

Supporting Information for

The SspB adaptor drives structural changes in the AAA+ ClpXP protease during *ssrA*-tagged substrate delivery

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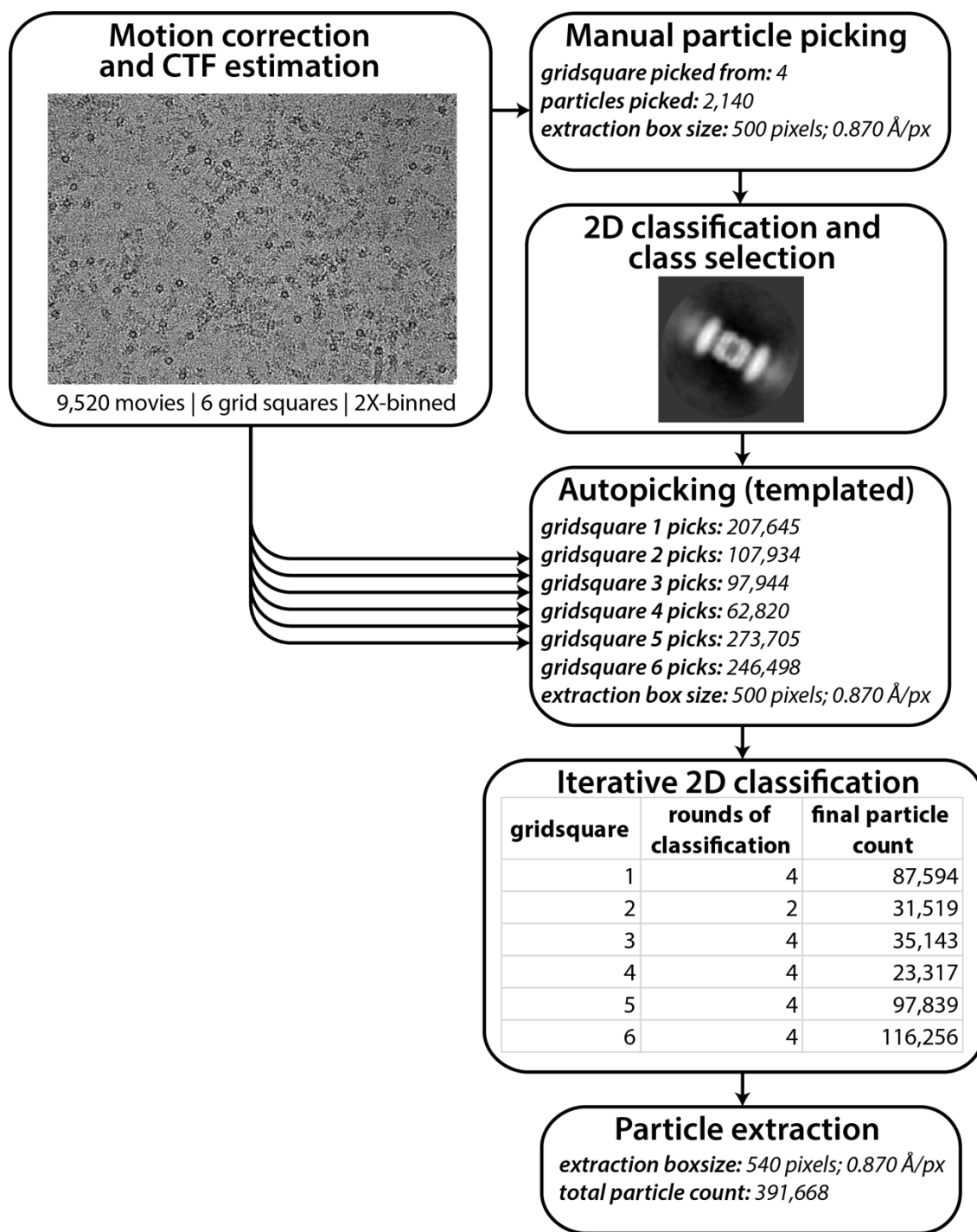


Fig. S1. Pre-processing workflow. Initial micrograph and 2D particle processing. Job types and key parameters are noted.

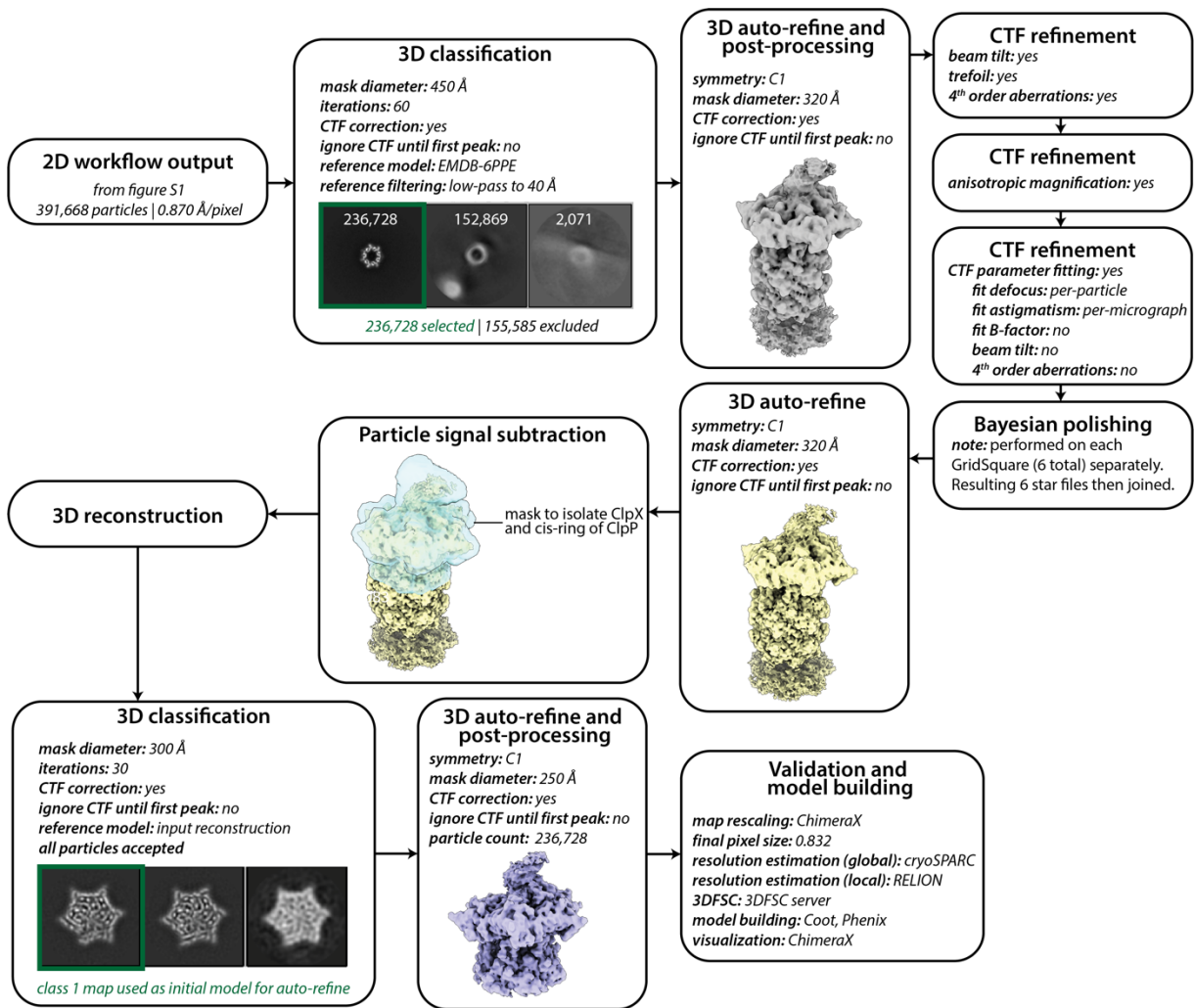


Fig. S2. RELION 3D processing workflow. Job names, job details, and non-default parameters are noted in each box.

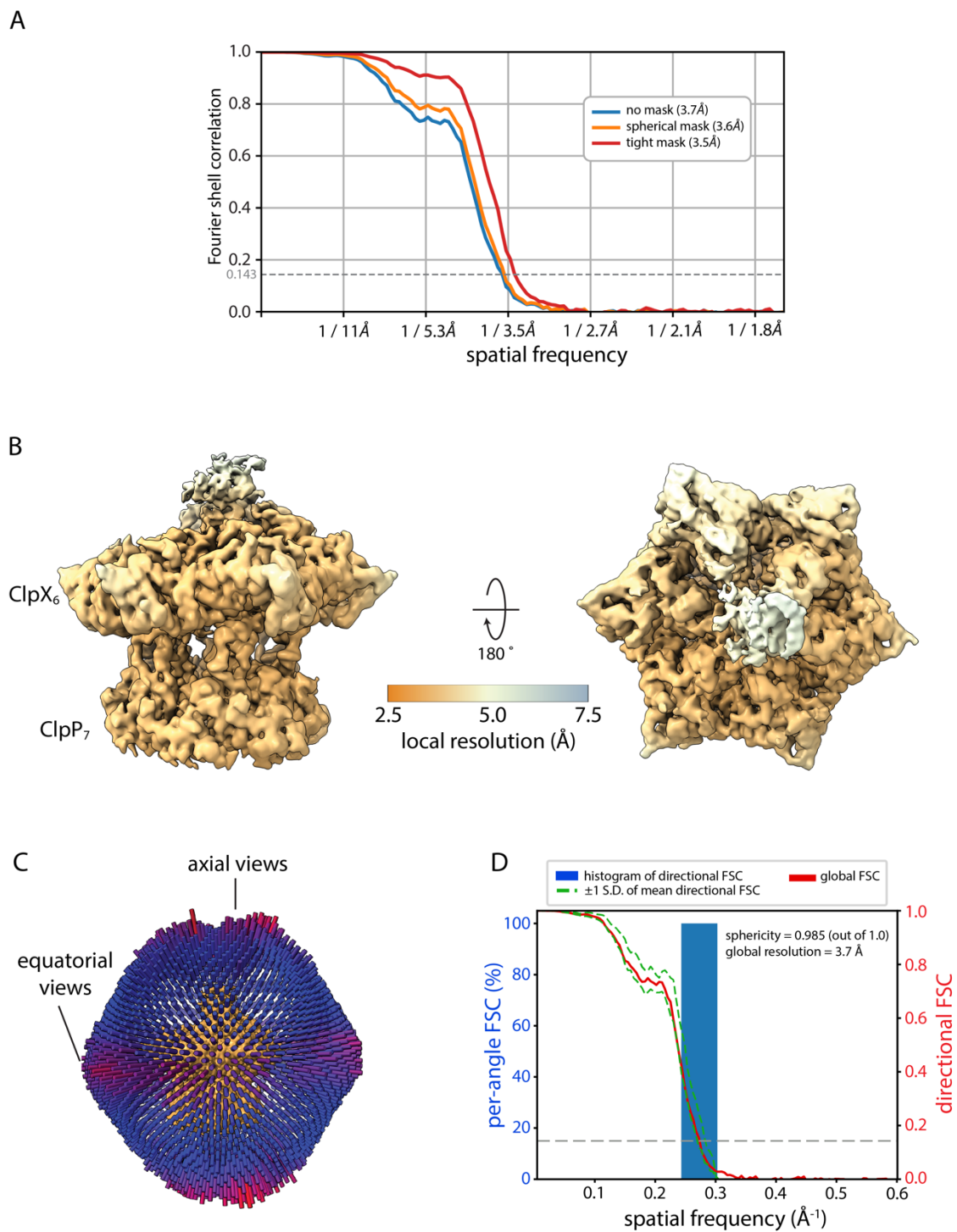


Fig. S3. Map assessment and validation. (A) Global resolution estimation by GSFSC implemented in CryoSPARC. (B) Local resolution assessment as estimated by RELION. (C) Projection angle distribution and directional FSC as estimated by the 3DFSC server (1).

Table S1. Cryo-EM data collection, refinement, model building, validation, and processing.

Sample and data deposition information	
Nucleotide added	ATP γ S
PDB ID	8ET3
EMDB ID	28585
EMPIAR ID	11349
Data collection	
Microscope	Titan Krios G3i
Camera / mode	Gatan K3 / counting
Magnification (nominal)	105,000 X
Accelerating voltage (kV)	300
Total electron dose (e-/Å ²)	76
Defocus range (μ m)	-1.0 to -2.5
Micrographs collected	9,520
Pixel size (super resolution)	
initial (Å)	0.435
calibrated (Å)	0.416
Model composition	
Non-hydrogen atoms	53,287
Protein residues	3,639
Ligands	4 ATP γ S, 2 ADP
Model refinement	
Refinement package	Phenix
Map-to-model cross correlation	0.78
RMS deviation bond lengths (Å)	0.002
RMS deviation bond angles (°)	0.633
Model validation	
MolProbity score	0.89
Clash score	1.51
C-beta outliers (%)	0
Rotamer outliers (%)	0
Ramachandran favored (%)	99.69
Map reconstruction	
Image processing package	RELION 3.1
Extracted particles	
for 2D classification	996,546
for 3D classification	391,668
final count	236,728
Resolution (Å)	
0.143 GSFSC unmasked	3.7
0.143 GSFSC spherical mask	3.6
0.143 GSFSC tight mask	3.5
3DFSC sphericity (unmasked)	0.985

Movie S1. Morph between the protease•substrate•adaptor complex (PDB 8ET3) and recognition complex (PDB 6WRF) as viewed from subunit **E** of ClpX (orange cartoon representation). As SspB (green surface representation) docks with ClpX (cartoon representation and surface outline) in the protease•substrate•adaptor complex, ClpX subunit **F** (gray cartoon representation) moves up to contact SspB and the IGF loop of ClpX subunit **E** moves from one binding cleft of ClpP to the adjacent, formerly empty, cleft.

Movie S2. Morph between the recognition complex (PDB 6WRF) and protease•substrate•adaptor complex (PDB 8ET3) as viewed from subunit **F** of ClpX.

SI Reference

1. Y.Z. Tan *et al.*, Addressing preferred specimen orientation in single-particle cryo-EM through tilting. *Nat. Methods* **14**, 793 (2017).