Skysong Innovations
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Droplet Injector for Time-Resolved Crystallography with XFELs

Background

Protein X-ray crystallography has become one of the most successful structural biology techniques since the first three-dimensional structure of myoglobin, a protein, was revealed. Many important scientific discoveries have been made using X-ray crystallography, including understanding insulin's mechanism of action, mass production of penicillin, understanding sickle cell anemia, the structure of DNA, and HIV inhibitors. NAD(P)H: Quinone oxidoreductase 1 (NQO1) is a flavoenzyme that is associated with cancer, Alzheimer's' and Parkinson's disease, making it an attractive target for drug discovery and analysis through X-ray crystallography. The development of bright X-ray radiation sources such as 3rd generation synchrotrons and hard X-ray free electron lasers (XFELs) has made it possible to determine structures of weakly-diffracting biomacromolecular crystals at room temperature. These two X-ray sources are characterized by significant differences in pulse duration, peak brilliance, and repetition structure, and require the development of different approaches to sample handling.

Serial femtosecond crystallography (SFX) methods have been developed with the increasing availability of XFELs in recent years to obtain room-temperature structural information from crystals that are too small, weakly scattering, or radiation damage-sensitive to be probed at synchrotrons. Droplet injection strategies are a promising tool to reduce to large amount of sample consumed in SFX measurements at XFELs with continuous injection approaches.

Invention Description

Researchers at Arizona State University have developed a sample injector with a mixing chamber and droplet generator for time-resolved crystallography with XFELs. This technology includes a new modular microfluidic droplet injector (MDI) design that was successfully applied to deliver microcrystals of human NAD(P)H: Quinone oxidoreductase 1 (NQO1) and phycocyanin. This technology constitutes a robust sample-conserving injection method for SFX studies on protein crystals that are difficult to obtain in amounts necessary for continuous injection, including the large sample quantities required for time-resoled mix-and-inject studies.

In initial tests, a full data set of protein crystals for NQO1 was collected with droplet injection, showing the first room temperature structure of NQO1 at an XFEL.

Potential Applications

- Protein X-ray crystallography for smaller sample sizes

Benefits and Advantages

- Up to 4-fold sample consumption savings
- Robust sample-conserving injection method
- Fully 3D-printed components
- Droplet injection parameters can be optimized based on duration and amplitude of employed electrical stimulus & phase delay of droplet