Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) or amyotrophic lateral sclerosis (ALS) are an extremely serious and growing social problem. These debilitating conditions are strongly linked with age and are characterized by progressive neurodegeneration and dementia. One of the hallmarks of these disorders is accumulation of impaired proteins which in healthy cells are efficiently removed by proteolytic systems, mainly 20S proteasome. This giant enzyme constitutes a cell cleaning system removing mutated, misfolded and oxidatively damaged proteins. Unfortunately, the enzyme efficiency declines with aging. Therefore, the activation of proteasome by either genetic means or use of natural or synthetic compounds could be a promising therapeutic strategy to delay the onset of age-related neurodegenerative diseases. However, so far little is known about the mechanism of 20S stimulation by small molecules, hence the lack of good leading structures for the design of activators. The possible approach to establishing them may be the use of the structures of the proteasome complexes with its natural proteinaceous regulators. It was shown that short peptides based on the 19S and PA200 regulators and comprising their *C*-terminal HbYX (hydrophobic-tyrosine-any residue) motif, are able to stimulate the 20S proteasome. The results of our studies also proved this concept – during the implementation of the previous Opus grant we have discovered a new group of very efficient proteasome activators, whose sequences were derived from the *C*-terminal fragment of another natural proteasome regulator, Blm10 protein (a yeast ortholog of PA200). The capability of these peptides to stimulate 20S proteasome was proved in tests with the standard fluorogenic substrates, as well as with model proteins (α-synuclein, Tau441). However, the membrane permeation assay showed that Blm analogs in their current form are unable to enter the cells. Their drawback is also low proteolytic stability. Improvement of these two parameters is indispensable for proposing a new leading structure for the design of effective proteasome activators, capable of reducing the accumulation of impaired proteins in cells.

Therefore, **the main goal of this project is the design of proteasome stimulators permeable to cell membranes and stable under proteolytic conditions**. To achieve this goal, we will implement several tasks. Firstly, we will select 3-5 sequences from the pool of our Blm-type activators and modify these sequences by introduction of non-natural amino acids, either commercially available or obtained as a result of a chemical synthesis. We will also introduce mimetics of the peptide bond, which should render greater proteolytic stability of the resulting peptidomimetics. Then, we will determine the influence of the obtained compounds on the catalytic activity of the human 20S proteasome using standard fluorogenic substrates. To evaluate the ability of the most active compounds to cross the cell membrane we will synthesize analogs labeled with a fluorescent dye and use a fluorescence microscopy to observe their internalization. Next, we will determine proteolytic stability of the cell permeable compounds in plasma. We also plan to assess if the obtained compounds can stimulate 20S proteasome for more efficient degradation of aggregation-prone proteins. If encouraging results are obtained in *in vitro* studies, the modulators will be tested in the *D. melanogaster* Alzheimer's disease model to check their influence on deficits in associative learning and mortality.

Studies we are going to perform should allow to develop small peptidomimetic molecules which are devoid of two major drawbacks limiting therapeutic application of peptides: the relatively short circulating plasma half-life and the lack of cell permeability but preserve their capability to counteract proteasome impairment. Such interventions have the potential to restore proteostasis in patients suffering from age-related disorders connected with increased accumulation of impaired proteins, including the most common in aging societies Alzheimer's and Parkinson's neurodegenerative diseases.