Among periodontal diseases the most popular is periodontitis (PD). The disease is initiated when bacterial plaque accumulates on the subgingival tooth surface and causes chronic inflammatory condition. It leads to periodontal ligament degradation, alveolar bone resorption, formation of deep periodontal pockets and damage of tooth-supporting structures. Severe forms cannot be completely treated, and advanced stages of the disease are permanent. Moreover, PD has been associated with adverse pregnancy outcomes, cardiovascular disease, rheumatoid arthritis, pulmonary disease and diabetes. PD affects up to 90% of the adult world population. Thus, it has serious impact on public health and medical expenditure.

The major etiological agent of PD is a bacterial consortium formed by *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola.* Considered as the keystone pathogen is *P. gingivalis*, which produces a large number of virulence factors responsible for disease formation and progression. The most important and the best known are proteolytic enzymes (called proteases), gingipains, responsible for initial colonization, acquisition of nutrients and evasion of host defense.

Some studies revealed the presence of the other putative proteolytic enzymes genes in *P. gingivalis* genome. One of them is a functional protease, designated Tpr, described initially by us in a previous study. This enzyme is potential important virulence factor which plays a key role in host-pathogen interactions, and is thus an attractive target for further studies. We showed that Tpr degrades several physiologically relevant substrates such as fibrinogen and fibronectin which are abundant in gingival crevicular fluid, as well as key elements of the innate immune system. Therefore, we hypothesize that Tpr may be involved in *P. gingivalis* virulence, not only via nutrient generation, but also via evasion of host defense.

Interestingly, its distant amino acids sequence identity suggests that Tpr may belong to the calpain family of cysteine proteases, which is unusual in prokaryotes. Tpr autoprocessing and activity is dependent on the calcium presence. We also investigated the possible inhibition of the Tpr by human cysteine protease inhibitors, cystatins. Surprisingly, we were able to observe efficient inhibitory effect by cystatins A, C and D. It is the first case of inhibition of bacterial protease by the cystatins. It is definitely worthwhile to verify the mechanism of this unusual interaction, especially given that such natural protease inhibitors may provide directed treatment against proteolytic enzymes for efficient periodontitis treatment.

An important milestone in the characterization of any protein, and in particular enzymes is the determination of the protein's 3D structure. In our project, we plan to perform detailed structural studies to elucidate mechanisms of calcium-dependent autoprocessing and enzymatic activity of Tpr at the atomic level. Furthermore, it is worth to investigate interactions between human-derived natural cysteine protease inhibitors, cystatins, with bacterial calpain-like protease Tpr as it is a very novel, previously undescribed aspect of the potential role of cystatins in maintaining homeostasis in the periodontium and oral cavity. Therefore, we will perform kinetic and enzymatic studies to describe nature of this interaction.

To sum up, we will thoroughly examine the Tpr structure, substrate specificity, and in particular inhibition by cystatins. The groundbreaking character of this project rests in the fact that very little is known about others than gingipains proteases of *P. gingivalis*. All results will expand our knowledge of the *P. gingivalis* virulence factors and pathogenicity. As gingipains are the major virulence factors of *P. gingivalis*, we believe that characterization of another protease can lead to new therapeutic approaches through blocking overall proteolytic activity of this periodontal pathogen.