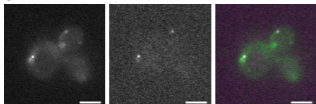
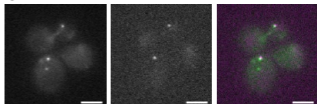


**a**

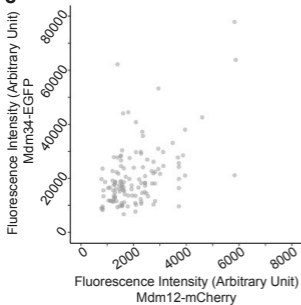
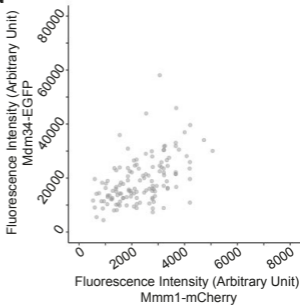
Mdm34-EGFP

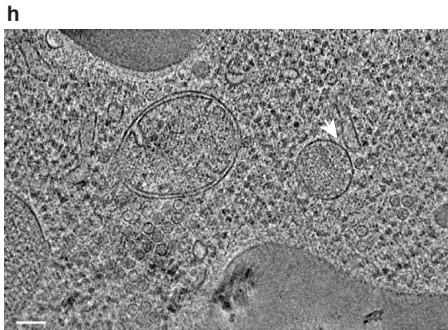
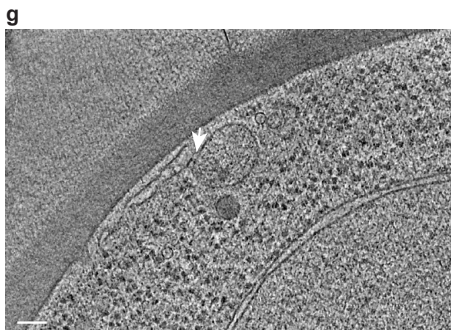
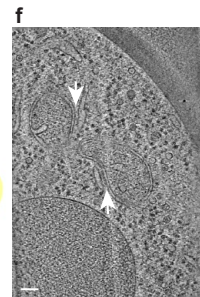
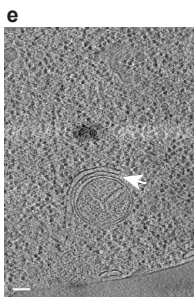
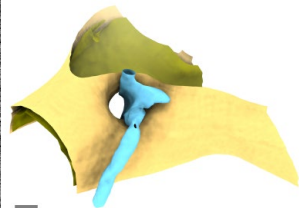
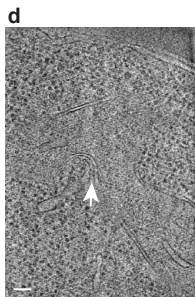
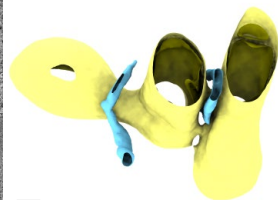
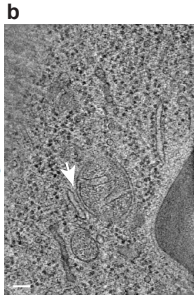
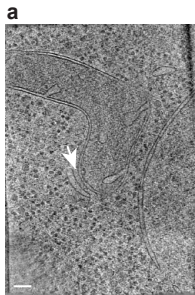
Mdm12-mCherry

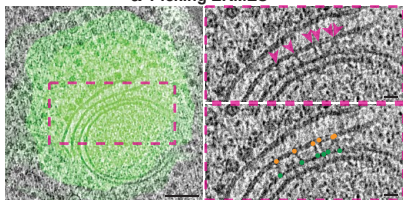
Mdm34-EGFP  
Mdm12-mCherry**b**

Mdm34-EGFP

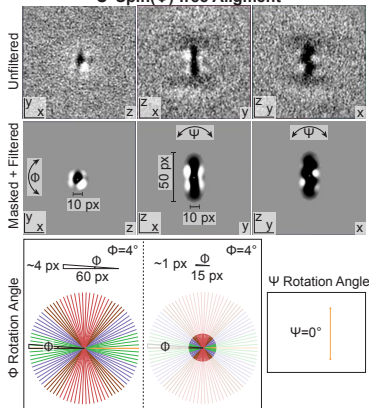
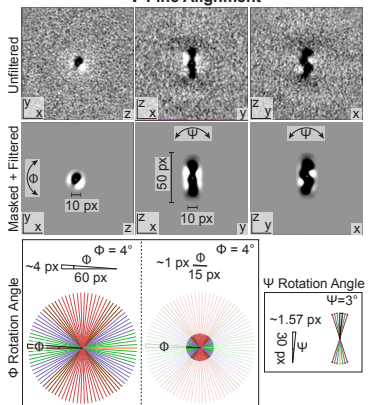
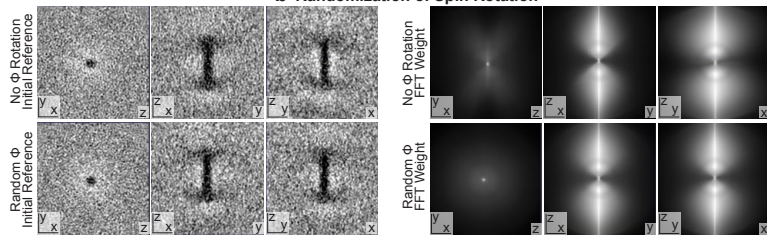
Mmm1-mCherry

Mdm34-EGFP  
Mmm1-mCherry**c****d**

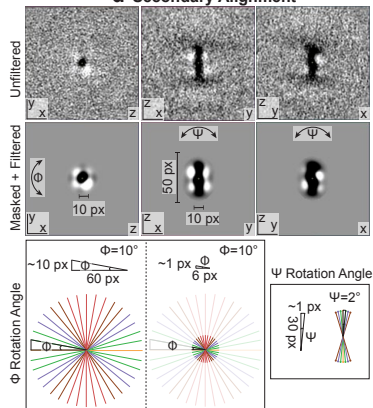
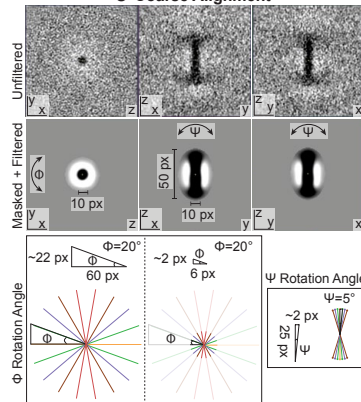
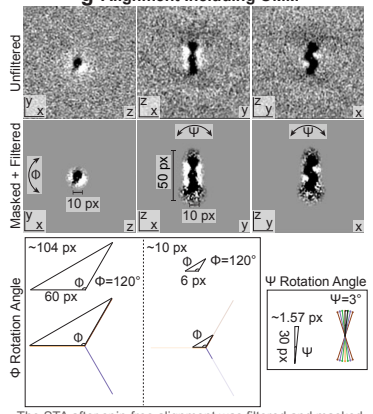
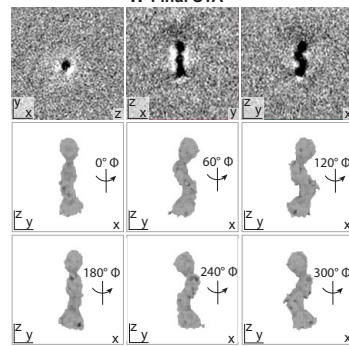


**a Picking ERMES**

ER and OMM proximal positions of bridging structures found in tomograms at locations of Mdm34-mNeonGreen cryo-FM signals were manually clicked as two points using Dynamo and stored as dipole models. The centres of these dipoles were used as extraction points for STA using subTOM

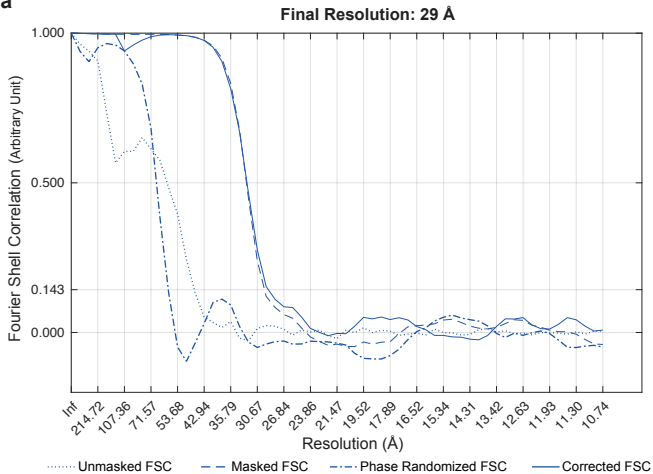
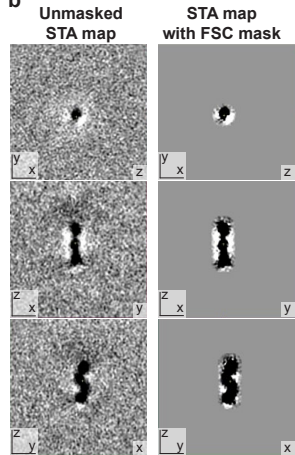
**e Spin( $\Phi$ )-free Alignment****f Fine Alignment****b Randomization of Spin Rotation**

Randomization of spin rotation around z-axis to minimize missing wedge dominant alignments

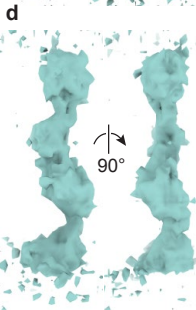
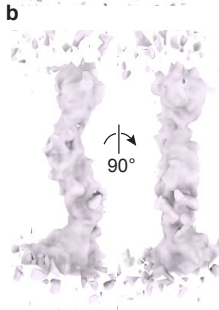
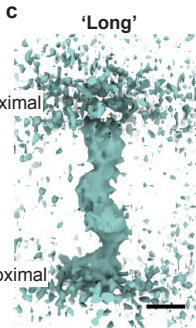
**d Secondary Alignment****c Coarse Alignment****g Alignment Including OMM****h Final STA**

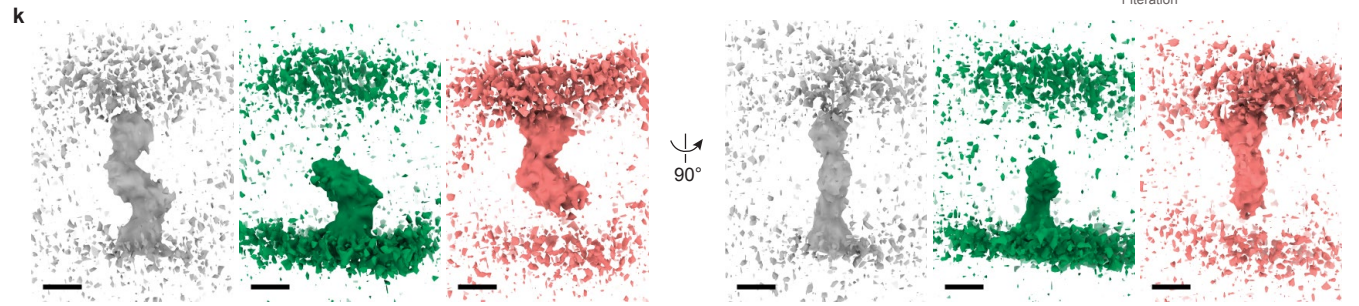
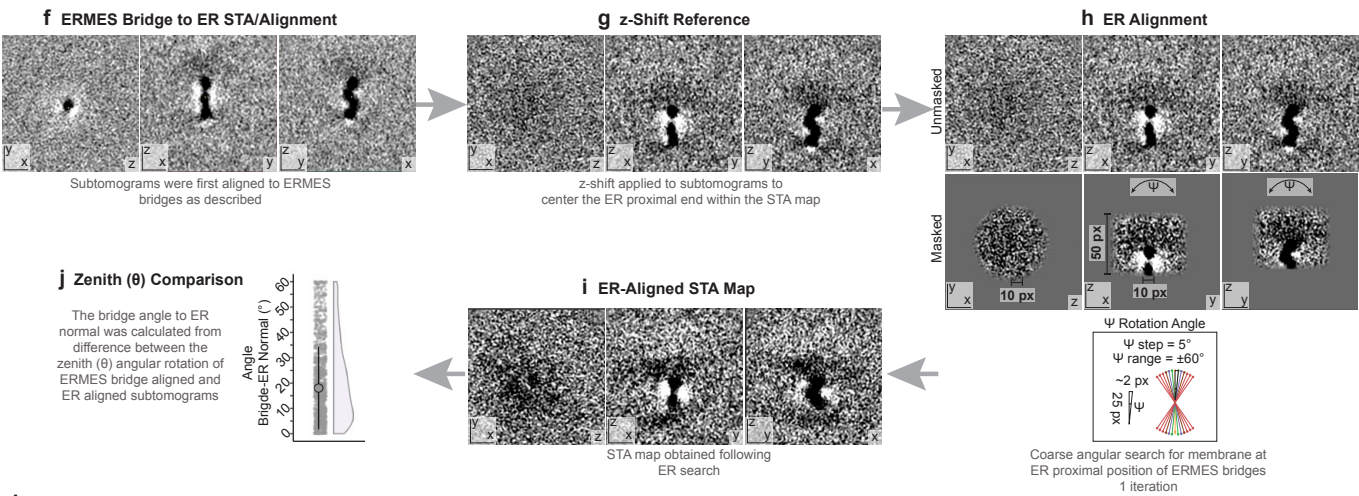
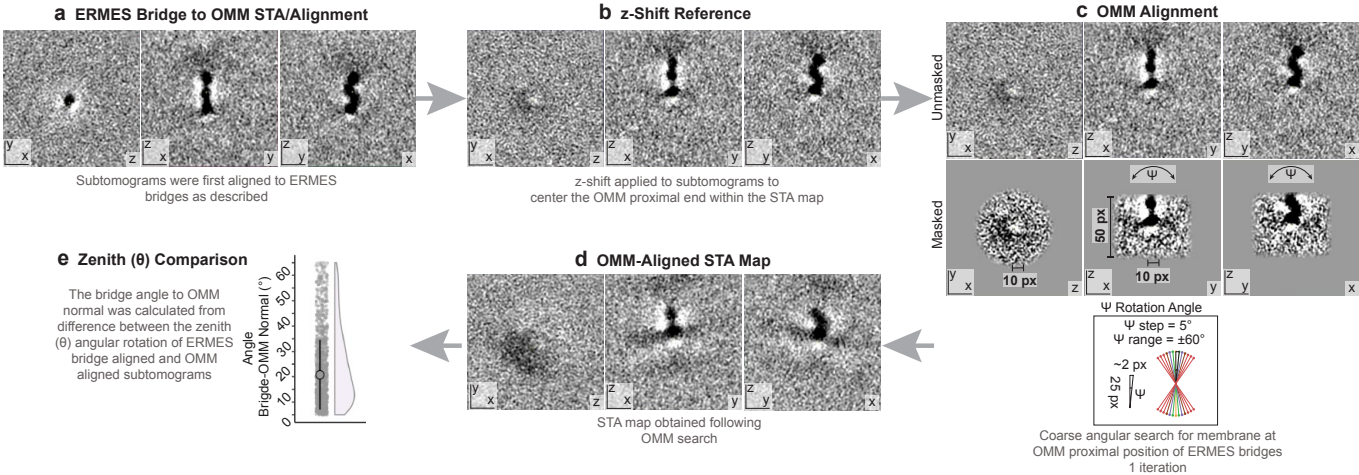
The STA after spin-free alignment was filtered and masked to produce a template for 3 iterations of a fine angular search alignment

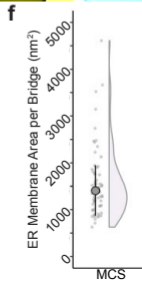
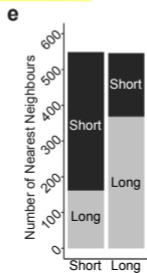
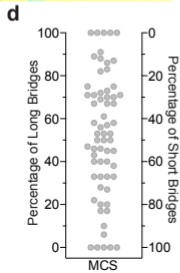
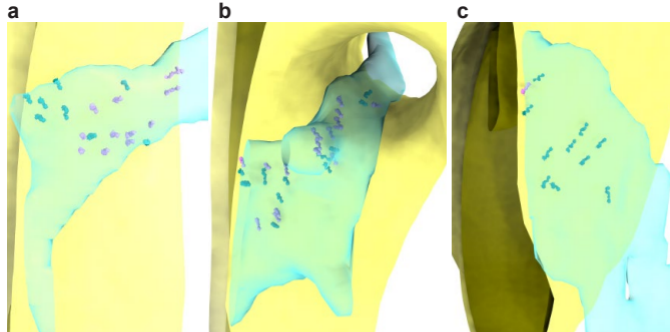
The STA after spin-free alignment was filtered and masked to partially include the OMM as a template for 3 iterations of a coarse angular search alignment

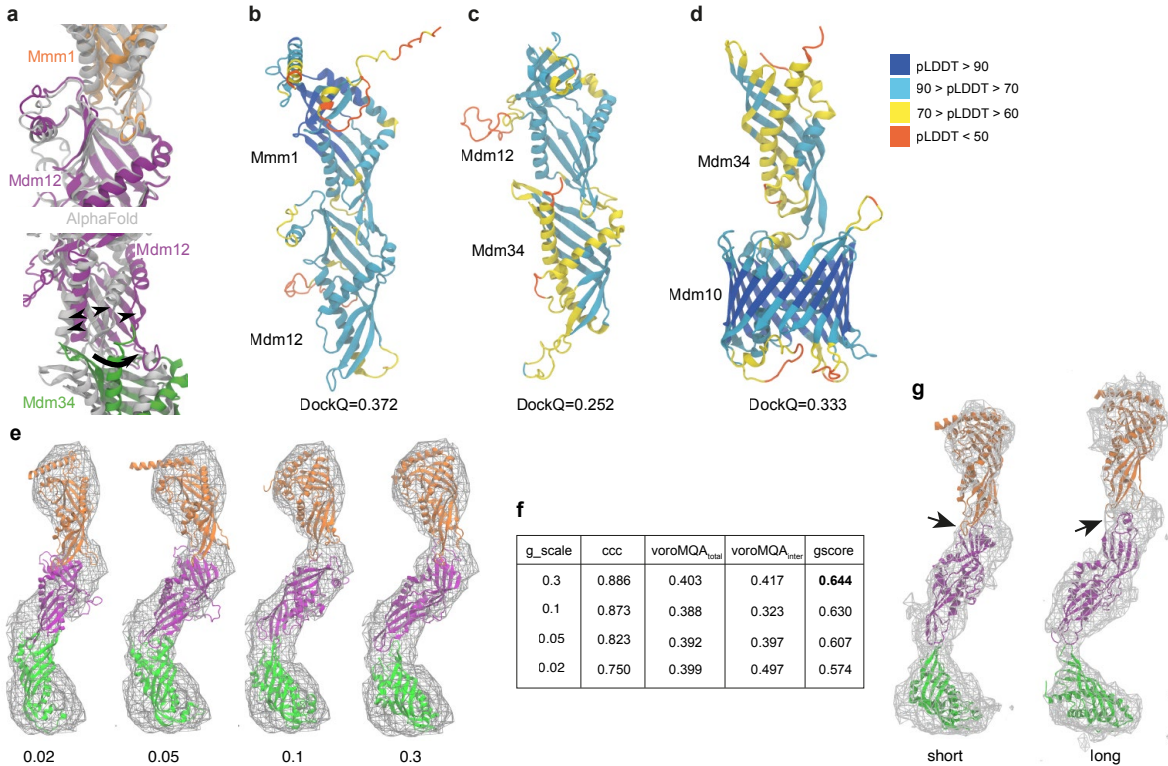
**a****b**



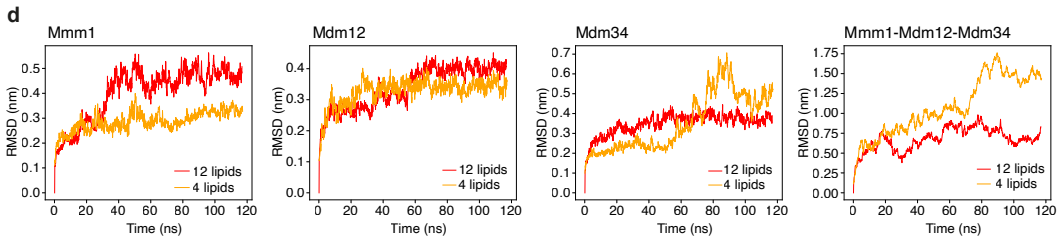
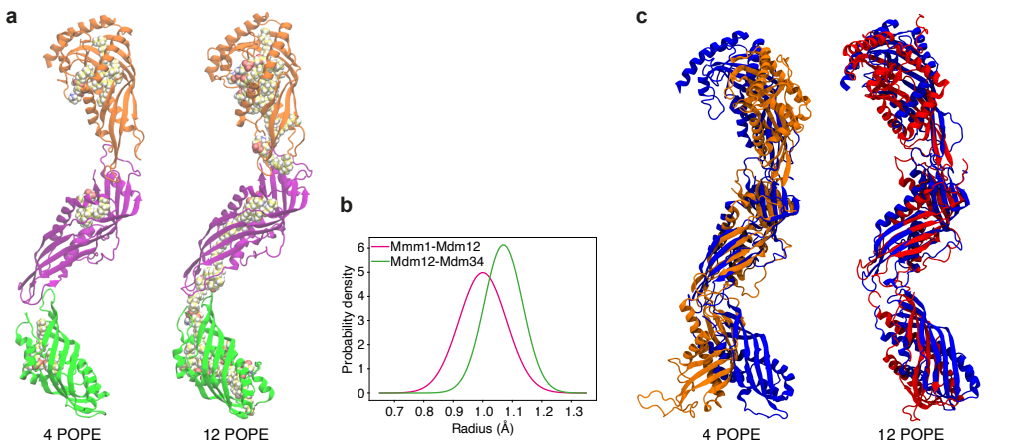


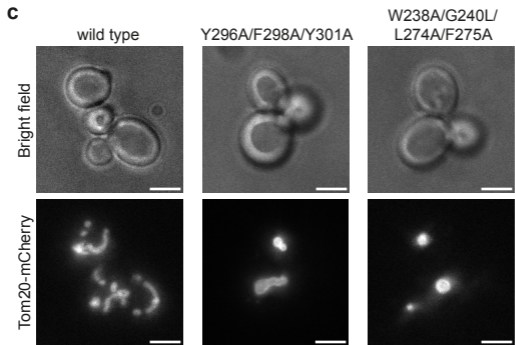
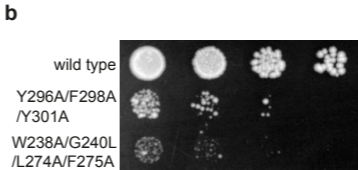
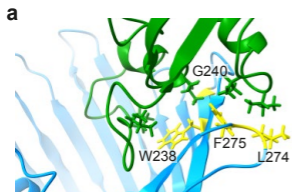












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## ERMES bridges

EMD-16873

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### Cryo-ET data collection and processing

Microscope	Titan Krios
Magnification	33000 x
Voltage (kV)	300
Detector	Gatan K3 in superresolution mode
Energy filter	yes
Electron exposure per tilt series ( $e^-/\text{\AA}^2$ )	approx. 140 - 150
Defocus range ( $\mu\text{m}$ )	-3.5 to -6.0
Pixel size ( $\text{\AA}$ )	1.342
Tilt range (min, max, increment)	$-56^\circ$ , $+56^\circ$ , $1^\circ$
Tilt scheme	Dose-symmetric
Frame number	4
Tomograms used for STA (no.)	51
Initial subtomograms (no.)	1133
Final subtomograms (no.)	1098
Map resolution ( $\text{\AA}$ ) at FSC threshold 0.143	29
Map resolution range ( $\text{\AA}$ )	-