mRNA is a single-stranded molecule which brings the information about protein structure (after copying it form DNA into RNA in transcription process) to ribosomes that use it for protein biosynthesis (translation). At one of mRNA ends, so called the 5' end, there is a cap structure which takes part in mentioned processes in addition to being responsible for the stability of mRNA in cellular environment full of RNA-degradating enzymes. Because of this important biological role of the cap structure, **modified cap analogs having new properties may serve as good tools for influencing the processes of translation, RNA degradation or RNA transport and changing them in a desired way.**

mRNA is built by a chain of molecular units called nucleosides that are joined with each other by single phosphates. The natural cap structure consists of modified nucleoside, 7-methylguanosine, attached to the first nucleoside of the 5' end of mRNA via a chain of three phosphates. So dinucleotide cap analogs comprise of three elements: 7-methylguanosine, which is responsible for the specificity of interactions with proteins, the typical nucleoside of RNA, guanosine, and triphosphate bridge that joins these two elements and is a target of cleavage by enzymes like DcpS or Dcp2.

The primary aim of the project is to synthesize a series of novel cap analogs modified within oligophosphate bridge and to explore their new properties when they exist as single molecules or after their incorporation at the 5' end of RNA. The results of biological experiments will enable to estimate the potential applicability of the new analogs both in basic studies and in therapies of genetic diseases.

Dinucleotide cap analogs will be synthesized in so-called "click" reaction – a high-yielding, quick and fast method of coupling two characteristic chemical groups which produces a structure called triazole ring. It is planned to take advantage of this synthetic approach to join two parts of the cap, each modified with one of this characteristic chemical group, in order to obtain dinucleotide cap analogs containing triazole within oligophosphate bridge. Our intent is to obtain compounds differing i.a. in the length of the phosphate chain and the position of the triazole moiety and thus representing different types of modifications. **This new class of compounds is the first example of utilizing click chemistry strategy to introduce a triazole moiety within oligophosphate chain of a cap structure.**

Then, we plan to use the obtained compounds in biological studies to investigate the influence of this novel type of modification on the resistance to hydrolysis by DcpS enzyme. The protein cleaves the cap species that remain after RNA degradation. The planned experiments are to identify analogs that are stable in the presence of this enzyme. It is important considering their potential inhibition activity towards DcpS. There is a high correlation between the inhibition of DcpS and the containment of disease progression in Spinal Muscular Atrophy (SMA). This is why it is worth exploring this direction of biological characterisation. It is crucial that these inhibitors will act selectively not to disrupt other cellular processes. Here, the decreased affinity to cap-binding proteins, like eIF4E, is desired. The result of our preliminary studies indicate there are at least several types of modifications (we mean triazole introduced within oligophosphate bridge) of cap structure that are responsible for increased resistance to degradation by DcpS and decreased affinity to eIF4E. On the other hand, increased affinity to eIF4E indicates the potential applicability as small-molecular translation inhibitors in anticancer therapies. Our aim is to find the connections between the types of modifications and arising properties so that in future it would be easier for other scientists to design similar compounds useful for mentioned applications.

Also, **selected cap analogs will be incorporated at the 5' and of RNA**. We intend to apply co-transcriptional capping strategy, i.e. perform in vitro transcription (the synthesis of RNA from the DNA template conducted in a tube by polymerase – an enzyme naturally conducting this process in cells) using cap analogs. Resulting modified transcripts which code luciferase will be introduced into cells to study the efficiency of translation using luciferase reporter system. The analogs incorporated into these transcripts will be selected on the basis of biological characterisation of dinucleotide analogs – only analogs that interact with eIF4E similarly or stronger than m7GpppG may take part in the initiation of protein biosynthesis. To determine the yield of translation we will measure the lever of light emission resulting from the reaction of luciferin conversion catalyzed by the luciferase. This will enable us to estimate the functionality of our analogs as cap mimics in biophysical studies on cap-dependent processes.