

Matriptase is an enzyme that cleaves bonds in proteins (peptide bonds), in a process called hydrolysis. This enzyme is anchored in the membrane of some cells, where it performs its functions, and its physiological activity is regulated by molecules called endogenous inhibitors. This enzyme plays a key role in maintaining tissue integrity and is important in the normal functioning of the skin. On the other hand, increased matriptase activity has been observed in some cancers, so the enzyme is investigated as a potential therapeutic target. Despite years of research, there are still many unanswered questions about the biology of this enzyme.

Since matriptase plays its function in the ACTIVE form, it is critical to study its activity, rather than expression level. Existing chemical tools to investigate active matriptase are not selective and cross-react with other enzymes, such as hepsin, so their utility is limited. Therefore, one of the main goals of our research is to develop a selective inhibitor and chemical marker for study of ACTIVE matriptase. Inhibitor will reduce matriptase activity, while chemical probe will label matriptase and enable observation of the enzyme in cells under a confocal microscope. This probe will be equipped with a fluorescent tag responsible for the illumination of the enzyme upon binding to the probe.

In our work, we will focus on the study of macrocyclic compounds, which are large molecules with a cyclic structure. These compounds show great advantages over linear derivatives due to their increased stability and selectivity. Unfortunately, it is difficult to obtain cyclic inhibitors, because they break down in synthesis conditions. Hence, there is a lack of tools for rapid selection of leading sequences for macrocyclic compounds for target enzyme. To address this problem, in this project, we propose to develop an innovative library of macrocyclic substrates for high-throughput screening to facilitate the selection of leading sequences for inhibitors and probes for proteases, including matriptase. These substrates, if recognized by the enzyme, will emit fluorescence that will be proportional to the hydrolysis/cutting rate. With this, we will select the optimal structure that will be converted to the desired molecules for matriptase studies.

Our probe may find applications for early diagnosis and monitoring treatment of diseases caused by matriptase activity, but also our study may facilitate a search for leading sequences in drug development. Since high levels of matriptase are associated with cancer progression our study may be helpful for searching tools for early diagnosis and precise tumor resection.

In this project, we will for the first time synthesize a library of macrocyclic substrates, optimize the structure of macrocyclic compounds for the study of matriptase activity, and test their use in biological assays, including study on selected cancer cell lines.

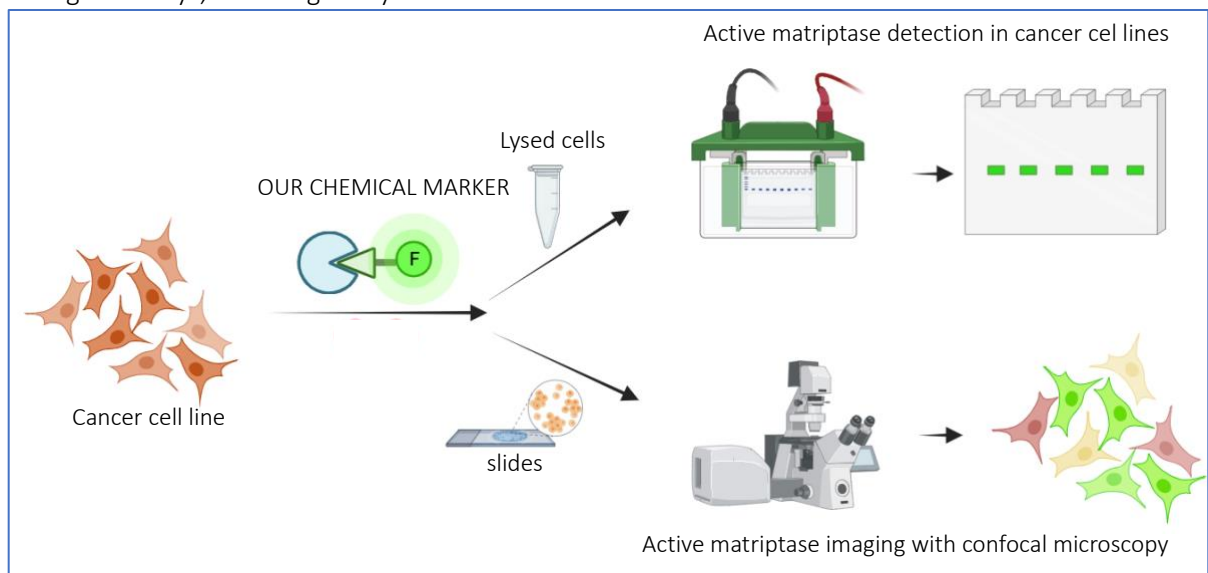


Figure 1: Schematic plan of the study