For centuries before their reaction nature and mechanism were discovered, humanity used various biocatalysts. Since ancient times, biocatalysts were successfully used for vine-making, brewery, bakery, diary, soy sauce production, *etc.* However ubiquitous in daily life, it was not until the beginning of XIXth century that the first scientific reports suggested mechanisms of these processes and in 1878 the term "enzyme" was proposed. Today, we estimate to know more than three thousand enzymes. The well characterized, commercially available enzyme preparation led to predictable and reliable processes, which improved the product quality.

Recently, cold-adapted enzymes, psychrophilic and psychrotropic, start to compete with mesophilic and thermophilic ones in various branches of industry, especially food and pharmaceutical ones. With the increasing availability of cold-adapted enzymes the knowledge about them also expands, but the enzymes' mechanism of adaptation to the cold has not been completely characterized. It is difficult to carry out in-depth analysis of this issue, because at the moment only about 40 crystal structures out of 120 thousands of all macromolecules depositions in Protein Data Bank are of cold-adapted enzymes.

The development of industry is dependent on the implementation of new technologies, with enzymes as catalysts of chemical reactions. Knowing as much as possible about their catalytic activity and specificity of their action, reactions catalyzed with adequate reaction rate may be designed, obtaining desired product with high efficiency, and when using cold-adapted enzymes with lower energy costs.

The aim of this project is to determine the crystal structures of several cold-adapted enzymes, including four hydrolases and two transferases. Analysis of the obtained crystal structures of these enzymes, in *apo* forms or mutants, as well as complexes with the substrates/ products and inhibitors, will allow the characterization of their catalytic sites and structural features involved in their adaptation to catalyze the reaction at low temperature.

The study will be based on: obtaining crystals of selected cold-adapted enzymes, collecting the diffraction pattern of these crystals using X-ray synchrotron radiation, processing diffraction data from dozens of measurements, the solution and refinement of these structures, their validation and deposition in the Protein Data Bank (PDB). The investigated cold-adapted enzymes will be mainly produced by two teams, we cooperate with prof. M. Turkiewicz from the Institute of Biochemistry of the Lodz University of Technology and prof. J. Kur from the Department of Microbiology, Gdansk University of Technology. Nonetheless, some of the studied proteins will have to be overexpressed in our laboratory with procedures developed by these teams. Out of six planned to investigate enzymes, we have already established the crystallization conditions for four: β-D-galactosidase from *Paraccocus* sp. 32d, β-D-galactosidase from *Arthrobacter* Sp.32cB, aminotransferase from *Psychrobacter* sp. B6 and MTA phosphorylase from metagenomic library of Antarctic soil, and for three of them we have already determined the structural data of the native forms.

Understanding the crystal structures of enzymes active at low temperatures will enable identifying the structural features responsible for this property. It will also enable comparison with their analogues from mesophilic and thermophilic organisms. Information obtained from crystal structures of these cold-adapted enzymes will enrich the basic knowledge of enzymes and will also enable designing enzymes for specific properties, a specific substrate specificity and the ability to act at low temperatures, which can potentially be used in biotechnological processes.