Changing concepts: Crystallization for serial and time-resolved crystallography

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Serial crystallography methods at both X-ray free-electron lasers (Chapman, 2011) and third generation synchrotron sources (Gati, 2013; Stellato, 2014) are now well established, making it possible to determine the structures of proteins – from small to very large – that only form very small crystals or that are extremely radiation sensitive. Serial crystallography methods are also very well suited for 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. In case of such experiments one deliberately strives to grow micron- or sub-micron sized crystals of proteins that would otherwise form larger crystals as well. Small crystals not only allow for uniform laser excitation of all unit-cells in the X-ray beam, but for future mix-and-diffuse studies of reactions that cannot be photo-induced, since the small dimensions allow for fast enough diffusion of substrate into the crystals, such that enzymatic reactions can be induced homogenously at a defined time delay to the X-ray probe. Size homogeneity of the crystals and, since the amount of sample is limited, a high yield of crystals and optimized sample delivery methods are very important in this regard. Here promising new crystallization methods will be shown, as well as a pipeline for (nano-) crystal characterization prior to the crystallographic experiment and an overview on strategies to reduce sample consumption. Yet another paradigm shift that will be addressed is related not to the size, but to the internal quality of protein crystals: In the past it was attempted to grow as highly ordered protein crystals as possible, however recently it could be shown that certain disorder in protein crystals can be exploited to directly obtain the molecular transform from crystallographic experiments (Ayyer, 2016).

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