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

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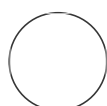
## 🌐 Liposome binding

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### ABSTRACT

This protocol details methods for LRRK2-liposome binding experiments analyzed by co-sedimentation or confocal fluorescence microscopy, respectively.

### ATTACHMENTS

[iuudbv9p.docx](#)

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**PROTOCOL integer ID:**  
68884

**Keywords:** Liposome binding, Co-sedimentation, Fluorescence microscopy

## Co-sedimentation analysis

- 1 Prepare samples in Beckman microfuge tubes with [M] 300 nanomolar (nM) LRRK2 or RCKW, [M] 20 micromolar ( $\mu$ M) PS liposomes in the absence or presence of [M] 1 millimolar (mM) GMPPNP.



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



- 3 Centrifuge samples at [S] 49000 rpm ( [S] 100000 x g ) for [D] 00:20:00 in a Beckman Optima TLX ultracentrifuge. 20m



- 4 Collect Supernatants and Pellets.

### Note

**Note:** Pellets were resuspended with the same volume of protein buffer as the supernatant.

- 5 Analyze samples by SDS-PAGE and coomassie blue staining.

## Confocal fluorescence microscopy analysis

30m

- 6 For PS liposome binding. Prepare samples in PCR tubes with [M] 20 micromolar ( $\mu$ M) Rhod-PE labeled PS liposomes and different concentrations of LRRK2 as indicated in the main text.



6.1 For GC/PS nanotube binding. Prepare samples in PCR tubes with [M] 20 micromolar ( $\mu\text{M}$ ) Cy5-PE labeled GC/PS nanotubes and [M] 100 nanomolar (nM) GFP-LRRK2.



7 Immediately deposit [V] 6  $\mu\text{L}$  [V] 10  $\mu\text{L}$  samples of step 6 on [D] 35 mm glass bottom dishes and incubate at [T] 37  $^{\circ}\text{C}$  for [T] 00:30:00 .



30m

#### Note

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

8 After incubation, capture the images with a spinning disk confocal (SDC) microscopy at [T] Room temperature on a Nikon Ti-E inverted microscope using the Improvision UltraVIEW VoX system (Perkin-Elmer).

