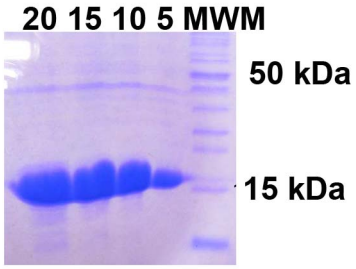
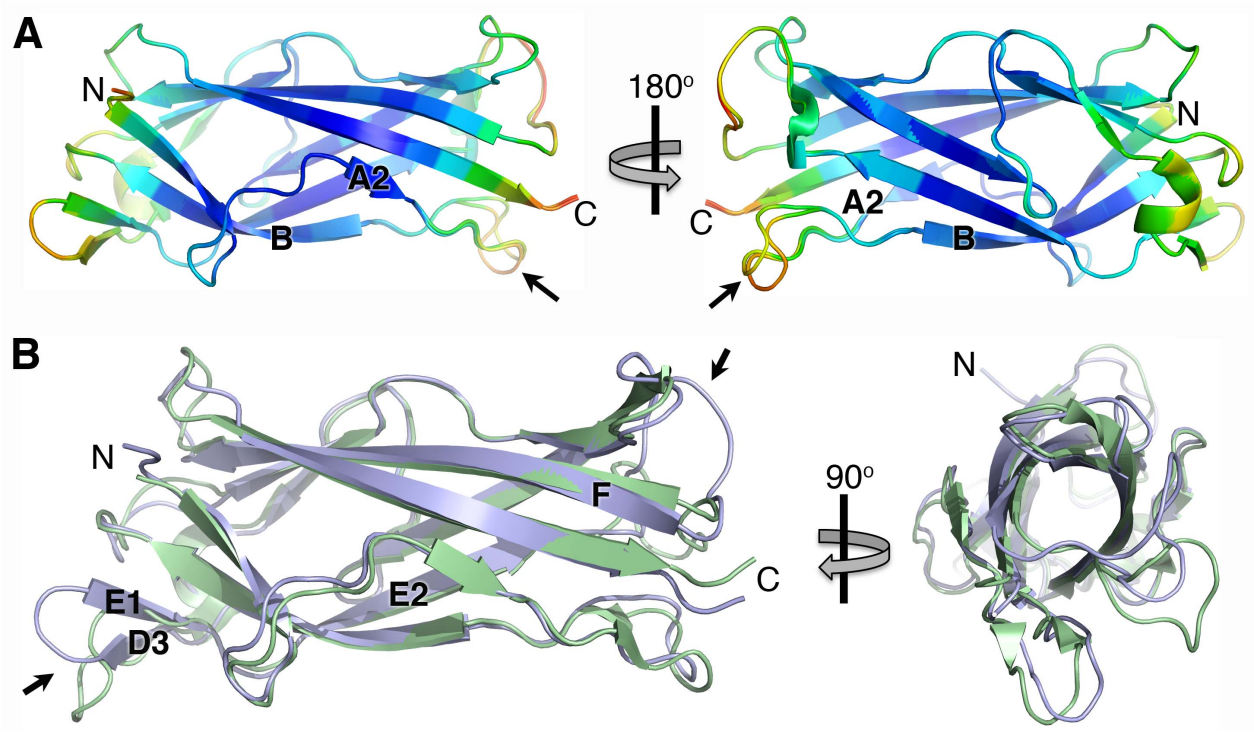
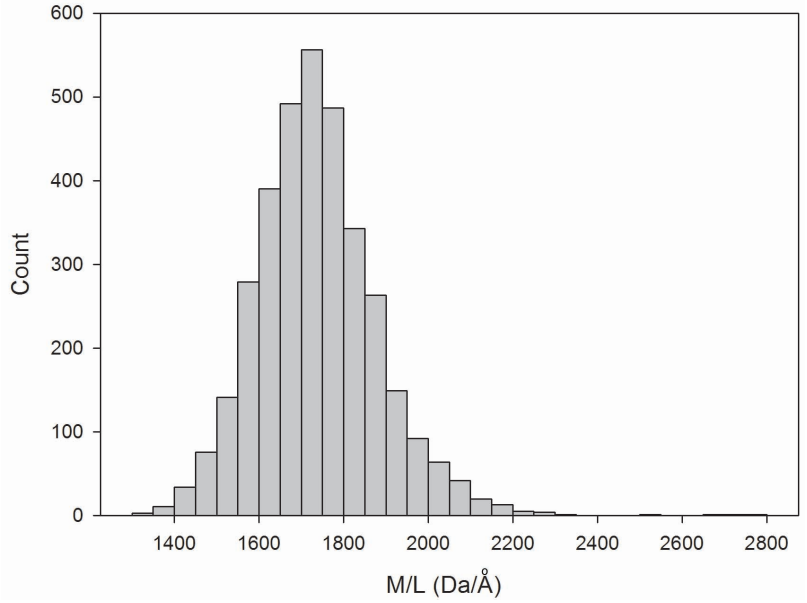


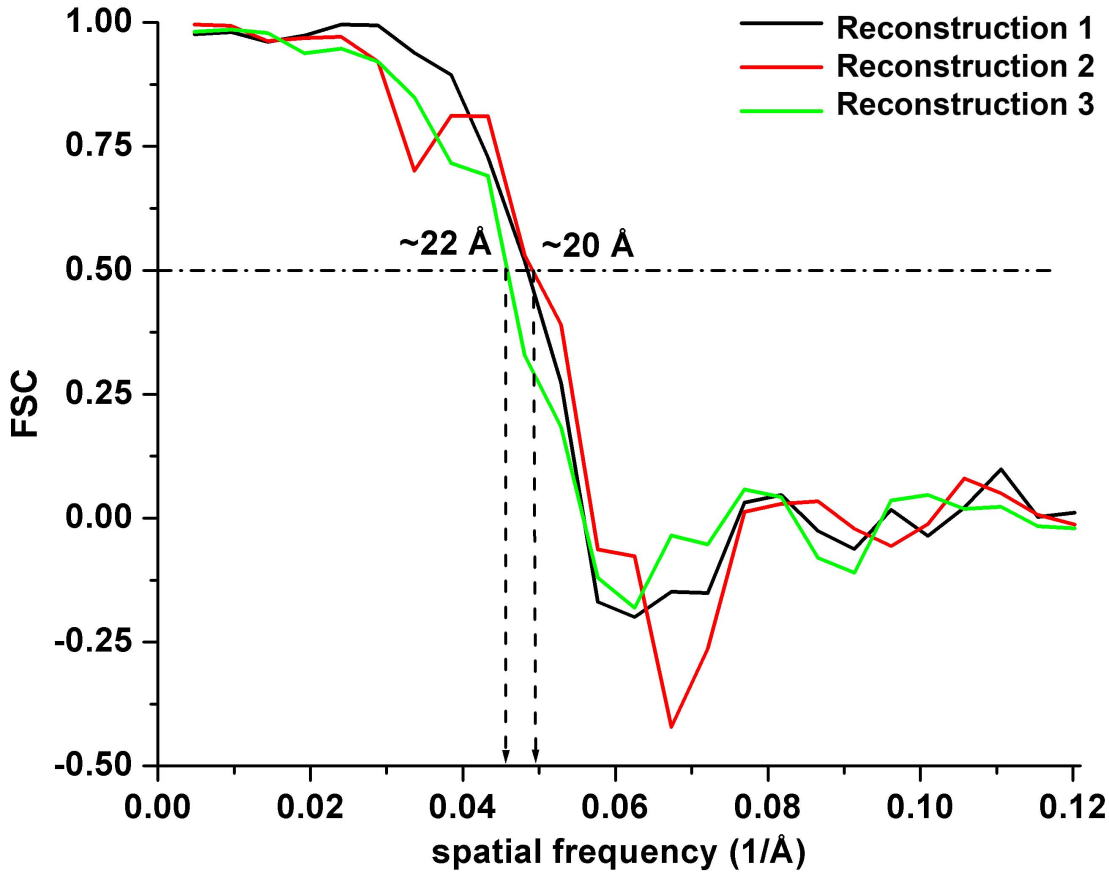
Supplementary Figure S1. Coomassie-stained SDS-PAGE showing purity of CS1 pilus preparation. Volumes (μl) of purified CS1 pili loaded are listed above each lane. MWM, molecular weight markers.



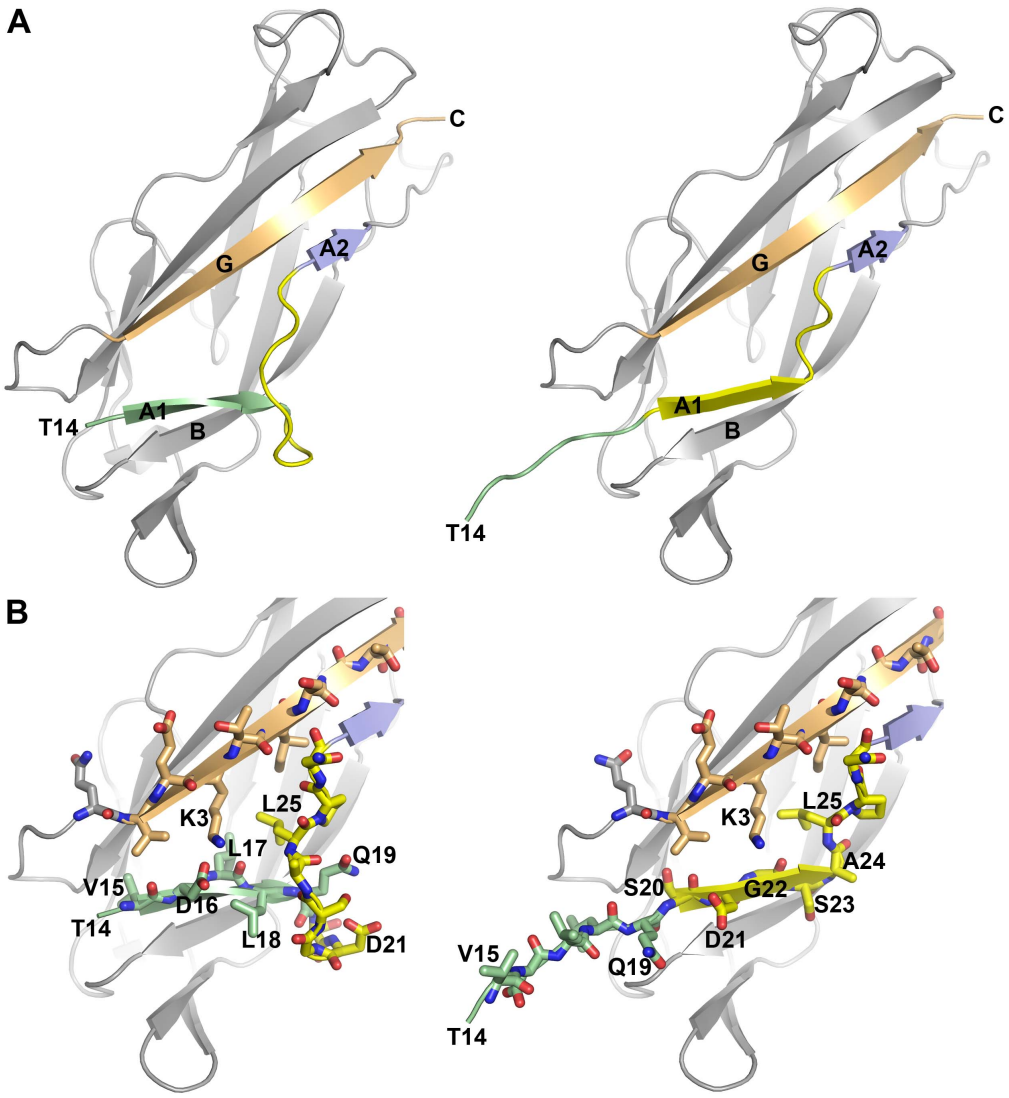
Supplementary Figure S2. Comparison of CooA and CfaB structures. (A) Superpositions of $CooA_{dsc}$ chains A and B show their close similarity, with only a slight deviation at the A2-B loop, indicated by the arrow. The root mean square deviation (RMSD) is 0.8 Å for all equivalent atoms. Molecules are colored by temperature factors: dark blue, 10; red, 30. **(B)** Superposition of $CooA_{dsc}$ (chain A, light blue) and CfaB (PDB code 3F84, green). RMSD for all Cα carbons, 2.3 Å. Arrows indicate loops that differ between the two structures.



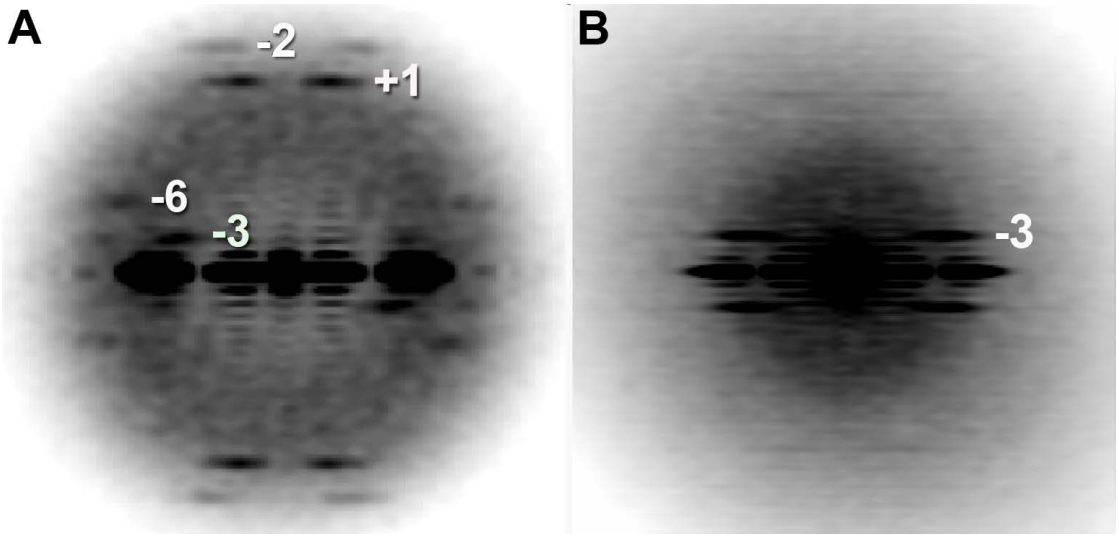
Supplementary Figure S3. STEM analysis of CS1 filaments. STEM mass histogram for CS1 filaments. M/L is mass per unit length.



Supplementary Figure S4. FSC to assess similarity between the 3D reconstructions and the pseudo-atomic resolution models. The Fourier shell correlation (FSC) 0.5 criterion confirms that the reconstructions and the corresponding filament models match well.



Supplementary Figure S5. Model for extension of the CooA N-terminal segment to span the gap between the heads of subunit *n* and the tails of *n+1* in model 3. (A) Ribbon diagram of $CooA_{dsc}$ (left) and proposed extension of the N-terminus for subunits in CS1 model 3 (right). $CooA_{dsc}$ strand A1 (residues 14-19) is colored light green, strand A2 (residues 28-30) is light blue and the intervening loop is yellow. Strand G (the Nte) is colored orange. **(B)** Close-up of strand A interactions in the $CooA_{dsc}$ crystal structure (left) and the extended model (right). To generate the extended model shown on the right, residues 14-19 in β-strand A1 were "mutated" in silico to residues 19-24, residues 19-24 were deleted and new Ala24 was bonded to Leu25 using COOT. Regularization was performed in COOT to optimize bond lengths and angles. This extended model is not expected to require further conformational adjustments because: (i) backbone hydrogen bonds between strands A1 and B are maintained; (ii) interactions between A1 and G are likely to malleable, as G is the Nte, which replaces the chaperone donor strand; and (iii) the amino acid changes in A1 are either conservative and/or occur on exposed residues (Thr14, Asp16, Leu18 and Gln19 side chains are all exposed and replaced with Gln19, Asp21, Ser23 and Ala24, respectively), or are not conservative but are nonetheless reasonable (Val15 with a buried side chain is replaced by Ser20, whose side chain could hydrogen bond with backbone atoms on strand G; Leu17, also with a buried side chain, is replaced by Gly22 with no side chain).



Supplementary Figure S6. CS1 pilus heterogeneity is enhanced in cryoEM specimens. Power spectra of **(A)** negatively stained (a, n=10,443) and **(B)** frozen hydrated (n=53,540) CS1 pili.