The correlations of the structure and function of RNAs is very well established. To change biological functions of RNAs, including human pathogenic RNAs, determination of structure of the native RNAs is fundamental. That process is relatively simple for *in vitro* RNA. Determination of the *in vitro* secondary structure of RNA is based on chemical, enzymatic and microarrays mappings as well as on thermodynamic rules folding of RNA. Folding of RNA is based on nearest-neighbor model and applies the thermodynamic parameters for duplexes and no-helical structural motifs determined in 1M sodium chloride buffer. Determination of RNA structure but in cellular condition (*in vivo, in cellulo*) is very difficult and long-lasting process and at present time is mostly based on chemical mapping in the cells and analysis of mapping with Next-Generation Sequencing (NGS).

In this project we propose:

(**1**) determine thermodynamic parameters concerning principles of folding of RNA in *in vivo-like* conditions to predict folding of any RNA in cellular conditions. To achieve this goal, we will conduct RNA thermodynamic studies *in cellular buffer.* Based on our preliminary results, we postulate on early stage of investigations to test two potential cellular buffers. One would be mammalian cell culture medium with low glucose (Dulbecco's Modified Eagle's Medium low glucose, DMEM low glucose) and second DMEM diluted human blood serum. Composition of both fluids (salts of mono- and divalent cations, amino acids, glucose) are very similar. A drastic difference concerns concentration of proteins which in serum is 175 time higher.

(**2**) implement *cellular* thermodynamic parameters into RNAstructure program (we propose *cRNAstructure* name for that new program). That will allow to compare thermodynamic stability and folding of the same RNAs in *in vitro* and *in cellular* conditions,

(**3**) compare folding of the same RNAs determinate by cRNAstructure and structures solved based on chemical mapping of RNAs in the cells followed by NGS analysis. For that group of studies we will particularly focus on segment 8 of influenza virus vRNA (vRNA8).

Presented project is very significant for two major seasons: (1) determination of native structure of RNAs will allow to better understand it biological function as well as correlation of structure and function of RNAs in the cells, (2) many RNAs are directly related with human diseases and for application of oligonucleotides (antisense oligonucleotides, siRNA, CRISPR-Cas9 system) as well as small molecules as potential therapeutics the knowledge about folding of pathogenic RNAs is absolutely necessary.

At present time all thermodynamic parameters, included used in various computer programs design to predict folding of RNAs, were determined in standard 1M sodium chloride buffer. In this project we postulate to determine those RNA thermodynamic parameters in cellular buffer. That include parameters for RNA duplexes and structural motifs carrying internal loops, bulge loops, dangling ends, hairpins, multibranch loops, coaxial stacking.

We postulate, for the first time, to determine thermodynamic parameters concerning folding of RNA in cellular buffer. The next, those parameters will be implemented into computer program RNAstructure which is world-wide used to predict folding of RNAs. Implementation into RNAstructure thermodynamic parameters determine in cellular buffer will allow predict native-like structure of RNA. The knowledge of folding of human pathogenic RNAs will allow to rational design potential therapeutics, particularly based on oligonucleotides and small molecules.