On-Grid Purification and Sample Preparation for Cryo EM
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Graphene Oxide Monolayer Affinity Capture

PABA-GO-NTA Monolayer on Lacey Carbon Grid

Grid Preparation
1) Langmuir-Schaefer transfer of PABA-GO-NTA monolayer with IPA/Water
2) 20 mM NiSO₄ incubation
3) 3X water droplet wash
4) Incubate BSA (10 μL, 0.1 mg/mL) face up, 5 min
5) 3X water droplet wash

Sample Preparation
Clarified bacterial lysate buffer:
- 20 mM HEPES pH 7.4,
- 100 mM NaCl,
- protease inhibitor

1) Incubate 5 μL face up, 2 min
2) Vitrobot -1 offset, 2X 6 sec blot, 80% humidity

Graphene Oxide Monolayer Affinity Capture

GroEL Capture from Lysate

Class Averages (2682 particles)

EM Density Map (8.1 Å, gold standard)

Lipid-PEG Monolayer Affinity Capture

- Addition of antifouling PEG brush
- Blocks protein adsorption to lipid monolayer

**DSPE-PEG(2K)-TrisNTA**

**Grid Preparation**
1) 95% DSPE-mPEG(350) : 5% DSPE-PEG(2K)-TrisNTA
2) Lipid-PEG monolayer compression to 50 mN/m
3) Langmuir-Schaefer transfer to lacey carbon grid
4) 20 mM NiSO₄ incubation
5) 3X water droplet wash

**Sample Preparation**
Clarified bacterial lysate in buffer: 20 mM HEPES pH 7.4, 100 mM NaCl, Protease inhibitor
1) Incubate inverted over 25 μL, 5 min, 4°C, rocker
2) 3X buffer droplet wash
3) CP3 plunger, 2.0 - 2.5 sec blot, 80% humidity

Lipid-PEG Monolayer Affinity Capture

Ca2+/Calmodulin-dependent Protein Kinase II (CaMKII-6xHis)
Lipid-PEG Monolayer Covalent Capture

DSPE-PEG(5K)-DBCO Monolayer on Lacey Carbon Grid

- **Diversify capture tools**
- **Improve purification specificity**
- **Robust incubations/washes**

**Grid Preparation**

1) DSPE-mPEG(350) : DSPE-PEG(2K)-NTA (5%:95%)
2) Lipid-PEG monolayer compression to 50 mN/m
3) Langmuir-Schaefer transfer to lacey carbon grid

**Sample Preparation**

Clarified bacterial lysate in buffer: 20 mM HEPES pH 7.4, 100 mM NaCl, 1 mM DTT, protease inhibitor

1) Incubate inverted over 25 uL
2) 3X buffer droplet wash
3) CP3 plunger, 2.0 - 2.5 sec blot, 80% humidity

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Q/A
Thank you
GO-NTA Sheets Characterization

(A) Pressure-area isotherm for GO-NTA sheets at the air-water interface, dispersed at 67 ng/mL in water at 20 °C. GO-NTA sheets compressed at a rate of 500 mm2/min. (B) SEM images taken 1.0 keV, with 5 μm scale bar and (C) AFM images of GO-NTA after LS-transfer onto Si wafers from a subphase of pure H2O (5 μm scale bar). (D) SEM images taken at 0.5 keV (5 μm scale bar) and (E) AFM of GO-NTA after LS-transfer onto Si wafers from a subphase of IPA/H2O (5 μm scale bar). (F) TEM image of GO-NTA monolayers after L-S transfer from a subphase of IPA/H2O onto TEM grids; Inset: Selected area electron diffraction analysis of GO-NTA monolayer.