ESRF	**Experiment title:** SAXS structure of SARS-CoV-2 virulence factor Nsp1: a step forward to unveil its pathogenic mechanism	**Experiment number**: MX2385
Beamline: BM29	**Date of experiment**: from: 11 June2021 to: 12June 2021	**Date of report**:
Shifts: 3	**Local contact(s)**: Mark Tully	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

The data was collected by the Local Contact, Mark Tully.

Report:

A total of three samples were analysed in the BM19, however it was only possible to obtained good dataset for only the full-length native protein. SAXS data were collected at the ESRF BioSAXS beamline BM29 [1]. An inline HPLC system (Shimadzu) was used [2] coupled directly to the BM29 in vacuum. The sample was loaded into vials and automatically injected onto the SEC, Superdex 200 10/300 GL (GE Healthcare), equilibrated with the corresponding buffer at room temperature via an integrated syringe system SAXS data were collected using X-rays of wavelength of 0.9919 Å and a sample-to-detector distance of 2.81 m corresponding to q ranges of 0.08– 4.5 nm^{-1}. About 1200 frames (at 2 sec/frame) were collected for each sample, where q is the magnitude of the scattering vector given by $q = 4\pi/\lambda \sin(\theta)$, with 2θ the scattering angle.

The beamlines integrated automated processing pipeline integrated each individual frame [3] before buffer subtraction and primary data processing was carried out using Scatter IV [2]. The SEC-SAXS was unable to completely separate the protein from the aggregates leading to overlapping peaks. To determine the number of distinct scatterers and extract the individual components the program BioXTAS RAW, which encompasses a single value decomposition (SVD) with evolving factor analysis (EFA) tool was used [4, 2].

Full-length native protein was analysed by SAXS in order to confirm the different oligomeric states obtained by SEC. Therefore, the sample carried forward for in-depth analysis was the native condition using SEC elution. The protein show similar SAXS scattering profiles and the Guinier plots of each scattering profile provide a linear fit for a monodisperse solution (Fig. 1A), and the radius of gyration (Rg) was calculated from this approximation, given a Rg 24.56 Å, and a I_0= 18. The Kratky plot, shows that the protein is correctly

folded and it shows the main peak position for globular particles (Fig. 1B). Based on the pair distance distribution function [P(r)] determined by the indirect Fourier transform from the scattering profile curves using GNOM [21], it was possible to determine the molecular mass of the sample and compare it with the calculated theoretical molecular mass determined from the known sequence. Pair-distance, P(r), distribution function, shows a single maximum at a Dmaximum of 78 Å, is the largest non-negative value that supports a smooth distribution function (Fig.2).

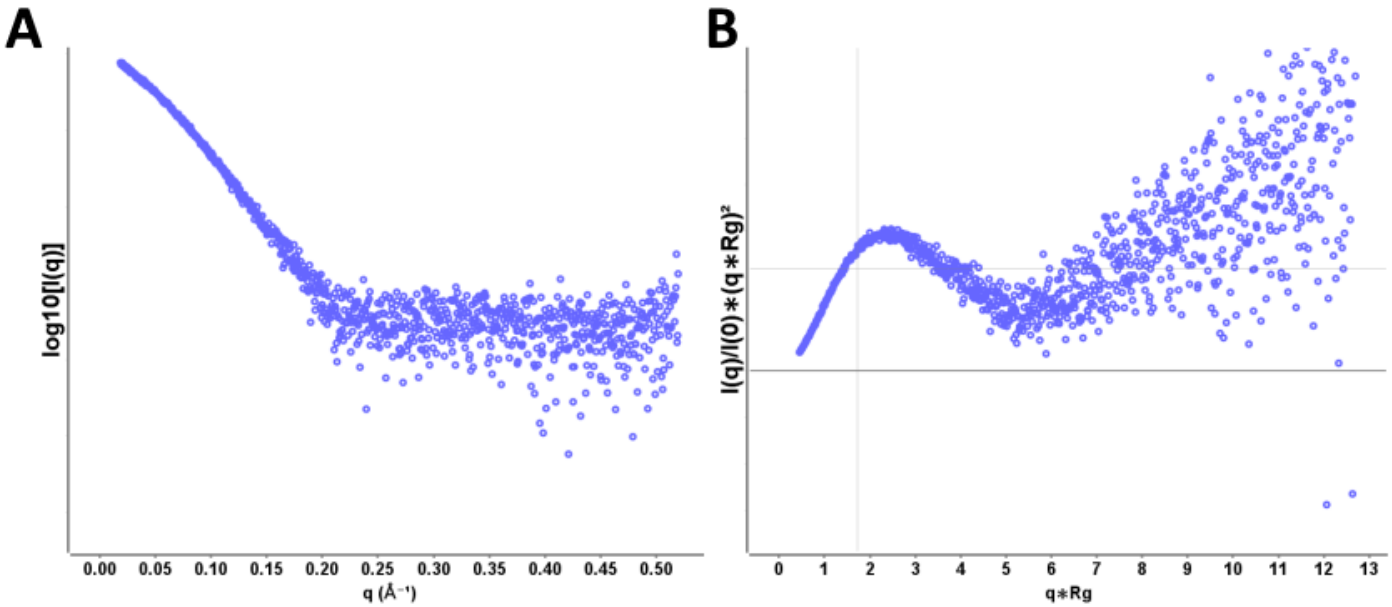

Fig. 1. **A.** Log_{10} SAXS intensity versus scattering vector, q. Plotted range represents the positive only data within the specified q-range. **B.**Dimensionless Kratky plot. Cross-hair marks the Guinier-Kratky point (1.732, 1.1), the main peak position for globular particles.

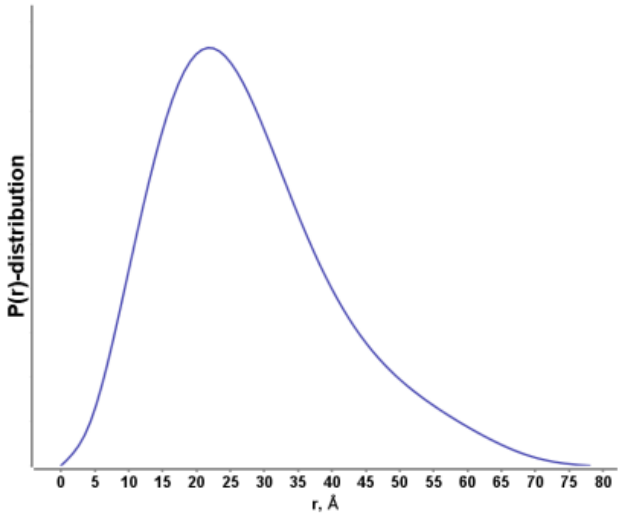

Fig. 3 Pair-distance, P(r), distribution function. Maximum dimension, dmax, is the largest non-negative value that supports a smooth distribution function.

References:

[1] Pernot, P., Round, A., Barrett, R., De Maria Antolinos, A., Gobbo, A., Gordon, E., Huet, J., Kieffer, J., Lentini, M., Mattenet, M., Morawe, C., Mueller-Dieckmann, C., Ohlsson, S., Schmid, W., Surr, J., Theveneau, P., Zerrad, L. & McSweeney, S. (2013). J. Synchrotron Rad. 20, 660-664.

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