Experimental report

13/09/2023

Proposal: **8-02-989**

Council: 10/2022

Title: Investigating the Kinetics of SARS-CoV-2 Membrane Fusion via Stopped-Flow SANS

Research area: Biology

This proposal is a new proposal

Main proposer: **Sebastian JAKSCH**

Experimental team: Sebastian JAKSCH

Local contacts: Sylvain PREVOST

Samples: DPPC in D2O

Instrument	Requested days	Allocated days	From	To
D22	2	0		
D33	2	2	21/06/2023	23/06/2023

Abstract:

The corona virus SARS-CoV-2 and the resulting COVID-19 disease have caused serious harm worldwide, in terms of both public health and socio-economic impacts. Despite the ongoing vaccination campaign, the appearance and rapid spread of new variants show that, without an effective treatment, the virus will remain a significant threat. It is therefore important to investigate and understand the mechanisms that make this virus so infectious - the fast and effective transmission between different hosts. After entering a new host, the first stage of a viral infection begins with the penetration of host cells, a process mediated by membrane fusion. We therefore propose a stopped-flow SANS experiment with contrast matched, reconstituted viral membrane vesicles and reconstituted human respiratory cell vesicles to observe the fusion kinetics. The experiment will provide us with both the timescale of the membrane fusion, and will also demonstrate under which conditions membrane fusion is either promoted or suppressed. The results will be important for the development of antiviral agents and pre-exposition prophylaxes.

Experimental Report for 8-02-989

Sebastian Jaksch

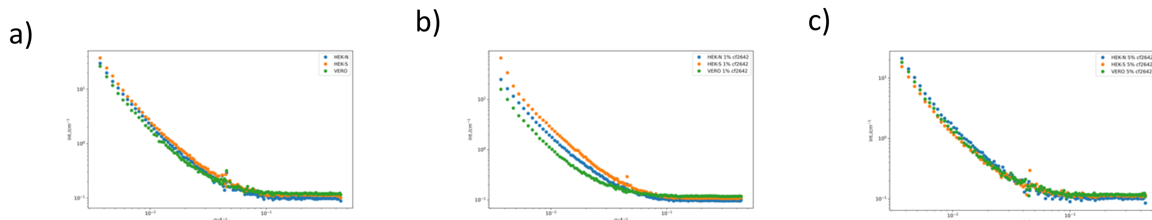
Local Contact: Sylvain Prevost
Beamtime: 21.-23.06.2023

During this experiment we proposed to investigate the impact of the antiviral drug cf2642 supplied by a collaborator (Joachim Bugert, Institut für Mikrobiologie der Bundeswehr) on the fusion kinetics of vesicles derived from plasma membranes of cell cultures that expressed either the SARS-Cov2 spike protein or the corresponding human ACE2 receptor protein. To that end we planned to mix purely deuterated phospholipids directly with the extracts from the plasma membrane as done before (https://arxiv.org/abs/2303.10746).

However, the commercial deuterated phospholipids were degraded due to reasons that could not be ascertained, so that no stable vesicle solution could be formed. The supplier commented that all quality procedures had been followed during production. Currently we are unable to state what happened to the sample to degrade.

In contrast, the self-prepared, protonated samples worked as planned, which allowed at least to investigate the structure impact of the antiviral drug. Representative scattering curves are shown below. We were able to show that the drug is primarily located in the membrane, without disturbing the overall vesicle structure of the sample. In addition, the drug seems to promote water uptake into the membrane.

Support by the instrument responsible and laboratory support staff was excellent and is much appreciated.



Scattering curves of the vesicles derived from cell cultures expressing ACE2 (Vero), Spike (HEK-S) and control (no spike, HEK-N) for no added drug (a), 10 uM added drug (b) and 50 uM added drug (c). In all cases vesicles with a size of 100 nm and a shell thickness of 5-6 nm describe the data well. Fixing all know parameters and fitting only the SLD shows an increase in SLD in the shell for higher drug concentrations. Since the drug has a SLD comparable to the phospholipid around $1x10^{-6}$ $Å^{-2}$ this can indicate an increased uptake of D_2O into the membrane.