mRNA-based technologies in medicine have been developed since the 1990s. Thanks to significant technological advances, mRNA is becoming an increasingly widely used tool in the development of vaccines and gene therapies. Despite many advantages over the conventional methods used so far, one of the main problems of mRNA-based therapies is the innate low stability of mRNA molecules. Regardless of the pathway, the process of mRNA degradation in mammalian cells is initiated by deadenylation, the removal of the poly(A) tail located at the 3' end of most mature eukaryotic mRNAs. The poly(A) tail acts as one of the major regulators of mRNA fate, including protecting mRNA coding regions from degradation, as well as playing an important role in translation initiation through complex protein-RNA interactions. Both these features, coupled with the non-coding nature of the poly(A) tail, make it an ideal target for beneficial modifications to enhance mRNA stability and translational efficiency, without interfering with the activity of the ribosomal machinery responsible for protein biosynthesis. In recent years, several studies have been presented on mRNA modification to manipulate the stability and translational potential of mRNA, which inspired my research.

The first part of this dissertation concerned the development of a biophysical method to study the structure-activity relationship in the interaction of poly(A) tail analogs with a protein that specifically binds the 3' end structure of mRNA (PABP). To develop the method, I used the sensitive microscale thermophoresis technique, which allows studying of a wide spectrum of molecular interactions. The developed method was used to study interactions between PABP and nearly thirty oligonucleotide analogs of the poly(A) tail, featuring selected chemical and structural modifications. Through detailed studies, I was able to determine the effect of a given modification on a specific interaction with PABP, stemming not only from the type of modification used, but also its location in the protein-binding pocket. I also carried out enzymatic resistance studies of aforementioned poly(A) analogs involving a specific deadenylase CNOT7. I selected poly(A) analogs showing simultaneously high affinity for PABP and resistance to enzymatic degradation. Translation studies in cell lysates were then carried out with chosen oligonucleotides, which showed that the compounds I selected exhibited inhibitory potential and specifically inhibited the translation process. Based on this part of the research, I have drawn a number of conclusions about the influence of the structure of poly(A) fragments on their affinity for PABP. Among other things, these studies proved that all kinds of chemical modifications of the poly(A) chain are best tolerated when the modified subunit is located at the end of the sequence bound by the protein. For this reason, in the remainder of my research I focused on making modifications within the 3' end of the RNA itself.

I devoted the second part of my doctoral project to developing a method that allows chemo-enzymatic modification of the 3' end of synthetic mRNAs. This modification was aimed at increasing the stability of mRNA molecules by improving their resistance to degradation by 3'-5' exonucleases, while maintaining their ability to interact with the PABP protein. The improved stability contributes to the extended lifespan of modified mRNAs in cells and results in increased production of mRNA-encoded proteins. The modification method I developed involves enzymatic ligation of chemically 5'-phosphorylated modified dinucleotides to the 3' end of any RNA chain. Chemical modifications were introduced into the structure of the dinucleotides, which were identified during the first part of the study as optimal from the point of view of interaction with PABP and enzymatic stability. The modification method was used to obtain reporter mRNA molecules exhibiting increased stability. Modified mRNAs were then tested in three different cell lines for their translational potential. Two types of mRNA modifications were identified that resulted in up to several times increased protein production in the cells.

The methods I have developed can contribute to further research on poly(A) mRNA tail modifications. Biophysical method that allows the study of the interaction between PABP and poly(A) analogs will find application in further studies of potentially beneficial poly(A) modifications. Moreover, the method of chemo-enzymatic modification of the 3' end of RNA can be used in obtaining any RNA molecules with increased stability, especially mRNAs with therapeutic potential.