



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

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## Abstract

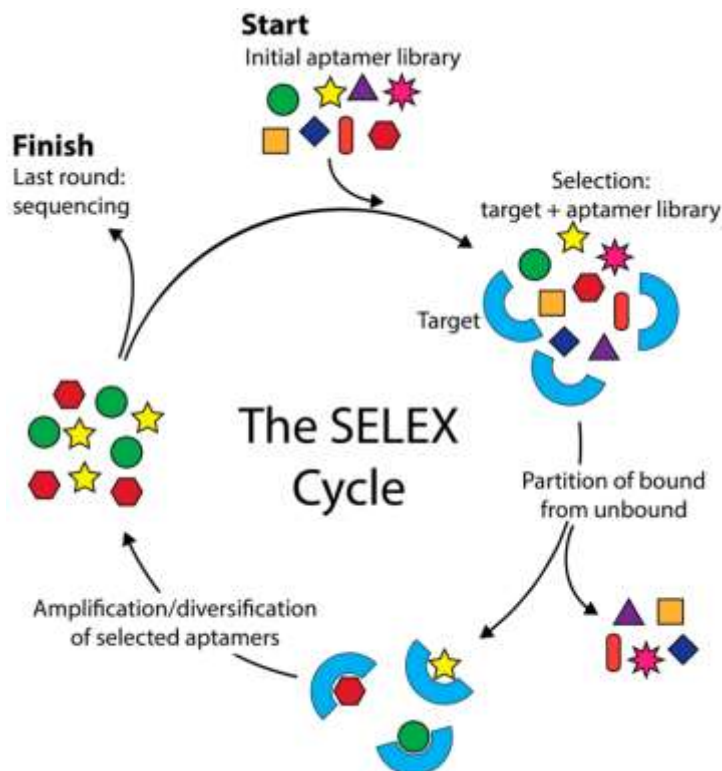
Considering the introduction of aptamers as a new generation of analyte identifiers, this class of materials can be used in diagnostic systems because aptamers are easier to produce, more sensitive, higher accuracy, less sensitive to environmental factors, easier to handle and can be used. A special type of aptamer that has sequence rich in guanine base can create a special 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, oxidize a chromogenic substrate and produce a colored 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 in the vicinity of the target analyte creates aptamers in several consecutive cycles. The aim of this study was the introduction a novel approach for obtaining DNA aptamers for detection a ligand such as aflatoxin M1 in bioinformatically manner in replacing to SELEX for obtaining the specific oligoaptamers against aflatoxin M1. For this purpose, the structure of the selected oligoaptamers were predicted using some molecular simulators and bioinformatically techniques. The results of these molecular simulations suggested G-quadruplex aptamers with suitable affinity for binding to aflatoxin M1 in colorimetric assays.

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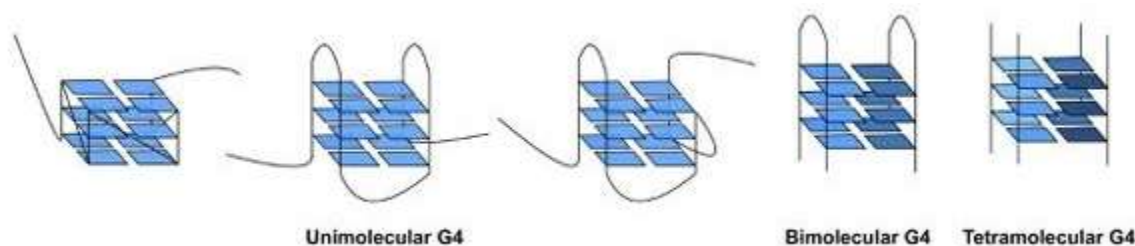
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## 1. Introduction

Aptamer has been introduced as a recognition element and capable for diagnostic and targeted therapeutic applications [1]. Indeed, aptamers are mainly DNA or RNA oligomers which can interact with difference targets such as micro and macro molecules with highly selectivity [2]. Aptamers versus others recognition elements have lots of advantages; they have higher stability, low immunogenicity, portability, reusability and easy to use [3-5]. Generally, Aptamers obtained by an *in vitro* selection technique called systematic evolution of ligands by exponential enrichment (SELEX) (Figure 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 major subtype of aptamers which could form different structures like hairpin, stem loop, G-quadruplex and etc. [7]. G-quadruplex (G4) is a unique structure and it's formed when the oligomers had G-rich sequences. There are different structures of G4 conformations, such as unimolecular G4, bimolecular G4, and tetramolecular G4[8] (**Figure 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 webserver, QGRS<sup>1</sup> Mapper calculate G-Score of each oligomer sequence and a higher G-score equals a higher probability of G4 conformation [9]. In addition, two online webserver, 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]. There are some literatures indicated the application of in silico methods to optimize DNA based aptamer and affinity predication such as artificial intelligent or Auto Dock vina softwares [12-15]. Meanwhile some

<sup>1</sup> Quadruplex forming G-Rich Sequences

other studies suggested the use of in-silico methods with caution due to several errors probably occur during predictions and optimizations [16, 17]. As mentioned before, aptamer could be used in therapeutic and diagnostic approaches, like a biosensor for detection of a specific ligand. Several platforms have been developed for the use of aptamers in biosensors [18]. In colorimetric approach, signals detectable with a naked eye and it could be quantifying with an OD<sup>2</sup> measurement by a microplate reader or a UV-visible spectrophotometer [19]. For example, in a colorimetric aptasensor based on gold nanoparticles, its color can be appearance when the aptamer bind to its analyte due to their high affinity, after that gold nanoparticles aggregated and then it produced a purple color [20]. In another approach, a G-quadruplex aptamer could be produced a color in a single step, in which the aptamers bind to their specific ligands and then they change to G4 conformations which have peroxidase activity such as horseradish peroxidase enzyme. In this case aptamers could produce colors if there is any chromogenic substrate such as tetramethylbenzidine (TMB) in reactions[19, 20]. Sometimes the oligomer aptamers which obtained by SELEX have low affinity and need post-SELEX optimizations [13]. Because of time consuming of SELEX to obtain ideal aptamers we can used in-silico methods to achieve the best oligomers [22]. There is different strategy for obtaining and optimizing oligomers as aptamers using an in-silico method; however, this study describe a methodology to choose aptamers 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 oligoaptamer for aflatoxin M1 as a ligand by Autodock Vina software.

## 2. Experimental Procedure

### 2.1. Oligonucleotides

To obtained original oligoaptamers which used to 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 and aptasensor and aflatoxin M1 and aptamer-based biosensor and aflatoxin M1.

### 2.2. Online Webservers and Softwares

#### 2.2.1. UNAFold Web Server and RNA Secondary Structure

UNAFold<sup>3</sup> web server (Mfold<sup>4</sup>)<sup>5</sup> and RNA secondary structure were used to predict the secondary structure of the oligonucleotides which obtained by searching the publications. For M. fold, the FASTA<sup>6</sup> format of sequences were used with default condition of predicted situation. In RNA secondary structure, the sequence type changed from RNA to DNA mode and default condition of predicted situation was used, too. From M. fold, the JPG and Vienna file of the structure which had the least  $\Delta G$  was saved and the result of RNA secondary structure was saved, too.

#### 2.2.2. QGPRS Mapper

For enrolling QGPRS Mapper<sup>7</sup>, the input sequences were the queries in FASTA format and the highest G score and overlaps were recorded for each sequence.

#### 2.2.3. RNA Compressor

RNA compressor<sup>8</sup> is used for predicted 3D structures of oligomers. RNA compressor is used for RNA sequences so all DNA sequences were mutated to RNA. In the first step used Vienna file of secondary structure of Mfold and all thymine bases were replaced with uracil by Notepad<sup>++</sup> software. Input sequences were put in the query form and saved as the files of each Oligomers.

<sup>2</sup> Optical density

<sup>3</sup> Unified Nucleic Acid Folding

<sup>4</sup> Multiple fold

<sup>5</sup> <http://www.unafold.org/mfold/applications/rna-folding-form.php>

<sup>6</sup> Fast-All

<sup>7</sup> <https://bioinformatics.ramapo.edu/QGRS/index.php>

<sup>8</sup> <https://rnacomposer.cs.put.poznan.pl/>

### 2.2.4. Discovery Studio Software

After receiving the results from RNA compressor, Discovery Studio Software<sup>9</sup> was used for changing the RNA sequences to DNAs. All uracil bases substituted by thymine bases and changed to sugar of all bases from ribose to deoxyribose.

### 2.2.5 PubChem and Open Bable

To achieved to 3D structure of aflatoxin M1 (as a target) PubChem online webserver<sup>10</sup> was used and saved the SDF<sup>11</sup> file. In the next step, SDF file was converted to PDB<sup>12</sup> file by Open Bable<sup>13</sup> software.

### 2.2.6. Autodock Tools

The aflatoxin M1 was put as the target in Autodock tools software<sup>14</sup> and merged the nonpolar hydrogens and edited its surface charge (kollman and gasteger) and then it saved as a PDBQT<sup>15</sup> file as the output. In addition, Autodock Tools software was used for oligomers as a macromolecule and merged nonpolar its hydrogens and added its surface charges and then saved as PDBQT file. In the other hand, it determined the binding grid as a binding site.

### 2.2.7. Autodock Vina

Autodock Vina<sup>16</sup> was used to evaluate the docking interaction between different oligoaptamers and the target. The score of interactions were 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 as primarily aflatoxin-specific aptamers for analysing with the softwares have been described above.

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

No.	Sequence (5' → 3')	Ref. no.
1	ATCCGTCACACCTGCTCTCGAATAGAGTACTCGATACTGACTTTATTTACCGTCTGGTGTGGCTCCCGTAT	[23]
2	ATCCGTCACACCTGCTCTCCTCGAAGTACTGACCCCTCCTCCAAGTCAACTTGTGGTGTGGCTCCCGTAT	[23]
3	ATCCGTCACACCTGCTCTTGGGGTTATTACTCGTGAGATTGGGAATAGGTTACATGGTGTGGCTCCCGTAT	[23]
4	ATCCGTCACACCTGCTCTTGTAGGGTTCCCACCAATTCAGTTCGGTTAAACCATGGTGTGGCTCCCGTAT	[23]
5	ATACGGGAGCCAACACCAAAGTAAGATCATCACCCGGACGCGGACATAATAGGAGAGCAGGTGTGACGGAT	[23]
6	ATCCGTCACACCTGCTCTAACTTACACATAATTCTAGGTTACATCTTGCTCATATGGTGTGGCTCCCGTAT	[23]
7	ATACGGGAGCCAACACCACCAACATTATCAGAGTATGTTACTTATAGTGGTGGCAGAGCAGGTGTGACGGAT	[23]
8	ATACGGGAGCCAACACCACGTCAACAATTATCAATGAGAAGCGGCTTATGAGGAGAGCAGGTGTGACGGAT	[23]
9	ATACGGGAGCCAACACCACATCGACTTACGAATCAACGCGTTTATTATTGGTTCAGAGCAGGTGTGACGGAT	[23]
10	ATCCGTCACACCTGCTCTGTGTACGCCGATTTACGTTTCTAGCAATTGCTATGTGGTGTGGCTCCCGTAT	[23]
11	ATCCGTCACACCTGCTCTACACTCCGCACGATCTTTTTTAGAACGCGTACCCGTTGGTGTGGCTCCCGTAT	[23]
12	ATACGGGAGCCAACACCAGGTGCGGTAAGGTTGCCCCAAAGCTTATATACTCAAGAGCAGGTGTGACGGAT	[23]
13	ATACGGGAGCCAACACCCTTGGTTAACCAATCAAACCGGACAACGAAGGGTCCAGAGCAGGTGTGACGGAT	[23]
14	ATACGGGAGCCAACACCAACAACCTAGATGTTCTGATAACACGAATCGTTTGAAGAGCAGGTGTGACGGAT	[23]
15	ATACGGGATCTAACACCAAGAACCTGGGGTTAAAAAACAGGAGTATGGATGCAGAGCAGGTGTGACGGAT	[23]
16	ATACGGGAGCCAACACCAAGTAACACACGCGGACCAGAAATACATCCCCCGTAGAGCAGGTGTGACGGAT	[23]

<sup>9</sup> <https://discover.3ds.com/discovery-studio-visualizer-download>

<sup>10</sup> <http://pubchem.ncbi.nlm.nih.gov/>

<sup>11</sup> Spatial Data File

<sup>12</sup> Protein data bank

<sup>13</sup> [http://openbabel.org/wiki/Main\\_Page](http://openbabel.org/wiki/Main_Page)

<sup>14</sup> <https://autodock.scripps.edu/>

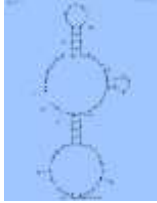

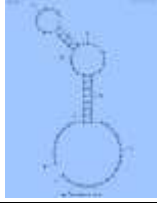

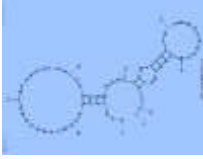

<sup>15</sup> Protein Data Bank, Partial Charge (Q), & Atom Type (T) format









<sup>16</sup> <https://vina.scripps.edu/>

17	ATCCGTCACACCTGCTCTCAATCTGAAATATTGCAAGCAGTGCTCACAATTTGTTGGTGTGGCTCCCGTAT	[23]
18	ATCCGTCACACCTGCTCTCCCGGGCTCCGTTTATTAGCAGACTTTGGCGGAATTGGTGTGGCTCCCGTAT	[23]
19	ATCCGTCACACCTGCTCTGGCATTAGTATTCCATAGCCGGCCAAGTCTATGTAGTGGTGTGGCTCCCGTAT	[23]
20	ATCCGTCACACCTGCTCTGACGCTGGGGTTCGACCCGGAGAAATGCATTCCCCTGTGGTGTGGCTCCCGTAT	[23]
21	ACTGCTAGAGATTTCCACAT	[23]
22	ATCCGTCACACCTGCTCTGACGCTGGGGTTCGACCCGGAGAAATGCATTCCCCTGGTGTGGCTCCCGTAT	[24]
23	TAACACGAGACACTGCTAGAGATTTCCACATTTCTCTTGTTC	[25]
24	GTTGGGCACGTGTTGTCTCTCTGTGTCTCGTGCCCTTCTAGGCCACA	[26]
25	ACTGCTAGAGATTTCCACATGCTGAGGCCGCTCTCTAGCAGTAAAA	[27]
26	ACTGCTAGAGATTTCCACA	[28]
27	ATCCGTCACACCTGCTCTGACGCTGGGGTTCGACCCG GAGAAATGCATTCCCCTGTGGTGTGGCTCCCG TAT	[29]
28	ATCCG CAACCTGCTCTGACGCTGGGGTTCGACCCGGAGAAATGCATTCCCCTGTGGTGTGGCTCCCGTAT	[30]
29	TCTCACTGCTAGAGATTTCCACAT	[31]
30	ATCCGTCACACCTGCTCTGACGCTGGGGTTCGACCCG	[32]
31	ACTGCTAGAGATTTCCA	[33]
32	ATCCGTCACACCTGCTCTGACGCTGGGGTTCGACCCGGAGAAATGCATTCCCCTGGGTGTGGCTCCCGTAT	[34]
33	ATCCGTCACACCTGCTCTGACGCTGGGGTTCGACCCGGAGA	[35]
34	CAACGCCAGTCAGTATCTTATATGCTATACTGGCTGGTGTG	[36]
35	CCGGCGGATGCTAATTGCAGAGCAGGTGTGCCGG	[35]
36	AAAA-ACACTATGTAGTGGTGT	[35]
37	GTTGGGCACGTGTTGTCTCTGTGTCTCGTGCCCTTCGCTAGGCCACA	[36]
38	GGGATGTGAGGTGGCTCTCGT	[37]

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

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	ATCCGTCACACCTGCTCTTGGGGTTATTACTCGTGAGATTGGGAATAGGTTACATGGTGTGGCTCCCGTAT	19		
7	ATACGGGAGCCAACACCACCAACATTATCAGAGTATGTTACTTATAGTGGTGGCAGAGCAGGTGTGACGGAT	15		
5	ATACGGGAGCCAACACCAAGTAAGATCATCACCCGGACGCGGACATAATAGGAGAGCAGGTGTGACGGAT	19		

12	ATACGGGAGCCAACACCAGGTGCGGTAAGGT TCGCCCTAAAGCTTATATACTCAAGAGCAGGTGTGACGGAT	13		
15	ATACGGGATCTAACACCAAGAACCTGGGGTTAA AAAAACAGGAGTATGGATGCAGAGCAGGTGTGACGGAT	16		
18	ATCCGTACACCTGCTCTCCCCGGCGTCCGTTTATTAGC AGACTTTGGCGGAATTGGTGTGGCTCCCGTAT	18		
8	ATACGGGAGCCAACACCACGTCAACAATTATTC AATGAGA AGCGGCTTATGAGGAGAGCAGGTGTGACGGAT	20		

According to the results obtained from QGPRS Mapper, 2D structures of oligomers (Table2), 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-quadruplex aptamer for aflatoxin M1 detection. These findings were also reported for selection of the 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 No. 5 sequence was predicted and obtained by using online software (RNA compressor). In 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 potential get G-Score by edit the sequence, so we modified this sequence to GGGATGTGAGGTGGCTCTGGTGG and finally evaluated these sequences in docking interaction (Figure 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 due to its most use in various studies and it had a short length. The oligomer no. 38 had the ideal 2D structure (stem-loop) and also it had multiple guanines. The modified sequence from the sequence no. 38 had a changing process done randomly in such a way that its 2D structure did not change and it get more G-Score than the original state. On the other hands, also the obtained results demonstrated that these changes were in a way that the modified sequence had a 2D structure similar to the sequence no. 38; therefore, it gained a G-score equal to 18. In docking process, the modified oligomer had higher interaction score when compared to the sequence no. 38.

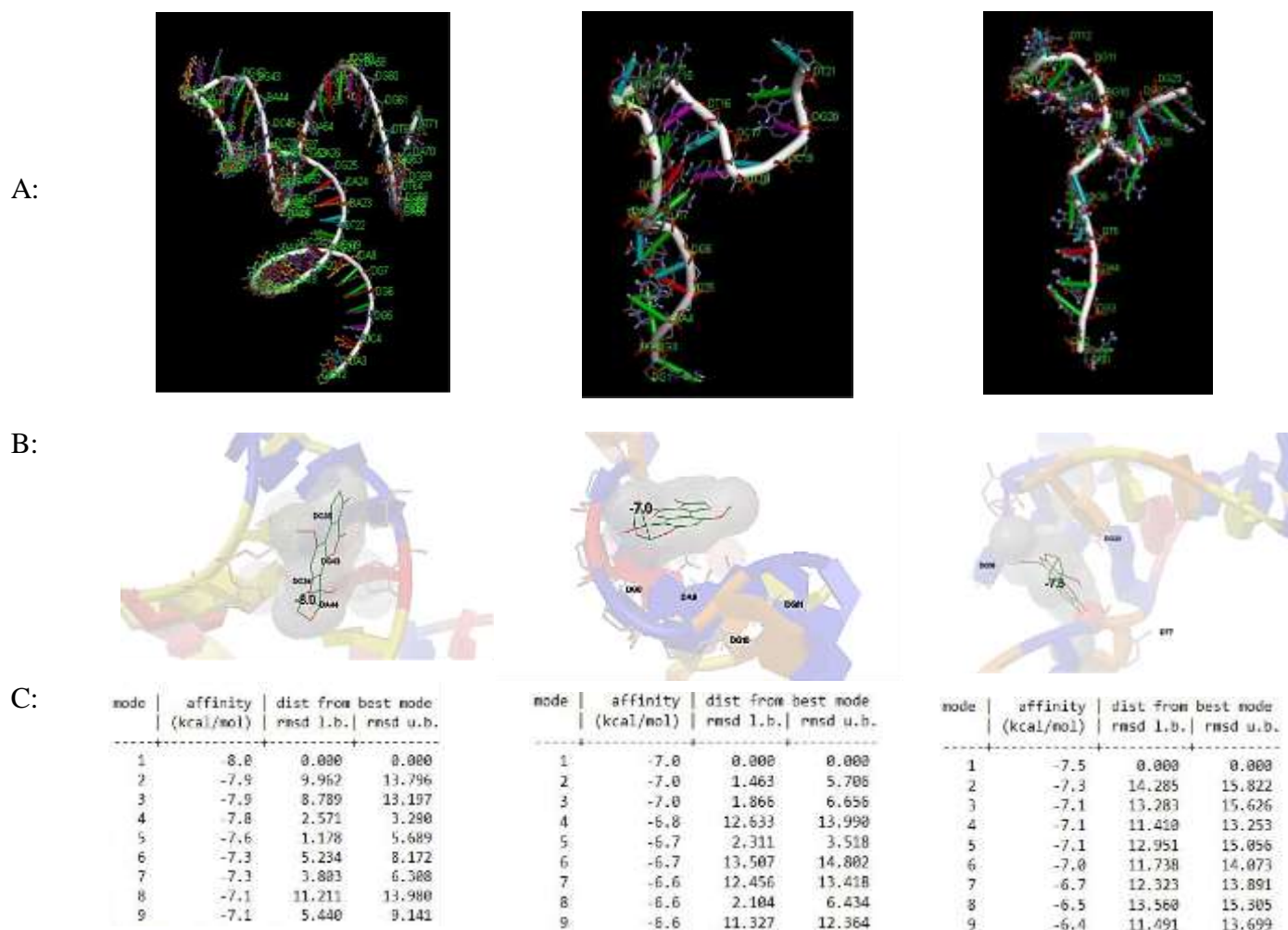


Figure 3. Docking interaction from vina Autodock simulator; A: left, sequence no. 21, middle, sequence no. 38, and right, the modified sequence; B: 3D structures of oligomers; and C: virtual interaction of the aptamers and aflatoxin M1.

#### 4. Conclusions

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

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#### Conflict of Interest

The authors declare that they have no conflict of interest.

## References

- [1] R. Liu, F. Zhang, Y. Sang, I. Katouzian, S.M. Jafari, X. Wang, *et al.*, "Screening, identification, and application of nucleic acid aptamers applied in food safety biosensing." *Trends in Food Science & Technology*, vol. 123, p. 355-75, 2022.
- [2] M. Xie, F. Zhao, Y. Zhang, Y. Xiong, S. Han, "Recent advances in aptamer-based optical and electrochemical biosensors for detection of pesticides and veterinary drugs." *Food Control*, vol. 131, p. 108399, 2022.
- [3] S. Arshavsky-Graham, R. Urmann K, Salama, N. Massad-Ivanir, J-G. Walter, T. Scheper, *et al.*, "Aptamers vs. antibodies as capture probes in optical porous silicon biosensors." *Analyst*, vol. 145, p. 4991-5003, 2020.
- [4] N.M. Danesh, H.B. Bostan, K. Abnous, M. Ramezani, K. Youssefi, S.M. Taghdisi, *et al.*, "Ultrasensitive detection of aflatoxin B1 and its major metabolite aflatoxin M1 using aptasensors: A review." *TrAC Trends in Analytical Chemistry*, vol. 99, p. 117-28, 2018.
- [5] A. Rafati, A. Zarrabi, S. Abediankenari, M. Aarabi, P. Gill, "Sensitive colorimetric assay using insulin G-quadruplex aptamer arrays on DNA nanotubes coupled with magnetic nanoparticles." *Royal Society open science*, vol. 5, p. 171835, 2018.
- [6] C. Tuerk and L. Gold, "Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase." *Science*, vol. 249, p. 505-510, 1990.
- [7] R. Stoltenburg, C. Reinemann, and B. Strehlitz, "SELEX--a (r)evolutionary method to generate high-affinity nucleic acid ligands." *Biomolecular Engineering*, vol. 24, p. 381-403, 2007.
- [8] E. Puig Lombardi and A. Londoño-Vallejo, "A guide to computational methods for G-quadruplex prediction." *Nucleic Acids Research*, vol. 48, p. 1-15, 2020.
- [9] O. Kikin, L. D'Antonio, and P.S. Bagga, "QGRS Mapper: a web-based server for predicting G-quadruplexes in nucleotide sequences." *Nucleic Acids Research*, vol. 34, p. W676-W82, 2006.
- [10] D.H. Mathews, D.H. Turner, R.M. Watson, "RNA Secondary Structure Prediction." *Current Protocols in Nucleic Acid Chemistry*, vol. 67, p. 1-9, 2016.
- [11] M. Zuker, "Mfold web server for nucleic acid folding and hybridization prediction." *Nucleic Acids Research*, vol. 31, p. 3406-3415, 2003.
- [12] Z. Chen, L. Hu, B.T. Zhang, A. Lu, Y. Wang, Y. Yu, *et al.*, "Artificial Intelligence in Aptamer-Target Binding Prediction." *International Journal of Molecular Sciences*, vol. 22, 2021.
- [13] M. Song, G. Li, Q. Zhang, J. Liu, Q. Huang, "De novo post-SELEX optimization of a G-quadruplex DNA aptamer binding to marine toxin gonyautoxin 1/4." *Computational Structural Biotechnology Journal*, vol. 18, p. 3425-3433, 2020.
- [14] S. Soon and N. Aina Nordin, "In silico predictions and optimization of aptamers against Streptococcus agalactiae surface protein using computational docking." *Materials Today: Proceedings*, vol. 16, p. 2096-100, 2019.
- [15] X. Yu, Y. Wang, H. Yang, X. Huang, "Prediction of the binding affinity of aptamers against the influenza virus." *SAR and QSAR in Environmental Research*, vol. 30, p. 51-62, 2019.
- [16] J. Dickerhoff, K.R. Warnecke, K. Wang, N. Deng, D. Yang, "Evaluating Molecular Docking Software for Small Molecule Binding to G-Quadruplex DNA." *International Journal of Molecular Sciences*, vol. 22, 2021.
- [17] B. Queen, A. Mohammed, "Using Bioinformatics and NCBI Tools to Sequence and Structure Analysis of Transcription Factor 7 Like 2 Gene (TCF7L2) in Iraqi Diabetic Mellitus Type II Patients." *Journal of Applied Sciences and Nanotechnology*, vol. 2, p. 1-10, 2021.
- [18] K. Banu, B. Mondal, B. Rai, N. Monica, R. Hanumegowda, "Prospects for the application of aptamer based assay platforms in pathogen detection." *Biocybernetics and Biomedical Engineering*, vol. 42, p. 934-949, 2022.
- [19] A. Rafati, N. Dorosti, and P. Gill, "Smartphone-based technology for nanomolecular detection of aflatoxin B1 by aptamer-conjugated magnetic nanoparticles." *World Mycotoxin Journal*, vol. 15, p. 159-69, 2022.



- [20] H. Marofi, A. Rafati, P. Gill, "Aptamer-Based Magnetic Nanoprobe for Quantitative Measurement of Chloramphenicol in Milk through Portable Reader." *Journal of Medical Signals & Sensors*, vol. 13, p. 136-143, 2023.
- [21] D.K. Nguyen and C-H. Jang, "Ultrasensitive colorimetric detection of amoxicillin based on Tris-HCl-induced aggregation of gold nanoparticles." *Analytical Biochemistry*, vol. 645, p. 114634, 2022.
- [22] S.J. Lee, J. Cho, B-H. Lee, D. Hwang, J-W. Park, "Design and Prediction of Aptamers Assisted by In Silico Methods." *Biomedicines*, vol. 11, 2023.
- [23] S. Malhotra, A.K. Pandey, Y.S. Rajput, and R. Sharma, "Selection of aptamers for aflatoxin M1 and their characterization." *Journal of Molecular Recognition*, vol. 27, p. 493-500, 2014.
- [24] X. Guo, F. Wen, N. Zheng, M. Saive, M.L. Fauconnier, J. Wang, "Aptamer-Based Biosensor for Detection of Mycotoxins." *Frontiers in Chemistry*, vol. 8, p. 195, 2020.
- [25] S.H. Jalalian, M. Ramezani, N.M. Danesh, M. Alibolandi, K. Abnous, S.M. Taghdisi, "A novel electrochemical aptasensor for detection of aflatoxin M(1) based on target-induced immobilization of gold nanoparticles on the surface of electrode." *Biosensors and Bioelectronics*, vol. 117, p. 487-492, 2018.
- [26] S. Karapetis, D. Nikolelis, and T. Hianik, "Label-Free and Redox Markers-Based Electrochemical Aptasensors for Aflatoxin M1 Detection." *Sensors*, vol. 18, 2018.
- [27] J.L. Liu, M. Zhao, Y. Zhuo, Y.Q. Chai, R. Yuan, "Highly Efficient Intramolecular Electrochemiluminescence Energy Transfer for Ultrasensitive Bioanalysis of Aflatoxin M1." *Chemistry*, vol. 23, p. 1853-1859, 2017.
- [28] Y-H. Pang, L-L. Guo, X-F. Shen, N-C. Yang, C. Yang, "Rolling circle amplified DNAzyme followed with covalent organic frameworks: Cascade signal amplification of electrochemical ELISA for alfatoxin M1 sensing." *Electrochimica Acta*, vol. 341, p. 136055, 2020.
- [29] H.K. Kordasht and M. Hasanzadeh, "Specific monitoring of aflatoxin M1 in real samples using aptamer binding to DNFS based on turn-on method: A novel biosensor." *Journal of Molecular Recognition*, vol. 33, p. e2832, 2020.
- [30] Q. Qiao, X. Guo, F. Wen, L. Chen, Q. Xu, N. Zheng, *et al.*, "Aptamer-Based Fluorescence Quenching Approach for Detection of Aflatoxin M (1) in Milk." *Frontiers in Chemistry*, vol. 9, p. 653869, 2021.
- [31] L. He, Z. Shen, J. Wang, J. Zeng, W. Wang, H. Wu, *et al.*, "Simultaneously responsive microfluidic chip aptasensor for determination of kanamycin, aflatoxin M1, and 17 $\beta$ -estradiol based on magnetic tripartite DNA assembly nanostructure probes." *Microchimica Acta*, vol. 187, p. 176, 2020.
- [32] X. Guo, F. Wen, Q. Qiao, N. Zheng, M. Saive, M.L. Fauconnier, *et al.*, "A Novel Graphene Oxide-Based Aptasensor for Amplified Fluorescent Detection of Aflatoxin M (1) in Milk Powder." *Sensors*, vol. 19, 2019.
- [33] T.N. Kulikova, A.V. Porfireva, G.A. Evtugyn, T. Hianik, "Electrochemical Aptasensor with Layer-by-layer Deposited Polyaniline for Aflatoxin M1 Voltammetric Determination." *Electroanalysis*, vol. 31, p. 1913-1924, 2019.
- [34] M. Khodadadi, A. Malekpour, M.A. Mehrgardi, "Aptamer functionalized magnetic nanoparticles for effective extraction of ultratrace amounts of aflatoxin M1 prior its determination by HPLC." *Journal of Chromatography A*, vol. 1564, p. 85-93, 2018.
- [35] A.K. Pandey, Y.S. Rajput, D. Singh, R. Sharma, "Prediction of shorter oligonucleotide sequences recognizing aflatoxin M1." *Biotechnology and Applied Biochemistry*, vol. 65, p. 397-406, 2018.
- [36] T. Chalyan, L. Pasquardini, D. Gandolfi, R. Guider, A. Samusenko, M. Zanetti, *et al.*, "Aptamer- and Fab'-Functionalized Microring Resonators for Aflatoxin M1 Detection." *IEEE Journal of Selected Topics in Quantum Electronics*, vol. 23, p. 350-357, 2017.
- [37] R. Liu, F. Zhang, Y. Sang, M. Liu, M. Shi, X. Wang. "Selection and Characterization of DNA Aptamers for Constructing Aptamer-AuNPs Colorimetric Method for Detection of AFM1." *Foods*, vol. 11, 2022.