



AUG 25, 2023

OPEN ACCESS



DOI:
dx.doi.org/10.17504/protocols.io.5qpobj29l4o/v1

Protocol Citation: Xinbo Wang, Pietro De Camilli 2023. Fluorescence recovery after photobleaching (FRAP). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.5qpobj29l4o/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Aug 19, 2022

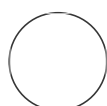
🌐 Fluorescence recovery after photobleaching (FRAP)

Xinbo

Wang^{1,2}, Pietro De Camilli^{1,2}

¹1. 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;

²2. Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815



xinbo.wang

ABSTRACT

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

ATTACHMENTS

[iuufbvk9p.docx](#)

Last Modified: Aug 25, 2023

PROTOCOL integer ID:
68888

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 (μ M) liposomes (labeled with trace amounts of rhodamine-PE) and [M] 1 millimolar (mM) GMPPNP.



- 2 Immediately deposit [V] 6 μ L - [V] 10 μ L samples of step 1 on a [L] 35 mm glass bottom dish and incubate at [T] 37 $^{\circ}$ C for [D] 00:30:00.



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 [T] 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 [D] 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