

Cryogenic sample environment for biological small-angle X-ray scattering

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Cryogenic sample temperatures have in recent years revolutionized protein crystallography and electron microscopy of biological matter. Sample environments which allow measurements in vitreous ice also have great potential for improving Small angle X-ray scattering (SAXS) experiments from aqueous solutions.

Aqueous solutions in the amorphous solid state of the solvent allow greater radiation doses and handling of the sample solution without containers or sample cells interacting with the probing radiation. Radiation damage originates largely from oxidation by ionized small molecules. The radiation damage observed in solution scattering arises from non-specific aggregation of the solutes, for which the method is very sensitive [1]. Stopping the diffusion of the solute molecules by cold temperatures can potentially give even greater increase in radiation resistivity in SAXS than in high resolution methods. Another potential application for frozen solutions is stopping biochemical reactions involving conformational changes, allowing measurement of the intermediate reaction products in samples prepared offline.

During 2007, a prototype of a cryogenic sample environment for solution SAXS (CryoSAXS) has been constructed at EMBL Hamburg. Simultaneously, efforts on determining optimal sample preparation methods and conditions have been started in collaboration with EMBL Heidelberg.



Figure 1: The CryoSAXS equipment being tested at the beamline X33

The CryoSAXS experimental setup consists of a cold-finger liquid nitrogen bath cryostat mounted on an XZ vacuum manipulator stage and a sample chamber with isolatable vacuum system and an on-axis megapixel-class digital camera for visual sample inspection during measurement. The sample holder can accommodate samples of various sizes with a suitable adaptor, ranging from macroscopic 3 x 5 mm samples used in experiments at DORIS III, to 300 micrometer diameter samples more suitable to 3rd generation sources. The vacuum manipulator stage and the on-axis camera allow accurate alignment of sub-millimeter samples to the beam. The system is also designed to be transportable and independent of specific beamline equipment, allowing experiments to be made on any suitable SAXS beamline. The motors, sensors and the camera are controlled by custom software running on a Linux laptop, sending commands via CAN and Ethernet buses.

The formation of amorphous solid water is dependent on two processes, the nucleation rate and the rate of cooling. Large sample volumes prepared by slow cooling require cryoprotectant concentrations close to 50% v/v for crystallization to be inhibited, making this approach somewhat undesirable. Increasing pressure during cooling reduces the nucleation temperature and allows for lower concentration of cryoprotectant to be used. The method of high-pressure freezing is widely used in electron cryomicroscopy of vitreous sections [2], and our efforts in adapting this method to preparation of protein solution samples have produced some promising preliminary results.

Tests of the CryoSAXS equipment were made during summer 2007 at the EMBL Hamburg BioSAXS beamline X33 (Figure 1). Based on the results obtained, several improvements on the setup and the sample preparation methods were made during the latter part of the year. This work was supported by EU FP6 Design study SAXIER, grant number RIDS 011934.

References

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- [2] J. Dubochet, Trends in Cell Biology. 5, 366-368 (1995)