CRYO MOUNTING OF LYSOZYME CRYSTALS

Lysozyme crystals are fished from the mother solution using nylon loops suitable for data collection with synchrotron radiation. Before fishing, loops are glued to a magnetic base that allows their handling by the robotic mounting system available at the XRD1 beamline of Elettra. The height of the loop pin and the type of base used are specific for each automatic mounting system. Crystal mounting and freezing can be done in-house, with no need to transport fragile crystallization experiments, thus reducing the risk of damage. Frozen crystals, stored in liquid nitrogen, are transported to the beamline where diffraction data are collected.

When crystals are frozen in liquid nitrogen within their mother solution, it is important to avoid the formation of crystalline ice. Ice formation in the large channels of protein crystals would damage the ordered arrangement of proteins, reducing the crystal diffraction power. In addition, small ice crystals in the mother solution would yield a powder diffraction pattern ("ice rings" on the area detector) and hamper data analysis. To avoid ice formation, before freezing, crystals are dipped in a 20% glycerol solution with the same composition as their mother solution.

1. Fill an insulated container (polystyrol box) with liquid nitrogen and cool down a carousel suitable for handling by the automated mounting system of the Elettra XRD1 beamline (Figure). If required, add liquid nitrogen. Temperature of the carousel should be stable before introducing the samples.

Figure: Carousel suitable for the *Sample Changer* system present of the XRD1 beamline of Elettra. Each carousel contains up to 10 crystals.

ATTENTION: While working with liquid nitrogen, pay attention to avoid splashing the freezing liquid to hands and eyes. Face and hands should be protected using suitable personal protection equipment. In addition, nitrogen is an asphyxiating gas, therefore the area should be properly ventilated.

2. Select a single crystal to be mounted. Select a loop with suitable dimensions: the loop should be slightly bigger than the crystal. A large amount of mother solution in the loop would increase background scattering.

3. In a centrifuge tube, dispense 20 μL of glycerol using a pipette for viscous liquids. Open the well containing the selected crystal, avoid touching the drop. Aspirate 80 μL of the reservoir solution contained in the crystallization experiment, add them to the glycerol and thoroughly mix the cryo-protectant solution. These operations should be performed quickly to avoid drying and precipitation of the crystal containing drop.

4. Prepare a 1-2 μL drop of the cryo-protectant solution and dispense it next to the crystal drop on the coverslip, avoiding mixing the two drops.

5. Attach the selected loop to the specific magnetic wand. The magnetic wand should be completely dried. With the loop, fish the crystal from the drop and dip it into the cryo-protectant drop. Work quickly: fish the crystal out of the cryo-protectant solution and immediately dip it in the liquid nitrogen. The magnetic wand reduces the risk of cold burns on the operator's hands.

6. Keeping the crystal in the cold liquid, place the crystal in the vial already positioned in the carousel. Once all carousel positions are filled, transfer it to the transport dewar (dry shipper).