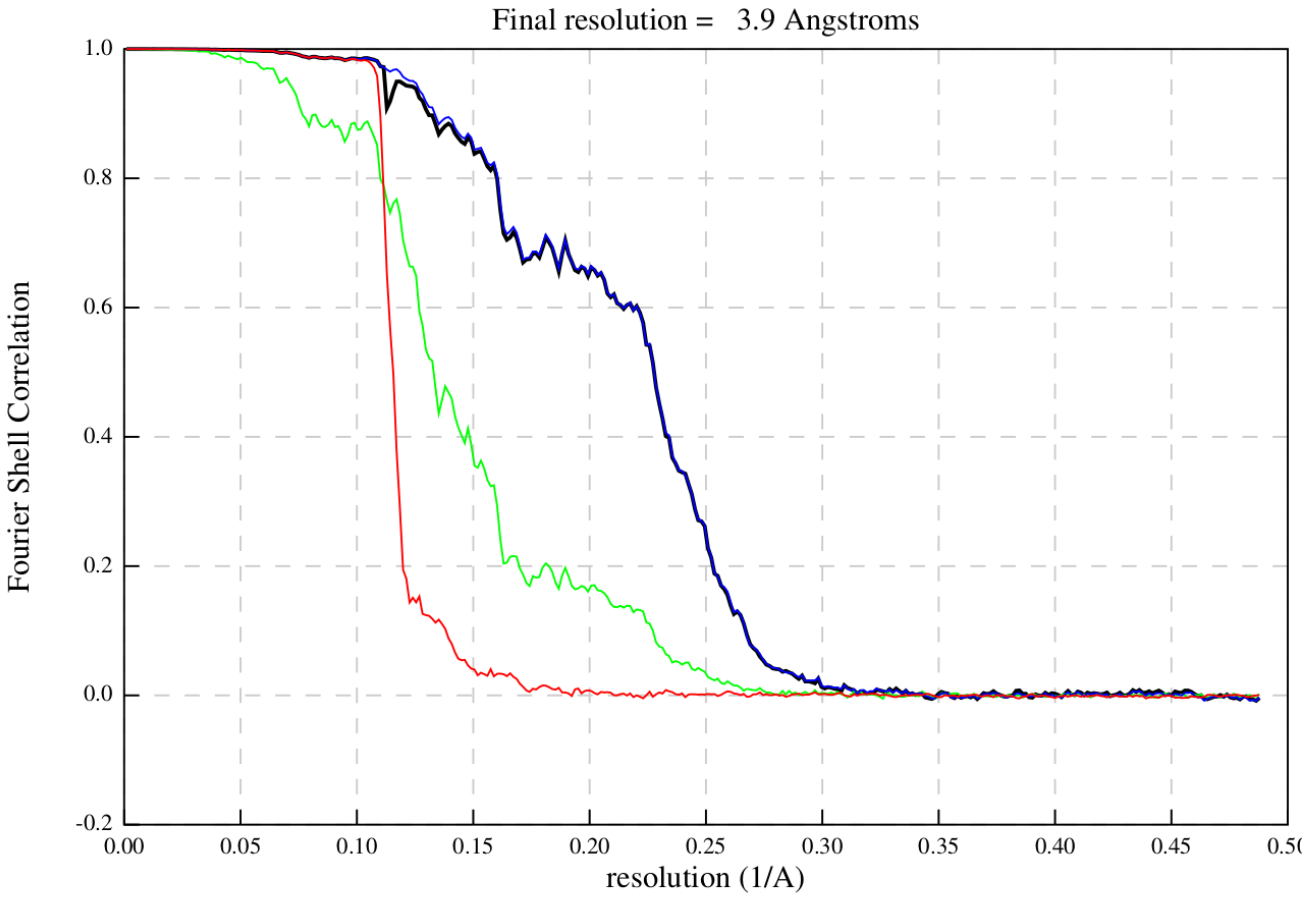


FIGURE 5. Fourier shell correlation correlation corrected (black line), unmasked maps(green), masked maps (blue) and phase randomised maps (red) showing that the resolution (FSC 0.143) is 3.9 Å.