Supporting Information

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SI Results

Conservation of Glu^{373}. Alignment of BamA P5 sequences from a diverse array of Gram-negative species highlights Glu^{373} as one of several particularly well-conserved residues within the polypeptide transport-associated (POTRA) P5 domain (Fig. S1). It is unlikely that this residue is critical for the POTRA fold, because glutamate at this position within the POTRA domain is not conserved in the POTRA consensus sequence (Fig. S1). Additionally, Omp85 homologs involved in related but distinct processes, such as the hemagglutinin transporter FhaC from *Bordetella pertussis* (Fig. S1), do not demonstrate sequence conservation at the position analogous to Glu^{373} in their β-barrel-proximal POTRA domains. These findings indicate this residue is critically important specifically in the structure or function of the BamA P5 domain.

Stability of BamA^{E373K}. The stability of BamA^{E373K} is examined most easily in strains carrying the *bamDR197L* suppressor because these strains are haploid. When these strains are lysed in sample buffer, displayed on SDS/PAGE, and then detected by Western blot, BamA^{E373K} in the suppressor strain is as stable as BamA in a wild-type strain (compare the leftmost and rightmost lanes in Fig. 1*B*, *Right*). However, when BamA is purified from cell extracts, some proteolytic cleavage (which releases an N-terminal fragment) occurs (Fig. 2*A*, *Right*).

In diploid strains in which wild-type *bamA* is provided *in trans*, the wild-type and mutant proteins cannot be distinguished simply (Fig. 2*A*, *Left*). However, the same level of proteolysis is observed, as evidenced by the released N-terminal fragment (Fig. 2*A*, *Right*). In other words, the *bamDR197L* suppressor has no effect on the stability of BamA^{E373K}.

A Complex Containing BamA^{E373K} Cannot Be Stabilized by Crosslinking. The periplasmic outer membrane protein chaperone SurA interacts transiently with BamA. Although SurA does not copurify with the β-barrel assembly machine (Bam) complex under native conditions, addition of the crosslinker dithiobis(succinimidyl)propionate (DSP) stabilizes this interaction and permits copurification of BamA and SurA (Fig. S4). Indeed, SurA can be crosslinked to both BamA^{WT} and BamA^{E373K} to a similar extent, suggesting that the interaction between BamA^{E373K} and SurA occurs normally. However, the interaction between BamA^{E373K}B and BamCDE is not stabilized by DSP, because the amount of copurified holocomplex is not increased in the presence of crosslinker in either a *bamD*$^{+}$ or *bamDR197L* background (Fig. S4). This finding argues that the BamA–BamD interaction that is disrupted by the E373K mutation is not restored by *bamDR197L*.

SI Materials and Methods

In Vivo Crosslinking of the Bam Complex. Strains DPR821, DPR822, and DPR990 were grown in 200 mL of LB medium supplemented with 125 μg/mL ampicillin to OD_{600} = ∼0.8. Cells were harvested by centrifugation (5,000 × *g*, 10 min). Cell pellets were washed in 20 mL of 20 mM KH_2PO_4 (pH 7.2) and 150 mM NaCl, resuspended in 10 mL of same buffer, and incubated with rocking for 15 min at 37 °C. DSP dissolved in DMSO was added to the cell suspension at a final concentration of 80 μg/mL, and the cells were incubated with rocking for 30 min at 37 °C. The reaction was quenched by addition of 1 M Tris·HCl (pH 7.4) to a final concentration of 20 mM. Cells were harvested by centrifugation, and affinity purification was performed as described in the text.

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E. coli          RFYVRKIRFEGN-DTSKDAVLRREMRQMEGAWLGSDLVDQGKERLN----RLGF-FETVDTDTQRVPGS--PDQVDVVYKVKER
V. harveyi       RIYVRDIRFTGN-NSTKDEVLRREMRQMEGSWLNSKSIETGKTRLN----RLGY-FENVEVQTVRVPGS--DDQVDLVYSVKEA
H. influenzae    RLTVRQLRFEGN-TVSADSTLRQEMRQQEGTWYNSQLVELGKIRLD----RTGF-FETVENRIDPINGS--NDEVDVVYKVKER
B. aphidicola    RYFVNKINFRGN-ELTQDIVLRREMKQIEGEWFNLKLIELGIKSLE----KLKF-LSDITVQKEILFNK--ENGVDITYTLKEQ
B. pertussis     RVYVRRIQIGGN-TRTRDEVVRREMRQQEAAWYDAGDIKVSRDRVD----RLGY-FNEVNVKTDPVPGS--PDQVDVNVDVKEK
P. aeruginosa    RAYVNRINFRGN-TKTEDEVLRREMRQMEGGWASTYLIDQSKARLE----RLGY-FKEVNVETPAVPGT--DDQVDVNYSVEEQ
C. crescentus    RVYVDRIDIVGN-TRTLDYVLRRELEVAEGDAYNRVLVDRSKNNMR----RLGF-FKEVEIEDAPG-SA--PDRTSLRVKVEEQ
B. japonicum     RTYIERINVRGN-TRTRDYVIRREFDLSEGDAYNRALVDRAERRLK----NLDF-FKSVKITTEPG-SS--SDRVILIVDLEEK
B. henselae      RAYVQRIEIRGN-EKTRDYVIRREIDLNEGDAYNQTLVQRAKRRLE----SLGF-FKAVNISMVPT-DQ--PDQIILVVDVLEA

FhaC P2          GWLIDGKPLEG---TRDRMMVFSAMPGWQDKVLNVFDIDQAIYNIN----NGGK-TGNITIVPADEYGYSYLDLQLQRRALPRV

POTRA motif      -VKVKKINIEGNLKKTKDEVLRRELELKPGDVFNREKLEKDIEALRDYYLNLGYFFADVKVEPEPDP-----GGVDLTIKVDEG
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Fig. S1. Sequence alignment of POTRA P5 domain sequences from a subset of Gram-negative bacterial species. The asterisk indicates the conserved glutamate residue (Glu^{373} in *Escherichia coli*). The consensus POTRA motif and POTRA 2 of FhaC from *Bordetella pertussis* are shown also. Alignments were generated using ClustalW.

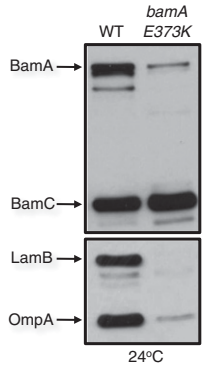

Fig. S2. Outer membrane protein levels in a *bamAE373K* mutant grown at the permissive temperature. Derivatives of JCM320 containing either p*bamA*$^{+}$ or p*bamAE373K* and lacking the inducible chromosomal allele of *bamA* were grown at the permissive temperature for *bamAE373K* (24 °C) to stationary phase. Whole-cell extracts were prepared by boiling, and samples were subjected to SDS/PAGE and Western blotting.

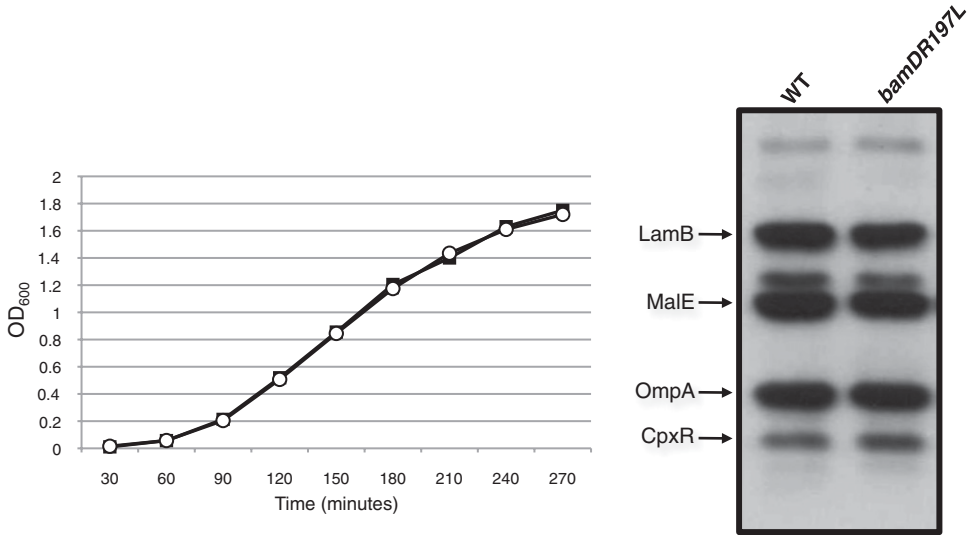

Fig. S3. Growth rate and steady-state protein levels in a *bamDR197L* mutant. (*Left*) Overnight cultures of JCM158 (closed squares) and DPR909 (open circles) were subcultured into LB medium and grown to late-exponential phase at 37 °C. (*Right*) Whole-cell extracts of JCM158 and DPR909 were prepared by boiling after cells were harvested at OD_{600} = ~1. Samples were subjected to SDS/PAGE, and antibodies were used to detect outer membrane proteins (LamB and OmpA), a periplasmic protein (MalE), and a cytoplasmic protein (CpxR).

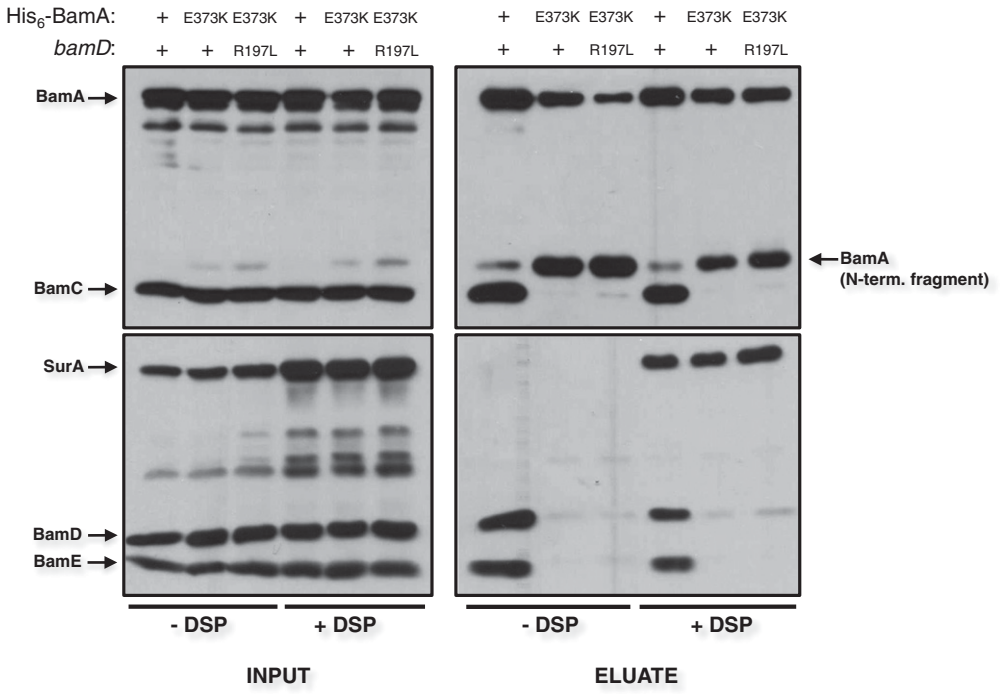

Fig. S4. In vivo crosslinking of BamA^{E373K}. DSP crosslinking, and affinity purification were performed in *bamD*$^{+}$ and *bamDR197L* strains. The complex was purified using His$_6$-tagged BamA or BamA^{E373K}. Samples were subjected to SDS/PAGE and immunoblotting for the proteins indicated. Protein levels are shown before (*Left*) and after (*Right*) purification.

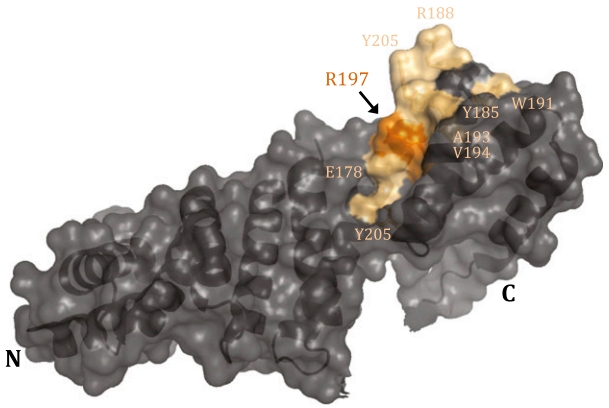

Fig. S5. Conserved residues in the BamD C-terminal domain. Phylogenetic analysis reveals that Arg^{197} (dark orange) is surrounded by a highly conserved patch of residues (light orange). This figure was generated using PyMol.

Table S1. Strains and plasmids

Strain/plasmid	Genotype and relevant features	Reference
E. coli K-12 strains		
MC4100	F-*araD139 (argF-lac)U169 rpsL150 relA1 flb5301 deoC1 ptsF25 thi*	1
XL1-Red	*endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac mutD5 mutS mutT* Tn10	Stratagene
JCM158	MC4100 *ara*$^{r/-}$	2
JCM320	JCM158 Δ*bamA* Δ(λ*att-lom*)::bla P_{BAD} *bamA araC*	3
DPR434	JCM320 pZS21	This study
DPR437	JCM320 pZS21::*bamA*$^{+}$	This study
DPR439	DPR437 *nadA*::Tn10 Δ(λ*att-lom*)	This study
DPR662	JCM320 pZS21::*bamAE373K*	This study
DPR682	DPR662 *bamDR197L*	This study
DPR808	JCM320 pZS21::*bamAE373R*	This study
DPR821	JCM158 pHis-BamA	This study
DPR822	JCM158 pHis-BamA^{E373K}	This study
DPR881	DPR662 *nadA*::Tn10 Δ(λ*att-lom*)	This study
DPR909	JCM158 *bamDR197L*	This study
DPR959	JCM158 *bamA101*	This study
DPR960	DPR909 *bamA101*	This study
DPR967	DPR808 *bamDR197L*	This study
DPR989	DPR909 pHis-BamA	This study
DPR990	DPR909 pHis-BamA^{E373K}	This study
Plasmids		
pZS21	Expression vector; λ P_L-driven expression, Kan^{r}	4
p*bamA*	pZS21::*bamA*$^{+}$	5
p*bamAE373K*	pZS21::*bamAE373K*	This study
p*bamAE373R*	pZS21::*bamAE373R*	This study
pHis-BamA	pET22-42::His_6-*bamA*	4
pHis-BamA^{E373K}	pET22-42::His_6-*bamAE373K*	This study
pBamE-His	pET22-42::*bamE*-His_8	6
pSK38	pETDuet-*bamB-bamA*	7
pCH121	pETDuet-*bamB-bamAE373K*	This study
pSK46	pCDFDuet-*bamC-bamD*	7
pCH123	pCDFDuet-*bamC-bamDR197L*	This study

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5. Kim S, et al. (2007) Structure and function of an essential component of the outer membrane protein assembly machine. *Science* 317:961–964.
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Table S2. Oligonucleotides used in this study

Primer name	Sequence (5′ to 3′)
BamAE373K-forward	5′-GCGTCAGATGAAAGGTGCATGGCTGGGGAGCGAT-3′
BamAE373K-reverse	5′-GCCATGCACCTTTCATCTGACGCATTTCGCGACG-3′
BamAE373R- forward	5′-GAAATGCGTCAGATGCGTGGTGCATGGCTGGG-3′
BamAE373R- reverse	5′-CCCAGCCATGCACCACGCATCTGACGCATTTC-3′
BamDR197L- forward	5′-CGTCGTTAACCTCGTAGAAGGCATGTTGCGCGACTA-3′
BamDR197L- reverse	5′-GCCTTCTACGAGGTTAACGACGGCAACCCAT-3′