Monitoring the crystallization of two enzymes in real time by dynamic light-scattering

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Obtaining well-diffracting crystals is often a bottelneck of biocrystallographic studies. It is increasingly important in serial crystallography which requires a reproducible production of microcrystals that are homogneous in size and diffraction quality. In order to gain a better control over the crystallization process, we used an instrument called the XtalController. This recent technology gives access to the full monitoring of crystallization assays using dynamic light scattering and videomicroscopy, and integrates a crystallization chamber with temperature and humidity regulation, as well as piezo injectors that allow the modification of the mother liquor composition during the experiment [1]. We exploited this technology to study the crystallization of two enzymes, the tRNA CCA-adding enzyme of *Planococcus halocryophilus*, a cold-adapted bacterium from the permafrost, and the hen egg white lysozyme in the presence of a synthetic chemical nucleant, the crystallophore Tb-Xo4. Using the XtalController, we were able to detect early nucleation events and drive the crystallization system toward growth conditions yielding crystals with excellent diffraction properties using cycles of dissolution/crystallization [2]. This work illustrates the potential of XtalController technology for the rational production of samples for crystallography, ranging from nanocrystals for electron diffraction, microcrystals for serial or conventional X-ray diffraction, to larger crystals for neutron diffraction.

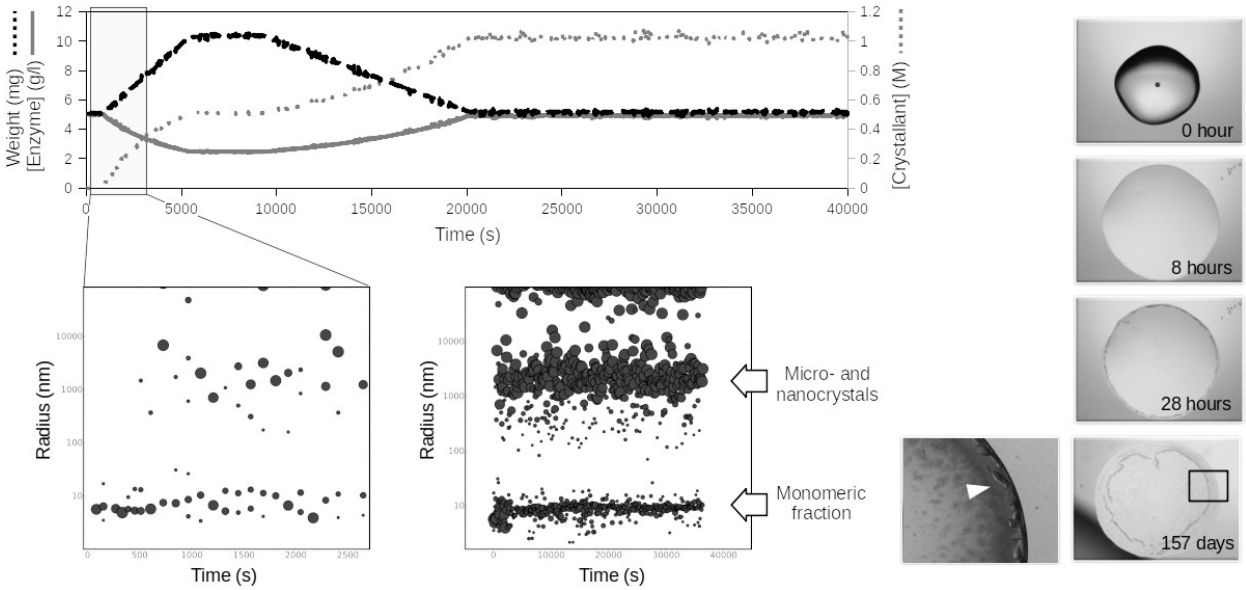

Figure 1. Monitoring the crystallization of a psychrophilic CCA-adding enzyme in the Xtal Controller. Record of crystallization parameters (top left) showing the variation of the weight of the crystallization drop upon crystallant addition or evaporation (black dotted line), enzyme concentration (gray line) and crystallant concentration (gray dotted line) over time. Evolution of particle sizes in the drop followed by dynamic light scattering (bottom left). Micrographs showing the evolution of crystallization drop (right panel). Microcrystals appear after a few days at the drop edges and grow slowly to a size of >0.5 mm (inset).

[1] Meyer, A., Dierks, K., Hilterhaus, D., Klupsch, T., Mühlig, P., Kleesiek, J., Schöpflin, R., Einspahr, H., Hilgenfeld, R. & Betzel, C. (2012). *Acta Cryst. F,* **68**, 994.

[2] de Wijn, R., Rollet, K., Engilberge, S., McEwen, A.G., Hennig, O., Betat, H., Mörl, M., Riobé, F., Maury, O., Girard, E., Bénas, P., Lorber, B. & Sauter, C. (2020). *Crystals*, **10,** 65.

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