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Cryo-EM sample preparation for RCKW:DARPin complex

Forked from Preparation of LRRK2 RCKW cryo-EM grids

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

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Abstract

This is Leschziner's Lab protocol for making cryo-EM grids for RCKW:DARPin complex.

Materials

LRRK2 Buffer:

- [M] 20 millimolar (mM) HEPES pH 7.4
- [M] 150 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 150 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 start

Decide which protein concentration to use, and create the proper LRRK2 buffers in order to obtain the right salt concentration (150 mM NaCl).

Freezing Grids

20s

1 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.

2 Dilute samples to desired concentration in the **LRRK2 buffer**. Make sure final salt is at 150 mM NaCl.
For best results, make 8 µL samples, good for freezing 2 grids. This is to minimize time spent outside of storage buffer, reducing aggregation.

3 Apply protein to grids and plunge freeze (3-4 uL)
We used a Vitrobot (FEI) to blot away excess sample and plunge freeze

4 Store grids in liquid nitrogen until ready for imaging.