Experimental report

02/09/2022

Proposal: DIR-208 **Council:** 4/2020

Title: Small-angle neutron scattering to study the interaction of the SARS-CoV-2-S2 peptide with lipid membranes

Research area:

This proposal is a new proposal

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Experimental team:

Local contacts: Olga MATSARSKAIA
Anne MARTEL

Samples: D lipids

Instrument	Requested days	Allocated days	From	To
D22	3	3	28/08/2020	29/08/2020
			10/02/2021	11/02/2021

Abstract:

Experimental report - DIR-208

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. This report focuses on the SANS part of these experiments, which are also featured in the report for experiment DIR-211.

Experimental results. A preliminary characterisation of the interaction between liposomes and was performed on D11 (D22 and D11 were switched between DIR-211 and DIR-208). Here, different ratios of FPs and liposomes were mixed and the stability of the liposomes was assessed (Fig. 1). This allowed us to select appropriate, optimal conditions for later experiments (Fig. 2).

In **Fig. 2 (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.

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. 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).

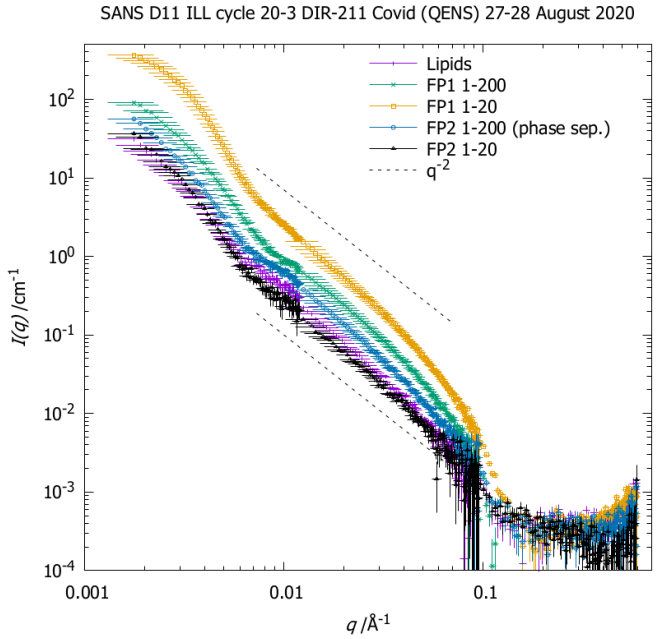

Fig. 1: Different FP1/2:liposome ratios. The increasing forward scattering indicates changing liposome interactions in the presence of higher concentrations of FP.

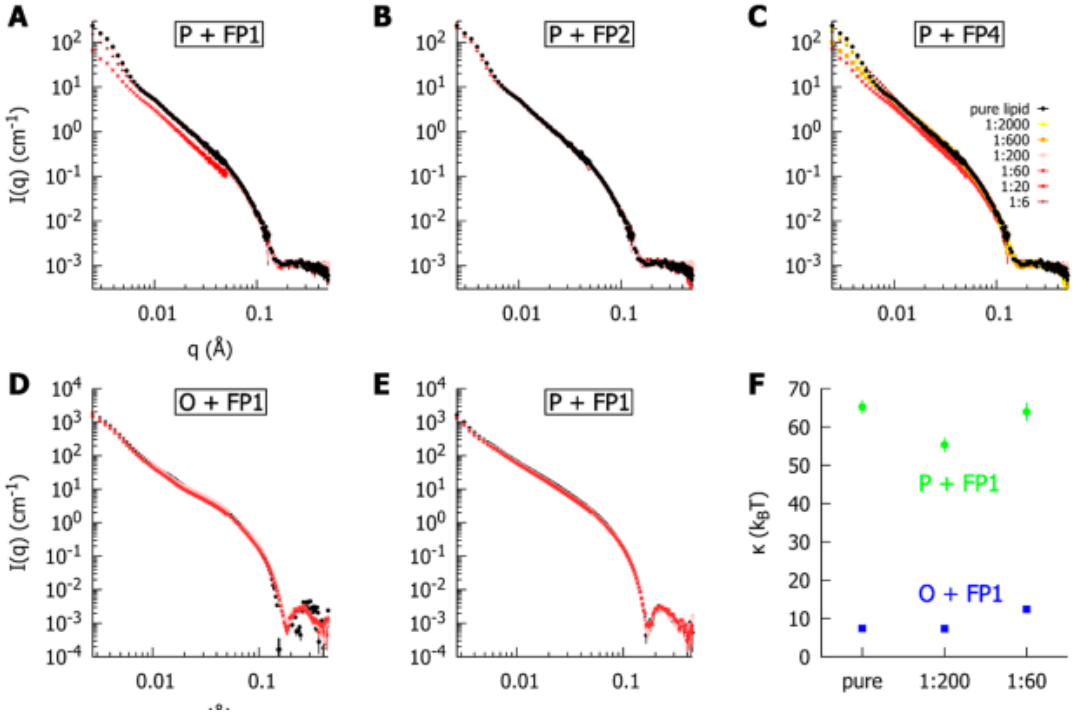

Fig. 2: SANS and NSE data on the effects of fusion peptides on model liposomes (see text for details).