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A model of SARS-CoV-2 SPIKE transmembrane domain linked to THE HR2 region: structural organisation and possible role in membrane fusion

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The spike (S) protein of SARS-CoV-2 effectuates membrane fusion and virus entry into target cells. Its transmembrane domain (TMD) is a homotrimer of α-helices anchoring the spike in the viral envelope. Although S-protein models available to date include TMD, its precise configuration has only been given brief consideration. Understanding viral fusion entails realistic TMD models, while no reliable approaches towards predicting the 3D structure of transmembrane (TM) trimers exist. Immediately upstream of the TMD is a domain known as HR2. Apart from being involved in the complex refolding of the spike, HR2 is believed to interact with the viral envelope, as it is brought into contact with the target membrane, which has been observed for HIV's protein gp41 from the same family of fusion proteins. Here, we adopted diverse computational tools to model the spike TMD (S-TMD) based solely on its primary structure and used the resulting model to study a larger element of the fusion machinery, TMD linked to HR2, i.e., an anchor plus a "membrane-fixing factor".

We performed amino acid sequence pattern matching and compared molecular hydrophobicity potential (MHP) distribution on the helix surface against TM homotrimers with known 3D structures. Eventually, the TMD of the tumour necrosis factor receptor 1 (TNFR-1) was selected for template-based modelling. Adjusting so-called "dynamic MHP portraits" and residue variability motifs, we iteratively built an all-atom homotrimer model of the S-TMD, whereof each helix possessed two overlapping interfaces interacting with either of the remaining helices. The interfaces included conservative residues like I1216, F1220 and M1229. The stability of this model was tested in all-atom molecular dynamics (MD) simulations in a POPC bilayer mimicking the viral envelope and compared to several alternative configurations, including a recent NMR structure of a trimerised peptide.

Our model trimer remained extraordinarily tightly packed over a microsecond-range MD and retained its stability when palmitoyl chains were added at cysteine residues located downstream in accordance with experimental data. Overall, the resulting model of S-TMD conforms to the known basic principles of TM helix packing.

S-TMD was then linked to the HR2 region, modelled based on an NMR structure of the same domain in SARS-CoV's spike. Given that HR2 is a canonical coiled with an angle between helical axes of ~17°, whilst in our model trimer this angle amounted to ~41°, a hinge area between the two domains was required, implying they are not part of a continuous coiled coil encompassing both domains. This is in agreement with the predicted functional versatility of the N-terminal aromatic-rich region in the TMD and C-terminal residues in HR2, which would also entail structural flexibility. Models of

monomeric and trimeric HR2 both on its own and linked to the TMD were subjected to MD simulations to evaluate the behaviour and mutual influence of the two domains when part of one molecule, possibly relevant to understanding viral fusion. Trimeric HR2 remained intact in water, and so did trimeric HR2 linked to TMD in a model POPC bilayer: throughout the MD simulation, both domains remained stable. Previously SARS-CoV-2's spike HR2 had been experimentally demonstrated to have limited affinity for lipid bilayers, and our MD simulations agree with these observations: on its own, monomeric HR2 did not remain membrane-bound. Linking it to a monomer of TMD, on the other hand, resulted in more frequent contacts with the bilayer. In both cases, HR2 tended to form hairpin-like structures, intramolecular contacts seemingly preferable to contacts with the membrane. Thus, even though the TM anchor significantly increases the ability of HR2 to be adsorbed on a model membrane mimicking the viral envelope, such interaction is still insufficient to consider HR2 as a peripheral membrane domain. We also conducted Monte Carlo (MC) simulations with an implicit hydrophobic slab mimicking a membrane, and data derived therefrom corroborate the results we obtained in the course of MD. In the lowest energy states, individual monomeric HR2 did not land onto the membrane interface. Meanwhile, the corresponding MC-states identified for monomeric HR2 linked to TMD corresponded to the protein anchored in the hydrophobic slab via the latter, but HR2 still failed to adsorb on the membrane surface, although it interacted with the hydrophobic medium rather strongly compared to HR2 without the TMD. It is thus likely that other factors than the TMD come into play for proper binding of HR2 to the membrane to take place during the early stages of fusion.

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