

Proteasome is a multi-subunit and multi-activity enzyme involved in an ubiquitin-dependent turnover of cytoplasmic and nuclear proteins. By catalysing degradation of proteins, the ubiquitin-proteasome pathway is deeply involved in regulation of cellular physiology, initiating or terminating various biochemical processes such as: differentiation, proliferation, apoptosis, gene transcription and signal transduction. It is also involved in removing of misfolded or damaged proteins, and supports the immune system by generating antigenic peptides.

A functional ubiquitin-proteasome system is essential for all eukaryotic cells and therefore any defects in its functioning have potential pathological consequences. It bears the histological significance of neurodegenerative disorders, among which are Alzheimer, Huntington and Parkinson diseases and amyotrophic lateral sclerosis (ALS). The malfunction of the proteasome activity is also connected with various cancers like lung cancer, colon cancer or myeloid leukemia. The proteasome appears thus as a very promising therapeutic target.

So far, the only proteasome-targeting agents used in clinics are two competitive inhibitors (bortezomib and carfilzomib), which have found pharmaceutical application in the fight against multiple myeloma and mantle-cell lymphoma. However, they directly block the enzyme's active site causing non-selective degradation of all proteasome substrates and triggering apoptosis.

Because inhibition of the proteasome by directly blocking its active (orthosteric) centers is not selective enough, our research group is trying to determine proteasome allosteric binding sites. Allosteric modulators of the proteasome activity may provide a more precise and substrate-specific regulation of the enzyme catalytic performance than competitive inhibitors known so far. Allosteric ligands exert their impact on the enzymes activity through long-distance conformational changes. In comparison with classic orthosteric ligands, allosteric compounds may enable not only inhibition but also stimulation of the proteasome. Such modulators may enable treatment of diseases connected with both down- and up regulation of the proteasome activity. Induction of apoptosis by synthetic proteasome inhibitors is a potential treatment strategy for cancer, whereas proteasome-activating compounds could be more effective in preventing consequences of the oxidative stress – the condition common to neurodegeneration processes.

This project inscribes to the current trends in development of efficient modulators of enzymes and receptors, which are more and more often of an allosteric type. However, the main problem in designing proteasome allosteric modulators is lack of information about the proteasome allosteric sites and this knowledge is absolutely necessary for developing new drugs, enabling to overcome civilization diseases. Our research group is trying to solve the issue by establishing the place of binding of low-molecular mass activators and inhibitors by means of X-ray crystallography. We try to find out if the proteasome binding sites are specific for the type of modulator (inhibitor/ activator) and how many allosteric signalling pathways are localized on the surface of the 20S proteasome. Defining the allosteric sites could provide sequence and structural requirements for a design of effective inhibitors and/or activators of the proteasome activity and will shed light on the mechanisms of the proteasome allosteric regulation.