

Structural basis of periplasmic bridge protein docking and nucleotide-dependent remodelling in lipopolysaccharide transport

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Abstract

The outer membrane of Gram-negative bacteria is assembled and maintained by the lipopolysaccharide (LPS) transport machinery, which extracts LPS from the inner membrane and conveys it across the periplasm via a protein bridge. How the inner-membrane transporter, LptB₂FGC, engages the soluble bridge component, LptA (named LptH in *Pseudomonas aeruginosa*), and how this junction responds to the ATPase cycle remain unresolved. Here, we determine cryo-electron microscopy structures of the *P. aeruginosa* LptB₂FGC-LptH complex in nucleotide-free and AMP-PNP-bound states at 3.24 Å and 3.05 Å resolution, respectively. The structures show that LptH augments the terminal beta-strand of LptC, thereby completing a continuous beta-jellyroll conduit. ATP analogue binding closes the LptB dimer and the transmembrane LPS-binding cavity while remodelling the LptF-LptC junction at the base of the assembled bridge. Molecular dynamics simulations show that the LptC-LptH connection remains intact but becomes less compact in the ATP-bound ensemble. Native mass spectrometry further reveals that thanatin preferentially disrupts LptH-LptH and LptC-LptH assemblies. Together, these findings establish the architecture of the transporter-bridge junction and reveal how nucleotide-driven closure remodels an already assembled periplasmic conduit while preserving bridge continuity.

Introduction

The asymmetric outer membrane is a defining feature of Gram-negative bacteria and a major determinant of their intrinsic resistance to antibiotics. Its outer leaflet is enriched in lipopolysaccharide (LPS), a large amphipathic glycolipid that limits the entry of toxic compounds and contributes to envelope integrity, host interactions and virulence^{1–4}. LPS is synthesised at the cytoplasmic face of the inner membrane, flipped to the periplasmic leaflet and then transported to the cell surface by the essential Lpt machinery. This trans-envelope system comprises the inner-membrane ABC transporter LptB₂FGC, the periplasmic bridge protein LptA, and the outer-membrane LptDE translocon^{1,4–6}. Additional auxiliary factors contribute to LPS transport efficiency and the system's overall stability. For example, proteins such as LptM and YedD have been implicated in facilitating these processes^{7–9}.

The prevailing bridge model proposes that the β-jellyroll domains of LptF, LptC, LptA and LptD form a continuous hydrophobic groove through which LPS moves from membrane to membrane¹⁰. In *Escherichia coli*, LptA assembles as a head-to-tail β-jellyroll oligomer that spans the periplasm and forms a continuous transport bridge between inner and outer membrane complexes. However, the exact stoichiometry, the number of LptA molecules required to span the periplasm, remains unknown¹¹. Spin-label EPR spectroscopy shows that LptA and LptC undergo conformational changes upon LPS binding, with LptA displaying increased rigidity in internal regions while remaining destabilised at the termini^{12,13}. LptA binds LptC with higher affinity than it self-oligomerises, supporting a model where Lpt bridge assembly is nucleated at LptC and extended by LptA oligomerisation¹⁴. In *Pseudomonas aeruginosa*, the LptA orthologue LptH retains a β-jellyroll fold but shares limited sequence identity with LptA and may

oligomerise with different stoichiometry *in vivo*^{6,15,16}. Functional complementation analyses confirm that LptH is essential for LPS transport and cell viability in *P.aeruginosa*^{15,17,18}.

Structural and functional studies of LptB₂FGC have established several principles of LPS extraction. LPS enters a central cavity between LptF and LptG from the outer leaflet of the inner membrane; the transmembrane helix of LptC breaks the pseudo-symmetry of the transporter and improves coupling between ATP hydrolysis and LPS movement; and ATP binding promotes nucleotide-binding-domain closure and collapse of the membrane cavity^{19–26}. Work in Enterobacteria has also identified a gate at the LptF-LptC junction that controls the movement of LPS from the transporter onto the bridge^{19–21,24,25}. Yet available structures have generally lacked the soluble bridge component, leaving uncertain how transporter motions are propagated into an assembled periplasmic conduit.

The periplasmic interfaces of the Lpt pathway also represent promising targets for pharmacological intervention. The antimicrobial peptide thanatin binds LPS and several Lpt proteins, disrupts β -strand-mediated interactions among LptC, LptA and LptD, and selects resistance substitutions in LptA^{16,27–29}. More broadly, peptidomimetic and macrocyclic antibiotics can disable LPS transport by targeting protein-protein contacts or trapping LPS within the trans-envelope machine^{30,31}. Whether thanatin dismantles distinct Lpt assemblies with a defined hierarchy, however, is not known. Here we combine native mass spectrometry (MS), cryo-electron microscopy (cryo-EM), conditional complementation and molecular dynamics simulations to define the *P. aeruginosa* LptB₂FGC-LptH complex in nucleotide-free and nucleotide-bound states. The results reveal the atomic basis of LptH docking, show that ATP-dependent closure of the transporter is accompanied by remodelling of the bridge junction, and identify differential stability among thanatin-sensitive periplasmic interfaces.

Results

LptH forms a stable complex with LptB₂FGC

To investigate how the periplasmic component LptH interacts with LptB₂FGC, we expressed *P. aeruginosa* LptH in *E. coli* strain BL21(DE3) and purified it following established protocols¹⁶. We expressed LptB₂FGC in ClearColi BL21(DE3) cells, an *E. coli* strain engineered to produce lipid IVA, a truncated version of LPS that lacks the polysaccharide moiety and contains four acyl chains instead of the characteristic hexa-acylated enterobacterial lipid A. We then purified the proteins following established protocols²², incorporating an additional delipidation step to remove bound lipids, particularly lipid IVA²⁶.

We next incubated the LptB₂FG(C) complex first with LptH in a 1:1 ratio and analysed the resulting solution by native MS after buffer exchange into 200 mM ammonium acetate supplemented with the dendritic oligoglycerol detergent ([G1]-OGD). In the mass spectrum, we observed the charge-state series corresponding to LptB₂FG, as well as a new charge-state series with a mass corresponding to the

LptB₂FGC-LptH complex. Notably, we and others previously showed that *P. aeruginosa* LptH exists in a monomer-dimer equilibrium in solution^{15,16}. Consistent with this, we also observed a lower-intensity charge state series corresponding to the LptB₂FGC bound to a dimeric LptH. Overall, these data suggest that all the available LptB₂FGC complexes are saturated with LptH (Fig. 1a). Importantly, no relevant charge states corresponding to LPS- or lipid IVA-bound LptB₂FG or LptB₂FGCH were observed, suggesting that the predominant species in solution are substrate-free.

Architecture of the nucleotide-free LptBFGC-LptH complex

Guided by native MS results, we reconstituted delipidated LptB₂FG(C) complexes into nanodiscs, incubated the reassembled transporter with purified LptH, and subjected it to single-particle cryo-EM analysis. Two-dimensional class averages of the LptB₂FGC–LptH complex revealed well-defined transmembrane features and a distinct periplasmic extension (Fig. S1). Build-and-Retrieve processing³² yielded a reconstruction at 3.24 Å (Fig. 1b,c). Side-chain density was well defined throughout the transmembrane domains and nucleotide-binding domains, and the periplasmic β-jellyroll domains of LptF, LptG, LptC and LptH could be modelled. Density for LptH encompassed residues 12–200. LptF and LptG each contribute six transmembrane helices arranged in a pseudo-symmetric architecture, defining the central membrane cavity of the transporter. The lateral interface between LptF TM5 and LptG TM1 (interface 1) is open towards the outer leaflet, whereas the reciprocal interface between LptG TM5 and LptF TM1 (interface 2) is closed (Fig. S2). Notably, the transmembrane helix of LptC is not resolved in our map, likely due to conformational flexibility.

Importantly, LptH remains stably associated with LptB₂FGC in the absence of LPS. Moreover, LptB₂FGC adopts a conformation similar to the previously determined apo state²⁶, demonstrating that formation of the proximal LptC–LptH connection does not require LPS occupancy or nucleotide binding. Recent single-molecule studies in *E. coli* showed that LPS facilitates formation and increases the lifetime of trans-envelope Lpt bridges³³, suggesting that substrate availability influences the assembly or stability of the complete transport machinery. Our structure complements these observations by demonstrating that the transporter-proximal LptC–LptH connection can assemble independently of LPS loading. Thus, the nucleotide-free structure captures an apo, preassembled state of the proximal Lpt bridge. This also indicates that nucleotide engagement is not required for LptH/A recruitment, but instead acts on an already assembled transporter–bridge junction.

On the periplasmic side, LptH binds the distal end of LptC and extends the beta-jellyroll groove formed by LptF and LptC (Fig. 1b,c). Rather than forming a linear extension, the connected β-jellyroll domains generate a continuous, highly twisted architecture that rotates by approximately 180° from LptF through LptC to LptH. Despite this pronounced curvature, the hydrophobic surfaces of the three β-jellyroll domains remain aligned, generating a continuous hydrophobic groove extending from the inner-membrane transporter into the proximal periplasmic bridge. The resulting arrangement therefore forms a twisted β-jellyroll conduit in which successive strands preserve the hydrophobic track while

progressively redirecting it away from the membrane. This arrangement provides a continuous pathway that shields the hydrophobic acyl chains of LPS from the aqueous periplasm. In the nucleotide-free structure, however, the groove is relatively narrow and contains no detectable LPS density, suggesting that the captured apo conformation represents a substrate-free state of the LPS transport pathway.

β-strand augmentation defines an essential LptC-LptH junction

The cryo-EM reconstruction resolves the LptC–LptH interface in sufficient detail to define the molecular basis of bridge-protein docking. LptC and LptH associate through edge-to-edge pairing of the C-terminal β-strand of LptC with the N-terminal β-strand of LptH, thereby extending the two β-jellyroll domains into a continuous antiparallel β-sheet. This arrangement brings their hydrophobic grooves into register and extends the transporter-proximal conduit away from the inner membrane. The interaction closely resembles the β-strand augmentation observed between other β-jellyroll components of the enterobacterial Lpt pathway, including the LptF–LptC and LptA–LptA junctions^{19,34,35}.

The interface is stabilised by both backbone hydrogen bonding and complementary side-chain packing. LptH residues Ile10, Val12, Ala14, Ala17 and Leu19 form a compact hydrophobic surface against Met180, Leu182, Val186 and Val192 of LptC (Fig. 2a). This core is surrounded by a network of polar and charged residues. Gln13, Asp15, Glu18, Asp20, Asp21 and Lys22 of LptH are positioned near Arg193, Gln189, Arg187, Asn185, His181, Arg179, His190 and Asp177 of LptC, providing additional contacts across the paired strands and adjacent loops. Together, these interactions provide a structural explanation for the stable association of LptH with LptB₂FGC observed by native MS.

Sequence comparison with orthologues from five other Gram-negative species revealed limited conservation of individual residues at the interface (Fig. 2b; Fig. S3 and S4). Nevertheless, the interaction preserves the edge-to-edge β-strand augmentation characteristic of Lpt β-jellyroll assemblies. Recognition therefore appears to depend less on strict conservation of individual side chains than on preservation of strand complementarity, surface shape and hydrophobic character. This view is consistent with previous mutational studies of the C-terminal region of LptC, in which deletion of residues 177–191 disrupted LptA–LptC complex formation³⁶. To examine the importance of the structurally defined interface in cells, we introduced charge-disrupting substitutions into the contacting surfaces of LptC and LptH. Quintuple variants of LptC (A161R/A162R/L182R/V186R/H190R) and LptH (I10R/V12R/Q13R/L19R/D20R) failed to complement the corresponding conditional strains under non-permissive conditions (Fig. 2c). Taken together, these findings provide structural and functional evidence that LptH/A docks onto LptC through a combination of hydrophobic and polar interactions, completing the β-sheet groove of the Lpt bridge. This docking configuration is critical for stable bridge assembly and continuity of the LPS transport pathway.

Thanatin disrupts LptH assemblies in a defined sequence

Because the LptC–LptH interface is formed by an exposed β -strand edge, we asked whether thanatin, an antimicrobial peptide known to target β -jellyroll interactions within the Lpt pathway, could disrupt it. Specifically thanatin^{27,29,37–39} was found to interact with *E. coli* LptD and LptA proteins, disrupt the interaction network between LptC, LptA, and LptD and, more recently, LptH dimerisation. To probe the effect of this antimicrobial peptide on the *P. aeruginosa* LptB₂FGCH₂ complex, we incubated the pre-formed LptB₂FGC-(LptH)₂ complex with increasing amounts of thanatin ranging from 0–10 μ M and analysed them by native MS (Fig. 3). Remarkably, at lower peptide concentrations, the LptB₂FGCH₂ species was readily depleted while LptB₂FGCH remained detectable. At higher concentrations, the transporter-associated LptH decreased only slightly at the highest thanatin concentration tested. The order of disappearance indicates that the LptH–LptH contact is more susceptible to thanatin than the LptC–LptH junction under the conditions of the native mass spectrometry experiment.

MD simulations reveal the sequence of thanatin-mediated interface disruption

To investigate the molecular basis for the sequential loss of LptH-containing species observed by native MS, we compared the dynamics of the LptC–LptH, LptH–LptH and LptH–thanatin interfaces in independent MD simulations. LptH retained its β -jellyroll architecture in each system, indicating that the observed differences were not caused by global unfolding. The LptC–LptH interface buried the largest solvent-accessible surface area, $11.09 \pm 0.45 \text{ nm}^2$, whereas the LptH–LptH interface was substantially smaller, $5.18 \pm 0.56 \text{ nm}^2$. The predicted LptH–thanatin complex displayed an intermediate buried interface area of $8.02 \pm 0.74 \text{ nm}^2$ (Fig. S5). These simulations therefore suggest that the LptH–LptH association is the least extensive of the three interfaces and provide a structural rationale for its preferential disruption by thanatin in the native MS experiments above. Together, the experimental and simulation data support a model in which thanatin initially competes with or destabilises the comparatively weaker LptH–LptH interaction before disrupting the more extensive LptC–LptH interface at higher peptide concentrations.

ATP analogue binding closes the transporter while preserving the assembled bridge

To determine how nucleotide engagement affects the transporter-bridge assembly, we incubated LptB₂FGC-LptH with the non-hydrolysable ATP analogue AMP-PNP and determined a cryo-EM reconstruction at 3.05 Å resolution (Fig. 4a,b and Fig. S6). The map resolves the nucleotide-binding and transmembrane domains at high resolution, with lower local resolution in the distal beta-jellyroll domains. Two AMP-PNP molecules are bound at the closed LptB dimer interface, identifying a nucleotide-engaged pre-hydrolysis state. Unlike previous nucleotide-bound LptB₂FGC structures, the reconstruction also contains the transporter-associated LptH molecule and therefore permits direct comparison of transporter closure with the conformation of the bridge junction. The overall LptC–LptH

connection is retained in the AMP-PNP-bound reconstruction. Thus, nucleotide binding does not dissociate the soluble bridge component but instead acts on an already assembled conduit.

The AMP-PNP-bound structure reveals a closed dimeric conformation of LptB₂ with clear density for two AMP-PNP molecules at the nucleotide-binding sites (Fig. 4c, 4d). The binding pocket is formed by residues from both LptB protomers. From one subunit, residues K14, Y13, T44, T43, Q85, K42, N38, V18, and R16 coordinate the nucleotide, positioned within conserved Walker A, Walker B, and Q-loop motifs that mediate ATP recognition and hydrolysis. At the dimer interface, residues S139 and E142 from the opposing LptB stabilise nucleotide binding. Notably, R296 from the cytoplasmic loop of LptG, or R297 from the equivalent position in LptF, projects toward the bound nucleotide, suggesting cross-domain communication via a mechanism analogous to the coupling helix observed in other ABC transporters. These interactions are consistent with the conserved architecture of ABC-transporter nucleotide-binding sites and with functional analyses of the Lpt ATPase cycle^{25,40}.

Moreover, AMP-PNP binding engages the coupling helices between TM2 and TM3 of LptF and LptG, driving a rigid-body inward rotation of the transmembrane domains that collapses the central LPS-binding cavity. Consistent with a post-expulsion state, no LPS density is observed^{20,21}. Both interfaces 1 and 2 adopt a closed configuration, with TM1 and TM5 helices from opposing subunits packed in parallel. As with other nucleotide-bound LptB₂FGC structures²¹, the transmembrane helix of LptC is not resolved, despite strong density for its periplasmic β -jellyroll domain. This likely reflects TM LptC disengagement from interface 1, thereby facilitating TMD compaction.

Nucleotide binding remodels the LptF-LptC bridge junction

Alignment of the nucleotide-free and AMP-PNP-bound structures shows that closure of the membrane cavity is transmitted into the periplasmic assembly. The dominant motion is a concerted rotation of the LptF and LptG transmembrane domains, accompanied by displacement of the LptF β -jellyroll and its connecting helices. The distal LptC-LptH β -sheet remains assembled (Fig. S7 and S8).

The largest local difference occurs at the LptF-LptC junction, where the β -jellyroll domains separate relative to the nucleotide-free state (Fig. 5 and Fig. S7 and S8). Concomitant rearrangements involve the helix linked to LptF TM3 and the loop connecting TM4 to the β -jellyroll (residues 242–255), which approaches LptG TM1. These changes narrow the connection back to the membrane cavity while altering the entrance to the periplasmic groove. The structural comparison is therefore consistent with a gated junction between the extraction chamber and the bridge, as proposed from earlier structural, biochemical and genetic analyses of enterobacterial LptB₂FGC^{16,25–28,30,31}. In particular, nucleotide engagement has been shown to be allosterically coupled to opening of the LptF β -jellyroll, with a much more limited response in LptG⁴¹, while solid-state NMR revealed increased conformational dynamics of the LptC transmembrane helix upon ATP or LPS engagement⁴². Our structures extend these observations to an assembled transporter–bridge complex, showing that nucleotide-dependent

remodelling is concentrated at the LptF–LptC junction while the distal LptC–LptH β -sheet remains intact.

In striking contrast to the LptF–LptC junction, the LptC–LptH segment remains comparatively unchanged following nucleotide binding. This asymmetric structural response suggests that ATP-dependent conformational changes are concentrated near the energy-transducing inner-membrane transporter, while the more distal LptC–LptH connection remains stably assembled. Together, the structures support a model in which nucleotide binding closes the cavity of the LptB₂FG transporter and selectively remodels the entrance to the periplasmic bridge without disrupting the bridge itself. A substrate-bound structure will be required to establish how this nucleotide-dependent gating event coordinates LPS entry into and movement along the continuous hydrophobic groove.

MD supports nucleotide-coupled remodelling of the assembled bridge

To test whether the conformational differences observed by cryo-EM persist in a membrane environment, we performed three independent 500 ns MD simulations of the apo complex and of the nucleotide-bound complex, replacing AMP-PNP with ATP. Both systems reached stable C α -RMSD plateaus after initial equilibration and retained the overall architecture of the transporter and periplasmic bridge. ATP therefore did not globally destabilise LptB₂FGC–LptH but instead altered specific collective and interfacial properties (Fig. S9-S11).

The orientation of the LptC–LptH assembly relative to the membrane showed distinct hinge-angle distributions in the apo and ATP-bound trajectories, indicating that nucleotide engagement reshapes the orientational ensemble of the periplasmic chute. Consistent with this, the buried solvent-accessible surface area at the LptC–LptH interface decreased from 11.50 ± 0.58 nm² in the apo simulations to 9.60 ± 0.47 nm² in the ATP-bound simulations. Residue-contact occupancies remained broadly conserved, indicating that ATP loosens the interface without disrupting the β -sheet connection (Fig. S9-S11). These simulations therefore extend the static structures by identifying a stable yet more flexible nucleotide-bound bridge, in which the LptC–LptH connection remains intact. Together with the cryo-EM structures, these results show that nucleotide-dependent changes are concentrated at the LptF–LptC junction without disrupting continuity of the distal bridge.

Discussion

Our study defines how the *P. aeruginosa* Lpt transporter engages its periplasmic bridge component and how nucleotide binding remodels the transporter–bridge junction. Native MS shows that periplasmic component LptH assembles readily with LptB₂FGC, while cryo-EM reveals how this interaction completes the transporter-proximal segment of the LPS conduit. This finding distinguishes assembly of the transporter-associated bridge segment from substrate-dependent stabilisation of the complete

trans-envelope Lpt machinery and suggests that bridge assembly can precede LPS loading. The preservation of LptH in both nucleotide-free and AMP-PNP-bound structures further indicates that nucleotide engagement remodels an already assembled complex rather than triggering recruitment or dissociation of LptH.

A central feature of the complex is the edge-to-edge pairing of the C-terminal β -strand of LptC with the N-terminal β -strand of LptH, which extends the two β -jellyroll domains into a continuous antiparallel β -sheet. This β -strand augmentation is a recurring architectural principle in the Lpt pathway and provides a structural basis for how divergent bridge proteins can maintain function despite limited sequence conservation. In the present structure, a compact hydrophobic core and flanking polar contacts stabilise the interface, accounting for the stable complex observed by native mass spectrometry. The genetic data are consistent with this interface being important in vivo. This interpretation is further supported by prior structural work showing edge-to-edge junctions within the enterobacterial Lpt system, including the LptF–LptC interface and the head-to-tail LptA oligomer observed in crystal and solution studies^{19,34–36}.

Thanatin further emphasises the relative hierarchy of LptH assemblies. Native MS shows that the assembled bridge is not uniformly susceptible to disruption: thanatin preferentially perturbs LptH/A self-association before the more extensive transporter-proximal LptC–LptH/A interface. The simulations provide a structural explanation for this behaviour: the LptH–LptH interface buries less surface area than the LptC–LptH junction, whereas the LptH–thanatin interface is intermediate. Together, these results suggest that thanatin preferentially exploits the weaker LptH–LptH association before disrupting the stronger transporter-proximal junction at higher concentrations. This sequence is consistent with a model in which the peptide perturbs bridge-protein interactions rather than globally unfolding LptH.

The AMP-PNP-bound structure shows that nucleotide engagement remodels the inner-membrane transporter without dissociating the assembled bridge. Closure of the LptB dimer is coupled to collapse of the transmembrane cavity and a local rearrangement at the LptF–LptC junction, whereas the LptC–LptH segment remains attached. This is, to our knowledge, the first nucleotide-bound LptB₂FGC structure that preserves the transporter-associated bridge and therefore directly links ATP-induced transporter closure to bridge remodelling. The present structures further place this gating event within the context of an assembled periplasmic conduit. This directly visualises the long-range coupling between the cytoplasmic ATPase and the periplasmic bridge that earlier genetic and biochemical studies had inferred^{43,44}. Because the structure is substrate-free, the precise relationship between this nucleotide-dependent rearrangement and LPS entry into the bridge remains to be established. This interpretation is in line with previous structural and biochemical studies that established the role of LptC in controlling directional transport and defining the route from the membrane cavity into the bridge^{19–21,25,26}.

The MD simulations are consistent with this interpretation. ATP does not destabilise the complex globally; instead, it alters the orientational ensemble and interfacial properties of the bridge-proximal assembly. In particular, the LptC–LptH interface becomes slightly less buried in the ATP-bound

trajectories, while contact patterns remain largely preserved. These data support a model in which nucleotide engagement produces a more compliant assembled bridge, capable of transmitting conformational changes from the transporter to the periplasmic conduit without breaking continuity. The comparison with the earlier *P. aeruginosa* LptB₂FGC structures also supports the idea that this species uses a comparatively compact transporter cavity and a distinct bridge geometry relative to enterobacterial systems²⁶.

Taken together, our results suggest that the Lpt system operates as a coupled machine in which bridge assembly and nucleotide-dependent transporter remodelling are coordinated. LptH docking completes the transporter-proximal bridge; thanatin preferentially destabilises the weaker LptH–LptH interaction, and ATP binding remodels the transporter while preserving bridge attachment. The AMP-PNP-bound complex therefore represents a nucleotide-engaged pre-hydrolysis state of the assembled transporter, compatible with a checkpoint-like model in which closure of the extraction chamber is coupled to controlled access to the periplasmic bridge. A substrate-bound intermediate state will be needed to establish the direction and timing of LPS movement through this assembly.

Declarations

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Author contributions

Conceptualisation of project: F.F., C-C.S. and J.R.B.; Methodology: Y.W., M.C., H.W.T., K.W., C.A.L., T.J.E., F.I., P.J.S., C.V.R., F.F., C-C.S., and J.R.B.; Investigation: Y.W., M.C., H.W.T., K.W., C.A.L., T.J.E., F.I., P.J.S., C.V.R., F.F., C-C.S., and J.R.B.; Funding acquisition: F.F., P.J.S., F.I., C.V.R., C-C.S., and J.R.B.; Project administration: P.J.S., F.I., F.F., C-C.S., and J.R.B.; Supervision: P.J.S., F.I., C.V.R. and J.R.B.; Writing—original draft: F.F., C-C.S. and J.R.B.; Writing—review and editing: Y.W., F.F., F.I., C.V.R., C-C.S., and J.R.B. All authors commented on the final version of the manuscript.

Competing Interests

C.V.R is founder and consultant of OMass Therapeutics. All other authors have no competing interests

Data availability

Cryo-EM maps and coordinates have been deposited in the EMDB and PDB, respectively, with accession numbers Apo: PDB 37ZP, EMDB EMD-78670; AMP-PNP: PDB 37ZQ, EMDB EMD-78671.

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Figures

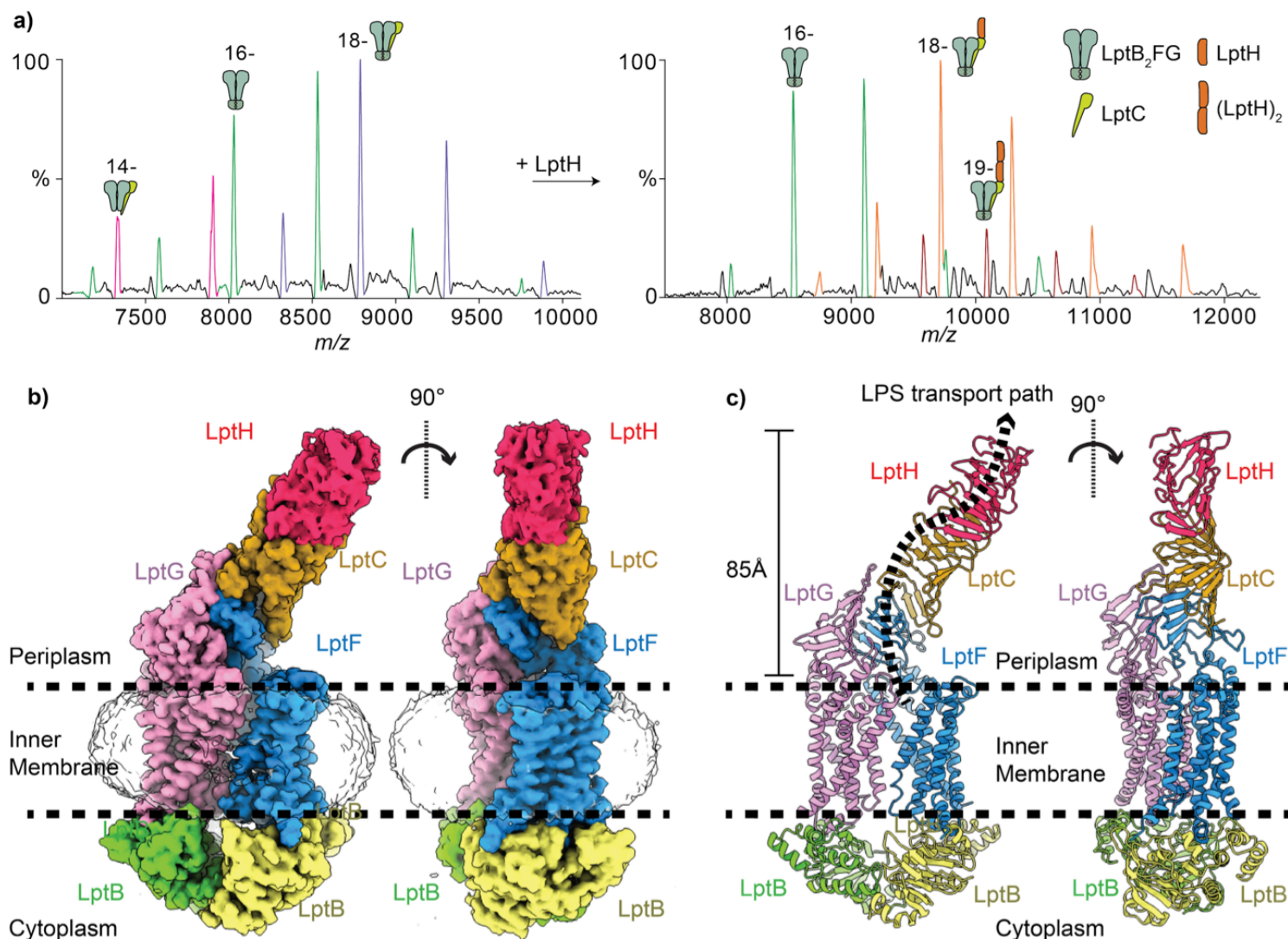


Figure 1

LptH forms a stable complex with LptB₂FGC. a) Native mass spectra of LptB₂FG/LptB₂FGC (left) with the addition of LptH (right), the data indicate that up to two molecules of LptH can bind to LptB₂FGC. b) Side views of the 3.24 Å cryo-EM map of the LptB₂FGCH viewed in the membrane plane. (c) Ribbon diagrams of LptB₂FGCH viewed in the membrane plane.

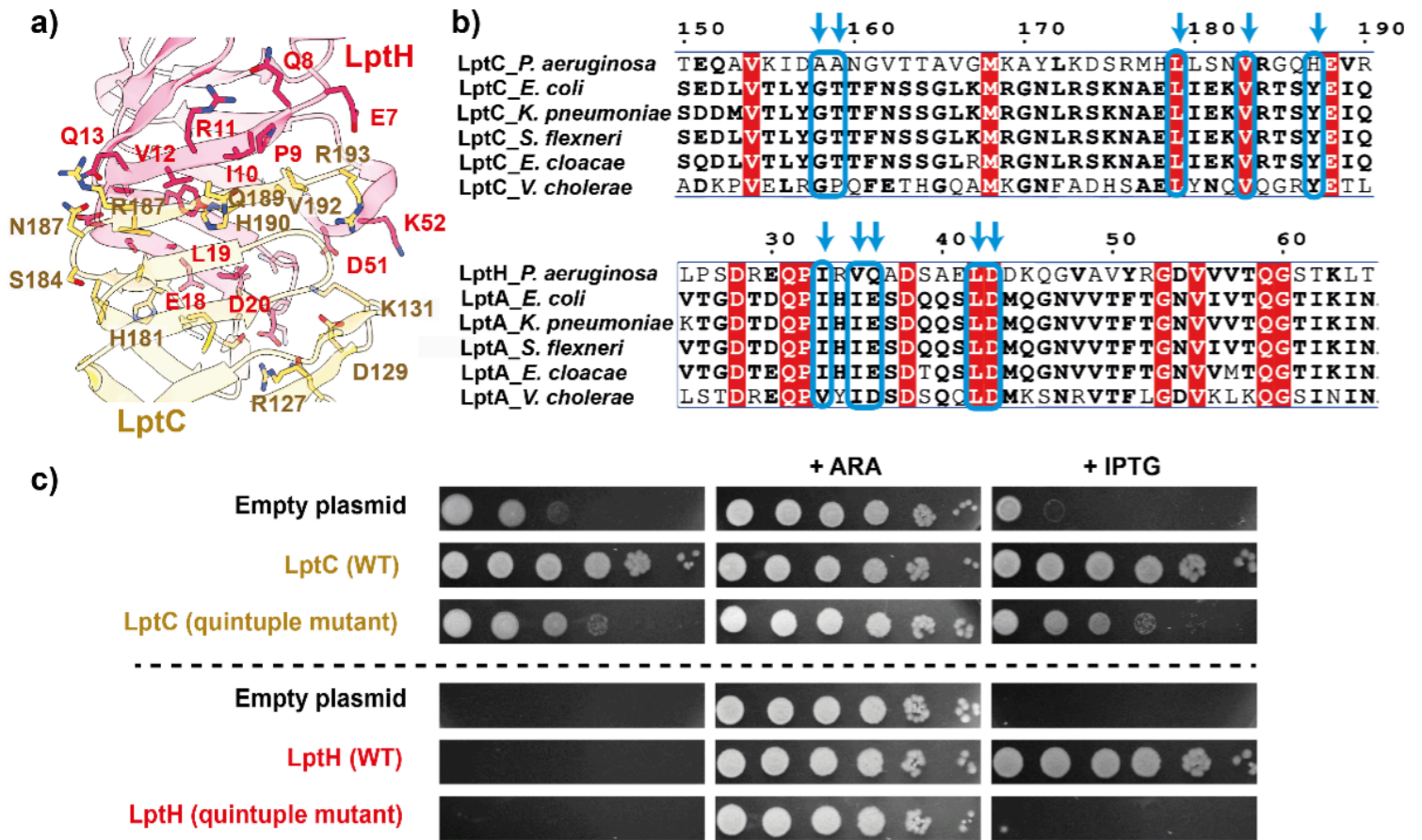


Figure 2

Disruption of the *LptB₂FGCH* complex by mutation a) Zoom-in view of the LptC-LptH binding interface showing the key interacting residues. b) Sequence alignment of *P. aeruginosa* LptC and LptH with their orthologues from *E. coli*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Enterobacter cloacae*, and *Vibrio cholerae*. Red colour indicates conserved residues, highly similar residues are shown in bold. Cyan circles and arrows indicate the residues chosen for the preparation of conditional mutants. Full sequences are shown in the Supplementary Information. c) Plating efficiency of the *P. aeruginosa* LptC (upper panel) and LptH (lower panel) conditional mutants carrying the empty plasmid pME6032 or its derivatives which express the cognate wild type protein (WT) or the protein variant with five amino acid substitutions (LptC-A161R-A162R-L182R-V186R-H190R or LptH-I10R-V12R-Q13R-L19R-D20R). Exponential-phase cultures in MH with 0.5% arabinose (ARA) were normalised to OD₆₀₀ = 1 in saline, and 5 µL of the 10⁻¹ to 10⁻⁶ dilutions were spotted onto MH agar supplemented with or without 0.5% ARA or 0.5 mM IPTG. Pictures were taken after 16 hours of incubation at 37°C and are representative of four biological replicates (n = 4).

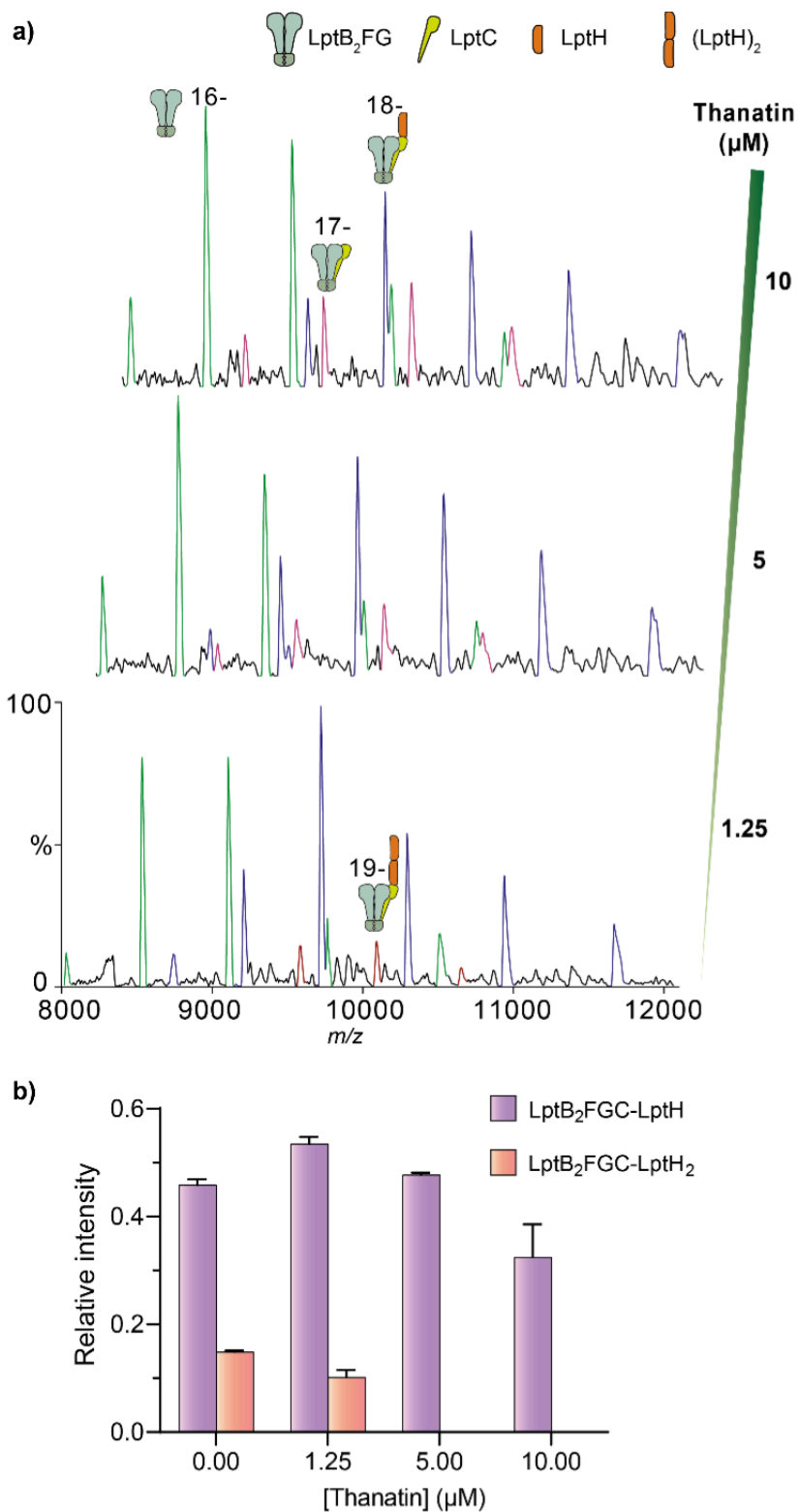


Figure 3

Thanatin sequentially disrupts LptH–LptH and LptC–LptH interactions. Native mass spectra of the pre-formed *P. aeruginosa* LptB₂FGCH₂/A₂ complex acquired in the presence of increasing concentrations of thanatin (0–10 μM).

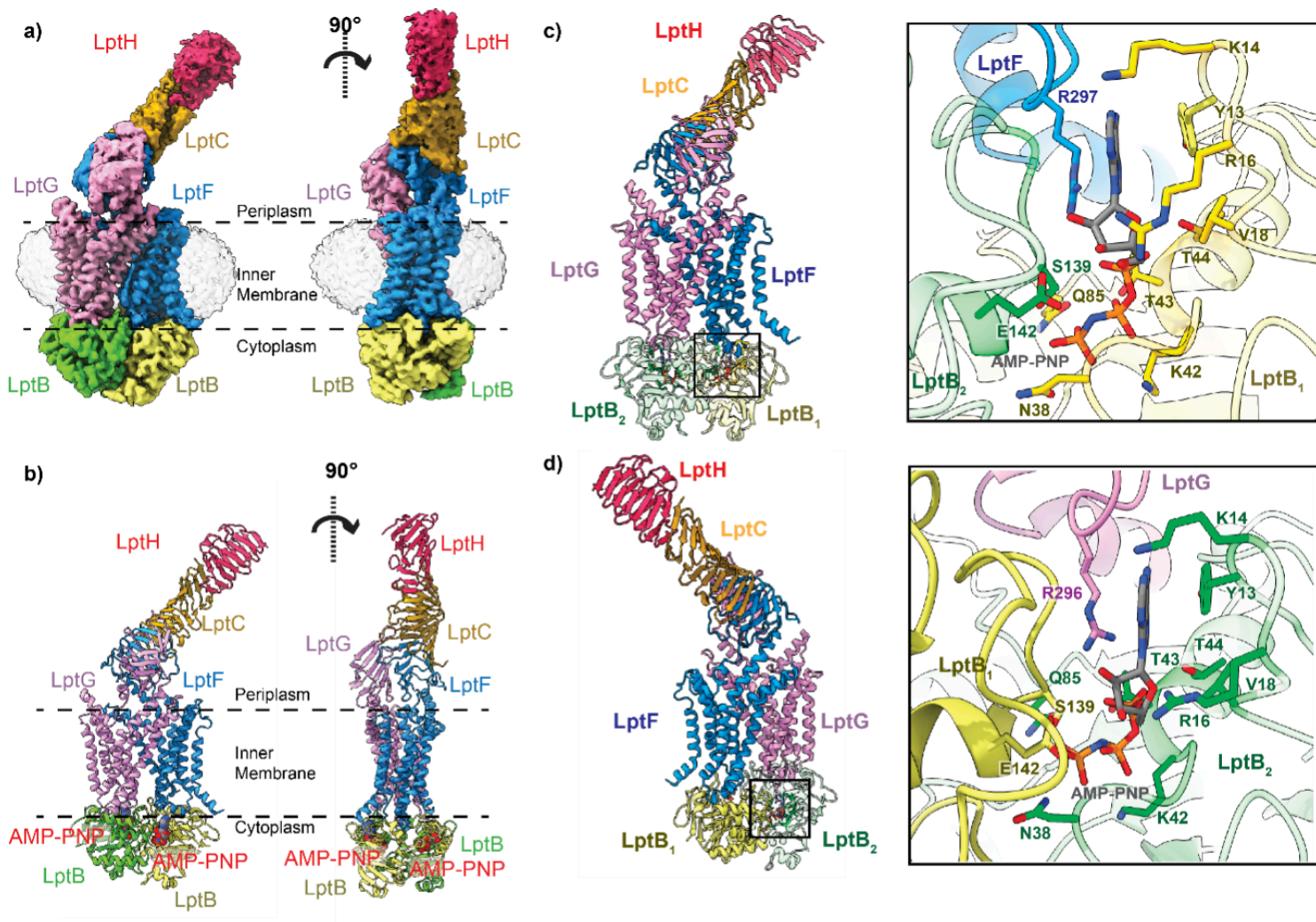


Figure 4

Cryo-EM structure of the *LptB*₂FGCH-AMPPNP complex. (a) Side views of the 3.05 Å cryo-EM map of the *LptB*₂FGCH-AMPPNP complex viewed in the membrane plane. *LptH*, *LptC*, *LptF*, *LptG*, and the two *LptB* molecules are coloured red, orange, blue, magenta, green, and yellow, respectively. The lipid nanodiscs surrounding the transmembrane domain are highlighted in grey. (b) Ribbon diagrams of *LptB*₂FGH viewed in the membrane plane. Two AMP-PNP molecules are shown as grey spheres.

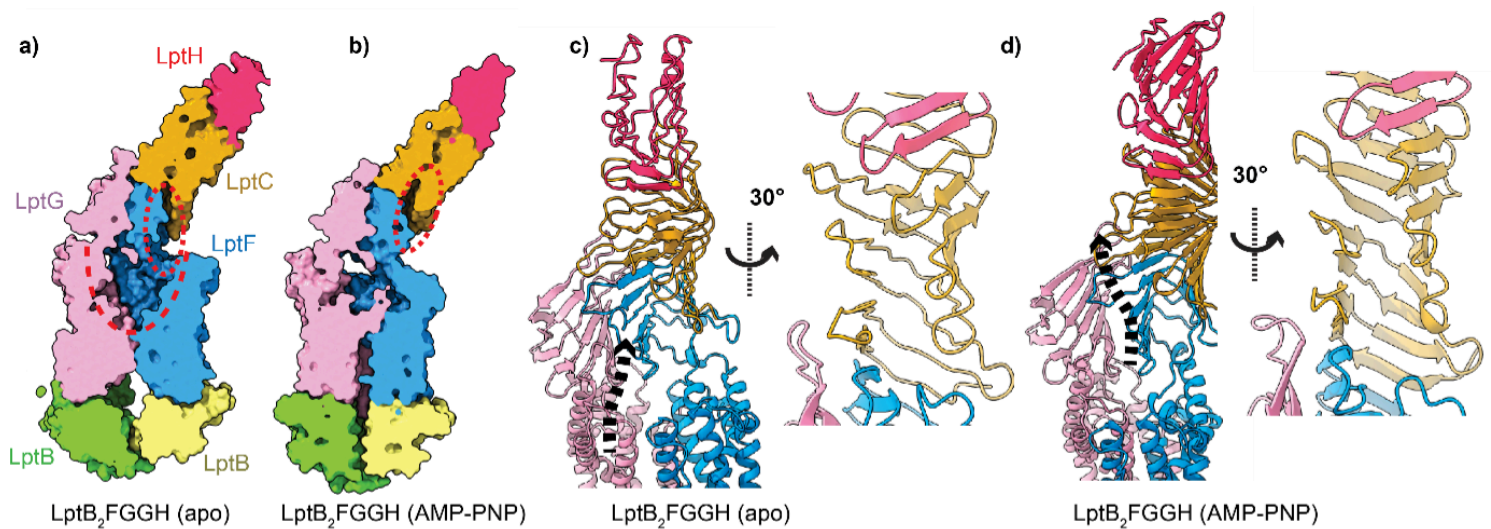


Figure 5

Nucleotide-dependent remodelling of the bridge entrance. a,b, Cross-sections through the nucleotide-free and AMP-PNP-bound LptB₂FGC-LptH complexes, showing closure of the transmembrane cavity and changes at the LptF-LptC junction. c,d, Structural superposition highlighting displacement of LptF, LptC and the connecting elements.

Supplementary Files

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