Negative Stain Electron Microscopy of Microtubules

Negative staining is a rapid, qualitative method for analyzing microtubule structure at the EM level. Because negative staining involves deposition of heavy atom stains, structural artifacts such as flattening of the cylindrical microtubule and opening up of microtubules into flat sheets are common. Cryo-electron microscopy, where microtubules are flash frozen in a thin film of vitreous ice and imaged without staining, is currently regarded as the best method to view native microtubule structure at high resolution. Nevertheless, negative staining is very useful because of its ease, rapidity and lack of requirement for specialized equipment other than that found in a regular EM facility.

I. Solutions and Supplies 0.5% (w/v) Uranyl Acetate (prepare by dissolving 50 mg UA in 10 ml of ddH_2O. Add water to tube containing UA, cover tube with foil and rotate in coldroom for several hours till fully dissolved. Filter through a 0.22 µm filter that has be prerinsed well with ddH_2O. Filtered stain stored at 4iC in a foil-wrapped tube can be used for >1 year.)

Filter strips (prepared by cutting Whatman #1 filter paper into small slivers)

Grids (200 mesh copper grids that have been formvar coated, carbon coated)

Rinse (ddH_2O with 5 mM EGTA or as appropriate)

II. Negative Staining Protocol

1. Glow discharge formvar and carbon coated grids just before use to increase their hydrophylicity.

2. Place sample on the grid (1-3 µl, sufficient to cover the grid surface).

3. ~10 sec later slowly pipet on 20 µl of UA stain using a P-20. While pipeting onto the grid, gently absorb stain on the opposite side using a filter paper sliver. The staining procedure should take ~30s-1'.

4. Allow the grid to dry after absorbing as much stain as possible with the filter paper and examine the grid as soon as possible, preferably on the same day. If there are problems with stain precipitation, or with general stain background then rinses prior to staining may be necessary (see below).

We have found this straightforward negative staining procedure to work very well with stable microtubules such as taxol or GMPCPP-stabilized microtubules. We do not recommend using UA dissolved in 50% methanol, since we had irreproducible results with this stain formulation. If there is a lot of salt or sucrose/glycerol in the buffer, then washing the sample prior to staining may be necessary. If dynamic microtubules undergoing polymerization at 37iC need to be examined, then a rinse with warm BRB80 may be necessary to remove the large amount of unpolymerized tubulin before applying stain.

Rinsing: There are several methods for rinsing the grid surface prior to applying stain. One method is to apply sample to the grid, allow adsorption for ~10 sec, hold the grid tilted downward and drop 2-3 large drops of rinse (either ddH_2O + 5 mM EGTA for removing interfering salts/buffer components/sucrose/glycerol or warm BRB80 for removing unpolymerized tubulin) over it and then apply the stain. Alternatively, rinsing can be done by placing a large drop of the rinse solution on parafilm and slowly drawing the grid, with the sample-coated surface facing the parafilm, over the surface of the rinse solution drop. The stain can then be applied as above.

Dilute samples can be concentrated on the grid by adsorption for longer times (1'-3'). Negative staining is relatively quick, so try both direct staining and rinsing prior to staining for the specific reaction conditions being analyzed.