Type IV pili are surface-exposed filaments which extend from a variety of bacterial pathogens and play a major role in pathogenesis, motility and DNA uptake. Here we present the crystal structure of a complex between a cytoplasmic component of the type IV pilus biogenesis system from *Thermus thermophilus*, PilM, in complex with a peptide derived from the cytoplasmic portion of the inner membrane protein PilN. PilM also binds ATP, and its structure is most similar to the actin-like protein FtsA. PilN binds in a narrow channel between the 1A and 1C subdomains in PilM; the binding site is well conserved in other Gram-negative bacteria, notably *Neisseria meningitidis*, *P. aeruginosa* and *V. cholerae*. We find no evidence for the catalysis of ATP hydrolysis by PilM; fluorescence data indicates that the protein is likely to be saturated by ATP at physiological concentrations. In addition, binding of the PilN peptide appears to influence the environment of the ATP binding site. This is the first reported structure of a complex between two type IV pilus biogenesis proteins. We propose a model in which PilM binds ATP and then PilN as one of the first steps in the formation of the inner membrane platform of the type IV pilus biogenesis complex.

Pili, or fimbriae, is a general term for the thin filaments which extend from the surface of Gram-negative and Gram-positive bacteria (1). They play an important role in adhesion of bacteria to surfaces and also contribute to other processes, notably motility and DNA transformation. Type IV pili (TFP) form the most widely distributed group, particularly prevalent in Proteobacteria, including major human pathogens such as *Neisseria meningitidis* and *Pseudomonas aeruginosa* (2,3). TFP harbour tip adhesins which mediate cellular attachment to host tissue receptors (4), play a crucial role in colonization (2) and DNA competence (5-7). They are 60-70 Å in width but reach lengths of several microns when extended from the bacterial surface. They possess great mechanical strength and, uniquely for bacterial pili, are capable of rapid retraction, generating powerful motor forces in the process (8,9). TFP are composed principally of a polymer of a pilin protein subunit, assembled noncovalently into a helical structure (8,9). Based on sequence and structural characteristics, TFP can be classified into two subtypes, termed type IVa and type IVb (10). Both type IVa and IVb pilin subunits consist of an N-terminal α-helix, which is more highly conserved in sequence, attached to an α/β globular domain. Models for the structures of assembled TFP have been proposed, based on a combination of crystallographic and electron microscopic data (11,12). However, despite detailed information on the structure and composition of TFP, the molecular details of their assembly and disassembly in *vivo* are unclear at present.

Around a dozen different proteins are directly implicated in TFP biogenesis, although the absolute number depends on the bacterium in question. A cartoon showing the principal type IV pilus biogenesis proteins in *Thermus thermophilus* is shown in Fig 1. Pelicic has noted that the proteins associated with type IVb pilus biogenesis are more diverse than their type IVa counterparts (13). Aside from the pilin protein itself (PilA4 in Fig 1), other core components, common to both type IVa and type IVb pilus biogenesis, comprise an assembly ATPase (14,15) (PilF in Fig 1) and an outer membrane secretin responsible for mediating the passage of the pilus fiber across the outer membrane (16) (PilQ in Fig 1). There is also a separate ATPase, PilT, which is dedicated to pilus retraction (17). *pilM*, *pilN*, *pilO*, *pilP* and *pilQ* are found consecutively on the chromosome, and appear to be specifically
associated with type IVa pilus biogenesis (13). *pilM, pilN* and *pilO* have been shown to be essential for TFP-dependent DNA uptake in *Thermus thermophilus* (7) and also for TFP assembly in *Neisseria meningitidis* (18). PilO and PilN are integral membrane proteins with a single transmembrane helix, predicted to form a heterodimer in the periplasm (19). Currently little is known about PilM, except that it is a cytoplasmic protein and has orthologs among the actin-like proteins EpsL (20), MreB (21) and FtsA (22). Work carried out in *Pseudomonas aeruginosa* has established that PilM, N, O and the lipoprotein PilP function as a complex, presumably spanning the inner membrane (3). This observation has parallels with work conducted on the type II secretion system (T2SS), where there is evidence for an analogous transmembrane complex (23). Structural similarities have been noted between the T2SS and TFP biogenesis component proteins; for example, the periplasmic domain from PilO has a ferridoxin-like fold which is similar to that adopted by the T2SS protein EpsM (19). A second T2SS protein, EpsL, also forms a ferridoxin-like fold in the periplasm (24), and has recently been shown to associate with the pseudopilus protein EpsG in vivo (25). EpsL has a cytoplasmic domain which binds to the ATPase EpsE and stimulates its enzymatic activity (26). There is therefore a link from ATP hydrolysis by EpsE, through EpsL, to the assembly of pseudopilin components in the periplasm which is linked to secretion in the type II system. Such an observation begins to address a fundamental problem in our understanding of both systems: how is energy, released by ATP hydrolysis in the cytoplasm, transferred to the process of (pseudo)pilus formation in the periplasm? Here we describe the determination of the crystal structure of PilM from *Thermus thermophilus* in complex with a peptide derived from PilN. From an analysis of the structure and binding measurements, we propose that PilM binding to ATP and PilN are crucial steps in the assembly of the type IV pilus biogenesis platform at the inner membrane.

**EXPERIMENTAL PROCEDURES**

*Cloning and Purification of PilM*—The *pilM* gene was cloned into the pET15b vector (Novagen) using the In-Fusion Advantage PCR Cloning system (Clontech), carried out according to the manufacturer’s guidelines. The pET15b vector was linearized using by digestion with *NdeI* and the *pilM* gene amplified from *Thermus thermophilus* HB8 genomic DNA using the primers: 5’-CCGCGGCGCAATATGTTCTAAAGCTTAGCTAG and 5’-GATCCTCGAGCATATGCTAATCAAGGGGCTCAG. The expression construct (*pilM*-15b) coded for a protein with a 6x-histidine tag followed by a thrombin cleavage site at the N-terminus. For expression, *pilM*-15b was transformed into T7 Express cells (NEB) and a single colony was inoculated into 50 ml of 2X-YT (27) medium containing 100 μg/ml of ampicillin and the cells were grown for 16 hours at 37°C. The overnight culture was diluted into 4 litres of fresh 2X-YT medium and the cells were grown until the absorbance of the culture at 600 nm was 0.6-0.8. At this stage, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM and the cells were harvested after 3-4 hours at 37°C. Two litres of cells were resuspended in 50 ml of buffer A (50 mM Tris/HCl pH 8.0, 200 mM NaCl) containing 0.350 mg DNAase, 40 mg lysozyme and 1X complete protease inhibitor (EDTA free; Roche). The cells were then lysed by sonication, the debris removed by centrifugation at 15,000g and the supernatant passed through a 0.4 μm filter. Ni-NTA resin (4 ml; Qiagen), equilibrated in buffer A, was added to the filtered supernatant and the suspension was incubated at 4°C for 2 hours. The resin was packed into an empty column (Bio-Rad) and washed with 3 column volumes each of buffer A containing 20, 30, 40 and 50 mM of imidazole respectively. The resin was rinsed with buffer B (50 mM Mes/NaOH pH 6.5, 200 mM NaCl, 5% glycerol, 10 mM MgCl₂ and 5 mM ATP) containing 50 mM imidazole and purified PilM was eluted using buffer B containing 250 mM imidazole. The PilM preparation was then concentrated and further purified using a Superdex 200 10/300 GL column (GE Healthcare). For fluorescence studies, PilM was purified in buffer B without ATP. For purification of SeMet-PilM, *pilM*-15b was transformed into B843(DE3) cells (Novagen) and grown in a proprietary selenomethionine enriched medium (Molecular Dimensions). After addition of IPTG, the cells were grown at 25°C for 16 hours. Purification of Se-Met-PilM was carried out in a similar manner to the
native protein, except that buffer B was supplemented with 1 mM DTT.

Crystallization of PilM- SeMet-PilM (6 mg/ml) was mixed with a two-fold molar excess of synthetic PilN peptide (MIRLNLLPKNLRRRV; Invitrogen) and incubated for 10 minutes at 20°C. 20 μl of chymotrypsin (1 mg/ml) was added to 300 μl of the SeMet-PilM/PilN complex mixture and incubated for 30 minutes at 20°C. The chymotrypsin-treated sample was then loaded onto a Superdex 200 10/300 GL column (GE Healthcare), equilibrated with buffer B supplemented with 1 mM DTT. The fractions corresponding to the SeMet-PilM peak were concentrated to 10 mg/ml and used for crystal trials. For crystallization, 250 nl of the PilM/N complex was mixed 250 nl of well solution containing 0.1 M Tris/HCl pH 8.0, 0.2 M magnesium chloride and 20% (w/v) PEG 6000 (PACT premier, Molecular Dimensions). Cryoprotection was achieved by washing the crystal consecutively in 12.5% (v/v) and 25% (v/v) glycerol solutions (0.1 M Tris/HCl pH 8.0, 0.2 M MgCl₂, 20% (w/v) PEG 6000, 0.05 M Mes/NaOH pH 6.5, 0.2 M NaCl, 10 mM ATP and 12.5% (v/v) or 25% (v/v) glycerol).

Structure Determination and Refinement- Multiple wavelength data for MAD phasing were collected from a single crystal of SeMet-substituted PilM. Data were processed using the automated data reduction package XIA2 (28), which uses XDS (29,30). Spacegroup identification was assisted by POINTLESS (31). Phasing was carried out using CRANK (32), from the CCP4 suite (33). Se site identification used SHELXD-2006 (34), as implemented within CRUNCH2 (35). Phasing used the package BP3 (36,37) and hand selection employed SOLOMON (38). The FOM at this stage was 0.44. Density modification and automated model building were carried out using PHENIX (39), which built an almost complete model (330 residues). This was followed by some minor manual rebuilding using the graphics package COOT (40).

At this stage, electron density for ATP/Mg²⁺ and the PilN peptide was readily apparent, and they were added to the model. Maximum likelihood refinement was carried out using REFMAC5 (41) and water molecules fitted using COOT. The final model for PilM was complete except for residues 1-10 at the N-terminus, 250-274 between α7 and α8, and 376-377 at the C-terminus. In addition, Tyr248 and Glu308 were built as Ala, due to lack of electron density for the side chains. Electron density was continuous for the PilN peptide up to and including Pro8, but weakened considerably thereafter. The protein composition of the crystal was not analyzed, so it is unclear whether the portions of PilM and PilN missing from the electron density map are due to disorder in the crystal or loss following limited proteolysis with chymotrypsin. Data reduction and refinement statistics are summarized in Table 1. Stereochemical parameters for the final structure were examined using PROCHECK (42), and were within or better than the tolerance limits expected for a structure at 2.2-Å resolution.

Fluorescence Binding Assay- Measurements were carried out in a 100 μl quartz cuvette at 20°C using a Cary Eclipse fluorescence spectrophotometer (Varian). ATP or the PilN peptide (as used for crystallization) were added to 100 μl of PilM (100 μM) and MANT-ATP (Invitrogen) in 50 mM Mes/NaOH pH 6.5, 200 mM NaCl, 5% (v/v) glycerol and 10 mM MgCl₂, and incubated for 3 minutes before measurement. Excitation and emission wavelengths used were 356 and 448 nm.

RESULTS

Inspection of sequence alignments of PilM with related proteins, FtsA, EspL and MreB, suggested that PilM might retain the ability to bind ATP, in common with FtsA and MreB. Consequently, ATP was included in the latter stages of purification and during crystallization trials. Crystals of PilM from Thermus thermophilus were obtained under these conditions but they diffracted poorly, to a maximal resolution of 8 Å. We reasoned that a likely binding partner for PilM would be the transmembrane protein PilN, as evidence from studies in Pseudomonas aeruginosa implicate it as part of a PilMNOP complex (3). We therefore incorporated a synthetic peptide, corresponding to the most highly conserved region of the short part of PilN predicted to lie within the cytoplasm, into the crystallization trials: this proved more successful in obtaining better quality crystals, which diffracted to a resolution of 2.2 Å (Table 1).

In common with other bacterial actin-like proteins, PilM forms a two domain structure; following a previous designation for FtsA (22), we divide each domain into two subdomains, 1A/C and 2A/B (Fig 2A). Electron density was obtained for a bound ATP
molecule and a Mg$^{2+}$ ion between the 1A and 2B domains. We did not find any evidence for ATP hydrolysis by PilM from a phosphate release ATPase assay (data not shown) and there was no evidence of cleavage of the phosphoester bond between the β and γ phosphates in ATP from the electron density map. At a structural level, PilM exhibited the greatest similarity to FtsA (rmsd backbone atoms 2.5 Å): all four subdomains overlaid well, although some difference in the relative orientation of the 1C subdomain was apparent (Fig 2B). PilM eluted from a size exclusion column with an apparent molecular mass of 42 kDa and examination of the crystal packing, using PISA (43), was consistent with the protein forming a monomer in solution. The cytoplasmic portion of EpsL, which corresponds to the region of homology with PilM, is also reported as being monomeric (20). By contrast, the polymerization of MreB is well established (44,45), and there is also a report of polymerization by FtsA (46). This may be a distinguishing feature between the type IV pilus biogenesis/type II secretion and the FtsA/MreB sub-families of actin-like proteins.

During model building electron density was observed for the first 8 residues of the PilN peptide, which binds in a narrow channel between the 1A and 1C subdomains (Fig 2A and Fig 3A). The 1C domain is not found in actin but does occur in FtsA (Fig 2B) (22) and PilM. This domain has been shown to be directly involved in the recruitment by FtsA of other proteins involved in septation (FtsL and FtsN) (47,48). The type II secretion protein EpsL lacks the 2B domain, and hence is unable to bind ATP, but otherwise aligns well with the PilM structure (Fig 2C; rmsd backbone atoms 2.8 Å). A particularly interesting feature of the superposition of EpsL with PilM lies in the co-location of the EpsL C-terminus with the PilN peptide (Fig 2D). The PilM structure terminates at the end of α11, whereas the last 12 residues of the C-terminus of cyto-EpsL fold down into the equivalent location of the PilN binding site. Where the polypeptide chains most closely coincide, there is even apparent sequence conservation between EpsL (N232,L233,L234) and PilN (N5,L6,L7; Fig 4A). After 15-20 residues from this point, EpsL and PilN are both predicted to form a single transmembrane helix which links these cytoplasmic domains to their periplasmic counterparts (19,20,24,49).

PilN binds in an extended conformation within a narrow crevice on the surface of PilM (Fig 3A). Analysis of the binding interface using the PISA server (43) gave a total of 933Å$^2$ of solvent-accessible surface area on the PilN peptide which is buried on formation of the complex; the equivalent figure for PilM is 667Å$^2$, giving a total of 1600Å$^2$. This value is comparable to figures obtained for other protein-protein complexes (50); indeed, it is rather higher than equivalent values for Fab-peptide antigen complexes, which are probably a better basis for comparison (eg (51)). This observation is explained by the fact that 73% of the solvent accessible surface area of the PilN peptide is buried on formation of the complex. In addition, there is a high degree of structural complementarity between the PilN peptide and its binding site (Fig 3A). The extended conformation of the peptide ensures that alternating residues are buried within the binding site, specifically Ile2, Leu4 and Leu6 (Fig 3A). These structural features help to explain the apparently high affinity of PilM for the PilN peptide: the PilM-PilN complex was prepared by addition of the PilN peptide to PilM, followed by size exclusion chromatography and concentration before initiation of crystal trials. The survival of the PilN peptide in its bound state through these procedures implies a low off-rate for dissociation from the complex.

Alignments of PilN sequences from a variety of Gram-negative bacteria demonstrated that the highest continuous segment of conserved sequence lies at the immediate N-terminus, and coincides with the portion of the peptide recognized by PilM (boxed in Fig 4A). A example of an interaction which we would anticipate is conserved in other Gram-negative organisms is Asn5 from PilN, which forms hydrogen bonds to main chain atoms from residues Val168 and Leu170 in PilM (Fig 3B). Conversely, Arg371 from PilM, which is highly conserved in PilM sequences (Fig 4B) forms a hydrogen bond to the carbonyl oxygen of Leu4 on PilN (Fig 3B). Some hydrophobic residues in PilM lining the binding pockets for Ile2, Leu4 and Leu6 in PilN are also well conserved, specifically Leu83, Phe175 and Leu208 (Fig 4B). The short β-strand 8 contains a well conserved sequence from Pro167 to Val172 which interacts with Asn5 and Leu7 within the PilN peptide, both of which are highly conserved in PilN sequences. For a more general view of sequence conservation within the PilM family, we used the
program CONSURF (52,53) to map sequence conservation onto the PilM surface around the PilN binding site (Fig 3C). The results provide a clear indication of the sequence conservation around the PilN crevice. This analysis also revealed a patch of highly conserved residues on the reverse side of the complex, between domains 1C and 2A (Fig 3D). It is interesting to note that this part of the structure coincides with the equivalent region contacted by a fragment from the EpsE ATPase, when examined in complex with the PilM ortholog EpsL (49). Whether this region is indeed involved in binding to other components of the type IV pilus biogenesis system remains an open question at present.

ATP is bound in a deep cleft on the PilM surface, with the triphosphate moiety buried more deeply than the ribose and adenine rings (Fig 5A). Several well conserved residues are packed around the ATP binding site; for example, Lys26 is located adjacent to the α and β phosphates (Fig 5B), and is highly conserved in other PilM sequences (Fig 4B). A Mg$^{2+}$ ion is coordinated between the β and γ phosphates of ATP, and also by Glu19 (Fig 5B). Asp14 in FtsA appears to play a similar role, and it is noteworthy that Asp is a more common substitution at this position in other PilM sequences (Fig 4B). Although it does not coordinate the Mg$^{2+}$ ion, Asp197 influences the electrostatic environment and, again, is well conserved in PilM sequences. The edge of the first 310 helix in the structure, specifically Gly314 and Gly315, forms a close contact with the ribose and adenine rings (Fig 5B). In general, the conformation and recognition of ATP by PilM is most similar to that of FtsA (22) although, as noted above, we were unable to identify any ATP hydrolysis activity associated with PilM.

Fluorescence binding experiments were carried out to examine the binding of ATP and PilN to PilM, using the ATP analog 2'(3')-O-(N-methylanthraniloyl) adenosine 5'-triphosphate (MANT-ATP). Competition experiments were carried out by titration of ATP into a solution of PilM plus MANT-ATP. Displacement of MANT-ATP from PilM led to a linear increase in fluorescence with ATP concentration, up to the point where all ATP binding sites are saturated at approximately 100 μM (Fig 6A). Thereafter, the fluorescence remains constant with ATP concentration. The results are consistent with MANT-ATP undergoing a quench in fluorescence on binding to PilM, which is then recovered on displacement by ATP. Binding behaviour was independent of the MANT-ATP concentrations used (Fig 6A). The data are consistent with ATP having a $K_D$ considerably less than 1 mM, indicating that PilM is likely to be saturated with ATP under physiological conditions prevailing inside the cell. Titration of the PilN peptide into the MANT-ATP/PilM complex resulted in a quench in MANT-ATP fluorescence of around 5-10%. Again, titration behaviour showed a saturable response, consistent with high affinity binding of the PilN peptide to the MANT-ATP/PilM binary complex (Fig 6B). The result indicates a structural response within the PilM-ATP binary complex on binding of PilN which affects the ATP site.

**DISCUSSION**

The results presented here provide the first detail at the atomic level of the interaction between two TFP biogenesis proteins, PilM and PilN. They form essential components of a large and complex biogenesis machine which spans the inner and outer membranes in Gram-negative bacteria, and delivers a variety of different functions, ranging from DNA uptake or competence, to cellular attachment and motility (13,54). PilM and PilN are known to be essential for natural competence in *Thermus thermophilus* (7); they have also been shown to be essential for TFP formation in other organisms—*Neisseria meningitidis*, for example (18). Both PilN and the structurally related protein PilO are each predicted to have a single transmembrane-spanning helix at their N-terminus, and form a heterodimer in the periplasm (19). It is striking, however, in alignments of PilN sequences from different bacteria, that the highest conservation lies in the short stretch of residues within the cytoplasm (Fig 4A). Our structure provides an elegant explanation for this observation, as this region coincides precisely with the part of the PilN peptide recognized by PilM in our structure. Furthermore, the distribution of well conserved residues between the 1A and 1C subdomains in PilM provides additional supporting evidence that the PilM-PilN complex from *Thermus* is likely to be a good model for the assembly in other bacteria, notably *Neisseria spp* and *Pseudomonas aeruginosa*, where extensive work on TFP biogenesis has been carried out.
Our results suggest a sequence of events for assembly of PilM and PilN into the inner membrane type IV pilus biogenesis complex (Fig 7). PilM would first bind to ATP, as our results indicate that the ATP binding site is likely to be saturated under physiological conditions (Fig 6A). We would then expect the PilM-ATP binary complex to associate with the N-terminal PilM-binding sequence on PilN. Our PilM structure is most closely related to the cell division protein FtsA and the motility-related protein MreB; both these proteins are thought to act through polymerization, in an actin-like manner (44-46). We found no evidence, however, from the physical properties of PilM or its crystal packing that it polymerizes in a similar way. In this respect at least, PilM seems to be more closely related to the monomeric T2SS protein EpsL (20). Structural similarities between the periplasmic domains of EpsL and PilN have led Sampaleanu et al. to suggest that EpsL functions in a similar fashion to the PilM-PilN complex (19). Our results would add some weight to this hypothesis. PilM therefore seems to be a curious combination of a protein which is structurally more closely related to FtsA and MreB, but is functionally closer to EpsL and its role in the T2SS.

Our structure has revealed one function for PilM- to bind the N-terminus of PilN- but also hints that recognition of other TFP biogenesis proteins may be important. The observation of a patch of conserved residues on the opposite side of the PilN binding site suggests binding to another component (Fig 3D). One possibility is that this might be the ATPase PilF, by analogy with the interaction of the cytoplasmic domain of EpsL with a domain from the ATPase EpsE (49). We note, however, that although evidence suggests that the equivalent ATPase PilF is involved in DNA uptake (55), it is reported that pilF mutants in *Thermus thermophilus* are piliated (56). This is in contrast to results from *Neisseria*, for example, where PilF has been shown to be absolutely required for TFP assembly and expression (18). In this case there may be risks, therefore, in extrapolating between results obtained in different bacteria. Nevertheless, our work has shown that in vitro assembly of at least some parts of the TFP machinery is possible, and offers a powerful approach to the study of this important macromolecular machine.

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**FOOTNOTES**

We gratefully acknowledge the assistance of Dr James Sandy on Diamond beamline IO2. Abbreviations used are: TFP, type IV pilus; T2SS, type II secretion system.

**FIGURE LEGENDS**

**Fig. 1.** Type IV pilus assembly in *Thermus thermophilus*. The pilin monomer contains an N-terminal signal sequence which is cleaved by the inner membrane pre-pilin peptidase PilD. The processed pilins assemble into the pilus fiber and emerge from the cell through the PilQ secretin. PilW may assist in the assembly and maturation of the secretin. PilF and PilT are cytoplasmic ATPases which provide energy for pilus assembly and retraction respectively. PilC, PilN and PilO are integral inner membrane proteins. PilM is thought to be part of the multi-protein inner membrane complex which also includes PilN and PilO.

**Fig. 2.** Structure of *T. thermophilus* PilM and comparison with other bacterial actin-like proteins. *A*. Ribbon plot of PilM (magenta), overlaid with the PilN peptide (blue) and ATP. The four sub-domains are labelled according to the original assignment for FtsA (22). *B*. Overlay of PilM and ligands (same colours and orientation as part A) with FtsA (cyan; PDB accession 1E4G) *C*. Overlay of PilM and ligands (same colours and orientation as part A) with EspL (green; PDB accession 2BH1) *D*. Detail of the PilN peptide (blue) and the C-terminus from EspL (20,49), with some residues from the latter labelled.

**Fig. 3.** Recognition of PilN by *T. thermophilus* PilM. *A*. View of the PilN binding site with a PilM electrostatic surface (blue positive, red negative charge), generated using CCP4MG (57), and the PilN peptide superimposed. *B*. Details of the PilM-PilN interaction, with PilM in magenta and PilN in blue. Protein-peptide hydrogen bonds and key residues are indicated. *C & D*: sequence conservation mapped onto the PilM structure using CONSURF (52). Maroon indicates high, white medium, and cyan low sequence conservation respectively. *C*. View of the PilN peptide binding site. *D*. View of the opposite side of the molecule from C, showing the cleft between domains 2A and 1C which is lined with well conserved residues. Images for C and D were generated using CHIMERA (58).
Fig. 4. Alignment of PilN and PilM sequences. A. PilN N-terminus sequences. The transmembrane-spanning region, as predicted by TMPRED (www.ch.embnet.org/software/TMPRED_form.html), is indicated by the shaded rectangle. The region of high sequence conservation, recognized by PilM, is boxed. Organisms and sequences used were *Thermus thermophilus* (Q721W7), *Pseudomonas aeruginosa* (Q51352), *Xanthomonas campestris* (Q8P5V4), *Legionella pneumophila* (Q5WXY0), *Neisseria meningitidis* (Q4W563) and *Vibrio cholerae* (C6YKU1). B. PilM sequences, aligned using CLUSTALW (59), with some manual modification. Numbering is for the *Thermus* sequence. Organisms and sequences used were *Thermus thermophilus* (Q5SIJ0), *Pseudomonas aeruginosa* (Q51351), *Xanthomonas campestris* (Q8P5V3), *Legionella pneumophila* (Q5ZX06), *Neisseria meningitidis* (Q9JY02) and *Vibrio cholerae* (A1EMB3). Underlined residues had weak or no density and were not included in the PilM/N crystal structure.

Fig. 5. Recognition of ATP by PilM. A. PilM surface with ATP structure superimposed. B. View of the ATP binding site, with specific interacting residues indicated. Some parts of the structure have been removed for clarity. Figure generated using CCP4MG (57).

Fig. 6. Fluorescence measurement of ATP and PilN binding to PilM. Binding of ATP or PilN was detected through changes in MANT-ATP fluorescence; in each case, ATP or PilN were titrated into a solution of PilM (100 μM) plus varying concentrations of MANT-ATP. F₀ and Fᵢₐₓ are fluorescence measurements at zero and infinite concentrations of ATP or PilN; F represents fluorescence measurements at all other concentrations. A) ATP titration; MANT-ATP concentrations used were 50 μM (♦), 75 μM (■) and 100 μM (▲). B) PilN titration; MANT-ATP concentrations used were 50 μM (♦) and 100 μM (▲).

Fig. 7. Model for the interaction of PilM with PilN and PilO.
### Table 1
**Data collection and refinement statistics**

| Space group       | P4₃2₁2                                                                 |
|-------------------|------------------------------------------------------------------------|
| Unit cell parameters | a,b=50.8Å, c=366.1Å                                                   |

#### X-ray source and wavelength (Å)

| X-ray source and wavelength | Peak DLS² IO2 (0.9798) | Inflection DLS IO2 (0.9800) | Remote DLS IO2 (0.9793) |
|-----------------------------|------------------------|-----------------------------|-------------------------|
| Resolution range (Å)        | 91 – 2.16 (2.21-2.16)² | 92 – 2.22 (2.28-2.22)   | 92-2.32 (2.38-2.32)   |
| Multiplicity                | 13.3 (11.9)            | 13.4 (14.0)                | 26.8 (28.1)            |
| Significance (<I>/sd)       | 16.3 (3.6)             | 16.6 (4.1)                 | 23.1 (5.5)             |
| No. unique reflections      | 27,534 (1,990)         | 25,402 (1,819)             | 22,348 (1,564)         |
| Completeness (%)            | 100.0 (100.0)          | 100.0 (100.0)              | 100.0 (100.0)          |
| $R_{merge}$ (%)             | 9.2 (66.4)             | 9.0 (65.0)                 | 9.6 (74.1)             |

#### Refinement Statistics

| Resolution range (Å) | 50-2.20 (2.26-2.20) |
|----------------------|---------------------|
| $R_{cryst}$          | 23.7                |
| $R_{free}$ (5% data) | 27.9                |

Nonhydrogen atoms

| All | 2,743 |
| Water | 88 |

Mean overall B (Å²) 38.9

RMSD from ideal values

| Bond distance (Å) | 0.028 |
| Bond angle (degrees) | 2.3 |

PDB Accession Code 2YCH

²values in parentheses refer to the outer resolution shell

Diamond Light Source

³$R_{merge} = \frac{\sum_{hkl} \sum_{sym} |I-<I>|}{\sum_{hkl} I}$
Figure 1
Figure 2
Figure 3
Figure 5
Figure 6

A

\[ \frac{(F-F_0)}{(F_{\text{inf}}-F_0)} \]

vs.

[ATP] (μM)

B

\[ \frac{(F-F_0)}{(F_{\text{inf}}-F_0)} \]

vs.

[PiN] (μM)
Figure 7
