Neisseria meningitidis use Type IV pili (T4P) to adhere to endothelial cells and breach the blood brain barrier, causing cause fatal meningitis. T4P are multifunctional polymers of the major pilin protein, which share a conserved hydrophobic N terminus that is a curved extended $\alpha$-helix, $\alpha_1$, in X-ray crystal structures. Here we report a 1.44 Å crystal structure of the N. meningitidis major pilin PilE and a $\sim$6 Å cryo-electron microscopy reconstruction of the intact pilus, from which we built an atomic model for the filament. This structure reveals the molecular arrangement of the N-terminal $\alpha$-helices in the filament core, including a melted central portion of $\alpha_1$ and a bridge of electron density consistent with a predicted salt bridge necessary for pilus assembly. This structure has important implications for understanding pilus biology.

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Neisseria meningitidis (Nm) is one of the very few extracellular bacterial pathogens able to cross the blood
brain barrier after having invaded the bloodstream from
the nasopharynx. In the bloodstream an unusually tight
interaction between the bacteria and the brain microvasculature
endothelial cells is established, leading to cortical plaque
formation that results in opening of the blood brain barrier and
bacterial invasion of the brain1–3. This interaction is mediated by
Type IV pilis (T4P), long thin polymers of pilin proteins that
interact with two endothelial cell receptors, CD147 and the β2
adrenergic receptor (β2AR). The interaction with CD147 is
responsible for bacterial adhesion and the interaction with the β2AR induces signalling in endothelial cells6. While the host cell
signalling events that lead to disruption of the blood brain barrier
are somewhat well-defined, the mechanism by which T4P initiate
this process is poorly understood and would benefit from a
detailed structure of the N. meningitidis T4P.

X-ray crystal structures of full-length Type IV pilin proteins
have been obtained by dissociating intact pilus filaments with
detergent, revealing a canonical structure with an extended gently
curving β-sheet (β1), the C-terminal half of which is embedded in a globular C-terminal domain8,9,12. The N-terminal half of the β-helix, β1N, protrudes from the globular domain and is comprised of hydrophobic residues, with the
exception of a threonine or serine at position 2 and an invariant
alanine at position 6, is highly conserved in Type IV pilins of the IVa class9. Two helix-breaking residues, Pro22 and Gly42, which are conserved in Type IV pilins of the IVa class,
introduce kinks in β1 and are responsible for its curvature. An
additional glycine at position 14 is also conserved. β1N has dual
functions in T4P biogenesis: it anchors the globular domain in the
inner membrane before pilus assembly13, and it interacts with adjacent β1Ns in the assembled pilus, forming a staggered helical
array in the filament core8,9,14,15. The conserved Glu5 is critical
for T4P assembly14,16–22 and models of T4P, based in part on the
crystal structure of the full-length pilin subunit from Neisseria
gonorrhoeae, PilE (Ng PilE), place this side chain in a salt
bridge with the positively charged N-terminal amine of its
neighbouring molecule in the otherwise hydrophobic core of the
filament8,9,17,14,23.

N. meningitidis PilE (Nm PilE) is 78% identical to Ng PilE and
100% identical in β1 (Fig. 1a,b). Both pilins share a ~20 amino-
acid hypervariable region, located between two conserved
cysteines, imparting antigenic variability to these pilis24–26. In
N. gonorrhoeae PilE the hypervariable region is located on a
β-hairpin that lies on top of the globular domain β-sheet1,9,11,14.

A cryo-electron microscopy (cryoEM) reconstruction of the Ng
T4P was determined at 12.5 Å resolution and an atomic model
was built by fitting the full-length Ng PilE structure into the
cryoEM density9. The PilE globular domains fit well into the
reconstruction, exposing the hypervariable region prominently on
the filament core. PilE subunits are related in the Ng T4P
reconstruction by an axial rise of 10.5 Å and an azimuthal
rotation (twist) of 100.8°. The subunits are held together largely
by hydrophobic interactions among the N-terminal β-helices but
these helices were not resolved due to the limited resolution
cryoEM map. The importance of the hydrophobic interactions
was corroborated by biochemical studies, which showed that
Ng T4P require detergent to dissociate into pilin subunits9,11,14.

The interactions between subunits are very strong, requiring
temperatures of 60 °C to denature the filaments14, consistent with
their ability to withstand tensile forces of 100 pN or more27–31.
Yet both Ng and Nm T4P have been shown to undergo a force-
induced conformational change that exposes an epitope, EYYLN,
along the length of the pilus32. EYYLN is located at the end of
β1 (resides 49–53) in Ng PilE, a site that is buried by
subunit:subunit interactions in the Ng T4P model and only
accessible at the tips of the pilis9. Consistent with this model, anti-
EYYLN antibodies normally bind to the tips of Nm and Ng T4P
but not along its length32,34. Yet when Ng T4P are placed under
force using optical or magnetic tweezers or molecular combing
they become longer and narrower29. This force-induced
conformational change exposes the anti-EYYLN epitope all
along the length of the pilus, allowing antibody to bind.

Similarly, Brissac et al. showed that anti-EYYLN antibodies can
bind along the length of Nm T4P when Nm are adhered
to endothelial cells, a property that is dependent on expression of
the minor pilin, PilX32. It is unclear how these T4P extend in a
reversible manner without completely disrupting the extensive
hydrophobic interactions among the N-terminal β-helices in the
core of the filament. Another poorly understood phenomenon
of T4P from at least one species, Geobacter sulfurreducens, is their
ability to facilitate long-range electron transport, a property that
is thought to occur via overlapping π–π orbitals of aromatic
residues in β1 (refs 35,36). Higher resolution structures may provide a
molecular basis for these and other remarkable properties of T4P.

Here we report the 1.44 Å X-ray crystal structure of Nm PilE and a
cryoEM reconstruction of the Nm pilus at higher resolution, ~6 Å, than any T4P structure reported previously.

Portions of the N-terminal β-helices are clearly resolved in this
reconstruction, allowing their precise placement, which provides evidence of a salt bridge between Phe1:N and the Glu5 side chain,
and reveals a melting of the central portion of β1N. This structure
provides a molecular framework for understanding key aspects of
T4P biology.

Results

Structure of the major pilin PilE from N. meningitidis. N.
meningitidis ΔN-PilE, comprising residues 29–161 of PilE
from the high adhesive SB variant of N. meningitidis strain 8013
(ref. 37) was expressed with an N-terminal His-tag. This PilE
variant, when expressed as a maltose-binding protein fusion on
Staphylococcus aureus, induces recruitment of the β2AR
in endothelial cells38. ΔN-PilE crystals were grown in the P212121
space group and the structure was solved by molecular
replacement with the full-length PilE structure from N.
gonorrhoeae (Ng PilE, 2H1Z), which shares 78% sequence
identity with N. meningitidis PilE (Nm PilE; Fig. 1a). Data
collection and refinement statistics are reported in Table 1. Nm
ΔN-PilE is a globular protein with an N-terminal β-helix corresponding to β1C, the C-terminal half of β1 (Fig. 2a),
eMBEDDED IN A FOUR-STRAIGHTED PARALLEL-BETA SHEET (β1 TO β4, RESIDUES 77–121). BETWEEN β1C AND THE β-SHEET IS THE β1-LOOP (RESIDUES 54–76), AN EXTENDED SEGMENT WITH A SINGLE-TURN β-Helix AND A 3₁₀ Helix THAT FORMS ONE EDGE OF THE GLOMERULAR DOMAIN. TWO RESIDUES IN THE β1-LOOP, SER63 AND SER69, ARE POST-TRANSLATIONALLY MODIFIED IN THE NATIVE PILE39,40. ON THE OPPOSITE SIDE OF THE β-SHEET, THE POLYPEPTIDE CHAIN EXITS β4 AND FORMS A β-HAIRPIN (β5–β6) THAT LIES ATOP THE β-SHEET, FOLLOWED BY AN IRREGULAR SEGMENT THAT PROTRUDES FROM THE GLOMERULAR DOMAIN SURFACE THEN WRAPS UNDER IT, ENDING IN AN EXTENDED C TERMINUS AT THE EDGE OPPOSITE THE β1-LOOP. CONSERVED DISULFIDE-BONDED CYSTEINES AT THE END OF β4 (CYST120) AND CLOSE TO THE C TERMINUS (CYST154) DEFINE THE BOUNDARIES OF THE D-REGION CONTAINING THE β-HAIRPIN. THE MOST PROTRUDING SEGMENT OF THE D-REGION, THE β-HAIRPIN LOOP AND β6 PLUS THE BEGINNING OF THE LOOP THAT FOLLOWS β6, IS HIGHLY VARIABLE IN SEQUENCE AMONG N. meningitidis TYPE IV PILINS AND IS REFERRED TO AS THE HYPERVARIABLE REGION (RESIDUES ~127 TO 146)38. THE β-HAIRPIN LOOP HOOKS OVER THE TOP OF THE β-SHEET AND ACROSS

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the β1–β2 loop; Ala129 at its apex forms a main chain hydrogen bond with Trp57 at the beginning of the ab-loop. Another N terminally truncated PilE structure, from N. meningitidis strain MC58, is available in the Protein Data Bank (PDB ID 4V1J). MC58 PilE is 89% identical in sequence to the SB pilin variant of strain 8013 PilE described here, differing mostly in the hypervariable region. PilE 4V1J crystallized in a different space group than ours, P6, and was solved to 1.43 Å resolution. This structure superimposes well on our strain 8013 PilE structure (root mean square deviation for main chain atoms, 1.1 Å, Supplementary Fig. 1). Small differences in the backbone conformations occur mainly in the hypervariable region at the end of the β6 strand of the b-hairpin and in the loop following this strand. The MC58 pilin has an Ala142 in place of the very prominent Lys140 seen in our 8013 PilE structure. Electron density is absent for a single residue, Ala131, at the tip of the b-hairpin loop in 4V1J, and the b3–b4 loop conformation differs very slightly between the two pilins.

Not surprisingly, the Nm PilE structure is highly similar to Ng PilE (Fig. 2a, b), superimposing with a root mean square deviation of 3.1 Å for all main chain atoms. Since the N-terminal 54 residues are identical in both proteins the missing 29

Figure 1 | Sequence alignment between N. meningitidis and N. gonorrhoeae PilE and other Type IV pilin-like proteins. (a) Amino-acid sequence alignment between N. meningitidis (Nm) and N. gonorrhoeae (Ng) PilE, with secondary structures indicated above and below the sequences, respectively, based on the crystal structures of Nm ΔN-PilE (residues 29–161, this study) and full-length Ng PilE.(1) A grey bar represents z1N, which is absent in the Nm ΔN-PilE structure. The secondary structure of Nm PilE z1 within the T4P filament is indicated at the top of the panel based on the cryoEM density map. The amino-acid alignment was performed using CLUSTAL OMEGA with manual adjustments and visualized using ESPript. Identical residues are shown in white font on a red background. Residues of interest are boxed and the conserved disulfide-bonded cysteines are connected. (b) N-terminal amino-acid sequence alignment among Type IV pilins from Gram negative and Gram positive bacteria, Type II secretion pseudopilins and archaeal flagellins. Conserved residues are boxed and/or highlighted. Glycines are also highlighted in the archaeal flagellins although their positions are not conserved.

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N-terminal residues in Nm ΔN-PilE monomer are also expected to be α-helical, forming a continuous α-helix with α1C, as seen in the full-length Ng PilE structure. The conserved Pro22 and Gly42 introduce kinks in Ng PilE, giving α1 a gentle S-shaped curve (Fig. 2b). Both proteins share essentially the same secondary structure elements in the globular domain (Figs 1a and 2). The only notable main chain deviations are in the position of the β3–β4 loop, which bends away from the β-strand in Nm PilE to a greater degree than it does in Ng PilE, and in the β-hairpin, which is more twisted in Nm PilE and has a two-residue insertion in its loop that allows it to contact the αβ-loop.

CryoEM reconstruction of the N. meningitidis T4P. To better understand the role of N. meningitidis T4P (Nm T4P) in host cell adhesion and signalling, we generated a cryoEM reconstruction. Not only are the Nm T4P filaments quite flexible, but image analysis indicated substantial variability, mainly in the twist (Methods). The Iterative Helical Real Space Reconstruction (IHRSR)\(^\text{41}\) approach was used to obtain the reconstruction, which after sorting converged to a rise of 10.3 Å and a rotation of 100.8° for the subset of segments used. The cryoEM map, with an estimated resolution of ~6 Å, shows well-defined density for the PilE globular domain, with a central depression and a protruding ridge on one side (Fig. 3a). Clear connectivity between the globular domains is observed between subunits in the right-handed 4-start (+4) helix, and to a lesser extent in the left-handed 3-start (−3) and right-handed 1-start (+1) helices. Rod-like density is present in the core of the filament consistent with the protruding N-terminal α-helical segments of PilE, α1N (Fig. 3b, c). This density shows that the α1N segments are staggered in a helical array in the filament core and partially tilted relative to the filament axis, with their N termini close to the centre of the filament and their C termini lying at a larger radius. Surprisingly the α-helical density is not continuous, being considerably weaker just before it connects with the globular domains (red asterisks in Fig. 3c). This weak density indicates loss of α-helical order and increased flexibility in a central segment of α1 in the intact pilus.

Atomic model of the N. meningitidis T4P. As a first step in building an Nm T4P model, the full-length Ng PilE structure was fit as a rigid body into the Nm pilus cryoEM density map to determine a global fit for Nm PilE that included α1N. While the Ng PilE globular domain, which includes residues ~28-53 of α1 (α1C), fits nicely into the map, the kink in α1N proximal to Pro22 drives the N-terminal end of α1N well away from the rod-like density corresponding to this subunit (Fig. 3c). Thus, we built a model for the Nm T4P by separately fitting the Nm ΔN-PilE structure (residues 29–161) and α1N from the full-length Ng PilE structure, separated into two segments, α1:1–14 and α1:24–28, into the cryoEM map. These α-helical segments were then joined by an extended non-helical segment, α1:15–23 (Fig. 3d).

The Nm T4P model was built iteratively as described in Methods. The final model was selected based on its fit to the electron density map, minimal steric clashes between subunits, and close proximity of the Glu5 side chain with the N-terminal amine of Phel in adjacent subunits. Refinement statistics are shown in Table 2. The diameter of the Nm T4P reconstruction and filament model is ~ 60 Å. The Nm ΔN-PilE crystal structure within the pilus model fits nicely into the cryoEM map (Fig. 4a, Supplementary Movie 1), having undergone only minor conformational adjustments during fitting and refinement including movement of the β3–β4 loop to bring it into proximity with an adjacent subunit (Fig. 4b). The ridge of density located on one side of the globular domain is occupied by the hypervariable segment of the D-region, the β-hairpin loop, β6 and the loop following β6, with Lys140 prominently displayed at its most protruding point. Lys140 is critical for pilus bundling and T4P-mediated bacterial aggregation, a phenotype associated with the high adhesive variant of N. meningitidis strain 8013 (ref. 42). The long axes of the globular domains run along the 4-start helix, each strand accentuated by the hypervariable ridges (Fig. 4a). The hypervariable region is implicated in binding to and remodelling of endothelial cells and was predicted based upon homology with Ng PilE to be surface-exposed\(^\text{38}\). Four key residues on Nm PilE mediate interactions of Nm T4P with β2AR and activation of endothelial cells: Thr130, Lys140, Asp143 and Lys144 (ref. 38). Thr130 lies on the β-hairpin β5–β6 loop near the top of the protruding hypervariable ridge whereas Lys140, Asp143 and Lys144 are located near the bottom of this ridge (Fig. 4a,b). The distance between these two binding sites on one PilE subunit (~26 Å) is approximately equal to the distance between these sites in neighbouring subunits in the 4-start helix. Thus, β2AR may bind to individual pilin subunits but may also recognize a conformational epitope spanning two subunits.

Pilin subunits are connected in the Nm T4P by contacts between the globular domains, between the globular domains and the α1s, and between the α1s themselves. The staggered globular domains along the 1-start helix have limited contact, between the β3–β4 loop on one subunit and the αβ-loop on the next (Fig. 4c).

Table 1 | Nm ΔN-PilE data collection and refinement statistics.

| Data collection | Nm ΔN-PilE |
|-----------------|------------|
| Beamline        | 55RL 7-1   |
| Wavelength (Å)  | 0.9753     |
| Space group     | P2_1212_1  |
| Cell a, b, c (Å) | 43.5, 46.3, 48.4 |
| Cell α, β, γ (°) | 90.0, 90.0, 90.0 |
| Resolution (Å)  | 1.44       |
| Completeness (%)| 98.7 (88.1) |
| No. of unique reflections | 118934 |
| Rmerge (%)      | 6.0 (60.7) |
| R-factor_obs (%)| 5.5 (53.9) |
| I/σ(I)          | 21.6 (2.8) |
| Wilson B value (Å²) | 18.7 |
| Mosaicity (%)   | 0.5        |
| Refinement      |            |
| Resolution limits (Å) | 33.43–1.44 |
| Z                | 4          |
| No. of reflections used | 18030 |
| R cryst (%)     | 18.7       |
| R free (%)      | 21.0       |
| No. of non-hydrogen atoms: |         |
| Protein         | 1037       |
| Water           | 156        |
| Avg B factor (Å²): |         |
| Protein         | 16.5       |
| Water oxygen    | 24.8       |
| RMSD bond lengths (Å) | 0.004 |
| RMSD bond angles (°) | 0.679 |
| Ramachandran plot |            |
| Favoured (%)    | 94.2       |
| Allowed (%)     | 5.8        |
| Outlier (%)     | 0          |
| PDB ID          | 5W18       |

RMSD, root mean squared deviation.

*Values in parenthesis represent the highest resolution shell.
The bottoms of each globular domain fit into a gap between two globular domains in the next turn of the 1-start helix: the β2–β3 loop of the upper subunit contacts the tip of the globular domains in the next turn of the 1-start helix: the β1–β2 loop of the upper subunit contacts the tip of the globular domains in the next turn of the 1-start helix: the β1–β2 loop of the upper subunit contacts the tip of the globular domains in the next turn of the 1-start helix. The bottoms of each globular domain fit into a gap between two lower subunits, and the D-region of the upper subunit contacts both the top of α1C and the β1–β3 loop of the other lower subunit (Fig. 4c). These interactions, mediated by mostly polar and charged residues, connect the globular domains in each consecutive turn of the 1-start helical strand, and would block antibody access the EYLN epitope at the end of α1C. The interactions in the core of the filament are more extensive and involve mainly hydrophobic residues. The primary contact points between the β1s occur along the 1-start helical strand: the continuous α-helical segments α1:24–41, which are an integral part of their respective globular domains, alternate along the 1-start helix of the pilus filament with the ordered segments of α1N (residues 1–14) from subunits in the next turn up in the 1-start helix (Fig. 4d, e). Each α1:1–14 inserts between two α1:24–41s of the 1-start helical turn below it, contacting both α1:24–41 and the globular domain for one of the subunits and α1:24–41 alone for the adjacent subunit. The most N-terminal portion of α1:1–14 extends to the helical turn below this one to contact the C-terminal residues of α1 at the EYLN epitope (Fig. 4e). Thus, each subunit contacts three consecutive turns of the 1-start helix—the top one via globular domain interactions, the one below that via α1:1–14 interactions with α1:24–41 and globular domains, and the one below that via α1:1–14 interactions with the EYLN epitope of α1. The hydrophobic interactions in the α-helical core of the T4P provide the remarkable stability of the pilus, which are resistant to heat and chemical denaturation and require treatment with detergent in order to dissociate them.

The α-helical core of the N. meningitidis T4P. In our Nm T4P model the α1 segment 24–53 is curved but runs roughly parallel to the filament axis while α1:1-14 is tilted with its N terminus to dissociate them.

Figure 2 | X-ray crystal structure of N. meningitidis ΔN-PilE and comparison with the full-length structure of Ng PilE. Two views of (a) Nm ΔN-PilE and (b) Ng PilE pilins are shown: a ‘surface view’ predicted to form the outer face of the T4P filament, and a 70° rotation. The αβ loop is shown in green and the D-region is shown in magenta, with the hypervariable region within the D-region outlined in the lower panel. Secondary structures are indicated. Nm ΔN-PilE residues Ser63 and Ser69 are post-translationally modified in the native pilin, as are the corresponding Ser63 and Ser68 shown in the full-length Ng PilE structure. The Ng PilE N-terminal α-helix, α1, is kinked at Pro22 and Gly42. The N-terminal half of α1, α1N, is absent in the Nm ΔN-PilE structure, but is identical in sequence and thus expected to share the same conformation as in Ng PilE.
membrane (Fig. 6a). This salt bridge appears to be maintained in the assembled pilus and may also contribute to filament stability.

The cryoEM map shows that α1 is non-helical between the helix-breaking residues Gly14 and Pro22. The loss of α-helical order at α1:15–23 compared with the all-helical conformation seen in the Ng PilE crystal structure lengthens α1 and effectively removes the Pro22-induced kink. By eliminating the α-helical structure for α1:15–23 we were able to place α1:1–14 into the rod-like EM density, at a position quite different from that predicted for Ng T4P (Fig. 3c,d). Yet the α-helices are not particularly tightly packed, leaving gaps in the filament core and a channel ~11 Å in diameter that winds through the filament (Figs 3b and 5a). This channel could accommodate flexibility in α1:15–23 and in the pilus filament itself.

Discussion

Our 1.44 Å crystal structure of Nm ΔN-PilE, together with our ~6 Å resolution cryoEM reconstruction of the Nm T4P allowed us to generate a model for this pilus that closely resembles our earlier 12.5 Å Ng T4P structure (Supplementary Fig. 2) but reveals new structural features that inform pilus biology. The non-helical conformation revealed for residues 15–23 of α1 is surprising given that this region is α-helical not only in the Ng PilE monomer structure but also in the other full-length Type IV pilin structures from Pseudomonas aeruginosa, Dichelobacter nodosus and G. sulfurreducens all of which possess S-shaped α-helices with kinks at Pro22 and Gly/Pro42. These full-length Type IV pilin structures were obtained by dissociating purified pili into pilin subunits with the detergent octyl β-D-glucopyranoside (OG), which disrupts hydrophobic interactions and solubilizes αN. In bacteria αN is anchored in the inner membrane prior to pilus assembly. The α-helical conformation would shield the polar nitrogens and oxygens in the αN backbone from the hydrophobic phase of the lipid bilayer.

Table 2 | Refinement statistics for the Ng T4P filament model.

| RMS deviations |          |
|----------------|----------|
| Bonds (Å)      | 0.009    |
| Angles (°)     | 1.04     |

| Ramachandran plot |         |
|-------------------|---------|
| Favoured (%)      | 75.5    |
| Allowed (%)       | 23.9    |
| Outliers (%)      | 0.6     |
| Rotamer outliers (%) | 0.0   |
| Cβ deviations    | 0       |

RMS, root mean square.
(and also from the acyl chains of βOG during crystal growth). Thus, the melting of the helix at α1:15–23 appears to be induced on integration of the pilus subunits into the pilus filament, perhaps to facilitate packing of α1N (Fig. 6a). The conserved helix-breaking residues Gly14 and Pro22 may destabilize the α-helical secondary structure in this region, allowing it to unfold during pilus assembly. Interestingly, the Type II secretion pseudopilins also have Gly14 and Pro22 (Fig. 1b) and thus may also adopt a non-helical conformation in this region within the pseudopilus. Similarly, although the Type IVb pilins lack the proline at position 22 they have glycines at positions 11, 14 and 19, which may destabilize this region in a manner similar to that of Gly14/Pro22. In contrast, archaean flagellins contain an N terminus that is homologous to α1N in the Type IV pilins but possess no proline in this segment, and glycines are not in conserved positions (Fig. 1b). A ∼4 Å resolution cryoEM reconstruction of an archaean flagellar-like filament from Igniococcus hospitalis was recently determined, which shows well-defined density corresponding to a straight, fully α-helical α1N (ref. 45). Thus, the loss of helical order seen for Nm T4P may be a shared feature of the T4P and Type II secretion pseudopili but is not necessarily a universal feature of filament-forming proteins containing a Type IV pilin N-terminal domain.

The new Nm T4P model with its non-helical α1 segment provides a molecular basis with which to understand the reversible force-dependent polymorphism observed for both.
Nm and Ng T4P²⁹,³². Ng T4P can be stretched to approximately three times their length when subjected to forces of ~ 100 pN, with a corresponding narrowing of the filament diameter²⁹. This force-induced conformational change exposes the EYYLN epitope at the end of α1, which is normally buried in the pilus and only accessible at the tip (Figs 4c–e and 6). Similar expression of this epitope is induced for Nm T4P upon binding to endothelial cells³². The mechanism by which Nm and Ng T4P can extend in a reversible manner was not apparent from the lower resolution Ng T4P model, in which the α1s are all-helical and twisted around each other in the filament core⁶. The new Nm T4P model can be envisioned as a coil-like structure: each turn of the coil, which follows the 1-start helical path, is comprised of the globular domains connected by a small number of electrostatic/polar interactions and by extensive hydrophobic interactions with intervening α1Ns (α1:1–14) from the subunits in the next turn of the coil; each consecutive turn of the coil is held together axially by electrostatic and polar interactions between the globular domains and by α1, which acts as a tether between each turn (Figs 4d,e and 6b, left panel). Shear forces on the pili may disrupt the weaker axial interactions between globular domains in consecutive turns of the coil, and extend residues α1:15–23, stretching the pilus while maintaining the lateral interactions between globular domains, α1:24–41 and α1:1–14 along the coil (Fig. 6b, right panel). Thus, force would produce a spring-like motion separating the coils to elongate the pilus. Such a motion was described recently for the P pilus of the donor chaperone pilus family based on the 3.8 Å resolution cryoEM reconstruction⁴⁶. The architecture of the P pilus is markedly different from that of the T4P: elongated P pilin subunits are connected along the coils by donor-strand exchange of their N-terminal extensions, which insert into a groove in the next subunit to complete an Ig fold⁴⁷; polar interactions hold the coils of the P pilus together in the axial direction. Yet P pilus, like the Nm and Ng T4P, can extend under force in a reversible spring-like manner, providing an interesting example of convergent evolution.

In our Nm T4P model α1:15–23 spans ~ 18 Å. In a fully extended conformation these nine residues could potentially span ~ 32 Å, which would take the axial rise per subunit from 10.3 Å to ~ 24 Å, an increase of ~ 230%. The melting of a few residues flanking 15–23 could further increase the filament length. Such an extension might separate the globular domains between consecutive turns of the coil enough for antibody to access to the EYYLN epitope, while maintaining the lateral hydrophobic interactions along the coil, between the globular domains, α1:24–41 and α1:1–14. Whereas the accompanying increase in rise per subunit can occur in the P pilus by simply tilting the subunits, the more complex interactions holding the Type IV pilins may also shift relative to each other, changing the register of the α1 interactions to increase the rise per subunit, transiently resulting in thinner longer pili. Upon release of force, α1N would collapse to the more compact and stable conformation seen in the Nm T4P model and interactions between the coils would reform. This conformational elasticity would allow N. meningitidis to remain attached via T4P to endothelial cells in the brain vasculature, and N. gonorrhoeae cells to remain attached to the lining of the urethra under shear forces from blood and urine flow, respectively. Of note, the force-induced stretching of Nm T4P would separate the β2AR-binding residue Thr130 from the Lys140/Asp143/Lys144 patch in adjacent subunits (Fig. 4b). Such a change may provide a signal to activate β2AR and cortical plaque formation, leading to loosening of the endothelial cell junctions and opening of the blood brain barrier. The conformational flexibility of the Nm T4P is likely...
responsible for the structural heterogeneity observed during processing of the cryoEM images, which precluded a higher resolution reconstruction.

The Nm T4P structure may also be relevant in considering the mechanism for charge transfer in the related T4P from \textit{G. sulfurreducens}. The \textit{G. sulfurreducens} Type IV pilin is unique

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**Figure 6 | Models for Type IV pilus assembly and force-induced conformational change.** (a) T4P assembly model. (1) Before their incorporation into pilus filaments pilin subunits are anchored in the inner membrane with $\alpha_1$ in an all-helical conformation to shield backbone oxygens and nitrogens from the acyl phase of the lipid bilayer. (2) Pilin subunits dock into a growing pilus filament, attracted in part by charge complementarity between Glu5, represented by the (−) charge, and the positively charged N-terminal amine (+) on the terminal subunit in the growing pilus. (3) The pilus filament is extruded out of the membrane a short distance by the pilus assembly machinery, opening up a gap at the base of the pilus for a new subunit to dock. As the newly added pilin is extruded out of the inner membrane $\alpha_{1:15-23}$ (red) melts in order for $\alpha_{1:1-14}$ to pack into the filament core in an $\alpha$-helical conformation. (4) Another subunit docks into the gap at the base of the pilus. The EYYLN epitope at the top of $\alpha_1$ is shown in orange. This epitope is exposed at the tip of the pilus and buried along its length. (b) Model for the force-induced conformational change of Nm and Ng T4P. Left panel: in its relaxed T4P conformation subunits are held together by lateral interactions between the globular domains along the +1-start helix and alternating $\alpha$-helices ($\alpha_{1:1-14}$) from the next turn up, as well as by axial interactions among the globular domains in consecutive turns of the +1-start helix (represented by the double-headed arrows in the schematic of the coil). The central portion of $\alpha_1$, $\alpha_{1:15-23}$ (red), is non-helical but compact. The EYYLN epitope (orange) is only exposed at the tip of the pilus. Right panel: under stress, the axial interactions are disrupted and $\alpha_{1:15-23}$ becomes fully extended but the lateral interactions between the globular domains and $\alpha_{1:1-14}$ are maintained for the most part, allowing a spring-like extension of the pilus to expose the EYYLN epitope all along its length.
in its absence of a globular domain: this 61 amino-acid pilin has an extended curved α1, as seen for Ng T4P, followed by an unstructured 8 amino-acid C terminus12. This pilin shares 50% identity with Nm PilE in its N-terminal 30 amino acids, including Gly14 and Pro22 (Fig. 1b), thus it may also have a non-helical α1 segment when incorporated into a pilus. The G. sulfurreducens pilin contains 6 aromatic residues, Phe1, Tyr24, Tyr27, Tyr32, Phe51 and Tyr57, five of which are also present in Nm PilE. These residues are implicated in charge conduction in G. sulfurreducens T4P, as a mutant, Aro-5, with alanine substitutions for all aromatics except Phe1, makes pil but shows a 10-fold reduction in conductivity36,48. The authors proposed metallic-like charge conductivity through closely spaced π–π stacked aromatic side chains, similar to electron transport through polyaniline or polycetylene nanostructures48. This model is supported by X-ray microdiffraction data for wild type conductive G. sulfurreducens T4P, which show a 3.2 Å peak representative of a repeating feature within the pilus; this peak is absent in the Aro-5 mutant35. Since the spacing of the aromatic residues in existing T4P models is considerably larger than 3.2 Å, non-computational models for the G. sulfurreducens T4P have been proposed that bring the core aromatic residues Phe1, Tyr24 and Tyr27 within π–π stacking distance (3–4 Å) to facilitate long-range electron transport through the pilus35,50. Such energy-minimized models have a comparable rise to Ng T4P (~10 Å) but a much smaller twist (55–60°) and are more compact50. While feasible, these models assume an all-helical α1. In our Nm T4P model with its non-helical α1 segment, the aromatic residues are not within π–π stacking distance, with most being >8 Å away (Cy–Cy) from their closest neighbour. Phe1, Tyr24 and Tyr27 form an aromatic band that spirals up the filament axis (Fig. 5d), whereas residues 32, 51 and 57, corresponding to the remaining aromatic amino acids in the G. sulfurreducens pilin, occupy more peripheral positions. Phe1 lies at approximately the same level along the Nm T4P filament axis as the Tyr27 of three subunits preceding (protomers P and P-3, respectively) and ~6 Å from Tyr24 (Fig. 5d), distances that are incompatible with π orbital overlap and the 3.2 Å diffraction peak seen for G. sulfurreducens T4P. The spacing of the aromatic side chains is constrained in Nm and Ng T4P by the presence of the globular domain, which places a lower limit on the subunit rise and rotation. The absence of a globular domain for the G. sulfurreducens Type IV pilins means that they can pack more closely in the pilus, with smaller rise and rotation values. These parameters, together with a non-helical, partially extended portion of α1, may provide more realistic G. sulfurreducens T4P computational models that aromatics except Phe1, Tyr24 and Tyr27, and perhaps other aromatic side chains, within 3–4 Å of each other to form a continuous chain of π–π stacked aromatic side chains for charge transfer. Interestingly, reduction of the solution pH from 10.5 to 2 results in a 100-fold increase in electrical conductivity for G. sulfurreducens T4P and a corresponding increase in the intensity of the 3.2 Å diffraction peak35. A possible explanation for this apparent increase in order is that the low pH disrupts the Phe1-Glu5 salt bridge, freeing the Phe1 side chain to optimize its orientation for charge transfer.

In conclusion, the 6 Å structure of the Nm T4P provides a framework for understanding Nm pilus interactions with host cell receptors as well as more general aspects of T4P biology including assembly, function and biophysical properties. The highly conserved T4P N-terminal domain is all-helical in X-ray crystal structures of full-length Type IV pilins, which most likely represents its state in the inner membrane. On the basis of these crystal structures and low resolution EM reconstructions it has been assumed until now that α1 is all α-helical in the intact pilus as well. We have shown that the N-terminal segment is actually partially melted in the assembled pilus. The melting of this region helps to explain pilus heterogeneity, flexibility and elasticity, including T4P’s ability to stretch under force, and in the case of G. sulfurreducens T4P, to conduct charge. N-terminal sequence similarity between Nm PilE and other Type IV pilins and Type II secretion pseudopilins suggest that this α1N melting may be a conserved feature, yet one that does not extend to the archaeal flagellins. The Nm T4P cryoEM reconstruction shows the limitations of secondary structure predictions, which can never be more than 80–90% accurate as they fail to account for contextual influences on protein structure. It also illustrates the value of obtaining the highest resolution possible for macro-molecular assemblies, as key structural features can be missed with rigid body fitting of atomic structures of isolated subunits. Such features can have important biological implications for the protein complexes.

Methods

N. meningitidis PilE expression and purification. The gene fragment encoding PilE resides 29–161 (AN-PilE) was cloned from genomic DNA of N. meningitidis 8013 (high adhesive strain, SB)55, using the primers Nm-PilE-EcoRIF-PCR (5’-CGG ATATTGCGGCGGCAAGATTTTGGCAACGCTCATC-3’) and Nm-PilE-PstI-PCR (5’-CGAAGTCGACTGATTTTTCCTCCTGCTC-3’), and inserted into the pMAL-p2x plasmid (NEB) at the EcoR1 and PstI cloning sites. The pilE gene was transferred into the PET15b vector (Novagen) restriction sites NdeI and BamHI using primers PilE-FP (5’-CGATTTTCATTAGGCAGCTC-3’) and PilE-AP (5’-CCCGGATCTTTAAGCTGACGATG-3’), which flank an N-terminal segment, the Nm-pilE was expressed in E. coli BL21(DE3) SHuffle strain (NEB) and grown at 16 °C for 22 hrs in LB, 0.4 mM IPTG. Cells were lysed by sonication in lysis buffer (20 mM Tris-HCl, pH 8, 5 µg/ml lysozyme, 1 mg/ml EDTA, free to 0.266. A composite annealed omit map. Diffraction data for AN-PilE were collected as the SSRL Beamline 7.1 by remote access using Blu-Ice software52. Raw data were processed and scaled and structure factor amplitude determinants were derived by data processing suite XDS53. Matthews coefficient calculations using the CCP4 program MATHIEUS_COE54,55 indicated that the asymmetric unit contains one AN-PilE molecule with 39.3% solvent content. The crystal structure was solved by the molecular replacement method using the program Phaser56 in CCP454,57. The Ng PilE crystal structure (PDB ID 2HI29), with the N-terminal 29 residues removed, was used as the model. Molecular replacement yielded an unambiguous solution with z-scores REF2 = 9.8, and TREF = 14.5. The model residues were changed to those of Nm PilE in COOT58 where necessary and rigid body refinement was carried out following 20 cycles of restrained refinement using REFMAC59 which brought Rwork to 0.197 and Rfree to 0.271 and 0.317, respectively. The AN-PilE model was examined and adjusted in COOT using difference maps. The modified model was further refined using the ‘Improve omit of maps by atoms update and refinement’ tool in ARP/wARP60 bringing the Rwork to 0.197 and the Rfree to 0.266. A composite annealed omit map was calculated using CNS52 and all the residues and water oxygens were validated with theomit map, to give Rwork of 0.211 and Rfree of 0.238. At this point the coordinates were validated and deposited in the protein data bank (PDB ID 4XPJ). Neutron diffraction employing the wPENX suite62 to refine the AN-PilE model, and used this program to re-refine the AN-PilE structure with options ‘Automatically add hydrogens to model’ and ‘Update waters’, reducing the R-factors to 0.187 and 0.201 (Rwork and Rfree respectively) and 0.197 and 0.201 (Rwork and Rfree respectively). These AN-PilE coordinates were deposited to the PDB under the accession number 5JW8. Data collection and refinement statistics are reported in Table 1.

Preparation of N. meningitidis T4P for cryoEM. N. meningitidis 8013 cells were grown on GCB plates63, resuspended in solubilization buffer (20 mM Tris-HCl, pH 8, 500 mM NaCl, 10 mM DTT, 10 µg/ml lysozyme) and loaded onto 3.7% agarose gels64. Proteins were eluted using electrophoresis buffer (50 mM Tris-HCl, pH 8, 10 mM NaCl, 0.5 mM dithiothreitol) and 50 mM dithiothreitol.

ΔN-PilE crystallization, data collection and refinement. ΔN-PilE crystals were grown by the hanging drop vapour diffusion method at 293 K. Initial crystallization conditions were obtained from the high throughput screening laboratory at the Hauptman-Woodward Medical Research Institute65. The mother liquor was dialysed against 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0.1 mM EDTA and 0.1% pluronic acid (Roche) and centrifuged at 10,000 g for 60 min. A 1:1 ratio of protein and reservoir solution (0.1 M ammonium sulphate, 0.1 M Mops pH 6.3, 3 mg/ml PEG 4000) was necessary and rigid body refinement was carried out following 20 cycles of restrained refinement using REFMAC59 which brought Rwork to 0.197 and Rfree to 0.271 and 0.317, respectively. The AN-PilE model was examined and adjusted in COOT using difference maps. The modified model was further refined using the ‘Improve omit of maps by atoms update and refinement’ tool in ARP/wARP59 bringing the Rwork to 0.197 and the Rfree to 0.266. A composite annealed omit map was calculated using CNS52 and all the residues and water oxygens were validated with the omit map, to give Rwork of 0.211 and Rfree of 0.238. At this point the coordinates were validated and deposited in the protein data bank (PDB ID 4XPJ). Neutron diffraction employing the wPENX suite62 to refine the AN-PilE model, and used this program to re-refine the AN-PilE structure with options ‘Automatically add hydrogens to model’ and ‘Update waters’, reducing the R-factors to 0.187 (Rwork) and 0.201 (Rfree). These AN-PilE coordinates were deposited to the PDB under the accession number 5JW8. Data collection and refinement statistics are reported in Table 1.
ethanolamine, pH 10.5) and vortexed to shear the pili from the cells. Cells were removed from the sheared sample by two rounds of centrifugation at 4,000 g for 10 min, followed by the addition of ammonium sulfate to 10% saturation and incubation at 16 h at 16°C. Pelleted cells were collected by centrifugation (13,000 g, 60 min, 4°C) and resuspended in solubilization buffer. A second round of purification was performed by addition of ammonium sulfate to 10% saturation and precipitation. The final protein concentration was 0.4 μg/mL in 20 mM ethanolamine, pH 10.5.

**CryoEM and image processing.** Samples (2 μL) were applied to plasma-cleaned lacey carbon grids and vitrified using a Vitrobot Mark IV (FEI, LLC). Images were collected at an operating voltage of 300 keV on an FEI Titan Krios equipped with a Falcon 2 direct electron detector. The magnification used provided a sampling of 1.05 Å per pixel. A total of 2,295 images were collected from a single grid using the EPU automated system on the Krios, and these were reduced to 541 images after discarding those with no filaments or extensively aggregated filaments, poor contrast transfer function (CTF) values, or images that were stored containing even ‘chunks’, where each chunk represents a set of frames corresponding to a dose of ≥20 electrons per Å². The full dose image stack was used for the estimation of the CTF, using CTFFIND3 (ref. 64), as well as for box-filling the images using the e2helixboxer routine within EMAN2 (ref. 65). The SPIDER software package66 was used for almost all other operations. Phase reversals in the images were corrected using the PHENIX suite62. B-factors were fixed during refinement due to the weak density in this region. In models where residues 1 and 2 were included in the map, the corresponding authors on request.

**Data availability.** The Nm AN-PilE was deposited in the PDB under accession number 5J58. The Nm T4P reconstruction was deposited in the Electron Microscopy Data Bank under accession number EMD-8287, and the corresponding atomic filament model was deposited in the PDB under accession number 5KUS. The data that support the findings of this study are available from the corresponding authors on request.

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