**Electron microscopy (EM) analysis of LRRK2-Nanotube assembles**

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

Solutions to prepare: Low salt buffer: 20mM HEPES 7.4, 90mM NaCl, 2.5mM MgCl2, 7% Glycerol, 2mMDTT, 20μM GDP.

**Protocol:**

***Negative stained EM analysis***

1, Prepare samples in a PCR tube with 300nM LRKK2, 20μM lipid nanotubes and 1mM GMPPNP.

2, Incubate samples at 370C for 30 minutes.

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

4, Place the discharged grids on a piece of Parafilm.

5, After incubation, apply 6μL of the samples to the grid and adsorbed on the grid for 5 min at room temperature.

6, Blot the grid with filter paper and stained with 2% uranyl acetate for 40 seconds.

7, Dry the grid with filter paper.

8, Images were collected 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***

1, Dialyze freshly purified LRRK2 into the Low salt buffer.

2, After dialysis, incubate LRRK2 (2μM) with the kinase inhibitor MLi-2 (5μM) for 10 min on ice.

3, Add 20μM lipid nanotubes into the mixture above and further incubate for 1 hour at room temperature in the presence of 1mM GTP.

**Note:** The total volume of the mixture we used was 12μL.

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

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

6, Sample-loaded grids were plunge-frozen in liquid ethane-propane mixture using a Vitrobot Mark IV (FEI) with the following parameters: blot force, 0; blot time, 1 s; wait time, 30 s; drain time, 0 s; humidity, 100%.

7, Cryo-EM micrographs were collected 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).

8, Data collection was performed with the SerialEM software. Movies were recorded 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 to -3 µm. 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 2.58 s total exposure time. The initial drift and beam-induced motions was corrected using MotionCor2*.*