ONLINE METHODS

***E. coli* strains and Growth conditions.** *E. coli* strains used in this study are listed Table S1.

Cells were cultured in LB (1% tryptone, 1% NaCl, and 0.5% yeast extract). Unless otherwise specified, ampicillin (amp) (50 μg / ml), chloramphenicol (Cm) (10 μg / ml), or kanamycin (Kan) (30 μg / ml) were added for selecting transformants and for keeping plasmid in the strains. For incorporation BPA into the protein *in vivo*, we used XB (1% tryptone, 1% NaCl, and 0.1% yeast extract).

To create the BamD-depletion strain, we amplified four DNA fragments coding 5′ UTR of BamD (- 540 to - 40 bp), kanamycin cassette, AraC-pBAD, and BamD (1 to 500 bp), respectively (Figure S13). Table S describes the pair of primers and template DNA to amplify each DNA fragment. These four DNA fragments were combined by the overlap PCR method and then electroporated into MC4100a harboring pKD46 coding λ Red recombinase[1]. Transformants were selected-for in the presence of kanamycin and 0.1% (w/v) L-arabinose.

Culturing of BamD-depletion strain was as follows, BamD-depletion strains harboring pBamD variants were inoculated into LB (+ amp + Kan + 0.02% (w/v) L-arabinose) and cultured for 10h at 37°C. The saturation culture was shifted into the LB (+ amp + Kan) and incubated for 10hr at 37°C. The saturation culture was diluted into the LB (+ amp + kan + 0.5% (w/v) D-Glucose) as to set OD_{600} = 0.02, and then incubated at 37°C till OD_{600}=0.8. We diluted the culture by same media to set OD_{600} = 0.02, and then incubated for 2.5h at 37°C.

Plasmids. Plasmids and primers used in this study are listed Table S2 through S6. Cloning of specific target gene ORFs into specified vectors was performed via SLiCE *in vitro* recombination [2].

Protein Purification. Expression of each subunit of the BAM complex and OmpC proteins harboring 6xHis epitope tag was performed as following; plasmids encoding each specific gene were transformed and proteins were expressed in BL21(DE3)* (Invitrogen). For BamB, BamC, BamD, and OmpC, transformants were grown in LB +amp at 37°C until reaching an OD_{600} of 0.5, at which point expression was induced by the addition of 1 mM IPTG for 3 hours. For BamD containing BPA, we transformed pHIS-BamD (X) amber and pEVOL-BpF [3]. Transformants were grown in XB (+ amp + Cm) till OD_{600} =0.5, and then we added 1 mM BPA, 0.05% (w/v) L-arabinose, and 1 mM

IPTG, incubated for 3 hr.

All of cells were pelleted by centrifugation at 6,000 x g for 10 min. Pellets were resuspended in Buffer A (150 mM NaCl, 20 mM NaPO4) and lysed via sonication. Lysates were clarified by low-speed centrifugation of 1100g for 10 min at 4°C. For BamB, BamC, and BamD (BPA variants too) proteins supernatants were again clarified by high-speed centrifugation, 17000 x *g* for 15 min at 4°C. High-speed clarified supernatants were then applied $Ni2^{+}$-NTA-affinity column chromatography. $Ni2^{+}$-NTA resin were washed by Buffer A with 50 mM imidazole and then proteins were eluted Buffer A containing an imidazole step gradient, with steps of 100, 150, 200, 250, 300, 350, and 400 mM. Peak fractions were collected as purified fraction. OmpC, low-speed pellets were resuspended in Buffer A containing 0.5% Triton X-100 and centrifuged for 11,600 x *g* for 10 min at 4°C. Pellets were denatured in Buffer A with 6 M urea and mixed at 25°C for 90 min, followed by centrifugation of 11,600 x *g* for 10 min at 4°C. Supernatant was then filtered through 0.45 μm cellulose acetate filter (ADVANTEC), and then applied Ni_2^{+}-NTA-affinity column chromatography. $Ni2^{+}$-NTA resin were washed by Buffer A with 6 M urea and 50 mM imidazole and then OmpC was eluted with Buffer A with 6M urea and 200 mM imidazole. Purified proteins were then diluted with glycerol to a final concentration of 10% snap frozen in liquid nitrogen and stored in -80°C. BamA was purified as detailed previously [4].

Isolation of *E.coli* Microsomal Membrane (EMM). *E. coli* crude membrane fraction were isolated as previously described [5]. In brief, pelleted cells were resuspended in sonication buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA) and lysed via sonication on ice. After low-speed centrifugation (1,100 x *g*, 5 min, 4°C), supernatant was centrifuged again (15,000 x *g*, 10 min, 4°C). Pelleted membranes were resuspended in SEM buffer (250 mM sucrose, 10 mM MOPS-KOH, pH 7.2, 1 mM EDTA) and flash frozen. Protein concentrations of EMM were calculated by OD_{280} measurements of 100 times diluted in 0.6% SDS (OD_{280}=0.21 was set to be 10 mg/ml).

EMM assembly assay. The EMM assembly assay was performed as previously described [5]. Substrates were prepared via *in vitro* transcription by SP6-RNA polymerase followed by in vitro translation in rabbit reticulocyte lysate supplemented with ^{35}S-methionine [6]. EMMs were resuspended in Assembly Assay buffer ([pH 7.2] 10 mM MOPS-KOH, 2.5 mM KH2PO4, 250 mM sucrose, 15 mM KCl, 5 mM MgCl2, 2 mM methionine, 5 mM DTT, 1% w/v BSA, 0.09% v/v Triton X-100), and incubated with ^{35}S-labelled substrate at 30°C for indicated time points. Assembly reactions were halted by

moving to ice for 5 minutes. The EMMs were then harvested via centrifugation at 15,0000 x *g* for 5 min at 4°C and washed with SEM buffer. After SEM buffer wash, trimeric protein assembled EMM pellets were solubilized in 1.5% DDM containing Blue native (BN)-PAGE Lysis Buffer (25 mM imidazole-HCl, pH 7.0, 50 mM NaCl, 50 mM 6-aminohexanoic acid, 1 mM EDTA, 7.5% (w/v) glycerol) on ice for 20 minutes. Solubilized proteins were clarified by centrifugation of 15,000g for 10 min at 4°C, followed by BN-PAGE analysis as described previously (Gunasinghe et al., 2018).

Peptide library was synthesized by ABclonal Biotechnology, and dissolved in DMSO at a concentration of 30 mM. The peptide inhibition screen of EspP was performed as stated above, but with the addition of 0.1 mM inhibitory peptide to the Assembly Assay buffer and incubated with EMMs for 5 minutes prior to the addition of ^{35}S-labelled substrate protein. Following harvesting EMMs were treated with 100 μg/mL PK on ice for 20 minutes. Digestion was halted by 2 mM PMSF. The EMMs were then harvested via centrifugation at 15,0000 x *g* for 5 min at 4°C and washed with SEM buffer. After SEM buffer wash, EspP assembled EMM proteins were analyzed by SDS-PAGE and radio-imaging. EMM assembly of trimeric proteins inhibited with the addition of 0.25 mM peptide and analyzed as above.

Sequence conservation analysis

For conservation analysis, we obtained homologous sequences of representative structure-known beta barrel outer membrane proteins from reference bacterial proteomes in UniProt (The UniProt Consortium, 2019) using phmmer and jackhammer HMMER 3.2.1 (http://hmmer.org). We basically used phmmer and used jackhammer if sufficient sequences cannot be collected. Best hit homologous sequences are collected in each organism having BamD homolog. We identified 31, 94, 25, 95, 19, 27, 44, 43, 23, 42, 187, 22, 132, 46, 58, 68, 39, 245, 261,115, 190 and 255 sequences for OmpA, OmpW, PagP, OmpX, OmpT, EspP, Hbp, NanC, OMPLA, Tsx, FadL, OmpG, OmpC, OmpF, PhoE, LamB, BtuB, Cir, FecA, FepA and FhuA, respectively (sequence identity less than 60%, expect for OmpX. In case of OmpX, sequence identity is less than 80% due to the small number of sequences). Also, homologous sequences of Tom40, Mdm10, VDAC1 and Sam50 are collected from reference eukaryotic proteomes in a similar way (86, 92, 77 and 136 sequences, respectively). Multiple alignments were generated by MAFFT [7] and then sequence logos of -5th transmembrane strands were created using WebLogo 3 [8].

***in vivo* assembly analysis by FLAG-OmpC.** We introduced a FLAG epitope tag into the N-terminal region of OmpC, immediately behind the cleavage site for the SEC-signal

sequence [9], for two reasons: (i) to distinguish between endogenous OmpC and the OmpC variants, since (ii) the use of OmpC-deletion strain for the experiment comes with issues of diminished OM integrity. The expression of FLAG-OmpC mutants was controlled by using a plasmid-based pBADara promoter cassette, to avoid the chronic effect of constitutively expressed mutant proteins (Figure 3A). We transformed pBAD-FLOmpC variants into MC4100A. Transformants were cultured in LB (+ amp) till OD_{600}=0.5 and then induced protein expression by 0.1% arabinose for 3 hr. *E. coli* cells were harvested and prepared total cell lysate or EMM.

Ni-NTA Substrate Pulldown. Here 50 μg of purified BAM complex protein was resuspended in 500 μL Buffer A (150 mM NaCl, 20 mM NaPO4) and incubated in the presence of DMSO or 0.5 mM inhibitor peptide for 12 hr with rotation at 4°C. After, 3 μL of radiolabelled OmpC was added to each tube and incubated on ice for 5 minutes, followed by the addition 25 μL Ni-NTA agarose beads with rotation at 4°C for 30 min. Beads were pelleted via centrifugation at 3000 x *g* for 30 seconds at 4°C and subsequently washed with 500 μL Buffer A containing 20 mM imidazole. Protein was eluted from the beads with 500 μL Buffer A containing 400 mM imidazole. Protein was precipitated via TCA precipitation and subjected to analysis by SDS-PAGE and imaged via phosphorimaging.

***In vitro* photo-crosslinking.** 32 μg of purified BamD-His_8 variants containing BPA were diluted up to 20 μL by Buffer A. 40 μL of radiolabeled OmpC was added to each tube and incubated at 25°C for 10 minutes in dark condition. Each mixture was transferred to lid of microtube and irradiated UV using B-100AP (UVP) for 7 min on ice. 10% (w/v) SDS solution was added to a final concertation of 1% at 95°C for 10 min to denature proteins. Protein mixtures were diluted with 0.5% TritonX-100 buffer (50 mM Tris-HCl pH 7.0, 150 mM NaCl, 0.5% Triton-X100), and purified with Ni-NTA agarose beads. Elute fractions were concentrated by TCA precipitation and analyzed by SDS-PAGE and radio-imaging.

Antibody Shift Assay. Antibody shift assay was performed as previously described with modification [10,11]. In brief, solubilized EMMs were incubated with 3 μL of anti-BamC antibodies for 45 min at 4°C. Samples were clarified via centrifugation at 13,000 x *g* for 10 minutes at 4°C, and subjected to BN-PAGE.

Urea Extraction of Intermediate. Assembly Assay was performed as stated above.

Following wash with SEM, the EMMs were resuspended in SEM with 6 M urea and incubated at 37°C for 1 hour, then 50 mM Tris HCL pH 8.0 was added. Membrane fractions were collected via high-speed centrifugation (100,000 x *g*, 45 min, 25°C), then washed with SEM buffer twice. Membrane pellets were solubilized for 20 minutes at 4°C in 1.5% DDM containing BN-PAGE lysis buffer and subjected to BN-PAGE analysis.

Neutron Reflectometry. Neutron reflection (NR) was carried out on the SOFIA reflectometer at the Materials and Life Science Experimental Facility (MLF) at J-PARC, Japan [12,13]. The reflectivity at the interface between the Si substrate and water solution was measured at 3 incident angles (0.3 °, 0.75 ° and 1.8°). The neutrons were introduced from the substrate side to illuminate the interface and the reflection intensity was normalized by the transmission intensity through the substrate to achieve the reflectivity with the Q range of up to 0.2 $Å^{-1}$.

Analysis of the NR profiles was performed using the MOTOFIT analysis software [14]. The software uses a conventional method, optical matrix method [15] to model the reflectivity profiles. Briefly, the layer is divided into several sublayers and then a characteristic matrix is evaluated for each sublayer, from which the whole reflectivity can be calculated exactly. The same layer with multiple datasets with three different isotopic contrasts is fitted simultaneously until the satisfactory fit is obtained (smallest χ^2 value in the genetic algorithm). All models include a 15 Å oxide layer on the surface of the silicon substrates and subsequently there are three layers of Ni, gold and Ni-NTA on the top of oxide layer. Protein layers will assemble outwards from the Ni-NTA layer.

The best-fit model from MOTOFIT gives information about the thickness, density and roughness of each layer. When a layer is composed of just two components, a chemical species or its fragment *s* and water *w*, the scattering length density (SLD) is given by:

$$\rho_{layer} = \phi\rho_s + (1-\phi)\rho_w$$

where ρ_w and ρ_s are the scattering length densities of the two components, respectively and φ is the volume fraction of species s in the layer. If more than one chemical species is present, then ρ_s is determined by the volume fraction of each species in the layer.

Statistical analysis. For all densitometry measurements we utilized “imagequant” quant (ImageQuant TL, cytiva) and statistical analysis and graphing was performed with Microsoft Excel software.

SUPPLEMENTAL TABLES

Supplementary Table 1: Strains used in this study.

E. coli strain name	**Genotype**	**Reference**
BL21 (DE3)*	F^-, ompT, hsdSB(rB^- mB^-), gal(λ cI 857, ind1, Sam7, nin5, lacUV5-T7gene1), dcm(DE3)	Invitrogen
MC4100A	F^- *lac*ΔU169 araD139 rpsL150 thi flbB5301 deoC7 ptsF25 relA1 ara+	[16]
BamD depletion strain	MC4100 ara+ P_{bamD}:: (kan, araC+ , P_{BAD})	This study

Supplementary Table 2: Plasmids for *in vitro* translation.

Plasmid name	Synthesized protein	Vector	Primers for construct	RE Site	Template DNA, source, or method
pTnT-EspP	EspP	pTnT	pTNTEspP-f / pTNTEspP-r	XhoI/XbaI	gblock, SLiCE
pTnT-OmpA	OmpA	pTnT	pTnTOmpA-f / pTnTOmpA-r	XhoI/XbaI	K-12 gene, SLiCE
pTnT-OmpC	OmpC	pTnT	--	XhoI/XbaI	(Gunasinghe et al., 2018)
pTNT-OmpF	OmpF	pTnT	pTnTOmpF-f / pTnTOmpF-r	XhoI/XbaI	K-12 gene, SLiCE
pTnT-LamB	LamB	pTnT	pTnTLamB-f / pTnTLamB-r	XhoI/XbaI	K-12 gene, SLiCE
pTnT-OmpC-W77A	OmpC W77A	pTnT	OmpCW77A-f / OmpCW77A-r		Quick change mutagenesis
pTnT-OmpC-E78A	OmpC E78A	pTnT	OmpCE78A-f / OmpCE78A-r		Quick change mutagenesis
pTnT-OmpC-E78K	OmpC E78K	pTnT	OmpCE78K-f / OmpCE78K-r		Quick change mutagenesis
pTnT-OmpC-Y79A	OmpC Y79A	pTnT	OmpCY79A-f / OmpCY79A-r		Quick change mutagenesis
pTnT-OmpC-K276E	OmpC K276E	pTnT	OmpCK276E-f / OmpCK276E-r		Quick change mutagenesis
pTnT-OmpC-F276A	OmpC F276A	pTnT	OmpCK276A-f / OmpCK276A-r		Quick change mutagenesis
pTnT-OmpC-F280A	OmpC F280A	pTnT	OmpCF280A-f / OmpCF280A-r		Quick change mutagenesis
pTnT-OmpC-F280V	OmpC F280V	pTnT	OmpCF280V-f / OmpCF280V-r		Quick change mutagenesis
pTnT-OmpC-E281A	OmpC E281A	pTnT	OmpCE281A-f / OmpCE281A-r		Quick change mutagenesis
pTnT-OmpC-E281K	OmpC E281K	pTnT	OmpCE281K-f / OmpCE281K-r		Quick change mutagenesis
pTnT-OmpC-Y286A	OmpC Y286A	pTnT	OmpCY286A-f / OmpCY286A-r		Quick change mutagenesis

pTnT-OmpC-P294A	OmpC P294A	pTnT	OmpCP294A-f / OmpCP294A-r		Quick change mutagenesis
pTnT-OmpC-Y325A	OmpC Y325A	pTnT	OmpCY325A-f / OmpCY325A-r		Quick change mutagenesis
pTnT-OmpC-Y365A	OmpC Y365A	pTnT	OmpCY365A-f / OmpCY365A-r		Quick change mutagenesis
pTnT-OmpC-β-sigAAA	OmpC G352A, L354A, F359A	pTnT	OmpCBsigAAA-f / OmpCBsigAAA-r		Quick change mutagenesis
pTnT-OmpF-V279A	OmpF V279A	pTnT	OmpFV279A-f / OmpFV279A-r		Quick change mutagenesis
pTnT-OmpF-Y285A	OmpF Y285A	pTnT	OmpFY285A-f / OmpFY285A-r		Quick change mutagenesis
pTnT-LamB-V333A	LamB V333A	pTnT	LamBV333A-f / LamBV333A-r		Quick change mutagenesis
pTnT-LamB-Y339A	LamB Y339A	pTnT	LamBY339A-f / LamBY339A-r		Quick change mutagenesis

Supplementary Table 3: Plasmids for recombinant protein expression.

Plasmid name	Expressed protein	Vector/Promoter	Primers for construct	RE site	Template DNA, source, or method
pET22-BamAL4H8	BamA His8 at loop4	pET22b/ T7	--		(Ding et al., 2020)
pHIS-BamB	His6-TEVsite-BamB	pHIS2-parallel/ T7	--		[17]
pHIS-BamC	His6-TEVsite-BamC	pHIS2-parallel/ T7	--		(Chen et al., 2021)
pHIS-BamD	His6-TEVsite-BamD	pHIS2-parallel/ T7	--		(Chen et al., 2021)
pET15b-OmpC	His6-TEVsite-OmpC	pET15b/ T7	pTnTOmpC-f / pTnTOmpC-r	XhoI/XbaI	K-12 gene, SLiCE
pET15b-OmpC Y286A	His6-TEVsite-BamD	pET15b/ T7	OmpCY286A-f / OmpCY286A-r		Quick change mutagenesis
pBAD-FLOmpC	FLAG-OmpC-FLAG	pBAD/ areBAD	FLAGOmpC1-f /FLAGOmpC1-r, FLAGOmpC2-f /FLAGOmpC2-r		K-12 gene, SLiCE
pBAD-FLOmpC F280A	FLAG-OmpC F280A	pBAD/ areBAD	OmpCF280A-f / OmpCF280A-r		pBAD-FLOmpC, Quick change mutagenesis
pBAD-FLOmpC Y286A	FLAG-OmpC Y286A	pBAD/ areBAD	OmpCY286A-f / OmpCY286A-r		pBAD-FLOmpC, Quick change mutagenesis
pBAD-FLOmpC F280A Y286A	FLAG-OmpC F280A Y286A	pBAD/ areBAD	OmpCY286A-f / OmpCY286A-r		pBAD-FLOmpC F280A Quick change mutagenesis
pHIS-BamD (X) amber	His6-TEVsite-BamD (X) BPA	pBAD/ areBAD	BamD(X)ambFwd/ BamD(X)ambRev		pHIS-BamD, Quick change mutagenesis

Supplementary Table 4: Plasmids for *in vivo* protein expression.

Plasmid name	Expressed protein	Vector/Promoter	Primers for construct	RE site	Template DNA, source, or method
pAp-BamD	BamD	pTnT/ BamA	BamDSL-f / BamDSL-r	XbaI/SalI	K-12 gene
pAp-BamD-His8	BamD-His8	pTnT/ BamA	BamDSL-f / BamDCHis8SL-r	XbaI/SalI	K-12 gene
pAp-BamD-His8 Y62A	BamD-His8 Y62A	pTnT/ BamA	BamDY62A-f / BamDY62A-r		pAp-BamD-His8, Quick change mutagenesis
pAp-BamD-His8 R197A	BamD-His8 R197A	pTnT/ BamA	BamDR197A-f / BamDR197A-r		pAp-BamD-His8, Quick change mutagenesis

Supplementary Table 5: Primers

Primer name	Sequence 5'->3'
pTnTl-f	ACTTAATACGACTCACTATAGGCTA
pTnT-r	GGATCCAAAAAACCCCTCAAGACCC
pSp64-f	ACCTTATGTATCATACACAT
pSp64-r	ACAGCTATGACATGATTACG
pTNTEspP-f	tgttctttttgcactcgagATGGATGTAACGCCGGTCATTAC
pTNTEspP-r	ccgcccgggtcgactctagaTCAGAACGAGTAACGGAAAT
pET15bOmpC-f	tgccgcgcggcagccatatgGAAGTTTACAACAAAGACGG
pET15bOmpC-r	ttagcagccggatcctcgagTTAGAACTGGTAAACCAGAC
pTnTOmpA-f	tgttctttttgcactcgagATGGCTCCGAAAGATAACACCTGGTACACTG
pTnTOmpA-r	ccgcccgggtcgactctagaTTAGAACTGGTAAACGATACCCACAGCAAC
pTnTOmpF-f	tgttctttttgcactcgagATGATGAAGCGCAATATTCTGGCAGTGATCG
pTnTOmpF-r	ccgcccgggtcgactctagaTTAGAACTGGTAAACGATACCCACAGCAACGGTGT
pTnTLamB-f	tgttctttttgcactcgagATGGTTGATTTCCACGGCTATGCACGTTCCGGTATTGGTTG
pTnTLamB-r	ccgcccgggtcgactctagaTTACCACCAGATTTCCATCTGGGCA
OmpCW77A-f	GACCGGTTACGGCCAGgcaGAATATCAGATCCAGG
OmpCW77A-r	CCTGGATCTGATATTCtgcCTGGCCGTAACCGGTC
OmpCE78A-f	CGGTTACGGCCAGTGGgcaTATCAGATCCAGGGCA
OmpCE78A-r	TGCCCTGGATCTGATAtgcCCACTGGCCGTAACCG
OmpCE78K-f	CGGTTACGGCCAGTGGaaaTATCAGATCCAGGGCA
OmpCE78K-r	TGCCCTGGATCTGATAtttCCACTGGCCGTAACCG
OmpCY79A-f	TTACGGCCAGTGGGAAgcaCAGATCCAGGGCAACA
OmpCY79A-r	TGTTGCCCTGGATCTGtgcTTCCCACTGGCCGTAA
OmpCK276A-f	CCTGGGTTGGGCGAACgcaGCACAGAACTTCGAAG
OmpCK276A-r	CTTCGAAGTTCTGTGCtgcGTTCGCCCAACCCAGG
OmpCK276E-f	CCTGGGTTGGGCGAACgaaGCACAGAACTTCGAAG
OmpCK276E-r	CTTCGAAGTTCTGTGCttcGTTCGCCCAACCCAGG
OmpCF280A-f	GAACAAAGCACAGAACgcaGAAGCTGTTGCTCAGT
OmpCF280A-r	ACTGAGCAACAGCTTCtgcGTTCTGTGCTTTGTTC
OmpCF280V-f	AACAAAGCACAGAACgtgGAAGCTGTTGCTCAG
OmpCF280V-r	CTGAGCAACAGCTTCcacGTTCTGTGCTTTGTT
OmpCE281A-f	CAAAGCACAGAACTTCgcaGCTGTTGCTCAGTACC
OmpCE281A-r	GGTACTGAGCAACAGCtgcGAAGTTCTGTGCTTTG
OmpCE281K-f	CAAAGCACAGAACTTCaaaGCTGTTGCTCAGTACC

Primer name	Sequence 5'->3'
OmpCE281K-r	GGTACTGAGCAACAGCtttGAAGTTCTGTGCTTTG
OmpCY286A-f	CGAAGCTGTTGCTCAGgcaCAGTTCGACTTCGGTC
OmpCY286A-r	GACCGAAGTCGAACTGtgcCTGAGCAACAGCTTCG
OmpCP294A-f	CGACTTCGGTCTGCGTgcaTCCCTGGCTTACCTGC
OmpCP294A-r	GCAGGTAAGCCAGGGAtgcACGCAGACCGAAGTCG
OmpCY325A-f	GATGTTGGTGCTACCgcgTACTTCAACAAAAAC
OmpCY325A-r	GTTTTTGTTGAAGTAcgcGGTAGCACCAACATC
OmpCY365A-f	AGCTCTGGGTCTGGTTgccCAGTTCTAAGTCGA
OmpCY365A-r	TCGACTTAGAACTGggcAACCAGACCCAGAGCT
OmpCBsigAAA-f	CTGGGTCTGGTTgccgcggcgTAAGTCGACCCGGGC
OmpCBsigAAA-r	GCCCGGGTCGACTTAcgccgcggcAACCAGACCCAG
OmpFV279A-f	AACAAAACGCAAGACgcgCTGTTAGTTGCGCAA
OmpFV279A-r	TTGCGCAACTAACAGcgcGTCTTGCGTTTTGTT
OmpFY285A-f	CTGTTAGTTGCGCAAgcgCAGTTCGATTTCGGT
OmpFY285A-r	ACCGAAATCGAACTGcgcTTGCGCAACTAACAG
LamBV333A-f	ACCAAGTGGTGGACCgcgGGTATTCGCCCGATG
LamBV333A-r	CATCGGGCGAATACCcgcGGTCCACCACTTGGT
LamBY339A-f	GGTATTCGCCCGATGgcgAAGTGGACGCCAATC
LamBY339A-r	GATTGGCGTCCACTTcgcCATCGGGCGAATACC
FLAGOmpC1-f	aacaggaggaattaaccATGAAAGTTAAAGTACTGTC
FLAGOmpC1-r	cttatcatcatcatccttataatcAGCAGCGTTTGCTGCGCCTG
FLAGOmpC2-f	gattataaggatgatgatgataagGAAGTTTACAACAAAGACGG
FLAGOmpC2-r	TACCAGCTGCAGATCTCGAGtcgacTTAGAACTGGTAAACCAGAC

Supplementary Table 6: Primers for BPA introduction into BamD

X	BamD(X)ambFwd	BamD(X)ambRev
27	GGGGTCAAAGGAAGAAtagCCTGATAATCCGCCAA	TTGGCGGATTATCAGGctaTTCTTCCTTTGACCCC
41	TTACGCGACTGCACAAtagAAGCTGCAGGACGGTA	TACCGTCCTGCAGCTTctaTTGTGCAGTCGCGTAA
43	GACTGCACAACAAAAGtagCAGGACGGTAACTGGA	TCCAGTTACCGTCCTGctaCTTTTGTTGTGCAGTC
49	GCAGGACGGTAACTGGtagCAGGCAATAACGCAAC	GTTGCGTTATTGCCTGctaCCAGTTACCGTCCTGC
53	CTGGAGACAGGCAATAtagCAACTGGAAGCGTTAG	CTAACGCTTCCAGTTGctaTATTGCCTGTCTCCAG
60	ACTGGAAGCGTTAGATtagCGCTATCCGTTTGGTC	GACCAAACGGATAGCGctaATCTAACGCTTCCAGT
62	AGCGTTAGATAATCGCtagCCGTTTGGTCCGTATT	AATACGGACCAAACGGctaGCGATTATCTAACGCT
64	AGATAATCGCTATCCGtagGGTCCGTATTCGCAGC	GCTGCGAATACGGACCctaCGGATAGCGATTATCT
65	TAATCGCTATCCGTTTtagCCGTATTCGCAGCAGG	CCTGCTGCGAATACGGctaAAACGGATAGCGATTA
70	TGGTCCGTATTCGCAGtagGTGCAGCTGGATCTCA	TGAGATCCAGCTGCACctaCTGCGAATACGGACCA
77	GCAGCTGGATCTCATCtagGCCTACTATAAAAACG	CGTTTTTATAGTAGGCctaGATGAGATCCAGCTGC
79	GGATCTCATCTACGCCtagTATAAAAACGCCGATT	AATCGGCGTTTTTATActaGGCGTAGATGAGATCC
89	CGATTTGCCGTTAGCAtagGCTGCCATCGATCGTT	AACGATCGATGGCAGCctaTGCTAACGGCAAATCG
98	CGATCGTTTTATTCGCtagAACCCGACCCATCCGA	TCGGATGGGTCGGGTTctaGCGAATAAAACGATCG
101	TATTCGCCTTAACCCGtagCATCCGAATATCGATT	AATCGATATTCGGATGctaCGGGTTAAGGCGAATA
105	CCCGACCCATCCGAATtagGATTATGTCATGTACA	TGTACATGACATAATCctaATTCGGATGGGTCGGG
109	GAATATCGATTATGTCtagTACATGCGTGGCCTGA	TCAGGCCACGCATGTActaGACATAATCGATATTC
114	CATGTACATGCGTGGCtagACCAATATGGCGCTGG	CCAGCGCCATATTGGTctaGCCACGCATGTACATG
117	GCGTGGCCTGACCAATtagGCGCTGGATGACAGTG	CACTGTCATCCAGCGCctaATTGGTCAGGCCACGC
121	CAATATGGCGCTGGATtagAGTGCGCTGCAAGGGT	ACCCTTGCAGCGCACTctaATCCAGCGCCATATTG
124	GCTGGATGACAGTGCGtagCAAGGGTTCTTTGGCG	CGCCAAAGAACCCTTGctaCGCACTGTCATCCAGC
136	CGATCGTAGCGATCGCtagCCTCAACATGCACGAG	CTCGTGCATGTTGAGGctaGCGATCGCTACGATCG
138	TAGCGATCGCGATCCTtagCATGCACGAGCTGCGT	ACGCAGCTCGTGCATGctaAGGATCGCGATCGCTA
148	TGCGTTTAGTGACTTTtagAAACTGGTGCGCGGCT	AGCCGCGCACCAGTTTctaAAAGTCACTAAACGCA
149	GTTTAGTGACTTTTCCtagCTGGTGCGCGGCTATC	GATAGCCGCGCACCAGctaGGAAAAGTCACTAAAC
153	TTCCAAACTGGTGCGCtagTATCCGAACAGTCAGT	ACTGACTGTTCGGATActaGCGCACCAGTTTGGAA
158	CGGCTATCCGAACAGTtagTACACCACCGATGCCA	TGGCATCGGTGGTGTActaACTGTTCGGATAGCCG
164	GTACACCACCGATGCCtagAAACGTCTGGTATTCC	GGAATACCAGACGTTTctaGGCATCGGTGGTGTAC
168	TGCCACCAAACGTCTGtagTTCCTGAAAGATCGTC	GACGATCTTTCAGGAActaCAGACGTTTGGTGGCA
176	GAAAGATCGTCTGGCGtagTATGAATACTCCGTGG	CCACGGAGTATTCATActaCGCCAGACGATCTTTC
181	GAAATATGAATACTCCtagGCCGAGTACTATACAG	CTGTATAGTACTCGGCctaGGAGTATTCATATTTC
184	ATACTCCGTGGCCGAGtagTATACAGAACGTGGCG	CGCCACGTTCTGTATActaCTCGGCCACGGAGTAT

X	BamD(X)ambFwd	BamD(X)ambRev
186	CGTGGCCGAGTACTATtagGAACGTGGCGCATGGG	CCCATGCGCCACGTTCctaATAGTACTCGGCCACG
192	AGAACGTGGCGCATGGtagGCCGTCGTTAACCGCG	CGCGGTTAACGACGGCctaCCATGCGCCACGTTCT
196	ATGGGTTGCCGTCGTTtagCGCGTAGAAGGCATGT	ACATGCCTTCTACGCGctaAACGACGGCAACCCAT
200	CGTTAACCGCGTAGAAtagATGTTGCGCGACTACC	GGTAGTCGCGCAACATctaTTCTACGCGGTTAACG
204	AGAAGGCATGTTGCGCtagTACCCGGATACCCAGG	CCTGGGTATCCGGGTActaGCGCAACATGCCTTCT
232	GATGAATGCGCAAGCTtagAAAGTAGCGAAAATCA	TGATTTTCGCTACTTTctaAGCTTGCGCATTCATC
233	GAATGCGCAAGCTGAAtagGTAGCGAAAATCATCG	CGATGATTTTCGCTACctaTTCAGCTTGCGCATTC
237	TGAAAAAGTAGCGAAAtagATCGCCGCAAACAGCA	TGCTGTTTGCGGCGATctaTTTCGCTACTTTTTCA

Supplementary Table 7: Structural Data of this study

Protein Name	PDB ID	PDB DOI	Citation
OmpX	1QJ8	10.2210/pdb1QJ8/pdb	[18]
OmpA	1BXW	10.2210/pdb1BXW/pdb	[19]
OmpW	2F1T	10.2210/pdb2F1T/pdb	[20]
PagP	3GP6	10.2210/pdb3GP6/pdb	[21]
OmpT	1I78	10.2210/pdb1I78/pdb	[22]
EspP	2QOM	10.2210/pdb2QOM/pdb	[23]
HBP	3AEH	10.2210/pdb3AEH/pdb	[24]
NanC	2WJQ	10.2210/pdb2WJQ/pdb	[25]
PldA/OmpLA	1QD5	10.2210/pdb1QD5/pdb	[26]
Tsx	1TLW	10.2210/pdb1TLW/pdb	[27]
FadL	1T16	10.2210/pdb1T16/pdb	[28]
OmpC	2J1N	10.2210/pdb2J1N/pdb	[29]
PhoE	1PHO	10.2210/pdb1PHO/pdb	[30]
OmpF	1BT9	10.2210/pdb1BT9/pdb	[31]
LamB	1MPM	10.2210/pdb1MPM/pdb	[32]
BtuB	1NQE	10.2210/pdb1NQE/pdb	[33]
CirA	2HDI	10.2210/pdb2HDI/pdb	[34]
FhuA	1BY3	10.2210/pdb1BY3/pdb	[35]
FepA	1FEP	10.2210/pdb1FEP/pdb	[36]
FecA	1KMO	10.2210/pdb1KMO/pdb	[37]
PapC	2VQI	10.2210/pdb2VQI/pdb	[38]
yeast-Tom40	6ucu	10.2210/pdb6UCU/pdb	[39]
yeast-Mdm10	7btx	10.2210/pdb7BTX/pdb	[40]
human-VDAC1	3EMN	10.2210/pdb3EMN/pdb	[41]
yeast-Sam50	7btx	10.2210/pdb7BTX/pdb	[40]

Supplementary Table 8: Characterization of BamA molecules in the membrane layer

Layer	t (Å)	SLD ($\times 10^{-6}$ Å^{-2})			Φ (%)			σ (Å)
		D_2O	GMW5	H_2O	BamA	POPC	Solution	
Cr	79.7±0.8	3.03	3.03	3.00	-	-	-	12.1±0.2
Au	251.8±5.1	3.88	3.79	3.40	-	-	-	13.0±0.1
NTA	8.0±0.7	5.00	4.28	0.43	-	-	66.5±3.5	10.0±0.1
His_6	6.0±0.5	4.94	4.46	2.50	-	-	25.3±7.4	4.0±0.1
β-Barrel	56.9±1.5	3.88	2.93	0.18	17.3±0.6	35.1±1.6	47.6±2.9	10.1±0.1
P3-5	29.5±0.8	5.96	4.48	-0.29	10.7±0.9	3.4±1.7	85.9±2.6	7.0±0.1
P1-2	31.2±0.8	6.14	4.73	-0.30	11.2±0.8	-	90.0±2.5	13.8±0.1

t: thickness; SLD: scattering length density; Φ: volume fraction; σ: roughness; P3-5: POTRA3, POTRA4 and POTRA5; P1-2: POTRA1 and POTRA 2.

Supplementary Table 9: Characterization of BamAD molecules in the membrane layer

Layer	t (Å)	SLD ($\times 10^{-6}$ Å^{-2})			Φ (%)				σ (Å)
		D_2O	GMW5	H_2O	BamA	POPC	BamD	Solution	
Cr	78.5±2.6	3.09	3.01	3.00	-	-	-	-	10.0±0.1
Au	254.6±3.6	3.88	3.87	3.60	-	-	-	-	13.1±0.1
NTA	9.6±1.0	5.24	4.05	0.46	-	-	-	64.8±5.9	10.1±0.1
His_6	7.1±0.8	5.00	4.44	2.50	-	-	-	25.8±7.0	4.0±0.1
β-Barrel	58.7±0.4	3.94	2.56	0.18	18.4±1.1	35.4±5.5	-	46.2±6.7	12.6±1.8
P3-5	29.7±0.8	5.41	4.36	-0.11	11.0±0.8	3.3±0.5	8.8±1.1	76.9±2.0	6.1±0.1
P1-2	30.3±0.4	6.09	4.66	-0.30	11.2±2.9	-	-	88.8±2.9	13.7±0.1

t: thickness; SLD: scattering length density; Φ: volume fraction; σ: roughness; P3-5: POTRA3, POTRA4 and POTRA5; P1-2: POTRA1 and POTRA 2.

Supplementary Table 10: Characterization of BamAD-OmpC molecules in the membrane layer

Layers[a]	t (Å)	SLD ($\times 10^{-6}$ Å^{-2})			Φ (%)					σ (Å)
		D_2O	GMW5.3	H_2O	BamA	POPC	BamD	OmpC	Solution	
Cr	79.8±3.4	3.08	3.07	3.02	-	-	-	-	-	16.6±2.4
Au	253.0±5.6	3.85	3.72	3.69	-	-	-	-	-	15.3±1.7
NTA	9.8±1.5	5.28	4.20	0.45	-	-	-	-	68.3±2.4	9.9±0.1
His_6	9.4±0.5	4.86	4.37	2.31	-	-	-	-	22.7±1.7	4.0±0.1
β-Barrel	56.8±1.5	3.78	2.82	0.22	18.3±1.2	35.9±1.5	-	-	45.8±2.7	6.0±0.1
P3-5	42.5±1.6	5.81	4.54	-0.09	11.7±2.9	3.3±0.9	9.0±3.3	8.0±1.2	76.0±7.3	12.1±0.1
P1-2	28.5±0.9	6.09	4.82	-0.28	10.7±0.9	-	-	-	89.3±0.9	5.0±0.1

t: thickness; SLD: scattering length density; Φ: volume fraction; σ: roughness; P3-5: POTRA3, POTRA4 and POTRA5; P1-2: POTRA1 and POTRA 2.

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