An Automated in situ Diffraction Platform for Serial Crystallography Studies at Room Temperature

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Watching how macromolecules carry out their functions at physiological temperatures is critically important for mechanistic understanding of biochemical reactions and biological processes. However, due to severe X-ray radiation damage at room temperature, X-ray data collection in protein crystallography is mostly conducted at cryogenic temperatures that inevitably hinder the dynamic studies of protein structures. While injector-based serial crystallography has made great strides at X-ray free electron laser facilities in the last decade(1), widely applicable serial diffraction methods that afford reasonable sample economy and gentle sample delivery are direly needed for probing protein structural dynamics at room temperature for many systems that cannot be produced in large quantities (grams!) and/or susceptible to air exposure or mechanical stress. To address this challenge, my laboratory has developed an in situ serial diffraction platform based on crystal-on-crystal devices with automation supported by both hardware and software components (2, 3). We have extensively tested this platform at the BioCARS beamline of the Advanced Photon Source where tens of thousands of diffraction images are collected from protein crystals grown on chip (3, 4). In my presentation, I will demonstrate how we grow crystals on chip and how we conduct large-scale in situ diffraction experiments. I will also present our scientific findings from DNA photolyases and photoreceptors enabled by this technology.

References

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