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

**Authors:** Xinbo Wang1,2 and Pietro De Camilli1,2

1Departments of Neuroscience and of Cell Biology, Howard Hughes Medical Institute, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, Connecticut 06510, USA;  
2Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

**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*.*