Fluorescence recovery after photobleaching (FRAP)

Xinbo Wang, Pietro De Camilli

1. Departments 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;
2. Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

ABSTRACT

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

ATTACHMENTS

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Protocol status: Working

We use this protocol and it's working

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

   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.

   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.