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Baig, Hasan; Madsen, Jan

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Logic and Timing Analysis of Genetic Logic Circuits using D-VASim

Hasan Baig and Jan Madsen
Department of Applied Mathematics and Computer Science
Technical University of Denmark
{haba, jama}@dtu.dk

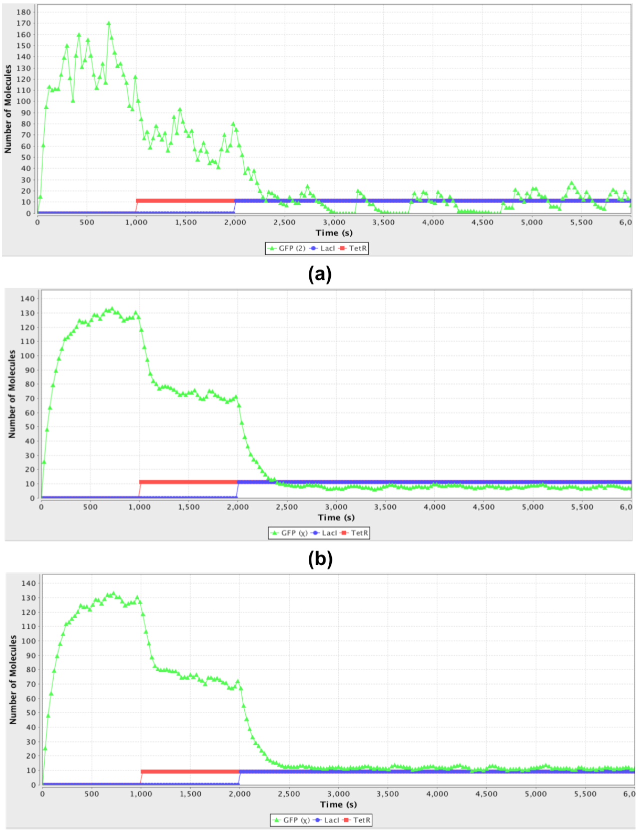
1. INTRODUCTION

In microelectronics, timing analysis is a very crucial requirement for ensuring the correct operation of a logic circuit. For the genetic logic circuits, the timing analysis may likely become an essential design characteristic. In digital electronics, circuits are made up of digital logic gates, which themselves are composed of transistors [1]. The transistors, typically used in the composition of digital logic gates, have well-defined threshold voltage value [1], which categorizes the logic level-0 and 1. To date, the timing characteristics, like propagation delay, hold time, setup time etc., are all well characterized. This is however not the case for genetic logic gates, where each of them are composed of different species and promoters, which may result in different threshold concentration values. Furthermore, digital logic gates have the same physical quantity, i.e., voltage, as their input and output. On the contrary, genetic logic gates usually have different species, which act as input to control the regulation of other specie, which acts as output. Besides this, the signals in electronic circuits propagate in separate wires, which does not directly interfere with each other. However in genetic circuits, signals are molecules, drifting in the same volume of the cell, and hence easily merge with the concentration of other compounds, resulting in crosstalk with the neighbouring circuit components. These facts make the timing analysis of genetic circuits quite challenging.

Similar challenges were encountered when the field of microelectronic circuits design was immature in its early days. Today, some of these challenges are solved through the enhancement in the fabrication process; others have been addressed through the development of advanced electronic design automation (EDA) tools. The advancement in the genetic design automation (GDA) tools can also help addressing these challenges, which may result in the reduction of design complexity of genetic logic circuits. D-VASim (Dynamic Virtual Analyzer and Simulator) is one such tool, which is developed for the simulation and analysis of genetic logic circuits [2]. A new feature of logic and timing analysis has been introduced in D-VASim. This feature is introduced to help users in verifying the logic function of a genetic logic circuit by extracting the observed logic function from the simulation data. During the analysis, a user may apply all the possible input combinations to find out the correct logic behavior of a circuit. The key challenge in determining the correct Boolean logic function from the analog simulation data is to categorize the input concentrations levels into logic-0 and logic-1. As mentioned earlier, this is similar to digital electronic circuits in which a certain threshold value of input voltage differentiates the logic levels 0 and 1 [1]. The threshold value for the concentration of input species must also be identified, which significantly effects the concentration of output specie of a genetic logic circuit.

2. METHODOLOGY

We performed the preliminary timing analysis on the genetic logic circuit models developed by Myers [3] using iBioSim [4]. Fig. 1 shows the results from running the stochastic simulation of the genetic NAND gate, one (Fig. 1(a)) and fifty times (Fig. 1(b) and (c)), respectively. The unit of species' concentration used for these models is the "number of molecules" [3]. Fig. 1(a) shows that both of the inputs are triggered to 11 molecules, TetR after 1000 time units (s) and LacI after 2000 time units (s), and that the output is highly stochastic, which makes it difficult to determine the input threshold value. A smooth output curve is obtained by plotting the average of 50 runs, as shown in Fig. 1(b) and (c).



(a)

(b)

(c)

Fig. 1. Preliminary analysis of a threshold value for the genetic NAND gate using iBioSim.

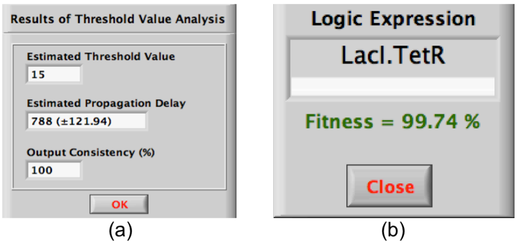
In Fig. 1(b), it can be observed that keeping the input concentration to 11 molecules causes the average output concentration to cross the level of input concentration. If we reduce the input concentration further to 10 molecules, the average output concentration stays above the level of input concentration, as depicted in Fig. 1(c). We performed the same analysis with different concentration levels on different logic circuits. Based on this analysis we define the threshold value as "*the minimum concentration of input species, which causes the average production of output specie to cross the level of threshold* *input concentration*". D-VASim obtains the threshold value of a genetic logic circuit iteratively; by automating this process of gradually increasing the level of input concentrations to a next user-defined level and observe if it effects the concentration of output specie. Another important parameter in obtaining the correct logic function of a genetic circuit is the *input-output propagation delay*. The input-output propagation delay can be defined as "*the time from when the input concentration reaches its threshold value until the time that corresponds to the instant when the output concentration crosses the same threshold value*". For instance, assume that the initial concentrations of the input species, of a two-input genetic AND gate, are zero (logic-0). The input-output propagation delay is the time between the instant when both of these input species are triggered to a significant concentration level (threshold level, logic-1) and the instant when the concentration of output specie reaches to the same significant level of concentration (threshold level, logic-1). Therefore, for the single-gate genetic circuits, the input-output propagation delay can also be termed as a *gate delay*. Similar to electronic circuits, where the multiple transitions of inputs go undetected when they occur either in one clock cycle or in a time less than the gate delay; the genetic circuits may also be expected to have such behavior. Hence, in order to obtain the correct behaviour of a genetic circuit, all the possible input combinations must be applied each after this propagation delay. The propagation delay in Fig. 1(b) is about 400 time units.

3. EXPERIMENTATION AND RESULTS

D-VASim requires some user-defined parameters to perform threshold value and propagation delay analysis. It begins by asking *Start at* value, which specifies the concentration of input specie(s), from which the tool should start its threshold analysis. The user is also required to specify *Increment of* and *Stop at* parameters. *Increment of* denotes the value with which the input concentration is increased for each iteration. *Stop at* value specifies the input concentration at which the algorithm should stop the analysis of the threshold value. When D-VASim estimates the possible threshold value, it verifies this value by iterating the model for the user-defined *No. of iterations*. During this iterative verification process, D-VASim obtains the average propagation delay. It also identifies how consistent the average output for the estimated threshold value is.

The algorithm requires an initial assumption of the propagation delay value. It is already mentioned earlier that the propagation delay value is critical in extracting the correct logic behavior of a circuit model. Thus, it is necessary for D-VASim to wait until this time value has lapsed before switching on to the next combination of inputs while searching for an appropriate threshold level. Since the propagation delay value is unknown for the automatic analysis, D-VASim begins the analysis with an assumed value and later estimates the approximate one. Assuming a higher value increases the estimation time but gives a more accurate estimation of the threshold value and propagation delay.

Fig. 2(a) shows the threshold value analysis results obtained by D-VASim for the genetic AND gate [3]. D-VASim also estimates the standard deviation (enclosed in braces in the *Estimated Propagation Delay* value box) of propagation delay for all number of iterations. These results indicate that an input concentration value of 15 (default units obtained from SBML model) is a threshold value for this model. Any value below is considered as logic-0 and above as logic-1. When the concentration of both the inputs are kept above 15 units, the output specie is supposed to be triggered approximately after 788 (±121.94) time units. The *output consistency* specifies that the average output, during iterative analysis, remained 100% consistent for the estimated threshold value and propagation delay.



Results of Threshold Value Analysis

Estimated Threshold Value: 15

Estimated Propagation Delay: 788 (±121.94)

Output Consistency (%): 100

Logic Expression

LacI.TetR

Fitness = 99.74 %

Fig. 2. Outcomes of D-VASim automated analysis. (a) Results of threshold value and propagation delay analysis (b) Behavior of a model in terms of Boolean expression.

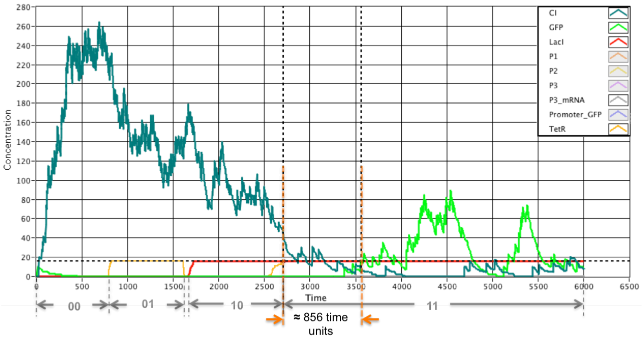


Fig. 3. Simulation traces for genetic AND gate in D-VASim.

These results can be verified in the simulation traces shown in Fig. 3. It can be observed clearly in this figure that when both the inputs, LacI and TetR (see [3] for circuit details) are triggered to logic-1 (threshold concentration level ≥ 15 molecules at ~2700 time units), the output specie (GFP) turns to logic-1 (crosses this threshold value) approximately after 856 time units, which lies under the standard deviation calculated by D-VASim.

When all the possible input combinations are applied with an estimated propagation delay, a logic verification option can be executed. Fig. 2(b) shows the Boolean expression obtained for this genetic circuit model from the simulation data. D-VASim also calculates the percentage fitness of an obtained Boolean expression in the entire simulation data. This tells that the extracted Boolean expression of this model satisfy 99.74% of the simulation data. The rest 0.26% are those instants where the output of an AND gate is low when both of its inputs are high (see Fig. 3 after 3500 time units).

4. SUMMARY

The capability of timing and logic analysis of genetic circuits may help users to characterize the timing constraints of genetic components. With D-VASim, it is also possible to perform the timing and logic analysis on the intermediate components of complex genetic circuits. We will explore this feature further by analysing the timings on recently published genetic circuits [5].

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