Sample Cells for High Pressure Biological X-Ray Solution Scattering

CNF Project Number: 1940-10

Principal Investigator & User: Richard E. Gillilan

Affiliation(s): Macromolecular Diffraction Facility of the Cornell High Energy Synchrotron Source (MacCHESS), Cornell High Energy Synchrotron Source; Cornell University

Primary Source(s) of Research Funding: National Science Foundation DMR-1829070, National Institutes of Health 1-P30-GM124166-01A1 and NYSTAR

Contact(s): reg8@cornell.edu

Website: https://www.chess.cornell.edu/macchess/hp_more

Primary CNF Tools Used: VersaLaser VLS3.50 engraver/cutter tool

Abstract:

Over 80% of the biomass of Earth consists of organisms living under extreme conditions of temperature, pressure, and chemical environment. As a potentially rich source of new tools and deeper understanding of the history and limits of life, there is much renewed interest in conducting biophysical and structural biological measurements under these types of conditions. We have developed an X-ray transparent, biologically compatible sample environment for conducting small angle X-ray solution scattering at hydrostatic pressures of up to 400 MPa (58,000 psi). The disposable, low-volume cells are designed to allow pressure equalization between the sample and pressurizing medium without excessive flexing of the thin X-ray transparent windows. The current design has been routinely and successfully used at the Cornell High Energy Synchrotron's High Pressure Biology facility (HP-Bio) since late 2019, including a remote "hands-on" training course in April 2021.

Summary of Research:

Though we surface dwellers are rarely aware of it, high hydrostatic pressure is the norm for most of the biomass on Earth. Organisms living in the deep ocean and subsurface experience pressures that can reach beyond 100 MPa (14500 psi). Biomolecules do not merely compress in response to external pressure; they rearrange their structure in informative ways to minimize total volume, including that of the surrounding water [1]. X-ray solution scattering (SAXS) is well-suited to study such changes, but special design considerations are necessary to handle the high pressure.

We recently introduced an easy-to-use SAXS system capable of maintaining up to 400 MPa of hydrostatic pressure on biological samples [2]. The system uses X-ray transparent single crystal diamond windows combined with a quick sample change mechanism (Figure 1A,B). To prevent the pressurizing medium (water) from mixing with the biological samples, we designed disposable laser cut PMMA cells with thin, X-ray transparent 7 μm polyimide film windows. At 400 MPa, the specific volume of water has declined by a significant amount, 11.6%. The chemically inert silicone grease used to seal the cells can flow, allowing pressure between the sample and external pressurizing water to equalize. The cells have also been adapted successfully to hold standard 1.5 mm glass sample capillaries for use in studying phase changes in deep sea lipid samples (Winnikoff and Budin, personal communication, 2021).

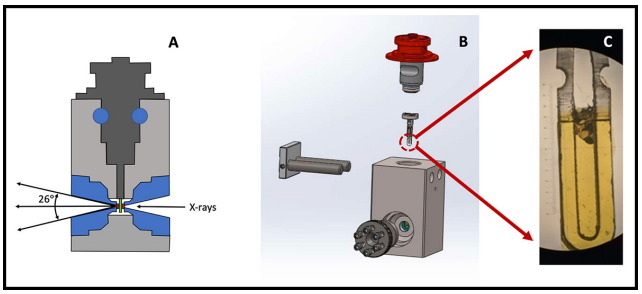

Figure 1: High pressure small-angle solution scattering system. X-rays enter the cell from the right (A) traveling through a single-crystal diamond window, passing through a sealed plastic sample cell at center and emerging from the second diamond window scattered at angles up to 26°. Water is used as a pressure medium. A locking pin and piston-style o-ring system allows easy access to the disposable inner PMMA cell (B), which has 7 µm polyimide windows to minimize X-ray scatter and a grease seal to allow for pressure equalization.

References:

[1] Ando N, Barquera B, Bartlett DH, Boyd E, Burnim AA, et al. 2021. The Molecular Basis for Life in Extreme Environments. Annu. Rev. Biophys.

[2] Rai DK, Gillilan RE, Huang Q, Miller R, Ting E, et al. 2021. High-pressure small-angle X-ray scattering cell for biological solutions and soft materials. Journal of Applied Crystallography. 54(1): 111-22.