Fluctuations in cellular zinc levels are recognize by metal-responsive transcription factor 1 (MTF-1), a zinc finger protein (ZFP), responsible for regulation of metal response genes such as metallothioneins (MTs) or zinc transporters. In mammals, under normal condition MTF-1 is shuttling between the cytoplasm and nucleus, however; it can be activated directly by elevated level of cellular zinc or indirectly by release of zinc from MTs under heavy metal load or oxidative stress. These lead to ZFs saturation causing MTF-1 export to nucleus where it binds to metal regulatory response element (MRE) to induce target gene expression The activation of MTF-1 upon zinc binding is characterized by an extremely complicated mechanism. So far, studies based on the activation of ZF motifs from MTF-1 protein under increasing concentration of cellular Zn(II) ions gave ambiguous results. There is only the evidence that ZFs from 1 to 4 constitute major role during DNA-binding, while the ZFs 5 and 6 are zinc sensors. On the other hand, other studies suggested that the linker between ZFs is essential zinc sensor to mediate metal-responsive transcription. We believe that molecular mechanism of signal transduction and MTF-1activation strongly depend on the differential affinity of the ZF motifs and their saturation under cellular free Zn(II) fluctuations. Therefore, the main goal of this project is to determine influence of structural and thermodynamic factors that govern activation of human MTF-1 (hMTF-1) in relation to Zn(II) concentration. We aim to perform DNA-binding studies of the ZF domain from hMTF-1 under controlled Zn(II) fluctuations and stress conditions (exposure to heavy metals, oxidants) to get better understanding of metal sensing mechanism. Besides, ZF domain, MTF-1 protein is composed of a unique cysteine-rich motif which is highly conserved among human, mouse, cow, pufferfish but is not present in zebrafish MTF-1 sequence suggesting a more specialized function of cysteine-rich motif in higher eukaryotes. Our preliminary results obtained for the shorter fragment derived from the C-terminal part of the hMTF-1 protein (cysteine-rich region) show that this motif is capable of binding Zn(II) ion with an affinity falling within the range of cellular Zn(II) fluctuations. This may suggest possible role for cysteine-cluster as a Zn(II)-dependent structural switch. On the basis of obtain results we will explore the biophysical and functional properties of hMTF-1 cysteine-rich region of unknown nature. In conclusion, the scope of this research project will focus on a series of biophysical tests on hMTF-1 protein fragments (zinc finger domain and cysteine-rich motif), including stability and metal binding study using spectroscopic measurements. Also fluorometric characterization of interaction with DNA molecule under controlled Zn(II) concentration as well as stress conditions will be accessed.