**Liposome binding**

**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 LRRK2-liposome binding experiments analyzed by co-sedimentation or confocal fluorescence microscopy, respectively.

**Protocol:**

***Co-sedimentation analysis***

1, Prepare samples in Beckman microfuge tubes with 300nM LRRK2 or RCKW, 20μM PS liposomes in the absence or presence of 1mM GMPPNP.

2, Incubate samples at 370C for 30 minutes.

3, Centrifuge samples at 49,000 rpm (100,000xg) for 20 min in a Beckman Optima TLX ultracentrifuge.

4, Collect Supernatants and Pellets

**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***

1-1, For PS liposome binding. Prepare samples in PCR tubes with 20μM Rhod-PE labeled PS liposomes and different concentrations of LRRK2 as indicated in the main text.

1-2, For GC/PS nanotube binding. Prepare samples in PCR tubes with 20μM Cy5-PE labeled GC/PS nanotubes and 100nM GFP-LRRK2.

2, Immediately deposit 6-10μL samples of step 1 on 35-mm glass bottom dishes 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).