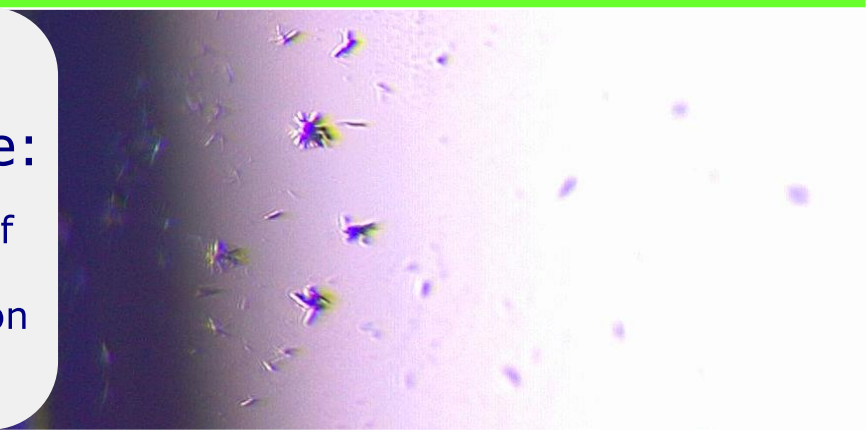
Hot Topic

From Crystals to Structure:

Micro Crystallization in the Pursuit of Natural Products Structure Elucidation



Application Note

Natural Products: Crystallization and Structure with < 0.1 mg of material

The Challenges

Knowing accurately the molecular structure of a certain compound is of great importance. Research facilities and the industry use many analytical techniques to understand and determine the structure of their compounds studied. Among these tools, single crystal X-ray diffraction (SC-XRD) plays an important role for structural elucidation. Though the challenge always is to obtain crystalline material of a certain size (> 0.01 x 0.01 x 0.01 mm^3, i.e., 10 x 10 x 10 μm^3) that can be used for X-ray diffraction experiments. Powerful Synchrotron facilities might help when the crystal size is somehow smaller. Though there is a limit on the minimum size needed: ~ > 1 µm (1000 nm). Electron diffraction (ED, also know as micro-ED) is a very promising technique, as the crystalline material needed must be below 1 µm (1000 nm) in all dimensions. In other words, ED allows us to measure nano crystals for structural elucidation purposes, where the crystal size is in the range of 10-1000 nm. Powders usually used in X-ray powder diffraction experiments (XRPD) become now suitable for ED experiments.

Though not all powders are crystalline. Furthermore, the crystallinity of the powder might not be "good" for ED diffraction experiments. In analogy to SC-XRD experiments, the crystals needed for ED experiments should be of "good quality". Though the most important question that needs to be address is: Is it possible to obtain nano crystalline material for ED experiments if < 1 mg of sample is available?

Real sample and goal of the project

A company that extracts natural products from fungi provided us various samples. Each vial provided had < 1 mg of a "honey like / gel like" material (see Fig. 1).

With this reduce amount of sample, it is a challenge to grow crystals that could be suitable for any type of diffraction experiments. Furthermore, what if the sample is not a pure material (100 % purity)? In fact, that is the next big challenge, because any material, including any impurity, that might be present in the sample could crystallize. Moreover, with ED any nano crystals produced can be analysed and will deliver a structure.

The customer's goal was to structurally characterize the natural products extracted from fungi and provided (as in Fig. 1) to Crystallise! AG. To reach that goal, with so little amounts of sample, Crystallise! AG has tackled a implemented a new approach: Direct crystallization on a TEM grid (3 mm Ø) using as low as possible amounts of sample.

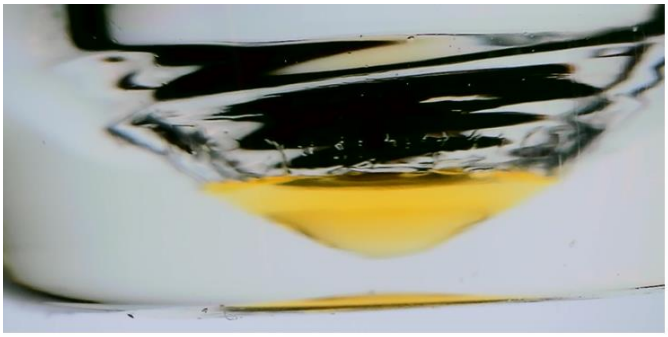


Figure 1. Sample as received, provided by the customer (< 1 mg)

Experiments and Results

After performing "micro-filtrations" and "micro extractions" of the sample, Crystalllise! AG was able to crystallize material on the TEM grids for direct measurements using an electron diffractometer ELDICO *ED-1*.

In general, the crystallization experiments were done with some **40-80 µg of sample**, using a volume of some 50 µL.

The results were very interesting. Various impurities were crystallized: NaCl, $NaNO_3$ and even Polyethylene (PE). By identifying each of these impurities, the crystallization procedure could be optimized further and further until the desired natural product was obtained in a crystalline form.

The final outcome: the structure of a new natural product was successfully obtained. A new macro-cycle (hexa-peptide) was fully characterized with electron diffraction experiments. Figure 2 shows the crystals of the natural product that were grown directly on the TEM grid.

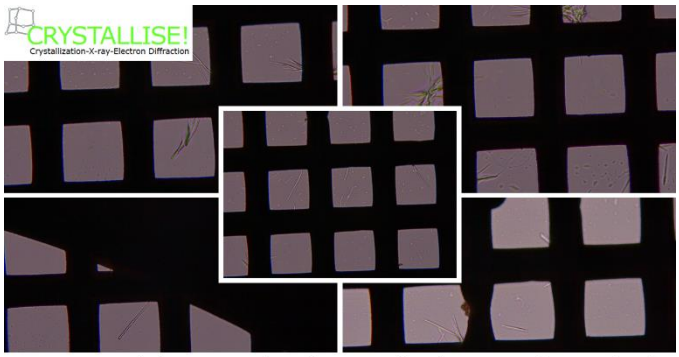


Figure 2. Crystals from a Natural Product grown directly on a TEM (3 mm Ø) grid.

Direct crystallization on a TEM grid can be done using less than 100 µg of sample. An electron diffraction measurement (see Fig. 3) on one of these crystals, produced the structure of the unknown, new natural product.

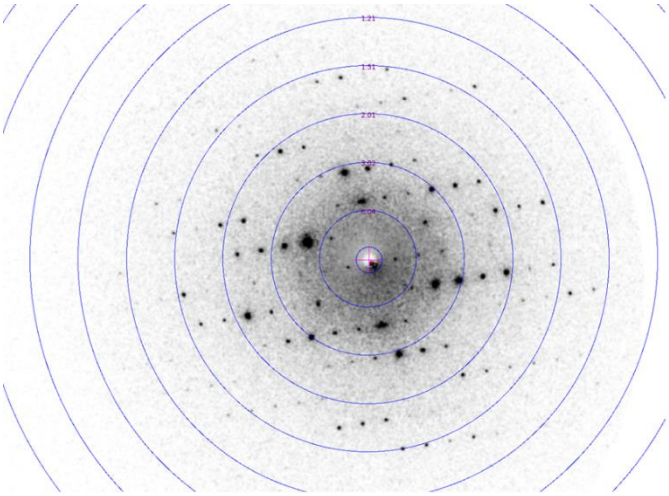


Figure 3. Diffraction pattern obtained using ED

Conclusions

The direct crystallization on the TEM grids is very promising for structural elucidation using electron diffraction techniques.

A mixture of components totalling < 1 mg of sample was successfully treated for the crystallization and characterization of a new natural product.

The procedure used, can be applied to any sample where the amount of material available is extremely low.

If crystallization on the grid is achieved, ED is a powerful technique for the structural characterization of the compound(s) studied.

Fields of applications

The field of natural products seems to be a very promising area to use this approach. Crystallise! AG has recently done the crystallization and structural characterization of some natural steroids, and this family of fungi natural products presented here. Furthermore, we have been recently contacted by a research facility working with the isolation of natural products from insects.

In general, the field of applications are those areas where a small amount of material is present, for example metabolites or others. Moreover, we believe that this procedure can be applied even when mixtures of materials are present. In our latest example, inorganic salts (and impurities) were crystallized first before achieving the crystallization of the natural product.

The approach can also be used for the identification of impurities. This is important because it might help to understand the physical properties of the sample studied. But most importantly, it brings a lot of knowledge for further experiments that need to be performed.