

One of the important and still valid problems is the maintaining of the spatial structure of biological macromolecules (nucleic acids or proteins) at high pressures. In particular, this problem concerns protein enzymes and their potential biotechnology applications. For these enzymes, it is important to maintain both the quaternary (multimer) and the tertiary structure under extreme conditions, where both the decay of oligomer structures and the spatial structure can potentially take place.

The aim of this project is to investigate the high-pressure behavior of selected model proteins (xylanase, glucose isomerase) both in the crystal and in solution. The xylose/glucose isomerase from *Streptomyces rubiginosus* is a tetramer made up of four identical subunits (homotetramer) and catalyses the reversible isomerization reaction of simple sugars (xylose and glucose). Another enzyme selected for research is the single-domain protein - xylanase from *Trichoderma longibrachiatum*, which catalyses the reaction of random hydrolysis of internal (beta-1,4-D-xyloside) bonds in xylane, which is the main component of plant hemicellulose.

The structures of these proteins have already been known, but the planned high-pressure studies of these structures are already new. These proteins will be crystallized and obtained crystals will be used for diffraction experiments at high pressures (up to 2 GPa), using synchrotron radiation, will be carried out. The obtained diffraction data will be used to solve and refine their crystal structures. Parallel to diffraction studies, analyzes of the structure of these proteins in solution under high pressures will be carried out using the technique of small-angle scattering of synchrotron radiation. This method will allow investigation of potential structural changes in xylanase and glucose isomerase occurring in solution.

X-ray diffraction and SAXS experiments will be conducted both on beam lines in European synchrotrons and at the National Synchrotron Radiation Centre "SOLARIS", Jagiellonian University in Krakow. Currently, in NSRC "SOLARIS" is constructed the new beam line SOLCRY5 dedicated to protein crystallography (PX) and small-angle scattering of synchrotron radiation (SAXS) experiments. At SOLCRY5 beam line will also be able to study the structure of biomacromolecules at high pressures. As part of the SAXS and PX studies planned in this project, it will be also possible to verify the research capabilities of the SOLCRY5 beam line.