

Enzymes are biocatalysts that speed up chemical reactions. These reactions occur in cells, which form a crowded environment. Cells, apart from water and ions, contain many other molecules such as nucleic acids, proteins, ribosomes, lipids, and metabolites. In addition, the cellular environment is compartmentalized by phospholipid membranes. Membranes are used for cell protection, transport of compounds, and organising cells' interior space. All in all, a specific biochemical reaction occurs with background macromolecules that may occupy 30% of volume in mammalian cells. Apart from physically restricting the space for the enzyme of interest, the crowders and membranes also nonspecifically interact with the enzyme and its substrates. The confinement and interactions may affect the reaction rates as compared to dilute water conditions. In addition, such conditions also affect the binding and effectiveness of enzyme inhibitors that are used as drugs.

However, for ease of interpreting the results, in a laboratory test-tube, enzymes are often studied in dilute buffer solutions, which do not reflect the physiologic conditions and cell context. So in order to understand what controls enzymatic activity and obtain realistic reaction rates or inhibitor efficiencies, we will perform enzymatic assays accounting for complex surroundings.

Proteases are an important class of enzymes that cleave peptide bonds and are involved in many biological functions. Also viral genomes encode proteases that cleave the viral protein chain precursors after they are translated by the host cell machinery. Since these proteases are key to viral replication, they have been viable drug targets. Drugs inhibiting proteases of the human immunodeficiency virus and hepatitis C virus (HCV) have been used in antiviral combination therapies in humans. The main protease of the SARS-Cov-2 virus has been also used as a target to design inhibitors that are in clinical trials. However, to design an effective inhibitor that could become an antiviral drug, we have to understand the details of how the viral proteases catalyze the reactions in the conditions mimicking the host cells.

We will investigate the reactions catalyzed by two enzymes encoded by the HCV genome. This virus causes liver inflammation which can lead to serious liver damage and cancer. There is currently no effective vaccine against hepatitis C although it is estimated that nearly 200 mln people world wide are affected by HCV. Treatment is available but diagnosis is low because new infections are asymptomatic and the HCV mutates becoming resistant to known therapies.

One of the HCV enzymes that we will focus on is called the NS3/4A protease and is a known drug target with several drugs approved for clinical use in the last decade. The second one is the NS2 protease with no approved drugs as yet, but it is a possible target to consider. These two proteases work at the endoplasmic reticulum membranes of the host cell. **Our goal is to determine how membrane environment and macromolecular crowding affect the activity of these two hepatitis C virus proteases crucial for the viral replication.** We will also investigate how the membrane and crowded surroundings affect the efficiency of two drugs binding to NS3/4A. As membrane mimics we will use micelles, lipid vesicles and nanodiscs, which are nanoscale lipid bilayers. As crowder molecules we will use synthetic polymers such as polyethylene glycol, polysucrose, polyglucose and proteins. The efficiencies of the reactions will be monitored by various techniques such as fluorescence spectroscopy, electrophoresis, chromatography, and microcalorimetry.

Since the HCV genome constantly mutates, drug-resistant variants are a problem. Therefore, investigations of HCV proteases and understanding their reactions in various conditions will help propose new inhibitors. We hope to understand how these two HCV proteases benefit by functioning and associating with the endoplasmic reticulum membrane. We will also contribute to developing the analysis methods used to determine the reaction parameters in crowded and lipid conditions.