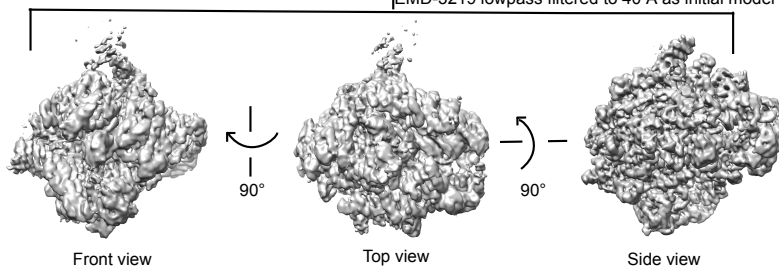


134,512 particles from 2069 micrographs



EMD-3219 lowpass filtered to 40 Å as initial model

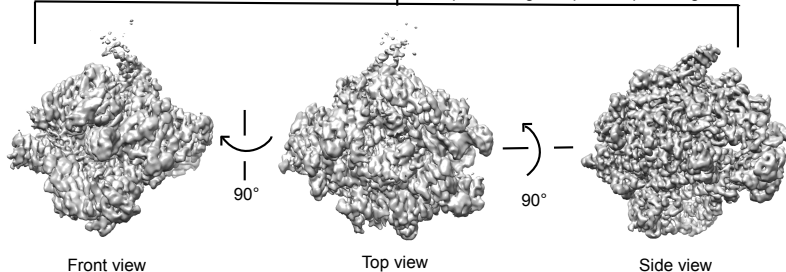


Front view

Top view

Side view

Movie processing and particle polishing



Front view

Top view

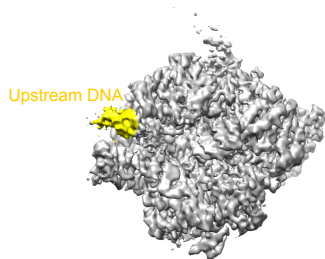
Side view

Focused classification and refinement on upstream DNA

Focused classification and refinement on α -amanitin binding pocket

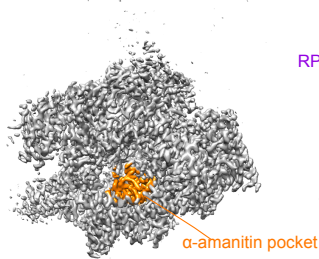
Focused classification and refinement on stalk

Postprocessing B-factor -138 Å²



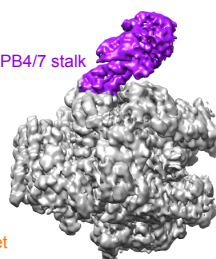
Upstream DNA

3.6 Å 67,808 particles



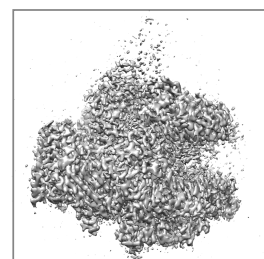
α -amanitin pocket

3.5 Å 115,728 particles

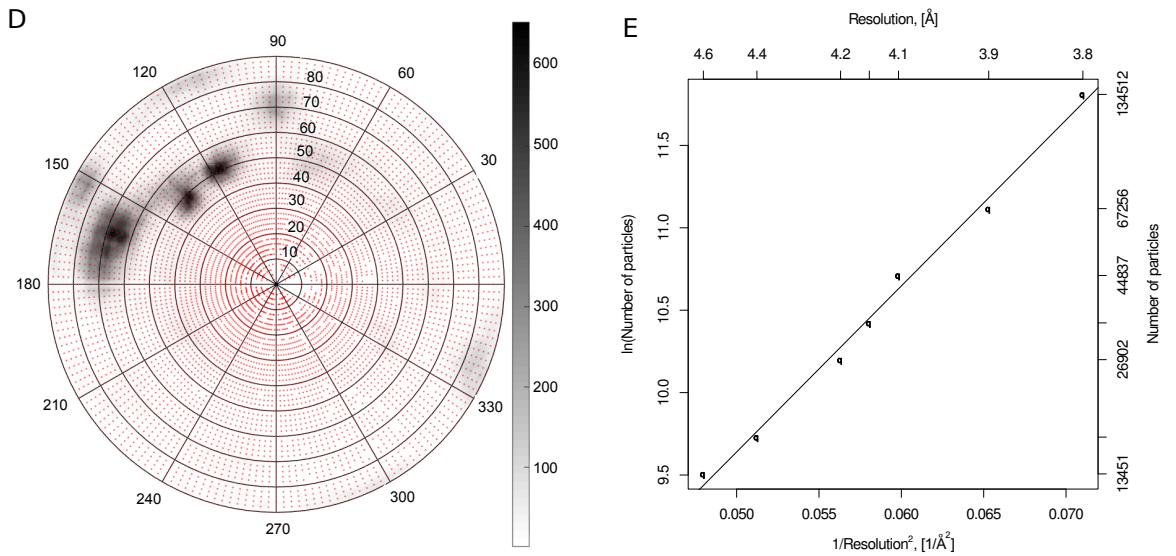
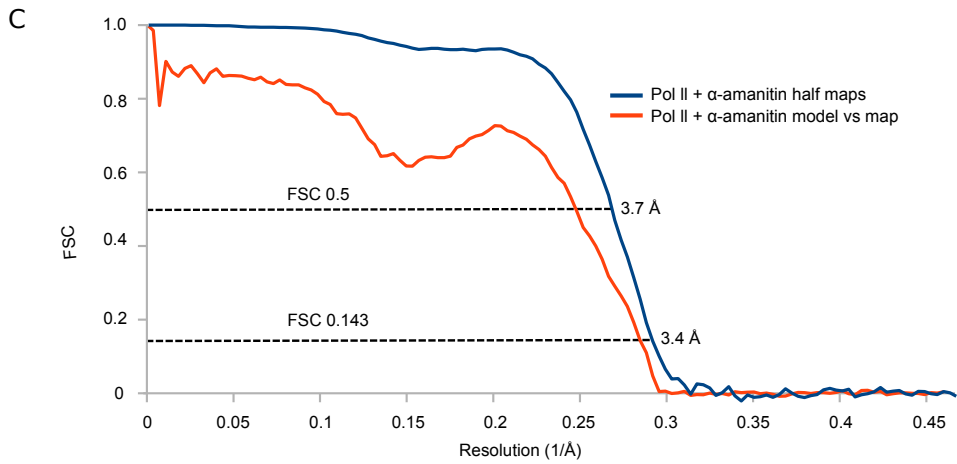
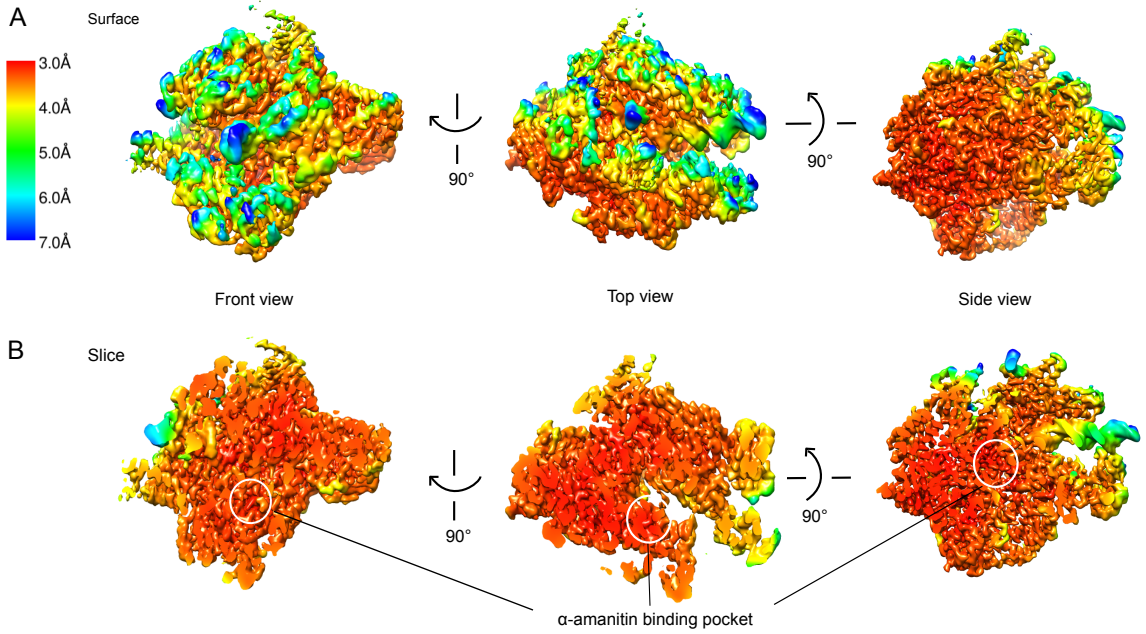


RPB4/7 stalk

3.6 Å 78,847 particles



3.4 Å 134,512 particles



Supplemental Figure 1 | Cryo-EM data processing.

The structure of bovine Pol II (EMD-3219) was low-pass filtered to 40 Å and used as the initial reference model. Semi-automatically picked particles were used for 3D refinement. Data processing with 3D refinement, movie processing and particle polishing gave a final reconstruction at a nominal resolution of 3.4 Å. Focused classifications and refinements were performed on upstream DNA, α -amanitin and its binding pocket, and the Pol II stalk subcomplex RPB4-RPB7.

Supplemental Figure 2 | Local resolution of the cryo-EM density map.

(A) Three views of a surface representation of the final cryo-EM density map colored according to local resolution.

(B) The same views as in (A) but sliced open to reveal the very high resolution at the active center of the polymerase and around the α -amanitin binding pocket.

(C) FSC plots for the cryo-EM reconstruction and for the model versus the cryo-EM reconstruction.

(D) Angular distribution of single particle images. Black shading indicates the number of particles assigned to a given view, while red dots indicate represented views.

(E) Resolution versus number of particles plot using random particle subsets with logarithmic and squared reciprocal axes. The slope of the linear fit indicates an overall B-factor of 101 Å²

Supplementary Table 1 Cryo-EM data collection, refinement and validation statistics

<i>Sus scrofa</i> Pol II EC bound by α -amanitin (EMDB-3981; PDB 6EXV)	
Data collection and processing	
Magnification	130,000x
Voltage (kV)	300
Electron exposure ($e^-/\text{\AA}^2$)	35
Defocus range (μm)	1.0-3.0
Pixel size (\AA)	1.07
Symmetry imposed	C1
Initial particle images (no.)	207,410
Final particle images (no.)	134,512
Map resolution (\AA)	3.4
FSC threshold	0.143
Map resolution range (\AA)	3.0-7.0
Refinement	
Initial model used (PDB code)	5FLM
Model resolution (\AA)	3.7
FSC threshold	0.5
Model resolution range (\AA)	3.0-7.0
Map sharpening B factor (\AA^2)	-138
Model composition	
Non-hydrogen atoms	32,710
Protein residues	3,907
Ligands	α -amanitin (1)
B factors (\AA^2)	
Protein	53.97
Ligand	56.58
R.m.s. deviations	
Bond lengths (\AA)	0.007
Bond angles ($^\circ$)	0.983
Validation	
MolProbity score	1.77
Clashscore	5.2
Poor rotamers (%)	0.5
Ramachandran plot	
Favored (%)	91.88
Allowed (%)	8.06
Disallowed (%)	0.05
