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Bioinformatical Prediction of G-quadruplex Aptamer for Detection of a Ligand in Practice

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ABSTRACT

Considering the introduction of aptamers as a new generation of analyte identifiers, this class of materials can be used in diagnostic systems because aptamers are easier to produce, more sensitive, higher accuracy, less sensitive to environmental factors, easier to handle and can be used. A particular type of aptamer that has a sequence rich in guanine base can create a unique nanostructure called G-quadruplex. The creation of this structure gives the aptamer an enzyme property so that it can act like an enzyme in the vicinity of it, oxidise a chromogenic substrate and produce a coloured signal. The main way to produce aptamers is a laboratory technique called SELEX (Systematic evolution of ligands by exponential enrichment), in which a mixture of different oligo libraries near the target analyte creates aptamers in several consecutive cycles. The aim of this study was to introduce a novel approach for obtaining DNA aptamers for detecting a ligand such as an aflatoxin M1 in bioinformatically manner in replacing SELEX for obtaining the specific oligo aptamers against aflatoxin M1. For this purpose, the selected oligoaptamers' structures were predicted using molecular simulators and bioinformatic techniques. The results of these molecular simulations suggested G-quadruplex aptamers with a suitable affinity for binding to aflatoxin M1 in colourimetric assays.

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1. Introduction

Aptamer has been introduced as a recognition element capable of diagnostic and targeted therapeutic applications [1]. Indeed, aptamers are mainly DNA or RNA oligomers that can interact with different targets, such as micro and macro molecules, with high selectivity [2]. Aptamers, versus other recognition elements, have many advantages; they have higher stability, low immunogenicity, portability, and reusability and are easy to use [3-5]. Generally, Aptamers are obtained by an in vitro selection technique called systematic evolution of ligands by exponential enrichment (SELEX) (Fig. 1) [6].

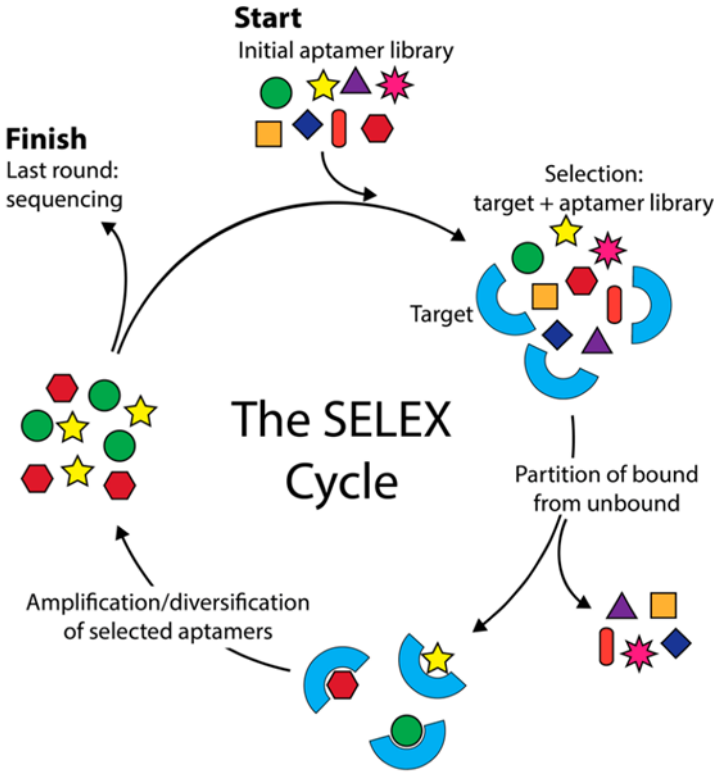


Figure 1: Schematic diagram of a SELEX process,

The library of random oligomers is incubated with the target. In the next step, the target is washed to remove non-linked oligomers, and finally, the linked oligomers (aptamers) are amplified and entered into the next cycle. A minimum of 12 cycles is required. DNA-based aptamers are a major subtype of aptamers that could form structures like hairpin, stem-loop, G-quadraplex, etc. [7]. G-quadraplex (G4) is a unique structure that is formed when the oligomers have G-rich sequences. There are different structures of G4 conformations, such as unimolecular G4, bimolecular G4, and tetramolecular G4[8] (Fig. 2).

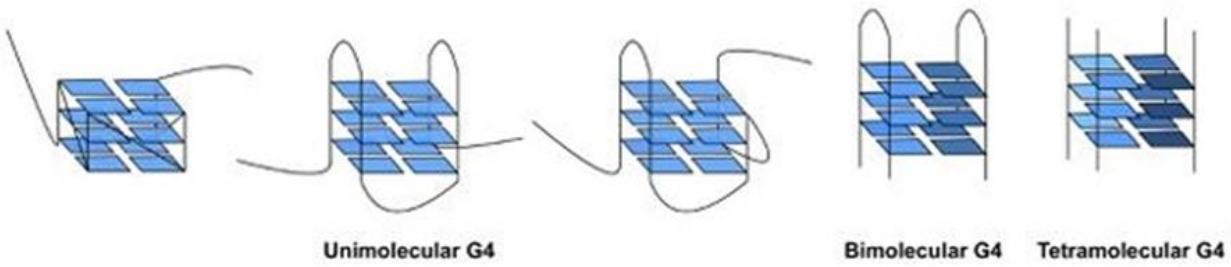


Figure 2: different G-quadruplex structure. Unimolecular G4s with different backbone arrangements (parallel, anti-parallel and mixed); Bimolecular quadruplex; Tetramolecular quadruplex [8]

G4 conformation is the predictable structure based on the number of guanines in the oligomer sequence and their secondary structure [8]. Online web server, quadruplex forming G-Rich Sequences (QGRS) Mapper calculate the G-Score of each oligomer sequence, and a higher G-score equals a higher probability of G4 conformation [9]. In addition, two online webservers, M-fold and RNA structure, predict secondary structures of oligomers that give the ability to estimate the number of loops or stem-loops structures likely to exist in the oligomers [10, 11]. Some literature indicated the application of in silico methods to optimise DNA-based aptamer and affinity predication, such as artificial intelligence or Auto Dock vina software [12-15]. Meanwhile, some other studies suggested using in-silico methods with caution due to several errors that probably occur during predictions and optimisations [16, 17]. As mentioned, aptamer could be used in therapeutic and diagnostic approaches, like a biosensor for detecting a specific ligand. Several platforms have been developed for using aptamers in biosensors [18]. In the colourimetric

approach, signals detectable with the naked eye could be quantified with an Optical density (O.D.) measurement by a microplate reader or a UV-visible spectrophotometer [19]. For example, in a colourimetric aptasensor based on gold nanoparticles, its colour can appear when the aptamer binds to its analyte due to its high affinity; after that, gold nanoparticles aggregate and then produce a purple colour [20]. In another approach, a G-quadruplex aptamer could create a colour in a single step, in which the aptamers bind to their specific ligands. Then they change to G4 conformations with peroxidase activity such as horseradish peroxidase enzyme. In this case, aptamers could produce colours if there is any chromogenic substrate, such as tetramethylbenzidine (TMB) in reactions [19, 20]. Sometimes, the oligomer aptamers obtained by SELEX have low affinity and need post-SELEX optimisations [13]. Because of the consumption of SELEX to obtain ideal aptamers, we can use in-silico methods to achieve the best oligomers [22]. There are different strategies for acquiring and optimising oligomers as aptamers using an in-silico method; however, this study describes a methodology to choose aptamers that could be formed to G-quadruplex conformations which they have introduced by former studies and predicted the secondary structure and estimated G-score with different online web server for obtaining of a former step to increase the affinity of aptamer and G-score for predicting a new oligo aptamer for aflatoxin M1 as a ligand by Autodock Vina software.

2. Experimental Procedure

2.1 Oligonucleotides

To obtain original oligo aptamers used as a recognition element to detect the aflatoxin M1, different publications [23-37]. For obtaining the articles, the search query was the same, including phrases such as aptamer and aflatoxin M1, aptasensor and aflatoxin M1, and aptamer-based biosensor and aflatoxin M1.

2.2 Online Webservers and Software

2.2.1 UNAFold Web Server and RNA Secondary Structure

Unified Nucleic Acid Folding (UNAFold) web server Multiple folds (Mfold)[1] and RNA secondary structure were used to predict the oligonucleotides' secondary structure, which was obtained by searching the publications. For M. fold, the Fast-All (FASTA) format of sequences was used with the default condition of the predicted situation. In the RNA secondary structure, the sequence type changed from RNA to DNA mode, and the predicted situation's default condition was also used. From M. fold, the JPG and Vienna file of the structure with the least ΔG was saved, and the result of RNA secondary structure was also saved.

2.2.2 QGPRS Mapper

For enrolling QGPRS Mapper[2], the input sequences were queried in FASTA format, and the highest G scores and overlaps were recorded for each sequence.

2.2.3 RNA Compressor

RNA compressor[3] is used to predict the 3D structures of oligomers. RNA compressor is used for RNA sequences, so all DNA sequences were mutated to RNA. In the first step, the Vienna file of the secondary structure of Mfold and all thymine bases were replaced with uracil by Notepad^{++} software. Input sequences were put in the query form and saved as the files of each oligomer.

2.2.4 Discovery Studio Software

After receiving the results from the RNA compressor, Discovery Studio Software[4] was used to change the RNA sequences to DNAs. All uracil bases are substituted by thymine bases and changed to sugar of all bases from ribose to deoxyribose.

([1]) http://www.unafold.org/mfold/applications/rna-folding-form.php
([2]) https://bioinformatics.ramapo.edu/QGRS/index.php
([3]) https://rnacomposer.cs.put.poznan.pl/
([4]) https://discover.3ds.com/discovery-studio-visualizer-download

2.2.5 PubChem and Open Bable

A PubChem online webserver was used to achieve the 3D structure of aflatoxin M1 (as a target), and the SDF file was saved. In the next step, the Spatial Data File (SDF) file was converted to a Protein Data Bank (PDB) file by Open Bable[1] software.

2.2.6 Autodock Tools

The aflatoxin M1 was put as the target in Autodock tools software[2] and merged the nonpolar hydrogens and edited its surface charge (Kollman and Gasteger), and then it saved as a Protein Data Bank, Partial Charge (Q), & Atom Type (T) format (PDBQT) file as the output. In addition, Autodock Tools software was used for oligomers as a macromolecule, merged nonpolar hydrogens, added its surface charges, and then saved as a PDBQT file. On the other hand, it determined the binding grid as a binding site.

2.2.7 Autodock Vina

Autodock Vina[3] was used to evaluate the docking interaction between oligo aptamers and the target. The score of interactions was recorded.

3. Results and Discussion

Thirty-nine distinct nucleotide sequences were selected from the papers, including aptamers against aflatoxin M1 (Table 1), and they used primarily aflatoxin-specific aptamers for analysis with the software described above.

QGQRS Mapper examined the ability of oligomers, and the aptamers that could form G-quadruplex configurations were separated. After that, the second structure of these aptamers was obtained using M-fold and RNA structure (Table 2).

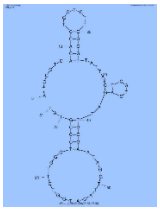
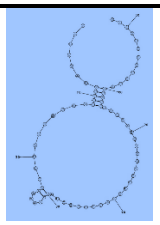
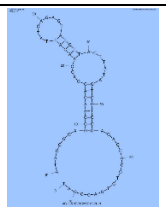
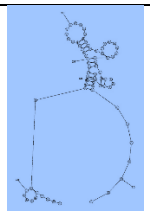
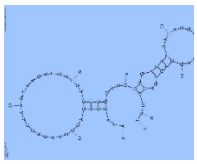
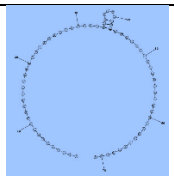
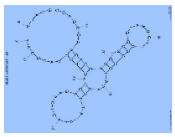
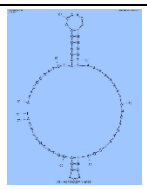
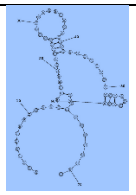
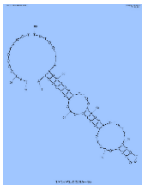
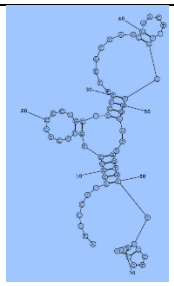
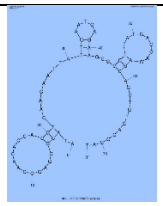
According to the results obtained from QGPRS Mapper, 2D structures of oligomers (Table 2), according to the loop-stem structures and thermodynamically stability of the selected oligomers, sequence No. 5 seem to have a better ability to be selected as a G-quadraplex aptamer for aflatoxin M1 detection. These findings were also reported for selecting suitable aptamers for several target ligands, avoiding lengthy experimental SELEX and instead using computational modelling to predict aptamer sequences [5, 19, 20]. As a result, the third structure of the No. 5 sequence was predicted and obtained using online software (RNA compressor). On the other hand, the sequence of ACTGCTAGAGATTTTCCACAT was the most used oligomer as the aflatoxin M1 aptamer in different studies [23, 25, 27, 31]. In addition, the sequence of GGGATGTGAGGTGGCTCTCGT had multiple guanines and in the second structure had stem-loop configuration; hence it had the potential to get G-Score by editing the sequence, so we modified this sequence to GGGATGTGAGGTGGCTCTGGTGG and finally evaluated these sequences in docking interaction (Fig. 3). The results from the simulations demonstrated that three oligomers could be selected as the G-quadruplex aptamers for targeting and of aflatoxin M1. For instance, oligomer no. 21 was the aptamer because it is used most in various studies and has a short length. The oligomer no. 38 had the ideal 2D structure (stem-loop) and multiple guanines. The modified sequence from sequence no. 38 had a random changing process, so its 2D structure did not change, and it got more G-Score than the original state. On the other hand, the results also demonstrated that these changes were due to the modified sequence having a 2D structure similar to sequence no. 38; therefore, it gained a G-score equal to 18. In the docking process, the modified oligomer had a higher interaction score when compared to sequence no. 38.

([1]) http://openbabel.org/wiki/Main_Page
([2]) https://autodock.scripps.edu/
([3]) https://vina.scripps.edu/

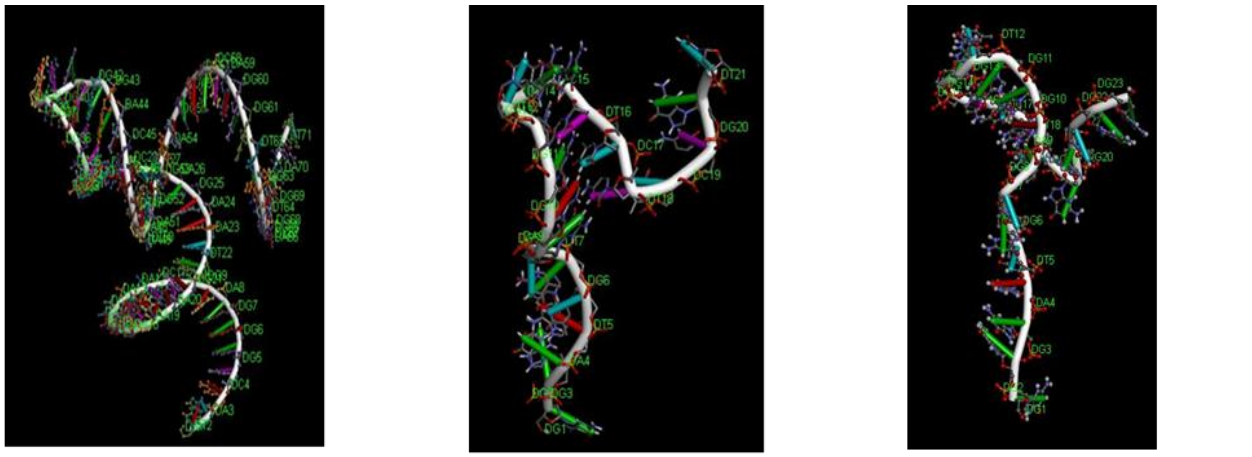
Table1: Sequences introduced in different studies to be used as aflatoxin M1 specific aptamer

No.	Sequence (5'→3')	Ref.
1	ATCCGTCACACCTGCTCTCGAATAGAGTACTCGATACTGACTTTATTTACCGTCTGGTGTTGGCTCCCGTAT	[23]
2	ATCCGTCACACCTGCTCTCCTCGAACTAGTTAGACCCTCCTCCAAGTCAACTTGTGGTGTTGGCTCCCGTAT	[23]
3	ATCCGTCACACCTGCTCTTGGGGTTATTACTCGTGAGATTGGGAATAGGTTACATGGTGTTGGCTCCCGTAT	[23]
4	ATCCGTCACACCTGCTCTTGTAGGGTTCCCACCCAATTCAGTTCCGTTAAACCATGGTGTTGGCTCCCGTAT	[23]
5	ATACGGGAGCCAACACCAAAGTAAGATCATCACCCGGACGCGGACATAATAGGAGAGCAGGTGTGACGGAT	[23]
6	ATCCGTCACACCTGCTCTAACTTACACATAATTCTAGGTTACATCTTGCTCATATGGTGTTGGCTCCCGTAT	[23]
7	ATACGGGAGCCAACACCACCAACATTATCAGAGTATGTTACTTATAGTGGTGGCAGAGCAGGTGTGACGGAT	[23]
8	ATACGGGAGCCAACACCACGTCAACAATTATTCAATGAGAAGCGGCTTATGAGGAGAGCAGGTGTGACGGAT	[23]
9	ATACGGGAGCCAACACCACATCGACTTACGAATCAACGCGTTTATTATTGGTTCAGAGCAGGTGTGACGGAT	[23]
10	ATCCGTCACACCTGCTCTGTGTACGCCCGTATTTACGTTCCTAGCAATTGCTATGTGGTGTTGGCTCCCGTAT	[23]
11	ATCCGTCACACCTGCTCTACACTCCGCACGATCTTTTTTAGAACGCGTACCCGTTGGTGTTGGCTCCCGTAT	[23]
12	ATACGGGAGCCAACACCAGGTGCGGTAAGGTTCGCCCTAAAGCTTATATACTCAAGAGCAGGTGTGACGGAT	[23]
13	ATACGGGAGCCAACACCACTTGGTTAACCAATCAAACCGGACAACGAAGGGTCCAGAGCAGGTGTGACGGAT	[23]
14	ATACGGGAGCCAACACCAACAACCTAGATGTTCTGATAACACGAATCGCTTCGAAGAGCAGGTGTGACGGAT	[23]
15	ATACGGGATCTAACACCAAGAACCTGGGGTTAAAAAAACAGGAGTATGGATGCAGAGCAGGTGTGACGGAT	[23]
16	ATACGGGAGCCAACACCAAGTAACACACGCGGACCAGAAATACATCCCCCCGTAGAGCAGGTGTGACGGAT	[23]
17	ATCCGTCACACCTGCTCTCAATCTGAAATATTGCAAGCAGTGCTCACAATTTGTTGGTGTTGGCTCCCGTAT	[23]
18	ATCCGTCACACCTGCTCTCCCCGGCGTCCGTTTATTAGCAGACTTTGGCGGAATTGGTGTTGGCTCCCGTAT	[23]
19	ATCCGTCACACCTGCTCTGGCATTAGTATTCCATAGCCGGCCAAGTCTATGTAGTGGTGTTGGCTCCCGTAT	[23]
20	ATCCGTCACACCTGCTCTGACGCTGGGGTCGACCCGGAGAAATGCATTCCCCTGTGGTGTTGGCTCCCGTAT	[23]
21	ACTGCTAGAGATTTTCCACAT	[23]
22	ATCCGTCACACCTGCTCTGACGCTGGGGTCGACCGGAGAAATGCATTCCCCTGGTGTTGGCTCCCGTAT	[24]
23	TAACACGAGACACTGCTAGAGATTTTCCACATTTCTCTTGTTCC	[25]
24	GTTGGGCACGTGTTGTCTCTCTGTGTCTCGTGCCCTTCCTAGGCCCACA	[26]
25	ACTGCTAGAGATTTTCCACATGCTGAGGCCGCTCTCTAGCAGTAAAA	[27]
26	ACTGCTAGAGATTTTCCACA	[28]
27	ATCCGTCACACCTGCTCTGACGCTGGGGTCGACCCG GAGAAATGCATTCCCCTGTGGTGTTGGCTCCCG TAT	[29]
28	ATCCG CAACCTGCTCTGACGCTGGGGTCGACCCGGAGAAATGCATTCCCCTGTGGTGTTGGCTCCCGTAT	[30]
29	TCTCACTGCTAGAGATTTTCCACAT	[31]
30	ATCCGTCACACCTGCTCTGACGCTGGGGTCGACCCG	[32]
31	ACTGCTAGAGATTTTCCA	[33]
32	ATCCGTCACACCTGCTCTGACGCTGGGGTCGACCCGGAGAAATGCATTCCCCTGGGTGTTGGCTCCCGTAT	[34]
33	ATCCGTCACACCTGCTCTGACGCTGGGGTCGACCCGGAGA	[35]
34	CAACGCCAGTCAGTATCTTATATGCTATACTGGCTGGTGTTG	[36]
35	CCGGCGGATGCTAATTGCAGAGCAGGTGTGCCGG	[35]
36	AAAA-ACACTATGTAGTGGTGT	[35]
37	GTTGGGCACGTGTTGTCTCTCTGTGTCTCGTGCCCTTCGCTAGGCCCACA	[36]
38	GGGATGTGAGGTGGCTCTCGT	[37]

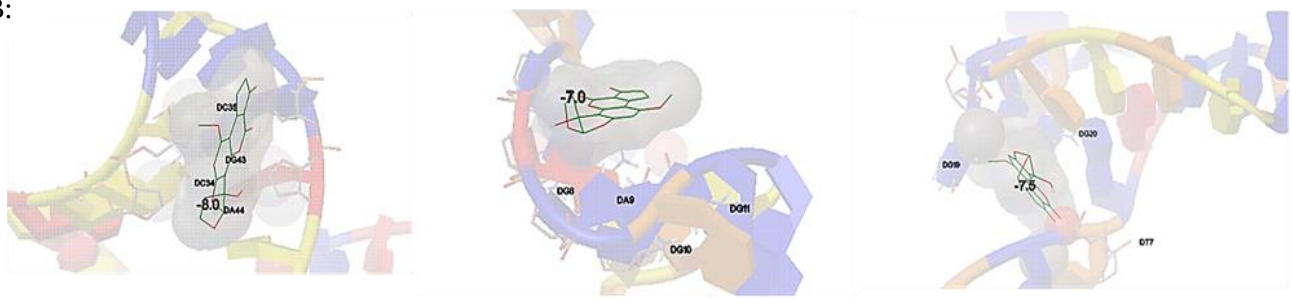
Table2: The sequences with G-Score and their secondary (2D) structures via M-Fold and RNA structure.

No	Sequence (5'→3')	G-Score	2D structure (M-Fold)	2D structure (RNA structure)
3	ATCCGTCACACCTGCTCTTGGGGTTATTACTCGTGAGA TTGGGAATAGGTTACATGGTGTTGGCTCCCGTAT	19		
7	ATACGGGAGCCAACACCACCAACATTATCAGAGTATGTT ACTTATAGTGGTGGCAGAGCAGGTGTGACGGAT	15		
5	ATACGGGAGCCAACACCAAAGTAAGATCATCAC CCGGACGCGGACATAATAGGAGAGCAGGTGTGACGGAT	19		
12	ATACGGGAGCCAACACCAGGTGCGGTAAGGT TCGCCCTAAAGCTTATATACTCAAGAGCAGGTGTGACGGA T	13		
15	ATACGGGATCTAACACCAAGAACCTGGGGTTAA AAAAACAGGAGTATGGATGCAGAGCAGGTGTGACGGAT	16		
18	ATCCGTCACACCTGCTCTCCCCGGCGTCCGTTTATTAGC AGACTTTGGCGGAATTGGTGTTGGCTCCCGTAT	18		
8	ATACGGGAGCCAACACCACGTCAACAATTATTCAATGAGA AGCGGCTTATGAGGAGAGCAGGTGTGACGGAT	20		

A:



B:



C:

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-8.0	0.000	0.000
2	-7.9	9.962	13.796
3	-7.9	8.789	13.197
4	-7.8	2.571	3.290
5	-7.6	1.178	5.689
6	-7.3	5.234	8.172
7	-7.3	3.803	6.308
8	-7.1	11.211	13.980
9	-7.1	5.440	9.141

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.0	0.000	0.000
2	-7.0	1.463	5.706
3	-7.0	1.866	6.656
4	-6.8	12.633	13.990
5	-6.7	2.311	3.518
6	-6.7	13.507	14.802
7	-6.6	12.456	13.418
8	-6.6	2.104	6.434
9	-6.6	11.327	12.364

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.5	0.000	0.000
2	-7.3	14.285	15.822
3	-7.1	13.283	15.626
4	-7.1	11.410	13.253
5	-7.1	12.951	15.056
6	-7.0	11.738	14.073
7	-6.7	12.323	13.891
8	-6.5	13.560	15.305
9	-6.4	11.491	13.699

Figure 3: Docking interaction from vina Autodock simulator; A: left, sequence no. 21, middle, sequence no. 38, and right, the modified sequence; B: 3D structures of oligomers; and C: virtual interaction of the aptamers and aflatoxin M1.

4. Conclusions

Selection of the specific aptamers, particularly G-quadruplex aptamers (with duality in function after an interaction with its ligand), was always one of the essential steps for the study that aimed to detect colourimetrically a target molecule. Here, we introduced a novel approach to obtain the DNA aptamer for detecting a ligand such as an aflatoxin M1 in a bioinformatic manner because of the time-consuming cost of the SELEX method. This study demonstrated that the specific oligo aptamers against aflatoxin M1 could theoretically be predicted using simulation software such as the ones described here. Then their findings could be simplified in practice by selecting one suitable oligomer as the specific aptamer for the design and development of colorimetric assays.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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