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

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Protocol status: Working

We use this protocol and it's working

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Abstract

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

Troubleshooting

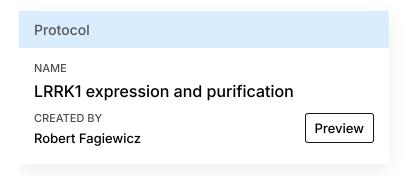
Before start

Make



Protein purification and buffer exchange

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



2 Prepare LRRK2 buffer exchange. Keep it at 4°C.

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
- 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

- We used UltraAuFoil Holey Gold 2/2 200 mesh grids and plasma cleaning them in the Solarus II (Gatan) using the QuantiFoil Au preset.
- Dilute the sample to the desired concentration using the LRRK2 exchange buffer. We used 6 micromolar (μ M).
- 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