During the current SARS-CoV-2 pandemic, it is obvious to everyone that viral infections are very serious problem. The research object of the project is the influenza A virus, and more precisely its RNA. About a billion people suffer from seasonal flu each year, and about 0.5 million die of complications from the flu. It is estimated that in 1918 and 1919 from the Spanish flu died between 50 and 100 million people. Due to frequent mutations in the RNA of the influenza virus, it is necessary to prepare new vaccines every year, and actually used anti-flu drugs are low effective. Presented research project is focused on viral RNA (vRNA) as molecular target for small molecule (ligand) inhibitors of influenza virus replication. The influenza virus genome is built with eight vRNA segments. Bioinformatic analysis of vRNA of many thousands of influenza virus strains showed nearly 90% structural conservation of vRNA. For many vRNA motifs it is close to 100%, indicating that there are fragments of RNA that can be targeted by therapeutic tools to achieve inhibition of influenza virus replication. Such therapeutic tools should have inhibitory activity against any strain of influenza virus. The planed research focus on ten conserved structural motifs of vRNA crucial for virus replication. The research project is very complex, innovative and multidisciplinary, and may lead to the selection of ligands serving as universal therapeutics for inhibition of influenza virus replication. The molecular mechanism of action of ligands that inhibit the replication cycle of the virus and new functions of vRNA related to its structure will also be discovered.

In details, the research project includes the following research tasks:

1/ high-throughput screening (HTS) of over 18,400 ligands library will be used to identify those that strongly bind to one of 10 conserved RNA structural motifs selected from segments 8, 7 and 5 of vRNA. The analysis of ligand binding will be carried out based on the FID method, using displacement by a specific ligand a fluorescent indicator from a particular vRNA motif. Ligand binding analysis will be performed at three levels. The first level is the analysis of entire ligand library. The next level is the repeated analysis of the originally selected ligands under stringent selection conditions. The third level of analysis will be carried out in MDCK cells infected with the influenza virus with luminescent properties, by having a luminescent marker gene in the segment 3,

2/ for the selected ligands, their dissociation constants for binding to the influenza RNA structural motifs and to the entire vRNA segments and their ribonucleoprotein (vRNP) complexes will be determined. In addition, the binding sites of selected ligands to viral RNA will be determined,

3/ for some of the best ligands, the structure of the vRNA-ligand complex will be studied based on molecular dynamics methods. They will allow for the valuation of complex structure and on chemical modification the structure of ligands to make them bind even more strongly with the target RNA, and thus become better inhibitors of influenza virus replication,

4/ tests for cytotoxicity will be carried out on a pool of selected ligands. These measurements will be preceded by the inhibition studies of influenza virus strain A/California/04/2009 (H1N1) in MDCK and A549 cells. The effect of the ligand on the viral titer will be determined. The dependence of the ligand concentration used and the time of addition on the inhibitory effect will be investigated.

5/ extensive molecular research will be carried out on the mechanism of inhibition of influenza virus replication by small molecules. The effect of ligands on the formation of viral RNAs, viral proteins and virion assembly will be determined. These studies will help to understand the molecular mechanism of inhibition and increase the knowledge on biological processes responsible for the influenza virus replication. This knowledge is very important not only to understand the molecular mechanisms of the proliferation of the influenza virus, but it will allow makes conclusions leading to more effective inhibition of its proliferation,

6/ for 4-5 the most effective inhibitors of influenza virus replication will be tested on laboratory mice. The tests will be carried out in accordance with the applicable rules of work on laboratory animals by professional personnel. The research will allow to evaluate the therapeutic efficacy of the ligands selected for research, as well as to perform a histopathological analysis of their effect on laboratory model mice.

The presented project involves very extensive basic research that can lead to effective, universal and cheap inhibitors of influenza virus proliferation targeting viral RNA. Postulated research can also be used as pipeline model for similar investigations on the inhibition of the proliferation of other strongly pathogenic RNA viruses, for example: SARS-CoV-2, Zika or HIV.