Figure EV1. Main and putative *Myxococcus xanthus* T6SS gene clusters.

A The cluster of conserved T6SS genes (MXAN\_4800–MXAN\_4813) in the genome of *M. xanthus*.

B Locus number of putative PAAR-repeat proteins.

C Domain architecture as predicted with PFAM 30.

D Gene neighborhood of putative PAAR-repeat proteins (green). Distant homologs to proteins known to have a role in the function of T6SS in other organisms are highlighted: VgrG (red) and DUF2169-containing proteins (blue). All gene clusters share similar set of homologs of less known proteins (shades of gray).
Figure EV2. Images of Myxococcus xanthus T6SS at different defoci.

A A T6SS structure captured by correlated cryo-PALM/ECT in our previous study [15] using a defocus of $-10\ \mu m$ during data collection.

B A similar structure captured in a cryotomogram of an M. xanthus cell with a defocus of $-6\ \mu m$. Tomography slices through full 3D reconstructions of intact cells are shown in both cases.

Data information: Scale bar in (B), 20 nm, applies to (A) and (B).
Figure EV3. Determination of the helical symmetries of *Myxococcus xanthus* T6SS extended and contracted sheaths by autocorrelation.

A, B Autocorrelation plots of the sub-tomogram average of extended (A) and contracted (B) sheaths, respectively. Translational search: \(-20\) to \(+20\) voxels, with increments of 1 voxel (voxel dimensions: \(7.8 \text{ Å} \times 7.8 \text{ Å} \times 7.8 \text{ Å}\)); rotational search: \(360^\circ\), with increments of \(1^\circ\). Blue to red colors indicate low to high correlation.