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Protocol status: Working
 We use this protocol and it's working

Created: Aug 19, 2022

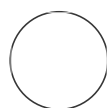
🌐 Electron microscopy (EM) analysis of LRRK2-Nanotube assemblies

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ABSTRACT

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

ATTACHMENTS

[iuugbv9p.docx](#)

MATERIALS

Solutions to prepare:

Low salt buffer:

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

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Negative stained EM analysis

- 1 Prepare samples in a PCR tube with [M] 300 nanomolar (nM) LRRK2, [M] 20 micromolar (μ M) lipid nanotubes and [M] 1 millimolar (mM) GMPPNP.



- 2 Incubate samples at [T] 37 °C for [D] 00:30:00 . 30m



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

- 4 Place the discharged grids on a piece of parafilm.

- 5 After incubation, apply [V] 6 μ L of the samples to the grid and adsorbed on the grid for [D] 00:05:00 at [T] Room temperature . 5m

- 6 Blot the grid with filter paper and stained with 2% uranyl acetate for [D] 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



31s

(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%.

- 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 Å (super-resolution pixel size is 0.549 Å) and a defocus range of -  1 µm to -  3 µm .

Note

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