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Electron microscopy (EM) analysis of LRRK2-Nanotube assembles

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We use this protocol and it's working

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Abstract

This protocol details methods for the analysis of LRRK2-Nanotube assembles by negative stained EM and Cryo-EM.

Attachments

DOCX

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21KB

Materials

Solutions to prepare:

Low salt buffer:

	A	B
	HEPES (7.4)	20 mM
	NaCl	90 mM
	MgCl2	2.5 mM
	Glycerol	7%
	DTT	2 mM
	GDP	20 µM

Negative stained EM analysis

1 Prepare samples in a PCR tube with [M] 300 nanomolar (nM) LRKK2, [M] 20 micromolar (µM) lipid nanotubes and [M] 1 millimolar (mM) GMPPNP.

2 Incubate samples at 37 °C for 00:30:00 . 30m

3 Glow-discharge carbon-coated grids (25 mA, 00:00:45) during the sample incubation time. 45s

4 Place the discharged grids on a piece of parafilm.

5 After incubation, apply 6 µL of the samples to the grid and adsorbed on the grid for 00:05:00 at Room temperature . 5m

6 Blot the grid with filter paper and stained with 2% uranyl acetate for 00:00:40 . 40s

7 Dry the grid with filter paper.

8 Collect images using a Talos L 120C TEM microscope at 80 kV with Velox software and a 4k × 4K Ceta CMOS camera (Thermo Fisher Scientific).

Cryo-EM analysis 1h 14m 14s

9 Dialyze freshly purified LRRK2 into the Low salt buffer.

10 After dialysis, incubate LRRK2 ([M] 2 micromolar (µM)) with the kinase inhibitor MLi-2 ([M] 5 micromolar (µM)) for 00:10:00 On ice . 10m

11 Add [M] 20 micromolar (µM) lipid nanotubes into the mixture above and further incubate for 01:00:00 at Room temperature in the presence of [M] 1 millimolar (mM) GTP. 1h

Note

Note: The total volume of the mixture is 12 µL .

12 Glow-discharge C-flat™ holey carbon gold grids (CF-1.2/1.3-3Au) (15mA, 00:00:45) during the sample incubation time, then place the discharged grids on a piece of parafilm. 45s

13 After incubation, apply 4 µL of the samples to the grid.

14 Plunge-freeze sample-loaded grids in liquid ethane-propane mixture using a Vitrobot Mark IV (FEI) with the following parameters: blot force, 0; blot time, 00:00:01 ; wait time, 00:00:30 ; drain time, 00:00:00 ; humidity, 100%. 31s

15 Collect cryo-EM micrographs on a Titan Krios transmission electron microscope (Thermo Fisher Scientific) operating at 300 kV, equipped with a post column GIF quantum energy filter and a Gatan K3 Summit DED camera (Gatan, Pleasanton, CA, USA).

16 Perform the data collection with the SerialEM software. Record movies in super-resolution mode with a physical pixel size of 1.098 A˚ (super-resolution pixel size is 0.549 A˚) and a defocus range of - 1 µm to - 3 µm .

Note

The total dose of ~60.6 e− Å−2 was attained by using a dose rate of ~23.5 e− pixel−1 s−1 across 43 frames for 00:02:00 00:00:58 total exposure time. The initial drift and beam-induced motions was corrected using MotionCor2.