ESRF	**Experiment title:** CryoEM structure Determination of the Vault Particle from *Dictyostelium discoideum*	**Experiment number**: MX-2018
Beamline:	**Date of experiment**: from: 27/03/2018 to: 30/03/2018	**Date of report**: 14/05/18
Shifts:	**Local contact(s)**: Eaazhisai Kandiah	*Received at ESRF:*

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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 x110000 (pixel size of 1.07 Å/pixel). We used an electron dose of 7.7 electrons/pixel/second and each movie contains 30 frames recorded in 6 seconds. The whole dataset has 3643 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 heterogenitydue 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 3.4 Å resolution in the CTF estimation (FIGURE 1).

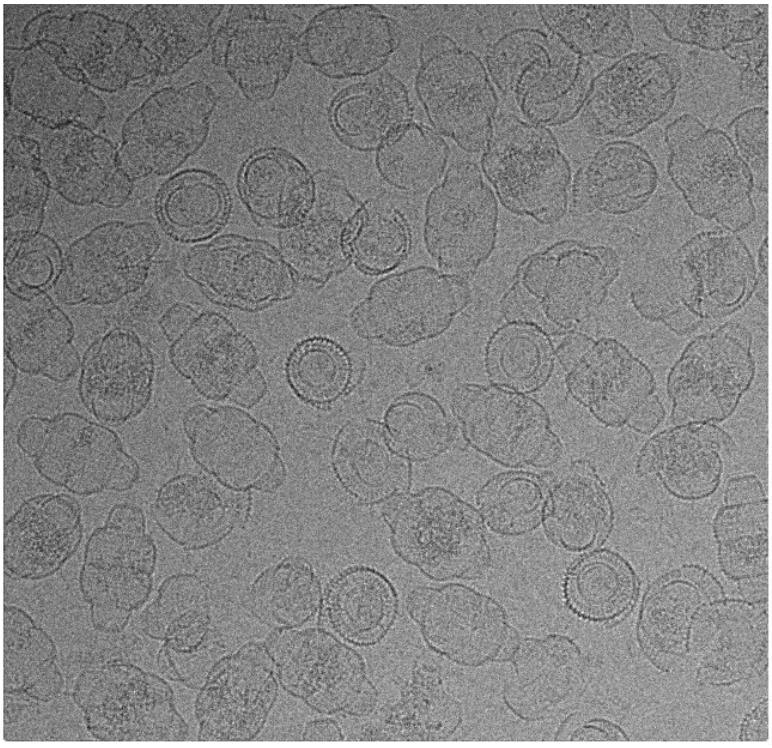
- **Status and progress of evaluation**

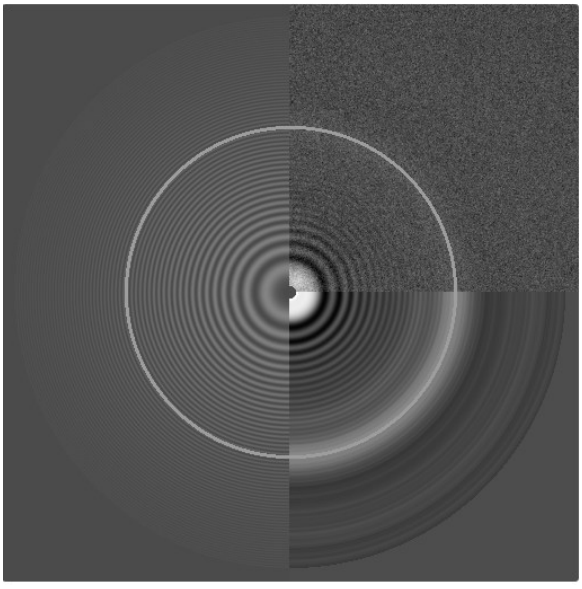
A total of 115327 particles were automatically picked with 700 x 700 box size in pixel and extracted with a pixel size of 1.07 Å. All particles were subjected for RELION 2.1 to two-dimensional (FIGURE 2) and three-dimensional classification. 18013 particles were selected and refined using D13 symmetry. 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 (FIGURE 3) has been obtained to 3.92 Å of resolution.

- **Results**

Now we have a map where we are able to trace the main chain of the MVP monomers, but at the present resolution we cannot define with confidence the residue side chains. We believe that an additional data collection will be necessary in order to achieve higher resolution (>3.5 Å). Taking into account the CTF estimation of our movies, we have information until 3.4 Å of resolution.

We consider that just collecting another dataset like this one, we will have enough particles to obtain a reconstraction near the 3.5 Å. New grids are ready and we just need additional time in the electron microscope.





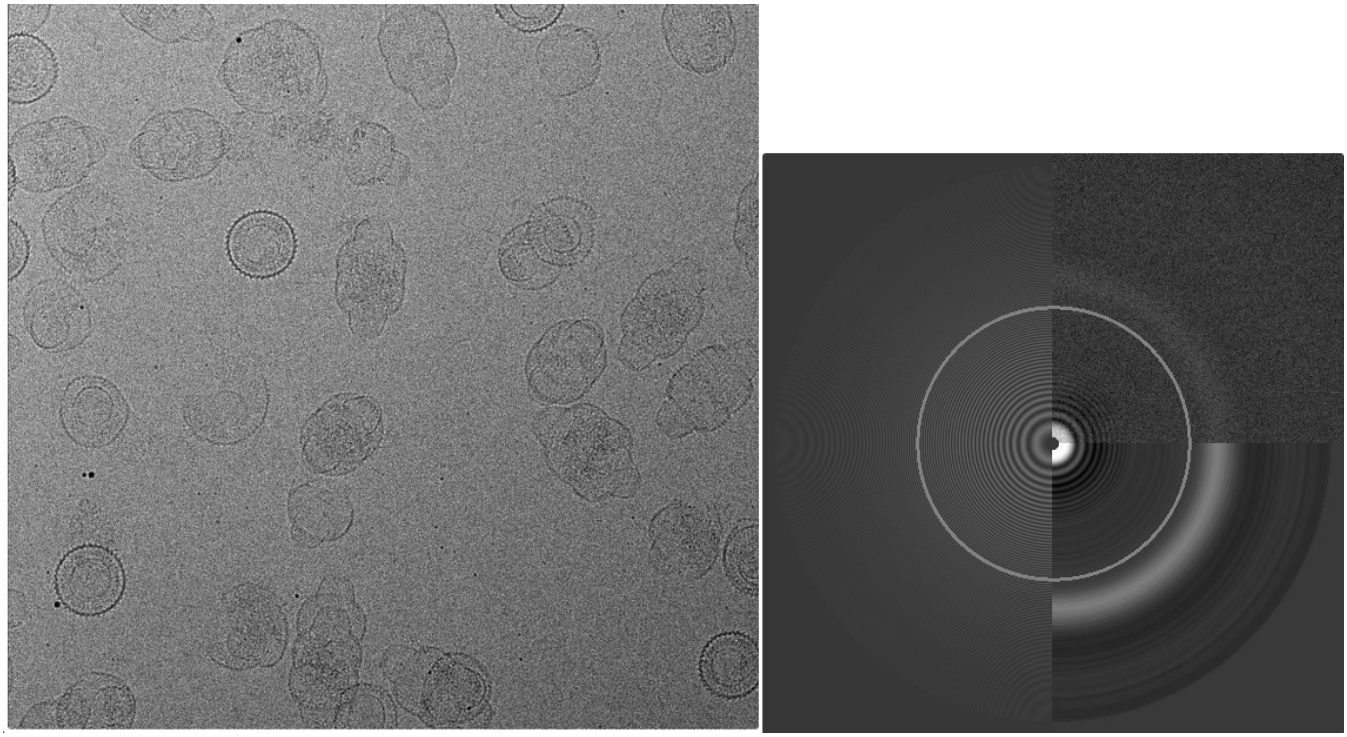


FIGURE 1. Cryo-micrographs and Fourier transforms of *D. discoideum* vaults acquired with a FEI Titan Kryos microscope equipped with a K2. Thon rings can reach at close to 3.4 $Å^{-1}$.

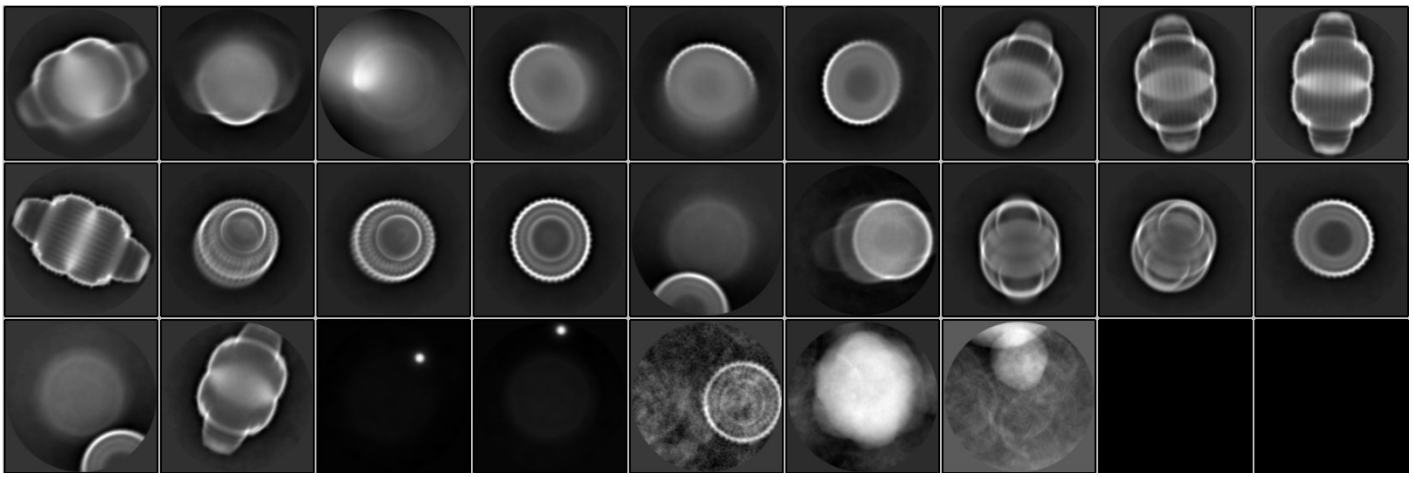


FIGURE 2. 2D classes of *D .discoideum* vaults obtained using the software Relion 2.1(Scheres, 2012).

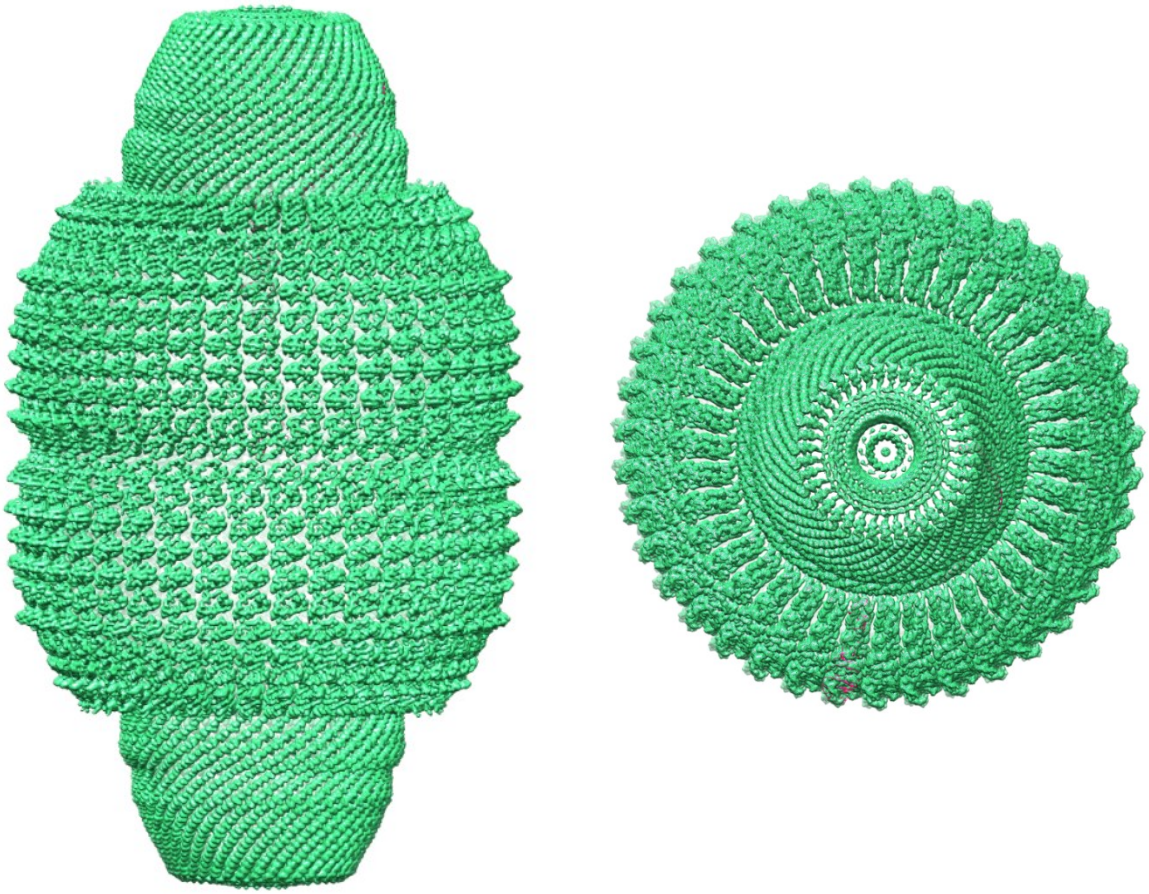


FIGURE 3. Lateral (left) and top (right) views of a 3D reconstruction of the *D. discoideum* vaults at 3.9 Å of resolution. They are in D13 symmetry

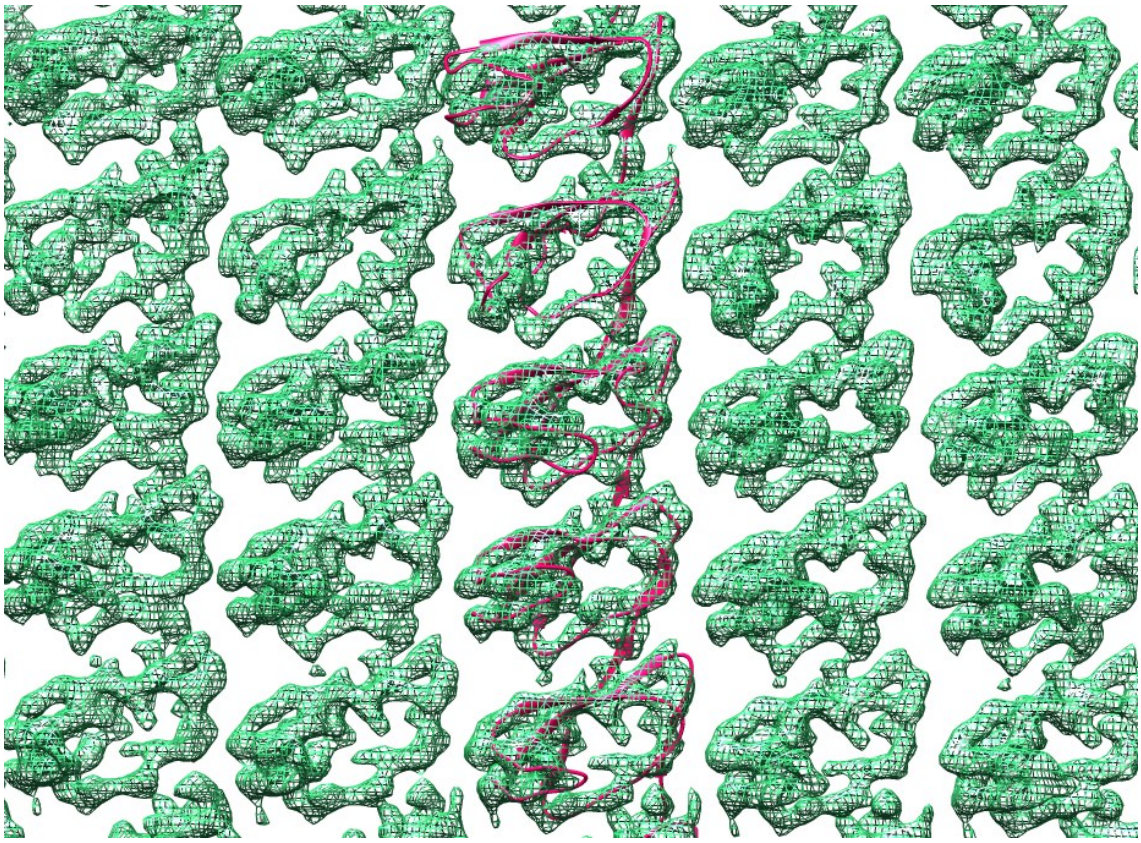


FIGURE 4. Magnified view at R3 to R7 domain of density map with the crystal structure of one MVP fitted in the density (PDBid: 4HL8).

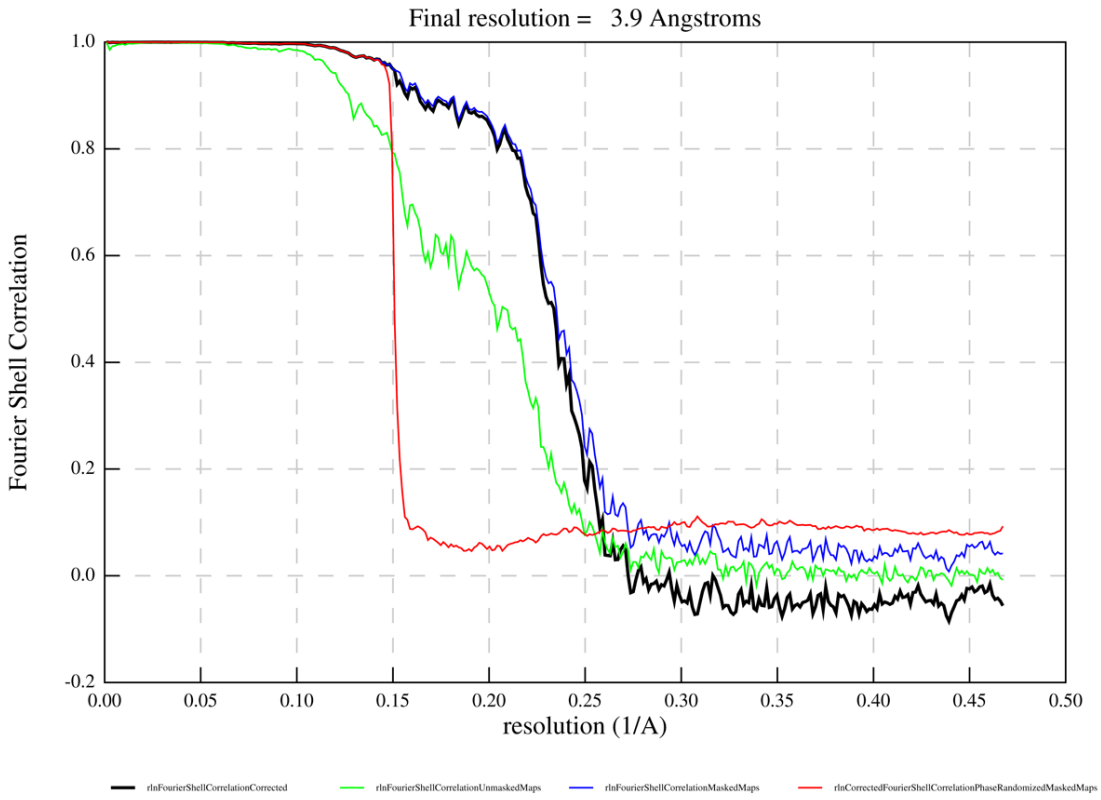


FIGURE 5. Fourier shell correlation correlation corrected (black line), unmasked maps(green), masked maps (blue) and phase randomised maps (red) showing that the resolution (FSC 0.143) is 3.9 Å.