

MS08-2-3 On-the-fly crystallization for nucleation studies by serial femtosecond crystallography at the European X-ray Free-Electron Laser

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Abstract

The study of nucleation or early crystallization events, while being very relevant for structural biology or even pharmaceutical applications, is a demanding topic with X-rays. Capturing highly dynamic processes at the desired moment in time while trying to keep the sample undamaged and preserving its quality remains a challenging hurdle. Recent studies were published using cryo-transmission electron microscopy, cryo-STEM tomography or dynamic light scattering (DLS) [1-3], but early stage crystallization remains not completely understood. The In-line crystallization method, presented here, is a novel concept for a rapid crystallization system to overcome some of the current limitations with X-rays and using the Serial Femtosecond Crystallography (SFX) method at the European X-Ray Free-Electron Laser (EuXFEL). This system allows the crystallization of lysozyme within one minute inside a capillary, with a nucleating agent (Tb-Xo4) and in conditions suitable for the use of a liquid jet with a Gas Dynamic Virtual Nozzle (GDVN), compatible with megahertz data collection at the scientific instrument SPB/SFX. In-line crystallization opens up two key possibilities: firstly, to be able to systematically control and optimize the size of crystals during an experiment. Ultimately, this control provides the very exciting possibility to systematically observe nucleation of protein crystals with XFEL pulses. Secondly, the possibility to reduce clogging of crystals in the sample delivery system, which is a key limitation to the good running of a SFX experiment using GDVN. The commercially available nucleating agent Tb-Xo4 from Polyvalan (<https://crystallophore.fr/>) is a lanthanide complex which was developed at the IBS (Grenoble, France) primarily as a phasing agent. Its nucleating property was then characterized in various assays [4, 5]. Our injection setup is optimized to allow for rapid nucleation of lysozyme at low salt concentrations via the addition of the nucleating agent Tb-Xo4. For lysozyme, such fast crystallization is difficult to achieve with other precipitants such as poly-ethylene glycol or salts while retaining good injectability of the liquid. The effect of Tb-Xo4 on lysozyme was already studied using DLS [3] and showed an immediate effect by triggering nucleation within seconds even in pure water in the absence of any other crystallant. The in-line crystallization allows a rapid and controlled crystallization mainly suited for nucleation and early crystallization studies while still using the full advantage of the European XFEL: high brilliance and high repetition rate. Optical microscopy, electron microscopy and early X-ray data supporting this setup will be presented here.

References

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