Porphyromonas gingivalis, black-pigmented (due to heme accumulation) Gram-negative anaerobic bacterium plays a key role in initiation and progression of a severe form of gum disease, chronic periodontitis. The bacterium is armed in an array of virulence factors used to corrupt the host immune system. In this way *P. gingivalis* colonizing the tooth surface below the gum line creates an environment supporting the growth of other bacteria, which turns into pathogens under bad influence of *P. gingivalis*. To accomplish this mission *P. gingivalis* needs to sense environmental clues and respond appropriately. To this end, like any other bacteria *P. gingivalis* is using two component systems (TCS), composed of a receptor protein with a histidine kinase (HK) domain in the cytoplasmic membrane and a second protein in cytoplasm, referred to as response regulator (RR). A signal received by HK leads to autophosphorylation and then a phosphate group is transferred onto a conserved Asp residue on RR, which dimerizes and binds DNA thus affecting gene expression. In this project we aim to investigate the TCS of *P. gingivalis* called PorXY. In yet unknown manner this system regulates secretion and post-translational modifications of circa 30 proteins, including all known *P. gingivalis* proteinaceous virulence factors, transported to the bacterial cell surface by Type IX Secretion System (T9SS). In many respect PorXY is unique among the bacterial TCS. Whilst PorY seems to be canonical HK, PorX shows unique features distinguishing it from the RR described to date. PorX does not possess a DNA-binding domain and instead of directly regulating genes expression, it interacts with SigP, the extracytoplasmic function (ECF) sigma factor, which binds to the promoter regions of T9SS genes. PorX also interacts with one of structural elements of T9SS, an inner membrane protein (IM) named PorL, however the role of this interaction is unknown. The C-terminal domain of PorX was annotated as an alkaline phosphatase belonging to the PglZ family. However, in our preliminary research we have shown unambiguously that PorX does not possess alkaline phosphatase activity but, instead, it has phosphodiesterase activity and cleaves 5′-phosphoguanylyl-(3′,5′)-guanosine (pGpG), one of the least known signal nucleotide in bacteria. Deletion of the *porX* gene leads to white phenotype of *P. gingivalis* colonies, which is an effect of impaired heme acquisition due to hindered secretion of hemoglobin degrading enzymes (gingipains) and heme-binding proteins by T9SS. With diffracting crystals of dimeric PorX we are about to solve the atomic structure of PorX. We also purified all auxiliary proteins interacting with PorX (SigP, PorL, PorY) as recombinant proteins in the amount and quality required for functional and structural studies.

Based on the solid preliminary results in this project we will: (*i*) determine the substrate specificity of PorX; (*ii*) elucidate catalytic mechanism of PorX activity; (*iii*) investigate the role of PorX in regulation of expression of T9SS components; (*iv*) examine the influence of PorX phosphorylation on the phosphodiesterase activity and interactions with auxiliary proteins; (*v*) identify cytoplasmic proteins, which interact with PorX; and (*vi*) determine structures of PorX complexes with SigP, PorL and PorY. The results of these studies will shed new light on the mechanisms regulating *P. gingivalis* virulence. This may facilitate the future development of novel, more effective methods of treatment and/or prevention of periodontitis and systemic diseases associated with periodontitis.