Human organism possesses variety of mechanisms of prevention from infections or tumor cells. The most frequent are immune cells, like cytotoxic T lymphocytes, “natural killer” cells and neutrophils equipped with cytotoxic granules. These granules contain proteolytic enzymes which hydrolyze a set of internal substrates in cells to be eliminated. One of the enzymes involved in this process are granzymes with GrA as one of the most important granzyme. In this dissertation a set of chemical tools for GrA is designed, synthesized and used in enzyme activity determination in cells.

In this study in order to obtain an enzyme, a method of recombinant GrA protein expression in HEK293 FreeStyle cells was optimized. Next, substrate specificity of GrA at S4-S1 pockets was determined with well established HyCoSuL approach as well as defined substrate library with unnatural amino acids. Incorporation of these two methods allowed to choose the most favorable amino acid residues for investigated enzyme pockets and synthesize specific compounds for GrA: substrates, inhibitor and fluorescent probe. The design and synthesis of internally quenched fluorescent substrates that also includes residues accommodating in prime pockets led to obtain enzyme-activable chemical reagent for future GrA investigation. Methods such as SDS-PAGE and confocal microscopy were used to establish optimal enzyme-probe bonding conditions and to determine the localization of the protease in cells. Active GrA was detected in lysates and living cells of NK92 cell line and human neutrophils indicating that the first fluorescent probe for GrA can be applied in the cell-based research. Internally quenched fluorescent probe was used to investigate the active enzyme within neutrophils. Additionally, the exact localization of GrA in neutrophil granules was observed. The possible role of GrA in two types of cell deaths: netosis and pyroptosis was presented. In both cases, the relocalization of the protease from granules to nuclear region suggested its contribution in those models of cell death. The increased activity of GrA can be associated with the hydrolysis of such proteins as histones and lamins, associated with the induction of chromatin decondensation, as well as gasdermins and pro-interleukin-1β in the initial phase of pyroptosis.