Bacteria survive in a highly heterogenous environment and are challenged by fluctuating biotic and abiotic factors which can generate oxidative stress. Due to that bacteria have evolved myriad mechanisms to sense and respond to stress conditions. The most well-known strategy is to change gene expression in response to environmental variation. Transcription factors or alternative sigma factors are activated under stress conditions, and bind to their target genes to activate or inhibit transcription of the regulated genes. Post-translational modifications (PTMs) of proteins also play an important role in the regulation of stress response, however their precise role in stress resistance remain largely unknown. One of the PTMs that is emerging as a significant regulatory mechanism is AMPylation. This process involves the covalent addition of an adenosine monophosphate (AMP) to a protein resulting in a modified protein with altered activity. Proteins capable of catalyzing AMPylation, termed AMPylators, are comparable to kinases. Recent discovery brings forward a new family of AMPylators – highly evolutionarily conserved pseudokinases from the selenocysteine-O family (SelO), which appear to be key players in the regulation of oxidative stress response and in the maintenace of redox homeostasis in cells. Because the physiological function of SelO and its mechanism of action is only partly understood this project will shed light on biological functions of bacterial homologue of a newly discovered SelO AMPylases, *E. coli* YdiU protein. The YdiU protein is a pseudokinase that is widely found in three kingdoms of life and is highly conserved from *E.coli* to human (50% in full-length sequence identity). Over 15,000 proteins contain a predicted YdiU. However, the *in vivo* and *in vitro* function of the YdiU has not been characterized until recent discovery of SelO AMPylase, making it one of "the top ten most wanted unknown unknows". Most species of bacteria contain a YdiU protein, and previous transcriptomic data showed an increase in YdiU during the expose to various stress conditions implying a relationship between YdiU and bacterial stress resistance. Recent studies revealed that YdiU acts as an AMPylator and transfers AMP from ATP to Ser, Thr, and Tyr residues on protein substrates. In addition to auto-AMPylation, two proteins involved in redox homeostasis were found to be potential AMPylated substrates of YdiU. Among potential substrates are SucA, the bacterial homolog of the E1 component of the α-ketoglutarate dehydrogenase complex and glutaredoxin (grx). The discovery that YdiU acts as an enzyme that mediates protein modification significantly highlight into the functions of YdiU family proteins. However it is still unknow how AMPyalation regulates the activities of substrates and whether YdiU modifies other protein substrates resulting in their function changes as part of other regulatory pathways and how these molecular changes translate into conditionally adaptive or deleterious phenotypes. The aim of this project is to expand the knowledge about the SelO signalling pathway in *E. coli*, by making use of an unexpected instability of the *E. coli* ydiU mutant, which leads to the emergence of suppressory mutations, compensating the lack of functional YdiU protein in a cells. Identification of mutated genes implies their connection with cellular energy metabolism or response to stress, or with membrane biogenesis. The latter may be associated with so called functional membrane microdomains (FMMs), which resemble eukaryotic lipid rafts and whose role in membrane functions in bacteria has been recognized only recently. Two parallel approaches will be used in the proposed work. (i) Identification and phenotypic analysis of ydiU mutation suppressors. Detection of supressory mutations in the ydiU mutants provides a unique opportunity to identify proteins other than Grx and SucA, that may be involved in the YdiU-dependent regulatory pathway of response to oxidative stress or to find out pathways that may overcome the requirement for functional YdiU for bacterial cell fitness. The identification and characteristic of such proteins is the goal of this task (ii) Identification of possible YdiU signalling pathway components and associated proteins in the detergent resistant membrane fraction . The goal of this task is to find, in the fraction of a detergent-resistant membranes (DRMs), proteins that could be directly involved in the YdiU-signalling pathway or associated with this pathway by regulatory connections.

Because the physiological function of SelO and its mechanism of action is only partly understood this project will shed light on biological functions of bacterial homologue of a newly discovered SelO AMPylases, *E. coli* YdiU protein. This may result in the discovery and characterization of novel proteins involved in evolutionarily ancient regulatory pathways that have significant role in the response to oxidative stress. The resistance to oxidative stress is one of the major adaptations that permits bacteria to survive in changing environmental conditions while using energy from respiratory processes, and protects bacterial pathogens from harmful effects of immune cells respiratory burst at infection. Therefore, results of these studies will be important not only for understanding the mechanisms of fundamental adaptive processes in bacteria in general, but should also contribute to the better understanding of processes responsible for the adaptation of bacterial pathogens to successful infection of their hosts.