The project's goal is to demonstrate that the uptake of host-derived vitamin B12 is required for *Mycobacterium tuberculosis* to maintain the synthesis of envelope lipids that are essential for survival within the host cell and thus can be exploited in designing *"Trojan horse"* conjugates in which B12 will act as molecular carrier improving antitubercular compound delivery through the tubercle bacilli cell envelope.

Until the SARS-CoV-2 pandemic tuberculosis, the disease caused by the bacillus *Mycobacterium tuberculosis*, was the main cause of death from a single infectious agent worldwide. The global increase in the frequency of extensively drug-resistant cases of tuberculosis constantly reminds us of the need for research on *M. tuberculosis* metabolism to find new weak points with the potential to be exploited in anti-TB drug design. *M. tuberculosis* is a facultative intracellular pathogen of macrophages and other immune cells. This created at least two hurdles in the path of anti-TB drug penetration from blood to its intrabacterial target. First is a highly impermeable mycobacterial cell envelope, and second is a variety of host cell types, tissues, and lesions to travel before reaching the pathogen. Therefore along with designing new anti-TB compounds, new ways of drug delivery should be explored to increase effective dose, prevent drug-tolerance, and shorten the treatment. Among them "Trojan horse" conjugation has emerged recently as a promising class of novel modification of antibiotics. According to the mythological origin of the term, in this method, an antibiotic is conjugated with a targeting moiety that is usually an essential nutrient for bacterial survival, which bacterium has to take up from the growth environment and bind to specific receptors on the bacterial outer membranes to mediate cargo delivery by specific bacterial uptake systems. Whereas traditional antibiotics primarily rely on nonspecific passive diffusion to enter bacteria following a concentration gradient, "Trojan horse" conjugates gain specific bacterial entry through active transport, which results in intracellular accumulation of the conjugates against their concentration gradient, selective antimicrobial activities among different bacteria, and reduced human toxicity.

In our project, we would like to explore the potential of vitamin B12 (cobalamin, Cbl) as a carrier for antimycobacterial compounds. Initially, in our preliminary study, we demonstrated *M. tuberculosis* inability to synthesize endogenous B12 and sole dependence on exogenous B12 uptake which represents the „Achilles' heel" of tubercle bacillus metabolism within the host and is the primary condition considering its use as a „Trojan horse" carrier. The uptake of vitamin B12 is required for *M. tuberculosis* long-term survival within the host which is the second, necessary condition however, the metabolic mechanism by which B12 contributes to the pathogenesis of tuberculosis remains poorly understood. Based on our preliminary data we hypothesize that vitamin B12 may be the key regulator of *M. tuberculosis* cell wall virulence lipids synthesis, which allows tubercle bacilli to survive in and escape from the host phagocyte.

Therefore, in the first part of the project, by the use of the whole mRNA sequencing (RNA-Seq) of *M. tuberculosis* cultured on various carbon sources with or without vitamin B12, we would like to identify in detail all potential B12-dependent metabolic pathways of tubercle bacillus. By constructing *M. tuberculosis* mutants of B12-dependent metabolism, studying their growth in various conditions *in vitro* and within human phagocytes, and isolating and analyzing *M. tuberculosis* virulence lipids we would like to explain the metabolic mechanism of B12 requirement for tubercle bacillus lipid synthesis and long-term survival within the host.

Next, the second part of the project aims to synthesize B12 bioconjugates of currently used antitubercular drugs (Bedaquiline, Rifampin, Ethambutol, Amikacin) as well as two novel 1H-benzo[d]imidazole derivatives whose antimycobacterial activity was demonstrated in our previous studies and verify *in vitro* and within the host macrophages whether conjugation with vitamin B12 improves their activity against *M. tuberculosis*. By the use of antibiotics of various chemical properties and structures, we would like to verify what antibiotic's physical, chemical, and structural properties are more prone to use in conjugation with B12 that will be beneficial concerning drug penetration into mycobacterial cell. The activity of the synthesized bioconjugates will be tested *in vitro* on a laboratory strain of tubercle bacillus in various metabolic conditions in relation to the effect of non-conjugated drug molecules. After cytotoxicity tests, the action of selected promising B12-antibiotic conjugates will be next tested on tubercle bacilli residing inside human phagocytes. Considering the very limited number of effective drugs used in tuberculosis treatment, improving drug transport using drug carriers that can transport cargoes against their concentration gradient might be, a game-changing method allowing expansion of the anti-Tb drugs assortment not only by new classes of compounds but also by existing antibiotics that are not used in therapy due to low penetration of tubercle bacillus envelope, low solubility and/or the need for administering in high doses that posed a danger of serious side effects.