The outbreak of the COVID-19 pandemic and the continued increase in the number of new coronavirus variants demonstrate the importance of developing new, effective antiviral drugs. Two proteins encoded by the SARS-CoV-2 virus – M^{pro} and PL^{pro} - represent effective targets for antiviral therapy. Their activity is essential for virus replication and infection of human cells. Inhibition of the activity of these two proteins leads to inhibition of viral infection. Many methods have been applied to identify potential compounds that inhibit the activity of viral proteins (known as inhibitors). Significant progress has been made in the development of M^{pro} inhibitors - a compound designed by Pfizer, Paxlovid, has been approved as a drug. PL^{pro} represents a more challenging drug target for researchers. This difficulty is due to the substrate preference of SARS-CoV-2 PL^{pro} and its functions. This enzyme blocks the body's defense mechanisms against this pathogen, allowing the virus to successfully invade host cells. To date, only a few compounds have been identified as potential inhibitors of PL^{pro} in cellular assays. The main limiting factor in conducting tests on virus-infected cells is the biosafety level requirements of the laboratory where such tests are performed (BSL-3), which significantly limits the number of compounds that can be tested. However, assays performed with recombinant proteins do not reflect the conditions in the cell, which means that the inhibitors identified in these types of assays are very often ineffective in cellular assays. To address this obstacle, the purpose of this project is to develop a cell-based assay that allows assessing SARS-CoV-2 PL^{pro} inhibitory potency in the BSL-2 settings. To achieve this goal, an activity-based probe will be designed. This compound should selectively bind to the PL^{pro} and enable detection of this complex. The structure of the probe will be based on a protein recognized by the PL^{pro} - ubiquitin.

The implementation of the above research goal will involve modification of Ub C-terminal motif. To select amino acids that can be incorporated into C-terminus, PL^{pro} substrate preferences will be determined. The activity and selectivity of the designed activity-based probe will be evaluated using purified recombinant enzymes and cell-based assays. In the final step of the research, a cellular assay will be developed to measure PL^{pro} protease inhibitor activity using an engineered Ub-based probe.

The developed cellular assay to measure intracellular inhibitory activity of SARS-CoV-2 PL^{pro} inhibitors will allow the elimination of compounds that are toxic or membrane impermeable. Its utilization should significantly accelerate the antiviral drug development efforts. Furthermore, the designed probe can be used to measure the activity of PL^{pro} protease in SARS-CoV-2 infected cells, allowing a more accurate understanding of its function.