INORGANIC OR BIOLOGICAL CRYSTALS?

First of all, before deciding on how to distinguish whether the crystal is inorganic (salt) or biological (protein), take a picture of the crystal before you destroy the crystal or make it disappear.

The most definitive test is to obtain an x-ray diffraction pattern of the crystal. A diffraction pattern of a protein crystal may look like that in figure 1. Want to take a less direct approach? Read on.

The following are ways to differentiate a biological crystal from an inorganic crystal.

1. Dehydration

A biological crystal typically has very significant solvent content and will dehydrate when removed from the drop or the drop is allowed to evaporate from around the crystal (figure 2). Inorganic crystals typically do not possess large solvent channels and have very little solvent content. Removing an inorganic crystal from the drop or allowing the drop to evaporate from around the crystal will typically not destroy or change the appearance of the inorganic crystal.

2. Physical Manipulation

A biological crystal behaves more like an ordered gel than a hard crystal and will powder, crumble, or break easily when touched with a probe such as a Micro-Tool or needle (figure 3). Inorganic crystals can also break apart, but they require more force and typically make a click or crunching sound when breaking apart under the force of a probe. The inorganic crystals are typically more dense than protein crystals and once broken, the pieces fall quickly and stay put on the bottom of the drop.

3. Birefringence

A biological crystal, unless it is cubic, will be weakly birefringent under cross polarizers (figure 4). Inorganic crystals are typically strongly birefringenet under cross polarizers. Some plastic plates and materials are also birefringent so this test is more easily performed and interpreted in an all-glass environment or in a plate made from a low birefringent plastic.

4. Dye absorption

A biological crystal typically has large solvent channels which will accommodate a small molecule dye. Small molecule dyes can travel into these solvent channels and color the crystal (figure 5). Inorganic crystals do not possess such solvent channels and will not absorb the small molecule dye. Dyes are chemicals and have solubility limits. So it is possible that a crystal grown in a reagent of high relative supersaturation may have a reagent concentration that will precipitate the dye or even crystallize the dye. Most dyes under such conditions will crystallize into needles or whiskers and of course be colored. So before adding dye, take a picture or memorize the location of crystals in the drop in case the crystal itself forms crystals. Finally, diluting the drop with dye can sometimes decrease the relative supersaturation in the drop to the point where the biological crystal will dissolve. Once the drop equilibrates again with the reservoir, the crystal may reappear and it may appear in a location different from the original crystal. For dyes, consider Izit (HR4-710) from Hampton Research.

5. Control Experiment

Using the same sample buffer, same reagent, same volumes and same hardware, set an experiment identical to the one that produced the crystals, except leave the sample out of the experiment. Simply replace the sample with the sample buffer. Don't get lazy and use water instead of sample buffer because the sample buffer may be a variable in the formation of the crystals. If a crystal forms that appears visually similar to the original crystal you likely have an inorganic crystal in the original setup.

6. Run a Gel

Collect, wash, and dissolve the crystals. Run the sample on SDS-PAGE. If a band indicates the presence of your sample, there is a high probability that your original crystals are biological.

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figure 1
X-ray diffraction pattern from a protein crystal.

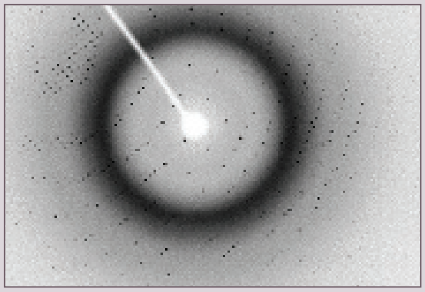
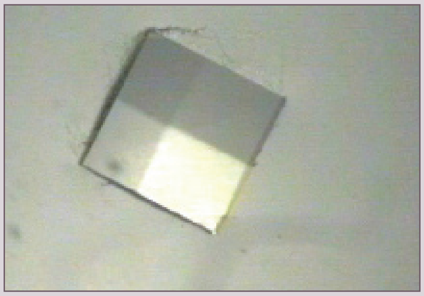


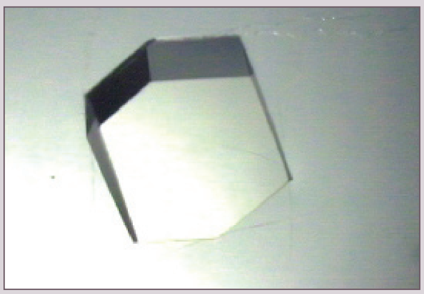
figure 2
Below is a happily hydrated protein crystal. On the next page is an unhappy, dehydrated protein crystal, removed from the drop.



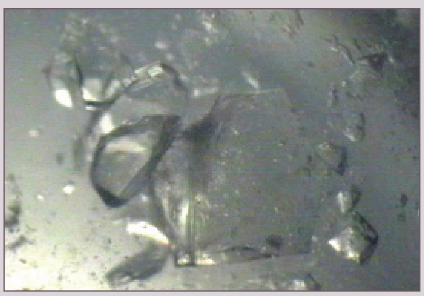
Dehydrated Protein Crystal

figure 3

A protein crystal before and after being bullied by a probe.



Before: Pre-Crush



After: Crush

figure 4

A weakly birefringent biological crystal.

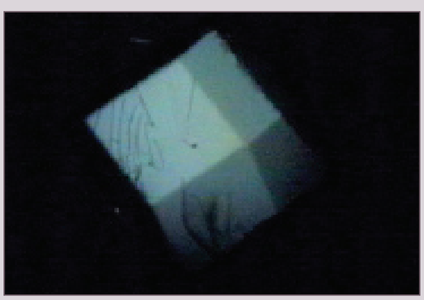


figure 5

Protein crystal stained with a dye.