Serial and Parallel Diffraction from protein nanocrystals

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We performed experiments at the P03 beamline of PETRA III to characterize protein nanocrystals by powder and serial diffraction and to investigate novel methods for structure determination using high brilliance synchrotron sources. These experiments are part of a research program to develop methods to obtain high-quality structural information from tens of thousands of nano- or micro-crystals, using X-ray free-electron lasers [1] or high-brilliance synchrotron radiation [2], as an alternative to current approaches of protein microcrystallography. In particular, serial crystallography provides the means to rapidly collect and analyse [3] data from thousands (even millions) of single crystals. In our scheme, the assembled set of 3D structure factors is equivalent to a complete dataset collected from a single crystal. In the case of synchrotron experiments, we aim to collect data at a low dose per crystal, and combine data from many crystals to obtain high-resolution structural information at room temperature. We are developing sample flowing methods, strategies for recording diffraction patterns, and determining the radiation sensitivity of small crystals irradiated for microsecond exposures.

For our PETRA III experiments we prepared crystals of the proteins Proteinase K and Cathepsin B. These crystals ranged in size from 1 µm to 20 µm width. We also used strongly scattering iron oxide nanoparticles as a test sample. We set up two different sample delivery methods. For the protein crystals, the sample was loaded into a fixed quartz capillary to obtain a high crystal concentration for static diffraction measurements. For the iron oxide nanoparticles we used instead a micrometer-diameter liquid jet [4, 5, 6]. The exposure of a particular sample in the liquid jet is limited by the time it takes a particle to traverse the focused X-ray beam. In our measurements the flow rate was about 10 m/s and the X-ray beam focus was 20 µm. Therefore the exposure time is limited to 2 µs per object. The flow rate and diameter of the liquid stream was optimised to deliver optimum dose to the sample given X-ray beam size and intensity we had.

We successfully collected diffraction patterns from Proteinase K microcrystals (see Fig. 1) loaded in a glass capillary using different integration times, ranging from 0.1 s to 1 s. Comparing diffraction patterns acquired using several exposure times we obtain an estimation of crystal damage and signal levels as a function of delivered dose. We will use this to study the radiation damage of small protein crystals under different conditions and compare it to the effects of damage in macrocrystals, as well as to the FEL diffraction experiments.

We also collected data on iron oxide nanoparticles flowing in the liquid jet, showing that good quality data can be acquired at a synchrotron in this configuration. We find that a key metric is the signal to background, which depends on the ratio of the crystal transit time to the detector collection time. Fast detectors allow the rejection of background by carrying out peak detection on individual frames. The results show that it should be possible to acquire diffraction from flowing protein microcrystals either with a slower jet or a more intense X-ray beam.
In the second part of our beamtime we will collect more data in the same fixed capillary geometry in order to get better statistics. In order to get a more systematic study of radiation damage, we are then planning to use a cryostream to keep the sample cold and to compare the powder patterns acquired in this condition to patterns collected at room temperature. We will also try to acquire diffraction patterns from protein crystals flowing in the liquid jet. Finally, we will test other sample delivery systems, such as thin-walled liquid cells loaded with protein crystals.

Our experiments have been a first step in developing the methodology of serial crystallography at high brightness synchrotron sources. This will offer a way to obtain high-quality data from crystals that are too small for conventional protein crystallography, and provide a new strategy for high-throughput measurements.

References