A microfluidics-based approach for serial time-resolved crystallography

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Methods in serial crystallography have shown success for performing time-resolved experiments to visualize macromolecular dynamics in real-time. Such experiments however, require the production of large quantities of isomorphous and uniformly-sized crystals in order to merge diffraction data from numerous crystals and thereby achieve high signal-to-noise structure factor amplitudes. Further, high-throughput data collection is needed to acquire a sufficient amount of data to solve structures over an array of time delays. To meet these needs, we present a microfluidics approach for macromolecular crystallization and room temperature *in situ* serial data collection that can be utilized at synchrotron or X-ray free electron laser (XFEL) beamlines, and is suited for time-resolved crystallography experiments. Here, ~1000 crystals are grown in a 1 m long glass capillaries inside nanoliter aqueous droplets emulsified in fluorinated oil and stabilized by a surfactant. Droplets of two different sizes (1:5 volume ratio) are generated and are alternatively loaded into the capillary where the smaller droplet contains a 50:50 protein:precipiant mixture and the larger droplet acts as the reservoir containing the mother liquor. Small droplet volumes induce a negative feedback mechanism that causes the growth of one crystal-per-drop with ~35 μm size and uniform characteristics. Crystals are delivered via a syringe pump containing fluorinated oil that acts as a mobile phase into a thin-walled plastic sample cell that has low background scatter for data collection. Utilizing hen egg-white lysozyme (HEWL) we demonstrate the potential of this microfluidics approach for application to a wide range of time-resolved crystallography experiments.