Experimental report

23/10/2021

Proposal: DIR-207 **Council:** 4/2020

Title: Studying HAT-mediated cleavage mechanisms of the SARS-CoV2 'S' Proteinand hACE-2 by neutron reflectometry

Research area:

This proposal is a new proposal

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Samples: Natural lipids
Proteins (spike and ace2 receptor)
18A peptide for nanodisc preparation

Instrument	Requested days	Allocated days	From	To
FIGARO	2	2	14/09/2020	16/09/2020

Abstract:

Experimental report DIR-207
Studying HAT-mediated cleavage mechanisms of the SARS-CoV-2 *S*-protein and hACE2 by neutron reflectometry

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October 23, 2021

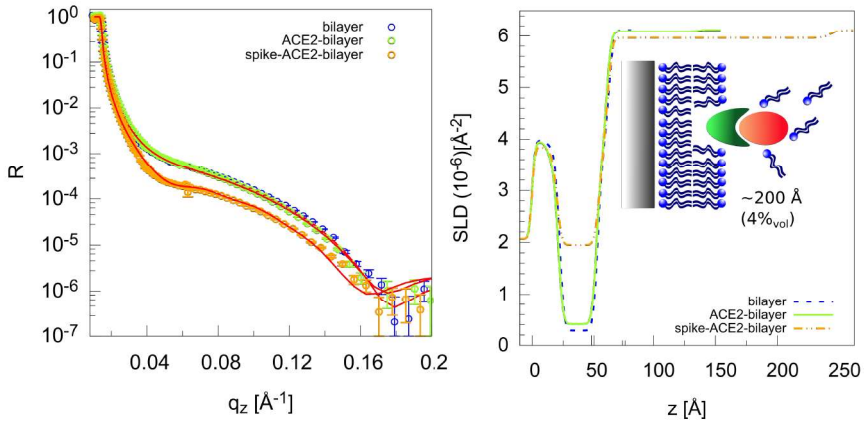

Figure 1: Summary of the reflectivity data (left) and the obtained SLD profiles (right) for the SLBs hPOPC:hPOPS:ACE2 bilayer interacting with the spike protein. The cartoon represents our interpretation of the ACE2-spike binding domain and the lipid stripping observed in the hydrogenated dataset, which is potentially due to the stabilisation of a closed protein form by unsaturated lipid binding.

The proposal DIR-207 for neutron reflectometry has been granted with **2 days beamtime** on Figaro. The proposed experiment aims at investigating the binding process of the SARS-CoV-2 virus, responsible for the Covid-19 disease outbreak, to mammalian cells. As reported by recent studies [15], the surface of the SARS-CoV-2 virus contains a transmembrane protein, called the 'S'-protein, which mediates its entry pathway into cell host (Figure 2). The extracellular domain of this protein has two characteristic subunits: the S1, which mediates the receptor binding, and the S2, responsible for the membrane fusion. In particular, the C-terminal subdomain of the S1 subunits binds to the ACE2 receptor, abundantly detected on the surface of lung and small intestine cells [7]. To mimic the viral entry process, we have designed a model system constituted by a solid supported lipid bilayer (SLB) with embedded receptor hACE2, which is put in contact with the spike protein HexaPro[5] synthesized by the *Membrane and Immunity Team* at the IBS (research group of Prof. Franck Fieschi). The HAT-cleavage was not investigated in this experiment.

bilayer, and no quantification was possible in both contrasts. If present, its coverage must be below the detection limit (<1%), which might indicate a binding specificity of this protein for the hACE2.
Interestingly, the data obtained for h-bilayer data suggest a **reduction of the surface coverage upon spike addition** (enhancement of water hydration in the tail region from 8 to 27%). Since no detergent was present in the spike solution (300 μL), as it contains only the soluble part of the protein[5], **we are trying to rationalise the hypothesis that the spike degrades the bilayer** by stripping lipid molecules from the surface. Recent literature reports the binding of fatty acids (linoleic acid) to the open conformation of the spike to form a more stable, enveloped conformation[16]. **Ongoing scientific discussion with the *Membrane and Immunity Team* at the IBS (Prof Franck Fieschi) and Nathan Zaccai at Cambridge University**.

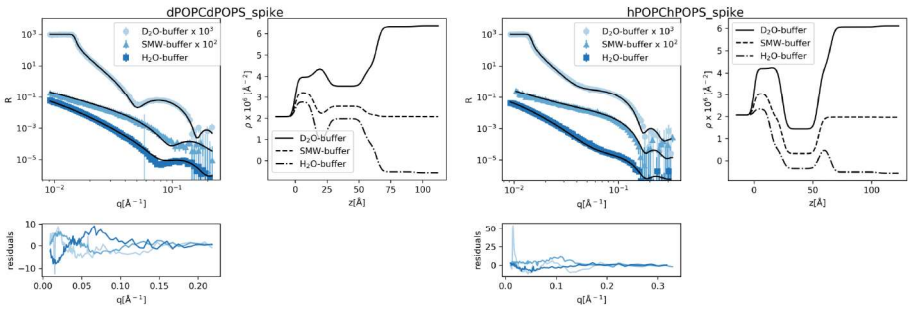

Figure 6: NR data of empty (no hACE2) POPC:POPS bilayers upon spike injection.

	Headgroups		Acyl chains	
	deut	hydrog	deut	hydrog
Thickness [Å]	10 ± 1	10 ± 1	29 ± 1	30 ± 1
Solvent fraction	0.51 ± 0.03	0.56 ± 0.04	0.17 ± 0.02	0.27 ± 0.03

Table 3: NR data for empty dPOPC:dPOPS and hPOPC:hPOPS upon spike injection. The SLBs were modeled as 5-layer-system (Figure 4).

hACE2-loaded bilayers

The SLBs prepared from loaded LPDs (with hACE2) were modelled as a sequence of 5 layers. **Unfortunately, the hACE2 fraction could not be quantified from the data analysis**, which we could explain by two different scenarios: i) its very low content in the outermost part of the bilayer, ii) its binding at high surface curvature (side of the membrane), corresponding to areas of low beam illumination. The thickness of the headgroup region was comparable to those of bilayers prepared from empty LPDs (7-8 Å), as well as their solvent content. The same holds for the acyl chains (32±1 Å and <10%$_{vol}$ solvent.)

	Headgroups		Acyl chains	
	deut	hydrog	deut	hydrog
Thickness [Å]	7 ± 1	8 ± 1	33 ± 1	31 ± 1
Solvent fraction	0.56 ± 0.03	0.46 ± 0.01	0.01 ± 0.01	0.10 ± 0.03

Table 4: NR data for loaded dPOPC:dPOPS and hPOPC:hPOPS. The SLBs were modeled as 5-layer-system.

Interestingly, the addition of the spike to the hACE2-loaded SLBs could be successfully described by its binding to the outermost layer (hACE2-spike layer in

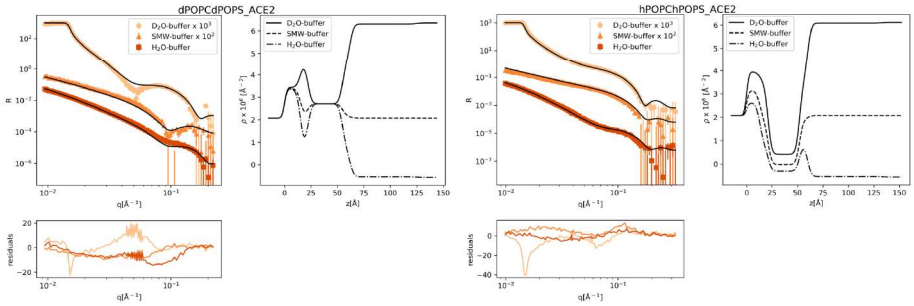

Figure 7: NR data of loaded POPC:POPS bilayers.

Figure 4), with a thickness of about 200 Å and very high hydration (96% solvent fraction). The protein was modeled according to structural information extracted from recent literature[2].
This result suggests the specificity of the hACE2-spike binding, which has a characteristic very high affinity[21, 20].
As for the case of the unloaded SLBs, the hydrogenated samples show lipid stripping upon spike injection, with no visible effect in the deuterated samples. The reason for this different behavior of hydrogenated and deuterated samples is not clear, yet, and need further investigation by complementary techniques (adsorption studies by Langmuir isotherms and quartz crystal microbalance).

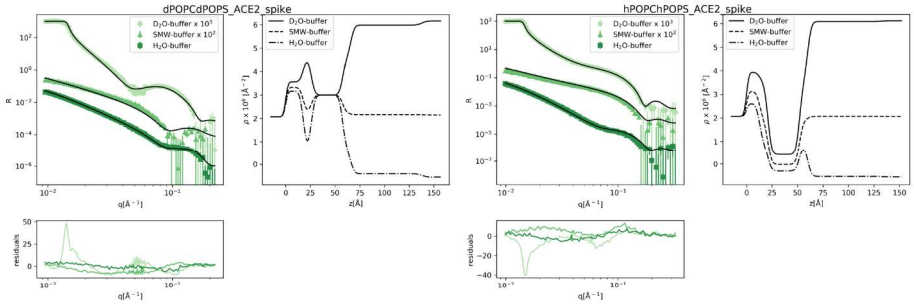

Figure 8: SLB from loaded POPC:POPS LPDs after interaction with the spike protein.

	Headgroups		Acyl chains		ACE-spike	
	deut	hydrog	deut	hydrog	deut	hydrog
Thickness [Å]	9 ± 1	9 ± 1	31 ± 1	33 ± 3	200 ± 10	180 ± 12
Solvent fraction	0.59 ± 0.05	0.58 ± 0.05	0.01 ± 0.01	0.34 ± 0.03	0.96 ± 0.02	0.95 ± 0.02

Table 5: NR data for loaded dPOPC:dPOPS and hPOPC:hPOPS upon spike injection. The SLBs were modeled as 5-layer-system.

SLBs from natural lipid extracts mimicking plasma membranes

The first SLB samples from natural LPDs had an extremely poor surface coverage, below the detection limit of the technique (data not reported). This was expected from the SEC results (Figure 3) and it suggests the important role of cholesterol on the formation of the peptide discs. Mixtures of natural lipids with lower cholesterol content (below 20%) could be successfully form LPDs (*Luchini et al., manuscript in preparation*), and systematic preparations with increasing cholesterol concentration will be object of our complementary studies.