# A Functional Interaction Between Y674-R685 Region of the SARS-CoV-2 Spike Protein and the Human α7 Nicotinic Receptor

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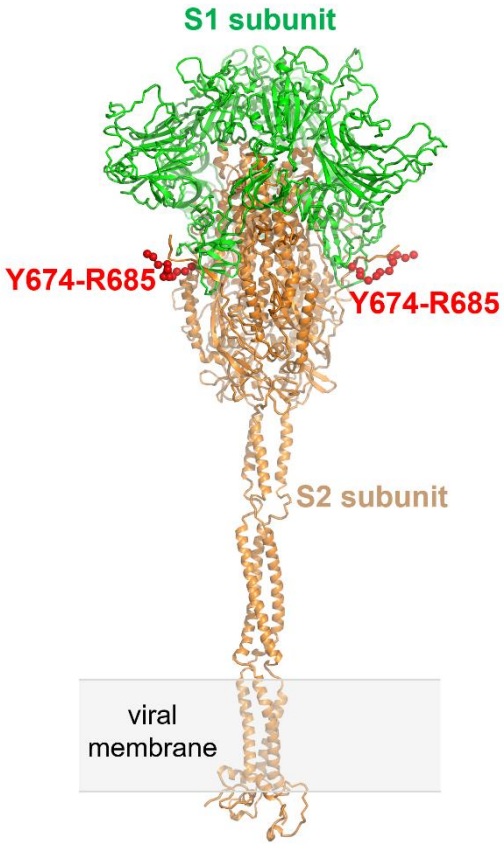
## Supplementary Figures

**Supplementary Figure 1.**

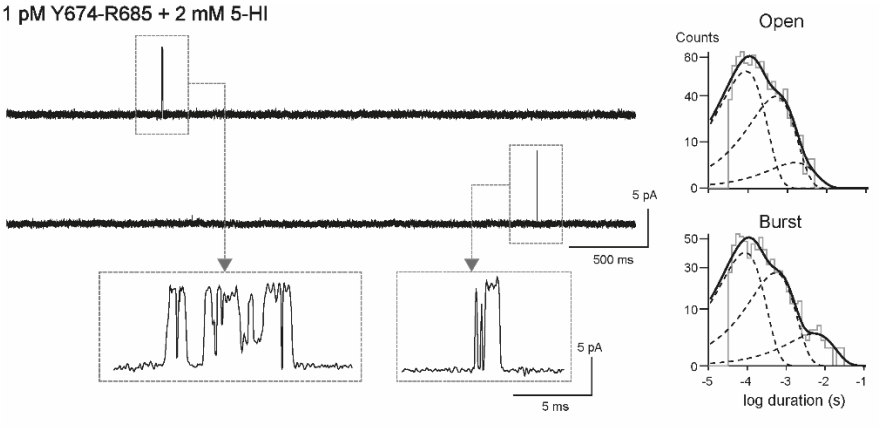
**Overview of the three-dimensional structure of the S protein from SARS-CoV-2.** The model shown here represents the complete SARS-CoV-2 S protein in the closed state after furin cleavage. This model was taken from (Casalino et al., 2020). The S1 and S2 subunit are coloured in green and orange, respectively. The glycans were omitted for simplicity. The Y674-R685 region (proposed to interact with nAChR) is shown with red spheres.



**Supplementary Figure 2.**

**Activation of the human α7 nAChR by 1 pM Y674-R685 in the presence of the type I PAM, 5-HI.**

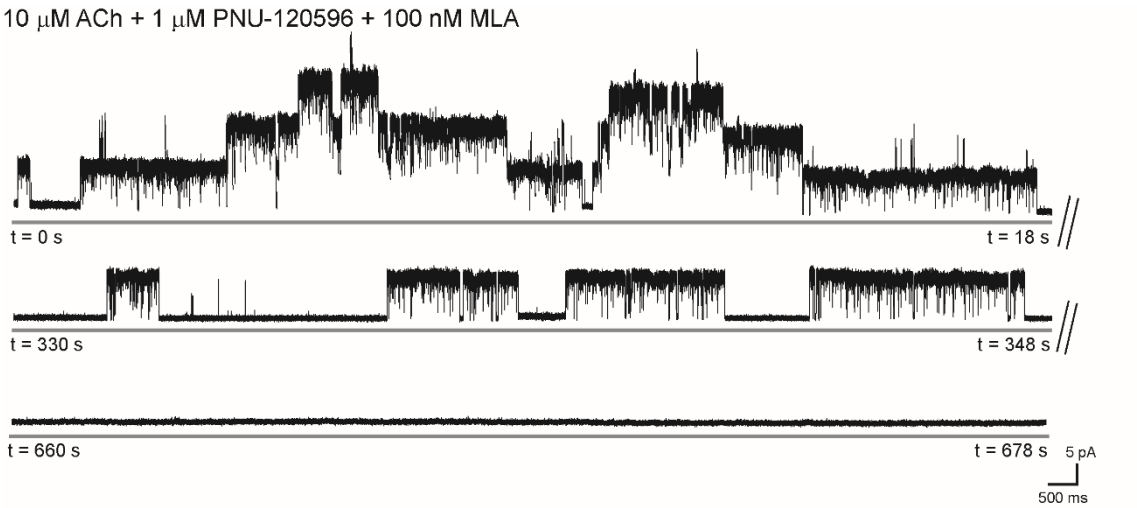
Single-channel currents were recorded from cells expressing the human α7 nAChR in the presence of 2 mM 5-HI as a PAM and 1 pM Y674-R685. Membrane potential: -70 mV, Filter: 9 kHz. Burst duration histograms were constructed with data from 18 different patches. The resulting mean burst duration was similar to that of recordings in the presence of 5-HI and ACh as the agonist (Fig. 6).



**Supplementary Fig. 3.**

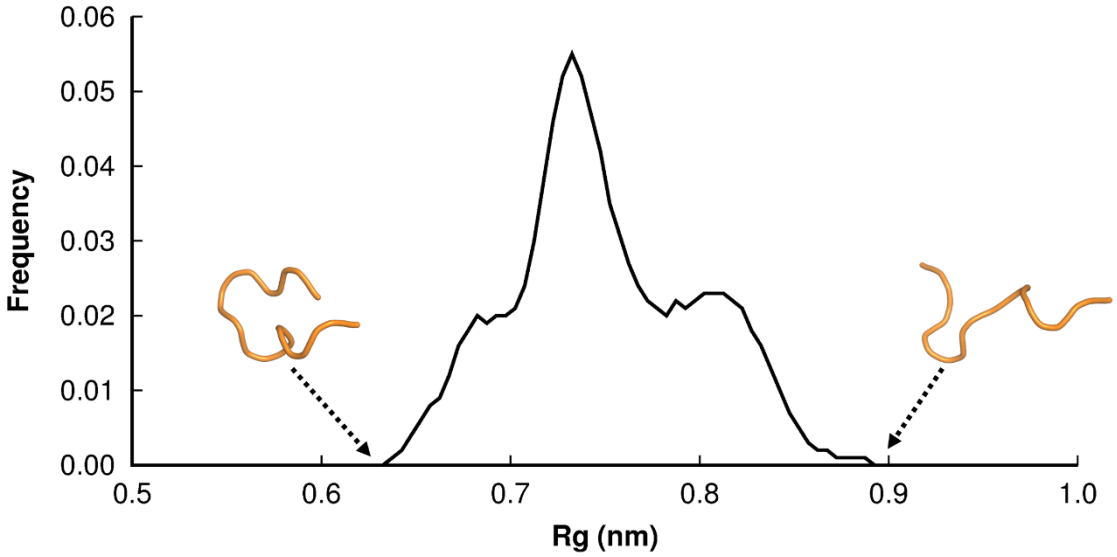
**Effect of the competitive antagonist MLA on single α7 channels activated by 10 μM ACh and potentiated by 1 μM PNU-120596 measured in real time.**

To measure the effect of MLA on single channels of human α7 nAChR activated by 10 μM ACh and potentiated by 1 μM PNU-120596 in real time we used a patch clamp pipette in which the tip was filled with the buffer solution containing 10 μM ACh and 1 μM PNU-120596 and the shaft with the same solution but including MLA (100 nM). At the beginning of the recording, high channel activity was observed, which appeared as multiple levels of channels due to the opening of more than one channel at the same time (upper trace). As the recording progressed, channel activity decreased (middle trace), and the clusters showed the same duration as in the absence of MLA. After about 10 min, channel activity was completely inhibited (lower trace). The traces correspond to different times of the same representative recording. Membrane potential: -70 mV, Filter: 3 kHz.



**Supplementary Figure 4.**

**Radius of gyration (Rg) distribution for the Y674-R685 region of the SARS-CoV-2 S protein when bound to human α7 nAChR from our previous molecular dynamics (MD) simulations** (Oliveira et al., 2021). Examples of the most compact and extended conformations adopted Y674-R685 within the agonist binding site.



**Supplementary Figure 5.**

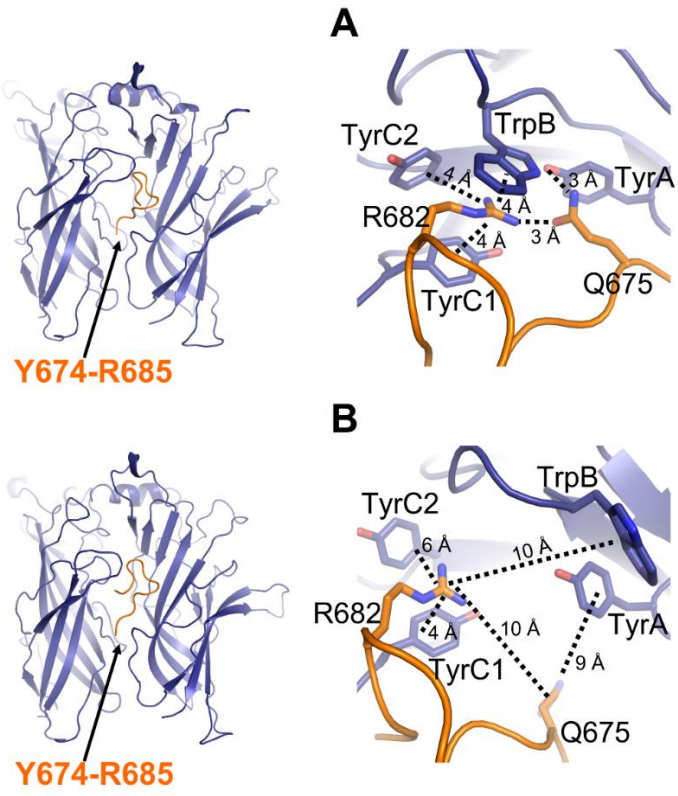
**Examples from our previous MD simulations of conformations adopted by the Y674-R685 fragment when bound to the human α7 nAChR.**

MD simulations of Y674-R685 bound to the human α7 nAChR show favourable binding (Oliveira et al., 2021).

Comparison of conformations from these simulations in which the most important interactions with conserved aromatic residues are either present (A) or absent (B).

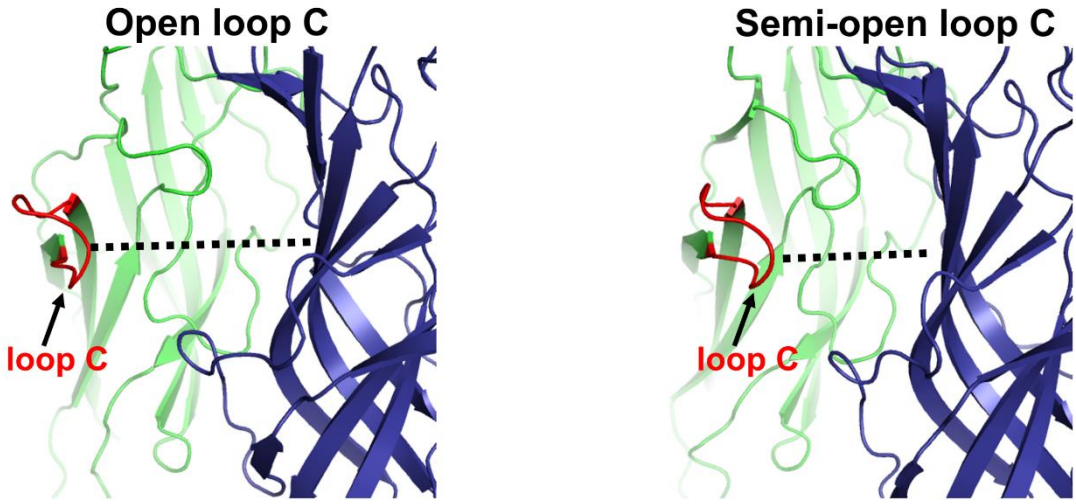
(**A**) Example of a conformation in which the interactions between the Y674-R85 and TrpB, TyrC1, TyrC2 and TyrA are present.

(**B**) Example of a conformation in which the interactions between the Y674-R685 and TrpB, TyrC2 and TyrA are absent. The figures on the left show the overall view of the Y674-R685:α7 complex. The figures on the right represent a close-up view of interactions formed by R682 and Q675 within agonist binding site. The α7 receptor and Y674-R685 are coloured in dark blue and orange, respectively. Interactions between sidechains of R682 and Q675 and the aromatic rings of TrpB (α7W171), TyrC1 (α7Y210), TyrC2 (α7Y217) and TyrA (α7Y115) are shown with dashed lines.



**Supplementary Figure 6.**

**Example of an open and semi-open conformation of loop C from our previous simulations of the Y674-R685:α7 nAChR complex** (Oliveira et al., 2021). The loop C is highlighted in red, and the principal and complementary subunits of the receptor are coloured in green and dark blue, respectively. The Y674-R685 fragment was omitted for simplicity.



**Supplementary Table 1**

***Y674-R685 Inhibition of α7 nAChR channels activated by ACh.***

| Y674-R685 concentration | Open (ms) | Burst (ms) | Cluster (ms) | n |
|---|---|---|---|---|
| | | | | |
| 0 | 148.0 ± 11.9 | 550.1 ± 37.7 | 3048 ± 516 | 3 |
| 1 pM | 66.3 ± 6.5 (p=0.0000770) | 187.2 ± 16.5 (p=0.0000109) | 878 ± 161 (p=0.000458) | 4 |
| 1 nM | 64.2 ± 3.0 (p=0.000294) | 209.9 ± 16.5 (p=0.000138) | 684 ± 48 (p=0.00139) | 3 |
| 1 µM | 45.7 ± 11.2 (p=0.000415) | 62.9 ± 13.2 (p=0.0000296) | 90.5 ± 23.5 (p=0.000581) | 3 |
| 10 µM | 9.9 ± 2.6 (p=0.00000277) | 11.2 ± 3.0 (p=0.000000841) | 14.1 ± 4.4 (p=0.0000663) | 4 |

Single-channel currents elicited by 10 µM ACh and potentiated by 1 µM PNU-120596 were recorded in the absence (control) or presence of Y674-R685 at the indicated concentrations. The open, burst and cluster durations were obtained from the corresponding histograms.
n indicates the number of recordings for each condition.
p corresponds to the resulting p value of the Student-t test in which each parameter was compared to that in the absence of Y674-R685.

**Supplementary References**

Casalino L, Gaieb Z, Goldsmith JA et al (2020) Beyond shielding: the roles of glycans in the SARS-CoV-2 spike protein. ACS Cent Sci 6 1722-1734. https://doi.org/10.1021/acscentsci.0c01056

Oliveira A, Ibarra AA, Bermudez I et al (2021) A potential interaction between the SARS-CoV-2 spike protein and nicotinic acetylcholine receptors. Biophysical journal 120(6) 983–993. https://doi.org/10.1016/j.bpj.2021.01.037