

Antibiotic resistance becomes an increasing danger of the contemporary world. The World Health Organization reports that if no actions are taken, by 2050 as many as 10 million deaths may be caused by bacterial infections. Moreover, around the world, there are already many pathogens that are difficult to eradicate due to their natural resistance to existing antibiotics. The presence of current drugs side effects is also a serious problem. Therefore, it is of extreme importance to look for new effective antibacterials that at the same time would be safe for humans. The project offers a universal, specifically targeted weapon against pathogens. Simultaneously, the proposed antibacterial would not be harmful for a host organism and its symbiotic microbiome. Accordingly, the objective of the project is the development of a new approach to fight bacteria and tackle antibiotic resistance. This will be achieved by targeting pathogenic microorganisms using RNA enzymes (ribozymes) that are able to recognize and cleave other RNA molecules (substrates) highly specifically. Cleavage of a specific messenger RNA (mRNA) that contains a template for synthesis of a protein essential for bacteria life should inhibit the growth of pathogens.

This project assumes an interdisciplinary approach to solve the set problem. Theoretical bioinformatic tasks will consist of: 1) analysis and alignment of selected mRNA sequences from the tested strains of *Escherichia coli*, 2) designing the constructs containing structures of ribozymes, 3) computational predictions of structures of substrate:ribozyme complexes, and 4) statistical analysis. The experimental path combines methods of biochemistry, molecular biology, microbiology, and biophysics. The level of binding of substrate:ribozyme complexes will be investigated. Then, the efficiency of mRNA substrate cleavage by the designed ribozymes will be tested. RNA molecules are highly sensitive to degradation. Hence, to protect their structure against ubiquitous enzymes present in the bacterial cell, ribozymes will be hidden in more stable constructs. This special system will increase the stability of ribozymes and the effectiveness of the cleavage, which will speed-up the inhibition of bacteria growth. These results will be checked at two levels: 1) from the outside, by monitoring the killing rate, and 2) from inside, by measuring mRNA substrate level after cleavage. Since the ribozyme will act as an antibiotic, bacteria might form defense mechanisms to survive, and generate mutations in the mRNA sequence. Hence, a formation of potential resistant mutants will also be studied.

The project objective is a brand-new approach to fight against antibiotic-resistant bacteria, so it will put great revelatory value into science. Aside from obtaining an innovative, universal and specific potential way to deal with resistant bacteria, the results of the project will provide insights into bacterial response to the presence of ribozyme in their cells and mechanism of formation of potential mutations, which can provide the basis and a good starting point for future similar research.