Poster

Droplet microfluidics for uniform microcrystallization and rapid mixing

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Time-resolved serial crystallography demands the production of millilitres of micron-sized crystals, alongside robust strategies for reaction initiation. This necessitates the crystallographer to transition from vapour diffusion to batch methods [1]. Droplet microfluidics has traditionally been employed for crystallization screening and generating large crystals suitable for standard, rotation-based crystallography under cryogenic conditions. Here, we report its application in generating uniformly small microcrystals and rapidly mixing substrates with crystals [2]. Droplets are rapidly generated with low sample consumption (~1.7 µl dead volume), and droplet volume is used to engineer crystal size, yielding crystals as small as 3 µm for lysozyme and 2 µm for Pdx1, a protein involved in vitamin B6 biosynthesis [3]. A seeding strategy was used for Pdx1, due to the improbability of nucleation with picolitre droplet volumes. Homogenous batches of small microcrystals ensure rapid substrate diffusion during mixing experiments and thereby provides a robust strategy for initiating reactions in slurries of microcrystals with sharp temporal resolution. Serial synchrotron data were collected from droplet-grown crystals using the SOS chip on ID29 at ESRF, yielding comparable data quality to batch controls.

Fast mixing is also needed. The transport of droplets within microchannels introduces circulations, facilitating rapid convective-diffusive mixing, suitable for triggering time-resolved experiments. Our mixing times scale with droplet volume and velocity, achieving mixing times approaching 1 millisecond, all while operating at frequencies comparable to current synchrotron and XFEL facilities. Our current PDMS prototypes are compatible with UV-Vis spectroscopy analysis of reaction intermediates in time-resolved *in crystallo* spectroscopy. Future endeavours will focus on the outstanding challenge of interfacing droplets with X-rays, leveraging fabrication strategies to develop an X-ray transmissible device suitable for *in situ* time-resolved experiments at a serial beamline.

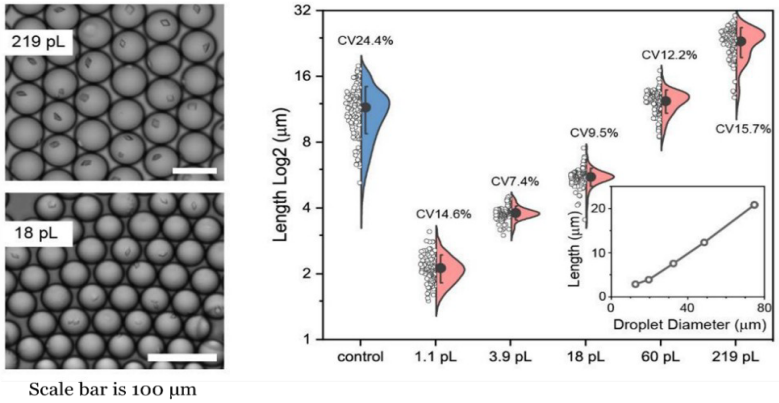

Figure 1. Droplet volume defines crystal size, allowing size tuning to crystals suitable for time-resolved experiments whilst ensuring uniformity.

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