Modified nucleosides present in mRNA and tRNA nucleic acids are important elements of the cellular machinery responsible for effective and accurate protein biosynthesis. The major players of this machinery are modified nucleosides located at the coding region of mRNAs and the anticodon stem-loop domain (ASL) of cytosolic and mitochondrial (mt) tRNAs, directly attending the codon-anticodon interactions on the ribosome. Since mitochondria produce ~90% of the energy required by the cell, the structural perturbation of mt-tRNAs were found to cause several human diseases (e.g. MERRF, MELAS, LHON).
In contrast to tRNAs, mRNA molecules contain a rather small number of modifications, but their importance in the regulation of cellular processes has been clearly demonstrated. Some of them, called epigenetic, were recently discovered to be dynamic and can play a critical regulatory role in protein biosynthesis.

Project goals and research description. Based on the chemically synthesized model oligomers, we focus on two complex problems addressing the role of mRNA and mt-tRNA modifications in translation process. The first subject of our interest is epigenetic modifications derived from 5-methylcytidine: 5-hydroxymethylcytidine (hm^5C), 5-formylcytidine (f^5C) and 5-carboxycytidine (ca^5C) identified in coding region of mRNA. It has been suggested that they play a regulatory role in the translation at the mRNA level, but there is no systematic research to date. Within the Project, we plan to perform the biophysical and structural characterization of suitably modified oligonucleotides to evaluate the impact of structurally distinct epigenetic cytidines on RNA properties and functionality.
Furthermore, we plan to find the metabolic pathway of hm^5C-RNA, which explains the reduced amount of this modification in cancer cells as compared to normal cells. Since hm^5C regulates translation, decreased level of hm^5C should have important biological or pathogenic consequences in the formation of cancer cells. In the Project, we hypothesize a possible hm^5C metabolic pathway and plan to verify the proposed thesis through *in vitro* experiments with a synthetically obtained hm^5C oligomer and a set of three enzymes (A3A, hSMUG1 and APE1) involved in cellular hm^5C-RNA transformation.

The second goal of our Project concerns two pathogenic nucleosides identified at the position 37 of hmt-$tRNA^{Met}$, resulting from mutation A4435→G in human mt-DNA and subsequent enzymatic methylation G_{37}→m^1G_{37}. Both pathogenic mutations cause severe mitochondrial dysfunction in some patients associated with hypertension, type 2 diabetes or Leber's hereditary optic neuropathy (LHON). Recently, we have proved that the replacement of conserved A_{37} to G_{37} and next to m^1G_{37} alters the thermal stability of ASL hairpin motif, particularly in the case of G_{37}-containing ASL, which was predicted to form a super-stable tetraloop hairpin [*Chem.Commun.,* **2021**, *57*, 12540]. Within the project we plan to use appropriate oligonucleotide models for biochemical and structural studies to assess the properties of damaged hmt-ASL^{Met} molecules.

Reasons for attempting a particular research topic. Determining the effects of m^5C, hm^5C, f^5C and ca^5C mRNA modifications on translation process, including cancer cells is important for several reasons. Firstly, protein biosynthesis is a fundamental process in every cell and its disruption leads to severe cell dysfunctions. In particular, increased amounts of hm^5C was observed in such important organs as the brain and heart. Moreover, the hm^5C-regulatory role can be associated with cancer cells formation. Finally, the biological role of the f^5C and ca^5C modifications has not been investigated so far. Studies involving pathogenic human mt-$tRNA^{Met}$ seems essential to understand the molecular reasons for mitochondria dysfunctions and clinical symptoms reported for patients affected by the mutation A4435→G. We believe that a detailed understanding of how pathogenic modifications alter the structure/properties of RNA molecules will facilitate the development of new therapies for human diseases in the future.

Substantial results expected. Biophysical and structural studies of RNA oligomers modified with m^5C, hm^5C, f^5C and ca^5C epigenetic modifications will broaden our understanding of their contribution to hybridization properties, base pairing discrimination and local/global structural changes. The obtained data will allow us to elucidate the differences between the modified cytidines which are important in the context of regulatory processes in the cells. Confirmation of the hypothesized hm^5C metabolic pathway in cancer cells will provide new insight into the role of post-transcriptional modifications in the control of gene expression in cancer cells and/or shed new light on the unknown regulatory function of the used A3A and hSMUG1 enzymes. The structural studies of the pathogenic hmt-$ASLs^{Met}$ supported by ribosome binding experiments will allow us to understand the contribution of defective nucleosides to the pathogenesis of mitochondrial diseases at the molecular level. In addition to the above-mentioned results, we will develop several novel procedures of modified oligonucleotides syntheses which will be helpful not only in the implementation of the Project tasks.