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Lipopolysaccharide assembly in the bacterial outer membrane revealed by X-ray crystallography

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Lipopolysaccharide (LPS), also termed endotoxin, is a main component of the external leaflet of the outer membrane (OM) in Gram-negative bacteria. It serves as a natural barrier against harsh environments and toxic compounds, including antibiotics, and partially confers drug-resistance in bacteria [1]. LPS is also a powerful activator of inflammation and innate immune responses in mammalian cells. For the discovery of LPS receptor, Toll-like receptor 4 (TLR4), Bruce A. Beutler won the Nobel Prize in Physiology or Medicine in 2011. As LPS is essential to most Gram-negative bacteria, intervening in LPS biogenesis offers great opportunities for developing novel antibiotics against pathogens. However, for a long time, it is unclear how LPS is transported across the periplasm (the space between the inner and outer membranes) and assembled in the external leaflet of the OM in Gram-negative bacteria. With the funding support from the Ministry of Science and Technology of China, National Natural Science Foundation of China and the Strategic Priority Research Program of the Chinese Academy of Sciences, structural biologist Yihua Huang and his colleagues at the Institute of Biophysics, Chinese Academy of Sciences, recently determined the crystal structure of the LptD/E complex from the pathogen *Shigella flexneri* [2]. This new membrane protein complex structure provides crucial insights into an essential step of LPS biogenesis and also provides us a novel target for developing new antibiotics against Gram-negative pathogens.

The biogenesis of LPS, an amphiphilic molecule, starts with its synthesis in the cystoplasm, followed by flipping into the external leaflet of the bacterial inner membrane by the flippase MsbA. Eventually, LPS will be transported across the periplasm and assembled into the bacterial OM, which involves seven essential lipopolysaccharide transporting (Lpt) proteins LptA-LptF [3]. It is believed that the OM-localized LptD/E complex is responsible for the translocation and insertion of LPS into the OM, which is the final step of LPS biogenesis [4].

After extensive protein expression screening of LptD/E homologues from various Gram-negative bacterial strains, Huang and colleagues narrowed down to three LptD/E homologues whose expression gave sufficient amount of protein for crystallization, and the group finally determined the LptD/E complex structure from *shigella flexneri* to 2.4 Å resolution [2]. The complex structure, for the first time, reveals a unique two-protein 'plug-and-barrel' architecture with LptE fully embedded into the barrel formed by the C-terminal region of LptD (Figure 1A). The LptD barrel, composed of twenty-six antiparallel β strands, is the most strand-containing outer membrane protein observed to date. As the largest single-protein β-pore, the LptD β barrel has a wide opening of 65 Å by 35 Å on the periplasmic side, which is sufficiently large to accommodate Ra LPS. More importantly, Huang and colleagues discovered that the first two strands (β1 and β2) of the twenty-six stranded β barrel are distorted, presumably due to the presence of two prolines within the sequence (Figure 1B). The distortion of the first two strands weakens their interactions with the respec-

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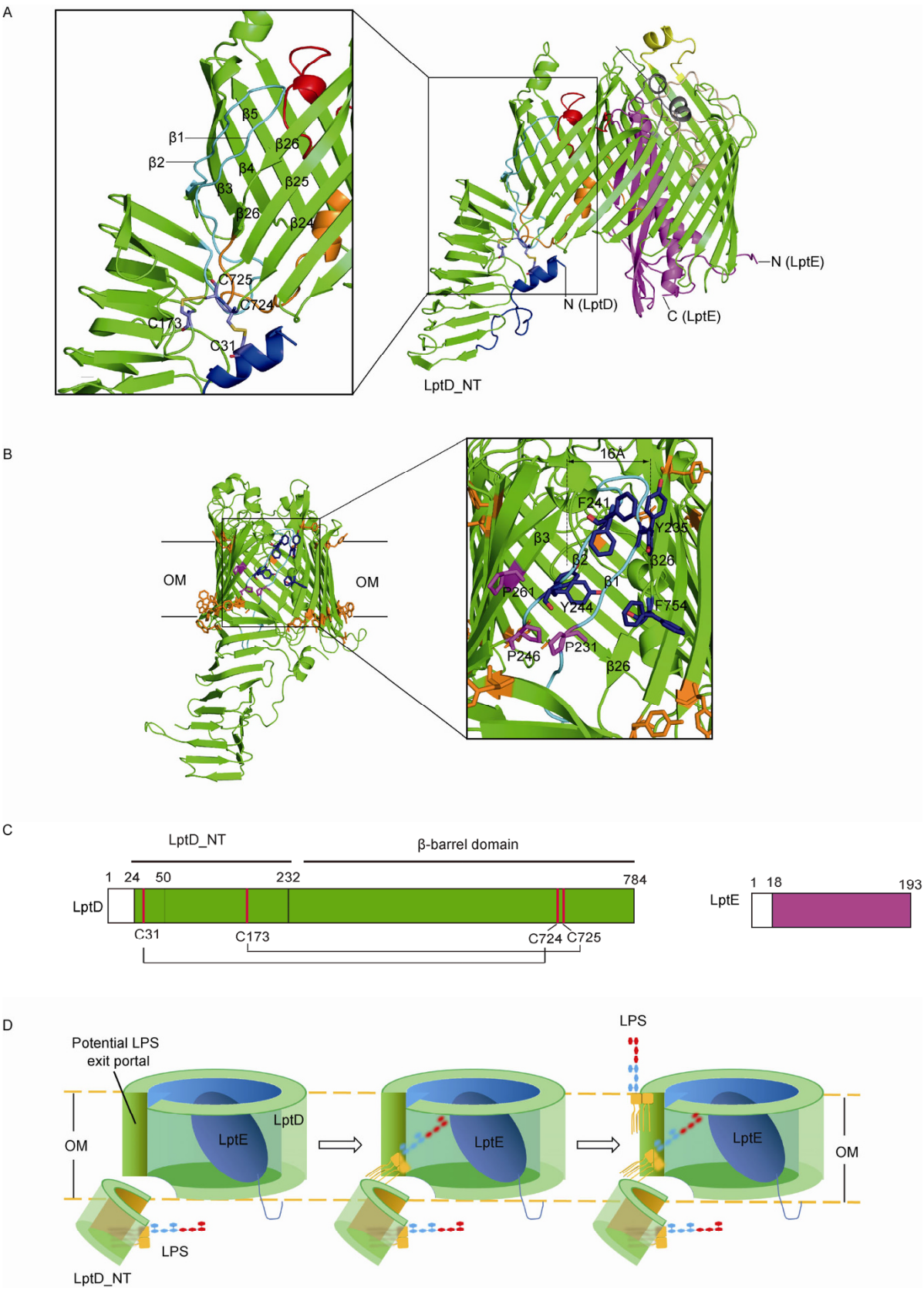

Figure 1 Crystal structure of LptD/E complex. A, Cartoon representation of the LptD/E complex. LptD and LptE are colored in green and magenta, respectively. The two pairs of disulfide bonds in LptD are labeled and highlighted in cyan with stick mode. B, LPS exit portal on the barrel wall (for clarity, LptE was omitted). C, Schematic structure of LptD and LptE. D, A proposed model for LPS insertion into the bacterial OM.

tive neighboring strands by reducing the regular inter-strand hydrogen bonding formation (Figure 1B), allowing them to be highly dynamic as also demonstrated by their higher average B factors than the rest of the β barrel. Based on these observations, Huang and colleagues proposed that strands β1 and β2 together function as a door, which creates a 16 Å wide gate between the LptD β barrel and the outer membrane, allowing lateral dislocation of LPS from the barrel into the outer leaflet of the OM (Figure 1B). The N-terminus of LptD forms a β-jellyroll that extends into the periplasm. Interestingly, the interior of the β-jellyroll is highly hydrophobic, suggesting that the β-jellyroll ushers LPS translocation across the periplasm by encompassing the hydrophobic moiety of LPS (Figure 1A).

Another striking feature of the LptD/E complex is the presence of two pairs of nonconsecutive disulfide bonds in LptD: residues Cys31 and Cys173 form disulfide bonds with residues Cys724 and Cys725, respectively, confirming previous functional analysis (Figure 1A and C). The disulfide bonds within LptD seem to be crucial in drawing the N-terminal β-jellyroll domain close to the C-terminal β-barrel domain of LptD and covalently stabilizing the inter-domain interactions. This might be critical for efficient transfer of LPS from the β-jellyroll into the β-barrel of LptD. On the basis of the the LptD/E complex structure and the interior surface property of the barrel lumen, the authors also proposed that the hydrophobic moiety and hydrophilic portion of LPS may enter into the lipid phase and the hydrophilic LptD barrel, respectively, upon leaving the β-jellyroll, and the whole molecule is inserted into the outer leaflet of the OM via the potential LPS exit portal (Figure 1D).

Since LPS export and OM insertion are essential for bacterial viability under most conditions, the proteins involved in this process represent an excellent set of drug targets against pathogenic bacteria. A group of protegrin I based peptidometic compounds have been shown to target LptD, and the lead compound is active against the opportunistic pathogen *P. aeruginosa* [5]. The crystal structure of the LptD/E complex will, thus, open an avenue for the development of new antibiotics that target the bacterial OM.

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