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Structure and dynamics of the SARS-CoV-2 envelope protein

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Motivation and Aim: SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) is a strain of coronavirus, which caused the COVID-19 (coronavirus disease 2019) pandemic. SARS-CoV-2 virus particle contains four main structural proteins: S ("Spike") is responsible for attachment and fusion with the host cell; M ("Membrane") plays the key role in assembly of virions; N ("Nucleocapsid") packages RNA; E ("Envelope") promotes assembly of virions, suppression of the stress response of the host cells, efficient transport of the virions along the secretory pathway and demonstrates ion channel activity. Coronaviral E proteins are small, integral membrane proteins of 75-109 amino acids, which have at least one helical transmembrane domain (TMD) and a long amphipathic region comprising one or two -helices at the C-terminus. SARS-CoV-2 E protein α consists of 75 amino acids, and its sequence is 95 and 36 % identical to those of SARS-CoV and MERS-CoV E proteins respectively. It's also known that E proteins may undergo palmitoylation and glycosylation, although the role of these modifications are not quite clear. In infected cells, E protein is localized at the intracellular trafficking endoplasmic reticulum (ER)-Golgi region and mostly at the intermediate compartment between ER and ER-Golgi intermediate compartment (ERGIC) [1]. Currently, the experimental structure of the full-length wild-type SARS-CoV-2 E protein is not available, and the influence of above-mentioned modifications on it has not been studied. Thus, the goal of the present study was to characterize the structure and dynamics of the SARS-CoV-2 envelope protein in the membrane depending on its contents and post-translational modifications.
Methods and Algorithms: The protein behavior was simulated using molecular dynamics. The Martini force field [2] was used for coarse-grained simulations, and the CHARMM36m force field [3] was used for all-atom simulations. All MD simulations were run with GROMACS [4].
Results: We have extensively characterized the structure and dynamics of the E protein in monomeric form, and studied its behavior in the pentameric assembly. In particular, we show that TMD has a preferable orientation in the membrane, while H2 and H3 reside at the membrane surface (Fig. 1). Orientation of H2 is strongly influenced by palmitoylation of cysteines Cys40, Cys43, and Cys44. Glycosylation of Asn66 affects the orientation of H3. We also observe that the E protein both generates and senses the membrane curvature, preferably localizing with the C-terminus at the convex regions of the membrane.
Conclusion: Our results highlight the role of the E protein in the infection process and explain the low abundance of the E protein in assembled virions. Localization of the protein to curved regions may be favorable for assembly of the E protein oligomers,

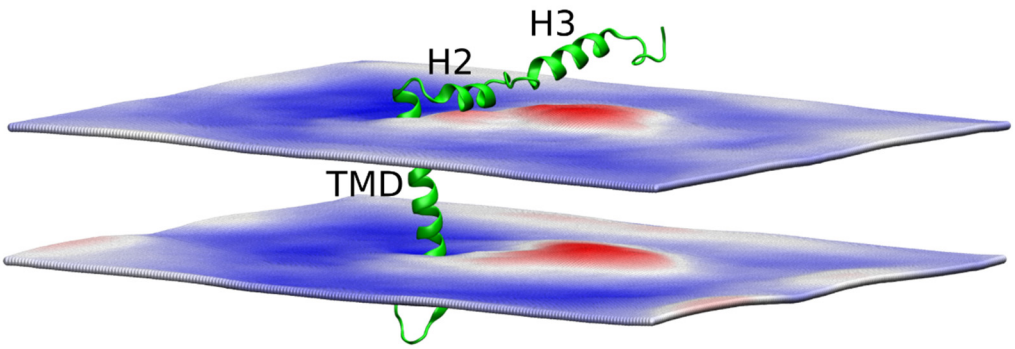

Fig. 1. Induction of membrane curvature by the SARS-CoV-2 envelope protein. Time-averaged position of the bilayer boundaries is shown. The white color of the bilayer corresponds to the unperturbed position of the membrane, blue – lowered, red – elevated

whereas induction of curvature may facilitate the budding of the viral particles. Flexibility of the C-terminus may be needed for interactions with the host proteins. Overall, these findings are in accordance with experimental observations, while providing a detailed description of the E protein structure and paving the way for development of therapies targeting the E protein [5].

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References

1. Ruch T.R., Machamer C.E. The coronavirus E protein: assembly and beyond. *Viruses*. 2012;4(3):363-382.
2. Marrink S.J. et al. The MARTINI force field: coarse grained model for biomolecular simulations. *J Phys Chem B*. 2007;111(27):7812-7824.
3. Huang J. et al. CHARMM36m: an improved force field for folded and intrinsically disordered proteins. *Nature methods*. 2017;14(1):71-73.
4. Abraham M.J. et al. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*. 2015;1-2:19-25.
5. Kuzmin A. et al. Structure and dynamics of the SARS-CoV-2 envelope protein monomer. *Proteins*. 2022;90(5):1102-1114.