

Identification of specific sphingolipid -BamA using coarse-grain molecular dynamics simulation of the *C.crescentus* outer membrane



Mariadelia Argüello Acuña¹, Jahmal J. Ennis¹, Jesse Sandberg¹, Ezry Santiago-McRae¹, Eric Klein^{1,2}, Grace H. Brannigan^{1,3}.

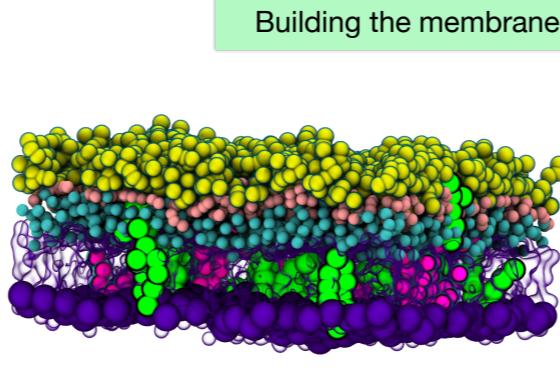
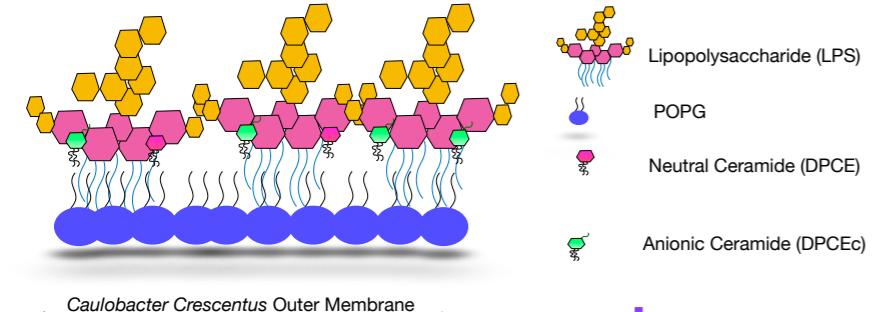
RUTGERS
UNIVERSITY | CAMDEN

¹Center of Computational and Integrative Biology (CCIB), Rutgers University-Camden, Camden, NJ, USA, ²Dept Biology, Rutgers Univ, Camden, USA, ³Dept Physics, Rutgers Univ, Camden, NJ, USA.

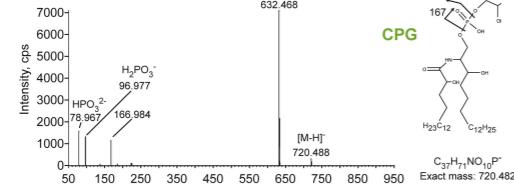
Abstract

Sphingolipids (SLs) have been primarily studied in eukaryotic cell membranes, where they modulate fundamental cellular processes ranging from apoptosis to cytoskeletal reorganization. It has recently been discovered that many bacteria also synthesize sphingolipids, but their role in bacterial membranes is poorly understood. One example of a gram-negative bacteria with sphingolipids in its outer membrane is *C. crescentus*. Previous studies showed that the absence of SLs in the outer membrane increases resistance to cationic polymyxin antibiotics, permeability, and susceptibility to antimicrobials like bacitracin while inducing an unfolded-protein stress response. The machinery responsible for the folding of many outer membrane proteins (OMPs) is the β -barrel assembly machinery (BAM complex). In the present study, we used coarse-grained molecular dynamics (CG-MD) simulations to understand the possible interactions between SLs and the BAM complex to determine if SLs regulate BamA activity by direct interaction. The *C. crescentus* BamA protein was homology modeled in two conformations: open- and closed-gate. Martini models of the rough lipopolysaccharide (RLPS), smooth lipopolysaccharide (SLPS), and anionic SLs of *C. crescentus* were built. We carried out CG-MD simulations of BamA embedded in a model of a *C. crescentus* bacterial membrane in the closed and open conformations. Afterward, the density distribution of SLs with respect to BamA was analyzed, using the CG-MD analysis toolkit Nougat from Brannigan Lab.

Background



The simulation system was set up by insane.py⁶. Besides coarse-grained LPS, we also add to insane.py the anionic version of ceramide. The composition of the membrane was LPS:DPCE: DPCEc upper leaflet in a ratio of 90:5:5 and POPG: DPCE:DPCEc lower leaflet in a ratio of 90:5:5. The box dimensions were 15 x 15 x 20 nm.



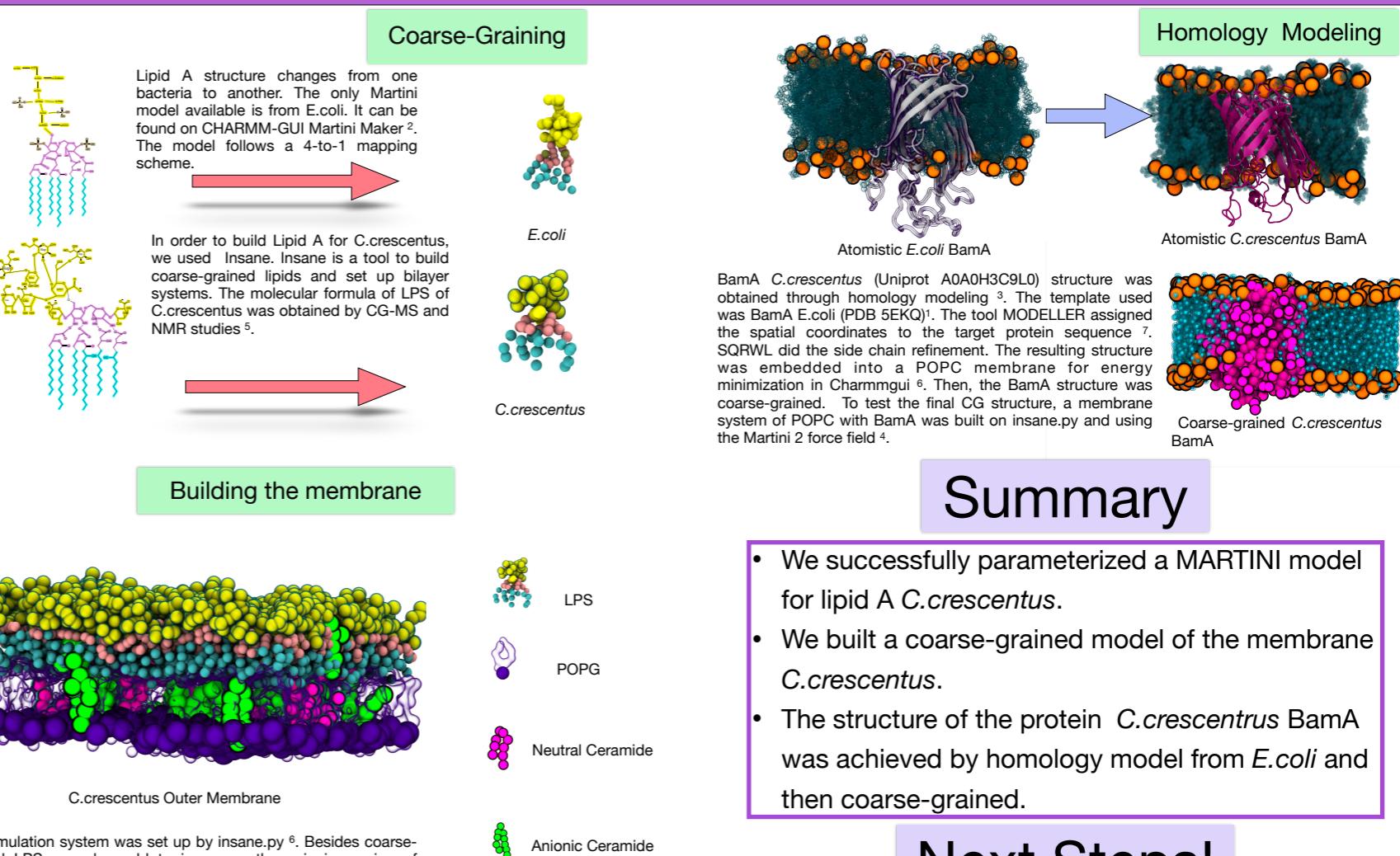
Structural determination of anionic sphingolipids was performed by MS/MS by analysis⁸.

Aims

- Aim 1. Build a martini model of Caulobacter Lipid A.
- Aim 2. Build a coarse-grained bacteria membrane of *C.crescentus*.
- Aim 3. Generate the structure of BamA of *C.crescentus*.

Simulation Methods

- We coarse-grained lipid A and anionic ceramide using martini 2.2 and the script insane.py⁴.
- The simulation used Gromacs 2016.1 and is still running, using Berendsen thermostat at 313K.
- The BamA structure *C.crescentus* was obtained through homology modeling. The template used the X-Ray diffraction structure of BamA *E.coli* (PDB 5EKQ)¹.



Summary

- We successfully parameterized a MARTINI model for lipid A *C.crescentus*.
- We built a coarse-grained model of the membrane *C.crescentus*.
- The structure of the protein *C.crescentus* BamA was achieved by homology model from *E.coli* and then coarse-grained.

Next Steps!

- Build coarse-grained membrane systems with SLPS:RLPS:DPCE: DPCEc in the upper leaflet and POPG:DPCE: DPCEc, with the BamA.
- Quantify specific interactions between ceramide and BamA.

Acknowledgements

- Rutgers Office of Advanced Research Computing (OARC).
- NRT Award :NSF DGE 2152059.
- NSF MCB -2224195.

References

- Bakelar et al. SCIENCE, 2016.
- Hsu et al. Journal of Computational Chemistry, 2017.
- Marks et al. Journal of Bacteriology, 2010.
- Marrink et al. J.Phys.Chem.B, 2017.
- Ravenscroft et al. Journal of Bacteriology, 1992.
- Wassenaar et al. Journal of Chemical Theory and Computation.
- Webb et al. Current protocols in bioinformatics, 2016.
- Zik et al. Cell Reports, 2022.