Supplementary Materials for

Disulfide Rearrangement Triggered by Translocon Assembly Controls Lipopolysaccharide Export

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Materials and Methods

Bacterial strains and growth conditions

For most experiments, the wild-type strain used is MC4100 [*F⁻ araD139 Δ(argF-lac) U169 rpsL150 relA1 flbB5301 ptsF25 deoC1 ptsF25 thi*]. The LptE-limiting strain used that makes varying levels of LptE according to growth phase is AM689 [MC4100 *ara*$^{+}$ *lptE::kan* λ_{att}*(*P_{BAD}*-lptE)*] (*4*). The *lptD*$_{\Delta 330\text{-}352}$ (*lptD4213*), *lptE*$_{\Delta 100\text{-}101/P99R}$ (*lptE6*) and *lptD*$_{\Delta 529\text{-}538}$ mutant strains used are NR698 (*14*), GC190 [MC4100 *ara*$^{+}$ *ΔlptE2::kan* pBAD18*lptE6*] (*15*) and MC4100 *ΔlptD2::kan* pET23/42*lptDΔ529-538* (*6*), respectively. The *ΔdsbA* and *ΔdsbC* strains used are NR1216 and NR1217 (*8*), respectively. For experiments involving the *dsbA*$_{P151T}$ mutant, the wild-type strain used is HK295, a MC1000 derivative [*F⁻ Δara714 galU galK Δ(lac)X74 rpsL thi*] and the *dsbA*$_{P151T}$ mutant used is HK348 [HK295 *zin::Tn10 dsbA*$_{P151T}$] (*16*). Luria-Bertani (LB) broth and M63/glucose minimal broth and agar were prepared as described previously (*21*). Arabinose (0.2% w/v) was added for the growth of AM689 (*4*) and GC190 (*15*). Growth of strains was carried out at 37 ^{o}C unless explicitly indicated. When appropriate, kanamycin (25 μg/ml) and carbenicillin (50 μg/ml) were added. Amino acids were added at 50 μg/ml when indicated.

Plasmid construction

To construct pET23/42*lptD-FLAG*$_3$, a cassette containing the coding sequence of the $FLAG_3$ tag was inserted into pET23/42*lptD-His* (*4*) to replace the original His_8 tag. Briefly, the entire pET23/42*lptD-His* template was amplified by PCR (using primers 5'-AGATCATGATATCGACTATAAAGACGATGATGACAAATAATTGATTAATACC TAGGCTGC-3' and 5'-ATAGTCGATATCATGATCTTTGTAGTCGCCGTCGTGATC TTTATAATCGCGCGCCAAGGC-3') and the resulting PCR product mixture digested with DpnI for > 1 h at 37 ^{o}C. NovaBlue (Novagen) cells were transformed with 1 μl of digested PCR product and plated onto LB plates containing 50 μg/ml carbenicillin. For each construct, plasmids from six colonies were isolated and sequenced.

To generate LptD Cys mutant constructs containing the $FLAG_3$ tag, pET23/42*lptD*$_{XXXX}$*-His* constructs were first made via site-directed mutagenesis using relevant primers (*8*) and pET23/42*lptD-His* (*4*) as the initial template. Briefly, the entire template was amplified by PCR and the resulting PCR product mixture digested with DpnI for > 1 h at 37 ^{o}C. NovaBlue (Novagen) cells were transformed with 1 μl of digested PCR product and plated onto LB plates containing 50 μg/ml carbenicillin. For each construct, plasmids from six colonies were isolated and sequenced. The resulting pET23/42*lptD*$_{XXXX}$*-His* constructs were used in the same protocol above to generate pET23/42*lptD*$_{XXXX}$*-FLAG*$_3$ constructs.

Growth of AM689 for OM analysis

10-ml cultures were grown overnight at 30 ^{o}C in LB broth containing 0.2% arabinose. Cultures were pelleted and washed twice in equal volume of LB broth. Fresh LB cultures (1.5-l) either containing 0.2% arabinose were inoculated with the washed cells to an initial OD_{600} of ~0.01 and were grown at 30 ^{o}C until OD_{600} reaches ~0.25 (~4 h) and ~0.5 (~5 h). The amount of cells equivalent to that in a 500-ml culture of OD_{600}

~0.5 was pelleted by centrifugation at 5000 x *g* for 20 min and subjected to OM analysis (see below).

Isolation of OM for analysis of LptD oxidation states

Strains MC4100, AM689, NR1216, NR1217, NR698, GC190 and MC4100 *ΔlptD2::kan* pET23/42*lptDΔ529-538* were used for OM analysis. These strains contain a single copy of *lptD* expressed from the chromosome or plasmid. Strains MC4100 containing p($lptD_{CCCC}$) or p($lptD_{CCSS}$) (pET23/42*lptD* or pET23/42$lptD_{CCSS}$, respectively (*8*)), MC4100, NR1216, HK295 and HK348 containing pET23/42*lptD-FLAG*$_3$ were also used for OM analysis experiments. These strains contain two copies of *lptD*, one expressed from the chromosome and the other expressed from pET23/42. OM analysis is performed as previously described (*8*). Briefly, cells were pelleted by centrifugation at 5000 x *g* for 20 min and then resuspended in 5 ml Tris-B buffer (10 mM Tris.HCl, pH 8.0) containing 20% (w/w) sucrose, 1 mM phenylmethylsulfonyl fluoride (Sigma), 50 µg/ml DNase I (Sigma) and 50 mM iodoacetamide (IAM, Sigma). Cells were lysed by a single passage through a French Press (Thermo Electron) at 8,000 psi. ~8 ml of cell lysate was layered onto a two-step sucrose gradient (top – 4 ml Tris-B buffer containing 40% (w/w) sucrose, bottom – 1 ml Tris-B buffer containing 65% (w/w) sucrose) and centrifuged at 39,000 rpm for 16 h in an Beckman SW41 rotor in an ultracentrifuge (Model XL-90, Beckman). OM fragments (~0.5 ml) were isolated from the 40%/65% interface by puncturing the side of the tube with a syringe. 1 ml of 20 mM Tris.HCl, pH 8.0 was added to the OM fragments to lower the sucrose concentration to below 20% (w/w). The OM fragments were then pelleted in a microcentrifuge at 18,000 x *g* for 30 min and then resuspended in 200-250 µl TBS containing 5 mM IAM. Protein concentration of these OM preparations were determined using Bio-Rad D_C protein assay after precipitating in 10% TCA and resolubilizing in TBS containing 2% SDS. The same amount of OM (based on protein content) for each strain was analyzed by non-reducing SDS-PAGE and immunoblotted using antibodies directed against LptD and LptE.

Pulse-chase analysis

Strains MC4100, NR1216, HK295 and HK348 containing pET23/42*lptD-FLAG*$_3$ were used in pulse-chase experiments. Pulse-chase experiments were essentially carried out according to published protocols (*18*). Briefly, a 5-ml culture was grown to OD_{600} ~0.5 in M63/glucose minimal media supplemented with eighteen amino acids (minus methionine and cysteine) at 37 ^{o}C. The culture was pulse-labeled with [^{35}S]-methionine (100 µCi/ml final concentration) (American Radiolabeled Chemicals) for 2 min and then chased with cold methionine (5 mM) at 37 ^{o}C. At the indicated time point during the chase, a 800 µl culture aliquot was transferred to a 1.5-ml tube containing 80 µl of trichloroacetic acid (TCA, 70% in water), incubated on ice for 20 min. Precipitated proteins were pelleted at 18,000 x *g* for 10 min at 4 ^{o}C, washed with 700 µl ice-cold acetone, and then solubilized in 80 µl 100 mM Tris.HCl, pH 8.0 containing 1% SDS and 20 mM *N*-ethylmaleimide (NEM, Sigma). The sample was sonicated for 30 s to aid solubilization. Following that, 800 µl of ice-cold IP buffer (50 mM Tris.HCl, pH 8.0 containing 150 mM NaCl, 2% Triton X-100, 1 mM EDTA) was added and the sample was centrifuged at 18,000 x *g* for 10 min at 4 ^{o}C. 700 µl of the supernatant was transferred to another 1.5-ml tube containing 2.5 µl of anti-FLAG® M2 magnetic beads

(Sigma). The beads were washed and pre-equilibrated with 3 x 1 ml IP buffer before use. The mixture was incubated on a rotary shaker for 1 h at 4 ^{o}C, and the beads were washed with 3 x 800 µl of ice-cold high salt buffer (50 mM Tris.HCl, pH 8.0 containing 1 M NaCl, 1% Triton X-100, 1 mM EDTA) and 1 x 800 µl ice-cold 10 mM Tris.HCl, pH 8.0 using a magnetic separation rack (New Englands Biolabs). 60 µl 2X SDS non-reducing sample buffer was then added to the beads and the mixture heated for 10 min at 100 ^{o}C to elute the bound proteins. 15 µl of eluted sample was applied to SDS-PAGE directly. For reduction of disulfide bonds, 0.5 µl β-mercaptoethanol (β-ME, Sigma) was added to 20 µl eluted sample and heated for 5 min at 100 ^{o}C before loading. 4-20% Tris.HCl polyacrylamide gels were used (running conditions: 150 V for 120 min). The gel was then dried and exposed to phosphor storage screens for autoradiography.

Seminative pulse-chase analysis

Strain MC4100 containing pET23/42*lptD-FLAG$_3$* was used in seminative pulse-chase experiments. A 5-ml culture was grown to OD_{600} ~0.5 in M63/glucose minimal media supplemented with eighteen amino acids (minus methionine and cysteine) at 30 ^{o}C. The culture was pulse-labeled with ^{35}S-methionine (100 µCi/ml final concentration) (American Radiolabeled Chemicals) for 2 min and then chased with cold methionine (5 mM) at 37 ^{o}C. At the indicated time point during the chase, a 800 µl culture aliquot was transferred to a 1.5-ml tube and pelleted at 18,000 x *g* for 1 min at 4 ^{o}C. The cell pellet was resuspended in 100 µl lysis buffer (20 mM Tris.HCl, pH 8.0 containing 150 mM NaCl, 1% SDS, 0.5 mg/ml lysozyme, 1 mM phenylmethylenesulfonylfluoride (PMSF, Sigma), 1 mM EDTA and 40 mM NEM). After incubated for 2.5 min at room temperature, 1 ml of ice-cold IP-2 buffer (50 mM Tris.HCl, pH 8.0 containing 150 mM NaCl, 2% n-octyl-β-glucoside (OG, Anatrace), 1 mM EDTA) was added and the sample was centrifuged at 18,000 x *g* for 30 min at 4 ^{o}C. 950 µl of the supernatant was transferred to another 1.5-ml tube containing 2.5 µl of anti-FLAG® M2 magnetic beads (Sigma). The beads were washed and pre-equilibrated with 3 x 1 ml IP-2 buffer before use. The mixture was incubated on a rotary shaker for 2 h at 4 ^{o}C, and the beads were washed with 4 x 800 µl of ice-cold wash buffer (50 mM Tris.HCl, pH 8.0 containing 1 M NaCl, 2% OG, 1 mM EDTA) using a magnetic separation rack (New Englands Biolabs). 30 µl of ice-cold elution buffer (50 mM Tris.HCl, pH 8.0 containing 150 mM NaCl, 2% OG, 1 mM EDTA, 250 µg/ml $FLAG_3$ peptide (Sigma)) was then added to the beads and the mixture incubated for 10 min at 4 ^{o}C to elute the bound proteins. An equal volume of 2X SDS non-reducing sample buffer was added and the sample split into two – one applied to seminative SDS-PAGE directly and the other heated for 10 min at 100 ^{o}C before loading. 10% Tris.HCl polyacrylamide gels were used (Running conditions: 150 V for 75 min, 4 ^{o}C). The gel was then dried and exposed to phosphor storage screens for autoradiography.

Trypsin digestion following seminative pulse-chase experiment

Trypsin digestion was performed according to published protocols (*5*). Samples that were eluted off the M2 magnetic beads were treated with 50 µg/ml trypsin for 4 h at room temperature. An equal volume of 2X SDS non-reducing sample buffer was added and the samples heated for 10 min at 100 ^{o}C before SDS-PAGE. 4-20% Tris.HCl polyacrylamide gels were used (running conditions: 150 V for 120 min). The gel was then dried and exposed to phosphor storage screens for autoradiography.

Bioinformatics

The list of non-identical LptD sequences was collected by BLAST. Using the *E. coli* LptD sequence as a start, an initial BLAST hit list was collected. The weak hits were then used as the new reference sequence to BLAST search for more LptD sequences. By doing this recursively for over 20 rounds, thousands of LptD sequences were collected. This initial list was then filtered to remove identical sequences and partial genes (usually containing deletions on one or the other end of the gene). The final list contains 1056 non-identical LptD sequences.

Antibodies

Monoclonal α-His conjugated to horseradish peroxidase was purchased from Qiagen. Monoclonal α-FLAG conjugated to horseradish peroxidase was purchased from Sigma. α-LptD (*12*), α-LptE (*5*) and α-DsbA (*22*) antisera were already described.

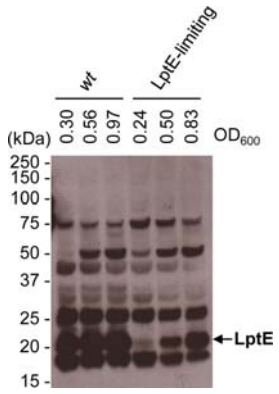

Fig. S1

A previously-reported LptE-limiting strain (*5*) makes different levels of LptE protein (lower than the wild-type strain) at various stages of growth even under non-depletion conditions. α-LptE immunoblot analysis of whole cell lysates obtained from wild-type (*wt*) or the LptE-limiting strain. Cells were sampled at early-, mid- and late-log phase (OD_{600} ~ 0.25, 0.5 and 0.9 respectively). Positions of relevant molecular weight markers are indicated in kDa.

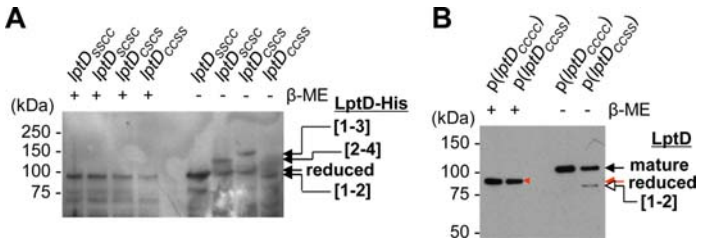

Fig. S2

The $LptD_{CCSS}$ double cysteine-less mutant protein migrates slightly faster than reduced LptD during non-reducing SDS-PAGE. Like wild-type LptD ($LptD_{CCCC}$), the other LptD mutants lacking two cysteines but are able to make disulfide bonds between the N- and C-terminal domains (e.g. $LptD_{SCSC}$ and $LptD_{CSCS}$) display slower mobility than reduced LptD on a gel (*8*). The mutant lacking the first two cysteines ($LptD_{SSCC}$), a protein containing only one disulfide between adjacent residues Cys_{724} and Cys_{725} (**[3-4]**) in the C-terminus, runs at the same position as reduced LptD. (*A*) α-His immunoblot analysis of OM fragments obtained from wild-type cells expressing $LptD_{SSCC}$-His, $LptD_{SCSC}$-His, $LptD_{CSCS}$-His or $LptD_{CCSS}$-His (*8*). (*B*) α-LptD immunoblot analysis of OM fragments obtained from *wt* cells expressing a second copy of LptD from a plasmid (p(*lptD*$_{CCCC}$) or p(*lptD*$_{CCSS}$)). Where indicated, β-mercaptoethanol (β-ME) was used to reduce disulfide bonds.

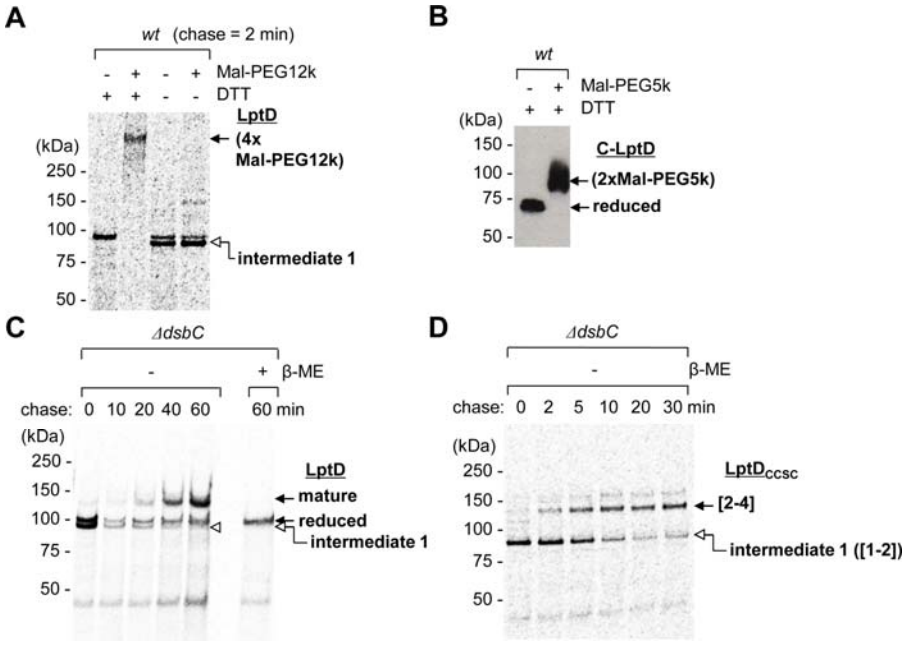

Fig. S3

A disulfide bond between Cys_{724} and Cys_{725} may be formed in intermediate **1**. Whether the **[3-4]** bond exists, however, does not affect the proposed pathway for LptD assembly (since subsequent reduction of this disulfide bond is a prerequisite for rearrangement to **[2-4]**-LptD; see Fig. 3C). (*A*) Maleimidyl-PEG (Mal-PEG12k) alkylation of [^{35}S]-labeled intermediate **1** in a wild-type background (from Fig. 2A). Intermediate **1** could not be chemically modified by Mal-PEG12k unless it had been pre-treated with reducing agent dithiothreitol (DTT), suggesting that the **[3-4]** disulfide bond may be formed in addition to the **[1-2]** bond. (*B*) Maleimidyl-PEG (Mal-PEG5k) alkylation of the C-terminal domain of LptD (C-LptD-$FLAG_3$) in a wild-type background. The C-LptD protein could be modified by Mal-PEG5k when pre-treated with DTT, indicating that the inability to modify Cys_{724} and Cys_{725} in intermediate **1** in (*A*) was not because these cysteine residues are sterically inaccessible. (*C*) [^{35}S]-Met pulse-chase of newly-synthesized LptD-$FLAG_3$ in a *ΔdsbC* background. Removing DsbC significantly slowed down the rate of disulfide bond rearrangement from intermediate **1** to the mature form of wild-type LptD (compare to Fig. 2A), suggesting that DsbC can act as a reductant during LptD assembly. (*D*) [^{35}S]-Met pulse-chase of newly-synthesized $LptD_{CCSC}$-$FLAG_3$ in a *ΔdsbC* background. In the absence of DsbC, disulfide bond rearrangement from the **[1-2]**-$LptD_{CCSC}$ species to the **[2-4]**-$LptD_{CCSC}$ species occurred at a rate comparable to the rearrangement in the presence of DsbC for wild-type $LptD_{CCCC}$ (compare to Fig. 2A). Because the $LptD_{CCSC}$ does not (cannot) contain the **[3-4]** bond, these data suggest that DsbC may be involved in reducing that disulfide bond in wild-type intermediate **1** (likely **[1-2][3-4]**-LptD) in order to allow rearrangement to take place. Where indicated, β-mercaptoethanol (β-ME) or DTT were used to reduce disulfide bonds.

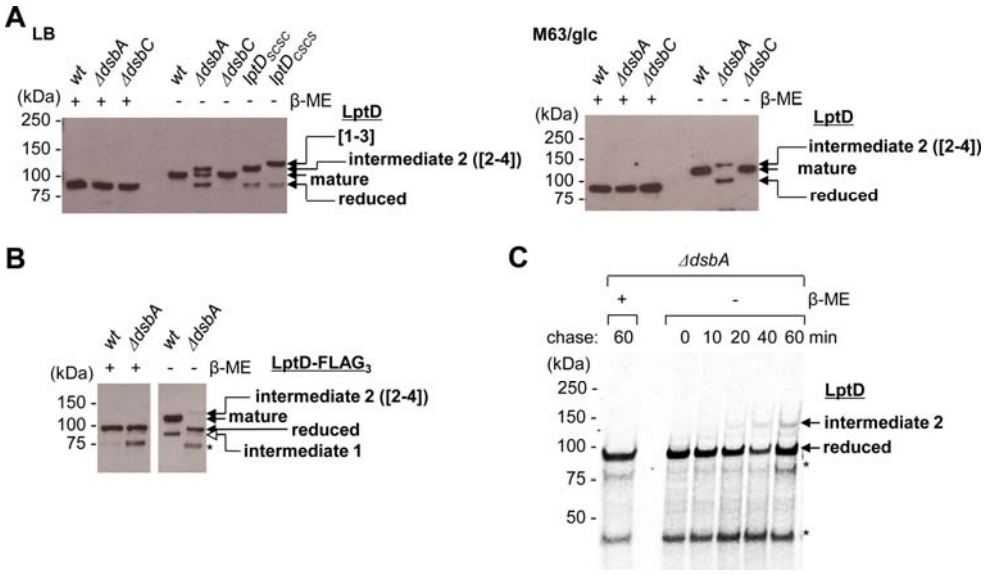

Fig. S4

DsbA is required for the formation of intermediate **1** (**[1-2]**-LptD), and thus the efficient assembly of mature LptD. Without formation of the **[1-2]**-LptD intermediate, the pathway to form mature **[1-3][2-4]**-LptD is extremely inefficient. (*A*) α-LptD immunoblot analysis of OM fragments obtained from *wt*, *ΔdsbA* and *ΔdsbC* cells grown in rich (LB) or minimal medium (M63/glc). OM fragments from $lptD_{SCSC}$ and $lptD_{CSCS}$ (*8*) were loaded as position markers for LptD species containing either the Cys_{173}-Cys_{725} (**[2-4]**) or Cys_{31}-Cys_{724} (**[1-3]**) disulfide bonds, respectively. (*B*) α-FLAG immunoblot analysis of OM fragments obtained from *wt* and *ΔdsbA* strains containing pET23/42*lptD-FLAG*$_3$ grown in minimal media. (*C*) [^{35}S]-Met pulse-chase of newly-synthesized LptD-$FLAG_3$ in a *ΔdsbA* background. Bands denoted by asterisks represent LptD degradation products.

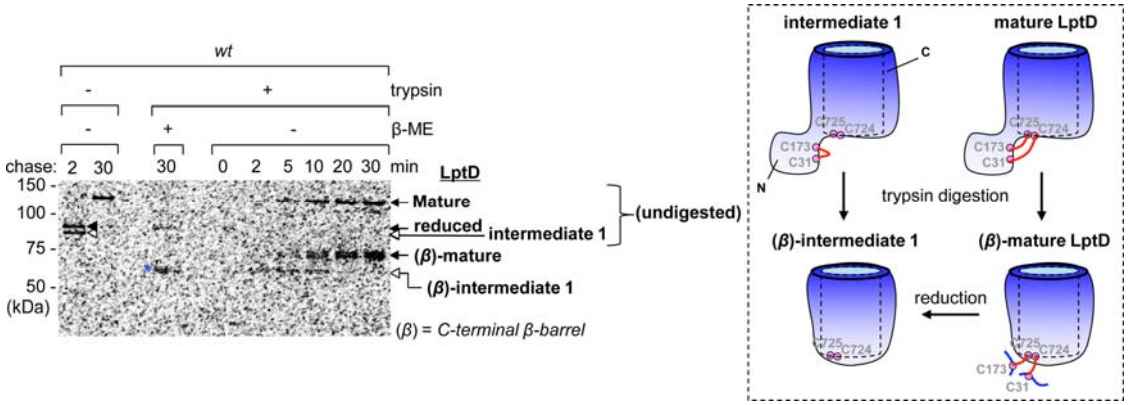

Fig. S5

Folding of the LptD β-barrel domain is slow and precedes disulfide bond rearrangement. Samples from Fig. 2B were subjected to limited trypsin digestion and analyzed by denaturing SDS-PAGE. A stable fragment resistant to trypsin digestion appeared early during the chase, and disappeared with the concomitant appearance of a second slightly larger trypsin-stable fragment. Trypsin digestion of purified mature LptD/E complex showed that a fragment that corresponds to the folded β-barrel of LptD is stable (*5*); thus, the larger trypsin-stable fragment was obtained from [^{35}S]-pulse-labeled mature LptD and corresponded to the folded β-barrel of LptD ((*β*)-mature) containing disulfide cross-linked tryptic peptides (through **[1-3]** and **[2-4]**, see schematic on the right). The smaller trypsin-stable fragment was identified as the folded β-barrel of intermediate **1** ((*β*)-intermediate **1**) obtained from [^{35}S]-pulse-labeled LptD intermediate **1** by comparing the gel mobility of this species to the mobility of the reduced β-barrel band (denoted by asterisk) obtained from reduction of the β-barrel of mature LptD. The time-dependent appearance of the two trypsin-stable fragments is consistent with formation first of a folded intermediate **1** (giving rise to a trypsin-resistant (*β*)-intermediate **1**) that is then converted to folded mature LptD (giving rise to a trypsin-resistant (*β*)-mature) via disulfide bond rearrangement. Graphical representations of LptD species before or after trypsin digestion are shown on the right.

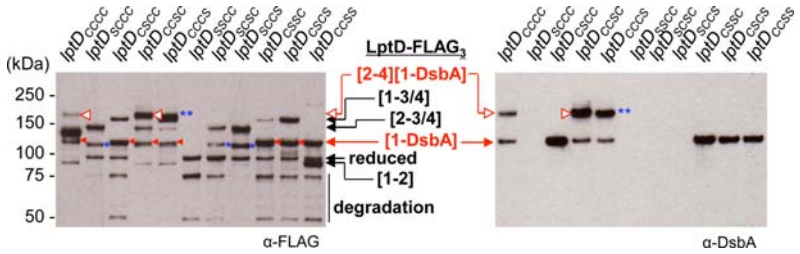

Fig. S6

The *dsbA*$_{P151T}$ mutation results in the accumulation of mixed-disulfide adducts between DsbA and Cys$_{31}$ of LptD. α-FLAG immunoprecipitation using *dsbA*$_{P151T}$ cells containing pET23/42*lptD-FLAG*$_3$ or single/double Cys mutants. Samples were analyzed by α-FLAG and α-DsbA immunoblot under non-reducing conditions. All LptD Cys mutant proteins that contain Cys$_{31}$ could be trapped in an intermediate with DsbA (**[1-DsbA]**-LptD, closed red arrowheads). The LptD$_{CCSC}$ and LptD$_{CCCS}$ proteins were also trapped in a second DsbA adduct via Cys$_{31}$. LptD$_{CCSC}$ exists predominantly as **[2-4]**-LptD in a wild-type *dsbA* background (*8*), which became cross-linked to DsbA in a *dsbA*$_{P151T}$ background (**[2-4][1-DsbA]**-LptD, open red arrowhead). In addition to **[1-3]**-LptD, LptD$_{CCCS}$ also exists as **[2-3]**-LptD in a wild-type DsbA background (*8*), which became cross-linked to DsbA in a *dsbA*$_{P151T}$ background (**[2-3][1-DsbA]**-LptD, double asterisks). Single asterisks denote degradation products from LptD containing one disulfide bond.

A

Cysteine motifs in LptD	Cys_{31}	Cys_{173}	Motifs at $Cys_{724/5}$	
			CysCys/ CysXaaCys	Single Cys
Present	236	1028	491	526
Missing	820	28	39	
Total	1056			

B

Organisms	Cys_{31}	Cys_{173}	$Cys_{724/5}$
Candidatus Solibacter usitatus	ILAVSAAAQVN	GFVTNCK---MPQ	VQYNTN--CCGF--SV
Campylobacter jeluni	------FMLGT	SAVSSCN---VED	YTYQRK--CWNY--SL
Helicobacter pylori	-----GALDAK	MSASGCS---IDN	ANYQRK--FLSF--NL
Candidatus Liberibacter solanacearum	--ETSSNKKVI	GTYTACAKCVQSP	LSYQND--CTTF--NI
Mariprofundus ferrooxydans	-----MLLLLH	VLYSTCP---ADE	LQYKHP--CWTA--GV
Candidatus Odyssella thessalonicensis	---SASVNAQP	GVYSPCDVCKADP	LRHTNE--CLVT--TF
Oceanibulbus indolifex	------FALCVA	AAVTSCKVCEDGE	LSYNNE--CVQV--DL
Asticcacaulis excentricus	FFADEIDLTAP	ALFTPCDLCVKNN	VIYKDD--CARF--EL
Sphingomonas sp.	--AQQLNEPQT	AAYTGCSV--EDS	IAYEDD--CLTL--GF
Roseomonas cervicalis	------F-----	VLYSACDLCAEDP	LTYEDE--CLIF--DT
Francisella cf._novicida	KALADDLAWVK	GYITSGD---PYD	LQYNAK--SWAV--RA
Marinomonas sp.	--SATELDWFP	GFYTTCE---PGS	IGFENC--CVKA--QF
Candidatus Blochmannia floridanus	DNSHKYSDHAP	GKFSTCT---IDN	IQYFTP--CWIF--SI
Acinetobacter sp.	IKEAYPGQEFF	ATYTTCP---PGQ	VNYESC--CWGI--SV
Escherichia coli	**--ASQCMLGVP**	**GSFTSCL---PGS**	**VQYSSC--CYAI--RV**
Pseudomonas aeruginosa	SADYSHLDWIP	GTYTRCE---PSS	FEYDSC--CWKL--RL
Polynucleobacter necessarius	SANTVLLPDRG	ATYSTCT---PQN	LEWNRD--CWTF--RG
Lautropia mirabilis	----MPLQLAP	GSFTSCK---PDD	VEYARD--CWAV--RV
Bdellovibrio bacteriovorus	------TVMSS	ADYTACT---NCP	AQFKRPGDCMMI--TF
Thermodesulfovibrio yellowstonii	-----FFIARI	ATFSTCE---PEP	IKYTQS--CWAT--NI
uncultured_Desulfobacterales bacterium	SNESLSMPQAL	GHFTTCE---GIL	ILYDNPCKCWGF--NL
Sulfurihydrogeninium yellowstonense	------F-----	GEFSGCP---FDQ	FDWNRG--CWSL--SF
Hydrogenobacter thermophilus	-----FMILSA	GDITTCP---PDK	LDYTGA--CWSL--GV
Thermovibrio ammonificans	--MRIGLPTVT	GEYTPCS---HSC	LTVNRG--CWSG--VL
Calditerrivibrio nitroreducens	------FLLQP	ATISACS---GDV	ILYKSE--CWNL--GF
Hippea maritima	------FLLFI	GIITTCK---CQD	FLYQED--CWGI--GL
Geobacter metallireducens	------FAPVFL	GTFTTCD---AER	VEYRHQ--CWSV--SV
Thermodesulfatator indicus	------FILVCP	TTITTCDICKNGK	FEYRTK--CWWG--VI
Desulfobacca acetoxidans	--WAEEVEIFK	CVVTTCD---ADR	LIFQRQ--CWGV--SL
Desulfobulbus propionicus	RYENPPRIEAE	GWVVTCKLHPGEV	LRYIQP--CWSV--EV
Desulfatibacillum alkenivorans	--WALTVLLFA	ASLTTCD---PDD	LIYEAA--CWAI--DL
Desulfovibrio sp.	--GGAHAQQPQ	AKITVCD---GDT	IIYNHQ--CFKVIGRT

Fig. S7

Cys_{173} and Cys_{725} are very highly conserved among LptD homologs. (*A*) Conservation of the four cysteine residues in *E. coli* LptD across 1056 LptD homologs that are non-identical. (*B*) Sequence alignments of 32 LptD homologs that are <30% identical depicting only the regions around the cysteine residues in *E. coli* LptD. *E. coli* LptD is shown in green.

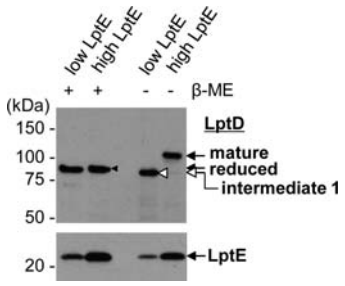

Fig. S8

Increasing LptE levels in cells results in conversion of intermediate **1** accumulated under LptE-limiting conditions to mature LptD. α-LptD and α-LptE immunoblot analyses of OM fragments obtained from cells that express different levels of LptE according to the growth phase. OM fragments were isolated from cells grown to early-log phase (low LptE) and mid-log phase (high LptE) (see Fig. S1).