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Microtechnologies for Structural Genomics

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Tips for Using the MicroRT™ System for Room- and Low-Temperature Crystallography

Our MicroRT system replaces conventional glass capillaries and makes collecting data at room temperature as easy as at low temperatures. By allowing rapid room-temperature screening and early weeding out of poorly diffracting crystals, MicroRT can increase your overall diffraction throughput by a factor of five or more.

- To prepare a sample, start by injecting your environment-stabilizing solution into the polyester tube using a syringe needle. Try to **inject the solution all the way down to the sealed end**, so there's no gas bubble between the end and the liquid plug. This will help the liquid plug to stay put even if the sample is roughly handled, and eliminate plug motion caused by temperature changes.

- **Be generous with your plug size** (say, 10-40 μl), especially if you want the crystal to remain fully hydrated for several hours. There is some evaporation (roughly 80 nl/hour) through the very thin polyester wall.

- Mount your crystal on a MicroMount or MicroMesh that's been inserted into one of our B1 or B2 goniometer bases. Remove excess liquid, but don't let the crystal dry out entirely or it may fall off the mount.

- Working under a magnifier or microscope, **draw the polyester tubing down over the crystal and mount and onto the goniometer base**, being careful not to bump the crystal. To make a better seal (required only if the crystal will sit for several hours), apply a tiny amount of grease (e.g., Dow-Corning #976V high vacuum grease) or oil to the tube-capturing tip of the goniometer base.

- Align the crystal manually or using an auto-alignment system, just as you would for a MicroMount without the tube. **Alignment is much easier than for crystals in glass capillaries** since the crystal doesn't contact the tubing wall and there's no optical distortion.

- After you've collected a desired number of frames, slide the polyester tubing off. You can then soak the crystal in any desired solution or replace the liquid plug with another solution (e.g., to vapor diffuse in a small molecule or to dehydrate the crystal), and then draw the tubing back over the crystal and take more frames.

- Once you've collected your room temperature frames, just pop off the tubing, plunge the MicroMount in your favorite liquid cryogen, and you're ready to collect low-temperature data.

If you want store your crystals for longer than 12-24 hours, replace the polyester tube with any 2 mm ID glass or thick-walled plastic tubing.

Our goniometer bases are compatible with all standard hardware including plastic caps/vials. The MicroRT system can also be used with conventional nylon loop mounts. However, since the loops can have random positions and orientations relative to the pin and tube axis, there's a greater chance of bumping the loop and crystal when the tubing is slid in place.

Dehydrating Crystals Using the MicroRT™ System

Dehydration has been used as a tool for inducing structural changes in protein crystals since the earliest days of protein crystallography. Max Perutz, John Kendrew and Hugh Huxley used it in their studies of hemoglobin and myoglobin in the 1940's and 1950's. Francis Crick was supposed to work on dehydration when he jointed Perutz's group, but suffered a famous distraction.

Dehydration remains a powerful (and underutilized) tool for improving or at least modifying the diffraction properties of protein crystals. Dehydration removes excess solvent, tightens packing of protein molecules, and reduces the size of solvent channels. As a result, it sometimes improves crystal order and diffraction resolution at room temperature, and can make successful flash cooling easier, especially for crystals with large initial solvent contents. Protein crystals usually undergo structural transformations when sufficiently dehydrated, yielding alternative crystal packings that may be difficult or impossible to achieve directly during crystal growth. Of course, dehydration often severely degrades diffraction properties, but original crystal order can usually be fully recovered just by rehydrating.

The MicroRT system is the basis for an easy, controlled and reliable way to dehydrate crystals. The method is based on the fact that **saturated solutions of salts, when placed in an enclosed container at constant temperature, will maintain a fixed relative humidity that depends on the salt**. By equilibrating a crystal via vapor diffusion with a series of salt solutions, the solvent content can be reduced in a controlled and reproducible way, without the osmotic shock and other problems that occur when crystals are directly soaked in high salt or high PEG solutions. Here's the procedure:

- Prepare a series of saturated salt solutions corresponding to the desired relative humidities to be tested. As-grown protein crystals typically have relative humidities of 95-98%, so a good set of humidities to test is 93%, 86%, 79%, 75%, and 68%.

- Inject a plug of 10-20 ml of the first saturated salt solution into the sealed end of a MicroRT polyester tube, and draw the tube over your crystal and onto the goniometer base, as described on the other side. If your crystal is to be flash cooled, do your cryoprotectant soak before starting dehydration.

- Take single frames periodically to characterize how the diffraction changes as the crystal dehydrates. The equilibration time depends on the crystal size (but not on the equilibrium r.h.); for 100-200 μm crystals, equilibration will take 1-2 hours.

- After equilibration with the first solution, just pop off the tubing, pop on another one filled with a salt solution that gives a lower final r.h., and take more frames. Repeat until you've explored a desired range of humidities. Then pop on the tube with the salt solution (or the original stabilizing solution) that gives the best diffraction, equilibrate, and flash cool.

- You should have little liquid remaining around the crystal, so there's no need for additional cryoprotection. Some salt crystals may form around your crystal, but the diffraction spots from these can be easily eliminated from your analysis.

Here is a partial list of saturated salt solutions and their relative humidities at 25°C. You can find a more extensive list at our website, www.mitegen.com, in our Technical Notes section.

Salt	**g/ml**	**r.h.**	**Salt**	**g/ml**	**r.h.**
KNO_3	>0.38	93%	$(NH_4)_2SO_4$	>0.79	79%
$(Ba)Cl_2$	>0.38	90%	NaCl	>0.37	75%
KCl	>0.40	86%	$CuCl_2$	>0.76	68%