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

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Fluorescence recovery after photobleaching (FRAP)

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ABSTRACT

This protocol details methods of the FRAP analysis of LRRK2-induced liposome tubules *in vitro*

ATTACHMENTS

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Keywords: Photobleaching, Fluorescence, LRRK2

Fluorescence recovery after photobleaching (FRAP)

40m 30s

1 Prepare LRRK2-liposome mixtures in a PCR tube with [M] 300 nanomolar (nM) GFP-LRKK2, [M] 20 micromolar (µM) liposomes (labeled with trace amounts of rhodamine-PE) and [M] 1 millimolar (mM) GMPPNP.

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. 30m

Note

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

3 Perform FRAP experiments with a Spinning disk confocal (SDC) microscopy at Room temperature on a Nikon Ti-E inverted microscope using the Improvision UltraVIEW VoX system, with the settings as:

3.1 Acquire the time-lapse images at every 00:00:15. 15s

3.2 Acquire three images before bleaching.

3.3 Bleach three ROIs with a 488 nm laser for 500 ms.

3.4 Acquire post-bleach images up to 00:10:00 at 00:00:15 intervals. 10m 15s