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Protocol status: In development
We are still developing and optimizing this protocol

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LRRK2-RCKW:E11DARPin:MLi-2 image processing

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ABSTRACT

Protocol for data processing of 8000 to 12000 micrographs for LRRK2-RCKW:E11 DARPin:MLi-2 complexes. Datasets are collected with an electron microscope equipped with a direct detector. It focused primarily on the tetrameric and monomeric population of LRRK2. This protocol allows us to obtain maps from 2.7 to 3.0 for tetramers and 3.0 - 3.6 A for monomers. All this processing was done using cryoSPARC.

MATERIALS

CryoSPARC v4[1, 2]

Topaz 0.2.5[3]

ChimeraX[4]

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1 After movie alignment and CTF estimation (Patch Motion Correction and Patch CTF Estimation jobs in CryoSPARC), discard the micrographs with an estimated resolution worse than 3.5 Å.

2 Pick your particles with Cryosparc's Blob picker.

2.1 Set particle diameter to 240-300 Å. This range diameter willl include from monomers to tetramers.

2.2 If using Cryosparc Live, calculate preliminary 2D classes to assess if you should proceed with your data collection.

2.3 At least 2 or 3 LRRK2-shaped 2D class averages after collecting 200-300 movies it's a good signal to keep collecting.

3 Extract your particles, binning them to a pixel size of 3.74 Å and choosing a box size that encloses the whole LRRK2 (binning from 400 to 100 Å).

4 Perform rounds of 2D classification in CryoSPARC and select only the particles belonging to

“good” 2D class averages.

Note

In the initial rounds, if in doubt of selecting a 2D class, it is OK to select it. You may rescue good particles later.
The selection of “good quality” 2D classes depends on the user. Take into consideration the recognition of LRRK2 domains and the visualization of secondary structure elements.

5 Train a particle-picking model using Topaz wrapper in Cryosparc using your best particles previously obtained. Keep in mind you need to train a model for tetramers and another for monomers.

Note

Choose as much variety of views of your protein as you may have in your previous 2d classes.

5.1 Some parameters worth playing with are the Expected number of particles, Minibatch size, the Number of epochs and the Learning rate.

6 Extract the particles with a pixel size of around 4 Å using Topaz Extract and Extract from Micrographs. This second step might take a few hours, depending on the number of micrographs you might have.

7 Clean your particle dataset with several rounds of 2D classification in CryoSPARC.
Select the particles belonging to “good” 2D class averages.

8 Remove bad particles by running an Ab-initio job in Cryosparc and discarding the particles belonging to the worst class, if it does not display LRRK2 features at all.

Note

It is a quick way of further cleaning the dataset.

8.1 Try several Ab-initio jobs with different classes. Analyze in Chimera the maps to decided which ones you will use in the next steps.

9 Extract the best particles you have so far in CryoSPARC with your preferred box size and using your original A/px.

10 Re-run a new 2D classification to remove the last junk particles you might have

11 Run a new Ab-initio just with one class. You will be using likely this map as your reference volume for next steps.

12 Tetramer's particle processing: Ab-inito was used as an input for NU-Refinement, where we imposed a D2 symmetry.

12.1 Monomer's particles: We used its Ab-initio as an input for NU-Refinement, using C1 symmetry

13 Tetramer's particles were expanded based on the volume symmetry and used in subsequent jobs.

14 A mask was created using NU-Refinement for tetramer's volume. The map was opened in Chimera. With Volume Eraser tool, three of the protomers were erased, leaving just one of them.

14.1 The map showing one protomer was uploaded to cryoSPARC using Import 3D volumes job.

14.2 From this map, we created a mask using Volume tools job. For that, we also input threshold mask (previously picked in Chimera, where we see a continuous density for our map without adding noise), Lowpass Filter, Dilate mask and Add soft padding. You might play with these parameters to see which one are the best for your case.

15 Using the map obtained at NU-Refinement job and the mask previously created, we run a local refinement.

16 Using as an input the particles and mask from local refinement, we run a 3D Variability job for exploring both discrete and continuous heterogeneity in our dataset.

16.1 After 3D Variability job, a 3D Variability display job was run. We run it first as a simple mode. It output a simple linear "movie" of volumes along each dimension. You should visualize your results through UCSF Chimera or similar.

17 A new 3D Variability job was created, using this time intermediate mode. (Here particles are sorted along each variability dimension, and then split into overlapping subsets, weighted by their position along the variability dimension.)

18 Subset particles showing less heterogeneity between them were used as an input for a local refinement (C1 symmetry), also taken as an input mask created on step 14 and the consensus map obtained in the NU-Refinement job.

Notes

19 Note 1: consider retraining your Topaz model with the particles of the best map you have and repeat image processing. Consider

Note 2: Transfer your particles to Relion and perform 3D classification with and without alignment and 3D Refine might help to improve your maps.

Note 3: Consider also cryoDRGN.