ESRF	**Experiment title:** Coherent Scattering from Protein Crystals	**Experiment number**: SC3289
Beamline:	**Date of experiment**: from: Oct. 24th, 2011 to: Oct. 31st, 2011	**Date of report**: Feb. 29th 2012
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Report:

Protein dynamics has attracted increasing attention from the biologists and physicists in the past twenty years. The fast timescale dynamics, such as the atom fluctuation, and side-chain rotations, is frequently studied by NMR and Neutron Spin Echo (NSE) [1]. The large domain motions are slow dynamics in μs-ms timescale or slower and the studies remains in a relatively blank area. These collective motions result in the diffuse scattering around the Bragg spots in the protein crystal diffraction pattern. In this work, we focused on measurement of the diffuse scattering from protein crystals using the coherent X-ray scattering.

The hen egg white lysozyme was crystallized using counter-diffusive method in quartz capillaries [2]. The protein crystals were exposed to coherent X-ray beam without further handling, and all measurements were done at room temperature. The X-ray coherent scattering of protein crystals was measured at the ID-10 beamline in hutch C. The beam size is 10μm×10μm and the flux is 1.58×10^9 ph/sec at the energy of 8.04 keV. The protein crystal sample was mounted on the goniometer head at the centre of rotations. A CCD detector mounted at 20cm downstream from the sample. By rotation of the protein crystal, a few Bragg peaks appeared in the CCD detector. Then calculation was done to evaluate the feasibility of the Bragg peak, so that it can be detected by the Maxipix detector located 366cm downstream from the sample. Once a feasible Bragg spot was found, then the CCD detector was removed, the Maxipix detector was moved to the calculated location, a fly-pass was equipped between the sample and detector to minimize the absorption by air.

The (110) reflection of the tetragonal lysozyme crystals was studied intensively in this experiment. The X-ray beam was radiated perpendicularly onto the <110> growing facet of the crystal. The rocking curves measurement was done for two purposes. One is to evaluate the quality of the crystal. The narrower the rocking curve is, the less mosaic the crystal is. The other one is for the time-resolved measurement. The crystal was rotated to reach the maximum of the Bragg reflection. The rocking curve width is about 16 arcsec for tetragonal lysozyme crystalized in capillary. A time-resolved measurement was done using 4ms exposure and the scan was repeated for a few thousand times.

The technique we used in this experiment is the X-ray Speckle Visibility Spectroscopy [3], which is modified from the visible light speckle visibility spectroscopy [4] and XPCS. The speckle visibility function is defined by

$$V_2(T) = \beta^{-1} [\langle I^2\rangle_T/\langle I\rangle^2 - 1],$$

where β is the contrast I is the pixel intensities of our interests. The visibility calculated as a function of exposure time is used to extract the dynamic information from the diffuse scattering around the Bragg peak.

Figure 1 shows an example of visibility function of the diffuse scattering. The decrease in visibility as a function of exposure time suggests that there are some motions in the sample. However, to correlation the dynamic information to the exact motions is the major obstacle in the research.

Because the experiment was done at room temperature, the protein crystals were experiencing much stronger radiation damage than in experiments at cryo-cooled temperature. Figure 2 shows the radiation damage effect in the lysozyme crystals. The intensity of the reflection decreases in rocking curves scans (in Figure 2a). Bragg spot deforms and splits when radiation damaged (in Figure 2b). Figure 2c is a microscopic view of damaged lysozyme crystal. The horizontal holes were driven by the coherent X-ray beam.

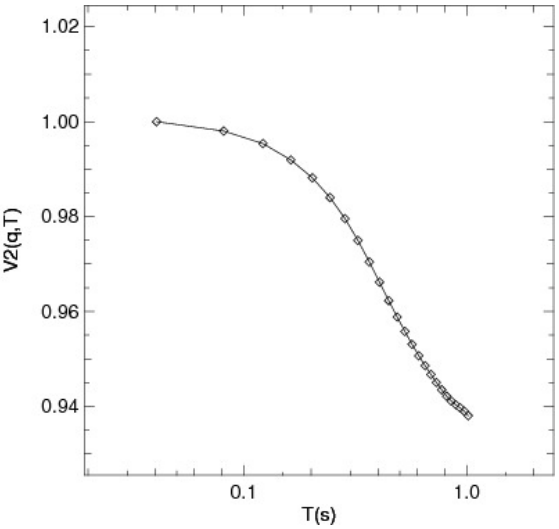

Figure 1 The normalized speckle visibility function $V_2(T)$ of the diffuse scattering around the lysozyme (110) Bragg spot.

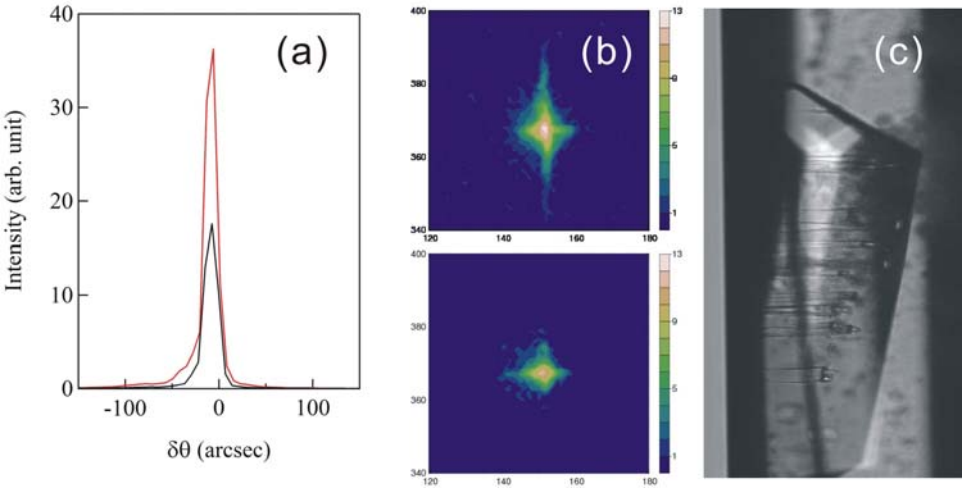

Figure 2 (a) The rocking curves of the (110) reflection were taken at the same position of the tetragonal lysozyme crystal. Red curve is from the first rocking curve scan, and the black curve is from the second rocking curve scan. (b) The Bragg spot on the top is taken after 30sec X-ray radiation, the Bragg spot on the bottom is taken after 140sec X-ray radiation. (c) The picture of the whole crystal is taken under a microscopy.

During this beamtime, we have developed an experiment protocol for the X-ray coherent scattering from the protein crystals. The rocking curve studies evaluated the quality of the protein crystal. The radiation damage of the protein crystals were also investigated by the behaviour of the rocking curves and the Bragg peak image. We have managed to take time-resolved measurement before observing signs of radiation damage. The XSVS analysis showed evidences of motion from the diffuse scattering near the centre of the Bragg peak. Cryo-cooled sample should be measured in future experiment to evaluation the amount of diffuse scattering coming from the mosaicity of the crystal comparing to that from the motion of protein molecules. Our ultimate goal is to measure the collective motion of motor proteins, such as kinesin and myosin, in crystal.

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