Influenza virus type A is still serious treat for human health and life. Each year seasonal flu epidemic occurred. Every year about billon peoples get sick from seasonal influenza virus and 3-5 millions of them become seriously ill and up to 0.5 million dye due to influenza complications. Epidemic flu called *Spanish Flu* in 1918-1920 caused death ca 20 million peoples which is twice such much as due to IWW military operations. Some of current flu viruses, such as H5N1 and H7N9, have pandemic potential which occur when animal flu is transmitted to human. Frequent mutations of influenza virus genome comes from viral RNA polymerase which mechanism of processing (without correction) allow to incorporate mutations. It is always high need for vaccine for new strains and especially pandemic strains.

Influenza genome is formed by eight single stranded RNAs of negative polarities. Cycle of virus is on each stage dependent on RNA. Recent studies demonstrate that folding of virus RNAs is crucial to understand influenza RNA structure-function relationship and based on that designing more efficient influenza virus proliferation inhibitors is possible.

Goal of the project is to compare *in vitro* and *in vivo* secondary structures of selected segments of RNA influenza virus as well as RNA conserved structural motifs presented in those segments. Based on that information concerning structure and interactions of viral RNAs we propose to perform influenza virus proliferation inhibition experiments in MDCK cell lines using antisense oligonucleotides (ASO), small ligands and siRNAs as potential influenza inhibitors. We propose to focus on: (1) determination how different biomolecules involved in virus cycle (especially NP) and cell conditions influence on structure of influenza RNA of selected segments. We propose: comparison of RNA secondary structure: in cells (*in vivo*), (ii) in isolated from cells RNP complex, (iii) in RNA obtained by deproteinization of RNP in native conditions, (iv) in RNA transcribed *in vitro*, (2) determine the structure of vRNA on different stage of virus cycle, (3) study the participation of secondary structure of influenza (+)RNA in regulation of replication, pre-mRNA maturation and translation (4) design effective ASOs, siRNAs and small molecule ligands for inhibition of influenza virus proliferation, (5) determine molecular mechanism of virus cycle disruption by inhibitory ASOs and ligands, (6) design and determine inhibitory properties of conjugates created by joining the best ASOs and ligands. We expected that application of conjugates increase specificity of inhibition and facilitate cell membranes penetrations.

The studies will be performed on vRNA, cRNA and mRNA of segments 5, 7 and 8 of A/VietNam/1203/2004 (H5N1) (*in vitro*) and A/California/04/2009 (H1N1) (*in vivo* and *in vitro*) viruses. The chosen segments gives the opportunity to compare mRNA from splicing process (segment 8 and 7) and mRNA as uninterrupted transcript (segment 5). All three segments are particularly important for influenza virus proliferation. Segment 8 encodes unstructural proteins NS1 and NS2 (NEP) necessary for replication. Segment 7 encodes proteins M1 and M2 necessary for budging of virion. Segment 5 encodes NP protein which together with virus vRNA and three subunits of viral polymerase forms replication-transcription complex RNP.