Experimental report

23/02/2022

Proposal: 8-05-466 **Council:** 10/2020

Title: The severity in COVID19 symptoms is inversely correlated with serum cholesterol levels. Is this relatedto impaired function of HDL?

Research area: Other...

This proposal is a new proposal

Main proposer: Marite CARDENAS

Experimental team: Yubexi CORREA
Sarah WALDIE
Trevor FORSYTH

Local contacts: Samantha MICCIULLA

Samples: HDL
SARS-CoV-2 Spike protein, incl 2 other variants
films made of dDMPC and dDMPC-20%dcholesterol

Instrument	Requested days	Allocated days	From	To
FIGARO	3	3	18/06/2021	21/06/2021
D17	3			

Abstract:

A recent study has shown that the severity in the COVID symptoms is inversely correlated with serum cholesterol and lipoprotein levels. The lipoprotein profile is complex in humans and vary largely among individuals. Individuals with high triglyceride and high cholesterol have lipoprotein structures that are quite distinct from healthy individuals. At the same time, the SARS-CoV-2 Spike protein was shown recently to bind cholesterol and lipoproteins. In a recent director discretion beamtime at D17, we observed clear binding of the Spike protein to cholesterol containing model membranes. Moreover, we observed differences in the reflectivities upon co-incubation with high density lipoprotein (HDL) from healthy individuals with and without spike protein at model membranes containing cholesterol. This implies that the spike protein changes the mode of HDL action and its capacity to exchange lipids. In this proposal, we aim at understanding the role of cholesterol in the membrane for the HDL-spike protein interaction as well as on the type of spike protein.

Experimental Report Figaro (8-05-466)

We have been studying the interaction between model membranes, Spike protein, and HDL because there is a relationship between the levels of cholesterol and covid severity. In our previous experiment, we found that spike protein removes lipids from model membranes composed of dDMPC: cholesterol (80:20). However, we did not know what kind of lipids the spike protein prefers to remove, but it seems there is a preference when the cholesterol is present in the membrane. For that reason, our goal in this experiment was to see if the removal either increase or decrease when we have model membranes composed of dDMPC only.

To do this, we prepared supported lipid bilayers composed of deuterated 1,2-dimyristoyl-D54-3-sn-glycerophosphatidylcholine (dDMPC) and perdeuterated cholesterol (dCholesterol) at 80:20 mol% as model membranes and characterized in three isotopic contrasts at 37 ° C using neutron reflection at the D17 reflectometer. After characterization in DTBS, HTBS and cmSi we incubated six independent model membranes with either: The SARS-CoV-2 Spike protein, HDL, and a mixture of HDL and SARS-CoV-2 Spike protein. Upon 5 h incubation time, the bilayers were rinsed and fully characterized in DTBS, HTBS, and CmSi (see table 1).

Table1. Surface composition

Surface	Composition
1	dDMPC + HDL
2	dDMPC +Spike
3	dDMPC + Spike +HDL
4	dDMPC:hChol +HDL
5	dDMPC:hChol +Spike
6	dDMPC:hChol +HDL+Spike

It is important to mention that during the analysis we realized we had some problems with the fitting. We think surfaces 4, 5, and 6 were not clean enough, and in figure 1 for surface 4 in D_2O, the minimum is slightly shifted to low q in comparison with the other surfaces which suggests there is a layer of dirt on the surface. Therefore, we decided to fit the data corresponding to surfaces 1, 2, and 3 (dDMPC).

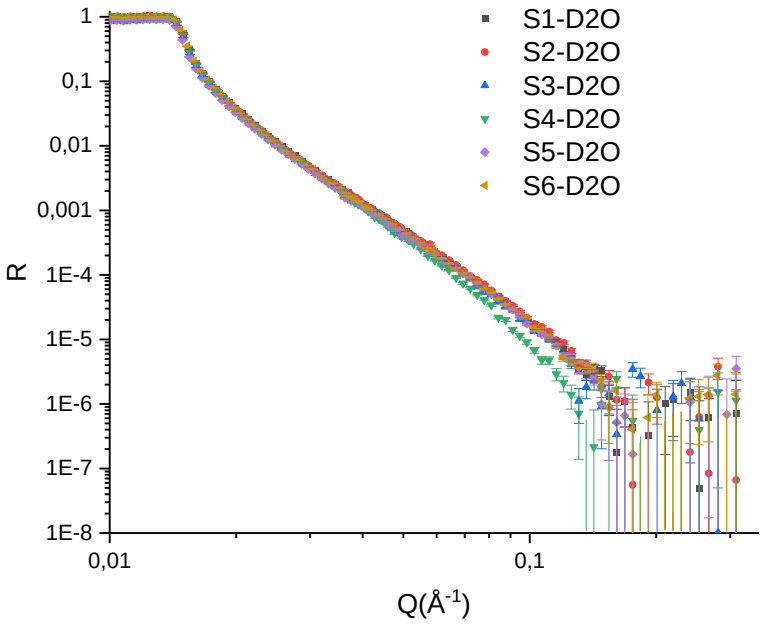

Figure 1. Neutron Reflection profile of the bare surfaces in D_2O (raw data).

The results obtained by neutron reflectometry suggest that the spike protein removes fewer lipids when cholesterol is not present in the membrane (Figure 2). Co-incubation of spike protein and HDL affected the lipid exchange process of the membrane as was seen previously.

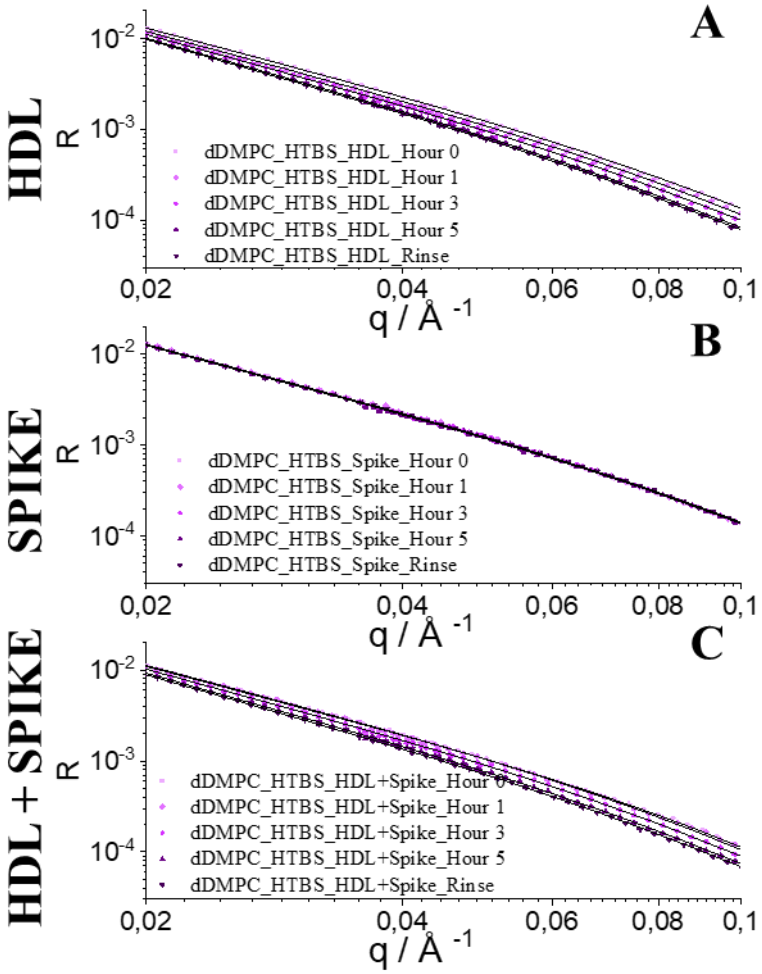

Figure 2. Neutron Reflection profiles of the kinetics including best fits (Line) and raw data (symbol). for the model membranes exposed to HDL (A), Spike protein + HDL (B), and Spike protein (C) in h-TBS at 37 °C after 5 hours of incubation and upon rinsing with excess h-TBS.

Finally, we can kindly suggest improving the method of cleaning for future experiments. We think using just organic solvent rinsing is not enough to remove dirt. So, next time is recommended to perform cleaning with the Pirahna solution.