Figure S1. Sorting histogram of the PaK and Ng T4P reconstructions showing the variability in the rise and rotation. (A) A histogram of the rise and twist distribution of PaK T4P using a reference-based sorting procedure with models having a variable rise (± 1.5 Å) and twist (± 2 °). **(B)** The animated gif shows the averaged power spectra computed from the segments in the histogram bins 1, bin 5 and bin 9 as shown in (A). **(C)** A histogram of the rise and rotation distribution of Ng T4P using a reference-based sorting procedure with models having a variable rise (± 0.5 Å) and twist (± 1 °). **(D)** The animated gif shows the averaged power spectra computed from the segments in the histogram bin 1, bin 5 and bin 9 as shown in (C).
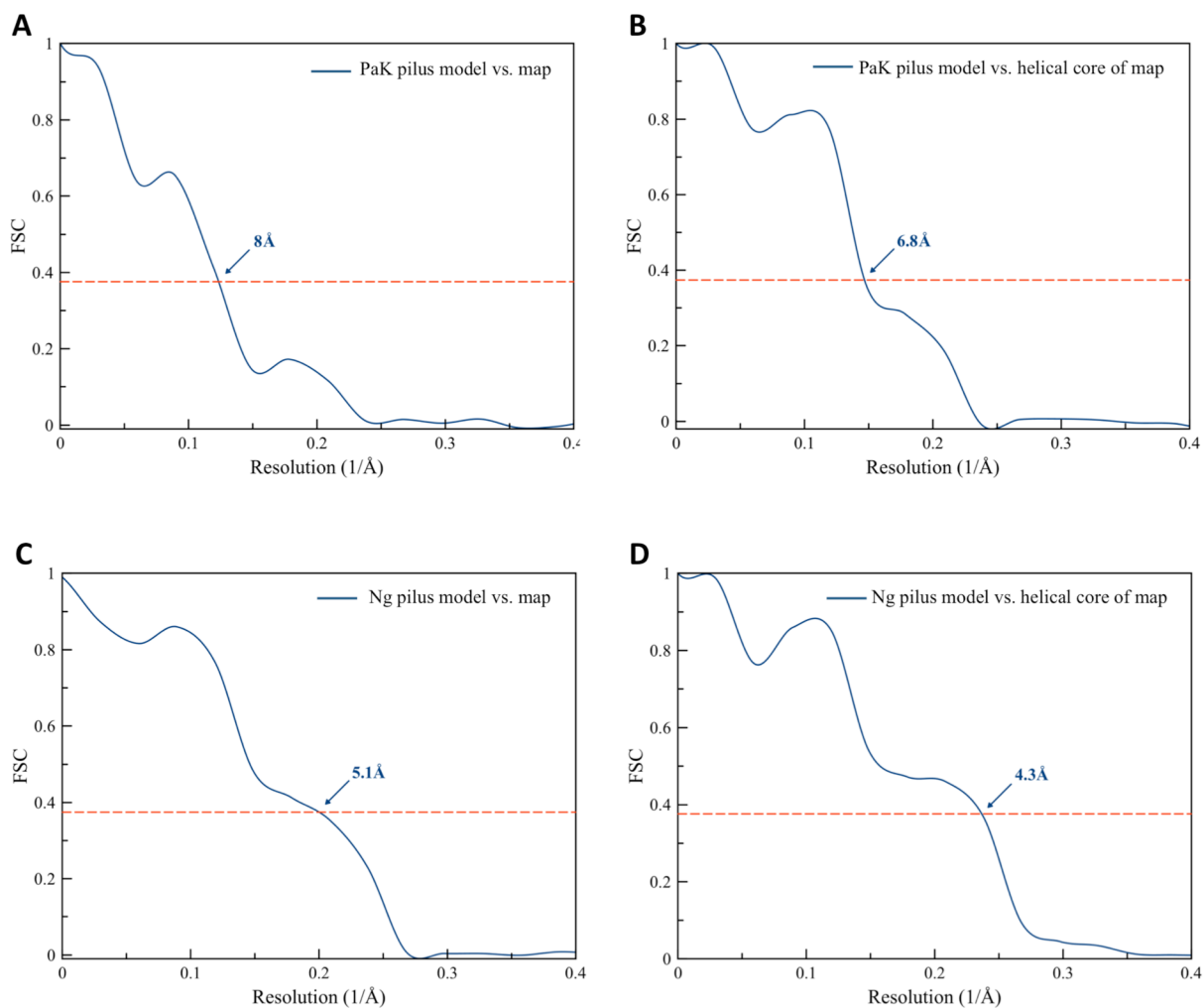
Figure S2. Resolution of the *P. aeruginosa* and *N. gonorrhoeae* Type IV pilus structures, derived from Fourier Shell Correlation (FSC) calculation between the refined atomic model and the map. (A) The FCS between the PaK pilus model and the map is 0.38 at 8 Å resolution. **(B)** The FSC between the PaK pilus model and the α-helical core of the map is 0.38 at 6.8 Å resolution. **(C)** The FCS between the Ng pilus model and the map is 0.38 at 5.1 Å resolution. **(D)** The FSC between the Ng pilus model and the α-helical core of the map is 0.38 at 4.3 Å resolution.