BIPHASIC DROPLET-BASED SAMPLE DELIVERY OF PROTEIN CRYSTALS FOR SERIAL FEMTOSECOND CRYSTALLOGRAPHY WITH AN X-RAY FREE ELECTRON LASER

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ABSTRACT

Serial femtosecond crystallography (SFX) laser pulses facilitate the structure determination of smaller crystals than can generally be studied by traditional synchrotron crystallographic methods. One limiting factor of SFX is the large volume of crystal suspension needed to obtain a full data set allowing structure determination. The current method of continuous sample stream injection into an X-ray free electron laser (XFEL) requires milliliters of sample and wastes the majority of the protein crystals grown under time consuming conditions. We propose the utilization of aqueous droplets as an efficient method for protein crystal delivery into an XFEL for structure determination by SFX.

KEYWORDS: Microfluidics, Droplets, X-ray Crystallography

INTRODUCTION

Traditionally, protein structure has been determined by X-ray crystallography performed at a synchrotron. Membrane proteins have been difficult to analyze by traditional crystallographic methods for several reasons: growing large enough crystals to withstand long, high-intensity X-ray pulses is time consuming, if not impossible for certain proteins, and smaller crystals that are easier to grow are destroyed before diffraction patterns can be collected [1]. Serial femtosecond X-ray crystallography (SFX) utilizes a high-intensity X-ray free electron laser (XFEL) with extremely short pulses to obtain diffraction patterns of small protein crystals before they are destroyed [1].

Currently, a continuous stream of crystal suspension is injected into the XFEL [2]. Since the femtosecond laser operates at a frequency of 120 Hz, the time between the pulses results in unused sample injected. The unused sample cannot be recovered and therefore becomes waste. The process to crystallize even a small amount of large protein complexes is resource intensive, so a suitable method of sample conservation is necessary. To accomplish this goal, we aim to generate aqueous sample-containing droplets in an oil carrier phase and synchronize these droplets to the pulses of the laser. This method would effectively replace the wasted crystals with non-precious oil when the laser is off, and we show the first experimental evidence of coupling a microfluidic device to the injector used in an SFX experiment.

EXPERIMENTAL

Microchips made from polydimethylsiloxane (PDMS) with a flow-focusing channel geometry [3] were fabricated using traditional photolithography and soft lithography methods [4]. A 2 cm piece of fused silica capillary (100 µm inner diameter) was inserted into the outlet channel between two oxygen plasma treated PDMS pieces. A PEEK cross-connection fitting (150 µm inner diameter) (IDEX, USA) was used as another method of droplet generation. Reservoirs and GP1 pressure regulators (Proportion-Air, USA) were used to supply liquids and pressure, respectively. The droplet generator was connected to a gas dynamic virtual nozzle (GDVN) [2] by a fused silica capillary (100 µm inner diameter). The GDVN

was connected to a helium gas tank by a separate fused silica capillary and was sealed into a vacuum chamber during the experiments. Imaging was accomplished *via* a high-speed camera (Fastcam SA4, Photron, USA). An Elveflow pressure pump was used to control the applied pressure, and flow-check valves were installed on the oil lines to prevent backflow for experiments at the LCLS beam line.

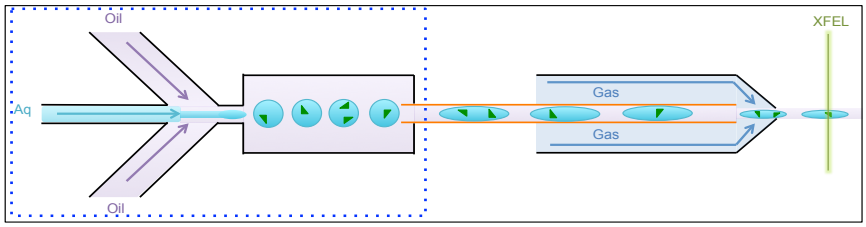

Figure 1: Schematic of droplet generation on-chip (inside the blue dotted rectangle) and off-chip interfaced with the XFEL instrument. Left: droplet generation in the PDMS microfluidic device. Middle: Transfer of crystal suspension droplets to a silica capillary (orange indicates the coupled silica capillary). Right: The silica capillary is used as the inner portion of the GDVN injecting a liquid stream into the XFEL chamber. (Figure not to scale.)

RESULTS AND DISCUSSION

The droplet generator has two oil-phase inlets and one aqueous-phase inlet meeting at a cross-shaped intersection by pressure-driven flow (Figure 1). Due to opposing intermolecular forces, the aqueous dispersed phase does not mix with the oil carrier phase at the intersection and, under certain parameters, will form spherical droplets of dispersed phase in carrier phase. The droplets and carrier oil are then pushed along by the bulk flow through the outlet and into an interfaced injector. The currently employed SFX injector, a GDVN, uses a fast-flowing sheath gas to jet the liquid stream at the nozzle tip [2]. From there, the liquid stream travels a few micrometers before the X-rays strike the stream.

Initial proof of concept experiments used a PDMS microchip in which aqueous droplets in an oil carrier phase were generated and successfully sent into a fused silica capillary. Figure 2 shows (a) droplet generation, (b) transfer to the capillary, and (c) successful droplet generation with crystals of the membrane protein photosystem I.

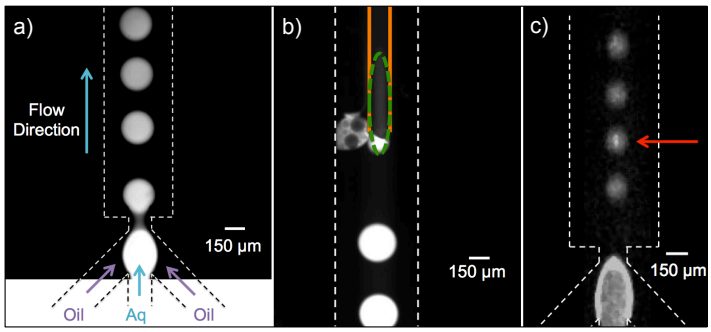

Figure 2: a) Fluorescein droplets generated on-chip. b) Fluorescein droplets entering a capillary (orange) for downstream injector coupling. The entering droplet is outlined in green. c) Droplets generated with a photosystem I crystal suspension. The arrow indicates a crystal within the droplet. All images were captured using fluorescence microscopy, and dashed white lines outline the microchannels.

Furthermore, using a PEEK cross connected to a GDVN, droplets were generated at a frequency of 9 Hz and injected (Figure 3a) using pressures of 69.6 psi for a representative protein crystal buffer and 70.3 psi for the oil phase, resulting in a sample flow rate of 21 µL min^{-1}. A pressure of 100 psi was applied to the GDVN to facilitate sheath gas flow, and the liquid was jetted into a vacuum chamber (Figure 3 b,c).

At the LCLS beam line, droplets containing granulo virus were also generated using the PEEK microfluidic cross fitting. Droplets were generated (Figure 3d,e) and injected into the beam line chamber *via* a GDVN. Pressures of 35.9 psi for the crystal buffer, 58.0 psi for the oil phase, and 100 psi for the sheath gas were applied. The droplet frequency was 9 Hz and the sample flow rate was 5.5 µL min^{-1}, a notable reduction compared to the typical 10-15 µL min^{-1} of the GDVN alone.

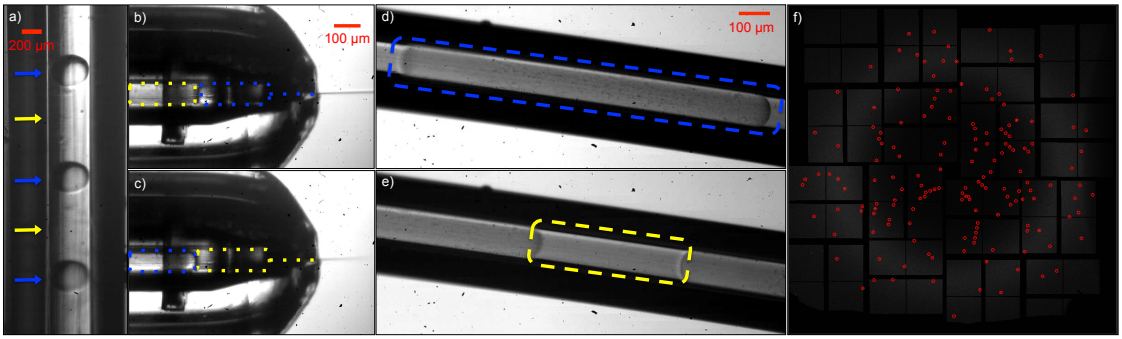

Figure 3 a) In PEEK tubing, droplets of crystal buffer in an oil carrier phase. Crystal suspension and oil are indicated by blue and yellow, respectively. b-c) High-speed brightfield images of droplets injected through a GDVN. Droplets of crystal suspension jetting (b), shown by the darker line leaving the nozzle, and oil sputtering (c), shown by the blurry line. d-e) Hydrophobically-treated fused silica capillary (100 µm inner diameter) containing aqueous droplets of granulo virus in an oil carrier phase. A single droplet (d) and the oil between two droplets (e) are shown. f) Diffraction pattern of granulo virus recorded during droplet generation. Red circles mark peaks determined by Cheetah analysis software. Hit rate was ~ 1 % with a resolution up to 2.6 Å.

CONCLUSION

In summary, we show that droplets of crystal suspension can be jetted into the XFEL with a 45% decreased sample flow rate and can yield high resolution diffraction patterns (Figure 3f). Further optimizations of droplet generation flow rate and synchronization with XFEL pulses will further reduce sample consumption. Overall, microfluidic droplets are a promising method of sample reduction for SFX.

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REFERENCES

[1] "Femtosecond X-ray protein nanocrystallography," H. N. Chapman, P. Fromme, A. Barty, T. A. White, R. A. Kirian, *et al.*, *Nature*, **470** (2011).

[2] "Liquid sample delivery techniques for serial femtosecond crystallography," U. Weierstall, *Philos. Trans. R. Soc., B*, **369**, 1647 (2014).

[3] "Formation of dispersions using 'flow-focusing' in microchannels," S. L. Anna, N. Bontoux and H. A. Stone, *Appl. Phys. Lett.*, **82**, 3 (2003).

[4] "High throughput protein nanocrystal fractionation in a microfluidic sorter," B.G. Abdallah, S. Roy-Chowdry, J. Coe, P. Fromme, and A. Ros, *ACS Nano*, **87**, 8 (2015).

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