Ribonucleic acids (RNAs) are one of the most important biomolecules. RNA biological functions are very different and largely related to their structure. In RNA, apart from the canonical bases, there are over 140 modified nucleotides. The most common are N6-methyloadenosine and pseudouridine. Modified nucleotides affect the structure and biological functions of RNA. The combination of RNA structure and its biological properties makes knowledge about the structure of RNAs containing modified nucleotides very required. In addition to many approaches of chemical mapping of RNA structure, there is also a method of predicting RNA folding based on knowledge of the thermodynamic rules of interactions of RNA within various structural motifs. Appropriately designed computer programs are used for this prediction, and RNAstructure program is one of the best.

The aim of the research project is to determine the thermodynamic parameters essential for folding of RNA containing the following natural RNA modifications: pseudouridine, N1-methylpsuedouridine, 5-methoxyuridine and 5-methylcytidine. The collected thermodynamic parameters will be implemented into the RNAstructure program. The next stage of the project will be the chemical mapping of several RNAs containing the selected modified nucleotides and the comparison of RNA structures generated with the modified RNAstructure program and determined experimentally with chemical mappings. Project is also important for other reasons. We can assume that due to vaccination world is slowly recovering from the SARS-CoV-2 virus pandemic. The most effective vaccines from Pfizer and Moderna contained spike mRNA in which all uridines were replaced with N1-methylpseudouridine. Earlier studies have shown that the introduction of N1-methylpseudouridine into vaccine IVT mRNAs most significantly increases mRNA expression and stability of mRNA in the cellular environment and indicated the best immunological parameters. Other modified nucleotides that also showed very promising results were: pseudouridine, 5-methoxyuridine and 5-methylcytidine. This was also the reason why the mentioned modified RNA nucleotides were selected for investigation in project.

The research plan includes the following steps: (1) synthesis of the necessary modified phosphoramidites and RNA oligonucleotides containing pseudouridine, N1-methylpsuedouridine, 5-methoxyuridine and 5-methylcytidine at specific positions, (2) measurements of the thermodynamic stability of complementary duplexes and duplexes containing modifications within nonhelical motifs of RNA. For each type of RNA modification, the next step is calculation of the respective thermodynamic parameters and theirs introduction into the RNAstructure program, (3) chemical mapping of two large fragments of the 28S subunit of the human ribosomal RNA (rRNA). Both model rRNAs contain 5 and 13 pseudouridine residues respectively, and are selected from the region of the rRNA where autocatalytic peptide bond formation on the ribosome takes place. Based on the results of the chemical mapping, the RNAs secondary structure will be determined and compared with the structure generated with the modified RNAstructure program. The comparison of both secondary structures will also serve to improvement, if necessary, of the modified thermodynamic parameters of the RNAstructure program, (4) because the determined thermodynamic parameters will also apply to *vaccine RNAs*, we will also study the structure of such RNAs. The selected RNAs are: subgenomic RNA M from SARS-CoV-2 virus (fragment approximately 800 nt long) and segment 4 from influenza RNA (fragment mRNA encoding the surface protein - hemagglutinin, approximately 1800 nt long). For the first model RNA, structures will contain one from four selected modifications, while for segment 4 RNA, only the structures containing pseudouridine and N1-methylpseudouridine, respectively. Next, their structures generated with RNAstructure and determined based on chemical mapping will be compared, (5) determining the structure of small RNA fragments containing modified pseudouridine by NMR and by crystallographic methods. These studies are aimed at determining the interactions responsible for the significant stabilization of RNA structures by pseudouridine.

Overall, the project is important for understanding biological functions and the effects of modifications on RNA structure. The aspect of *vaccine RNAs* is extremely important also, as the effectiveness of the Pfizer and Moderna vaccines indicates that this is the right direction for the future development of IVT mRNA vaccines.