The recent significant progress in the fields of genetics, molecular biology and biotechnology has given new opportunities to diagnose and treat diseases that were previously considered incurable with the currently available pharmaceutical strategies. The class of compounds that perfectly enroll in the above trend are aptamers, the diverse group of single-stranded oligonucleotides whose sequence determines folding into a peculiar secondary and tertiary structure. The presence of these structural elements determines the aptamer spatial arrangement, a crucial factor that allows the oligonucleotides to bind and modulate their target protein activity. It was observed that aptamers affinity and specificity, similarly to antibodies, are predominantly settled by tertiary interactions. The examples of great structural diversity of aptamers are oligonucleotides RE31, RV66 and Toggle-25t, which adopt G-quadruplex structure with additional duplex fragment, parallel G-quadruplex with 5' and 3' flanking regions, and a stem–loop with an internal bulge architecture, respectively. Aptamers found wide range of applications, starting from being an attractive therapeutic tool, through constituting efficient drug delivery systems, up to elements of diagnostic platforms. Despite numerous potential applications of aptamers and a number of clinical trials involving these oligonucleotides, so far there is only one compound, named Macugen, approved by Food and Drug Administration for the treatment of neovascular age-related macular degeneration. The above illustrates the level of complexity of factors that should be taken under consideration during aptamers clinical translation. It is worth stressing that aptamers exhibit great variety of advantages over antibodies like better thermal and shelf stability, shorter time of preparation and minor batch-to-batch variation, ease of introduction of chemical modifications, a large diversity of potential targets without requirement for them to have immunogenic character, and above all, the ability to distinguish between closely related molecules. The broad range of benefits resulting from the use of aptamers as therapeutics encourages scientists to make attempts to develop new aptamers with more favorable therapeutic properties.

One of the crucial limitation in modulating of the affinity and binding of native aptamers toward target protein is limited diversity of chemical functionality of modified nucleotides in comparison to amino acids. The major advance in the field was development of alteration of nucleotide residues (SOMA-DNAs), which better mimic the amino acid residues and could have a significant influence on improvement of aptamers binding strength due to the presence of additional hydrophobic interactions. The above gave the foundation and impulse to create the novel modifications, synthesized for the first time in our Laboratory, which were named Slow Off-Rate unlocked nucleic acids, SUNAs. Combination of increased flexibility of unlocked nucleic acid (UNA) moiety with protein-like side chain of base is expected to enhance protein-aptamer interactions. The other modification that could improve protein-aptamer interaction is thiolation of internucleotide linkages, which consists of replacement of one oxygen with sulfur atom. The introduction of sulfur in the C4 position of uridine, which gave the rise to 4-thiouridine residue modification (s4U), was also said to improve aptamer affinity towards target protein. Furthermore, it was also proven that enhancement of oligonucleotide-protein interaction could be achieved via alteration of ribose ring with a 2′-fluoroarabinose moiety(2'-FANA), characterized by different sugar conformation.

Taking the above into account, in this project, we propose to extend knowledge about utility of above mentioned modifications to develop RE31, RV66 and Toggle-25t variants with enhanced affinity towards target proteins. Detailed recognition of the processes and mechanisms at the molecular level could allow for the designing of new versions of well-known inhibitors, which would simultaneously result in the improvement of therapeutic methods. An important expectation is that the studies will provide comprehensive hints on improvement of aptamers with different spatial arrangements in order to increase their affinity towards target protein via introduction of modified nucleotide residues. It is worth noting that our research is the first attempt to use SUNAs as tool to modulate affinity and selectivity of aptamers possessing different structures and modes of action.