EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

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.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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:** CHIKV nsP1 in complex with m^7GMP cap structure	**Experiment number**:
Beamline: CM01	**Date of experiment**: from: 07/03/2022 to: 09/03/2022	**Date of report**: 24/08/2022
Shifts: 6	**Local contact(s)**: Dr. Eaazhisai Kandiah	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Rhian Jones AFMB CNRS UMR 7257

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Report:

Cap structures are universally present in eukaryotic mRNA 5' termini, and are critical for RNA stability, processing and translation. Many viruses such as Chikungunya (CHIKV) encode viral enzymes that append cap structures to the viral RNA to mimic host mRNA and hijack the host machinery. The aim of this experiment was to solve the structure of CHIKV nsP1 capping pore covalently bound to the 7-methyl guanosine monophosphate (m^7GMP) cap0 structure. NsP1 forms a covalent complex with the cap0 via catalytic histidine 37 prior to transfer to the 5' terminal phosphate of the viral RNA. The experiment was performed by Eaazhisai Kandiah, who communicated with us via Skype about the experimental set-up. Four replicate grids of nsP1 with the substrates (Quantifoil R2/2 gold 300 mesh with a graphene oxide coating) that had been pre-screened on the TALOS Artica at the CNB Madrid were loaded into the microscope. The ATLASes corresponded well to those aquired pre-transportation, and data collection was set up with multi-acquisition from two of the grids to have enough squares for collection, due to uneven graphene oxide coverage over the grids.

An automated data collection was set up with the following parameters; 4 images were collected per hole at a magnification of 105,000 in super-resolution mode (corresponding to a super-res pixel size of 0.42 Å/pix and a physical pixel size of 0.839Å/pix), spot size 4, defocus range: -0.8-2.5μm, exposure time: 1.8s distributed over 44 frames, with a dose of 1.01e^-/$Å^2$ per frame.

A total of 13,385 movies were collected from the first grid, and 11,295 from the second. All data was processed on the fly using the CM01 pipeline; monitored via the ExiMX interface. Movie drift was first corrected using MotionCorr2 and CTF correction performed with gCTF and CTFFind4. Particles are clearly visible at up to -0.8um defocus.

Data were transferred to the lab, and the movies were processed from within cryoSPARC. The processing was carried out by a collaborator (Michael Hons), and the data are also being processed in Relion.

3D classification yielded two major classes, one that exhibits dimsnsions typically observed for nsP1 dodecamers, and one where the monomer capping domains are inclined by 8^{o} relative to the centre of the ring, resulting in an overall dilation of the pore (0.3nm for the inner pore and 0.5nm for the overall ring) (Figure 1). In both cases, some density corresponding to an m^{7}GMP moiety is observed near the catalytic histidine, but the connection between the alpha phosphate and the histidien is unclear, and this data seyt may benefit from another aqusition from grids made with the SAH cofactor present. Nonetheless, this dataset has provided us with important insights into the structural dynamics of the nsP1 capping pathway.

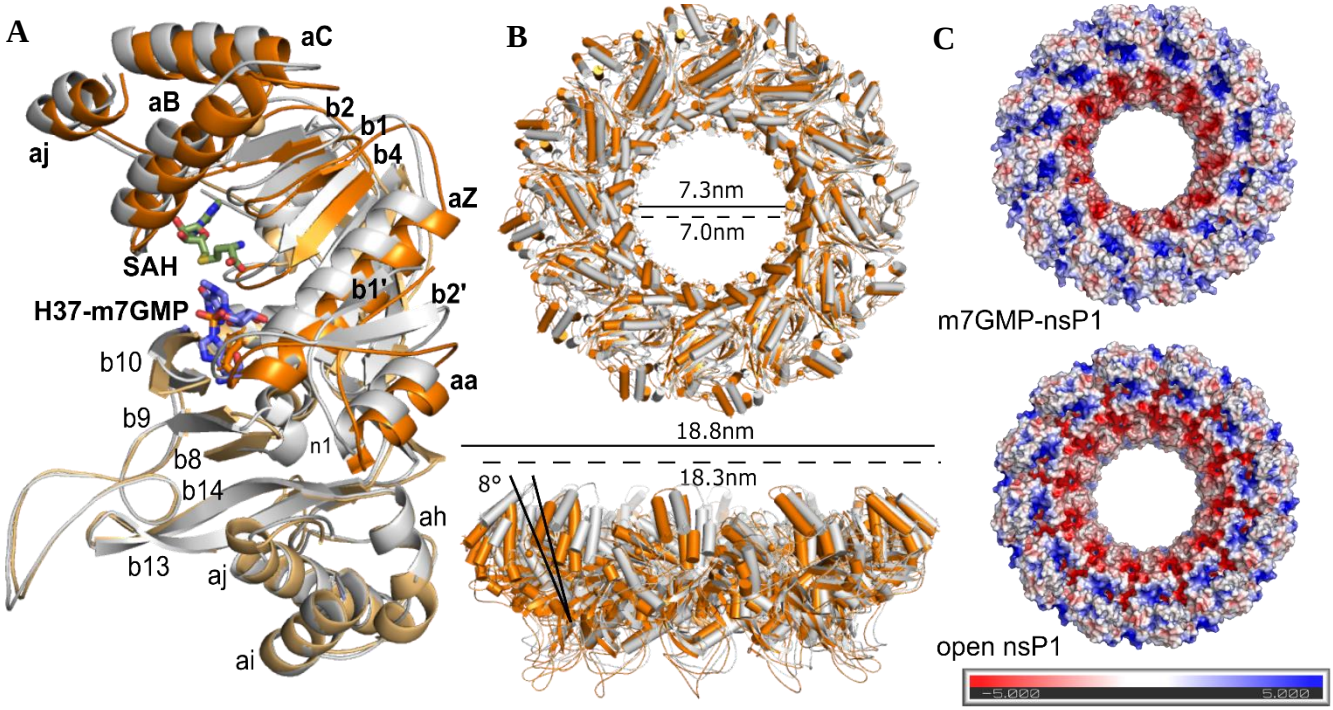

Figure 1: Differences in conformation between the 'open' (orange) and 'closed' forms of the nsP1 rings observed in this data collection. In all other previous data collections, only the closed form of the ring has been observed. A) Motions at the level of individual protomers is concentrated in the capping domain (coloured dark orange). B) In the context of the ring, this corresponds to an inclination by 8o and an opening of the ring. C) In turn, this changes the pattern of charges observed at the surface of the nsP1 rings.