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

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Cryo-EM sample preparation for full length LRRK2(I2020T):MLi-2/GZD-824-E11 DARPin complex

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ABSTRACT

Guide for cryo-EM grid preparation of FL-LRRK2 bound to Type-I/Type-II inhibitors

MATERIALS

Vitrobot (Thermo Fisher Scientific)

SAFETY WARNINGS

Handle liquid nitrogen with gloves and face shields equipped all the time. Handle liquid ethane with face shields all the time.

BEFORE START INSTRUCTIONS

Before starting cryo-EM grid preparation section: Set up Vitrobot at 95% humidity and 4C. Put new paper blot

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89960

Keywords: ASAPCRN, cryo-EM, LRRK2, inhibitors

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Protein purification

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

Protocol

NAME
LRRK1 expression and purification

CREATED BY
Robert Fagiewicz

PREVIEW

Sample preparation

2 Prepare LRRK2 buffer. 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 (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 Thaw E11 DARPin and spin it down. Measure its concentration.

5 Dilute inhibitors (diluted in 100% DMSO) to an intermediate desired concentration using LRRK2 buffer.

6 Based on the initial and final LRRK2 concentration, calculate the necessary volume of E11 DARPin to get a proportional ratio LRRK2:E11 DARPin 1:1.25 in the sample. After that, add either MLi-2 or GZD-824. Dilute to your final desired LRRK2 concentration using LRRK2 buffer (150 mM NaCl).

Note

In our samples, final concentrations were:
5 uM LRRK2
6.25 uM E11 DARPin
Either MLi-2 or GZD-824 at a final concentration 20 uM and 40 uM, respectively.

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

cryo-EM grid preparation

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

9 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).

10 Store grids in liquid nitrogen until ready for imaging