European XFEL Workshop

Opportunities for microfluidic devices at Free-Electron Lasers

Microfluidic Droplet Generators and Mixers for Serial Crystallography

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Serial femtosecond crystallography (SFX) with X-Ray Free Electron Lasers (XFEL) has evolved as a powerful technique for crystallography for proteins over the past years. Despite the recent advances in the field, limitations remain due to the restrictions in growing protein crystals sufficiently small in size (ideally sub-µm) for SFX, the requirement of highly concentrated crystal suspensions in the order of several milliliters, the limited tools for substrate-based time-resolved crystallography studies as well as the lack of *de novo* phasing approaches. The field of microfluidics has developed tools providing solutions to current challenges in SFX. To address the loss of precious protein crystals in liquid-jet injection technology typically achieved with gas dynamic virtual nozzles (GDVNs), we propose microfluidic droplet generation synchronized with the repetition frequency of currently available XFELs. Aqueous droplets of crystal suspensions can be intersected by an immiscible oil phase, thereby reducing the overall amount necessary for continuous injection with a GDVN dramatically. We demonstrate that microfluidic droplet generation can be coupled to traditional GDVNs and applied this approach to SFX of granulovirus. In addition, we explore microfluidic mixing based on hydrodynamic focusing and fast diffusive mixing for SFX. Mixing devices were developed both with photolithography as well as 3D printing approaches achieving sub-ms mixing times at flow rates compatible with GDVNs. Optimization of device geometry and flow rates allow the measurement of reaction time points ranging from several ms up to seconds. This mixing approach has been applied to study the reaction of the enzyme 3-deoxy-D-manno-2-octulosonate-8-phosphate synthase with its substrates phosphoenolpyruvate and arabinose-5-phosphate.