The cell surface of most Gram-negative bacteria contains lipopolysaccharide that is essential for their viability and drug resistance. A 134-kDa protein complex LptB2FG is unique among ATP-binding cassette transporters because it extracts lipopolysaccharide from the external leaflet of the inner membrane and propels it along a filament that extends across the periplasm to directly deliver lipopolysaccharide into the external leaflet of the outer membrane. Here we report the crystal structure of the lipopolysaccharide transporter LptB2FG from Klebsiella pneumoniae, in which both LptF and LptG are composed of a β-jellyroll-like periplasmic domain and six α-helical segments in the transmembrane domain. LptF and LptG form a central cavity containing highly conserved hydrophobic residues. Structural and functional studies suggest that LptB2FG uses an alternating lateral access mechanism to extract lipopolysaccharide and traffic it along the hydrophobic cavity toward the transporter’s periplasmic domains.
he asymmetric outer membrane (OM) comprises phospholipids in the inner leaflet and lipopolysaccharide (LPS) in the outer leaflet and is essential for Gram-negative bacteria1–3. In particular, LPS plays a critical role in drug resistance and pathogenesis4–6. LPS typically contains three components: lipid A, core oligosaccharide, and O-antigen polysaccharide (Fig. 1a). The O-antigen can contain up to 200 sugars, which contribute to bacterial resistance to the host immune system4–5. The lipid A and the core oligosaccharide are synthesized at the cytoplasmic side of the inner membrane (IM) and then flipped to the periplasmic side by MsaB6. The precursor of the O-antigen units are synthesized independently in the cytoplasm and transported by Wzx into the periplasmic side of the IM, where they are polymerized and ligated to lipid A core oligosaccharide by Waal to form LPS1,7. Translocating millions of these large amphipathic LPS molecules from the IM across the aqueous periplasm and correctly inserting them into the outer leaflet of the hydrophobic OM during cell division is a formidable challenge4–7.

Seven LPS transport proteins, LptA-G, are responsible for transporting LPS from the IM to the cell surface8–12 (Fig. 1a). Structural and functional studies reveal that the carboxyl (C) terminus of LptC interacts with the amino (N) terminus of LptA, and that the C terminus of LptA interacts with the N terminus of LptD forming a bridge to transport LPS across the periplasm9,12–15. The two-protein complex, LptD/E11,16–21, catalyzes the insertion of LPS into the OM through the intramembranous barrel and lateral opening of LptD12,22–25. The ABC transporter LptB2FG complex has a molecular weight of ~134 kDa, and contains two transmembrane domains (TMDs), LptF and LptG, and two nucleotide-binding domains (NBDs), LptB8,26 (Fig. 1a, b). All three components of LptB2FG are essential for LPS transport in Escherichia coli8,10,12. LptB hydrolyzes ATP to provide energy for LPS extraction and transport27. Unlike other ABC transporters which transport their substrates across the membrane, the LPS transporter LptB2FG extracts LPS from the periplasmic side of the IM and passes it to the periplasmic domain of the IM protein LptC7. However, the LPS extraction mechanism remains unknown.

To understand the structural basis of how ABC transporter LptB2FG extracts and transports LPS, we have solved the crystal structure of the LptB2FG complex from Klebsiella pneumoniae. Functional assays on lptFG-depleted E. coli NR1113 strain8 suggest that the transporter LptB2FG extracts LPS from the periplasmic leaflet of the IM to the transporter's internal cavity and reorients it toward the periplasmic domain of LptF or LptG. These features are different from other structurally characterized ABC transporters.

**Results**

**Purified LptB2FG has ATPase activity.** The purified LptB2FG transporters have ATPase activity (Supplementary Fig. 1), and our functional assay showed that the His-, Flag-, and Myc-tagged LptB2FG can complement lptFG-depleted strain NR1113 (Supplementary Fig. 2). Both the native LptF and LptG genes have been deleted from E. coli NR1113, but they are covered by an arabinose-inducible copy of the lptFG operon at the att site. When LptF and LptG are depleted by omitting the inducer arabinose from the growth medium bacterial growth ceases8. The kanamycin-resistant plasmid pTRC99a-Kan containing lptBFG was used as the template for lptBFG mutagenesis and expression to complement bacterial growth of E. coli NR1113 (see “Methods” section). These data are consistent with the finding that LptB with the C-terminal His tag displays its ATPase activity in vitro26 and can rescue lptF-depleted E. coli cells27.

**Overall structure of LptB2FG.** LptB2FG of K. pneumoniae was expressed, purified (Fig. 1b), and crystallized (see “Methods” section). The crystals belong to space group P212121, with the cell dimensions a = 105.25 Å, b = 210.52 Å, c = 258.94 Å, and α = β = γ = 90°. Heavy-atom derivatives were obtained by soaking the crystals in potassium tetranitroplatinate (II) (see “Methods” section). The crystals belong to space group P212121 with the cell dimensions a = 105.25 Å, b = 210.52 Å, c = 258.94 Å, and α = β = γ = 90°. Heavy-atom derivatives were obtained by soaking the crystals in potassium tetranitroplatinate (II) (see “Methods” section).

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The two periplasmic β-jellyroll domains are shifted to one side of the TM5F-TM1G interface (Fig. 2a, b). TM1F(G) crosses the IM at an angle of ~67° or 53° to the other, approximating with twofold rotational symmetry (Fig. 2a, b). Overall, the dimension of the LptB2FG transporter is of ~86 Å in width and 128 Å in length. The two periplasmic β-jellyroll domains are shifted to one side of the transporter thus generating a periplasmic opening at the side of the TM5F-TM1G interface (Fig. 2c, d). 

Details of the structure determination and the model building are provided in the “Methods” section. The structure was determined to a resolution of 3.7 Ångström (Å) by the single-wavelength anomalous dispersion (SAD) using data sets collected at 1.0723 Å, and the sequence content of the crystals was 77%, which helped to generate a clear experimental electron density map (Supplementary Fig. 5). 

Data collection and refinement statistics

| Data collection<sup>b</sup> | LptBFG-Pt | LptBFG-Hg | LptBFG-sulfur | LptBFG-Se<sup>a</sup> |
|----------------------------|-----------|-----------|---------------|-----------------------|
| Space group                | \(I2I_2I_2\) | \(I2I_2I_2\) | \(I2I_2I_2\) | \(P2_12_12_1\) |
| Cell dimensions            | \(a, b, c\) (Å) | \(a, b, c\) (Å) | \(a, b, c\) (Å) | \(a, b, c\) (Å) |
| \(\alpha, \beta, \gamma^\circ\) | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 |
| Wavelength (Å)             | 1.07226   | 1.00068   | 1.77120       | 0.9795                |
| Resolution (Å)             | 29.96–3.70 (3.90–3.70) | 22.99–5.14 (5.27–5.14) | 29.15–4.23 (4.64–4.23) | 29.96–6.00 (6.71–6.00) |
| \(R_{merge}\) (%)         | 25.2 (>100.0) | 40.9 (>100.0) | 21.1 (72.4) | 22.0 (>100.0) |
| CC<sub>1/2</sub> (%)       | 100 (94.6) | 99.5 (76.0) | 100 (99.6) | 99.8 (84.9) |
| \(I/\sigma(I)\)            | 15.5 (1.7) | 6.0 (1.1) | 26.4 (7.9) | 10.7 (1.5) |
| Completeness (%)           | 99.8 (99.8) | 99.0 (99.3) | 97.3 (90.1) | 99.2 (100.0) |
| Redundancy (%)             | 120.8 (117.5) | 26.2 (27.9) | 173.6 (81.8) | 19.4 (20.2) |
| Phasing                    | 29.96–3.70 | 4 | 0.332 |
| Sites (Pt)                 | 20311 |
| Figure of merit            | 0.29/0.32 |
| Refinement                 | 29.96–3.70 |
| No. reflections            | 20311 |
| \(R_{merge}/R_{free}\)    | 0.29/0.32 |
| No. atoms                  | 8422 |
| Protein                    | 4 |
| Ligand/ion                 | 2 |
| Water                      | 0 |
| B-factors                  | 112.40 |
| R.m.s. deviations          | 0.009 |
| Bond lengths (Å)           | 1.160 |
| Ramachandran statistics    | 96.8 |
| Allowed (%)                | 3.2 |
| Outliers (%)               | 51.75 |
| PDBe code                  | 30 |

The closest entry with partial similarity to LptF or LptG is the sorting nexin 9 (PDB code 3DYT) with a Z score of 5.4, and a root mean squared deviation (RMSD) of 7.1 over 140-aligned Ca atoms. In addition, this ABC exporter has two large periplasmic domains not found in other ABC exporter structures.

The well-known ABC transporters, MalFGK<sub>2</sub>–37, PglK<sub>38</sub>, and MsbA<sub>6</sub>, transport maltose, vitamin B-12, lipid-linked oligosaccharide, and lipid A core oligosaccharide across the IM, respectively. In contrast, the ABC exporter LptB2FG does not transport its substrate across the IM and sheds light on this transport mechanism.

Transporter LptB2FG has lateral gaps in the IM. LptF and LptG each form half of the transporter in the IM by contributing TM1-6, with TM1 of each subunit interfacing with TM5 of the other, approximating with twofold rotational symmetry (Fig. 2a, b). TM1F(G) crosses the IM at an angle of ~67° or 53° to the membrane plane (Fig. 3a, b). This feature separates TM1F-5G and TM5F-1G at their base, leaving visible gaps in the electrostatic potential surface map between interfacing segments (Fig. 3c, d), forming the lateral gates of the transporter LptB2FG. There are five interactions within TM1F-5G (F_V32/G_I325, F_V32/G_Q324, F_V32/G_L331, F_L35/G_P328, and F_I25/G_F317) and one interaction within TM5F-1G on the periplasmic side (F_S318/G_K40) (Fig. 3a, b), indicating that the lateral gate TM1F-5G is in a closed form and the lateral gate TM5F-1G is in an open form.

The cavity of LptB2FG. TM 1-6 of LptF and LptG form a cavity (Fig. 4a), which expands into the periplasm where the TM...
segments bend outwards. This cavity is 25 Å in length and 8 Å in width at its widest points (Supplementary Fig. 6). The periplasmic entrance to the cavity is surrounded by the periplasmic loops and periplasmic domains of LptF and LptG. Despite LptF and LptG sharing only 17% amino-acid sequence identity, the structures of the TMDs are strikingly similar, with an RMSD of 2.15 Å for 160-aligned Cα atoms. However, the periplasmic domain of LptG is shifted ~40 Å from that of LptF (Fig. 4b), which opens the cavity to the periplasm at the lateral gate TM5F-1G side, while the lateral gate TM5F-1G is open. The LptB 2FG structure presented here may represent an open conformation of the lateral gate of TM5F-1G (Figs. 2c, d and 3a, b). The IM section of the internal cavity is very hydrophobic (Supplementary Fig. 7), while the section above the IM is highly positively charged (Supplementary Fig. 6c). The residues positioned inside of the cavity show a higher degree of conservation than those positioned outside of the transporter (Supplementary Figs. 7 and 8). There is an extra electron density in the cavity that could not be assigned with any confidence (Supplementary Fig. 9). We speculate that the cavity of LptB 2FG may bind LPS (Supplementary Fig. 6c), as LPS is very hydrophobic at the lipid A and highly negatively charged near the inner core (Fig. 1a). Positively charged residues are required for LPS-specific binding in other membrane proteins.39 The highly conserved residues K34 (TM1G) and R136 (TM3G) of LptG are found at the upper cavity of the transporter (Fig. 3b) and around this extra electron density. We hypothesized that the residues K34 and R136 may be involved in LPS extraction and transport. Functional assays indeed revealed that the double glutamic acid substitution K34E/R136E significantly affected the bacterial growth. In contrast, LptG residues, K40 and K41, are not conserved, and functional assays showed that the double-mutant K40E/K41E did not affect cell growth (Figs. 3c, d and Supplementary Fig. 7). We also expected that the internal hydrophobic cavity is important for LptB 2FG’s function.

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**Fig. 2** Crystal structure of the *K. pneumoniae* lipopolysaccharide transporter LptB 2FG. a Cartoon representation of LptB 2FG. LptF, LptG, and the two LptB molecules are shown in cyan, yellow, green, and blue, respectively. b The LptB 2FG complex rotated by 180° along the y-axis relative to the left panel. c The electrostatic potential map of LptB 2FG. There is a lateral gate between TM1G and TM5F. The most positive potential is colored in blue and the most negative potential is colored red. d The LptB 2FG electrostatic potential map rotated by 180° along the y-axis relative to the left panel. There is a lateral opening between TM1F and TM5G.
conserved hydrophobic residues F26 and L62 of LptF are located in the central cavity and around the extra electron density (Fig. 3b and Supplementary Fig. 7). We speculate that these two residues may be involved in LPS transport. A functional assay showed that the double-mutant LptF F26D/L62D was lethal (Fig. 3c, d). The double-mutant LptF F26D/L62D was cultured in the presence of 0.2% L-arabinose, and the mutants of G_K34E/R136E, G_K40E/K41E, and F_F26D/L62D by western blotting. The bacterial cells for western blotting were cultured in the presence of 0.2% L-arabinose.

**Both LptF and LptG periplasmic domains are critical.** The periplasmic domains of LptF and LptG adopt β-jellyroll folds, which together comprise 10 β-strands in a manner that resembles the periplasmic β-jellyroll domain of LptC (Supplementary Fig. 11). The periplasmic domains of LptF and LptG take opposite side-by-side orientations, with both LptF and LptG connecting to the hydrophobic cavity of LptB2FG (Fig. 2 and Supplementary Figs. 6–8).

Two-point mutations on the C-terminal strand of LptA disrupt LptA oligomerization and another mutation in the C-terminal region of LptC disrupts the LptA-LptC interaction, resulting in bacterial death. To test whether the C-terminal strands of the periplasmic domains of LptF and LptG are also as important as that of LptA and LptC, we generated single proline substitutions at the C-terminal β-strands of LptF and LptG (F_D229P, F_Q231P, F_T225P, and F_R223P) and at the C-terminal β-strands of LptG periplasmic domain (G_G228P, G_W230P, and G_T225P, and G_S223P) (Fig. 4b). Functional assays showed that all the mutants severely impaired the bacterial growth, except for F_T225P (Fig. 4c, d). Additionally, double proline substitutions (F_D229P/Q231P, F_T225P/R223P, G_G228P/W230P, and G_T225P/S223P) cause significant cell growth defects (Supplementary Fig. 2), suggesting these periplasmic domains are important for the function of LptF and LptG in LPS transport.
Interactions of LptF and LptG with LptB. The NBDs of ABC transporters hydrolyze ATP and drive the conformational changes of the TMDs to translocate their substrates across membranes through the coupling helices. The LptF-coupling helix (residues E84, V87, M88, H89, C91, and I93) interacts with LptB residues H73, R77, Y82, F90, R91, and R150, while the LptG-coupling helix (residues R86, S87, E88, V91, A94, and F97) interacts with LptB residues L72, Y82, E86, F90, R91, V102, and R150 (Fig. 5 and Supplementary Fig. 12a). Recent work confirmed LptB-F90, L72, M73, and I105 interact with coupling helices of LptF (V87) and LptG (S95) by UV-dependent crosslinking. Interestingly, the LptB2FG crystal structure also suggests other interactions between TM1F/G and LptB through highly conserved residues (Fig. 5 and Supplementary Fig. 8).

Fig. 4 LptB2FG cavity and the periplasmic domains. The color scheme is the same as in Fig. 2. a The transmembrane domains of LptB2FG, top view. Both LptF and LptG have six TM segments respectively, forming a cavity. b The superimposition of LptF and LptG from K. pneumoniae. The proline substitutions are shown in sphere. c Functional assays of the single proline mutants of periplasmic domains of LptF and LptG according to E. coli amino-acid sequences. NR1113 cells were transformed with empty vector (pTRC99a_Kan, negative control) or the vector encoding LptBF(Flag)G(Myc) (positive control) or its single proline mutant derivatives. All bacterial cultures were adjusted to an OD600 nm of 0.5 with fresh medium, serially dilution 1:10 as indicated on the top of the figure and then replica plated in agar plates containing kanamycin but in the absence of L-arabinose. The positive control (LptBF(Flag)G(Myc) in NR1113), the negative control (empty plasmid in NR1113), LptF mutants F_D229P, F_Q231P, F_R223P and F_T225P, and LptG mutants G_S223P, G_T225P, G_G228P, and G_W230P were used in the functional assays. Double proline substitutions (F_D229P/Q231P, F_T225P/R223P, G_G228P/W230P, and G_T225P/S223P) are reported in Supplementary Fig. 2. d The protein expression levels of the mutants shown by the western blotting. The LptF and LptG protein expression of the negative control (empty plasmid), the positive control (plasmid with LptBF(Flag)G(Myc)), LptF mutants D229P, F_Q231P, F_R223P, and F_T225P, and LptG mutants G_S223P, G_T225P, G_G228P, and G_W230P were detected. The bacteria cells for western blotting were cultured in the presence of 0.2% L-arabinose. All mutants in Figs. 3 and 4 and Supplementary Fig. 2 are based on E. coli amino-acid sequence. The difference in residues is listed here: LptF-D229 (N229_K. pneumoniae), LptG-T225 (M225_K. pneumoniae).
In this structure, the aromatic side chain of LptB-F90 interacts with hydrophobic residues of LptG-(Y10 and V91) and LptF-(Y6, V87, and M88) (Fig. 5c, d). Disrupting this hydrophobic interaction by substituting the aromatic residue with alanine (LptB-F90A) was lethal, whereas an aromatic residue substitution LptB-F90Y had no impact27. Single mutants of other LptB residues R150A and L93F that interacted with the coupling helices of LptF (E84, V87, and M88) and LptG (E88 and V91) also affected the LPS transport41.

The structure of LptB in this LptB2FG crystal form is in a nucleotide-free state. Superimposition of the LptB protomer structure of LptB2FG with the

E. coli
LptB protomer structure complexed with ATP (PDB code 4QC2) reveals conformational changes within the Walker A, Q-loop, signature motif, Walker B, D-loop, and H-loop (Supplementary Fig. 12b). Superimposing the dimeric

E. coli
LptB-ATP structure with the dimeric

K. pneumoniae
LptB structure reveals a shift of 13 Å by the second LptB subunit through translocation (Fig. 5a).

Discussion
The crystal structure of the LptB2FG complex uniquely represents ABC transporters like itself and LolCDE, which both possess the ability to extract large amphiphiles from the external face of the IM in advance of distinctly different periplasmic transport steps toward the OM. As an ABC exporter, this LptB2FG structure has large periplasmic domains. The structure register was validated by sulfur and selenomethionine anomalous data, as well as by functional assays from ourselves and other groups41. The extraction of LPS from the periplasmic leaflet of the IM and its transport to LptC by the LptB2FG complex utilizes ATP hydrolysis, as does the transport of LPS to LptA by LptC41. The crystal structure of LptB2FG shows that the coupling α-helices of LptF and LptG are located within a highly conserved groove of LptB (Fig. 5b). It also reveals that residues Y6, L7, I4, and of TM1F and residue Y10 of TM1G interact with residues of LptB (Fig. 5b–d). Notably, LptF and LptG’s coupling helices connect TM2F(G) and TM3F(G) within the LptB2FG cavity, and TM3F(G) is further connected to the periplasmic domains (Fig. 2a, b and Supplementary Fig. 12a). It has been shown previously that hydrolysis of ATP induces conformational changes in the LptB groove structure27. Speculatively, the binding of ATP by LptB would induce a similar conformational change to that seen in the

E. coli
form (moving the opposite protomers of LptB into close contact)
and play a critical role for LptB$_{2FG}$ to extract LPS from the IM and transport LPS (Fig. 6).

The structure of multidrug ABC exporter Sav1866 complexed with ADP is in an outward-facing conformation, while the cavity surface in the IM is hydrophilic. The structure of the human sterol transporter ABCG5/ABCG8 is in an inward-facing conformation, and the structure of MsbA in complex with AMP-PNP is in an outward-facing conformation (Supplementary Fig. 13). All the previously reported ABC transporters are proposed to translocate their substrates across membranes by alternating inward- and outward-facing conformations, harnessing the energy of ATP binding and hydrolysis at their nucleotide-binding domains. The LptB$_{2FG}$ structure reveals that the LptB$_{2FG}$ cavity opens to the periplasm, while the LptB$_{2FG}$ transporter has separations between TM5F-1G, as well as TM1F-5G. The LptB$_{2FG}$ structure in complex with ATP shows that the lipid A is buried in the external leaflet of the OM. We propose that the LptB$_{2FG}$ transporter uses alternating lateral gates (TM5F-1G and TM1F-5G) in the periplasmic LptCAD subunits (Supplementary Fig. 4), indicating that the periplasmic packing reveals a $\beta$-jellyroll extension between neighboring LptF subunits (Supplementary Fig. 4), suggesting that the periplasmic domain of LptF plays a critical role for forming the LPS transport protein complex and transporting LPS.

Both periplasmic domains of LptF and LptG may be involved in transporting LPS as almost all single or double proline substitutions of the periplasmic domains of LptF and LptG kill bacteria (Fig. 4), suggesting that either lateral gate TM5F-1G or TM5G-1F of LptB$_{2FG}$ may extract LPS from the periplasmic leaflet of the IM. The LptB$_{2FG}$ structure presented here may represent only one of the transporter’s conformational states, where it takes LPS from the TM5F-1G opening to the cavity and then delivers LPS to the periplasmic domain of LptF. We speculate that LptB$_{2FG}$ may adopt another conformation, which allows the transporter to obtain LPS similarly, but from the lateral gate TM1F-5G to deliver LPS to the periplasmic domain of LptG.

The LptB$_{2FG}$ electron density map contains an unassigned density within the central hydrophobic cavity. Functional assays showed that double-mutants LptG$_{K34E/R136E}$ and F$_{26D/L62D}$ within the cavity result in severe cell growth defects, suggesting that this complex may extract LPS from the IM to the central cavity. This is consistent with a report that LptB$_{2FG}$ of *Burkholderia cepacia* is unable to export LPS lacking 4-amino-4-deoxy-C-arabinose modification with the lipid A or core oligosaccharide, while the LptD$_{31H}$ mutant can export the unmodified LPS. Bioinformatics analysis suggests that residue LptG$_{D31}$ is structurally equivalent to LptG$_{K34}$ of *K. pneumoniae*, occupying the same position in the cavity. Additionally, LptC and LptA could not extract LPS directly from the IM. Taken together, these data suggest that the ABC transporter LptB$_{2FG}$ extracts LPS from the IM and helps to transport it to the OM. The LptB$_{2FG}$ structure now reveals that the periplasmic LptCAD $\beta$-jellyroll track is likely continuous with the LptF$_{2FG}$ $\beta$-jellyroll motifs that interface between LptCAD and the LPS lipid A moieties buried in the external leaflet of the IM.

**Fig. 6** Mechanistic model for LPS transporter LptB$_{2FG}$. LPS transporter LptB$_{2FG}$ may extract and transport LPS through a cycle of ATP-free state (resting stage), ATP-binding state, and ATP hydrolyzed state. The dimeric LptB molecules move closer to bind ATP when ATP enters into the active site of LptB (step 1). This triggers the conformational changes of TM5F-1G, which fully opens the lateral gate TM5F-1G of the transporter to extract the lipid A of LPS from the IM into the internal hydrophobic cavity (lateral opening). The lipid A is then pushed to the periplasmic domain of LptF when ATP is hydrolyzed, while the lateral gate TM5F-1G may be closed and the lateral gate TM1F-5G may be fully open to extract LPS and finally the LPS may be pushed to the periplasmic domain of LptG (step 2). The highly conserved hydrophobic residues and positively charged residues in the cavity may be involved in LPS extraction and transport. Next, ADP is released and LptB$_{2FG}$ returns to the ATP-free state (step 3) and the cycle repeats.
A continuous hydrophobic tunnel is thus expected to connect the internal leaflet of the OM with the external leaflet of the IM. LptB2-catalyzed ATP hydrolysis is expected to drive LPS transport by staging unique conformational changes felt at the interface, where LPS molecules are disposed orthogonally between the β-jellyroll track and the external leaflet of the IM. The mechanics of LPS transport is thus restricted within the external leaflet and periplasmic domains of the LptB2FG complex, with the impulse coming from the cytoplasm in the absence of any associated transmembrane lipid flipping events.

In summary, our structural and functional studies suggest that LptB2FG uses an alternating lateral access mechanism for LPS extraction from the IM external leaflet and into an interior LPS-binding cavity. LPS is then extruded orthogonally into the periplasmic LPS-binding cavity. LPS is then extruded orthogonally into the periplasmic

The E. coli wild-type LptB2FG and the mutants of LptF F26D/L62D and LptG K34E/R136E were expressed and purified using the same protocol as that of the LptB2FG of *K. pneumoniae*, and purified by size-exclusion chromatography using a column HiLoad 16/600 Superdex 200 pg column in 20 mM Tris–Cl, pH 7.8, 150 mM NaCl, 5% glycerol, and 0.05% DDM. The chromatograms of wild-type LptB2FG, mutants LptF F26D/L62D and LptG K34E/R136E were integrated and compared (Supplementary Fig. 10).

### Crystalization and data collection

The LptB2FG crystallization trials were performed with 1 μl of protein and 1 μl of reservoir solution and the sitting-drop vapor diffusion technique at room temperature. The best crystals of LptB2FG were obtained in 0.1 mM MES pH 6.5, 0.1 M sodium chloride, 0.1 M lithium sulfate, and 24% PEG 300 within 8 days. Crystals were harvested after 3–4 weeks and cryoprotected by supplementing the crystallization solution with 20% glycerol, before being flash frozen in liquid nitrogen. Platinum derivatives were obtained by soaking the crystals for 4 h in the crystallization solution with 2 mg ml⁻¹ potassium tetraniortoplplatinate (II) K₂Pt(NO₃)₄. The data sets were collected at beamline 103, Diamond Light Source, UK, at the platinum L3 edge. The data were processed using XIA2 with DIALS and scaled using AIMLESS. Anisotropic diffraction was observed and correction was applied during data processing. Crystals belonged to space group *P₂₁₂₁₂₁*, with unit-cell dimensions: *a* = 105.5 Å, *b* = 210.8 Å, *c* = 258.9 Å, and *α* = *β* = *γ* = 90°. The SeMet incorporated LptB2FG crystals from *S. exneri* were obtained in 0.1 M sodium acetate trihydrate, 0.1 M MES pH 6.5 and 28% v/v PEG 400 for 21 days. The wavelength 0.9795 Å was used for SeMet crystals at beamline 103. The SeMet crystals belonged to space group *P₂₁₂₁₂₁*, with the cell dimensions *a* = 110.15 Å, *b* = 124.53 Å, *c* = 398.09 Å, and *α* = *β* = *γ* = 90° (see “Methods” section and Table 1).

The LptB2FG sulfur anomalous data were collected at 1.7712 Å and the data were processed using XIA2 with DIALS and scaled using AIMLESS. LptB2FG co-crystallization with nucleotide (ATP, ADP, AMP, and AMP-PNP) was attempted; however, no nucleotide-binding structure was obtained.

### Structure determination and model building

The platinum positions in the Pt-derived crystals of the *P₂₁₂₁₂₁* space group were determined by the SAD method using SHELLS suite and were successful in locating platinum sites but did not result in a readily interpretable map. Data from selenomethionine crystals were collected across three isomorphous crystals were combined to increase the anomalous multiplicity to around 60-fold with a diffraction limit of 3.7 Å and this produced a map where the 12 transmembrane helices were clearly visible along with the electron density for the LptB domains. Anisotropy correction of the raw data set was performed using the STARANISO web server (http://staraniso.globalphasing.org/cgi-bin/staraniso.cgi) with a surface threshold of 1.2I/σ(I) and approximate vector 0.1 *a* + 0.6 *b* + 0.8 *c*.

The phasing and density modification of this anisotropy corrected data were performed with PHENIX resulting in a significantly more interpretable map than the main chain of a single copy of the complex clearly visible along with some side-chain density, particularly around the transmembrane region. Four-ordered platinum sites were found with an overall figure of merit of 0.33. The very high solvent content of these crystals (75%, *V*ₐwat = 5.4 Å⁻³) aided density modification significantly. This initial density modified map is shown along with the final model and anomalous density map for the platinum position (Fig. 4b). The best data we collected can reach to 3.3 Å, but the completeness of the high-resolution shell was dropped to 10% after the anisotropy correction. We used this data for the initial structure building, while we performed the final refinements using the 3.7 Å data. The best electron density map with the refined structure is shown in Supplementary Fig. 5.

Two molecules of high-resolution models of LptB (PDB code 4QC2) were placed into the density at the base of the transmembrane region and adjusted to fit by using COOT. The transmembrane regions of LptF and LptG were relatively straightforward to build using standard helices and registry was assigned using the STARANISO web server. The peaks in the anomalous density map for the platinum position were generated using PHENIX. The initial registry assignment of the structure was performed using the peaks in the sulfur Fourier maps with 10 peaks at 3 sigma (Supplementary Fig. 3a). The side chains of the LptB2FG structure is further registered using the SeMet sites identified from the anomalous map of *S. flumeni*, as the amino acid sequences of LptFG from *S. flumeni* share conserved methionine residues with that of the *K. pneumoniae* (Supplementary Figs. 3c, 15–17). In addition, the platinum sites are found near methionine, arginine, and histidine residues as would be expected for this metal. Model refinement was performed using BUSTER with each chain assigned amino acids a rigid body for TLS. NCS was performed to restrain the two LptB domains. We used the 3.7 Å data for the final refinement. The final model has *Rmerge* and *Rfree* of 0.29/0.32, respectively, and further statistics are given in Table 1.

### Site-directed mutagenesis and functional assays

All single or double mutations were generated following the site-directed mutagenesis protocol of Liu and Naismith. The mutations were amplified by PCR using *Q5* hot start high-fidelity DNA polymerase. To avoid ampicillin already used to select the *E. coli* lptB deletion strain RR1113, the pTRC99a plasmid's ampicillin resistance gene was replaced by a kanamycin-resistance gene. Named as pTRC99a-Kan, this plasmid...
was used as the template for the LptBFG mutagenesis, which included an octa-

histidine tag (8 x His) at the C-terminus of the LptB.

The constructs for LptF and LptG tags inserted at 130 of LptF (LptF-230-Flag), a c-Myc tag at residue 228 of LptG (LptG-228-Myc) or a Flag tag at residue 138 of LptF (LptF-138-Flag), and a c-Myc tag at 144 of LptG (LptG-144-Myc) to generate two plasmids, which were named LptF2BG228 and LptFB138G144, respectively. All mutations have been confirmed by DNA sequencing.

Primers for generating the LptF and LptG tags are listed as below:

K12_F230_Flag_F: GATACAAAGTACGACGATATACAA CACGGACATCT

K12_F138_Flag_F: GATACAAAGTACGACGATATACAA CACGGACATCT

K12_G228_Myc_F: GAACAAAAATCATCATCAGAAGAGATCG ACTT

K12_G144_Myc_F: GAACAAAAATCATCATCAGAAGAGATCG ACTT

CGGTGAACTCCTAGACG

K12_G228_Myc_R: CCTCTTCTGAGATGAGTTTTTGTTC GCCGCTCA

K12_G144_Myc_R: CCTCTTCTGAGATGAGTTTTTGTTC GCCGCTCA

These single or double mutants were transformed into the E. coli lptFG-depleted NR1113 strain respectively. The transformed E. coli cells were grown on LB agar plate supplemented with antibiotics (kanamycin 50 µg ml⁻¹) and 0.2% L-arabinose at 37 °C for 12 h. Single colonies of each transformation were inoculated into 5 ml LB medium supplemented with antibiotics and 0.2% (w/v) L-arabinose. The cells were cultured in an incubator at 200 rpm and at 37 °C for 12 h. Subcultured cultures were used for the functional assays. The E. coli NR1113 strain with the empty plasmid pTRC99a was used as the negative control, while the NR1113 strain with the plasmid pT7c99a-Kan-LptBFG-Flag/G(Myc) or the NR1113 strain in 0.2% L-arabinose was used as the positive control. For functional assays, the cells were harvested and washed twice and then diluted in sterile LB medium to OD₆₀₀ nm of 0.5. Ten-fold serial dilution functional assays were performed. The dilution range was from 10⁻¹ to 10⁻⁶, and 5 µl of the diluted cells were dripped onto the LB agar plates containing kanamycin 50 µg ml⁻¹. Cell growth was observed after overnight culture at 37 °C. All the assays have been performed in triplicate.

Western blot. The protein expression levels of LptF and LptG were determined by western blotting. An aliquot of 0.5 ml of overnight cultures of transformed NR1113 cells with wild type or LptFG mutants was inoculated into 50 ml LB supplemented with antibiotics (kanamycin 50 µg ml⁻¹) and 0.2% L-arabinose. The cells were cultured at 37 °C for 6 h and harvested by centrifugation. The cells were re-suspended in 1 ml buffer containing 20 mM Tris-Cl, pH 7.8, and 150 mM NaCl supplemented with Complete protease inhibitor tablets (Roche), 1 µg ml⁻¹ DNase (Sigma-Aldrich) and 1 mM PMSF. The cells were lysed by sonication for 45 s on ice. The MesA membrane fraction was harvested and solubilized with 2% DDM for 20 min at room temperature. The un-dissolved debris was removed by centrifugation at 13,000g for 10 min at 4 °C. The supernatant was loaded to a Ni²⁺-NTA column and washed with a buffer containing 0.05% DDM, 20 mM Tris-Cl pH 7.8, 150 mM NaCl, and 30 mM imidazole. The protein was eluted with 0.05% DDM, 20 mM Tris-Cl pH 7.8, 150 mM NaCl, and 300 mM imidazole. The eluted samples were mixed with 4x SDS-PAGE loading buffer and incubated at 98 °C for 10 min. The samples were centrifuged at 13,000 x g for 1 min, and 10 µl of each sample was loaded onto 12% Bis-Tris Plus SDS-PAGE Gel (Invitrogen) for the immunoblot analysis.

ATPase activity assay. ATPase activity was performed using ATPase/GTAPase Activity Assay Kit (Sigma). E. coli C43 (DE3) cells harboring lptBFG (or lptBFG mutant) plasmid or LoLCDE plasmid were cultivated in 1 L LB medium. The protein overexpression and the cell collection were performed using the same protocol as described in the protein purification and expression method section. Cells were disrupted twice by passing a cell disruptor at 30,000 psi. The membrane fraction was harvested by the ultra-centrifugation at 100,000g for 30 min and solubilized in 1% DDM followed by an ultra-centrifugation. The supernatants of each sample were loaded onto a gravity column containing pre-balanced 200 µl of Ni²⁺-NTA beads. The LptBFG or LoLCDE complex was washed with 15 column volumes of wash buffer (50 mM imidazole 20 mM Tris-Cl pH 8.0, 300 mM NaCl, and 0.05% DDM), and eluted using the elution buffer (250 mM imidazole, 20 mM Tris-Cl pH 8.0, 300 mM NaCl, and 0.05% DDM). The protein concentration of all samples was determined with detergent compatible Pierce BCA Protein Assay Kit (Thermo Scientific) according to the manufacturer’s instruction. Briefly, 2.5 µl of purified protein was diluted to 25 µl for the BCA assay. The albumin (BSA) was used as the standards. An aliquot of 200 µl of working reagent (made by mixing reagent A and reagent B at 5:1 volume ratio) was added to each sample and incubation at 37 °C for 30 min. The absorbance at the 562 nm was measured and the protein concentration of each sample was determined.

The ATPase activity assay was performed in 96-well plates. An aliquot of 1 µl of each sample was mixed with 4 µl 0.5% DDM TBS and 5 µl assay buffer was prepared (ATPase/GTAPase Activity Assay Kit) to make 10 µl of ATPase activity assay sample. The phosphate standards and blank control for colorimetric detection was prepared according to the manufacturer’s instructions of ATPase/GTAPase Activity Assay Kit (Sigma). An aliquot of 30 µl reaction mix (made by 20 µl assay buffer plus 10 µl 4 mM ATP solution) was added into each ATPase activity assay sample. After incubation at room temperature for 15 min, 200 µl reagent (ATPase/GTAPase Activity Assay Kit) was added into each sample to terminate the reaction and all samples were incubated for additional 30 min. The absorbance at 600 nm was measured. All assays were repeated six times. ATPase activities of all samples were determined using the mean value of the samples according to the linear regression of standards.

Data availability. The atomic coordinates and structure factors of LptBFG are deposited at the Protein Data Bank under access code 5L75. Other data are available from the corresponding authors on reasonable request.

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Author contributions
C.D. and H.D. conceived the project. H.D., Z.Z. and X.T. designed primers and generated the constructs for protein expression, and performed the functional assays. H.D., Z.Z., and X.T. expressed, purified, and crystallized the LptB2FG. H.D., Z.Z., N.G.P. and C.D. undertook data collection and structure determination. N.G.P. and C.D. did the structure refinements. C.D., H.D., Z.Z. and N.G.P. wrote and X.T. revised the manuscript.

Additional information
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