Investigating microcrystal electron diffraction

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In the field of single crystal X-ray diffraction one often feels limited whenever the crystal size is not optimal. Lately electron microscopy has benefitted by the revolution in detector technology. This has also led researchers to explore microcrystal electron diffraction (MicroED) using a transmission electron microscope with nanometer to micrometer sized single crystals. In recent years, the method has been shown to work for solving a few crystal structures of proteins in the Gonen lab. It has been ascertained that the data is of sufficient quality for structure solution using the direct methods and molecular replacement and refinement using standard X-ray crystallographic software. We have now tried electron diffraction with crystals of proteins (RNA polymerase, lysozyme, metalloproteins), small peptides (vasopressin) and small molecules. Micro-crystals of the proteins studied were grown either by using a robotic crystallization screening method in the sitting-drop geometry or by the hanging drop method. A uv microscope was used to ensure that the micro crystals were indeed protein. Grids were prepared by either a manual, slow and gentle blotting method *or by* using a Vitrobot. A glove box was used to manually freeze anaerobic metalloprotein crystals on grids. Low-dose (~0.08 e/Å^2/s) electron diffraction data was collected on samples at liquid nitrogen temperatures, using the continuous rotation method. The screening for microED is time intensive as there are several challenges including crystal deterioration during vitrification, salt crystals forming during grid freezing, sensitivity to radiation, icing on grids, crystals being too large and absorbing electrons, no diffraction and inability to find multiple crystals as data set from one crystal tends to be incomplete. In addition when we eventually collect data, the merging R-factors are higher than what we typically see for X-ray diffraction. The ability to measure weak reflections as from high resolution protein crystal diffraction is very crucial and the detector features seems to be crucial for good microED data. The detector should include ultra-fast readout, linear behavior within a broad dynamic range for each pixel, high signal-to-noise ratio and single-electron sensitivity. A comparison of the resolution of the diffraction data, completeness, tilt range, structure solution and refinement software used for microED structure determination of vasopressin hormone and small organic compounds will be presented.