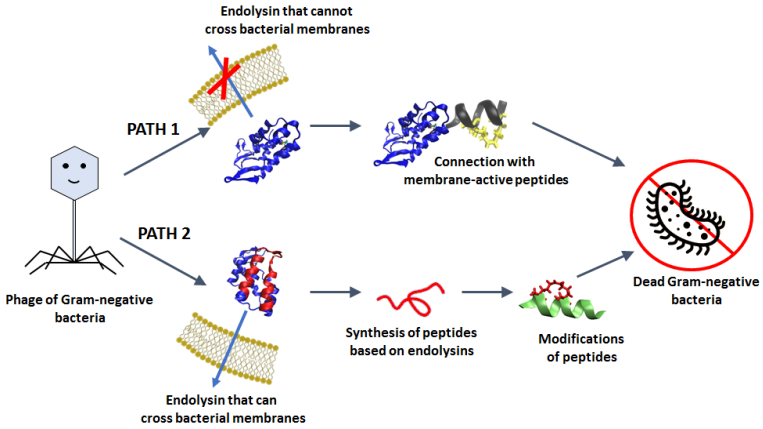
Combating antibiotic-resistant pathogens has become one of the greatest challenges facing researchers in the modern world. Overuse of antibiotics has caused a sharp increase in antibiotic resistance, especially now, during and after the COVID-19 outbreak. As a result, it is essential to develop new effective antibacterial drugs against multidrug-resistant strains of bacteria. It has become a priority to find new options to combat Gram-negative bacteria, which have been recognized by the World Health Organization as a serious threat to human health and life.

Bacteriophage-derived endolysins have been proposed as an alternative antimicrobial therapy. These are enzymes that work by breaking bonds within peptidoglycan, one of the main components of the bacterial cell wall. Many studies involving these enzymes are currently underway. While the better-developed examples of therapeutic endolysins are those directed against Gram-positive bacteria, Gram-negative bacteria were once thought to be resistant to the way endolysins work. The assumption of Gram-negative pathogens insensitivity to endolysins was due to the presence of an outer membrane in their cell walls. Such a membrane acts as a natural barrier, preventing endolysins from reaching the peptidoglycan from outside the cell. This old paradigm has been largely challenged by the introduction of modified endolysins, such as by combining them by genetic engineering methods with peptides that interact with the outer membranes of bacteria. In addition, some endolysins were found active against Gram-negative bacteria. Such endolysins contain helical peptides in their structures, with a large number of basic amino acids, which interact with the negatively charged membrane elements in Gram-negative bacteria.

This project proposes to **exploit phage endolysins as sources of novel antibacterial agents that interact with membranes**. It was planned to 1) combine, by chemical methods, endolysins incapable of passing through the outer membrane of bacteria with peptides that degrade the bacterial wall and 2) use fragments of endolysin sequences that exhibit membrane activity against Gram-negative bacteria to identify new peptides with antibacterial properties. In addition, peptide modifications are planned to enhance the membrane activity and biostability of the proposed compounds. The focus is on modifications ensuring the stability of the helices.



Scheme showing the scientific goal of the project

To design, obtain, and investigate the activity of the proposed molecules, a number of experimental techniques from organic chemistry, biochemistry, biotechnology, biophysics and microbiology will be used. It is planned to check the effect of designed molecules on bacterial membrane permeability and ability to bind to its elements. The structures of the peptides in the presence of bacterial membrane mimics (micelles, liposomes) will be studied. In addition, the stability of the obtained molecules in solutions containing natural enzymes that have the ability to degrade peptides will be checked. Most importantly, their activity against Gram-negative bacterial strains and toxicity against red blood cells and eukaryotic cells will be tested. The possibility of bacteria acquiring resistance to the proposed potential antibiotics will also be investigated. Finally, the most promising molecules will be tested on mice with bacteria-infected wounds.

This project will open up new possibilities for using phage endolysins as sources of biologically active molecules. The strategies developed in the implementation of the proposed tasks may lead to the discovery of new pathways for designing antibiotics against Gram-negative bacteria.