Metallothioneins (MT) are a family of small proteins rich in sulfur and metal ions. This family consists of proteins with a similar structure found in all cells but with different preferences. This protein was discovered in 1957 as a molecule that binds cadmium, a toxic element that does not play any significant role in mammals. For years, it was considered a protein whose role is to detoxify the body from unnecessary and dangerous cadmium and other harmful metal ions. As metallothionein was researched, it turned out that its natural role is to bind zinc and that heavy metals displace it, hence their presence in exposed organisms. The binding of zinc by metallothionein is different from other zinc proteins. It was found that seven zinc ions are bound with different affinities. Due to both the incomplete saturation of MT and the binding of zinc with different strengths, they fulfill the role of a cellular zinc buffer. They can simultaneously donate zinc to other proteins and bind excess when is needed. Several studies have shown that this protein also participates in copper binding; however, the function of these proteins concerning this element is currently almost unknown. So far, very modest studies on the interaction of copper with MT have been carried out. Practically only copper-MT (binary) complexes were investigated. Meanwhile, a growing body of evidence shows that the natural forms of copper MT complexes are mixed species containing zinc and copper. So far, no one has attempted to describe both the formation of such complexes and their properties or functions. Given that MT is involved in cellular zinc homeostasis, it is very likely that copper binding is of a similar regulatory nature. However, it is too early to speak about it with full conviction. The study of such mixed complexes is technically very complicated due to the reactivity of the MT itself, the copper ions, and the presence of zinc. Therefore, the project's primary goal is to describe the formation of mixed species with zinc. An essential part of the research is a structural investigation using mass spectrometry, which is currently the only method to study such complex systems with high resolution. During the project implementation, we aim to find preferentially formed mixed complexes and determine the affinities of zinc and copper ions in those species. Above all, we aim to describe the buffering properties of mixed zinc/copper complexes. To do so, the transfer of these metals between mixed complexes and proteins or motifs that naturally bind these elements in the cell will be examined. It will allow us to explain the role of mixed complexes in the cellular regulation of copper and zinc. Moreover, we will try to answer whether the regulation of one metal ion occurs synergistically from the other or not. By implementing this project, we will overcome another milestone in exploring these challenging proteins to study by showing their structure and describing their features. Principle investigator of the project has almost twenty years of experience working on MT and is an expert in the biochemistry of zinc and copper.