Supplemental methods

Outline of the EM reconstruction:

ParM filament images were extracted and from the electron micrographs and straightened. The digitized images were corrected for the phase of the Contrast Transfer Function (CTF). (The amplitude of the CTF was corrected after the refinement.) To adopt our previous procedures used for cryo electron micrographs without any change for negatively stained filaments, the contrast of the images was inverted. An averaged power spectrum was calculated from 33 ParM filaments (Fig. S1). It showed clear layer lines. From this the selection rule was assigned as either \( l = -17n + 37m \) (“right handed helix along the long pitch helix”) or \( l = 17n + 37m \) (“left handed helix”) (Stewart, 1988). The true pitch was about 89 nm. Note that the handedness was not determined yet at this stage. We initially assumed the selection rule as \( l = -17n + 37m \) (right handed helix like f-actin) and reconstructed a three dimensional structure by using helical reconstruction (Stewart, 1988). The layer lines up to the 37th order (24 Å) were included. This initial structure was further refined (see below).

The handedness was determined independently as last step after using the initial refined structure to calculate a reference projection (see “Determining the handedness”). This showed the correct selection rule to be \( l = 17n + 37m \) (left handed helix opposed to the originally assumed right handed helix). Therefore we had to mirror our initial structure to obtain the final structure of ParM.

Refining the structure:

The refinement of the structure (Fig. S2) was performed as described (step 1 in Narita and Maéda, 2007). The map from the helical reconstruction was used as initial
By using the correlation maps (Fig. S2c), the polarity of each image could be determined (Narita and Maéda, 2007). Only filaments with clear polarity and clear correlation pattern on the correlation maps (Fig. S2c) were selected. The final structure was reconstructed from 26 filaments including 2,085 protomers. The CTF amplitude was corrected as described (Narita and Maéda, 2007) after the iteration converged.

The final resolution (23 Å) was evaluated by Fourier Shell Correlation (Fig. S3).

**Determining the handedness:**

To determine the handedness, tilt pairs of the same electron micrographs were taken, consisting of an untilted image and another tilted by 30 degrees (Fig. S4). 6 pairs of filaments that were parallel to the tilt axis was extracted (Fig. S5c, d).

A reference projection set (Fig. S5b) was calculated from the initial refined structure (Fig. S5a) with the selection rule \( l = -17n + 37m \). Then correlation maps between the extracted images (Fig. S5c, d) and the reference projection set were calculated (Fig. S5e, f). The peaks obtained shifted by tilting and the handedness was determined by the direction of this shift relative to the azimuth angle. If the handedness of the reference was correct, the shift should be plus. Yet we found the actual shift to be minus (Fig. S5g, Table S1) for all of the six ParM filament pairs. It indicated that the true handedness is left handed, opposite to that of the reference model and the true selection rule therefore is \( l = 17n + 37m \).

The same procedure was performed on actin filaments as a control. Tilt pair images of negatively stained actin filaments were taken and 6 pairs of filaments were extracted. A three-dimensional structure of the actin filament determined from cryo electron micrographs (described in result as step 1, Narita and Maéda, 2007) with well-known
handedness (right handed) was used as reference. The peak shift was plus for all of the six pairs (Table S1), indicating the handedness of the reference was correct.

**Constructing an atomic model of the ParM filament:**

The crystal structure of ParM-ADP (van den Ent et al., 2002) was docked into our final EM map by Situs (Wriggers and Birmans, 2001). The fitting with the largest correlation value was selected. Then energy minimization was performed by NAMD (Phillips et al., 2005) without any constraint from the EM map. To evaluate if the atomic model is correct, the model was compared with the EM map by Fourier Shell Correlation (van Heel, 1987). Each non-hydrogen atom in the atomic model was replaced by a Gaussian density distribution with a radius of 4 Å. The 200 % volume contour in the EM map was defined as zero and the pixels with negative values were set to zero. Then the Fourier Shell Correlation (van Heel, 1987) was calculated between the EM map and the map from the atomic model (Figure S6). The two maps agreed very well up to 23 Å resolution, the evaluated resolution of the EM map.

**Figure legends**

Figure S1. A averaged power spectrum calculated from 33 ParM filaments. Each filament had a length of 267 nm.

Figure S2: Illustration how the three-dimensional structure of the ParM filament was determined and refined. The procedures are identical to the method described previously for f-actin containing filaments (Narita and Maeda, 2007). (a) Examples of negatively stained ParM filament images. (b) A set of projections was calculated from the reconstructed image in the previous cycle. A helically reconstructed ParM
structure was used as the initial reference. (c) Shows the correlation maps calculated from (a) and (b). (d) Correlation peaks determined from the correlation maps (c). Each white point indicates the axial and azimuth position of one ParM protomer. (e) Averaged images of extracted short segments at different azimuth angles. (f) A new structure produced by back-projection of the averaged images in (e). This structure was used as a reference for the next cycle of refinement. Cycles were repeated until the structure converged.

Figure S3. Evaluation of the resolution of the obtained 3D electron density map (Frank, 2002). We divided the images of the ParM filaments into two groups and reconstructed two 3D maps, which were compared by Fourier Shell Correlation (van Heel, 1987). The resolution was estimated to be 23 Å, with a threshold of 0.5.

Figure S4. An example of tilt pairs to determine the handedness of the ParM structure. (a) The image without tilt. (b) The image with 30 degrees tilt. The tilt axis was the horizontal axis.

Figure S5: Determining the handedness. (a). Our initial refined ParM structure with the selection rule \( l = -17n + 37m \) (right handed helix) (b). A set of projections from each azimuth angle was calculated. (c, d). An example of tilt pairs of ParM filaments. The tilt axis is parallel to the filament. (c). Without tilt. (d). With 30 degrees tilt. (e). The correlation peaks between (b) and (c). (f). The correlation peaks between (b) and (d). (g). (e) was shifted by 30 degrees (blue) and by -30 degrees (red) and superposed on the peaks from the tilted image (f, green). The minus-shifted peaks (red) fit better to the green peaks than the plus-shifted peaks (blue) do.
Figure S6: Fourier Shell Correlation between the atomic model and the EM map.

Table S1: Orientation of the peak shift by tilting. “-” and “+” means the peak shift by tilting was minus and plus, respectively.

Fig. S7: Filamentous particles like ParM spontaneously align parallel to each other when concentrated above a certain threshold. They can be further oriented by shear flow into a glass capillary and by strong magnetic fields. Well oriented gels are the most appropriate specimen for X-ray fiber diffraction analysis, because the observed diffraction patterns can be treated as the cylindrical average of the diffraction pattern from the individual filaments. A good indication on how well the filaments are oriented in the capillary can be seen in a polarizing microscope before taking a X-ray diffraction pattern.

Shown are photographs of very well oriented ParM gels in a glass capillary taken with a polarizing microscope with crossed polarizing plates with the analyzer either at 45° (a) or 90° (b) relative to the capillary.

Fig. S8: X-ray diffraction pattern of oriented gels of ParM-AMPPNP (left half) and ParM-GMPPNP filaments (right half). The disorientation parameter for ParM-GMPPNP was 2.8°, whereas that of ParM-AMPPNP was about 3.8° causing the layer lines to look broader and more smeared out due to the larger overlap of Bessel terms.

Fig. S9: Stopped flow data covering a wide time range up to 500 s. Note that the light scattering intensity for both ParM-GTP (blue symbols) and ParM-Mix filaments
(green symbols) jumped up to a value of about 35% in the first 2 frames, whereas for ParM-ATP (red symbols) the initial light scattering intensity was only about 5%. This indicates, that when adding GTP there is a very rapid initial polymerization of ParM within the first two second of mixing and too fast for us to resolve at the present stage, followed by a slower phase, which is still very rapid compared to ATP. The peak light scattering intensity is about a factor of 2 higher for ParM-ATP than for ParM-GTP. We attribute this to the formation of some larger aggregates, when ATP is rapidly mixed with ParM monomers. This phenomenon is not apparent if either GTP or the Mix is injected to ParM. The ParM concentration was 6 µM and the nucleotide concentration was 5 mM in total.

Fig. S10: A stopped flow experiment using GTP. The ParM concentration was 6 µM and the GTP concentration was 5 µM (red symbols) and 1 µM (blue symbols). Note that the slope of the decrease are similar for both curves.

Fig S11: The relationship between average polymerization rates from 5 to 10 individual filaments at each data point and the ParM concentration in the presence of 5 mM ATP (blue) and 5 mM GTP (red) and 8 % PVA. The intersection of the linearly approximated curves determines the critical concentration. Note that the error in this measurement is larger than for light scattering experiment (Fig. 2c).

**Literature**


