The raising number of antibiotic-resistant bacterial strains has become one of the major health problems of modern times. Refractory bacterial infections cause severe medical burden worldwide, resulting in considerable number of deaths every year. Among antibiotic-resistant infections caused by e.g. *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, Klebsiella pneumoniae* and *Enterobacteriaceae* family, WHO lists *tuberculosis* as one of the most challenging. In 2016, 10.4 million people developed an active tuberculosis, from which 1.8 million died. Moreover, it is estimated that one-third of human population is latently infected with *M. tuberculosis*. In the latent infection, tubercle bacilli may survive within human macrophages and dendritic cells in inactive (dormant) form, causing no apparent symptoms. When the immune system is compromised (e.g. during HIV infection or immunosupressive therapy), Mtb becomes activated. Treatment of mycobacterial infections is long and often requires combination of several drugs. Unfortunately, multidrug-resistant Mtb (MDR) strains are growing in number.

To help reduce bacterial pathogenicity more knowledge concerning their cell biology as well as resistance mechanisms is needed. The lack of new antibiotics development, forces scientific world to look for the new strategies in fighting against pathogens, thus metal-based antimicrobial therapy has now gained pace. One of the most promising targets for novel antimicrobial therapies against *mycobactrium* strains are metal-sensing transcriptional regulators (e.g. ArsR-like) and the proteins they regulate e.g. metal efflux pumps. Moreover, ArsR "metal-sensing" transcriptional regulators that controls complex system of metal homeostasis in the cell are linked to key virulence factors, what makes them even more promising targets for the antibacterial drug development.

The aim of this project is to understand the interaction of Zn(II) with "metal-sensing", *mycobacterial* transcriptional regulator from ArsR-family and to explore its biological role in zinc homeostasis and involvement in regulation of gene transcription in *mycobacteria*. Our main goal is to answer the following questions: How do mutations in metal-sensing domain of SmtB/BigR4 protein (ArsR-family) affect the interaction of Zn(II) with the native protein and Zn-domains itself? To answer this question, we plan to use two complementary approaches, in vitro and in vivo. We will use both, metal sensing domains and whole native proteins and their mutants to determine thermodynamic properties, structure and dynamics of metal complexes (potentiometry, NMR, ITC) as well as their interaction with the DNA. Our in vitro results will be verified by in vivo studies: we will compare wild-type bigR4 and its mutants as well as allelic replacement of BigR4 gene with SmtB in *M. smegmatis* strains to determine the binding sites of SmtB/BigR4 along the chromosome. This truly multidisciplinary and novel approach will allow us to create comprehensive picture of Zn-dependent SmtB metal-sensing machinery in *Mycobacterium*. This project combines the advantages of bioinorganic chemistry approach, solution NMR techniques and genetic engineering/microbiological experiments, making possible to fully understand and describe at molecular level the interaction of metal binding domains and whole native proteins with metals. The uniqueness of the scientific approach and the combination of state-of-the-art methodologies render this project timely, highly desirable and novel.