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Preparation of ATP13A2 cryo-EM grids

Sue Sim[1], eunyong_park [1]

[1]University of California, Berkeley

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S Sue Sim

ABSTRACT

Preparing cryo-EM grids using purified ATP13A2 samples

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Preparing samples 10m

1 Spin purified and concentrated ATP13A2 sample (5-7 mg/mL) after SEC at **17000 x g, 4°C, 00:10:00** 10m

2 Keep protein on ice

3 Plasma clean grids

3.1 Used gold holey carbon grids (Quantifoil R 1.2/1.3, 400 mesh) and PELCO easiGlow Glow Discharge Cleaning System (0.39 mBar, 25-30 mA, 40-45 sec)

4 Apply 3 µL of protein sample to grids and plunge freeze

4.1 Used Vitrobot Mark IV (FEI) operated at 4°C and 100% humidity and Whatman No.1 filter paper to blot samples

4.2 Our blotting settings were 3.5-4.5 second blot at force 25, but every Vitrobot is slightly different. Use optimal settings for your machine.

5 Store grids in liquid nitrogen until ready for imaging.

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