Supplementary Materials:

Materials and Methods

Figures S1-S6

Tables S1-S6

Movies S1-S2

References (*35-40*)

Supplementary Materials for:

The structure of the β-barrel assembly machinery complex

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Figures S1-S6

Tables S1-S6

Movies S1-S2

References (*35-40*)

Materials and Methods:

Expression of recombinant BamABCDE complex

A single plasmid (pJH114) containing all five Bam proteins (BamA, B, C, D, and E) was obtained from Harris Bernstein and used for expression and purification (*11*). The plasmid was transformed into BL21(DE3) cells (NEB), plated onto LB-carbenicillin agar plates (Teknova) and incubated overnight at 37°C. A single colony was used to inoculate a 5-mL LB-ampicillin culture and incubated overnight at 37°C. The overnight culture was then used to inoculate a 50 mL starter culture of LB-ampicillin which was allowed to grow to saturation. The cells were then centrifuged, washed three times with 1x PBS and then re-suspended in 12 mL 1x PBS. The resuspended cells (1 mL) were then added to twelve 2 L baffled flasks containing 1 L of 2xYT medium supplemented with ampicillin (50 μg/mL). These cultures were incubated at 37°C with shaking at 180 rpm, grown to an OD_{600} between 0.8-1.0, and then induced with 0.5 mM IPTG. Cultures were grown an additional 4 hours at 37°C before harvesting. Cell were either used immediately or flash frozen and stored at -20°C.

Purification and crystallization

Cells were resuspended in lysis buffer (1x PBS supplemented with DNase I (10 μg/ml) and PMSF (500 μM)) and lysed with three passes through an Emusiflex C-3 high pressure homogenizer (Avestin) at 18,000 psi. The cell lysate was then centrifuged at 6000 x *g* for 10 min at 4°C to remove cell debris, and the resulting supernatant was centrifuged at 200,000 x *g* for 90 min at 4°C to isolate cell membranes. The membranes were then re-suspended in solubilization buffer (1x PBS, 1% DDM, and 37 mM imidazole) using a dounce homogenizer and stirred at medium speed overnight at 4°C. The solubilized sample was then centrifuged again at 200,000 x *g* for 60 min at 4°C and the supernatant collected.

Solubilized BamABCDE complex was purified by affinity chromatography using a 5 mL HiTrap Nickel column (Qiagen) and an ÄKTA Pure system (GE Healthcare). The column was equilibrated with Buffer A (1x PBS, 0.03% DDM, and 37 mM imidazole) and the sample automatically loaded using the sample pump with an in-line air sensor. Protein was eluted with a linear gradient of 37-500 mM imidazole using Buffer A and Buffer B (1x PBS, 0.03% DDM, and 1 M imidazole). Fractions containing BamABCDE were pooled, concentrated to ~2 mg/mL, and passed through a 16/60 Sephacryl S-300 HR column (GE Healthcare) at a flow rate of 0.5 mL/min using 25 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.6% C_8E_4. All five Bam proteins (BamA, B, C, D, and E) eluted from the gel filtration column as a single monodisperse peak as verified by SDS-PAGE analysis. Fractions containing BamABCDE were pooled and concentrated to ~12 mg/mL.

Broad crystallization screening was performed using hanging drop method on a Mosquito LCP crystallization robot (TTP Labtech) with commercially available crystallization screens. An initial hit was improved by additive screening using the AdditiveHT screen (Hampton Research) with final crystals grown at 22°C in 100 mM Tris-HCl, pH 8.5, 200 mM $MgCl_2$, 10 mM $MnCl_2$, and 8% PEG 4000.

Data collection, structure determination, and modeling

Crystals were harvested by quick transfer directly into a cryoprotectant solution containing 20% glycerol and flash-cooled in liquid nitrogen. Diffraction data were collected to 3.4 Å resolution at the SER-CAT beamline (ID22) at the Advanced Photon Source at Argonne National Laboratory and processed using HKL2000 (*35*). The structure was solved by molecular replacement using Phaser (*36*) within PHENIX (*37*) using previously reported crystal structures of the Bam components. Search order was key here for success, first starting with the BamCD complex (PDB ID 3TGO) followed by the barrel domain of BamA (PDB ID 4C4V), POTRA5 and then POTRA4 of BamA (PDB ID 3Q6B). BamE (PDB ID 2KM7) and POTRA1 (PDB ID 3EFC) were then placed based on density within a difference (F_o-F_c) map. After several rounds of building and refinement, POTRA2 and 3 (PDB ID 3EFC) were then manually placed in weak density followed by rigid body refinement for all components for final placement. The structure was refined to R/R_{free} values of 0.22/0.27. All model building and refinement were performed using COOT (*38*) and PHENIX (*37*), respectively. Final placement of side chains was based on evaluation of $2F_o$-F_c, F_o-F_c, and feature-enhanced (FEM) density maps (*39*). RMSD analysis was performed within PyMOL (Schrödinger) for C-α atoms using default settings. Surprisingly, BamB was not found within our crystal structure despite it being present in our purification. The absence of BamB was confirmed by analyzing crystals and our initial sample of the complex by SDS-PAGE analysis, which also were lacking BamB presumably due to proteolysis during storage/incubation.

To see how BamB interacts with the BamACDE complex, we modeled BamB into our complex using the previous reported BamAB crystal structure (PDB ID 4PK1) to produce the modeled structure of the fully assembled BamABCDE complex. Here, we were able to place BamB in our structure by performing a superposition of the two structures along POTRA3 of BamA. Analysis of interacting interfaces was performed using the PDBePISA (*40*). All figures were made with PyMOL (Schrödinger) and annotated and finalized with Adobe Illustrator.

Data Collection	**BamACDE**
λ (Å)	1.0
Space group	C2
Mol/ASU	1
a, b, c (Å)	234.85, 109.23, 103.99
α, β, γ (º)	90, 95.04, 90
Resolution (Å)	50 - 3.4 (3.52 - 3.4)
Completeness (%)*	99.6 (99.9)
Redundancy*	6.3 (6.4)
Wilson B-factor ($Å^2$)	133
R_{sym}*	0.12 (1.00)
I / σ (I)*	21.3 (1.6)
Refinement	
Resolution (Å)	30 - 3.4
No. reflections	36,000
R/R_{free}	0.23/0.28
r.m.s. deviations	
Bonds (Å)	0.004
Angles (º)	1.082
No. Protein atoms	9409
B-factors ($Å^2$)	
Protein	158
Ramachandran Analysis¥	
Favored (%)	84.6
Allowed (%)	15.0
Outliers (%)	0.4
PDB code	-

¥ Performed using Molprobity.
* Indicates statistics for last resolution shell shown in parenthesis.

Table S1. Data collection and refinement statistics.

BamC	Group	Location	BamD	Group	Location	Distance [Å]
Hydrogen bonds						
LYS 32	O	Unstructured	TYR 77	OH	Helix 3	2.78
SER 36	N	Unstructured	TYR 80	OH	Helix 3	2.87
SER 36	OG	Unstructured	LYS 81	NZ	Helix 3	3.36
TYR 41	OH	Unstructured	ASP 162	OD2	Helix 7	2.86
TYR 41	OH	Unstructured	ARG 166	NH2	Helix 7	2.83
ALA 48	N	Unstructured	ASP 207	O	Loop 8	3.4
ALA 48	O	Unstructured	ARG 212	NH1	Helix 9	3.61
GLU 49	O	Unstructured	ARG 212	NH1	Helix 9	3.52
HIS 51	N	Unstructured	ASN 241	OD1	C-term.	2.77
HIS 51	O	Unstructured	ASN 241	ND2	C-term.	3.63
THR 61	O	Unstructured	ASP 204	N	Helix 8	3.8
SER 62	OG	Unstructured	ARG 203	O	Helix 8	3.06
TYR 65	OH	Unstructured	ARG 141	NH1	Helix 6	3.51
ALA 66	O	Unstructured	ARG 152	NH1	Helix 6	3.57
THR 70	OG1	Unstructured	THR 164	OG1	Helix 7	2.96
GLY 74	O	Unstructured	THR 161	OG1	Helix 7	3.71
GLY 77	N	Unstructured	ASP 162	OD2	Helix 7	2.91
ILE 82	O	Unstructured	TYR 107	OH	Helix 5	2.99
PRO 85	O	Unstructured	GLN 70	NE2	Helix 3	3.23
LEU 151	N	N-domain	GLN 44	OE1	Helix 1	3.37
ALA 192	N	N-domain	GLN 158	OE1	Loop 6	3.39

Additional interface residues (**BamC**)			
TYR 31	GLN 34	VAL 35	GLY 37
ASP 38	ALA 40	LEU 42	ALA 44
ALA 45	PRO 46	LEU 47	LEU 50
ALA 52	PRO 53	ALA 54	MET 56
ILE 57	LEU 58	PRO 59	VAL 60
GLY 63	ASP 64	ILE 67	PRO 68
GLY 72	SER 73	ALA 75	VAL 76
LEU 80	ASP 81	ARG 83	PRO 84
ALA 86	GLN 87	ARG 150	ASP 152
ASP 190	ALA 191	GLN 195	GLU 200

Additional interface residues (**BamD**)			
GLN 40	GLN 41	LEU 43	ASP 45
GLY 46	ASN 47	TRP 48	GLN 69
LEU 73	ASP 74	ASN 104	TYR 110
MET 111	LEU 114	MET 117	ALA 118
ASP 121	GLY 126	PHE 144	SER 148
VAL 151	PRO 155	TYR 159	THR 160
LYS 165	LEU 167	VAL 168	PHE 169
LYS 171	ASP 172	LEU 174	GLU 199
LEU 202	TYR 205	PRO 206	THR 208
GLN 209	THR 211	LEU 215	LYS 233
LYS 236	ILE 237	ALA 240	ASN 244

Table S2. Summary of interactions between BamC and BamD. This is a summary of all interactions between BamC and BamD as analyzed by PDBePISA.

BamD	Group	Location	BamE	Group	Location	Distance [Å]
Hydrogen bonds						
TRP 191	N	Helix 8	GLN 34	OE1	β1	3.21
VAL 192	N	Helix 8	GLN 34	OE1	β1	2.75
ALA 193	N	Helix 8	GLN 34	OE1	β1	3.64
ASN 196	ND2	Helix 8	LEU 63	O	Loop 3	3.09
GLN 224	O	Helix 9	ARG 78	NH1	β2	3.64
GLN 224	O	Helix 9	ARG 78	NH2	β2	3.76
MET 225	O	Helix 9	GLN 88	NE2	β3	2.37
GLN 226	O	Helix 9	LYS 107	NZ	β4	2.48
GLN 226	OE1	Helix 9	SER 111	N	C-term.	3.64
GLN 230	NE2	Helix 10	ASP 66	OD1	Loop 3	2.67
GLN 230	NE2	Helix 10	PRO 67	O	Loop 3	3.22
Salt bridges						
LYS 233	NZ	Helix 10	ASP 66	OD1	Loop 3	3.59

Additional interface residues (**BamD**)			
ARG 188	GLY 189	ALA 190	VAL 195
GLU 199	LEU 202	MET 218	TYR 222
TYR 222	MET 227	ALA 229	VAL 234
ILE 237			

Additional interface residues (**BamE**)			
ARG 29	PRO 30	ILE 32	GLY 35
MET 64	SER 65	PHE 68	THR 70
THR 72	PHE 74	TYR 75	VAL 76
GLN 80	THR 90	LEU 110	

Table S3. Summary of interactions between BamD and BamE. This is a summary of all interactions between BamD and BamE as analyzed by PDBePISA.

interface residues (**BamC**)			
GLY 55	MET 56	ILE 57	LEU 58
PRO 59			

interface residues (**BamE**)			
PRO 67	PHE 68	GLY 69	

Table S4. Summary of interactions between BamC and BamE. This is a summary of all interactions between BamC and BamE as analyzed by PDBePISA.

BamA	Group	Location	BamD	Group	Location	Distance [Å]
Hydrogen bonds						
Ile 352	O	POTRA 5	ARG 197	NH2	Helix 8	3.32
GLY 356	N	POTRA 5	ASP 136	OD2	Loop 5	2.83
ASP 357	O	POTRA 5	ASP 136	N	Loop 5	2.93
ASP 358	O	POTRA 5	ARG 132	NH1	Loop 5	2.94
THR 359	O	POTRA 5	GLN 125	NE2	Loop 5	3.1
THR 359	O	POTRA 5	ARG 135	NH1	Loop 5	2.31
ASP 362	OD2	POTRA 5	TYR 177	OH	Helix 7	2.84
ARG 366	NH1	POTRA 5	TYR 185	OH	Helix 7	3.35
GLU 373	N	POTRA 5	TYR 185	OH	Helix 7	3.68
Salt bridges						
ASP 358	OD1	POTRA 5	HIS 139	NE2	Loop 5	3.47
ASP 358	OD2	POTRA 5	HIS 139	NE2	Loop 5	3.55
GLU 373	OE1	POTRA 5	ARG 197	NE	Helix 8	2.97
GLU 373	OE1	POTRA 5	ARG 197	NH2	Helix 8	3.8
GLU 373	OE2	POTRA 5	ARG 197	NE	Helix 8	3.28
GLU 373	OE2	POTRA 5	ARG 197	NH2	Helix 8	2.55
ASP 481	OD1	P. Loop 2	ARG 188	NH2	Helix 7	2.91

Additional interface residues (**BamA**)			
ALA 95	SER 96	ARG 120	VAL 121
GLY 122	GLU 123	ARG 160	ARG 350
LYS 351	ARG 353	PHE 354	GLU 355
SER 360	LYS 361	ALA 363	ARG 367
MET 369	ARG 370	GLN 371	MET 372
GLY 374	GLN 446	TRP 449	LEU 450
GLY 451	THR 452	TYR 477	VAL 480

Additional interface residues (**BamD**)			
PRO 63	PHE 64	ARG 94	ARG 97
LEU 98	THR 101	LEU 119	ASP 120
SER 122	LEU 124	PHE 128	VAL 130
ASP 131	ASP 134	PRO 137	GLN 138
ARG 141	GLU 178	SER 180	VAL 181
TYR 184	GLU 187	ALA 190	VAL 192
ALA 193	ASN 196	GLY 200	ARG 203

Table S5. Summary of interactions between BamA and BamD. This is a summary of all interactions between BamA and BamD as analyzed by PDBePISA.

BamA	Group	Location	BamE	Group	Location	Distance [Å]
Hydrogen bonds						
TYR 348	OH	POTRA 5	TYR 37	N	Loop 1	3.44
TYR 348	OH	POTRA 5	TYR 37	O	Loop 1	3.66
TYR 348	OH	POTRA 5	LEU 59	O	α2	3.82
GLU 373	O	POTRA 5	GLN 34	NE2	β1	2.9
GLY 374	O	POTRA 5	GLY 35	N	β1	3.26
SER 408	OG	POTRA 5	THR 61	OG1	Loop 3	3.34
ASP 410	OD2	POTRA 5	THR 61	N	Loop 3	2.44
ASP 410	OD2	POTRA 5	THR 61	OG1	Loop 3	2.97
GLN 411	NE2	POTRA 5	THR 61	OG1	Loop 3	3.22
PRO 518	O	P. Loop 3	TYR 28	N	N-term.	3.46
GLU 521	OE2	P. Loop 3	TYR 28	OH	N-term.	3.48

Additional interface residues (**BamA**)			
ASN 345	ARG 346	VAL 349	ARG 350
ARG 370	MET 372	ALA 375	TRP 376
PRO 409	ASP 481	VAL 483	PHE 517
ILE 519	ASN 520		

Additional interface residues (**BamE**)			
VAL 27	ARG 29	PRO 30	ASP 31
ILE 32	ASN 33	ASN 36	THR 39
ASP 42	ALA 56	TYR 57	GLY 60
PRO 62	LEU 63	PHE 77	ARG 78
GLN 79	HIS 83		

Table S6. Summary of interactions between BamA and BamE. This is a summary of all interactions between BamA and BamE as analyzed by PDBePISA.

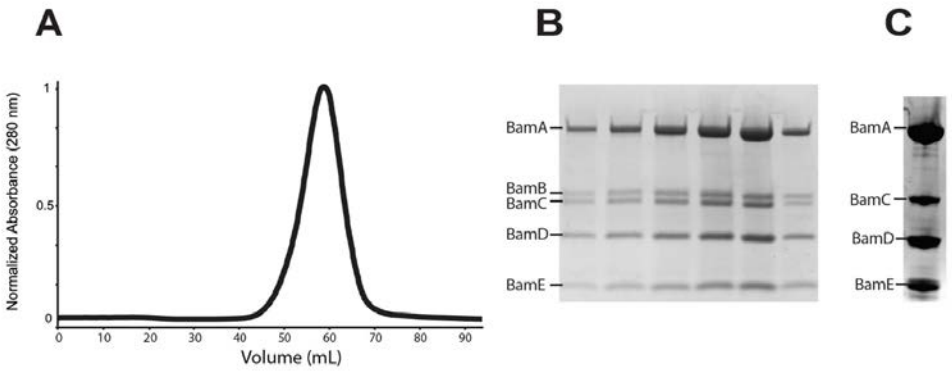


Figure S1. Purification of the BAM complex. A. Gel filtration profile of the BAM complex using a 16/60 Sephacryl S-300 HR column (GE Healthcare). **B.** SDS-PAGE analysis of peak fractions obtained from gel filtration in panel A. Fractions were pooled and concentrated for crystallization. **C.** SDS-PAGE analysis of pooled fractions from gel filtration after incubation at 4º C for ~10 days. BamB is no longer observed and presumed to be degraded.

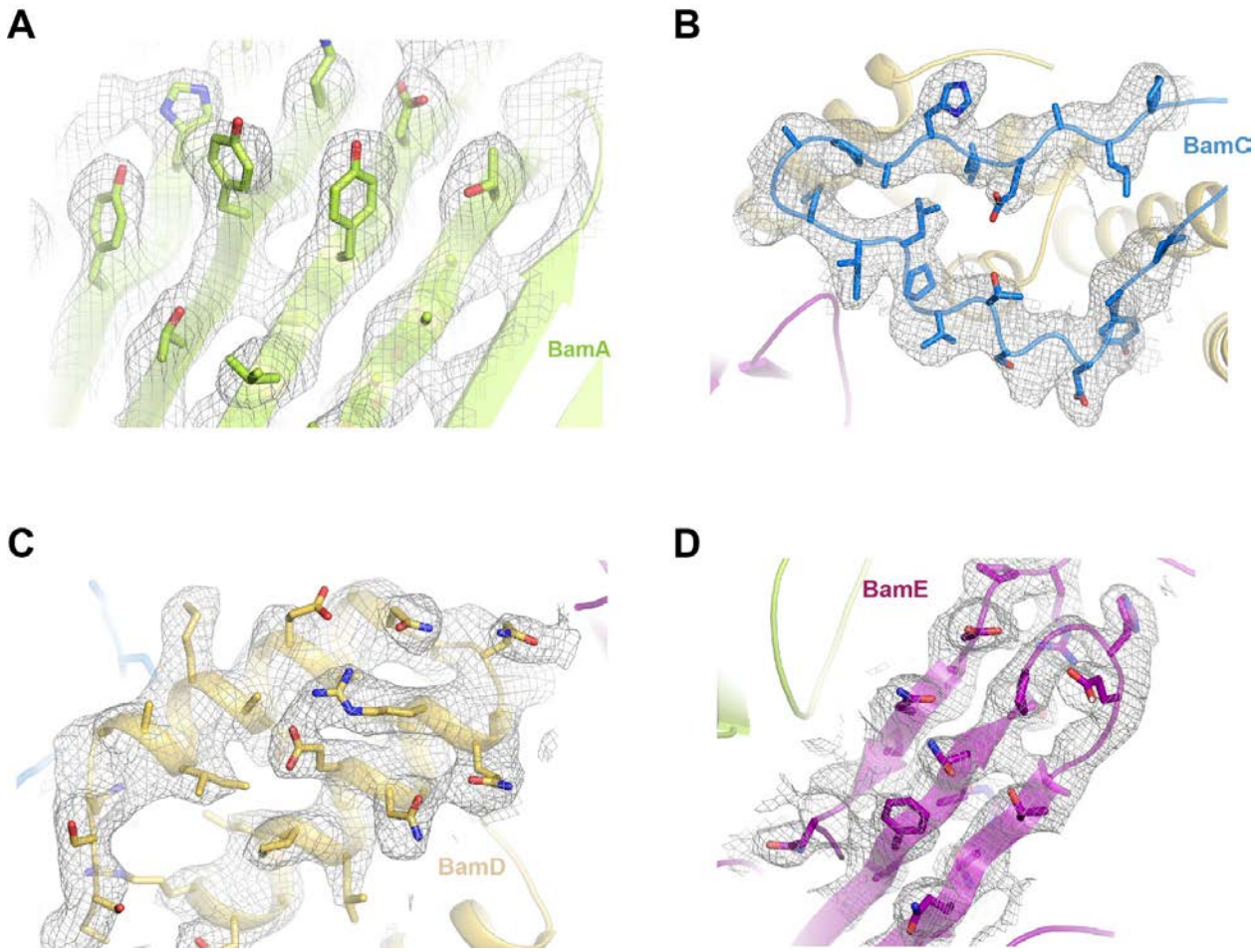


Figure S2. Representative electron density for each Bam component. A. Density (gray mesh) for BamA (green) along strands β4-β7 of the β-barrel domain. **B**. Density along residues 49-60 of BamC (blue). **C**. Density along residues 211-244 of BamD (gold). **D**. Density along residues 29-35 and 77-89 of BamE (purple). In all panels, a feature-enhanced map (FEM) is shown at 1.0 σ.

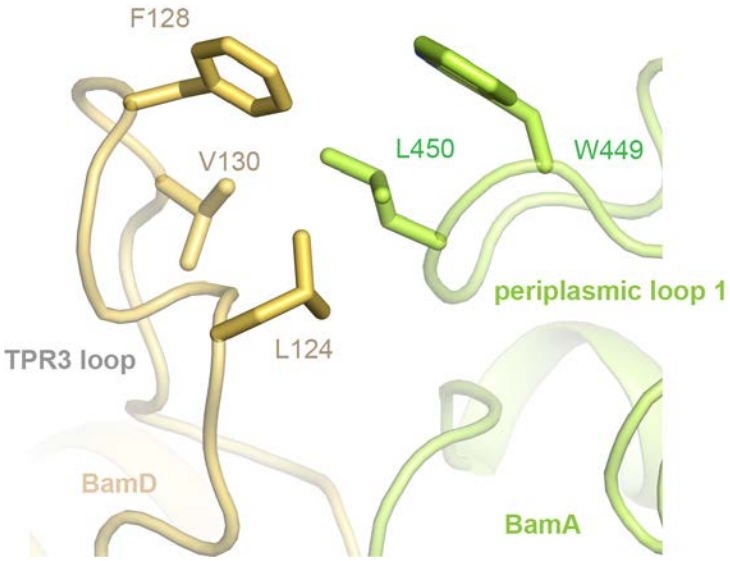


Figure S3. Interaction of periplasmic loop 1 of BamA with TPR3 loop in BamD. Zoomed view of the interaction between the TPR3 loop of BamD (gold) with periplasmic loop 1 of BamA (green). This interaction is largely a hydrophobic interaction mediated by L124, F128, and V130 of BamD and W449 and L450 of BamA.

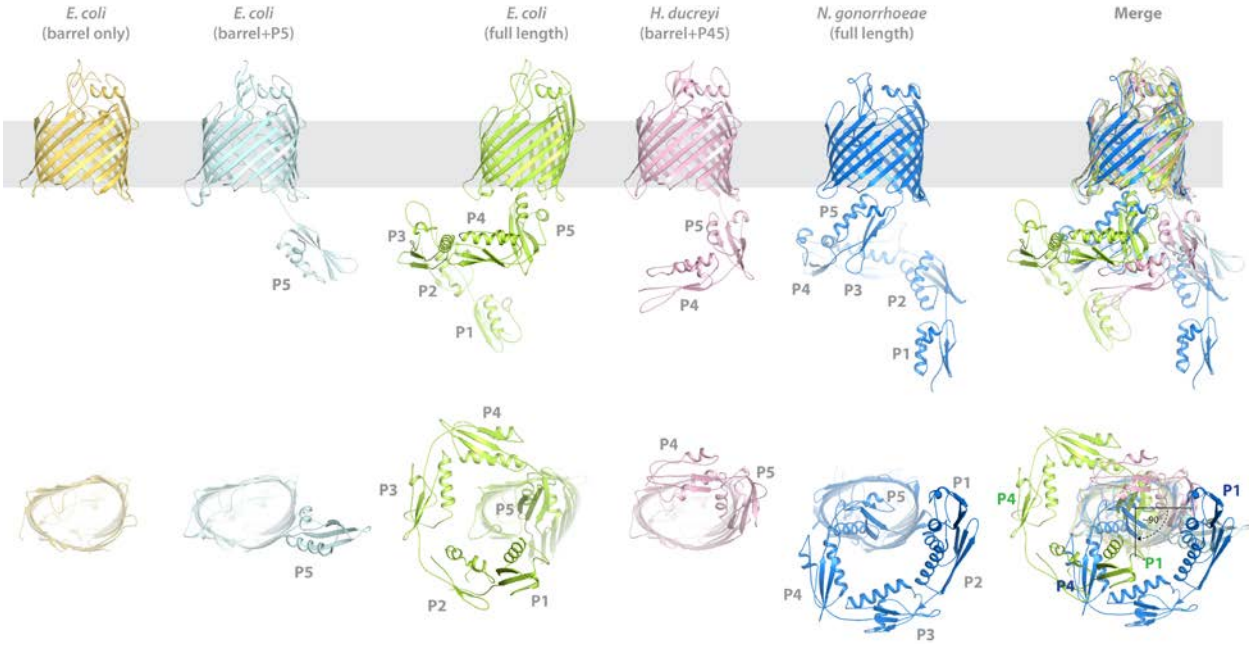


Figure S4. Comparison of all BamA structures and POTRA domain conformations. Shown here is a structural comparison of all known BamA structures containing the membrane domain from a membrane view (top row) and view from the periplasm (bottom row). Three structures are from *E. coli* with barrel only (gold, PDB ID 4N75), barrel with POTRA5 only (cyan, PDB ID 4C4V), and full length reported here (green). Two other structures of BamA have been previously reported from *H. ducreyi* containing barrel with POTRA4 and 5 only (pink, PDB ID 4K3C) and a full length structure from *N. gonorrhoeae* (blue, PDB ID 4K3B). Compared to *N. gonorrhoeae*, the POTRA domains of *E. coli* BamA undergo ~90 clockwise twist (bottom of Merge panel).

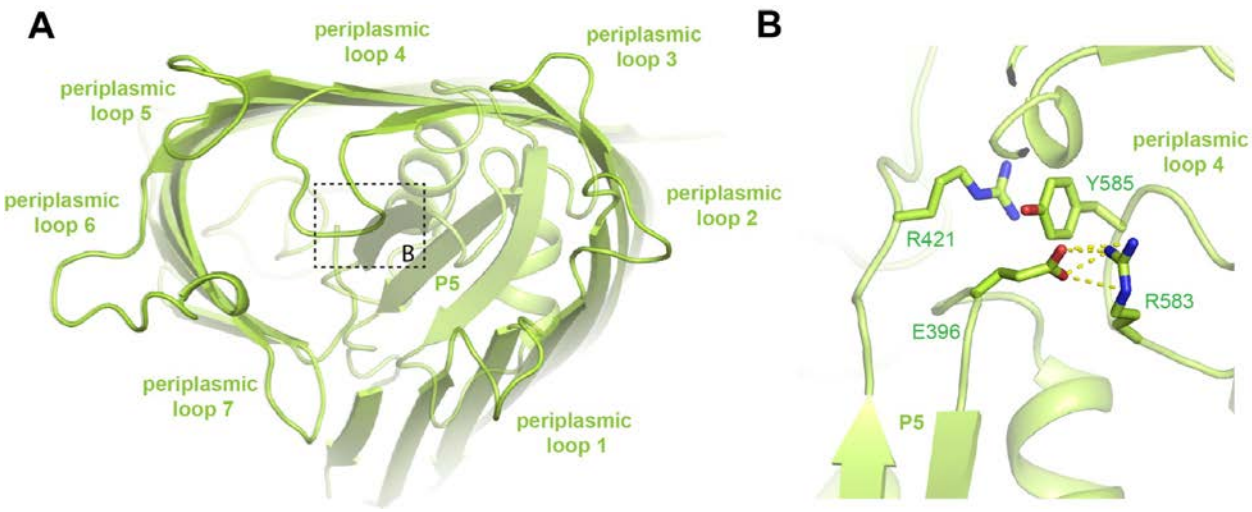


Figure S5. Periplasmic loops of BamA. A. Seven periplasmic loops shown here are thought to play a role in the function of BamA. They were fully resolved in our crystal structure with many of them making contacts either with POTRA 5 or with other Bam components (Figure S3). **B**. Highlighted here is the interaction of periplasmic loop 4 with POTRA5 where two interactions are key. The first is a salt bridge between residues E396 and R583 and the second pi stacking between R421 and Y585.

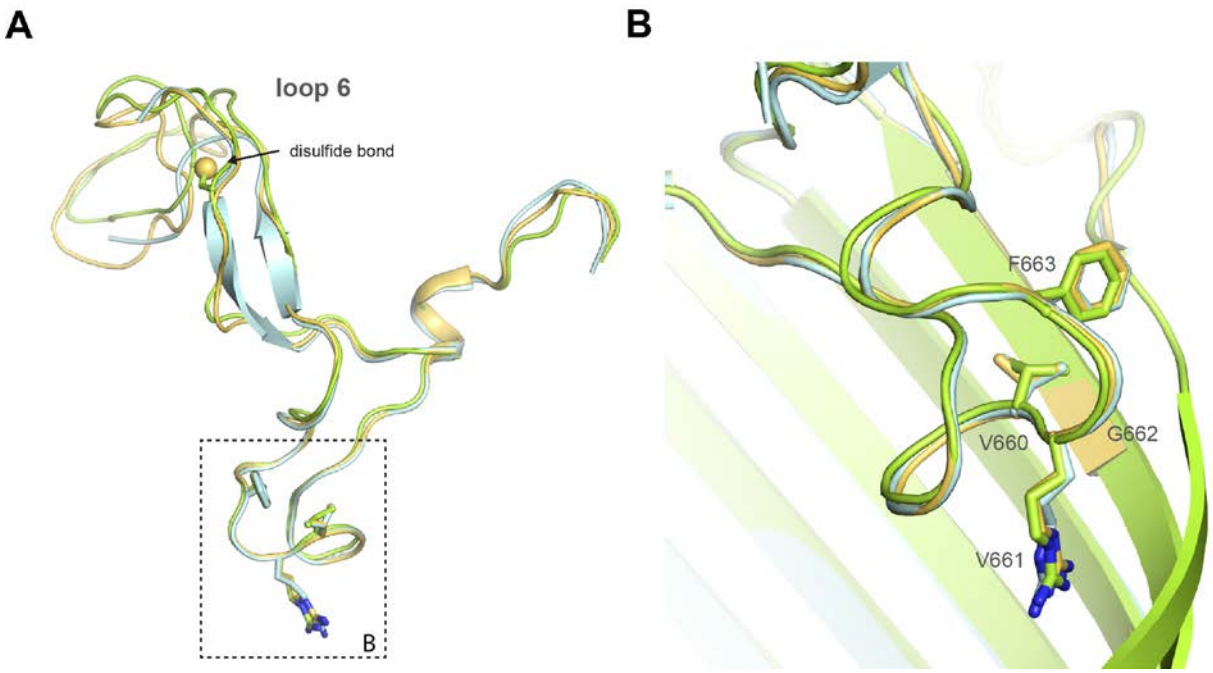
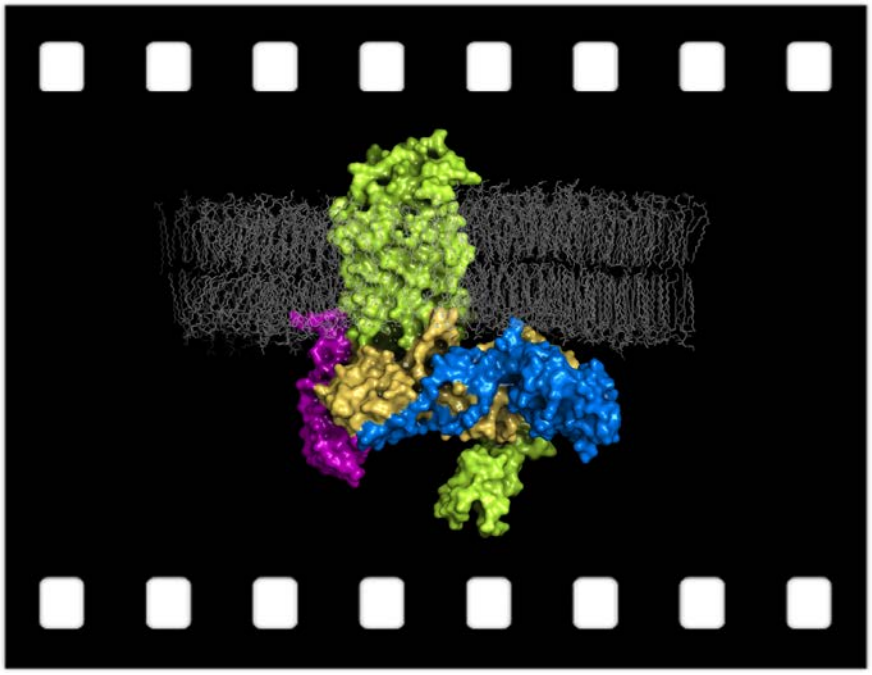
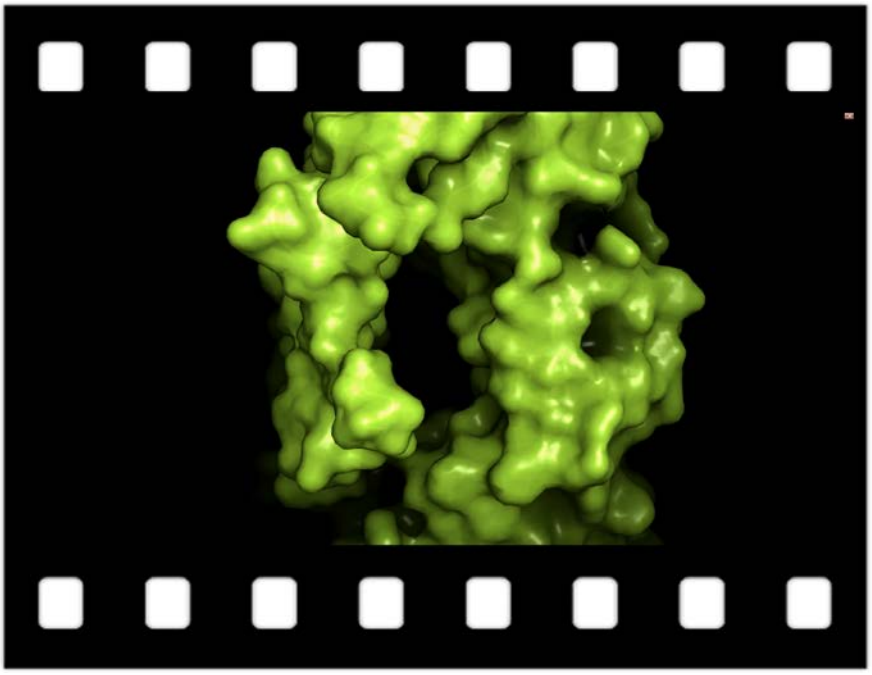


Figure S6. Conformation of loop 6 and the VRGF motif in BamA. A. Shown here is the entire loop 6 (residues 641-709) from an alignment of our structure (green) with PDB ID 4C4V (cyan) and PDB ID 4N75 (gold). The conformation of the full length of the loop is unchanged with the except that in our structure, the top of the loop is fully resolved, including the disulfide bond formed between C690/C700 (gold sphere), similar to that which as observed in PDB ID 4N75. **B**. Zoomed view showing that the conserved VRGF motif remains unchanged despite significant conformational changes to the barrel domain upon binding BamCDE.



Movies S1. Overview of the BAM complex structure. In this study, we report the structure of the fully assembled BAM complex, formed from our crystal structure of BamACDE and the previously reported crystal structure of BamAB. In this movie, we show the overall conformation of the BAM complex and highlight interactions between the Bam components.



Movie S2. Morph of the β-barrel domain of BamA. In our structure of the BamACDE complex, we observe an unprecedented shift of the β-barrel domain of BamA. In this movie, we depict the morph between these observed conformational changes compared to previously reported crystal structures.