Nature webinar: Determining protein and small molecule structures with Microcrystal Electron Diffraction		
1	How to prepare the sample in powder form such as ibuprofen that show in the slides on the grid?	Grinding it between 2 glass slides. Apply some to the grid, remove the excess. Or dissolve it in low-boiling solvent and let it evaporate on the grid.
2	Which software do you use for indexing and scaling?	We (Thermo) process our data with Dials, others are happy with XDS. Because of the flat Ewald sphere, it's best to use a program that can index on a full sweep of data, not single images. It is important to fix (and accurately know) the distance to the detector. That can be calibrated with gold nanoparticles (powder diffraction). It's also important to center your crystal accurately, rotate with a constant speed and minimise or correct lens distortions.
3	How do you screen crystallization drops for crystals appropriate for micro-ED?	For screening of nano-crystals we have to just take some of the crystallization slurry and stain it on an EM grid. If we see something promising in neg stain, we then move into cryo for imaging. Method based on the Single Harmonic Generation (e.g. Formulatrix: combined effect of SHG and UV-TPEF imaging is so precise that SONICC can detect microcrystals (<1 um).
4	Do you use any cryoprotectant prior to freezing your grid?	You could do it as you won't lose the contrast as you are in diffraction mode (in contrary to SPA where you are in imaging mode). As we are freezing thin sample, the usual flash freezing used for CryoEM SPA workflow make the usage of cryoprotectant superflous.
5	How many data set are required at least to build a 3D model	It depends on the symmetry of course: we could solve paracetamol structure with 40% complete data. By combining 2 to 5 acquisition we can solve the whole structure. Highly sample dependent for small molecules around 1-10. For proteins usually more.
6	Will a video of the webinar be made available?	Our webinars are available here: https://www.cryo-electronmicroscopy.com/cryo-em-webinars/
7	Molecular Replacement was mentioned but what if there is no model available?	Protein microED: Anomalous scattering signal is too weak at the energies used, except perhaps the Uranium K-edge (116 keV) in case of isomorphous replacement with (UO2)2+ ions. But old fashioned Multiple Isomorphous Replacement (MIR) should work. Including SeMet substitutions and Iodonation of tyrosines. The expected phasing power of each metal is smaller than for X-ray crystallography.

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8	The pictures of your grids with protein crystals look very pristine and clean. We are having trouble transferring protein crystal samples to the grid and getting a good freeze with no ice on the grids. Do you have any tips for sample transfer and blotting?	We are using very long blot times, especially when the xtal buffer is very viscous.
9	How can we analyze three dimensional crystals too small to be adressed by X-Ray Crystallography structural analysis?	Fast atomic-resolution 3D structural information. Diffraction data from nanocrystals in minutes.
10	Which technique can address polymorphic crystals, or mixtures of small crystals where XRD is not working?	Instant productivity. Nanocrystals as small as 100 nm can be readily analyzed, removing the burden of growing large crystals (as with X-ray crystallography). Also reduces the amount of sample material required. Mixtures of different polymorphs and compounds can be analyzed.
11	I'm already involved in a membrane protein structural analysis using cryoEM single particle analysis, and I wish to acquire a TEM for SPA. I have many projects who could benefit from MicroED: will I have to choose between a specific microscope for SPA and a specific microscope for MicroED?	2-in-1 solution. MicroED and single particle analysis (SPA) can be performed on the same cryo-electron microscope. This solution is compatible with new microscopes but is also retrofittable on existing units.