Among RNA viruses, influenza, SARS (including SARS-CoV-2), Ebola, Zika, MERS, Dengue and RSV are constant human threats causing epidemics and global pandemics. Influenza A virus (IAV) causes seasonal epidemics of flu. There are about 1 billion flu cases annually including 290 000 - 650 000 deaths. Currently, two antiinfluenza proteins are target as therapeutic i.e. the matrix 2 (M2) ion channel protein and neuraminidase (NA). There is an urgent need for developing new detection and antiviral strategies due to the emerging drug resistance and viral RNAs seems to be optimal as biomarkers and therapeutic targets.

IAV belongs to family of Orthomyxoviridae and possess segmented to eight segments, single-stranded RNA genome with minus polarity. Viral RNA secondary structural motifs includes: duplexes, single stranded regions, many types and sizes of internal loops and bulges, dangling ends, hairpins, various kinds of multibranch loops. Viral and cellular RNAs may also contain higher-order motifs such as G-quadruplexes (G4s), i-motifs and pseudoknots (PKs). Many of those RNA structural motifs were selected as targets for therapeutic treatments by various type oligonucleotide tools and small molecules (SMs) in different human RNA related diseases.

There is a growing knowledge about the structure of IVA RNA and the structure-function relationship. Several groups, including our, made a big progress in experimentally probing the structure of vRNA, mRNA *in vitro, in virio* and *in cellulo*. However, RNA motifs important for virus cycle are still discovered and studied intensively studied towards solving theirs structure and biological function. Viral RNA genome undergoes fast and frequent mutations. IAV conserved structural RNA motifs gives opportunity to modulate or inhibit virus replication; however, precise tools for that activates are still desired. G-quadruplexes and pseudoknots are already detected in IAV. Unique structures of both those RNA motifs require more advanced oligonucleotides tools and small SMs for specificity and selectivity concerning therapeutic treatment. On the other hand, these RNA structural motifs are much difficult to study due to complex structure and theirs “therapeutic targeting” is much more challenging.

The general goal of the project is studies of dynamic conserved RNA structural motifs of IAV, mostly pseudoknots and G-quadruplexes, for better understanding of their function and inhibition/modulation of IAV replication with novel peptide nucleic acids (PNA). That knowledge would be important for learning influenza virus biology and designing of new, efficient and universal antivirals. Traditional antisense peptide nucleic acids (asPNAs) are useful as probes and diagnostic tools due to the superior binding affinity toward target nucleic acids through duplex formation and chemical stability against nucleases and proteases. Chen research group has developed nucleobase modified double-stranded RNA-binding PNAs (dbPNAs) that show sequence and structure specific binding to dsRNAs over single-stranded RNAs (ssRNAs). Chen and Kierzek groups have recently applied a 10-mer dbPNA-neamine conjugate with superior binding and inhibitory activity in targeting IAV RNA panhandle structure. We believe that combining asPNAs and dbPNAs (designated as daPNAs) will result in further enhanced binding affinity and specificity that can be further facilitated by conjugation with SMs. In consequence, result in better antiinfluenza therapeutics.

We propose use several daPNAs targeting many conserved of IVA RNA motifs, particularly the pseudoknots and G-quadruplexes. Moreover, high-throughput screening (HTS) of small molecules libraries will allow select the best ligands for PNA-SM conjugates preparation. Binding of PNAs to selected RNA structural motifs will be investigated with various biophysical methods. Finally, the inhibition/modulation effect of the most effective PNAs and PNA-SM conjugates will be investigated in cell cultures and model mouse using various methods.

For research we chose influenza strain A/California/04/2009 (H1N1) which is a circulating presently strain with pandemic potential and A/WS/1933 H1N1 (WSN) and A/Puerto Rico/8/34 (H1N1) (PR8), for selected researches.