Gram-negative Bacterial Cell Wall

--Outer Membrane Proteins: many are selective pores, or "porins"

--Inner Membrane Proteins
A few references on membrane protein structure:


The classical protein folding patterns

Antiparallel 🆕

Parallel 🆕/アメリカ

Antiparallel 🆕

(Also, 🆕+アメリカ)
Outer Membrane Proteins (OMPs). Several structures of specific "porins", as well as integral membrane domains or anchors of other proteins, are known.
LamB (Maltoporin), an integral outer membrane protein; a trimer
One subunit of LamB (Maltoporin)
Membrane proteins consisting of β-barrels

<table>
<thead>
<tr>
<th>Protein</th>
<th>n</th>
<th>S</th>
<th>$R$ (Å)</th>
<th>$\alpha$ (deg)</th>
<th>Oligomeric state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gramicidin A (native)</td>
<td>1</td>
<td>6</td>
<td>3.4</td>
<td>77</td>
<td>Head-to-head dimer traversing the membrane</td>
</tr>
<tr>
<td>Nanotube</td>
<td>1</td>
<td>–</td>
<td>5.6</td>
<td>90</td>
<td>Stack of about eight cyclic octapeptides through the membrane</td>
</tr>
<tr>
<td>OmpX</td>
<td>8</td>
<td>8</td>
<td>7.2</td>
<td>37</td>
<td>Monomer without channel</td>
</tr>
<tr>
<td>OmpA</td>
<td>8</td>
<td>10</td>
<td>7.9</td>
<td>43</td>
<td>Monomer without channel</td>
</tr>
<tr>
<td>OmpT</td>
<td>10</td>
<td>12</td>
<td>9.5</td>
<td>42</td>
<td>Monomer without channel</td>
</tr>
<tr>
<td>OmpLA $^c$</td>
<td>12</td>
<td>12</td>
<td>10.6</td>
<td>37</td>
<td>Monomer without channel</td>
</tr>
<tr>
<td>TolC</td>
<td>12</td>
<td>20</td>
<td>13.6</td>
<td>51</td>
<td>Single β-barrel composed of a trimer, forms a channel</td>
</tr>
<tr>
<td>α-Haemolysin</td>
<td>14</td>
<td>14</td>
<td>12.3</td>
<td>37</td>
<td>Single β-barrel composed of a heptamer, forms a channel</td>
</tr>
<tr>
<td>Porin Rhodobacter capsulatus</td>
<td>16</td>
<td>20</td>
<td>15.5</td>
<td>43</td>
<td>Trimer of parallel β-barrels forming three channels</td>
</tr>
<tr>
<td>Porin OmpF (PhoE, OmpC)</td>
<td>16</td>
<td>20</td>
<td>15.5</td>
<td>43</td>
<td>Trimer of parallel β-barrels forming three channels</td>
</tr>
<tr>
<td>Porin Rhodobacter blasticus</td>
<td>16</td>
<td>20</td>
<td>15.5</td>
<td>43</td>
<td>Trimer of parallel β-barrels forming three channels</td>
</tr>
<tr>
<td>Porin Paracoccus denitrificans</td>
<td>16</td>
<td>20</td>
<td>15.5</td>
<td>43</td>
<td>Trimer of parallel β-barrels forming three channels</td>
</tr>
<tr>
<td>Porin Omp32</td>
<td>16</td>
<td>20</td>
<td>15.5</td>
<td>43</td>
<td>Trimer of parallel β-barrels forming three channels</td>
</tr>
<tr>
<td>Maltoporin (two species)</td>
<td>18</td>
<td>20</td>
<td>17.1</td>
<td>40</td>
<td>Trimer of parallel β-barrels forming three channels</td>
</tr>
<tr>
<td>Sucrose porin</td>
<td>18</td>
<td>20</td>
<td>17.1</td>
<td>40</td>
<td>Trimer of parallel β-barrels forming three channels</td>
</tr>
<tr>
<td>FhuA</td>
<td>22</td>
<td>24</td>
<td>19.9</td>
<td>39</td>
<td>Monomer clogged by a removable polypeptide domain</td>
</tr>
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<td>FepA</td>
<td>22</td>
<td>24</td>
<td>19.9</td>
<td>39</td>
<td>Monomer clogged by a removable polypeptide domain</td>
</tr>
</tbody>
</table>

$^a$ All sheets are antiparallel except for native gramicidin A. The topologies are always all-next-neighbor.

$^b$ The radius is calculated for a circular cross section. The angle $\alpha$ can vary by $\pm 15^\circ$ around the barrel (Fig. 1).

$^c$ This enzyme exists as a monomer in the membrane and becomes active on dimerization.
Aromatic "bands" on exterior of porins interface to the lipid bilayer

"Bands" of aromatic residues around circumference of structure.
What is currently known about the structures of the integral membrane parts of bacterial outer membrane proteins:

Topology: an even number of antiparallel beta strands which follow the order in the polypeptide (no crossover strands).

Number of strands range from 8 to 22 in known examples.

Hydrophobic residues on the exterior in the membrane-spanning segment; aromatic bands at levels corresponding to extremities of hydrophobic layer of membrane.

Many porins are trimers; there is a pore within each protomer of the trimer. Some are monomers.

Most integral outer membrane proteins end in an Aromatic-X-Aromatic sequence motif. This motif triggers the periplasmic unfolded protein response in E. coli\(^1\).

Outer membrane proteins also have a high frequency of Aromatic-X-Aromatic sequence motifs within their sequences. This motif is recognized by a molecular chaperone that facilitates outer membrane protein folding\(^2\).

--Outer Membrane Proteins: many are selective pores, or "porins"

--Inner Membrane Proteins
Inner membrane proteins: consider a simple (structurally) example, the potassium channel.
Potassium channel from *Streptomyces lividans*

--an integral membrane protein with sequence similarity to all known K+ channels

--$10^4$-fold selectivity of $\text{K}^+$ over $\text{Na}^+$ (1.33 vs 0.95 Å ionic radii)

--$10^8$ ions/sec
First structure of pore-forming fragment: solved at nominal resolution of 3.2 Å
Structure: a tetramer; each protomer has two long helices and a short "pore helix".

The tetramer has external "aromatic bands" around the periphery where they would be at the top and bottom of the lipid bilayer.
Difference Fouriers from crystals soaked in rubidium and cesium to identify ion binding sites

Rubidium 4.0 Å  
Cesium 5.0 Å
Structure suggests two contributions to stabilization of monovalent cation:
1. Aqueous cavity stabilizes ion in hydrophobic interior
2. “Pore” helices stabilize electrostatically.
How to get higher resolution data?

Crystallize in complex with Fab fragment of a monoclonal antibody. Complex behaves much like a water-soluble protein.

Complex between two subunits of channel and two Fab fragments.  

Crystal packing.
Higher level of detail at 2.0 Å resolution

Experimental map

Proposed model of K⁺ translocation
Another couple of examples: proteins that "couple" transport of two substrates.
Two proteins of the "Major Facilitator Superfamily":
Lactose permease (LacY)
Glycerol-3-phosphate transporter (GlpT)
Resolution of Structures

LacY ~3.5 Å

GlpT ~3.3 Å
LacY: a monomer with 12 helices in a pseudo-twofold symmetry.
Model for the conformational switch of LacY
GlpT: also a monomer with 12 helices and a pseudo-dyad within molecule
Schematic model for GlpT mechanism