Human coronaviruses (CoVs) cause infections with a diverse range of courses, from common cold, to severe respiratory diseases. The recent outbreak of coronavirus disease 2019 (COVID-19) pandemic has become a global health threat. It is caused by Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2). In this interdisciplinary project, we focus on the SARS-CoV-2 papain-like protease (PLpro), a crucial viral enzyme. Enzymes are biological molecules that significantly speed up the rate of chemical reactions that take place within cells. P**roteases** are enzymes, which cut the proteins into smaller pieces (up to single amino acids). Because of its importance in processing other SARS-CoV-2 proteins and weakening the human immune response, it is an excellent target for potential drugs. PLpro is present also in other CoVs and has been studied in the case of previous epidemics. However, such enzymes from different CoVs' species exhibit considerable differences. Thus, **we aim to compare the PLpro of SARS-CoV-2 and other CoVs, especially the most related SARS-CoV**. Moreover, this protein exhibits structural similarity to human ubiquitin C-terminal hydrolase L1 (UCH-L1). UCH-L1 belongs to unique group of proteins which are knotted (meaning they form knots, such as tied on a piece of a rope). Thus, it is also of great importance to understand differences between these two enzymes (unknotted and knotted). This knowledge will be crucial for designing safe drugs, blocking only viral proteins, while not affecting the human ones. With all this information, **we aim to find new chemical compounds, selectively blocking action of PLpro, and thus acting as safe, potential anti-SARS-CoV-2 drugs.**

Achievement of such a goal requires a non-standard approach and development of new techniques, as well as the highest quality and accuracy of research. We will use our unique experience in investigation of enzymes and will combine knowledge from different fields, as the project will be conducted by our interdisciplinary team that involves chemists, physicists, bioinformaticians, pharmacists and a medical doctor.

The project has many facets, that include various bioinformatics and drug design methods. At the beginning we will characterize and compare the SARS-CoV-2 protease to proteases from other coronaviruses, such as SARS-CoV and MERS-CoV. Both of these viruses caused epidemics in recent years. In order to go beyond static structures that can be obtained from databases, we will perform computer simulations of protein's behavior in water solution that mimics its natural environment. This will give us an insight into the dynamics of the protein and also provide its structurally different representations that we will utilize in drug design steps of the project. In this part, we will focus on finding the most potent candidate for PLpro inhibitor by searching within the group of known drugs. Importantly, in our project we focus not only on the target viral protein, but also we take into account similar human proteins. Namely, we will use human UCH-L1 protein to check the drug's toxicity against human cells. Using docking methods, we will establish binding strength of the compounds to PLpro and UCH-L1. The drugs with the highest affinities to PLpro, and lowest to UCH-L1, will be selected for further experimental verification.
Moreover, the goals of this project include also the creation of an online database containing information about potential drugs blocking/inhibiting PLpro. The database will hold not only the data we will obtain from our analysis, but also the available information published by others.

Understanding SARS-CoV-2 PLpro on a molecular level will allow us to gain valuable insight into its mechanisms and unique features. Comparing this enzyme with analogous ones from better known CoVs will provide information which characteristics of the previously studied proteins may be translated to our target. This in turn will facilitate the process of designing new anti-SARS-CoV-2 drugs. Determination of differences between viral PLpro and human UCH-L1 will be crucial for the design of drugs selective only for the former protein, and thus lacking toxicity.

The information gathered during this project will not only lead to development of potential antiviral agents, but also will contribute to expanding our understanding of the coronaviruses. Additionally, creation of an online database of potential PLpro inhibitors will provide a valuable asset for global research community. The database of potential SARS-CoV-2 PLpro inhibitors will allow for the long-term goal of this project – that is to provide the global pharmaceutical and medicinal community with knowledge that will help combat COVID-19 pandemic. Thus the project responds to the challenges posed in the EXPRESS CALL TO FUND RESEARCH ON COVID-19. However, provided tools will go beyond and will be of greatest importance in case of future epidemics.