Neurodegenerative diseases related to the aggregation of amyloidogenic proteins in the brain tissue (like Alzheimer's and Parkinson's disorders) are serious medical, economic, and social problems. Due to the lack of drugs and appropriate therapies, neurodegenerative amyloidosis remains incurable and lethal. The subject of my dissertation is cystatin C (hCC), which belongs to a wide group of amyloidogenic proteins. Its pathogenicity is related to an inherited point mutation in the gene, which leads to the overproduction of an easily oligomerizing variant L68Q and disease called hereditary cystatin C amyloid angiopathy. My doctoral research aimed to prove that copper ions (commonly present in the brain tissue) influence the structure and aggregation of cystatin C.

During realization of my PhD project I prepared expression vectors and overproduced in the *E. coli* bacterial system three new cystatin C mutants - H86A, H90A and H86_90A. Their biological activity and structures proved to be very similar to those of the wild type (WT) protein. The circular dichroism studies with the temperature gradient showed reduced conformational stability of the H90A mutant. It results in a significantly increased tendency to dimerization. The point mutation (H90A) was introduced in the AS loop which, according to previous research, should not affect the process of three-dimensional domain swapping. However, the removal of the His90 residue destabilized the protein chain which suggests a possible, but yet undescribed, role of the AS loop in the cystatin C dimerization.

I investigated the influence of copper(II) ions on the structure and conformational stability of hCC variants using circular dichroism and molecular filtration chromatography. The presence of $Cu^{2+}$ ions does not affect the protein structure, but prolonged incubation leads to dimerization (especially in the cases of WT and H90A proteins). Additionally, the interactions with copper(II) ions significantly reduce the thermal stability of the wild type protein and the H90A variant. Similar data for hCC WT and H90A proteins suggests that the presence of the His86 residue has a key role in the cystatin C - copper(II) ions interaction. Removal of the mentioned residue (in cases of H86A and double H86_90A variants) decreased the sensitivity of both mutants to the presence of copper in the solution.

The EPR studies showed four complexes formed by copper(II) ions in an aqueous solution containing the monomeric form of cystatin C (stabilized against dimerization). However, only two of them involve studied His86 and His90. NMR spectra confirmed the interaction of the copper(II) ions with both histidine residues in the AS loop but also indicated

the presence of number of additional, non-specific interactions with copper in different parts of the protein.

Fibrillation of cystatin C in the presence of copper ions proved that ions do not affect the morphology of protein fibers. The exception was the H90A mutant which, under weaker shaking conditions (300 rpm) and in the presence of copper chloride, formed mainly large protein aggregates among which I observed small fibrillar structures interacting with thioflavin T.

Another experiment performed on WT protein at a low protein concentration, proved that copper influences the most likely the first stages of the oligomerization process. The protofibrillary structures in the sample with the addition of copper appeared several hours earlier than in the control sample. $Cu^{2+}$ probably stabilize dimeric and/or low-oligomeric soluble forms of the protein, which leads to a delay in the aggregation but does not affect the structure and morphology of the formed fibrils.

The last stage of my PhD research was the crystallization of overproduced proteins in the presence of copper ions to establish the metal binding site. Unfortunately, dimerization and further protein oligomerization induced by $Cu^{2+}$ led to rapid precipitation of protein deposits in the crystallization droplets and prevented the formation of crystals. However, I obtained three crystal structures of the H90A (monomeric and dimeric) and H86_90A variants without the presence of copper ions. Both H90A structures are deposited in the Protein Data Bank under 7PU2 (monomer) and 7PU3 (dimer) codes. The structure of the H86_90A protein was not deposited due to the poor quality of the model.

In conclusion, copper(II) ions – cystatin C interactions are weak and rather unspecified. However, one of the most preferred binding sites includes tested His86 and His90. My research confirmed that the presence of copper(II) leads to dimerization and further oligomerization of cystatin C. The major roles of $Cu^{2+}$ ions in this process are inducing dimerization and probably stabilizing soluble, small protein oligomers.