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Rotary Shadowing Macromolecules

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I'm somewhat amazed by how many varied techniques there are for rotary shadowing, and how few seem to agree with what works for us. So, here is our method.

We shadow biological molecules from the connective tissue matrix, usually ranging in size from 16 to 300 kilodaltons. Many are linear but some are globular. We spray the molecules in solution with 70% glycerol. The other 30% is 100 microgram/mL of protein, preferably in a volatile buffer such as 1% acetic acid or 0.1M ammonium bicarbonate, pH 7.8. Other buffers can be used, but salt crystals can be a big problem. Spraying is done using an air brush, and we spray onto freshly cleaved mica discs cut from sheets using a hole punch. The sample is dried in vacuum, though we often accumulate 20 samples, so some drying occurs outside vacuum.

Before coating, we are careful to pre-pump the vacuum chamber and heat the guns thoroughly to out-gas them, then vent the jar with nitrogen, introduce the samples, and pump again. (Our system uses a turbo-molecular pump.) We can vary the angle of the rotary stage from outside the chamber, so we tilt the specimen away from the gun and again outgas thoroughly so that little vacuum loss is seen during evaporation. This is very important, as gas is introduced to the rods whenever the system reaches atmospheric pressure. It is also important to keep the system clean, as outgassing a dirty gun takes much longer than outgassing a clean gun.

We try to complete evaporation without entering the 10^{-5} range, and we begin in the 10^{-7} range. We evaporate at a slow rate, usually taking 3 to 5 minutes to complete a run, which also seems to improve resolution. Final film resolution is proportional to *vacuum: the better the vacuum the smaller the grain size*. We use a quartz monitor for controlling the amount of Platinum - Carbon coming from the electron beam gun, but we also use a folded piece of filter paper placed 90 degrees relative to the source, and monitor the color, which should be dark gray (not black, not brown). We evaporate at 6 degrees relative to the sample as the sample rotates.

Following Pt-C deposition, we then tilt the sample to 90 degrees relative to a resistance carbon source and evaporate a backing film of carbon onto the mica. The thickness of the film is monitored with a piece of folded filter paper placed 90 degrees relative to the carbon source, and the correct amount is a just visible tan color (not gray) on the filter paper. (Our film thickness monitor is not sensitive enough to monitor carbon deposition.) We find this carbon film absolutely necessary for sample stability, perhaps because we use so little Pt-C. However, too much carbon will certainly affect final resolution, losing edge detail.

Finally, the replicas are exposed to the vapors of 1% acetic acid in a petri dish for about a half-hour prior to floating in distilled water. This acid treatment is very useful in helping to release the replicas from the mica (so that they float off as one intact film). We use high-transmission 600 mesh grids to support the films.

For many years we evaporated Platinum wire from carbon rods using a resistance source. We wound 2.3 cm of wire around a cylindrical jig which was the same diameter as the sharpened

carbon rods (about 1 mm). Prior to coiling the wire, we passed it through a alcohol burner flame until it was orange, which made it more malleable and perhaps cleaned it a bit. The coil was transferred to the resistance source, spanning the intersection of two rods (therefore the site of most resistance and primary heating) held together with moderate spring tension. The carbon rods with accompanying Pt coil were at 6 degrees and about 11 cm away from the mica discs. Using a welders plate (Fullam #12511), we observed the metal as current was increased through the rods, and just after the wire melted, the current was turned up just a bit more and left there until the Pt was seen to evaporate completely. It was necessary to observe this through the welders plate to get a good shadow and to know when to turn the current down to avoid too much carbon. We evaporated a backing film of pure carbon from a second source oriented at 90 degrees from a distance of 11 cm so that the rods just started to spark, counted to 1.5 seconds, at which time the amount of carbon was probably about right (as judged by filter paper color and a bit of luck). ■