Influenza virus type A is a dangerous virus for human health and life. Each year seasonal flu affects about billon peoples and 3-5 millions of them become seriously ill while up to 0.5 million die due to influenza complications. Some of current flu viruses subtypes have pandemic potential and pandemic flu is much more serious and deadly spreading very easily worldwide. 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 dependent on RNA on each stage. 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.

Project concerns influenza virus RNA type A (A/California/04/2009) and we propose to focus on segment 8 vRNA(-) which is the smallest influenza RNA containing 875 nucleotides. There are two major aims of that proposal: (1) developing a new next-generation sequencing (NGS) method to determine *in vivo* structure of RNAs. This NGS approach does not require treatment of RNAs in the cells with reactive and nonspecific exogenous reagents. New method (PVP-NGS) is based on spontaneous cleavage of internucleotide bond of the singe stranded regions of RNA enhanced by polyvinylpyrolidone (PVP), (2) studies of the cotranscriptional folding of influenza vRNA in *in vitro*, in virus/MDCK lysate and in virus infected MDCK cells in presence of antisense oligonucleotides. Large difference in RNA transcription and RNA folding rates results in cotranscriptional folding of RNA. In consequence, ASO could bind to only partially transcribed vRNA and result in RNA cleavage and/or structural rearrangement. Since many years, we are working on determination of secondary structure of several vRNAs and inhibition of influenza virus proliferation with antisense oligonucleotides. We assume that for large RNAs (such as influenza vRNA) understanding process of cotranscriptional RNA folding is essential to solve the mechanism of RNA folding in the cells. Moreover, knowledge about cotranscriptional folding of RNA is crucial to design very active antisense oligonucleotides. We propose to perform cotranscriptional folding of full length vRNA8 but also products of segmental folding of vRNA8 which will be obtained by inducted termination transcription of vRNA8. To study folding of viral RNA in the MDCK cells we propose a new method of structural next generation sequencing (NGS) based of stability of RNA encoded into its structure.

Cotranscriptional folding of vRNA in presence of ASO and theirs influence on proliferation influenza virus is major aim of that project. Presence of ASO during vRNA transcription may results in formation of *thermodynamically stable transient intermediates*, which can results in missfolding of vRNA native structure required to influenza virion formation. The investigations proposed in this project can results in developing a new way of designing of ASOs to reach maximum virus proliferation inhibition. Perhaps, the most therapeutically active would be ASOs directed on double stranded regions of the conserved fragments of vRNA which would result in inhibition of target RNA folding into native structure.

However, this project is focused on vRNA8 but similar investigations could be performed on remaining segments of vRNA as well as viral mRNAs and cRNAs. Moreover, this strategy of investigations would be possible to apply to study cotranscriptional folding of almost any human RNA involved in RNA related diseases.

Results of this project will be new knowledge on structure and cotranscriptional folding of influenza virus RNA *in vitro*, lysate and MDCK cells. Understanding of mechanism of cotranscriptional folding of vRNAs will be very helpful for efficient design of antisense oligonucleotides targeting vRNAs and resulting in influenza virus proliferation inhibition. Process of cotranscriptional folding of influenza RNA was never investigated so far and we are certain that missfolding of influenza virus RNA native structure will result in inhibition of influenza virus proliferation. Results of proposed investigations will have basic meaning to influenza virus studies and understanding influenza virus biology from viral RNA point of view including pandemic strain of influenza virus.