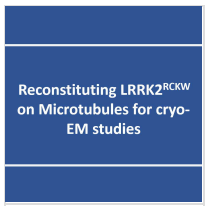

May 23, 2022

Reconstituting LRRK2^{RCKW} on Microtubules for cryo-EM studies

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This protocol contains a short instruction for reconstituting LRRK2^{RCKW} on microtubules for cryo-EM studies with or without kinase inhibitors present.

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LRRK2, microtubule interaction, Microtubule, ASAPCRN

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Originally used without kinase inhibitors 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

Kinase inhibitor step was added for "Structural basis for Parkinson's Disease-linked LRRK2's binding to microtubules" by Snead, Matyszewski, Dickey et al.

Buffers needed:

Polymerization buffer

- [M]**1 X BRB80**
- [M]**1 millimolar (mM) DTT**
- [M]**1 millimolar (mM) GTP**
- [M]**1 millimolar (mM) MgCl2**
- [M]**10 micromolar (µM) Taxol**
- [M]**10 %** of either Glycerol (for low protofilament sized microtubules) or DMSO (for higher protofilament sizes) (DMSO was used in the original publication, and glycerol was used in Snead, Matyszewski, Dickey et al.)

LRRK2 Reaction buffer

- [M]**20 millimolar (mM) HEPES pH 7.4**
- [M]**80 millimolar (mM) NaCl**
- [M]**0.5 millimolar (mM) TCEP**
- [M]**2.5 millimolar (mM) MgCl2**
- [M]**10 micromolar (µM) Taxol**

[M]**1 X BRB80 (usually made as a 5X solution)**

- [M]**80 millimolar (mM) PIPES-KOH pH 6.8**
- [M]**1 millimolar (mM) MgCl2**
- [M]**1 millimolar (mM) EGTA**

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'.

Instruction 1h 0m 44s

1 Add purified LRRK2^{RCKW} and unpolymerized bovine tubulin dimer in a 2:1 molar ratio into the polymerization buffer (2 LRRK2^{RCKW}s for each tubulin dimer). (Recommended total size: **10 µL**)

Note: Concentration of LRRK2^{RCKW} has to be at least **2.5 micromolar (µM)** to see filaments occur. Has been tested with multiple variants.
For Snead, Matyszewski, Dickey et al, all filaments were formed at **4.5 micromolar (µM)** LRRK2^{RCKW} and **2.25 micromolar (µM)** tubulin dimer.

Note: NaCl concentration at this step should remain at around **90 millimolar (mM)** or less, often imposing a limit on LRRK2^{RCKW} concentration allowed to be used. Higher salt concentration will prevent or reduce filament formation.

1.1 If incubating with LRRK2 kinase inhibitors, add those before adding tubulin to LRRK2^{RCKW} and allow to incubate for at least **00:05:00** . 5m

In Snead, Matyszewski, Dickey et al, MLi-2 was added at **5 micromolar (µM) (final concentration after adding tubulin)** .

2 Allow the mixture to polymerize at **Room temperature** for at least **01:00:00** . 1h

3 Prepare cryo-EM grids. 45s
Recommended grids to use: Lacey Carbon on copper, 300 mesh, made by EMS. Glow

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discharged right before plunge freezing for **00:00:45** at **20 mA** current.

4 Dilute the sample 3-fold in the LRRK2 reaction buffer. (Recommended mixture: **4 µL sample** + **8 µL LRRK2 buffer**).

This step reduces glycerol to be within acceptable levels for cryo-EM.

This will reduce the effective concentration of components, but the dilution of $LRRK2^{RCKW}$ and tubulin might be non-linear due to filaments bundling to each other. The minimum concentration mentioned in Step 1 only applies to incubation step.

5 Apply diluted sample to grid and plunge freeze using your usual Vitrobot settings. (Ex. **4 µL sample** , blotted for **00:00:04** at blot force 20 for our particular Vitrobot (FEI); conditions might vary from one machine to another). 4s

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