According to World Health Organization (WHO), *Mycobacterium tuberculosis* (tubercle bacillus), a human pathogen and the causative agent of tuberculosis (TB), claimed ~1.7mln lives worldwide. TB has been diagnosed in about 10 mlns of people, including 1mln children. Current TB treatment requires demanding therapy with a combination of at least four drugs taken for several months. The increasing prevalence of drug resistant and multidrug resistant TB is worrying. According to WHO, urgent interventions are needed to improve the coverage and quality of diagnosis, treatment and care for people with drug-resistant TB. **Emerging drug and multi-drug resistance amongst *M. tuberculosis* strains must be counteracted with drug discovery.**

Pyrazinamide (PZA) is the first-line anti-tuberculosis drug that has a unique ability to target and kill mycobacteria in a metabolically silent "persister" state. This has historically resulted in shortening the duration of anti-tuberculosis chemotherapy from previous 9 to current 6 months. PZA was previously found to inhibit the quiescent malaria parasite as well as being effective against *Escherichia coli* persisters. PZA is a prodrug, hydrolyzed intracellularly to pyrazinoic acid (POA) by pyrazinamidase PncA, an enzyme frequently mutating in PZA-resistant strains, making it impossible to use PZA as an autonomous antibiotic. Due to its bacteriocidal activity towards metabolically silent bacteria, PZA is considered the only indispensable first-line drug used for TB treatment in current and future regimens. The mode of bactericidal activity of this compound in *M. tuberculosis* have remained elusive on a molecular level. According to the scientific reports, mutations conferring resistance to pyrazinamide are found in proteins forming essential protein complexes. PZA/POA interferes with: the ribosome – by inhibiting the activity of RpsA protein, the RNA degradosome – by inhibiting the activity of PNPase, and with the fatty acid synthesis complex – by disturbing the interactions between FAS1-FabH-FAS2 systems. The project's PI has been recently involved in reporting, for the first time, the composition of the RNA degradosome protein complex [Plocinski et al. Nucleic Acids Research, 2019]. **The current project is set to reveal the molecular target of pyrazinamide, responsible for its bactericidal activities.** By exploiting a large number of advanced techniques from the field of biochemistry, structural biology (protein crystallography and cryo- Electron Microscopy), molecular modeling and synthesis of POA derivatives; **we are going to search for novel compounds inhibiting activities of the key bacterial enzymes e.g. PNPase. We are also going to implement high-throughput screening of commercially available libraries of compounds, as a source of alternative inhibitors of studied here processes.** The proposal will evaluate potency and usefulness of new PZA/POA derivatives as anti-tuberculosis drugs. On the other hand, **the proposal will address such ill-defined areas of basic research as the interplay between the RNA degradosome and ribosomes, and the involvement of both protein complexes in the process of trans-translation – an essential yet underexplored process,** necessary for error correction during protein synthesis.