Standard Experimental Report
(All fields are mandatory)

Proposal title: **SARS-CoV-2 orf7a and orf8 proteins and their Zn-mediated interaction with BST2**

Proposal number: 20210695
Beamline: FAME-UHD
Shifts: 18
Date(s) of the experiment: 12/4/2022 - 19/4/2022
Date of report: 13/02/2023

- Objective & expected results (less than 10 lines): **-**

The goal of the proposed XAS measurements was to provide insights on the binding mode of BST2, orf7a and orf8 proteins with Zn(II) ions. In particular, the results of the experiments will allow determining the conditions in which Zn(II) ion coordinate orf and BST2 proteins and how the presence of BST2 affects the coordination mode of orf7 and orf8.

- Results and the conclusions of the study (main part): **-**

The SARS-CoV-2 open reading frames ORF7a and ORF8 code for virion non-structural (accessory) proteins, orf7a and orf8, respectively, of yet unknown function. The orf7a protein is common to all SARS-CoV type coronaviruses and is highly conserved, while orf8 is remarkably different from proteins coded by genes ORF8 and ORF8b of human SARS-CoV-1. Orf7a is expressed in the host cell and is likely to be involved in the inhibition of the intracellular (at endoplasmic membrane) process of virion immobilization before and after virion vesiculation. On the basis of sequence similarities, one can argue that also the orf8 protein can be involved in the same inhibition process, strengthening the inhibition of virion immobilization.
Tethering processes are mainly performed by proteins of the tetherin family, also known as BST2 or cluster of differentiation 317 (CD317). BST2 is expressed in many cells in the interferon-dependent antiviral response pathway and is able to block viral replication by trapping enveloped viral progeny on the surface of infected cells, leading to virus internalization and degradation.
In this proposal, we performed X-ray Absorption Spectroscopy (XAS) measurements aimed at studying the interaction of orf7a and orf8 with the tetherin protein known as bone marrow stromal antigen 2 (BST2). The BST2 dimerization process is strongly influenced by the presence of divalent cations, such as Zn(II), as they may interfere in disulfide Cys bond formation. Due to the low concentration of the samples, we focused the experimental session on orf7a samples.
In Figure 1, we compare the XANES spectra of BST2 with the Zn in buffer. The difference between the two spectra clearly demonstrates that BST2 is able to bind Zn.

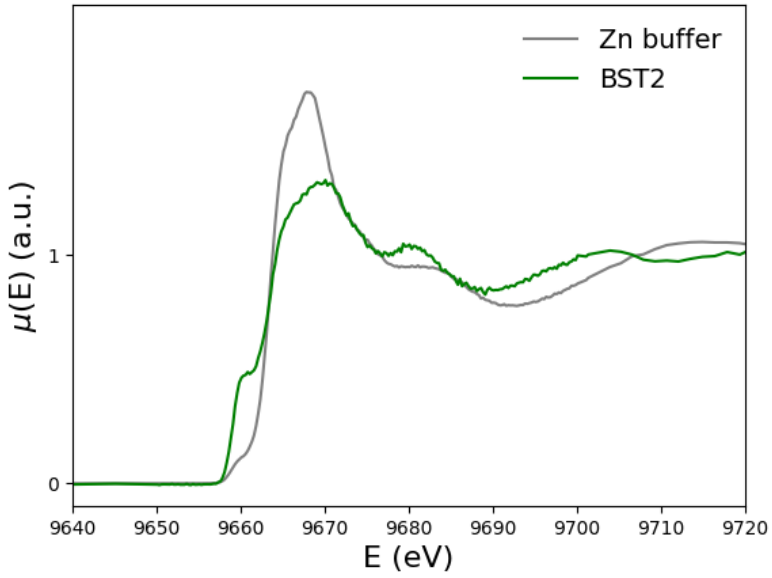

Figure 1 - XANES spectra of Zn in buffer (in gray) and BST2 (in green).

In Figure 2, we compare the XANES spectra of orf7a in two different variants, 16-81 and 15-82, with the Zn in buffer. The difference between the spectra clearly demonstrates that both variants of orf7a are able to bind Zn.

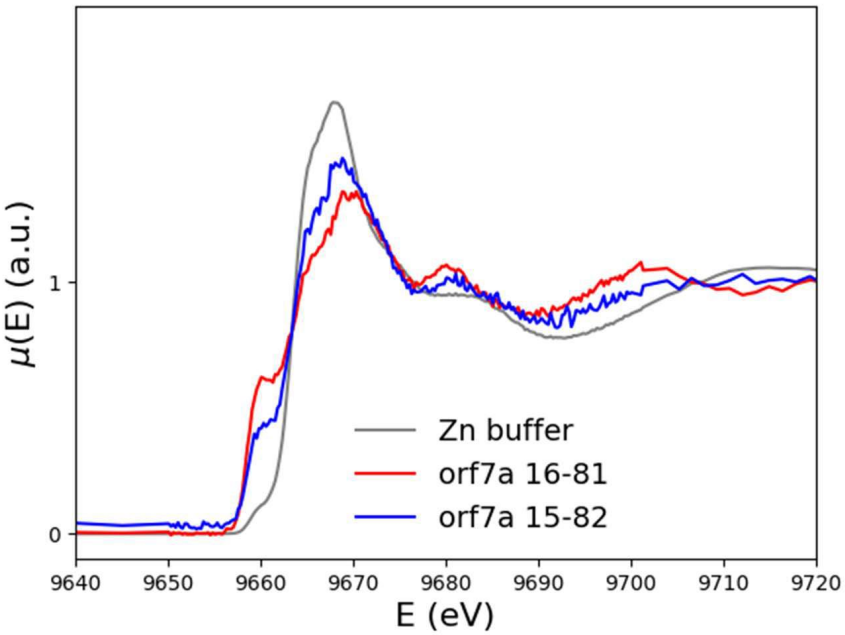

Figure 2 - XANES spectra of Zn in buffer (in gray) and orf7a.

Finally, in Fig.3 we show instead the XANES spectra of Zn-orf8a in two different lengths, 15-82 and 16-81, and two different preparation protocols. The differences observed between the

XANES lead us to hypothesize that the interaction with Zn, even considering the same ORF7a fragment, varies when measurements are performed on precipitated protein or on protein in solution. This is in agreement with our previous findings coming from measurements performed at FAME-UHD [Petrosino 2021].

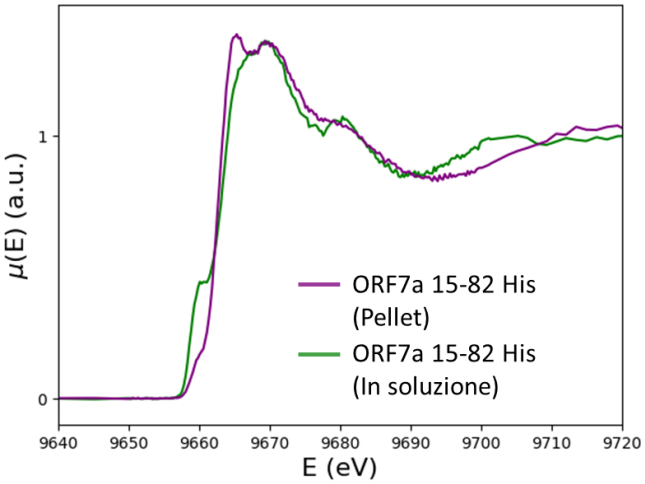

Figure 3 - XANES spectra of Zn-orf7a in two different lengths, 15-82 and 16-81, and two different preparation protocols.

The quantitative analysis of the XAS data is still ongoing and different possible Zn-orf7a binding sites are being built with the help of numerical simulations. Moreover, the XAS experiments we performed also provided valuable information on orf7a/BST2 Zn coordination and on the sample preparation protocols. The measurements collected in this report will have to be complemented by similar measurements performed on Zn-orf8-BST2 complexes.

- Justification and comments about the use of beam time (5 lines max.): **-**

We made use of all the assigned beamtime in order to determine the best experimental conditions in terms of sample preparation protocols and metal and protein concentrations in order to obtain reproducible coordination mode between Zn(II) ions and the considered proteins. The process was particularly challenging due to the low protein and metal concentrations.

- Publication(s): **-**

M. Petrosino *et al.* (2021). *Chemistry Open*, **10**, 1133-1141.