Renewed interest in microED
Recent blogs highlighting the technique of micro-electron diffraction (microED) and its utility in the structural determination of small molecules have significantly increased its visibility in the scientific community. These posts were based on the publications of Gruene et al. and Jones et al., who showed that structural details can be extracted from individual nanocrystals, even in a heterogeneous mixture.

Not only was the required size of the sample considerably smaller than with X-ray crystallography (XRD), but the speed with which the structure was determined was also remarkable. Within just a few hours, researchers were able to solve the structure of a powder (e.g. acetaminophen found in a commercially available combination pain killer). This is an exciting and extremely promising advancement that is poised to reshape the very landscape of drug discovery.

A commercially available sample of progesterone analyzed via microED. The resulting atomic structure was determined at a resolution of 1 Å. Figure adapted from Jones, C.G. et al. The CryoEM method MicroED as a powerful tool for small molecule structure determination. ACS Cent. Sci., v. 4(11), p. 1587–1592. 2018.

Acetaminophen structure determined from a commercial microcrystalline blend. Individual crystals (B) are too small for XRD, but diffraction intensity can be clearly visualized with microED (C). Figure adapted from Gruene T. et al. Rapid Structure Determination of Microcrystalline Molecular Compounds Using Electron Diffraction. Angewandte Chemie, v. 57(50), p. 16313-16317. Dec. 10, 2018.
**The microED method**

MicroED’s unique requirements set it apart, not just from other cryo-EM techniques, but from traditional crystallography as well. It necessitates small crystals (<200 nm in size), which are typically significantly easier to create than large XRD crystals (>50 µm) but can also be cut (or milled) from these larger crystals using a focused ion beam (FIB). Crystals >200 nm have increased secondary scattering, which convolutes the data. Sample preparation also varies between small molecule samples and protein samples. Small molecule crystals are usually dry, they can be handled, and they are often even measured, at room temperature. When reducing the size of large crystals composed of small molecules, the sample can easily withstand mechanical grinding or can simply be crystallized spontaneously out of solution using evaporation.

Protein crystals, however, are typically kept in water to retain their hydrated native states. These samples are subsequently flash-frozen (vitrified) in order to avoid sample damage due to crystalline ice formation. These samples are sensitive to changes in humidity or to the buffer and may disintegrate at the slightest touch. Therefore, it is recommended to use the FIB-milling approach on vitrified samples to reduce large protein crystal size.

Finally, due to the unique dimensions of the crystal, a small beam size and good stage stability are key. Fortunately, Thermo Scientific™ EM stages are capable of a full range of motion from -70° to +70° while maintaining crystal illumination. Additionally, low-dose imaging (1.5–3.0 el/Å²) is preferable, as this minimizes sample damage and the beam strength does not impact the diffraction pattern resolution.

<table>
<thead>
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<th>Creation of small crystals (small molecule)</th>
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<tr>
<td>• Grinding of larger crystals</td>
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<td>• Evaporation of solvent</td>
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<th>Creation of small crystals (protein)</th>
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<tr>
<td>• Vitrified to preserve structure</td>
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<tr>
<td>• Larger crystals reduced in size via FIB milling</td>
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**Axis of rotation for microED diffraction pattern collection. Stability is key in order to keep the small nanocrystal illuminated.**
Proof of concept: paracetamol/acetaminophen
While the structure of acetaminophen had previously been determined with microED, we wanted to prove that we could replicate these results in-house.

Example of microED structure determination performed in-house on Thermo Scientific instrumentation.

This process highlights some of the key benefits of microED analysis. First, microED is orthogonal to NMR, mass spectrometry, etc., and carries the added benefit of easy sample preparation. Only ~10⁻¹² grams of the sample were required to obtain the structure shown, and this nanocrystal could be extracted from a mixture consisting of filler or other compound constituents. MicroED is also incredibly fast, continuously collecting a 70-degree range of data in a matter of seconds.

The data processing procedure, performed in the open-source Diffraction Integration for Advanced Light Sources (DIALS) software, is outlined below. It is important to note that the full structure could be determined from a dataset that was only 43% complete; additional data from other crystals could be used to further enhance these results.

MicroED and Structural Biology
While most microED results are comparable in quality to equivalent XRD experiments, the electron diffraction measurements are achieved with far smaller quantities of material. Below is a comparison of several characterization techniques applied to the structure of granulin. This protein is found on the crystalline surface of granulovirus particles and is responsible for the virus’s longevity outside of a host.

**X-ray crystallography with Synchrotron**
- Large crystal needed (>2 µm)
- 21 granulin crystals used for structure determination
- 1.7 Å resolution

**X-ray crystallography with X-ray free-electron laser (XFEL)**
- Small crystals can be used
- Large quantity needed to solve structure (83,000 native crystals)
- 2.0 Å resolution
- XFEL is expensive and access is restricted

**MicroED**
- Small crystals needed
- 5 granulin crystals used for structure determination
- 2.8 Å resolution

Electron density map (wireframe) of granulin obtained at 2.8 Å resolution using microED. The corresponding molecular structure is overlaid. Atomic resolution is possible, as indicated in the inset (red).
Using microED, researchers from Thermo Fisher Scientific were able to obtain the structure of granulin at a 2.8 Å resolution from merely 5 nanocrystals. While this resolution is not quite as good as more expensive crystallographic techniques, a substantially smaller quantity of material was necessary. However, looking to the future, further improvements to the resolution achieved with microED are possible with instrument and method optimization.

What can microED do for you?

- **Target Discovery**
- **Lead Discovery**
- **Lead Optimization**
- **Pre-clinical Development**
- **Clinical Development**
- **Registration**
- **Marketing & Sales**

**Structural Biologist**
You cannot get crystals large enough for standard crystallographic analysis.
You want to reduce time-to-structure for enhanced impact (large crystals often have an extended optimization period).

**Medicinal Chemist**
You need routine structure confirmation, particularly on compounds that are resistant to standard NMR and MS analysis.

**Analytical Chemist**
You would like structural determination for sample-limited and patentable compounds.
- Example: metabolites (MetID), naturally derived compounds

**Physicist**
You are interested in determining crystal form, polymorphism and the composition of mixtures.

Find out more at pharmadrugdiscovery.com