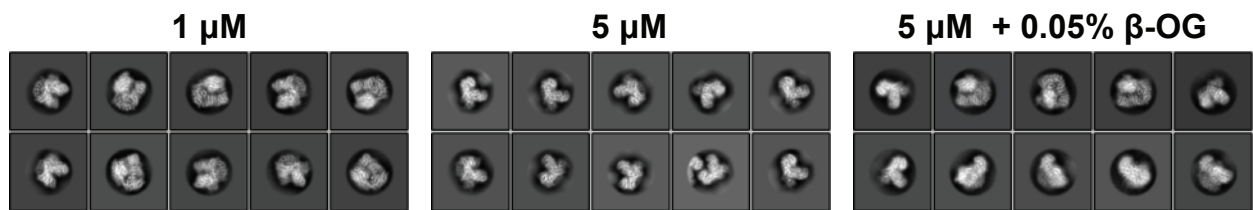


A 2D classification: 890,658 picks, 2.3 Å/pixel



B 3D classification (1 class), re-center and re-extract
3D auto-refine: 490,560 particles

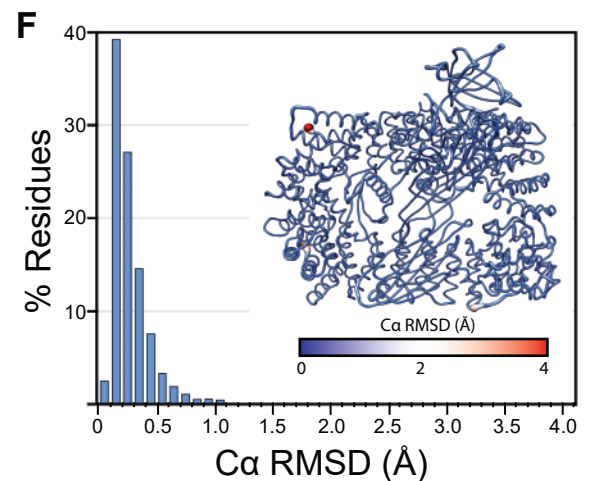
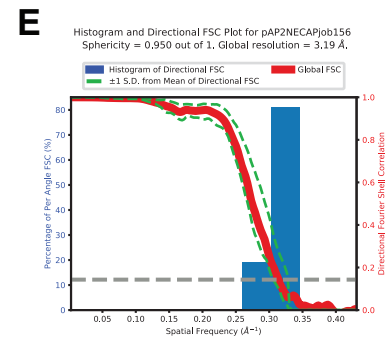
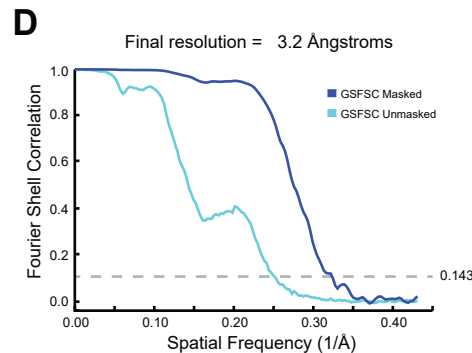
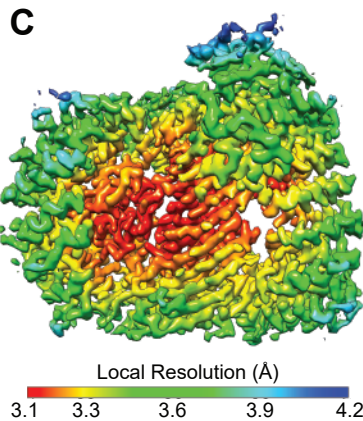
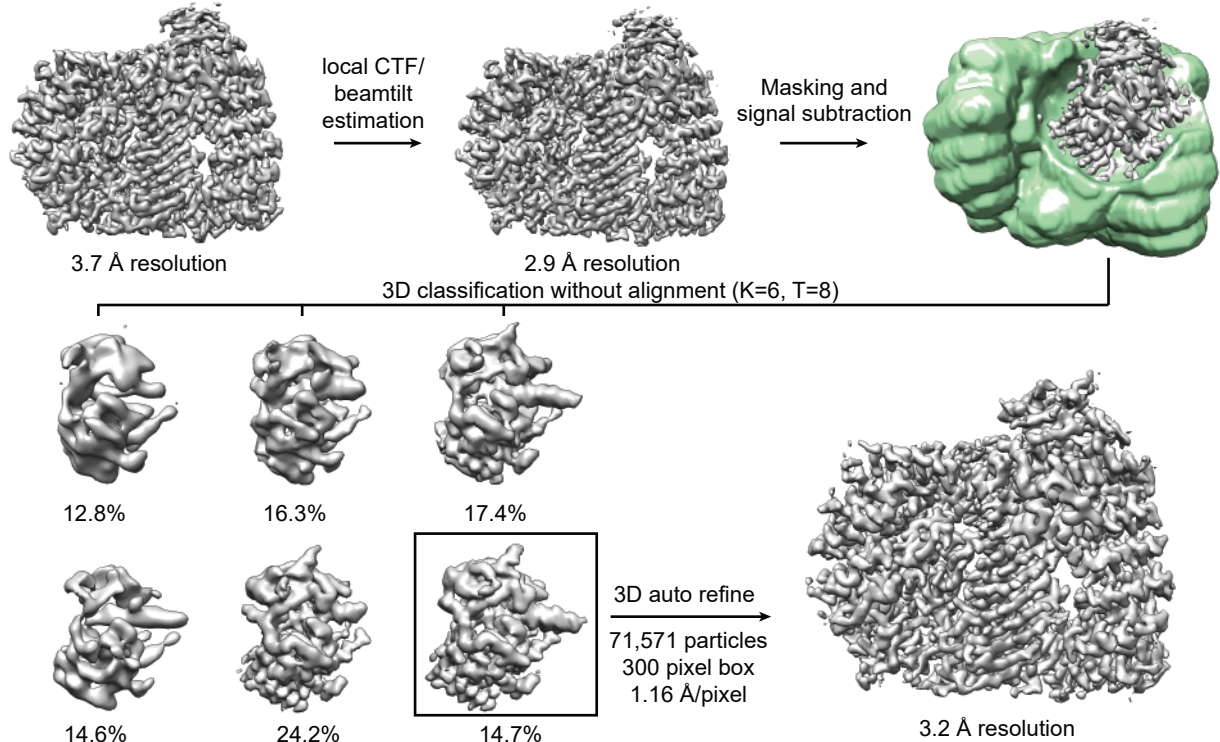


Figure 1—figure supplement 1. Classification, signal subtraction, and refinement of phosphoAP2 bound to NECAP

A. Representative 2D class averages demonstrating different particle orientation preferences under different grid preparation conditions.

B. Processing workflow for 3D classification and refinement of phosphoAP2-NECAP using the Relion 3 software suite.

C. Cryo-EM map colored by local resolution (Relion 3).

D. Fourier Shell Correlation (FSC) plot

E. 3D FSC plot. An overall sphericity of 0.95 shows that resolution is nearly uniform in three dimensions.

F. Histogram plot of Ca RMSD of top ten molecular models from Rosetta refinement. Inset shows molecular model colored and scaled by RMSD.