Experimental report

21/08/2022

Proposal: DIR-211 **Council:** 4/2020

Title: Membrane dynamics influenced by fusion peptides of respiratory syndrome coronaviruses

Research area:

This proposal is a new proposal

Main proposer: Tilo SEYDEL

Experimental team: Judith PETERS
Tatsuhito MATSUO

Local contacts: Michael Marek KOZA
Jacques OLLIVIER
Ingo HOFFMANN
Armando MAESTRO
Olga MATSARSKAIA
Tilo SEYDEL
Anne MARTEL
Lionel PORCAR
Sylvain PREVOST
Ralf SCHWEINS
Orsolya CZAKKEL
Francesca NATALI
Tatsuhito MATSUO

Samples: Lipids
D2O
Artificial viral fusion peptides

Instrument	Requested days	Allocated days	From	To
IN13	10	10	08/02/2021	18/02/2021
IN15	4	4	28/08/2020	01/09/2020
			22/09/2020	24/09/2020
IN16B	4	4	08/02/2021	12/02/2021
IN5	3	3	23/08/2020	26/08/2020
D11	3	3	27/08/2020	28/08/2020

Abstract:

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Scientific background. Coronavirus disease-2019 (COVID-19), a potentially lethal respiratory illness caused by the coronavirus SARS-CoV-2, emerged in the end of 2019 and continues to spread aggressively across the globe. A thorough understanding of the molecular mechanisms of cellular infection by coronaviruses is therefore of utmost importance. Since the fusion between viral and host membranes is a crucial step in viral infection, we investigated the role of selected SARS-CoV-2 Spike fusion peptides (named FP1-FP4), and the influence of calcium and cholesterol, on this fusion process using model membranes. Structural information from specular neutron reflectometry (NR) and small angle neutron scattering (SANS), complemented by dynamics information from quasi-elastic (QENS) and neutron spin−echo (NSE) spectroscopy, revealed strikingly different functions encoded in the Spike fusion domain and allows us to establish a tentative fusion mechanism mediated by the FPs.

Experimental results. In this report, we present selected information deduced from our measurements. For more details, we refer to our **publication** (Santamaria, A. et al. (2022). Strikingly different roles of SARS-CoV-2 fusion peptides uncovered by neutron scattering. *Journal of the American Chemical Society*, *144*(7), 2968-2979).

In **Fig. 1 (A-C)**, SANS profiles of "P" liposomes (35% cholesterol) in the presence of FP1, FP2 and FP4, respecitvely, are shown. The legend in Fig. 1C shows the peptide:lipid molar ratios. FP3 showed the weakest effects and is therefore not featured here. FP1 and FP4 have the most pronounced effects. Due to the preservation of the overall amount of membrane and the decreasing low q intensity, we assume that FP1 may lead to lipid disk formation, indicating a possible perforation of the liposomes. Figs. 1D and 1E show the effects of FP1 on "O" liposomes (without cholesterol) and on P liposomes, and these effects are less pronounced than those of FP4 on P liposomes. In Fig. 1F, the bending rigidities of O and P liposomes are displayed. Small quantities of FP1 soften the membranes, while larger amounts of FP1 render O liposomes more stiff. This latter effect is traced back to the formation of multilamellar membranes.

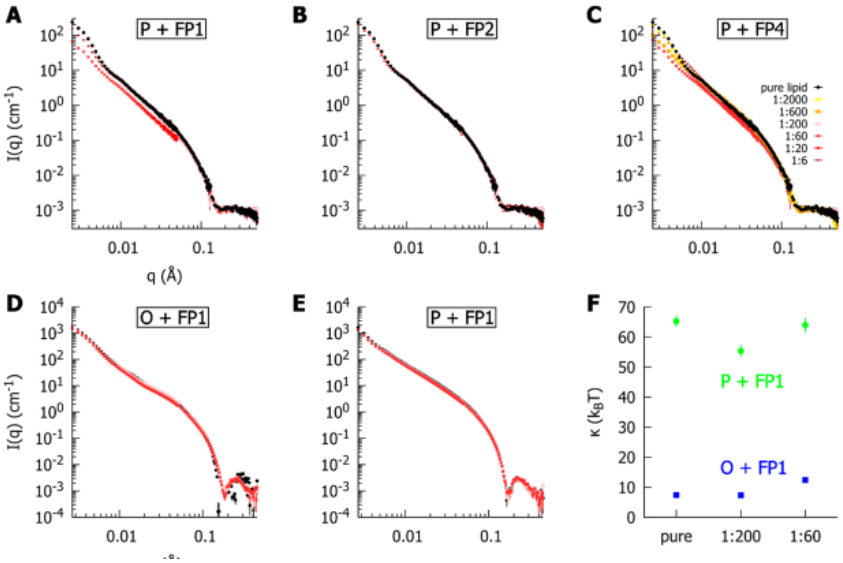

Figure 1. SANS and NSE data on the effects of fusion peptides on model liposomes (see text for details).

Table 1 shows lipid diffusion coefficients and residence times derived from QENS data. Higher FP1 and FP2 concentrations

	D ($\times 10^{-5}$ cm^2/s)	t (ps)
pure lipids	1.93 ± 0.03	1.7 ± 0.1
lipids + FP1(1:200)	1.97 ± 0.03	1.65 ± 0.1
lipids + FP1(1:20)	2.36 ± 0.04	1.9 ± 0.1
lipids + FP2(1:200)	1.97 ± 0.02	1.65 ± 0.1
lipids + FP2(1:20)	1.62 ± 0.02	1.3 ± 0.1

(1:20 peptide:lipid ratio) lead to a faster diffusion compared to pure liposomes without added FPs, which may complement the finding that FP1 induced lipid reorganization within the membrane.

Fig. 2 shows NR results on the interaction of FP1 with a cholesterol-containing model membrane. Notably, this interaction is Ca^{2+}-dependent: the addition of Ca^{2+} leads to an insertion of FP1 across the membrane, as seen in the increase of the transmembrane FP1 volume fraction. Upon chelating Ca^{2+}, FP1 inserts itself only into the outer leaflet of the membrane. No insertion of FP1, FP2 and FP4 into the inner leaflet was observed.

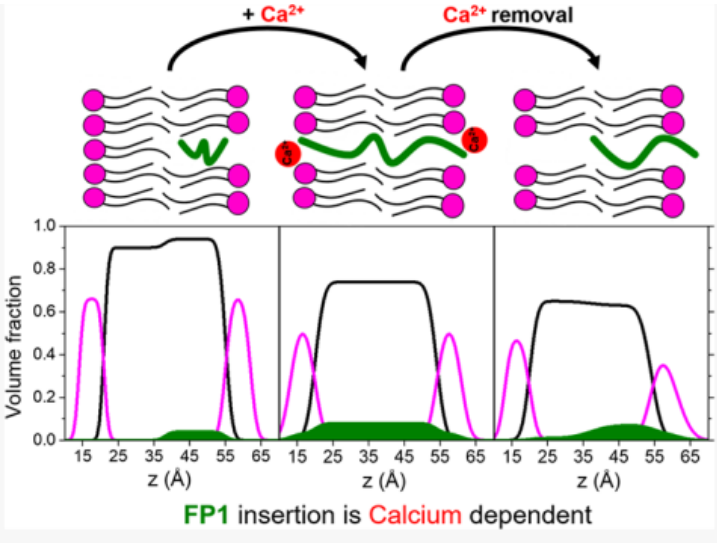

Figure 2: Schematic of NR results on the insertion of FP1 into a model membrane (see text for details).

Conclusions. We observe surprisingly different roles and functions of the four FPs investigated. Our data (see our publication for details) allow us to propose a fusion mechanism between SARS-CoV-2 and host cell membranes: FP1 initiates binding, and FP4 bridges the viral and the host membranes. Infection of the host cell ensues. FP2 and FP3 show overall weaker effects and may be tentatively attributed structural roles. We note that our results are not only of interest in the context of the current COVID-19 pandemic, but also lay the foundation for powerful interdisciplinary research of viruses and virus-host interactions.