Expanded View Figures

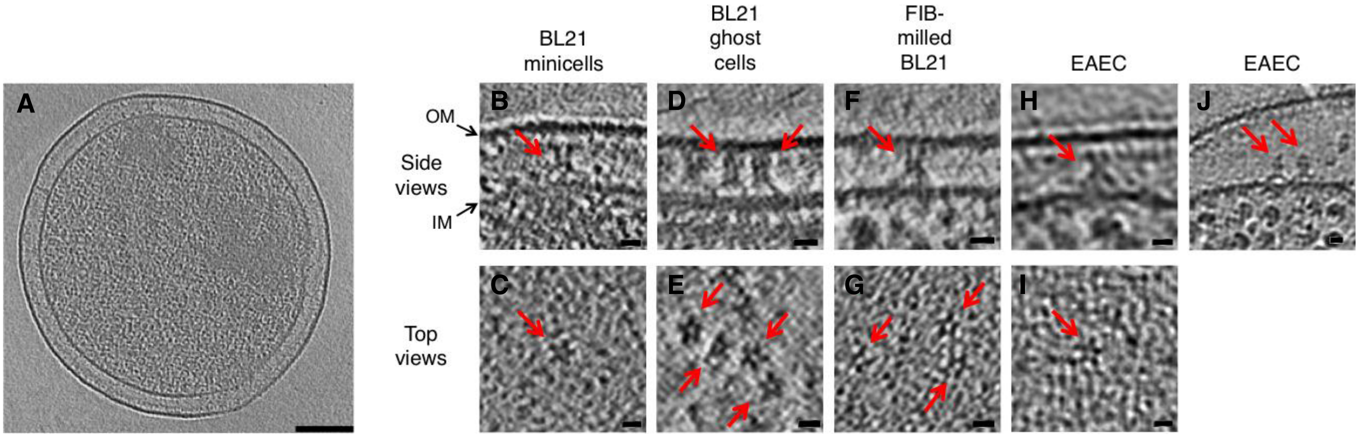

Figure EV1. The TssJLM complex exhibits a C5 symmetry in *Escherichia coli* BL21 and in EAEC.

- A Slice (13.8 nm) through an engineered BL21 minicell with a diameter of ~450 nm. Scale bar 100 nm.
- B Slice (9.7 nm) showing a side view of a TssJLM particle embedded in the cell envelope of a BL21 minicell. The particle spanned the periplasm and resembled an inverted letter Y.
- C Slice (9.7 nm) showing a top view of a TssJLM particle embedded in the membrane of a BL21 minicell. The particle resembles a 5-branched star.
- D Slice (9.7 nm) showing a side view of a particle embedded in the cell envelope of a BL21 ghost cell.
- E Slice (9.7 nm) showing a top view of a particle embedded in the cell envelope of a BL21 ghost cell.
- F Slice (9.7 nm) showing a side view of a particle embedded in the cell envelope of a FIB-milled BL21 cell.
- G Slice (13.8 nm) showing a top view of a particle embedded in the cell envelope of a FIB-milled BL21 cell.
- H Slice (9.9 nm) showing a side view of a particle embedded in the cell envelope of an EAEC cell in which TssJLM was heterologously overexpressed.
- I Slice (9.9 nm) showing a side view of a particle embedded in the cell envelope of a wild-type EAEC cell.
- J Slice (9.9 nm) through the distorted periplasm of an EAEC in which TssJLM was heterologously overexpressed. The particles seen in side view were detached from the OM but still attached to the periplasmic side of the IM.

Data information: Slices in panels (B–J) show side views (upper rows) and top views (lower rows) of TssJLM embedded in the cell envelope of BL21(DE3) (expressing TssJLM) and EAEC cells, as indicated by red arrows. The outer and inner membranes are indicated (OM and IM). Scale bars 10 nm.

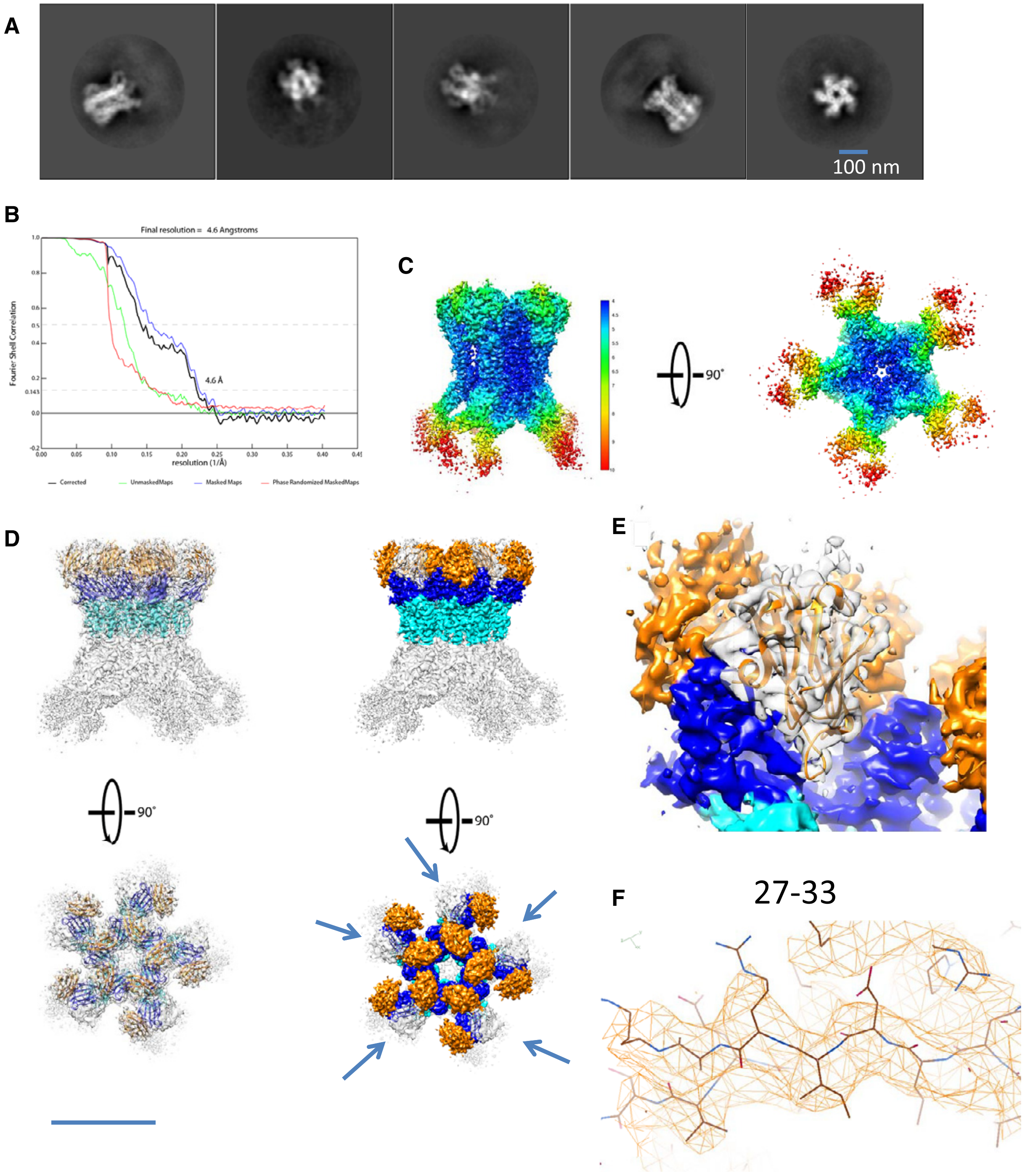

Figure EV2. The cryo-EM reconstruction of the core of the membrane complex (MC).

A 2D classes of the subtracted SPA cryo-EM density corresponding to the core of the MC

B FSC curve of the core complex as calculated using post-process

C 3D representation of the core 3D reconstruction coloured according to the local resolution. Two views are shown.

D Fitting of 10 TssM-TssJ pseudoatomic models from the crystal structure (4Y7O) in the core cryo-EM density locally sharpened. The cryo-EM density was coloured according to the fitted structure. The five additional densities are highlighted by arrows pointing at them. Two views of the complexes are shown. In orange is the TssJ, in blue the beta sheet-rich region and in cyan the alpha helical domain.

E Transparent representation of the cryo-EM density in which the third TssJ was fitted (ribbon diagram in orange). The colouring follows that in (D).

F Representative cryo-EM density, corresponding to the TssJ′ sequence 27–33, with the fitted pseudoatomic model in stick form.

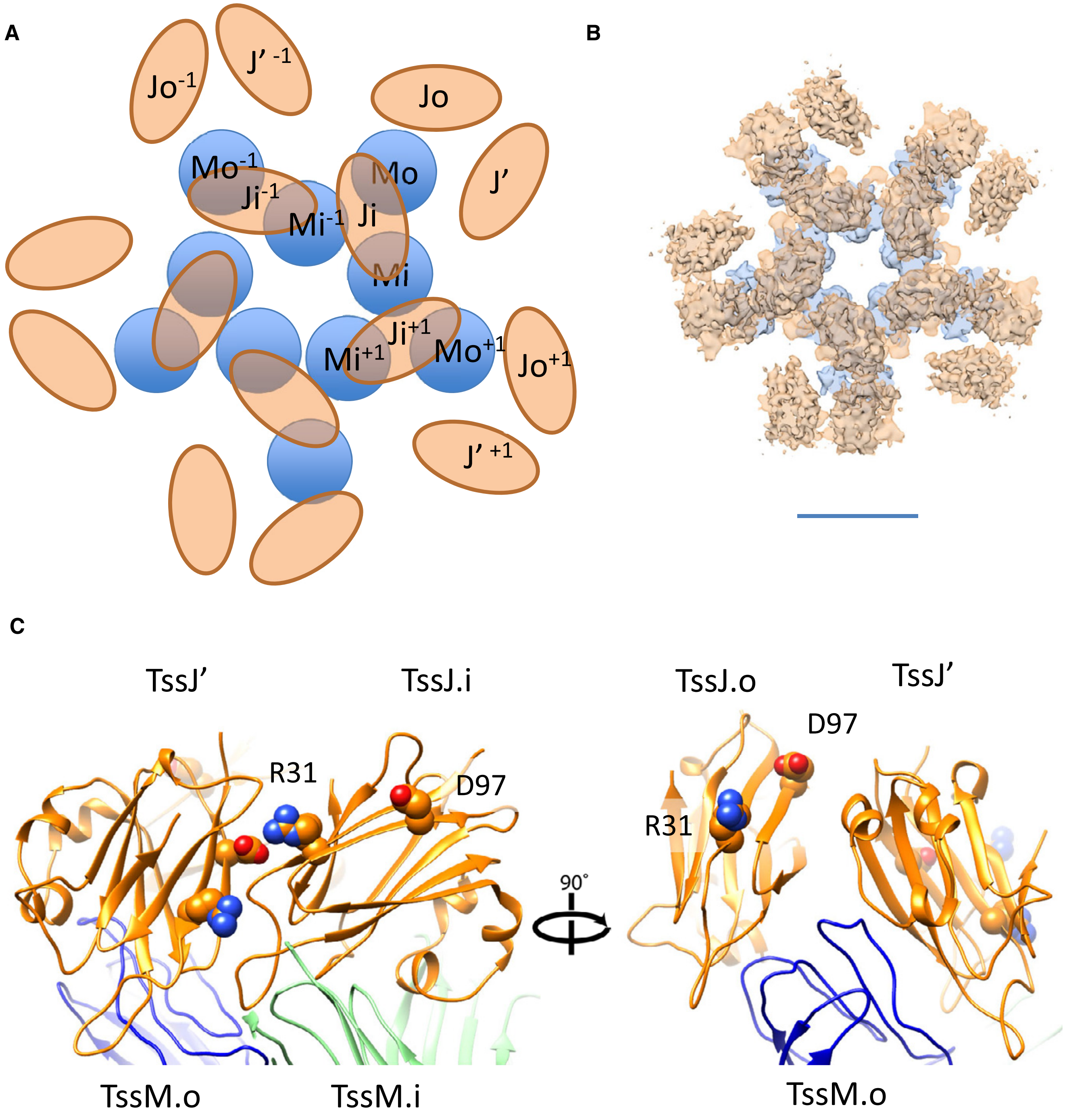

Figure EV3. The TssJ-TssM assembly.

A Schematic diagram of the TssM-TssJ assembly, labelled according to the nomenclature used throughout this paper.
B Superimposition of two slices through the MC complex corresponding to TssJ in orange and TssM in light blue, mimicking the diagram in (A).
C TssJ.o–TssJ′ interface (in orange) with the two residues involved in the interaction as an atom diagram and labelled.

Figure EV4. The TssM pseudoatomic model.

A Comparison between the TssM from the PDB (4Y7O) in cyan and the refined TssM on the cryo-EM density in orange.
B Comparison of the pseudoatomic model of TssM in the internal (green) and in the external (blue) pillars.
C Interaction interfaces between the internal TssM.a and the external TssM.A within the complex.
D Interaction interfaces between the internal pillars TssM.a and TssM.a^{+1}. The two pillars are twisted at 76° with respect to one another.
E Interaction interfaces between the internal pillar TssM.a and the external TssM.A^{+1}. The two pillars are twisted at 68° with respect to one another.
F Weak C-terminal density of TssM (1109–1129) in pink.
G The unsharpened density map of the whole complex at a contour level of 0.0055. The TssM foot domain (in blue) between amino acids 382 and 570 predicted structure by RaptorX fits into the density. The two feet converge upon arriving at the membrane level. The rest of the pseudoatomic model of TssM is in pink.

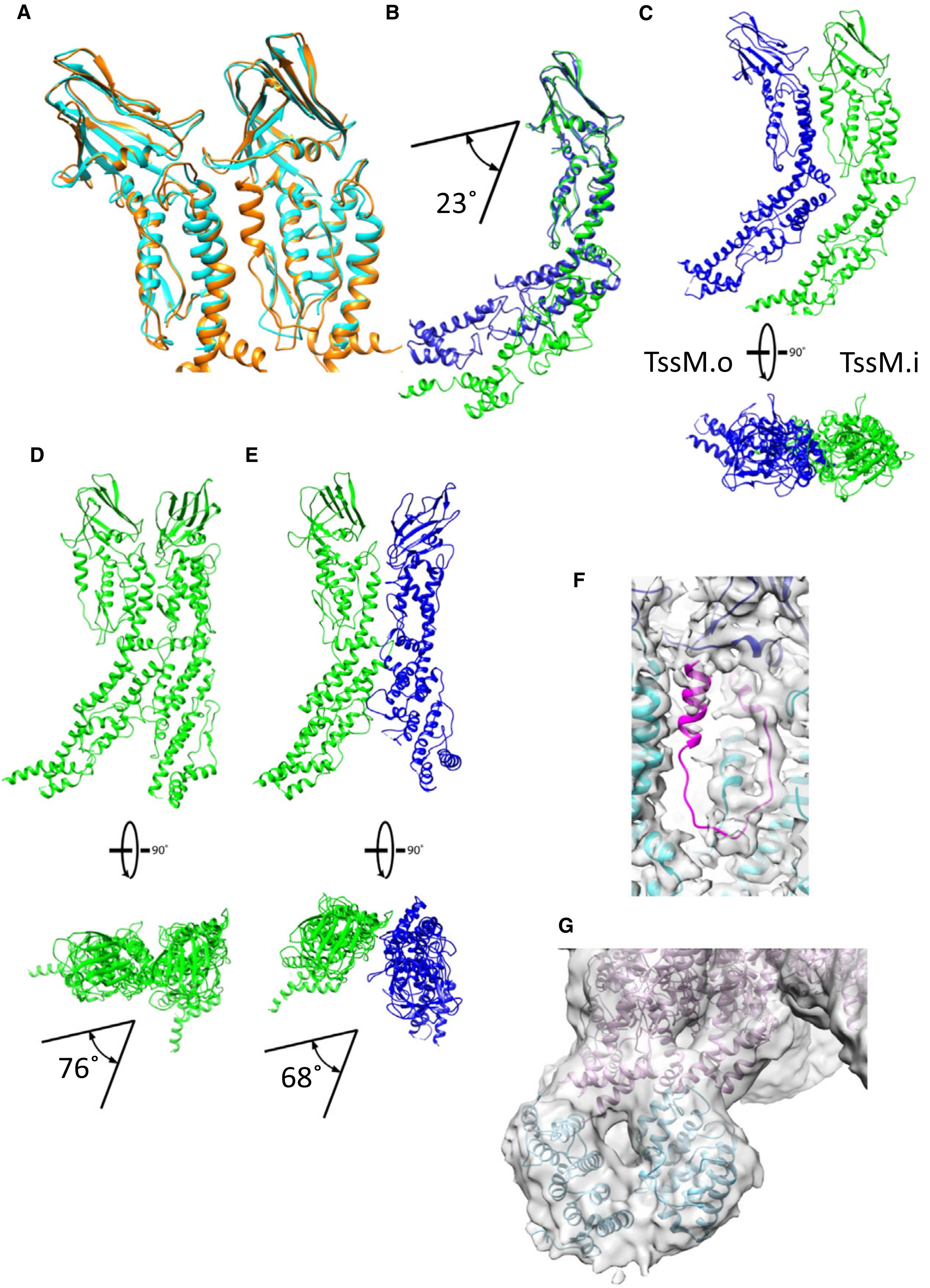

Figure EV4.