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Multi-input DNA-based Logic Gates for Profiling the MicroRNA Biomarkers of Hepatitis-C Viral Infection

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Abstract

Hepatitis C is a serious disease that cannot easily be diagnosed at the early stages of this illness. Recently, microRNA are introduced as an efficient biomarker for detecting it. Real-time PCR (The Polymerase Chain Reaction) is the most popular method to profile the microRNA expression. However, it is costly, time-consuming and its accuracy is not sufficient for the very few levels of these biomarkers. Recently, DNA logic gates are used to detect the microRNA expression volume. We presented a DNA-based logic system to detect the pattern of hepatitis C viral infection. The higher number of microRNAs that are detected means the diagnosis is more reliable, and the possibility of a false positive is less. The proposed system has no limits on the number of inputs. We tested it with 20 microRNAs of hepatitis-C viral infection. The results show that this gate can detect complicated patterns with higher fan-in, lower complexity/cost, better response time, and higher accuracy rather than real-time PCR.

Keywords: DNA logic gate, MicroRNA, Hepatitis-C.

1. Introduction

Hepatitis C is a serious liver disease that has been called a silent disease because people with this disease may not be aware of it. Most people who get infected develop a chronic or lifelong infection that over time, it can cause serious health problems including liver failure, liver damage, and even liver cancer [1]. Hepatitis C viral (HCV) infection is more prevalent than hepatitis B virus infection (HBV) and more than one million new cases of hepatitis C infection are reported every year, worldwide [2]. So the early diagnosis of this disease causes the treatment process will begin sooner. Diagnostic tests for hepatitis C can be divided into two general categories; serological assays and molecular assays. Serological assays detect antibodies related to the hepatitis C virus and it is called the anti-HCV test [3]. Antibodies are chemicals released during the infection in the bloodstream of the patient. The test results usually take a few days to several weeks. Anti-HCV is divided into a non-reactive or negative antibody test and a reactive or positive antibody test. A negative antibody test shows a person does not have Hepatitis C but the result is not reliable. The test needs to be repeated if a person has been exposed to the hepatitis C virus in the last six months. A positive antibody test means that a person has been infected. However, this result does not mean that you have been infected with hepatitis C. It may be antibodies that existed in the blood even if the person has already been infected with the virus and the virus has been cleared of the body. Therefore, the person will need an additional test called the RNA test or PCR (The Polymerase Chain Reaction) test. This test is reliable, which means that the negative result shows the person does not have hepatitis C and the positive result shows the infection in the body of a person. If the person has been infected, the pattern of RNA expression changes and the results of the PCR test will be positive [1]. So, the patient should be treated.

The PCR is a powerful technique for amplifying or copying specific pieces of DNA. PCR can characterize, analyze, and synthesize any specific DNA or RNA. This method requires two primer molecules to get the copying process started. The primers are short chains of the four different chemical components that make up any strand of genetic material (A, T, C, and G). These primers are similar to small pieces of DNA or RNA (MicroRNA). Therefore, it is not suitable for early biomarker detection. Over the past years, it found that

DNA molecules could be used for a new kind of computation. DNA-based computing is a fast-developing interdisciplinary research area that makes a wonderful bridge between biology and computation sciences and technologies [4] and [5]. This technology is a branch of computing in which DNA molecules/strands are utilized as the computation elements instead of conventional electronic devices such as transistors and gates.

A DNA-based computer is a set of DNA strands that can be programmed to solve problems. Due to the incredible data density in DNA and the high level of parallelism, it could be hoped that the DNA molecules are used as the computational elements [6]. DNA-based computing was firstly used by Leonard Adelman [4] in 1994 to solve the Hamiltonian problem. Adelman showed that DNA molecules could solve complex problems with a very high degree of parallelism and at a much shorter time than silicon computers. Later, this method was set aside because, despite the maximum use of DNA parallelism, there was no constructive design method, and a large number of strands were needed to solve large issues.

DNA-based computation also can be used to construct logical gates [7] in which binary calculations are performed by chemical reactions between input and internal strands of the gate. Various styles are proposed for DNA-based logic gate design, which is divided into two categories: Enzyme-based and enzyme-free. The enzyme-free design style is more popular because of design simplicity and lower experimental cost. Enzyme-free nucleic acid devices can perform Boolean logic gates by detecting biomolecular inputs such as DNA or RNA. Enzyme-free nucleic acid devices produce fluorescence and convert nucleic acid sequences into an electronic signal through an optical response [7]. It uses a Toehold domain to control the DNA displacement process (Toehold mediated).

The most important application of DNA logic gates is the diagnosis in medicine. These circuits can be used for detective and treatment of diseases. Researchers predict that soon, new diagnostic methods and possibly new therapies will be presented by using DNA-based computation in live cells [7].

This system evaluates the symptoms of diseases. If it does not detect conditions associated with the disease, the answer is "No" and in the case of the existence of the disease, the answer is "YES". In this case, the system can produce a DNA sequence with a therapeutic role and affect the expression of genes. In addition, this system will be able to adjust the drug release rate based on the severity of the disease.

We need to define an appropriate biomarker to diagnose a disease with the help of DNA computations. A biomarker is an indicator that shows the presence or progression of a particular condition. It can be used to diagnose a disease or monitor the efficacy of the treatment. The discovery of new biomarkers reduces the time and costs associated with drug development and the success rate of translating experimental drugs into clinical treatments increases [8].

One of the most useful biomarkers for diagnosing diseases is microRNAs. They are a class of non-coding RNA molecules ranging from 19 to 24 nucleotides and regulate more than 30% of all genes [7]. It should be noted that microRNAs play an important role in regulating genes involved in processes such as development, differentiation, and control of cell growth. Therefore, the change in expression of each of them can cause serious problems in the cells.

In this paper, we want to identify the changes in RNA expression using the DNA logic gates. This method has high sensitivity and accuracy and it can diagnose this disease at early stages. We designed a high fan-in DNA-based system to detect the biomarkers pattern of hepatitis C viral infection. High fan-in means a large number of microRNAs at various levels. Detection of a higher number of microRNAs makes diagnosis more reliable and reduces the probability of false positives. This paper is organized as follows. In the next section, a summary of previous work in the field of DNA calculations and logical gates of DNA are expressed. In section 3, explanations are given on the rational DNA gates for medical applications. Section 4 explains the proposed logical system for the diagnosis of hepatitis C viral infection. Simulation results are presented in sections 5 and finally, section 6 concludes the paper.

2. Related work

The ability to build high-controlling DNA architectures allows designers to discover new applications in different fields. In the following section, some studies related to DNA-based computation and DNA-based logical gates are expressed. The Seesaw Logic Gates, presented in [9], [10], and [11], offers an appropriate logic gate architecture for large circuits, which has a high level of scalability compared to previous methods. Deiters and Hemphill represent a DNA-based AND gate to detect microRNA expression patterns of complex diseases like cancer in live mammalian cells [7]. Since the excessive expression of miR-21 and miR-122 is involved in cancer and the replication of the hepatitis C virus, in [7] a special diagnosis is presented with two inputs miR-21 and miR-122.

In [12], a small set of the DNA-based logic gate is presented, which is produced fluorescent output by the Boolean logic gates functions AND NAND OR NOR XOR and XNOR. The proposed system can be used for diagnosis and treatment at the molecular level [12]. In [13], a linear DNA logic gate was designed to detect the microRNA based on the Toehold mediated strand displacement and the fluorescence resonance energy transfer (FRET). Two biomarkers microRNA-195 and microRNA-21 have been selected as the logical input for heart failure and fluorescent as the logical output [13].

In [14], for the first time is used to calculate DNA logic and measure DNA length. Also created a set of logic gates with unlabeled DNA input including the logical gates AND, OR, INHIBIT, and the 2 to 1 encoder [14]. Also in [15], two OR and AND gates are serially connected, which respond to MicroRNA inputs and produce a small molecular output.

To date, many of the encrypted microRNAs with some classes of viruses, such as the Hepatitis C virus (HCV) virus, have been identified. Hepatitis C is a positive-chain RNA virus that is a major cause of chronic liver injury in humans [16]. Hepatitis C virus is a type of contagious infection that causes liver inflammation by attacking the liver. It is transmitted through the connection with contaminated blood and the use of a common syringe in injectable drug addicts. Most people with this virus do not feel any signs and symptoms, and they may not even know that the virus is in their bodies. This chronic liver disease caused by the

hepatitis C virus is one of the major causes of hepatocellular carcinoma (HCC) [17]. Early diagnosis of diseases, such as viral infection, makes it possible to cure the disease before developing it. The most sensitive test for detecting RNA viruses is HCV RNA testing using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).
But the required equipment and materials for this method are costly and it is complex and less reliable because of the short length of microRNAs [18]. We are looking for a method with higher speed and less error. In this paper, a high fan-in DNA-based system is represented to detect the biomarkers pattern of hepatitis C viral infection. The biomarkers are microRNAs that play an important role in regulating the internal conditions of the liver. The output of the diagnosis system is a strand containing fluorescent that produces light and shows the change in the expression level of microRNA patterns. There- fore, the system diagnoses viral infection. High fan-in means this system can detect a large number of microRNAs at various levels. In addition to the lack of restrictions on increasing the number of inputs, this system can detect microRNAs simultaneously that have been reduced due to illness and those whose levels of expression have increased. As a special case, an implementation of this gate for the detection of 20 microRNAs introduced in scientific papers as biomarkers of this infectious disease are accurately simulated and investigated. This gate does not have errors in existing experiments and has a higher rate of diagnosis, which leads to early diagnosis of disease and the survival of many patients.

3. The Application of DNA-based Logic Gates in Medicine

As before mentioned, in [7] a DNA-based gate with miR-21 and miR-122 inputs was proposed to detect patterns of cellular microRNA expression in live cells. To display the output of the DNA computation, a photochemically activated logic gate is used with fluorescence output. The advantage of fluorescent output is that it is simple to see and low cost. In addition, it can be observed without cell lysis or RNA purification.
Figure 1, represents the 2-inputs logic gate designed in [7]. It is necessary that some sub-strands in both inputs of AND gate should be the same. Therefore, they can react to the structure of this gate. These sub-strands are called Toehold. Each input strand consists of two sub-strands as the toehold at the beginning and end of the strand, each one consisting of a combination of six nucleotides. In order to construct the AND gate presented in [7], they need to produce middle strands (M122 for miR-122 and J21 for miR-21). If the input strand exists (input = `1'), a strand, which has the same Toe-hold with other input, is generated by an extra reaction. Otherwise if (input = `0'), it is not produced. Therefore, the AND gate does not react and the output of the circuit remains zero. However, designing a two-input gate in two-floor increases the number of strands. In addition, additional reactions will increase the complexity of the system.

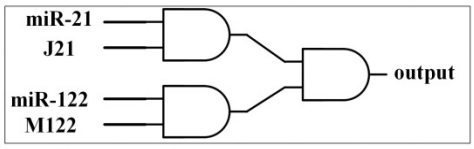

Figure 1: Schematic representation of the proposed AND gate in [7].

Figure 2 represents the implementation of the two-input AND gate. In this figure, colored parts are considered as Toehold. It is shown for miR-21 with blue and green colors and for miR-122 with orange and red colors. The sub-strands with the same color react together. The red part is the same toehold that has to be in both inputs of the gate to react with the gate. However, this is not possible naturally. Therefore, there is a need for additional strands (J21 and M122). The blue circle Q represents the BlackHoleQuencher2 and the red circle F shows TAMRA Fluorophore. Finally, the release of the strand containing F activates fluorescence and produces light.

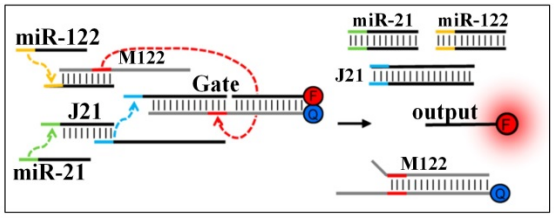

Figure 2: Implementation details of two-inputs AND gate presented in [7].

In the method presented in [7], the auxiliary inputs with the value of '1' and additional logic gates are needed which increases the number of levels and the additional reactions. Therefore, this method has more complexity and excessive overhead. This is one of the main disadvantages of this method. Moreover, this logic gate cannot be extended easily. Since a large number of microRNAs should be identified for the detection of diseases such as cancer and viral infection, high-fan logic gates are required.
In addition, in 2017, in [15], a work similar to paper [7] is presented with some changes. In this paper, with a serial connection of two-gate OR and AND, a logic DNA system is designed that can produce a small molecular output signal. The benefits of small molecular outputs are compared with acid nucleic or proteins that can be synthesized freely and expand the use of modular DNA computations. Figure 3, shows the overview of the proposed gate design [15] and details of its implementation.

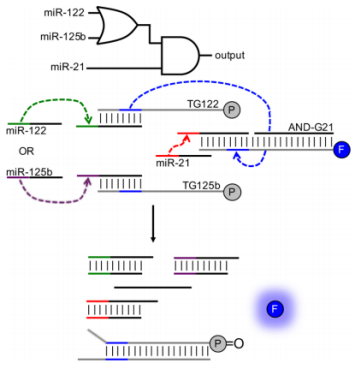

Figure 3: the overview of OR-AND gate [15]. A) Electronic representation of the gates. B) Schematic representation of the DNA implementations.

Conventionally, the output of an OR logic gate will be '1' when t least one of the inputs is '1'. so, the OR gate output is produced by miR-122 and TG122 reactions, or miR-125b and TG125b reactions, or both. The OR gate output reacts as one of the AND gate inputs with the third input, miR-21. The AND logic gate output, in the presence of both inputs, will be '1'. So, the final output in the presence of miR-21 and at least one of inputs of OR gate, will be '1' and will be produced a small molecular signal at the output which produces light by releasing fluorescence. The main advantage of this method is removing the auxiliary strands of [7] that lead to a simpler and faster gate.

4. The Proposed High Fan-in DNA Logic Gate

In this paper, a DNA-based system is presented to detect the pattern of expression of the hepatitis C biomarkers and save the lives of many patients with early diagnosis and treatment. When all inputs of gate exist, the reaction between the input strands of microRNA and the gate strands takes place, and a fluorescent strand is produced at the output. As mentioned before similar sub-strands must be existed in inputs strands of [7] while such conditions do not exist in real situations. Therefore, the authors of [7] used auxiliary gates between inputs and the main gate with an input always `1' to overcome this problem. Auxiliary input strands and gates will increase the complexity of the gate. The presented gate does not require these auxiliary strands. In Figure 4-A, subsections of logic gate inputs presented in [7] (miR-21 and miR-122) are shown. As shown in Figure 4-B, in the method [7], the right subsection of miR-21 (GT *) must be a complement of the left subsection of miR-122 (Z) to react two strands together. Naturally, this is not possible because the structure of the microRNAs cannot be changed. Therefore, two additional logic gates and two auxiliary inputs are required for the two-input AND.

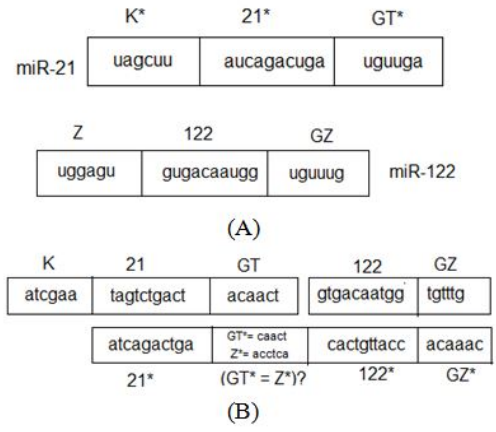

Figure 4: (A) Subsections of logic gate inputs. (B) The structure of a two inputs logic gate in [7].

Figure 5 shows the presented two inputs DNA logic gate that does not require any auxiliary inputs.

Figure 5: Symbolic view of the proposed two-input DNA logic gate.

In this gate, the first #6 nucleotides of the miR-122 are joined to the complement of miR-21 and separated from the miR-122. This separation is shown by a small distance between Z and 122 in Figure 6.

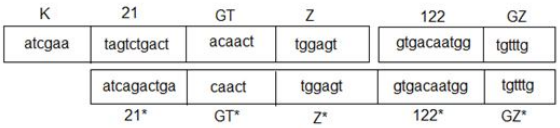

Figure 6: The structure of the proposed two-input logic gate.

Figure 7 represents the operation of the proposed structure of a two-input gate step by step. In Figure 7-A, miR-21 entered into the system, it joins to the upper part of the gate strand (K, 21, GT) and separates double-strand miR-21 from the gate. It should also be noted that the sub-strand Z is also separated from the gate by splitting the miR-21 from the gate because it does not connect to the rest of the gate. In Figure 7-B, another input (miR-122) enters into the system to generate the double-strand miR-122 and releases the output strands (122, GZ, Flour).

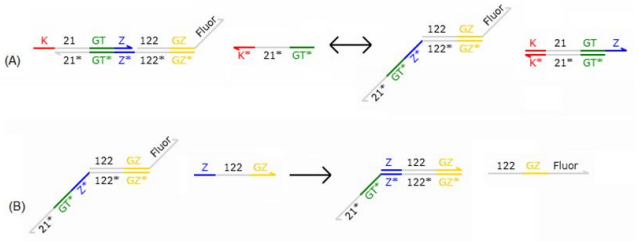

Figure 7: (A). Enter the miR-21 strand to the system. (B). Enter the miR-122 strand to the system.

When both inputs (miR-21 and miR-122) exist in the system, a chain of reactions is completed and the output strand is released. The proposed diagnosis system does not depend on the structure of input microRNAs and can use any microRNA as an input. In addition, the number of input microRNAs can increase because the function of this system does not limit the microRNAs. Consequently, the mentioned gate design can be easily extended to the design of multi-input gates. This paper provided a 20-input logic system Using the structure of the presented two-input gate. The inputs are miR-21, miR-221, miR-155, miR-122, miR-141, miR-100, miR-181, miR-34, miR-130, miR-199, miR-301, miR-27, miR-92, miR-320, miR-10, miR-200, miR-20, miR-618, miR-134 and let7-g. These inputs according to [8] and [19] are biomarkers of the hepatitis C virus that one of them is Down-regulated (let7-g) and the rest is up regulated. The expression of Down-regulated biomarkers decreases and the expression of Up-regulated biomarkers increases in the cells during the viral infection.

It should be noted that the structure of the system should not be changed for new inputs because the proposed system does not depend on the structure of the input strands. The gate strand is defined based on the inputs in the template that specifies the proposed structure.

The inputs strands and the presented 20-input system are shown in Table 1. When all of 20-input exist in the system, the fluorescent output is released and the risky situation will be alarmed.

Table 1: Input strands of the presented 20-input logic gate.

miRNA	Expression change	miRNA	Expression change
miR-21	UP	Let-7g	DOWN
miR-221	UP	miR-301	UP
miR-155	UP	miR-27	UP
miR-122	UP	miR-92	UP
miR-141	UP	miR-320	UP
miR-100	UP	miR-10	UP
miR-181	UP	miR-200	UP
miR-34	UP	miR-20	UP
miR-130	UP	miR-618	UP
miR-199	UP	miR-134	UP

The advantages of the proposed system are the elimination of the auxiliary inputs and additional gates, the reduction of the gate complexity and number of the strands, and gate extension for more inputs for real applications. Other advantages of this method are increasing the speed of detection and automate-ability of diagnostic system design specified for each person. The microRNAs that changes are the same in an illness for all patients, but the changes of expression microRNAs are not the same. Each person may have a pattern of change. Therefore, the customization of the diagnostic system for each person is an appropriate option for diagnosis.

5. Simulation and Results

We modelled and simulated the high fan-in logical system (20 inputs) using the Visual-DSD tool (v0.14) [20] that is a well-known toolbox for DNA strand displacement simulation provided by Microsoft Research and has been used in numerous researches [21]. This tool simulates the reactions between the DNA strands and generates the results such as reactions graph, reaction time, and concentration of output strands. Since the DNA and RNA strands are similar and just differ in just one nucleotide, Visual-DSD uses the same method to simulate both of them. Both DNA logic gates simulated and compared; the extension of the presented gate in [7] and the proposed gate in this paper. The inputs of both gates are 20 biomarkers of the hepatitis C virus (miR-21, miR-221, miR-155, miR-122, miR-141, miR-100, miR-181, miR-34, miR-130, miR-199, miR-301, miR-27, miR-92, miR-320, miR-10, miR-200, miR-20, miR-618, miR-134 and let7-g), one of them is down-regulated (Let7-g), and the rest are up-regulated.

In both gates, the concentration of 70nM to 100nM is considered as logical `1' of input (presence of input), and a concentration of 0 to 30nM is considered as a logical `0' of input. In addition, the concentration of 1000nM is selected for gate concentration. The output is a strand containing a fluorescent section that can be detected by Elisa or any fluorimetric device.

The proposed gate reactions may be reversible because the generated strand is not stable. The released strand of the first-step reaction is combined with the gate-strand and returns the gate to its initial state and does not allow the reaction to continue. Although the first input of the gate is consumed, the output has not been generated. Our analyses and simulations show that the probability of reverse reactions very low such and can be concluded the concentration of the final strand will be acceptable. However, to resolve this problem, for the first few inputs, a strand called sink is defined that removes the intruder causing the problem from the reactions. In addition, by releasing the input strand, increases the number of reactions and produces more output. The number of sink strands required will be discussed, later. NOT used to define the down-regulated string (Not-Let7-g). In the presence of Let-7-g the NOT strand (complement of Let-7-g) is combined with the total concentration of Let-7-g and the reaction is stopped. Otherwise, the complementary string will be combined with the other strands and they will participate in the production of output.

In Table 2, the functional correctness of the proposed gate and Hemphill & Deiters gate will be discussed. In this table, columns L and C represents the logical and absolute value of output concentration (in Nano-Mols), respectively. This table shows the results for 4 values of inputs (00000h, 00001h, FFFFEh, and FFFFFh) in hexadecimal format. In the second column, the condition is checked that all inputs do not exist and the input strand has a logical value of "00000". In the third column, one of the inputs is ON (mir-134) and the rest is OFF "00001" and in the fourth column, one of the inputs is OFF (mir-134) and the rest is ON "FFFFE". The final column shows the condition in which all the inputs are ON. Logic and Sub-columns *con.* shows the logical value and concentration level of the corresponding DNA strand.

Table 2: The functional correctness test.

Input	"00000"		"00001"		"FFFFE"		"FFFFF"	
Data Type	L	C (nM)	L	C (nM)	L	C (nM)	L	C (nM)
[7]	'0'	6	'0'	6	'0'	10	'1'	71
Our gate	'0'	7	'0'	7	'0'	10	'1'	75

According to Table 2, can be concluded that both of the gates have the correct function and the logical value of output will be `1' only when the inputs are ON ("FFFFF"). Figure 8 shows the response time of the proposed logic gate and Hemphill & Deiters gate with input "FFFFF". Response time defined as the time required to reach the highest concentration of output. The red curve indicates the proposed gate and the green curve represents the method of [7]. As shown in figure 8, the proposed gate reaches the highest concentration of output in the threshold value, sooner than method [7], so the velocity of the proposed gate is higher than [7]. The main reasons are the simplicity of the proposed gate and the low number of reactions for producing the final string compared to the method [7].

The maximum concentrations of output and gate strands for the proposed gate and [7] are examined in Nano-Mols in Table 3. It is worth noting that these parameters depend on the concentration of input strands. Therefore we evaluated these parameters for two states (State1 and state2). In this table, CIH and CIL show the concentration of the input strands corresponding with the logic '1' and logic '0', respectively, and CG represents the concentration of the internal gate strand. In each column, a certain concentration for the input '1', input '0', and the gate concentration are considered. The maximum concentration of output strand is investigated for both gates in these conditions.

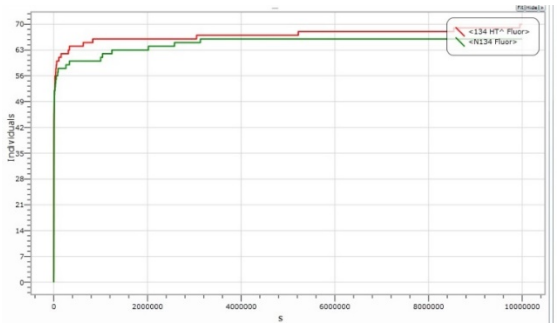

Figure 8: Concentration level of the output strands for the proposed gate (red curve) and the method [7] (green curve).

Table 3: Maximum Output Concentration for Different Input Concentrations in Logic Mode '1'.

Signal	Concentration Levels Signal (nM)	
	State 1	State 2
C_{IH}	100	1000
C_{IL}	10	100
C_G	1000	10000
Output of the proposed gate	75	747
Output of Gate in [7]	71	707

In Table 3, it can be seen that in different concentrations of the input (columns of the Table), the maximum concentration of output for the proposed gate is higher than the maximum concentration of output for the gate [7]. It should be noted that the number of samples is considered 10000. The output concentration of the 20-input proposed gate and the 20-input gate of [7] has been checked in constant time. The results of these gates are shown in Table 4. In each column, a different concentration of the input and gate strands is considered and the output concentration for our gate and gate [7] is shown in a constant time. It is worth noting that the concentrations are represented in nano-Mols.

Table 4: Output concentration in constant time.

Signal	Concentration Levels Signal (nM)	
	State 1	State 2
C_{IH}	100	1000
C_{IL}	10	100
C_G	1000	10000
Output of the proposed gate	65	680
Output of Gate in [7]	62	646

By comparing the results of Table 4, we find that independent of the input concentrations, at a constant time; our gate has a higher concentration of output strands. This indicates that the speed of our gate is greater than the gate [7]. In this table, C_{IH} and C_{IL} show the concentration of the input strands corresponding with the logic '1' and logic '0', respectively, and C_G represents the concentration of the internal gate strand.

In Table 5, the time to reach a constant concentration of output is investigated for the proposed gate and gate [7]. In each column, the different concentration of the input strands and the gate strands is considered and a constant concentration of output (C_{OUT}) is specified, proportional to it. The arrival time of our gate and gate [7] to this output concentration, is calculated.

Table 5: Time to reach the constant output concentration.

Concentration (nM)				Time (nM)	
C_{IH}	C_{IL}	C_G	Concentration C_{OUT}	Our gate	Gate of [7]
100	10	1000	71	1.34E+5	1.52E+6
1000	100	10000	670	5.34E+3	3.2E+4
10000	1000	100000	5978	1.19E+3	3.2E+3

As shown in Table 5, for different concentrations of input, the time to reach the constant concentration of output for the proposed gate is less than gate [7]. Therefore, the speed of the proposed gate is higher. In addition, by increasing the concentration of inputs and gate strand, the speed of reaching the logic value '1' increases for both gates. Improving proposed gate speed relative to the gate [7] is shown in table 6 for different concentrations of inputs.

Table 6: Improving the proposed gate speed relative to the gate [7] for different input concentrations.

State	Concentration (nM)			Our gate speed ratio
State 1	100	10	1000	11.36
State 2	1000	100	10000	6.16
State 3	10000	1000	100000	2.70

In Table 6, we see that the speed rate of improving the proposed gate relative to the gate [7] is decreased by increasing the concentration of the input string and gate. As mentioned before, sink strands were used to solve the problem of the reversibility of reactions. In table 7, the effect of the number of sink strands on the reaction speed is shown. The concentration of input strands is considered 100nM as logical value `1', 10nM as logical value `0', and 1000nM as the concentration of gate strand. In each column, has been shown the time to reach the constant concentration of output with a specific sink number for the proposed gate.

Table 7: Effect of number of sink strands on reaction speed.

NSink number of sink	Constant output concentration	Time (nM)	Speed ratio
0	64	32:50E + 5	X
1	64	13:50E + 5	2.4 X
2	64	13:00E + 5	2.5 X
3	63	40:00E + 5	8.12 X
4	64	35:00E + 4	9.28 X
19	65	1749.98	1857.16X

According to Table 7, the speed of the proposed gate increases with the increasing number of sinks strands. The maximum number of sink strings we can have for the 20 inputs gate is 19(N-1). As we said before, one of the reasons for the higher speed of the proposed gate is the less number of strands and reactions to release the output strand than method [7]. In Tables 8 and 9, the number of inputs, gate strands, and reactions are shown for the proposed gate and the gate [7]. Note that our gate is considered without a sink string.

According to table 8, the number of our gate strands ($N_{our-gate}$) is less than the number of Deiters gate strands ($N_{Deiters}$) for the different number of gate inputs. Also, by decreasing the number of gate inputs (N_{in}), the difference between the number of strands in our gate and Deiters gate is decreased.

Table 8: Compare the number of gate strands with different inputs.

N_{in}	Number of gate strands	
	$N_{our\text{-}gate}$	$N_{Deiters}$
4	12	16
16	48	64
17	51	68
18	54	72
19	57	76
20	60	80

Table 9: Compare the number of reactions in the gates with different inputs.

N_{in}	Number of gate strands	
	N_R	N_{RD}
4	13	14
16	61	74
17	65	79
18	69	84
19	73	89
20	77	94

In table 9, for the different number of gate inputs (Nin), the number of our gate reactions (NR) is lower than the number of Deiters gate reactions (NRD). In addition, in each column, the difference between the numbers of reactions in these gates is decreased by the decrease in the number of gate inputs. It should be noted that the number of strands and the number of reactions in our gate decreases in the presence of sink strings.

According to Tables 8 and 9, the additional strands and reactions in the gate [7] do not exist in our gate, and due to the fewer number of strands and reactions, the overhead and the complexity of our gate will be decreased.

As has been said, recently, DNA-based logic gates have been used detect to microRNA expression levels that are more accurate and faster than previous methods, and have not required high cost and advanced laboratory equipment. Hence, in this paper, a 20-input DNA-based logic gate was presented that was compared with the 20-inputs logic gate [7]. Based on the simulation results, it was observed that due to the less complexity and the presence of additional strands and gates, our presented gate is faster. It should be noted that with the definition of sink strands, this speed is also improved. In addition, due to the less complexity and simplicity of structure, it is easier to expand our gate.

3. Conclusion

Early diagnosis of Hepatitis C viral infection can be very beneficial and it can cause early treatment. Therefore, the probability of saving the patient's life increases. The best way for disease diagnosis is to use microRNAs. In recent years, DNA logic gates are used to detect the microRNA expression level as a biomarker. It is less costly, more accurate, and faster than previous methods. Therefore, with the early diagnosis of diseases, many human beings can be saved. In the method [7], similar sub-strands should exist in input microRNAs that do not meet normal microRNAs. The authors of [7] used auxiliary input strands to overcome a problem that will increase the complexity of the gate. This paper presents a high fan-in DNA logic system with specific microRNA inputs to detect hepatitis C viral infection. It is possible to use this DNA-based logic gate for blood plasma and cell samples and it should be noted that the nature of the samples does not affect the results. If all inputs are present, the disease in the human body is diagnosed. The simulation results demonstrate the accuracy, efficiency, and speed of our proposed gate. In addition, this gate has less cost, complexity, and error probability due to the low number of required strands and reactions. In the future of this research, we can design a multi-threshold logic system that recognizes different levels of biomarkers because the change rate of disease biomarkers is different. In addition, a new design for improving the Not Logical Gateway can be provided.

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