MS25-04 | Serial Protein Crystallography in a S/TEM

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Serial crystallography, where diffraction snapshots of a large ensemble of randomly oriented crystals are taken, evades the cumulative damage inherent to rotation diffraction techniques. This approach has facilitated the use of sub-micron crystals in latest-generation X-ray sources, making large classes of small, radiation-sensitive systems such as recalcitrant protein crystals or nano-porous materials amenable to crystallographic structure solution [1]. On the other hand, electron radiation provides the advantage of a more favorable ratio of elastic scattering to damaging energy deposition by three orders of magnitude over X-rays, enabling another viable path to study such small, sensitive crystals, using transmission electron microscopes (TEMs) [2].

We present a new scheme for high-speed, low-dose protein nano-crystallography in a scanning TEM (S/TEM), which combines the benefits of both serial and electron approaches in order to achieve ultimate dose efficiency, while providing a high level of automation and ease of operation. Combining automated real-space mapping of protein crystal positions and morphologies with hardware-synchronized beam positioning and diffraction detection using a hybrid-pixel camera, diffraction snapshots from hundreds of crystals can be acquired per second, at a hit rate exceeding 60%.

Results on lysozyme and granulin samples are presented, both of which were solved at a resolution better than 2Å using mostly standard X-ray software, along with a thorough discussion of the data acquisition and analysis pipeline.

[1] Chapman *et al.*, Nature 470, 73 (2011)
[2] Shi *et al.*, eLife 2, e01345 (2013)