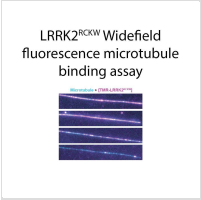

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LRRK2RCKW Widefield fluorescence microtubule binding assay

David M. Snead[1,2]

[1]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093;

[2]Department of Biochemistry and Molecular Biology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, 21205

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Mariusz Matyszewski

This assay uses TMR labeled LRRK2 or LRRK1 RCKW to measure binding to microtubules in vitro.

Created by David Snead. Adapted to protocols.io by Mariusz Matyszewski.

As used in Snead, Matyszewski, Dickey et al. 2022.

DOI

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David M. Snead 2022. LRRK2RCKW Widefield fluorescence microtubule binding assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygxz7bdv8j/v1

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LRRK2, imaging, ASAPCRN, microtubule, binding

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Similar setup as in:

Deniston CK, Salogiannis J, Mathea S, Snead DM, Lahiri I, Matyszewski M, Donosa O, Watanabe R, Böhning J, Shiau AK, Knapp S, Villa E, Reck-Peterson SL, Leschziner AE (2020). Structure of LRRK2 in Parkinson's disease and model for microtubule interaction.. Nature.
https://doi.org/10.1038/s41586-020-2673-2

Also see:
https://dx.doi.org/10.17504/protocols.io.ewov14qykvr2/v1

Image analysis:

Image analysis was performed with ImageJ. Average TMR-LRRK2RCKW fluorescence intensity per microtubule was calculated from a 1 pixel-wide line drawn along the long axis of the microtubule; overall average background fluorescence intensity was subtracted. These background-subtracted intensities were averaged over all microtubules per field of view, normalized by microtubule length, to yield a single data point. Eight fields of view at each concentration of LRRK2RKCW were then averaged.

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Recommended Equipment and Setup:
This single-molecule imaging experiment was originally performed using total internal reflection fluorescence (TIRF) microscopy with an inverted microscope (Nikon, Ti-E Eclipse) equipped with a 100x 1.49 N.A. oil immersion objective (Nikon, Plano Apo), and a MLC400B laser launch (Agilent), with 405 nm, 488 nm, 561 nm and 640 nm laser lines. Excitation and emission paths were filtered using single bandpass filter cubes (Chroma), and emitted signals were detected with an electron multiplying CCD camera (Andor Technology, iXon Ultra 888). Illumination and image acquisition were controlled with NIS Elements Advanced Research software (Nikon), and the xy position of the stage was controlled with a ProScan linear motor stage controller (Prior).

Required Buffers:

Streptavidin Buffer:

- [M]**0.5 mg/mL Streptavidin**
- [M]**30 millimolar (mM) HEPES pH 7.4**
- [M]**2 millimolar (mM) MgOAc**
- [M]**1 millimolar (mM) EGTA**
- [M]**10 % Glycerol**

Wash Buffer:

- [M]**30 millimolar (mM) HEPES pH 7.4**
- [M]**50 millimolar (mM) KOAc**
- [M]**2 millimolar (mM) MgOAc**
- [M]**1 millimolar (mM) EGTA**
- [M]**10 % Glycerol**
- [M]**1 millimolar (mM) DTT**
- [M]**200 micromolar (µM) Taxol**

LRRK2 Buffer:

- [M]**20 millimolar (mM) HEPES pH 7.4**
- [M]**80 millimolar (mM) NaCl**
- [M]**0.5 millimolar (mM) TCEP**
- [M]**5 % Glycerol**
- [M]**0.5 millimolar (mM) MgCl2**
- [M]**20 micromolar (µM) GDP**

For hazard information and safety warnings, please refer to the SDS (Safety Data

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Sheet).

Please take notice of the buffer preparation in section 'Materials'.
Make sure that you have labeled taxol-stabilized microtubules available. See the protocol here.

Create microscope slides: 1h 11m

1 Adhere Biotin-PEG-functionalized coverslips (Microsurfaces) to a microscope slide using double-sided scotch tape, creating 4 channels per slide.

2 Add the **streptavidin buffer** to each channel and incubate for **00:03:00** . 3m

3 

Wash twice with **Wash buffer**.

4  3m

Add a 1:150 dilution of taxol-stabilized microtubules (**19 µL** per channel) and incubate for **00:03:00** .
See https://dx.doi.org/10.17504/protocols.io.bp2l6bdedgqe/v1 for making taxol-stabilized microtubules.

5 

Wash twice with **LRRK2 buffer**. Add more buffer if necessary to prevent drying out.

Prepare LRRK2: 1h 11m

6 Make sure to use TMR labelled protein. See https://dx.doi.org/10.17504/protocols.io.ewov1nq5ogr2/v1 for labeling protocol.

7  10m

Create a working aliquot of LRRK2 (or LRRK1) in the desired concentration (ex. **25 nanomolar (nM)** - **50 nanomolar (nM)**) in the LRRK2 buffer at **Room temperature** (recommended volume of **25 µL**). If adding inhibitors, add them

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now with DMSO. Incubate for **00:10:00** at **Room temperature** .

Adding LRRK2 and imaging: 5m

5m

8

Add LRRK2^{RCKW} sample to the channel (**19 µL**). Incubate for **00:05:00** . Prepare next step while waiting.

9 Image slide. We imaged using multiple fields of view along the flow chamber with the objective in widefield illumination, with successive excitation at 488 nm (15% laser power, 100 ms exposure) and 561 nm (25% laser power, 100 ms exposure).
Check guidelines for image analysis notes.

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