Abstract of dissertation

Fluorophosphate labeled (oligo)nucleotides as tools for studying biological processes

Molecular probes are valuable research tools used in biophysical studies. These probes allow researchers to understand how cellular processes work, from enzymatic transformations to intermolecular interactions, and to study structural changes in more complex structures such as proteins and nucleic acids.

The aim of the PhD project was to produce nucleotide and oligonucleotide biophysical tools to study biological processes related to structural changes of oligonucleotides and enzyme functions. The chosen chemical modification was a fluorine atom, which was introduced within the (oligo)nucleotide structure as the fluorophosphate moiety. Fluorine is not found in natural organic compounds, but its presence significantly alters the physicochemical properties of synthesized molecules, so nearly 20% of all therapeutics posses this atom in their structure. Moreover, fluorine is active in nuclear magnetic resonance spectroscopy, hence fluorine-labeled compounds are used as molecular probes in ^{19}F NMR monitoring of biological processes.

In this PhD project the chemical synthesis of nucleotides and oligonucleotides labeled with fluorophosphate at the 5' ribose position was developed. A number of fluorinated oligonucleotides, which differed in the length of the polymer chain (from 6 to 24) and the type of nucleobases were obtained. Much attention has been devoted to their applications as molecular probes in the ^{19}F NMR. Signal changes on NMR spectra allowed to monitor such biological processes as formation of duplexes, G-quadruplexes and *i*-motif structures of DNA. Moreover, nucleotide single mismatches in DNA duplexes were distinguished and interactions between nucleic acids and protein or small-molecule ligand were monitored. The utility of the designed probes to study structural changes of oligonucleotides as a function of variations in acid-base conditions or temperature was also demonstrated.

The PhD project also consists of a section devoted to the search for inhibitors of DcpS and Fhit proteins. Fluorophosphate analogs of nucleotides turned out to be substrates for the both enzymes, where fluoride ions were one of the products. This discovery enabled the development of a high-throughput screening (HTS) method based on fluorescence detection of fluorides. Using the developed HTS assay, a library of 141 nucleotide-derived compounds was searched, the best inhibitors were selected and their IC_{50} parameters were determined.