Enzymes represent a group of proteins, which act as catalysts of living organisms processes. Hydrolases are enzymes, which catalyze the hydrolysis of a chemical bond. Their action can be depicted in a following way: AB + H_2O → A-H + B-OH. In turn, glycosidases represent a subgroup of hydrolases and are in charge of glycosidic bond hydrolysis. Glycosidic bond is a type of covalent bond that joins a carbohydrate (sugar) molecule to another molecule, e.g. to another sugar (oligo- and polysaccharides), to an amino acid (glycoproteins), to a lipid (glycolipids), to an amine (nucleic acids), to a quercetin (rutin), etc. The proper action of glycosidases is vital for living organisms functioning. Inappropriate activity of some glycosidases most often is the cause of serious dysfunction called lysosomal storage diseases, that result from defects in lysosomal function. Monitoring of these glycosidases activities is crucial for quick and proper diagnosis and, when possible, efficient therapy. Besides diagnostics of lysosomal diseases, glycosidases have been investigated and employed in different fields of science, medicine and technology, since their action leads to release of biologically active molecules from respective glycosides. For example, it is extremely important to control β-glucosidase activity during the production of biofuels from the renewable lignocellulosic biomass sugars.

The purpose of my research is to design, prepare and investigate fluorescent indicators of β-glycosidases' activity, evaluate of the mechanism of action of these indicators in order to find sensitive, selective, reproductive and applicative method for monitoring of β-D-glucosidase, β-D-glucuronidase and *N*-acetyl-β-D-glucosaminidase activity.

Fluorescent enzyme activity indicators generally contain the inactivated fluorophore(s) linked with a specific, most often chiral, structural fragment fitting to the enzyme active site. Enzyme action releases the fluorophore and thus enzyme activity can be correlated with the rate of increase of fluorescence intensity signal. I have focused my attention on the fluorophores in which the excited-state intramolecular proton transfer (ESIPT) can occur. The fluorescence, characterizing such compounds, is distinguished by a substantial red-shifted emission with abnormally high Stokes shift and low susceptibility to self-reabsorption effect which enables linear dependence of fluorescence intensity on concentration in a wider range than in the case of common fluorophores like 4-MU and fluorescein. This enables linear dependence of fluorescence intensity on concentration in a wider range to be determined.

Among the ESIPT fluorophores I chose 4'-substituted derivatives of flavonol (3-hydroxy-2-phenylchromen-4-one), with electron-withdrawing (fluoro, chloro, cyano) and electron-releasing groups (metoxy and dimethylamino). Two other fluorophores are derivatives of 3-hydroxychromen-4-one, substituted at C2 carbon by furan-2-yl and tiofuran-2-yl groups. Such a spectrum of fluorophores will allow me to select the most efficient one. These fluorophores will be synthesized using Algar-Flynn-Oyamada reaction in the conditions modified by our group. For the synthesized fluorophores the fluorescence measurement conditions will be tested in order to gain the highest fluorescence quantum yields. Next, the respective glycosyl donor will be coupled to the fluorophore forming glycosides-target enzyme indicators, which will be exposed to the action of specific glycosidase. The course of enzymatic cleavage will be monitored by the rise of red-shifted fluorescence. For adequate interpretation of the experimental findings, kinetics of the enzymatic hydrolysis will be investigated at different conditions in which indicators exhibit highest sensitivity. Several approaches will be tested in order to improve the fluorescence efficiency of the studied fluorophores and general reproducibility of the method. The most important approach to fluorescence enhancement will be based on the Metal Enhanced Fluorescence (MEF) phenomenon.

Keeping all above considerations in mind, this project will end with a highly sensitive, selective and reproductive method for monitoring of the β-glucosidase, β-glucuronidase and β-*N*-acetylglucosaminidase activities. This project will also shed some light on the mechanism of action of the tested indicators and glycosidases action. Importantly, the developed approach and optimized conditions will be able to be applied for determination of the activities of other hydrolase enzymes.