SUPPORTING INFORMATION

The small molecule nitazoxanide selectively disrupts BAM-mediated folding of the outer membrane usher protein

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Table S1. Strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Reference or source
Strains[a]		
BW25113	*LacI^q rrnB ΔlacZ hsdR514 ΔaraBAD ΔrhaBAD*	(1)
BW25113Δ*bamB*	BW25113 *bamB::kan*	(2)
BW25113Δ*bamC*	BW25113 *bamC::kan*	(2)
BW25113Δ*bamE*	BW25113 *bamE::kan*	(2)
MC4100	F-*araD139 (argF-lac)U169 rpsL150 relA1 flb5301 deoC1 ptsF25 thi*	(3)
MC4100*bamA101*	MC4100 *ara$^{r/-}$ bamA101*	(4)
Plasmids		
pMON6235Δcat	Cloning vector, P_{ara}, Amp^r	(5)
pTRYC	Cloning vector, P_{trc}, Clm^r	(6)
pTRYC-NdeI		This study
pACYC184	Cloning vector, P_{trc}, Clm^r, Tet^r	(7)
pFJ20	pMON6235Δcat derivative encoding PapC	(8)
pMJ3	pMON6235Δcat derivative encoding His-tagged PapC	(9)
pJP1	pMON6235Δcat derivative encoding FLAG-tagged PapC	This study
pJH114	pTRC99a/NdeI derivative encoding BamABCDE-His	(10)
pBAD18::*bamB*	pBAD18 derivative encoding BamB	(11)
pBamE-His	pET22-42 derivative encoding BamE-His	(12)
pBamABCDE	pTRYC derivative encoding BamABCDE-His	This study
pBamA	pTRYC derivative encoding BamA	This study
pBamB	pTRYC derivative encoding BamB	This study
pBamE	pTRYC derivative encoding BamE-His	This study
pFJ29	pACYC184 derivative encoding the whole pap operon	(8)

[a]All strains are *E. coli* K-12.
Amp^r, ampicillin resistance; Clm^r, chloramphenicol resistance; Tet^r, tetracycline resistance; P_{ara}, arabinose-inducible promoter, P_{trc}, IPTG-inducible promoter.

Table S2. Oligonucleotides used in this study

Primer name	Sequence (5' - 3')
Bam mutant verification	
bamBUPFwd	5’-CTGAAAACCCTTGATACCAT-3’
bamBDOWNRev	5’-ATAAATCATCAGACAACGC-3’
bamCUPFwd	5’-GCCATTACACAACAAACTAT-3’
bamCDOWNRev	5’-GTTTGCGTATCATATCAGAA-3’
bamEUPFwd	5’-AAGTCACACGTAATACACT-3’
bamEDOWNRev	5’-AGCGAATAAATAACAGACAG-3’
bamBUPFwd	5’-CTGAAAACCCTTGATACCAT-3’
Bam cloning	
bamE-HisFwd	5’-GCGCGCGAATTCATGCGCTGTAAAACGCT GACTGC-3’
bamE-HisRev	5’-GCGCGCAAGCTTTTAGTGGTGGTGGTGGT GTGGTG-3’
pTRYC/NdeIFwd	5’-CACACAGGAAACAGCATATGGAATTCGA GCTCGG-3’
pTRYC/NdeIRev	5’-CCGAGCTCGAATTCCATATGCTGTTTCCTGTGTG-3’
bamFwd	5’-GATATACATATGGTTAGGAAGAACGCAT AATAACG-3’
bamRev	5’-TATATACCCGGGTTAGTGGTGATGATGGTGTGATGAT-3’
BamAREVXmaI	5’-CTTATTACCCGGGCACTTACCAGGTTTTACGATG-3’
PapC 3X FLAG	
PapCflFW_tail	5'-GACTACAAGGACCACGACGGTGACTACAAGGACCACGACATCG ACTACAAGGACGACGACGACAAG GGGGAAAAAACGACAAAC-3'
PapCflFW_short	5'-GGGGAAAAAACGACAAACAGAAATTTCACATGGAGTCGCTTTTA TCTGTTCCGTGCCATT-3'
PapCflRev_tail	5'-CTTGTCGTCGTCGTCCTTGTAGTCGATGTCGTGGTCCTTGTAGT CACCGTCGT GGTCCTTGTAGTCGTTGTAGCGGCTCTGCTC-3'
PapCflRev_short	5'-GTTGTAGCGGCTCTGCTCCTGGCTTCCCTGATAGTCAGC-3'
no-SCAR mutagenesis ($BamD_{P100S}$)	
sgRNA-target-F	5'-TATTCGGATGGGTCGGGTTAGTTTTAGAGCTAGAAATAGCAAG-3'
sgRNAtarget-R	5'-TAACCCGACCCATCCGAATAGTGCTCAGTATCTCTATCACTGA-3'

CPEC rev 2	5'-CCAATTGTCCATATTGCATCA-3'
CPEC2F	5'-CGGCGTCACACTTTGCTAT-3'
gamR	5'- TTTATAACCTCCTTAGAGCTCGA-3'
pKDseq5	5'-CAGTGAATGGGGGTAAATGG-3'
sgrnaR	5'-GCCTGCAGTCTAGACTCGAG-3'
sgrnaA	5'-AGCTTTCGCTAAGGATGATTT-3'
BamDP100S	5'-TGCCGTTAGCACAGGCTGCCATCGATCGTTTTATTCGCCTTAATA GCAC CCA TCCGAATATCGATTATGTCATGTACATG-3'

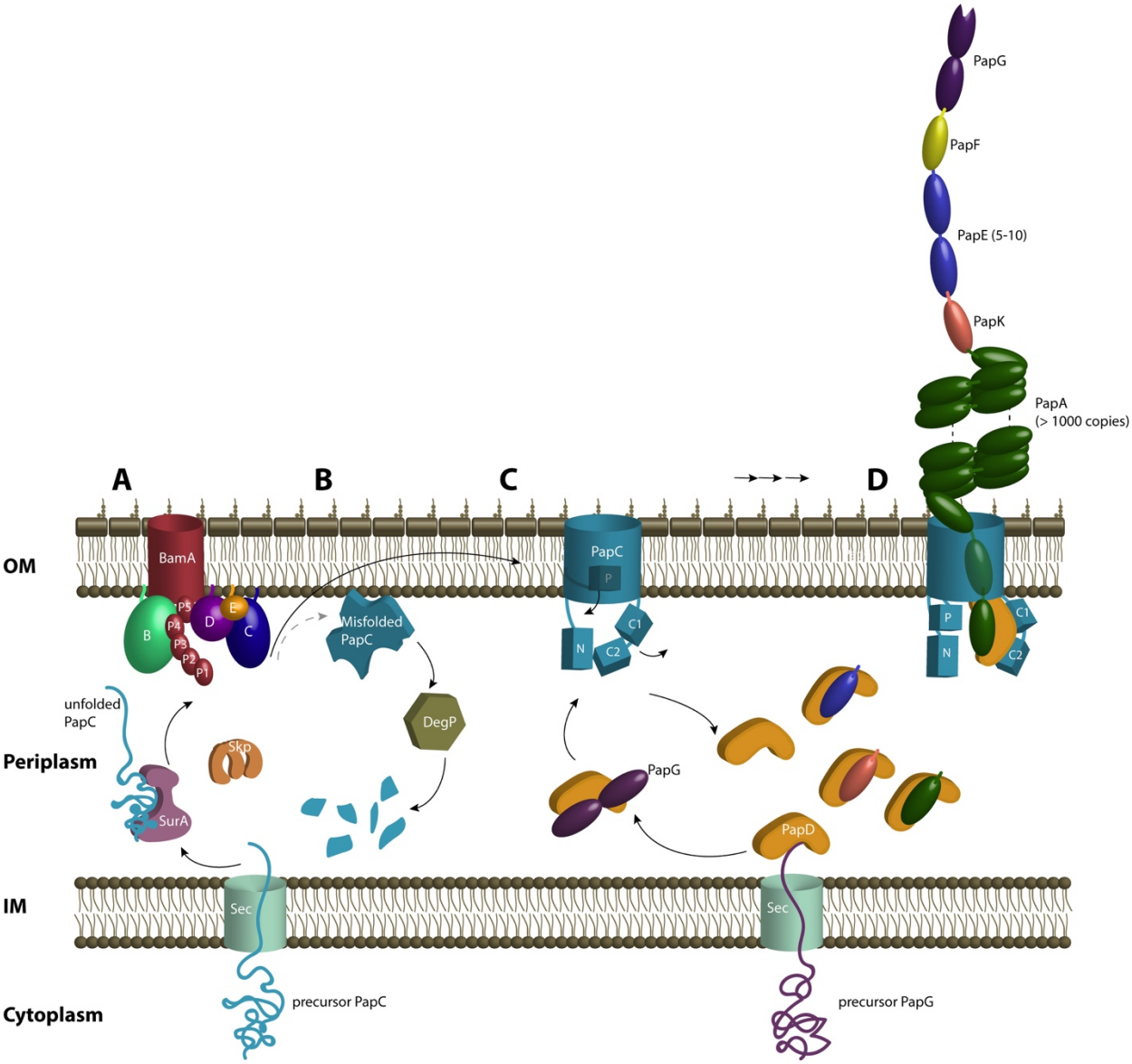

Figure S1. Model for BAM complex-mediated folding of the PapC usher and P pilus assembly by the CU pathway. (A) In *E. coli*, the BAM complex consists of BamA, an OM ß-barrel protein with five N-terminal POTRA domains (P1–P5), and the lipoproteins BamB–E. Nascent OMPs such as PapC are translocated across the inner membrane (IM) via the Sec pathway into the periplasm. Chaperones such as SurA, Skp, and DegP recognize unfolded OMPs in the periplasm and transport them to the BAM complex. The BAM complex then catalyzes the proper assembly and insertion of the protein into the OM. The usher follows a SurA-BamB folding pathway. (B) Substrates that fall off-pathway are targeted for degradation by periplasmic proteases such as DegP. (C) The usher (PapC) comprises an OM β-barrel channel domain, a plug domain (P), and N- and C-terminal periplasmic domains (N, C1 and C2). Upon entering the periplasm via the Sec pathway, nascent pilus subunits form binary complexes with the pilus chaperone (PapD), which facilitates subunit folding. Periplasmic chaperone-subunit complexes are recruited by the usher in a sequential manner. The usher catalyzes the exchange of chaperone-subunit for subunit-subunit interactions to build the pilus fiber and provides the channel for secretion of the fiber to the cell surface.

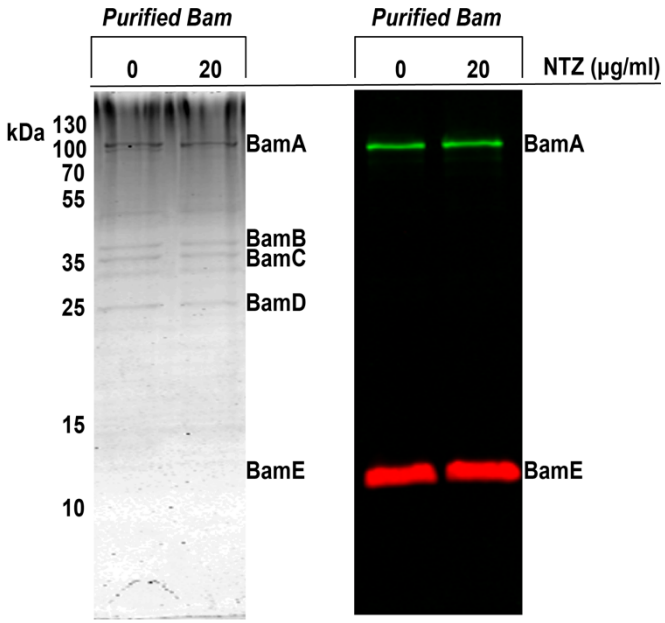

Figure S2. Effect of NTZ on BAM complex formation. Strain BW25113/ pBamABCDE-His_6, expressing the BAM complex with a His-tagged BamE, was grown in the presence of 0 or 20 μg/ml NTZ, OM fractions were isolated, and the detergent-extracted complex was captured by nickel affinity chromatography. Proteins eluted from the column were then analyzed by staining with Commassie blue (left panel) or immunoblotting with anti-His-tag and anti-BamA antibodies (right panel).

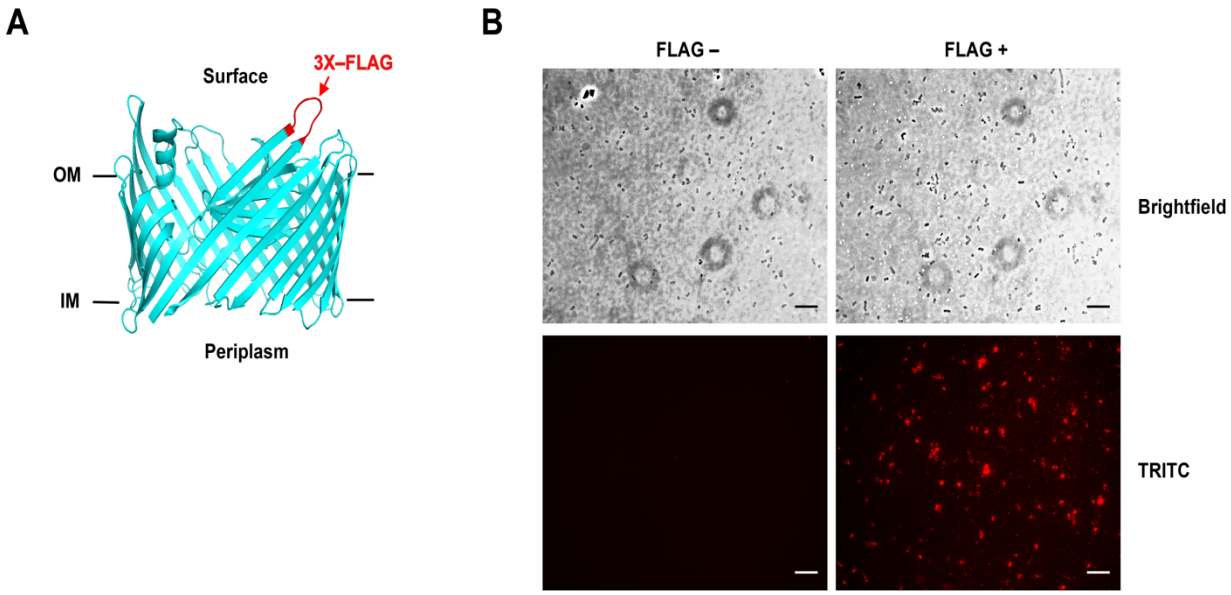

Figure S3. Detection of the PapC usher in intact bacteria. (A) Structure of PapC [PDB ID: 2VQI] showing surface-exposed loop between ß-strands 3 and 4 where the 3X FLAG tag was inserted. (B) Intact bacteria from cultures of BW25113/pMJ3 ($PapC_{His}$; FLAG-) or BW25113/pJP1 ($PapC_{FLAG}$; FLAG +) were labeled with a PE-conjugated anti-FLAG antibody to detect PapC levels in the OM and the bacteria were imaged by fluorescence microscopy at 100X magnification. Representative brightfield (top panels) and TRITC-filter (bottom panels) images are shown. Scale bar represents 10 µm.

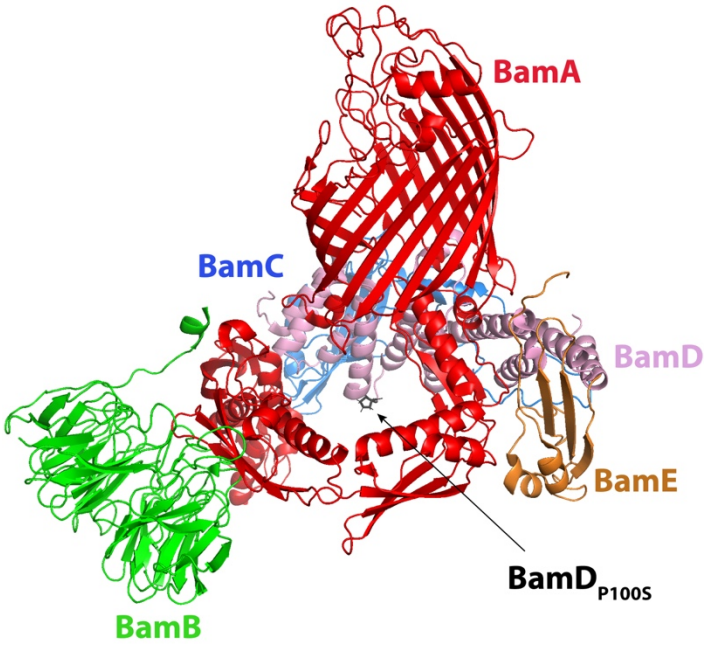

Figure S4. The $BamD_{P100S}$ mutation. Structure of the BAM complex (PDB ID: 5LJO) showing location of the $BamD_{P100S}$ mutation.

References

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