The versatile β-barrel membrane protein
William C Wimley

The β-barrel membrane protein is found in the outer membranes of bacteria, mitochondria and chloroplasts. Approximately 2–3% of the genes in Gram-negative bacterial genomes encode β-barrels. Whereas there are fewer than 20 known three-dimensional β-barrel structures, genomic databases currently contain thousands of β-barrels belonging to dozens of families. New research is revealing the variety of β-barrel structures and the variety of functions performed by these versatile proteins.

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Abbreviations
OM outer membrane
OMP outer membrane protein
PDB Protein Data Bank
TM transmembrane

Introduction
Recent studies in genomics, proteomics, structural biology and genetics have begun to reveal the versatility and ubiquity of the β-barrel membrane protein. Although unified by many common structural features, β-barrel membrane proteins carry out diverse functions in diverse organisms. Their distribution extends from many families of bacteria to eukaryotes, all of which have β-barrel membrane proteins in their genomes. The variety of known functions performed by β-barrels is expanding and now includes all the functional categories listed in Table 1. The common structural features of the few β-barrels of known structure have been reviewed in detail elsewhere (e.g. [1,2]). Here, the focus will be on the diversity of distribution, function and architecture of β-barrel membrane proteins to draw attention to the many β-barrels whose three-dimensional structures are yet to be determined.

Structure and architecture
The number of possible designs for stable transmembrane (TM) β-sheets is limited by the physical constraints imposed by the nonpolar core of the bilayer membrane [3]. All TM proteins must fold into structures that are roughly cylindrical across the bilayer and that expose predominantly nonpolar sidechains to the membrane. Most importantly, any TM structure must involve all membrane-buried backbone polar groups in hydrogen bonds [3]. The only TM β-sheet structure known to satisfy these constraints is the β-barrel, in which the TM β-strands are laterally hydrogen bonded in a circular pattern. In a β-barrel membrane protein, these interstrand hydrogen bonds are the dominant stabilizing interaction [4] because they occur in the nonpolar bilayer milieu. The extensive nonlocal backbone hydrogen bonds between strands also greatly rigidify the core of the barrel structure [5]. The contribution of each hydrogen bond to stabilization has been estimated to be as high as 0.5 kcal/mol/residue [3,6,7], which is very significant because even the smallest β-barrels have at least 80 amino acids in the membrane. As a result of these hydrogen-bonding interactions, β-barrels make very stable structures that do not readily unfold in membranes [8,9].

Currently, fewer than 20 unique TM β-barrel structures have been solved. A gallery of β-barrels is shown in Figure 1. Schulz [10] outlined a set of simple rules that describe the structural features of all β-barrels of known structure. In brief, all are cylindrical, closed barrels with an even number of TM β-strands that are connected in a β-meander topology with alternating tight turns and longer loops. This architecture suggests that the β-hairpin is the basic structural subunit of a TM β-barrel. Typically, one side of the membrane has only tight turns, as shown in Figure 1. The hydrophobic, lipid-exposed surface of a β-barrel is approximately 27 Å thick and includes two bands of aromatic residues that are found in the interfacial region of the bilayer, ± 10 Å from the bilayer mid-plane [11]. Membranes are spanned by β-strands of 9–11 residues with a tilt of 20–45° out of the TM axis. The smallest known barrels contain 8 TM strands; this may be the minimum possible size [12] because of packing constraints in the barrel interior. The largest barrels of known structure, the TonB-dependent importers, contain 22 strands each. However, there is good evidence for the existence of β-barrels that are much larger [13].

The membrane-interacting surfaces of β-barrels are cryptically encoded in their amino acid sequences, with a dyad repeat pattern of alternating residues oriented either toward the lipid bilayer or toward the barrel interior. As expected, the composition of the lipid-exposed surfaces of β-barrels is very similar to that of the lipid-exposed surfaces of helical membrane proteins, see Figure 2, with an abundance of phenylalanine, tyrosine,


**Table 1**

<table>
<thead>
<tr>
<th>Functional category</th>
<th>One example</th>
<th>Structure (PDB accession)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonspecific porin</td>
<td>OmpF</td>
<td>2OMF</td>
</tr>
<tr>
<td>Facilitated transporter</td>
<td>Maltoporin</td>
<td>1MPR</td>
</tr>
<tr>
<td>Energy-dependent transporter-efflux</td>
<td>TolC</td>
<td>1QJ8</td>
</tr>
<tr>
<td>Energy-dependent transporter-influx</td>
<td>FepA</td>
<td>1FEP</td>
</tr>
<tr>
<td>Protein secretion pore</td>
<td>PulD</td>
<td>NA</td>
</tr>
<tr>
<td>OM usher pore</td>
<td>PapC</td>
<td>NA</td>
</tr>
<tr>
<td>Adhesin</td>
<td>OmpX</td>
<td>1QJ8</td>
</tr>
<tr>
<td>Lipase</td>
<td>OmpLA</td>
<td>1QD6</td>
</tr>
<tr>
<td>Protease</td>
<td>OmpT</td>
<td>1178</td>
</tr>
<tr>
<td>Mitochondria protein import pore</td>
<td>Tom40</td>
<td>NA</td>
</tr>
<tr>
<td>Protein pore-forming toxin</td>
<td>α-Hemolysin</td>
<td>7AHL</td>
</tr>
</tbody>
</table>

*For each category, I give one example for which some detailed information is known. These are broad functional categories. Some of them include multiple distinct families of proteins with hundreds of known members [25]. A PDB accession number is given for those examples for which a three-dimensional structure is known. NA, not available.

Tryptophan, valine and leucine, and the strong exclusion of polar and charged residues [11**,14,15]. The abundance of aromatic residues is especially striking at the bilayer interfaces, where they constitute about 40% of the lipid-exposed amino acids [11**]. Despite the fact that many β-barrels contain a water-filled channel, or a mobile hatch or plug domain, the abundant interior residues are predominantly the small or polar amino acids glycine, threonine, serine, asparagine and glutamine. Tyrosine is also relatively abundant, whereas the other hydrophobes are scarce [11**].

Although Schulz’s rules [10] describe β-barrels of known structure very well, the sparse sampling of β-barrels to date means that many families of β-barrels with different architectures and topologies are yet to be described. Some examples of these other families are discussed below.

**The bacterial ‘OMPome’**

Gram-negative bacteria have two bilayer membranes, an inner phospholipid membrane containing only α-helical membrane proteins and an outer phospholipid/lipopolysaccharide membrane containing only β-barrel membrane proteins. How many outer membrane (OM) β-barrel genes are in the genomes of Gram-negative bacteria? In the *Escherichia coli* genome, there are about 60 proteins, or 1.5% of the total, annotated as known, ‘probable’ or ‘putative’ outer membrane proteins (OMPs). This characterization is based mainly on sequence similarities to known proteins. Recognizing other TM β-barrels can be done using physical principles, but this is a more difficult task because the lipid-exposed surfaces of β-barrels are cryptic, obscured in the inside-outside dyad repeat pattern of the TM β-strands (Figure 2). Several groups have recently developed algorithms to identify β-barrels using either composition/architecture-based methods [11**,16**] or neural networks [17**]. See Figure 3 for an example of one analysis. For Gram-negative bacterial genomes, all of these algorithms find an asymmetric distribution of β-barrel scores or ranking from which identification can be made with moderate accuracy. Wimley [11**] found that the highest scoring 2.5% of the genes of *E. coli*, about 105 proteins, encoded almost all known β-barrels and only a few false positives. Similarly, Zhai and Saier [16**] found 118 proteins (2.8% of the genome) that scored high enough to be considered putative β-barrels. The overlap between these two putative β-barrel sets is greater than 90%. Using a neural network analysis, Casadio and co-workers [17**] identified about 75 proteins, or 1.8% of the *E. coli* genome, as strong candidate β-barrels. All three groups found that one-third or more of the potential β-barrels in the *E. coli* genome are unknown or hypothetical proteins. Application of the same selection criteria to the genomes of less well annotated Gram-negative bacteria produces very similar β-barrel abundances, from about 2.1% of the approximately 5500 genes of *Pseudomonas aeruginosa* [11**] to 3.5% of the 3900 genes of *Yersinia pestis*. *Hemophilus influenzae*, one of the smallest Gram-negative genomes (with ~1700 genes), has approximately 40 proteins (or 2.5% of the total genome) that are potential β-barrel membrane proteins (W.C. Wimley, unpublished data). In summary, multiple independent analyses indicate that 2–3% of the genes in all Gram-negative bacteria encode β-barrel OMPs.

Recent proteomic analyses have begun to measure the number of OMPs that are actually expressed in Gram-negative bacteria. Molloy et al. [18] analyzed the OM proteome of *E. coli* using 2D gels and mass spectrometry, and identified about 20 proteins, or 0.5% of the genome, that are highly abundant OMPs. Maddock, Andrews and colleagues [19] have been analyzing the OM proteome of another Gram-negative bacterium, *Caulobacter crescentus*. By improving methodology and extending the pI range of separation, they were able to identify about 50 proteins (1.5% of the genome) in their OM preparations that are thought to be OM β-barrels [20**]. Importantly, as many as one-third of these observed OMPs are annotated as hypothetical proteins or are not annotated with any known function. Nonetheless, most of these are predicted by computational means (e.g. [11**]) to be OM β-barrels.

**Folding and insertion**

Tamm and colleagues [21] have shown that the insertion and folding of OmpA, an eight-stranded β-barrel, into phospholipid vesicles is a spontaneous and cooperative process. A similarly cooperative assembly of β-sheets has been noted for small, hydrophobic model peptides [6] and for pore-forming toxins [13**,22]. An all-or-none insertion process makes sense for β-barrels in light of their network
of nonlocal hydrogen bonds. The manner in which β-barrels are inserted into bacterial OM is not well understood. Spontaneous insertion of β-barrels into membranes is unlikely to be the major pathway for several reasons. First, β-barrels must transit the inner membrane without inserting into it to be correctly inserted into the OM. Second, although it is easy to visualize a β-barrel inserting a set of tight β-hairpins across a membrane, β-barrels in bacteria often have the opposite topology, with their long loops on the outside of the OM and the tight turns of the β-hairpins on the periplasmic surface. OM β-barrels are first secreted across the inner membrane into the periplasmic space by virtue of an N-terminal signal sequence that targets the protein to the Sec translocation machinery [23]. In the periplasmic space, there are chaperones, such as the SurA and skp proteins of *E. coli*, that stabilize unfolded OMPs and are essential for proper insertion into the OM [21]. There is also at least one OMP, called Omp85 in *Neisseria*, that is conserved in all Gram-negative bacteria and has been shown to be essential for OM biogenesis, perhaps through a role in the insertion and folding of OMPs [24]. This folding pathway offers a possible explanation for the strict segregation of β-barrel and α-helical membrane proteins in Gram-negative bacteria. Proteins destined for either membrane can use the Sec translocation machinery in the inner membrane because they use distinct pathways for subsequent membrane targeting and insertion. Segregation is by structural...
class, such that α-helical membrane proteins, with their long contiguous hydrophobic helices, move laterally from the translocation machinery directly into the inner membrane lipids [23], whereas β-barrel OMPs, with their cryptically encoded hydrophobic surfaces, are translocated through the inner membrane into the periplasmic space and then subsequently integrated into the OM.

Large oligomeric β-barrel pores

Among the β-barrels for which there currently are no three-dimensional structures, there is a broad assortment that are multimeric OM pores required for the secretion of proteins across the OM, or the secretion and assembly of filamentous structures such as pili. In any species of Gram-negative bacteria, there are dozens of such secretion pathways [25**], many of which are important for pathogenicity. The proteins required for filamentous structures are often found in a plasmid-encoded operon that also contains a specific OM usher protein. As an example, the E. coli OM usher PapC is required for P pilus assembly [26]. A typical OM usher protein [25**,27], PapC is approximately 840 amino acids long and is predicted to span the OM.
with a large C-terminal β-barrel domain of between 18 and 24 TM β-strands [25**]. The tight turns of the β-hairpins are on the external surface of the OM [26]. Their N-terminal domains are periplasmic ‘plugs’ that regulate transport through the pore. PapC monomers assemble in the OM into multimeric structures containing 10–12 subunits and a 20–30 Å central pore [26].

Also identified are ‘autotransporter’ proteins and ‘two-partner secretion’ pores [25**], which have a similar architecture, including a C-terminal β-barrel domain that assembles into an oligomeric OM pore [28,29] and an N-terminal periplasmic domain that regulates transport. Like the usher proteins, these pores allow the specific transport of a single protein species. The transported protein is usually encoded in the N-terminal part of the pore sequence, if it is an autotransported protein, or as an upstream gene in an operon. Electron microscopy revealed a 20 Å pore with sixfold symmetry in the structure of the Neisseria IgA protease autotransporter [28]. Transport of the C-terminal protease domain was shown to occur through a common, central channel [28]. Interestingly, the C-terminal β-barrel of the two-partner secretion pore FhaC most likely spans the membrane with an odd number of β-strands, probably 17 or 19 [30]. Although this feature has not yet been observed in the few known β-barrel structures, it could be present in other β-barrels or families of barrels.

These families of multimeric β-barrel pores are abundant in bacteria [25**,31] and are important in bacterial pathogenicity. Homologs of two-partner secretion pores are even found in eukaryotes [25**]. Currently, the best structural information we have for them is from electron microscopy [26,27,29], which has shown that all of these multimeric β-barrel pores are roughly symmetrical, often have 6- or 12-fold symmetry, and have a single, central pore. It is not currently known if these multimeric β-barrel pores are formed by rings of closed monomeric β-barrels or if they consist of a single large β-barrel made from the TM β-hairpins of the subunits.

Architectural nonconformists

The secretins comprise another distinct family of multimeric OMP secretion pores. They function as one of the terminal OM pores of the general secretion pathway [25**,27]. Secretin pores are observed to be large in electron microscopy images, from 50 to 100 Å in diameter [27,32,33]. A dodecameric pore structure has been proposed for the meningococcal secretin PilQ [32] and for the C-terminal domain of the secretin protein PilD [34]. Also, electron microscopy analysis has revealed a two-domain architecture for the secretin complexes, with a membrane-spanning ring domain and a periplasmic ‘cup’ domain [32]. Despite the large pore, the periplasmic domains keep secretins closed to nonspecific solute flow. Most importantly for this discussion, the architecture...
of secretin pores is very different from all other known β-barrels. Their TM β-strands are not found in the obvious strand-turn-strand β-hairpin structures that are always present in the other known β-barrels. Instead, the TM β-strands of secretins are isolated single strands in the sequence bracketed by longer loops that are presumably on both sides of the membrane [25**]. Thus, the secretins are not identified as β-barrels using algorithms that are based on known β-barrel structures (e.g. [11**,16**,17**]). Based on their unusual architecture, it remains possible that the secretin pores are not β-barrels at all.

Mitochondria and chloroplasts

It has been hypothesized that some, perhaps most, of the integral OMs of mitochondria and chloroplasts are β-barrels because these membranes are relics of the organelles’ evolutionary history as symbiotic intracellular Gram-negative bacteria. Based on structure predictions and experimental studies of individual proteins, it is now clear that the OMs of these organelles contain both α-helical and β-barrel membrane proteins. For example, the abundant mitochondrial voltage-dependent anion channel (VDAC) has long been thought to be a β-barrel [35] with about 14–16 TM β-strands [36,37], as has the functionally similar chloroplast protein OEP24 [38]. The detailed work of Pfanner and others [39,40,41**] has shown that the protein translocase of the outer mitochondrial membrane (TOM) is a complex of membrane proteins with a central pore composed of oligomers of the β-barrel protein Tom40 [39]. However, the Tom40 β-barrel pore is closely associated with several integral membrane proteins that are anchored to the membrane by membrane-spanning α-helices [40]. Similarly, Toc75, the pore of the chloroplast OM translocase, is a β-barrel [42], whereas the other subunits of the translocase are anchored in the membrane by TM α-helices [42].

Schleiff et al. [43**] have recently performed a genomic and proteomic analysis of the chloroplast OM. By using a combination of structure prediction, target sequence detection, pI calculation and manual screening, they identified a list of approximately 900 proteins in Arabidopsis (3.1% of the genome) that are potentially chloroplast β-barrels. They also performed a preliminary proteomic analysis of the chloroplast OM, which identified a small set of proteins including both β-barrel and α-helical membrane proteins. Preliminary application of similar multistage screening to the genome of the yeast Saccharomyces cerevisiae revealed at least 100 proteins, or about 1.5% of the genome, that are potentially mitochondrial β-barrels (WC Wimley, unpublished data).

The image that emerges from these analyses is that the OMs of mitochondria and chloroplasts do contain β-barrel membrane proteins, but, during their long evolutionary history as intracellular organelles, some α-helical membrane proteins have also been targeted to these membranes. Despite recent advances in mitochondrial proteomics [44], the exact proportion of α-helical and β-barrel proteins in mitochondrial OMs is currently not known.

Other bacteria

The single membranes of classical Gram-positive bacteria and archaea apparently do not contain β-barrel membrane proteins [25**]. However, some other bacteria that are not proteobacteria, (i.e. not classical Gram-negatives) do contain β-barrels. In a recent review of bacterial OM transporters, Saier and colleagues [25**] showed that members of some β-barrel transporter families are found in non-proteobacterial families with inner and outer membranes. These families include Deinococcus, Chlamydia and Cyanobacteria. Also, the cell walls of some ‘acid-fast’ bacteria, including those belonging to the Treponema and Mycobacteria families, are coated with a mycolic-acid-rich lipid layer attached to the cell wall that functions as a protective barrier. These waxy OMs can be 50–100 Å thick, compared to about 30 Å for a phospholipid bilayer, due to the very long chains of the mycolic acid groups. In all of these dual-membrane organisms, there are large protein pores of approximately 20 Å diameter that span the OM [45–47]. It has been proposed that these protein pores, such as major surface protein A (MspA) from Mycobacterium smegmatis and the major outer membrane proteins (MOMP)s of Chlamydia, are β-barrels [45–47]. This idea is supported by the fact that these pores span the outer lipid layer without long hydrophobic α-helical sequences and that they have a high potential for TM β-sheet formation [11**]. However, the exact nature of the pore structure remains to be elucidated.

Pore-forming toxins

Some secreted bacterial toxins exert their cytotoxic effects by assembling into membrane-spanning β-barrel pores [48]. The list includes α-hemolysin of Staphylococcus aureus [49], which assembles into the heptameric 14-stranded β-barrel pore that is shown in Figure 1. The large family of cholesterol-dependent cytolysins (e.g. perfringolysin [13*]) has also been shown to assemble into very large β-barrel pores, as much as 300 Å in diameter [13*]. In these proteins, each monomer contributes two β-hairpins to the pore’s single barrel [13*]. Several multirpart protein toxins, such as the anthrax toxin [50], also have a multimeric, β-barrel pore-forming subunit through which the other protein toxin subunits cross the membrane. An interesting structural theme that unifies the β-barrel toxins is that the TM β-barrel is formed from sequences that are present as long loops that are normally folded back into the monomeric or inactive protein. After assembly of the multimeric ‘prepro’ structure, the loop sequences undergo a large concerted conformational switch in which the tip of the
loop inserts into the membrane to become one of the β-hairpins of the barrel [2,13]. The formation of the hydrogen-bonded network of the β-barrel makes insertion essentially irreversible.

Conclusions
The β-barrel membrane protein is found in the OMs of bacteria, mitochondria and chloroplasts, and in the TM pores of some secreted protein toxins. Here I have reviewed the diversity of their function, architecture and distribution. It is hoped that future studies will bring to light the diversity of their three-dimensional structure as well.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
**■ of outstanding interest


This paper describes the formation of the mitochondrial translocase of the outer membrane transporter involved in the secretion of the Bordetella pertussis filamentous hemagglutinin. J Biol Chem 2000, 275:30202-30210.

The versatile β-barrel membrane protein

The β-barrel membrane protein, such as the porin from the outer membrane of Escherichia coli, is a well-studied example of a β-barrel protein. These proteins are characterized by a repeating pattern of antiparallel β-strands that form a cylindrical structure. The β-barrels are typically involved in the transport of molecules across biological membranes, and their structural features have been extensively studied.


41. Model K, Prinz T, Ruiz T, Rademacher M, Krimmer T, Kuhlbrandt W, Pfanner N, Meisinger C: Protein translocase of the outer mitochondrial membrane: role of import receptors in the structural organization of the TOM complex. J Mol Biol 2002, 316:657-666. This paper describes the formation of the mitochondrial translocase pore and how pore formation is modulated by some of the other members of the TOM complex. This paper nicely illustrates the interplay between the β-barrel pore protein Tom40 and its associated α-helical membrane protein partners.


43. Schleiff E, Eichacker LA, Eckert K, Becker T, Minus O, Stahl T, Soll J: Prediction of the plant β-barrel proteome: a case study of the chloroplast outer envelope. Protein Sci 2003, 12:748-759. This paper is a bold attempt to identify potential β-barrel chloroplast OMPs from the genome of Arabidopsis. Using a hierarchical selection process, about 900 proteins, or 3.1% of the genome, were found to encode proteins that could potentially be β-barrels.


57. Chimiento DP, Mohanty AK, Kadner RJ, Wiener MC: Substrate-induced transmembrane signaling in the cobalamin transporter BtuB. Nat Struct Biol 2003, 10:394-401. A recent structural study of BtuB, a TolC-dependent cobalamin (vitamin B_{12}) importer, Gram-negative bacteria have many TolC-dependent importers. All are OM β-barrels that are energetically coupled to the inner membrane so that they can drive the import of rare cofactors. This study has revealed some important details of the structural transitions that are involved in transporter activity.