

## Standard Sample Preparation and Characterization for Serial Crystallography

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Serial crystallography (SX) has been a revolutionary technique in structural biology for more than a decade, providing insights into the structures and dynamics of biomolecules at room temperature. Using intense ultra-short X-ray pulses, SX made the collection of diffraction data from micron-sized crystals possible, avoiding radiation damage and allowing for the capture of transient states and intermediates in biological reactions. The success of SX experiments heavily relies on the quality of the sample.

Standard samples in SX research provide critical roles. First, the commissioning of new beamline devices needs well-characterized samples. By providing consistent and reproducible diffraction patterns, the standard samples help in the calibration of new devices and verification of their performance. Secondly, they are required for the validation of experimental setups, ensuring that all components, from sample delivery systems to data acquisition software, function correctly.

In this study, we present detailed protocols for the preparation of lysozyme, myoglobin, iq-mEmerald, and photoactive yellow protein (PYP) crystals. These proteins were selected as standard samples due to their robust crystallization properties and suitability for a wide range of SX experiments. Through the optimization of existing protocols, we achieved high-quality crystal samples with improved yield, specifically for SX applications.

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