Oral presentation

Combining 3D-Electron Diffraction with Scanning Electron Diffraction to investigate nanocrystals within a long acting injectable pharmaceutical formulation

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3D-Electron Diffraction (3D-ED) has captured the attention of many within the pharmaceutical industry due to the ability to determine crystal structures from sub-micron crystals [1]. Here, we combine 3D-ED with low dose Scanning Electron Diffraction (SED) to elucidate the crystal structure and unveil crystal defects from nanocrystals of cabotegravir (GSK1265744), a potent integrase strand transfer inhibitor developed for HIV treatment, which may be formulated as a long-acting injectable nanosuspension. This demonstrates the uses of multi-dimensional electron diffraction to reveal crystallographic information from pharmaceutical nanocrystals which may otherwise be difficult to characterise by bulk techniques such as X-Ray Powder Diffraction.

3D-ED was carried out using a Thermo Fisher Titan Krios G3i operated at 300 kV under cryogenic conditions. A diffraction pattern was recorded for each tilt increment over a range of ± 60 ° at a continuous tilt rate of 1 $°s^{-1}$. Diffraction patterns were recorded on a CETA camera with an exposure time of 0.5 s per frame, forming a tilt series of 240 diffraction patterns. The cumulative electron dose was 20 e $Å^{-2}$. The formulation was directly deposited onto Quantifoil carbon-coated 3 mm copper grids. 3D-ED was used to determine the unit cell consistent with previous work [2]. From this, a preliminary electron diffraction structure solution for cabotegravir was obtained. This was achieved using ab initio dual space methods implemented in SHELXD and kinematically refined in SHELXL to a preliminary R-factor of 22.4%. In addition, SED, a variant of 4D Scanning Transmission Electron Microscopy (STEM), is used to assess sub-nanocrystal structural variations. SED microscopy was carried out on a Thermo Fisher Spectra 300 microscope operated at 200 kV and diffraction patterns were collected on a QD Merlin medipix3 direct electron detector with a convergence semi-angle of ~ 0.5 - 1 mrad, 1 ms dwell time, and a beam current of 2 pA. These conditions resulted in a cumulative electron dose of ~10 e $Å^{-2}$ per scan. *Post-facto* analysis can be performed by producing virtual dark field images (VDFs), formed by plotting the intensity within a defined virtual aperture as a function of probe position. The summed diffraction signals in Fig. 1(b) – (c) are formed from the signal corresponding to the areas highlighted in Fig. 1(a), a virtual bright field image. Contrast within the crystals indicates the presence of strain whilst the related diffraction patterns suggest an orientation relationship between the two parts of the bi-crystal which can be further explored using the 4D dataset; we speculate that the similarity of the *a* and *b* lattice parameters give rise to a propensity for twinning.

In this presentation, we will show how structure solutions obtained using 3D-ED can be used to enhance analysis of SED data for quantitative defect analysis within molecular crystals, a relatively nascent field.

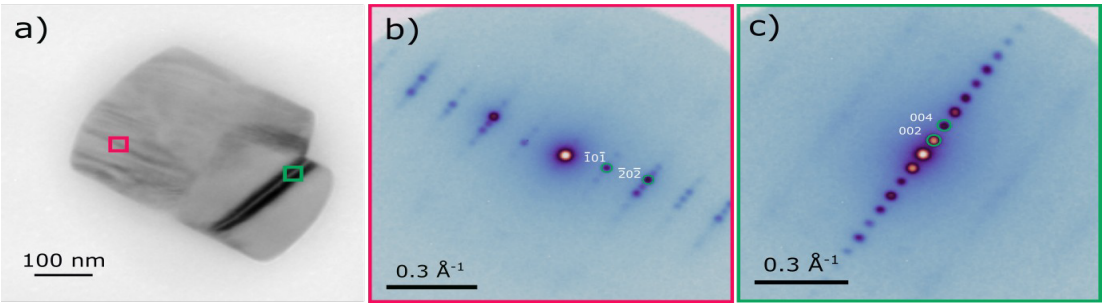

Figure 1. 3D-ED is used to solve the crystal structure of cabotegravir which is subsequently used for SED analysis. (a) a virtual bright field image of a chosen nanocrystal of cabotegravir on which SED microscopy was performed. Contrast indicates the presence of strain. Diffraction patterns (b) – (c) from different areas of the particle are related but indicate an interesting orientation relationship between the two crystals.

[1] Gemmi, M. *et al.* 3D Electron Diffraction: The Nanocrystallography Revolution. *ACS Cent. Sci.* **5**, 1315–1329 (2019).

[2] Johnstone, D. N., Copley, R. C. B., Graves, R. G., Brum, J. & Midgley, P. A. Multidimensional Electron Diffraction-Microscopy of Cabotegravir Nanocrystals. *Microsc. Microanal.* **25**, 1942–1943 (2019).

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