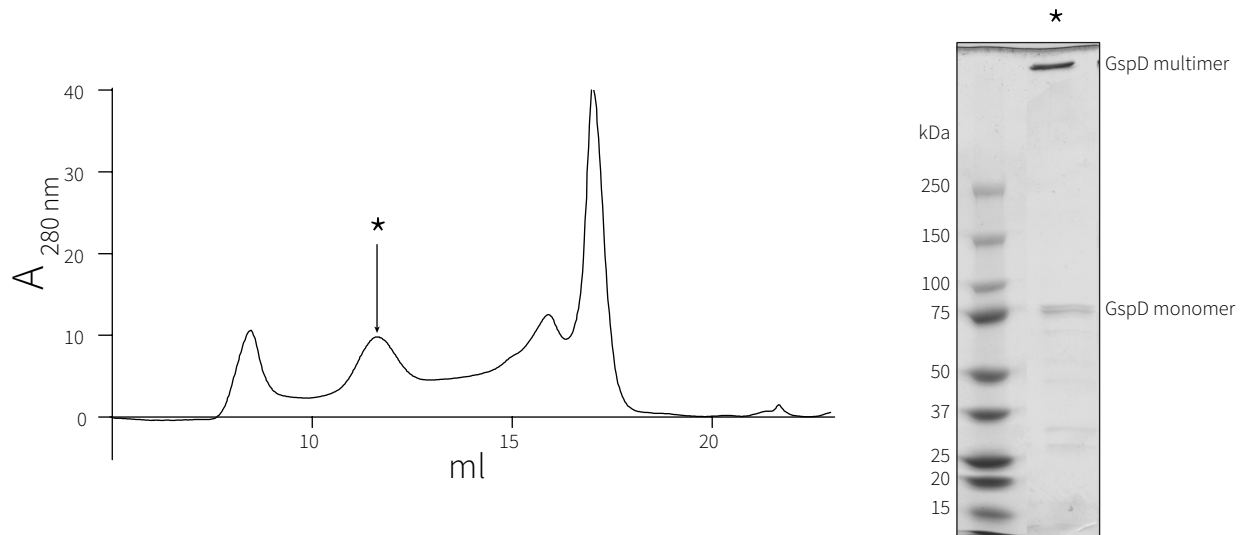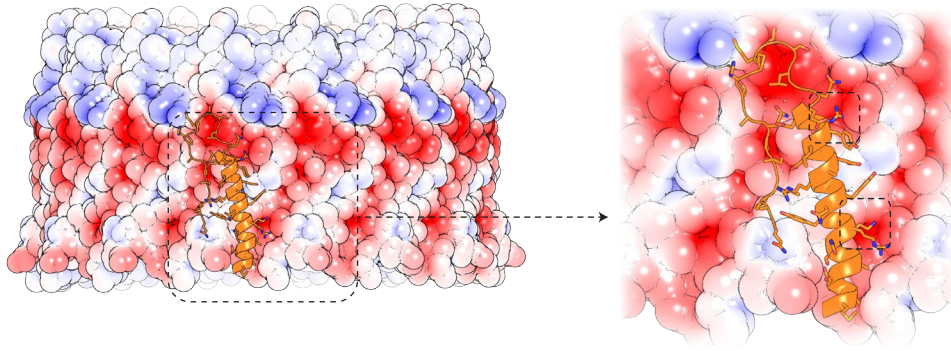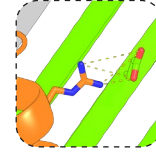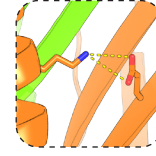Figure S1. Superose 6 10/300 size exclusion chromatography A_{280nm} trace of Amphipol A8-35 stabilised EPEC GspD. The peak corresponding to the multimeric protein (*) was subjected to SDS-PAGE to assess its purity. The heat stable GspD multimer and monomeric form can be observed.
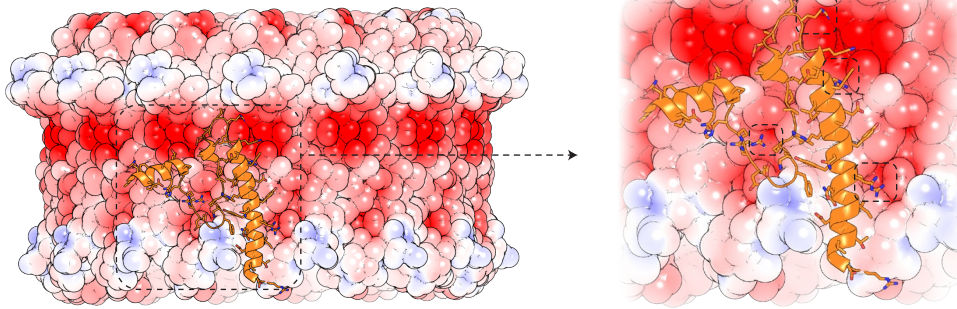
Figure S2A
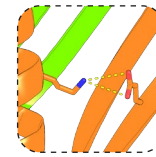
T2SS GspD *EPEC*

(A)Lys598-(A)Glu326

(A)Arg609-(O)Glu334

T2SS GspD *Vibrio cholerae*
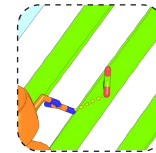
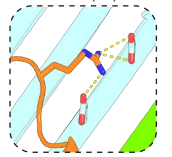(A)Lys599-(A)Glu323

(A)Arg616<(N)Glu547
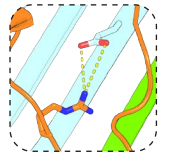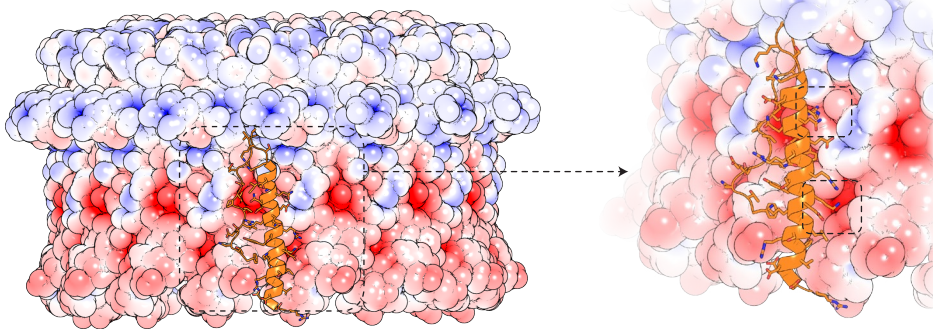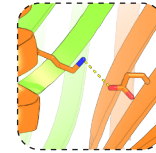(N)Glu549
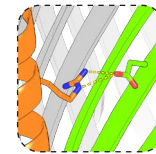
(A)Arg610-(O)Glu331

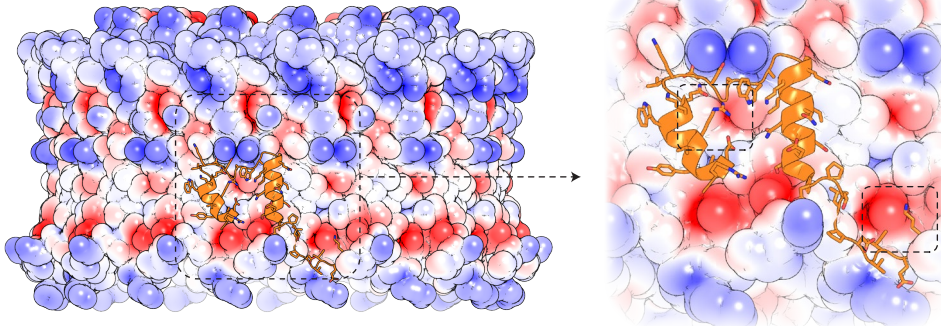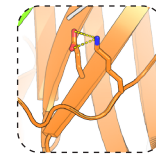(A)Arg633-(N)Glu543

T2SS GspD *E. coli* K-12

(A)Lys584-(A)Glu328

(A)Arg595-(O)Asp336

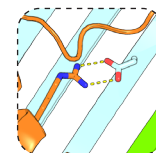T3SS InvG *Salmonella enterica*

(A)Lys516-(A)Glu305

(A)Lys551-(N)Asp455

Figure S2B

T2SS GspD *EPEC*

T2SS GspD *Vibrio cholerae*

T2SS GspD *E. coli* K-12
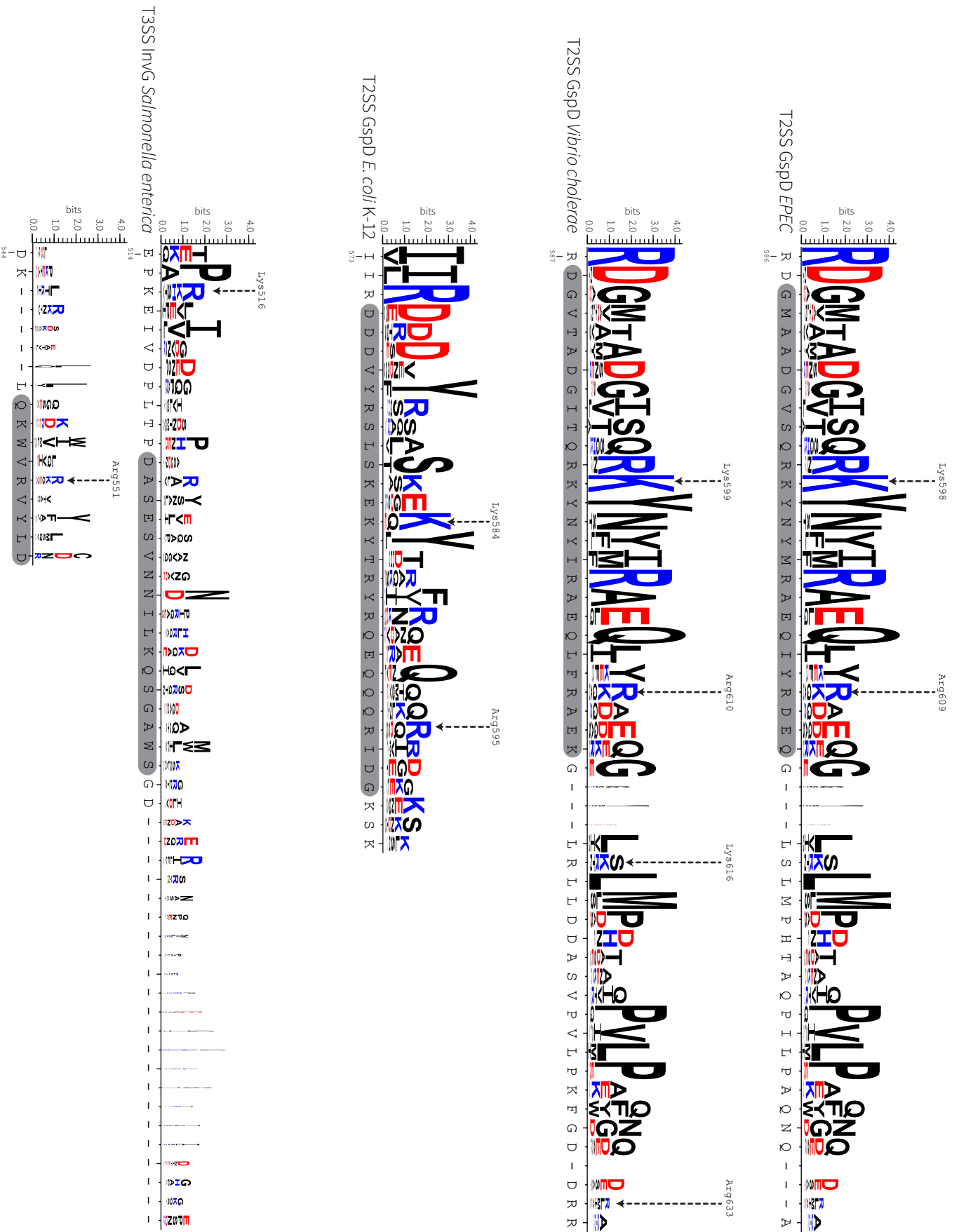
T3SS InvG *Salmonella enterica*

Figure S2. (A) Locking down the S-domain onto the T2SS secretin complex. The secretin multimeric β-barrel is locked together by two conserved salt bridges. The structures of the four known secretin barrels (EPEC T2SS GspD, *Vibrio cholera* T2SS GspD, E. coli K12 T2SS GspD, and *Salmonella enterica* T3SS InvG) are presented with their surface rendered with their electrostatic potentials shown (red = negative, blue = positive). The C-terminal alpha helix/helices are shown in orange. Potential salt bridges between the helix and the barrel. The subunits are colour coded (orange=chain A, green=chain O, blue=chain). The residues involved in the potential salt bridges are indicated and chain/subunit ids are shown in brackets. (B) Sequence logo of alignments of the Vibrio (581 sequences) and Klebsiella-type (363 sequences) T2SS secretins and the *Salmonella enterica* T3SS secretin (1543 sequences) C-terminal alpha helix regions. The sequence and helix location (in grey) are shown below the sequence logos . Residues involved in potential salt bridges to the β-barrel surface are indicated. Positive charges residues are shown in blue and negatively charged residues are shown in red.
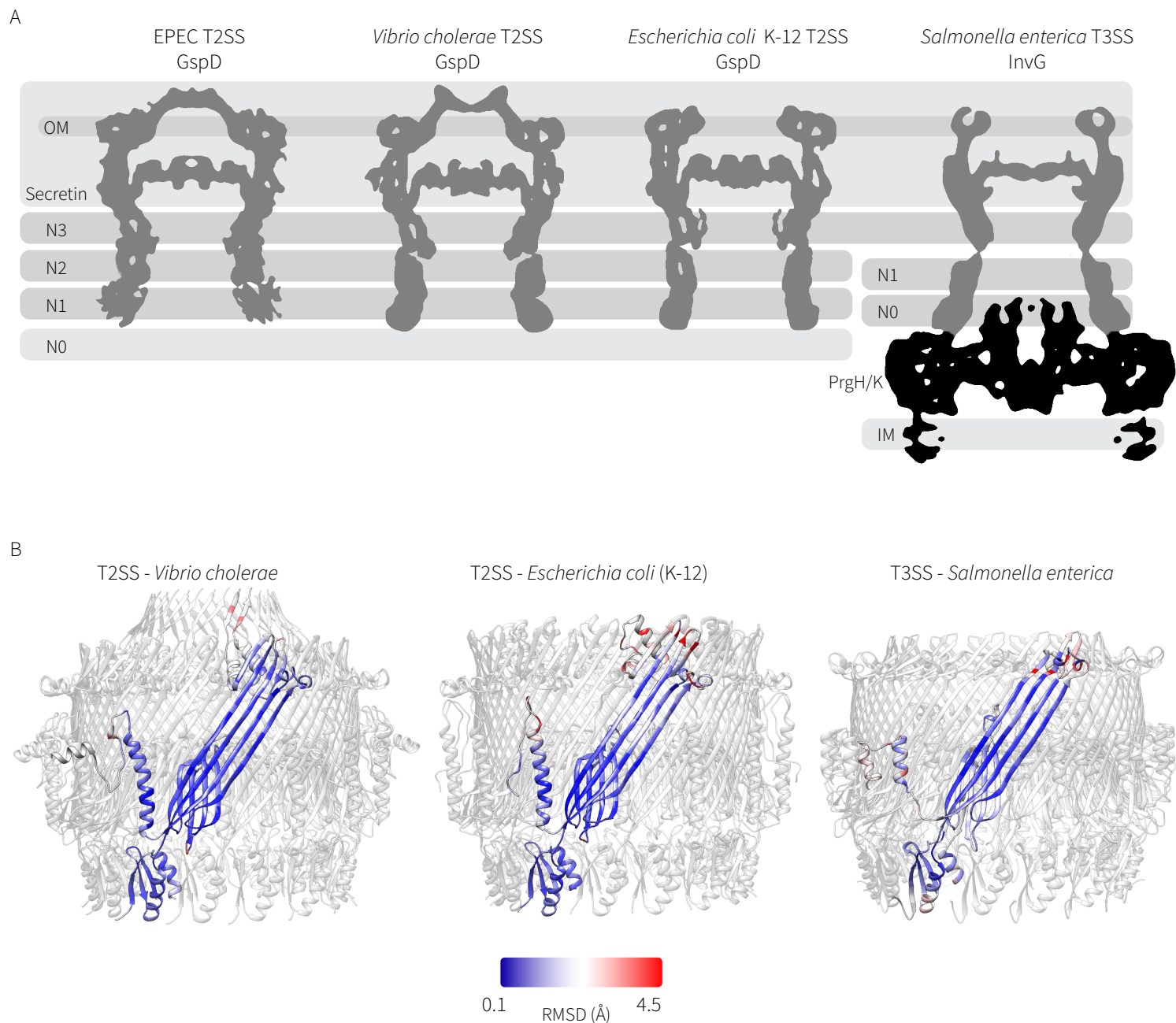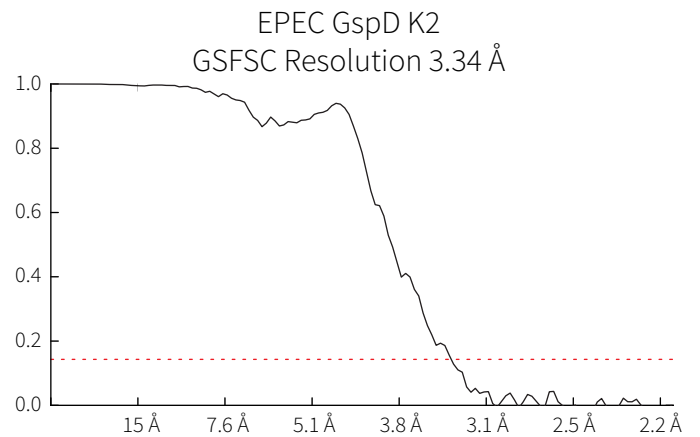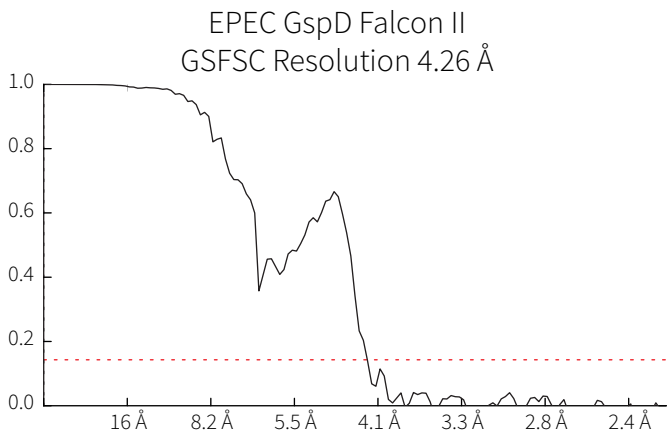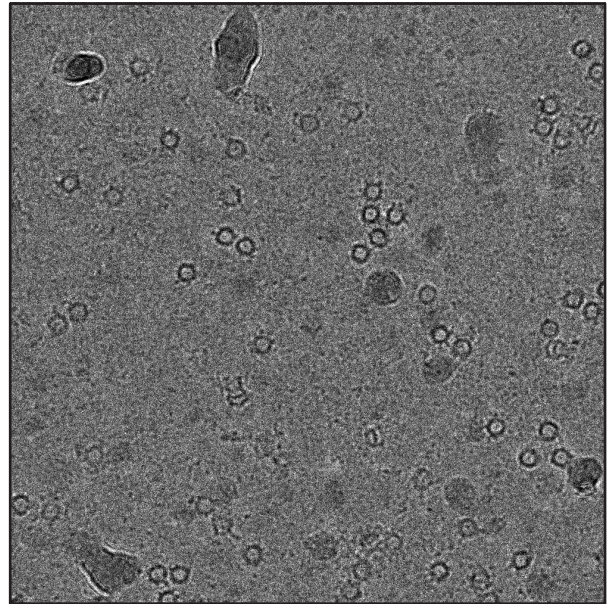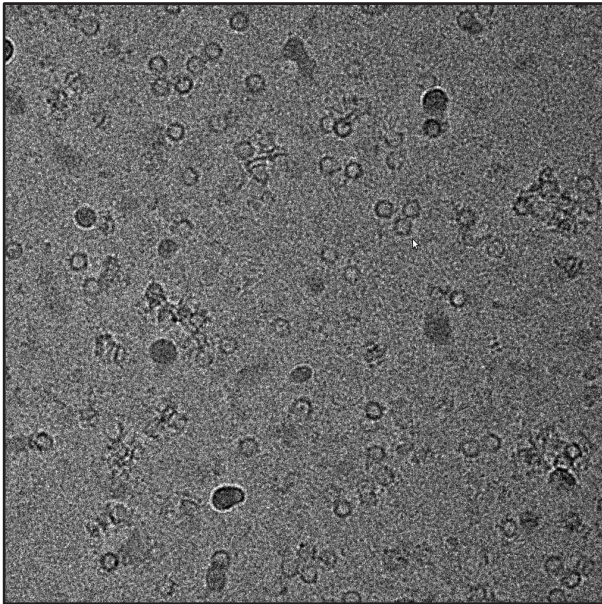
Figure S3

Figure S3. Comparison of secretin structures. **(A)** The electron density map for the EPEC secretin GspD (EMD-8779) is compared to the T2SS secretins from V. cholerae (EMD-6676) and E. coli K-12, (EMD-6675) as well as the T3SS secretin and associated basal body from S. enterica (EMD-8400). The maps were low-pass filtered to 7 Å and sliced through the axis of symmetry to aid in comparison. Relevant domains are highlighted where appropriate **(B)** Root-mean square deviation (RMSD) analysis comparing the T2SS secretins from V. cholerae (left) (PDB: 5WQ8) and E. coli K-12 (middle) (PDB: 5WQ7) and the T3SS secretin from S. enterica (PDB: 5TCQ) with the EPEC T2SS secretin from this study. A single chain from each secretin is coloured from blue to red, representing the C-α RMSD values from 0.1 to 4.5 Å. This was calculated in the UCSF Chimera (69) software package using the Matchmaker algorithm in conjunction with a BLOSUM-30 weighted sequence matrix.
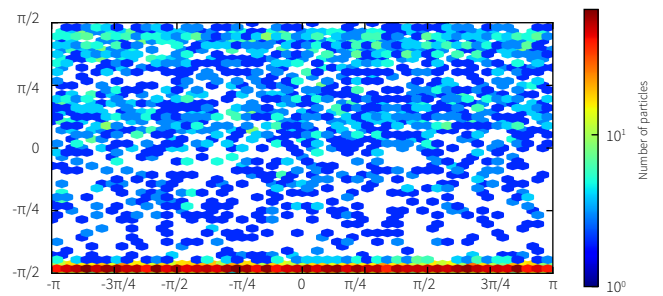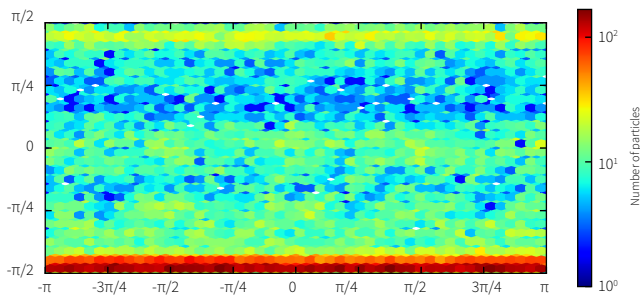
Figure S4. (A) Fourier Shell Correlation (FSC) plot of the final maps. Based on the FSC=0.143 criteria the maps shao a resolution of 4.26 Å and 3.34 Å. (B) Example raw micrographs showing the particles. (C) Plots showing the distribution of particle orientations over the azimuth and elevation angles of the final maps.