**Abstract for the general public**

Constantly growing antibiotic resistance of bacteria constitutes a non-trivial challenge for the scientific community nowadays and was defined by the World Health Organization as one of the major threats to human health around the world. Interestingly, bacterial cells are known to communicate with each other using organic signaling molecules what is considered as a potential treatment target representing microorganisms Achilles' heel. This communication was shown to play a crucial role in regulation of essential processes in bacteria, hence its interference represents promising research direction to overcome antibiotic resistance and biofilm formation. Such disruption of cell-to-cell communication, called quorum quenching (QQ), can be achieved by enzymatic degradation of signaling molecules and represents the topic of this proposal. By now, four catalytic groups of enzymes have been shown to act on bacterial signaling molecules including: lactamases, reductases, cytochrome oxidases and acylases. Among those, lactonases and acylases constitute the most promising ones from the perspective of harnessing their QQ potential, with preference towards acylases due to their irreversible mode of action in contrast to lactonases, higher specificity and final product being easier to metabolize. Nevertheless, their narrow specificity and limited robustness of other QQ enzymes for large scale industrial and medical utilization motivates the search of other templates for protein engineering.

Hence, the main focus of the project is to propose catalytically improved enzymes with QQ potential as an alternative to conventional antibiotic therapies or agents for industrial utilization to combat biofilms formation. The study will be conducted on prototypical QQ enzyme – *Pseudomonas aeruginosa* acyl-homoserine lactone acylase (PvdQ) and biotechnologically well-established *Escherichia coli* penicillin G acylase (EcPGA). Initially, we aim to screen preselected hotspot residues based on already designed smart library of EcPGA mutations, shortlist the most promising candidates and computationally explore their preference towards wide range of bacterial signaling molecules. Further, variants with the most interesting properties will be characterized experimentally. In parallel, we aim to describe a complete catalytic cycle of PvdQ and EcPGA enzymes and its preliminary designed variant, carrying three amino acids substitutions with experimentally validated improved catalytic properties using advanced computer simulations. We hypothesize, such investigation will broaden our understanding of key determinants responsible for QQ activity of studied enzymes and will significantly contribute to overcome remaining limitations and boost rational design of new promising variants.