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^7GTP and SAH substrates	**Experiment number**: mx2637
Beamline: CM01	**Date of experiment**: from: 21/01/2022 to: 23/01/2022	**Date of report**: 24/08/2022
Shifts: 6	**Local contact(s)**: Dr. Daouda Traore	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Juan Reguera AFMB CNRS UMR 7257

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 in complex with an S-adenosyl homocysteine (SAH) and 7-methyl guanosine triphosphate (m^7GTP) ligands. NsP1 uses these precursors to form a covalent complex with an m^7GMP cap0 structure that will eventually be transferred to 5' terminal phosphate of the viral RNA. The experiment was performed by Daouda Traore, 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 by Daouda. The ATLASes corresponded well to those aquired pre-transportation, and data collection was set up with multi-acquisition from three 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: 1-2.5μm, exposure time: 1.8s distributed over 40 frames, with a dose of 0.95e^-/Å^2 per frame yielding a total dose per movie of 38e^-/Å^2.

A total of 6035 movies were collected from the first grid, 2076 from the second and 2754 from the third. 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. Particles are clearly visible at up to -0.8um defocus.

Data were transferred to the lab, and the movies were processed from within Relion 3.1.3. Following motion correction (Motion Corr2) and CTF correction (CTFFind 4), 4461 of the 10865 movies were taken forward for processing following pruning for high astigmatism, or CTFs that did not exhibit Thon rings beyond 4-5Å. A very high number of micrographs were discarded due to a lack of graphene oxide coverage (and absence of particles) as multi-grid set-up in EPU does not allow for hole selection.

Using Relion's autopicking procedure, 561,683 particles were selected from the dose-weighted micrographs (roughly 126 per micrograph), and 470,236 were retained following 2D classification. 2D classification yielded classes that correspond mostly to single ring projections of nsP1 (purified in GDN detergent); an improvement on previous data collections in fos-choline detergent where both single rings and sandwiches of single rings (an artefact of association via the exposed detergent micelle) were observed. This improvement in data homogeneity facilitated processing, and 3D classification did not yield any major differences in species in the dataset and was just used to 'clean' particles and improve the final resolution of reconstructions.

Finally, 241,438 particles were 3D refined using c12 symmetry (no differences in the maps were observed in c1) to 2.8Å resolution (2.5Å post-processing, Figure 1).

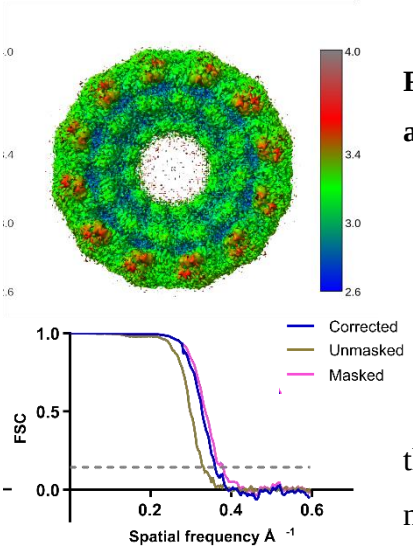

Figure 1: Local resolution plot of nsP1 rings bound to SAH and m^{7}GTP ligands and FSC. The dashed line represents the 0.143 threshold.

Contrary to structures collected with GTP or SAM ligands alone from the previous step of the nsP1 capping pathway (involving the methylation of GTP to produce m^{7}GTP), the ligands are much more clearly defined in all of the 12 active sites of the dodecamer (Figure2). We propose that this reflects the need for binding of both m^{7}GTP and SAH ligands simultenously within the active pocket for correct positioning of the m^{7}GMP moiety for transfer and organisation of the active site- we identify several key amino acids that change rotamers and contacts to the ligands relative to the SAM and GTP bound structures. This correlates with biochemical data that shows that SAH is needed as a cofactor for transfer of the m^{7}GMP cap from the m^{7}GTP to the protein.

Finally, unexpectedly we do not observe any transfer of the m^{7}GMP cap to the nsP1, despite the presence of magnesium and SAM cofactors. This was an unexpected result, but analysis of the active site suggests that the m^{7}GTP alpha phosphate group is too distant for nucleophilic attack, and that the leaving group is incorrectly positioned. We hypothesise that this structure corresponds to a metastable state (as observed for other guanylyltransferases), and could serve as a checkpoint in the nsP1 capping reaction. Indeed, subsequent

cryoEM data collections where the protein was incubated with both substrates and an RNA that is poorly capped show that the RNA serves as a stimulus for transfer of the m^7GMP cap to the protein. The PDB model and maps have been submitted to the PDB and EMDB (8AOW and EMD-15554), and we have submitted a paper for publication describing different structural states of the CHIKV nsP1 capping pathway.

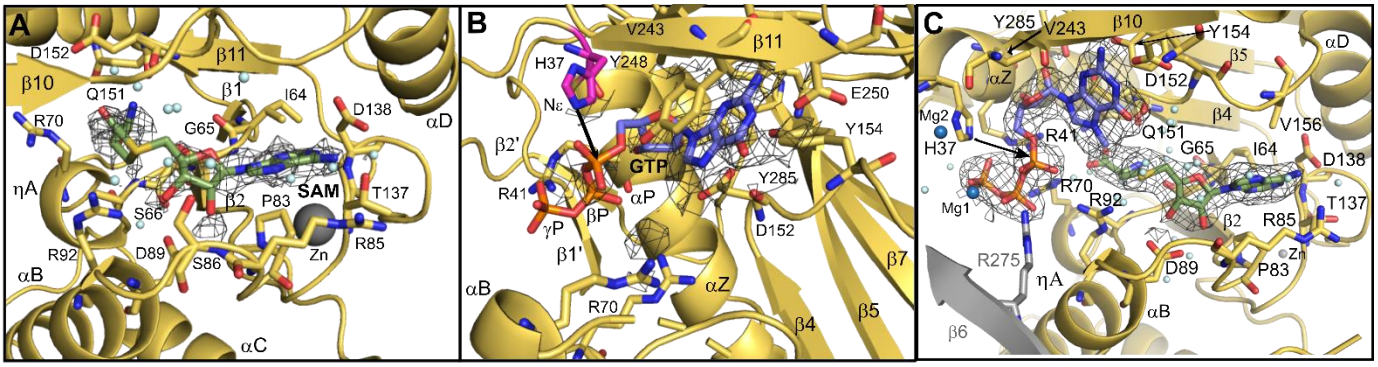

Figure 2: Comparison of ligand densities in c12 symmetrised maps for A) SAM bound nsP1 (CM01 data collection mx2261), B) GTP bound nsP1 and C) SAH and m^7GTP bound nsP1 (this experiment). The ligand density is substantially improved when both ligands are bound togther (all maps corresponding to the ligands are contoured at sigma level 2.0).