

Civilization diseases like cancer, diabetes or bacterial infections are one of the main reasons of people mortality independently on age and origins. Perfect therapy methods are still not found. Many hopes are concerned with discovery of diseases origin, in which usually participate tens or hundreds of biological macromolecules called enzymes. From this point of view one of the most important group of enzymes are proteases, which elevated or lowered level allows fast clinical diagnostics using specific markers and also gives a chance for reasonable fast drug discovery research based on protease activity. An outstanding reason for proteases investigations are available on the market drugs against cancer, diabetes and HIV, which are based on proteases activity inhibition. Unfortunately, these drugs can be used only toward limited number of diseases and many other proteases (in human there are around 650 proteases described so far) participating in different disorders in humans and other living organisms require further investigations.

Proteases are key players in development of inflammation, which is a biological process that delivers defensive cells to the unhealthy tissues. This protective response is generally manifested by the rapid recruitment of leukocytes and other blood cells from circulation to the site of injury or infection. So far multiple proteins and small molecules have been demonstrated to be involved in this process, with proteases and cytokines being among the key players in inflammation. Recent studies demonstrate that proteases act in the sophisticated network, which involve activity of many different proteolytic enzymes at the same time. Given the fact that more and more proteases are actively involved in inflammation, there is an urgent need for the development of new chemical tools, that relaying on enzymes activity could be used for an accurate monitoring of inflammation proteolytic network. In order to detect active form of protease, chemical tools called activity-based probes must be employed. Unfortunately, currently there is no technology available, which allows multiplex monitoring of many proteolytic enzymes in active form. Such visualization is beyond the capabilities of confocal microscopy, which allows for the reliable detection of up to four chemical probes in parallel. We propose completely new approach, in which we will use stable lanthanides isotopes as detection tags in activity-based probes, following by proteases visualization using recently introduced state-of the art technology, called mass cytometry. Due to minimal overlap in metal signals, currently, this technology allows monitoring of more than 40 different parameters in parallel. The ultimate goal will be to design and synthesize isotope labeled probes and their use for parallel analysis of the activity of eight proteases involved in inflammation, a goal impossible to achieve so far using any currently known technology.