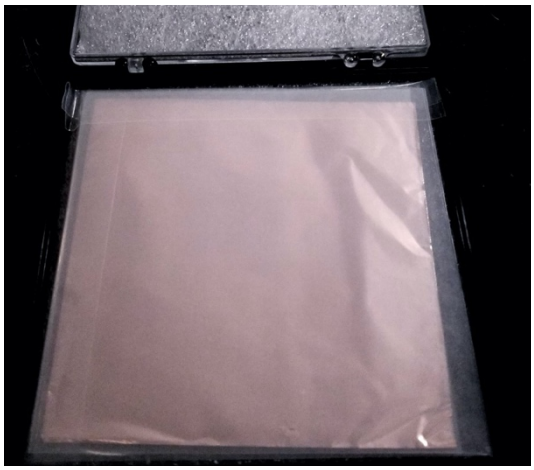
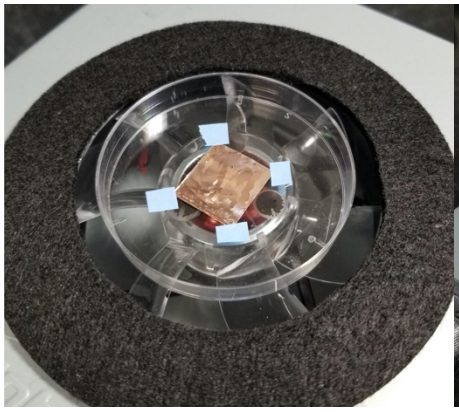
Procedure for Making Graphene Oxide TEM grids

This procedure is based on a published protocol by Han et al 2020, to prepare monolayer graphene oxide for cryoEM applications, be sure to cite this paper [1].

1. Make 1M solution of APS (22.8 g into 100 mL DI water). Pour solution into large Pyrex dish.



2. Cut out a section of graphene/copper film with enough area to cover the number of grids you intend to make. Use the small scissors to cut along the plastic film (use as a guide to make a straight line).



3. Place graphene/copper foil in spin coater, tape down corners. There is graphene on both sides of the copper foil, so it doesn't matter at this point which side is up.

4. Use the glass pasture pipette to apply a few drops of MMA EL 6 in center of graphene/copper foil. COVER BEFORE TURNING ON THE SPIN COATER!! Bring spin coater up to speed and let run for 1 minute. After spinning, clean the glass cover to remove the excess MMA.

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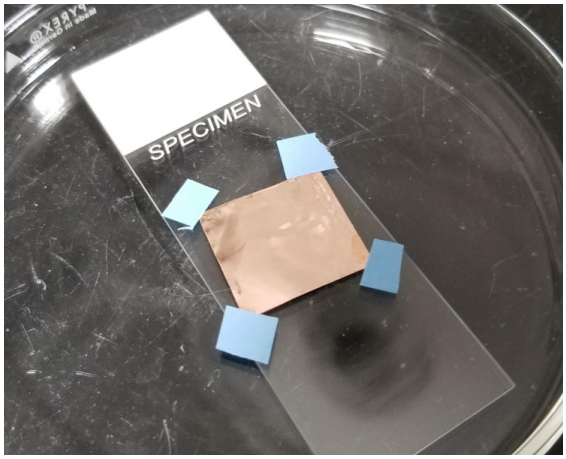
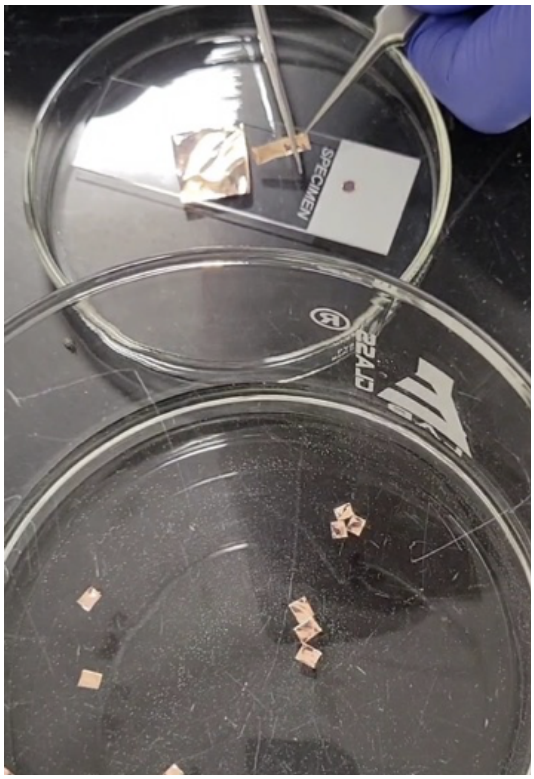
5. Place MMA/graphene/copper foil MMA side down on a glass slide and glow plasma clean with TergeoEM (use the Quantifoil Recipe) to remove the graphene layer on the backside of the graphene/copper foil.



6. After plasma cleaning, cut the film into squares slightly larger than a TEM grid, keeping track of which side the MMA film is on at all times!



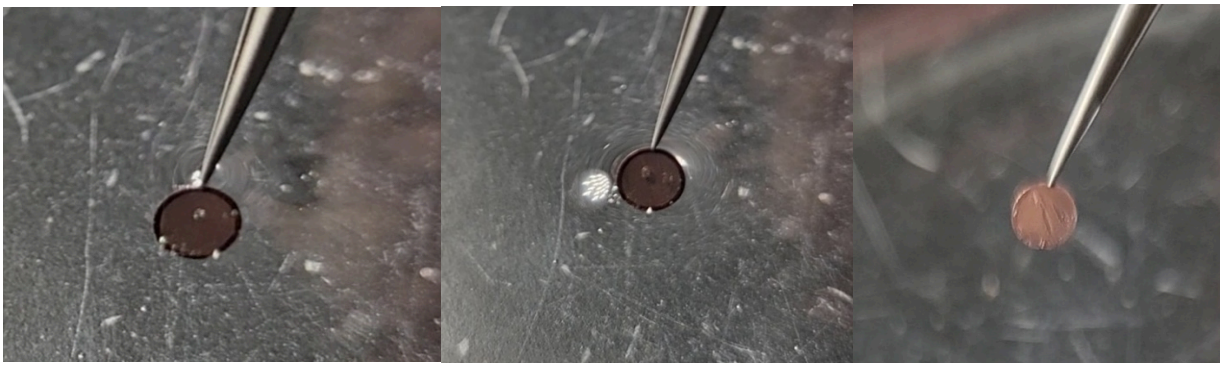
7. Float the newly cut pieces in 1 M ammonium persulfate (APS), exposed copper side down, to remove the copper substrate. This will take 1-2 hours. If, after 1 hour, some of the pieces still have a lot of copper, they were likely put on the surface of the solution upside down. Flip them over and let them sit for the remainder of the time or until no traces of copper are left on the films. The picture below shows a piece that was put in correctly (the large

bubbles on the right) and a piece that was put in upside down (the undissolved copper square on the left).

8. At this point you should have clear MMA/graphene films with the MMA layer facing up.

9. Using a glass slide, transfer the MMA/graphene films to DI water, floating them on the surface for 10 minutes.

10. Using a pair of fine tip forceps, pickup one TEM grid, and with a scooping motion transfer a film onto the carbon side of a TEM grid. Make sure that the film covers one side of the grid smoothly and completely. Allow the grids to air dry and then place them on a glass slide facing carbon side up.



11. Bake the dried grids on a hot plate at ~130 C for about 20 minutes. Allow to cool to room temperature.

12. Place the grids warm acetone for 45 minutes, use small Pyrex dish, keep the TEM grids carbon side up.

13. Transfer grids to fresh acetone for another 45 minutes or use pasture pipette to remove old acetone and add more. Do not allow the acetone to dry on the grids at any point during this step.

14. Transfer grids to isopropyl alcohol (IPA) for 30 minutes.

15. Remove grids from IPA and use filter paper to wick away standing IPA on grids, then place on a glass slide and heat on hot plate (100 C) for 20 minutes.

16. Grids can be stored at this point, place the TEM grids in the Dry Keeper.

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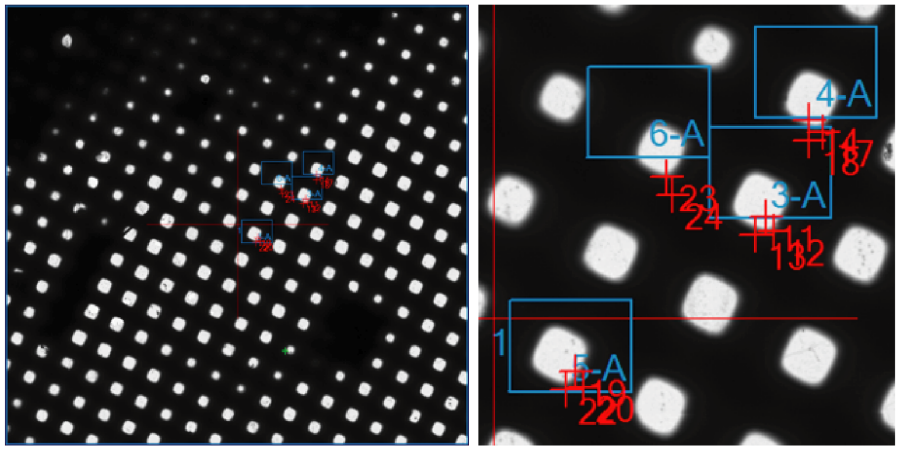
Day of cryo-sample prep

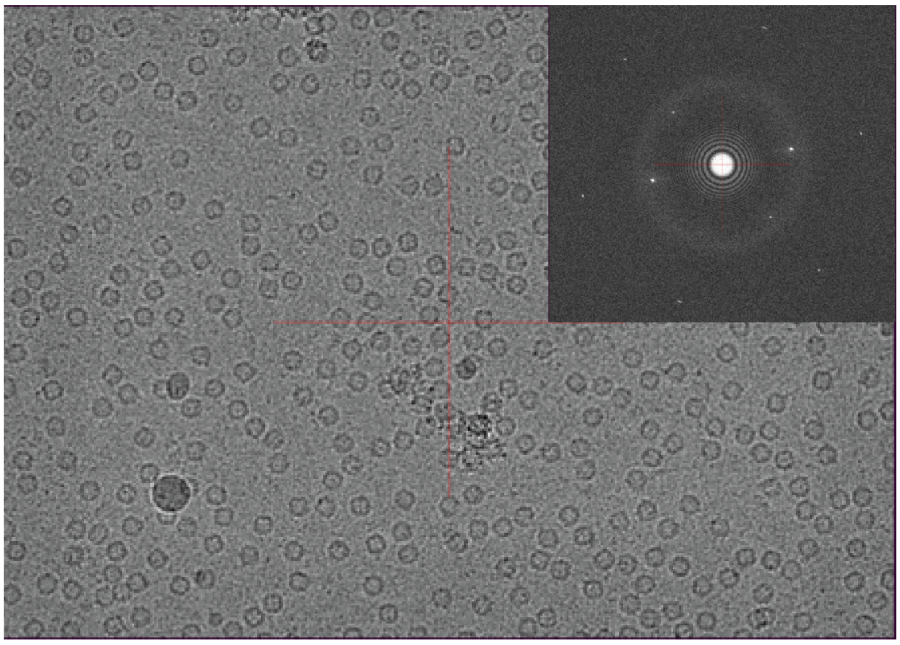
17. Use UV/Ozone cleaner (4-4.5 minutes) to make GO grids hydrophilic. Place GO TEM grids GO side up on a glass slide, and place inside the UV/Ozone. We recommend UV/Ozone cleaning immediately before preparing cryo-grids with the Vitrobot. DO NOT cleaner. GLOW DISCHARGE OR PLASMA CLEAN GRAPHENE-OXIDE GRIDS, THIS WILL RUIN THEM!

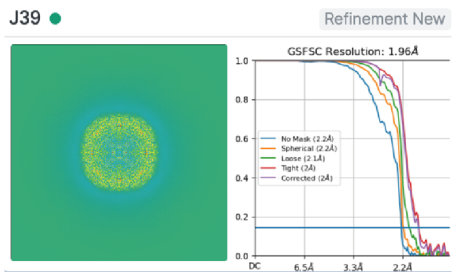
18. We recommend using a protein concentration similar to what you might use for a continuous carbon film grid prep if optimum conditions for GO grids are not yet known. For Mouse Apoferritin we typically use 1 mg/ml, which 1/5 of that used to prepare UltrAufoil TEM grids.

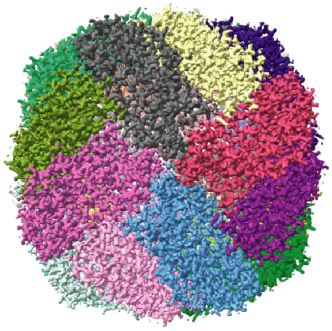
Results

CryoEM of Mouse Apoferritin on GO TEM grids prepared using this procedure. Full Montage of a GO TEM grid, data collected over grid squares 3A, 4A, 5A and 6A. Micrograph of Apoferritin and corresponding FFT, the 6-layer lines indicate single monolayer of GO. Mouse Apoferritin EM map at 1.96 Å resolution collected with the UNC Chapel Hill Talos Arctica.









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References

1. Han, Y., et al., *High-yield monolayer graphene grids for near-atomic resolution cryoelectron microscopy.* Proc Natl Acad Sci U S A, 2020. **117**(2): p. 1009-1014.