AMR-TB Goal and objectives

Tuberculosis (TB) is a global pandemic disease, one of the top 10 causes of deaths worldwide. Almost one third of the world's population is latent carriers of TB bacteria. Although TB is usually curable, its modern strains often exhibit multi-drug resistance which makes TB a lethal threat; the number of such cases constantly grows (currently estimated by the World Health Organization (WHO) as 480 000 people worldwide and counting). To overcome this challenge, the unified effort of specialists in different branches of fundamental and clinical sciences is required.

Antimicrobial resistance (AMR), the ability of microorganisms to overcome almost all of the antimicrobial treatments that we currently have, has been identified as one of the main challenges facing the 21st century, and it has become a critical problem across the globe, including in the developed world. Unless we step up our research efforts and find new approaches to deal with these bugs, it might not be long before we will find ourselves in a situation similar to times before the development of the penicillins, where simple infections turn out to have deadly consequences.

Isoniazid (INH) is the main and historically one of the first drugs used for TB treatment. It is known that INH enters the bacterium cell membrane by passive diffusion and then activates KatG (catalase-peroxidase enzyme) which results in a range of reactive species or radicals and isonicotinic acid. These species attack multiple targets, including mycolic acid synthesis, lipid peroxidation, DNA, and NAD metabolism. Mutations in KatG lead to INH resistance. However, the molecular mechanism of interactions between INH and KatG is still unclear and the mechanism of metabolic transformation of INH to isonicotinic acid is not fully understood. It is also known that the minimal inhibition concentration (MIC) of INH is positively correlated with the remoteness of the mutant aminoacid from the active centre of the catalase. [1-3]. Insufficient understanding of biomolecular mechanisms that are responsible for the therapeutic properties of isoniazid is a major fundamental obstacle preventing effective development of drugs capable of overcoming antimicrobial resistance of TB bacteria.

Our goal is to understand the mechanism of the interaction between the catalase KatG and INH and the cascade of biochemical processes resulting in the bacterial death. We will investigate how mutations in KatG influence the stacking in the active centre of enzyme and drug metabolism. Using this knowledge we will suggest possible modifications of the drug for treating tuberculosis.

Mutations of TB bacterium under INH treatment will be investigated by developing the theoretical models of INH-KatG reaction, metabolism of INH in bacterium cell, and comparison with obtained experimental data. Usually bacteriologists concentrate either on the fundamental microbiology of TB strains or on clinical treatment of the disease. The strength of our collaboration is in unique combination of both: clinical-experimental and theoretical investigations. Such multiscale approach involving micro- and macro-models of the system has never been completed in application to realistic bacteria and its mutants. Nevertheless, the time is ripe for building such models as multiscale modelling is a very actively developing area of research in various fields of physics, chemistry, and biology. This will be particularly beneficial for the antimicrobial resistance problematics as it requires paradigm changing new ideas since the traditional ways of developing new antibiotics struggle with producing new drugs quickly enough to overcome the resistance acquired by the bacteria.

The specific **objectives of the project** consist of the following.

1. The determination of minimal inhibition concentration of INH to 100 strains of *M. tuberculosis* with a wide spectrum of drug resistance (WP2).
2. Genome sequencing of the 100 strains obtained in objective 1 (WP1).
3. The molecular genetics and recombinant protein production of wildtype and mutant KatG orthologues based on the data obtained in objective 1 and 2 (WP1, WP3, WP4).
4. The investigation of enzymatic activity of KatG for various strains obtained from patients with AMR to INH (determined in objective 3) (WP2).
5. Using results of objective 4, the investigation of the catalase structure and its mutants and the creation of the genotype-phenotype map and fitness landscape of the bacterial strains (WP1).
6. Mathematical modelling of the metabolic pathway of INH transformation in the bacterium (normal and mutant) elucidated in objective 4, the prediction of the metabolic pathway at different mutations (WP3).
7. High performance molecular dynamics simulations of the interaction of INH and catalase based on the molecular structures obtained in objective 5 (WP1, WP2).
8. Relative fitness measurements of all strains sequenced in objective 2, correlation of results with objective 4 (WP4).
9. Studying the native dynamics of catalase using normal mode analysis for deducing the mechanisms of INH-KatG interaction, complementing and making more accurate the results of objectives 5 and 7 (WP2).
10. Based on the results of objectives 4-6 and 8-9 suggesting modifications of INH capable of overcoming AMR for treating TB (WP1-WP4).