The aim of this 5-year project is ***to understand the interaction and biological role of copper (and other divalent metal ions like zinc, cobalt, or nickel) with an unusual virulence factor: GroEL1 mycobacterial chaperonin.***

Mycobacterium genus comprises clinically relevant bacteria such as *M. tuberculosis, M. smegmatis*, *M. bovis*, and *M. leprae* – pathogens, which have a profound impact on human health and animal farming. The project will focus particularly on ***GroEL1 chaperonins from nontuberculous mycobacterium species with histidine-rich C-terminal tail (HRCT).*** Histidine amino acids are building blocks of proteins able to bind metals efficiently. The reason for tackling this specific problem is the lack of new, effective antibiotic treatments, that prompt the scientific community to look for new antibacterial strategies - one of them is to use of the antibacterial properties of metals. It was recently discovered, that the above proteins are essential for metabolic and energetic adaptation under cell stress conditions thus they are promising molecular targets of a new generation of medicines against bacterial infections. ***More detailed knowledge of bacterial cell biology, especially resistance mechanisms and its virulence factors is needed to overcome mycobacterial drug-resistant infections.*** To achieve this goal, studies on the thermodynamic properties of the complexes formed by the potential virulence factors and drugs, are required.

Our main goal in this project is to answer the following questions:

1. How does the binding of Cu(II) and other Me(II) ions to GroEL1 HRCT motifs emerge? What are the binding modes? What are the stabilities of pH-dependent metal complexes? How do different amino acids influence the binding? How do the mutations affect the binding?
2. What is the difference in the interaction between M(II) and HRCT domain itself and the longer protein domain with secondary and tertiary structure?
3. What are the structural and dynamic changes upon M(II)-interactions with the protein?
4. What are the exact structures of the M(II)-GroEL1 complexes?
5. How do *in vitro* experiments reflect *in vivo* observations?

Since it is well known, that metal ions play an essential role in the process of mycobacteria species pathogenesis, it is of high importance to investigate the metal ions' interactions with the potential protein targets. In this particular project, we will determine pH-dependent thermodynamic properties of HRCT-Me(II) complexes, characterize the structures and dynamics of M(II)-GroEL1, and perform living cell experiments to see the implications of GroEL1 protein deletion on cell survival in various conditions in *Mycobacterium smegmatis*. We want to propose a unique approach - the combination of (i) a bioinorganic chemistry approach on peptides, with (ii) advanced NMR (peptides/domains of the protein) giving us information about binding sites together with structure–dynamics relationship, (iii) crystallography, and (iv) *in vivo* studies on mycobacterial cells giving us the opportunity to create a comprehensive picture of studied systems. ***This is a truly multidisciplinary and novel approach, which will allow us to create a far-reaching picture of metal-dependent GroEL1 machinery in Mycobacterium.***

We will try to combine the advantages of the bioinorganic chemistry approach, solution NMR techniques, X-ray crystallography methods, and genetic engineering/microbiological experiments, making it possible to fully understand and describe at the molecular level the interaction of metal binding domains and whole native proteins with metals. The scientific approach, the combination of state-of-the-art methodologies, and the topic's timeliness render this project highly desirable, and novel.