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Structural insight into OprD substrate specificity

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OprD proteins form a large family of substrate-specific outer-membrane channels in Gram-negative bacteria. We report here the X-ray crystal structure of OprD from *Pseudomonas aeruginosa*, which reveals a monomeric 18-stranded β-barrel characterized by a very narrow pore constriction, with a positively charged basic ladder on one side and an electronegative pocket on the other side. The location of highly conserved residues in OprD suggests that the structure represents the general architecture of OprD channels.

In Gram-negative bacteria such as *Escherichia coli*, the uptake of the majority of water-soluble substrates is mediated by nonspecific porins such as OmpF. However, many Gram-negative bacteria (for example, pseudomonads) do not possess porins and consequently have a poorly permeable outer membrane. In the absence of porins, transport of the majority of small molecules is mediated by substrate-specific channels of the OprD family[1,2]. The prototype of the OprD family is OprD from *P. aeruginosa*, an opportunistic human pathogen responsible for many hospital-acquired infections[1]. Treatment of patients with *P. aeruginosa* infections is hampered by the high intrinsic antibiotic resistance of this bacterium, which is partly due to the low permeability of its outer membrane[3]. Besides mediating the specific uptake of basic amino acids[4], *P. aeruginosa* OprD also serves as the entryway for carbapenem antibiotics and is therefore important clinically[5]. Despite the importance of the OprD family for the functioning of many Gram-negative bacteria, no structural information is available for any member of this family. To gain insight into the mechanism of substrate transport by OprD family members, we have determined the X-ray crystal structure of *P. aeruginosa* OprD.

The OprD structure was solved by SAD using selenomethionine-substituted protein (see **Supplementary Methods** and **Supplementary Table 1** online for details). OprD crystallizes as a monomeric 18-stranded β-barrel (**Fig. 1a**); this contrasts with the predictions of topology programs such as TMB-PRED[6] and those of other studies, which suggested that the β-barrel would have 16 strands[7]. The monomeric nature of OprD in the crystals is unexpected, given the presence of the two short β-strands S5 and S6 in the OprD structure (**Fig. 1a**). These short β-strands are a structural hallmark of trimeric outer-membrane channels and thus suggest that OprD may form a trimer in the outer membrane. This notion was confirmed by the results of nondenaturing PAGE in mild detergents, which revealed the presence of monomers and oligomers, most probably trimers (**Supplementary Fig. 1** online). Thus, OprD may exist as a labile trimer within the outer membrane. To our knowledge, the low stability of the OprD trimer is so far unique, as all other trimeric outer-membrane channels are extremely stable. The X-ray structure also shows that it is highly unlikely that OprD functions as a serine protease, as has been proposed[8]. His156, Asp208 and Ser296, proposed to be the residues of the catalytic triad[9], are too far apart and located in the wrong positions to be part of a catalytic triad (**Fig. 1a**).

The interior of the OprD barrel shows two long loops, L3 and L7 (**Fig. 1a**), which fold inward to form a very narrow, roughly circular constriction (∼5.5 Å in diameter). The pore constriction lies at the interface of loop L3 (Ala127–Ser130), loop L7 (Asp295–Ile297 and Ser302–Asp307), strand S17 (Arg391) and strand S18 (residue Arg410) of the barrel wall (**Fig. 1a**). The OprD pore constriction is composed exclusively of polar side chains (of Ser130, Ser296, Ser302, Asp307, Arg391 and Arg410) and backbone carbonyl groups (of Gly129, Gly130, Ile297, Gln304 and Tyr305). It is predominantly negatively charged, but has arginine residues (Arg391 and Arg410)

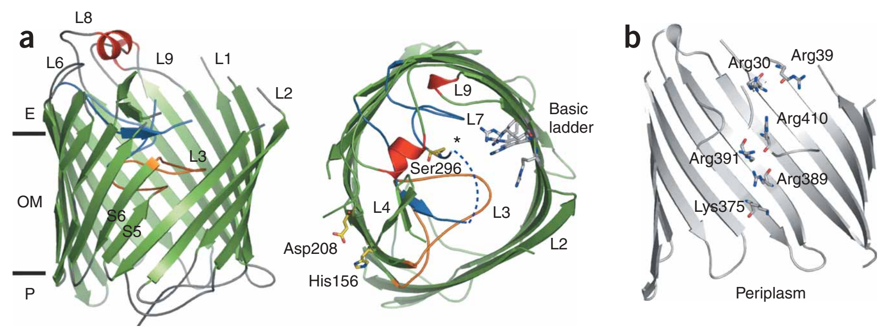

Figure 1 Structure of *P. aeruginosa* OprD. (**a**) Cartoons of OprD viewed from the side (left) and from the extracellular environment (right). Green, β-strands; gray, loops and turns; red, α-helices; orange and blue, pore-constricting loops L3 and L7, respectively. Loops have been smoothed for clarity. Dotted line in extracellular view represents the segment of loop L7 not visible in the structure. The short β-strands S5 and S6 are indicated. The residues in the basic ladder and residues His156, Asp208 and Ser296, which form the putative OprD catalytic triad[8], are shown as stick models. OM, outer membrane. (**b**) Side view of the pore showing the basic ladder.

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