Tuberculosis continues to be one of the major healthcare problems worldwide. The 2016 WHO report states there had been 1.5 million tuberculosis related deaths and approximately 9.6 million new tuberculosis infections throughout the year 2014. The increasing incidence of multidrug resistant and totally drug resistant tuberculosis has become a significant challenge in the eradication of the bacterium from human population using currently available drugs.

Drug-resistant TB is a serious public health issue in many developing countries as its treatment takes longer and requires more expensive drugs. MDR-TB is defined as resistance to the two most effective first-line TB drugs: rifampicin and isoniazid. Extensively drug-resistant TB (XDR-TB) is also resistant to three or more six classes second-line drugs. Even worse is the totally drug-resistant TB (TDR-TB) which is resistant to all currently known anti-TB drugs. Both XDR-TB and TDR-TB are extremely difficult to cure, as they do not respond to the standard six-month treatment. The length of therapy can exceed two years and requires application of expensive and toxic drugs.

Future generation antituberculosis drugs in order to be useful in the treatment of at least MDR-TB/XDR-TB should have a good safety profile, higher potency than existing drugs, a shorter required duration of therapy, effectiveness in treating MDR and XDR strains, and no antagonistic activity against other antituberculosis drugs. An antibacterial enzyme target should be essential for the microorganism and not present in the host. The proper identification and experimental validation of the mode of action is the first step in the choice of a new drug target. Several approaches can be used to identify a potential drug target such as gene expression profiling, activity based protein profiling (ABPP), pathway or phenotype analysis, or drug databases. Optimally, a drug target should be a protein, peptide or nucleic acid possessing “druggable” features, with potential to bind or interact with small molecules, antibodies or recombinant proteins. A good target should be essential for growth, survival as well as for pathogenesis, and its homologues should not be present in the host. The bacteria should be sensitive even to a slight decrease of the target protein molecule. Optimally, mutations in the bacterial genome should not compensate for the depletion of the target molecule.

The main goal of this project is a comprehensive evaluation of PAP I, poly(A) polymerase and PNP bifunctional protein displaying guanosine pentaphosphate synthetase and polynucleotide phosphorylase activities of tubercle bacilli as targets for new antimycobacterial drugs.

This aim will be accomplished by (i) verifying the essentiality of PAP I and PNP in tubercle bacilli, (ii) engineering of conditional mutants, (iii) determination of minimal PAP I and PNPase protein levels required for normal growth of tubercle bacilli, (iv) developing of in vitro enzymatic assays to test putative inhibitors of PAP I and PNPase.

The secondary goal of this project is to characterize in details the role of poly(A) polymerase and PNPase in DNA repair pathways in mycobacteria. This aim will be accomplished by (i) transcriptional profiling and proteome analysis of wild type and PAP I or PNP-depleted *Mtb* strains growing under various conditions including intracellular infection of human macrophages, (ii) testing sensitivity to various DNA damaging agents, (iii) in vitro and in vivo determination of PAP I and PNP interactions with other DNA repair proteins, various DNA and RNA substrates as well as identification of their enzymatic activities.

The project will provide a detailed description of PAP I and PNP as the putative drug targets in tubercle bacilli. Moreover it will be the first comprehensive characterization of an enzymatic and functional analysis of poly(A) polymerase in an important bacterial human pathogen. Additionally, for the first time PAP I and PNP will be validated both in vitro and in vivo in respect to their nucleotidyltransferase activities that have been proposed to be an important in DNA repair as well as RNA stability in mycobacteria.