In Quest of Optimal Technology to Obtain Selective Activity-Based Probes for Investigation of Proteases in Cell Death

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Proteolysis is one of the most important and ancient reactions in biology. Enzymes that catalyze this reaction are called proteases. Proteases as "good guys" perform many significant biological processes like cellular quality control, apoptosis, blood coagulation or signal transduction. However they can be also "bad guys" contributing to pathological events like cancer, diabetes, coagulopathies, inflammation, infectious or degenerative diseases. It is estimated that 5–10% of all pharmaceutical targets being pursued for drug development are proteases.

Despite significant progress in recent years, one of the biggest problems in the investigation of proteases is their similar activity and location. Due to the overlapping substrate specificity (preference in the recognition of natural amino acids) it is very hard to distinguish many major proteolytic enzymes families using chemical tools developed using classic screening technologies. This also very often limits discovery of selective drug or marker for specific activity monitoring. Classic examples here are cysteine proteases from caspase family, which play crucial role in controlling of several major metabolic pathways, especially apoptosis.

Our group has recently developed technology to obtain new types of ultrasensitive chemical tools (substrates, inhibitors, activity-based probes) for major families of medically important proteases. Using this novel, unique and very efficient technology called Hybrid Combinatorial Substrate Library (HyCoSuL) we have demonstrated that protease substrate specificity can be significantly enlarged by the use of unnatural amino acids in peptide sequence. An overview of major strategies to develop very active and selective chemical tools, which can be used for reliable investigation of activity and location of proteases in health and disease will be presented.

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