Increasing bacterial resistance to classical antibiotics stimulates the search for new substances with high antibacterial activity, low susceptibility to resistance and low human toxicity. At present, high hopes are being placed in the compounds structurally similar to natural nucleic acids. These are so called peptide nucleic acids (PNA). Their oligomers have the ability to bind to complementary strands of DNA or RNA so they can block the expression of the genes necessary for living bacteria, leading to inhibition of bacterial growth or death. PNAs are highly specific, so developing resistance against them would be a very difficult task for bacteria. All these qualities make PNA an antibiotic of the future, which would raise the fight against bacteria to a higher level.

Unfortunately, poor penetration of PNA into bacterial cells effectively hampers its development as an antibacterial. So far, attempts have been made to combine PNA with other compounds, such as peptides. It brought some improvements in the transport of PNA to bacteria, however insufficient to achieve high antibacterial efficacy. Therefore, we have proposed a strategy based on vitamin B_{12}, which attached to PNA, acts as a transporter of PNA to the inside of bacterial cells. Recently, we have shown that vitamin B_{12} introduces peptide nucleic acids into cells of *E. coli* and *S. typhimurium*. We do not know the route of transport used by the vitamin B_{12}-PNA conjugates. However, it seems that the most likely way of their infiltration into bacteria is via the protein transport system of vitamin B_{12}. There are premises for this, because in preliminary studies we have shown that one of the proteins forming this system called BtuB is essential for the transport of conjugates to *E. coli* cells.

The main objective of the project is to verify whether other proteins in the transport pathway of vitamin B_{12} to *E. coli*, such as BtuF, BtuC and BtuD, also participate in the transport of PNA combined with vitamin B_{12} into bacterial cells. In addition, we will investigate how the vitamin B_{12} and PNA conjugates interact with these proteins at the molecular level. Within the framework of the project, we will synthesize appropriately designed PNA sequences and their conjugates with vitamin B_{12}. To verify that the BtuF protein, as well as the BtuCD protein complex, are involved in the transport of vitamin B_{12}-PNA conjugates in *E. coli*, we will use mutant strains deprived of these proteins. These strains will also undergo transformation to obtain the red fluorescence gene, thereby gaining red fluorescence under UV light. The strains prepared in this way will be exposed to the vitamin B_{12}-PNA conjugates, which are designed to block the expression of the red fluorescence gene, which will result in a decrease in red fluorescence of the bacteria. To achieve this effect, PNA must be inside the bacterial cell. Thus, by measuring red fluorescence of bacteria after exposure to vitamin B_{12} and PNA conjugates, we will know whether PNA was introduced into bacterial cells. By comparing the fluorescence results in mutant and wild-type *E. coli* strains exposed to the vitamin B_{12}-PNA conjugates, it will be possible to infer whether the protein of which the mutant strain is devoid of is involved in the transport of these substances to bacteria. Experiments will be supplemented by theoretical studies in which we will examine the interactions between vitamin B_{12}-PNA conjugates and BtuF, BtuCD proteins at molecular level using docking techniques and atomistic molecular dynamics simulations.

Knowledge of the mechanism of transport of peptide nucleic acids using vitamin B_{12} pathway to bacterial cells will allow a deeper understanding of the proteins supporting the transport of vitamin B_{12} and their adaptability to the transport of vitamin B_{12} derivatives. This knowledge will also provide information on how to optimize the structure of the vitamin B_{12}-PNA conjugates to increase their transport efficiency which can translate into greater biological activity. This will also increase our knowledge of potential pathways for the introduction of antibacterials, such as PNA, into bacterial cells. Furthermore, the route of transport of vitamin B_{12} can be recognized as a non-invasive pathway for introducing other types of substances into bacterial cells with the use of vitamin B_{12}.