Summary:

My doctoral thesis concerns the structural and functional analysis of the complex of mitochondrial specialized chaperone proteins (including the Hsp70 - Ssq1 protein and the J-type protein - Hsc20) with the Isu1 protein, which is the molecular scaffold within which the biosynthesis of iron-sulfur clusters (FeS) takes place. The chaperones of the Hsp70 family play many important roles in the cell. In addition to the classical functions of chaperones, such as protecting proteins from aggregation or helping them disaggregate, they are also involved in the folding of protein precursors, the transport of polypeptides across membranes, and the modulation of protein interactions. Most Hsp70 proteins are multifunctional and their substrate specificity is determined by cooperation with J-domain proteins (JDP). The Hsp70 system operates in a specific cycle where the hydrolysis of ATP by Hsp70 results in a strong binding of the substrate. The next step, i.e. the release of the substrate, takes place when the nucleotide exchange factor (NEF) exchanges ADP for ATP, causing the Hsp70 conformation to change to favor dissociation of the substrate. In my research, I focused on a specific case, which is the chaperone proteins involved in the biosynthesis of iron-sulfur centers (FeS). The process of FeS biogenesis can be divided into two stages: (i) synthesis of the FeS cluster within the specialized scaffold protein (Isu1) and (ii) transfer of the FeS cluster from the Isu1 molecular scaffold to target proteins with the participation of Hsp70 system proteins. In eukaryotes, this system is located in the mitochondria. In the case of the baker's yeast *Saccharomyces cerevisiae*, which was my model research organism, the gene coding for the mitochondrial Hsp70 protein was duplicated. This led to the creation of a copy of the mtHsp70 protein called Ssq1, which is dedicated exclusively to the biogenesis process of FeS clusters. The high degree of specialization of this system makes it an excellent research model, because Ssq1 protein interacts with only one JDP (Hsc20) and its only substrate is Isu1.

The first challenge during my research was to develop a method for obtaining highly concentrated preparations of the Ssq1-Hsc20-Isu1 complex. For this purpose, I used a method in which I overproduced three proteins in *Escherichia coli* expression system (Isu1, Hsc20 and Ssq1, respectively, with the T239A mutation preventing ATP hydrolysis). This approach allowed me to obtain protein complexes already in cells. Nevertheless, the key step was preparative gel filtration, which allowed me to separate protein complexes based on their mass. The Ssq1-Hsc20-Isu1 complex prepared in this way was then used in deuterium exchange experiments combined with mass spectrometry (HDX-MS). The obtained results confirmed the important role of selected key amino acid residues known from the previous literature in the interaction between the Ssq1, Hsc20 and Isu1 proteins. At the same time, the obtained results allowed for the selection of further regions within the proteins that had not previously been identified as significant for the protein:protein interaction within the analyzed complex. On this basis, plasmid constructs with alanine substitutions of selected amino acid residues were prepared. Then, the mutated versions of the proteins were purified and analyzed in protein complex precipitation experiments. In my experiments, I used a version of the Isu1 protein with a GST (Glutathione-S-Transferase) tag, which I precipitated by adding reduced glutathione-coated agarose to the reaction mixture in order to bind the labeled protein and proteins interacting with it. Using the Isu1-GST fusion protein, I conducted a series of experiments with different variants of the Ssq1, Hsc20 and Isu1 proteins with introduced mutations within the regions involved in the formation of the Ssq1-Hsc20-Isu1 complex. This analysis included amino acid residues known from the literature as well as those newly identified during my research. The obtained results unequivocally confirmed that the regions within the Ssq1, Hsc20 and Isu1 proteins identified as significant in deuterium exchange mass spectrometry (HDX-MS) experiments are crucial for the formation of the Ssq1-Hsc20-Isu1 ternary complex.