doi:10.1016/j.jmb.2011.03.035 J. Mol. Biol. (2011) 409, 348–357

Contents lists available at www.sciencedirect.com

Journal of Molecular Biology

journal homepage: http://ees.elsevier.com.jmb

Crystal Structure of BamD: An Essential Component of the β-Barrel Assembly Machinery of Gram-Negative Bacteria

Cristina M. Sandoval, Susan L. Baker, Katarina Jansen, Sandra I. Metzner and Marcelo C. Sousa*

Department of Chemistry and Biochemistry, University of Colorado at Boulder, Boulder, CO 80309, USA

Received 2 February 2011; received in revised form 15 March 2011; accepted 16 March 2011
Available online 2 April 2011

Edited by R. Huber

Keywords:
BamD;
outer-membrane biogenesis;
TPR;
OMP assembly;
BAM complex

Folding and insertion of integral β-barrel proteins in the outer membrane (OM) is an essential process for Gram-negative bacteria that requires the β-barrel assembly machinery (BAM). Efficient OM protein (OMP) folding and insertion appears to require a consensus C-terminal signal in OMPs characterized by terminal F or W residues. The BAM complex is embedded in the OM and, in *Escherichia coli*, consists of the β-barrel BamA and four lipoproteins BamBCDE. BamA and BamD are broadly distributed across all species of Gram-negative bacteria, whereas the other components are present in only a subset of species. BamA and BamD are also essential for viability, suggesting that these two proteins constitute the functional core of the bacterial BAM complex. Here, we present the crystal structure of BamD from the thermophilic bacteria *Rhodothermus marinus* refined to 2.15 Å resolution. The protein contains five tetratricopeptide repeats (TPRs) organized into two offset tandems, each capped by a terminal helix. The N-terminal domain contains three TPRs and displays remarkable structural similarity with proteins that recognize targeting signals in extended conformations. The C-terminal domain harbors the remaining two TPRs and previously described mutations that impair binding to other BAM components map to this domain. Therefore, the structure suggests a model where the C-terminal domain provides a scaffold for interaction with BAM components, while the N-terminal domain participates in interaction with the substrates, either recognizing the C-terminal consensus sequence or binding unfolded OMP intermediates.

© 2011 Elsevier Ltd. All rights reserved.

Introduction

The outer membrane (OM) of Gram-negative bacteria is unique and essential for its survival. This semipermeable membrane is typically asymmetric, composed of phospholipids on the inner leaflet and, primarily, lipopolysaccharide on the outer leaflet.[1] Proteins associated with the OM can be divided into three groups based on their architecture: (i) lipoproteins, which do not contain transmembrane domains and are attached to the OM by lipid modification of their N-terminal cysteine residue;[2] (ii) OM proteins (OMPs), characterized by

*Corresponding author. Department of Chemistry and Biochemistry, 215 UCB, University of Colorado at Boulder, Boulder, CO 80309, USA. E-mail address: Marcelo.Sousa@colorado.edu.

Abbreviations used: BAM, β-barrel assembly machinery; OM, outer membrane; OMP, OM protein; TPR, tetratricopeptide repeat; rmBamD, *R. marinus* BamD; POTRA, polypeptide translocation associated; SAD, single anomalous dispersion; SeMet, seleno-methionine; Hsp, heat shock protein; TEV, tobacco etch virus.

0022-2836/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.

a transmembrane β-barrel architecture;[3] and (iii) periplasm-spanning export channels, whose trans-OM components can have β-barrel structures as in TolC[4] or a novel α-barrel architecture typified by Wza.[5,6]

Transmembrane β-barrel OMPs carry out a variety of functions in bacteria, including nutrient and waste product exchange with the environment, cell adhesion and fulfillment of structural and enzymatic roles.[3] Folding and insertion of OMPs in the OM is an essential process that requires a multiprotein complex in the OM known as the β-barrel assembly machinery (BAM).[7,8] In *Escherichia coli*, this complex is composed of BamA, a β-barrel OMP also known as Omp85/YaeT, and four lipoproteins, BamBCDE (previously known as YfgL, NlpB, YfiO and SmpA, respectively).[8,9] Periplasmic chaperones such as SurA assist in shuttling nascent OMPs to the BAM complex.[10] The process was recently reconstituted *in vitro*, indicating that SurA and BAM can mediate OMP folding and insertion into membranes.[11] *In vivo*, the efficiency of the process appears to depend on a consensus sequence at the C-terminus of β-barrel OMPs.[12,13] The last residue in OMPs is almost always F or W, and starting from the C-terminus, positions 3, 5, 7 and 9 are hydrophobic residues, with Y frequently occupying position 3.[13] Thus, it is likely that this signal is important for one or more mechanistic steps in OMP folding and insertion.

BamA is essential for viability,[8,14,15] and homologs are retained in mitochondria and chloroplasts where they also mediate insertion of β-barrel proteins.[16] BamD is the only BAM lipoprotein required for viability.[8,9,17,18] Both BamA and BamD are broadly distributed in Gram-negative bacteria,[19] which, together with their requirement for viability, suggest that they constitute the core of the BAM complex. The phylogenetic distribution of BamB, BamC and BamE is more restricted,[19] and individual deletion of their genes in *E. coli* results in membrane permeability defects but not loss of viability.[8,9]

The structural information necessary to understand the molecular mechanisms of OMP folding and insertion mediated by BAM is beginning to emerge. BamA contains a large periplasmic domain with five polypeptide-translocation-associated (POTRA) repeats in addition to its transmembrane β-barrel. Crystallographic and solution scattering data revealed a superhelical arrangement of the POTRA domains[20–22] thought to interact with nascent OMPs and perhaps nucleate folding of their β-strands.[20,21,23] Structures of the nonessential lipoproteins BamB and BamE have recently been reported.[24–27] However, structural data on the essential lipoprotein BamD have remained elusive, although sequence analysis suggested that the protein contains tetratricopeptide repeats (TPRs).[19] Here, we report the crystal structure of BamD from *Rhodothermus marinus* refined to 2.15 Å resolution and discuss the implications for the role of BamD in the BAM-mediated mechanism of OMP folding and insertion.

Results and Discussion

Crystal structure of BamD

A lipid-free, soluble form of BamD from the thermophilic bacteria *R. marinus* (rmBamD) was expressed in *E. coli* from a plasmid encoding the mature protein (amino acids 23–280) with the N-terminal cysteine mutated to alanine. The structure was determined using single anomalous dispersion (SAD) methods and seleno-methionine (SeMet)-substituted protein. Data collected from native crystals were then used to refine the structure to 2.15 Å resolution (Table 1). The final model contains residues 30–280 and is thus only missing seven amino acids from the N-terminus due to conformational flexibility.

Table 1. Data collection and refinement statistics

	Native BamD	SeMet BamD
Data collection		
Space group	*R*3[a]	*R*3[a]
Unit cell dimensions: $a=b$ (Å)	114.37	114.22
c (Å)	77.63	77.62
Wavelength (Å)	1.0000	0.9792
Resolution (Å)[b]	70.0–2.15 (2.23–2.15)	50.0–2.7 (2.80–2.70)
R_{sym}[c] (%)	7.3 (33.7)	8.5 (22.8)
I/σ	20.3 (2.7)	15.2 (3.7)
Data completeness (%)	99.7 (99.0)	97.7 (87.7)
Redundancy	2.8 (2.6)	3.6 (2.7)
Phasing		
FOM before DM[d]		0.36
FOM after DM		0.69
Refinement statistics		
Resolution (Å)	57.2–2.15 (2.26–2.15)	
Reflections in work set	19,492	
Reflections in test set	1032	
Protein atoms	2117	
Water molecules	152	
R_{work} (%)	16.9 (20.7)	
R_{free}[e] (%)	21.0 (27.9)	
Mean B-value, protein (Å^2)	37.1	
Mean B-value, water (Å^2)	41.5	
RMSD bonds (Å)	0.007	
RMSD angles (°)	1.007	

[a] The hexagonal axis setting was used.
[b] Values in parentheses are for the highest-resolution shell.
[c] $R_{sym}=\sum_h\sum_i|(I_i(h)-\langle I(h)\rangle|/\sum_h\sum_i I_i(h)$, where $I_i(h)$ is the ith measurement of reflection h, and $\langle I(h)\rangle$ is the weighted mean of all measurements of h.
[d] FOM, figure of merit; DM, density modification.
[e] $R_{work}=\sum|F_{obs}-F_{calc}|/\sum F_{obs}$, where F_{obs} is the observed structure factor amplitude, and F_{calc} is the structure factor calculated from the model. R_{free} is computed the same as R_{work}, but using the test set of reflections.

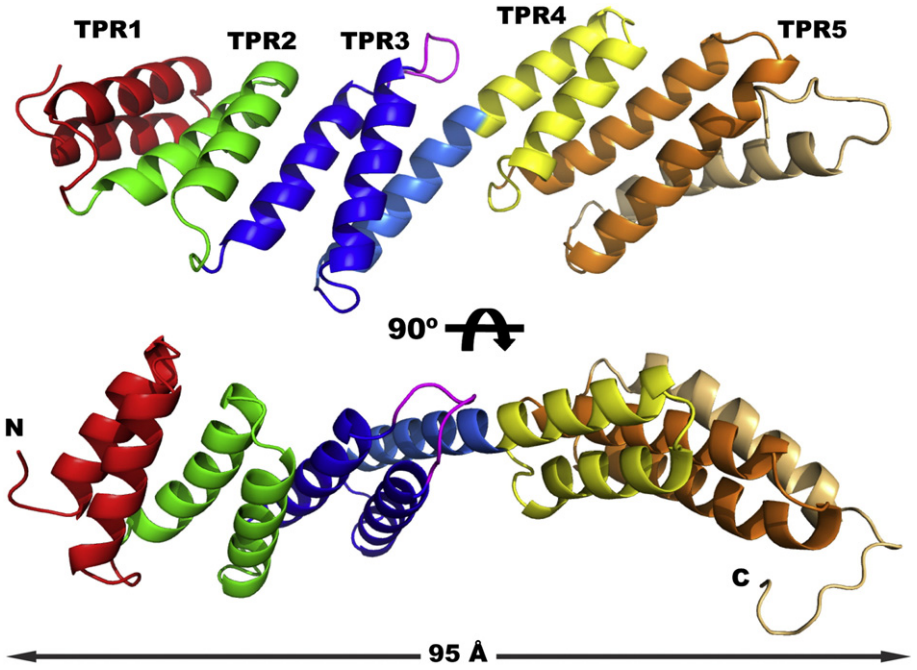


Fig. 1. Crystal structure of rmBamD. Two views of rmBamD related by a 90° rotation. The N-terminal domain contains TPRs 1–3 (red, green and blue, respectively) and is capped by a helix (light blue). The C-terminal domain contains TPRs 4 and 5 (yellow and orange) and the final capping helix (light orange). An extended loop in TPR3 is colored magenta.

The structure of rmBamD consists of five TPRs arranged in two offset tandem arrays (Fig. 1). TPRs are 34 amino acid degenerate sequences that fold into a helix–turn–helix motif.[28,29] TPRs are typically arranged in tandem arrays such that the two antiparallel helices (A and B) from one repeat pack against the A helix of the next motif. The tandem is frequently terminated by a capping helix. Packing several TPRs in this manner results in a right-handed superhelical structure.[30] In rmBamD, however, three N-terminal TPRs (TPR1–3; red, green and blue in Fig. 1) are capped by a helix that is continuous with the A helix of TPR 4 (light blue and yellow). As a consequence, the two C-terminal TPRs (TPR4–5; yellow and orange) are offset from the N-terminal three, breaking the superhelical arrangement. rmBamD is therefore a very elongated molecule measuring more than 95 Å between the N-terminus and the capping helix of TPR5 (light orange). In general, the turns connecting the A and B helices are short and fairly typical. TPR3, however, is notable by the extended loop that connects the two helices (magenta in Fig. 1).

Mapping the electrostatic surface potential on the structure of rmBamD reveals a striking distribution of charge (Fig. 2). One side of the molecule, harboring the turns linking the TPRs helices, is positively charged, whereas the opposite side displays a strong electronegative character. Interestingly, the offset between the N- and C-terminal domains creates a negatively charged semicircular groove at the bottom of rmBamD, approximately 24 Å in diameter, that may be important for interaction with other BAM components.

BamD across Gram-negative bacteria

BamD is essential in *E. coli*[8,17,18] and *Neisseria meningitidis*[31] and, together with BamA, is thought to constitute the core of the BAM. Consistent with its essential role, BamD homologs are ubiquitous in Gram-negative bacteria.[17,19] We used hidden Markov models to iteratively search the KEGG database for BamD homologs using jackhmmer (part of the HMMER package[32]). The alignment was manually edited in Jalview[33] to eliminate a few sequences that did not contain the invariant cysteine at the mature N-terminus that becomes lipidated in BamD.[34] This resulted in 607 sequences with broad representation of Gram-negative bacteria.

The length of BamD is approximately 240–300 amino acids (from the translational start) in most bacteria, with rmBamD being 280 amino acids long. In *E. coli*, BamD is 245 amino acids, and the alignments suggest that the protein is missing the last helix capping TPR5 in rmBamD (Fig. 3a). Conversely, BamD homologs in Pseudomonadales, including *Moraxella*, *Acinetobacter* and *Pseudomonas*, have an extended C-terminus that makes them 340–380 amino acids long. This extension does not have clear sequence similarity to any other protein and may represent additional TPRs.

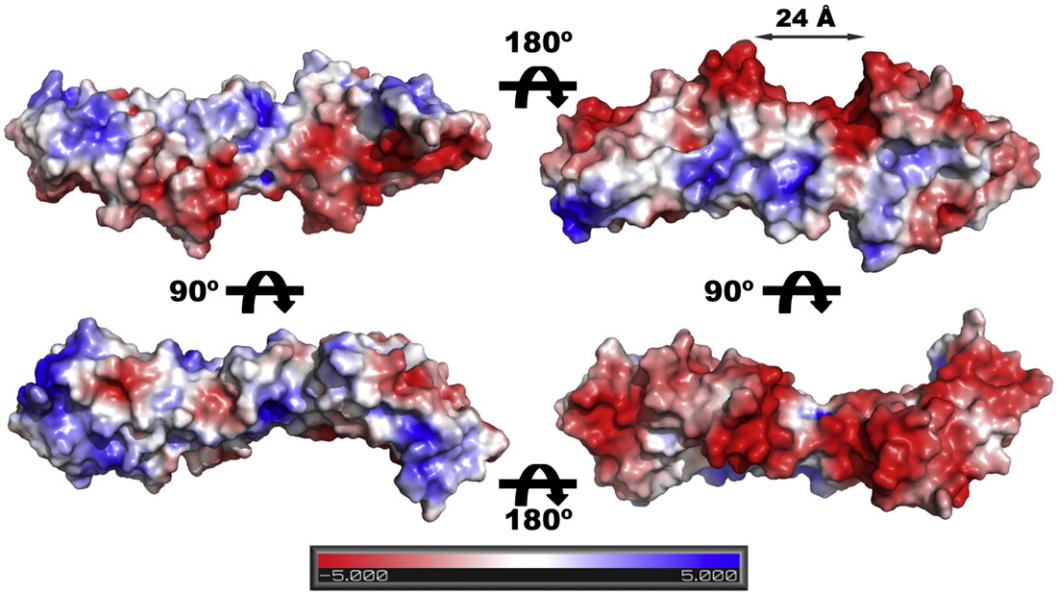


Fig. 2. Electrostatic surface potential of rmBamD. The distribution of positive (blue) and negative (red) surface potential is mapped to a surface representation of rmBamD. For reference, the orientation of the molecule on the left panels is the same as in Fig. 1. A negatively charged, semicircular groove between the N- and C-terminal domains 24 Å in diameter is labeled.

To evaluate conserved features in BamD, we subjected the aligned sequences to analysis with the ConSurf server, which uses Bayesian methods to score the rate of evolution at each aligned position.[35] Figure 3a shows the alignment of rmBamD with *E. coli* and *Neisseria* sequences for reference. The results mapped on the structure of rmBamD (Fig. 3b) show that the N-terminal domain is generally more conserved than the C-terminal domain. The extended loop connecting the A and B helices of TPR3 together with TPR4 forms a deep groove lined with conserved residues, suggestive of a protein–protein interaction surface. Consistent with this hypothesis, C-terminal truncations of *E. coli* BamD resulted in impaired binding to BamA as well as the accessory lipoproteins BamC and BamE.[9,17] These truncations map to the beginning of TPR5 in rmBamD and would not only eliminate the entire TPR5 but also likely result in destabilization of TPR4 as well. Therefore, we suggest that the extended loop of TPR3 together with the C-terminal domain of BamD harbor the determinants for interaction with other BAM components.

Potential roles for BamD in OMP biogenesis

The structure of rmBamD N-terminal domain is strikingly similar to a number of proteins involved in recognition of C-terminal targeting signal sequences (Fig. 4). The right-handed twist in the TPR tandem creates a concave face lined by the TPR's A helices. TPR domains in proteins such as the heat shock protein (Hsp)-organizing protein, Tom70 (a component of the mitochondrial translocase of the OM) and FKBP52 use this concave surface to recruit molecular chaperones Hsp70 or Hsp90 by binding their C-terminal tails in an extended conformation[36–38] (Fig. 4a–c). Similarly, the C-terminal peroxisomal targeting signal-1, present at the C-terminus of proteins destined to the peroxisome, is bound by PEX5 in the concave face of its TPR domain (Fig. 4d), although a second cluster of TPRs also contributes to the binding.[39] Notably, the targeting signal peptides are all bound in a similar extended conformation in these proteins. Since the structure of the N-terminus of rmBamD is very similar to that of TPR domains in

Fig. 3. Sequence conservation in BamD. A sequence alignment of BamD homologs (see the text) was analyzed with the ConSurf server. Conservation scores are normalized so that the average score is zero and the standard deviation is 1. Thus, rapidly evolving positions are negative (colored cyan) and slowly evolving positions are positive (colored purple), while positions with an average rate of evolution are colored white. (a) Aligned sequences of rmBamD (*Rhodothermus*), *E. coli* and *N. meningitidis* (*Neisseria*) colored by the ConSurf scores. Structural elements are labeled and highlighted in boxes colored as in Fig. 1. (b) Conservation scores mapped on a surface representation of the rmBamD structure showing a groove between the extended loop in TPR3 and the C-terminal domain lined with slowly evolving residues.

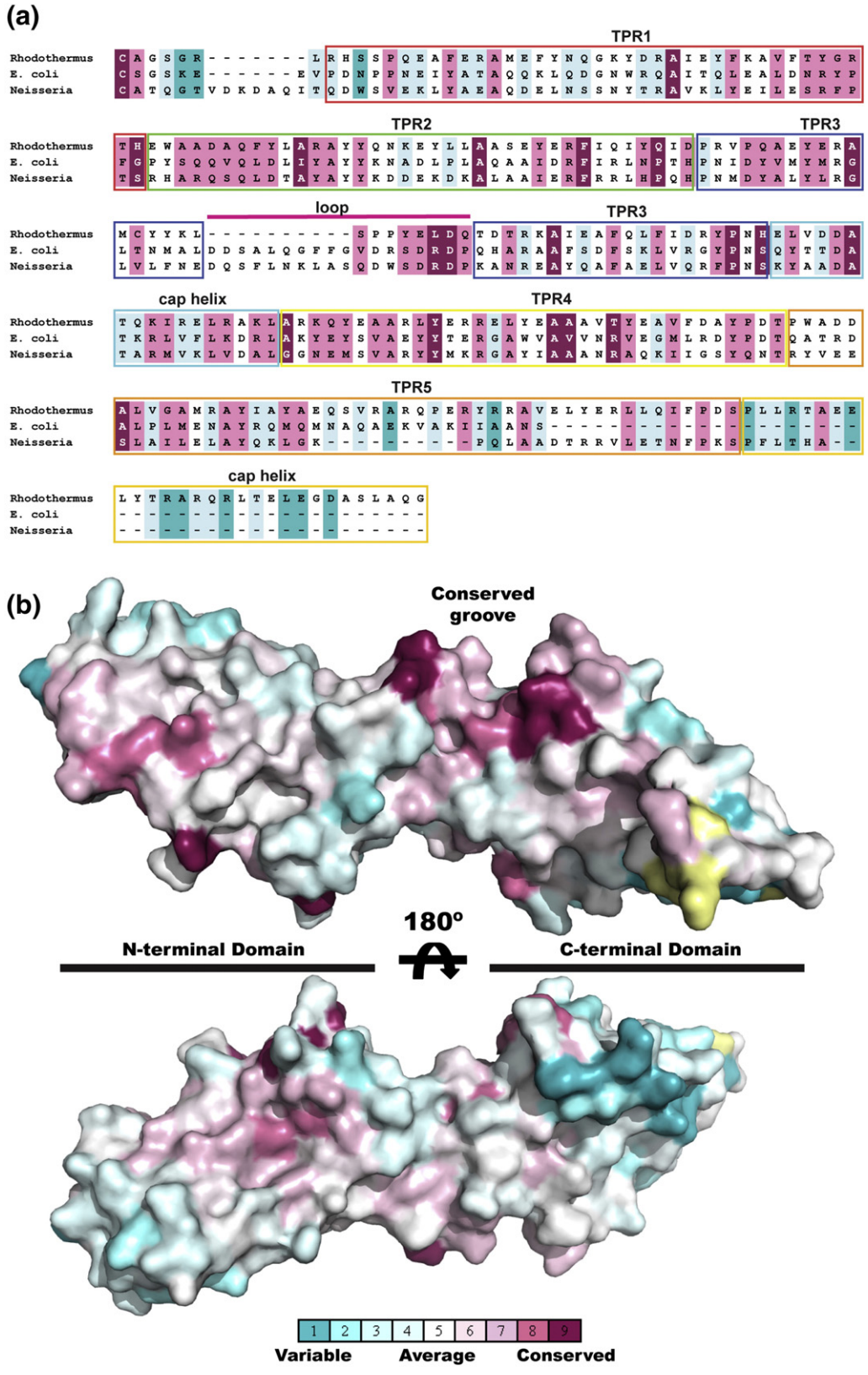


Fig. 3 (*legend on previous page*)

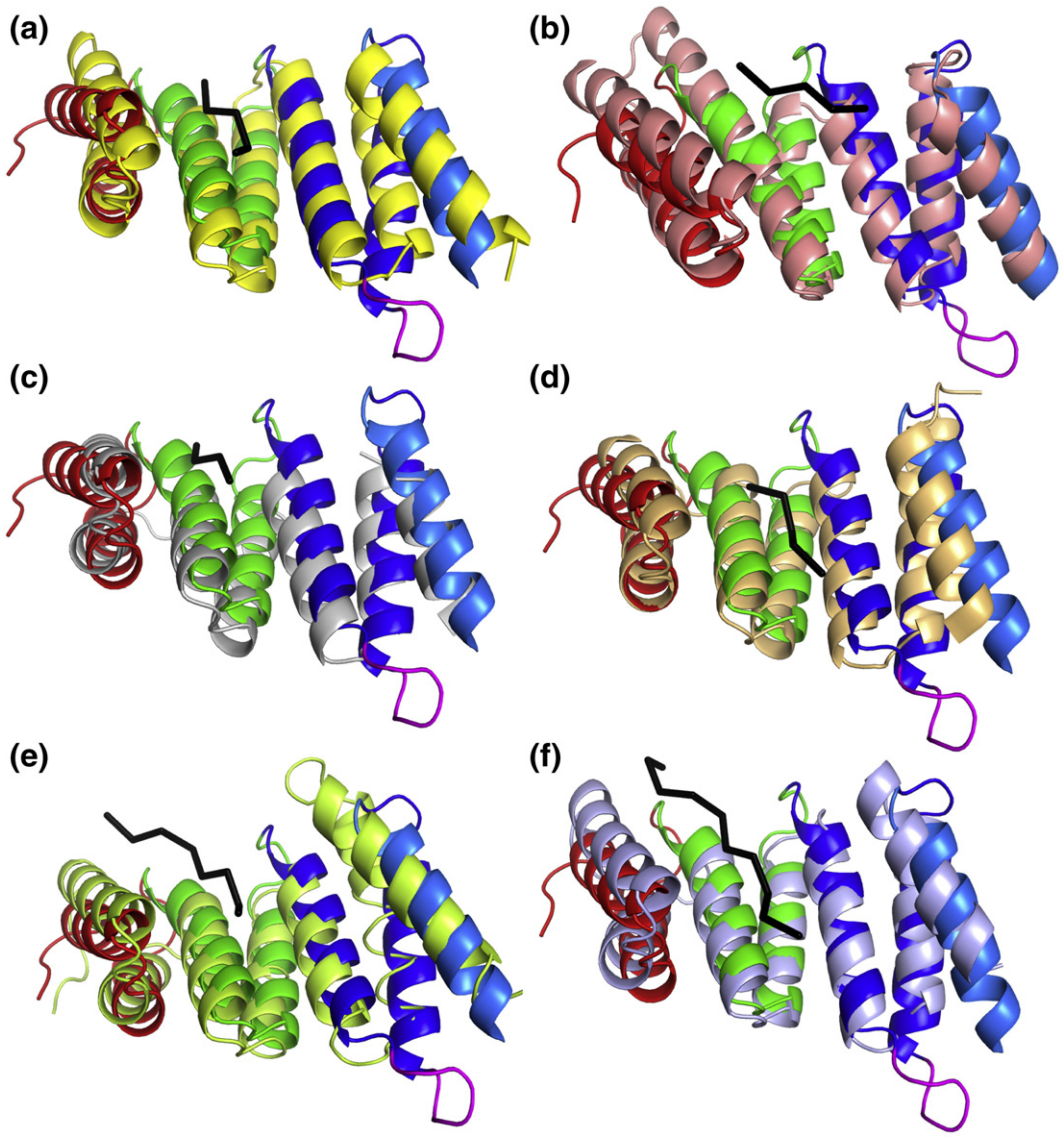


Fig. 4. The N-terminal domain of rmBamD is similar to proteins binding extended polypeptides. The N-terminal domain of rmBamD (colored as in Fig. 1) is superimposed to TPR domains of Hsp-organizing protein (a) (yellow), FKBP52 (b) (salmon), Tom70 (c) (gray) and PEX5 (d) (light orange), as well as the chaperones PcrH (e) (light green) and IpgC (light blue). All these proteins bind extended polypeptides (shown as black C^{α} traces) in the concave face of the TPR tandem.

these proteins (Fig. 4), BamD may have an analogous role, recognizing the C-terminal consensus sequence observed in the vast majority of OMPs: starting at the C-terminus, positions 1, 3, 5, 7 and 9 are hydrophobic amino acids, with 1 being almost always F or W and 3 being preferentially Y.[13] Given the conservation of this C-terminal signature in OMPs across species, it is likely that they are important for one or more mechanistic steps of OMP folding and insertion. BamD is ideally suited to recognize the OMP signal.

The N-terminal domain of BamD is also structurally similar to that of chaperones of the type three secretion system such as IpgC, SycD and PcrH[40–42] (Fig. 4e and f). Crystal structures of IpgC and PcrH in complex with their targets show the concave face of the chaperone 3–TPR tandem binding peptides that are 8–13 amino acids long in extended conformations, spanning the entire face of the TPR tandem (Fig. 4e and f).[41,42] By analogy to these proteins, it is possible that BamD binds unfolded OMPs in an extended conformation prior to folding and insertion into the OM. Interestingly, the receptor for β-barrel protein import in mitochondria (Tom70) also has TPR domains that interact with precursor β-barrels prior to translocation through

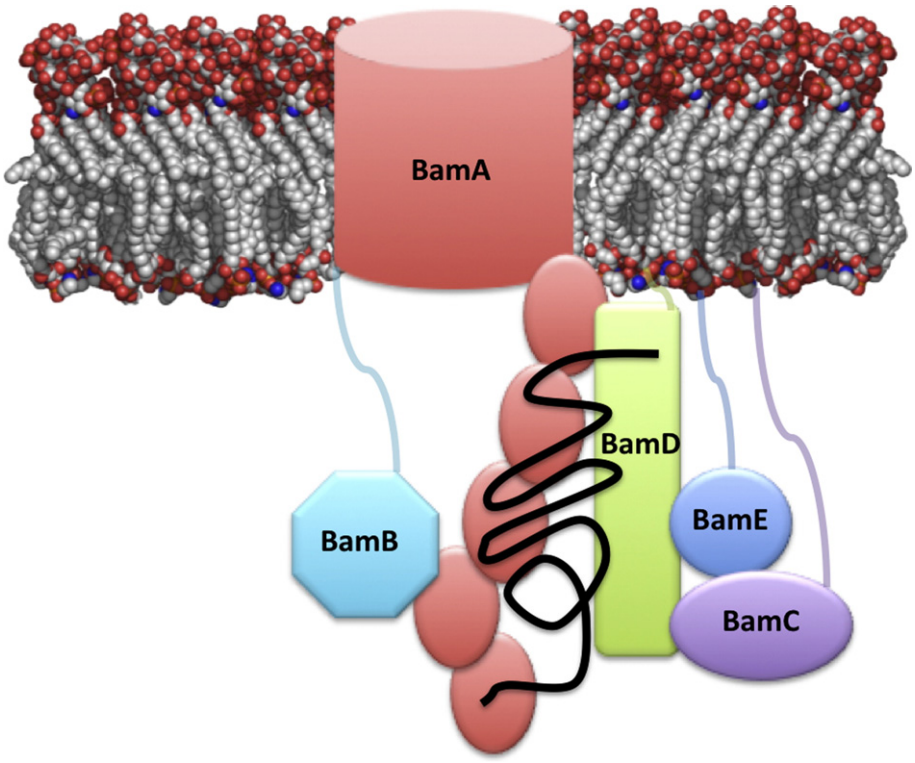


Fig. 5. Working model of the BAM complex. BamA and BamD constitute the core of the complex. BamA is proposed to recognize and help fold nascent OMPs with its five POTRA repeats (show as orange ovals). The N-terminal domain of BamD may assist in this folding role or may recognize the consensus sequence at the C-terminus of OMPs. BamC and BamE interact with the C-terminal domain of BamD and help stabilize the complex. BamB interacts with BamA independently of BamC, BamD and BamE.

the translocase of the OM pore, in addition to the TPRs binding Hsp70 and Hsp90 mentioned above.[43,44]

BamD is associated with the membrane by its lipidated amino-terminal cysteine.[34] All but seven amino acids at the N-terminus are defined in the structure of rmBamD. Therefore, the N-terminal domain must be proximal to the membrane. Conversely, BamC and BamE, the other two BAM lipoproteins known to interact directly with BamD, have disordered N-terminal extensions.[25,27,45,46] Both BamE and BamC are thought to bind the C-terminus of BamD.[9,17] Therefore, currently available data on the BAM complex components can be consolidated into a working model described in Fig. 5. BamA, with its periplasmic five-POTRA domain recognizes nascent OMPs[14] and may nucleate formation or β-strands or β-hairpins in its substrates.[20–23] BamA interacts directly with BamD, whose C-terminal domain provides a scaffold for the binding of BamC and BamE, which in turn stabilize the complex with BamA. C-terminal truncations of BamD are thus tolerated in *E. coli* but result in loss of BamC and weakened binding of BamE to the complex.[9,17] The N-terminal domain of BamD plays an essential role in the BAM function. We suggest that it may be required to recognize the C-terminal consensus signal in OMPs or perhaps interact with unfolded OMP precursors. BamB interacts directly with BamA independently of BamCDE.[9,11,17] As a nonessential component of BAM, BamB may increase the efficiency of the complex or assist in the folding and insertion of a specific subset of substrates, either recognizing their C-terminal OMP signal or nucleating β-strand formation.[24]

Materials and Methods

Protein cloning, expression and purification

The gene fragment coding for mature BamD (amino acids 23–280) was PCR amplified from *R. marinus* genomic DNA. The primers incorporated NdeI and XhoI restriction sites at the 5′ and 3′ ends of the fragment, respectively, and also mutated the N-terminal cysteine (C23) to alanine. The amplified fragment was ligated into a modified pET28 vector that incorporates an N-terminal His tag followed by the recognition sites for the tobacco etch virus (TEV) protease. The resulting plasmid (pMS716) thus drives the expression of a His-tagged mature rmBamD C23A. Upon

cleavage with TEV protease, the amino acid GHM from the tag remains fused to the N-terminus of the protein.

E. coli Rosetta (DE3) cells (Novagen) were transformed with plasmid pMS716 and were selected in LB agar plates containing 50 μg/ml kanamycin. A single colony was used to inoculate 100 ml of LB supplemented with 50 μg/ml kanamycin, and the culture was grown overnight. A 10-ml aliquot was then diluted into 1 l of LB with kanamycin and was allowed to grow at 37 °C to an OD_{600} of 0.6. Expression was induced by addition of 1 mM isopropyl-β-D-thiogalactopyranoside (Gold Bio Inc.) and incubation for 3 h at 37 °C. Cells were harvested by centrifugation, resuspended in buffer A [25 mM Tris–HCl (pH 8.0) and 150 mM NaCl] supplemented with a protease inhibitor cocktail (complete ethylenediaminetetraacetic acid-free; Roche) and frozen until used. After thawing, cells were lysed on ice by sonication. Cell debris were removed by centrifugation at 16,000 rpm for 30 min at 4 °C, and the supernatant was applied to a 10-ml Ni-NTA column (Qiagen) pre-equilibrated with buffer A. The protein was allowed to bind the Ni-NTA beads in batch for 2 h at 4 °C. The resin was collected in a column and was washed with 5 column volumes of buffer A, followed by a wash with 5 column volumes of buffer A containing 25 mM imidazole. The protein was then eluted with buffer A supplemented with 200 mM imidazole. TEV protease was added to the elution pool and was dialyzed overnight at 4 °C in buffer A supplemented with 10 mM DL-dithiothreitol (Gold Bio Inc.) to cleave the His tag. The cleaved protein was concentrated and loaded on a size-exclusion column (HiLoad 26/60 Superdex 200; Amersham Pharmacia Biotech) pre-equilibrated with buffer A and was eluted in the same buffer. Purified rmBamD protein was concentrated to 15 mg/ml and was stored at −80 °C until needed.

SeMet-substituted rmBamD was prepared as described above with the following modifications to the bacterial cultures. Overnight cultures of *E. coli* Rosetta (DE3) transformed with pMS716 were spun down and resuspended in 3 l M9 minimal medium supplemented with 50 μg/ml kanamycin and 4.0 g/l glucose. Cultures were grown at 37 °C to an OD_{600} of 0.4. Methionine synthesis was then inhibited by addition of 100 mg/l D-lysine, D-phenylalanine and D-threonine and 50 mg/l D-isoleucine and D-valine. The culture was also supplemented with 120 mg/l of SeMet (Acros Organics), and incubation continued at 37 °C until an OD_{600} was 0.6. Cultures were cooled on an ice bath for 10 min, and expression was induced by addition of 1 mM isopropyl-β-D-thiogalactopyranoside followed by incubation overnight at 20 °C. Protein purification was performed as describe above. Final protein concentration was 7.5 mg/ml.

Protein crystallization

Crystallization screening with purified rmBamD was carried out using numerous commercial and homemade screens at various protein concentrations. Several conditions yielded crystals, but best conditions appeared in Wizard screens (Emerald Biosystems). Further optimization was performed using the hanging-drop vapor diffusion method (2.0 μl of protein:2.0 μl of precipitant) at 16 °C. The best native crystals were obtained from a precipitant solution containing 3.25 M NaCl and 0.1 M Tris (pH 8.0) and a protein solution at 7.5 mg/ml concentration. Crystals were harvested, transferred directly into 4.0 M malonate (pH 7.0) and flash frozen in a stream of N_2 at 100 K. Crystals of SeMet-substituted rmBamD were obtained in 2.7 M NaCl, 0.1 M Tris (pH 7.5) and 0.2 M MgCl2. Cryo-protection was achieved in 4 M malonate as well.

Structure determination

A data set to 2.7 Å resolution collected at the peak wavelength for selenium from SeMet-substituted rmBamD crystals was used to solve the structure by SAD using the AutoSol module of PHENIX.[47] Three selenium sites were readily identified (rmBamD has three nonterminal methionines) and used to calculate SAD phases followed by density modification. The resulting electron density map was readily interpretable, and a partial model was automatically built into this map with the AutoBuild module of PHENIX. Manual rebuilding using Coot[48] interspersed with model refinement in PHENIX was carried out until no further improvement of the *R*-factor could be achieved. Amino acids 30–280 could be unambiguously modeled and refined. The final model statistics are shown in Table 1.

Accession numbers

Atomic coordinates and structures factors have been deposited in the Protein Data Bank with accession number 3QKY.

Acknowledgements

This research was supported by National Institutes of Health grant AI080709 to M.C.S. Part of this work was performed at the Advanced Light Source, funded by the Department of Energy. Structural biology research at the University of Colorado at Boulder is supported in part by the William M. Keck Foundation.

References

1. Nikaido, H. (2003). Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev.* **67**, 593–656.
2. Tokuda, H. & Matsuyama, S. (2004). Sorting of lipoproteins to the outer membrane in *E. coli*. *Biochim. Biophys. Acta*, **1693**, 5–13.
3. Tamm, L. K., Hong, H. & Liang, B. (2004). Folding and assembly of β-barrel membrane proteins. *Biochim. Biophys. Acta*, **1666**, 250–263.
4. Koronakis, V., Eswaran, J. & Hughes, C. (2004). Structure and function of TolC: the bacterial exit duct for proteins and drugs. *Annu. Rev. Biochem.* **73**, 467–489.
5. Dong, C. J., Beis, K., Nesper, J., Brunkan-LaMontagne, A. L., Clarke, B. R., Whitfield, C. & Naismith, J. H.

(2006). Wza the translocon for *E. coli* capsular polysaccharides defines a new class of membrane protein. *Nature*, **444**, 226–229.
6. Collins, R. F. & Derrick, J. P. (2007). Wza: a new structural paradigm for outer membrane secretory proteins? *Trends Microbiol.* **15**, 96–100.
7. Silhavy, T. J., Kahne, D. & Walker, S. (2010). The bacterial cell envelope. *Cold Spring Harbor Perspect. Biol.* **2**, a000414.
8. Wu, T., Malinverni, J., Ruiz, N., Kim, S., Silhavy, T. J. & Kahne, D. (2005). Identification of a multicomponent complex required for outer membrane biogenesis in *Escherichia coli*. *Cell*, **121**, 235–245.
9. Sklar, J. G., Wu, T., Gronenberg, L. S., Malinverni, J. C., Kahne, D. & Silhavy, T. J. (2007). Lipoprotein SmpA is a component of the YaeT complex that assembles outer membrane proteins in *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **104**, 6400–6405.
10. Sklar, J. G., Wu, T., Kahne, D. & Silhavy, T. J. (2007). Defining the roles of the periplasmic chaperones SurA, Skp, and DegP in *Escherichia coli*. *Genes Dev.* **21**, 2473–2484.
11. Hagan, C. L., Kim, S. & Kahne, D. (2010). Reconstitution of outer membrane protein assembly from purified components. *Science*, **328**, 890–892.
12. Robert, V., Volokhina, E. B., Senf, F., Bos, M. P., Van Gelder, P. & Tommassen, J. (2006). Assembly factor Omp85 recognizes its outer membrane protein substrates by a species-specific C-terminal motif. *PLoS Biol.* **4**, e377.
13. Struyve, M., Moons, M. & Tommassen, J. (1991). Carboxy-terminal phenylalanine is essential for the correct assembly of a bacterial outer membrane protein. *J. Mol. Biol.* **218**, 141–148.
14. Voulhoux, R., Bos, M. P., Geurtsen, J., Mols, M. & Tommassen, J. (2003). Role of a highly conserved bacterial protein in outer membrane protein assembly. *Science*, **299**, 262–265.
15. Voulhoux, R. & Tommassen, J. (2004). Omp85, an evolutionarily conserved bacterial protein involved in outer-membrane-protein assembly. *Res. Microbiol.* **155**, 129–135.
16. Gentle, I. E., Burri, L. & Lithgow, T. (2005). Molecular architecture and function of the Omp85 family of proteins. *Mol. Microbiol.* **58**, 1216–1225.
17. Malinverni, J. C., Werner, J., Kim, S., Sklar, J. G., Kahne, D., Misra, R. & Silhavy, T. J. (2006). YfiO stabilizes the YaeT complex and is essential for outer membrane protein assembly in *Escherichia coli*. *Mol. Microbiol.* **61**, 151–164.
18. Onufryk, C., Crouch, M. L., Fang, F. C. & Gross, C. A. (2005). Characterization of six lipoproteins in the σ^E regulon. *J. Bacteriol.* **187**, 4552–4561.
19. Gatsos, X., Perry, A. J., Anwari, K., Dolezal, P., Wolynec, P. P., Likic, V. A. *et al.* (2008). Protein secretion and outer membrane assembly in *Alphaproteobacteria*. *FEMS Microbiol. Rev.* **32**, 995–1009.
20. Kim, S., Malinverni, J. C., Sliz, P., Silhavy, T. J., Harrison, S. C. & Kahne, D. (2007). Structure and function of an essential component of the outer membrane protein assembly machine. *Science*, **317**, 961–964.
21. Gatzeva-Topalova, P. Z., Walton, T. A. & Sousa, M. C. (2008). Crystal structure of YaeT: conformational flexibility and substrate recognition. *Structure*, **16**, 1873–1881.
22. Gatzeva-Topalova, P. Z., Warner, L. R., Pardi, A. & Sousa, M. C. (2010). Structure and flexibility of the complete periplasmic domain of BamA: the protein insertion machine of the outer membrane. *Structure*, **18**, 1492–1501.
23. Knowles, T. J., Jeeves, M., Bobat, S., Dancea, F., McClelland, D., Palmer, T. *et al.* (2008). Fold and function of polypeptide transport-associated domains responsible for delivering unfolded proteins to membranes. *Mol. Microbiol.* **68**, 1216–1227.
24. Heuck, A., Schleiffer, A. & Clausen, T. (2011). Augmenting β-augmentation: structural basis of how BamB binds BamA and may support folding of outer membrane proteins. *J. Mol. Biol.* **406**, 659–666.
25. Kim, K. H., Kang, H. S., Okon, M., Escobar-Cabrera, E., McIntosh, L. P. & Paetzel, M. (2011). Structural characterization of *Escherichia coli* BamE, a lipoprotein component of the β-barrel assembly machinery complex. *Biochemistry*, **50,**, 1081–1090.
26. Kim, K. H. & Paetzel, M. (2010). Crystal structure of *Escherichia coli* BamB, a lipoprotein component of the β-barrel assembly machinery complex. *J. Mol. Biol.* **406**, 667–678.
27. Knowles, T. J., Browning, D. F., Jeeves, M., Maderbocus, R., Rajesh, S., Sridhar, P. *et al.* (2011). Structure and function of BamE within the outer membrane and the β-barrel assembly machine. *EMBO Rep.* **12**, 123–128.
28. D'Andrea, L. D. & Regan, L. (2003). TPR proteins: the versatile helix. *Trends Biochem. Sci.* **28**, 655–662.
29. Main, E. R., Xiong, Y., Cocco, M. J., D'Andrea, L. & Regan, L. (2003). Design of stable α-helical arrays from an idealized TPR motif. *Structure*, **11**, 497–508.
30. Allan, R. K. & Ratajczak, T. (2010). Versatile TPR domains accommodate different modes of target protein recognition and function. *Cell Stress Chaperones*,; [Epub ahead of print].
31. Volokhina, E. B., Beckers, F., Tommassen, J. & Bos, M. P. (2009). The β-barrel outer membrane protein assembly complex of *Neisseria meningitidis*. *J. Bacteriol.* **191**, 7074–7085.
32. Eddy, S. R. (2009). A new generation of homology search tools based on probabilistic inference. *Genome Inform.* **23**, 205–211.
33. Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M. & Barton, G. J. (2009). Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics*, **25**, 1189–1191.
34. Fussenegger, M., Facius, D., Meier, J. & Meyer, T. F. (1996). A novel peptidoglycan-linked lipoprotein (ComL) that functions in natural transformation competence of *Neisseria gonorrhoeae*. *Mol. Microbiol.* **19**, 1095–1105.
35. Ashkenazy, H., Erez, E., Martz, E., Pupko, T. & Ben-Tal, N. (2010). ConSurf 2010: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. *Nucleic Acids Res.* **38**, W529–W533.
36. Scheufler, C., Brinker, A., Bourenkov, G., Pegoraro, S., Moroder, L., Bartunik, H. *et al.* (2000). Structure of TPR domain–peptide complexes: critical elements in the assembly of the Hsp70–Hsp90 multichaperone machine. *Cell*, **101**, 199–210.

37. Wu, B., Li, P., Liu, Y., Lou, Z., Ding, Y., Shu, C. *et al.* (2004). 3D structure of human FK506-binding protein 52: implications for the assembly of the glucocorticoid receptor/Hsp90/immunophilin heterocomplex. *Proc. Natl Acad. Sci. USA*, **101**, 8348–8353.
38. Wu, Y. & Sha, B. (2006). Crystal structure of yeast mitochondrial outer membrane translocon member Tom70p. *Nat. Struct. Mol. Biol.* **13**, 589–593.
39. Gatto, G. J., Jr, Geisbrecht, B. V., Gould, S. J. & Berg, J. M. (2000). Peroxisomal targeting signal-1 recognition by the TPR domains of human PEX5. *Nat. Struct. Biol.* **7**, 1091–1095.
40. Buttner, C. R., Sorg, I., Cornelis, G. R., Heinz, D. W. & Niemann, H. H. (2008). Structure of the *Yersinia enterocolitica* type III secretion translocator chaperone SycD. *J. Mol. Biol.* **375**, 997–1012.
41. Job, V., Mattei, P. J., Lemaire, D., Attree, I. & Dessen, A. (2010). Structural basis of chaperone recognition of type III secretion system minor translocator proteins. *J. Biol. Chem.* **285**, 23224–23232.
42. Lunelli, M., Lokareddy, R. K., Zychlinsky, A. & Kolbe, M. (2009). IpaB–IpgC interaction defines binding motif for type III secretion translocator. *Proc. Natl Acad. Sci. USA*, **106**, 9661–9666.
43. Chan, N. C., Likic, V. A., Waller, R. F., Mulhern, T. D. & Lithgow, T. (2006). The C-terminal TPR domain of Tom70 defines a family of mitochondrial protein import receptors found only in animals and fungi. *J. Mol. Biol.* **358**, 1010–1022.
44. Mills, R. D., Trewhella, J., Qiu, T. W., Welte, T., Ryan, T. M., Hanley, T. *et al.* (2009). Domain organization of the monomeric form of the Tom70 mitochondrial import receptor. *J. Mol. Biol.* **388**, 1043–1058.
45. Albrecht, R. & Zeth, K. (2010). Crystallization and preliminary X-ray data collection of the *Escherichia coli* lipoproteins BamC, BamD and BamE. *Acta Crystallogr., Sect. F: Struct. Biol. Cryst. Commun.* **66**, 1586–1590.
46. Knowles, T. J., McClelland, D. M., Rajesh, S., Henderson, I. R. & Overduin, M. (2009). Secondary structure and ^{1}H, ^{13}C and ^{15}N backbone resonance assignments of BamC, a component of the outer membrane protein assembly machinery in *Escherichia coli*. *Biomol. NMR Assign.* **3**, 203–206.
47. Adams, P. D., Afonine, P. V., Bunkoczi, G., Chen, V. B., Davis, I. W., Echols, N. *et al.* (2010). PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **66**, 213–221.
48. Emsley, P. & Cowtan, K. (2004). Coot: model-building tools for molecular graphics. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **60**, 2126–2132.