Nucleoid associated proteins (NAPs) are the most abundant proteins involved in organization of bacterial DNA. They compact the chromosome by bending and/or wrapping the DNA around themselves or bridging the neighboring DNA strands. The most conserved NAP among bacteria is Heat-Unstable protein (HU) that interplays with Integration Host factor (IHF) in maintaining chromosome structure and regulating gene expression. Mycobacterial IHF (mIHF) is a unique NAP that is essential for *Mycobacterium smegmatis* viability and is supposed to be crucial for the survival of *M. tuberculosis* inside the macrophages during infection. Interestingly, mIHF in pathogenic species, such as *M. tuberculosis* and *M. bovis*, consists of an additional N-terminal domain (absent in *M. smegmatis*), which biological function remains unknown. The aim of this project is to analyze the role of mIHF in chromosome organization and dynamics during the mycobacterial cell cycle and to establish the biological function of an additional N-terminal domain of mIHF.

To address the objectives of the proposed project we will implement various molecular biology techniques and advanced microscopic methods. Time-lapse fluorescence microscopy techniques will allow to analyze mycobacterial chromosome organization in in real-time on a single cell level. We will be able to localize single particles of mIHF protein utilizing the high-resolution photo activated localization microscopy. Genome-wide methods, such as chromatin immunoprecipitation-sequencing and RNA-sequencing will provide information of the global arrangement of mIHF binding sites and enable to identify the genes possibly regulated by this protein.

Although the knowledge concerning bacterial chromosome has expanded in recent years, still little is known about the organization and dynamics of chromosomes in slow-growing, pathogenic bacteria, such as the genus *Mycobacterium*. Due to the fact that tuberculosis remains serious worldwide health problem and new multi-drug resistant strains are constantly emerging, the studies concerning NAPs, such as mIHF, are crucial to explore the new potential drug targets.