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

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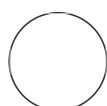
Liposome tubulation

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ABSTRACT

This protocol details methods for the LRRK2-induced liposome tubulation experiment and its analysis by confocal fluorescence microscopy and negative stained electron microscopy.

ATTACHMENTS

[iuuebv9p.docx](#)

Keywords: Liposome
tubulation, LRRK2, Electron
microscopy

Confocal fluorescence microscopy analysis

30m

- 1 Prepare the samples in a PCR tube with **1M 300 nanomolar (nM)** LRRK2 proteins (WT or mutant full length LRRK2 or RCKW), **1M 20 micromolar (μM)** liposomes with or without **1M 1 millimolar (mM)** GMPPNP (or other guanylnucleotides).



Note

Note: Liposome tubulation is sensitive to LRRK2 concentration. Too much protein results in more liposome aggregates.

- 2 Immediately deposit **6 μL** - **10 μL** samples of step 1 on a **35 mm** glass bottom dish and incubate at **37 °C** for **00:30:00**.



Note

Note: Drop some buffer in the dish to prevent samples from drying out due to evaporation during incubation.

- 3 After incubation, capture images with a Spinning disk confocal (SDC) microscopy at **Room temperature** on a Nikon Ti-E inverted microscope using the Improvion UltraVIEW VoX system (Perkin-Elmer).



Note

Note: Movies were collected from time **00:00:00**.

Negative stained electron microscopy (EM) analysis

31m 25s

- 4 Glow-discharge carbon-coated grids (25 mA, **00:00:45**).

45s

5 Place the discharged grids into a ± 35 mm glass bottom dish.

6 Prepare samples in a PCR tube with 300 nanomolar (nM) LRKK2, 80 micromolar (μ M) liposomes and 1 millimolar (mM) GMPPNP.



7 Immediately apply 6 μ L of the mixture to the grid and incubate the mixture at 37 °C for 30m
00:30:00



Note

Note: Drop some buffer in the dish to prevent samples from drying out due to evaporation during incubation.

8 Blot the grid with filter paper after incubation and stain samples with 2% uranyl acetate for 40s
00:00:40

9 Dry the grid with filter paper.

10 Take images using a Talos L 120C TEM microscope at 80 kV with Velox software and a 4k \times 4K Ceta CMOS Camera (Thermo Fisher Scientific).

