

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://www.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.



Experiment title: Cryoelectron microscopy studies on a disassembly intermediate of a non-enveloped nodavirus

Experiment number:
MX-2025

Beamline: CM01	Date of experiment: from: 17-04-2018 to: 19-04-2018	Date of report: 17-01-2019
Shifts: 9	Local contact(s): Eaazhisai Kandiah	<i>Received at ESRF:</i>

Names and affiliations of applicants (* indicates experimentalists):

Dr. Manidipa Banerjee, Kusuma School of Biological Sciences, Block 1A, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, Delhi 110016, India, Phone: 91-11-26597538, Fax: 91-11-26597530, Email: mbanerjee@bioschool.iitd.ac.in

*Kimi Azad, Kusuma School of Biological Sciences, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, Delhi 110016, India, Email: Kimi.Azad@bioschool.iitd.ac.in

Report:

Cryo-electron micrographs of incrementally heated Flock House Virus (FHV) particles collected at ESRF (Experiment # MX-2025) were utilized to generate an asymmetric reconstruction to a resolution of 4.7 Å (Figure 1, A and C) using RELION 2.1. The reconstruction represents the eluted particle, a disassembly intermediate of FHV. The asymmetric reconstruction has revealed several essential global as well as local conformation alterations that occur during non-enveloped virus disassembly, including loss of membrane penetrating peptides from the capsid, and opening of a pore at the 2-fold axis on one side of the capsid. The cryomicrographs also contained a small fraction (~2%) of puffed particles, another FHV disassembly intermediate representing a more advanced stage of uncoating and characterized by a prominent surface protrusion (Figure 1B). These particles were manually picked from the cryomicrographs and processed to obtain a 3D reconstruction at 26.2 Å resolution (Figure 1D). The puffed particle represents a unique disassembly intermediate, captured during genome release. We aim to achieve a high resolution, asymmetric

reconstruction of the puffed particle to understand detailed conformational changes occurring in the capsid during genome release.

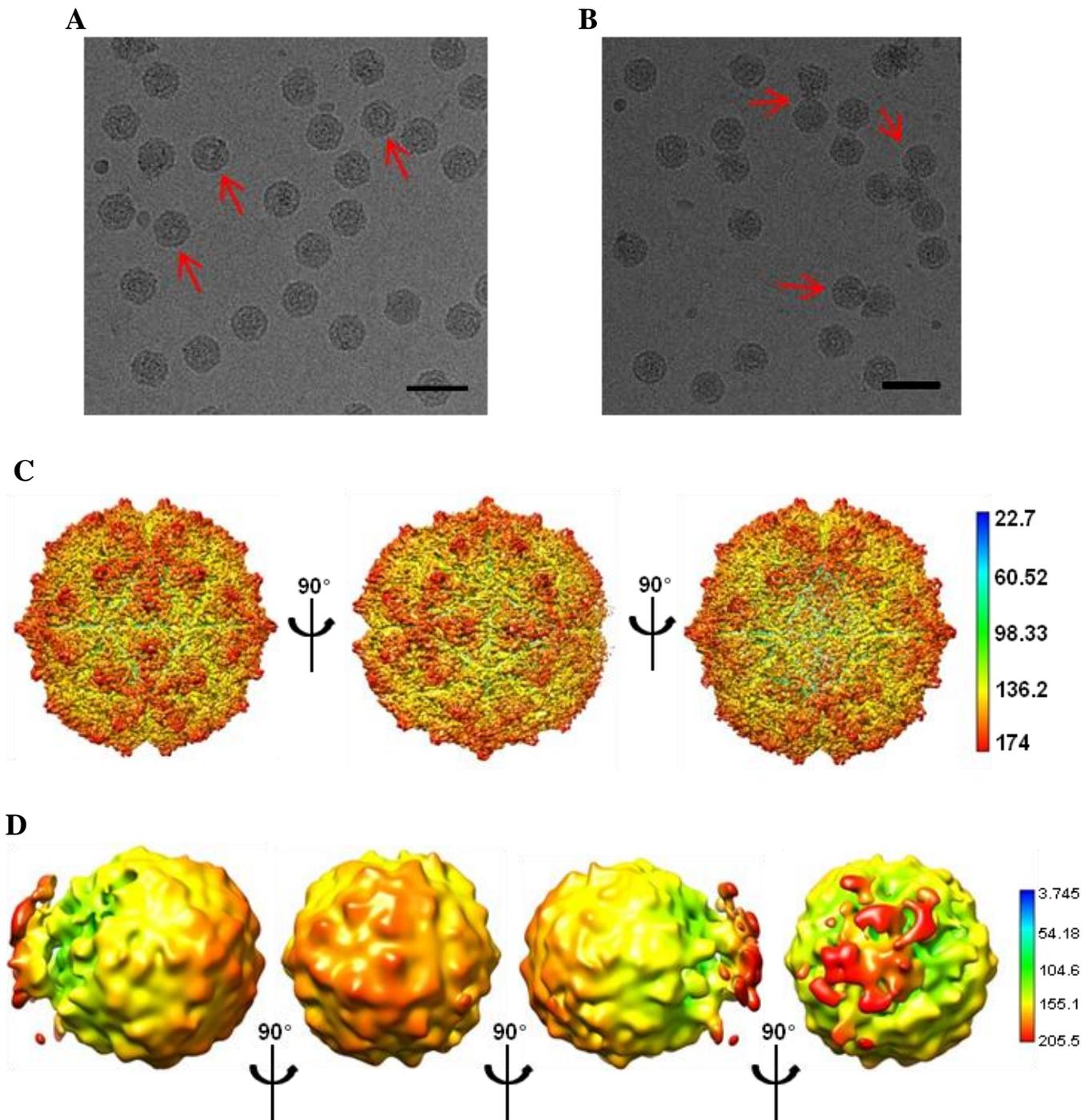


Figure 1: Asymmetric cryo-EM reconstruction of intermediate eluted and puffed particles. (A) Cryo-electron micrograph of frozen hydrated wildtype FHV particles heated to 70 °C. Red arrows point to the particles with considerably less dense centres. (B) Cryo-electron micrograph containing frozen hydrated FHV puffed particles (highlighted with red arrows). Scale bar = 50 nm. (C) Surface rendered, radially colored cryo-EM density map of reconstructed eluted particle (C) and puffed particle (D), in different orientations with the color key (right) displaying radial distance (in Å) from particle centre.