Novel Membrane Bending Mechanism of the Coronavirus Envelope (E) Protein

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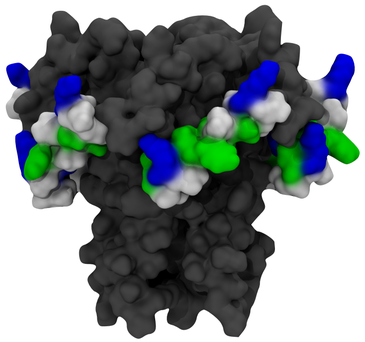
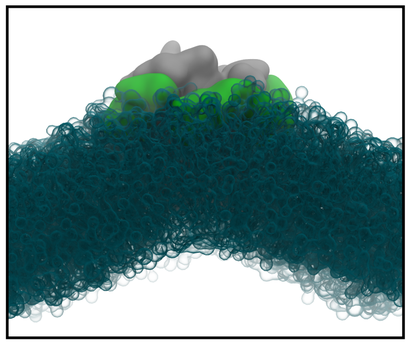
Introduction

- The Envelope (E) protein of the severe acute respiratory syndrome-related coronaviruses (including SARS-CoV and SARS-CoV 2) forms a homo-pentameric ion channel in the ERGIC of host cells and in the envelope of the mature virion
- It is known that the E protein induces membrane curvature, and that this deformation plays a key role in allowing the virus to escape its host cell and infect other cells[1]
- The precise mechanism is unknown
- Using Coarse-Grain Molecular Dynamics (CG-MD) simulations, we were able to replicate the deformation effect, observing persistent membrane curvature around the protein across a 25 μs simulation
- Understanding how the E protein induces membrane deformation may be significant in the fight to cure Covid-19 or mitigate its effects



Side view of the E protein pentameric viroporin (PDB ID 5X29). Residues 53 to 65 are colored to highlight the five-fold symmetry of the protein.



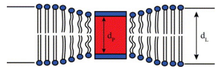
Still image rendered from Molecular Dynamics simulation trajectory showing membrane deformation local to the viroporin.

[1] Schoeman & Fielding, Virology, 2019

[2] Jensen & Mouritsen, Biochem Biophys Acta, 2004

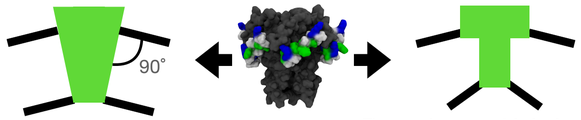
Approach & Methods

- Hydrophobic mismatch, a 'classical' physics mechanism for membrane deformation local to a protein inclusion, does not seem to apply in this case due to the asymmetric nature of the deformation



Hydrophobic mismatch, as depicted here[2], would engender a symmetric deformation of the outer and inner membrane leaflet.

- We instead investigated two alternative explanations: a shape-based mechanism prevalent in the literature, and a novel *asymmetric* hydrophobic mismatch mechanism



The **shape-based mechanism** relies on the explanation that membranes tend to sit normal to the protein surface. An asymmetric protein will cause induction of curvature as a result.

The novel **asymmetric hydrophobic mismatch mechanism** follows the classical hydrophobic mismatch model above, but includes a short TMD and a wide protein cap that prevents symmetric deformation.

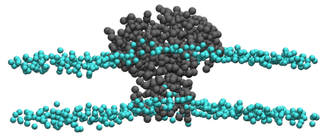
- To test which mechanism controlled the behavior of the E protein, we used CG-MD simulations to vary the length of lipids composing the membrane

If the shape-based mechanism is responsible for E's behavior, we would expect to see a similar deformation profile regardless of lipid length

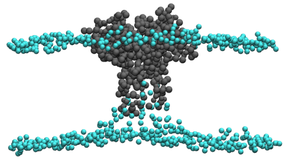
If the asymmetric mismatch mechanism is responsible, we would expect to see a reduction in curvature when shorter lipids are used and an increase in curvature when longer lipids are used

- We used the Martini 2.2 force field and Gromacs 2016 to perform several 25 μs CG-MD simulations of the E protein surrounded by membrane lipids of varying lengths and saturation levels

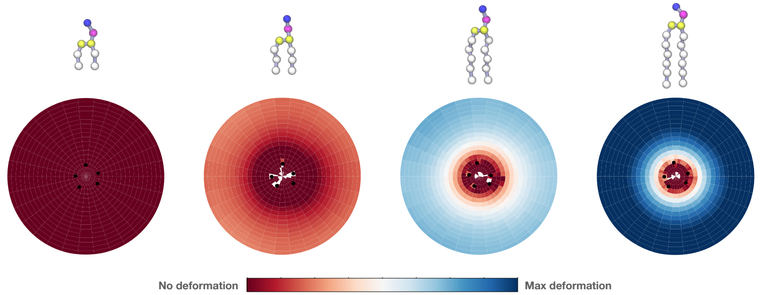
Results



Still image rendered from CG-MD trajectory of E protein surrounded by short lipid species DT. No significant membrane curvature detected.



Still image rendered from CG-MD trajectory of E protein surrounded by long lipid species DX. Considerable inner leaflet deformation detected.



(Upper) Martini representation of lipid species used to form the membrane, arranged shortest to longest.

(Lower) Top-down polar coordinate plots showing deformation of the inner leaflet relative to the bottom of E protein's TMD (black dots). **Dark red regions contain no membrane deformation. Pink, white, and blue represent regions containing increasing degrees of deformation.**

Unsaturated lipids (not shown) followed a similar trend.

Summary

- These results demonstrate that the novel **Asymmetric Hydrophobic Mismatch** mechanism explains E protein's ability to induce membrane curvature
- Neither the 'classical' hydrophobic mismatch nor shape-based explanations are sufficient to explain E's behavior
- To our knowledge, this is the first time an Asymmetric Hydrophobic Mismatch mechanism has been proposed and demonstrated