



	Experiment title: High-pressure protein crystallography of the hexagonal form of hen egg-white lysozyme	Experiment number: LS 2336
Beamline: ID27	Date of experiment: from: 24 Sep 2014 to: 27 Sep 2014	Date of report: 28 Feb 2015
Shifts: 9	Local contact(s): Volodymyr Svitlyk	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): Kamil F. Dziubek* LENS, European Laboratory for Non-Linear Spectroscopy, Via Nello Carrara 1, 50019 Sesto Fiorentino, Italy Michalina Aniola*, Andrzej Katrusiak, Ewa Patyk* Faculty of Chemistry, Adam Mickiewicz University, ul. Umultowska 89b, 61-614 Poznań, Poland		

Report:

The goal of the experiment was to study the structure of hexagonal polymorph of hen-egg white lysozyme (hHEWL) in the function of pressure. This most studied protein is known to crystallize in 5 crystal forms, but only two of them were investigated at high pressure. In particular, hHEWL is interesting due to its highest water content and structural differences in flexible loop regions, compared with all other crystal forms of HEWL.

For this experiment we used a purposefully designed diamond anvil cell with a large aperture (70 degrees) and 600 μ culets. The single crystals were fished out of the crystallization wells with a nylon loop and transferred immediately to an experimental chamber formed by a pre-indented gasket hole of 300 μ diameter and 150 μ height filled with the same reservoir solution slightly diluted with pure water. Ruby spheres were used for pressure measurement by ruby luminescence method. No focusing optics were applied and a parallel beam was collimated by slits to 50 μ in both directions, in order to reduce radiation damage of the sample. For the same reason an ultrashort wavelength of 0.3311 Å was applied. The diffraction images were recorded with the Perkin Elmer flat panel detector. Data collection was performed by the rotation method. We decided to irradiate successively different regions of the sample during data collection to mitigate the relatively fast degradation of crystals exposed to intensive X-rays beam at room temperature.

The crystals were therefore conceptually divided in several zones partitioning its projection visible in the compression cavity (Figure 1). The whole rotation range of 70 degrees was covered by 3 consecutive short cycles consisting of 23 exposures of 1 degree width. After accumulating each 23-frame sequence the crystal was translated in order to irradiate a fresh zone, and the rotation continued. The data were subsequently merged and exported to CrysAlisPro software suite.

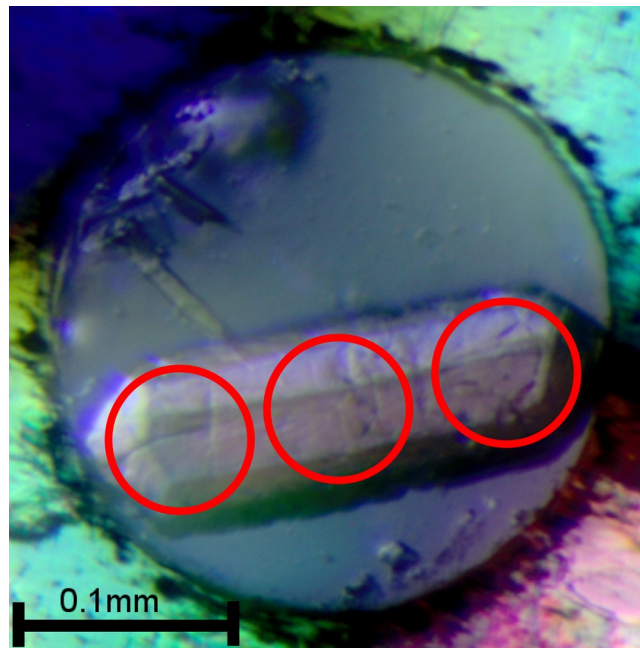


Figure1. Crystal of hHEWL at 1 GPa showing measured zones marked with red circles.

We have collected diffraction datasets at 0.5 GPa, 1.0 GPa and 1.5 GPa using three different crystals and the aforementioned experimental approach. Unfortunately, it turned out that there were only few usable crystals in the available crystallization boxes, as most of them gave very poor diffraction. We obtained a resolution average of about 2.5 Å. Each dataset consisted of approximately 44000 observations, representing 10000 unique reflections. However, after initial processing the data, we were not able to determine a high resolution structure. We suppose that the crystals were affected either during transport or loading procedure.

Unfortunately, the difficulties which marred the data collection hampered us from getting the convincing refinement results. Currently we are constantly working on processing the data sets. We also plan to optimize the conditions of the crystal growth in order to obtain better crystals.