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Image Processing and 3D Reconstruction

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Protocol status: Working

We use this protocol and it's working

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Abstract

This workflow was used to analyze a Krios dataset of the PI3KC3-C1/RAB1A Complex and generate a reconstruction of three distinct conformational states of the VPS34 lipid kinase domain.

Attachments



[852-2197.pdf](#)

37KB

Materials

Materials and Software

- cryoSPARC v3 software
- UCSF ChimeraX v1.5 or similar software
- High-performance computing cluster or powerful workstation for computational tasks



Data Import

- 1 Import the raw Cryo-EM data sets into cryoSPARC v3.

Motion Correction and Fourier Cropping

- 2 Apply motion correction to the super-resolution movies.
- 3 Perform Fourier cropping 2x on the motion-corrected data using cryoSPARC's implementation of Patch Motion Correction.

CTF Determination

- 4 Use cryoSPARC's Patch CTF Estimation for Contrast Transfer Function (CTF) determination.

Particle Picking and Training a Model

- 5 Manually pick single particles from a selection of micrographs covering a range of defocus values.
- 6 Train a particle-picking model using Topaz.

Particle Extraction and Binning

- 7 Extract particles with an appropriate box size (e.g., 400×400×400 or ~1.5x the diameter of a single PI3K complex) to ensure retention of delocalized CTF information.
- 8 Optionally, bin the extracted particles 4x to increase computational speed for subsequent processing.



Initial 2D Classification

- 9 Apply two-dimensional (2D) classification to the extracted particles.



- 10 Exclude obvious junk particles from further processing based on the 2D classification results.

Heterogeneous Refinement

- 11 Use a junk class from the early rounds of an ab initio run, along with a map generated using an apo PI3K model in UCSF Chimera using molmap at a resolution of 20 Å, for heterogeneous refinement.
- 12 Iterate this process for 3-4 rounds until a healthy substack of particles is evident by 2D classification.

Ab Initio Reconstruction and Particle Cleanup

- 13 Perform a three-class ab initio reconstruction for a final particle cleanup.
- 14 Should result in a clearly clean class of particles.

High-Resolution Refinement

- 15 Re-extract the particles at a full 400-pixel box size.
- 16 Perform homogeneous refinement using the ab-initio model and the clean particle stack to generate a high-resolution model and particle alignments for downstream classification.

3D Classification without Alignment

- 17 Conduct 3D classification without alignment using a large mask on the putative kinase domain and create 50 classes.

Selection of Classes for Further Analysis

- 18 Select the three most populated classes from the group of 50.



Heterogeneous Refinement of Selected Classes

- 19 Perform heterogeneous refinement on the selected classes.

3D-Variability Analysis

- 20 Perform 3D-variability analysis in cryoSPARC in cluster mode for each population showing strong density for the kinase domain.
- 21 Using the clusters containing the strongest density, perform non-uniform refinements on each.

Local Refinement

- 22 Conduct local refinement on three parts of the complex: VPS15 pseudokinase domain, RAB1A interface region, and BECN1/ATG14 BARA dimer domain.
- 23 For VPS34 Kinase containing classes - perform local refinement with a mask aligned to the particular pose of the kinase domain.
- 24 Create masks for these regions using UCSF ChimeraX Volume Tools.

Combining Refined Maps

- 25 Combine the locally refined maps, which correspond to distinct kinase conformations, using UCSF ChimeraX with the `vop maximum` command.

Model Building and Visualization

- 26 Utilize the combined maps for model building and visualization of the structures.