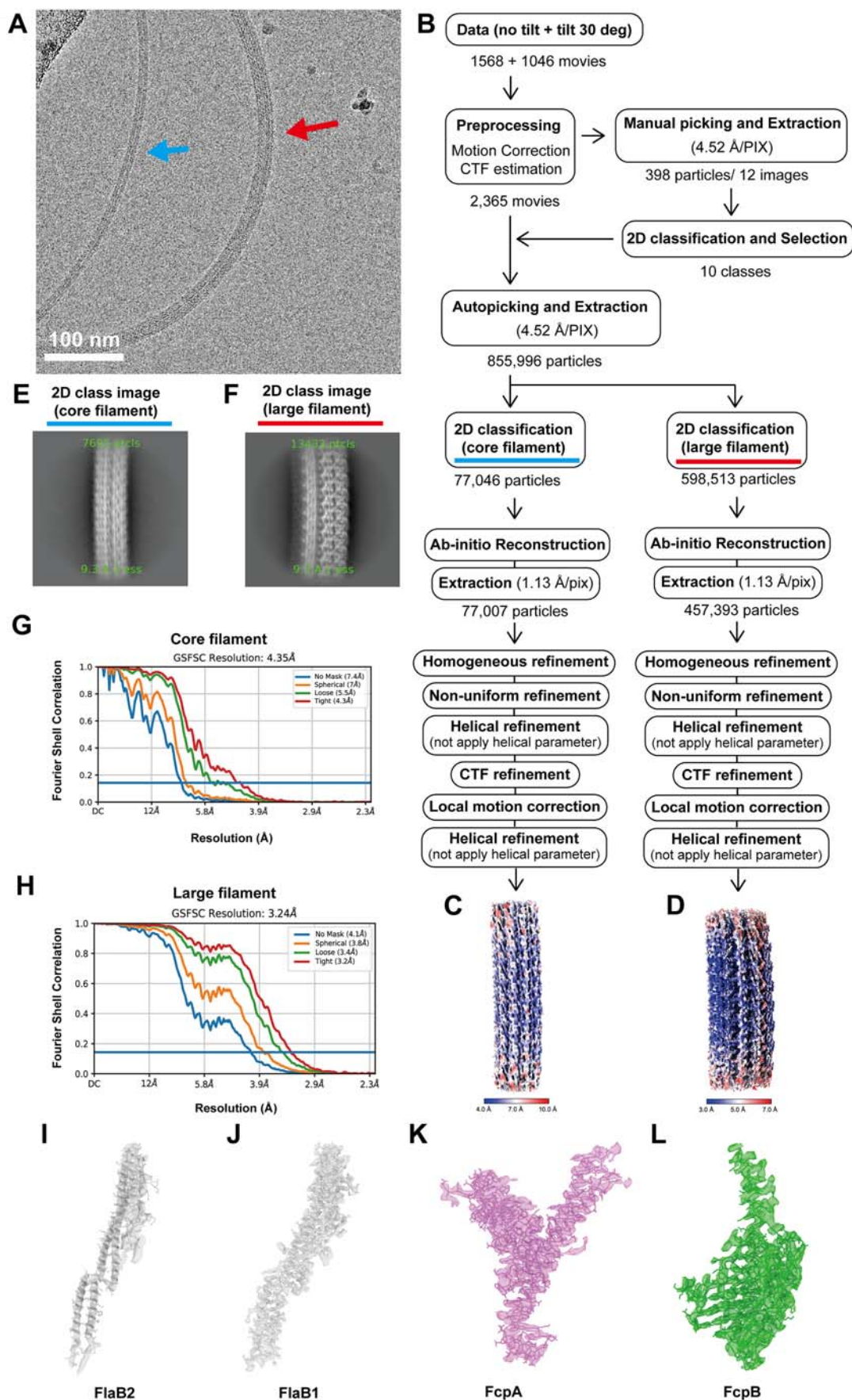


Expanded View Figures

Figure EV1. Summary of Cryo-EM data acquisition and image processing of periplasmic flagella (PFs) from wild-type strain.

(A) Cryo-EM image of purified PFs. Blue and red arrows indicate the unsheathed core filament and the sheathed filament, respectively. (B) Data-processing workflow. (C) Reconstructed image of the unsheathed core filament. (D) Reconstructed image of the sheathed filament. (E) 2D class image of the unsheathed core filament. (F) 2D class image of the sheathed filament. (G) Fourier shell correlation (FSC) analysis of the reconstructed unsheathed core filament, showing a global resolution of 4.35 Å. (H) FSC analysis of the reconstructed sheathed filament, showing a global resolution of 3.24 Å. (I-L) Fitted atomic models and corresponding density maps of FlaB2 (I), FlaB1 (J), FcpA (K), and FcpB (L).



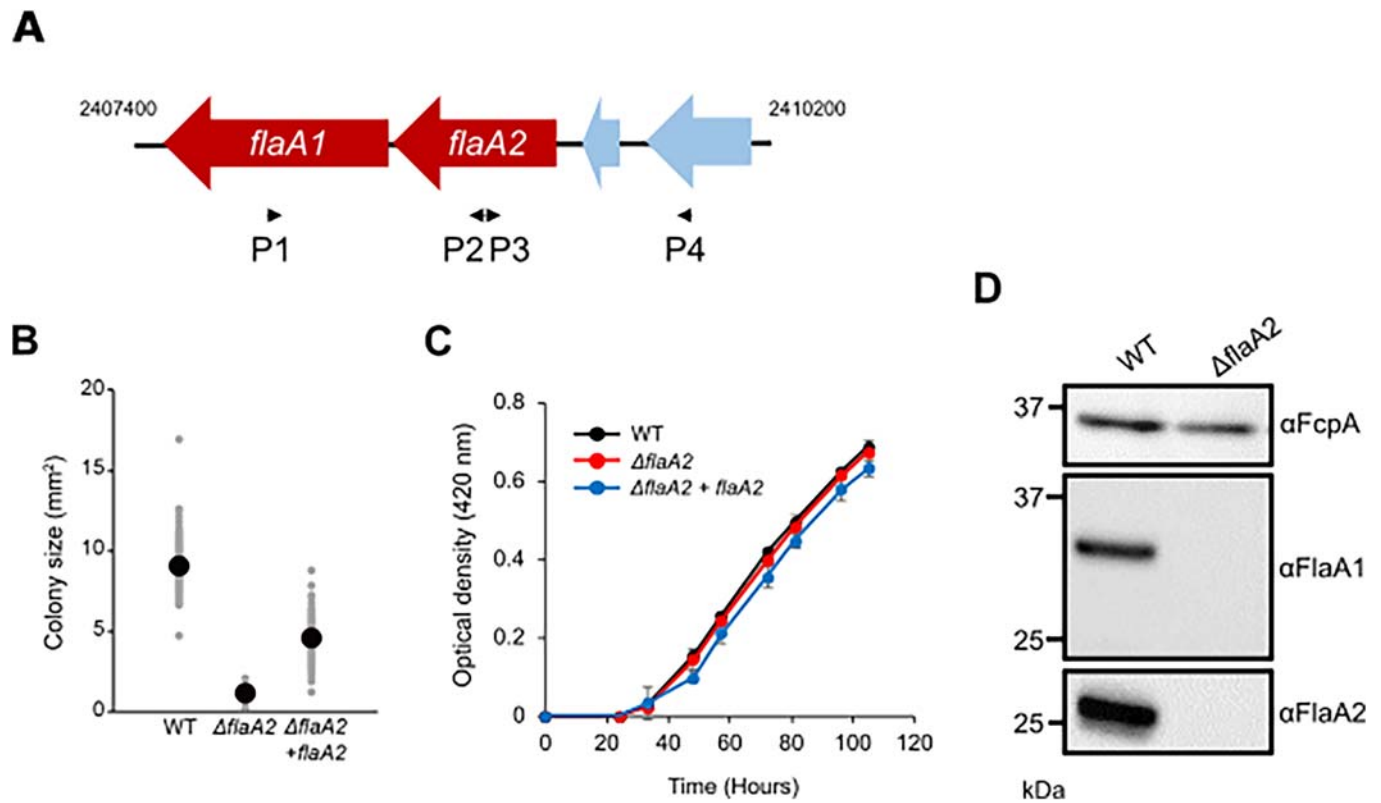


Figure EV2. Δ *flaA2* mutant expresses neither FlaA2 nor FlaA1.

(A) Schematic diagram of the genomic region (positions 2407400 to 2410200) of *L. biflexa* serovar Patoc strain Patoc I (Paris) (GenBank accession no. CP000786.1), showing the relative positions and orientations of *flaA2* and *flaA1*. The locations of primers used for constructing the *flaA2* deletion mutant (P1: 2407983–2408003, P2: 2408872–2408895, P3: 2408896–2408916, P4: 2409784–2409803) are indicated by black arrows. (B) Quantification of colony size of wild-type (WT), Δ *flaA2*, and *flaA2*-complemented (Δ *flaA2* + *flaA2*) strains. All individual data points are plotted, with mean values indicated by black circles. Data were collected from 131 colonies for WT, 194 colonies for Δ *flaA2*, and 176 colonies for *flaA2*-complemented strains, obtained from two independent biological replicates. Statistical significance was assessed using a two-tailed Student's *t*-test, assuming unequal variances (WT vs Δ *flaA2* $P = 6.94 \times 10^{-96}$, WT vs Δ *flaA2* + *flaA2* $P = 8.29 \times 10^{-75}$, Δ *flaA2* vs Δ *flaA2* + *flaA2* $P = 2.50 \times 10^{-94}$). (C) Growth of *L. biflexa* WT (black), Δ *flaA2* (red), and *flaA2*-complemented (blue) strains. Optical density (OD) at 420 nm was measured at 0, 24, 33, 48, 51, 72, 81, 96, and 105 h after inoculation. Measurements were terminated at 105 h (day 4), as cell sedimentation occurred after day 5. For each independent experiment, OD measurements were performed in triplicate, and the triplicate values were averaged to obtain a single data point. Data points represent the mean of three independent experiments, and error bars indicate the standard deviation. The culture medium for the *flaA2*-complemented strain contained 25 μ g/mL kanamycin. (D) Immunoblotting of whole-cell lysates from WT and Δ *flaA2* mutant using anti-FcpA, anti-FlaA1, and anti-FlaA2 antisera. Whole-cell lysates were prepared in two independent experiments, and each preparation was analyzed by immunoblotting; a representative blot is shown.

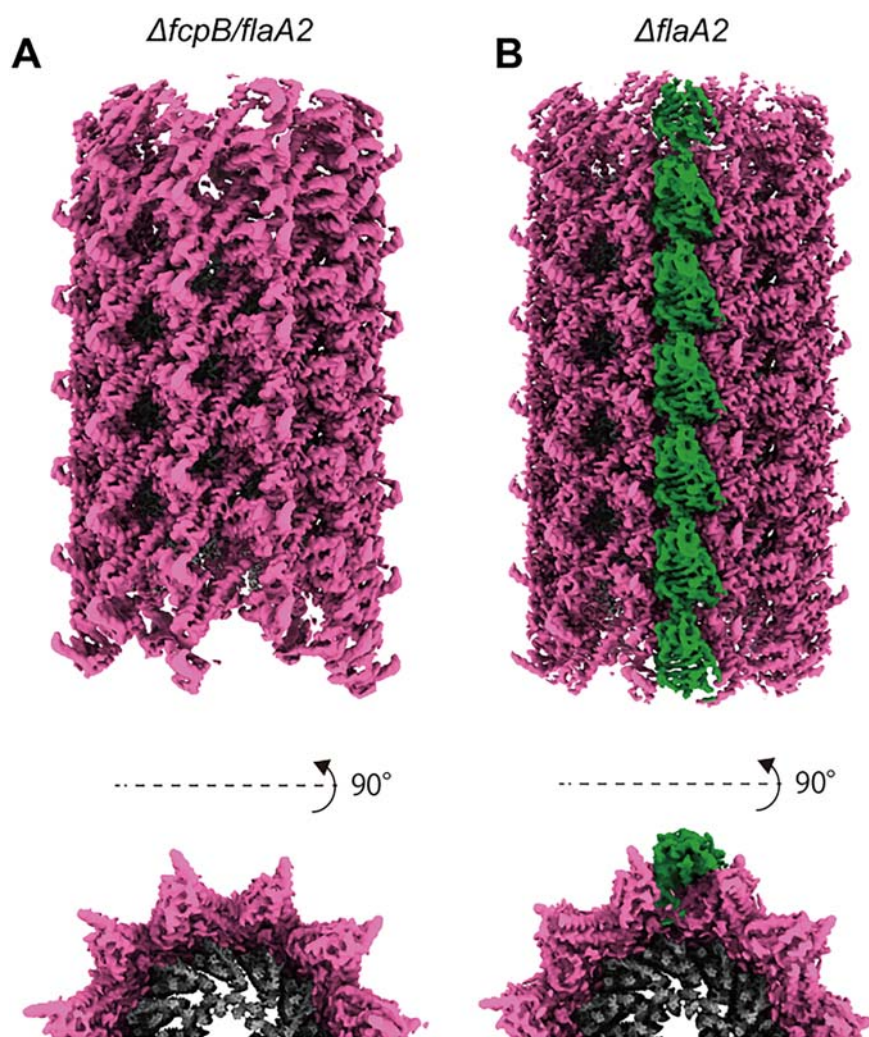


Figure EV3. Interaction of FcpB with FcpA.

(A) Reconstructed image of the periplasmic flagellum (PF) from $\Delta fcpB/flaA2$ mutant, which is uniformly covered by FcpA alone. The FlaB core filament (gray) and FcpA (magenta) are shown. (B) Reconstructed image of the PF from $\Delta flaA2$ mutant, uniformly covered by both FcpA and FcpB. FlaB (gray), FcpA (magenta), and FcpB (green) are shown. See Methods for details of reconstruction reproducibility.

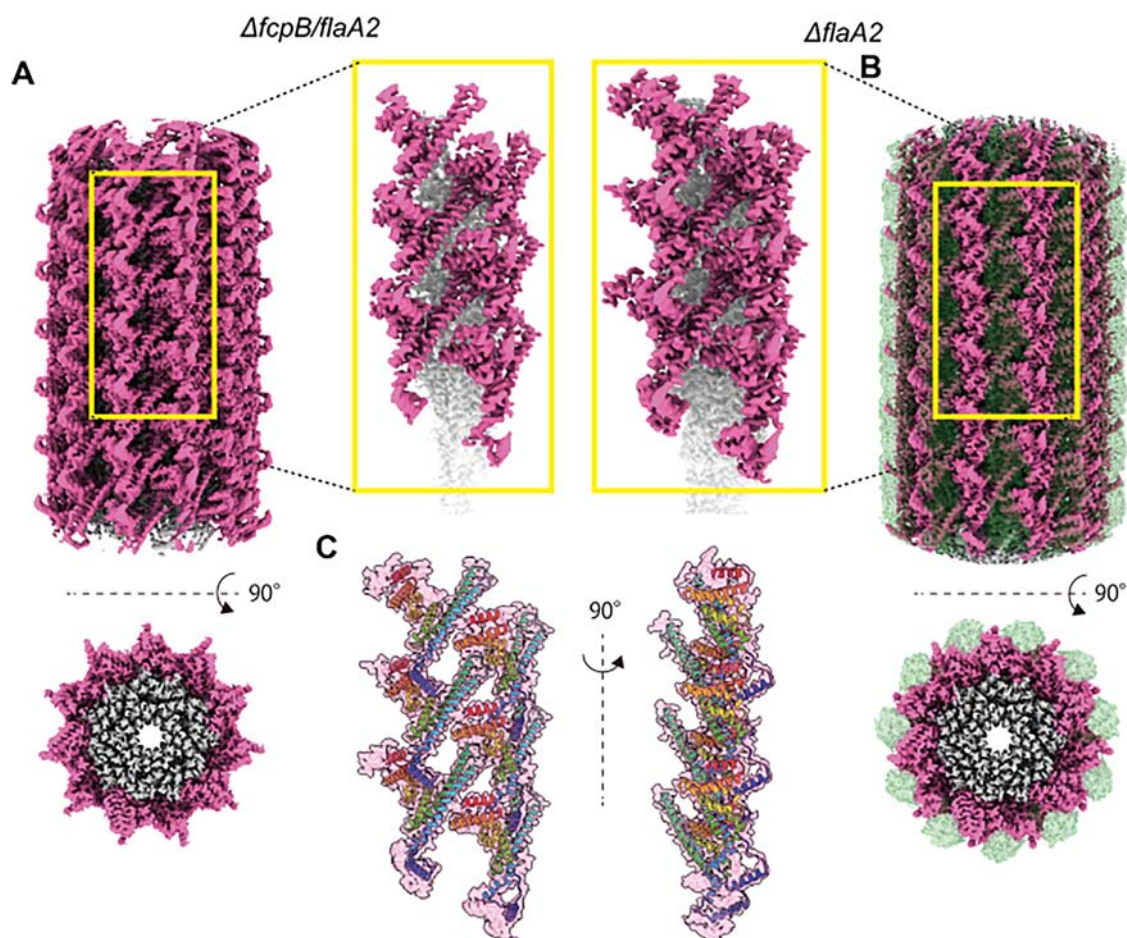


Figure EV4. Effect of FcpB on the structure of FcpA row.

(A) Reconstructed image of the periplasmic flagellum (PF) from $\Delta fcpB/flaA2$ mutant, which is uniformly covered by FcpA alone. The FlaB core filament (gray) and FcpA (magenta) are shown. A magnified view of the boxed region is shown on the right. (B) Reconstructed image of the PF from $\Delta flaA2$ mutant, uniformly covered by both FcpA and FcpB. FlaB (gray), FcpA (magenta), and FcpB (green) are shown. In the magnified view (left), FcpB is omitted to highlight FcpA structure. (C) Structural comparison of FcpA between the two mutants. The FcpA model from $\Delta fcpB/flaA2$ mutant (shown as a rainbow-colored ribbon, from panel A) is superimposed onto the FcpA density map from $\Delta flaA2$ mutant (magenta, from panel B). See Methods for details of reconstruction reproducibility.

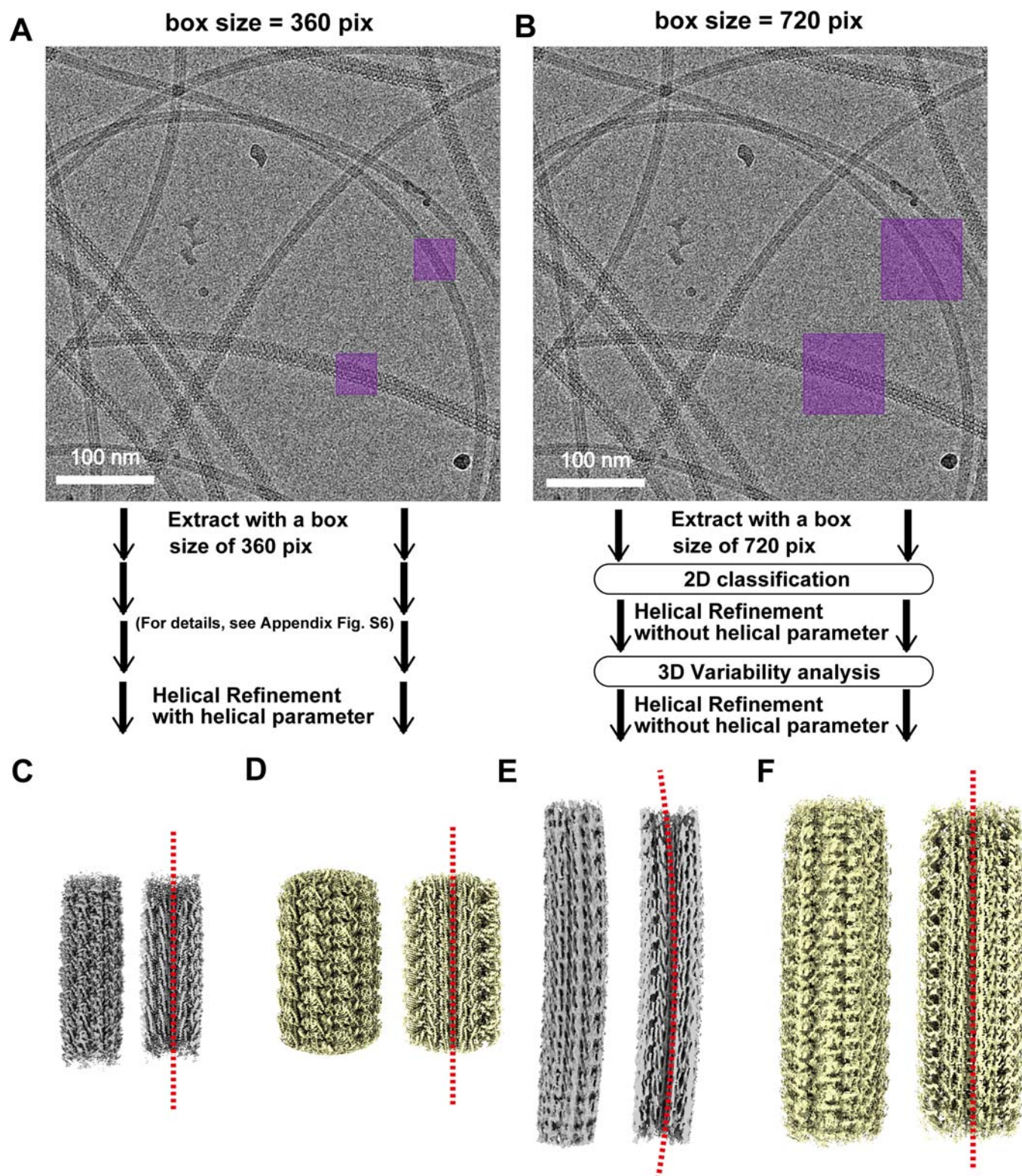


Figure EV5. Structural comparison of $\Delta flaA2$ mutant PFs processed with and without applying helical symmetry.

(A, B) Data-processing workflows for helical refinement with (A) and without (B) applying helical symmetry. The areas from which particles were extracted are indicated by purple squares. For details, see Appendix Fig. S6. (C-F) Reconstructed density maps and corresponding cross-sectional views of the core filament obtained with (C) and without (E) applying helical symmetry, and of the sheathed filament obtained with (D) and without (F) applying helical symmetry. Red dotted lines indicate the filament axis.