

Supplemental Materials and Methods

Random mutagenesis of *bamA* and identification of detergent-sensitive mutants. PCR Mutagenesis of *bamA* was accomplished by amplifying a fragment of the *bamA* ORF encoding the periplasmic domain of BamA with an error-prone polymerase provided in the GeneMorph II kit (Stratagene). Primers JCM51 (5'-AAAGAATTCTAGTTAGGAAGAACG-3') and JCM58 (5'-ATCTGACGCATTTTCGC-3') were used at 125 ng each with 50ng pZS21::*bamA* as template in a 50 μ l reaction. The PCR conditions were as follows: 95°C for 5 min followed by 30 cycles of 95°C for 30 sec, 50°C for 30 sec, and 72°C for 2 min, followed by a final 10-min extension step at 72°C. The product was digested with EcoRI and BsmBI (New England Biolabs), ligated into pZS21::*bamA* that had been previously digested with the same restriction enzymes, and transformed into JCM320. Transformants were selected on LB agar supplemented with arabinose and kanamycin. Detergent-sensitive *bamA* mutants were identified by simultaneously plating JCM320 (transformed with PCR-mutagenized pZS21::*bamA* described above) on LB agar lacking arabinose and containing SDS/EDTA (0.5%/1.0 mM).

Site-directed mutagenesis. Plasmid-borne *bamA* and *surA* mutant alleles were obtained by site-directed mutagenesis from parent plasmids (pZS21::*bamA* and pSurA) using the Stratagene QuikChange site-directed mutagenesis kit per the manufacturer's instructions using Platinum *Pfx* polymerase (Invitrogen) and oligonucleotide primers listed in Table S3. The PCR conditions used were as follows: 95°C for 5 min followed by 16 cycles of 95°C for 30 sec, 65°C for 30 sec, and 68°C for 7 min, followed by a final 10-min extension step at 68°C. PCR products were digested with DpnI (New England Biolabs) for 1 h at 37°C, used to transform DH5 α , then plated on LB agar with appropriate antibiotic and incubated at 37°C overnight. Plasmids were purified from resulting transformants and the mutations were confirmed by DNA sequencing.

Western blot analysis. Cultures were grown overnight in LB and then back-diluted 1:500 into fresh media. 1-mL samples were collected from cultures grown in each condition at $OD_{600} = 1$ unless otherwise noted. Harvested samples were normalized by optical density, pelleted ($5,000 \times g$, 10 min), and resuspended in SDS-PAGE sample buffer. Samples were then boiled for 10 min and subjected to electrophoresis through 12% SDS-PAGE. Previously described rabbit polyclonal antisera that recognize BamA (1:50,000 dilution), BamB (1:5,000 dilution), BamC (1:30,000 dilution), MalE (1:30,000 dilution), LamB/OmpA (1:30,000 dilution), OmpF/C (1:30,000 dilution), TolC (1:10,000 dilution), LptD (1:6,000 dilution), DegP (1:30,000 dilution), or SurA (1:8,000 dilution) were used for immunoblots. Protein bands were visualized using the ECL antibody detection kit (Amersham) and Hyblot CL film (Denville Scientific).

Affinity purification and *in vivo* crosslinking. Cells were grown in 200 mL of LB media supplemented with 125 $\mu\text{g}/\text{mL}$ ampicillin to $OD_{600} = \sim 0.8$ and harvested by centrifugation ($5000 \times g$, 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 $\mu\text{g}/\text{mL}$, 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, washed in 20 mL of 20 mM potassium phosphate (pH 7.2) and 150 mM NaCl, and resuspended in 10 mL of BugBuster solution (Novagen) containing lysozyme (5 $\mu\text{g}/\text{mL}$), DNase I (50 $\mu\text{g}/\text{mL}$), RNase I (50 $\mu\text{g}/\text{mL}$), and 1 mM PMSF. Cells were allowed to lyse by rocking for 15 min at room temperature. To remove cellular debris after lysis, the lysates were then centrifuged at $10,000 \times g$ for 10 min. Cleared lysates were transferred to separate tubes and 100 μl samples (“input”) were collected for SDS-PAGE. Lysates were incubated with 80 μl of Ni-NTA resin (Qiagen) for 20 min at room temperature. Resin from each sample was subsequently washed with 5 mL of 50 mM

potassium phosphate buffer (pH 8.0) containing 300 mM NaCl and 20 mM imidazole. Resin was pelleted and the wash buffer discarded. Bound proteins were then eluted from the resin with 500 μ l of 50 mM potassium phosphate buffer (pH 8.0), containing 300 mM NaCl and 200 mM imidazole. Input and eluate samples were resuspended in SDS-PAGE buffer and boiled for 10 min. The samples were used for SDS-PAGE and western blot analysis as described above.