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Development of Multi-Output DNA Constructs as Logic Gates Responsive to MicroRNA Concentrations

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Введение. In recent year DNA molecules were intensively used in constructing advanced nano construct-based logic gates by taking advantage of specificity and predictability of Watson-crick base pairing of DNA. These logic gates include AND, OR, AND NOT and XOR gates just to cite few. Indeed, there are still limitations in constructing more complex circuits using DNA as building blocks. We have created a molecular switch based on threshold binary DNAzymes that exhibit catalytic activity depending on the concentration of input and can perform in the same reaction mixture [1], [2].

Основная часть. The aim of this project is to develop YES (Bi-Dz) and 5 input (5i-DTh) binary gates. To be catalytically activated, YES gate requires one microRNA to reform its catalytic core. In contrast, 5 input gate activation depends on sequential process: four microRNAs must first unwind specific loops, allowing the fifth microRNA to reconstruct the catalytic core, micro-RNA-binding arms were designed to have different melting temperature using UNAFold application (https://www.unafold.org/). Both designs are activated by microRNA-92. The YES gate was designed to cleave the DAD1 RNA target (involved in preventing apoptosis and highly expressed in cancer cells), while the 5-input gate was designed to cleave GFP RNA target (fluorescent marker for visualization). YES gate is activated at low concentrations of the microRNA-92, whereas the 5-input gate is not. High concentrations of miRNA-92 lead to the disruption of the YES gate catalytic core and renders it non-functional. Conversely, high concentration leads to activate 5-input gate to cleave GFP. Cleavage assay of both DAD1 and GFP was observed using PAGE and the results were analyzed using a gel documentation system. It was shown that higher microRNA levels shift the activity toward GFP cleavage while diminishing DAD1 cleavage [3], [4], [5].

Выводы. Results showed that Both Yes and 5 input binary gates were able to cleave their RNA targets when they are separated. Similar graphs for cleaving RNA targets were also achieved when gates were together (mix cleavage assay). Our designed gates were effective in reaching our goal of this study.

Список использованных источников:

1. Macdonald, J., Li, Y., Sutovic, M., Lederman, H., Pendri, K., Lu, W., Andrews, B. L., Stefanovic, D., & Stojanovic, M. N. (2006). Medium scale integration of molecular logic gates in an automaton. Nano Letters, 6(11), 2598–2603. https://doi.org/10.1021/nl0620684

2. Deng, W., Xu, J. Y., Peng, H., Huang, C. Z., Le, X. C., & Zhang, H. (2022). DNAzyme motor systems and logic gates facilitated by toehold exchange translators. Biosensors and Bioelectronics, 217, 114704.

3. De Oliveira, A. G. G., Dubovichenko, M. V., ElDeeb, A. A., Wanjohi, J., Zablotskaya, S., & Kolpashchikov, D. M. (2021). RNA-Cleaving DNA thresholder controlled by concentrations of mIRNA cancer marker. ChemBioChem, 22(10), 1750–1754.

4. Nedorezova, D. D., Dubovichenko, M. V., Kalnin, A. J., Nour, M. a. Y., Eldeeb, A. A., Ashmarova, A. I., Kurbanov, G. F., & Kolpashchikov, D. M. (2023). Cleaving Folded RNA with DNAzyme Agents. ChemBioChem, 25(1).

5. Cramer, E. R., Starcovic, S. A., Avey, R. M., Kaya, A. I., & Robart, A. R. (2023). Structure of a 10-23 deoxyribozyme exhibiting a homodimer conformation. Communications Chemistry, 6(1).