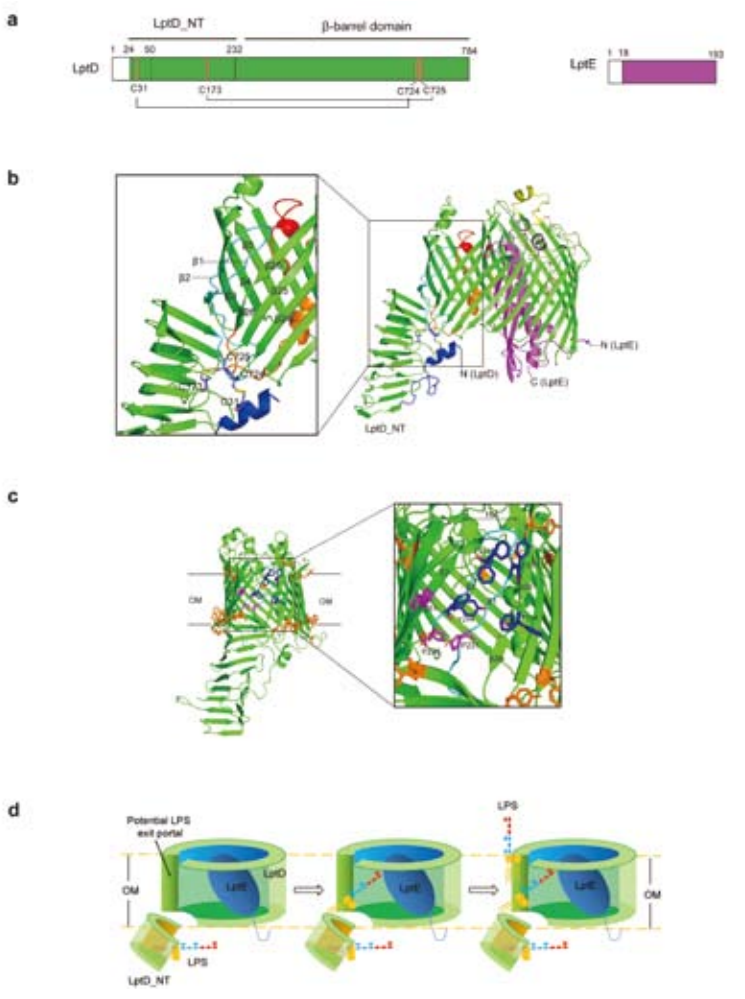
CAS Structural Biologists Detail How Lipopolysaccharides Transport and Insert in Gram-Negative Bacterial Outer Membrane

On July 3, 2014, the world leading scientific journal *Nature* published a breakthrough in lipopolysacharride biogenesis entitled "Structural basis for lipopolysaccharide insertion in the bacterial outer membrane". In this paper, a research team from the CAS Institute of Biophysics, led by structural biologist HUANG Yihua, announced their high-resolution structural determination of a membrane protein complex that is responsible for lipopolysaccharides' export and assembly in the outer membrane (OM) of Gram-negative bacteria.

This work is predicted to have important implications in the battle against Gram-negative infections. "Lipopolysaccharide (LPS) is not only the main components of bacteria OM, but also the primary cause of innate immunity and inflammation responses in humans. Thus, the research on LPS biogenesis has been carried out over a hundred years and is of fundamental importance", says Prof. HUANG, corresponding author of the paper.

Also termed endotoxin, LPS was first discovered by renowned German microbiologist Richard F. J. Pfeiffer at the end of the nineteenth century. A hundred years later, American scientists Bruce Beutler won the 2011 Nobel Prize



The LptD-LptE membrane protein complex crystal structure and model of LPS transfer and assembly at OM. a. Schematic structures of LptD and LptE; b. LptD-LptE membrane protein complex crystal structure; c. Features of the LPS exit portal on the LptD barrel wall; d. Proposed model for LPS insertion into bacterial outer membrane.

in Physiology or Medicine for the identification of LPS receptor, the Toll-like receptor 4 in mammalian cells. LPS is synthesized in cytoplasm, and thereafter, flipped to the external leaflet of an inner membrane protein, the flippase MsbA. The further export of LPS from the periplasm to the outer leaflet of OM was carried out by seven essential lipopolysaccharid transport proteins (LptA-F). Of them, the OM-localized LptD-LptE complex completed the final step of LPS biogenesis and, thus, represents an important drug target.

"The LptD-LptE complex structure is truly exciting. The high-resolution structure of the complex not only shed light on how LPS is transported across periplasm and finally assembled in the OM of Gram-negative bacteria, but also paved the way for developing new antibiotics against pathogens", says first author QIAO Shuai, a PhD student at Prof. HUANG's laboratory.

"Yes, structural biologists have many reasons to get excited about our structure. First, the complex structure reveals an unprecedented two-protein 'plug-and-barrel' architecture with LptE embedded into a 26-stranded barrel formed by LptD. Second, for the first time, scientists observed two pairs of non-consecutive, inter-domain disulphide bonds in a membrane protein. Third, the barrel formed by LptD is the most strand-containing and the largest β-barrel pore observed to date. Fourth, for the first time, we observed a β barrel with two strands adopting loop conformations. And, most importantly, we identified a potential LPS exit portal on the barrel wall, which gives clues how LPS is exported into the lipid bilayer", says Prof. ZHANG Kai (Xuejun Cai Zhang), co-author of the paper.

Currently, a group of peptidometic compounds based on the structure of protegrinI have been shown to target LptD, and a lead compound is revealed to be active against the opportunistic pathogen *P. aeruginosa*. The crystal structure of the LptD/E complex will open an avenue to new antibiotic strategies targeting the bacterial OM.

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