The Role of Lipid Packing in the Fusion Domain of SARS-CoV-2 on Fusion

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1. Background

- SARS-CoV-2 is the virus responsible for the COVID-19 pandemic
  - Over 760 million cases in the world and 6.8 million global deaths
- Membrane fusion is an integral part of the viral life cycle which is accomplished by it spike glycoprotein to deliver its genetic information into the target cells
- The fusion domain (FD) is responsible for initiating membrane fusion

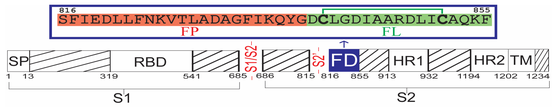


Figure 1: SARS-CoV-2 spike protein functional regions.[1]

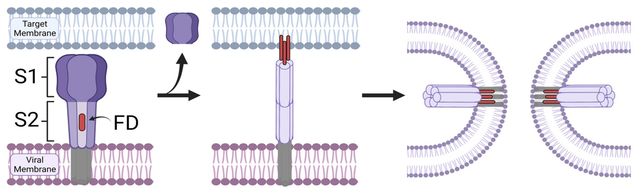


Figure 2: SARS-CoV-2 spike protein membrane fusion mechanism[1].

- The importance of the interaction of the FD and host cell membrane has been determined with simple lipid compositions[2]:
  - Linear increase in fusion with increasing anionic lipid concentration
  - Inverse relationship with lipid packing and membrane fusion
  - The FD prompted fusion in an environment resembling the late endosomal membrane

2. Hypothesis

There is a preference of fusion for cell lines with <u>larger anionic lipid concentrations</u>

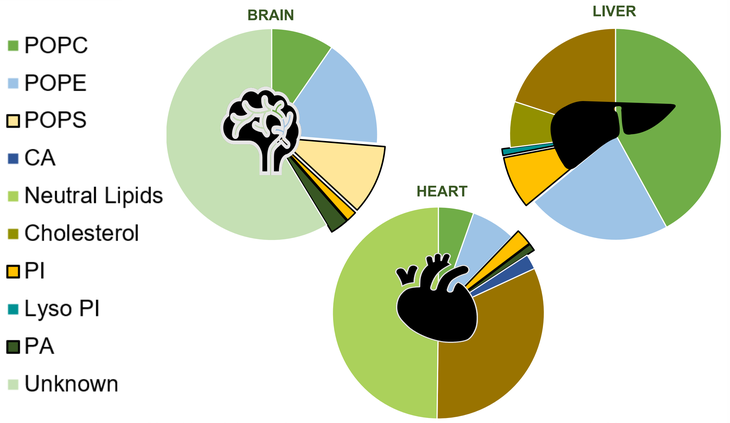
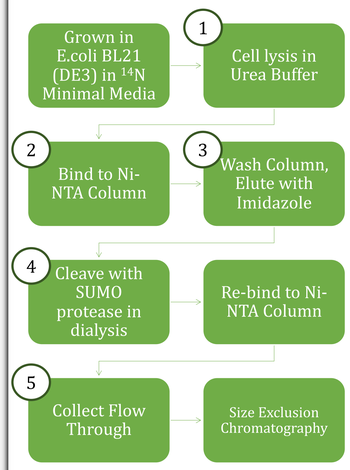


Figure 3. Pie charts of lipid breakdowns for Brain, Liver, and Heart cell types provided by Avanti Lipids

Therefore, the <u>brain cell type lipid composition</u> will display the greatest fusion

3. Protein purification

40 amino acid FD construct inserted into pET41 vector containing an N-terminal Hisx6-SUMO tags.



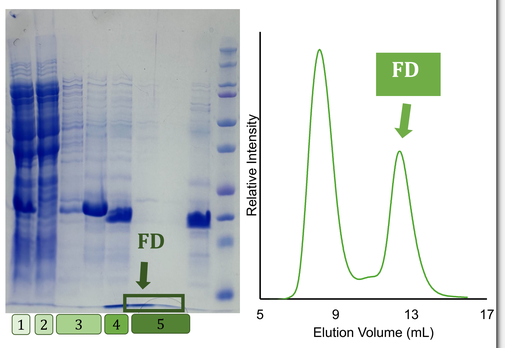


Figure 4: Tris Tricine SDS-PAGE Gel and corresponding trace from Size Exclusion Chromatography with Peptide Column

4. Fusion Assay

FRET based lipid mixing assay to assess the fusogenic properties of the complete FD

- pH induced fusion: The pH was dropped to pH 5 to resemble the endosomal pH
- Triton added to cause 100% signal

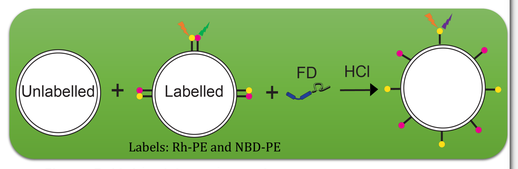
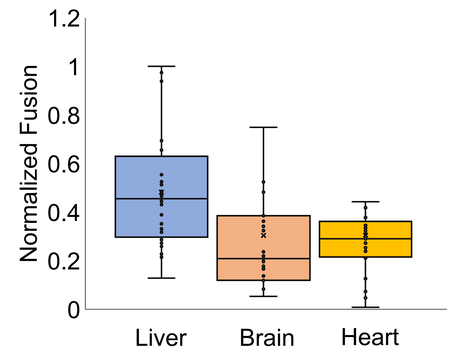


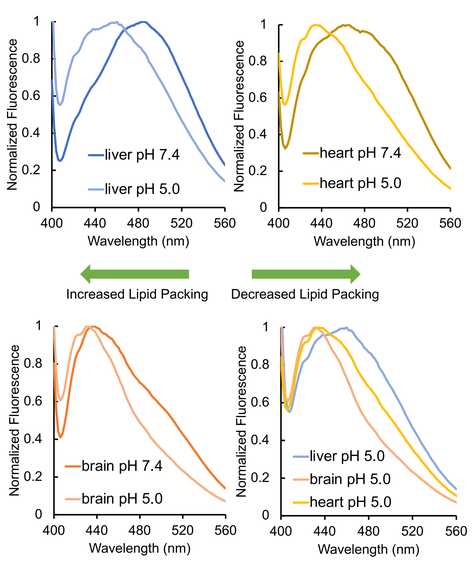
Figure 5: Lipid mixing schematic.



- The Liver cell line lipid composition displays the most fusion
- Followed by the heart and then the Brain
- Despite the heart lipid containing the highest amount of anionic lipid it has the lowest fusion

Figure 6: WT fusion with different lipid compositions via lipid mixing. (n≥26)

5. Lipid Packing



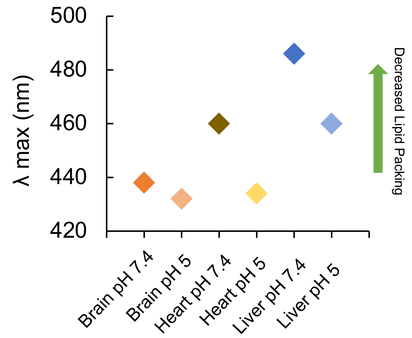


Figure 7: Lipid packing of LUVs of different compositions compared via C-Laurdan

C-Laurdan

- Fluorescent Probe that is sensitive to the lipid environment.
- Two emission peaks:
  - ~440 nm (⬆ Lipid Packing)
  - ~490 nm (⬇ Lipid Packing)

- The lowest amount of lipid packing is in the liver cell type lipid composition which is then followed by heart and then brain cell types
- Even with lowering the pH, which shows an increase in lipid packing, the trend is consistent

6. Conclusions and Future Work

<u>Our conclusions :</u>

- There is an inverse relationship between lipid packing and membrane fusion mediated by the FD
- Liver lipid compositions have the greatest membrane fusion and lowest amount of lipid packing
- Brain lipid compositions displayed less membrane fusion than the liver despite a higher anionic lipid concentration
- Lipid packing may be more important than anionic lipid concentrations for membrane fusion

<u>Moving forward</u> we plan to further investigate...

- The impact that these complex cell line lipids have on not just the fusion function of the FD but also the structure
- The interactions of these complex cell lines with mutants of the FD

6. References

(1) *Adapted from "Coronavirus Replication Cycle", by BioRender.com (2020). Retrieved from https://app.biorender.com/biorender-templates*
(2) *Birtles, D, Abbas, W, Lee, J. Bis(monoacylglycero)phosphate Promotes Membrane Fusion Facilitated by the SARS-CoV-2 Fusion Domain. (Under Review)*