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We use this protocol and it's working

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Image Processing and 3D Reconstruction

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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

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., 400x400x400 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.