Structure and mechanism of the Nap adhesion complex from the human pathogen *Mycoplasma genitalium*

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*Mycoplasma genitalium* is a human pathogen adhering to host target epithelial cells and causing urethritis, cervicitis and pelvic inflammatory disease. Essential for infectivity is a transmembrane adhesion complex called Nap comprising proteins P110 and P140. Here we report the crystal structure of P140 both alone and in complex with the N-terminal domain of P110. By cryo-electron microscopy (cryo-EM) and tomography (cryo-ET) we find closed and open Nap conformations, determined at 9.8 and 15 Å, respectively. Both crystal structures and the cryo-EM structure are found in a closed conformation, where the sialic acid binding site in P110 is occluded. By contrast, the cryo-ET structure shows an open conformation, where the binding site is accessible. Structural information, in combination with functional studies, suggests a mechanism for attachment and release of *M. genitalium* to and from the host cell receptor, in which Nap conformations alternate to sustain motility and guarantee infectivity.
The human pathogen *Mycoplasma genitalium*, a member of the *pneumoniae* cluster of mycoplasmas, binds to eukaryotic cells by means of its adhesion complex, the Nap. This complex is formed by two heterodimers, each consisting of proteins P110 and P140. In addition to their roles in cytadherence and motility, P110 and P140 are immunodominant proteins and constitute the main target of host antibodies during infection. Antibiotic resistance to human pathogens from the *pneumoniae* cluster is increasing at an alarming rate, making it necessary to explore novel therapeutic strategies. Anti-adherence molecules, aimed at preventing the establishment of infection, are attractive potential antimicrobial drugs. A deep understanding of the Nap structure and adhesion mechanism will facilitate the development of anti-adherence therapies. Recently, we determined the crystal structure of the extracellular region of P110 and demonstrated its binding to sialic acid receptors. Here, we address the structure and mechanism of the Nap adhesion complex and reveal an intricate interplay between P110 and P140.

**Results**

**Crystal structure of P140 and in complex with P110N.** Crystals were obtained from the extracellular region of P140 (residues 23–1351) (Fig. 1, Supplementary Figs. 1 and 2), both alone and in complex with the N-terminal domain of P110 (P110N: residues 23–827) (Fig. 2a, Supplementary Fig. 2). The structure of P140, for which there are no molecular models or experimental phases available, was determined by density modification techniques, starting with a mask derived from the subtomogram-averaged map of the whole Nap obtained by cryo-electron tomography (cryo-ET) (see Methods). With four heterodimers in the asymmetric unit, the P40–P110N crystals were refined at 2.65 Å resolution to a final model with agreement R and R\(_{\text{free}}\) factors of 18.7 and 22.4, respectively (Supplementary Table 1).

**Structural similarities of P140 and P110.** The structure of the extracellular region of P140, with a bulky N-terminal domain (residues 23–1243) and a small C-terminal domain (residues 1244–1351), has an overall shape resembling the capital letter P (Fig. 1a). The N-terminal domain consists of a seven-bladed (β-sheet) propeller and a “crown” formed by the clustering of the long polypeptide segments that emerge from the propeller (Supplementary Fig. 3). β-Sheets I to VI each have four strands, while β-sheet VII, the last β-sheet in the propeller that connects directly with the C-terminal domain, has only two strands (Fig. 1b). P140 and P110 share many features of domain organization and the topology of the secondary structural elements, suggesting a common ancestor, although the degree of conservation differs for the N-terminal and C-terminal domains (Supplementary Fig. 4). The N-terminal domains, with an RMSD of 3.5 Å between the Ca atoms of 359 structurally equivalent residues (~28%), are markedly different in the crown. In contrast, the C-terminal domains are closely related, with 74 equivalent residues (~71%) and an RMSD of 2.2 Å.

**Binding site analysis of the Nap.** The binding site for the sialylated oligosaccharides, identified in the structure of P110, is located at the interface between P140 and P110 in the crystal structure of the P140–P110N complex (Fig. 2a–d). Interaction of the two subunits changes the position of the sialic binding β-hairpin while the interfering loop (P140 residues 807–827) inserts into the binding pocket of P110N, sterically interfering with the binding of oligosaccharides to the complex (Fig. 2c–d). In agreement with this, surface plasmon resonance analysis shows that, in solution, sialylated compounds 3SL and 6SL (neuraminic acid forming an α2→3 or an α2→6 linkage to a lactose monosaccharide, respectively), which bind to P110 alone, do not bind either to P140 alone or to P140–P110 complexes (Supplementary Fig. 5). The structure of the P140–P110N complex suggests that P110 residues Gln460–Asp461, from the binding β-hairpin, and

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**Fig. 1 Structure of P140.** a P140 contains a large N-terminal domain and a smaller C-terminal domain, which is followed in sequence by a predicted transmembrane helix. The N-terminal domain has two distinct regions: the β-propeller (green) and the crown (blue). b Two views of the β-propeller, 70° apart. The first and last β-sheets of the propeller (I and VII, respectively) interact with the C-terminal domain and are structurally contiguous in the propeller ring. A large β-bulge (residues 69–77, in cyan) occludes the center of the propeller ring. The presence of only two strands in the last β-sheet (VII) constrains the interface between the N-terminal and C-terminal domains.
Arg600, close to the receptor-binding site, play an important role in the interaction with P140 (Fig. 2b, d). P110 variants carrying an R600A substitution or the triple substitution RQD (R600A, Q460A, and D461A) were introduced by transposon delivery into an *M. genitalium* P110 null mutant. Strains expressing the P110-RQD variant protein, which was barely detectable, showed a null binding capacity phenotype (Fig. 2g, Supplementary Fig. 6b). The variant protein P110-R600A was well expressed, but the strain presented no capacity for adherence and characterization of cell motility was not feasible.

**Single-particle cryo-EM of the P140–P110 extracellular region.** Using a sample of P140–P110 complexes, with the complete extracellular region included for both subunits (P140 residues 23–1351 and P110 residues 23–938), we performed single-particle cryo-electron microscopy (cryo-EM). We obtained a map with an overall resolution of 4.1 Å, although non-isotropic (Fig. 2e, Supplementary Table 2, Supplementary Figs. 7 and 8). The P140–P110N X-ray structure could be fitted as a rigid-body without modifications into the P140–P110 cryo-EM map with UCSF Chimera (Supplementary Table 3, Supplementary Fig. 7, Supplementary Table 3).
Supplementary Movie 1). Therefore, the structure of the P140–P110 complex found by cryo-EM corresponds to the conformation of the X-ray P140–P110N structure, where access to the sialylated oligosaccharides binding site is occluded (Supplementary Movie 1). In the P140–P110 cryo-EM map, there is no density for the C-terminal domain of P140, whereas the C-terminal domain of P110, which is absent in the P140–P110N complex, is visible, albeit with a weak density (Fig. 2e, Supplementary Fig. 7). This indicates a significant flexibility of the C-terminal domains with respect to the bulkier N-terminal domains. In the P140–P110 complex found by cryo-EM, the interface between subunits spans 2758 Å2 with 30 hydrogen bonds and an estimated Gibbs free energy of ~20 kcal/mol, resulting in a 100% probability of the formation of the complex (PIASA server17).

Motility analysis of mutants. Five mutations were introduced close to the interface between the N-terminal and C-terminal domains, to check if either adhesion or motility was affected. The five mutated residues (Y830A, R834G, D836L, W838F, and G839F) were chosen in the vicinity of the potassium-binding site found in P1106 (Fig. 2e, f, Supplementary Table 4). The P110–G839F strain showed no detectable levels of adhesins, while P110–D836L exhibited hemadsorption values similar to those of wild-type cells (Fig. 2g). The P110–Y830A and P110–R834G variants D836L exhibited hemadsorption values similar to those of wild-type cells (Fig. 2g). The P110–Y830A and P110–R834G variants exhibited also a fourfold increase in the resistance to complement compared to the wild-type strain (Supplementary Table 4). The P110–W838F mutant was severely impaired, with high Kd and Bmax values. Motility was examined by time-lapse microscopy to monitor the movements of individual cells for 120 s (Supplementary Fig. 6, Supplementary Table 5). In the P110–W838F mutant, cells were completely non-motile and, in agreement with this extreme phenotype, phase contrast images revealed the presence of large aggregates resulting from the inability of cells to spread. The Y830A, R834G, and D836L variants also exhibited altered gliding velocities, indicating that structural integrity at the interface between the N-terminal and C-terminal domains, away from the cell receptor-binding site, is critical for motility in M. genitalium.

Single-particle analysis of the Nap. Next, we performed single-particle cryo-EM using a purified sample of Nap complexes, obtained as previously described3 (see Methods). The purified Nap complexes contain full-length P140 and P110 proteins, including the transmembrane helices and cytoplasmic regions, which are required for formation of tetramers. Classification indicated that only ~15–20% of the images corresponded to complete Nap particles where the extracellular region was well defined. The best map, with an overall resolution of 9.8 Å, allowed accurate rigid-body fitting with Chimera16 of the P140 structure alone and the P110 structure alone, confirming the presence of a dimer of P140–P110 heterodimers in each Nap (Fig. 3a–c, Supplementary Fig. 9, Supplementary Table 2). The arrangement of P140–P110 heterodimers is essentially identical to the X-ray and cryo-EM P140–P110 structures, with the interactions between both subunits preventing access to the sialylated oligosaccharide-binding site. This is the “closed” conformation of the Nap (Supplementary Movie 2). C-terminal domains act like stalks connecting the large N-terminal domain of each subunit with the outer surface of the cell membrane. The two P110 subunits are almost parallel to the dimer axis and face away from each other, while the two P140 subunits of the Nap adopt a “V-shaped” arrangement with the C-terminal domains very close to each other (Fig. 3c). The distance between heterodimers is large (Fig. 3b, c), suggesting that their interaction is weak. This is in agreement with MALS measurements where only heterodimers are detected when mixing equimolecular amounts of constructs from the extracellular regions of P110 and P140 (Supplementary Fig. 10). Therefore, there are two interfaces in the Nap between the extracellular regions of P140 and P110, which we name the “tight” and “loose” interfaces (Fig. 3b, e). The cryo-EM map of the Nap shows density corresponding to the Nap transmembrane region and the positions of the transmembrane helices (their N-terminal ends) can be seen (Fig. 3a, c). The close proximity of the P140 C-terminal domains also brings the N-terminal ends of the corresponding transmembrane helices closer together. For P140 and P110, sequence analysis indicates the presence of one transmembrane helix containing one and two Engelman motifs (GXXG, with X any residue, in general hydrophobic), respectively, which are characteristic of high-affinity interactions in membrane helices18,19.

Cryo-electron tomography of the in situ Nap. The structure of the in situ Nap complex was also determined by cryo-ET from mildly lysed M. genitalium cells3. Classification of Nap volumes and subsequent sub-tomogram averaging provided an improved map for the most abundant class (~85%) at 15 Å resolution, in which the four subunits, two from P110 and two from P140, are clearly distinguished and present a nearly perfect twofold symmetry (Fig. 3d–f, Supplementary Fig. 11). The rigid-body fitting of the structures of P110 alone and P140 alone into the cryo-ET density with Chimera16 reveals major differences from the cryo-EM structure of the Nap (Fig. 3, Fig. 4a, Supplementary Table 3). In the cryo-ET structure, the longest axis of the four subunits runs parallel to the axis of twofold symmetry of the Nap. This
Fig. 3 The structure of Nap by single-particle cryo-EM and in situ cryo-ET. a–c Three different views of the single-particle cryo-EM from the Nap (beige surface) with the structures of P140 (blue) and P110 (yellow) fitted into the density. In a the crown, propeller, and C-terminal domain are indicated. b Top view of the Nap, depicting the loose and tight interfaces. An approximate twofold axis perpendicular to the cell membrane relates the pair of P110–P140 heterodimers of a Nap. Fitting of the P140 and P110 subunits is unambiguous. The interface between the P110 and P140 subunits, defined as tight in the cryo-EM structure of the Nap, is the same found in the cryo-EM structure of the P140–P110 heterodimer. The interfering loop and the binding site are indicated with a black star. c The lateral view along the loose interface shows the V-like shape adopted by the two P140 subunits. d–f Three views of the cryo-ET map of the Nap, with the structures of P140 and P110 accurately fitted. The membrane bilayer of the mycoplasma cell is clearly defined in the lower part of the lateral views (d, f). e The top view shows that the tight interface is wider in the cryo-ET “open” conformation than in the cryo-EM “closed” conformation of the Nap. f The lateral view along the loose interface shows an interaction between heterodimers.
implies that between the cryo-ET and cryo-EM structures there is a rotation of P110 relative to P140 in the heterodimer and an increase of 10 Å in the closest distance between the C-terminal domains of P140 subunits (Fig. 4b, c, Supplementary Fig. 12, Supplementary Movies 3 and 4). The loose interface between P140 and P110 subunits, which is very wide in the cryo-EM structure of the Nap, is narrower in the cryo-ET structure, with a few interactions between P140 and P110 (in particular, P140 residues 1175–1179 interacting with the P110 loop 750–755) that are absent in the cryo-EM Nap (Fig. 3c, f). The tight interface...
between P140 and P110, which is in the “closed” conformation in the cryo-EM structure of the Nap, undergoes major rearrangements in the cryo-ET structure, becoming on average ~7 Å wider (Fig. 3a, b, d, e). This widening “opens” the sialylated oligosaccharide-binding site, making it accessible for binding in the cryo-ET structure of the Nap. Therefore, the cryo-ET and cryo-EM structures of the Nap correspond to conformations that, with respect to the sialylated host cell receptors, are respectively “open”, with the binding site accessible and ready for binding, and “closed”, with the binding site occluded and not accessible for binding. In the cryo-ET map, the cell membrane surrounding the Nap is visible as a double layer that is perpendicular to the Nap binding. In the cryo-EM map, the cell membrane surrounding the Nap, undergoing the adhesion machinery of mycoplasmas from the M. genitalium (strain G37, residues 23–1331) was amplified from a synthetic clone (Supplementary Table 4). Protein production and purification for P140 and P100 followed the same protocol as for P110N.

Preparation of P140–P110 and P140–P110N complexes and SEC-MALS analysis. Central fractions of the Superdex 200 16/60 column (GE Healthcare) from P140 and either P110N or P110 were mixed in a 1:1 ratio to a final concentration of ~11 mg/ml (measured with an absorption coefficient of 1.32). Each mixture was incubated for 30 min at room temperature to obtain the heterodimeric complex. For protein oligomerization experiments, prior to sample injections, the multangle light scattering (MALS, Wyatt Technologies & Corporation) detector was normalized with 25 μl of BSA at 5 mg/ml. Then 150 μl of each purified P140, P110, and P140–P110 sample at 0.6, 2, and 0.5 mg/ml, respectively, were injected at 0.5 ml/min using a Superdex 200 increase 10/300 GL column (GE Healthcare) with running buffer at 0.25 mM Tris and 150 mM NaCl. All samples were purified by filtering through 0.22 μm filters.

Preparation of purified Nap complexes. A P110His strain was generated for purification of the Nap complexes from M. genitalium G37 cells (ATCC 33530). Produced by genetic engineering, the strain carries a 6xHis tag insertion in the MG192 gene. Four liters of the P110His strain grown in SP4 medium in suspension at 37 °C at 150 r.p.m. was harvested by centrifugation (16,000 × g, 30 min).

Discussion
Integration of the information obtained from the structural approaches shows that the Nap is composed of two P140–P110 heterodimers with a loose interface between them in the extracellular region, suggesting that subunits in the Nap are held together mainly by interactions in the intracellular and transmembrane regions. Each heterodimer of the Nap appears to undergo large structural rearrangements between the “open” and “closed” conformations, which are associated with attachment and release to cell receptors, respectively (Fig. 4b, Supplementary Fig. 12, Supplementary Movie 4). The “open” conformation corresponds to a state in which the P140 and P110 extracellular regions from the four subunits of the Nap interact only weakly with each other, allowing P110 to remain in a conformation that is ready to bind or is bound to sialylated cell receptors. The “closed” conformation corresponds to a state in which the tight interaction of the extracellular regions of P140 and P110 occlude the cell receptor-binding site. The “closed” conformation could occur rapidly to release the Nap from the sialylated cell receptor.

To avoid being trapped in the overall most stable state, the cycling between “open” and “closed” conformations would require a net input of energy in each Nap complex. The structural rearrangements in the Nap extracellular region can be transmitted to the cell interior through repositioning of the transmembrane helices. In vivo variants of Nap complexes indicate that structural rearrangements can play a critical role in both cell adhesion and motility. Results from this work, together with previous data, could form the basis for developing therapeutic strategies targeting the adhesion machinery of mycoplasmas from the pneumoniae cluster.

Methods
Cloning, expression, and purification of P110N, P140, and P110. The region corresponding to the MG_192 gene from M. genitalium (strain G37, residues 23–827, P110N) was amplified from a synthetic clone (Supplementary Table 6) using primers P110N-F and P110N-R as Forward and Reverse, respectively (Supplementary Table 4). This region corresponding to the MG_192 gene from M. genitalium (strain G37, residues 23–1331) was amplified from a synthetic clone (Supplementary Table 6) using primers P110F and P110R as Forward and Reverse, respectively (Supplementary Table 4). Protein production and purification for P140 and P110 followed the same protocol as for P110N.

Crystallization of P140 alone and the P140–P110N complex. Crystallographic data were acquired on a CrystalLographix four-circle diffractometer (Oxford Diffraction). Crystals of P140 alone and the P140–P110N complex were prepared by mixing 1 μl of P140–P110N complex at 10.95 mg/ml and 1 μl reservoir solution containing 4% PEG3350, 1.0 M MgTris Propane pH 7.5, and 0.2 M sodium sulfate hydrate at 20 °C in hanging drop in 24-well plates. Crystals from the P140–P110N complex were prepared by mixing 1 μl of P140–P110N complex at 10.95 mg/ml and 1 μl reservoir solution containing 4% PEG3350, 1.0 M magnesium chloride hydrate at 20 °C in hanging drop in 24-well plates. All crystals were flash-cooled in liquid nitrogen with 20% glycerol as a cryo-protectant.

X-ray data collection and structure determination. X-ray diffraction experiments were performed at the XALOC Beamline (ALBA, Spain). Data were processed with XDS21 using XDS21, Aimless and Pointless23 from the CCP4i suite of programs.24 P140 crystals belong to the space group C2 with six subunits in the asymmetric unit, while P140–P110N crystals belong to the space group P21 with eight subunits in the asymmetric unit and contain four heterodimers in the crystal asymmetric unit. For the P140–P110N crystals, a partial molecular replacement solution was obtained with Phaser software25 using the N-terminal domain of the available structures of P110 (PDB code 6RT3) as a search model. In these P140–P110N crystals, an initial mask was tentatively defined for P140 using the sub-tomogram averaged map of a whole Nap complex obtained from cryo-ET images of M. genitalium ghost cells. Density modification in the crystal of the P140–P110N complex, by iterative non-crystallographic symmetry averaging and solvent flattening followed by phase extension with DM,26 allowed us to obtain a rough density for P140, in which a few secondary and tertiary structural elements were recognizable. This density was sufficient to provide an initial molecular replacement solution for the P140 crystals (with six subunits in the asymmetric unit) and for crystals of the orthologous protein P1 from M. pneumoniae (now deposited in the PDB with code 6RC9) as a search model. In these P140–P110N crystals, the initial mask was tentatively defined for P140 using the sub-tomogram averaged map of a whole Nap complex obtained from cryo-ET images of M. genitalium ghost cells. Density modification in the crystal of the P140–P110N complex, by iterative non-crystallographic symmetry averaging and solvent flattening followed by phase extension with DM.26, allowed us to obtain a rough density for P140, in which a few secondary and tertiary structural elements were recognizable. This density was sufficient to provide an initial molecular replacement solution for the P140 crystals (with six subunits in the asymmetric unit) and for crystals of the orthologous protein P1 from M. pneumoniae (now deposited in the PDB with code 6RC9). Averaging within and between crystals, while updating and refining the masks, provided electron density maps parallel to the plane of P140 and P1. The weak selenium methionine anomalous data, available from both the P140 and the P140–P110N crystals, were used to confirm
Cryo-ET and sub-tomogram averaging. Ghost cells were prepared similarly as reported previously. In all cases, molecular graphics rendering and analyses were performed with either UCSF Chimera (resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, USA). In all cases, rigid-body transformations were used to fit X-ray structures into the cryo-EM density maps using the fit-in-map function in UCSF Chimera.

Single-particle cryo-EM. For single-particle cryo-EM, a 3.5 μl aliquot of 20 μg/ml purified P140–P110 heterodimer, or 25 μg/ml purified Nap complex, was applied to a hole grid in cryo-ET data and subsequently processed as described in ref. 36. Puromycin-resistant colonies were obtained using primers COMmg192-F and the specific mg192 forward primer (Supplementary Table 4). To reconstitute the mutated full-length MG_192 alleles, we conducted splicing by overhang extension (SOE) PCR using the specific primers COMmg192-F and the specific mg192 forward primer (Supplementary Table 4). To reconstitute the mutated full-length MG_192 alleles, we conducted splicing by overhang extension (SOE) PCR using the specific primers COMmg192-F and the specific mg192 forward primer (Supplementary Table 4).

Surface plasmon resonance. For binding assay experiments, a Biacore 3000 biosensor platform (GE Biosystems) equipped with a research-grade streptavidin-coated biosensor chip was used. The chip was docked into the instrument and preconditioned with three 1-min injections of 1 M NaCl in 50 mM NaOH. Both 35L-PAA-biotin and 6SL-PAA-biotin (Carbosynth) oligosaccharides were injected over the third and second flow cell, respectively, at 10 μg/ml diluted in HBS-P (150 mM NaCl, pH 7.4, 0.15 M NaCl, pH 6.0, 0.005% P20). The first cell was left blank to serve as a reference. The running buffer consisted of HBS-P at a flow rate of 30 μl/min and the immobilization levels acquired were ~160 and ~180 response units for 35L-PAA-biotin and 6SL-PAA-biotin, respectively. A series of diluted purified extracellular P140 and P140–P110 samples in HBS-P (1.25, 2.5, 5, 10, and 20 μg/ml) was injected over the cell surface at 30 μl/min. Interaction analysis were performed at 25 °C and the protein was allowed to associate and dissociate for 60 and 90 s, respectively, followed by a 30 s regeneration injection step of 0.05% SDS at 30 μl/min.

Strains, culture conditions, and primers. M. genitalium was grown in SP4 medium (Hardy Diagnostics) at 37 °C in tissue culture flasks. Mutants were isolated on SP4 agar plates supplemented with puromycin (3 μg/ml). All M. genitalium strains used in this work are listed in Supplementary Table 4. Escherichia coli XL1-Blue strain (Agilent, Santa Clara, USA) was used for cloning and plasmid amplification purposes. It was grown in LB broth or on LB-agar plates containing 100 μg/ml ampicillin. Primers used are listed in Supplementary Table 7.

DNA manipulation and mutant construction. Plasmid DNA was purified using Qiagen Plasmid Miniprep Kit (Thermo Fisher Scientific). PCR products and DNA fragments were recovered from agarose gels using the gel extraction kit (Qiagen) and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany), and digested using the corresponding restriction enzymes (Thermo Fisher Scientific) when necessary. For transformation of M. genitalium, plasmids were purified using the GenElute HP Midiprep Kit (Sigma-Aldrich, St. Louis, USA) following the manufacturer’s instructions. P110 variants were generated in a two-step PCR procedure using DNA from plasmid pTnPacP110-WT as a template. For each mutant, the first PCR round was performed using primer COMmg192-F and the specific mg192 reverse primer, or primer COMmg192-R and the specific mg192 forward primer (Supplementary Table 4). To reconstitute the mutated full-length MG_192 alleles, we conducted splicing by overhang extension (SOE) PCR using the specific ampiclon pair obtained for each mutant as a template (R600A, R803A, R834G, D836L, W838F, and G839F) and primers COMmg192-F and COMmg192-R. Then, the mutated P110 alleles were digested with Apol and Xhol and ligated into a similarly digested plasmid backbone. Finally, the plasmid backbone was ligated into the corresponding minitransposons. The P110 variant carrying the triple substitution RQ460A and D461A into the pTnPacP110-WT was cloned into the pTnPacP110 minitransposons, using primers Tnp3, RTPCR192-R, RTPCR192-R, and PacUp, ruled out the presence of additional mutations in the MG_192 sequence. These plasmids were transformed into a M. genitalium MG_192 null mutant to create the different P110 variant strains. Identification of the minitransposon insertion site in the individual clones was done by sequencing using the PacDown primer and chromosomal DNA as a template.

Transformation and screening. M. genitalium MG_192 null mutants were transformed by electroporation using 5 μg of plasmid DNA of the different minitransposons, as previously described. Puromycin-resistant colonies were

The sequence assignment to the P140 structure. Final models were traced with Coot and refined with Refmac5 (Supplementary Table 1).

Cryo-ET and sub-tomogram averaging. Ghost cells were prepared similarly as reported previously. Briefly, cells were grown in a 270-μl polystyrene box in both cases. All images were normalized to 1/e2 for zero-dose projection alignment without image alignment was performed. Constituent sub-tomograms from the respective 2D classes were summed together to create 3D classes. Molecular graphics rendering and analyses were performed with either UCSF Chimera or UCSC ChimeraX (resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, USA). In all cases, rigid-body transformations were used to fit X-ray structures into the cryo-EM density maps using the fit-in-map function in UCSF Chimera.

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picked, propagated, and stored at −80 °C. For screening purposes, strains were further propagated in 25-cm² tissue culture flasks with puromycin and lysed using 0.1 M Tris-HCl pH 8.5, 0.03% Tween 20, and 250 μg/ml Proteinase K for 1 h at 37 °C. Then, Proteinase K was inactivated at 95 °C for 10 min. M. genitalium lysates were screened by sequencing using the PacDown primer. By contrast, the MG₁₉₂ alleles were fully re-sequenced to rule out the presence of undesired mutations.

**Sequencing reactions.** Sequencing reactions were performed with the BigDye® v3.1 Cycle Sequencing Kit using 2.5 μl of genomic DNA, following the manufacturer’s instructions (Thermo Fisher Scientific). All sequencing reactions were analyzed using an ABI PRISM 3130xl Genetic Analyzer at the Servei de Genòmica i Bioinformàtica (UAB).

**SDS-PAGE.** Whole-cell lysates were obtained from mid-log phase cultures grown in 75 cm² flasks. Protein concentration was determined with the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific), and similar amounts of total protein were separated by SDS-PAGE following the standard procedures.

**Quantitative hemadsorption assay.** We used 10⁶ mycoplasma cells during the hemadsorption assay. Fluorescence-activated cell sorting (FACS) data were acquired using a FACSDiCalibur (Becton Dickinson, Franklin Lakes, USA) equipped with an air-cooled 488 nm argon laser and a 633 nm red diode laser and analyzed with the CellQuest Pro and FACS Diva software (Becton Dickinson). Hemadsorption was quantified using flow cytometry as previously described with few modifications. Binding of mycoplasma cells to red blood cells can be modeled in an inverse Langmuir isothermal kinetic function:

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M = \frac{N_{\text{max}}[\text{RBC}]}{K_m + [\text{RBC}]},
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where \(M\) is the percentage of cells bound, \(N_{\text{max}}\) is the maximum number of receptors, and \(K_m\) is the equilibrium dissociation constant. The plots represent the best-fitting curves to a series of hemadsorption measurements obtained from at least two biological repeats for each strain. We performed a double-gating strategy, using a preliminary FL3-H/FL2-H gate following an SSC-H/FL1-H gate, as described previously.

**Time-lapse microcinematography.** Gliding properties of the wild type and mutant strains were analyzed by time-lapse microcinematography as previously described. Samples from mid-log phase cell cultures were grown overnight on eight-well ibiTreat μ-slides (Bidi, Grazelfingen, Germany) filled with 200 μl of SP4 medium. Prior to the observation, medium was replaced with fresh SP4 pre-warmed at 37 °C and cell movement was examined at 37 °C and 5% CO₂ using a Nikon Eclipse TE 2000-E inverted microscope equipped with a CO₂ Microscope Cage Incubation System (Okolab, San Bruno, USA). Images were captured at 2 s intervals for a total of 2 min for all different strains. The frequency of motile cells was determined by examining approximately 250 isolated cells of each strain.

**Data availability**

Atomic coordinates and structure factors for the reported crystal structures of P140 and the P140-P110N complex have been deposited in the Protein Data Bank under accession codes 6RUT and 6S3U, respectively. The cryo-electron microscopy density for the 4.1 Å resolution of electron tomograms. The reconstruction, sub-tomogram averaging, and classification code referenced in the methods is available:

- (i) Acta Crystallogr. Sect. D Biol. Crystallogr. 66, 231–32 (2010).
- (ii) Acta Crystallogr. Sect. D Biol. Crystallogr. 66, 231–32 (2010).

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Author contributions
Conceived and designed the experiments: D.A., M.P.S., J.P., O.Q.P., I.F., and A.S.F. Performed the experiments: D.A., M.P.S., M.M.-S., D.V., M.R., M.S.W., A.S., J.R., L.S., S.T.-P., L.G.-G., and O.Q.P. Analyzed the data: D.A., M.P.S., M.M.-S., D.V., M.S.W., J.R., L.S., J.P., O.Q.P., I.F., and A.S.F. Contributed reagents/materials/analysis tools: E.Q., J.P., I.F., and A.S.F. Wrote the paper: D.A., M.S., O.Q.P., I.F., and A.S.F.

Competing interests
The authors declare no competing interests.

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