Instant protein crystallization induced by the nucleating agent crystallophore.

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Abstract

Time-resolved Serial Crystallography (TR-SX) allows to obtain structural dynamics information and observe biological macromolecules in action by capturing transient intermediates along a biological pathway. [1–4]

From an experimental point of view, serial crystallography brings new constraints on crystal preparation as it intrinsically requires a large amount of samples to make sure to collect a complete diffraction data set. Moreover optimal time-resolved experiments require crystalline samples with a narrow size distribution in order to ensure a uniform triggering of the reaction under study through the entire crystal volume. The ability to generate crystals on-demand may open new opportunities in TR-SX. First, it would enable the preparation of fresh crystals just prior to data collection Second, it may also limit common soaking issues such as bad substrate/fragment diffusion due to crystal packing or interferences with crystal contacts. Indeed, molecules used for fragment based drug discovery may be incubated with the protein prior to crystallization, as can the substrates or analogues be prepared with the crystallization solution thus ensuring a controlled triggering or inhibition of the targeted reaction. [5,6]

The crystallophore is a lanthanide complex with both nucleating and phasing properties. [7,8] The nucleating properties of the crystallophore have been challenged for the minute-scale production of crystals with the appropriate size for either SX experiments or electron diffraction of 3D nanocrystals (3D ED). As a proof-of-principle, we generated HEWL crystals with a distribution ranging from the nanometer to the micrometer size allowing their diffraction quality to be evaluated by means of electron diffraction and synchrotron serial crystallography, respectively. Finally, we used time-resolved small-angle X-ray scattering (TR-SAXS) to determine the time window associated with the crystal production assisted by the crystallophore.

(1) https://doi.org/10.3390/cryst11050521.

(2) https://doi.org/10.1038/s43586-022-00141-7.

(3) https://doi.org/10.1016/j.crstbi.2024.100131.

(4) https://doi.org/10.1107/S2059798323011002.

(5) https://doi.org/10.1107/S1600576719013517.

(6) https://doi.org/10.1038/s41596-022-00777-5.

(7) https://doi.org/10.1039/C7SC00758B.

(8) https://doi.org/10.1107/S1600576719006381.