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**Supporting information for article:**

# Crystals of SctV from different species reveal variable symmetry for the cytosolic domain of the type III secretion system export gate

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**Table S1** . MR solutions for $LscV_C$ produced by *Phaser*.

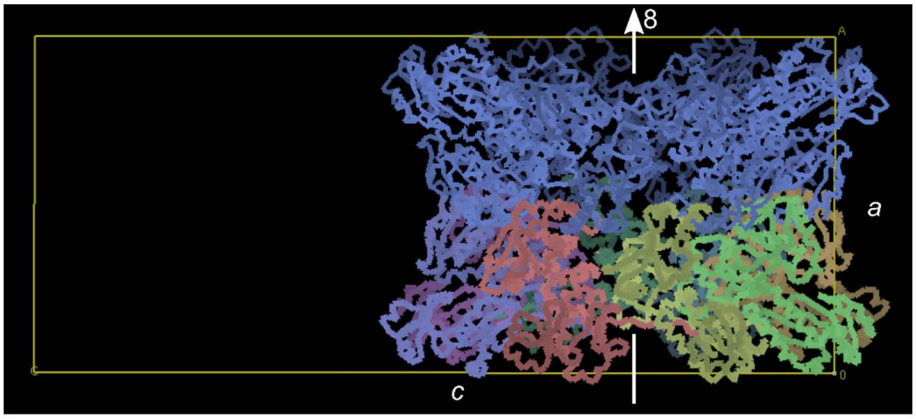
A monomer of $YscV_C$ was used as a search model (PDB: 7ALW).

| | **Search for 8 copies** | | | | **Search for 9 copies, solution #1** | | | | **Search for 9 copies, solution #2** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| #Copy | Euler angles | | | **TFZ** | Euler angles | | | **TFZ** | Euler angles | | | **TFZ** |
| 1 | 175.4 | 75.0 | 216.6 | **10.3** | 175.5 | 74.9 | 216.7 | **10.3** | 175.3 | 74.9 | 216.8 | **10.3** |
| 2 | 165.7 | 78.9 | 263.0 | **14.1** | 165.6 | 79.0 | 263.0 | **14.1** | 165.6 | 78.9 | 263.0 | **14.1** |
| 3 | 188.5 | 79.2 | 173.7 | **18.3** | 188.4 | 79.3 | 173.8 | **18.5** | 188.4 | 79.3 | 173.7 | **18.5** |
| 4 | 186.4 | 101.5 | 82.6 | **19.2** | 186.4 | 101.5 | 82.6 | **19.3** | 186.6 | 101.4 | 82.8 | **19.3** |
| 5 | 344.2 | 99.8 | 352.5 | **19.8** | 344.0 | 99.9 | 352.7 | **19.8** | 344.4 | 99.8 | 352.5 | **19.8** |
| 6 | 340.5 | 88.1 | 307.2 | **19.6** | 340.6 | 88.2 | 307.2 | **19.7** | 340.6 | 88.2 | 306.9 | **19.7** |
| 7 | 189.9 | 90.0 | 128.2 | **22.6** | 189.7 | 90.1 | 128.1 | **22.8** | 189.8 | 90.0 | 128.1 | **22.8** |
| 8 | 353.7 | 105.6 | 37.5 | **26.6** | 353.5 | 105.5 | 37.4 | **25.9** | 353.7 | 105.6 | 37.5 | **25.9** |
| 9 | | | | | 351.5 | 147.6 | 71.5 | **5.7** | 71.8 | 51.4 | 324.4 | **-** |

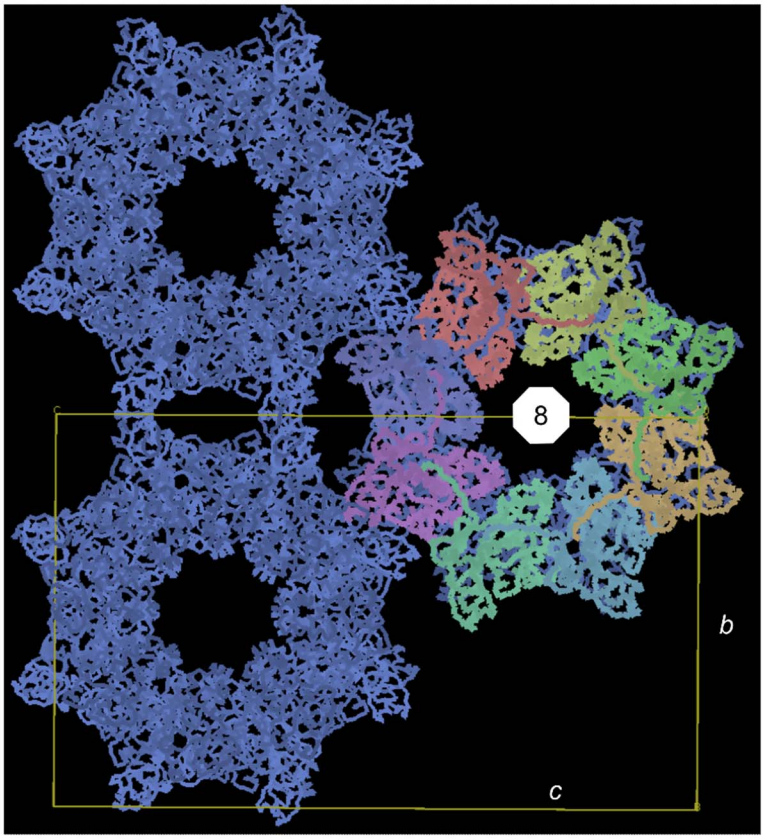
**Table S2** Anisotropy analysis of the diffraction data performed with the STARANISO server operated by Global Phasing Ltd. (https://staraniso.globalphasing.org/cgi-bin/staraniso.cgi).

The table gives the diffraction limits (in Å) and the principal axes of the ellipsoid fitted to the diffraction cutoff surface.

| | *a** | *b** | *c** |
|---|---|---|---|
| $YscV_C$:$YscX_{32}$:YscY<br>PDB ID: 7qij<br>Diffraction images: https://data.sbgrid.org/dataset/907/ | 4.907 | 3.895 | 3.851 |
| $LscV_C$ | 4.230 | 4.015 | 3.625 |
| $LscV_C$:$YscX_{32}$:YscY | 9.567 | 6.710 | 6.232 |
| $AscV_C$:$AscX_{31}$:YscY | 6.921 | 6.847 | 6.801 |




*(a)*




*(b)*

**Figure S1** Stacked octameric $LscV_C$ rings in the crystal after MR. (*a*) View along the *b* axis reveals that the 8-fold rotational symmetry of $LscV_C$ runs parallel to the crystallographic 2-fold *a* axis. The rings are stacked via their membrane-distal sides. (*b*) View along the *a* axis shows co-planarity of the octamers with the *bc* plane. Close symmetry-related molecules are shown.

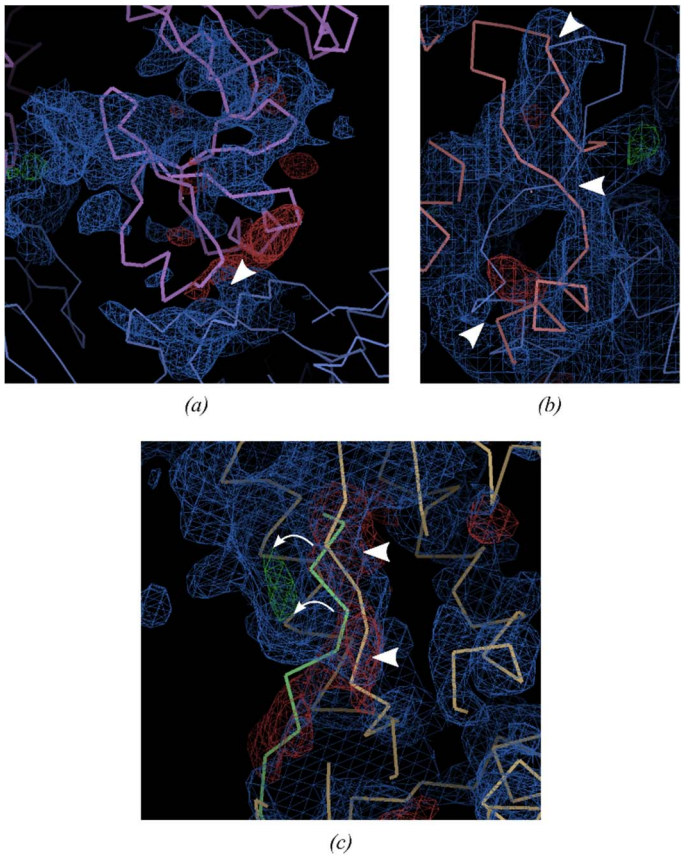



**Figure S2** Packing of $LscV_C$ in the crystal results in clashes indicated as arrowheads. (*a*) Too-close contacts between two adjacent octameric rings (magenta and light blue) occur at the periphery of the ring. SD2 (magenta) exhibits weakly defined electron density, indicating flexibility or wrong placement. (*b*) Clashes between stacked octamers occur at the SD4-SD4 interface (orange and light blue). (*c*) The N-terminal linker of one protomer (green) folds onto the neighboring subunit (yellow). Clashes could be alleviated by moving the linker into density on the left.

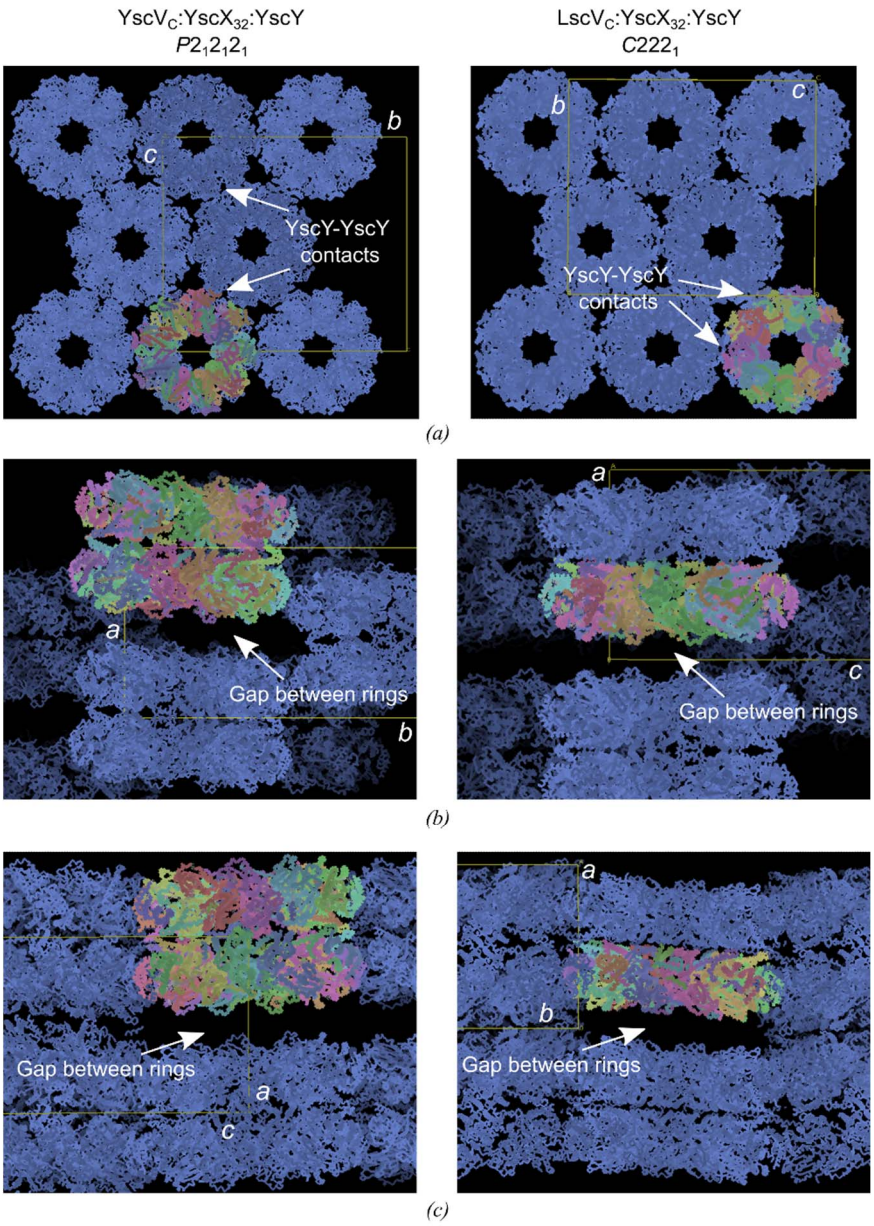



**Figure S3** Packing of YscV$_C$:YscX$_{32}$:YscY (left) compared to LscV$_C$:YscX$_{32}$:YscY (right). Views along the (*a*) shortest, (*b*) middle, and (*c*) longest axis of the unit cell. Crystal contacts are almost entirely made between two molecules of YscY on the periphery of the nonameric ring. Along the *a* axis, stacked nonamers are separated by larger solvent channels. Images of YscV$_C$:YscX$_{32}$:YscY reprinted from Gilzer *et al.*, 2022.

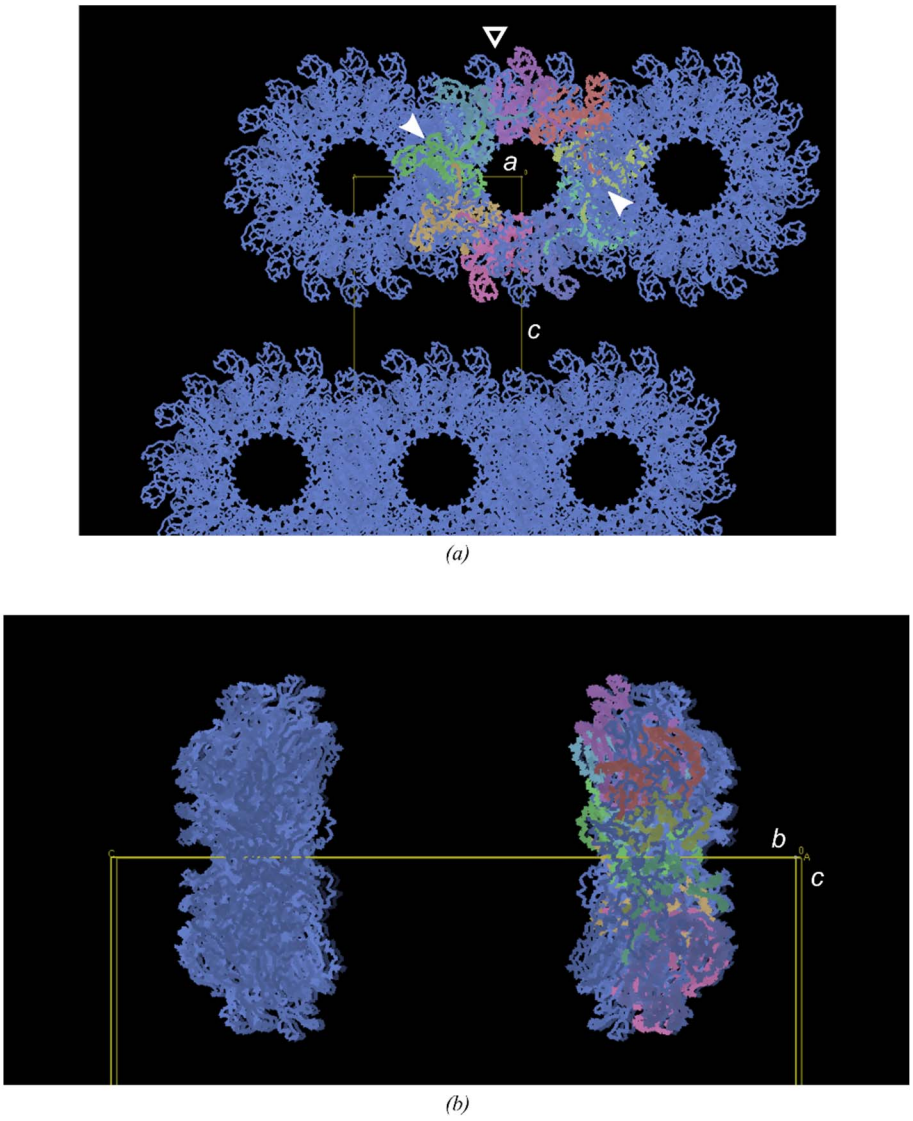



**Figure S4** Wrong MR solution for $AscV_C$:$AscX_{31}$:YscY produced when searching with a $YscV_C$ nonamer (PDB: 7ALW) as model. (*a*) View along the *b* axis shows large gaps along *c* and severe clashing of nonameric rings. Adjacent oligomers overlap with more than one protomer (white arrowheads) and a symmetry-related ring overlaps almost entirely (triangle). (*b*) View along the *a* axis presents large gaps between the placed model and a symmetry mate along the *b* axis.