Proteolytic enzymes, also known as proteases or peptidases, are responsible for peptide bonds hydrolysis, therefore for controlling the synthesis, activation and degradation of proteins. They play an important role in many biological processes including digestion, metabolism, maturation, aging and death. The nature of chemical reactions that proteases catalyze, demands tight control of their activity, in order to avoid excessive and dangerous hydrolysis of proteins needed for cell functioning. One of the proteolytic enzymes whose activity plays a key role in maintaining protein homeostasis is 20S proteasome (called constitutive proteasome) - a large protein enzyme complex. The primary function of proteasome is to degrade damaged, non-functional or unnecessary cellular proteins. Disorders in proteasome functioning can lead to many disorders for example Parkinson, Alzheimer and Huntington disease (resulting from the proteasome inability to hydrolyze pathological proteins) or cancer (caused by excessive enzyme activity). In eukaryotic cells, several form of proteasome are present. Under immune response the second form of proteasome is induced and it is called immunoproteasome. The structure of both forms of proteasome is slightly different, which causes that they exhibit different functions. The first known function of immunoproteasome is production of short peptide fragments called antigens that enable cell communication. Further studies revealed that this enzyme is involved in many biological processes including inflammatory proteins production, immune cell differentiation, arthritis, or cancer, but its precise function in mentioned processes has to be elucidated. Moreover, studies show that regulation of immunoproteasome activity may constitute an important factor in cell functioning.

Chemical compounds called markers (such as fluorescent substrates or inhibitors with chemical tags) are suitable tool for monitoring enzyme activity. One of the most important requirements for using these tools in biological research, is their selectivity toward enzyme of interest, which means that they should interact only with one protease in the cell. Since the proteasome is a multicatalytic complex, it is particularly important to have three selective substrates to monitor the activity of each subunit separately. Therefore, the main goal of this project is to design and synthesize selective fluorogenic substrates for each catalytic immunoproteasome subunit and 20S proteasome. The first step to achieve this goal will be to determine full substrate preferences of both proteasome forms, using fluorogenic substrate libraries containing natural and a wide variety of unnatural amino acids. The varied structure of amino acids side chains provides an ideal tool for enzyme-substrate interaction studies. In the second stage of the study, based on the obtained substrate preference profiles, selective substrates for each catalytic subunit of both enzymes will be designed and synthesized. Their selectivity will be investigated in the next step. The resulting substrates will constitute valuable tools for comparing both enzymes activities in diseases, which could help in selection of appropriate treatment. Moreover, the resulting peptide sequences can be utilized as leading structures in inhibitor design. This is important because proteasome peptide inhibitors have already been used as effective drugs in cancer therapy.