I.2. **Abstract**

Human HtrA2 protease belongs to the HtrA (*high-temperature requirement A*) family of serine endopeptidases. The HtrA family of proteins are widely expressed among prokaryotic and eukaryotic organisms, including humans with four known members. HtrA proteases degrade denatured, aberrant proteins and some native proteins which may have regulatory functions. Most HtrA's are localized extracytoplasmically and HtrA2 localizes to mitochondria. In the physiological state, HtrA2 is an important protein quality control factor, essential for maintaining mitochondrial homeostasis. Under conditions of cellular stress, it plays a pivotal role in promoting apoptosis. HtrA2 is involved in oncogenesis and neurodegenerative disorders (e.g. Parkinson's and Alzheimer's diseases) and is assumed to be a novel target in the therapy of cancer and neurodegenerative disorders. The HtrA proteases require activation and the structures of their active and inactive forms differ. To date, only crystal structure of the HtrA2 inactive form has been solved. HtrA2 is a homotrimer, whose subunits comprise N-terminal serine protease domain (PD) and a C-terminal PDZ domain which binds a substrate or activating peptide. In the inactive state, the access to the catalytic site is restricted by the interaction of the PDZ domain with the PD. Molecular mechanism of HtrA2 activation is not well understood.

The purpose of the research undertaken in this work was to investigate and describe the exact molecular changes occurring in HtrA2 during thermal and peptide-induced activation.

The results of this work confirmed that HtrA2 proteolytic activity was stimulated by temperature and that the PDZ domain removal caused activity increase. It was shown, for the first time, that the kinetics of the model peptide cleavage by HtrA2 and is typical for the allosteric enzymes. Stimulation of the HtrA2 activity by a set of the previously described activating peptides was confirmed. Furthermore, it was demonstrated that the peptides which had earlier been characterized only as binding to the isolated PDZ domain, activated HtrA2. Stimulation of HtrA2 protease activity by the tested peptides was efficient at the temperatures 25-45^{o}C. These results indicate the importance of the PDZ domain in the HtrA2 activity regulation.

To understand the molecular basis of the HtrA2 temperature-induced increase in activity, structural changes were monitored using a set of single tryptophan (Trp) HtrA2 mutants with Trps located at the PDZ-PD interface. The accessibility of each Trp residue to the aqueous medium was assessed by steady-state fluorescence quenching and these results in combination with mean fluorescence lifetimes and wavelength emission maxima indicated that upon increase of temperature HtrA2 structure relaxes, the PDZ-PD interface becomes more exposed to the solvent, and significant conformational changes involving both domains occur. To further the

knowledge of HtrA2 thermal activation, the dynamics of the PDZ–PD interactions during temperature increase was monitored using Tryptophan–Induced Quenching (TrIQ) method. The TrIQ method allows monitoring changes in protein structure due to the properties of tryptophan residue, which quenches the fluorescence of bimane derivatives. The intensity of the quenching depends on the distance of the fluorophore and Trp residue in the range of 5 to 15Å, which is ideal for assessing short-range interaction and small changes in protein structure. The TrIQ results suggested that during activation the PDZ domain changes its position versus PD inside a subunit, including a prominent change affecting the L3 regulatory loop of PD, and changes its interactions with the PD of the adjacent subunit (PD*), specifically with its L1* regulatory loop containing the active site serine. The α5 helix of PDZ was involved in both, the intra– and intersubunit changes of interactions and thus seems to play an important role in HtrA2 activation. The model presented in this work describes PDZ movement in relation to PD and PD*, resulting in an increased access to the peptide binding and active sites, and conformational changes of the L3 and L1* loops. The TrIQ method was also used to monitor molecular changes in HtrA2 during peptide-induced activation. Results obtained suggest strong induction of similar but not identical molecular changes.

In conclusion, results of this study provide an insight into the molecular basis of HtrA2 activation by temperature increase as well as by an allosteric peptide regulator binding to PDZ domain.