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Supplemental Information

In Situ Structure of an Intact Lipopolysaccharide-Bound Bacterial Surface Layer

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Supplemental Tables

Table S1: Cryo-EM data collection, refinement and validation statistics, Related to Figure 1.

	#RsaA$_{NTD}$:PS(EMDB: EMD-10389), (PDB: 6T72)
Data collection and processing	
Magnification	130,000
Voltage (kV)	300
Electron exposure (e–/Å^2)	43
Defocus range (μm)	-1.5 to -3
Pixel size (Å)	1.08
Symmetry imposed	C1
Initial particle images (no.)	129633
Final particle images (no.)	115776
Map resolution (Å)	3.7
FSC threshold	0.143
Map resolution range (Å)	3.68-4.5
Refinement	
Initial model used (PDB code)	None
Model resolution (Å)	3.7
FSC threshold	0.5
Model resolution range (Å)	3.7-4.5
Map sharpening *B* factor (Å^2)	-85.819
Model composition	
Non-hydrogen atoms	26278
Protein residues	3388
Ligands	210
B factors (Å^2)	
Protein	70.97
Ligand	147.87
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	1.515
Validation	
MolProbity score	1.23
Clashscore	4.29
Poor rotamers (%)	0.00
Ramachandran plot	
Favored (%)	97.92
Allowed (%)	2.08
Disallowed (%)	0.00

*based on 14 RsaA$_{NTD}$ subunits

Table S2: Strains, plasmids and oligos used in this study, Related to Method Details.

Strain, plasmid or oligo	Description, construction or sequence (5' – 3')	Reference or application
Strains		
Escherichia coli		
S17-1	*E. coli* 294::RP4-2(Tc::Mu)(KM::Tn*7*)	(Simon et al., 1983)
Alpha-select™	*deoR endA1 recA1 relA1 gyrA96 hsdR17*(r_K^- m_K^+) *supE44 thi*-1 Δ(*lacZYA-argFV169*) Φ80δ*lacZ*Δ*M15* F^-	Bioline
Caulobacter crescentus		
NA1000	*syn-1000*; previously called CB15N. This strain does not make the adhesive holdfast.	(Evinger and Agabian, 1977)
YB5754	NA1000 Δ*rsaA*	E. Quardokus, unpublished
YB1001	NA1000 Δ*rsaA* ::pNPTS138::*rsaA*TEV250	This study
Plasmids		
pNPTS138	pLitmus 38 derivative; with *nptI, sacB* and RK2 *oriT* sequences, and deleted *bla* gene; Km^R	M.R.K. Alley, unpublished
pNPTS138::*rsaA*TEV250	pNPTS138 parent vector containing 1000 bp fragment upstream of *rsaA*, *rsaA* with TEV protease site inserted at amino acid residue 250 and 500 bp downstream of *rsaA*	This study
pNPTS138Δ*rsaA*	A 471 bp upstream of *rsaA* and a 530 bp PCR product downstream of *rsaA* were cloned into pNPTS138.	(Hardy et al., 2010)
Oligo Name		
138rsaA1kbupF	AGCTTCTCTGCAGGATATTGGCCGGCAGTTGCACCG	*rsaA*TEV250 construct
138rsaA500dwnR	AATTCGTGGATCCAGATGGGCGGGCGACCAGCACG	*rsaA*TEV250 construct
rsaATEV250upR	TGGAAGTACAGGTTCTCCGAACCCGACACGCCCGA	*rsaA*TEV250 construct
rsaATEV250dwnF	CGTGTCGGGTTCGGAGAACCTGTACTTCCAGGGCA CCCTCTCGCTGACCACCGGC	*rsaA*TEV250 construct
662uprsaAF	TGGCTCCAGTCCGCCGGTTGC	*rsaA* sequencing primer
132uprsaAF	TATAGCGCTTTTCGGCGGGGG	*rsaA* sequencing primer
rsaA338F	CTACTCGAAGTTCGCTCAGG	*rsaA* sequencing primer
rsaA842F	CACCGCCAACAACGACACG	*rsaA* sequencing primer
rsaA1338F	GTGGCTCAAACGGCCGGC	*rsaA* sequencing primer
rsaA1838F	CGCTCGCGTCACGATCACCTC	*rsaA* sequencing primer
rsaA2338F	CGTGTTCAACCTGACCCTGTC	*rsaA* sequencing primer
rsaA2834F	GACAAGCTCGACCTCGTC	*rsaA* sequencing primer
564uprsaAR	CACCTAGGCTTGGGGAAGCTTCCG	*rsaA* sequencing primer
62uprsaAR	CAGCATTTTTCTAACCGGTACAGC	*rsaA* sequencing primer
rsaA447R	ACGAAACGCCCGTGTAGG	*rsaA* sequencing primer
rsaA942R	CAGGACGTCGGTGCCAGC	*rsaA* sequencing primer
rsaA1445R	GGCGGCGGTTTGGGTGAC	*rsaA* sequencing primer
rsaA1939R	AGACCGGTCGCCAGTTCG	*rsaA* sequencing primer
rsaA2445R	CGGTCGTGTTGGTGTCGG	*rsaA* sequencing primer
rsaA2938R	AGGTACTGAGCCAGGGTC	*rsaA* sequencing primer
FHindIIIrsaA	GACCTCCAGAAGCTTGGCCCAGTC	ΔrsaA PCR/sequencing
RsalIrsaA	GCCGTCGAAGTCGAGACCGCC	ΔrsaA PCR/sequencing
TEVprimerF	GAGAACTTGTACTTCCAGTCG	TEV protease site primer
M13R(-48)	AGCGGATAACAATTTCACACAGGA	pNPTS138 PCR/sequencing
M13F (-40)	GTTTTCCCAGTCACGAC	pNPTS138 PCR/sequencing