Novel sample delivery systems for serial femtosecond crystallography and time-resolved SFX experiments

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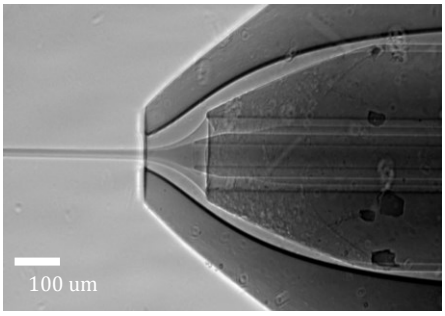
Serial femtosecond crystallography enables structure determination of biological macromolecules and their complexes from small but ubiquitous crystals that are unsuitable for conventional analyses. Diffraction patterns are obtained from single crystals in single shots from a pulsed X-ray source by the method of "diffraction-before-destruction." The sample must be replenished at the rate of the X-ray source. Liquid jets have been successfully used to deliver sample to the X-ray beam, starting from first experiments at the LCLS. However, these currently suffer from a high rate of sample consumption, high scattering background (especially when slowed down), and low reliability.

We developed a Double-flow focusing nozzle, which injects sample suspension into a stable ethanol sheath jet. This type of nozzle substantially reduces sample consumption (by about a factor of 5) and enables injection of samples even in high viscosity and high salinity buffers, which wouldn't run in a standard nozzle. Ice formation is prevented, substantially improving experimental efficiency.



Time-resolved crystallography combines structural analysis with chemical kinetics, since the structure of transient states and kinetic mechanisms can be determined simultaneously. The mix-and-inject approach is an emerging technique for time-resolved experiments. However, in contrast to light induced pump-probe TR-SFX, injection technology is the main bottleneck due to added complexity of fluid mixers mixing dynamics. Mix-and-inject experiments exploit diffusion of a substrate into sample crystals to initiate a reaction and the life-time of a transient reaction intermediate is determined by both the mixing dynamics and the time that the crystals need to travel from the mixing point to the x-ray interaction region. In order to increase the efficiency of current mixers, we developed novel 3D printed microfluidic mixers, which were tentatively characterized in our lab using fluorescence.