

## ABSTRACT FOR GENERAL PUBLIC

Ubiquitination is a process of protein modification, which involves attaching the small protein named ubiquitin to proteins undergoing modification (the so-called substrates). Ubiquitination is an extremely important process due to the fact that ubiquitin marks damaged, incorrectly structured or malfunctioning proteins, leading to their degradation. For the pioneering discovery of the process of controlled degradation of damaged or unnecessary cellular proteins involving the ubiquitin, three researchers Aaron Ciechanover, Avram Hershko and Irwin Rose received the Nobel Prize in Chemistry in 2004. Subsequent discoveries by researchers from many research centers worldwide have shown that the ubiquitination process also plays a key role in many other cellular processes. In turn, aberrations at any stage of this process are the cause of many diseases, including: cancers, neurodegenerative and infectious diseases as well as immunological or genetic disorders.

In addition to ubiquitin, a number of other proteins are involved in the process of ubiquitination. These are: ubiquitin activating enzymes (E1), ubiquitin conjugating enzymes (E2) and ubiquitin ligases (E3). These enzymes have the ability to regulate the activity and function of many proteins by interacting with each other. More than 30 ubiquitin conjugating enzymes and more than 600 ubiquitin ligases, which can ubiquitinate various substrates, have been described in humans. Ubiquitin ligases seem to be of particular interest in looking for new drugs, including anti-cancer drugs. An increased amount of ubiquitin ligases has been observed in various types of cancers. The main problem in scientific progress over drug development, e.g., inhibiting the activity of selected ubiquitin ligases, is the complexity of the ubiquitination process. To date, the exact biological role of many ubiquitin ligases has not been well described and their substrates have not yet been identified.

In the presented project, we plan to characterize the interaction network and the biological role of ubiquitin ligase Upf1 (Nam7) using baker's yeast *Saccharomyces cerevisiae* as a model organism. This protein has not yet been characterized in details, both in terms of its role in the ubiquitination process, as well as in other cellular processes. In humans, the disturbed function of the Upf1 protein is associated with the occurrence of some genetic diseases as well as some types of pancreatic cancers and soft tissues sarcomas. Our project focuses on the understanding of the mechanisms of Upf1 ligase activity, identification of ubiquitinated protein substrates, and the consequences of its action in ubiquitination processes, in relation to other biological processes, thus in the cell functioning.

To implement individual research tasks, we will use modern biochemical techniques based on the complementation of protein fragments, namely NanoBiT® and NubiCA, as well as standard biochemical techniques, molecular biology techniques and genetics. The optimized NanoBiT® system proposed in our project will enable the study of ubiquitination protein interactions in living cells on a large scale. The project will contribute to a better understanding of protein interaction networks and the consequences that these interactions have in the cell. On the other hand, understanding detailed molecular mechanisms and cellular functions may not only broaden the current state of knowledge, but in the long term, contribute to the development of drugs, for example, the anti-cancer drugs targeting selected ubiquitin ligases.