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# Membrane Proteins with β-Barrel Fold

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*Abstract:* Porins, the major proteins found in the bacterial outer membrane, exhibit an unusual hollow β-barrel structure. This motif constitutes the scaffold for a pore that facilitates the diffusion of solutes across the membrane. OmpF porin was the first membrane protein to be crystallized many years ago at the Biozentrum in Basel. Since then a wealth of structural information at high resolution has been acquired by X-ray crystallography. Porins from *E. coli* turned out to be extremely robust and easy to manipulate, allowing detailed and comprehensive structure–function analysis. In particular, insight was obtained into the role of the highly charged pore constriction in OmpF porin and the 'greasy slide', a string of aromatic residues, in maltoporin.

**Keywords:** Membrane protein · Porin · Protein crystallography · Simulation

## 1. Introduction

A quarter to a third of all proteins are associated with the cell membrane, as inferred from genome sequence analyses. This accounts for protein sequences that contain long hydrophobic segments indicative of transmembrane helices. The prevalence of membrane proteins with other secondary structure is largely unknown [1]. In this respect, the outer membrane of Gram-negative bacteria represents an interesting case. Most, if not all, of its proteins lack transmembrane helices and, instead, show the β-barrel fold, for recent reviews see [2].

Porins, the major class of outer membrane proteins, allow the passage of small solutes (nutrients, waste products) across the protective shield of the outer membrane (Fig. 1). They are rather tough and comparatively easy to overexpress, purify and crystallize [3]. Here, we give a short account of their structural features and how these relate to functional properties.

## 2. Crystallization

Compared to the wealth of structural information available for soluble proteins, the structures of only few membrane proteins are known to date. The reason for this lies in several peculiarities inherent to this class of proteins. First, they are notoriously difficult to overexpress and tend to disintegrate during solubilization. Having solved this problem, the solubilized and hopefully monodisperse protein may still not crystallize due to the lack of sufficiently large polar patches on the molecular surface. The hydrophobic protein surface, which is in contact with the membrane in the native state, will be shielded by (mostly disordered) detergent molecules. This prevents it from engaging in specific crystal contacts, although in rare cases also specific hydrophobic protein–protein contacts have been observed. Generally, all these difficulties can be overcome only by lengthy screening procedures. This involves screening for the most suitable protein variant (species, mutant), detergent and crystallization condition. A more rational approach is to increase the protein's hydrophilic surface by complex formation either with specific antibody fragments [4] or, if there are any, with soluble proteins which interact with the membrane domain of interest.

## 3. Architecture

As a general rule, protein chains traverse the membrane with regular secondary structure to avoid mismatch between polar main-chain atoms and the apolar environment of the membrane core. For proteins with β-structure, as an additional requirement, the polar main-chain atoms at the two edges of the β-sheet have also to be satisfied in their hydrogen-bonding capacity. This is most naturally fulfilled by rolling up the sheet to a β-barrel as depicted in Fig. 2 for the OmpF porin monomer [5]. For this fold to be stable in the membrane, no long stretches of hydrophobic amino-acid sequence are required. Instead, only every second residue (situated on the membrane exposed face of the strand) has to be apolar. It turns out that the minimal length of such a transmembrane strand is just seven residues [6].

All porins form tight homotrimers, probably to stabilize the hollow subunits. In contrast, the membrane anchor OmpA [7] and the siderophore transporters FhuA and FepA [8], which do not show open pores, exist as monomers. The β-barrel scaffold encompasses the aqueous pore that runs along the barrel axis and is constricted by one or more loops interacting with the inner wall of the β-barrel.

Porins can be classified into two groups depending whether they show

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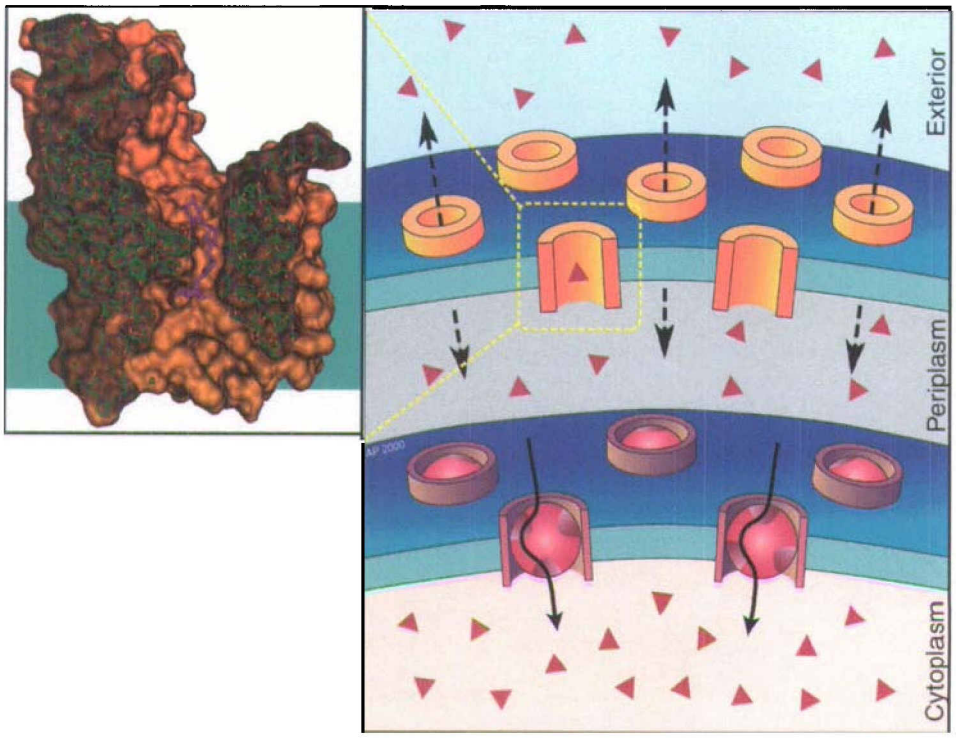


Fig. 1. Scheme of a bacterial cell surface. The cytoplasm (bottom) is surrounded by the tight plasma membrane. Specific substrates (triangles; *e.g.* sugars) are pumped into the cell by active transporters. In Gram-negative bacteria, additional protection is conferred by the outer membrane, which has pore proteins (porins) incorporated to allow solute exchange with the cell exterior (top). This process is driven simply by passive diffusion and results in equilibration between the exterior and the intermembrane space (periplasm). The small panel (left) shows a longitudinal section through maltoporin with a maltodextrin molecule bound at the channel constriction.

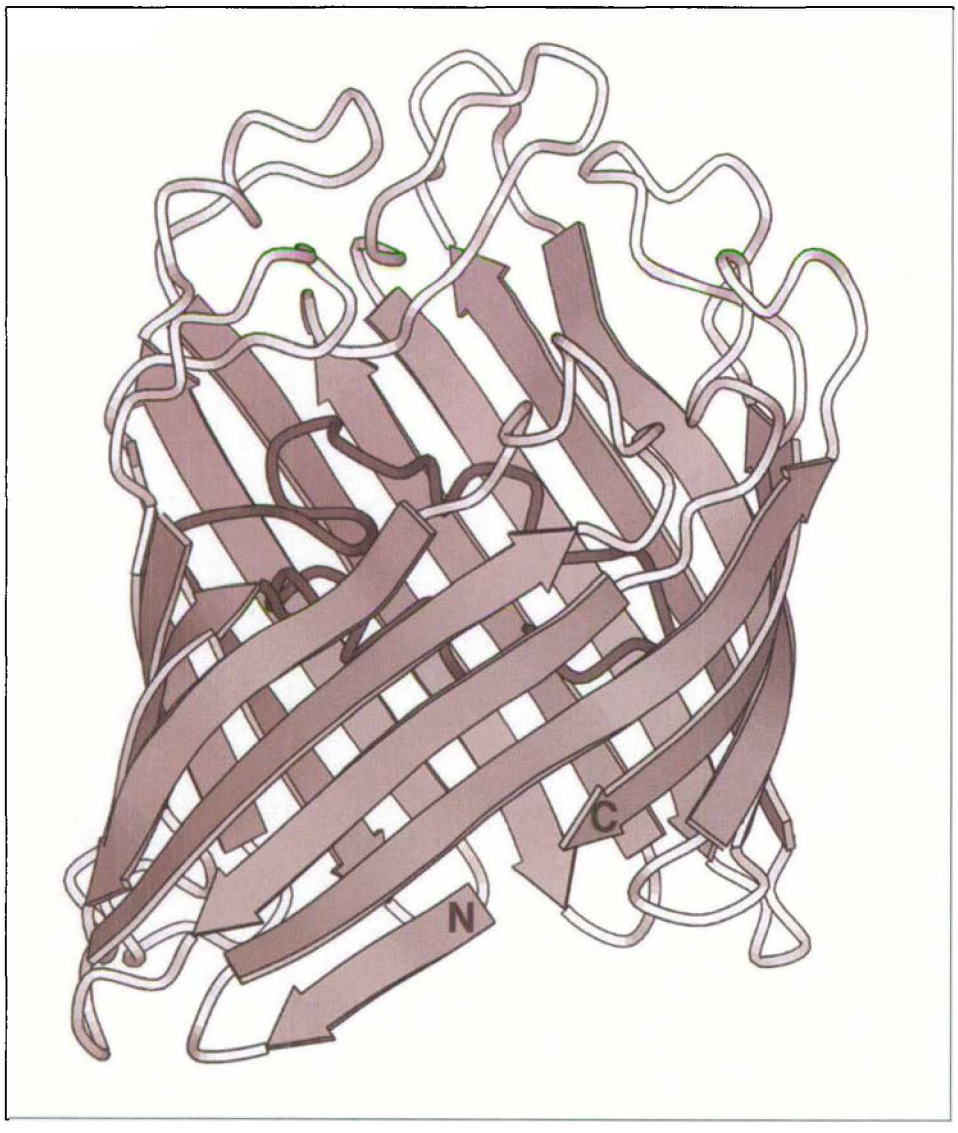


Fig. 2. Schematic sketch of the OmpF porin fold. Sixteen antiparallel β-strands (arrows) are arranged to form a β-barrel encompassing the pore.

substrate specificity or not. In both cases, solute translocation is driven by the concentration gradient over the outer membrane. Representatives of both groups have been studied and are discussed in the following.

## 4. Non-specific Porins

Sixteen antiparallel strands form the β-barrel of non-specific porins. This scaffold is modified by a loop (Fig. 2) that interacts with the inner face of the barrel wall and contributes to a constriction about half-way through. In OmpF porin from *E.coli*, but also in other non-related non-specific porins [9], the constriction is highly charged and shows a conspicuous segregation of basic and acidic residues (Fig. 3).

Tens of site-directed mutants have been produced to probe various functional aspects such as voltage gating, thermal stability, and determinants of ion flow [10]. It turned out that OmpF is an ideal object for such studies. Almost all mutants studied expressed well and crystallized isomorphously. The β-barrel appears to be an autonomous scaffold, which is not affected by tinkering with the channel constriction. Functionally, the variants can be characterized conveniently by measuring single channel conductance and ion selectivity upon insertion into artificial lipid [11].

Recently, we have focused on the role of the charges at the pore constriction

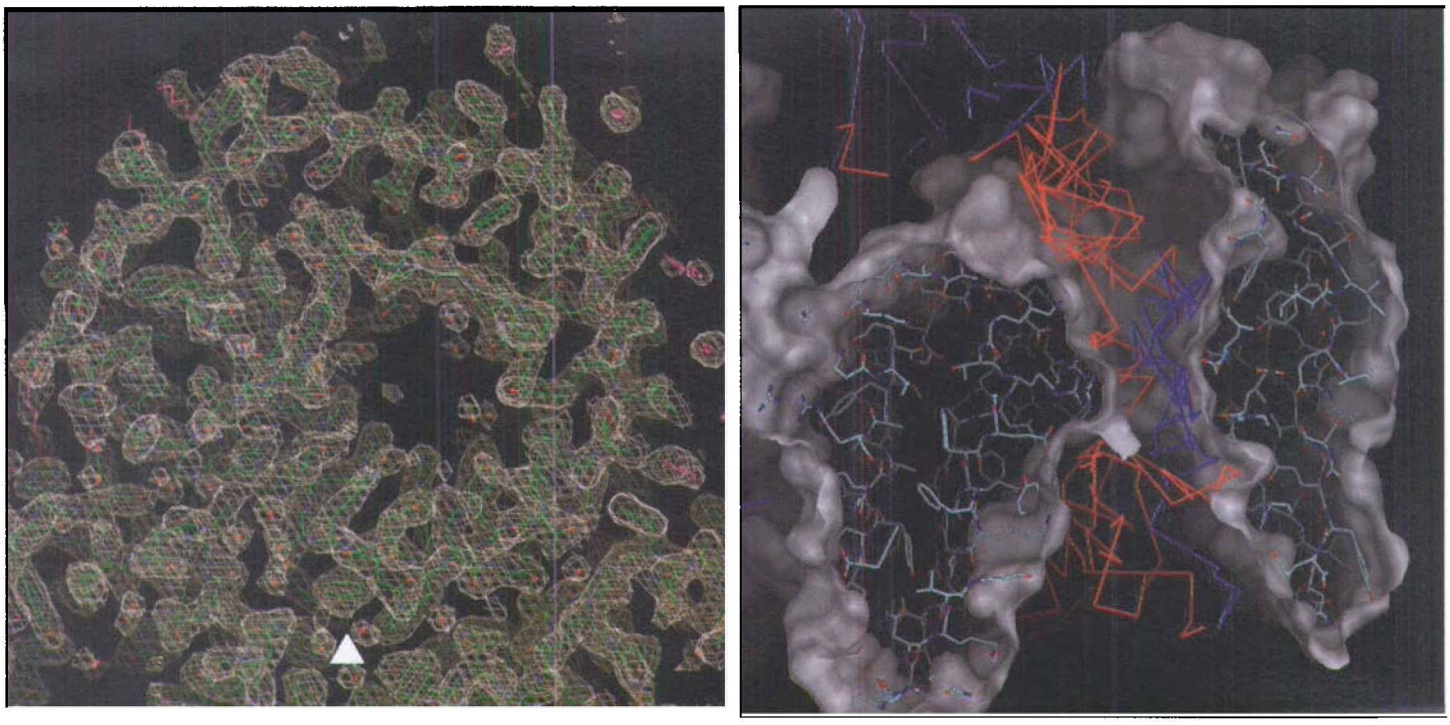

Fig. 3. (a) Cross-section through the OmpF trimer at the height of the pore constriction. The model is shown superimposed onto the electron density map ($2F_o$-$F_c$ coefficients, resolution 2.4 Å). The view is parallel to the symmetry axis (white triangle), only one monomer is shown in full. The transmembrane pore (three per trimer) is lined with charged residues (Arg, Asp, Glu). Some water molecules (red crosses) are found bound to pore lining. (b) Longitudinal section through the OmpF pore with the Brownian dynamics trajectories of an anion (blue) and a cation (red) superimposed. The two types of ions choose different routes according to the dipolar nature of the pore. Note the arginine cluster at the right side of the constriction and the two carboxylate groups at the left side. Reproduced with permission from [12].

[12][13]. As expected, these charges crucially determine cation over anion selectivity. Typically, deletion of one charge alters the selectivity by a factor of two. The charges also have a pronounced influence on channel conductance. For example, in mutant D113N/E117Q, which has two negative charges at the constriction removed, the conductance is reduced by a factor of two, although the pore structure is found virtually unchanged. The protein charges serve to increase the local concentration of counter ions at the constriction and, thereby, increase the efficiency of the pore. This was confirmed by Brownian dynamics simulations [12][13], in which ions were allowed to move in response to a stochastic force representing Brownian motion and an electrostatic force exerted by the protein charges (Fig. 4).

## 5. Maltoporin

Longer maltodextrin molecules, degradation products of starch composed of linear polyglucose chains, do not pass through the non-specific porins. For the passage of this important nutrient, maltoporin (LamB protein from *E.coli*), the prototype of a specific porin, has evolved. Characteristically, the translocation rate saturates with increasing substrate concentration indicating a binding site within the pore. The protein shows only very limited sequence similarity with *e.g.* OmpF.

Structure determination of maltoporin [14] turned out to be difficult. No useful heavy atom derivatives were found and no suitable model for molecular replacement was available. The solution was obtained by cyclic 3-fold averaging and phase extension starting at very low resolution (8 Å). The position of the local symmetry axis was determined by an almost forgotten Patterson correlation method [15]. Initial phases for averaging were obtained by placing the trimeric model of OmpF on the local axis.

The fold of maltoporin is again a β-barrel, this time composed of 18 strands (Fig. 4). Six contingent aromatic residues line the channel and form a smooth hydrophobic path from the channel vestibule through the constriction to the outlet. The start of this 'greasy slide', which is easily accessible from the channel vestibule, probably serves to align the sugar with the channel axis *via* non-specific hydrophobic interactions. This notion has been corroborated by kinetic analysis of respective mutants [16]. The center of the slide is part of the maltodextrin binding site as revealed by the crystal structures of respective complexes (Fig. 4). Since the affinity of this site is in the millimolar range, the channel will not be blocked under physiological conditions. Rather, the presence of a binding site at the strategic position of the channel constriction serves to enhance the local sugar concentration thereby facilitating translocation.

It is easy to conceive that the smoothness of the 'greasy slide' in conjunction with the non-specific nature of the hydrophobic interaction is of advantage for rapid translocation. However, there are also a large number of H-bonding interactions between the sugar hydroxyl groups and several charged residues at the channel lining. These are arranged in two tracks juxtaposed to the polar edges of the polysaccharide. During register shift of the sugar, the H-bonds that must be broken and formed several times during translocation are relayed from one protein side-chain to the next as revealed by a recent molecular simulation study employing the Conjugated Peak Refinement algorithm [17]. As a result the H-bonding capacity of the polar groups is constantly satisfied and no large energy barriers are encountered.

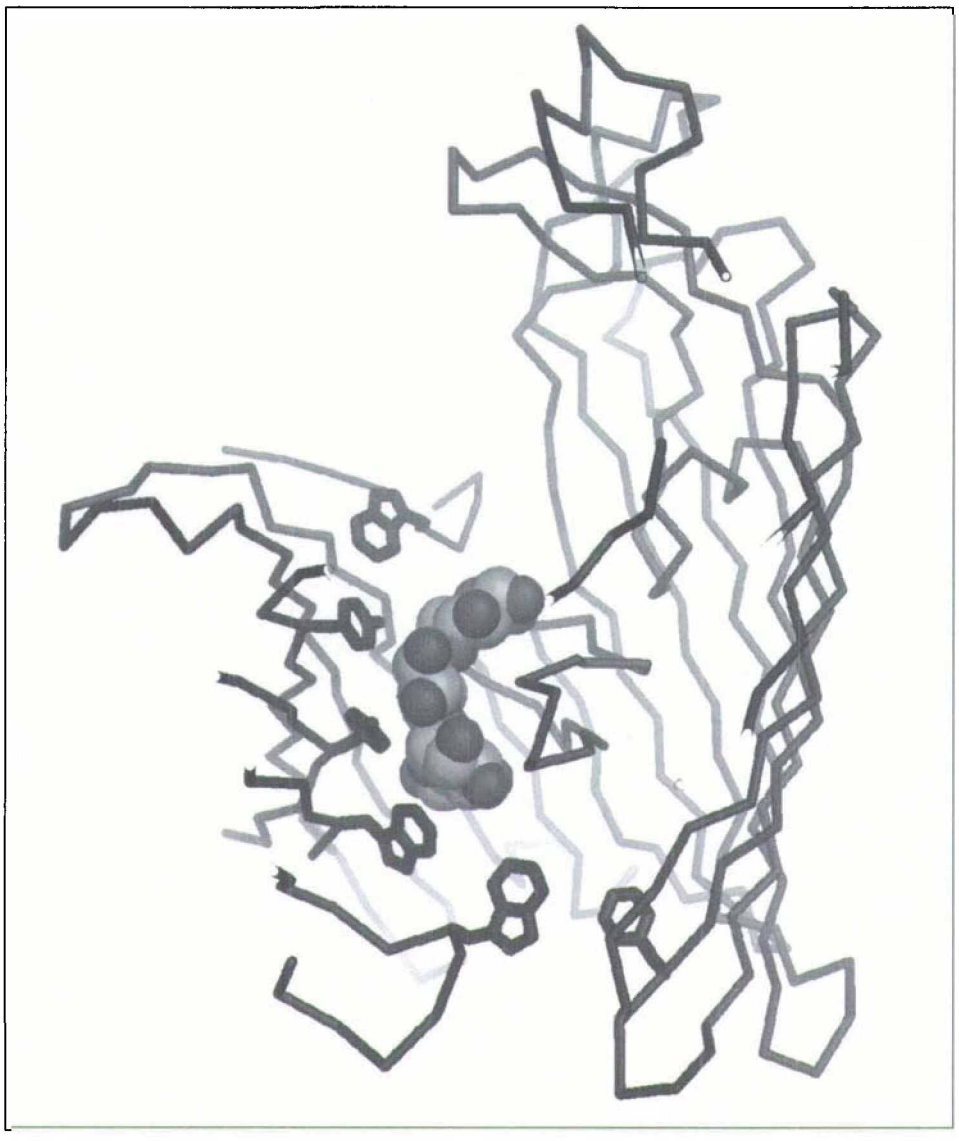

Fig. 4. Side-view of maltoporin with part of the backbone clipped off for clarity. The six aromatic residues of the 'greasy slide' are shown as ball-and-stick models. The bound maltotriose molecule (drawn in space-filling representation) is in van-der-Waals contact with the slide. Additionally, the sugar hydroxyl groups are bound to charged protein side-chains (not shown).

## 6. Conclusions

A wealth of structural and functional knowledge on porins has been gathered. The β-barrel framework has proven to be very robust and allows replacement of pore lining residues without disturbance of its structure. Engineering of tailor-made sub-nano devices with unique filtering or even enzymatic properties is now conceivable. In the bacterial outer membrane, the β-barrel motif shows to be extremely versatile, exemplified by the passive diffusion pores OmpF and maltoporin, but also by the iron siderophore transporters and the putative membrane anchor OmpA.

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