Influenza is an interesting subject of various biological research due to its serious threat to global public health. Overall, the influenza virus is a human and animal pathogen that causes seasonal epidemics and sporadic pandemics. The pandemic potential is a result of the high variability of its genome and is correlated with its pathogenicity, replication, or growth kinetics. These processes can be controlled by the RNA structure. The influenza virus belongs to the RNA viruses with segmented genome, that takes part in processes throughout the life cycle, highlighting its fundamental role in the virus biology. Furthermore, viral RNA (vRNA) is involved in many processes through activities that relate to its secondary and tertiary structures. To date, it has been found that the vRNA and mRNA sequences form complex secondary structures that are conserved among different viral strains. Additionally, it has been shown that specific motifs can have important functions during replication. Hence, the search for new potential targets within viral RNA seems to be a good direction in the development of alternative therapeutic strategies for viral infections.

Polymorphic nature of RNA allows it to adopt a variety of secondary structures depending on the sequence, the local environment, the presence of molecules, or chemical modifications. Among them, there are noncanonical ones called G-quadruplexes (G4s) that form within the G-rich sequences and are stabilized by Hoogsteen hydrogen bonds. Importantly, there is a growing interest in these unique structures within the viral genomes. The presence of G4s was confirmed in the genomes of RNA viruses, such as Zaire ebolavirus (EBOV), Zika virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Recently, our group reported studies concerning G4s in the influenza A virus (IAV).

Although a significant number of ligands binding G4s have been studied and some of them are considered potential therapeutic agents, there is still a need to design and develop new tools targeting well-defined secondary structural motifs. These findings encouraged us to develop antisense oligonucleotide (ASO)-based tools targeting the IAV G-rich sequences and examine their potential effect on influenza replication.

In this project proposal, we take into account the fact that the unique structural motifs (G4s) found within vRNA may play an important role in viral replication and so far have not been considered as a molecular target. Therefore, we propose: *i)* chemical synthesis of a series of ASOs, *ii)* determination of thermodynamic and structural properties of ASO/target sequences (part A), *iii)* and examination of the biological potential of selected ASOs as modulators of virus proliferation (part B).

More specifically, in the first stage of our project (part A) we will chemically synthesize unmodified and modified ASOs containing 2′-O-methoxyethylribose, locked nucleic acid, and peptide nucleic acid. We will also design and prepare gapmer variants of ASOs. Next, the thermodynamic and structural properties of ASO/the IAV target sequences will be determined using the UV melting method, circular dichroism (CD), and nuclear magnetic spectroscopy (NMR). Additionally, we will characterize their binding affinity and specificity by electrophoresis experiments and fluorescence spectroscopy. Based on these results we will gain more insights into ASO-induced stabilization/destabilization of G4s and choose “the most promising” ASOs for biological studies. In the second part (part B), we are planning to perform biological studies (*in vitro*) to investigate the effect of selected ASOs on virus replication. Briefly, we will examine the biostability of ASOs in serum using an electrophoretic technique. Finally, we will determine the cytotoxic effect of ASOs as well as ASO inhibitory effect on viral RNA copies number, and viral titers in cells infected with the influenza virus.

To the best of our knowledge, targeting the IAV G-quadruplexes with ASOs represents an attractive but unexplored strategy. We believe that investigations proposed in this project will provide valuable information related not only to the physicochemical properties of ASOs/the IAV target sequences but also to the potential applications of ASO tools against viral infection. Furthermore, we realize that the approach presented here can be applied to other RNAs from influenza virus (such as mRNA and cRNA), as well as to other RNAs from different viral genomes.

The presented project is focused on influenza virus genomic RNA, particularly G-rich regions, and the understanding of G4-mediated molecular mechanisms in viral replication. In addition, this project highlights the possibility of targeting viral conserved structural motifs (G4s) using ASO tools as an emerging anti-influenza approach.