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PROSPECTS OF USING BINARY DEOXYRIBOZYMES AS A BIOSENSOR FOR QUANTITATIVE ANALYSIS OF OLIGONUCLEOTIDE CONCENTRATIONS

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Introduction. Many diseases are associated with changes in the body's own nucleic acids – these include all viral infections – from COVID-19 to HIV, all types of cancer, as well as genetic diseases.

For the diagnosis and treatment of diseases, it is often necessary to know not only the qualitative, but also the quantitative content of markers. For example, patients with hepatitis C or HIV should regularly take a viral load test, and the diagnosis of some types of cancer involves determination of onco-marker concentration. For instance, some types of miRNAs in high concentrations are an indicator of the development of cancer, however, healthy cells also contain miRNA, but in lower concentrations [1].

Digital PCR can cope with all these tasks, but this method requires expensive equipment, highly qualified maintenance, and a large number of reagents. We propose an alternative method – DNAzymes specific for a certain concentration of the marker, constructed using threshold concept.

The main part. DNAzyme is a single-stranded DNA with a catalytic activity that is performed by the catalytic core (Dz core), and the latter is flanked within two binding arms for the substrate. By splitting the Dz core into two, it is obtained the binary (or split) DNAzyme. The binary DNAzyme used to design the constructs in this study, consists of two strands. Each of this strand has a substrate and analyte binding arms. The substrate is a synthetic double-labeled oligonucleotide with a fluorophore at the 5’ end and a quencher at the 3’ end. When a certain analyte is present, the sensor threads will complementarily bind to the analyte, recreating the Dz core (binary DNAzyme needs an activator to hold the two strands together in order to form the catalytic core). After that, the structure binds to the fluorescence substrate (F-sub), and subsequently its cleavage occurs. This cleavage separates the quencher and the fluorophore, resulting in a fluorescent output signal [2].

Thanks to the threshold concept, we have the opportunity to construct DNAzymes that are activated only in a certain concentration of the marker. So, it is possible to design DNAzyme with a single binding site (yes gate) and will be active only in low concentrations. However, an increase in binding sites, for example to 5, will lead to a complete assembly of the structure and activation of the DNAzyme only in the presence of a 5-fold increase in the concentration of marker molecules [3].

Aim: to develop a method for determining the concentration of markers using binary logic elements based on DNAzyme, which, depending on the concentration of the input signal, will switch the signal intensity.

As with any biosensor, the elements of molecular logic have a marker that they read, in this case we chose miRNA-92a as a test model because of the level of diversity of its expression in different types of cancer. The bioreceptor is binary DNAzymes, they complementarily bind to the marker, forming the Dz core, which cleave the targeted molecule (mRNA or F-sub). It results from it a fluorescence. We can choose different fluorophores for each gate, which fluoresce in different spectrum leading to the identification of which gate is activated by the concentration. Then the information is converted into a digital logic element, which gives us the opportunity to determine the maximum concentrations in the sample.

Conclusion. The introduction of a new technique can help increase the availability of diagnostics of oncological, viral, and genetic diseases, which will facilitate the detection of disorders at an early stage, and, accordingly, will enable more effective and safe treatment.

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