

Enzymes are biomolecular catalysts that speed up the rates of biochemical reactions to make them fast enough to sustain life. Even viruses contain genetic information on how to express the enzymes crucial for catalysis of the processes leading to viral replication. Importantly, these enzymes once inhibited preclude viral infections in humans. However, to design an effective inhibitor, and further antiviral drug, we have to understand how these enzymes work, i.e., understand at the molecular level the mechanisms by which they catalyze the reactions.

To understand how the enzymes work, we need to determine their reaction rates and obtain quantitative measures such as kinetic parameters. The knowledge of kinetic parameters will help us answer the following fundamental questions: what is the enzyme's catalytic mechanism; what is the enzyme's function in biochemical cycles and its global role in the metabolism; what controls enzyme's activity and how this activity can be hindered. Therefore, due to the described crucial importance of enzymes, in this project we will investigate the biochemical reactions catalyzed by various viral enzymes.

Another crucial aspect of understanding the enzymatic reactions is that these reactions occur in cells, which apart from water, are packed with many other molecules such as proteins, nucleic acids, ribosomes, lipids, metabolites etc. Up to now, typically, enzyme kinetic parameters are determined in dilute and homogeneous solutions, which do not reflect the reality of the cell context and are far away from the physiologic conditions. So in order to estimate realistic enzyme dynamics and kinetics we have to keep in mind that cells contain about 200-400 g/L of macromolecular crowders. Thus, it seems obvious that such complex environment has to be taken into account to obtain relevant biochemical information.

That is why in this project we will determine how the *crowded environment influences the conformations and kinetics of selected viral enzymes*. We will imitate the multi-component cellular environment by crowding agents of different molecular weights, charges and concentrations and by cell lysates. We will apply both *in silico* molecular dynamics simulations and *in vitro* enzymatic assays. We believe that the combination of computer simulations and wet lab experiments will shed more light on this challenging topic.

We will research three enzymes, termed proteases that cleave peptides. One of them will be trypsin, a known digestive enzyme, which will be used as a proof-of-concept control. The other two will be viral proteases; one encoded by the human immunodeficiency virus type 1 (HIV-1), and the other one by hepatitis C virus (HCV). These viral proteases are intrinsically dynamic. Also, they are viral drug targets, and HIV-1 and HCV viruses still infect, respectively, about 40 and 170 million people worldwide. The knowledge gained as a result of this project about the structural dynamics and kinetics of these two viral proteases *in conditions mimicking cellular environments* could help design more effective inhibitors leading further to antiviral drugs.

Overall, these studies will allow us to analyze and correlate enzyme dynamics and kinetics under crowded conditions imitating cellular environment. The results of the project will enhance our understanding of the mechanism of enzymatic activity of two viral enzymes that cleave peptides. This knowledge may help in further antiviral drug design.