Function of the membrane-proximal domains of the SARS-CoV-2 Spike protein in membrane fusion

Funding : 3-year PhD or engineer position funded by ANR, starting as soon as possible

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Cellular infection by SARS-CoV-2 begins with the fusion of its lipid envelope with the host cell membrane, a process mediated by the Spike (S) protein of the viral membrane. The S protein is composed of two subunits: S1, responsible for binding to the host cell, and S2, which drives fusion [1]. Fusion by S2 starts with the insertion of its fusion peptide FP1 into the target cell membrane, forming a molecular bridge. S2 then folds back onto itself, which brings the viral and cellular membranes in close apposition and triggers fusion. The final stages of S2-mediated fusion, leading to lipid bilayer merging and fusion pore opening, are less understood but likely involve membrane perturbation by FP1, possibly acting in conjunction with the transmembrane (TM) domain of S2 and its adjacent membrane-proximal (pTM) and cytosolic (Cyto) domains (Fig. 1).

We have recently found that FP1 mediates membrane fusion *in vitro* and that the presence of cholesterol and ceramide in the membrane strongly enhances FP1-induced fusion [2]. The membrane proximal domains of S2, pTM and Cyto, were also proposed to interact with cholesterol but the exact role of these protein-lipid interactions in S2-mediated fusion remains to be established [3].

This project aims to elucidate the molecular mechanisms of S2-mediated membrane fusion, with a particular focus on how its FP1 and membrane-proximal domains, pTM and Cyto, cooperate with each other and with specific lipids to induce fusion. To achieve this, we will use a combination of *in vitro* cell-free membrane imaging and docking/fusion assays, along with *in situ* observations of cell-cell fusion events. The project will be done in close collaboration with Grégory Lavieu's lab (https://u-paris.fr/lavieu-lab/).

Applicants should hold a Master's degree in biochemistry, biophysics, or molecular and cellular biology. To apply, please send a single PDF file containing a CV, a short statement of research interests and experience, and the names of 3 references with contact information to: david.tareste@inserm.fr.

References

[1] Jackson, C. B., Farzan, M., Chen, B. & Choe, H. Mechanisms of SARS-CoV-2 entry into cells. Nat. Rev. Mol. Cell Biol. 23, 3–20 (2021).

[2] Niort, K. et al. Cholesterol and Ceramide Facilitate Membrane Fusion Mediated by the Fusion Peptide of the SARS-CoV-2 Spike Protein. ACS Omega 8, 32729–32739 (2023).

[3] Corver, J., Broer, R., van Kasteren, P. & Spaan, W. Mutagenesis of the transmembrane domain of the SARS coronavirus spike glycoprotein: refinement of the requirements for SARS coronavirus cell entry. Virol. J. 6, 230 (2009).

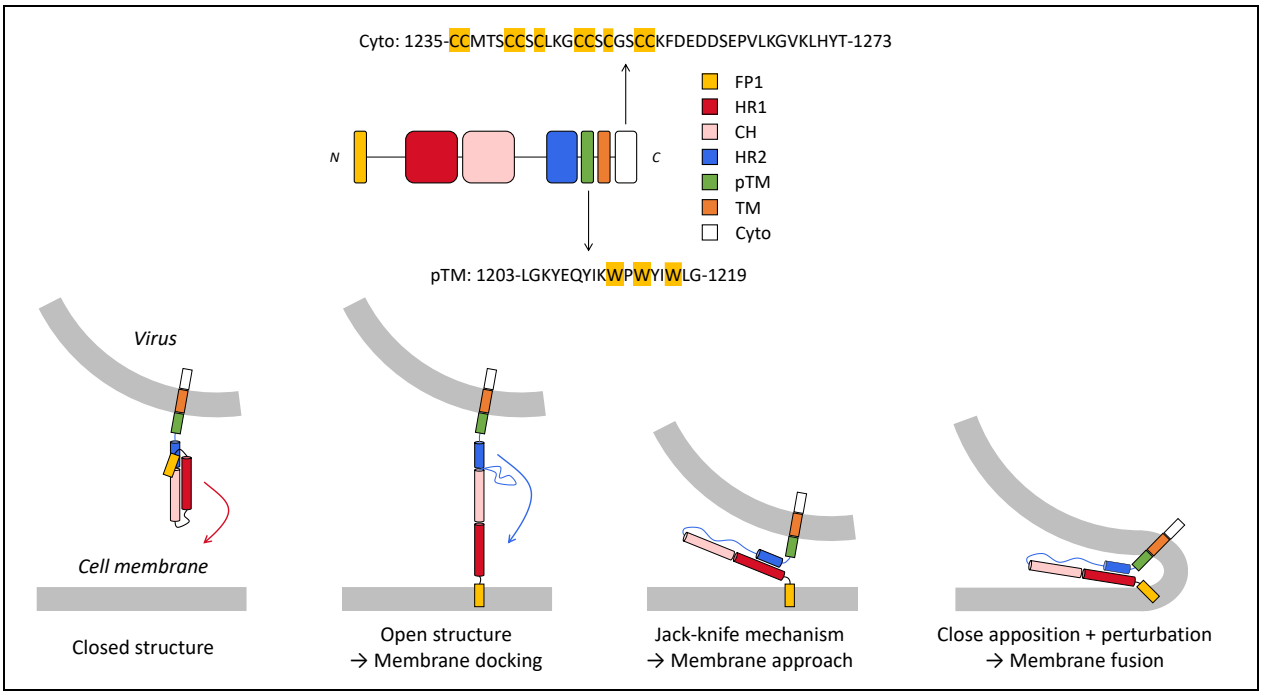

Figure 1. (Top) Molecular architecture of the S2 protein. (Bottom) Various stages of S2-mediated membrane fusion.