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

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LRRK2-RCKW: MLi-2: E11 DARPin cryo-EM sample preparation

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ABSTRACT

Protocol used to prepare LRRK2-RCKW: DARPin:MLi-2 complex and cryo-EM grid preparation.

BEFORE START INSTRUCTIONS

Make

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Protein purification and buffer exchange

1 His6-Z-TEV-LRRK2-RCKW was expressed and purified as described in a previous protocol.

Protocol

NAME
LRRK1 expression and purification

CREATED BY
Robert Fagiewicz

PREVIEW

2 Prepare LRRK2 buffer exchange. Keep it at 4ºC.
20 millimolar (mM) HEPES pH=7.4
150 millimolar (mM) NaCl
2.5 millimolar (mM) MgCl2
20 micromolar (µM) GDP
0.5 millimolar (mM) TCEP

3 Spin down purified LRRK2-RCKW (10000 rcf, 4°C, 10 minutes). Leave protein on ice afterward. For the best result, keep protein on ice and reduce the amount of time between spinning and freezing cryo-EM samples.

4 Exchange buffer using a spin desalting column (Zeba™ Spin Desalting Columns, 7K MWCO (Catalog number: 89877).

5 Spin down again the exchange buffer LRRK2-RCKW (10000 rcf, 4°C, 10 minutes) and measure the concentration. Leave protein on ice afterward

Expected result

The initial concentration range was 20-40 uM. The final concentration might be half of the initial one. Final volume might be 13-16 uL.

5.1 Thaw E11 DARPin and spin it down. Measure its concentration.

6 Dilute MLi-2 stock (diluted in 100% DMSO) to a desired concentration

7 Based on LRRK2-RCKW concentration, add the necessary volume to get a proportional ratio LRRK2:DARPin:MLi-2 1:1.25:3 and dilute to a final 10 micromolar (µM) LRRK2-RCKW concentration using exchange buffer (150 mM NaCl).

8 Incubate 10 minutes at RT. Afterward, keep it on ice until grid preparation.

E11 DARPin purification

9 E11 DARPin purified as described in the next protocol

cryo-EM sample preparation

10 We used UltraAuFoil Holey Gold 2/2 200 mesh grids and plasma cleaning them in the Solarus II (Gatan) using the QuantiFoil Au preset.

11 Dilute the sample to the desired concentration using the LRRK2 exchange buffer. We used 6 micromolar (µM).

12 Apply 3 to 3.5 microliters (µl) of sample and plunge freeze. We used a Vitrobot (FEI) to blot away excess sample and plunge freeze in ethane liquid. (In our case, we use 4 seconds as a time blot as 20 sec as a wait time and 4 as a blot force, but these parameters are slightly different from one Vitrobot to another. I would try with the Vitrobot parameters already tested in your machine first).

13 Store grids in liquid nitrogen until ready for imaging