Experimental report

15/02/2023

Proposal: DIR-257 **Council:** 4/2021

Title: Investigating the kinetics of SARS-CoV-2 membrane fusion via stopped-flow SANS

Research area:

This proposal is a new proposal

Main proposer: **Dominic HAYWARD**

Experimental team: Sebastian JAKSCH
Dominic HAYWARD

Local contacts: Ralf SCHWEINS
Sylvain PREVOST
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Samples: Natural lipid vesicles in deuterated phosphate buffered saline solution

Instrument	Requested days	Allocated days	From	To
D11	2	2	21/09/2021	23/09/2021

Abstract:

Experimental Report DIR-257

Responsible: Sylvain Prevost
Users: Dominic Hayward, Sebastian Jaksch

Date: 21-23 Sept. 2021

Short description
This experiment was a stopped flow experiment with contrast matched virus-like and human-host-like vesicles to investigate the fusion kinetics of SARS-Cov2 viruses with human cells in order to better understand the extremely infectious nature of SARS-Cov2.

In order to do this deuterated virus-like vesicles were mixed with protonated human-host-like vesicles in a contrast matched (80/20 H2O/D2O) PBS buffer at pH 7.4 in a stopped flow setup (see photo).

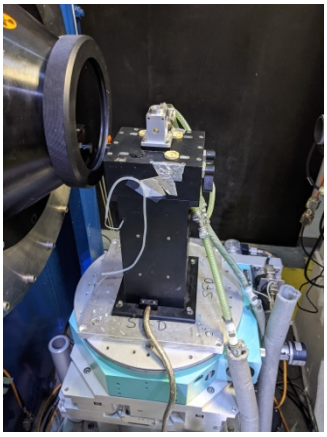
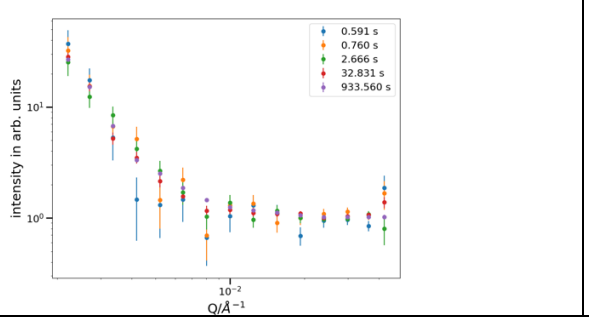


Figure 1. Photograph of the stopped flow setup on D11.

Investigating the forward scattering we wanted to follow the decreased scattered intensity over time in the contrast matched medium at low scattering vectors (4e-3 to 7e-3 $Å^{-1}$, see images).



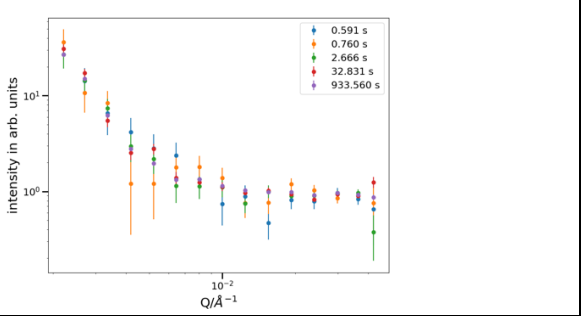


Figure 2. Scattered intensity of vesicles with (a) and without (b) spike protein mixed with human-host-like cells in a matched buffer for different times after mixing. The background is high due to the high hydrogen content (80% H2O) in the buffer. No clear time evolution is visible.

However, the scattered intensity did not vary more that the errorbars, so here no clear time evolution could be distinguished.

We therefore attempted fitting the exponent of the forward scattering using $I(q) = I_0 \times q^{\alpha} + bgrnd.$
This rendered the time evolution as shown in Fig.3.

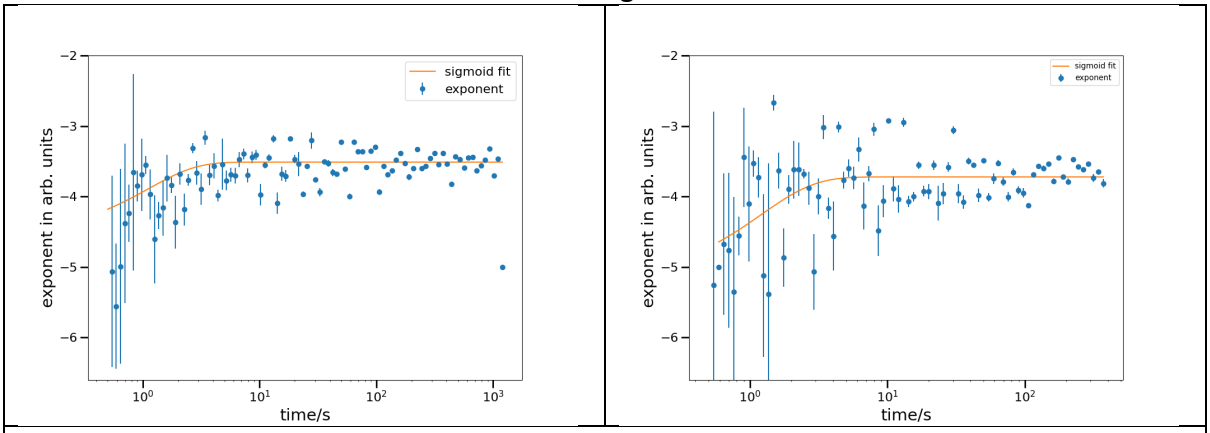


Figure 3. Exponent of the forward scattering exponent over time for vesicles with (a) and without (b) spike protein in the virus like vesicles as from data in Fig. 2.

However, also here we could find no time evolution and no difference between the two samples with and without spike protein, which was completely against expectation and literature data.

From the data we could finally concluded that under the given conditions, no fusion took place. This was due to the pH, which stabilized the colloidal suspension of vesicles.

Also, we gathered very helpful data as to which amount of deuterization is necessary, and about the best deuterization method of the samples.

Only with the help of the experiments performed here we were able to perform a second and improved experiment at ANSTO, which led to data which are currently being prepared for publication. Without the experiment done here (regarding deuterization, colloidal behavior, scattering contrasts etc.) the follow up experiment would not have been possible.

The instrument scientist, Sylvain Prevost, and the entire staff both in the facility and the support labs were extremely helpful, and their help before, during and after the experiment made it possible to understand which parameters were key to develop a working setup for such an experiment. This is gratefully acknowledged.