ABSTRACT

Influenza is highly contagious disease, which occurs seasonal as epidemic and occasionally as pandemic. Each year the World Health Organization registers 3-5 million cases of severe illness, and about 290. 000-650. 000 deaths. The pandemic potential of influenza virus is a result of high variability of its genome and is correlated with its pathogenicity, replication, growth kinetics as well as the viral life cycle including intracellular replication dynamics, RNA packaging, RNA editing and mRNA splicing. These processes can be controlled by the RNA structure.

Influenza A virus represents enveloped virus which genome consists of eight negative-sense viral RNA (vRNA) segments. The vRNA is transcribed into positive-sense complementary RNA (cRNA) and viral messenger RNA (mRNA) inside infected host cells. The vRNA with nucleoprotein and three polymerase subunits forms ribonucleoprotein complex (vRNP) which is separate replication and transcription machinery. RNA is used throughout the life cycle, highlighting its fundamental role in the influenza biology. Moreover, RNA is involved in many viral processes through activities which correspond to its secondary and tertiary structures. It is known that sequences of vRNA and mRNA form complex secondary structures that are conservative among influenza A virus strains. Furthermore, it has been shown that G-rich regions are present within the genome of various viruses (for example Zicka virus) and form non-canonical structures such as G-quadruplexes (G4), which can play important biological functions. Therefore, the knowledge on vRNA structure-function relationships is essential and the research on this topic should be continued.

In the following project, we are planning to take into consideration the fact that the RNA structure of influenza virus genome is still unknown and the unique structural motifs found within vRNA can play important role in virus biology. According to this, the main goal of project is to inspect the genome of influenza A virus for unique G-rich sequences with the potential to form RNA G-quadruplex structures and study their potential influence on the IAV replication cycle. For this purpose, we postulate bioinformatic analysis across the influenza virus genome to identify potential G4-forming sequences (PQSs). Next, we would like to answer the question: *whether these potential quadruplex-forming sequences (PQSs) are conserved among the IAV strains ?* Retention of conserved PQS regions in the IAV genomes supports a possibly critical role for these structured sites in the context of virus biology. Subsequently, we are planning to perform biophysical investigations of the ability of PQSs to the RNA G4 structures formation. More specific, we will perform thermodynamic and structural studies by various types of spectroscopy (ultraviolet-visible, circular dichroism, nuclear magnetic resonance) and electrophoretic techniques to determine the stability of RNA G-quadruplex structures, their dynamics as well as intermolecular interactions. Finally, we would like to study the potential of PQSs from influenza virus biology point of view. To this end, we will conduct *in vitro* biological studies using cells infected with influenza A virus. More specifically, we will implement the structure-disrupting mutations in the PQS regions of vRNA, and then analyze their effect on the ribonucleoprotein complex activity and virus replication cycles.

The proposed project enhances the current knowledge on the structure of influenza virus RNA. The results of our project will provide detailed information about the presence of G-rich sequences in the IAV genome and their structure as well as potential biological role which can play in the virus replication. In addition, the results of our investigations can be useful for the development of new antiviral approaches aimed at conservative structural motifs of vRNA.