RNA viruses pose a threat to the health and life of humans and many animals, and indirectly also to the global economy. Among many RNA viruses, several that cause epidemics and pandemic outbreaks are of particular concern. This group of viruses includes: influenza, SARS (including SARS-CoV-2), Ebola, Zika, MERS and Denga. The current SARS-CoV-2 pandemic has affected so far more than 165 million people and approximately 3.5 million have already died (Johns Hopkins University of Medicine, May 19, 2021). The current pandemic is also making the public aware of the high pandemic potential of Influenza A virus (IAV). According to WHO, about 1 billion people get seasonal flu each year, and about 600,000 die from complications from the disease. The Spanish flu (1918/19) is estimated to kill 50-100 million people. The genome of RNA viruses, including IAV and SARS, is undergoing mutations that result in the emergence of new variants and strains. For this reason, it is difficult to introduce universal therapies and vaccines that often have to be changed over time. For these reasons, understanding the biology of IAV and SARS-CoV-2 viruses and development of antisense therapies are very helpful in the context of more effective countering of both viruses.

The subjects of the research of the grant proposal are two RNA viruses: influenza A virus and SARS-CoV-2. Influenza A virus has (-)RNA, single-stranded and divided into eight genome segments. Whereas SARS-CoV-2 is a single-stranded genome (+)RNA virus. Despite the differences in the viral cycle of the two viruses, their replication is fully RNA dependent at all stages. In addition, many studies, including our own, have proven that the structure of IAV RNA and SARS-CoV-2 RNA contains numerous conserved motifs and is crucial in the biology of replication of both viruses. The overall aim of the project is biological, chemical and biophysical research to use peptide nucleic acids (PNAs) to form triplex (triple-stranded) structures with selected conserved double-stranded RNA (dsRNA) regions of influenza A and SARS-CoV-2 viruses. We also plan to perform high-throughput screening (HTS) of ligand libraries to select ligands which bind to selected RNA structural motifs of both viruses and perform virtual high-throughput screening (VHTS). Both of these groups of studies are aimed at finding the optimal PNA and ligand that bind to the same viral RNA structural motif and developing PNA-ligand conjugates capable of forming a specific dsRNA/PNA-ligand triplex with improved binding properties and increased antiviral activity.

The following research is proposed in the project:

1/ designing modified PNAs forming triplexes with helixes of conserved RNA motifs of IAV and SARS-CoV-2 and evaluation of PNAs binding to selected motifs. Modified PNA monomers will make formation of dsRNA/PNA triplex less sequence depended and at the same time thermodynamically more stable.

2/ performing high-throughput screening (HTS) and virtual high-throughput screening (VHTS) searching for ligands that bind strongly to selected IAV and SARS-CoV-2 RNA structural motifs. Both screenings will allow to select ligands suitable for PNA-ligand conjugates preparation.

3/ development of PNA-ligand conjugates, determination of their binding ability to viral RNAs and biophysical properties of complexes. PNA-ligand conjugates should possess dramatically enhanced binding abilities to target RNA comparing to “parent” compounds and in consequence antiviral activity. Performed analyses will allow to select the best candidates for cellular tests of their inhibitory properties against viral replication.

4/ evaluation of inhibitory properties of selected PNA, SM and PNA-ligands on proliferation of IAV and SARS-CoV-2 using IAV virus (A/California/04/2009(H1N1)) and non-infectious SARS-CoV-2 replicon constructed in PI group. We will determine the compound binding sites within RNA and assess specificity.

5/ evaluation of inhibitory activities of the most efficient and specific PNA-ligand conjugates on mouse model. We will select ca. 10 PNA-ligand conjugates for testing their antiinfluenza properties in mice. The research will allow to evaluate the therapeutic efficacy of the conjugates selected for research, as well as to perform a histopathological analysis of their effect on laboratory mice.