**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.

**Protocol:**

***Confocal fluorescence microscopy analysis***

1, Samples were prepared in a PCR tube with 300nM LRKK2 proteins (WT or mutant full length LRRK2 or RCKW), 20μM liposomes with or without 1mM GMPPNP (or other guanylnucleotides).

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

2, Immediately deposit 6-10μL samples of step 1 on a 35-mm glass bottom dish and incubate at 370C for 30 minutes.

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

3, After incubation images were captured with a Spinning disk confocal (SDC) microscopy at room temperature on a Nikon Ti-E inverted microscope using the Improvision UltraVIEW VoX system (Perkin-Elmer).

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

***Negative stained*** ***electron microscopy (EM) analysis***

1, Glow-discharge carbon-coated grids (25 mA, 45s).

2, Place the discharged grids into a 35-mm glass bottom dish.

3, Prepare samples in a PCR tube with 300nM LRKK2, 80μM liposomes and 1mM GMPPNP.

4, Immediately apply 6μL of the mixture to the grid and incubate the mixture at 370C for 30 minutes.

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

5, Blot the grid with filter paper after incubation and stain samples with 2% uranyl acetate for 40 seconds.

6, Dry the grid with filter paper.

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