Supplementary Methods:

Protein expression and purification

ParM (UniProt: PARM_ECOLX) and ParM mutants were expressed from plasmid *pJSC1* and its derivatives [25] in *E. coli* BL21-AI cells and purified as described previously [3,25]. Buffer MR was used in all experiments: 50 mM Tris-HCl, 100 mM KCl, and 1 mM $MgCl_2$, pH 7.0. *Wild type ParM and ParM(S19R, G21R):* ParM was purified by ammonium sulfate precipitation (at a final concentration of 10 % (sat.) ammonium sulfate) of the lysate, followed by addition of ATP to the re-suspended pellet. ParM filaments were pelleted by centrifugation at 100,000 *g*, and the resulting pellet containing pure protein was re-suspended in buffer and further purified by size exclusion chromatography on a Sephacryl S-200 column (GE Healthcare). *ParM(K258D, R262D):* the protein was purified using a 5 mL HiTrap Q HP column (GE Healthcare), and eluted at increasing KCl concentrations. Fractions containing ParM were pooled and further purified by size exclusion on a Sephacryl S-200 column (GE Healthcare) into buffer MR. Concentrated aliquots of pure protein were frozen and stored at -80 °C until further investigation.

Sample preparation for microscopy

ParM+AMPPNP and ParM+ATP: 10 μM ParM protein was incubated with 2 mM nucleotide in a total volume of 100 μL for 5 minutes at room temperature before cryo-EM sample preparation. The same procedure was used for polymerisation of the ParM S19R, G21R mutant. *ParM+ATP+vanadate and ParM+ADP+vanadate*: 10 μM ParM protein was incubated with 2 mM ATP or ADP and 4 mM sodium orthovanadate in a total volume of 100 μL for 2 hours or 5 minutes at room temperature before cryo-EM sample preparation. *ParM+ADP*: 400 μM ParM was incubated with 10 mM ADP in a total volume of 25 μL for 5 minutes at room temperature. *ParM(K258D, R262D)+AMPPNP*: 60 μM protein was incubated with 2 mM AMPPNP in a total volume of 100 μL for 5 minutes at room temperature. *ParM in vitro doublets*: 20 μM ParM protein was incubated with 2 mM AMPPNP in the presence of 2 % (w/v) PEG 6000 in a 100 μL for 5 minutes at room temperature. *ParM(D170A) over-expressing cells*: ParM was expressed to high levels for cryo-ET

using the plasmid pRBJ212 [ParM(D170A), *ptac* promoter] [1] transformed into *E. coli* B/R266 cells. Cells were grown in M9 medium at 30 °C and induced with 1 mM IPTG at an OD_{600} ~ 0.5. Samples were prepared 4 hours after induction. *Bacterial cells with different copy number plasmids containing the ParMRC locus*: *E. coli* strain B/R266 [26] was transformed with high (pDD19), medium (pKG321) or low-copy (pKG491) plasmids and grown in M9 medium supplemented with 100 μg/mL ampicillin at 30 °C [21]. Cells were grown to an OD_{600} ~ 0.4 – 0.6 (grown to logarithmic growth phase) before sample preparation for cryo-EM.

Cryo-EM grid preparation

Samples for cryo-EM were prepared by pipetting 2.5 μL of the sample onto a freshly glow-discharged Quantifoil Cu/Rh 200 mesh grids (R 2/2 for purified protein, and R3.5/1 for cellular tomography) and plunge frozen into liquid ethane in an Vitrobot Mark IV (FEI Company). Only for cryo-ET, 11 μL of sample were pre-mixed with 1 μL of protein-A conjugated with 10 nm colloidal gold (CMC, Utrecht). Plunged grids were transferred to liquid nitrogen and stored.

Electron microscopy data collection

Two-dimensional cryo-EM data was collected either using an FEI Krios microscope operated at 300 keV or a FEI Spirit microscope operated at 120 keV. High-throughput data was collected on the FEI Krios using EPU software (FEI Company) at an unbinned calibrated pixel size of 1.30 Å or 1.07 Å on a Falcon II direct electron detector. A combined total dose of 25-32 $e^-/Å^2$ was applied with each exposure that lasted 1 s. Images were collected at 1 – 6 μm underfocus. Tilt series data was collected on an FEI Krios equipped with a Quantum energy filter (Gatan) using SerialEM software [27], on a K2 direct electron detector operating in counting mode. Tilt series data were typically collected from ±60° with 1° tilt increment at 4 – 12 μm underfocus with a combined dose of about 120 $e^-/Å^2$ applied over the entire series.

Image processing and data analysis

Real-space helical reconstruction: An averaged power spectrum for each cryo-EM image was calculated using CTFFIND [28] and images showing clear Thon rings were retained. ParM filaments were extracted from the selected images using SPRING and EMAN2 [29,30]. The helical symmetry of each sample was accurately determined by comparing the power spectrum of the aligned segments with power spectra of re-projections of the calculated reconstructions. Experimentally determined helical parameters (ED Table 1) were used for refinement using the program segmentrefine3D in SPRING. The final volumes were compensated for the B-factor and filtered to the obtained resolutions (ED Table 1). Resolution of the structure was estimated using gold-standard FSC measurements in SPRING and additionally using ResMap [31]. Visualization of densities was carried out in UCSF Chimera [32]. *Atomic model building*: The atomic structure from PDB 4A62 [3] was fitted into the cryo-EM density of ParM+AMPPNP using MolRep [33]. Maximum-likelihood refinement of the atomic structure against the cryo-EM density was carried out in REFMAC5 [34] using standard protein stereochemistry and additional external restraints based on PDB 4A62, generated in ProSMART [35]. Model building was carried out in COOT and MAIN [36,37]. *Rigid body fitting:* ParM was divided into two sub-domains, based on the previous ParM+ADP X-ray structure (PDB 1MWM). Each sub-domain was declared as a rigid body and these were fitted into the ParM+ADP filament structure using REFMAC5. *Polarity assignment of ParM filaments in doublets*: First, images of ParM doublets were carefully selected based on image quality (as assessed by a visual inspection of power spectra), and by a visual assessment of the distance between the two filaments in the doublet. The assumption made from the appearance of the class averages was that images in which the distance between the centres of two ParM filaments in the doublet was maximum would show ParM filaments entirely in the same XY plane (the Z-axis being the path of the electron beam in the microscope). The two ParM filaments in all the doublets in these selected images were picked manually using EMAN2 [30]. The manual pick was used to extract short segments along each filament in the doublet. The extracted segments were aligned to re-projections of the high-resolution ParM+AMPPNP filament model using SPRING [29]. In five out of the six doublets analysed, the assigned directionality of ParM filaments was predominantly anti-parallel and in one case the assignment was mostly parallel. *Derivation of the doublet model*: The ParM doublet is not a true helical specimen, thus conventional helical reconstruction could not be carried out. This difficulty of

characterising higher-order filament structures of ParM filaments has been previously reported [20]. The average distance between the centres of the two ParM filaments in the doublet was found to be 65.1 Å by analysis of 1D line-profiles of all obtained class averages. Two copies of the high-resolution cryo-EM structure of the ParM+AMPPNP filaments were accordingly placed with their centres 65.1 Å apart in space in an anti-parallel orientation. The placement was repeated for all possible combinations of the azimuthal angles of both filaments. Re-projections of all these resulting volumes were aligned with all obtained class averages. As expected intuitively from an inspection of the class averages, models in which the thickest part of one ParM filament overlapped with the thinnest part of the other filament in the doublet had higher cross-correlation scores. We placed two copies of the atomic structure of the ParM+AMPPNP in the volume with the highest score. Since this was not a standard cryo-EM reconstruction meaning resulting atomic accuracy will be somewhat lower, we only used the Cα atoms for determining distances shown in ED Table 2. *Tomographic reconstructions*: Tilt series data were aligned using IMOD [38] and 3D reconstructions were conducted using the SIRT algorithm implemented in Tomo3D [39]. Visualisation of data was carried out using IMOD and UCSF Chimera [32].

Supplementary References:

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