Using Molecular Dynamics Simulations to elucidate a role for bacterial ceramides

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MS Defense

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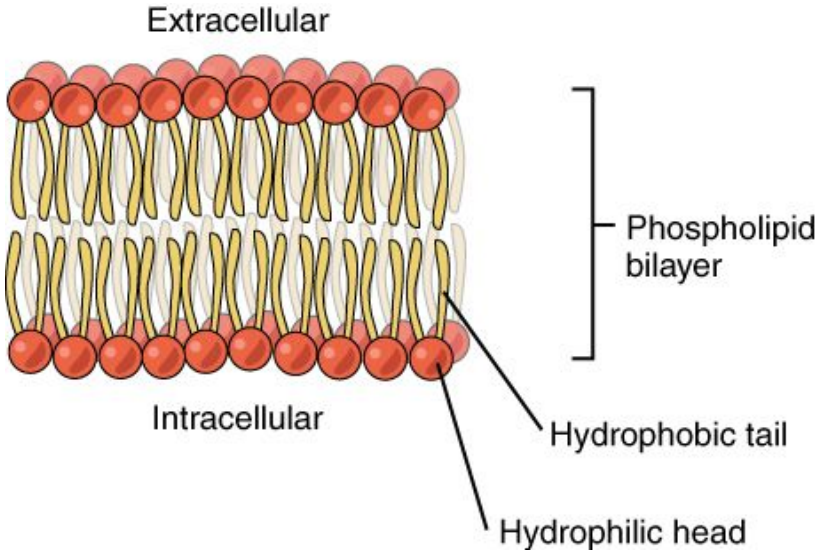
Outline

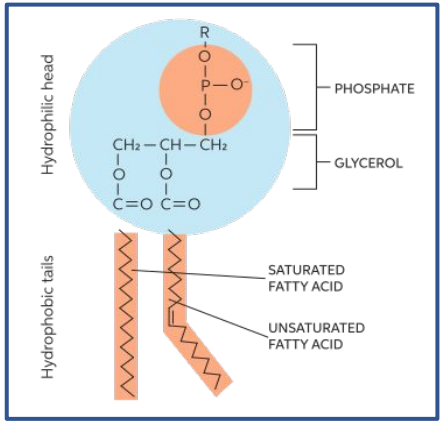
- Background
  - What are biological membrane lipid compositions?
- Motivation
  - What is the role of unique bacterial lipids?
- Methods
  - How do we study these lipids and their effects?
- Results
  - What did we find?
- Conclusion
  - What is next?

Background - Cell Membrane and lipids



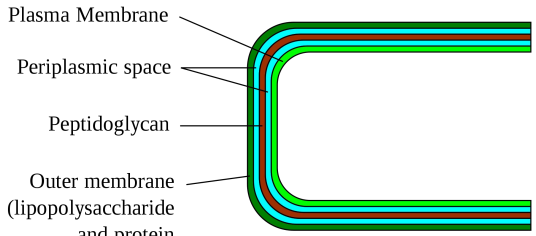
Typical bilayer membrane

- Commonly found lipid in membrane include Glycerophospholipid

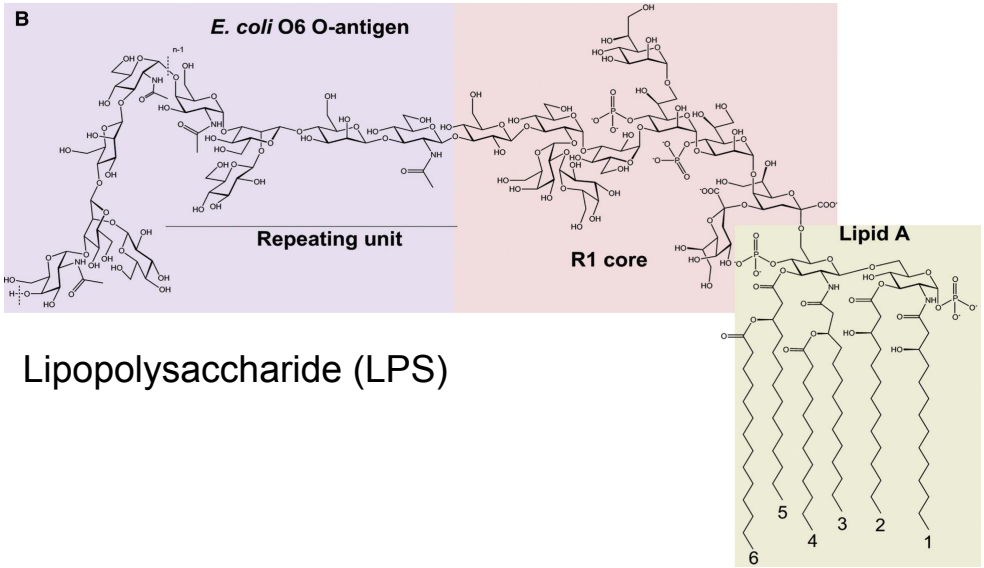


- Eukaryotic lipids typically contain 2 acyl chains
- Bacterial lipids may have more than 2 acyl chains

Background - Gram-negative bacteria



Gram Negative

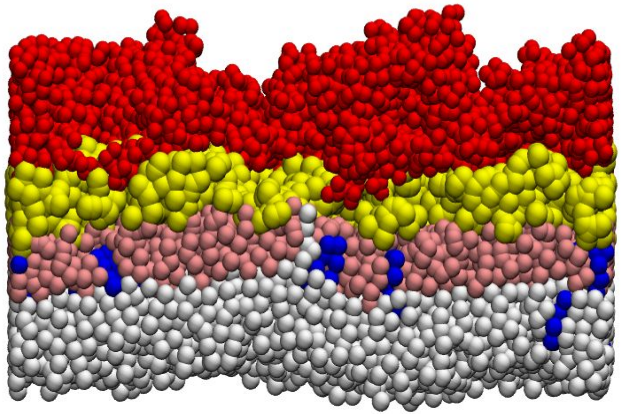


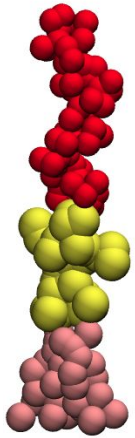
Lipopolysaccharide (LPS)

- Gram-negative bacteria have inner and outer membrane.
- Lipid A conserved between species
  - Number of acyl chains vary
- Core oligosaccharides consist of repeating units of sugars, such as keto-deoxyoctulosnate (KDO) and hexoses.
- O-antigen chain consists of several repeating units of sugar
- Large diversity in the sugar compositions of the O-antigen domain

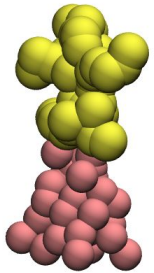
Background - Closer look at the outer membrane

- There are two types of LPS - Smooth and Rough
- Smooth LPS (SLPS)- contains all three domains
- Rough LPS (RLPS)- lacks the O-antigen





Smooth LPS (SLPS)



Rough LPS (RLPS)

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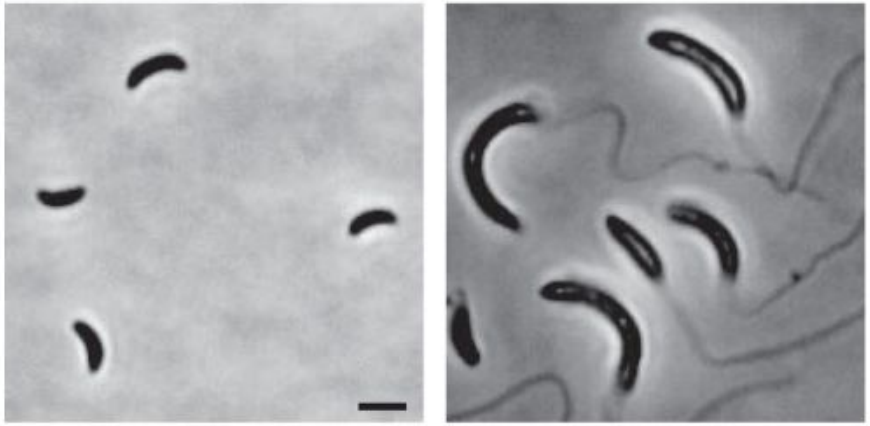
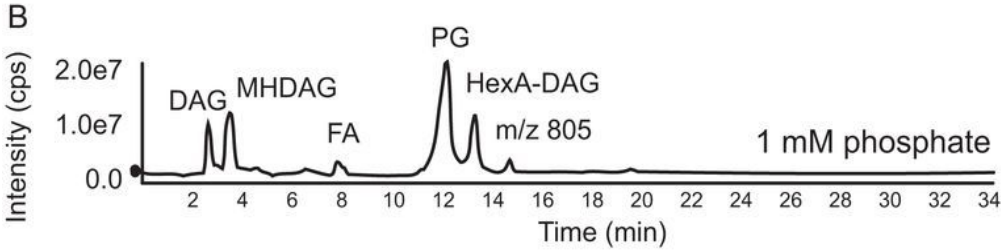
Motivation

- *Stankeviciute G, et al,. mbio 2019*
- *Caulobacter crescentus* is an oligotrophic Gram-negative bacterium
- Adapts to phosphate starvation by elongating and producing stalks



How are the cells able to elongate with limited phosphate?

Lipid Composition:



Stankeviciute G, *et al,*. mbio 2019

Motivation

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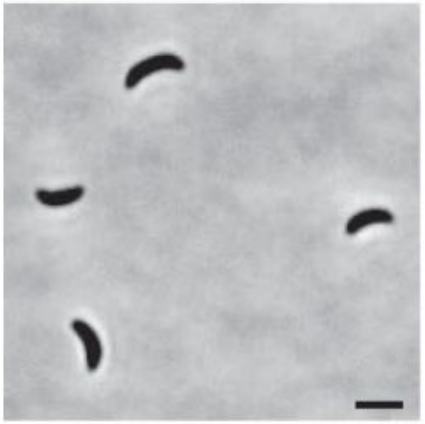
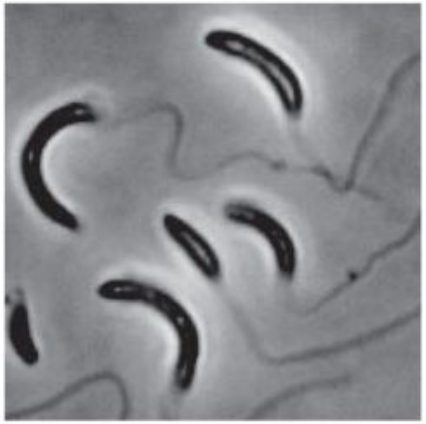
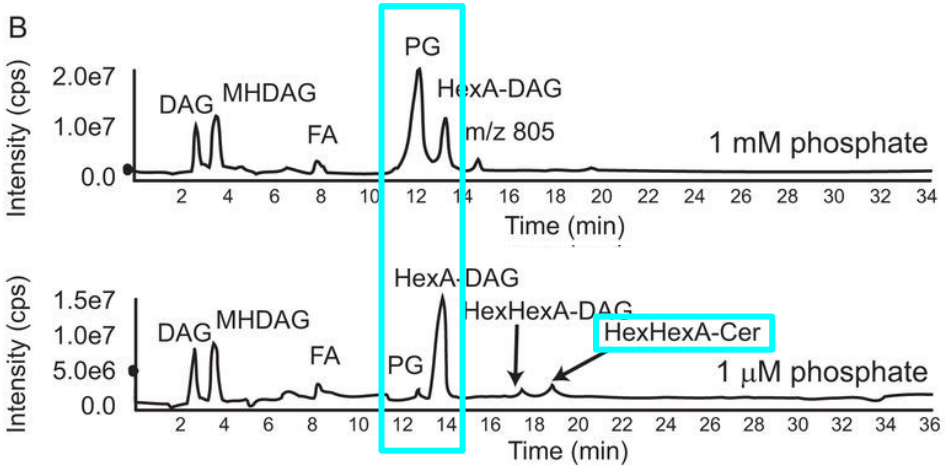


1 mM phosphate



1 μM phosphate

Lipid Composition:



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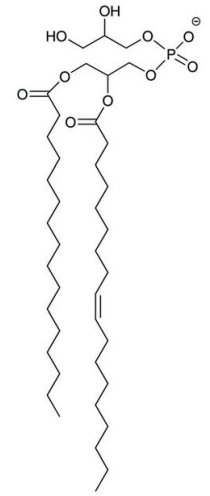
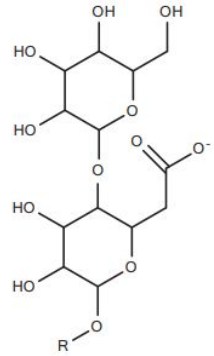
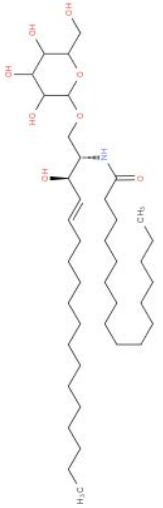


Phosphatidyl glycerol

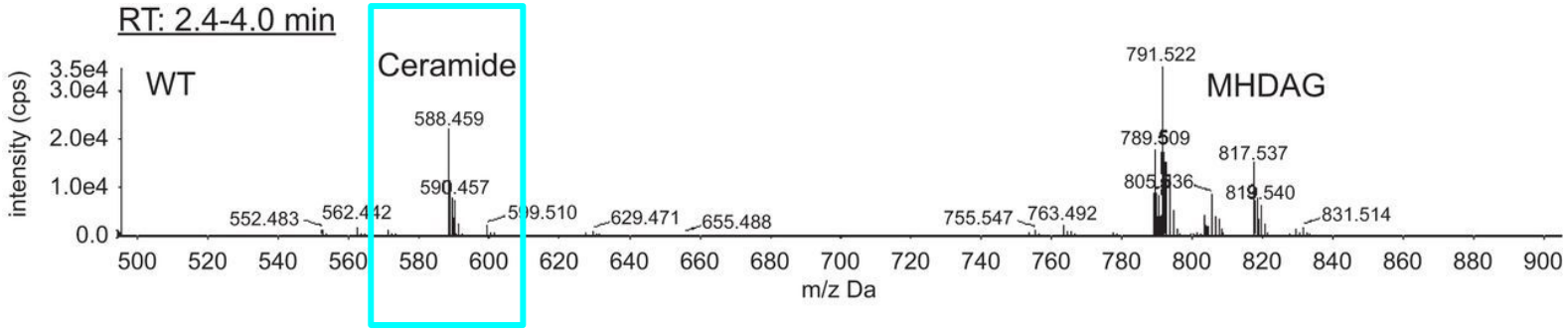


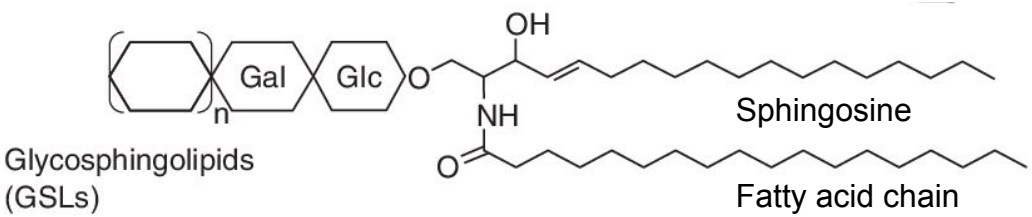
Hex-HexA-Cer
GSL-2

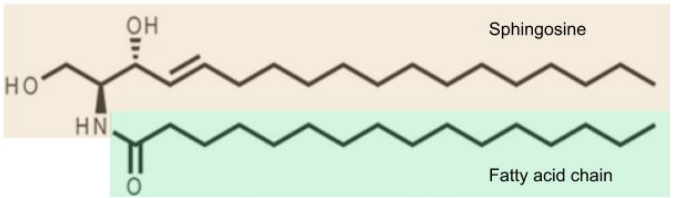


Caulobacter produces sphingolipids!

- Produces glycosphingolipids only in low phosphate condition



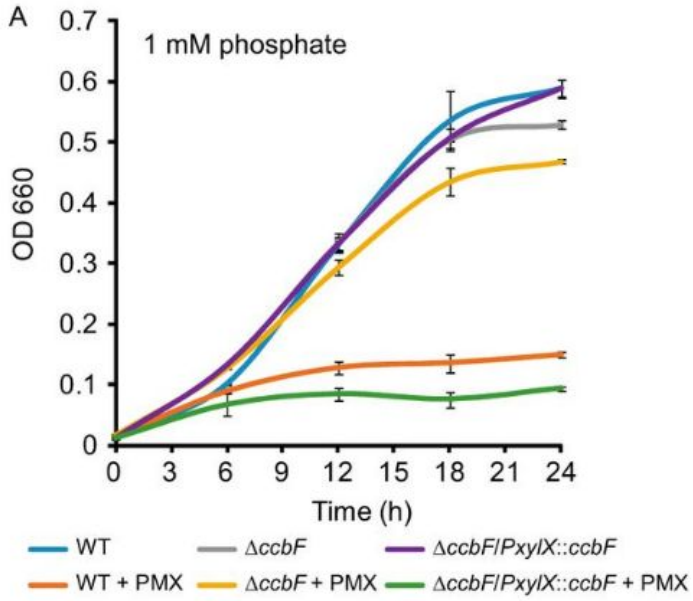




Ceramide

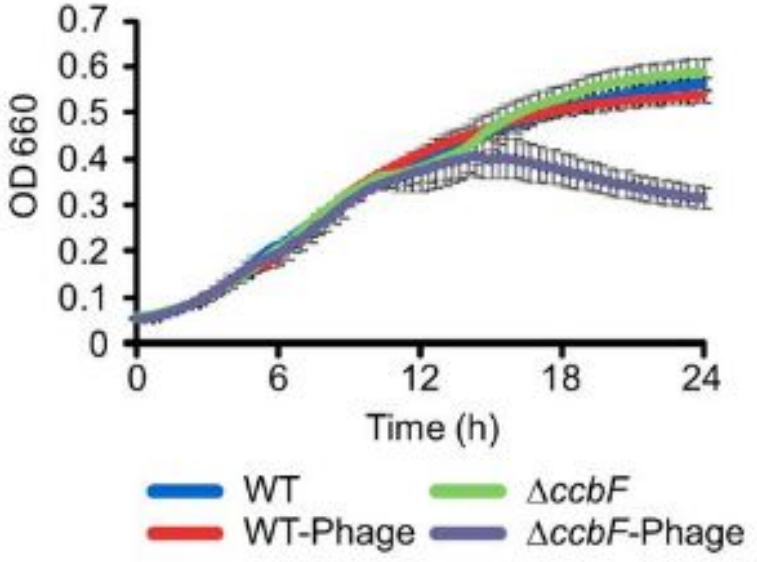
Stankeviciute G, *et al*,. mbio 2019

Antibiotic sensitivity



- ccb*F*: gene involved in the first step of ceramide synthesis
- *Caulobacter* deficient in ceramides is resistant to PMX

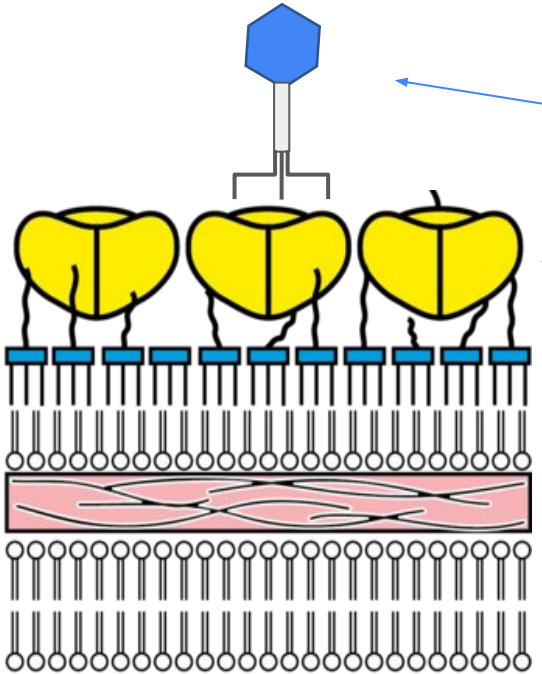
Phage sensitivity



- *Caulobacter* without ceramide show **more** phage binding!

Stankeviciute G, *et al*,. mbio 2019

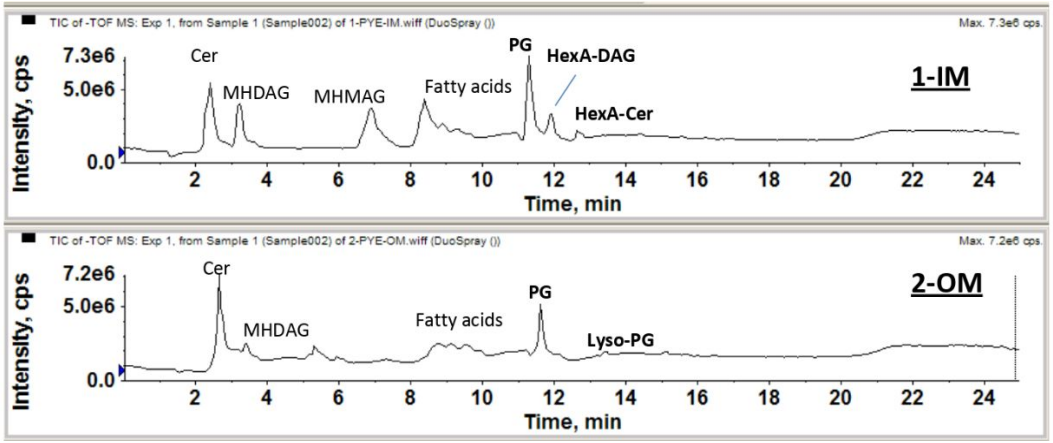
Ceramide and phage resistance



Phage

S-layer

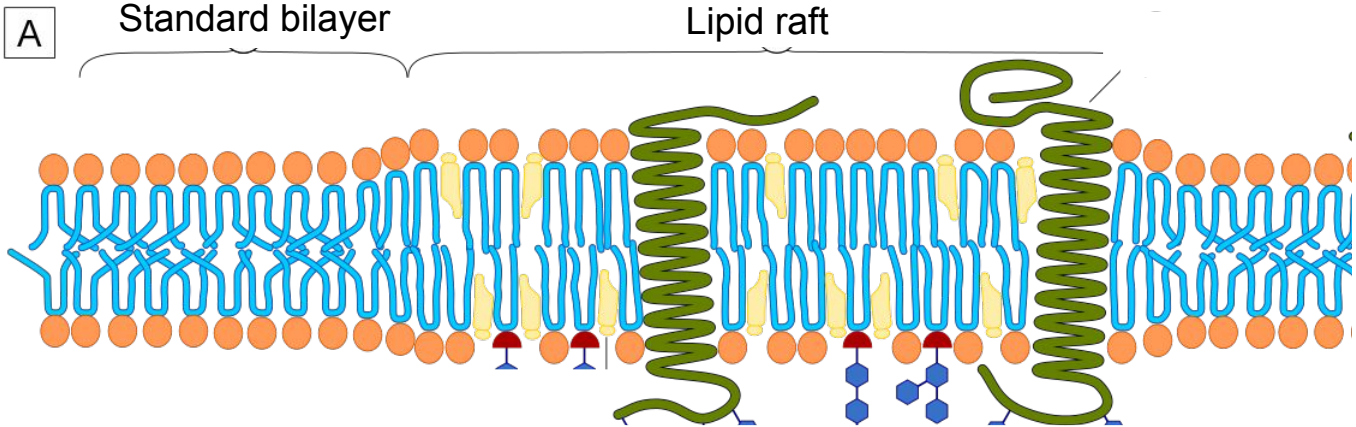
Outer membrane (LPS/GSL)



What does Ceramide do in the membrane?

Hypothesis

- Ceramide causes domain formation, lipid rafts, which effects lipid organization in the membrane
- Lipid Domains are enriched in cholesterol, sphingolipids and phospholipids
- Lipids within domains are saturated and more ordered, area per lipid decreases



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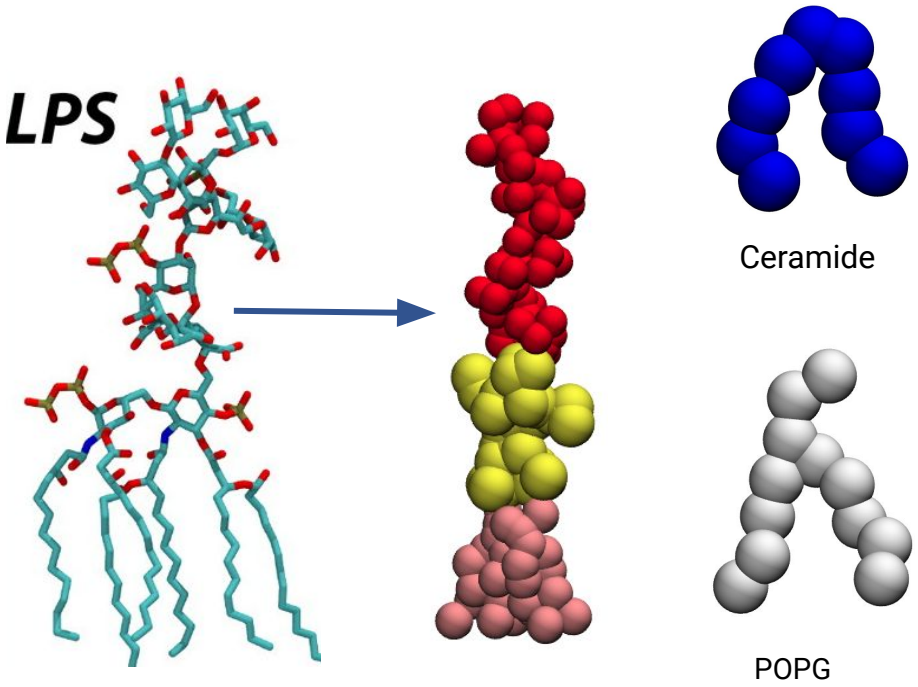
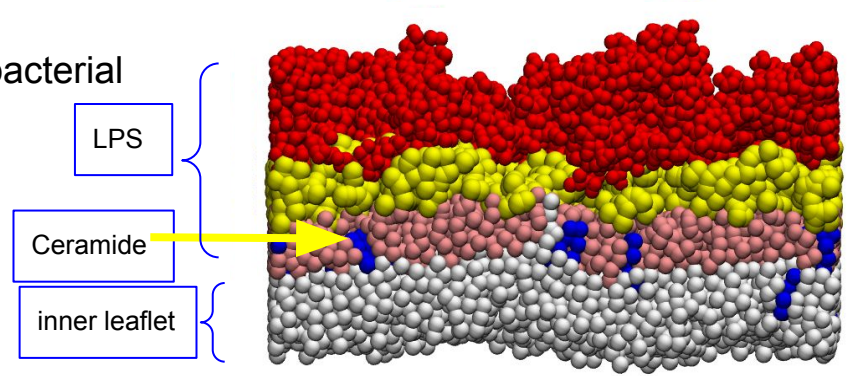
Our computational approach

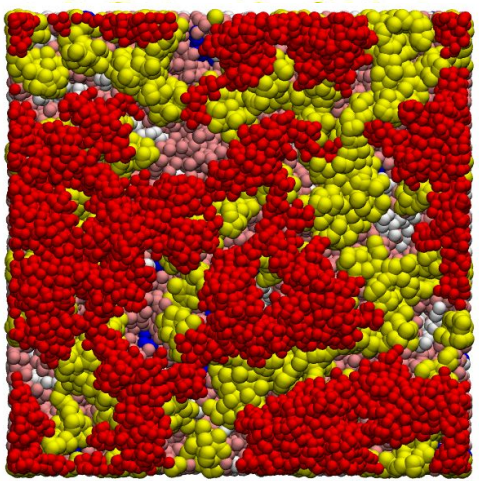
- Difficult to study membrane conformational changes experimentally
- Molecular Dynamics as computational microscope
- Coarse-Grained MD
- *E. coli* LPS



Membrane Systems and composition

- **Random Membrane**
  - Mimics native bacterial membrane





- Composition of outer leaflet:
  - 1:1:0.5 RLPS: SLPS:POPG
  - Ceramide concentrations of 0, 5, 10, and 20%

Membrane Systems and composition

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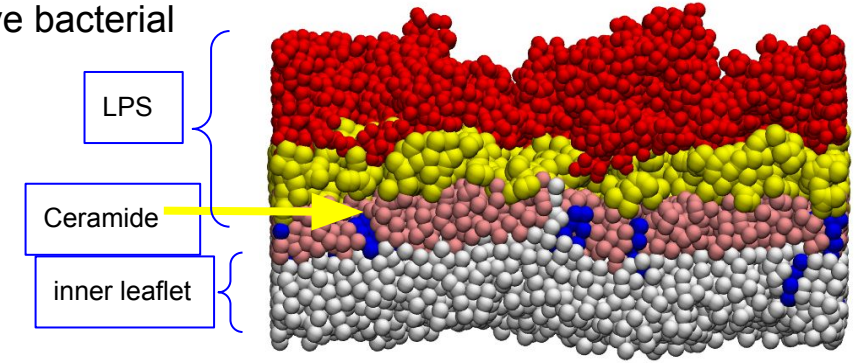
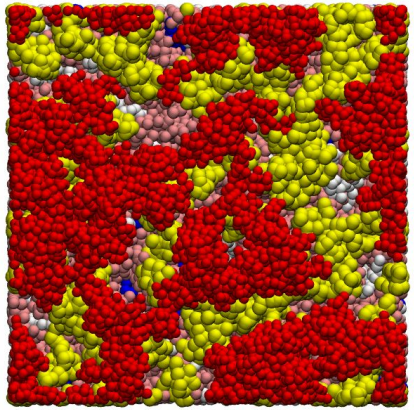
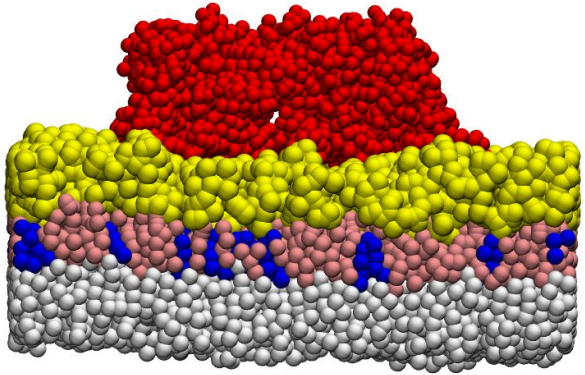
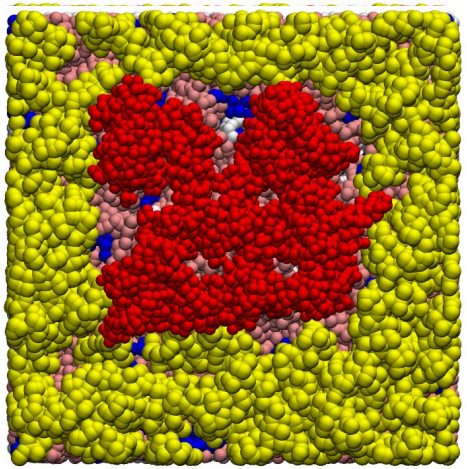




- **Artificial Membrane**
- Composition of outer leaflet
  - 1:3:0.5 SLPS: RLPS:POPG
  - Ceramide concentrations of: 0, 10, 15, 20 and 25%.





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Hypothesis: ceramide causes domain formation

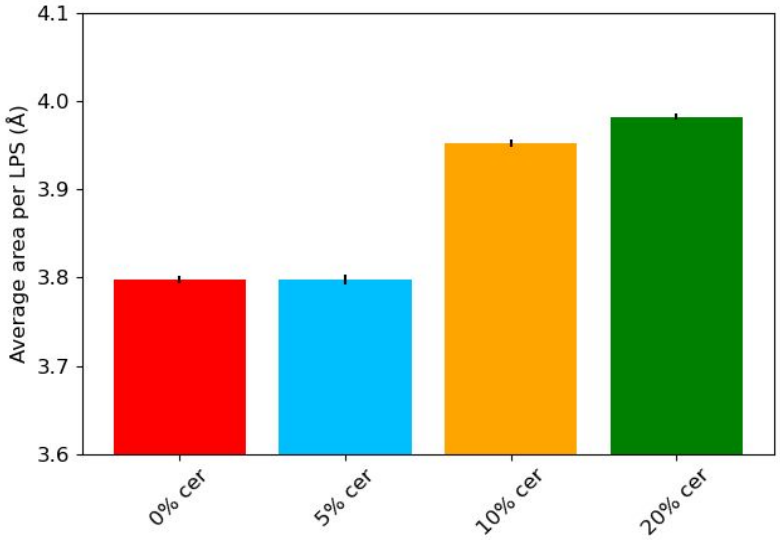
LPS is highly immobile, unable to see domain formation within simulation time.

Characteristics of lipid within domains:

- Decreases area per lipid
- Lipid within domains are more ordered
- Lipids are packed tightly together

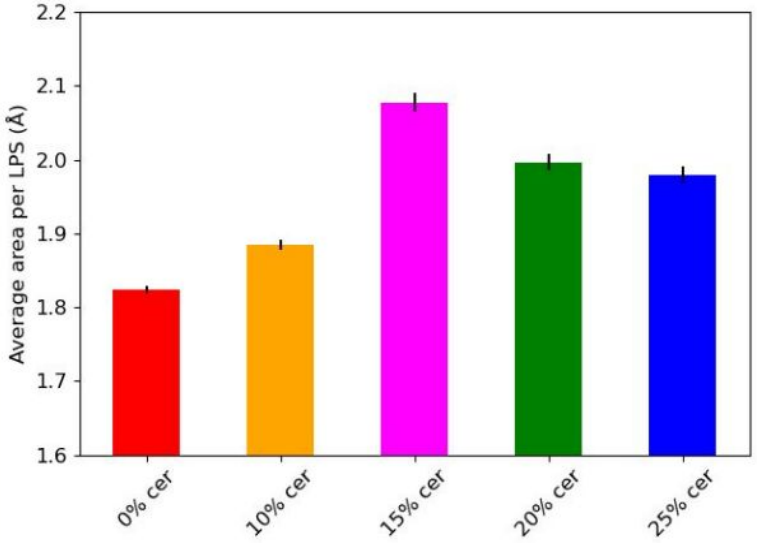
Area per LPS

Random membrane



- Area increased with increasing ceramide concentration

Artificial membrane



- Area increased with increasing concentration until 15% ceramide. 20 and 25% systems show no significant difference.

Order parameter

- To study mobility of acyl chain we calculated the order parameter

$$S = 1/2 < 3(cos\theta)^2 - 1 >$$

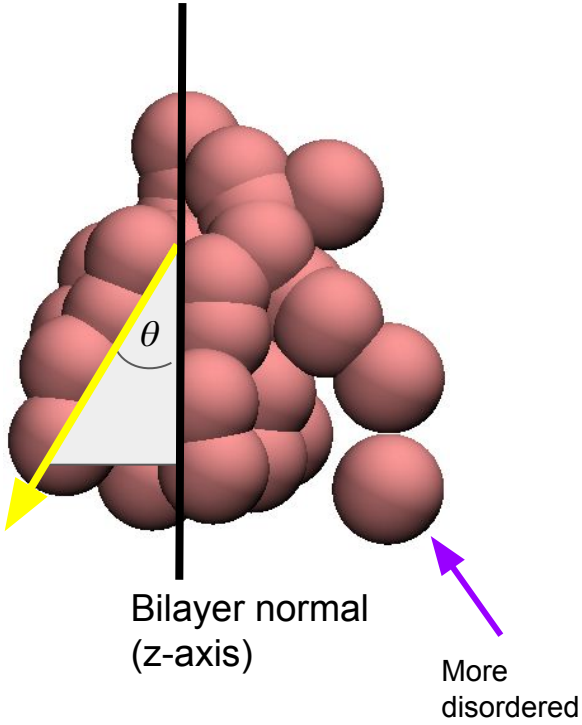
Θ - represents the angle between the acyl chain vector and the bilayer normal

Averaged over time and over all the LPS molecules

Value range from 0 to 1:

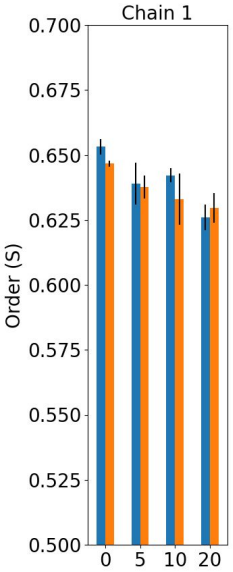
0 = Disordered

1 = Ordered

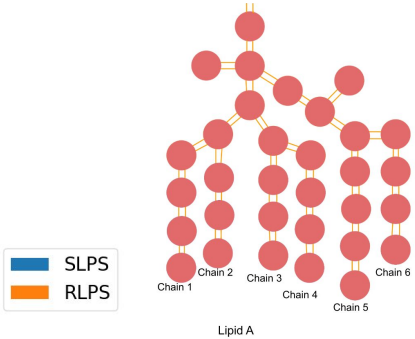


Order parameter

Random membrane

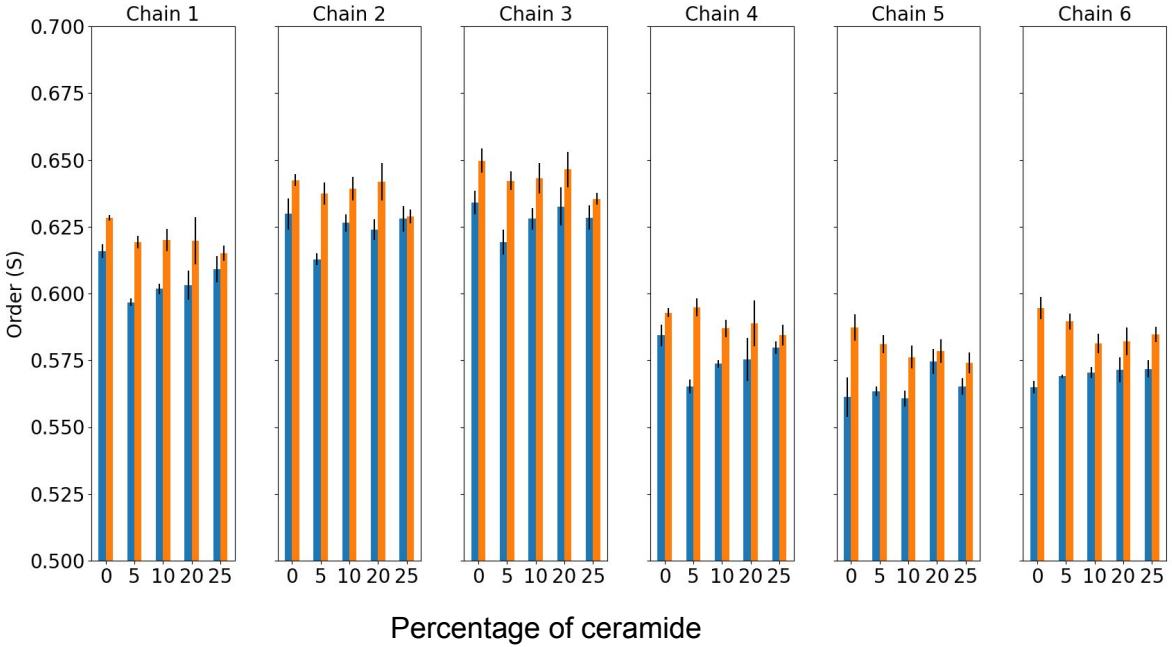


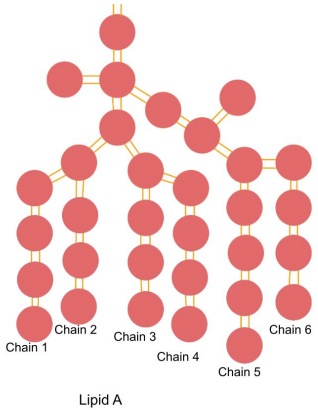
Percentage of ceramide



- Chain 6 was more disordered in both SLPS and RLPS
- Average order for chains 4 and 5 decreased with the addition of 10% and 20% ceramide
- There is no difference in the order of RLPS and SLPS within a given ceramide concentration.

Order parameter





- SLPS chain 1 order increased with increasing ceramide concentration.
- RLPS chains are more ordered compared to SLPS

Hypothesis: ceramide causes domain formation

Characteristics of lipid within domains:

- Decreases area per lipid
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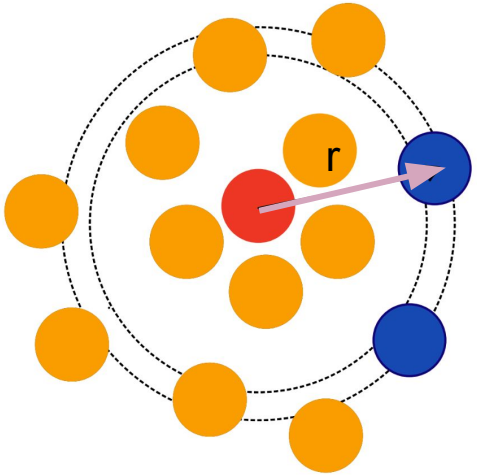
- Lipids are packed tightly together

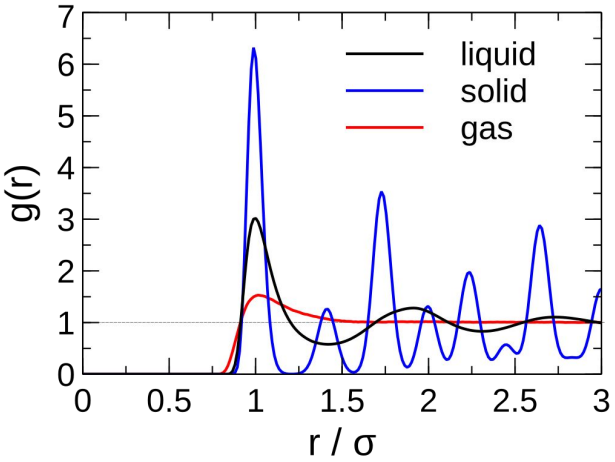
<u>Our findings:</u>

- Area per LPS increase, with increasing ceramide
- SLPS in artificial membrane,is more disordered
- RLPS is more ordered

Radial distribution function (rdf)

- Examining Lipid packing
- Rdf - measures the probability of finding pairs of atoms at distance of radius, r





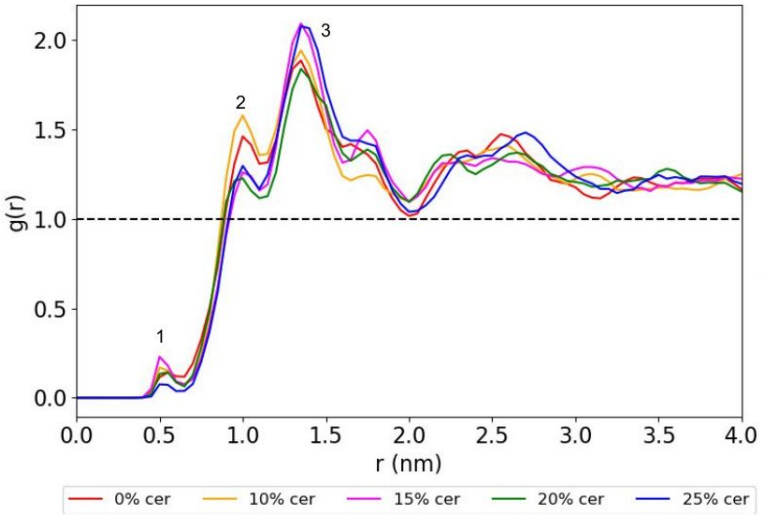
We measured the following pairs

- RLPS- RLPS
- SLPS-SLPS
- SLPS-RLPS

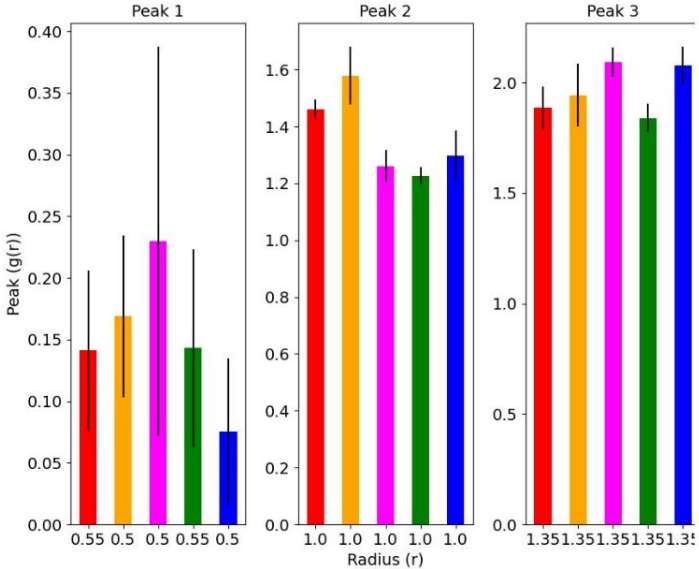
Radial distribution function (rdf):

Artificial membrane: RLPS-RLPS

Rdf distribution



The peak height for first three peaks

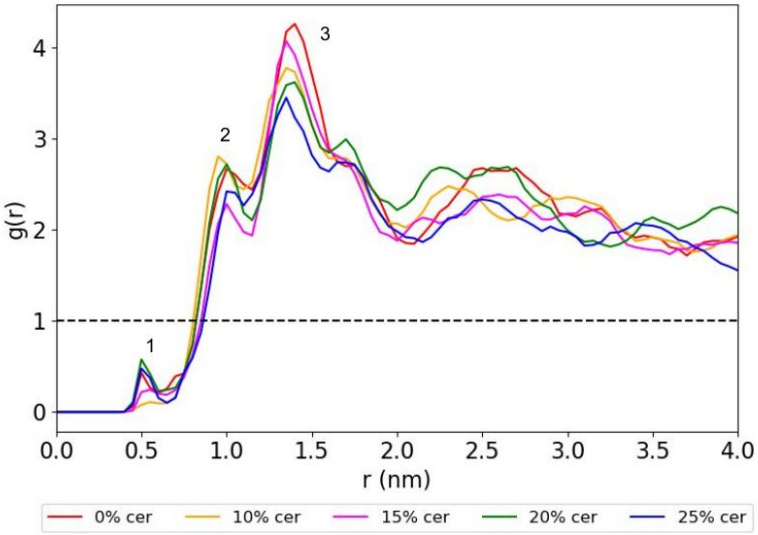


- More RLPS found closer together in system with 15% and 25% ceramide.

Radial Distribution (rdf):

Artificial membrane: SLPS-SLPS

Rdf distribution



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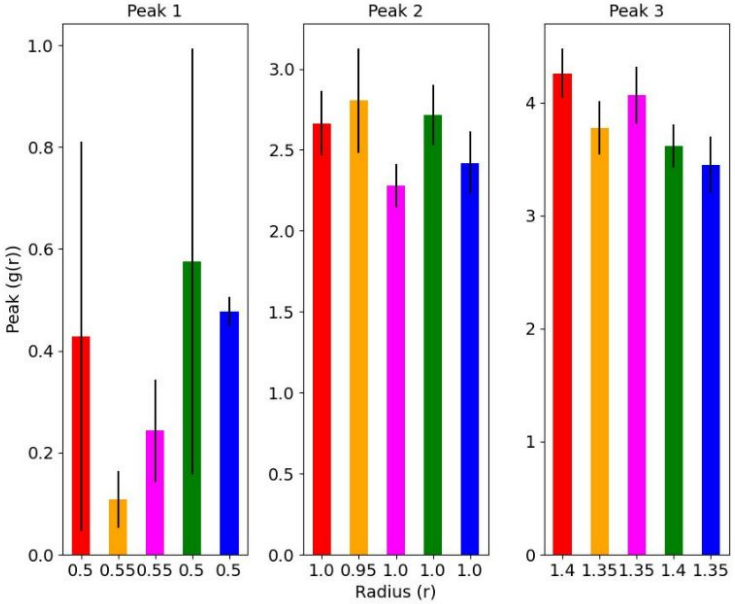


- More SLPS found closer together in system with 0% and 15% ceramide.

Hypothesis: ceramide causes domain formation

Characteristics of lipid within domains:

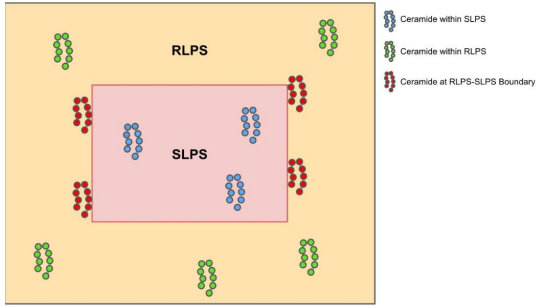
- Decreases area per lipid
- Lipid within domains are more ordered

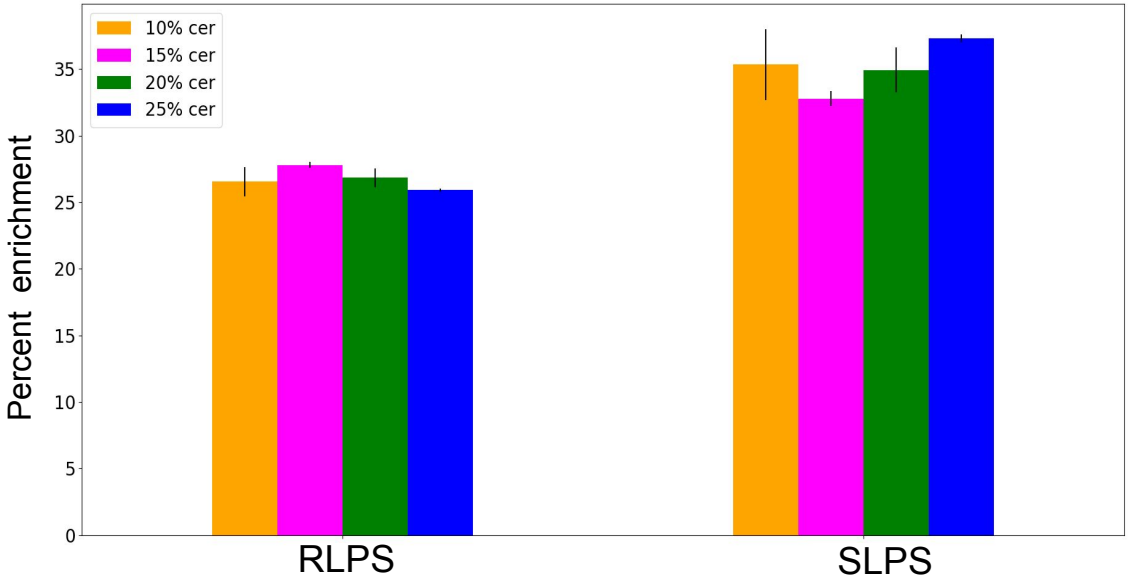
- Lipids are packed tightly together

<u>Our findings:</u>

- Increases area per LPS, with increasing ceramide
- SLPS around only SLPS is more disordered, from artificial membrane
- RLPS is more ordered
- RLPS in high ceramide concentration is closer to other RLPS
- SLPS-SLPS are likely to be closer in lower ceramide concentration

Ceramide Occupancy in artificial membrane





- SLPS region is enriched in ceramide

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Our findings:

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- SLPS around only SLPS is more disordered, from artificial membrane
- RLPS is more ordered
- RLPS in higher ceramide concentration is closer to other RLPS
- SLPS is likely to be closer in lower ceramide concentration
- Ceramide may favor interaction with the more disordered, SLPS

Summary

- Area per LPS increases in systems with 5, 10, and 15% ceramide, but area decreases in systems of 20 and 25% ceramide.
- Across ceramide concentrations, RLPS is more ordered compared to SLPS
- RLPS-RLPS were likely to be found closer to each other in systems with 15% and 25% ceramide but not in the lower concentrations
- Ceramide may favor interaction with more disordered, SLPS

Overall, we find that ceramide disrupts LPS packing.

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Conclusion - What's next?

- Our finding do not provide any insight into specific mechanisms to explain antibiotic and phage sensitivity, **yet!**

- Antibiotic sensitivity
  - Simulations including polymyxin B, LPS, ceramide should be studied - particularly with regards to calcium ion interaction in the core region of LPS.

- Phage sensitivity
  - Simulations including the surface protein might provide better insights.

  - Ceramide disrupts LPS packing, thus it may further impact the protein layer organization, that could leave 'pockets' in the outer membrane, preventing bacteriophages from attaching to the outer membrane.

Thank you!

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