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Fitting the Pieces of the β-Barrel Assembly Machinery Complex

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Supporting Information

ABSTRACT: β-Barrel membrane proteins are found in the outer membranes of mitochondria, chloroplasts, and Gram-negative bacteria; however, exactly how they are folded and inserted remains unknown. Over the past decade, both functional and structural studies have greatly contributed to addressing this elusive mechanism. It is known that a conserved core machinery is required for each organelle, though the overall composition varies significantly. The vast majority of studies that aimed to understand the biogenesis of β-barrel membrane proteins has been conducted in Gram-negative bacteria. Here, it is the task of a multicomponent complex known as the β-barrel assembly machinery (BAM) complex to fold and insert new β-barrel membrane proteins into the outer membrane. In this review, we will discuss recent discoveries with the goal of utilizing all reported structural and functional studies to piece together a current structural model for the fully assembled BAM complex.

The two types of fully integrated membrane proteins are those with either an α-helical or β-barrel fold. Both can serve many functions within the membrane, including nutrient import and export, signaling, motility, and adhesion. While α-helical membrane proteins can be found in nearly all membranes, β-barrel membrane proteins can be found only within the outer membranes (OM) of mitochondria, chloroplasts, and Gram-negative bacteria.[1−6] Despite their essential functions, exactly how these β-barrel outer membrane proteins (OMPs) are folded and inserted into the membrane remains unknown. Recent work has, however, identified conserved complexes responsible for the biogenesis of OMPs.[1,3−5,7,8] In Gram-negative bacteria, a multicomponent complex known as the β-barrel assembly machinery (BAM) complex was identified and isolated and later shown using *in vitro* assays to be necessary and sufficient for mediating the folding and insertion of OMPs into the OM.[9−12] Orthologous systems are also found in both mitochondria and chloroplasts, which share a conserved core machinery with Gram-negative bacteria.[1,3−6,8]

In Gram-negative bacteria, nascent OMPs are synthesized in the cytoplasm and transported across the inner membrane (IM) into the periplasm by the Sec translocon[13−16] (Figure 1). The periplasmic chaperones SurA and Skp then interact with the nascent OMPs and further escort them to the inner surface of the OM, delivering them to the BAM complex.[17−21] The BAM complex then folds and inserts the nascent OMPs into the OM. The exact mechanism for how the BAM complex accomplishes its role at the OM is still not well understood; however, recent studies have provided many invaluable clues. The BAM complex is composed of five components, including BamA, an OMP itself, and four lipoproteins called BamB, BamC, BamD, and BamE. Studies have shown that BamB and BamD interact directly with the periplasmic domain of BamA while BamC and BamE interact directly with BamD. Of the five components of the BAM complex, only BamA and BamD are necessary for viability[22] with BamB, -C, and -E single deletions affecting OMP content or OM permeability and susceptibility to certain antibiotics.[9,23,24] In this review, we focus on recent studies to help us piece together the first complete structural model of the fully assembled BAM complex. Our hope is that this model (Supplementary Model 1) will assist others in their studies and help in determining the true mechanism for the biogenesis of OMPs in Gram-negative bacteria.

BAMAB COMPLEX CRYSTAL STRUCTURE

BamA is the only fully integrated component of the BAM complex and is composed of an N-terminal periplasmic domain consisting of five polypeptide transport-associated (POTRA) repeats and a C-terminal 16-stranded β-barrel domain. Studies have shown that interaction with the other Bam components is mediated by the POTRA domains.[9,25] Crystal structures of the POTRA domains were the first structures of the BAM complex to be determined,[25−28] and recently, structures of the membrane-inserted β-barrel domain were determined,[29,30]

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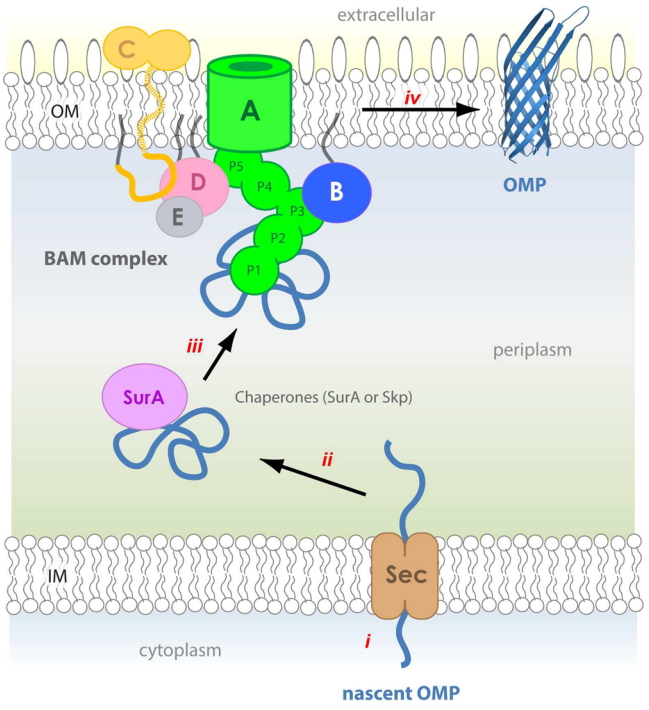

Figure 1. Biogenesis of β-barrel outer membrane proteins in Gram-negative bacteria. Nascent β-barrel outer membrane proteins (OMPs) are first synthesized in the cytoplasm and then transported across the inner membrane (IM) into the periplasm by the Sec translocon (*i*). Chaperones SurA or Skp then bind the OMPs (*ii*) and further escort them to the BAM complex located in the OM (*iii*). The BAM complex then folds and inserts the OMPs into the OM (*iv*).

including a full length construct from *Neisseria gonorrheae*.[31] Subsequent studies indicated that the β-barrel domain of BamA requires lateral opening into the membrane for function, which is mediated by a separation of strands β1 and β16.[32,33] However, whether substrate actually passes through the lateral gate during folding and/or insertion remains to be determined. While the structure of BamA has been determined with the POTRA domains in an open and closed state, here we will depict our models of BamA in the open state, which would allow barrel access to OMP substrates.

BamB is a lipoprotein, attached to the periplasmic side of the outer membrane by its N-terminal (NT) lipid anchor.[9,34,35] Although not essential for viability, deletions of BamB result in significant membrane defects as well as hypersensitivity to some antibiotics,[34] indicating an important role for BamB in membrane protein biogenesis. However, the molecular mechanism of BamB action is still elusive. The structure of BamB from *Escherichia coli* has been determined,[36−40] revealing an eight-bladed β propeller, with each propeller composed of four antiparallel β-strands consisting of WD40-like repeats. BamB is wider at one end than the other, giving the protein an overall wedge shape. The narrower end also has the longer loops housing some of the most conserved residues of BamB. The contacts observed in the crystal packing hinted that BamB may bind to unfolded OMPs by β-augmentation. This interaction was first observed in the crystal packing of the soluble POTRA domains[25,26,41] of BamA, suggesting that this may be a general method by which the components of the BAM complex interact with nascent polypeptide chains of substrate OMPs. However, this idea has yet to be fully accepted because crystal contacts are often mediated by $\beta-\beta$ interactions in crystallography and because few experimental data have yet been reported in support. If this is the case though, the eight-bladed β-propeller of BamB will provide a large surface area and multiple binding sites for the β-strands of OMP substrates.

Genetic and biochemical studies indicate that of all four lipoproteins in *E. coli*, only BamB and BamD interact directly with BamA.[22,35] Mutations that disrupt the BamA−BamB interaction have a phenotype similar to that of BamB deletion, suggesting the function of BamB is dependent on its ability to

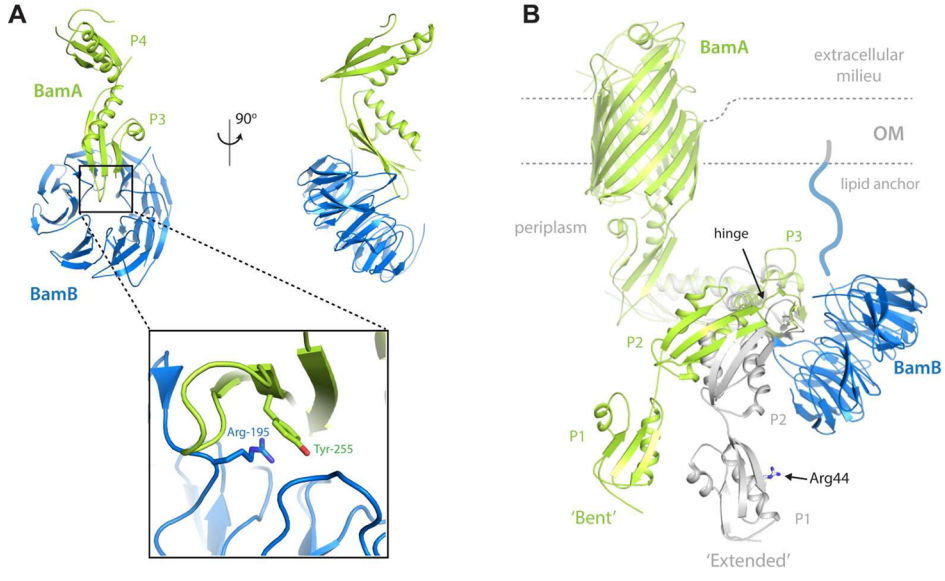

Figure 2. BamAB complex crystal structure. (A) Cocrystal structure of a fusion of POTRA3−5 of BamA (green) with BamB (blue) [Protein Data Bank (PDB) entry 4PK1]. The inset highlights an important interaction between Tyr255 of BamA and Arg195 of BamB. (B) Superposition of the BamAB crystal structure with the full length structure of BamA from *N. gonorrheae* ("bent") (PDB entry 4K3B) and the crystal structure of a soluble fragment of POTRA1−4 representing the "extended" conformation (gray) (PDB entry 3EFC). Residue Arg44 of BamA is shown as sticks, which has been shown to be important for the interaction with SurA.

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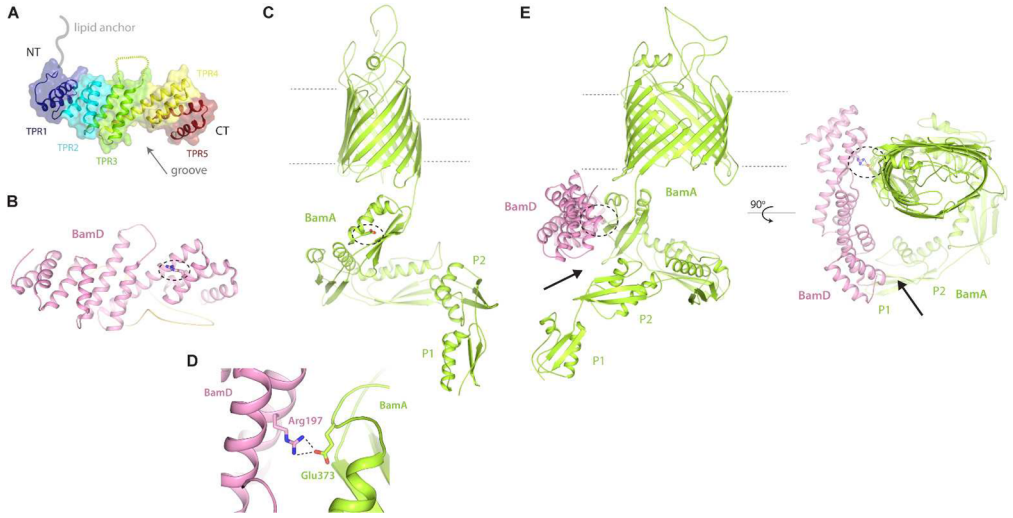

Figure 3. Modeling the BamA−BamD interaction interface. (A) Structure of BamD (PDB entry 3Q5M) with each tetratricopeptide repeat (TPR) colored from blue to red, with the disordered loop in TPR3 shown as a dashed line. (B) Structure of BamD (pink) (PDB entry 3Q5M) with residue Arg197 shown as sticks (dashed circle). BamC is shown as a gold ribbon and interacts with the majority of the opposite side of BamD. (C) Model of full length BamA (green ribbon) in the open conformation with residue Glu373 of POTRA5 shown as sticks (dashed circle). (D) Modeled interaction between BamA (green) and BamD (pink) that is hypothesized to be mediated by residues Glu373 (BamA) and Arg197 (BamD) via a putative salt bridge interaction. (E) Membrane view of the model of the BamAD complex with BamA rotated 90° clockwise along the *y*-axis with respect to panel A. BamD is positioned parallel to the membrane with the putative salt bridge indicated by a dashed circle. The right panel shows the extracellular view that is rotated 90° along the *x*-axis. The black arrows indicate the site of a putative interaction between BamD and POTRA2.

bind to BamA.[9,35,40] Copurification and site specific cross-linking identified residues on BamB that are involved in this interaction,[35] and these residues map onto two conserved loops between propellers. Pull-down experiments with POTRA deletions of BamA indicated POTRA2−5 are important for binding to BamB and mutation of residues in POTRA3 abolishes binding to BamB altogether.[25]

To understand the nature of the interaction between BamA and BamB at the molecular level, a crystal structure of a fusion construct of the two proteins was recently obtained (Figure 2).[42] Fusion constructs between BamB and the soluble periplasmic domains of BamA (fragments lacking the membrane domain) were required to create stable complexes of soluble BamAB. Although fusions of POTRA1−5, -3−5, or -4−5 were attempted, crystals were obtained for the POTRA3−5 fusion only. In the electron density resulting from these crystals, POTRA3 and -4 were visible, but there was no density for POTRA5, suggesting it was not ordered. The structure shows the interaction is mediated between POTRA3 of BamA and specific residues in the extended loop of BamB. The POTRA domain sits across the face of one of the blades of the BamB propeller and points a loop into the center of the BamB cylinder. Numerous loops of BamB undergo changes upon binding to BamA, including a relatively large (11 Å) movement of the conformationally flexible loop 17, suggesting an "induced fit" mechanism for BamB binding BamA. The interaction is stabilized by numerous hydrogen bonds between the two well-packed surfaces, as well as a critical cation−π interaction between the guanidinium group of Arg195 of BamB and the aromatic ring of Tyr255 of BamA (Figure 2A).

The structure is consistent with previous studies identifying residues of BamB that were important for BamA binding.[25,35] To support the interactions observed in the structure, disulfide engineering was conducted to cross-link cysteine residues within the interface and by a complementary pull-down approach in which a BamA depletion strain was used to demonstrate that mutation of the Tyr255 cation−π interaction destroyed complex formation.

Recent biochemical data using mutated and/or deleted components of the BAM complex suggest that *in vivo*, a surface-exposed loop of BamA undergoes conformational cycling during OMP insertion.[43] Furthermore, movement in the BamA POTRA domains around a hinge region between POTRA2 and -3 has been suggested on the basis of comparison of the available crystal structures and SAXS data of the BamA POTRA domains.[26,28] This movement would allow the POTRAs to extend from a "bent" fishhook-like conformation to a more "extended" conformation and may form part of a conformational cycle in BamA. Another study, however, which used ssNMR to look at full length BamA alone in lipid bilayers, suggested that the POTRA domains have limited flexibility, although it remains to be determined what effect sample preparation may have played here.[44] Still, it will be interesting to see the dynamics of the POTRA domains in the context of the entire BAM complex. It could be that the presence of the lipoproteins more efficiently catalyzes conformational changes within the POTRA domains of BamA, possibly explaining the difference in the efficiency of folding of certain OMPs when BamB is present.[24,34,45] Superimposing the structures along POTRA3 of the BamAB complex with full length BamA[31] and with the "extended" structure of POTRA1−4[25,26] shows BamB to be positioned near the POTRA2−3 hinge region (Figure 2B). When BamA is in the extended conformation, BamB may now be able to interact with POTRA2 and is therefore bound in a prime location within the complex to allow it to regulate the movement of the BamA POTRAs around the hinge[42] during a conformational cycle.

INTERACTIONS OF BAMD WITH THE POTRA DOMAIN(S) OF BAMA

BamD is the only essential lipoprotein of the BAM complex and interacts directly with BamA. BamD also interacts with the

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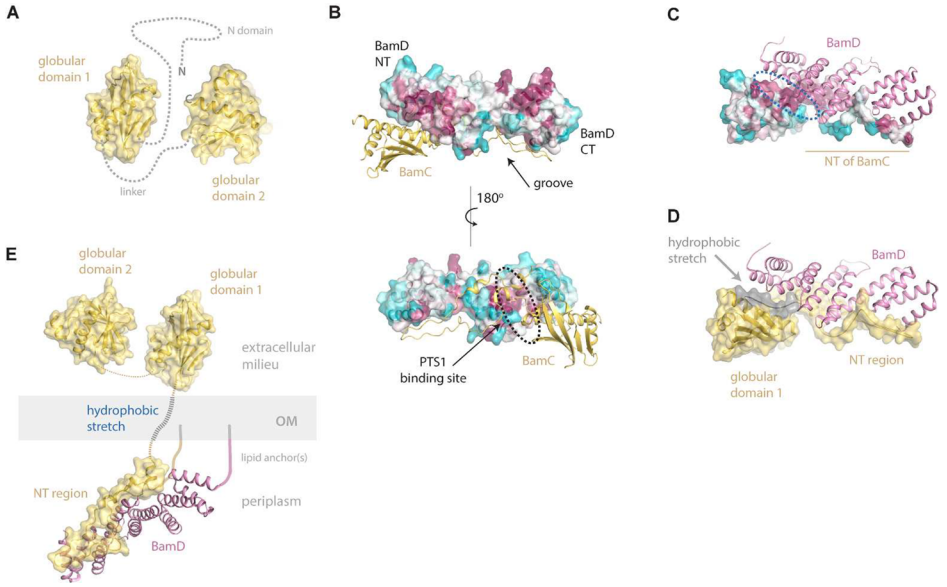

Figure 4. Structures of BamC, BamD, and the BamCD complex. (A) Structures of BamC (PDB entries 2YH6 and 2YH5) showing each globular domain. Dashed lines (gray) indicate the locations of the unstructured regions (N-terminal domain and linker). (B) BamCD structure (PDB entry 3TGO) with conservation analysis of BamD mapped onto the surface [highly conserved (maroon) to nonconserved (blue)] and BamC shown as a gold ribbon. The PTS1 binding site based on structural homology to the PEX5 structure is circled in black dashes on the back face of the BamCD structure. (C) Conservation analysis of BamC mapped onto the BamC surface with BamD show as a pink ribbon. The hydrophobic stretch of BamC is indicated by a blue dashed circle and in gray surface in panel D. (E) Model for how full length BamC may cross the bacterial OM. The N-terminal domain is anchored to the inner leaflet of the OM and interacts with BamD (pink), while the hydrophobic stretch of residues (thick gray striped line) mediates surface exposure of globular domains 1 and 2 (gold).

nonessential components BamC and BamE, which themselves have not been shown to directly bind BamA.[9,22,23] The crystal structures of BamD from *E. coli* and *Rhodothermus marinus* have been previously determined.[46−48] Both structures are highly similar to one another and reveal BamD is composed of an extended arrangement of five tetratricopeptide repeat (TPR) domains (Figure 3A). TPR domains are small helix−turn−helix motifs in which the α-helices are packed in an antiparallel conformation.[49] TPR tandems are present in other proteins, such as Tom70,[50] Hsp protein,[51,52] and PEX-5,[53] where the concave face formed from the TPR domains repeats is used to recruit their partner proteins by binding their short C-terminal (CT) peptide segments in extended conformations. Given BamD has been shown to bind to unfolded OMP substrates[54] and the interaction is dependent on the putative "β-signal" sequence at the CT of OMPs,[55] the TPR domains in BamD may bind this short signal sequence by the mechanism observed in these other TPR-containing proteins. Indeed, on the basis of structural similarity to a signal receptor involved in peroxisomal targeting, PEX5, which binds to a short peptide targeting sequence PTS1, the pocket in BamD formed between TPR1 and -2 was predicted to be involved in binding the CT targeting sequence of unfolded OMP substrates (Figure 4B).

Systematic POTRA deletions suggest that POTRA5 is required for the interaction of BamA with BamD.[25] However, there is little information available for the molecular basis of how BamD interacts with the POTRA domains of BamA. Primary work to study the interaction of BamD with BamA comes from genetic studies that identified a suppressor mutant of BamD, R197L, which could alleviate the conditional lethality of a BamA mutant, E373K[43,56] (Figure 3B,C). These studies led to the idea that the interaction of BamA with BamD may be mediated by a salt bridge (Figure 3D) and that disruption of this salt bridge might be ablating the BamA−BamD interaction, thereby leading to lethality. However, despite rescuing the normal phenotype, the suppressor mutant R197L of BamD could not be pulled down with the E373K mutant of BamA, giving rise to the hypothesis that these Bam components may need to be somehow activated to function properly.[43,56] Given that BamD is elongated, it is expected that it may lie in parallel with the membrane in the proximity of POTRA5 to form the putative salt bridge (Figure 3E). Other interactions between POTRA5 and BamD are almost certainly contributing, and other orientations of BamD are possible. In our model, TPR1 of BamD is in the relative proximity of the POTRA domains, particularly POTRA2, such that it is possible BamD may interact with other regions of the periplasmic domain of BamA.

BAMCD COMPLEX CRYSTAL STRUCTURE

Both lipoproteins BamC and BamE require BamD to copurify with BamA because they have no intrinsic affinity for BamA.[22,25] The BamC−BamD interaction has been shown to require at least the CT of the region of BamD.[22] An unusual feature of BamD is how the first three TPR repeats are offset from the last two, making for a highly extended molecule that presents a large surface area to interact with the various partner proteins in the BAM complex. The offset between the TPR repeats also produces a large groove on the BamD surface (Figures 3A and 4B).

BamC is composed of three main domains. At the extreme NT of the protein is a region of ∼75 amino acids that is disordered in solution followed by two globular helix-grip domains, separated from each other by a short flexible linker (Figure 4A). The structures of the NT and CT helix grip domains have been determined by X-ray crystallography and solution NMR.[39,57,58] Surprisingly, neither of these domains appears to be required for the interaction of BamC with BamD.

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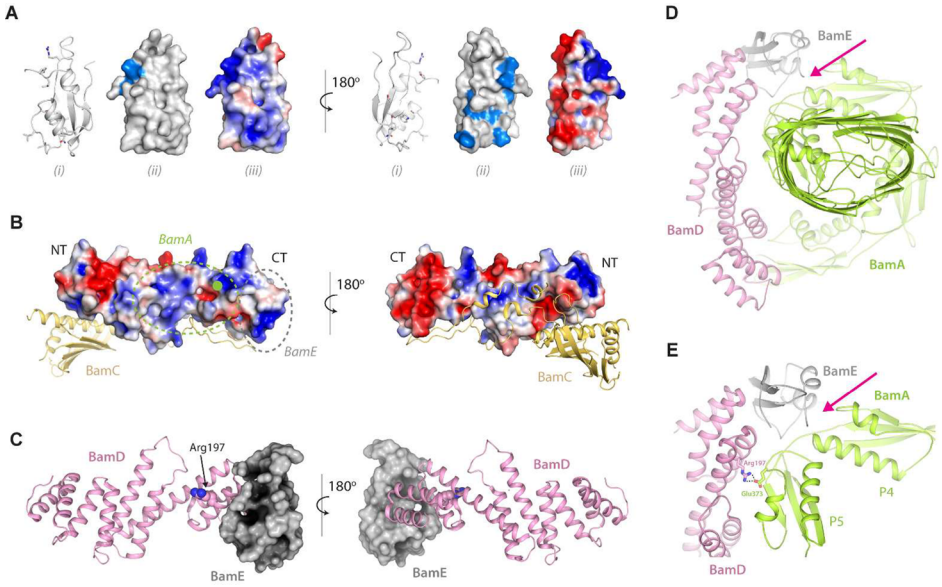

Figure 5. Modeling the BamD–BamE interaction interface. (A) Structure of BamE (PDB entry 2KM7) shown in ribbon (*i*), surface (*ii*), and electrostatic surface representation (*iii*). Residues important for the interaction with BamD are show as sticks (*i*) and colored blue in the surface representation (*ii*). The electrostatic surface representation shown in the right panel highlights the strongly electronegative surface (red) along the putative BamD binding surface. (B) Electrostatic surface representation of BamD. BamC is shown as a gold ribbon, and the location of residue Arg197 is indicated with a green sphere. The surface that mediates interaction with BamA is indicated with a green dashed oval, and the putative surface that mediates interaction with BamE is indicated with a gray oval, along the strongly electropositive surface (blue) of TPR5 of BamD. (C) Modeled BamDE complex. BamD is shown as a pink ribbon, and BamE is shown as a gray surface. Arg197 is shown as a sphere. (D) Model for the BamADE complex with BamA colored green, BamD pink, and BamE gray. (E) Close-up of panel D with the β-barrel domain of BamA removed for the sake of clarity. The magenta arrows indicate the site of a putative interaction between BamA and BamE.

A recent crystal structure of the BamCD complex and subsequent systematic truncation analysis revealed that it is the disordered extreme NT of BamC that is essential for the stoichiometric interaction between the two proteins.[59,60] The globular helix-grip domain makes very minimal contact with BamD. In contrast, the NT unstructured region binds as an elongated chain across one side of the surface of BamD and sits in the deep groove between TPR 3 and TPR 4 (Figure 4B), reminiscent of a "lasso" type loop across the entire CT face of BamD. Most of the highly conserved residues in BamC are in the NT domain and map onto the surface involved in the interaction with BamD (Figure 4C), giving additional support for the functional importance of this unstructured domain.

Interestingly, the pocket on BamD previously predicted to bind to extended peptides, based on similarity to PEX5 and the other TPR domain proteins,[53] is occluded in the BamCD structure. If indeed BamD does bind the putative β-signal peptides in a manner similar to that of PEX5 (Figure 4B), the BamCD complex structure could imply a regulatory role for the binding of BamC to BamD in modulating the ability of BamD to bind to OMP precursor proteins.

SURFACE EXPOSURE OF BAMC

Perhaps the most intriguing aspect of BamC comes from a recent study that suggests part of the structure is exposed on the surface of the bacterial cell.[60] Immunofluorescence microscopy was conducted on whole and permeabilized cells with anti-BamC sera, and BamC was found to be surface-exposed. Indirect whole cell enzyme-linked immunosorbent assay experiments were also used to independently show the same result. Furthermore, they were able to show that BamC that is surface-exposed is still bound as part of the BAM complex.

Given that the NT domain of BamC is the only domain necessary and sufficient[59] for interaction with BamD, a strictly periplasmic protein, it stands to reason that the NT part of BamC must reside in the periplasm, but what about the two globular, helix-grip domains of BamC? Protease shaving of whole cells coupled with mass spectrometry verified that the globular domains are indeed exposed on the bacterial cell surface but found no evidence that the NT region is surface-exposed. These results suggest a model in which the NT region of BamC is in the periplasm, bound via its lasso-like structure around the extended face of BamD, while the two globular domains are present on the surface. This model is dependent on a region of BamC crossing the bacterial OM. Conservation analysis shows that the majority of the highly conserved residues in the NT of BamC is involved in interactions with BamD (Figure 4C). However, a conserved short stretch of residues beyond the NT "lasso" region was identified that could ideally sit within the membrane because it was composed of largely hydrophobic residues (Figure 4D). It was concluded that this conserved hydrophobic stretch of residues somehow traverses the OM, linking the periplasmic NT to the surface-exposed helix-grip domains at the CT of BamC (Figure 4E). The crystal structure of BamCD may represent how the NT of BamC tightly binds BamD,[59] but in the absence of a membrane, the structure may not have captured how a hydrophobic region may behave in the presence of a lipid bilayer. Given that this hydrophobic sequence is not predicted to form an α-helix, exactly how this stretch may cross the OM is not known.

A model of BamC exposing large globular domains to the cell surface represents a unique topology among the bacterial lipoproteins studied to date, and the possible role, if any, of these extracellular domains in the function of the BAM complex remains to be determined. It has been suggested that they may

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be involved in binding and stabilizing the loop regions of nascent OMPs as they emerge from BamA during folding and/or insertion or that they may bind and stabilize BamA itself. The evolutionarily related SAM complex of mitochondria, which acts to insert OMPs into the mitochondrial OM, consists of three components: Sam50, which is related to BamA, and two peripheral components, Sam35 and Sam37, which have no sequence similarity to any of the Bam lipoproteins. Interestingly, in *Saccharomyces cerevisiae*, these two peripheral components are known to be exposed on the surface of the mitochondria.[61,62] It is tempting to postulate that the similar location within the SAM complex hints at a possible mechanistically conserved role for an outward-facing component during the biogenesis of OMPs.

INTERACTIONS OF BAMD WITH BAME

BamE is the smallest component of the BAM complex and the most highly conserved aside from BamA.[63] Despite this, BamE is not required for cell viability with mutants lacking BamE displaying only minor OM defects.[23] One function of BamE is to act to stabilize BamD binding to BamA. Glycine and cysteine scanning studies of BamE identified a set of residues important for BamD binding, all lying on one face of the protein[64] (Figure 5A). The electrostatics of this face are largely electronegatively charged. Masking out regions of BamD that interact with BamA and BamC indicates that BamE may interact with the electropositive region of TPR5 of BamD (Figure 5B). This would place BamE close enough to cooperatively enhance or stabilize binding of BamD to POTRA5 of BamA (Figure 5C–E). Interestingly, some residues in BamE that were found to be important for binding BamD were also important for binding to phosphatidylglycerol (PG).[64] Binding of BamE to PG did not eliminate binding to BamD and, in fact, may be cooperative and suggest another hidden function for BamE. *In vitro* studies indicate that PG enhances the insertion of OMP into the OM, so perhaps BamE contributes by localizing PG into the proximity of the BAM complex for added efficiency. BamE also appears to work with BamD to regulate the conformation of BamA.[43,65] The presence of BamE reduces the extracellular sensitivity of BamA to protease cleavage, while release or lack of BamE increases protease sensitivity.[65] One explanation for this may be that BamE helps modulate the lateral opening of BamA.[32] Perhaps BamE's affinity for PG might partially destabilize the membrane structure adjacent to BamA, inducing a structural change in BamA. Clarification of the role of BamE and the conformational states of BamA remains.

INTERACTION OF SURA WITH BAMA

SurA is the major periplasmic chaperone for delivering unfolded OM proteins to the BAM complex for insertion into the OM.[17,18] SurA is a member of the peptidyl-prolyl isomerase (PPI) family, consisting of two PPI domains. It is the first PPI domain that appears to be involved in primary chaperone function and BamA binding.[66] Data show SurA directly interacts with BamA primarily through POTRA1 as deletion of POTRA1 abolishes the ability to chemically cross-link to BamA. Studies show that BamA residues Ala18 and Arg44 are critically positioned for helping mediate SurA binding.[67,68] On the basis of the recent BamAB complex structure,[42] this would place SurA in the proximity of BamB at POTRA2 and -3, which are thought to act in concert within the OMP biogenesis pathway[25,67] (Figure 2B).

MODEL OF THE FULLY ASSEMBLED BAM COMPLEX

The structures of all the components of the BAM complex have now been reported, including complex structures of BamAB and BamCD (Table 1). Using these structures, along with models for the BamAD and BamDE complexes based on existing genetic and functional studies, here we have pieced together a working structural model for the fully assembled BAM complex from *E. coli* (Figure 6 and Supplementary Model 1). A few interesting observations can be taken from the model. For example, the only surface exposure is along BamA and the globular domains of BamC, while BamB, BamD, and BamE are fully within the periplasm. Also, BamD is likely oriented parallel to the membrane with TPR4 interacting with POTRA5 of BamA. As modeled, it also seems possible that the NT domain of BamD may also interact with other regions of the periplasmic domain of BamA such as POTRA2. Additionally, interaction of BamE along TPR5 of BamD puts BamE in the proximity of both POTRA4 and -5, which may bridge an additional interaction between BamD and BamA, thereby enhancing their apparent affinity for one another. Lastly, it is worth noting that the lateral opening site of BamA is located centrally within the BAM complex just above the POTRA domains, which would be ideal for mediating interaction with

Table 1. Reported Structures of Components within the BAM Complex

Bam protein	species	method	PDB entry	ref
BamA	*E. coli*	X-ray	4C4V	30
	E. coli	X-ray	4N75	29
	E. coli	X-ray	3OG5	28
	E. coli	X-ray	3Q6B	27
	E. coli	X-ray	3EFC	26
	E. coli	NMR	2V9H	41
	E. coli	X-ray	2QCZ	25
	E. coli	X-ray	2QDF	25
	N. gonorrheae	X-ray	4K3B	31
	Haemophilus ducreyi	X-ray	4K3C	31
BamB	*E. coli*	X-ray	3Q7M	38
	E. coli	X-ray	3Q7N	38
	E. coli	X-ray	3Q7O	38
	E. coli	X-ray	3P1L	37
	E. coli	X-ray	3PRW	36
	E. coli	X-ray	3Q54	46
	E. coli	X-ray	2YH3	39
	Pseudomonas aeruginosa	X-ray	4HDJ	40
	Moraxella catarrhalis	X-ray	4IMM	
BamAB	*E. coli*	X-ray	4PK1	42
BamC	*E. coli*	X-ray	3SNS	58
	E. coli	X-ray	2YH5	39
	E. coli	X-ray	2YH6	39
	E. coli	NMR	2LAE	57
	E. coli	NMR	2LAF	57
BamD	*E. coli*	X-ray	3Q5M	48
	E. coli	X-ray	2YHC	39
	R. marinus	X-ray	3QKY	47
BamCD	*E. coli*	X-ray	3TGO	59
BamE	*E. coli*	NMR	2KM7	64
	E. coli	NMR	2KXX	63
	E. coli	X-ray	2YH9	39

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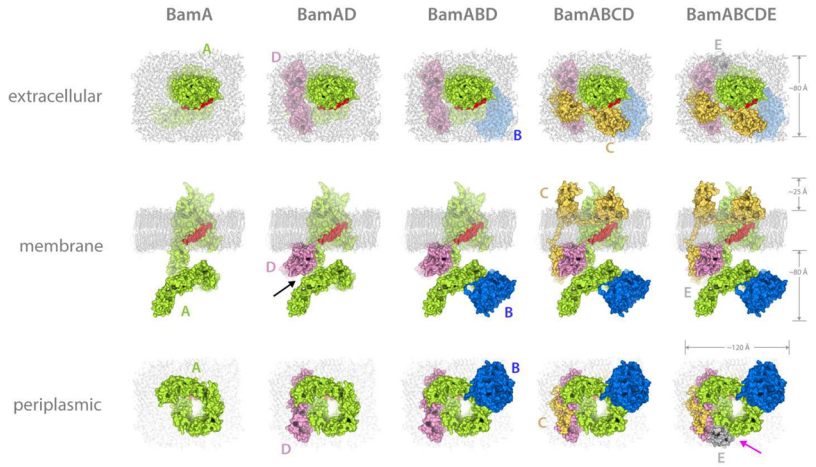

Figure 6. Model of the fully assembled BAM complex. BamA and models for the fully assembled BAM complex and subcomplexes are shown from three different views: extracellular, membrane, and periplasmic. BamA is shown in the open conformation that allows access by substrate OMPs. β-Strands 1 and 16 of BamA are colored red to indicate the site of lateral opening. The black arrow indicates the site of an additional putative interaction of the TPR1 domain of BamD with the periplasmic domain of BamA, possibly along POTRA2. The magenta arrow indicates the site of a putative interaction between BamE and POTRA5 of BamA, which may explain why interaction of BamD with BamE has been shown to enhance the interaction of BamD with BamA.

substrate OMPs as they are being folded and inserted into the OM.

SUMMARY AND FUTURE DIRECTIONS

We have pieced together a structural model for the fully assembled BAM complex; however, the mechanism for how it may function at the OM remains a topic of ongoing research. Currently, there are two leading mechanistic models that we term the BamA-assisted and BamA-budding models.[69] We have recently reviewed these and therefore will not discuss them further here. However, it should be noted that for such a complicated system like the BAM complex, more than a static structural model is needed to fully understand the mechanistic details. Therefore, as we have relied on structural, genetics, functional, and biochemical studies to piece together a model for the fully assembled BAM complex, we will continue to depend on these methods working in concert to piece together the true mechanism for how the BAM complex functions at the OM for the biogenesis of OMPs in Gram-negative bacteria.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.5b00852.

Protein Data Bank file (PDB)

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Notes

The authors declare no competing financial interest.

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