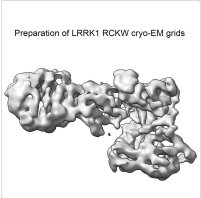

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Preparation of LRRK1 RCKW cryo-EM grids

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Protocol used to create LRRK1 RCKW grids for cryo-EM used in Snead, Matyszewski, Dickey et al.

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LRRK1 Buffer:

- [M]**20 millimolar (mM) HEPES pH 7.4**
- [M]**80 millimolar (mM) NaCl**
- [M]**0.5 millimolar (mM) TCEP**
- [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

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

Decide which protein concentration to use, and create the proper LRRK1 buffers in order to obtain the right salt concentration (80 mM NaCl).

Preparing Sample 10m

1 Spin down purified LRRK1 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 **LRRK1 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.

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In our publication, we used concentrations ranging from [M]**2 micromolar (µM)** to [M]**6 micromolar (µM)** . Lower concentrations were used for samples collected on using a tilted stage.

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

Each Vitrobot is slightly different, so use your preferred settings. We used **3.5 µL** of sample and a 4 second blot at force 20, but it is unlikely to work on other Vitrobots.

5 Store grids in liquid nitrogen until ready for imaging.

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