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INNOVATION IN ACTION: TURNING IDEAS INTO REALITY

Chapter 32

Molecular Docking Study of Aptamer-Based Biosensor for Detection of Shiga Toxin (Stx) Protein from Escherichia Coli O157:H7

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ABSTRACT

The increasing prevalence of pathogenic Escherichia coli (E. coli) in water and food sources poses a significant threat to public health, necessitating the development of rapid and accurate biosensor detection methods such as aptamer-based biosensors due to their high specificity and sensitivity. Aptamers are nucleic acids that can bind with high affinity and specificity to a range of target molecules. This research aims to investigate biophysical mechanisms by utilizing biophysics simulations such as molecular free energy calculation and molecular docking to elucidate the interaction between specific aptamer with E. coli protein such as Shiga Toxin (Stx). The methodology involves characterizing aptamer-E. coli interactions, identifying key aptamer structural features and docking analysis of binding process between aptamer and E. coli. In conclusion, this research bridges theory for future applications, providing a framework for developing advanced biosensing technologies, by using the in-silico strategies that allowed the detection of aptamer-target interaction during molecular docking processes.

Key Words: Escherichia Coli; Aptamer; Protein; Molecular free energy; Molecular docking

1. INTRODUCTION

Enterohemorrhagic E. coli (EHEC), especially the O157:H7 strain producing Shiga Toxin protein (Stx), can cause serious foodborne illnesses like bloody diarrhea and hemolytic uremic syndrome (HUS) (Liu et al., 2022) With its rising presence in food and water, rapid and accurate detection method is critical. This study focuses on designing aptamer and Stx protein structures, performing molecular docking, and calculating Minimum Free Energy (MFE) and docking scores using HDOCK.

2. LITERATURE REVIEW

Aptamer-based biosensors have emerged as a promising tool for pathogen detection due to their high specificity, sensitivity, and stability. Aptamers are single-stranded oligonucleotides that fold into defined architectures and bind to targets such as proteins with high affinity and specificity. Aptamer is built with three structures, primary structures are built with a long sequence of nucleotides (A, T/U, C, G). The primary structures are fundamental for the secondary and tertiary structures. Next, secondary structures, generally a two-dimensional structure can form various secondary structures like hairpins, loops and bulges which contribute to their overall 3D shape and binding capabilities. The evaluation of good stability of these structures is according to their lowest molecular free energy (MFE) value. Lastly, tertiary structure or commonly known as three- dimensional (3D) structure enables aptamers to recognize and bind to their specific targets in molecular docking and molecular dynamic simulation (Liang et al., 2024). Furthermore, the ligand-protein docking is to predict how a protein interacts with ligands of known 3D structure (Mohanty & Mohanty, 2023). Docking score is an algorithm designed to compute the binding affinity of a protein-ligand complex and as an evaluation for good docking process (Yang et al., 2022). Simulating the binding at the atomic level emphasizes how the aptamer strongly binds to target, which affects the biosensor's selectivity and sensitivity.

3. METHODOLOGY

3.1. Aptamer preparation

The structure of aptamer was retrieved from Protein Data Bank with PDB code: 2AU4. Aptamer was shuffled using shuffleseq tools from usegalaxy.eu web server with 1000 number of shuffles. The validation of the aptamer's structures sequence was based on their molecular free energy (MFE) produced by RNALfoldz tools. The best five sequences from a thousand were chosen, has the lowest MFE value as presented in Table 1. This study uses RNAfold WebServer as it provides an interactive graphical output of the MFE structure or aptamer secondary structure as shown in Figure 1. From that, RNAfold WebServer was used to transform the FASTA sequence format into dot-bracket format. Then, the five sequences of aptamer chosen are generated in RNAComposer software for tertiary or 3D structure in PDB file format as in Figure 1.

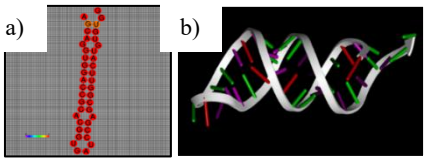

Figure 1: a) secondary structures b) tertiary structures of 2AU4 Aptamer for model 1

3.2. Target peptide/protein Escherichia coli preparation

The crystal structure of target protein Escherichia coli was retrieved from PDB code: 1C48 called as Shiga Toxin (Stx). Throughout this project, AlphaFold 3 were used as this software shows highly accurate prediction of the long peptide sequence structures. The 3D structures were visualized in Protein Data Bank (PDB) format using BIOVIA Discovery Studio software as shown in Figure 2 for analyse the Ramachandran plot. The Ramachandran Plot was used to confirm the structure of peptide generate by this software. Ramachandran Plot is a graphical representation of the dihedral angles, Phi (ϕ) and Psi (ψ) of amino acid residues in protein structures. It highlights regions for alpha helices (Q-I & Q-III) and beta sheets (Q-II), with some areas allowed, and others rarely used or unstable (Q-IV).

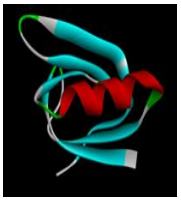

Figure 2: 3D structures of Stx in the forms of solid ribbon

3.3. Molecular docking of protein Escherichia coli and aptamer

Five chosen aptamers were docked with the protein Stx obtained from AlphaFold 3 as it shows stable structures as shown in Ramachandran plot, Figure 3. HDOCK server is a protein-protein and protein-DNA/RNA docking based on a hybrid algorithm of template-based modelling and ab initio free docking. The molecular docking process was using HDOCK server without any adding specification, and the results showed top ten possible docking residues prediction for each of five specific chosen aptamers and Stx protein in the PDB files format to be analyzed in the BIOVIA.

4. RESULTS & DISCUSSION

4.1. Structures of candidate aptamers and target protein Escherichia coli

Result from the Table 1, conclude that the aptamer model 1 has the lowest MFE values from RNAfoldz tools which is -24.0 kcal/mol and followed by model 2 and 3 which are -23.4 kcal/mol and -22.6 kcal/mol. However, for models 4 and 5, the MFE values are the lowest. These results suggest that all the 2AU4 aptamer sequences exhibit lower MFE, suggesting structural stability (Alkriz & Joujeh, 2024).

From the Ramachandran plot, Figure 3, Stx protein operated in AlphaFold 3 visualize that both regions, β-sheets (quadrant II) and right-handed α-helix (quadrant III) are the most prevalent structure in Stx. This suggest that Stx structures imparts stability to proteins and is crucial for their proper folding and function.

Table 1 Molecular free energy (MFE) values for the best five aptamer models from RNALfoldz tools

Aptamer Model	Aptamers sequences	MFE (kcal/mol)
1	AGCAGGUGGACCGCACGGUGAUCCGAGCGGUUCAUGUGUGG	-24.0
2	ACGGUUAGGGUGGUGGGGAACCACCACCGUUGAUCGCUGGG	-23.4
3	ACCCUCGAAACGGCGAGCGAGGGUGUCGCUGUUUGAGGGGU	-22.6
4	CUGGGGAGGCAAGCUGGGGACCGUAGCUUGCAGUCCUGGAU	-20.5
5	AGUAGCUGGAUGGCGACACUCAGUGGGCGUCGUGGUCGAGC	-20.0

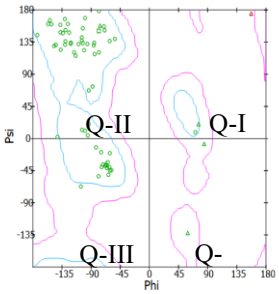

Figure 3: Ramachandran plot for AlphaFold 3

4.2. Molecular docking analysis

The analysis in 3.1 concludes that all of the 2AU4 aptamer structures are stable to be docking with AlphaFold 3's structures to estimate the binding affinity. The docking scores of the best prediction model in each of the five chosen aptamers with Stx are presented in Table 2, where the aptamers are ranked based on their docking scores. In general, the lower the rank, the better (Abd Halim et al., 2022). The results show that model 3 have the lowest docking scores of -267.50 as shown in Figure 4. This is followed by model 2 and 5 with the binding energy of -250.90 and -244.88 respectively. However, even though model 1 has the lowest MFE values, it has higher docking scores of -232.13 compared to model 3, 2 and 5.

Table 2 Docking scores for the best prediction model from five aptamer models from HDOCK

Aptamer model	Aptamer sequence	Docking scores
3	ACCCUCGAAACGGCGAGCGAGGGUGUCGCUGUUUGAGGGGU	-267.50
2	ACGGUUAGGGUGGUGGGGAACCACCACCGUUGAUCGCUGGG	-250.90
5	AGUAGCUGGAUGGCGACACUCAGUGGGCGUCGUGGUCGAGC	-244.88
1	AGCAGGUGGACCGCACGGUGAUCCGAGCGGUUCAUGUGUGG	-232.13
4	CUGGGGAGGCAAGCUGGGGACCGUAGCUUGCAGUCCUGGAU	-220.23

Figure 4: 3D structures of best aptamer model 3 (white) binding with target protein Shiga Toxin (blue/red) in molecular docking with -267.50 docking scores

5. CONCLUSION AND RECOMMENDATION

In this study, in-silico methods, including MFE calculation and molecular docking, were used to evaluate aptamers against the target protein of E. coli. The 2AU4 aptamer model 3, Figure 4, showed the lowest MFE (-23.4 kcal/mol) and docking score (-267.50), making it the best candidate for further Molecular Dynamics (MD) simulations to assess structural stability through RMSD and RMSF analysis. In conclusion, the strong docking results suggest this aptamer could be a useful detector in future aptamer-based biosensors, such as colorimetric sensors for detecting Shiga Toxin protein in E. coli O157:H7.

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