Experimental report

09/09/2022

Proposal: DIR-215 **Council:** 4/2020

Title: Interaction of CoV-2 peptide responsible for fusion (S2 peptide) withbiomimetic membranes by neutron reflectometry

Research area:

This proposal is a new proposal

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Samples: Peptides and natural lipid extracts

Instrument	Requested days	Allocated days	From	To
FIGARO	6	2	20/08/2020	22/08/2020

Abstract:

Experimental report of DIR215 on FIGARO

Interaction of CoV-2 peptide responsible for fusion (S2 peptide) with biomimetic membranes by neutron reflectometry (NR)

Scientific Background

β-Coronaviruses (CoVs) are single-stranded positive sense RNA viruses[1] that belong to class I viral fusion viruses. They can infect cells via direct membrane fusion mechanism[2]. Upon viral entry, copies of the genome are made in the cytoplasm, followed by expression of viral proteins and final assembly of functional viral particles, which are then released from the cell. The main structural components of β-coronaviruses include a lipid envelope, the Spike (S), Membrane (M), and Envelope (E) proteins, as well as the Nucleoprotein (N), which forms complexes with the viral RNA. Although it is already well-know that the S1 spike protein domain contains the receptor binding site for the angiotensin-converting enzyme 2, ACE-II[3], it has to be underlined that the S2 spike subunit contains a fusion domain that is responsible for triggering the fusion between lipid bilayers. After S1-ACE-II binding, a proteolysis-triggered conformational change in the C-terminal S2 subunit occurs[3]: S2 continues to be embedded in the viral membrane, but its heptad repeat (HR) 1 and 2 domains associate to form a six-helix bundle fusion core[4]. Proteolysis at the S2' site (at residue 816) subsequently frees the Spike protein fusion domain, which associates with the host cell and initiates membrane fusion. However, the molecular mechanisms driving this fusion process are not deeply understood. The aims of the experiments summed up here were to unravel the fusion mechanism, by simplifying the systems down to its core elements: 22 to 25 amino acids peptides derived from the fusion domain of the Spike (called fusion peptides, FP1, 2 and 4) were employed, and their interaction with cell membrane mimicking lipid monolayer was investigated.

Results

The results obtained from this beamtime have been complemented with studies with NR with lipid bilayers, SANS, QENS and NSE and published on JACS in 2022 by Santamaria et al[5]. Moreover, such results has been included in Andreas Santamaria's PhD thesis.

To better mimic the real picture of the infection, relying on the interaction of the Spike protein with the plasma membrane (PM), a model membrane that could well replicate the latter was exploited. This PM biomimetic model was made of natural phospholipids (hydrogenous and deuterated) extracted from yeast grown at the ILL D-Lab, and purified at the lipid platform of ILL (L-Lab). Deuterated cholesterol was provided by the ANSTO deuteration platform. The PM out-of-plane structure at Π=22mN·m^{-1} was investigated with NR. Four different isotopic contrasts were employed: hydrogenous PM in ACMW (8.1% *v/v* D_2O) and D_2O, and deuterated PM in ACMW (8.1% *v/v* D_2O) and D_2O. The same contrasts were employed to investigate FP interaction and insertion with the PM model. Data modelling was performed by simultaneously fitting all contrasts to obtain a single set of structural parameters that allowed us to determine the volume fraction of peptides partitioning into the monolayer. Two-layer models, which included the partition of the FPs into the aliphatic lipid tails and lipid polar headgroups, were adequate to describe the experimental data. Including in the model a third layer for the peptides yielded a worse fit of the data and suggested that the interaction of FP1, FP2 and FP4 with the membrane is not due to a physisorption of the peptides, but rather to their insertion directly into the lipid monolayer. The bound FP1 is found distributed across the entire PM monolayer, as it is present in both the lipid headgroups (6%) and aliphatic tails (7%) (see **Figure 1 C**). In contrast, FP2 and FP4 had a negligible presence in the acyl region, but interacted more strongly within the lipid headgroups region (17% for FP2 and 25% for FP4, **see Figure 1 F** and **I**), where significant decreases in hydration were observed.

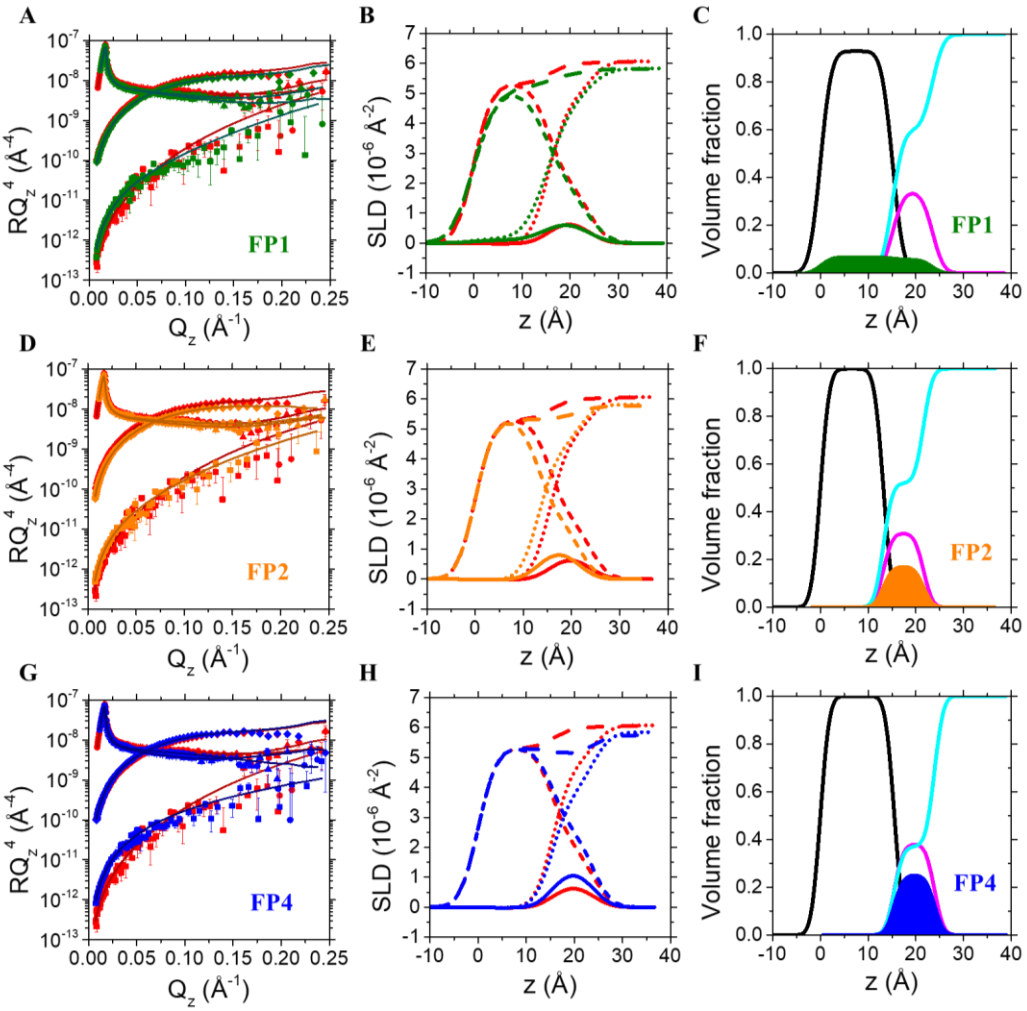

Figure 1 Experimental (symbols) and simulated (lines) NR profiles of PM monolayers in the absence (red) and presence of **A** FP1, **D** FP2 and **G** FP4 (green, orange and blue). Data at two isotopic contrasts (D_2O and ACMW) have been measured for hydrogenous (circle and squares) and deuterated (triangles and diamonds) lipid monolayers. Solid lines in **A**, **D** and **G** are simulated curves. SLD profiles corresponding to fits of the isotopic contrasts are plotted in **B**, **E** and **H**. Continuous, dot-dot, short and long dashed lines indicate the monolayer SLD profile in ACMW and D_2O with hydrogenous lipids, and in ACMW and D_2O isotopic contrast with deuterated lipids, respectively. Volume fraction profiles normal to the interface of PM monolayers highlighting the distribution of tails (black), heads (magenta), water (cyan), and **C** FP1 (green), **F** FP2 (orange) and **I** FP4 (blue).

References

[1] T. S. Fung and D. X. Liu, "Human Coronavirus : Host-Pathogen Interaction," pp. 529–560, 2019.

[2] T. Tang, M. Bidon, J. A. Jaimes, G. R. Whittaker, and S. Daniel, "Coronavirus membrane fusion mechanism offers a potential target for antiviral development," *Antiviral Res.*, vol. 178, no. April, p. 104792, 2020.

[3] M. Hoffmann *et al.*, "SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor," *Cell*, vol. 181, no. 2, pp. 271-280.e8, 2020.

[4] S. Xia *et al.*, "Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion," *Cell Res.*, vol. 2, no. February, 2020.

[5] A. Santamaria *et al.*, "Strikingly Different Roles of SARS-CoV-2 Fusion Peptides Uncovered by Neutron Scattering," *J. Am. Chem. Soc.*, 2022.