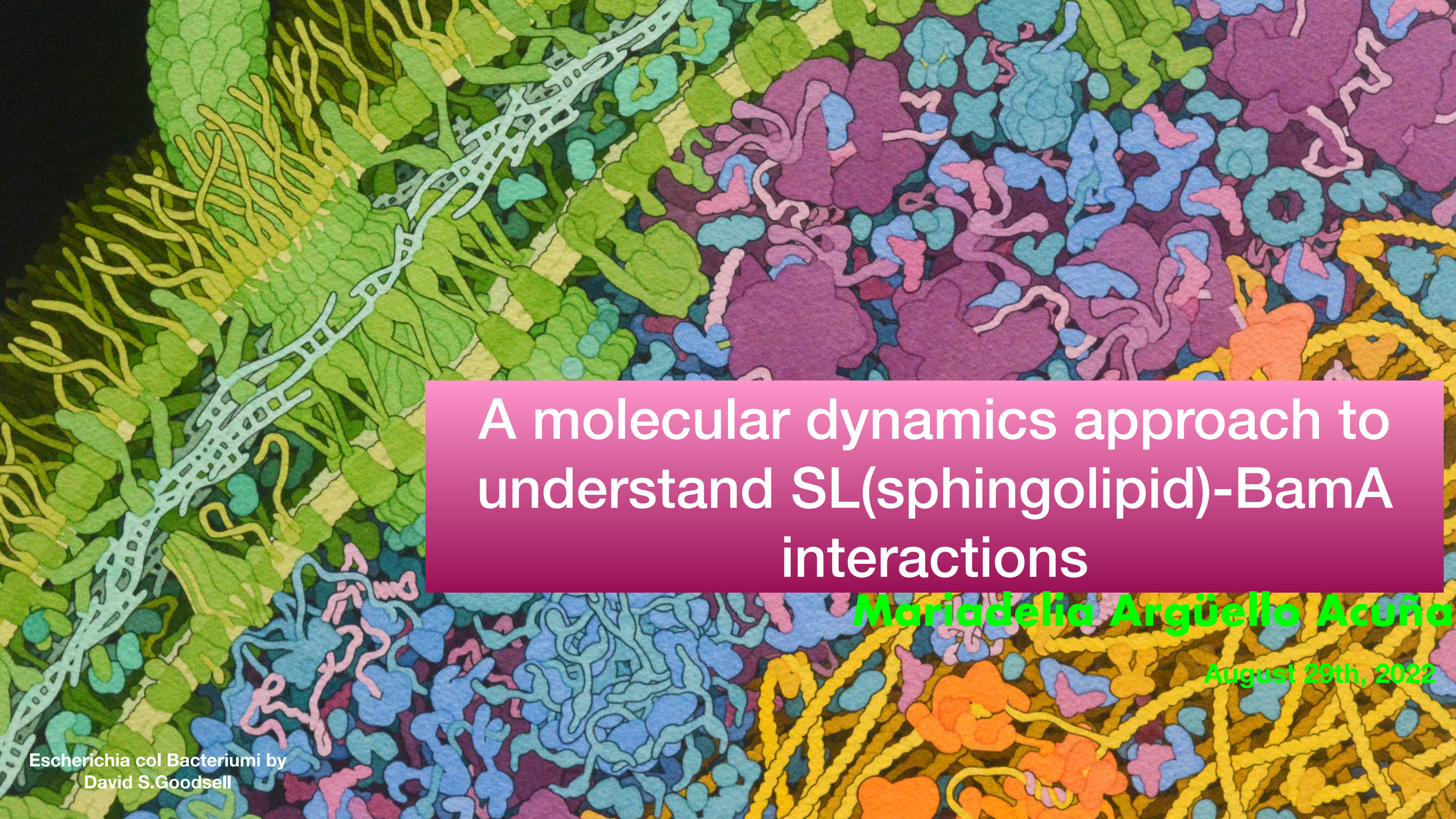


A molecular dynamics approach to understand SL(sphingolipid)-BamA interactions

Mariadelia Argüello Acuña

August 29th, 2022

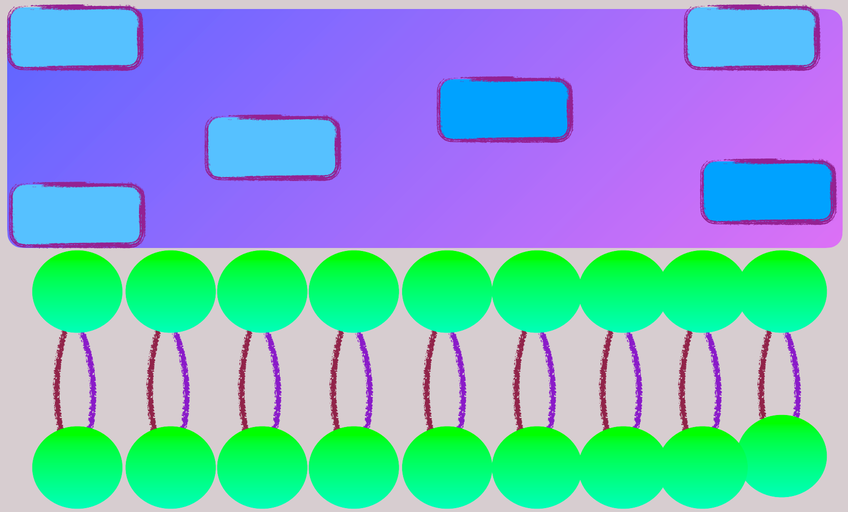
Escherichia col Bacteriumi by David S.Goodsell

Outline

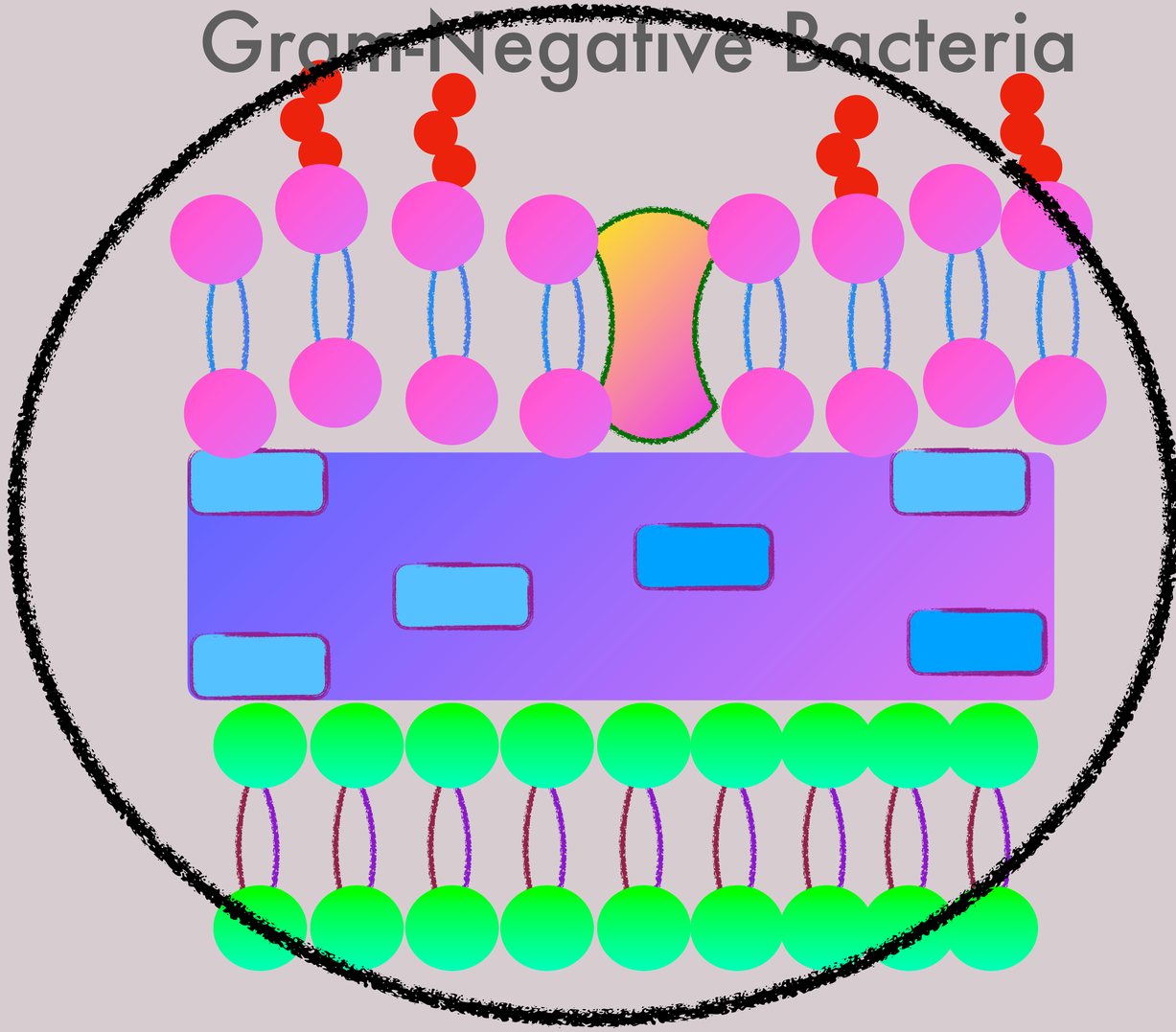
- Background
- Main Question
- Preliminary Work
- Back to Main Question and Hypothesis
- Projects aims (rationale and approach)
- Timeline
- Summary

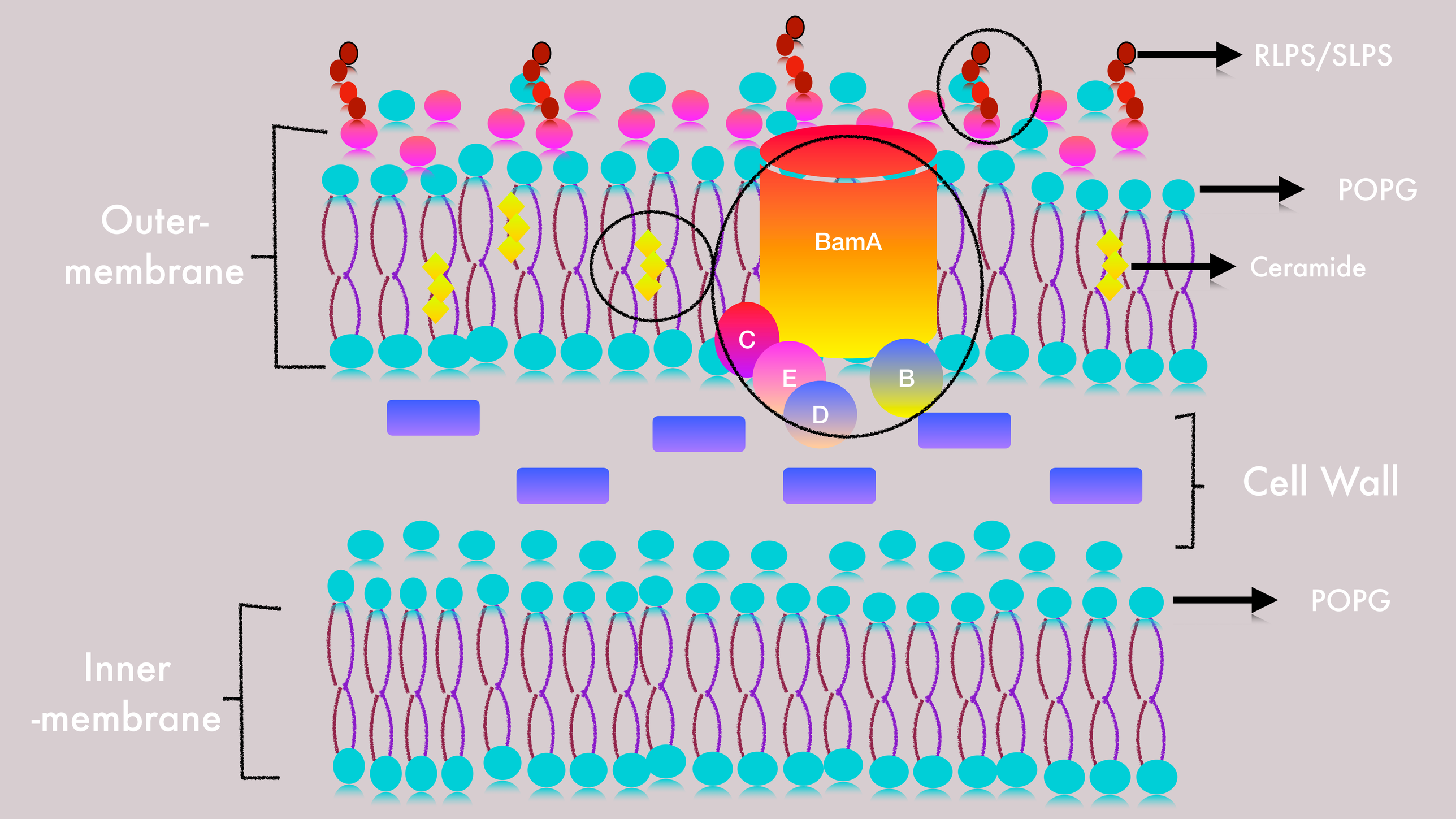
Bacteria Cell Wall

Gram-Positive Bacteria



Gram-Negative Bacteria




RLPS/SLPS
POPG
Ceramide
BamA
C
E
D
B
Outer-
membrane
Cell Wall
POPG
Inner
-membrane

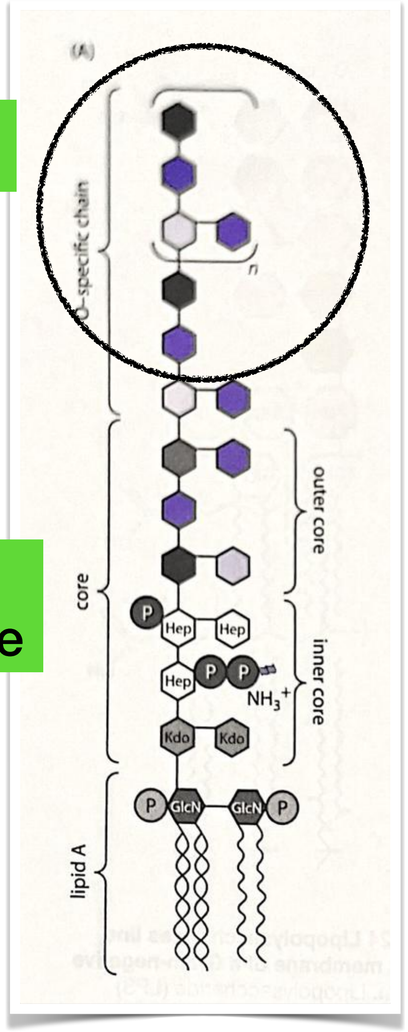
Lipopolysaccharide (LPS)

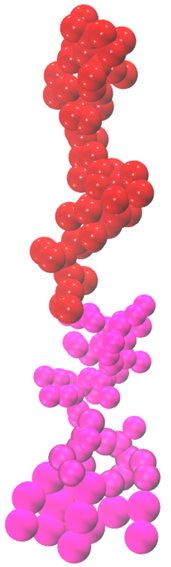
O-Antigen

Core oligosaccharide

Lipid A

RLPS





SLPS

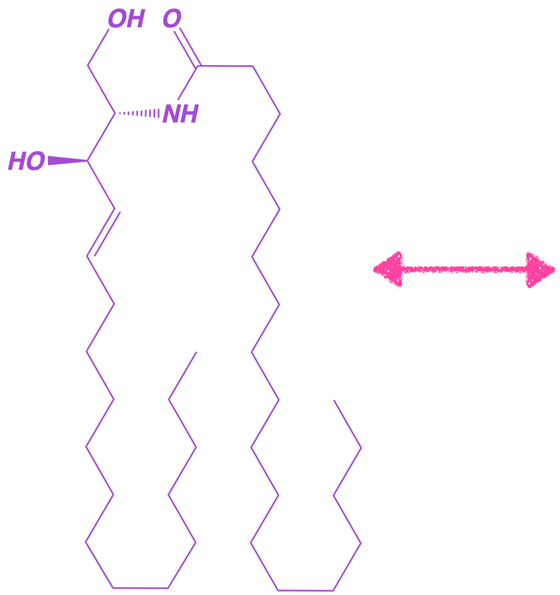
Purpose:Prevent passive diffusion of hydrophobic solutes like antibiotics and detergents.

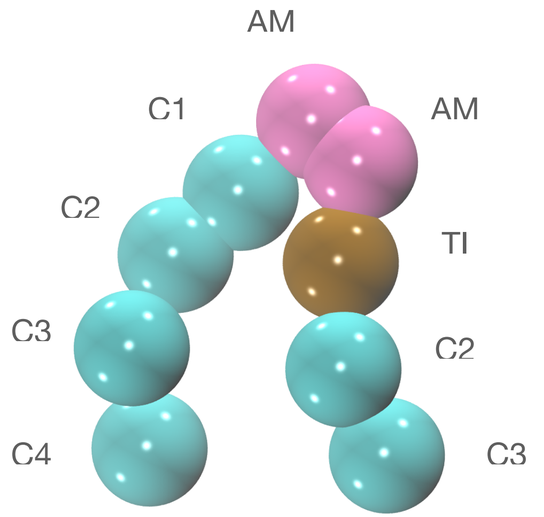
Sphingolipids(ceramide)

Hexosyl-hexuronosyl-ceramide glycosphigolipid (GSL)

Phosphate starvation

LPS from Shingomonadaceae family





Coarse-grain representation of ceramide

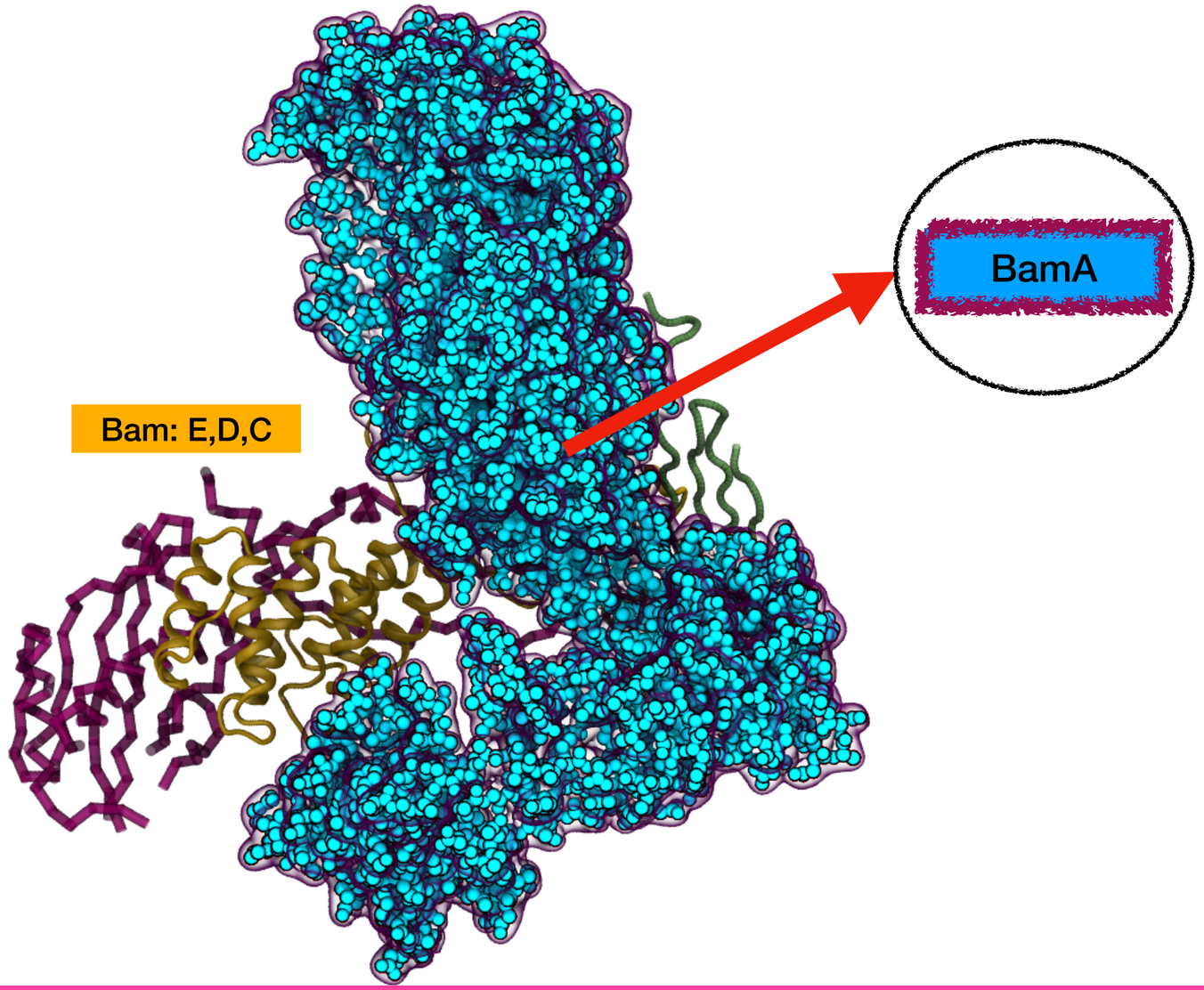
Caulobacter crescentus

Purpose on C.crescentus?

Klein Lab

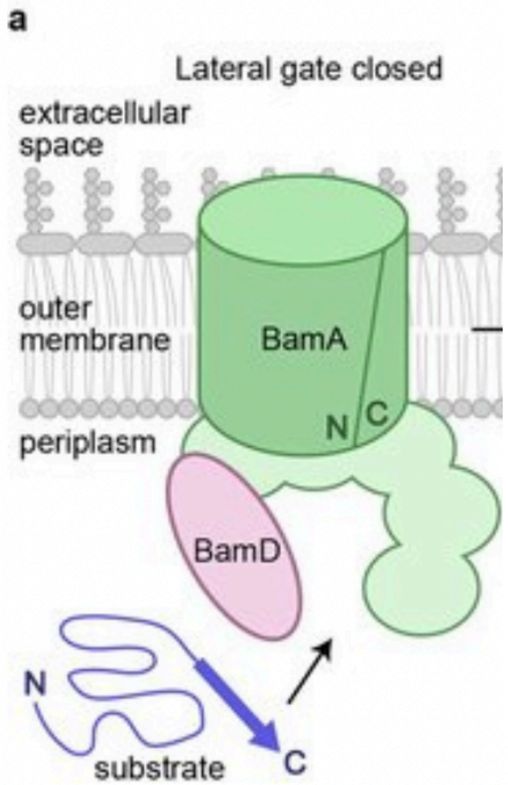
Brannigan Lab:Ceramide disrupts LPS packing

β-barrel assembly machinery (**BAM**)



Purpose:Folding and inserting OMPs into the OM.

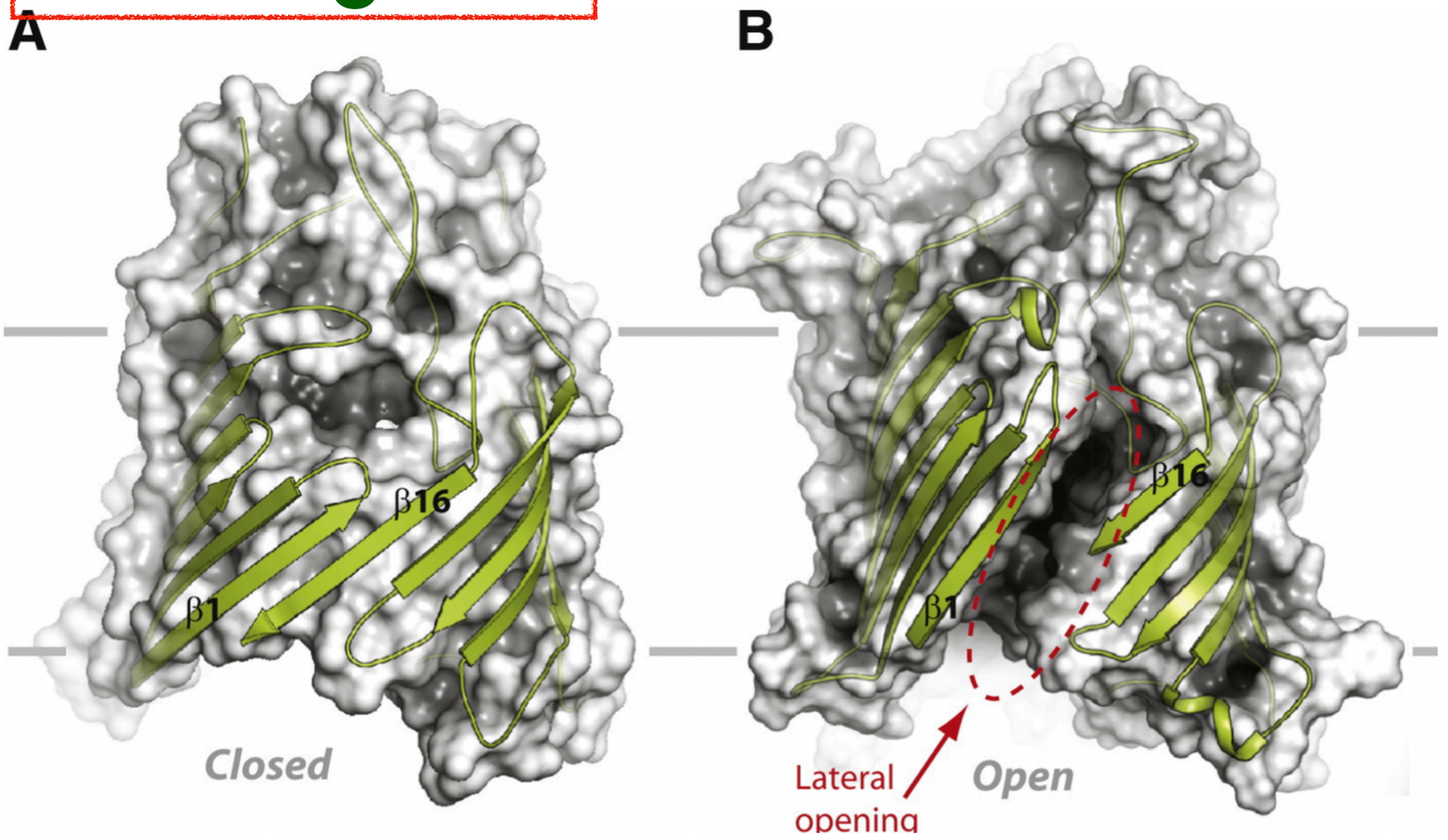
Proposed model of β-barrel assembly by BamA



From:https://www.researchgate.net/figure/Proposed-model-of-b-barrel-assembly-by-the-BAM-complex-a-The-substrate-is-recruited-to_fig2_342101074 [accessed 22 Aug, 2022]

What conformations does BamA takes on the OM?

Close gate



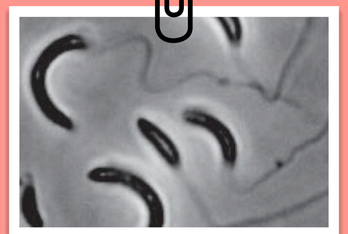
Open gate

From:https://doi.org/10.1016/j.str.2014.05.008.

Main Question

Where does ceramide binds to BamA ?

What is the motivation of my project?

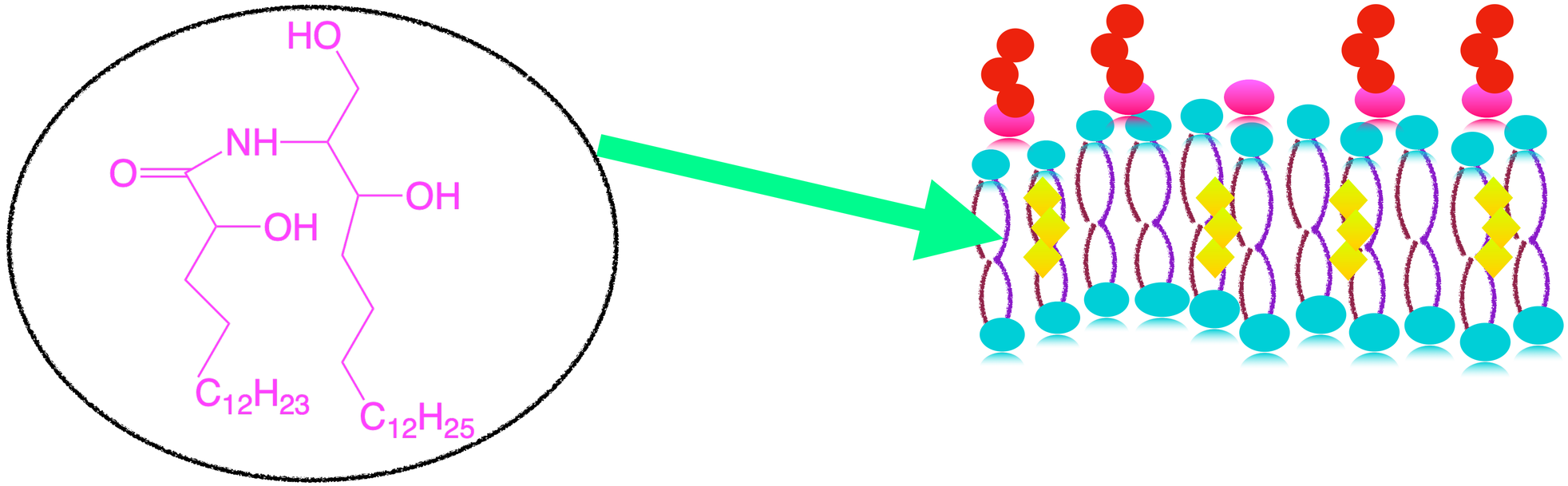


Klein Lab

Investigate how ceramides affect OM functionality and biophysical properties

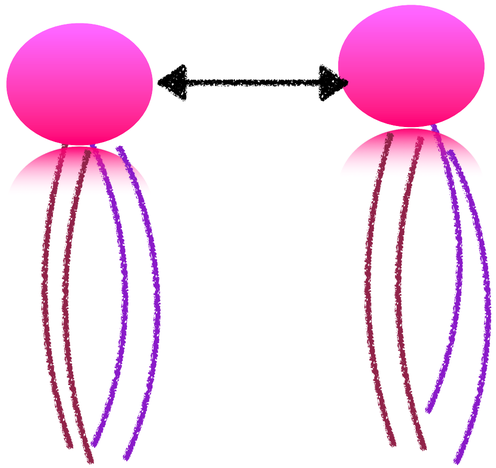
Preliminary Work

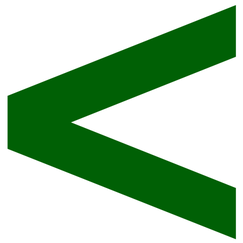
Characterize the role of SLs(ceramide) on the outer membrane structure.

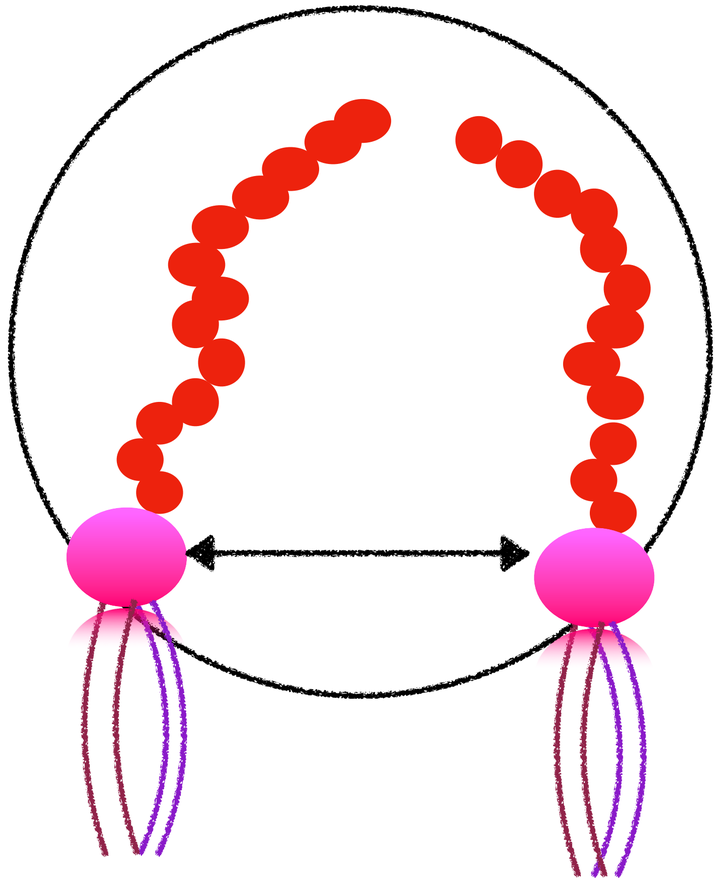


SLs (ceramide) disrupts LPS packing.

How do ceramides disrupt LPS packaging ?



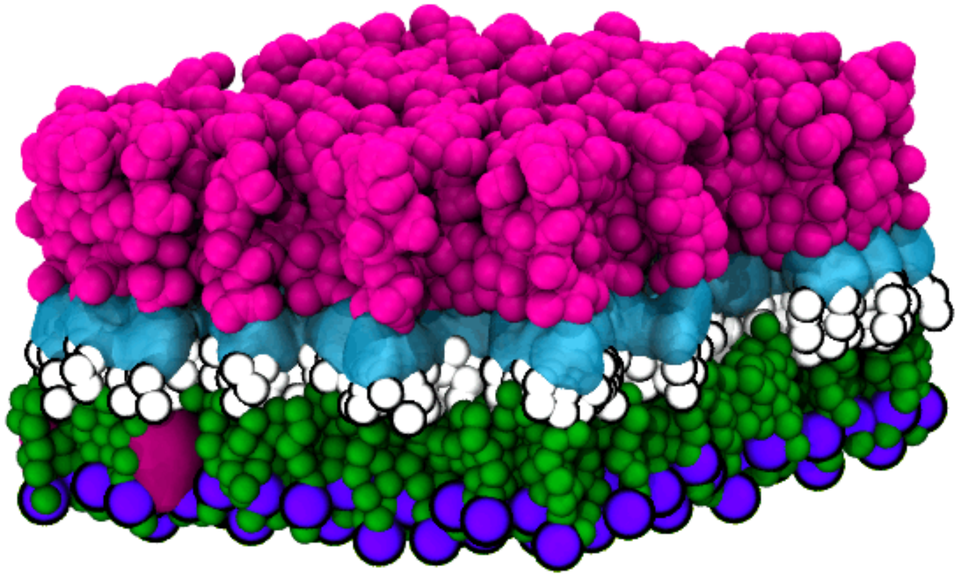




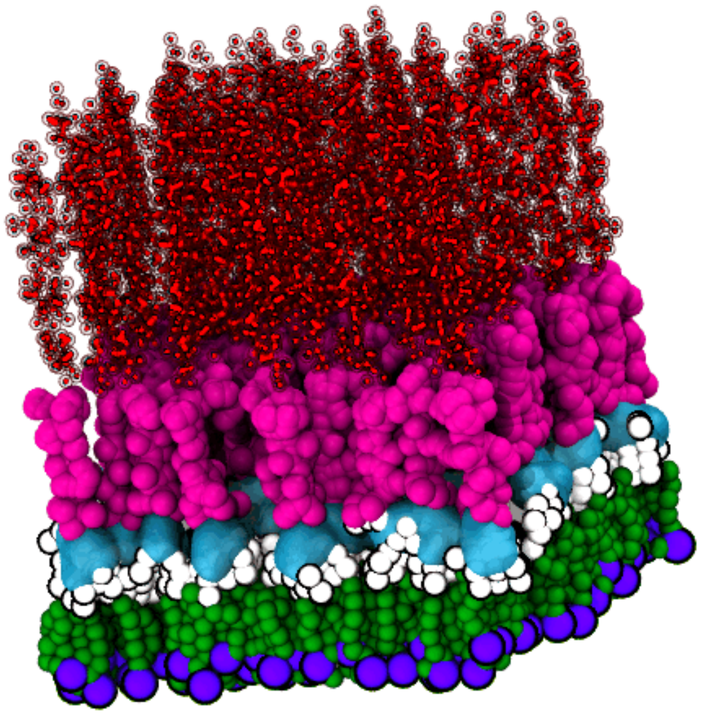
Area per lipid of RLPS

Area per lipid of SLPS

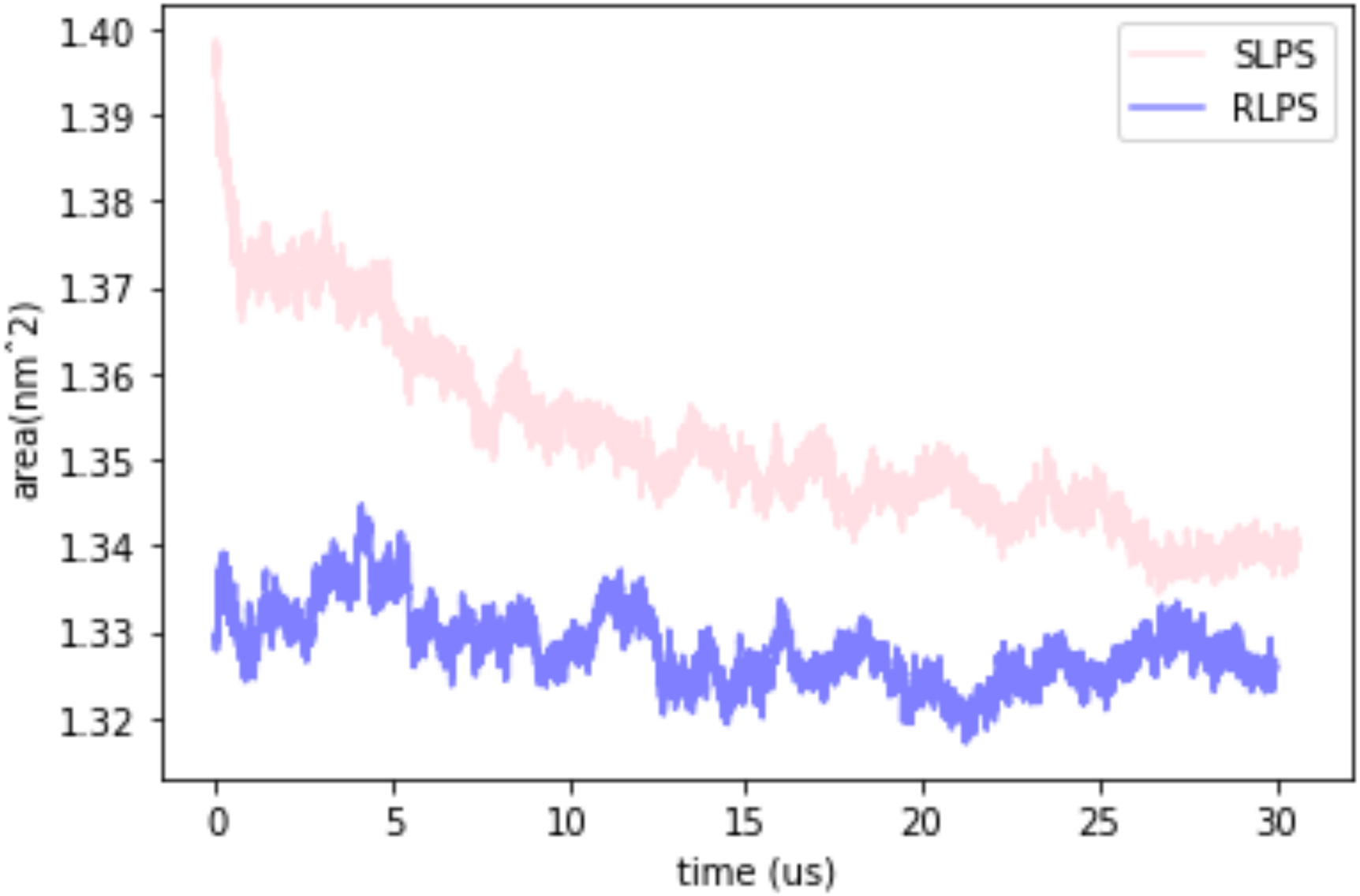
RLPS



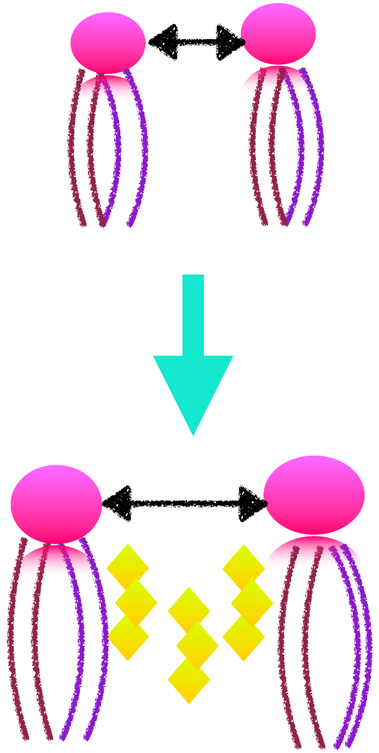
SLPS



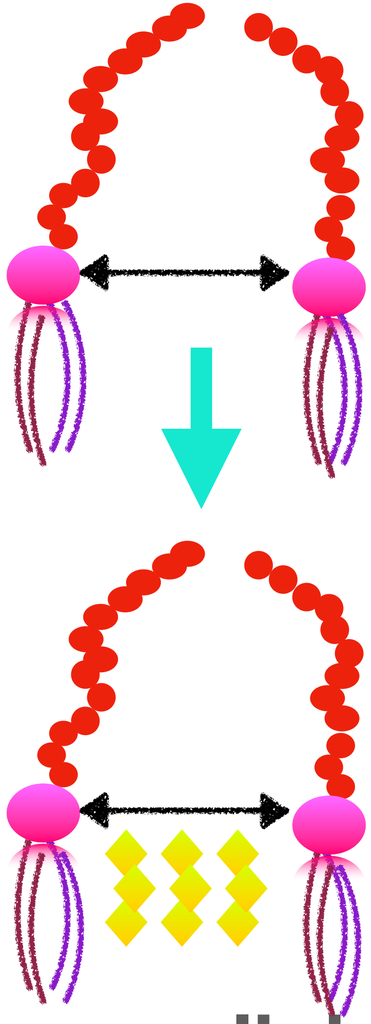
Packing evidence, SLPS is less condense



Change in area per lipid with ceramide



Area per lipid of RLPS increase



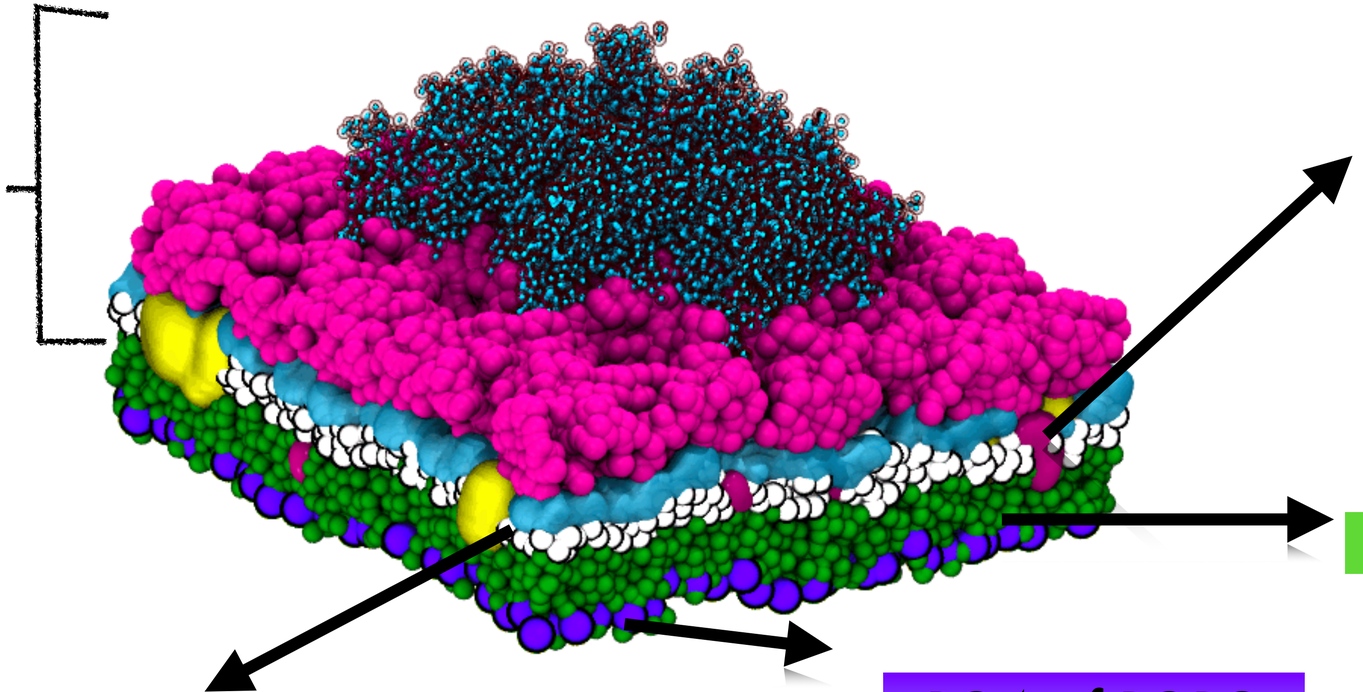
Area per lipid of SLPS same

Ceramide in two domains SLPS and RLPS

LPS (RLPS/SLPS)

RLPS

SLPS



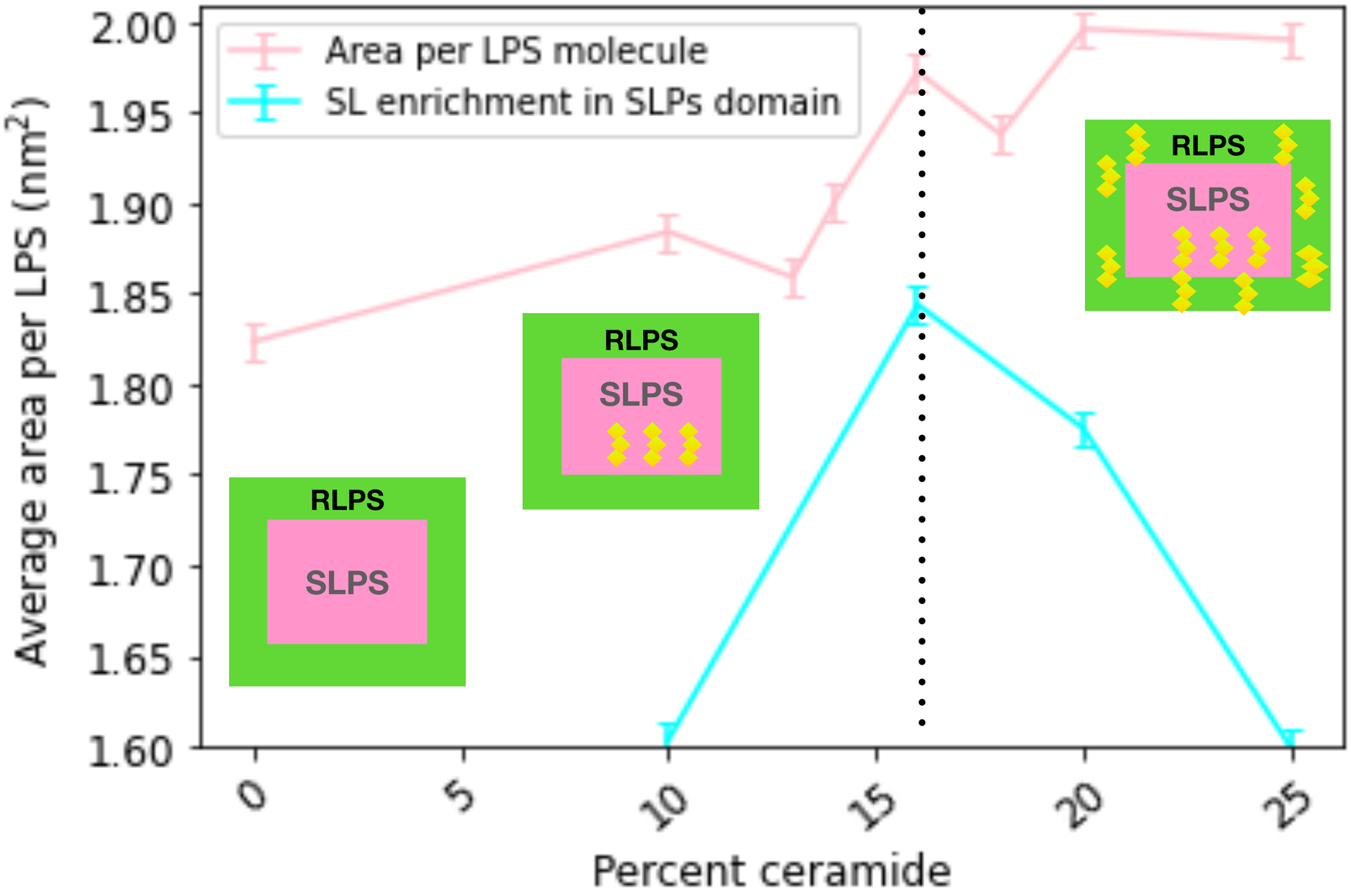
PODG

POPG

PO4- of POPG

Ceramide

Ceramide disrupts LPS packing



Main Question

Where does ceramide binds to BamA ?

Hypothesis

SLs regulates BamA complex activity by direct interactions with BamA protein.

Aims Project

Aim 1a. Modelling the caulobacter BamA protein and lipids to run the simulations

Aim 1b. Build lipids in insane to run the simulations

Aim 2. Run the simulations and analyze the density distribution of ceramide.

Aim 1a. Modelling the caulobacter BamA protein and lipids to run the simulations

Aim 1b. Build lipids in insane to run the simulations

Rationale

Aim 1a. Modelling the caulobacter BamA protein

- The chemical structure of BamA of E.coli differs from BamA of C.crescentus.
- Switch from E.coli system to a C.crescentus system.

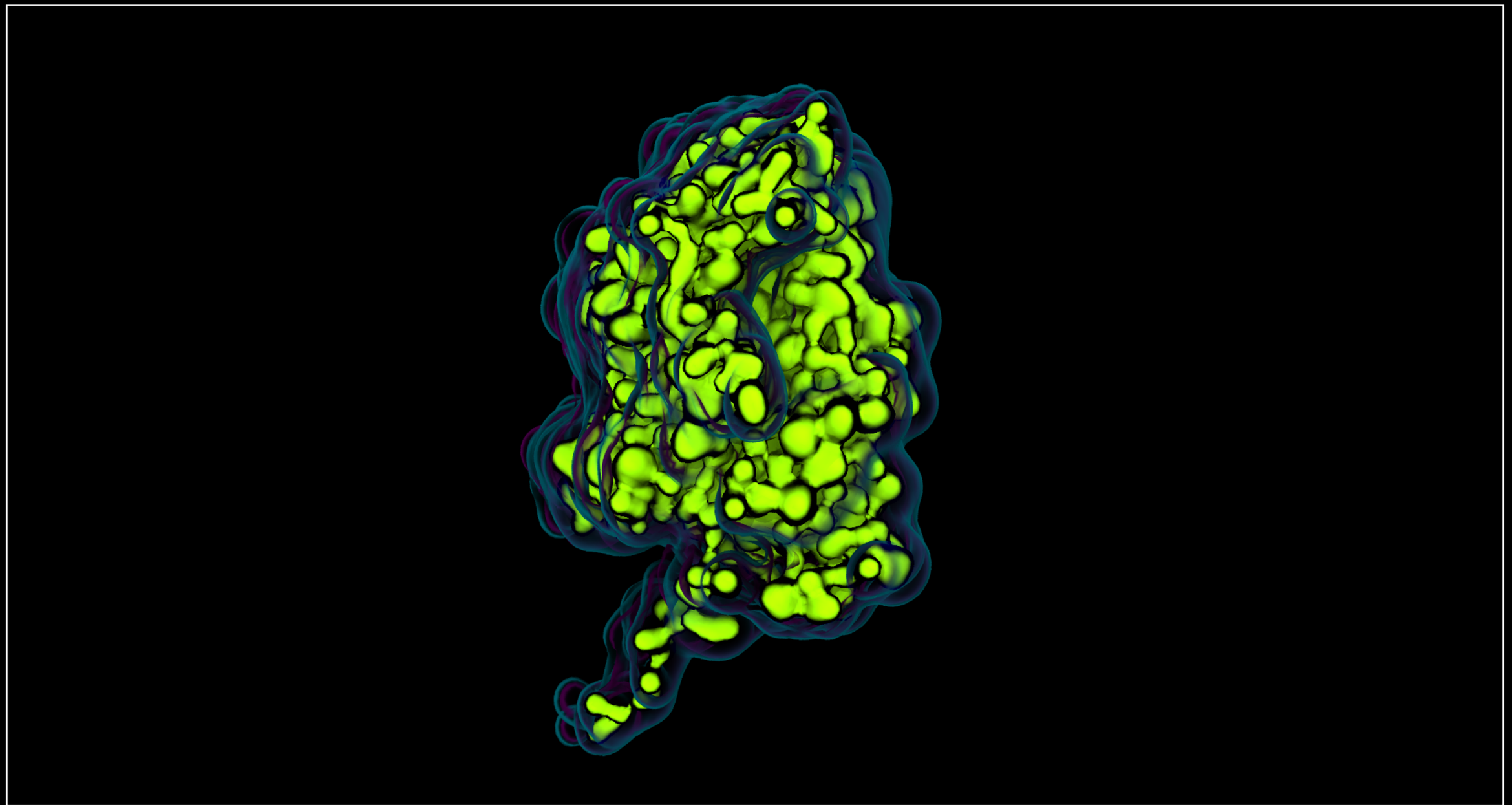
- Work with the most accurate/realistic system.

Approach

Aim 1a. Modelling the caulobacter BamA protein

- Build Caulobacter BamA protein from sequence A0A0H3C9L0.
- Assign the spatial coordinates to the just build BamA protein by MODELLER.
- Refinement of side chain by SQRWL and molprobity.
- Set up a Atomistic Simulation.
- Coarse grain the final structure.

BamA coarse grain of E.coli



Rationale

Aim 1b. Build lipids in insane to run the simulations

- The chemical structure of LPS of E.coli differs from LPS of C.crescentus.

- C. Crescentus OM presents anionic ceramide and neutral ceramide in the OM.

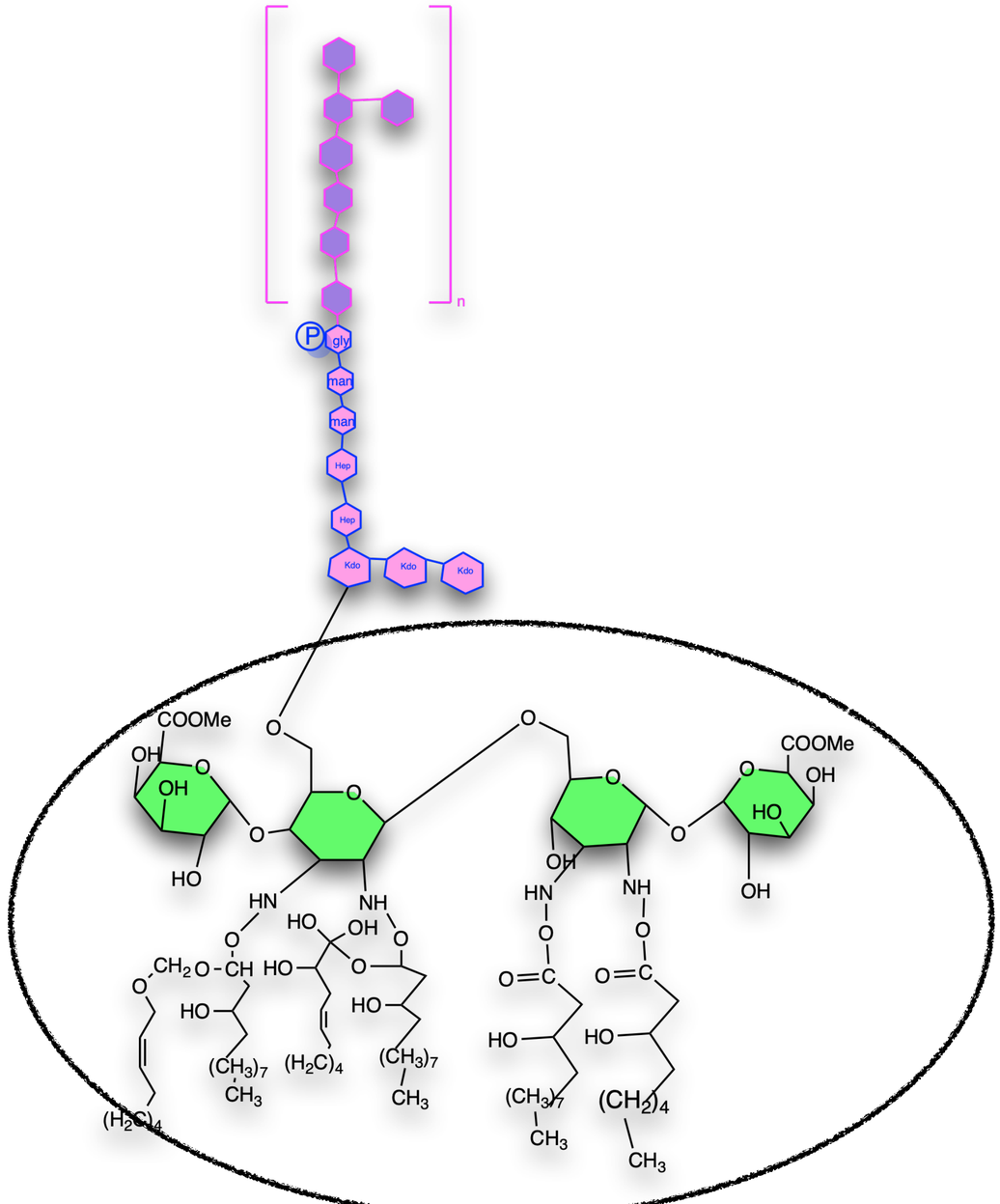
Approach

Aim 1b. Build lipids in insane to run the simulations

Add the chemical structure of RLPS, SLPS, anionic ceramide on insane.py

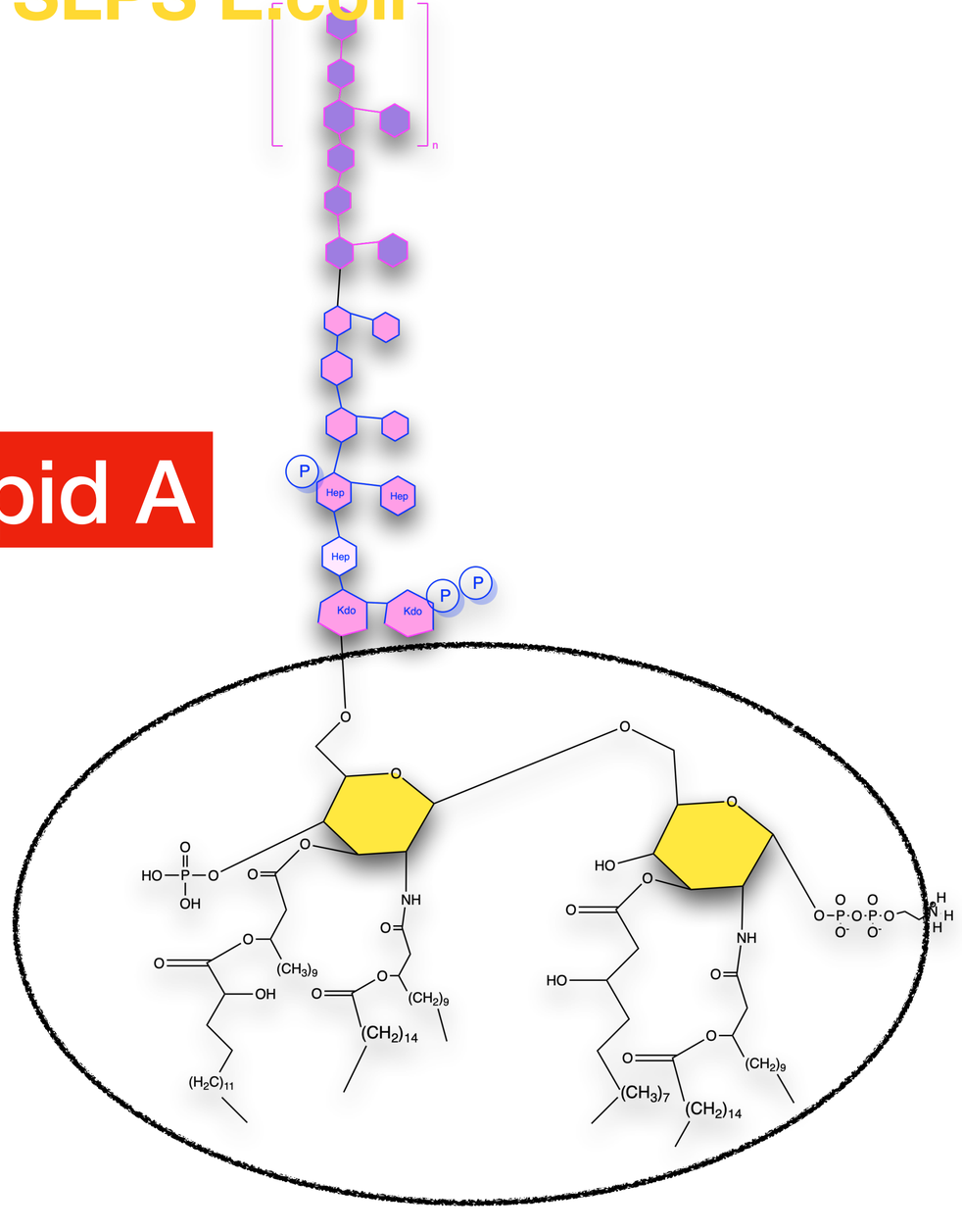
Differences in chemicals structures LPS

SLPS C.crescentus



SLPS E.coli

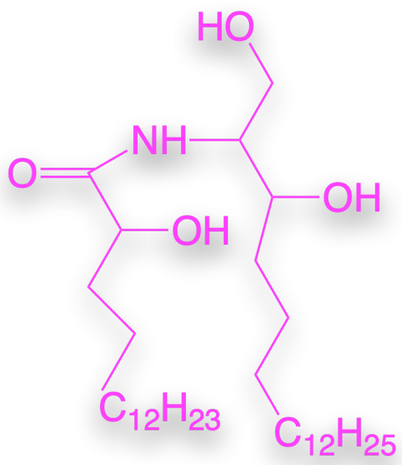
Lipid A



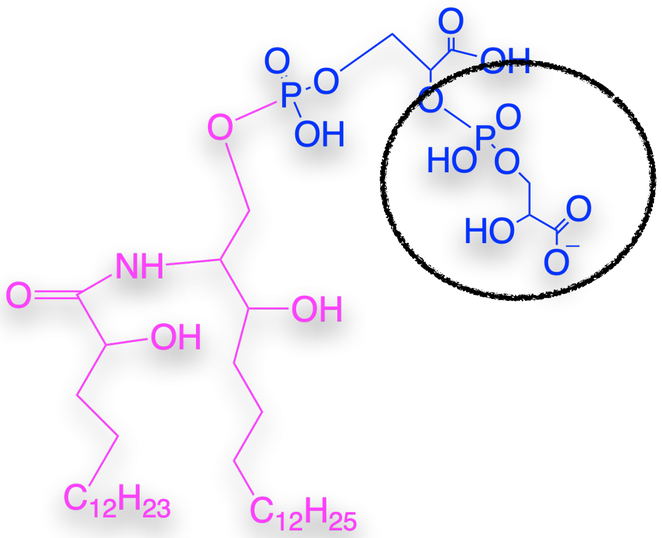
Differences in chemicals structures ceramide

Ceramide C.crescentus

Neutral

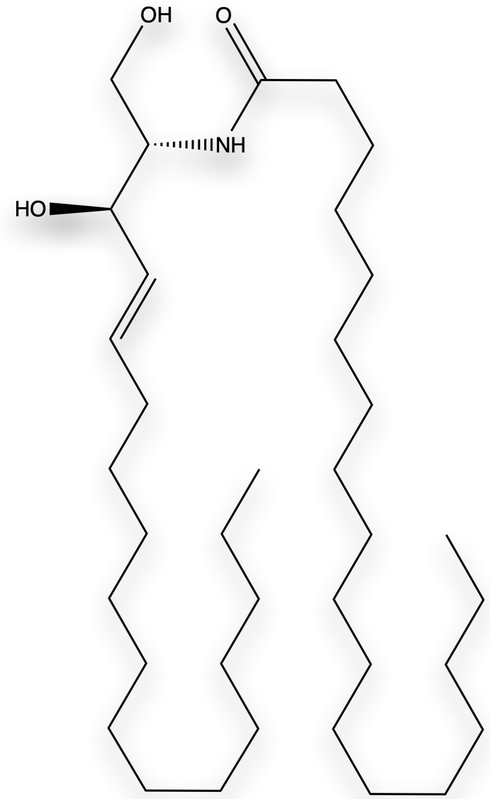


Anionic



HEADGROUP

Ceramide E.coli



Aim 2. Run the simulations and analyze the density distribution of ceramide.

Aim 2.Rationale

Aim 2. Run the simulations and analyze the density distribution of ceramide.

- To know where ceramide it is on the outer membrane.
- To know how ceramide it is distribute (bind) around BamA.

Aim 2.Approach

Aim 2. Run the simulations and analyze the density distribution of ceramide.

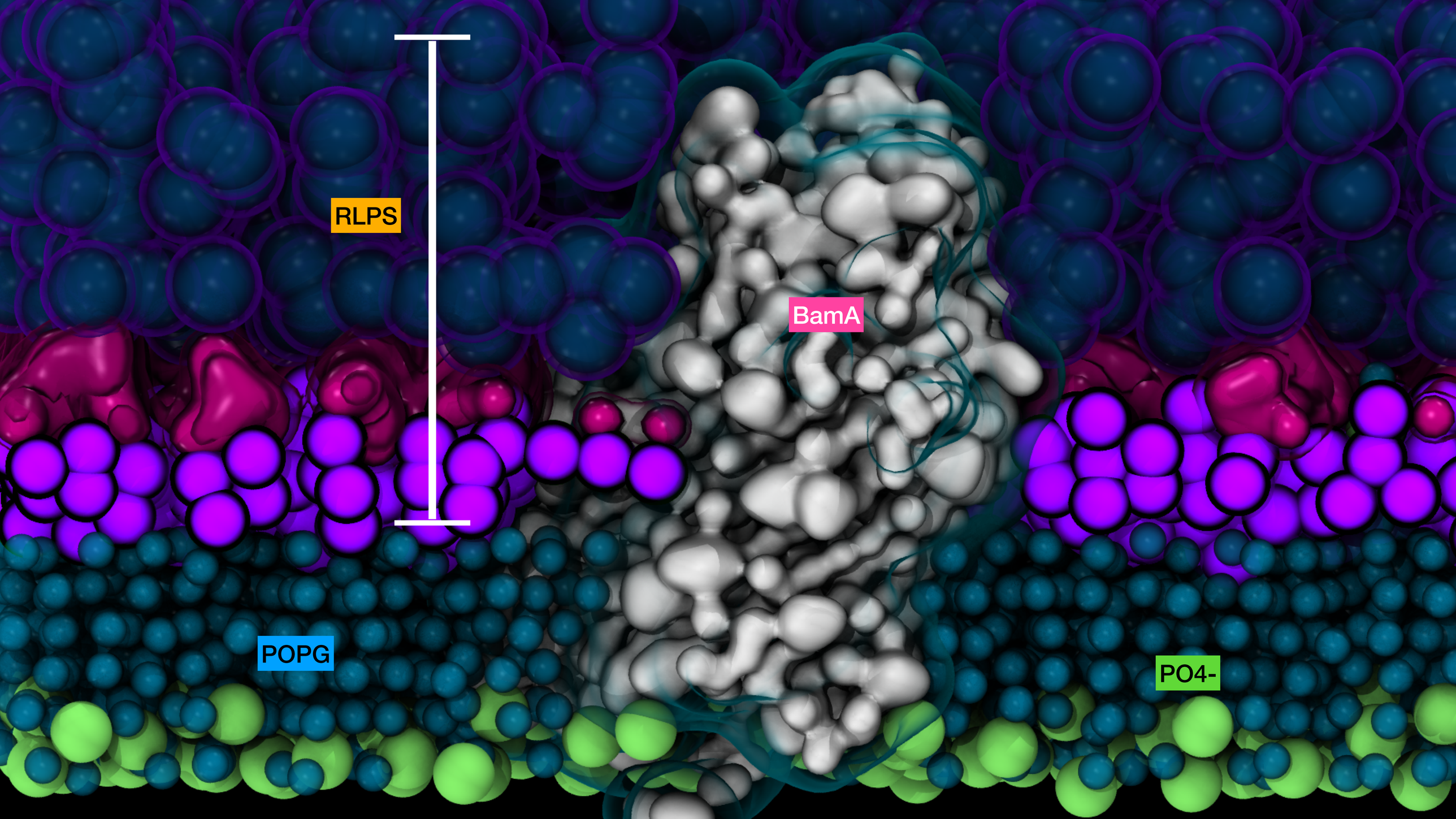
- Four sets of simulations to run :

RLPS:SLPS:POPG: Ceramide : Anionic Ceramide

2:2:1:1:X

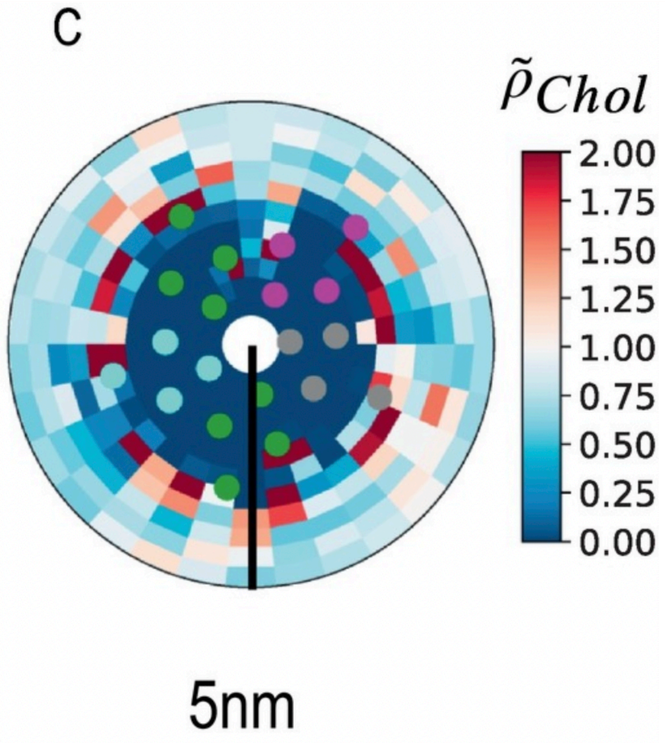
BamA open No AC	BamA close No AC
BamA open AC	BamA close AC

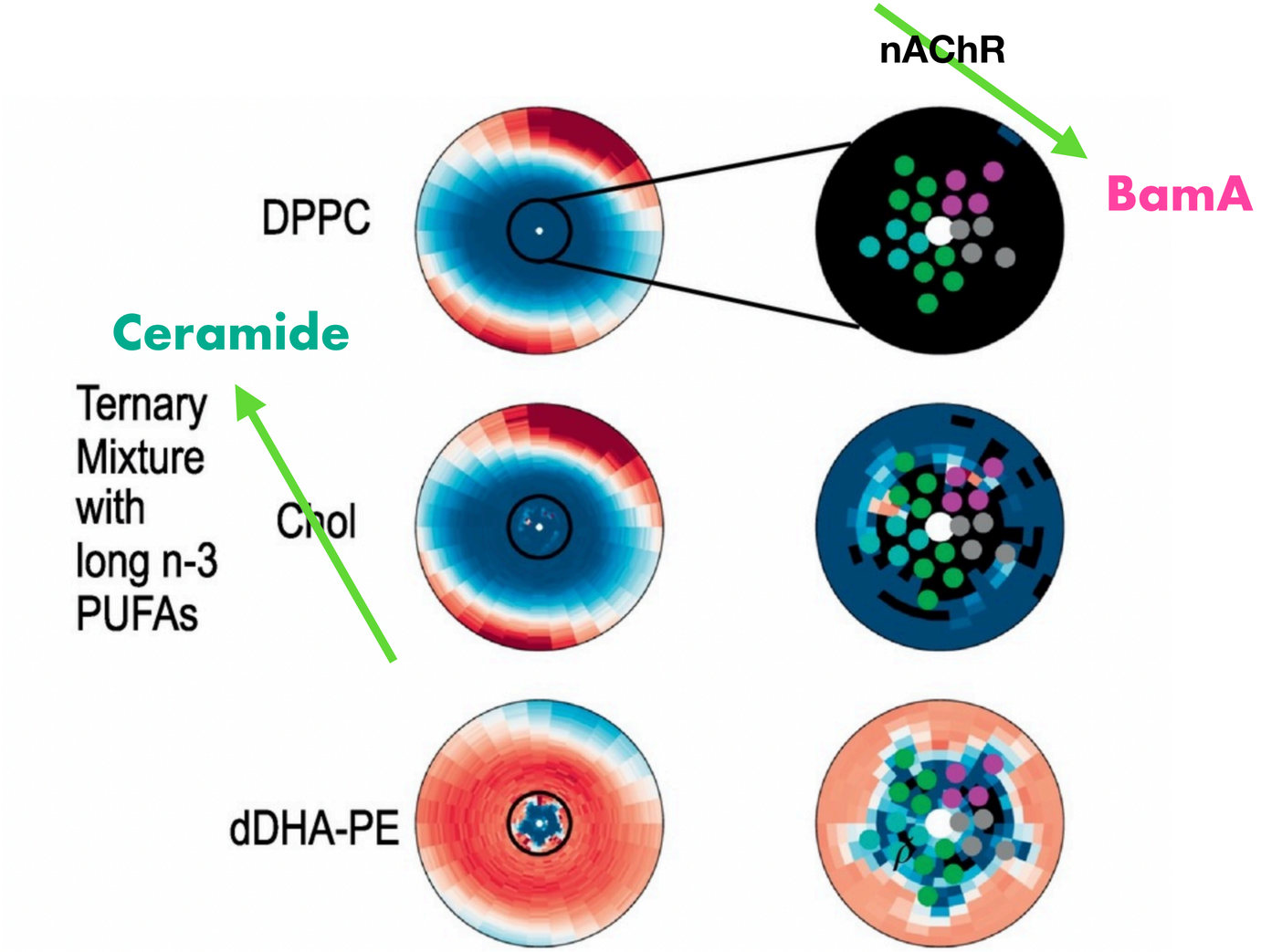
- Density distribution will be calculate with the software Nougat from Brannigan Lab.


RLPS
BamA
POPG
PO4-

Density Distribution by Nougat

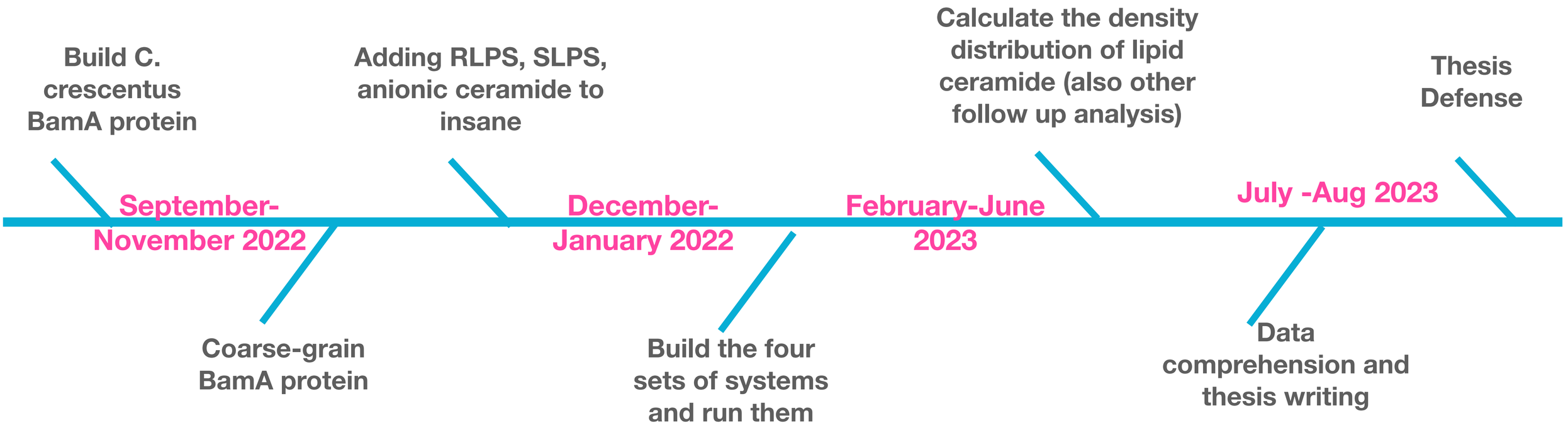
Normalized density (p) of beads of lipid (Chol) around system of study nAChR





From:Sharp L, Salari R, Brannigan G. Boundary lipids of the nicotinic acetylcholine receptor: Spontaneous partitioning via coarse-grained molecular dynamics simulation. Biochim Biophys Acta Biomembr. 2019 Apr 1;1861(4):887-896. doi: 10.1016/j.bbamem.2019.01.005. Epub 2019 Jan 18. PMID: 30664881; PMCID: PMC6392067.

Timeline


Build C. crescentus BamA protein
September-November 2022
Coarse-grain BamA protein
Adding RLPS, SLPS, anionic ceramide to insane
December-January 2022
Build the four sets of systems and run them
Calculate the density distribution of lipid ceramide (also other follow up analysis)
February-June 2023
July -Aug 2023
Data comprehension and thesis writing
Thesis Defense

Summary

Main Question: Where does ceramide binds to BamA ?

Hypothesis:SLs regulates BamA complex activity by direct interactions with BamA protein.

Aim 1a. Modelling the caulobacter BamA protein

Aim 1b. Build lipids in insane to run the simulations

Aim 2. Run the simulations and analyze the density distribution of ceramide.