Bacteria produce a variety of proteins that must be secreted outside the cell to play their biological role. Among them, many participate in pathogenesis being harmful to the attacked organism (including humans) and causing the development of different diseases. Since Gram-negative bacteria are enveloped with two biological membranes, the secretion process requires specific and usually complex proteinaceous machineries, called secretion systems, forming the channel in the membrane. To date, eleven different types of secretion systems have been discovered and they are extensively investigated because they seem to be perfect targets for the development of antimicrobial drugs.

Bacteria belonging to the phylum Bacteroidetes inhabit almost all ecosystems on Earth. They are especially numerous in soil, oceans, and freshwater but also in the gastrointestinal tract (GIT) of animals including humans. These bacteria often play a beneficial role, helping in the assimilation of different dietary compounds that could not be otherwise metabolized by humans. However, some members of Bacteroidetes might become harmful when escaping out of the GIT, others are pathogenic by nature. Both pathogenic and beneficial Bacteroidetes employ a diverse repertoire of proteins exposed on their outer membrane (OM) to adapt to the extracellular environment. Numerous are secreted by the Type 9 Secretion System (T9SS) which is found only in this phylum. However, many of these proteins are secreted in a T9SS-independent manner and the mechanism of their secretion to the surface is completely unknown. Among them are surface lipoproteins (SLPs) – molecules of various functions and activities which are attached to the OM via lipid anchor. Since SLPs play a fundamental role in the physiology of Bacteroidetes, understanding the mechanism of their secretion is of high importance. We identified the lipoprotein secretion system (LSS) in Bacteroidetes and the main aim of this project is to characterize this system both structurally and functionally.

A pathogenic member of Bacteroidetes, *Porphyromonas gingivalis*, was a model for the identification of LSS. This bacterium produces a variety of virulence factors contributing to the development of periodontitis and associated diseases including rheumatoid arthritis, cardiovascular disease, Alzheimer's disease, and aspiration pneumonia. Fimbriae - long fibers protruding from the OM are the important virulence factors of *P. gingivalis*. They allow for adhesion to other cells, both bacterial and human, and stimulate an inflammatory response. Fimbriae are assembled from subunits secreted as lipoprotein prosubunits. Therefore, we hypothesized that the system enabling their secretion will also support the secretion of other SLPs. In our mutagenesis studies, we identified LSS in *P. gingivalis* which turned out to be widespread in other members of Bacteroidetes.

In this project, we will determine the 3D structure of LSS to understand the mechanism of secretion on the atomic level. We will also determine the function of individual components of LSS and identify new proteins involved in the biosynthesis of lipoproteins. There are many lipoproteins that play their biological function inside the cell; thus, secretion is dependent on the specific signal in their amino acid sequence. Therefore, we will determine the lipoprotein export signal (LES) targeting for secretion via LSS. Finally, based on LES and mass spectrometry (MS) analysis we will identify all SLPs in *P. gingivalis*.

The results obtained in this project will contribute to a better understanding of the fundamental process of lipoprotein biogenesis and secretion in Bacteroidetes. Since SLPs are commonly used as vaccine agents, this project will facilitate the identification of new SLPs and therefore might contribute to the discovery of promising candidates for SLP-based vaccines. Moreover, the proteins involved in bacterial lipoprotein biogenesis and secretion are unique to bacteria and do not exist in higher organisms, making this pathway an attractive target for the development of novel antimicrobials.