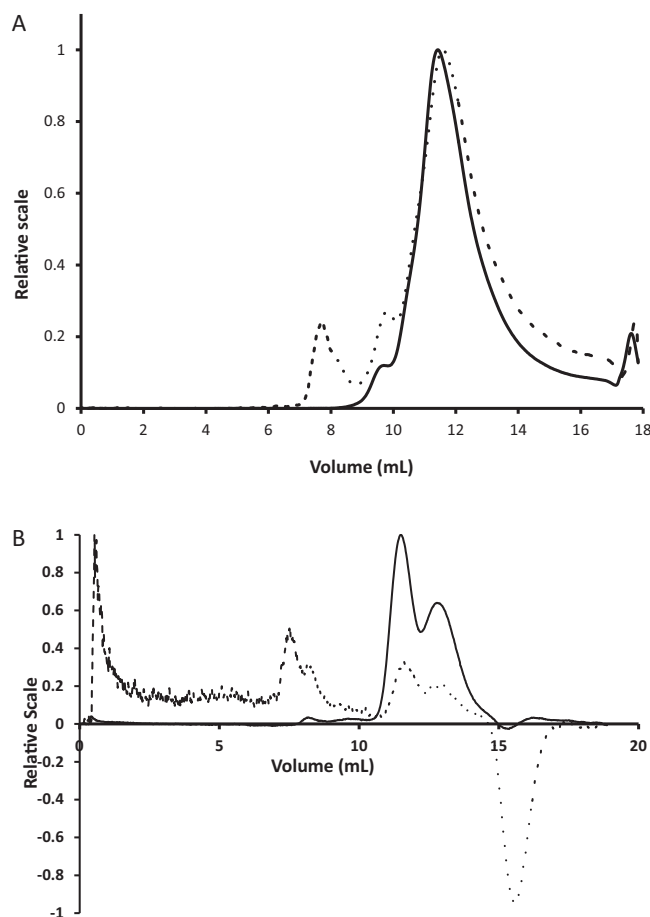# Supporting Information

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Fig. S1. Size exclusion chromatography combined with multiangle laser light scattering analysis. (A) TtPilN[49–207]. Purified TtPilN[49–207] was applied to a Superdex 75 10/300 GL gel filtration column running at a flow rate of 0.75 mL/min in 25 mM Hepes/NaOH (pH 7.0), 100 mM NaCl, and 5% glycerol. Samples eluting from the column passed through an in-line DAWN HELEOS-II laser photometer (wavelength, 658 nm) with a QELS dynamic light scattering attachment, and a UV absorption detector. Light-scattering intensity (dotted line) and eluant UV absorbance (solid line) were analyzed using ASTRA 6 software to give a weight-averaged molecular mass of 34 kDa. (B) TtPilMN complex. Experimental details are as for A, except that a Superdex 200 10/300 column (GE Healthcare) was used.
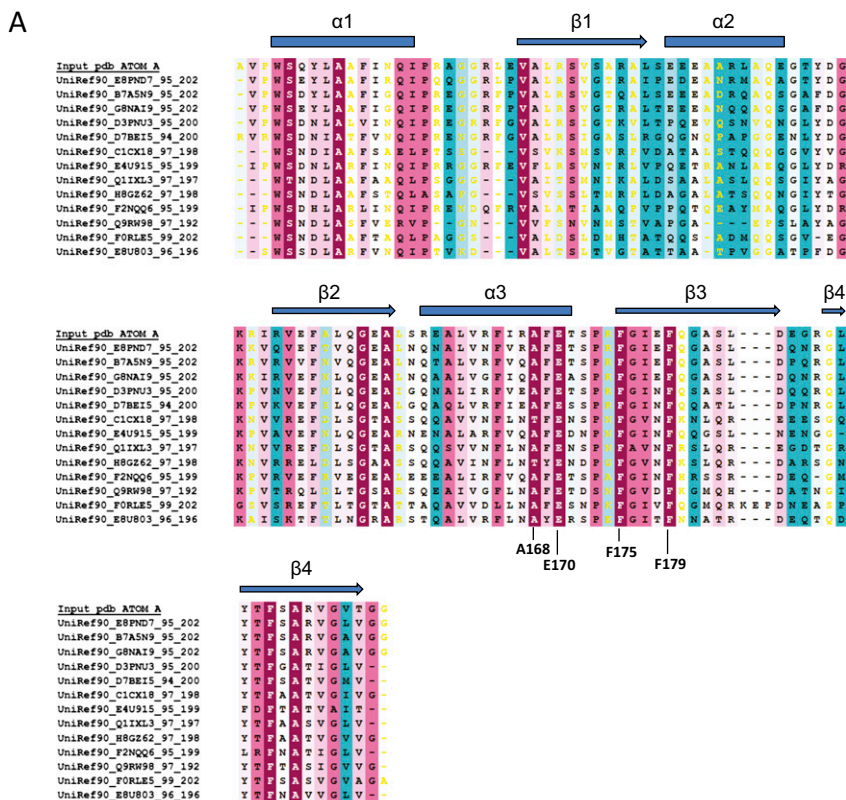
Fig. S2. Sequence alignments. (*A*) Alignment of PilN sequences with secondary structure. The alignment was generated using CONSURF (1); residue coloring is as used in Fig. 3*D*. The location of the selected highly conserved residues shown in Fig. 3*D* is also indicated. (*B*) Sequence alignment of *Thermus thermophilus* PilO (Target) against *Pseudomonas aeruginosa* PilO, as used for homology modeling. The sequence of *P. aeruginosa* PilO was derived from PDB entry 2RJZ. "h" denotes α-helix, and "s" denotes β-strand.

1. Ashkenazy H, Erez E, Martz E, Pupko T, Ben-Tal N (2010) ConSurf 2010: Calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. *Nucleic Acids Res* 38(Web Server issue):W529–W533.
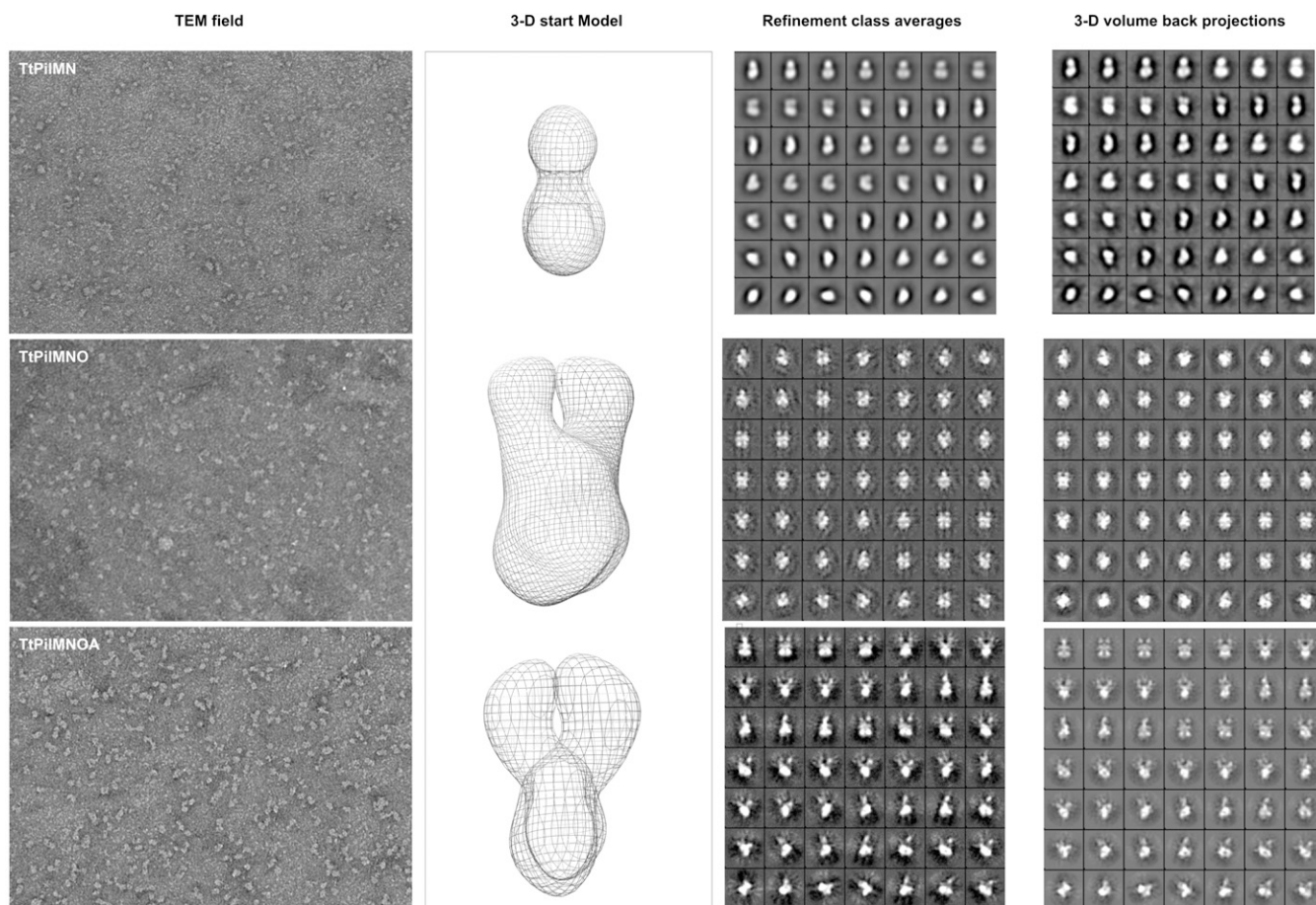
Fig. S3. Transmission electron microscopy (TEM) of TtPilMN, TtMNO, and TtMNOA4 complexes. Example TEM fields, 3D start models, class averages, and backprojections are shown in three rows for each of the three complexes. (Left-hand column) Example TEM fields of the stable purified complexes negatively stained in uranyl acetate and then viewed under cryogenic conditions. (Scale bar: 50 nm.) (Center column) Three-dimensional start models generated in EMAN2 using reference free MSA class averages and are displayed at 1σ in wire frame. (Scale bar: 5 nm.) (Right-hand columns) Two-dimensional projection class averages generated during the final round of the iterative refinement procedure (*Left*) compared with the corresponding back projections from the final refined 3D structure (*Right*). No masking was used in determination of these structures. The TtPilMN dataset was boxed into 64 pixel boxes; the TtPilMNO and TtPilMNOA datasets were boxed into 72 pixel boxes. See Table 3 for other details.
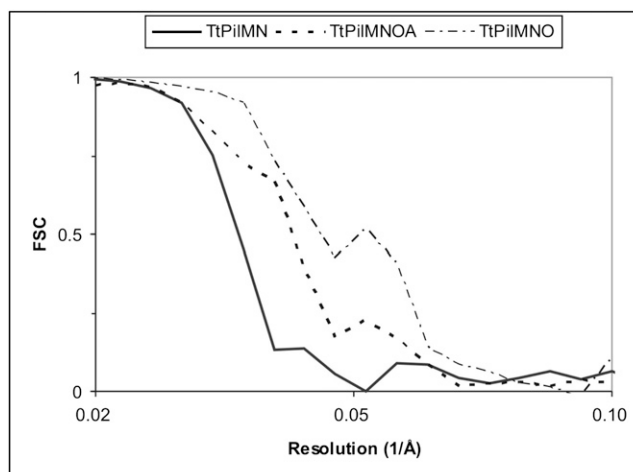
Fig. S4. Fourier shell correlation plots for the TtPilMN, TtMNO, and TtMNOA4 volumes.