Transmission electron microscopy for the evaluation and optimization of crystal growth

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Crystallization of protein samples remains the most significant challenge in structure determination by X-ray crystallography. Here we demonstrate the effectiveness of transmission electron microscopy (TEM) analysis to aid in the crystallization of biological macromolecules. We found the presence of well-order lattices with higher order Bragg spots, revealed by Fourier analysis of TEM images, as a good predictor of diffraction-quality crystals. Moreover use of TEM allowed 1) comparison of lattice quality among crystals from different conditions in crystallization screens; 2) detection of crystal pathologies that could contribute to poor X-ray diffraction, including crystal lattice defects, anisotropic diffraction and crystal contamination by heavy protein aggregates and nanocrystal nuclei; 3) qualitative estimation of crystal solvent content to explore the effect of lattice dehydration on diffraction; and 4) selection of high quality crystal fragments for microseeding experiments to generate reproducibly larger size crystals. Applications for X-ray free electron laser (XFEL) and micro electron diffraction (MicroED) experiments are also discussed.