Quick and dirty (but actually not dirty) negative staining protocol

Purpose

This protocol can help get you started on making your first negative stain grids. It works well for samples like ribosomes and apoferritin.

Set-up

1. Negative stain grids with continuous carbon (e.g. 20 nm)
2. Plasma cleaner, i.e. Gatan Solarus
3. Protein at appropriate concentration, 3 µl per grid
4. MilliQ water, ~500 µl
5. 2% uranyl acetate, 10 µl per grid
6. Whatman filter paper
7. Negative action tweezers, or positive action tweezers with clamps, at least 1 pair
8. 10 µl pipette and tips
9. Timer

Protocol

1. For each grid, prepare 3x 5 µl drops of MilliQ water followed by 2x 5 µl drops of 2% UA on a piece of parafilm.
2. Place desired number of grids on grid holder with carbon sides facing up.
3. Plasma clean with Gatan Solarus, using 'Carbon film standard' protocol, which uses H_2/O_2 (6.4:27.5 sccm) for 30 sec.
4. Pick up a grid with the tweezers, carbon side facing up.
5. Apply 3 µl sample onto carbon side of grid for 30 sec.
6. Touch the edge of the grid briefly on Whatman paper to blot away liquid, then immediately touch the carbon side of the grid to the first drop of water to pick it up.
7. Repeat blotting and picking up of subsequent drops without any incubation time, until the final drop of 2% UA is picked up. Allow to stain for 30 sec.
8. Blot away the stain from the grid and allow to dry on the tweezers before transferring to a grid box for storage.
9. Allow negative stain grids to dry for 30 min before loading them onto a microscope.

Notes

- MilliQ water drops help to wash away buffer components like phosphates and glycerol that can reduce staining quality. Buffered solution can be used instead of water in this step.

Additional / reading material

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5912373/