## Abstract

This dissertation thesis focuses on creating tools for the analysis and potential therapeutic intervention in the biological processes regulated by proteolysis. I focus on two important proteolytic enzymes: HIV-1 protease, which is indispensable for the polyprotein processing of the nascent virus and thus for the development of infectious viral particle, and glutamate carboxypeptidase II, a tumor marker and a neuropeptidase from the prostate and central nervous system.

Rational design of inhibitors of these therapeutically relevant enzymes serves two purposes: firstly, protease inhibitors were shown to be powerful drugs (HIV protease is in fact **the** example of successful drug development driven by structural biology). Secondly, and in the context of this thesis perhaps more importantly, inhibitors of medicinally relevant proteases might serve as tools for the elucidation of basic biological questions concerning regulation, timing and spatiotemporal control of such key processes as virus maturation or cancer development. The experimental work described in this thesis summarizes my results in both these areas.

*Human Immunodeficiency Virus Protease*

Human immunodeficiency virus (HIV), a causative agent of AIDS, has been estimated to kill close to 40 million people during the past four decades with 1.5 million dying the last year only. 35 million more are living with the infection and the disease is spreading with increasing speed in the less developed regions such as sub-Saharan Africa.

Because of its deadliness, during the early 90s of last century, with vaccination nowhere close to completion, scientific community along with pharmaceutical industrial waged unprecedented war against this disease. Their combined effort led to a clinical approval of more than thirty antiretrovirotics and gave rise to a so called highly active antiretroviral therapy (HAART) which dramatically improves the patients` lives as well as their life expectancy.

Among the primary targets chosen for combating HIV is one of the vital enzymes – HIV protease (HIV PR). This small homodimeric aspartyl protease became one of the most studied enzymes in the world. HIV PR plays a crucial role in the viral lifecycle by cleaving the polyproteins into the functional units and its inhibition hinders the viral maturation, making the particles non-infectious. Even thought HIV PR is well understood on the biochemical level – structure and enzymatic activity – its in-depth role in the biological process of viral

maturation is not well established. Our knowledge suggests that the cleavage must be strictly regulated, both in time and place, however how this is done, is not fully determined. Ever emerging resistances of HIV PR to the clinical drugs as well as not fully understood nature of the polyprotein processing makes it even now, more than 25 years after its discovery, an attractive target for many studies.

*Glutamate carboxypeptidase II*

Unlike AIDS a prostate carcinoma (PCa) causes more havoc in the developed world than in the third world countries. This pathological condition is the leading cause of deaths among all cancerous diseases in men with over 300,000 deaths reported annually. The most common treatment for PCa is a combination of tumor resection with chemotherapy. Systemic chemotherapy is by nature highly non-specific, targeting all dividing cells, and as such causes number of side effects and it is very often carcinogenic itself. To circumvent these issues scientists have been trying for more than quarter of a century to develop therapeutics that would be specifically directed to the uncontrollably proliferating cells. The most intensively pursued approach for a development of such a therapeutic is targeted drug delivery, i.e. attempt to find a difference between the malignant and normal cells on a protein level and exploit it.

Glutamate carboxypeptidase II could serve as such a potential target, since it is heavily overexpressed in PCa cells as well as on some solid tumor neovasculature. In addition GCPII is a membrane bound protease which internalizes upon ligand binding, making it an ideal candidate for targeted therapy and/or diagnostic. Strategies using both small agents, such as inhibitors, and monoclonal antibodies have been tested in the past with some of them entering late stages of clinical trials. However, the potential for further improvement is limitless and novel inhibitors with even stronger binding affinities are still needed.