# A Parallel Approach to Perform Threshold Value and Propagation Delay Analyses of Genetic Logic Circuit Models

Sanaullah,[*,†] Hasan Baig,[‡] Jan Madsen,[¶] and Jeong-A Lee[*,†]

†*Computer Engineering Department, Chosun University, Gwangju 61452, South Korea*

‡*Center for Quantitative Medicine, UConn Health, 263 Farmington Avenue, Farmington, CT 06030-6033, USA*

¶*Applied Mathematics and Computer Science Department, Technical University of Denmark, Kongens Lyngby 2800, Denmark*

E-mail: mr.raosanaullah@gmail.com; jalee@chosun.ac.kr

## Algorithm

**Algorithm.** Consider the time-scale plots drawn for the sample values from Table S1 shown in Figure S1. The algorithm triggers the input concentration to certain levels after a specific time delay and observes the output behavior of the circuits. It requires a specific input combination to determine the threshold value, which indicates that all the input combinations need to be checked one by one until the one that triggers the output concentration is found. For some circuits such as OR, NOR, AND, and NAND, the output transitions can be observed by triggering both the inputs to the same concentration level simultaneously. As such, the algorithm follows the pattern 00 to 11, and consequently, the estimation process is relatively faster for some circuits - for example, an AND gate.

Table S1: Sample values of the user-defined parameters for threshold value analysis

| **Parameters** | | **Values** |
|---|---|---|
| Input concentration | $C_{in}$ | 0 |
| Incremental value | $Inc$ | 2.75 |
| End concentration | $C_{inE}$ | 15 |
| Assumed time delay | $T_D$ | 800 |
| Settling time | $S_T$ | 200 |
| Number of iterations | $i$ | 10 |
| Amount of time to verify each iteration | $V_T$ | 1000 |
| User defined upper threshold value percentage | $OC_{DUTh}$ | 90 |
| User defined lower threshold value percentage | $OC_{DLTh}$ | 30 |

For a genetic AND gate, the case of the input combination "11" is shown in Figure S1. To determine whether the output concentration crosses the input concentration, we need the initial output protein concentration for the input logic combination "00." Therefore, both the inputs are maintained at zero concentration for the first 800 time units ($T_D$), and the average initial output concentration is obtained. After the specified time ($T_D$) has passed, the input concentration is incremented to the next level as indicated by line 32 in Algorithm S1. Notably, the algorithm also operates for the case where the average initial output concentration is high - for example, a NOT gate - by iteratively incrementing the input concentration until the output concentration falls below the input concentration level. The point $t1$ in Figure S1a is where the output protein concentration crosses that of the input. This is the *possible* threshold value of 5.5 molecules for the given example. Subsequently, the value for which the algorithm initiates a separate loop and simulates the circuit model for a defined number of iterations $i$, which is 10 in this case, needs to be verified. This loop is defined in lines 12-20 in Algorithm S1.

The input protein must only be triggered after the initial concentration of output is settled as shown in Figure S1b to measure the propagation delay correctly. At this time, the algorithm allows the user to specify a period of time called the settling time $S_T$, as mentioned before, after which the initial output is expected to become stable. Only after $S_T$ amount of time has elapsed does the algorithm trigger the inputs. The propagation

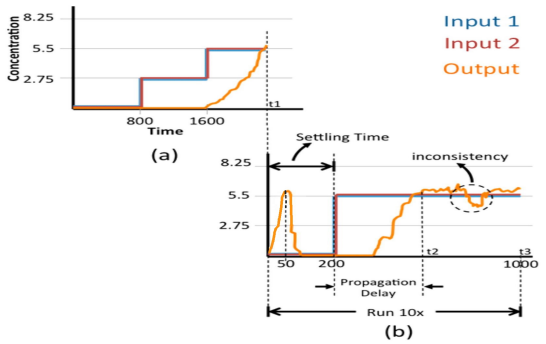


Figure S1: Figure a, shows the settling time and b, the concept of inconsistency of the threshold value and timing analysis of genetic logic circuits (time-scale plot) [1].

delay may be incorrectly estimated if a small value of $S_T$ is chosen; therefore, depending upon the complexity of the given circuit, the user should carefully select this value. In the example shown in Figure S1b, if instead of 200, a value of 50 time units is chosen for $S_T$, the algorithm would estimate the propagation delay to be zero. This is because, at 50 time units, the output concentration would already be above the threshold level.

The output data from all $i$ iterations (10 in this case) are averaged for estimating the average propagation delay and the inconsistency in the threshold values. The concept of inconsistency is illustrated in Figure S1b. It is calculated by averaging the output data that are less than the input concentration after the output crosses the input concentration for the first time. Thus, in Figure S1b, the consistency is estimated between the two points $t2$ and $t3$ and this time duration is specified by the parameter $V_T$, which is the amount of time to verify the model in each iteration $i$. From Table S1, the user-defined acceptance percentage of output consistency for the upper ($OC_{DUTh}$) and lower ($OC_{DLTh}$) threshold values is 90% and 30%, respectively. This indicates that, if 90% of the average output data consistently remain above the input concentration between the instants $t2$ and $t3$, then this percentage value is accepted as the upper threshold value. Similarly, if only 30% of the average output data remain above the input level between the two instants $t2$ and $t3$, then this percentage

**Algorithm S 1:** Threshold value analysis

```
1: procedure BEGIN
     INITIALIZE (C_in, Inc, C_inE, T_D, S_T, i, O_S, V_T, OC_DUTh, OC_DLTh)
     /*
     C_in = Initial input concentration at which the analysis should start from
     Inc = Increment Value: Value added to the previous concentration level until the concentration level reaches C_inE
     C_inE = End concentration of input at which the analysis should stop
     T_D = Assumed time delay
     S_T = Settling time
     O_S = Name of output specie
     i = Number of iterations to verify the consistency of results
     V_T = Amount of time to verify a model for each iteration i
     OC_DUTh and OC_DLTh = user-defined percentage acceptance of output consistency for upper and lower threshold
     values respectively
     /*
2:   for1 all possible input combinations do
3:    if (C_inC == 0 ) then   /* C_inC = current input concentration level*/
4:      Determine initial output concentration (C_Oinit)
5:    else
6:      while1 (C_inC <= C_inE) do
7:        while2 (T_C1 <= T_D) do    /* T_C1 = current time l */
8:          Execute Simulation
9:          if (C_OS > C_inC) then /* C_OS = output concentration of selected specie*/
10:           PT = C_inC    /* PT = Possible Threshold Value*/
11:           /* Verification process*/
12:           for2 number of iterations i do
13:             while3 (T_C2 <= V_T) do    /* T_C2 = current time 2*/
14:               Execute Simulation
15:               if (T_C2 >= S_T) then
16:                 Trigger the input to the value of PT
17:                Store the output concentration data in array
18:             end
19:             Take running average of all output i arrays
20:           end
21:           Estimate time delay (T_E) and consistency (OC_E)
22:           Terminate loop2
23:       end
24:       if (C_OS > C_inC) do
25:         if (OC_E > OC_DUTh) then
26:           Consider lower threshold value = 0 if not found already
27:           Return the results and terminate all loops
28:         else if (OC_E < OC_DLTh)
29:           Save lower threshold level and resume analysis
30:         else
31:           Resume analysis
32:       C_inC = C_inC + Inc
33:       T_C1 = T_C2 = 0
34:     end
35:    end
36:   end
37: end
```

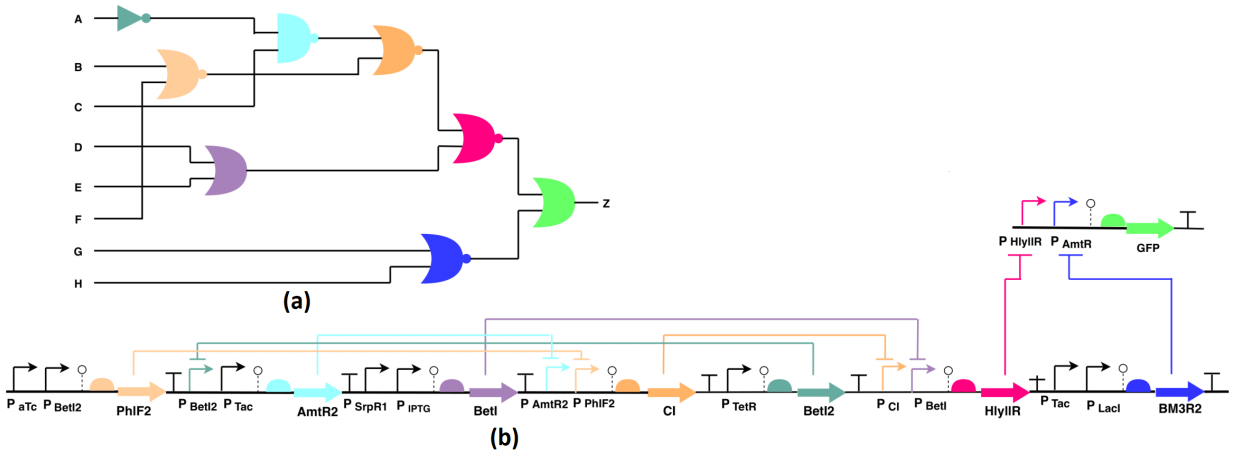


Figure S2: The 8-input circuit designed in iBioSim (a) Its schematic diagram and (b) the SBML model.

value is accepted as the lower threshold value as shown in lines 24-31 of Algorithm S1. The percentage output consistency is calculated using Equation 1.

$$\%\ output\ consistency = \frac{O_{t2-t3} - D}{O_{t2-t3}} * 100 \tag{1}$$

where $O_{t2-t3}$ is the average output data between $t2$ and $t3$ and $D$ is the deviation or the number of times the output data deviate from the expected threshold value. This deviation is defined differently for the two cases. When the initial input concentration is low, $D$ is the number of times the output is observed to be *less* than the possible threshold value as shown in Figure S1b and vice versa. If the user-defined output consistency threshold cannot be satisfied, the current results are discarded and the analysis is resumed from point $t1$.

## Genetic Circuit

The 8-input genetic circuit tested was designed in iBioSim[2]. Its schematic diagram and the SBML model is shown in Fig. S2.

# References

(1) Baig, H.; Madsen, J. Simulation Approach for Timing Analysis of Genetic Logic Circuits. *ACS synthetic biology* **2017**, *6*, 1169–1179.

(2) iBioSim. `https://github.com/MyersResearchGroup/iBioSim`.