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Preparation of LRRK2 RCKW cryo-EM grids

Forked from Preparation of LRRK2 RCKW trimer cryo-EM grids

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ABSTRACT

This is Leschziner's Lab updated protocol for making cryo-EM grids for LRRK2 RCKW. This protocol, when using lower protein concentration, results in better monomer and dimer formation than the old protocol.

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FORK NOTE

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MATERIALS TEXT

LRRK2 Buffer:

- [M]**20 millimolar (mM) HEPES pH 7.4**
- [M]**80 millimolar (mM) NaCl**
- [M]**0.5 millimolar (mM) TCEP**
- [M]**5 % volume Glycerol**
- [M]**2.5 millimolar (mM) MgCl2**
- [M]**20 micromolar (µM) GDP**

Note: please change salt as needed to maintain final salt of 80 mM NaCl

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).
Take proper precautions while freezing grids.

BEFORE STARTING

Decide which protein concentration to use, and create the proper LRRK2 buffers in order to obtain the right salt concentration (80 mM NaCl).

Preparing Sample 10m

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1 Spin down purified LRRK2 RCKW. **10000 rcf, 4°C, 00:10:00 , (can be faster)**
Leave protein on ice afterwards.

> For best results, reduce the amount of time between spinning and freezing samples.

Freezing Grids 20s

2 Plasma clean grids.
We used UltrAuFoil Holey Gold 1.2/1.3 300 mesh grids and plasma cleaned them in the Solarus II (Gatan) using the QuantiFoil Au preset.

3 Dilute samples to desired concentration in the **LRRK2 buffer**. Make sure final salt is at 80 mM NaCl.
For best results, make **10 µL** samples, good for freezing 2 grids. This is to minimize time spent outside of storage buffer, reducing aggregation.

> High concentrations favor dimers and trimers, while lower concentrations favor monomers.

3.1 **(Optional)** If using inhibitors, let them incubate on ice in the final LRRK2 buffer before plunge freezing.

4 Apply protein to grids and plunge freeze.
We used a Vitrobot (FEI) to blot away excess sample and plunge freeze

5 Store grids in liquid nitrogen until ready for imaging.

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