Using molecular dynamics simulations to elucidate a role for bacterial ceramides

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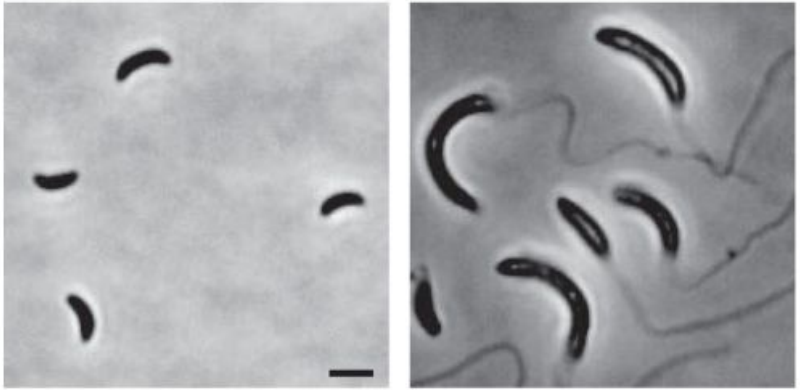
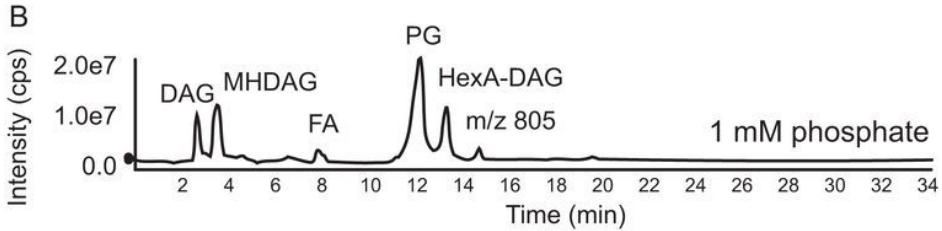
Background

- *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

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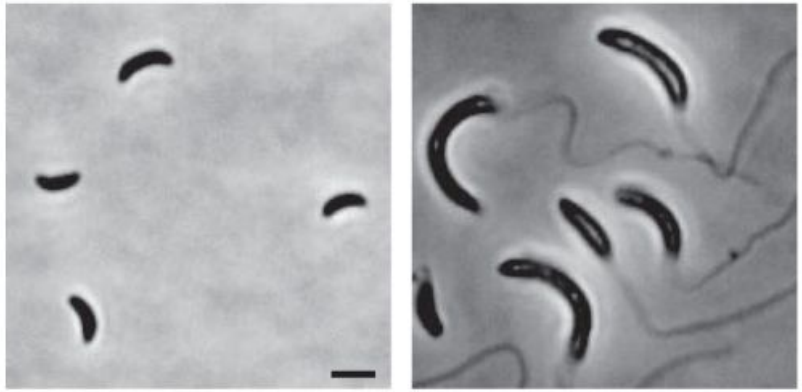

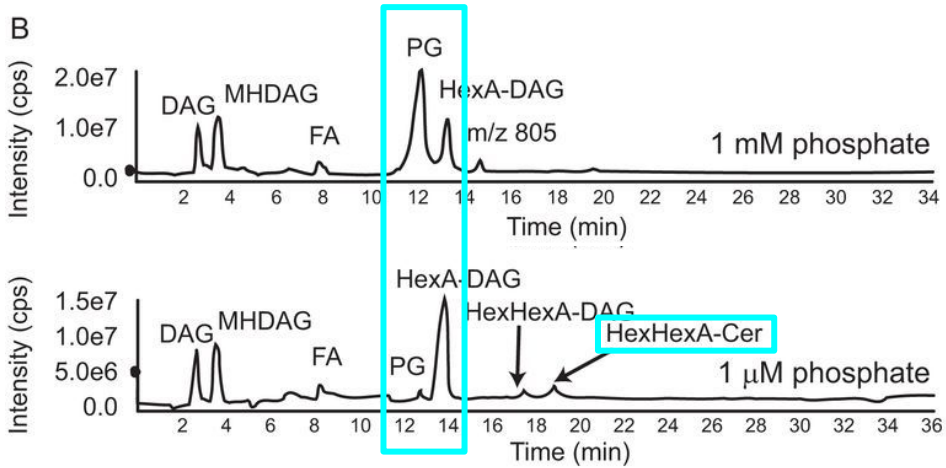

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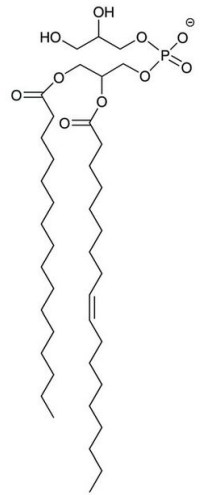
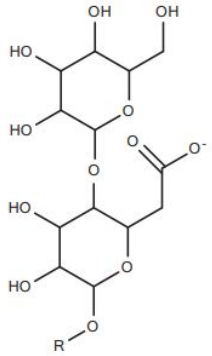
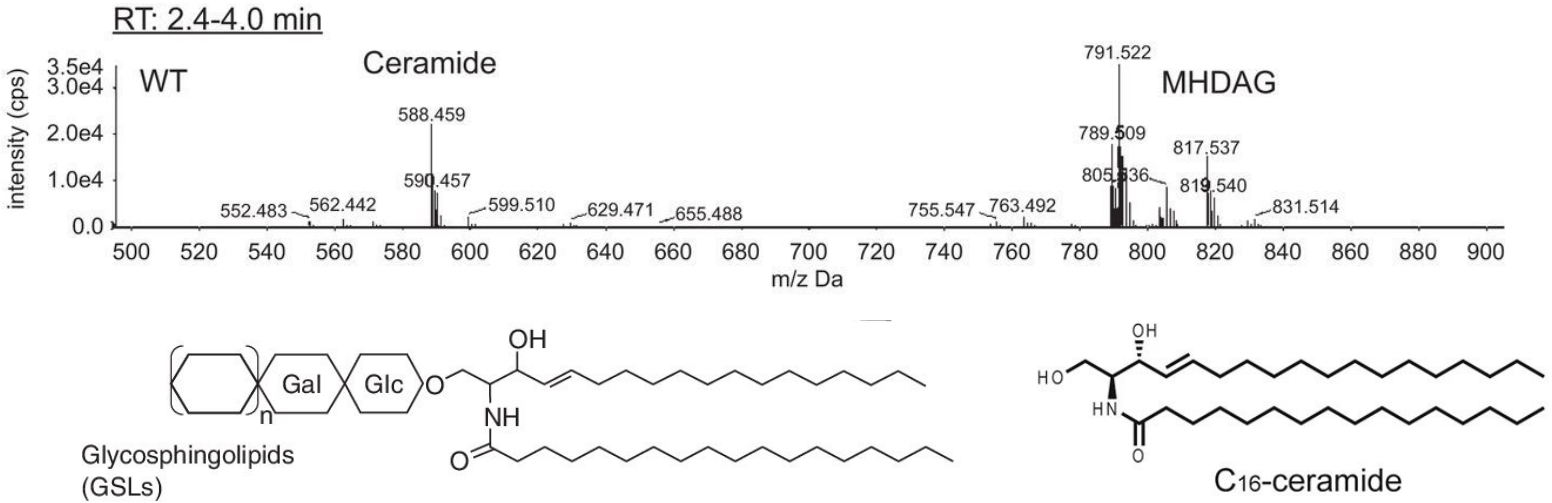




Caulobacter produces sphingolipids!

- Produces glycosphingolipids only in low phosphate condition



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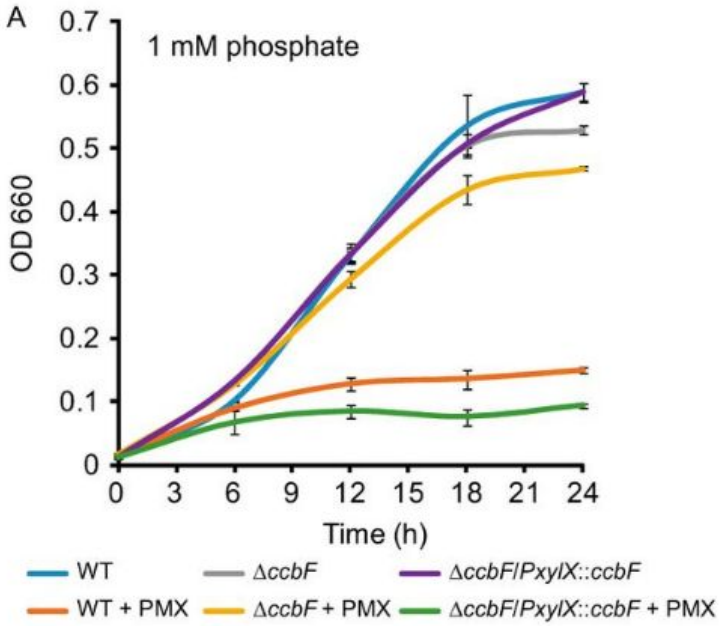
Antibiotic sensitivity



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

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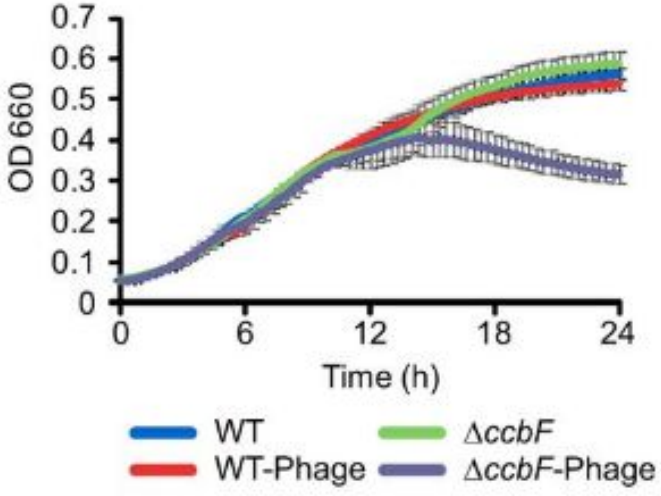
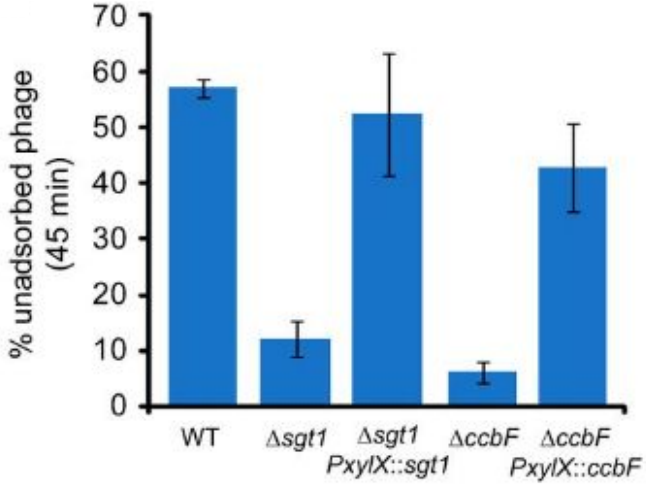
Phage sensitivity





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

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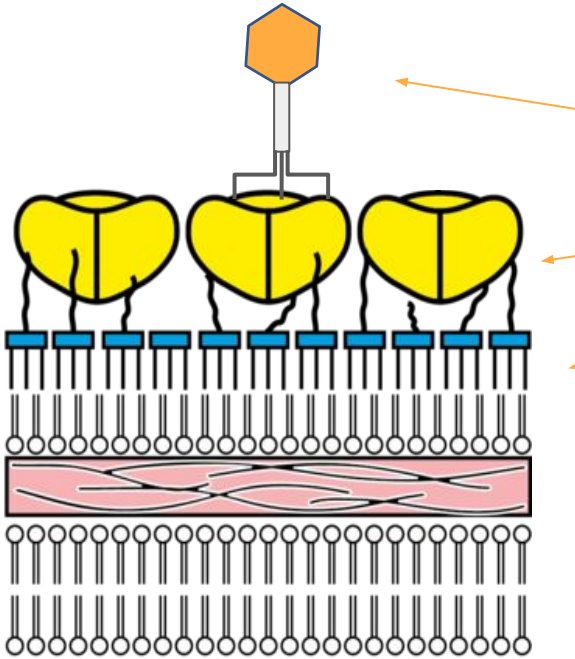
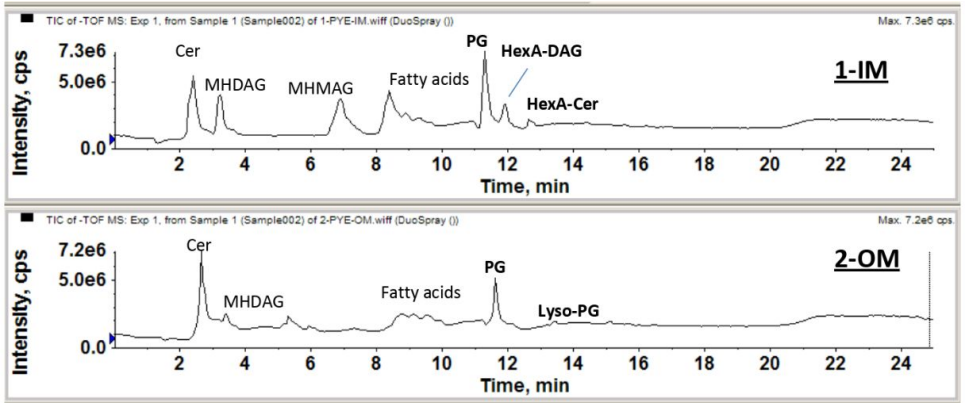
Ceramide and phage resistance



Phage

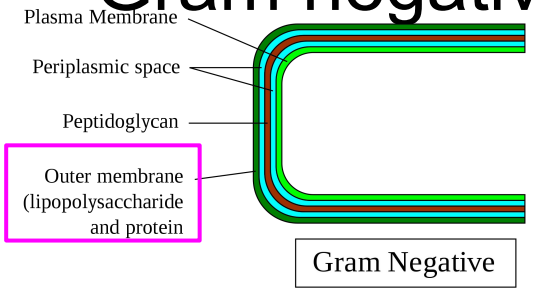
S-layer

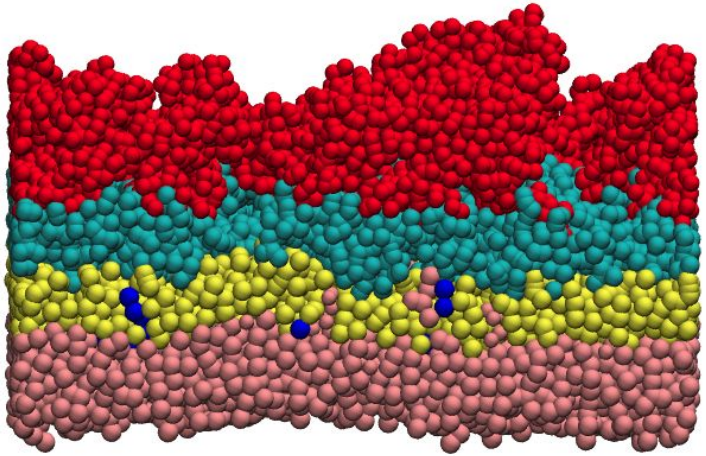
Outer membrane (LPS/GSL)



What does Ceramide do in the membrane?

Gram negative bacterial outer membrane





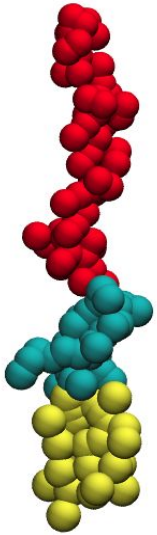
O- antigen

Core

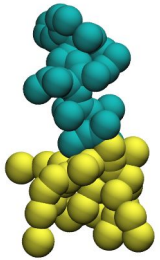
Lipid A

LPS

Phospholipids



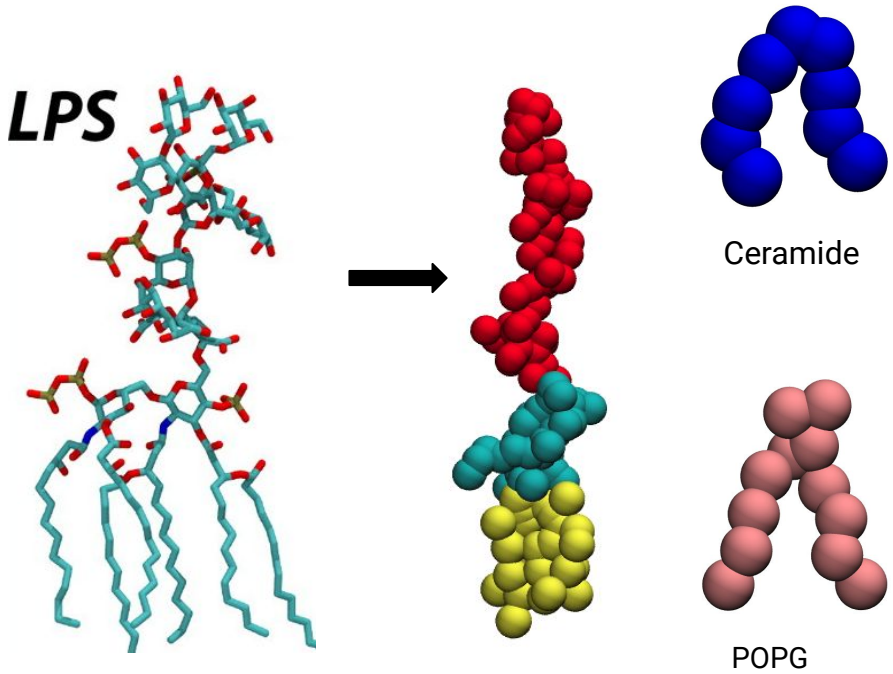
Smooth LPS (sLPS)



Rough LPS (rLPS)

Our computational approach

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

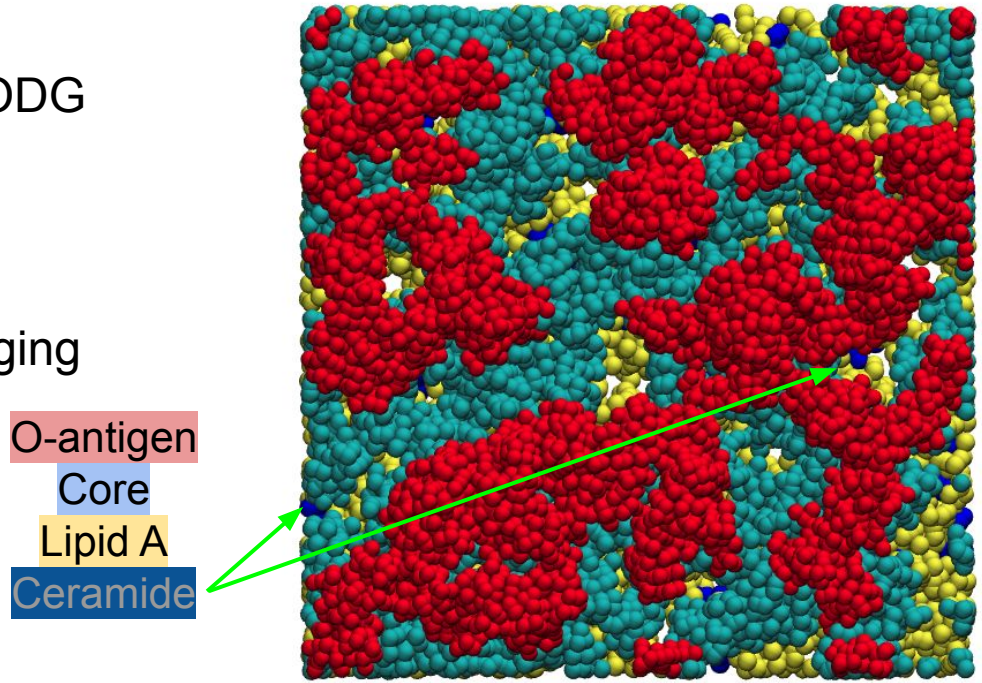


Membrane compositions

- Outer leaflet:
  1:1:0.5 rLPS:sLPS:POPG
- Inner leaflet: 95:5 POPG:PODG

- Simulations are set up with ceramide concentration ranging from 5-40%

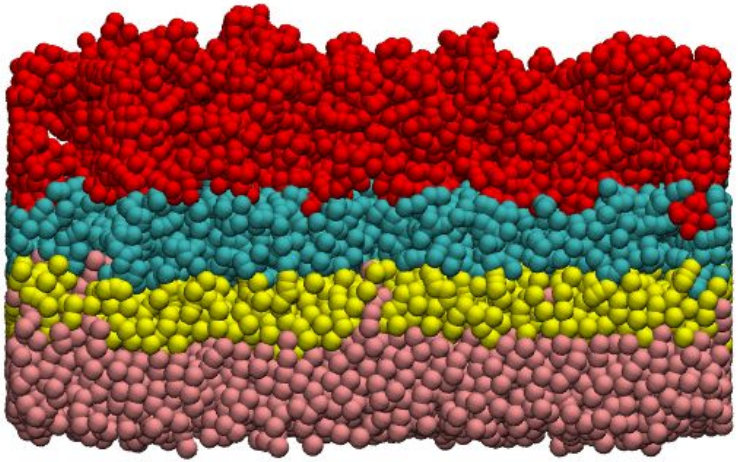
20% ceramide simulation - Extracellular view:



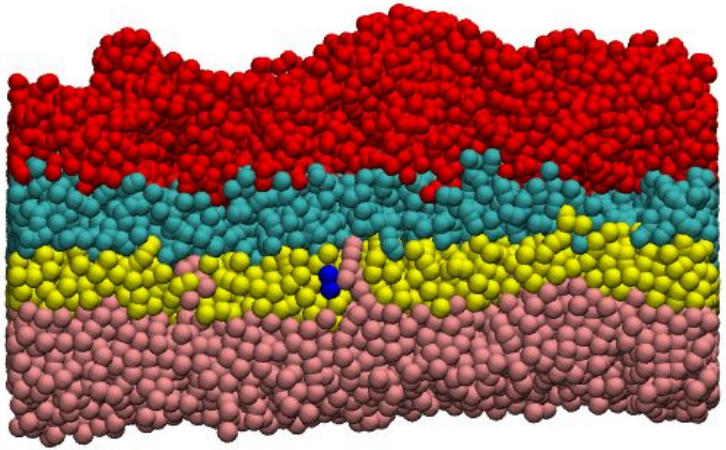
Observations from the simulations

Flexibility of O-antigen

No ceramide



5% ceramide



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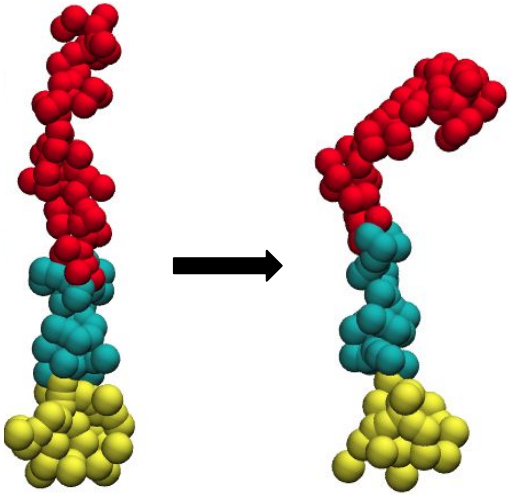
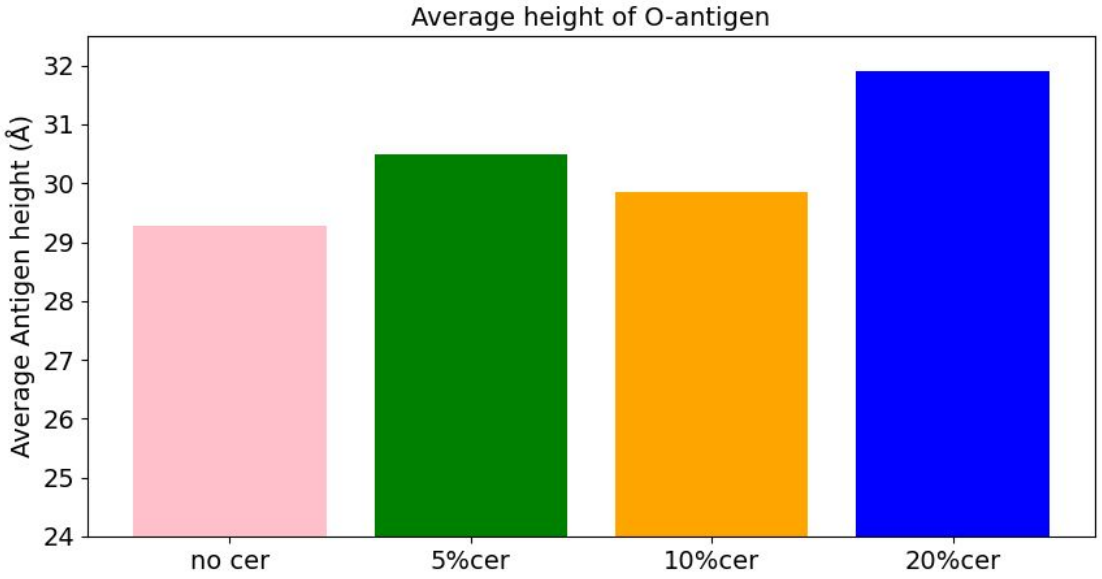
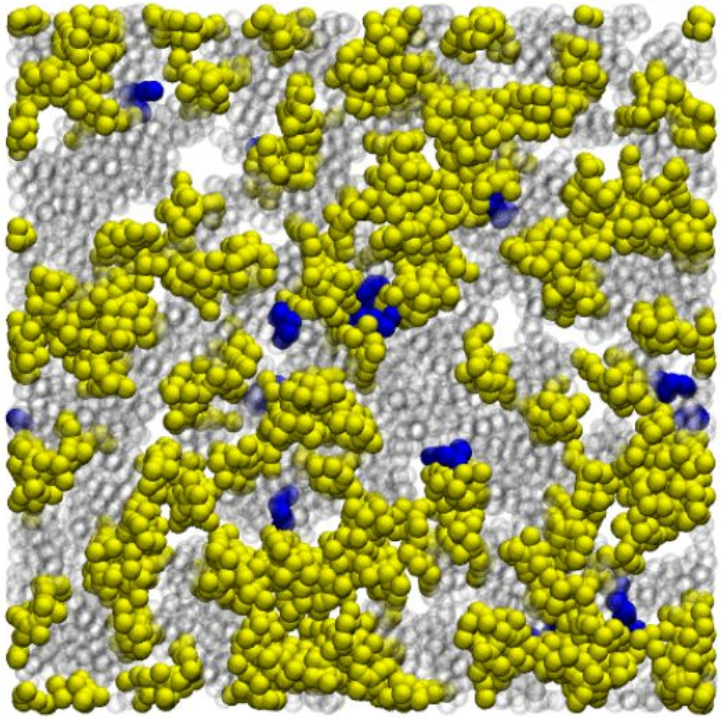




Preliminary data: one simulation for each condition
Simulation run for 30μs

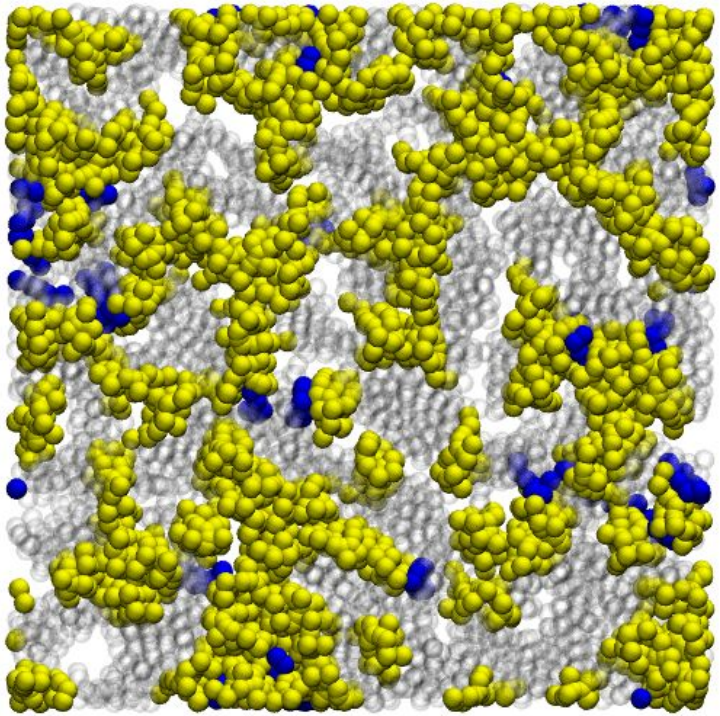
Ceramide may prefer to be in close to sLPS instead of rLPS

5% Ceramide

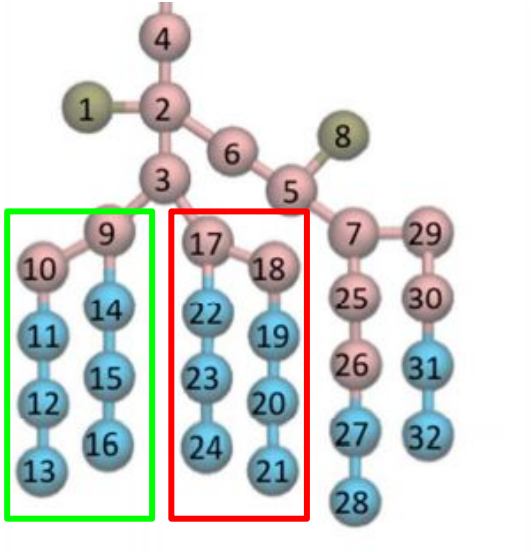


rLPS
sLPS:Lipid A
Ceramide

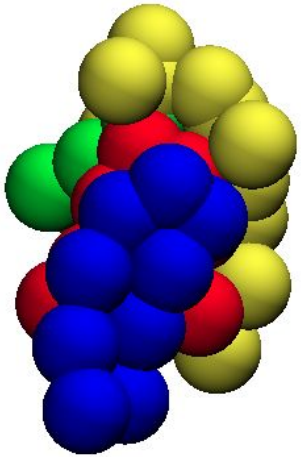
10% Ceramide



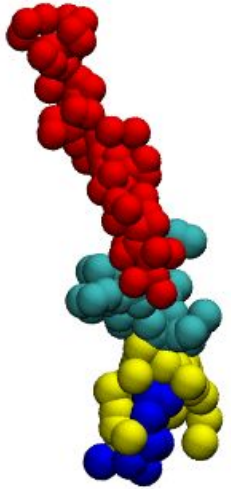
Ceramides might interact with lipid A at specific sites.



E.coli Lipid A structure



Lipid A, with double acyl chains colored in green and red.



Summary

- Ceramides are common, not unique to *Caulobacter*.
- Ceramides are modified in very diverse manners
- From the simulations:
  - O-antigen is flexible
  - Ceramide may prefer to interact with specific sites on lipid A
  - Ceramide may have a preference for sLPS vs rLPS.

Future work

- Re-analyse data using fully converged system (50μs).
- Analysis of ceramide-lipid A interaction.
- Replicates of simulations.

Thank you!

Klein Lab:

- Eric Klein
- Gabriele Stankeviciute
- Antonella Abou Samra
- Eliza Yost

Brannigan Lab:

- Grace Brannigan
- Liam Sharp
- Jesse Sandberg

- Ezry St.Iago-McRae (Fu lab)

RUTGERS