Summary of the project: ***The impact of model biological membranes on the structure and oligomerization process of human cystatin C.***

Amyloidogenic diseases (amyloidoses) are among the main problems faced by contemporary medicine. These diseases are characterized by the accumulation of insoluble proteins in various parts of the organism. Amyloidoses include such widespread diseases as Alzheimer's, Parkinson's, rheumatoid arthritis, and type II diabetes. The basic problem associated with amyloidosis is the lack of effective treatment for patients - only symptomatic therapy is available. It is well established that the insoluble forms of proteins formed during the course of amyloidoses arise from soluble monomers by their dimerization and oligomerization. However, the mechanism of this process remains a riddle. One hypothesis of amyloid formation points to biological membranes as a catalyst of the process and considers possible mechanisms of cellular toxicity of amyloidogenic proteins. Toxic effects on the cell may be exerted by insoluble amyloid deposits that accumulate on the surface of the cell causing its damage. The deposits may also accumulate toxic waste products. Another, proposed as toxic, form of the proteins are donut-shaped, soluble oligomers. They can insert themselves into the cell membrane, create channels and cause uncontrolled outflow of cell contents into the intercellular space. However, it is still not entirely clear which of the forms of the protein is more harmful (insoluble amyloid or soluble oligomer), and thus is the main reason for the emergence of the disease state. The debate is still dividing scientists all around the world.

One of the described amyloidogenic proteins is human cystatin C (hCC) - a small protein belonging to the family of cysteine proteinase inhibitors. In the physiological state, the protein is present mostly in the body fluids in monomeric form and regulates the activity of cysteine proteases. In disease states, hCC undergoes dimerization resulting in a loss of biological activity and inhibitory properties. In further stages, the protein oligomerizes and then aggregates in the form of amyloid deposits in the cerebral blood vessels. Studies have shown that cystatin C forms the donut-shaped oligomers during its oligomerization. The disease state associated with the presence of hCC aggregates is called hereditary amyloid cerebral angiopathy. It is characterized by severe damage to the blood vessels of the brain, massive and frequent brain hemorrhages and death of patients at an early age. An interesting feature of hCC is the ability to migrate through biological membranes. The highest concentrations of hCC are usually observed in physiological fluids, what allows the use of protein as a marker of kidney disease or atherosclerosis. However, the protein also plays an important inhibitory role inside the cell. So far, it has been shown that hCC can migrate across the cell membrane bidirectionally. However, this process has not yet been described in detail and it is not clear how the hCC penetrates the membrane on its own, or whether it needs help from transport proteins. It also has not been studied how cell membranes affect protein stability and its tendency to oligomerize. The aim of the project is to determine what is the impact of biological membranes on the structure and oligomerization process of human cystatin C. Studies using natural biological membranes are very difficult due to their high complexity, therefore in this project we will use micelles and liposomes, which are commonly used as mimetic (analogs) of biological membranes (including the cell membrane). As a part of the project, a number of such techniques as calorimetric, chromatographic, electrophoretic, fluorescence and spectroscopic techniques will be combined with theoretical methods. They will allow the analysis of the effect of the presence of biological membranes on such modifications of the hCC structure as dimerization or oligomerization. Liposomes (and eukaryotic cells) will be also used for the initial characterization of hCC transmembrane transport. The results of the project will allow broadening of the general knowledge regarding the interactions of proteins with biological membranes and the impact of these interactions on the structure of proteins and their oligomerization propensities.