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Supplemental Information

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Supplementary Information for:

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Supplementary Figures:

Supplementary Tables:

Supplementary Experimental Procedures

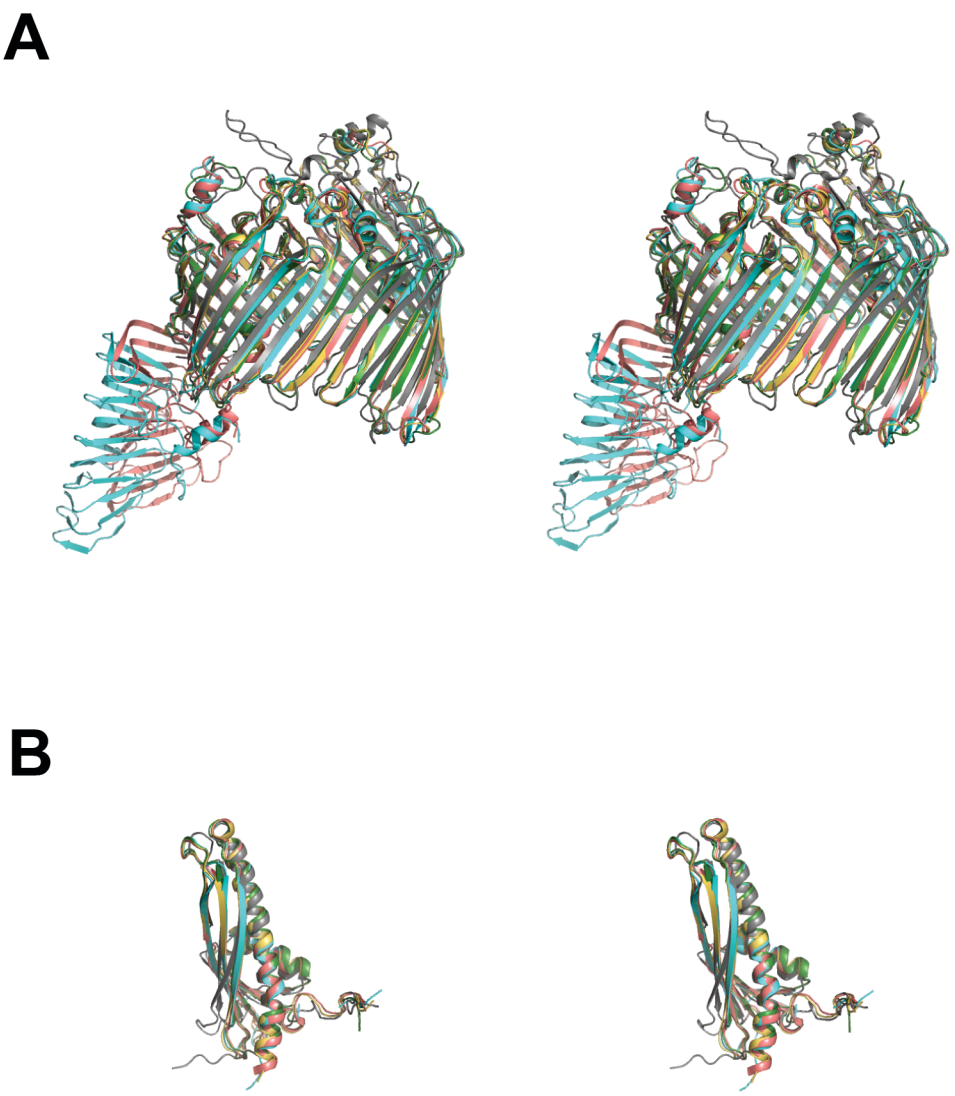

Figure S1 (Related to Figure 1)**: LptD and LptE structural alignments**. LptD (a) and LptE (b) structural alignment stereo diagrams: *Yp*LptDE (green), full-length *Kp*LptDE (cyan), *Pa*LptDE (gray), *Sf*LptDE (salmon) PDBID = 4Q35, *St*LptDE (yellow) PDBID = 4N4R

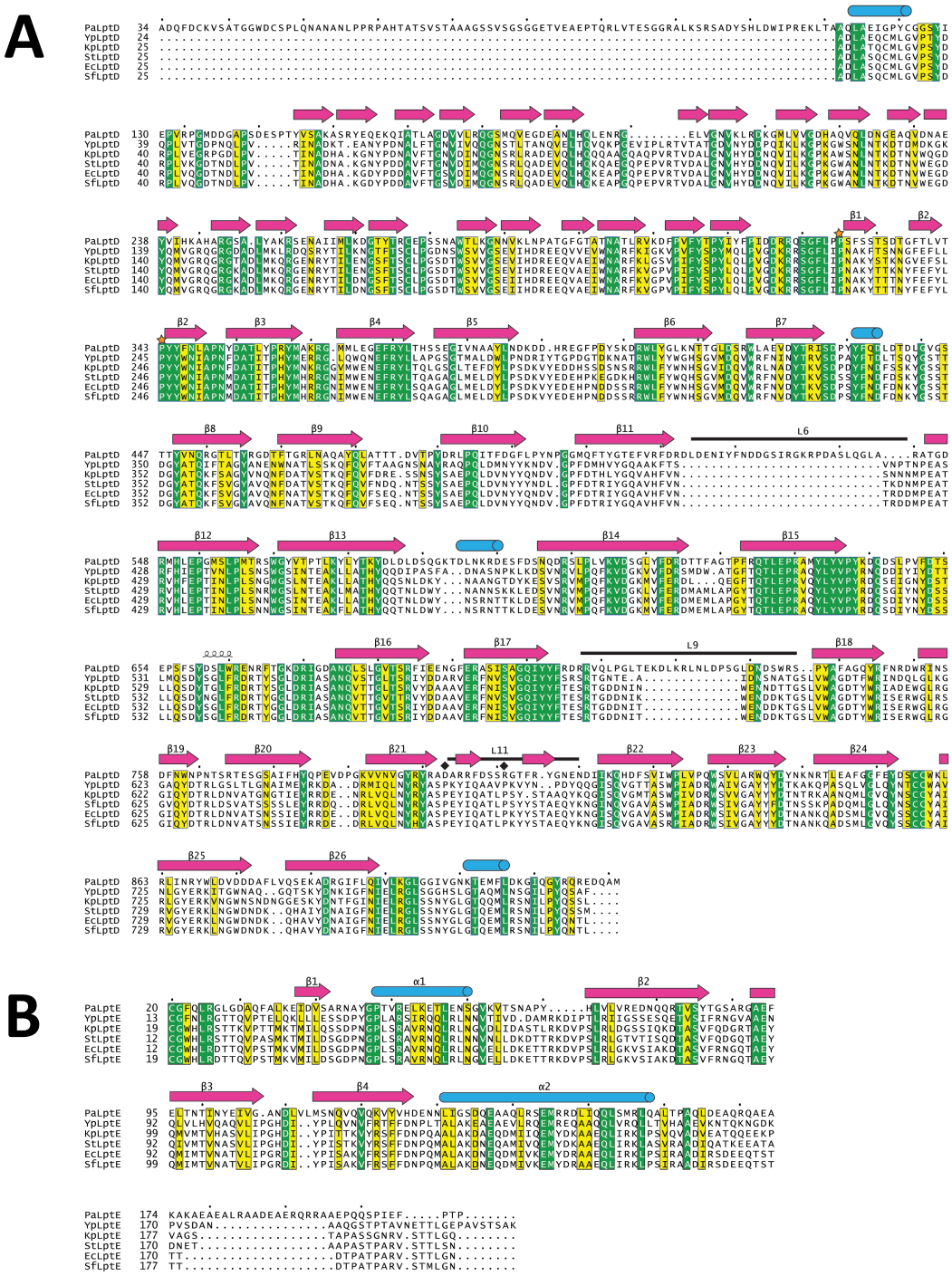

Figure S2 (Related to experimental procedures)**: Sequence alignment of known LptDE structures. (A)** LptD and **(B)** LptE. In **(A)** extracellular loops L6, L9, and L11 are indicated by a solid black line. Gold stars indicate the strictly conserved proline residues that are part of the lateral gate. Proline residues conserved in most LptD sequences but not in *Pa*LptD are indicated by black diamonds. In **(A)** and **(B)**, β-strands are shown in magenta, α-helices in blue, conserved residues are in green blocks, and residues with high sequence similarity in yellow. Alignments generated with Clustal Omega and ESpript.

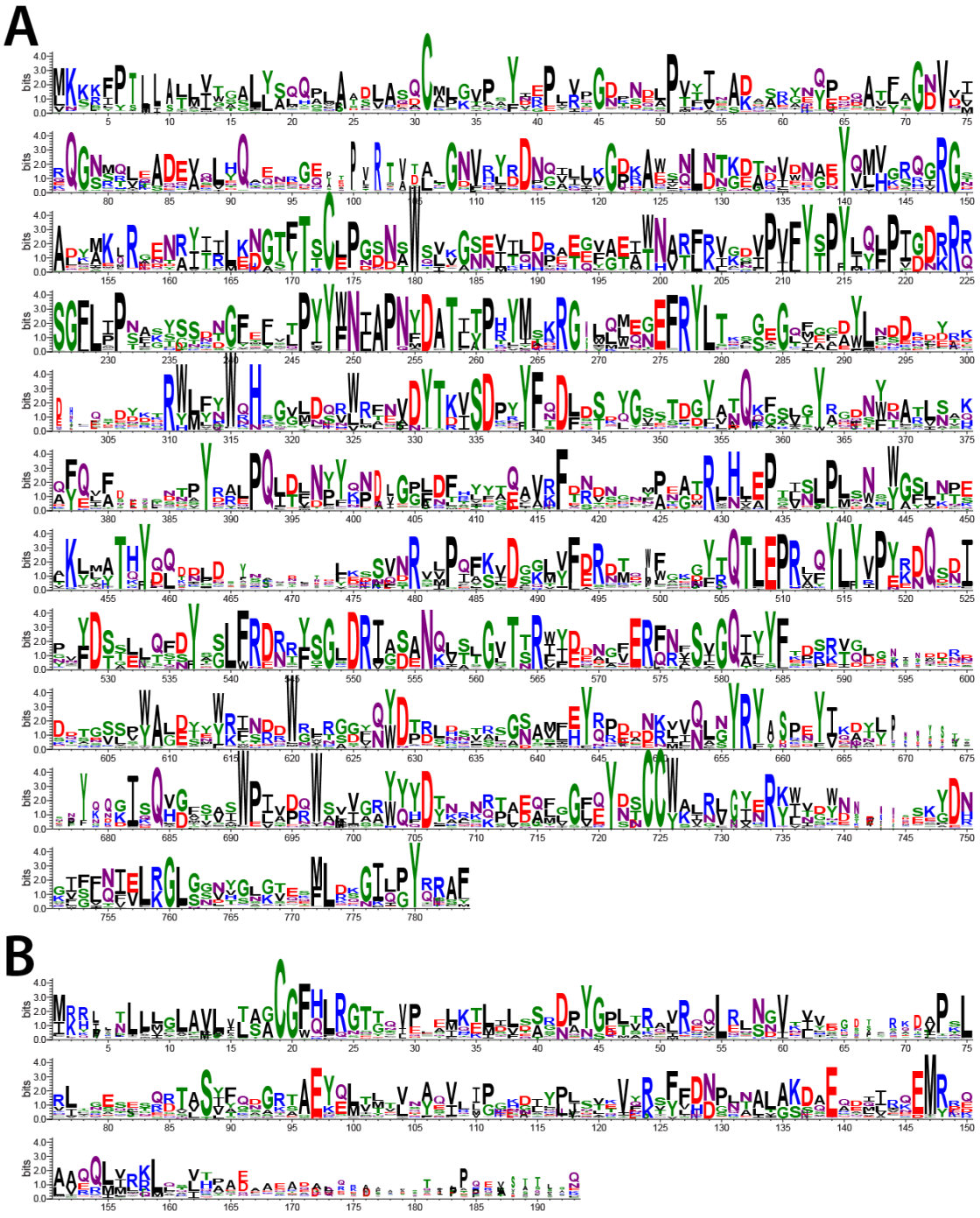

Figure S3 (Related to experimental procedures)**: Global sequence conservation in LptD and LptE.** A selection of the family of LptD and LptE sequences were analyzed for sequence conservation. **(A)** LptD sequences from 222 species were aligned. Insertions not found in the *E. coli* sequence were truncated, for clarity. **(B)** Alignment of LptE sequences from 281 species. Insertions not found in *E. coli* were truncated. Height of the letter represents prevalence of the residue at that position.

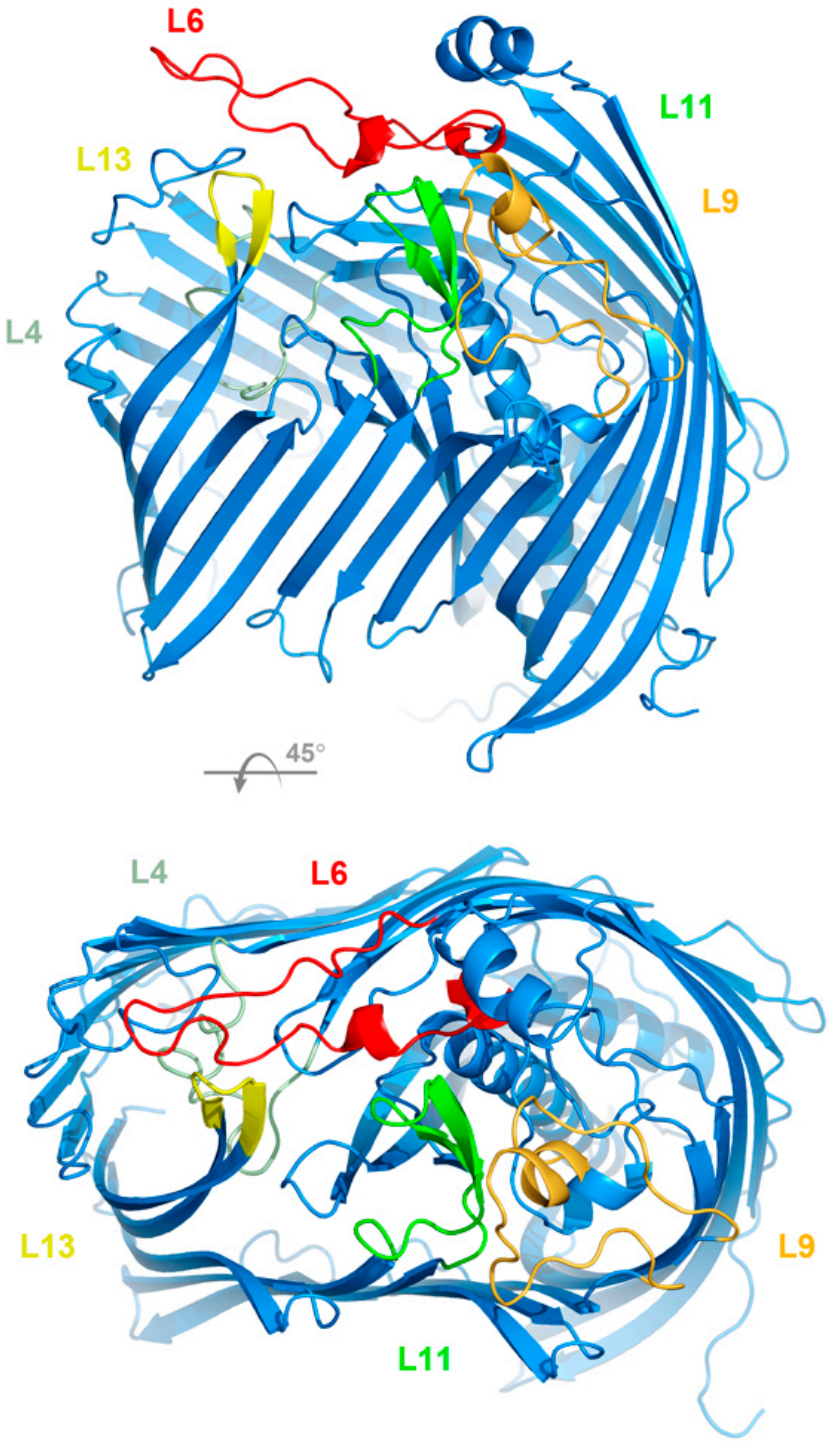

Figure S4 (Related to Figure 1)**: Side(3/4) view and top viewof *Pa*LptDE.** *Pa*LptDE is shown in blue, with several extracellular loops highlighted: L4 in gray, L6 in red, L9 in orange, L11 in green, and L13 in yellow. Compared to the four other LptDe structures, L6 contains a 23-residue insertion and L9 contains an 11-residue insertion and divergent sequence.

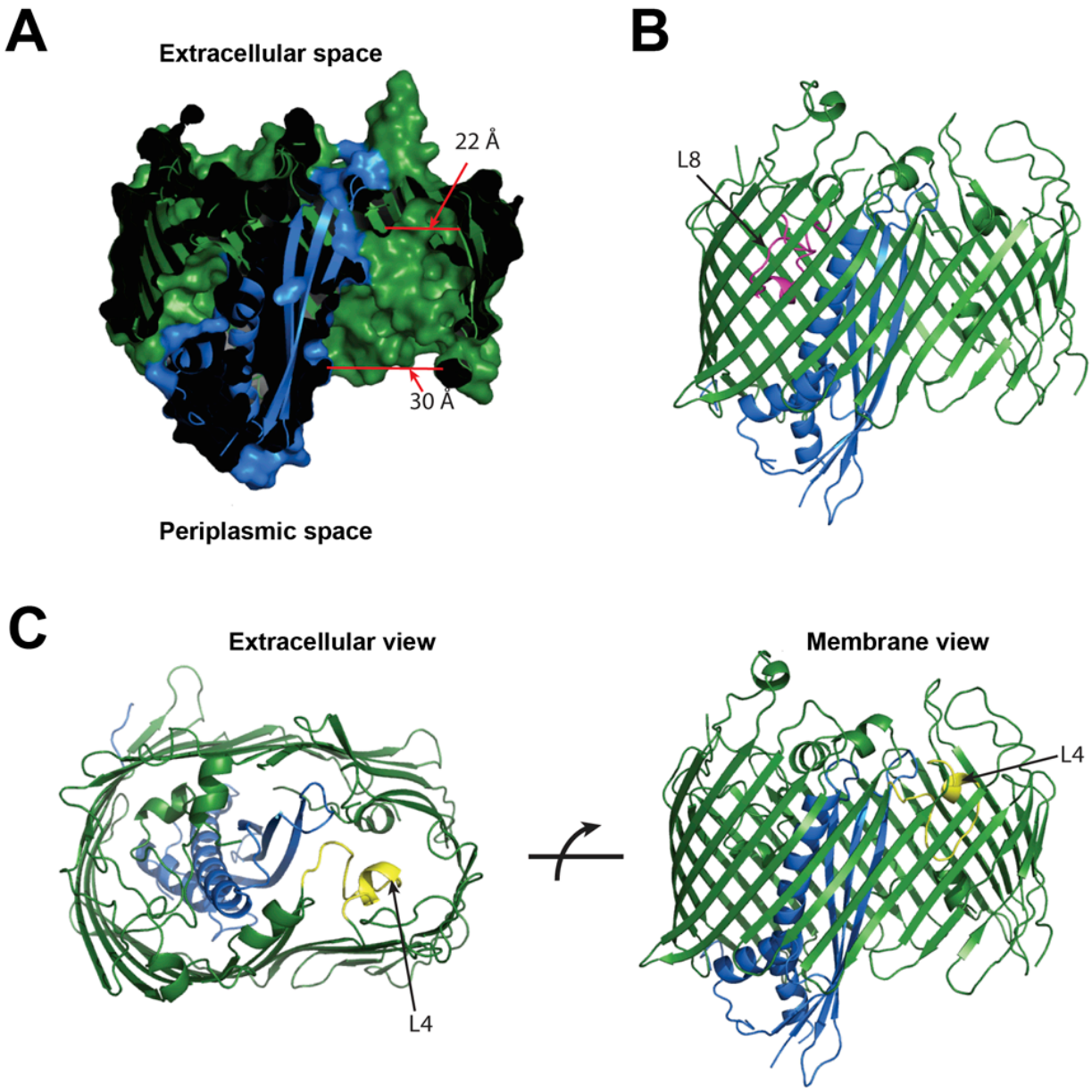

Figure S5 (Related to Figure 1)**: Positioning of LptE within the LptD lumen. a.** Cutaway surface of *Yp*LptD (green) and *Yp*LptE (blue) complex viewed from the membrane. The lumenal cavity tapers from 30 Å at the periplasm to 22 Å deep in the cavity near the extracellular space. **b.** L8 (magenta) of LptD (green) folds into the lumen of the barrel, causing LptE (blue) to reside at an angle within LptD. This results in a tapering of the lumenal cavity in the LptD barrel. View is the same as in a. **c.** L4 (yellow) of LptD (green) blocks the exit of LPS from the lumen into the extracellular space and likely moves to create an exit pore.

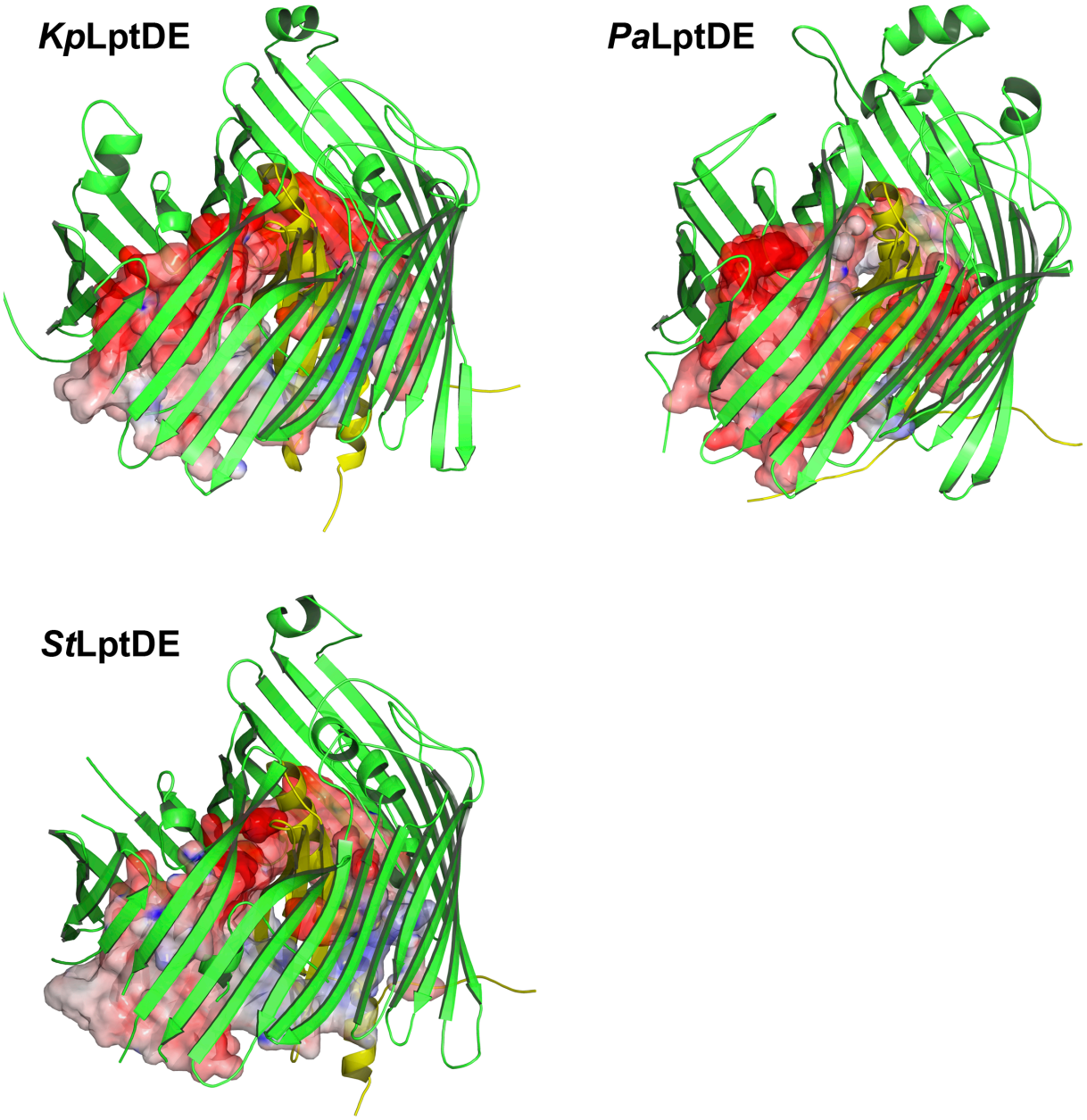

Figure S6 (Related to Figure 7)**: Hollow representations of LptDE structures.** The solid surface is a casting of the lumenal cavity of *K. pneumoniae*, *P. aeruginosa and S. typhimurium* (PDBID 4N4R) LptDE generated by populating all open areas with water molecules using the program Hollow. Positive areas are represented in blue (+20kT), neutral in white, and negative in red (-20 kT). The electrostatic surface was generated using APBS.

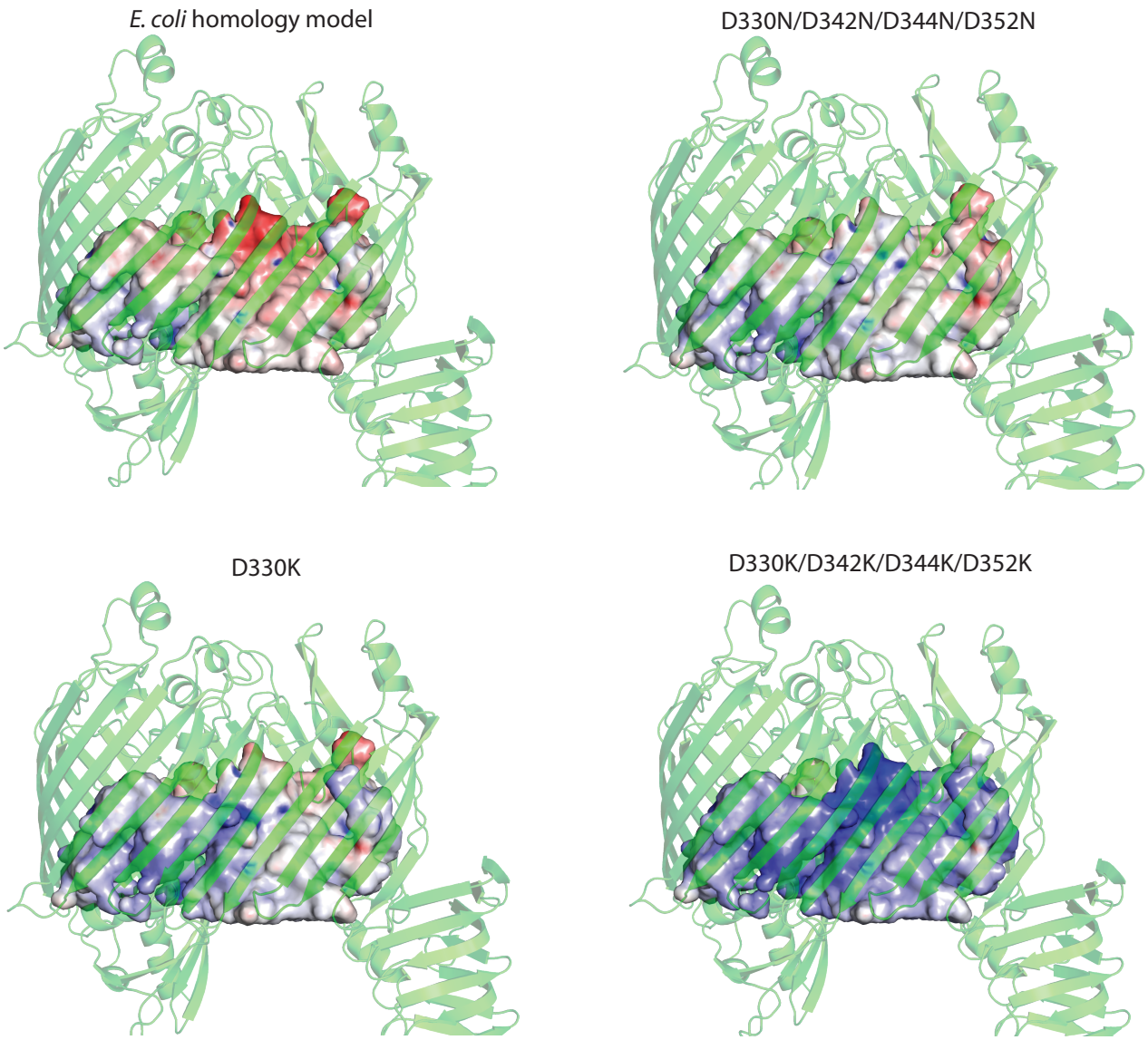

Figure S7 (Related to Figure 7)**:** ***In silico*** **modeling of charge mutations in the lumenal cavity.** A homology model of *E. coli* LptDE complex was generated from *Sf*LptDE (PDB ID 4Q35). Electrostatic charge of the lumen is mapped onto a surface representing non-protein occupied space within the lumen. Conserved aspartic acid residues were mutated *in silico* to asparagine or lysine residues. Lumenal electrostatics are mapped onto the lumen surface.

Supplemental Table 1 (Related to Figures 1 and 3): Length of LptD and LptE constructs used in this study

Construct	LptD residues	LptE residues	PDB code
*Yp*LptDE	225 – 780	21 – 207	5IXM
*Kp*LptDE	203 – 782	21 – 196	5IV8
Full-length *Kp*LptDE	25 – 782	20 – 196	5IV9
*Pa*LptDE	301 – 924	21 – 207	5IVA

Supplemental Experimental Procedures

Cloning, expression and purification of LptDE

Primer names and sequences used for LIC cloning are listed in the Table below. The modified pET9 vector, into which LptD was inserted, contained an N-terminal PelB signal sequence, a 10X His tag, a TEV site, and LIC cloning sites. The modified pCDF-1b vector for LptE expression contained the PelB signal sequence, LIC cloning sites, and a C-terminal 6X His tag. LptD and LptE for each species were co-expressed in BL21 (DE3) cells in TB media supplemented with 50μg/ml kanamycin and 25μg/ml streptomycin to stationary phase (3-4 days). The cells were grown at 21°C without IPTG induction.

To make the pBAD24_LptD_WT plasmid, *E. coli* LptD (residues 1 – 784) was cloned into pBAD24 using primers pBAD24_LptD_NcoI_for2 and pBAD24_LptD_HindIII_rev2. A C-terminal 10X histidine tag was subsequently inserted into this vector using primers pBAD_LptD_chis10_for and pBAD_LptD_chis10_rev to create pBAD24_LptD_WTcHis. This vector and its mutant derivatives were used in the LptD knock-out and localization assays.

Primer sequences and names/numbers for constructs used in this study			
Function:	**Gene:**	**Name:**	**Sequence (5'-3'):**
Expression	LptD_Yersinia	LptD_Yersinia225_pET9_for	TACTTCCAATCCATGTCTGGATTTCTGATTCCGAACGCC
	LptD_Yersinia	LptD_Yersinia_pET9_rev	TATCCACCTTTACTGTTATTAGAATGCGCTCTGATACGGCAG
	LptE_Yersinia	LptE_Yersinia_pCDF-SS_for	CTCCTAACACCTCGGGCTTTAACCTGCGTGGCAC
	LptE_Yersinia	LptE_Yersinia_pCDF-SS_rev	CATCCATCATCAATGGTGATGGTGATGGTGTTTGGCCGAGGTTGAGACC
	LptD_Klebsiella	LptD_Kleb_25f_NTT	TACTTCCAATCCATGGCCGATCTGGCAACCCAGTG
	LptD_Klebsiella	LptD_Kleb_203f_NTT	TACTTCCAATCCATGTTTAAACTGGGTAGTGTGCCGATTTTCTACAGC
	LptD_Klebsiella	LptD_Kleb_782r_NTT	TATCCACCTTTACTGTTATTACAGACTAGACTGGTACGGCAGGATATTGC
	LptE_Klebsiella	LptE_Kleb_20f	CTCCTAACACCTCGGGTTGGCATCTGCGTAGCAC
	LptE_Klebsiella	LptE_Kleb_196r	CATCCATCATCAATGGTGATGGTGATGGTGCTGGCCCAGCGTGGTG
	LptD_Pseudomonas	LptD_Pseud_301f_NTT	TACTTCCAATCCATGCTGCGTGTTAAAGATTTTCCGGTCTTCTATACC
	LptD_Pseudomonas	LptD_Pseud_924r_NTT	TATCCACCTTTACTGTTATTACATCGCCTGATCTTCGCGTTG
	LptE_Pseudomonas	LptE_Pseud_21f	CTCCTAACACCTCGGGCTTCCAACTGCGTGGTCTGG
	LptE_Pseudomonas	LptE_Pseud_207r	CATCCATCATCAATGGTGATGGTGATGGTGCGGGGTCGGAAATTCAATCGG
In vivo cloning	LptD_Escherichia	pBAD24_LptD_NcoI_for2	TCGTTACCATGGATGAAAAAACGTATCCCCACTCTCCTGG
	LptD_Escherichia	pBAD24_LptD_HindIII_rev2	ACTGTTAAGCTTTCACAAAGTGTTTTGATACGGCAGAATG
	LptD_Escherichia	lptD_Cm.F	ACCGTTTGTCACGCGCAACGTTACCGATGATGGAACAATAACCTGTGACGGAAGATCACT
	LptD_Escherichia	lptD_Cm.R	TAACCGCACTGCGGATTACGTGGTAAATCAACAAATCACATTACGCCCCGCCCTGCCACT
Mutagenesis	LptD_Escherichia	pBAD_LptD_cthis10_for	CCATCACCATCACCATCACCATTGAAAGCTTGGCTGTTTTG
	LptD_Escherichia	pBAD_LptD_cthis10_rev	TGATGGTGGCTGCCACCGCCACCCAAAGTGTTTTGATACGGC
	LptD_Escherichia	LptD_Ec_G227C	GTGACAAACGTCGCTCTTGTTTCTTGATCCCGAAC
	LptD_Escherichia	LptD_Ec_Y314C	TTCACGTCGTTGGTTATTCTGCTGGAACCACTCCG
	LptD_Escherichia	LptD_Ec_Y347C	GCTACTTCAATGATTTCGATAACAAGTGCGGTTCCAGTACTG
	LptD_Escherichia	LptD_Ec_T351C	TCGATAACAAGTACGGTTCCAGTTGTGACGGCTACGC
	LptD_Escherichia	LptD_Ec_D714C	CCAATGCTAACAAGCAAGCCTGCTCTATGTTAGGTGTGCAAT
	LptD_Escherichia	LptD_Ec_G718C	GCAAGCCGACTCTATGTTATGTGTGCAATACAGCTC
	LptD_Escherichia	LptD_Ec_I777C	AGAGATGCTGCGTTCGAACTGTCTGCCGTATCAAAACACT
	LptD_Escherichia	LptD_Ec_L758C_2	ACGCAATCGGCTTTAACATCGAATGTCGCGGCCTGAG
	LptD_Escherichia	LptD_Ec_M772C_2	GGTCTGGGTACGCAAGAGTGCCTGCGTTCGAACATTCTG
	LptD_Escherichia	LptD_p231a_f	TTTCTTGATCGCGAACGCCAAG

	LptD_Escherichia	LptD_p231a_r	CCAGAGCGACGTTTGTCAC
	LptD_Escherichia	LptD_p246a_f	GTTCTACCTGGCATATTACTG
	LptD_Escherichia	LptD_p246a_r	TCAAAGTAGTTGGTGGTG
	LptD_Escherichia	LptD_p231a_multi	GCGATGTTCCAGTAATATGCCAGGTAGAACTCAAAGTAG
	LptD_Escherichia	LptD_p246a_multi	CTCTGGTTTCTTGATCGCGAACGCCAAGTACAC
	LptD_Escherichia	LptD_r774e_f	AGAGATGCTGGAATCGAACATTCTGCCG
	LptD_Escherichia	LptD_r774e_r	TGCGTACCCAGACCGTAG
	LptD_Escherichia	LptD_r310e_f	CAGTTCACGTGAATGGTTATTCTACTGG
	LptD_Escherichia	LptD_r310e_r	TCATCGTTCGGGTGTTCATC
	LptD_Escherichia	LptD_e273q_e275q	TGGCAACATCATGTGGCAGAACCAGTTCCGCTACCTCTCCC
	LptD_Escherichia	LptD_e288q_d290n	GGGCGCTGGCTTGATGCAGCTGAACTATCTGCCTTCAGA
	LptD_Escherichia	LptD_d330n	GTGGCGTTTCAACGTCAACTACACCAAGGTCAG
	LptD_Escherichia	LptD_d342n_d344n	GCGATCCTAGCTACTTCAATAATTTCAATAACAAGTACGGTTCCAGT
	LptD_Escherichia	LptD_d352n	ATAACAAGTACGGTTCCAGTACTAACGGCTACGCAA
	LptD_Escherichia	LptD_d749n	GATAACGATAAACAACATGCGGTATATAACAACGCAATCGGC
	LptD_Escherichia	LptD_e273k_e275k	GTGGCAACATCATGTGGAAGAACAAATTCCGCTACCTCTCC
	LptD_Escherichia	LptD_e288k_d290k	GCGGGCGCTGGCTTGATGAAACTGAAGTATCTGCCTTCAGATAAA
	LptD_Escherichia	LptD_d330k	GTGGCGTTTCAACGTCAAGTACACCAAGGTCAGCG
	LptD_Escherichia	LptD_d342k_d344k	GGTCAGCGATCCTAGCTACTTCAATAAGTTCAAGAACAAGTACGGTTCCAGTACTGAC
	LptD_Escherichia	LptD_d352k	CGATAACAAGTACGGTTCCAGTACTAAGGGCTACGCAACGC
	LptD_Escherichia	LptD_d749k	GGGATAACGATAAACAACATGCGGTATATAAGAACGCAATCGGCTTTA

For purification, cells were resuspended in lysis buffer (50 mM Tris-HCl pH 7.5, 200 mM NaCl, 1 mM $MgCl_2$, 10 μg/ml DNaseI, 100 μg/ml 4-(2-aminoethyl)benzenesulphonyl fluoride (AEBSF)) and lysed by three passages through an Emulsiflex C3 (Avestin) homogenizer at 4°C. The lysate was centrifuged at 10,000*g* for 10 min to remove unlysed cells and the supernatant was incubated with 2% (v/v) Triton X-100 for 30 min at 4°C. Membranes were isolated from the lysate by centrifugation at 234,000*g* for 60 min at 4°C. The pellet was resuspended in buffer containing 50 mM Tris-HCl pH 7.5, 200 mM NaCl, and 20 mM imidazole and solubilized by constant stirring in 5% (w/v) Elugent (Millipore Merck) for 16 h at 4°C. Insolubile material was removed by centrifugation at 370,000*g* for 60 min at 4°C. The supernatant was filtered and applied to a 15-ml Ni-NTA column (Qiagen) equilibrated in 50 mM K_2HPO_4 pH 7.5, 200 mM NaCl, 10% (v/v) glycerol, 20 mM imidazole, and 0.1% (w/v) dodecyl maltoside (DDM) (Anatrace). Protein was eluted with 250 mM imidazole and peak fractions were pooled and dialysed against 25 mM Tris-HCl pH 8.0 containing 0.6 mM EDTA pH 8.0 for 16 h at 4°C. The sample was applied to a 10 mL Q-Sepharose column (GE Healthcare) equilibrated with 25 mM Tris HCl pH 8.0, 0.6 mM EDTA, and 0.1% DDM and eluted with a linear gradient of 0-0.6 M NaCl . Peak fractions were then concentrated and applied to a S-300HR Sephacryl size exclusion column (GE Healthcare) equilibrated in 20 mM Tris-HCl pH 7.5, 200 mM NaCl, and 0.8% C_8E_4.

ΔlptD strain and Phage lysate generation

First, pBAD24_LptD_WT was electroporated into *E. coli* strain NM1100 containing a chromosomal lambda Red system (Bougdour et al., 2008) and plated on LB agar containing 50 μg/ml ampicillin. After O/N incubation at 32°C, a single transformant was grown in LB broth containing 50 μg/ml ampicillin at 32°C to an OD_{600} of 0.6. These cultures were then placed in a shaking 42°C water bath for 15 min to induce lambda gene expression and then immediately cooled in an ice-water slurry. Cells were pelleted at 4°C and washed three times with ice-cold ultrapure water. Final pellets were resuspended in 1/100 of the original culture volume in ice-cold ultrapure water containing 10% (v/v) glycerol and either used immediately or frozen. In parallel, the chloramphenicol resistance cassette from pCAT19 (Fuqua, 1992) was PCR amplified using primers lptD_Cm.F and lptD_Cm.R. These primers contain 5' overhangs incorporating 40 bp of complementary sequence upstream and downstream of the *lptD* gene. The PCR product was electroporated into NM100 containing pBAD24_LptD_WT prepared as described above. After re-suspension in 1 ml of LB broth and recovery at 37°C for 1 hour, cells were spread on LB plates containing 50 μg/ml ampicillin, 10 μg/ml chloramphenicol, and 1% (w/v) arabinose. The plates were incubated O/N at 37°C to select for colonies carrying the chromosomal *lptD* deletion and pBAD24_LptD_WT. The chromosomal insertion of the chloramphenicol cassette at the *lptD* locus was verified by PCR.

The Δ*lptD* cells were grown at 37°C in LB medium containing 50 μg/ml ampicillin, 1% (w/v) arabinose, 0.01 M $MgSO_4$, and 0.005 M $CaCl_2$. At an OD_{600} of ~ 0.1-0.2 P1vir phage was added and the culture was grown 4 h at

37°C or until complete lysis was observed. A couple of drops of chloroform were added to lyse any remaining cells and the culture was centrifuged to remove debris. The supernatant was collected, two additional drops of chloroform were added, and the sample was stored at 4°C.

LptD localization assay

Glycerol stocks of MG1655 *E. coli* cells containing pBAD24_LptD_WTcHis and mutant variants were inoculated into 500 mL TB medium containing 50 μg/ml ampicillin and 0.2% (w/v) arabinose. Cultures were grown overnight at 37°C and harvested. Cell pellets were resuspended in 40 mL of buffer containing 50 mM Tris HCl pH 7.5, 200 mM NaCl, 0.4 mg DNAse I, and 4 mg AEBSF. The cell suspension was lysed via three passes through an Emulsiflex C3 (Avestin) homogenizer at 4°C. The lysate was centrifuged (7,000*g*, 4°C, 10 min) to remove unbroken cells and the supernatant was centrifuged at 234,000*g* for 1h at 4°C to collect membranes. The membrane pellet was resuspended in 15 mL of 50 mM Tris HCl pH 7.5 and 200 mM NaCl. Samples were mixed 1:1 with 4X LDS buffer (Invitrogen) containing 5% (v/v) β-mercaptoethanol, heated at 99°C for 20 min, and then centrifuged at 20,000*g* for 20 min. Aliquots (5 μl) of each sample were run on 4-12% Bis-Tris NuPAGE gels (Invitrogen) and transferred to PVDF membranes for immunoblotting. The membranes were blocked for 2 h in PBS containing 2% (w/v) BSA and 0.1% (v/v) Tween. An antihistidine HRP conjugated antibody (Sigma) was used to detect the C-terminal 10X histidine tag present in all of the LptD constructs. The blots were developed using SIGMA*FAST* 3,3'-diaminobenzidine solution (Sigma).

Protein	PaLptDE (5IVA)		PaLptDE + C-terminus (model)		KpLptDE(5IV8/5IV9)		YpLptDE (5IXM)		StLptDE (4N4R)		SfLptDE (4Q35)		EcLptDE (4RHB)	
Monomer	**LptE (B)**	**LptD (A)**	**LptE (B)**	**LptD (A)**	**LptE (B)**	**LptD (A)**	**LptE (H)**	**LptD (G)**	**LptE (B)**	**LptD (A)**	**LptE (B)**	**LptD (A)**	**LptE (B)**	**LptD (A)**
Nr of atoms														
in interface	292 (24.5%)	368 (7.7%)	313 (26.3%)	386 (7.9%)	289 (25.8%)	364 (8.4%)	292 (26.3%)	365 (8.6%)	315 (26.8%)	392 (9.0%)	300 (25.4%)	378 (6.2%)	307 (26.0)	396 (8.7)
on the surface	815 (68.4%)	3058 (64.3%)	813 (68.2%)	3125 (63.7%)	751 (67.1%)	2823 (65.3%)	771 (69.3%)	2795 (66.0%)	799 (67.9%)	2830 (64.8%)	780 (66.0%)	3961 (64.7%)	790 (66.9)	2888 (63.3)
Total	1192 (100.0%)	4755 (100.0%)	1192 (100.0%)	4909 (100.0%)	1119 (100.0%)	4324 (100.0%)	1112 (100.0%)	4237 (100.0%)	1176 (100.0%)	4370 (100.0%)	1181 (100.0%)	6126 (100.0%)	1181 (100.0)	4564 (100.0)
Nr of residues														
in interface	69 (45.4%)	105 (18.1%)	72 (47.4%)	111 (18.6%)	70 (47.9%)	116 (21.5%)	70 (49.6%)	111 (21.0%)	70 (46.4%)	114 (21.4%)	71 (47.3%)	117 (15.4%)	72 (48.0)	118 (21.2)
on the surface	151 (99.3%)	573 (99.0%)	149 (99.0%)	590 (98.8%)	140 (95.9%)	534 (98.9%)	141 (100.0%)	522 (98.9%)	142 (94.0%)	530 (99.6%)	145 (96.7%)	750 (98.9%)	145 (96.7)	546 (98.2)
Total	152 (100.0%)	579 (100.0%)	152 (100.0%)	597 (100.0%)	146 (100.0%)	540 (100.0%)	141 (100.0%)	528 (100.0%)	151 (100.0%)	532 (100.0%)	150 (100.0%)	758 (100.0%)	150 (100.0)	556 (100.0)
Accessible Surface area														
Buried (sqA)	2860.3 (28.0%)	2518.5 (8.1%)	3385.0 (33.1%)	3079.0 (9.9%)	3274.8 (34.9%)	3038.0 (10.4%)	3309.2 (34.7%)	3024.3 (10.5%)	3441.7 (34.7%)	3168.2 (11.0%)	3477.6 (34.8%)	3085.2 (7.8%)	3527.5 (35.2)	3168.5 (10.7)
Total (sqA)	10220.2 (100.0%)	30932.1 (100.0%)	10219.0 (100.0%)	31236.5 (100.0%)	9384.6 (100.0%)	29155.5 (100.0%)	9532.1 (100.0%)	28834.1 (100.0%)	9925.7 (100.0%)	28857.6 (100.0%)	99986.0 (100.0%)	39325.5 (100.0%)	10025.2 (100.0)	29500.9 (100.0)
Solvation E (kcal/mol)	-113.6	-423.4	-112.9	-436.4	-122.0	-362.0	-106.0	-382.1	-109.0	-378.2	-131.7	-560.1	-128.8	-386.1
H-bonds	35		29		29		29		46		40		31	
Salt bridges	17		21		21		22		42		24		19	
					core	full					core	full		
***Molec Vol A^3**	93,170		95,750		88,090	114,700	88,770		87,750		90,610	113,800	90,010	
***Molec Surf A^2**	36,310		36,650		32,500	43,830	33,460		32,900		34,280	43,650	33,410	
****Lumen Vol A^3**	-		9,695		8,972		7,710		8,030		9,380		8,290	
****Surface Area A^2**	-		4,096		4,241		3,964		4,011		3,967		3,385	

Table S2 (related to Figure 2)**: Surface area and volume analysis of known LptDE core structures.** The analysis was carried out with QtPISA (E.B. Krissinel and K. Henrick (2007) Inference of macromolecular assemblies from crystalline state, J.Mol.Biol. 372 774-797).

***Molecular volume and surface area of full-length and core complexes.** Analysis performed with UCSF Chimera (UCSF Chimera – a visualization system for exploratory research and analysis. (2004) Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenbaltt DM, Meng, EC, Ferrin TE. J Comput Chem. 13, 1605-12).

****Lumenal volume and surface area of known LptDE core complexes.** The lumenal cavities were identified and their volumes calculated with the Voss Volume Voxelator webserver (Voss, NR, Gerstein, M (2010) 3V: cavity, channel and cleft volume calculator and extractor).

Supplemental Experimental Procedures

Cloning, expression and purification of LptDE

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To make the pBAD24_LptD_WT plasmid, *E. coli* LptD (residues 1 – 784) was cloned into pBAD24 using primers pBAD24_LptD_NcoI_for2 and pBAD24_LptD_HindIII_rev2. A C-terminal 10X histidine tag was subsequently inserted into this vector using primers pBAD_LptD_chis10_for and pBAD_LptD_chis10_rev to create pBAD24_LptD_WTcHis. This vector and its mutant derivatives were used in the LptD knock-out and localization assays.

Primer sequences and names/numbers for constructs used in this study

Function:	Gene:	Name:	Sequence (5'-3'):
Expression	LptD_Yersinia	LptD_Yersinia225_pET9_for	TACTTCCAATCCATGTCTGGATTTCTGATTCCGAACGCC
	LptD_Yersinia	LptD_Yersinia_pET9_rev	TATCCACCTTTACTGTTATTAGAATGCGCTCTGATACGGCAG
	LptE_Yersinia	LptE_Yersinia_pCDF-SS_for	CTCCTAACACCTCGGGCTTTAACCTGCGTGGCAC
	LptE_Yersinia	LptE_Yersinia_pCDF-SS_rev	CATCCATCATCAATGGTGATGGTGATGGTGTTTGGCCGAGGTTGAGACC
	LptD_Klebsiella	LptD_Kleb_25f_NTT	TACTTCCAATCCATGGCCGATCTGGCAACCCAGTG
	LptD_Klebsiella	LptD_Kleb_203f_NTT	TACTTCCAATCCATGTTTAAACTGGGTAGTGTGCCGATTTTCTACAGC
	LptD_Klebsiella	LptD_Kleb_782r_NTT	TATCCACCTTTACTGTTATTACAGACTAGACTGGTACGGCAGGATATTGC
	LptE_Klebsiella	LptE_Kleb_20f	CTCCTAACACCTCGGGTTGGCATCTGCGTAGCAC
	LptE_Klebsiella	LptE_Kleb_196r	CATCCATCATCAATGGTGATGGTGATGGTGCTGGCCCAGCGTGGTG
	LptD_Pseudomonas	LptD_Pseud_301f_NTT	TACTTCCAATCCATGCTGCGTGTTAAAGATTTTCCGGTCTTCTATACC
	LptD_Pseudomonas	LptD_Pseud_924r_NTT	TATCCACCTTTACTGTTATTACATCGCCTGATCTTCGCGTTG
	LptE_Pseudomonas	LptE_Pseud_21f	CTCCTAACACCTCGGGCTTCCAACTGCGTGGTCTGG
	LptE_Pseudomonas	LptE_Pseud_207r	CATCCATCATCAATGGTGATGGTGATGGTGCGGGGTCGGAAATTCAATCGG
In vivo cloning	LptD_Escherichia	pBAD24_LptD_NcoI_for2	TCGTTACCATGGATGAAAAAACGTATCCCCACTCTCCTGG
	LptD_Escherichia	pBAD24_LptD_HindIII_rev2	ACTGTTAAGCTTTCACAAAGTGTTTTGATACGGCAGAATG
	LptD_Escherichia	lptD_Cm.F	ACCGTTTGTCACGCGCAACGTTACCGATGATGGAACAATAACCTGTGACGGAAGATCACT
	LptD_Escherichia	lptD_Cm.R	TAACCGCACTGCGGATTACGTGGTAAATCAACAAATCACATTACGCCCCGCCCTGCCACT
Mutagenesis	LptD_Escherichia	pBAD_LptD_cthis10_for	CCATCACCATCACCATCACCATTGAAAGCTTGGCTGTTTTG
	LptD_Escherichia	pBAD_LptD_cthis10_rev	TGATGGTGGCTGCCACCGCCACCCAAAGTGTTTTGATACGGC
	LptD_Escherichia	LptD_Ec_G227C	GTGACAAACGTCGCTCTTGTTTCTTGATCCCGAAC
	LptD_Escherichia	LptD_Ec_Y314C	TTCACGTCGTTGGTTATTCTGCTGGAACCACTCCG
	LptD_Escherichia	LptD_Ec_Y347C	GCTACTTCAATGATTTCGATAACAAGTGCGGTTCCAGTACTG
	LptD_Escherichia	LptD_Ec_T351C	TCGATAACAAGTACGGTTCCAGTTGTGACGGCTACGC
	LptD_Escherichia	LptD_Ec_D714C	CCAATGCTAACAAGCAAGCCTGCTCTATGTTAGGTGTGCAAT
	LptD_Escherichia	LptD_Ec_G718C	GCAAGCCGACTCTATGTTATGTGTGCAATACAGCTC
	LptD_Escherichia	LptD_Ec_I777C	AGAGATGCTGCGTTCGAACTGTCTGCCGTATCAAAACACT
	LptD_Escherichia	LptD_Ec_L758C_2	ACGCAATCGGCTTTAACATCGAATGTCGCGGCCTGAG
	LptD_Escherichia	LptD_Ec_M772C_2	GGTCTGGGTACGCAAGAGTGCCTGCGTTCGAACATTCTG
	LptD_Escherichia	LptD_p231a_f	TTTCTTGATCGCGAACGCCAAG
	LptD_Escherichia	LptD_p231a_r	CCAGAGCGACGTTTGTCAC
	LptD_Escherichia	LptD_p246a_f	GTTCTACCTGGCATATTACTG
	LptD_Escherichia	LptD_p246a_r	TCAAAGTAGTTGGTGGTG
	LptD_Escherichia	LptD_p231a_multi	GCGATGTTCCAGTAATATGCCAGGTAGAACTCAAAGTAG
	LptD_Escherichia	LptD_p246a_multi	CTCTGGTTTCTTGATCGCGAACGCCAAGTACAC
	LptD_Escherichia	LptD_r774e_f	AGAGATGCTGGAATCGAACATTCTGCCG
	LptD_Escherichia	LptD_r774e_r	TGCGTACCCAGACCGTAG
	LptD_Escherichia	LptD_r310e_f	CAGTTCACGTGAATGGTTATTCTACTGG
	LptD_Escherichia	LptD_r310e_r	TCATCGTTCGGGTGTTCATC
	LptD_Escherichia	LptD_e273q_e275q	TGGCAACATCATGTGGCAGAACCAGTTCCGCTACCTCTCCC
	LptD_Escherichia	LptD_e288q_d290n	GGGCGCTGGCTTGATGCAGCTGAACTATCTGCCTTCAGA
	LptD_Escherichia	LptD_d330n	GTGGCGTTTCAACGTCAACTACACCAAGGTCAG
	LptD_Escherichia	LptD_d342n_d344n	GCGATCCTAGCTACTTCAATAATTTCAATAACAAGTACGGTTCCAGT
	LptD_Escherichia	LptD_d352n	ATAACAAGTACGGTTCCAGTACTAACGGCTACGCAA
	LptD_Escherichia	LptD_d749n	GATAACGATAAACAACATGCGGTATATAACAACGCAATCGGC

	LptD_Escherichia	LptD_e273k_e275k	GTGGCAACATCATGTGGAAGAACAAATTCCGCTACCTCTCC
	LptD_Escherichia	LptD_e288k_d290k	GCGGGCGCTGGCTTGATGAAACTGAAGTATCTGCCTTCAGATAAA
	LptD_Escherichia	LptD_d330k	GTGGCGTTTCAACGTCAAGTACACCAAGGTCAGCG
	LptD_Escherichia	LptD_d342k_d344k	GGTCAGCGATCCTAGCTACTTCAATAAGTTCAAGAACAAGTACGGTTCCAGTACTGAC
	LptD_Escherichia	LptD_d352k	CGATAACAAGTACGGTTCCAGTACTAAGGGCTACGCAACGC
	LptD_Escherichia	LptD_d749k	GGGATAACGATAAACAACATGCGGTATATAAGAACGCAATCGGCTTTA

For purification, cells were resuspended in lysis buffer (50 mM Tris-HCl pH 7.5, 200 mM NaCl, 1 mM $MgCl_2$, 10 μg/ml DNaseI, 100 μg/ml 4-(2-aminoethyl)benzenesulphonyl fluoride (AEBSF)) and lysed by three passages through an Emulsiflex C3 (Avestin) homogenizer at 4°C. The lysate was centrifuged at 10,000*g* for 10 min to remove unlysed cells and the supernatant was incubated with 2% (v/v) Triton X-100 for 30 min at 4°C. Membranes were isolated from the lysate by centrifugation at 234,000*g* for 60 min at 4°C. The pellet was resuspended in buffer containing 50 mM Tris-HCl pH 7.5, 200 mM NaCl, and 20 mM imidazole and solubilized by constant stirring in 5% (w/v) Elugent (Millipore Merck) for 16 h at 4°C. Insolubile material was removed by centrifugation at 370,000*g* for 60 min at 4°C. The supernatant was filtered and applied to a 15-ml Ni-NTA column (Qiagen) equilibrated in 50 mM K_2HPO_4 pH 7.5, 200 mM NaCl, 10% (v/v) glycerol, 20 mM imidazole, and 0.1% (w/v) dodecyl maltoside (DDM) (Anatrace). Protein was eluted with 250 mM imidazole and peak fractions were pooled and dialysed against 25 mM Tris-HCl pH 8.0 containing 0.6 mM EDTA pH 8.0 for 16 h at 4°C. The sample was applied to a 10 mL Q-Sepharose column (GE Healthcare) equilibrated with 25 mM Tris HCl pH 8.0, 0.6 mM EDTA, and 0.1% DDM and eluted with a linear gradient of 0-0.6 M NaCl . Peak fractions were then concentrated and applied to a S-300HR Sephacryl size exclusion column (GE Healthcare) equilibrated in 20 mM Tris-HCl pH 7.5, 200 mM NaCl, and 0.8% C_8E_4.

ΔlptD strain and Phage lysate generation

First, pBAD24_LptD_WT was electroporated into *E. coli* strain NM1100 containing a chromosomal lambda Red system (Bougdour et al., 2008) and plated on LB agar containing 50 μg/ml ampicillin. After O/N incubation at 32°C, a single transformant was grown in LB broth containing 50 μg/ml ampicillin at 32°C to an OD_{600} of 0.6. These cultures were then placed in a shaking 42°C water bath for 15 min to induce lambda gene expression and then immediately cooled in an ice-water slurry. Cells were pelleted at 4°C and washed three times with ice-cold ultrapure water. Final pellets were resuspended in 1/100 of the original culture volume in ice-cold ultrapure water containing 10% (v/v) glycerol and either used immediately or frozen. In parallel, the chloramphenicol resistance cassette from pCAT19 (Fuqua, 1992) was PCR amplified using primers lptD_Cm.F and lptD_Cm.R. These primers contain 5' overhangs incorporating 40 bp of complementary sequence upstream and downstream of the *lptD* gene. The PCR product was electroporated into NM100 containing pBAD24_LptD_WT prepared as described above. After re-suspension in 1 ml of LB broth and recovery at 37°C for 1 hour, cells were spread on LB plates containing 50 μg/ml ampicillin, 10 μg/ml chloramphenicol, and 1% (w/v) arabinose. The plates were incubated O/N at 37°C to select for colonies carrying the chromosomal *lptD* deletion and pBAD24_LptD_WT. The chromosomal insertion of the chloramphenicol cassette at the *lptD* locus was verified by PCR.

The Δ*lptD* cells were grown at 37°C in LB medium containing 50 μg/ml ampicillin, 1% (w/v) arabinose, 0.01 M $MgSO_4$, and 0.005 M $CaCl_2$. At an OD_{600} of ~ 0.1-0.2 P1vir phage was added and the culture was grown 4 h at 37°C or until complete lysis was observed. A couple of drops of chloroform were added to lyse any remaining cells and the culture was centrifuged to remove debris. The supernatant was collected, two additional drops of chloroform were added, and the sample was stored at 4°C.

LptD localization assay

Glycerol stocks of MG1655 *E. coli* cells containing pBAD24_LptD_WTcHis and mutant variants were inoculated into 500 mL TB medium containing 50 μg/ml ampicillin and 0.2% (w/v) arabinose. Cultures were grown overnight at 37°C and harvested. Cell pellets were resuspended in 40 mL of buffer containing 50 mM Tris HCl pH 7.5, 200 mM NaCl, 0.4 mg DNAse I, and 4 mg AEBSF. The cell suspension was lysed via three passes through an Emulsiflex C3 (Avestin) homogenizer at 4°C. The lysate was centrifuged (7,000*g*, 4°C, 10 min) to remove unbroken cells and the

supernatant was centrifuged at 234,000*g* for 1h at 4°C to collect membranes. The membrane pellet was resuspended in 15 mL of 50 mM Tris HCl pH 7.5 and 200 mM NaCl. Samples were mixed 1:1 with 4X LDS buffer (Invitrogen) containing 5% (v/v) β-mercaptoethanol, heated at 99°C for 20 min, and then centrifuged at 20,000*g* for 20 min. Aliquots (5 μl) of each sample were run on 4-12% Bis-Tris NuPAGE gels (Invitrogen) and transferred to PVDF membranes for immunoblotting. The membranes were blocked for 2 h in PBS containing 2% (w/v) BSA and 0.1% (v/v) Tween. An antihistidine HRP conjugated antibody (Sigma) was used to detect the C-terminal 10X histidine tag present in all of the LptD constructs. The blots were developed using SIGMA*FAST* 3,3'-diaminobenzidine solution (Sigma).