Human body is colonized by numerous bacteria. Some of them are beneficial, while others cause diseases. Both groups depend on their hosts to provide them with the nutrients necessary for growth. Since most nutrients cannot diffuse through membranes, bacteria produce specialized uptake systems that can bind these compounds and transport them into bacterial cells. In the case of Gram-negative bacteria, which have two membranes, that transport usually occurs in a stepwise manner and is carried out by independent systems in the inner and outer membranes. A very important nutrient for the majority of bacteria is iron, which they need for proper functioning of numerous essential proteins. However, in the human body, almost all of the iron is bound by proteins either in an ionic form or in the form of heme. Heme is also an important nutrient for some bacteria because, even though they need it, not all of them are able to produce it. Therefore, many bacteria produce protein systems that can extract iron or heme from human proteins and deliver it to bacteria.

An important example of bacteria that require heme for growth is *Porphyromonas gingivalis*. *P. gingivalis* is a Gram-negative, pathogenic bacterium responsible for the development of periodontitis, which is an inflammatory disease of tooth-supporting tissues, also associated with the onset of numerous systemic diseases, including cardiovascular diseases, aspiration pneumonia, rheumatoid arthritis and Alzheimer's disease. *P. gingivalis* can bind and lyse erythrocytes to release hemoglobin from them and it can degrade hemoglobin and other heme-binding proteins to free up heme, which can then be bound by several proteins secreted by this bacterium and delivered to specific membrane transporters for import into the cell. One of the bacterial heme-binding proteins is called IhtB. This is a soluble protein that is attached to the surface of *P. gingivalis* via a lipid anchor and thus is called a lipoprotein. IhtB may be able to remove iron from heme, which would be a unique property among known surface heme-binding proteins. We determined that IhtB forms a complex with the IhtA protein. This protein is predicted to form a barrel in the outer membrane of *P. gingivalis* to facilitate the first step of heme uptake.

The main goal of this project is the structural and functional characterization of the IhtAB complex. To this end, we will obtain the three-dimensional structure of the IhtAB complex with bound heme, which will allow us to understand the mechanism in which the heme moiety is bound and transported. Since the acquisition of heme is a crucial aspect of the functioning of *P. gingivalis*, we will compare a strain that produces IhtAB with a strain unable to produce it. We will test whether they differ in growth rate, sensitivity to oxidative stress, proteolytic activity, biofilm formation, and outer membrane vesicle production. We will also determine whether IhtB can be released into the medium for more efficient heme binding. Finally, as in the natural habitat of *P. gingivalis*, IhtB may be exposed to higher temperatures and several proteases, we will assess the suitability of IhtB for heme scavenging based on its thermal stability, sensitivity to proteolysis and oligomeric state in a heme-bound form.

The results obtained in this project will lead to a better understanding of the heme acquisition mechanisms of *P. gingivalis*, as well as other bacteria utilizing similar systems for the uptake of various nutrients. Since both membrane transporters and surface lipoproteins are excellent candidates for vaccine development and transporters are also studied for drug delivery, the results of this project may in the future contribute to the development of novel therapeutic strategies against periodontitis and related diseases.