Supplemental Information: β-barrel proteins determine the effect of core oligosaccharide

composition on outer membrane mechanics

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Running Title: Molecular basis of bacterial envelope mechanics
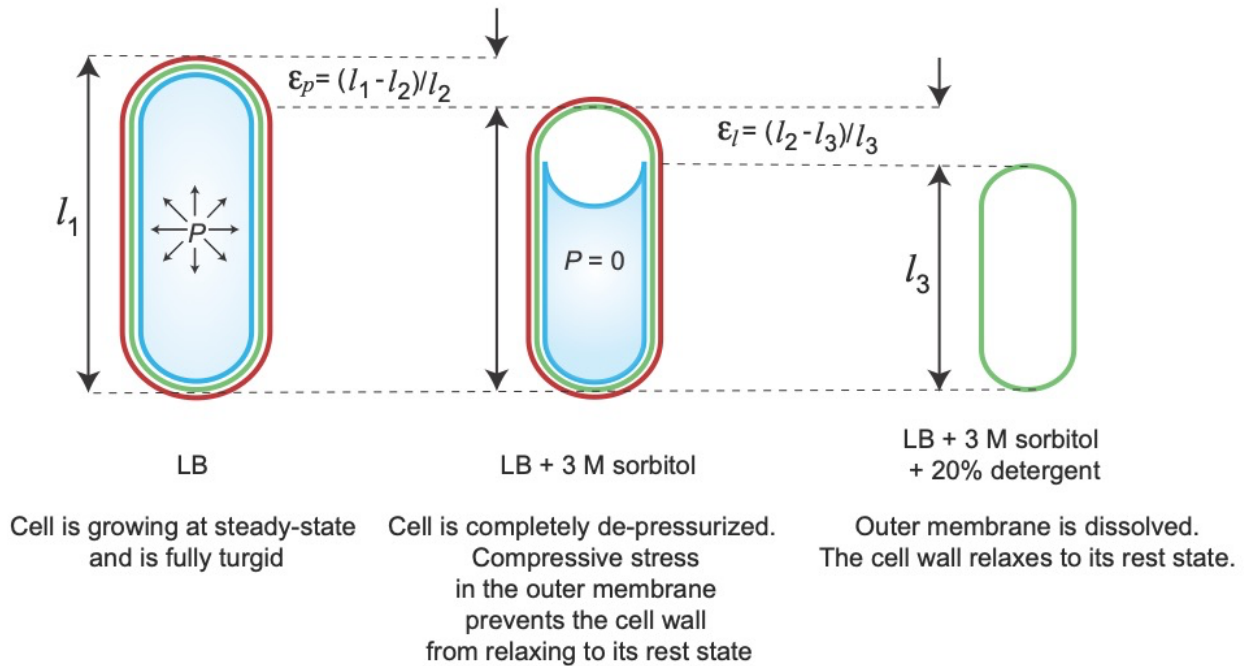
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Plasmolysis-lysis Assay

Figure S1. **Plasmolysis-lysis assay used to measure the ratio between the stiffness of the cell wall and the outer membrane.** Model of a fully turgid cell at a steady-state length (l_1). The cell is de-pressurized by a large 3M hypo-osmotic shock resulting in a plasmolysed cell whose length contracts (l_2). The strain resulting from this shock is calculated by: $\varepsilon_p = \frac{l_1 - l_2}{l_2}$. The cell is then treated with 20% detergent which dissolves the outer membrane allowing the cell wall to relax to its rest state (l_3). The strain resulting from this shock is calculated by: $\varepsilon_l = \frac{l_2 - l_3}{l_3}$. By treating the outer membrane and cell wall as parallel linear springs, relative stiffness is calculated by: $\frac{k_{om}}{k_{cw}} = \frac{\varepsilon_l}{\varepsilon_p(\varepsilon_l + 1)}$.
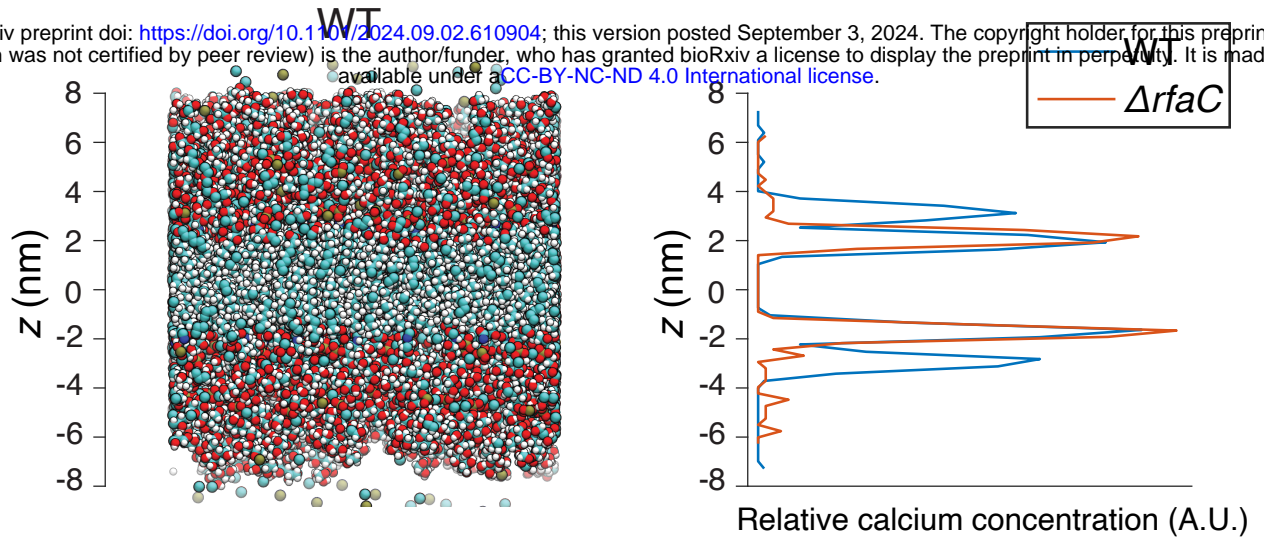
Figure S2. **Calcium distribution reflects the phosphate distribution in simulated lipopolysaccharide bilayers.** A) Simulated wild-type lipopolysaccharide bilayer. B) Calcium distribution across the thickness of the bilayer.
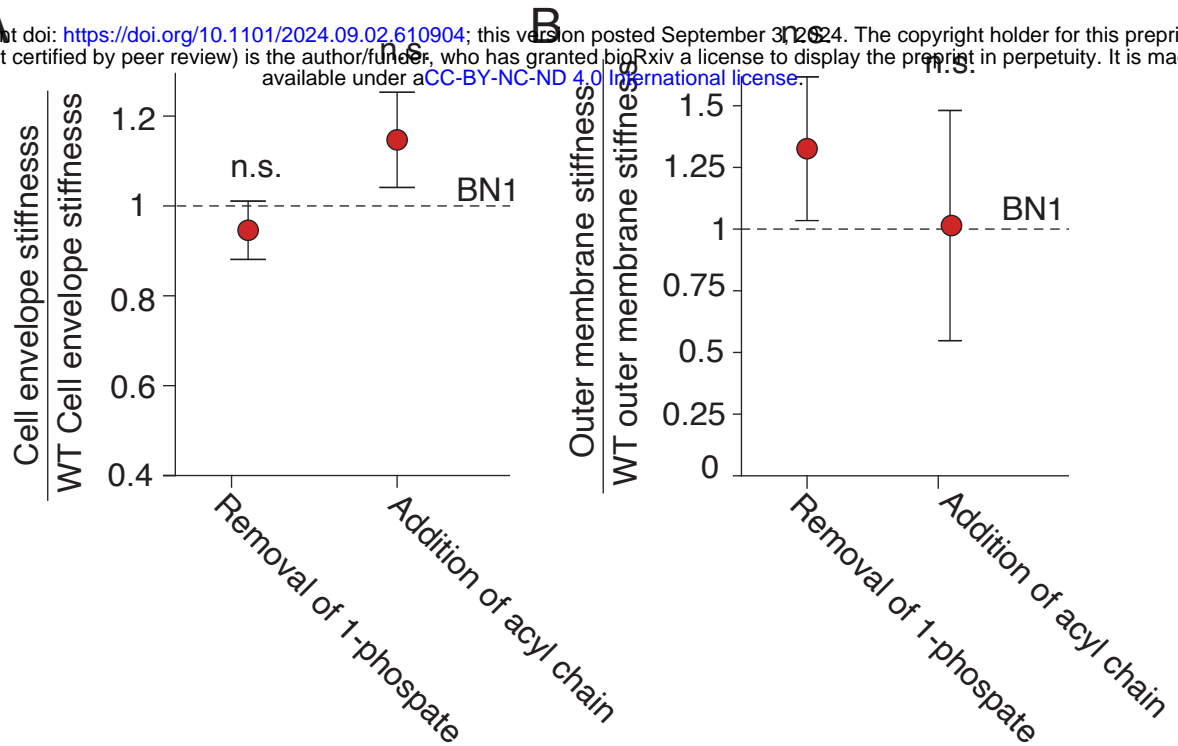
Figure S3. **Modifications to lipid A have weak effects on cell envelope stiffness.** A) Cell envelope stiffness of modified lipid A strains, normalized by wild-type (BN1) cell-envelope stiffness; n = 48, 54, 64, for BN1pE (removal of 1-phosphate), BN1pP (addition of acyl chain), and BN1 wild-type cells. B). Outer membrane stiffness of normalized by wild-type (BN1) cell-envelope stiffness; n = 45, 51, 87, for BN1pE, BN1pP, and BN1 wild-type cells.

Table S1. Strains used in this study.

Strain	Genotype	Relevant features	Source/Reference
DF065	F-lambda-*rph*-1	MG1655, wild type	*E. coli* Genetic Stock center (Yale)
DF153	DF065*, ΔrfaC::kan*	MG1655, *rfaC* deletion, KanR	
DF154	DF065*, ΔrfaF::kan*	MG1655, rfaF deletion, KanR	
DF155	DF065*, ΔrfaG::kan*	MG1655, rfaG deletion, KanR	
DF156	DF065*, ΔrfaJ::kan*	MG1655, rfaJ deletion, KanR	
DF005	Δ(araD-araB)567 ΔlacZ4787(::rrnB-3) λ- rph-1 Δ(rhaD-rhaB)568 hsdR514	BW25113, wild type	*E. coli* Genetic Stock center (Yale)
DF032	DF005*, ΔrfaC::kan*	BW25113, *rfaC* deletion, KanR	*E. coli* Genetic Stock center (Yale)
DF152	DF005*, ΔrfaF::kan*	BW25113, *rfaF* deletion, KanR	*E. coli* Genetic Stock center (Yale)
DF041	DF005*, ΔrfaG::kan*	BW25113, *rfaG* deletion, KanR	*E. coli* Genetic Stock center (Yale)
DF036	DF005*, ΔrfaJ::kan*	BW25113, *rfaJ* deletion, KanR	*E. coli* Genetic Stock center (Yale)
DF053	DF065*, ΔompA::kan*	MG1655*, ompA* deletion, KanR	
DF104	DF065*, Δlpp::kan*	MG1655, *lpp* deletion, KanR	
DF103	DF065*, Δpal::kan*	MG1655, *pal* deletion, KanR	
DF106	DF065*, ompA 1-192 ::kan*	MG1655, *ompA* 1-192 deletion, KanR	
DF049	DF005*, ΔompA::kan*	*BW25113, ompA* deletion, KanR	*E. coli* Genetic Stock center (Yale)

Strain	Genotype	Description	Source
DF102	DF005, *Δlpp::kan*	BW25113, *lpp* deletion, KanR	*E. coli* Genetic Stock center (Yale)
DF101	DF005, *Δpal::kan*	BW25113, *pal* deletion, KanR	*E. coli* Genetic Stock center (Yale)
DF110	DF005, *ompA 1-192 ::kan*	BW25113, *ompA* 1-192 deletion, KanR	
DF090	*F– araD139 Δ(argF-lac)U169 rpsL150 relA1 thi fib5301 deoC1 ptsF25 rbsR*	MC4100, wild type	Silhavy et al. 1984
DF043	DF090, *ΔompA::kan*	MC4100, *ompA* deletion, KanR	
DF006	W3110, ΔeptA, ΔlpxT, Δ*pagP*	BN1	Needham et al. 2013
DF008	BN1, pQLinkN-lpxE	*BN1pE,* AmpR	Needham et al. 2013
DF109	BN1, pQLinkN-pagL	*BN1pL,* AmpR	Needham et al. 2013
DF007	BN1, pQLinkN-pagP	*BN1pP,* AmpR	Needham et al. 2013
DF157	DF065, *ΔompA <>frt ΔrfaC::kan*	MG1655, *ompA* and *rfaC* double deletion, KanR	
DF158	DF065, *ΔompA <>frt ΔrfaF::kan*	MG1655, *ompA* and *rfaF* double deletion, KanR	
DF159	DF065, *ΔompA <>frt ΔrfaG::kan*	MG1655, *ompA* and *rfaG* double deletion, KanR	
DF160	DF065, *ΔompA <>frt ΔrfaJ::kan*	MG1655, *ompA* and *rfaJ* double deletion, KanR	

Table S2. Primers used in this study.

Name	Sequence	Primer type	Description
TS023	ATTCCGGGGATCCGTCGACC	FP	P1 - Kan FW
TS024	TGTAGGCTGGAGCTGCTTCG	RP	P2 - Kan RV
TS025	CAGTCATAGCCGAATAGCCT	RP	k1 - middle of kan cassette
TS026	CGGTGCCCTGAATGAACTGC	FP	k2 - middle of kan cassette
TS027	ATTGGTTTTTGCCCGGGT	FP	rfaC FW
TS028	AGTAGCACGAAATGGCGAATTATCTAC	RP	rfaC RV
TS029	AATATGTTCGTCAAATCCTGCC	FP	rfaF FW
TS030	GTCATAGTTCTCTGCTTGTAGCGC	RP	rfaF RV
TS031	ACAGCGCGTCAGATATTTAAG	FP	rfaG FW
TS032	TATCAACGCCAACATCACTCAGG	RP	rfaG RV
TS033	CAGGTTTCTGCACGAGCTA	FP	rfaJ FW
TS034	CTCAAAAAGGCGTTCGTAATAATCACC	RP	rfaJ RV
AA001	CGACCTGGACATCTACACTC	FP	ompA 1-192 FW
AA002	GTATAGGAACTTCAGAGCGC	RP	ompA 1-192 RV
AA003	TAAAGGTATCAAAGACGTTGTAACTCAGCCGCAGGCTTAAATTCCGGGGATCCGTCGAC	FP	homology to ompA 1-192 with stop codon, and homology to the Kan cassette
AA004	GAAGCAGCTCCAGCCTACACGTCAGTTATTCCTTACCCAGCAATGCCTGCAGATCCTGC	RP	homology to the Kan cassette