Specific binding of an anionic bacterial ceramides to BamA in model membranes

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Abstract

BamA is a beta-barrel outer membrane protein (OMP) that assists in folding other OMPs in Gram-negative bacteria. Because of its key role, high conservation, and location at the cell's surface, it is a promising target for therapeutic intervention and is worth further investigation. The exact mechanism that modulates the open and closed state of BamA's lateral gate is currently not well understood. Recently, a novel anionic ceramide, ceramide polyphosphoglycerate (CPG2), was found in the Gram-negative bacterium *Caulobacter crescentus*. Knocking down BamA or anionic ceramide synthesis yields similar phenotypes, including increased antibiotic susceptibility and induction of the unfolded-protein response, suggesting the two may be linked. Here, we computationally study anionic ceramide interactions with BamA using coarse-grained molecular dynamics. We found several superficial binding sites for anionic ceramide and measured their binding affinity to BamA in ternary mixed POPC, neutral ceramide and anionic ceramide model membranes using the density threshold affinity method.

Approach

- Coarse-grained molecular dynamics simulation of BamA in a ternary mixture of POPC, anionic ceramide, and neutral ceramide
- Calculate the density distribution of lipids around BamA
- Determine site of high anionic ceramide enrichment
- Quantitatively rank binding sites using density threshold affinity method[3]

$$\Delta G_{bind} = -RT \ln \frac{P_{occ,site}}{P_{unocc,site}} + RT \ln \frac{P_{occ,bulk}}{P_{unocc,bulk}}$$

- $P_{occ,site}$: Occupancy probability of lipid in the site
- $P_{occ,bulk}$: Occupancy probability of lipid in bulk patch

Methods

- System composition: BamA (PDBID: 5EKQ), 4% anionic ceramide, 4% neutral ceramide, 92% POPC
- Simulation software: Gromacs 2022[4]
- Force Field: Martini 2.2[5]
- Membrane Building Tool: Insane[6]
- Protein Coarse-Graining Tool: Martinize[7]
- Simulation Time: 40 μs
- Replicas: 10

BamA

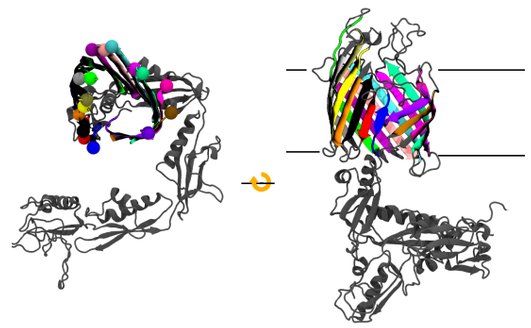


Fig 2. Visualization of BamA (PDBID: 5EKQ) topdown (left) and side view (right), each strand of the beta-barrel colored.

Background

- *Caulobacter crescentus* is a gram negative bacteria that was found to elongate in phosphate starved conditions (Fig 1), which normally limits lipid production.
- Under lipid A deficiency, *C. crescentus* can survive using a novel anionic ceramide[1,2].
- The role of anionic ceramide in the outer membrane of *C. crescentus* has not been determined.
- The Bam complex fold proteins in the outer membrane.
- Preliminary data shows that knocking out anionic ceramide or BamA cause similar phenotypic response.
- It is unclear if anionic ceramide has specific binding to BamA.

Research Questions

1. Does anionic ceramide bind to BamA?
2. What is the binding affinity for anionic ceramide sites on BamA?
3. What residues of BamA contact anionic ceramide?

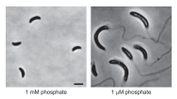
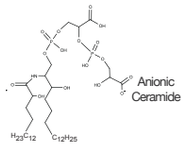
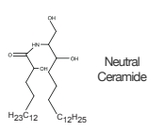


Fig 1. Image of *C. crecentus* in phosphate rich (left) and starved (right) conditions[1]





Results

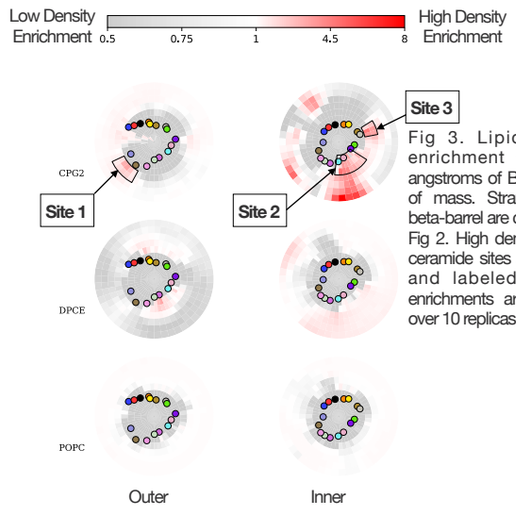


Fig 3. Lipid density enrichment within 40 angstroms of BamA center of mass. Strands of the beta-barrel are colored as in Fig 2. High density anionic ceramide sites are outlined and labeled. Density enrichments are averaged over 10 replicas

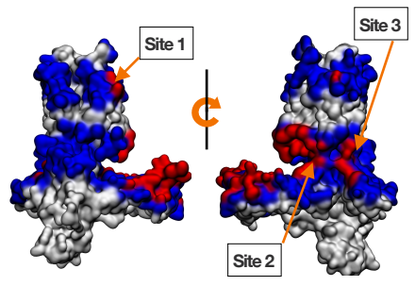


Fig 4. Contact Frequency between the anionic ceramide head group region and BamA residues in the beta-barrel. High contact frequency in red, low contact frequency in blue and no contact is grey.

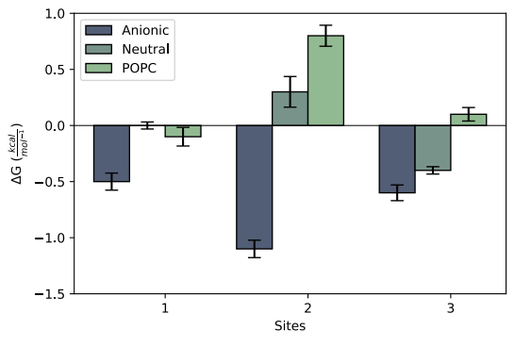


Fig 5. The free energy of binding for each lipid species in sites as in Fig 3. Error bars are the standard error between 10 replicas

Fig 6. Anionic ceramide high contact positive charged residues

Chain	Site	Residue	Contact Frequency	CF Rank
Bam A	1	K769	0.3	6
	2	R594	0.6	1
		R407	0.3	5
		K585	0.5	3
		K628	0.5	2
	3	R383	0.2	7
		K494	0.2	8
		R496	0.2	9
Bam D		R163	0.3	4

Summary

- Here we show that anionic ceramide has specific interactions with the beta barrel regions of BamA
- Anionic ceramide binds to site 2 with the highest affinity
- Anionic ceramide binds to positively charged residues in the loop regions
- POPC has no specific interactions with BamA
- Neutral ceramide does not compete with anionic ceramide

Acknowledgments

- Rutgers Office of Research and Computing (OARC)
- NCSA (Delta GPU)
- NSF DGE 2152059
- NSF MCB 2224195

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