Coronavirus Envelope (E) Protein: Lipid Sensitivity and Membrane Bending

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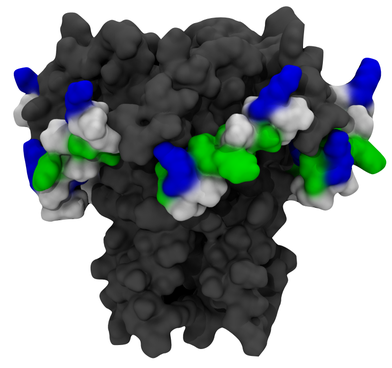
Abstract

The Coronavirus envelope (E) protein is a pentameric viroporin that is implicated in numerous viral processes including but not limited to assembly, budding, envelope formation, and pathogenesis. While much work has recently been done to characterize this protein's structure, function, and interactions with other proteins, its interactions with and effects on surrounding membranes are less well understood.

It is known that the viroporin loses ion-selectivity in an exclusively neutral lipid environment, but it is not clear what drives this behavior. In the present study we use coarse-grain molecular dynamics (CG-MD) simulations to identify stable binding sites for anionic lipid headgroups. We next plan to use all-atomistic molecular dynamics (AA-MD) simulations to investigate the effect of lipid charge on viroporin structure using the sites identified from CG-MD. Incorporation of anionic lipid species into the existing protein structure may explain the change in selectivity observed in previous studies, and could prove useful for groups pursuing drug development projects.

The E protein is also required for membrane curvature although the precise mechanism is unknown. Using CG-MD, we observe that the E protein bends the membrane in simulations with lipid species that have long acyl chains. We find this effect is limited when shorter lipid species are used, which suggests it results from asymmetric mismatch between the viroporin transmembrane domain (TMD) and the thickness of a typical host membrane. This result has implications for viral pathogenesis, as induction of membrane curvature is one of the important viral processes that leads to budding and further viral spread.

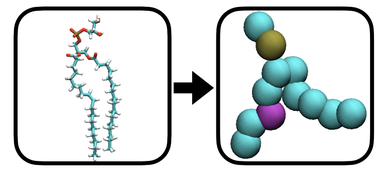


E protein viroporin (PDB ID 5X29) shown from the side. Residues are highlighted to show five-fold symmetry of the homo-pentamer. Lysine residues (blue) are positively charged at physiological pH.

Methods

- In order to achieve the time scale and lipid diffusion rates necessary, we use Coarse-Grain Molecular Dynamics (CG-MD) and the Martini 2.2 force field

All-Atomistic licorice representation of POPG



Coarse-Grain representation of POPG, with four non-hydrogen atoms mapped to one CG "bead"

- All simulations used Gromacs 2016 and ran for 25 μs using Berendsen thermostat at 313 K
- All simulations used lipid head group composition of 95% PC and 5% PG

Identification of Charged Lipid Binding Sites

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
- In addition to transporting K^+ and Na^+ the viroporin may also transport Ca^{2+}, activating the host inflammasome[1]
- The viroporin loses ion selectivity when placed in a neutrally charged lipid membrane[2]

Initial Observations

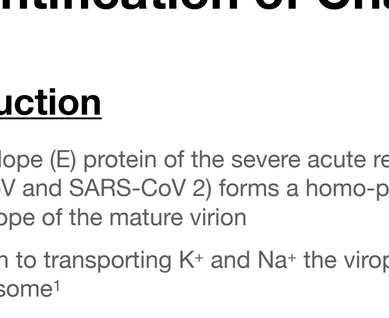
- E protein is unstable when simulated with AA-MD in an exclusively neutral lipid membrane
- Positively-charged lysine residues are located close to the TMD-ECD junction and may be attractive to anionic lipid head groups in the surrounding membrane

Approach

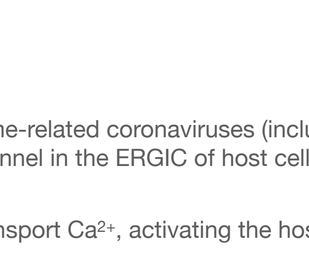
- We hypothesized that binding sites for charged lipid headgroups are located in the outer leaflet that may stabilize the structure and/or explain previous experimental results
- We measured normalized density of charged lipids in the annular region of the outer leaflet

Results

- Normalized density plots show clear enrichment of charged lipid POPG in annular region of outer leaflet
- Five-fold symmetric pattern of enrichment indicates presence of specific binding sites



Top-down polar coordinate plot showing normalized density of charged lipids in the outer leaflet. Black dots represent COM of E protein TMD helices. Dashed black line denotes approximate extent of E's protein cap.



Same plot as shown at left, but with charged lipid binding sites circled

Next Steps

- Back-map CG structure with 'bound' lipids to AA representation
- Measure binding affinity using Alchemical Free Energy Perturbation (AFEP)

Identification of Novel Membrane Bending Mechanism

Introduction

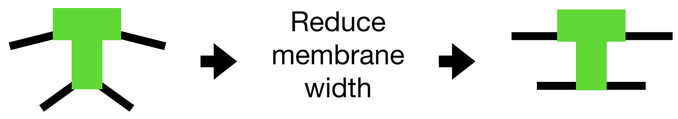
- It is known that the E protein induces membrane curvature, and that this deformation plays a key role in allowing the virus to escape its host cell and infect other cells[3]
- The precise mechanism is unknown

Initial Observations

- E protein's membrane deformation does not meet the definition of classical Hydrophobic Mismatch mechanism, which requires a symmetric membrane deformation around a protein inclusion[4]

Approach

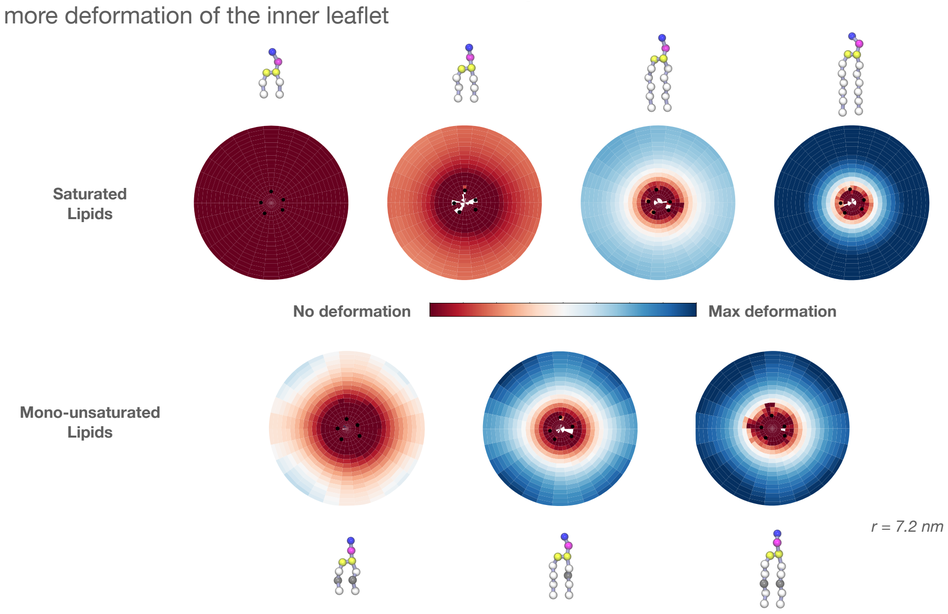
- We hypothesized that a novel *asymmetric* hydrophobic mismatch mechanism was responsible for membrane deformation and could be modulated by adjusting the width of the membrane local to the protein



In these cartoon representations green represents the E protein and the black lines represent the orientation of the lipid bilayer surrounding it

Results

- Results clearly indicate that shorter membrane widths yield less deformation and wider membranes yield more deformation of the inner leaflet



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.

Summary

- Binding sites for charged lipids have been identified in the outer leaflet annular region.
- These binding sites may stabilize the structure and/or explain previous experimental results
- The E protein bends thick membranes but not thin ones, suggesting an asymmetric mismatch mechanism.
- This mechanism may explain the critical role of the E protein in determining virus shape.

References & Acknowledgments

[1] Nieto-Torres, et al, Virology, 2015

[2] Verdiá-Báguena et al, Virology, 2012

[3] Schoeman & Fielding, Virology, 2019

[4] Jensen & Mouritsen, Biochim Biophys Acta, 2004

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