MS2-P3 Developing and optimizing serial crystallography for static and dynamic structural biology

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In time resolved studies, the use of micron- or sub-micron sized protein crystals not only allows for uniform laser excitation of all unit-cells in the X-ray beam, but for mix-and-inject (Schmidt, 2013) studies of enzymatic reactions that cannot be photo-induced. The small dimensions allow for fast enough diffusion of substrate into the crystals, such that those reactions can be induced homogenously at a defined time delay to the X-ray probe. This has strong implications for sample preparation: in case of such experiments one deliberately strives to grow micro- or even 'invisible' nano-crystals of proteins that would otherwise form larger crystals as well. Mix-and-inject experiments require furthermore a narrow size distribution of the crystals and fast mixing of the crystal suspension with substrate solution.

Serial crystallography methods, now established at both XFEL (Chapman, 2011) and third generation synchrotron sources (Gati, 2013; Stellato, 2014; Nogly, 2015) are very well suited for such time-resolved experiments (Tenboer, 2014; Barends, 2015), making it possible to reveal the dynamic nature of biological macromolecules and their interactions at near-atomic spatial resolution and on ultrafast timescales, even for extremely radiation sensitive samples.

Here we describe promising new crystallization methods for such experiments, the CFEL-pipeline for crystal characterization prior to the crystallographic experiment and novel sample delivery methods for reduced sample consumption, increased data collection efficiency and designed for mix-and-inject experiments.

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