For many years, it has been known that zinc, as a trace element, is essential for the proper functioning of living organisms. Both its deficiency and excess can lead to many disorders and even contribute to the development of cancer. Therefore, our cells have developed a range of mechanisms to maintain the correct level of this element. Changes in the concentration of available zinc ions within the cell serve as a signal that regulates the production level of many proteins, including those involved in controlling the amount of this element in the cell. However, most cellular zinc is bound to proteins. In these proteins, zinc can be responsible for enzymatic activity and maintaining proteins' proper, stable structure. It is also associated with proteins responsible for its transport or storage. An essential role of bound zinc is also mediating interactions between proteins and DNA-protein. Some proteins bind zinc very tight (enzymes), while others bind it much less strongly. For the latter, whether this element is bound may depend on the concentration of free zinc in the cell. The main goal of this project will be to examine the impact of naturally occurring changes in motifs commonly considered zinc-binding sites, such as zinc fingers and protein fragments rich in the sulfur-containing amino acid cysteine, on zinc interaction. The first stage of the project will be the chemical synthesis of peptides and the production of selected zinc-binding proteins. Next, we will investigate the impact of differences in the length of linkers located between the amino acids responsible for metal binding in zinc fingers. A thorough thermodynamic and structural analysis will allow us to understand how sequence changes affect the structure of zinc fingers, tune the overall stability of this motif, and how they affect their DNA-binding capacity. We hope these studies will help us answer the question of whether changes in the concentration of unbound zinc in cells can influence the spatial organization of certain zinc fingers and promote their interaction with DNA in a manner dependent on the availability of this metal. We will then examine how zinc fingers with commonly occurring zinc-binding amino acid arrangements differ from those naturally altered. Particularly interesting will be learning how these different zinc fingers behave in relation to DNA under the influence of changes in zinc ion concentration in the cell. Another group of proteins studied by our team for years are metallothioneins. They bind up to seven zinc ions with varying affinity and can both store excess zinc and donate it to other proteins when needed. In this project, we would like to focus on the role of a previously uncharacterized metallothionein-like protein, MT1HL1. It has fewer sulfur residues than other metallothioneins. During the project, we will investigate which metal ions interact with this protein and try to determine this protein's function and role in regulating cellular zinc and copper concentrations. The next stage of the project will be a closer look at the role of zinc in mediating protein-protein interactions in systems where metal binding is very strong and where it is weaker, as well as in complexes formed by two different proteins. We hope that the planned studies will also enable us to develop, as part of the final stage of the project, molecular tools useful for studying metalloproteins or utilizing zinc-binding motifs and domains.