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Improving aptamer affinity for tight binding human Thrombin

Moustapha A. Y. N. (ИТМО), **Эльдиб А.А.** (ИТМО)
Научный руководитель – кандидат технических наук, доцент Kolpashchikov D.M.
(University of Central Florida - Chemistry Department)

Введение. Encouraging advancement was made in the aptameric field to obtain greater stability and binding of the aptamer to its target using chemically modified nucleotide. It's only recently that significant progress has been made by using different strategies such as bivalent, Dimers or assemblies on nanoparticles aptamers. The aim of this project is to address the challenges of current aptamers that suffer from a high dissociation Rate Constant (K-off) that lowers the stability of the complex aptamer-protein. in an aqueous environment by using a rationally designed protective scaffold.

Основная часть. Aptamers are single-strand DNA (ssDNA) or RNA that bind target molecules with high affinity and specificity like nucleic acids, proteins, cells and even small organic molecules, with their ability to fold into three-dimensional structures. Aptamers are obtained from a processus that is called Systematic Evolution of Ligand by Exponential Enrichment (SELEX) which consist of 3 major steps: selection, partitioning and amplification that undergo for several cycles.[1] Aptamer often suffers from high k-offs, due to the surrounding aqueous environment that disrupts the complex even with high affinity. Designing a rational scaffold that will protect the complex will indeed lower the high k-off. We have chosen thrombin as a model cause aptamer specifically for this protein are the most studied aptamers.[2] TBA15 and TBA22 bind to exosites I and II respectively. We have designed a spider-like scaffold incorporating TBA15 and TBA22: one positioned at the center and the other at the edge to enable sandwich binding. Additionally, specific scaffold extremities are modified with sequences that will later be functionalized with hydrophobic moieties. The dissociation constant (kd) will be measured by Electrophoretic Mobility Shift Assay (EMSA) for both aptamers on the scaffold in comparison to the absence of a scaffold.

Выводы. Overall, our scaffold-based approach offers a novel solution to enhance the stability of aptamer-protein complexes by reducing the high dissociation rate in aqueous environments. This study provides a promising strategy for improving aptamer performance, paving the way for more robust and efficient aptamer-based applications.

Список использованных источников:

1. Chen X., Jia B., Lu Z., Liao L., Yu H., Li Z. Aptamer-Integrated scaffolds for biologically functional DNA origami structures // ACS Applied Materials & Interfaces. – 2021. – Vol. 13, No. 33. – P. 39711–39718.
2. Musumeci D., Montesarchio D. Polyvalent nucleic acid aptamers and modulation of their activity: a focus on the thrombin binding aptamer // Pharmacology & Therapeutics. – 2012. – Vol. 136, No. 2. – P. 202–215.