**Fluorescence recovery after photobleaching (FRAP)**

**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 of the FRAP analysis of LRRK2-induced liposome tubules *in vitro*

**Protocol:**

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

2, Immediately deposit 6-10μL samples of step 1 on a 35-mm glass bottom dish 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, FRAP experiments were performed 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:

Time-lapse images were acquired every 15s; Three images were acquired before bleaching; Three ROIs were bleached with a 488nm laser for 500ms; Post-bleach images were acquired up to 10 minutes at 15s intervals.