Research in Molecular Medicine

Evaluating the Anti-*Salmonella* Aptamer Using Bioinformatic Tools and Enzyme-linked Aptamer Assay Method

Fatemeh Bozorgmehr, Mehdi Zeinoddini*, Hamid Reza Rasouli Jazi

1. Department of Bioscience and Biotechnology, Faculty of Chemistry and Chemical Engineering, Malek-Ashtar University of Technology, Tehran, Iran.

Use your device to scan and read the article online

Citation Bozorgmehr F, Zeinoddini M, Rasouli Jazi HR. Evaluating the Anti-*Salmonella* Aptamer Using Bioinformatic Tools and Enzyme-linked Aptamer Assay Method. Research in Molecular Medicine. 2022; 10(3):125-136. https://doi.org/10.32598/rmm.10.3.3.1

https://doi.org/10.32598/rmm.10.3.3.1

Article Type:
Research Paper

Article info:
Received: 10 Jan 2022
Revised: 23 Mar 2022
Accepted: 25 Jun 2022

Keywords:
Salmonella, Diagnosis, Aptamer, Bioinformatic analysis, Enzyme-linked aptamer assay (ELAA)

ABSTRACT

Background: Identifying and quantifying food pathogens and environmental samples requires accurate, high-sensitivity, and specific methods. *Salmonella* is a zoonotic bacterium that can directly cause human diseases. This work aims to develop anti-*Salmonella* aptamer using bioinformatic tools and enzyme-linked aptamer assay (ELAA) to identify *Salmonella* and assess its affinity to simulated protein.

Materials and Methods: An aptamer sequence was chosen to attach to *Salmonella*'s surface protein (outer membrane protein A [OmpA]). Then, the aptamer's secondary and tertiary structures were achieved using the UNAFOLD and ROSETTA servers. The structure of OmpA was simulated by two methods: ab initio (using I-TASSER online server) and homology modeling (using MODELLER software and molecular dynamics simulation). In the next step, aptamer-protein binding was assessed via HDOCK software. Afterward, aptamer and biotin-labeled primers were synthesized to amplify specific sequences. Finally, the performance of aptamer to detect *Salmonella* was assessed by streptavidin conjugated with horseradish peroxidase (SA-HRP) using the ELAA method.

Results: This study showed that the aptamer binds to OmpA and is acceptable with a -283.3 docking score. The superiority of the selected aptamer to random aptamers was also reviewed and approved. After assuring the validation of an aptamer with bioinformatic analysis, the aptamer was cloned in the pTZ57R plasmid and then used as a template in PCR. After the optimization of PCR and ELAA, aptamer performance was successfully performed to identify *Salmonella*.

Conclusion: The designed aptasensor can be used for future investigations to detect *Salmonella*.

*** Corresponding Author:**
Mehdi Zeinoddini, Associate Professor.
Address: *Department of Bioscience and Biotechnology, Faculty of Chemistry and Chemical Engineering, Malek-Ashtar University of Technology, Tehran, Iran.*
Phone: *+98 (21) 22974604*
E-mail: *zeinoddini52@mut.ac.ir*

Introduction

Gram-positive and gram-negative bacteria, even at low concentrations, are responsible for infectious diseases [1]. *Salmonella* is a gram-negative bacteria that is the leading cause of contagious diseases and accounts for thousands of deaths worldwide [2]. Both classic zoonotic and, more importantly, the foodborne transmission of *Salmonella* spp. have been documented [3]. Typically, the isolation of *Salmonella* from swabs, food, and other environmental samples through the traditional culture approach involves several steps of priority to enrichment, selective enrichment, and growth on selective media to increase the sensitivity of the detection methods [4]. The performance of current detection technologies is limited because of their difficulty, high cost, time-consuming, and low sensitivity and availability [5]. Unfortunately, diagnosing *Salmonella* and other pathogens in complex instances, such as feces, food [6], and environmental samples, is challenging for several reasons. Monoclonal and polyclonal antibodies have recently been the most common compound ligands for pathogens capture. Designing simple, fast, and economical methods with minimal laboratory equipment is very suitable for researchers who can help detect bacteria. Gram-negative bacteria express outer membrane proteins (OMPs) such as outer membrane protein A (OmpA), porins (OmpC and OmpF), and lipoproteins that activate innate cells via toll-like receptors. OmpA is one of the significant OMPs that forms into the outer membrane via an N-terminal 8 transmembrane amphipathic β-barrel region [7]. In contrast, the C-terminal region is retained in the periplasm [8, 9]. Then again, DNA or RNA single strands molecules, called aptamers, capable of binding to non-nucleic acid molecules, are used to identify microorganisms.

Aptamers are attained through a technique known as the systematic evolution of ligands by exponential enrichment (SELEX). SELEX includes various stages such as incubation of oligonucleotide library, separation, amplification by polymerase chain reaction (PCR), and purification [10]. Aptamers are single-stranded oligonucleotides that can naturally be divided into three-dimensional (3-D) structures. They have the capability of connecting to bio surfaces precisely [11]. Aptamers can assume stable 3-D configurations, such as lops, triplexes, pseudoknots, G-quadruplexes, and staples in the aqueous phase. Aptamers may distinguish target molecules between secondary or tertiary structures. For example, compounds such as amino acids are combined for interaction using pseudoknots, internal rings, and unilateral bulges [12, 13]. It is possible to integrate aptamers with different operating systems that will change the signal from aptamers to the analyte or electrochemical signals that are measurable and depend on the concentration of the sample. Although the development of aptamers has been concentrated on medical applications, the latest environmental developments have also been reported. Production of the aptamer is commercially available from multiple suppliers that provide services to regulate the sensitivity and structure of the produced aptamer. However, no commercial testing is based on aptamer allocated to environmental pollution [14, 15]. As novel therapeutic agents, aptamers, also known as chemical antibodies, have several advantages over customary antibodies. Aptamers are smaller, can cause chemical changes, possess thermal and chemical stability [16], have lower-cost production, and are nonimmunological [17].

In this work, we studied the interactions of DNA aptamer with surface proteins of *Salmonella*. To ensure the effective binding between aptamer and OmpA, the protein modeling (with MODELER software & ab initio method, separately) and simulation (with molecular dynamic pathway) were performed. We selected a DNA aptamer that interacts explicitly with OmpA based on previous experimental studies. The tertiary structure of an aptamer was predicted, and the possibility of protein binding to the aptamer was examined via docking procedures. At the next step, aptamer was cloned, sequenced, and characterized for binding efficiency. This study aimed to assess aptamer to detect *Salmonella* using bioinformatic analysis and enzyme-linked aptamer assay (ELAA).

Materials and Methods

Single-stranded DNA aptamer modeling

Based on the previous SELEX-based enrichment studies for aptamer design (TATGGCGGCGTCACCCGACGGGGACTTGACATTATGACAG), this aptamer was selected as a candidate that could be bound to the outer membrane protein A (OmpA) of *Salmonella* bacteria [18]. This process was extracted by a SELEX procedure with 7 rounds over a pool of several millions of random sequences directed against the OmpA of *Salmonella*. In this study, the 3-D structure of the ssDNA molecule was obtained from the nucleotide sequence over 4 main steps (Figure 1). The ssDNA aptamer was initially transformed to RNA, after which this structure was modeled. We prepared this structure with the following steps to model the sequence of ssDNA aptamer. The first step of our approach was to predict the secondary structure, which was itself predicted by UNAfold (server [19],

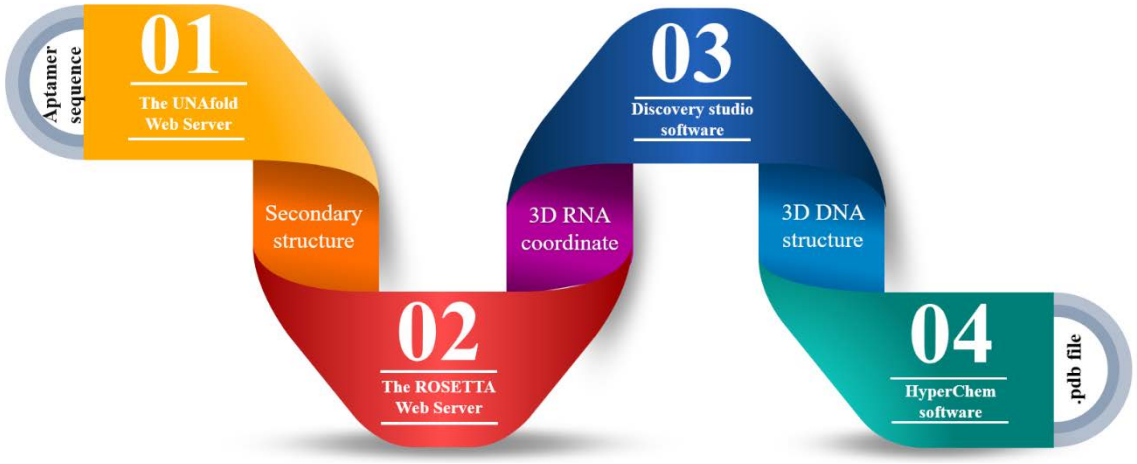

Figure 1. Schematic representation of the preparation of tertiary ssDNA structure

This approach consists of making the ssDNA secondary structure from the sequence using UNAfold (step 1), constructing 3D ssRNA models using ROSETTA server (step 2), transforming the 3-D ssRNA models into ssDNA models using Discovery Studio (step 3), and optimizing the 3-D ssDNA structures using HyperChem (step 4).

based on free energy minimization. In the next step, the thymidine nucleotides were replaced with uracil nucleotides in the DNA structure. This structure was used as an input for the ROSETTA server [20] to determine the tertiary structure of ssRNA (step 2). For this purpose, in the FARFAR2 section, the sequence and secondary structure of interest aptamer were submitted. After selecting 2000 structures, the best-suggested model was used for the following procedure. Then, the RNA spatial structure was converted to DNA using Discovery Studio software, version 16.1.0 (step 3). As the applied changes, the uracil nucleotides were replaced with thymidine, and the ribose sugars were transformed into deoxyribose sugars. In the final step, 5000 steps of the steepest descent minimization were executed by HyperChem software, version 8.0.10 (step 4). The output of this step (.pdb file) was saved and used for the subsequence analysis.

Homology modeling

The MODELER software was used for comparative or homologous modeling 3-D protein structures [21]. It performs protein structure modeling despite spatial constraints. In this research, this software was used to model the desired protein and evaluate its performance in binding with the selected aptamer. Based on the previous reports, OmpA has been introduced as one of the candidate targets for binding with aptamer [18]. For this purpose, the protein's amino acid sequence (from the UniProt [22]) was received in FASTA format. In the next step, to obtain homologous proteins with the template protein (OmpA), the sequence of the protein was done BLAST via the protein data bank (PDB) database [23]; three proteins (1qjp, 4rha, and 2k0l) which had the highest similarity with the OmpA were selected as a pattern. These proteins were chosen as the patterns according to the indices, such as the quality and resolution of the proposed model, overlapping of different protein sections with template protein, etc. After obtaining the PDB files of the template proteins, the alignment file was prepared using Clustal Omega [24]. In the next step, multiple template modeling was used, and the best model with the lowest DOPE (discrete optimized protein energy) score was used for molecular dynamics.

Molecular dynamics simulation

A molecular dynamic simulation was used to find suitable protein motifs orientation to another. It was carried out to confirm and improve the modeled protein by homology modeling through GROMACS with CHARMM36 all-atom force field and TIP3P as a selected water model. Protein was soaked in a cubic box of water molecules, and the periodic boundary conditions (PBCs) were applied to the system in all spatial directions. The box coordinates such that the protein is located in the center and extends 1 nm from each side. The charges on the protein were neutralized by adding $5Na^{+}$ ions. For energy minimization, 50000 steps were used in the steepest descent method. In addition, the particle mesh Ewald (PME) algorithm method was used for NVT (constant number of particles, volume, and temperature) equilibrium, and Parrinello-Rahman was used for NPT equilibrium, wherein the number of particles, pressure, and temperature are

all constant. After completing two equilibrium steps, the system correctly fits the desired temperature and pressure and is prepared to implement the molecular dynamics (MD) process for data collection. This step was carried out for 30 ns at 300 °K. The obtained .pdb file from this step was saved for subsequent analyses.

Ab initio modeling

For another modeling method, the amino acid sequence of the protein from the UniProt) [22] was received in FASTA format. The sequence was simulated in the De novo method by the I-TASSER [25] database. Then, the optimization of the prediction outputs of the I-TASSER server was done with PdbViewer software (SPDBV) [26]. The .pdb output file from this step was saved for the subsequent analyses.

Models validation

Ramachandran plots and Z-score points were used to compare and validate the accuracy of the predicted structures. The Ramachandran plots were constructed using the Ramachandran plot server [27] and the ProSA-web server [28] was utilized to calculate the Z-score.

Molecular docking

The interaction between the OmpA and aptamer was evaluated through online servers. The studies of the interaction of aptamer and simulated protein were carried out via the HDOCK server. One service of this server is to examine the protein-RNA/DNA interaction that can provide results in a limited time. To achieve this purpose, by providing input data for the receptor and ligand molecules, the server automatically predicts an interaction via a combination of template or free-based docking algorithms [29]. Regarding the analysis and validation of the binding results of the simulated protein through molecular dynamics and the non-randomness of the results that show the high affinity of the aptamer binding to the simulated protein, 100 random sequences were obtained by the server [30] and their secondary and tertiary structures were provided and saved in .pdb format. Then, the modeling of the tertiary structure, as explained in previous sections, was implemented on them, and the docking of each structure with the simulated protein was done.

Cloning of candidate aptamer

After screening and examining the aptamer function through bioinformatics analyses, the selected sequence was cloned into the pTZ57R plasmid. During the cloning process, amplified aptamer was used in ligation production. With the confirmation of PCR products, 5 µL of PCR product was added to 1 µL linear TA vector, 1 µL T4 buffer, 0.5 µL T4 ligase, 0.5 µL PEG, and 2 µL H_2O. The mixture was kept at room temperature for 2 hours. In the next step, 5 µL of the ligation mixture was added to 200 µL of competent cells of *E. coli* DH5 (prepared with the calcium chloride method). The mixture was incubated for 30 min on ice. After a heat shock at 42°C for 90 s, it remained again for two minutes on ice. After adding 100 µL of LB-broth, for 50 min shaking at 37°C with a thermocycler, the entire content was plated onto the LB agar plate containing ampicillin antibiotic at 37°C overnight. The next day, the recombinant plasmid was formed on the colonies on the plate containing antibiotics. To confirm the entrance of aptamer into the plasmid, we utilized colony PCR, digestion (with EcoRI and HindIII), amplification (with M13 universal primer), and sequencing.

Cell culture and plasmid extraction

The recombinant *E. coli* was primarily cultured in an LB broth medium (0.1 g tryptone, 0.5g yeast extract, 0.1 g sodium chloride, soluble in distilled water, pH 7.0) overnight at 37°C on a shaker incubator. The harvested cells were centrifuged at 9000×g and 25°C for 5 min, and their genomic DNA was isolated using a high pure DNA purification kit, according to the manufacturer's instructions. After plasmid extraction, the plasmid is used as a template.

Amplification with specific primers

In this study, we used a DNA aptamer to detect *Salmonella*. The aptamer was a single-stranded DNA containing 40 bps that was cloned in the TA vector. At the 5′ and 3′-end of this aptamer, 15 and 20 bases were designed for aptamer amplification using forward and reverse primers (Table 1). It should be noted that the forward primer was marked with biotin. The aptamer amplification was used by thermocycler and PCR technique, and the amplified sample was used to control testing the agarose gel 2.5% (with TAE).

Assessment of the functionality of aptamer with ELAA technique

To control the aptamer function, we screened its binding to the target molecule through the ELAA technique. This technique is equivalent to ELISA, which is used instead of an antibody from oligonucleotide. For this purpose, the bacterium was coated in ELISA strips. Afterward, the work step was done as follows: Incubation of bacterium in strips (14-15 hours), 3 hours incubation with blocking solution (3% BSA), washing (phosphate buffer saline

[PBS]) 3×5 min, 2 hours incubation with aptamer, washing (PBS) 3×5 min, 30 minutes incubation with SA-HRP, washing (PBS) 3×5 min and in the end, the detection steps with tetramethyl benzidine chromogen and peroxide substrate were conducted. In this step, formation or lack of blue color was recorded. Finally, H_2SO_4 was added to stop the reaction, and the absorbance was read at 450 nm.

Statistical analysis

Using SPSS software, version 27.0.1 a one-sample t-test was designed and compared with 100 random aptamers. For this purpose, the software tools calculate the means of the data after entering the data. Then, the software designed and analyzed the one-sample t-test using the statistical data's output. The test value was considered -283.32 in this study.

Results

Prediction of the tertiary structure of ssDNA aptamer

The DNA aptamer was 40 nucleotides in length. In Figure 2, the 2-D and 3-D structures are shown.

Protein modeling and MD simulation

One hundred models with different DOPE scores were obtained by MODELER software, version 10.1. The best model with the lowest DOPE score (Figure 3b) was used for molecular dynamics. After modeling the protein, a molecular dynamic simulation was performed (Figure 3c). The simulation results, including analysis of root mean square deviation (RMSD) and gyration radius, showed that the structure has become more stable with the progress of MD simulation. However, this short simulation aims to check the position and orientation of amino acids in the connection region of two motifs and reduce non-favorable dihedral angles (Figure 4). The root mean square fluctuation (RMSF) chart depicts the fluctuation of amino acids. Examining this chart in the point of motifs joint shows that there has not been a significant change in this part. RMSF showed the fluctuation of the various motifs of the protein. The orientation between two different motifs occurs from 230–250 residues because there is no significant fluctuation (Figure 4). The OmpA Rg value confirmed the stabilization of the process during the simulation time of 30 ns (Figure 4). I-TASSER models are ranked based on their C-scores. Among the offered models, the best model with the highest C-score (Figure 3a) was selected for validation analyses.

Comparison of protein's different models

Protein structures were assessed by the Ramachandran plot. The Ramachandran plot showed the simulated model through molecular dynamic simulation that 98%, 1.3%, and 0.7% of residues were in the highly, preferred

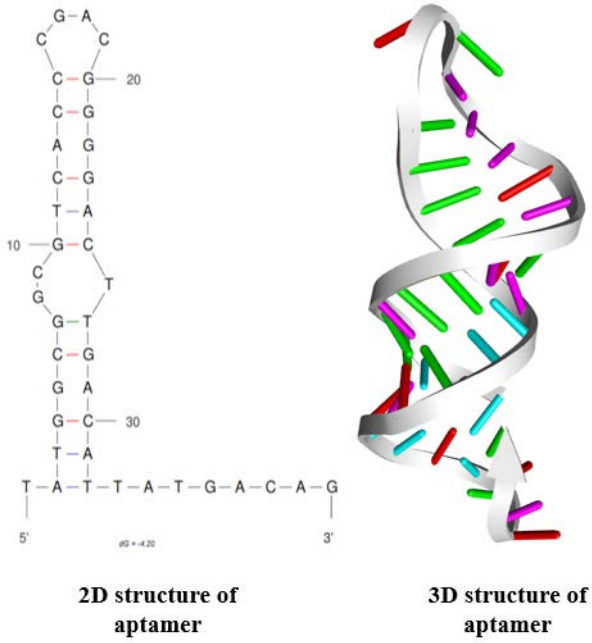

Figure 2. The 2-D and 3-D structures of ssDNA aptamer

The ΔG energy of folding for ssDNA aptamer is -4.20 kcal/mol. Nucleotides in 3-D structure are G (green), A (red), T (blue), and C (purple).

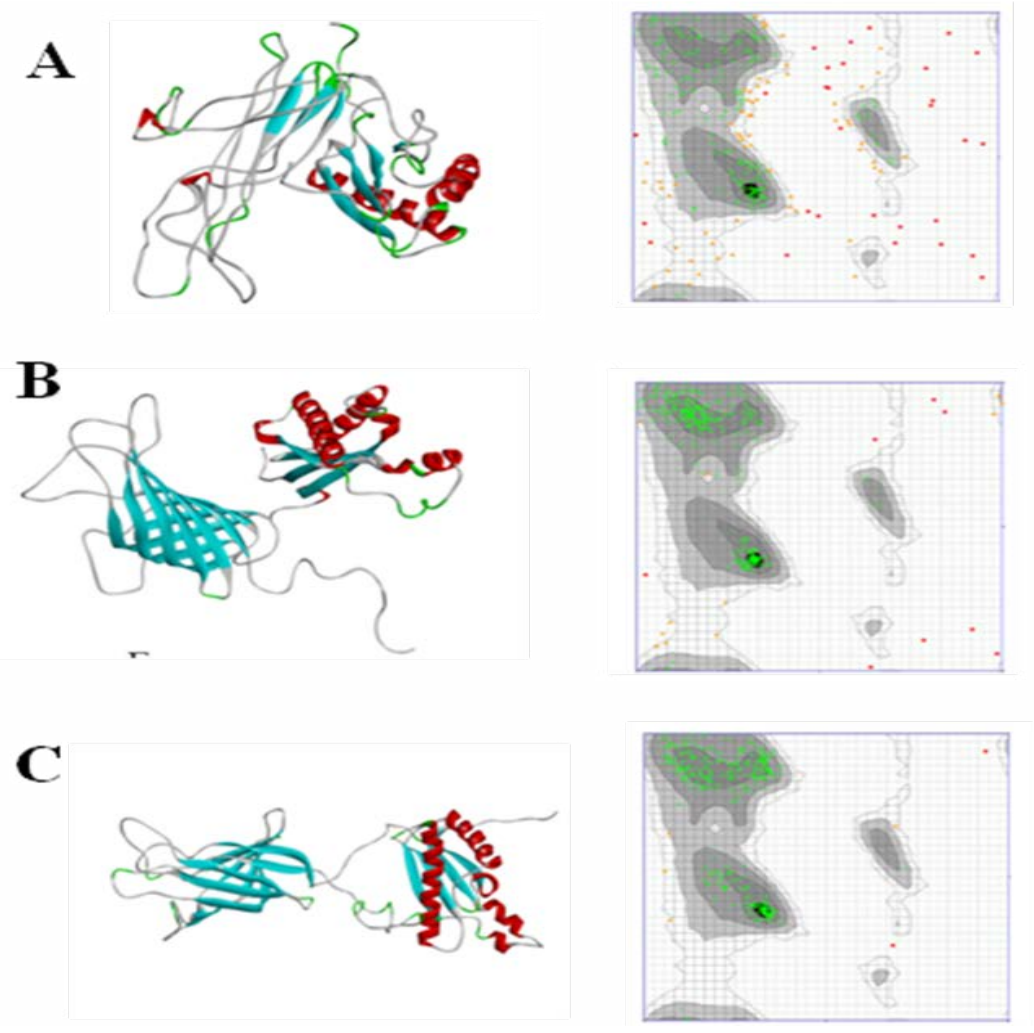

Figure 3. 3D structures of proteins and validation results

A) The structure of OmpA is predicted through I-TASSER and its Ramachandran plot, B) The structure of OmpA is predicted through MODELLER and its Ramachandran plot, C) The structure of OmpA is predicted through molecular dynamic simulation and its Ramachandran plot

and questionable observation, respectively (Figure 3c). The Ramachandran plot about modeling with MODELLER was reported at 93%, 4%, and 3%, respectively (Figure 3b). This data about the De novo simulation was reported at 66%, 23%, and 11%, respectively (Figure 3a). The ProSA-web results have shown a Z-score of -6.13, -5.88, and -4.78 for I-TASSER, homology-modeled OmpA, and molecular dynamic simulation, respectively. Figure 5 shows the data validation results. The structure simulated by the molecular dynamic simulation method shows the best quality.

Molecular docking

To investigate the interactions of OmpA with aptamer to identify the possible binding locations of the protein to the aptamer, we performed molecular docking computations using the HDOCK server. The HDOCK server presented the -283 docking score as the best (Figure 6). The server presented the results, which showed that the score of aptamer and simulated protein would be more suitable than those of all DNA random structures. Interactions between aptamer and OmpA are shown in Figure 6. This issue was confirmed by a 1-sample t-test analysis with SPSS software. The results of this analysis include the calculation of the mean of random structures that were compared with the aptamer docking score during the one-sample t-test analysis. The results showed a significant difference, with a $P<0.001$ (Table 2).

Aptamer production

After the approval of aptamer performance, it was selected for the following studies in the laboratory. Plasmid

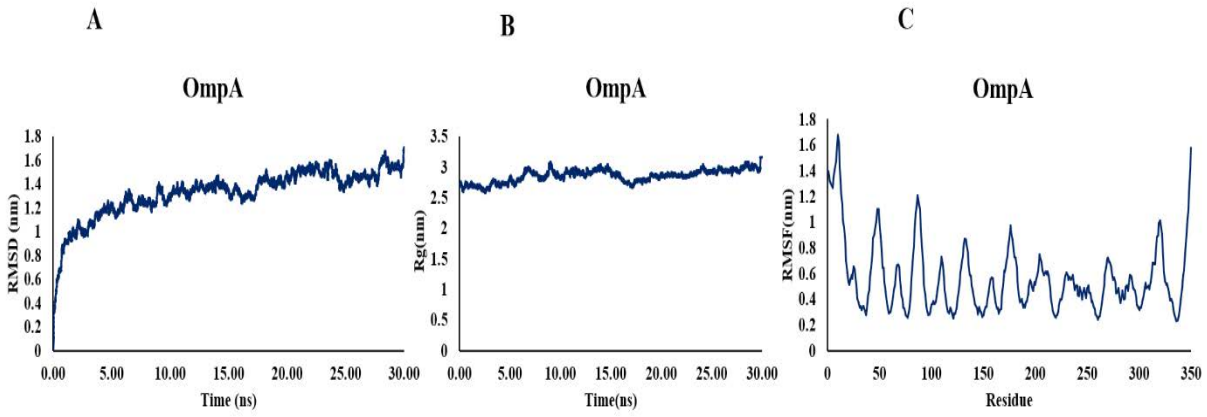

Figure 4. RMSD, Rg, and RMSF graphs of OmpA during 30ns MD simulation

pTZ57R-apta (Figure 7) was used for aptamer production. The insert fragment is shown in red. The aptamer amplification was performed using a biotinylated forward primer (Figure 7). The amplification of the DNA fragment was confirmed by agarose gel 2.5%. Also, the results of the enzyme digestion and amplification with the M13 primer on the agarose gel are shown in Figure 7. The results confirm the accuracy of the insert fragment to the TA vector. Finally, the sequence of the pTZ57R plasmid was also confirmed by the sequencing technique (data not shown).

Detection of *Salmonella*

To detect *Salmonella*, we used PCR products of a biotin-labeled forward primer (the reverse primer was not labeled). In ELAA optimization, we used bacterial immobilization and aptamer incubation steps at different times. The optimal times for bacterial immobilization and aptamer incubation were determined overnight and in 3 hours, respectively. One well was also used, without aptamer, as the negative control. According to the results, the aptamer sequence was specific for *Salmonella* (Figure 8), and the minimum aptamer concentration for this assay was approximately 100 ng. Also, increasing the concentration up to 600 ng does not affect the performance of the aptamer.

Discussion

The *Salmonella* genus displays the most foodborne pathogens isolated from food-producing animals responsible for zoonotic infections in humans and animal species [31]. Several methods with high sensitivity and

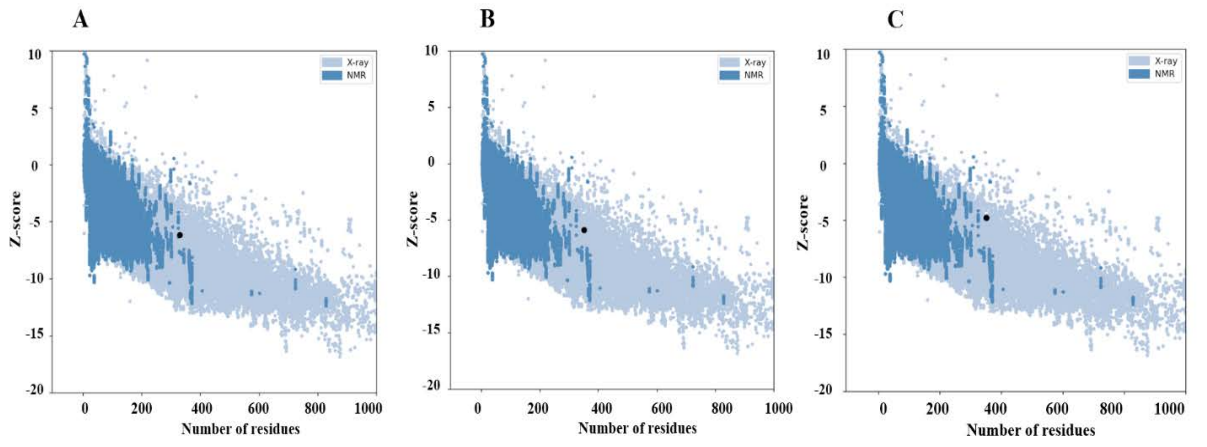

Figure 5. The plot exhibition of de novo, homology modeling, and MD simulation results
The black dot shows the similarity of the model with x-ray and nuclear magnetic resonance (NMR) structures.

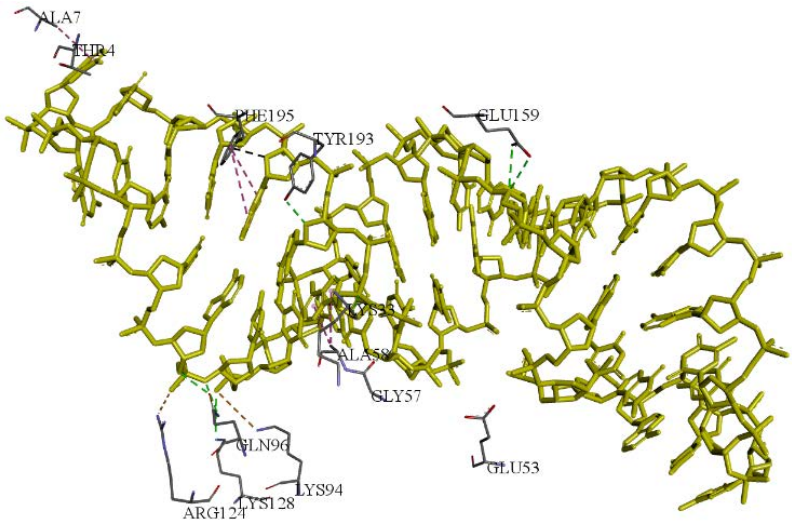

Figure 6. Schematic of interaction between OmpA and aptamer in Discovery Studio software

Seven hydrogen bonds (green), 2 electrostatic(orange), 5 hydrophobic (purple), and 1 other (black) interaction were observed in this binding.

specificity are used for *Salmonella* identification. Aptamers can be developed with high affinity and specificity to interact with desired targets [32]. Recently, numerous analytical and classical methods have been used to identify *Salmonella*. In one research conducted by Joshi et al., the specific DNA sequence for detection of *Salmonella* OMP was obtained after 7 times of screenings. Their study used the South-Western blot analysis and mass spectrometry to identify *Salmonella* bacterium [18]. This investigation claimed that anti-OmpA *Salmonella* aptamer could detect *Salmonella*. In recent years, several *Salmonella* OMPs have been investigated as diagnostic antigens, virulence factors, and vaccine candidates [33], and the function of their genes and the molecular structure of OMPs have been studied [34-36]. However, only a few OMPs have already been identified [37]. Based on the studies conducted by Joshi, OmpA was a candidate for the detection of *Salmonella* species [18].

In this study, we developed 3-D models of OmpA by comparing various in silico methods, such as homology modeling and ab initio, as well as molecular dynamics

Table 1. Sequences of primers and aptamer used in the study

Name	Sequence
Forward primer	Biotin-TATGGCGGCGTCACC
Reverse primer	5 CTGTCATAATGTCAAGTCCC3
Aptamer	5 TATGGCGGCGTCACCCGACGGGGACTTGACATTATGACAG 3

Table 2. The result of 1-sample t-test

N	Mean±SD	Mean±SE
Docking score 100	-249.0409±13.84609	-249.0409±1.38461

t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
				Lower	Upper
Docking score 24.757	99	0.000	34.27910	31.5317	37.0265

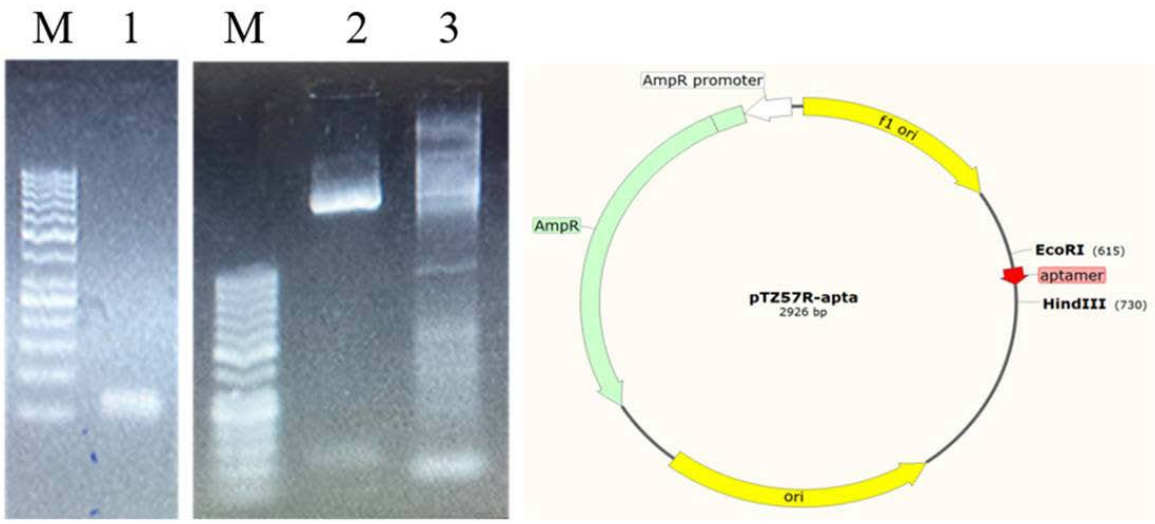

Figure 7. Agarose gel electrophoresis of aptamer amplification and recombinant pTZ57R vector (pTZ57R-apta) in SnapGene software

M: DNA ladder (50bps), 1: Aptamer, 2: Digestion of pTZ57R plasmid with EcoRI and HindIII (114bps), 3: Amplification of 91bps fragment (with universal primer).

simulation. Protein structures generally determine their biochemical functions [38]. The structure of proteins could be assessed by experimental and theoretical approaches. Bioinformatics methods are significant before the experimental determination of 3D protein structures [38, 39]. Researchers have claimed that using multiple patterns "naturally" increases the modeling accuracy with the variability and divergence of natural structures [40]. OmpA sequence served as a query for a BLAST search against a protein data bank (PDB) to find the template with a lower *E* value, higher query coverage, and maximum identity. The use of sequence alignment methods to recognize the relationship between the target sequence and one or more possible templates is the first step in predicting the structure [41]. Based on BLAST search and alignment generations, the best predicted 3-D model of the OmpA was applied to MD simulation. Protein structure modeling was also performed by I-TASS-

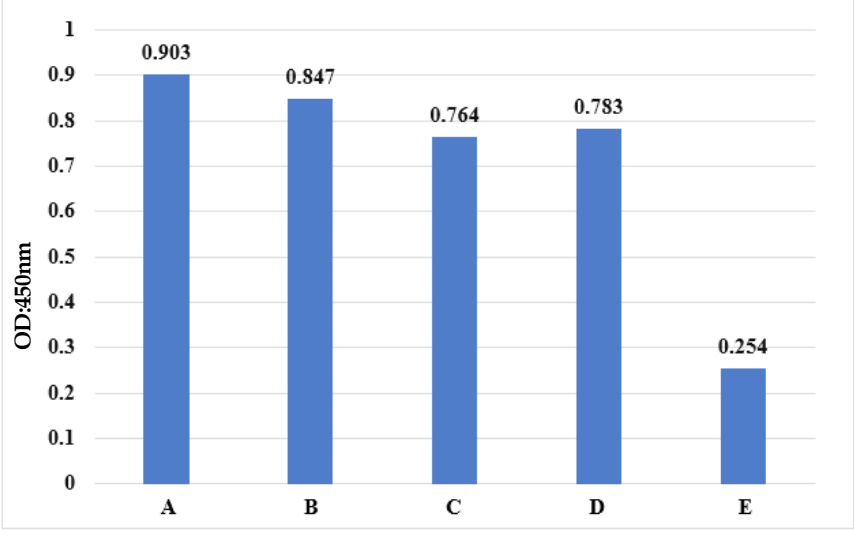

Figure 8. *Salmonella* detection using 100 (A), 200 (B), 300 (C), 600 (D) and 0 ng (E) aptamer by enzyme-linked aptamer assay (ELAA) technique at optimize condition

ER. The Ramachandran plot was used for the validation of structures. Further verification was carried out using the ProSA web server.

The results showed that molecular dynamics simulation protein with 98% of residues in the high observation and -4.78 Z-score could be the most suitable protein to continue bioinformatics analysis. Also, the RMSF results showed that the orientation of various protein motifs has occurred in residues from 230 to 250. Based on the previous studies, we selected a DNA aptamer with specific interactions with OmpA [18]. In particular aptamers, similar to monoclonal antibodies, interfere specifically with the target and detect or block the function of a target molecule. The achievement of the aptamer with the highest affinity for the target is solved with the SELEX procedure or through computational approaches [42]. To use the aptamer for bioinformatical analysis, ssDNA structure was achieved by the Rosseta server, whose use helped us to reduce the cost. Then, to investigate further interactions of aptamer with OmpA in terms of identifying the possible binding locations of the aptamer to the protein, we performed molecular docking computations using the HDOCK server. Tools such as HDOCK are used to analyze the propensity of thousands of ssDNA to a dedicated protein [43]. Docking results indicate the interaction of aptamer and protein with a -283 docking score. The previous experimental studies that illustrated the DNA molecules interacting with the outer membrane protein of *Salmonella* [18] were approved with the result of docking calculations. The probability of interaction is also confirmed through statistical analysis with SPSS software, and the difference was significant.

After confirming the results of the bioinformatical analysis, the cloning process was carried out, and the recombinant plasmid was used as a template in the PCR amplification. Finally, we have developed an enzyme-linked aptamer assay (ELAA) to detect OMPs of *Salmonella* using the selected aptamer. ELAA showed recognizable signals against *Salmonella*. This assay was also suitable for detecting *Salmonella* by minimum aptamer amount (100 ng). The results indicate the possibility of developing an aptasensor to detect *Salmonella* in the next investigation. In 2013, Dwivedi [44] used a DNA aptamer to identify *Salmonella* due to a magnetic capture. In this study, aptamers were marked, and DNA aptamer pools were classified after biotin labeling. In addition, an electrochemical sensor was made to detect *Salmonella*. Despite high accuracy, methods such as mass spectrometry and western blot techniques [18] require advanced experimental equipment and expert users. However, the successful use of the ELAA technique for Staphylococcus was performed and presented acceptable results [45]. Due to the advantages of this technique, such as ease of implementation, low cost, and speed of detection, this technique has been carried out on the bacterial strain of *Salmonella*. In this study, bioinformatical devices were studied to connect aptamer to *Salmonella* surface proteins before implementation of the laboratory phase. In the end, the performance of aptamer was determined using optimized PCR and ELAA techniques. In conclusion, these results indicated the accuracy of aptamer for OmpA *Salmonella* detection, and it could be used in future works as an aptasensor for investigation and utilization of *Salmonella* identification.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors contribution's

All authors equally contribute to preparing all parts of the research.

Conflict of interest

The authors declared no conflict of interest.

References

[1] Soundy J, Day D. Selection of DNA aptamers specific for live Pseudomonas aeruginosa. PLoS One. 2017; 12(9):e0185385. [DOI:10.1371/journal.pone.0185385] [PMID] [PMCID]

[2] Alzwghaibi AB, Yahyaraeyat R, Fasaei BN, Langeroudi AG, Salehi TZ. Rapid molecular identification and differentiation of common Salmonella serovars isolated from poultry, domestic animals and foodstuff using multiplex PCR assay. Arch Microbiol. 2018; 200(7):1009-16. [DOI:10.1007/s00203-018-1501-7] [PMID]

[3] Singh BR, Singh VP, Agarwal M, Sharma G, Chandra M. Haemolysins of Salmonella, their role in pathogenesis and subtyping of Salmonella serovars. Indian J Exp Biol. 2004; 42(3):303-13. [PMID]

[4] Birner P, Oberhuber G, Stani J, Reithofer C, Samonigg H, Hausmaninger H, et al. Evaluation of the United States Food and Drug Administration-approved scoring and test system of HER-2 protein expression in breast cancer. Clin Cancer Res. 2001; 7(6):1669-75. [PMID]

[5] Caygill JS, Davis F, Higson SP. Current trends in explosive detection techniques. Talanta. 2012; 88:14-29. [DOI:10.1016/j.talanta.2011.11.043] [PMID]

[6] Stevens KA, Jaykus LA. Bacterial separation and concentration from complex sample matrices: A review. Crit Rev Microbiol. 2004; 30(1):7-24. [DOI:10.1080/10408410490266410] [PMID]

[7] Pautsch A, Schulz GE. Structure of the outer membrane protein: A transmembrane domain. Nat Struct Biol. 1998; 5(11):1013-7. [DOI:10.1038/2983] [PMID]

[8] De Mot R, Vanderleyden J. The C-terminal sequence conservation between OmpA-related outer membrane proteins and MotB suggests a common function in both gram-positive and gram-negative bacteria, possibly in the interaction of these domains with peptidoglycan. Mol Microbiol. 1994; 12(2):333-4. [DOI:10.1111/j.1365-2958.1994.tb01021.x] [PMID]

[9] Park JS, Lee WC, Yeo KJ, Ryu KS, Kumarasiri M, Hesek D, et al. Mechanism of anchoring of OmpA protein to the cell wall peptidoglycan of the gram-negative bacterial outer membrane. FASEB J. 2012; 26(1):219-28. [DOI:10.1096/fj.11-188425] [PMID] [PMCID]

[10] Liu X, Zhang X. Aptamer-based technology for food analysis. Appl Biochem Biotechnol. 2015; 175(1):603-24. [DOI:10.1007/s12010-014-1289-0] [PMID]

[11] Takemura K, Wang P, Vorberg I, Surewicz W, Priola SA, Kanthasamy A, et al. DNA aptamers that bind to PrPC and not PrPSc show sequence and structure specificity. Exp Biol Med. 2006; 231(2):204-14. [DOI:10.1177/153537020623100211] [PMID]

[12] Bing T, Zheng W, Zhang X, Shen L, Liu X, Wang F, et al. Triplex-quadruplex structural scaffold: A new binding structure of aptamer. Sci Rep. 2017; 7(1):15467. [DOI:10.1038/s41598-017-15797-5] [PMID] [PMCID]

[13] Rasouli Jazi HR, Zeinoddini M, Arab SS. A novel in silico SELEX method to screen and identify aptamers against Vibrio cholerae. Curr Comput Aided Drug Des. 2023;19(6):416-424. [DOI:10.2174/1573409919666230126101635] [PMID]

[14] Díaz-Fernández A, Lorenzo-Gómez R, Miranda-Castro R, de-Los-Santos-Álvarez N, Lobo-Castañón MJ. Electrochemical aptasensors for cancer diagnosis in biological fluids-A review. Anal Chim Acta. 2020; 1124:1-19. [DOI:10.1016/j.aca.2020.04.022] [PMID]

[15] Kudłak B, Wieczerzak M. Aptamer based tools for environmental and therapeutic monitoring: A review of developments, applications, future perspectives. Crit Rev Environ Sci Technol. 2020; 50(8):816-67. [DOI:10.1080/10643389.2019.1634457]

[16] Pestourie C, Tavitian B, Duconge F. Aptamers against extracellular targets for in vivo applications. Biochimie. 2005; 87(9-10):921-30. [DOI:10.1016/j.biochi.2005.04.013] [PMID]

[17] Javaherian S, Musheev MU, Kanoatov M, Berezovski MV, Krylov SN. Selection of aptamers for a protein target in cell lysate and their application to protein purification. Nucleic Acids Res. 2009; 37(8):e62. [DOI:10.1093/nar/gkp176] [PMID] [PMCID]

[18] Joshi R, Janagama H, Dwivedi HP, Senthil Kumar TM, Jaykus LA, Schefers J, et al. Selection, characterization, and application of DNA aptamers for the capture and detection of Salmonella enterica serovars. Mol Cell Probes. 2009; 23(1):20-8. [DOI:10.1016/j.mcp.2008.10.006] [PMID]

[19] No author. DNA folding form [Internet]. 2023 [Updated 2023 November]. Available from: [Link]

[20] No author. NEW, ROSIE-2 beta apps, new cluster, shorter queue, improved performance [Internet]. 2023 [Updated 2023 November]. Available from: [Link]

[21] Webb B, Sali A. Comparative protein structure modeling using MODELLER. Curr Protoc Bioinformatics. 2016; 54:5.6.1-5.6.37. [DOI:10.1002/cpbi.3] [PMID] [PMCID]

[22] No author. Find your protein [Internet]. 2023 [Updated 2023 November]. Available from: [Link]

[23] No author. RCSB protein data bank (RCSB PDB) [Internet]. 2023 [Updated 2023 November]. Available from: [Link]

[24] No author. Multiple sequence alignment [Internet]. 2023 [Updated 2023 November]. Available from: [Link]

[25] No author. I-TASSER protein structure and function predictions [Internet]. 2023 [Updated 2023 November]. Available from: [Link]

[26] No author. Swiss-PdbViewer aka DeepView [Internet]. 2023 [Updated 2023 November]. Available from: [Link]

[27] No author. Zlab program in bioinformatics and integrative biology [Internet]. 2023 [Updated 2023 November]. Available from: [Link]

[28] No author. Proso web protein structure analysis [Internet]. 2023 [Updated 2023 November]. Available from: [Link]

[29] Yan Y, Tao H, He J, Huang SY. The HDOCK server for integrated protein-protein docking. Nat Protoc. 2020; 15(5):1829-52. [DOI:10.1038/s41596-020-0312-x] [PMID]

[30] No author. Random DNA sequence generator. [Internet]. 2023 [Updated 2023 November]. Available from: [Link]

[31] Jajere SM. A review of Salmonella enterica with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. Vet World. 2019; 12(4):504-21. [DOI:10.14202/vetworld.2019.504-521] [PMID] [PMCID]

[32] Zhang Y, Lai BS, Juhas M. Recent advances in aptamer discovery and applications. Molecules. 2019; 24(5):941. [DOI:10.3390/molecules24050941] [PMID] [PMCID]

[33] Isibasi A, Ortiz V, Vargas M, Paniagua J, González C, Moreno J, et al. Protection against Salmonella typhi infection in mice after immunization with outer membrane proteins isolated from Salmonella typhi 9, 12, d, Vi. Infect Immun. 1988; 56(11):2953-9. [DOI:10.1128/iai.56.11.2953-2959.1988] [PMID] [PMCID]

[34] Puente J, Alvarez-Scherer V, Gosset G, Calva E. Comparative analysis of the Salmonella typhi and Escherichia coli ompC genes. Gene. 1989; 83(2):197-206. [DOI:10.1016/0378-1119(89)90105-4] [PMID]

[35] Puente J, Verdugo-Rodríguez A, Calva E. Expression of Salmonella typhi and Escherichia coli OmpC is influenced differently by medium osmolarity; dependence on Escherichia coli OmpR. Mol Microbiol. 1991; 5(5):1205-10. [DOI:10.1111/j.1365-2958.1991.tb01894.x] [PMID]

[36] Puente JL, Flores V, Fernández M, Fuchs Y, Calva E. Isolation of an ompC-like outer membrane protein gene from Salmonella typhi. Gene. 1987; 61(1):75-83. [DOI:10.1016/0378-1119(87)90366-0] [PMID]

[37] Lin J, Huang S, Zhang Q. Outer membrane proteins: Key players for bacterial adaptation in host niches. Microbes Infect. 2002; 4(3):325-31. [DOI:10.1016/S1286-4579(02)01545-9] [PMID]

[38] Kleywegt GJ, Jones TA. Databases in protein crystallography. Acta Crystallogr D Biol Crystallogr.1998; 54(Pt 6 Pt 1):1119-31. [DOI:10.1107/S0907444998007100] [PMID]

[39] Lambert C, Leonard N, De Bolle X, Depiereux E. ESyPred3D: Prediction of proteins 3D structures. Bioinformatics. 2002; 18(9):1250-6. [DOI:10.1093/bioinformatics/18.9.1250] [PMID]

[40] Venclovas Č. Comparative modeling in CASP5: Progress is evident, but alignment errors remain a significant hindrance. Proteins. 2003; 53(Suppl 6):380-8. [DOI:10.1002/prot.10591] [PMID]

[41] Petrey D, Honig B. Protein structure prediction: Inroads to biology. Mol Cell. 2005; 20(6):811-9. [DOI:10.1016/j.molcel.2005.12.005] [PMID]

[42] Buglak AA, Samokhvalov AV, Zherdev AV, Dzantiev BB. Methods and applications of in silico aptamer design and modeling. Int J Mol Sci. 2020; 21(22):8420. [DOI:10.3390/ijms21228420] [PMID] [PMCID]

[43] Sepehri Zarandi H, Behbahani M, Mohabatkar H. In silico selection of Gp120 ssDNA Aptamer to HIV-1. SLAS Discov. 2020; 25(9):1087-93.[DOI:10.1177/2472555220923331] [PMID]

[44] Dwivedi HP, Smiley RD, Jaykus LA. Selection of DNA aptamers for capture and detection of Salmonella Typhimurium using a whole-cell SELEX approach in conjunction with cell sorting. Appl Microbiol Biotechnol. 2013; 97(8):3677-86. [DOI:10.1007/s00253-013-4766-4] [PMID]

[45] Bayraç C, Öktem HA. Evaluation of Staphylococcus aureus DNA aptamer by enzyme-inked aptamer assay and isothermal titration calorimetry. J Mol Recognit. 2017; 30(2):e2583. [DOI:10.1002/jmr.2583] [PMID]