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Liposome binding

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We use this protocol and it's working

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Abstract

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

Attachments

DOCX

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19KB

Co-sedimentation analysis

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

2 Incubate samples at 37 °C for 00:30:00 . 30m

3 Centrifuge samples at 49000 rpm (100000 x g) for 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 (µM) 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 (µM) Cy5-PE labeled GC/PS nanotubes and [M] 100 nanomolar (nM) GFP-LRRK2.

7 Immediately deposit 6 µL - 10 µL samples of step 6 on 35 mm glass bottom dishes and incubate at 37 °C for 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 Room temperature on a Nikon Ti-E inverted microscope using the Improvision UltraVIEW VoX system (Perkin-Elmer).