

Regulated cell death is a key process during which dangerous or nonfunctional cells are removed from an organism to maintain its homeostasis. To date the best characterized type of cell death is apoptosis. This process, being immunologically silent is a suicidal cell death as a result of caspases activation. In contrast to apoptosis, pyroptosis is highly inflammatory cell death. This process is triggered by various external stimuli including viral and bacterial infection, or by microparticles such as alum, or silica. Such infections result in the cell blebbing, membrane rupture, and the leakage of the cellular content into the external environment. In pyroptosis, viruses and bacteria induce the innate immune reaction, and the microparticles can lead to multiple diseases, including life style diseases. Given that it is of special importance to dissect the molecular mechanisms underlying pyroptosis, which could be further beneficial for the development of efficient treatment.

For a long time pyroptosis was considered as uncontrolled reaction of the cells against external stimuli. While this field has progressively expanded, it was discovered that this process is controlled by inflammatory caspases (-1, -4, -5 in humans, and -1, -11 in mice), which are specifically activated upon certain stimuli. What is more, these enzymes activate cytokines, which are further secreted outside the cells to combat infection. However, it took over 20 years to discover that these cytokines are exported into extracellular environment through pore membranes formed by gasdermin D protein. Since that time the field has rapidly expanded, however there are still some outstanding issues that need to be addressed. One of the biggest question is how other proteases, namely calpains and cathepsins contribute to this process. Currently some published results regarding their role in pyroptosis seem to be conflicting.

An ultimate goal of this project is to understand the true contribution of calpains and cysteine cathepsins to pyroptosis, especially their role in the activation/inactivation of gasdermin D and their proteolytic cross talk with inflammatory caspases. Demonstrating that cathepsins or calpains can cleave gasdermin D and directly trigger cell death would provide a completely new insight into this process. The unique aspect of this project is to use selective, small molecule fluorogenic substrates with aggregation induced emission (AIE) characteristic for the real-time imaging of selected proteases` activity in pyroptotic cells. These substrates can help to reveal which proteases are activated at the first place and how do they affect the activation of other enzymes. This kinetic cross-talk will be useful to determine the pyroptotic mechanism, which is absolutely required for the development of new therapeutics for inflammation-related diseases.