Graphene Oxide Grid Preparation Protocol

I. Materials:

- Graphene Oxide Dispersion in H_2O (2 mg/ml) - prod#1003729481
- 1.5 mL Microcentrifuge Tubes (or similar)
- Centrifuge
- Micropipettes and Tips (for volumes ranging from 5 to 200 μL)
- Cryo-EM Grids (CF or Quantifoil)
    - CF R 1.2/1.3 - cat#CFT213-100
    - Quantifoil R 1.2/1.3 - prod#N1-C14nCu40-01
- PELCO TEM Grid Holder Blocks - prod#16820-25
- PELCO easiGlow™ Glow Discharge Cleaning System - prod#91000S
- Anti-capillary Tweezers - prod#510-4NM
- Chien Staining Pad - prod#10523-2
- 90mm Whatman Circular Filter Paper - cat#1001 090

Optional:

- Ossila UV Ozone Cleaner - prod#L2002A3-US
- Methanol 99.9% - prod#MX0488-6
- Ethanol Anhydrous 200 proof- cat#15055
- Chloroform 99.5+% stab. with amylene - cat#043685.K2
- 100x20mm Glass Petri Dishes - ref#3160-102
- Serological Pipette and Glass Tips (for volumes around 10 to 25 mL)

II. Optional Preparation: Washing Grids in Organic Solvents (Quantifoil Grids Only)

1. Label three **clean** large glass petri dishes with “Methanol,” “Ethanol,” and “Chloroform,” respectively, and place a filter paper in the bottom of each.

2. Retrieve the Quantifoil R 1.2/1.3 grids and place them film side upward on the filter paper inside the “Methanol” petri dish. Handle grids carefully.

3. Place all materials inside a fume hood (Methanol, Ethanol, Chloroform, serological pipette, petri dishes).

4. Using a serological pipette, **slowly** pour 20 mL of Methanol into the petri dish with the grids. Correct any grids that have flipped over and ensure all are fully covered by the liquid. Place the lid on the petri dish and set a timer for 5 minutes.

5. While waiting, use a serological pipette to fill the “Ethanol” petri dish with 20 mL of Ethanol, ensuring all parts of the filter paper are submerged.

6. After 5 minutes in Methanol, transfer the grids directly to the “Ethanol” petri dish. Ensure all grids are submerged and upright, then set a timer for 10 minutes.

7. Do not fill the “Chloroform” petri dish until **after** the grids are placed on the dry filter paper. Once the 10 minutes elapses, carefully transfer the grids from the liquid Ethanol to the dry filter paper in the “Chloroform” petri dish.

8. Ensure all grids are facing film side up on the filter paper, then wait for the filter paper to completely dry (do **not** mix chloroform and ethanol). After the grids and filter paper are completely dry, use the serological pipette **with a glass tip** to add 25 mL of chloroform to the petri dish with the grids.

9. Cover the petri dish with its lid, leaving it partially open to allow for evaporation. Wait until all chloroform is evaporated before cleaning up and using the grids.

III. Procedure

A. Preparing the Graphene Oxide (GO) Stock

1. Combine 20 µL of GO suspension (concentration of 2 mg/mL) with 180 µL of ddH_2O in a labeled 1.5 mL microcentrifuge tube (note: this will create a mixture with a concentration of 0.2 mg/mL).

2. Mix together with a micropipette set to 180~200 µL volume (tip: reuse the same pipette tip and volume from adding the ddH_2O to mix).

3. Place the microcentrifuge tube opposite a balance inside a centrifuge and run for 15 seconds at 300 g.

4. Remove the microcentrifuge tube and balance from the centrifuge and place the former upright in a rack.

5. Extract ~75 µL of liquid from the **top surface** of the newly centrifuged GO mixture and transfer it to a new tube, labeled to show this difference. This will be applied to the grids.

B. Preparing the Grids

6. Retrieve your preferred grid type. It is recommended to use either CF R 1.2/1.3 grids from EMS or Quantifoil R 1.2/1.3 grids. Handle grids carefully.

7. Place the grids carefully on a **clean** PELCO TEM Grid Holder Block and insert them into a PELCO easiGlow™ Glow Discharge Cleaning System set to 15 mA of current with a 0.26 mBar vacuum for 35 seconds for CF grids (20 mA, 0.26mBar, and 80 seconds for Quantifoil).

8. Once complete, remove the grids and pick each one up with anti-capillary tweezers to suspend it above the counter. Ensure the film side is facing upward.

C. Functionalizing the Grids

9. Use a micropipette to apply 5 µL of the **top** GO mixture onto a grid, allowing approximately 1 minute between each grid for blotting later on.

10. Let the grids sit with the mixture on top for 12 minutes (incubation time). During this time, prepare a filter paper for blotting by folding it in half and turning it upside down to rest on the counter.

11. Use a micropipette to create 20 µL droplets of ddH_2O (3 for each grid) on a clean Chien Staining Pad. Try to do this close to when you will be blotting and washing to prevent evaporation.

12. Once a grid has incubated long enough, blot the film side on the filter paper. Then wash the film side in a water droplet and blot on the filter paper. Repeat the washing once more for the film side, then one last time on the bar side.

13. Allow the grid to dry while still on the tweezers for at least 5 minutes before transferring to a desiccator.

D. Freezing with GO Grids

14. After the grids dry, they are ready for plunge freezing as normal, since they have already been glow discharged. If the grids are stored for a long period of time, treat them in an Ossila UV Ozone Cleaner to restore the hydrophilicity. Do **NOT** glow discharge the grids again. This will damage the GO layer.

15. Use sample concentrations similar to Negative Staining (0.04~0.08 mg/mL) when freezing with these functionalized grids.