Monitoring Protein Crystal Reactions & Mixing Time in an Injector with UV-Visible Spectroscopy

SLAC NATIONAL ACCELERATOR LABORATORY

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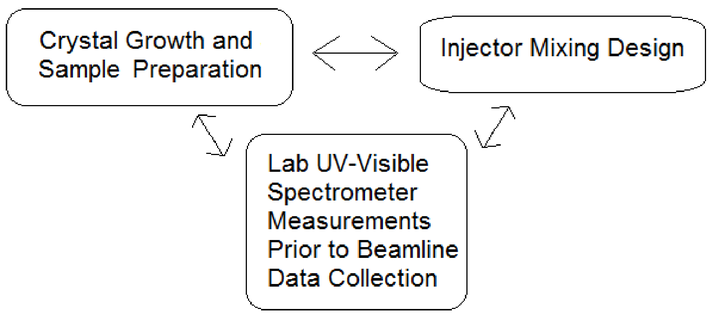
Introduction

The microfluidic electrokinetic sample holder (MESH) injector is tool for delivering numerous crystals into the X-ray beam for serial X-ray diffraction measurements at the synchrotron and XFEL.[1] The first goal is to obtain UV-Visible (UV-Vis) absorption spectra of the crystals exiting the MESH injector. A second goal is to monitor the UV-Vis absorption spectra of the solution during a fast color changing reaction to characterize the mixing rate within a mixing MESH injector. Once the mixing times for various injector setups are well characterized, our final goal is to use the UV-Vis absorption spectrometer to monitor the oxidation of cytochrome c oxidase (CcO) protein crystals as they exit the MESH mixing injector to optimize the experimental design for structural investigations of short-lived intermediates in this process.

Keywords: microfluidic electrokinetic sample holder (MESH) injector, crystallography, fluorescein dye, Cytochrome c oxidase (CcO) protein, UV-Visible (UV-Vis) Spectrometer

Research



Protein Crystals:

Cytochrome c oxidase (CcO) protein crystals were selected for this experiment. The enzyme is a large transmembrane protein that is found in bacteria and inside mitochondrion of eukaryotes.

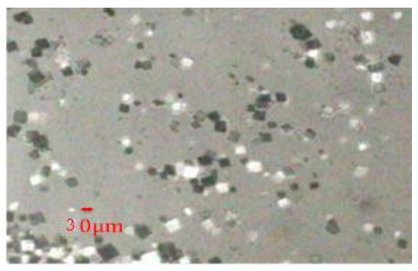
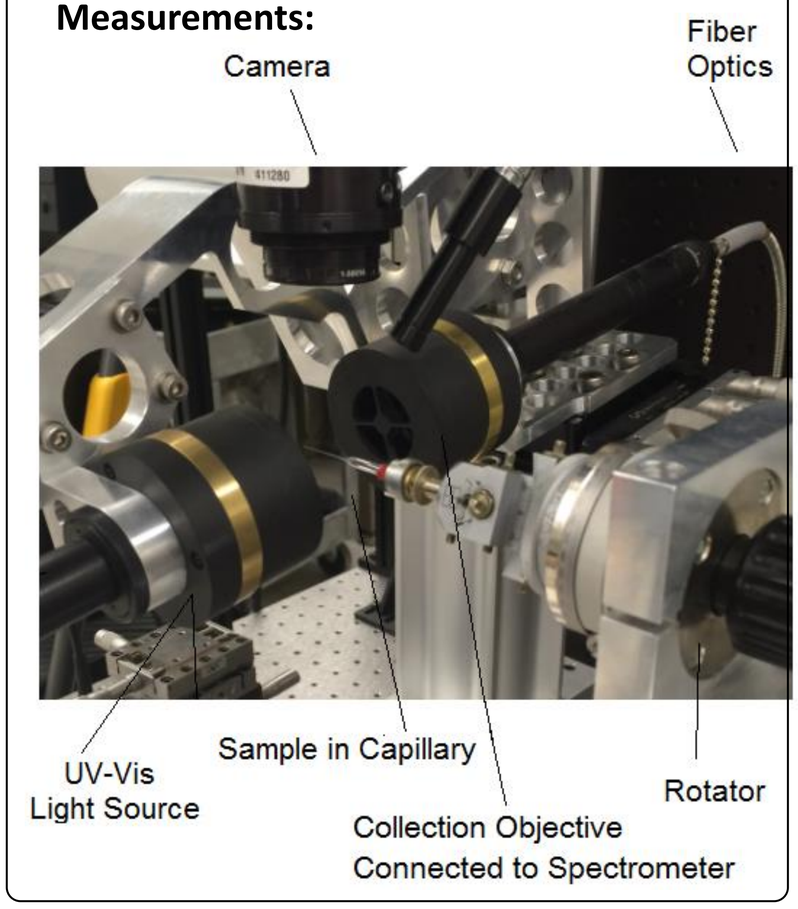


Figure (2). CcO crystals viewed with a microscope.

Lab UV-Visible Absorption Spectroscopy Measurements:



The UV-Vis microspectrometer was manually aligned and optimized with appropriately selected fiber optics.

CcO is reduced to a ferrous sample by addition of 1:10 volume 100mM dithionite to degassed CcO solution.
A strong understanding of the sample and the reaction prior to access to the beamline saves valuable beamline data collection time for users.

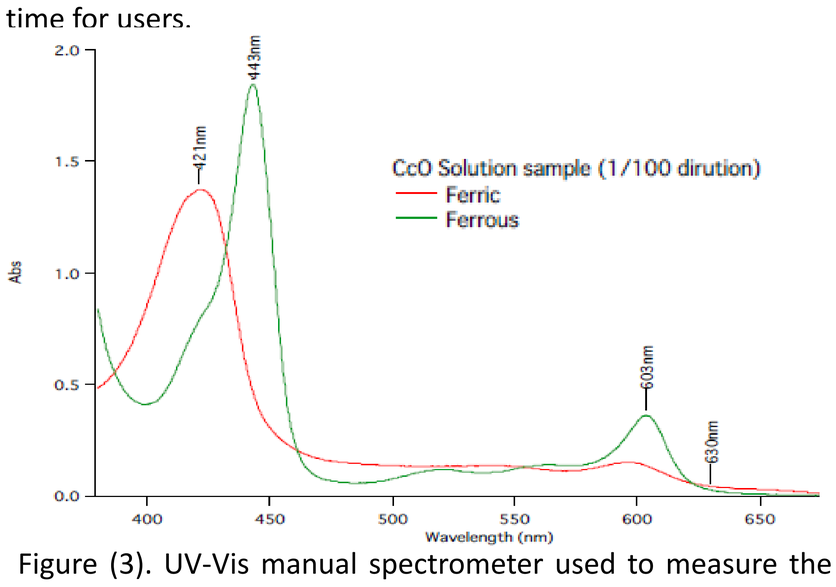


Figure (3). UV-Vis manual spectrometer used to measure the absorbance spectrums of oxidized and reduced forms of CcO.

Injector:

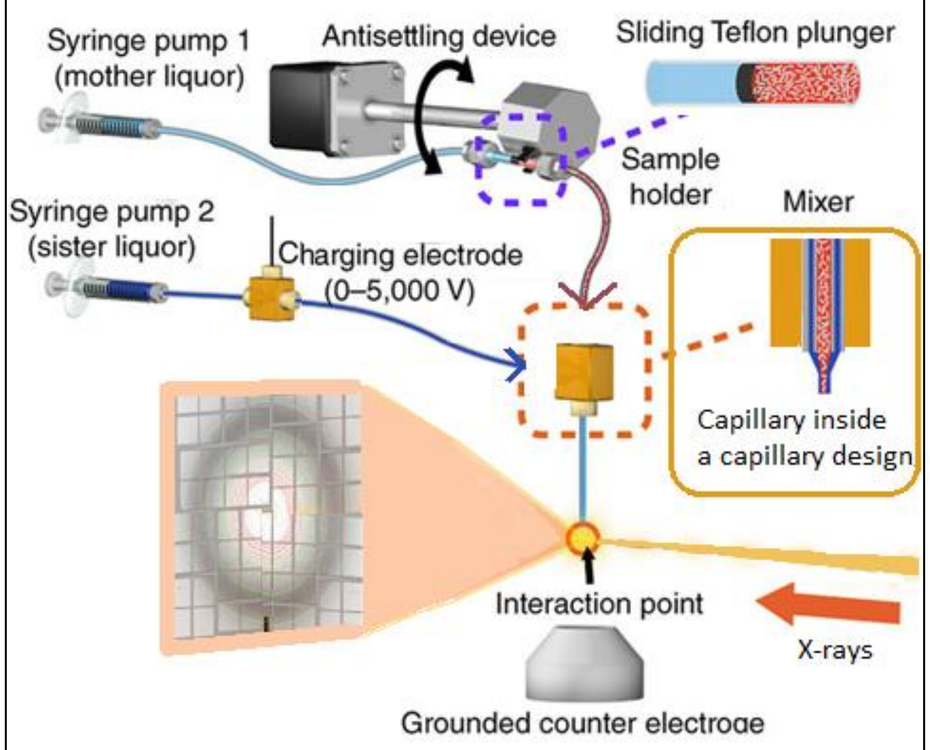


Figure (1). MESH injector for "in-atmosphere" experiments both at LCLS-MFX and SSRL BL12-2 and SSRL BL12-1. [1]

The MESH injector set-up consists of a continuous flow of microcrystals suspended in a mother liquor that gets mixed with a sister liquor. To monitor the reaction, fluorscein dye is used. Equal volumes of 11.4 mM fluorescein with 500 mM sodium iodide, a fluorescein quencher, are loaded in two separate syringes and delivered into the mixer capillary. The solutions in the MESH injector are charged and are propelled towards a grounded target as they exit the final injector capillary. Variable design parameters of the mixer include capillary diameter and the distance between the interaction point and the exit of the injector nozzle.
Fluorescein's absorption maximum is at 495 nm with a quick mixing time of around 1 ms for microfluidics. [2,3]

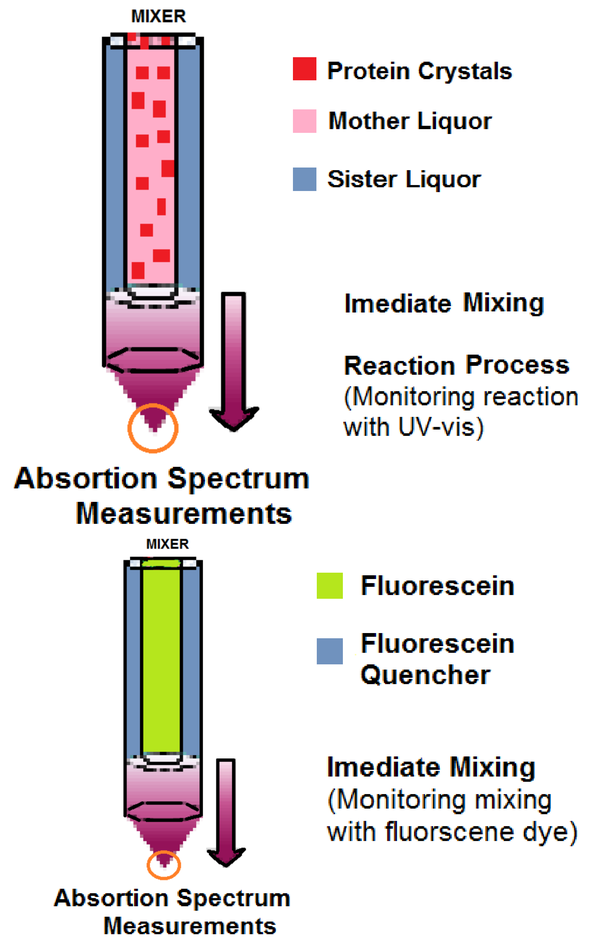


Figure (5). The mixer design of the MESH injector for the CcO reaction (top) and for monitoring the mixing time with fluorescein (bottom).

To optimize the mixing time, the diameters of the capillaries and the distance between the fluid interaction region within the injector and the exit point of the injector may be changed. The mixing should be quick and efficient in order to measure the reaction process with absorption spectra at the interaction point.
Serial X-ray crystallography will enable the structure of short-lived structure of CcO intermediates and as multiple crystals are used for the experiment, the X-ray dose to individual crystals is smaller. This reduces the radiation damage effects. At the interaction point, the crystals are exposed to X-rays to obtain the diffraction pattern measurements.

Conclusions

This technique may be further applied to different protein crystals and reactions. The injector allows observing reactions in protein crystals over millisecond intervals with a UV-Vis spectrometer. Quenching fluorescein dye will be used to determine the mixing time within injector setups. With a good understanding and control of the reaction mixing time and reaction rates prior to beamtime, users can be more efficient and attain the data they need during their beam time.

Acknowledgments

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1. Sierra, Raymond G., Cornelius Gati, Hartawan Laksmono, E. Han Dao, Sheraz Gul, Franklin Fuller, Jan Kern, Ruchira Chatterjee, Mohamed Ibrahim, Aaron S. Brewster, Iris D. Young, Tara Michels-Clark, Andrew Aquila, Mengning Liang, Mark S. Hunter, Jason E. Koglin, Sébastien Boutet, Elia A. Junco, Brandon Hayes, Michael J. Bogan, Christina Y. Hampton, Elisabetta V. Puglisi, Nicholas K. Sauter, Claudiu A. Stan, Athina Zouni, Junko Yano, Vittal K. Yachandra, S. Michael Soltis, Joseph D. Puglisi, and Hasan Demirci. "Concentric-flow electrokinetic injector enables serial crystallography of ribosome and photosystem II." *Nature Methods*, 2015. doi:10.1038/nmeth.3667.

2. Ilich, P. (2017). *Cite a Website - Cite This For Me*. [online] Facpub.stjohns.edu. Available at: http://facpub.stjohns.edu/ilichp/orgo_LAB/UV_VIS_lab/UV_VIS_Dana_2.htm .

3. Maillot, S., Carvalho, A., Vola, J., Boudier, C., Mély, Y., Haacke, S. and Léonard, J. (2014). Out-of-equilibrium biomolecular interactions monitored by picosecond fluorescence in microfluidic droplets. *Lab on a Chip*, 14(10), p.1767.

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