Development of LCP and MESH Crystal Injectors

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Introduction

Macromolecular crystallography through X-ray diffraction analysis creates a 3D understanding of protein structures within crystals at an atomic level. The goal of my project is to develop and test two types of crystal injectors new to LCLS and SSRL, the MESH and LCP injectors.

A crystal injector is a sample delivery device that produces a continuous liquid stream that contains micro-crystals. Efficiently collecting X-ray diffraction data from many small crystals, reduces radiation exposure to each crystal.

The LCP injector uses gas pressure to drive a piston that pushes crystals that flow in a viscous (LCP) delivery medium, while the MESH injector uses high voltage (HV) to produce an electro-spray that propels crystals within various solutions. Our goal is to combine use of the mixing MESH injector with UV-visible absorption spectroscopy (UV-vis ABS) to simultaneously monitor the color (electronic state) of protein crystals and collect X-ray diffraction data to determine the corresponding protein structure.

Keywords: Crystallography, crystal injectors, LCP, electro-spray, MESH

Research

1 MESH injector

The MESH injector is a new low flow delivery system for crystals in various solutions that uses high voltage (HV) to charge the carrier solution. A maximum of 5 KeV will be used. The charged crystal slurry travels from a syringe through a capillary. It exits the capillary and is propelled towards a grounded target. Before reaching the target, the sample is exposed to x-rays, creating diffraction patterns measured by an area detector.

The uniqueness of the MESH injector is that it is a low flow injector that does not require viscous media. Therefore reactions within crystals may be triggered through solution mixing. The timing for these reactions can be adjusted for a more optimal experimental setup.

In the mixing MESH injector, a shorter capillary is inserted within a wider capillary, so the inner liquid mixes with the outer liquid according to the difference in capillary length.

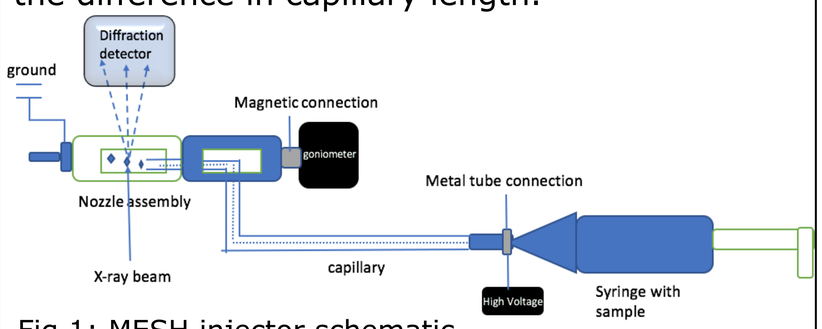

Fig 1: MESH injector schematic

2 LCP injector

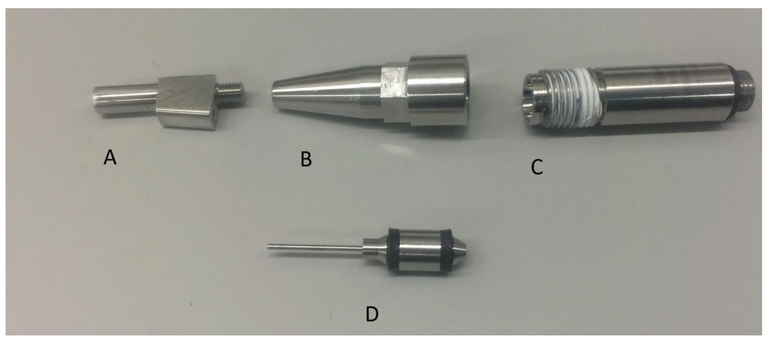

Fig 2: Main parts of the LCP injector in a disassembled state A) nozzle body B) reservoir C) hydraulic stage body D) piston

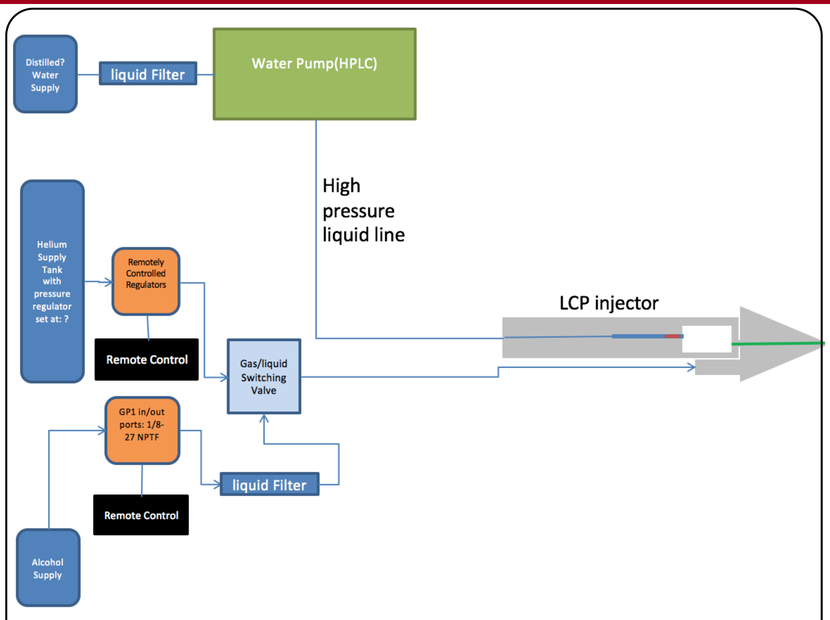

Fig 3: Schematic of LCP injector

The LCP injector was developed as an alternative to the Gas Dynamic Virtual Nozzle (GDVN) injector, which is incompatible with protein crystals that grow in viscous mediums or for crystals in limited supply. All crystal injectors are useful for laser pump-probe experiments, but the MESH and LCP injectors have lower sample consumption than the earlier developed GDVN injector.

LCP is a growth medium for membrane protein crystals, and was chosen as a delivery material to fill the high-viscosity injector because of its ability to grow well ordered crystals.

Injectors are commonly used with very radiation sensitive crystals. At LCLS only one shot (of approximately 40 femtoseconds) per crystal can be applied, requiring a constant flow of new crystals. The diffraction pattern is collected before the crystal experiences significant radiation damage but it is destroyed in the process (diffraction before destruction). Proteins that bind the common biological chromophore retinal are ideal for time-resolved measurements, since they can be activated with a flash of laser light. At SSRL, crystals exiting the LCP injector will be exposed to a continuous beam of X-rays.

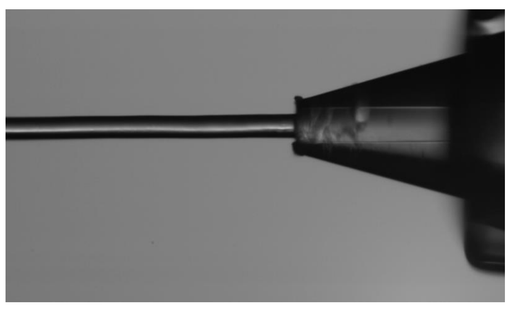

Fig 4: Photo of ideal straight LCP jet

3. UV-Visible Absorption Spectroscopy

UV-Vis ABS is useful to monitor the reactions occurring within crystals, and to verify the intermediate state of enzymes that change color during reactions. X-rays are used to obtain the structure of a sample, but even low doses of X-rays may cause local damage to the sample structure itself, such as photo-reduction of metal centers.

Absorbance: $A(\lambda) = \log_{10}[(I_R(\lambda) - I_D(\lambda)) / (I_S(\lambda) - I_D(\lambda))]$

The benefits of UV-Vis ABS include its speed and non-invasiveness, allowing for rapid monitoring of the protein state during X-ray diffraction experiments. To monitor the chemical state of metalloenzymes within the mixing MESH injector, reactions can be monitored using UV-Vis ABS. For our experiments, we will be setting up the mixing MESH injector at SSRL BL9-2 and will use an in-situ UV-Vis ABS system.

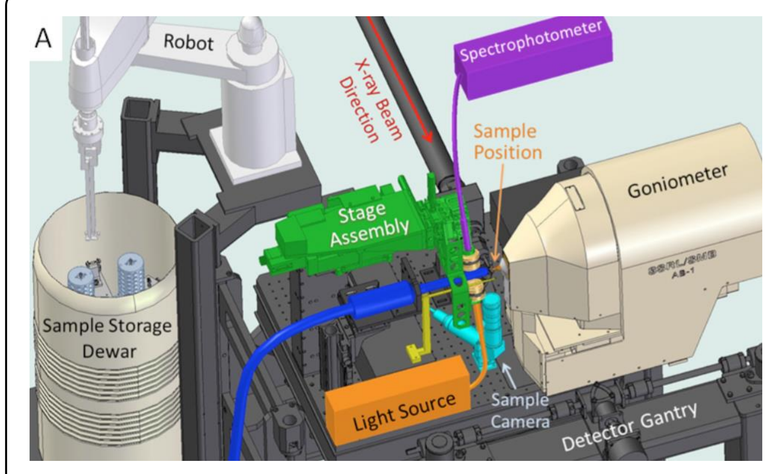

Fig 5: Schematic view of UV-Vis ABS incorporated into a crystallography beamline at SSRL

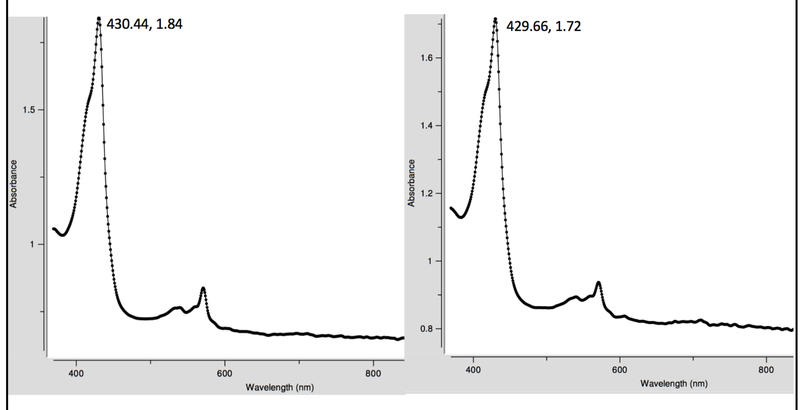

Fig 6, 7: Absorption spectra from a MET myoglobin sample before and after X-ray exposure using UV-Vis ABS system controlled by the Blu-Ice software. After radiation exposure, the MET myoglobin is reduced.

Conclusions

So far through my project I have gained substantial skills and knowledge on growing crystals, utilizing the UV-Vis ABS system using the Blu-ice software, and evaluating achieved sample spectra.

For the remainder of my internship, I will work on testing a new MESH injector setup and monitor the spectra of solutions and crystals exiting the injector while determining and optimizing mixing times. I will also work on setting up the LCP injector at SSRL BL12-2. Both the LCP and MESH injector hardware we are setting up are compatible with the MFX instrument at LCLS and can also be used at microfocus synchrotron beamlines such as SSRL BL12-2 and BL12-1 prior to LCLS beamtime.

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