An even closer look at the COVID-19 infection process

The highly penetrating and non-destructive nature of neutrons, coupled with their ability to provide information at the molecular level, has enabled neutron science to make essential contributions to humanity's persistent battle against viruses: HIV, influenza, hepatitis B and C and now, most recently, SARS-CoV-2, the virus responsible for the COVID-19 pandemic.

#EUROPEAN LARGE-SCALE FACILITIES #NEUTRONS #HEALTH

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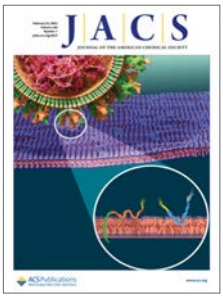
Following the emergence of SARS-CoV-2, a dedicated task force was immediately set up at the Institut Laue Langevin (ILL) to evaluate how the world's most intense neutron source and the expertise of its scientists could contribute to the collective effort to combat COVID-19. The pandemic and the creation of this task force led to a complete change in the research direction of a number of ILL scientists, including PhD student Andreas Santamaria. *"I started my PhD in July 2019 using reflectometry to study the interaction between proteins and model membranes. I had carried out eight months of research when the first lockdown started. I was then invited to join the recently-formed COVID task force and I've been working on COVID-19 ever since."*

A number of different research projects dedicated to SARS-CoV-2 have been advancing in parallel at the ILL and Santamaria has contributed to two of them. The results of the first, which used neutron reflectometry to investigate how lipid bilayer degradation is induced by the spike protein, were recently published in Nature Scientific Reports. *"Infection of human cells is caused by the SARS-CoV-2 spike protein binding to the ACE2 receptor. This first study was very interesting because you can see the whole spike protein and how it interacts with the lipid membrane, with and without ACE2,"* explains Santamaria. *"With this set-up, however, it is difficult to see what is happening at the molecular level. So, what we did in the second study was to simplify the system down to its core elements."*

The section of the SARS-CoV-2 spike protein that is involved in the initial penetration into the host cell can be subdivided into four short peptide segments called fusion peptides (FP1, FP2, FP3, FP4). Santamaria, in collaboration with researchers from the ILL and the Cambridge Institute for Medical Research in the UK, studied the molecular interaction of each of these



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The published article featured on the front cover of the Journal of the American Chemical Society showing the interaction between the virus and the host cell. The panel shows the studied peptides: FP1 in green, FP2 in orange, FP3 in yellow and FP4 in blue.

GIANT EFFECT

Dynamic light scattering, lipid monolayer, Langmuir trough and Brewster angle microscopy experiments were all carried out at the Partnership for Soft Condensed Matter (PSCM), a scientific and technical platform collaboratively established and run by the ILL and the European Synchrotron (ESRF). Circular dichroism spectroscopy experiments were carried out at the Molecular Chemistry Department (DCM), a research center jointly supported by the Université Grenoble Alpes (UGA) and the French National Centre for Scientific Research (CNRS).

four fusion peptides with model membrane systems, focusing in particular on FP1, FP2 and FP4. Natural lipids - extracted and separated at ILL's lipid deuteration platform, the L-Lab – were used to construct the model membranes as they more closely resemble the real environment and thus the cell membrane than synthetic lipids. *"Though the model that we used is much simpler, it enabled the specific function of each fusion peptide to be investigated,"* explains Santamaria.

The interaction between the peptides and model membranes was revealed by combining the complementary structural and dynamic information provided by four different neutron techniques at the ILL: specular neutron reflectometry and small angle, quasi-elastic and spin-echo neutron scattering. The results obtained, published this year in the Journal of the American Chemical Society, reveal the strikingly diverse roles of the different segments of the SARS-CoV-2 spike protein in the initiation of viral infection. FP1, for example, inserts into the host membrane forming fusion initiation points in a calcium ions-depending manner and enables lipids to mix between the viral and host membranes, whereas FP4 acts a bridge between the two membranes, facilitating their fusion into a single bilayer.

The results contribute towards a more thorough understanding of the molecular mechanisms of the SARS-CoV-2 infection process but there are also even wider potential implications. SARS-CoV-2 belongs to a family of single-stranded positive sense RNA viruses called β-coronaviruses, that includes SARS-CoV-1 and Middle Eastern respiratory syndrome coronavirus (MERS-CoV). *"All β-coronaviruses have spike proteins and though there are small differences between the viruses, the infection process is globally the same so this work helps the general infection mechanism of the entire family of viruses to be better understood,"* explains Santamaria.

"Strikingly Different Roles of SARS-CoV-2 Fusion Peptides Uncovered by Neutron Scattering".
J. Am. Chem. Soc., Santamaria et al. (2022)
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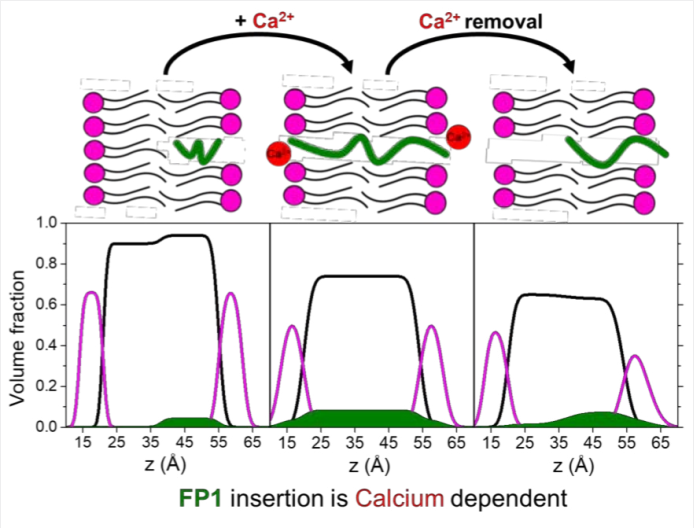
Neutron reflectivity data of bilayers with FP1, at different concentrations of calcium ions. Volume fraction profiles normal to the interface of bilayers highlight the distribution of aliphatic tails (black), hydrophilic headgroups (magenta), and FP1 (green). Increasing calcium concentration provokes FP1 to deeply insert in the membrane, while calcium removal causes FP1 to rearrange. Data from FIGARO at ILL.



Proposed fusion mechanism between SARS-CoV-2 and eukaryotic host membrane. The viral membrane bilayer is colored green, the eukaryotic host membrane in blue, and the S2' protein is in red. The direction of the protein is drawn with red arrows, while the direction of the lipids is drawn with black arrows. FP1and FP4 are represented as ovals, and the structured S2' protein as a circle (attached to the viral membrane). **(A)** FP1 forms a fusion initiation point on binding the host membrane. **(B)** The initiation point enlarges provoking lipids mixing between the viral and host membrane, leading to the growth of a hemifusion diaphragm. **(C)** FP4 bridges the two membranes together thereby facilitating the fusion of the two membranes into a single bilayer. Moreover, the two membranes coming together exclude the folded S2' from the growing synapse. **(D, E, F)** A hemifusion diaphragm is formed and lower free calcium concentrations lead to FP1 orienting itself like FP4, thereby providing further contact between the two membranes. **(G)** It is also possible that FP1 initiation points may form on both the viral and the plasma membrane. **(H)** The two membranes form a pore, and as in **(F)**, the pore expands as the Spike protein is excluded by the two membranes coming together due to the bridge function encoded in the Spike fusion peptides.