Dare to spin – well diffracting protein nanocrystals through on-vortex crystallisation

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Protein crystallisation has been extensively studied for X-ray and neutron diffraction. The hunt for optimised conditions yielding large single crystals has become the staple of protein crystallography labs around the world. Recently new advances in synchrotron beam lines and free electron X-ray lasers have opened the world of smaller (<40 µm a side) crystals being used for structure determination. Electron diffraction (3D ED / microED) brings altogether new challenges to the field. Not only can it utilise even smaller crystals, it even requires one dimension to be sub 1 µm to allow the electron beam to pass through. Additionally, it benefits from the other dimensions being significantly larger, since that allows for more protein to participate in the diffraction measurement - which in turn improves signal strength while reducing beam damage effects. Such ideal plate shaped crystals are often created by focused ion beam milling of larger crystals, but this approach is time consuming and requires specialised machinery. We have found that leaving behind the careful and slow techniques of X-ray protein crystallography and instead employing rapid crystallisation on a vortex mixer can offer surprisingly good control over crystal size in the range required for 3D ED. The optional addition of metal or PTFE beads to create shear forces capable of fragmenting larger crystals provides a feedback loop that seeds new crystal growth should the seed count be insufficient at first. With an optimized protocol taking only minutes, we have succeeded in creating solutions containing countless well diffracting crystal plates of a urate oxidase (UOX), a ribonucleotide reductase R2 and two arginine kinases amongst others. Diffraction data sets to 2 Å are commonly obtainable from these plates with both the diffraction quality and resolution improving with decreasing plate thickness. In the case of UOX the achievable resolution changed from 3 Å to 1.4 Å when switching to the new method.