EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

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 RNA substrate	**Experiment number**:
Beamline: CM01	**Date of experiment**: from: 26/10/2022 to: 28/10/2022	**Date of report**: 04/08/2023
Shifts: 6	**Local contact(s)**: Dr. Michael Hons	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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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 also append cap structures to the viral RNA during infection, allowing hijacking of the host translational machinery and for evasion of the immune response. Alphaviruses such as CHIKV encode a unique non-structural protein (nsP1) for viral RNA capping that forms a membrane associated dodecameric pore in viral replication organelles in the host cell. NsP1 caps the RNA via a non-canonical pathway where methylation of a GTP nucleotide precedes gunaylation of the enzyme and transfer of the m^7GMP moiety to the RNA. To date, at the ESRF we have obtained structures of nsP1 in complex with substrates corresponding to the methylation, guanylation and nsP1-covalent cap formation of the pathway, but we are lacking a structure of the protein in complex with an RNA substrate. The aim of this experiment was to solve the structure of nsP1 in complex with an RNA substrate corresponding to the first 15 nucleotides of the CHIKV genome, that we have demonstrated is capped by the enzyme *in vitro*.

The experiment was performed by Dr. Michael Hons, who communicated with us via Skype about the experimental set-up. Two replicate grids of nsP1 with the RNA in 5:1 molar ratio (Quantifoil R2/2 gold 300 mesh with a graphene oxide coating) that had been pre-screened on a TALOS Artica were loaded into the microscope, in addtion to two replicates with a 5:1 molar ratio of the RNA plus capping substrates (SAH amd m^7GTP). Michael set up one data acquisition from the grid with the RNA alone, and one from the RNA with the capping substrates using EPU multi-grid. The following parameters were used for the aquisition; 2 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 35 frames, with a dose of 1.02e^-/$Å^2$ per frame. The mutli-grid set up has been enormously useful to the nsP1 project, allowing us to collect from a sufficient number of holes in the case of poor graphene oxide coverage, or collection of different datasets within a single session now that the camera aquistion rates are so rapid.

A total of 13,385 movies were collected from the RNA+substrates grid, and 11,295 from the second grid (RNA only). The motion corrected movies were imported into cryoSPARC for patch CTF correction, where movies with excessive drift, bad ice or CTFs where Thon rings did not go beyond 4A were excluded, yielding 12,363 and 8309 movies respectively.

For the RNA only dataset, between 139,000-228,000 particles were taken forward from different 2D classifications, where a good distribution of partcile views were present in the dataset. Heterorefinement in cryoSPARC yielded only one major class, and different 3D classification and refinement strategies were performed with C1 and C12 symmetry with between 80-90,000 particles, yielding final reconstructions at 2.94Å (C12) and 3.4Å (C1) with non-uniform refinement. Unfortunately, all active sites in the dodecamer were empty, so we did not attempt to improve the resolution of the structure further using polishing or further focussed classification techniques.

For the RNA plus substrates dataset, between 120,000 and 180,000 particles were used for final reconstructions post 2D and 3D classification, from an initial 2 million picked particles. Again, unfortunately despite going to high resolution (2.7Å with non-uniform refinement and imposing C12 symmetry), no signal corresponding to the RNA could be found in any of the active sites.

We belive that it is likely that the enzyme binds to the RNA transiently, and that the capping reaction turns over rapidly in the presence of the substrates. In this case, we think that a structure with the RNA bound nsP1 will only be possible to achieve with cross-linking experiments, or by using an active site mutant. It is also possible that complexes with the other nsPs will reinforce binding of the RNA.

The data from the 3 mx cryoEM France BAG collections collected aside from this one have now been published in PNAS, where Dr. Michael Hons is a cofirst author (Structural basis and dynamics of Chikungunya alphavirus RNA capping by nsP1 capping pores, PNAS March 2023, vol.120.no.12 https://doi-org.insb.bib.cnrs.fr/10.1073/pnas.2213934120).

We thank the ESRF and all local contacts for their helpful advice and patience in data collections (particularly when working with graphene oxide grids!)