Implementation of a genetic logic circuit: bio-register

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Abstract We introduce an idea of synthesizing a class of genetic registers based on the existing sequential biological circuits, which are composed of fundamental biological gates. In the renowned literature, biological gates and genetic oscillator have been unveiled and experimentally realized in recent years. These biological circuits have formed a basis for realizing a primitive biocomputer. In the traditional computer architecture, there is an intermediate load-store section, i.e. a register, which serves as a part of the digital processor. With which, the processor can load data from a larger memory into it and proceed to conduct necessary arithmetic or logic operations. Then, manipulated data are stored back to the memory by instruction via the register. We propose here a class of bio-registers for the biocomputer. Four types of register structures are presented. In silicon experiments illustrate results of the proposed design.

Keywords Synthetic biology · Genetic logic gate · Bio-register · Bio-computer

Introduction

In the past decades, increasingly biological experts have valued design and construction of biological circuits as an important trend, which serves as a preliminary stage of developing the biocomputer. In which, the biological transistor is made from DNA and RNA instead of

traditional electrons (Bonnet and Yin 2013). It is regarded as a field of cross-disciplinary research. Both of the development in molecular biology and engineering can be applied in. Many scholars have been using the past well-developed ideas to explore biomolecular computing (Wang and Buck 2012; Miyamoto et al. 2013; Xie et al. 2011) in recent years. Logic circuits in synthetic biology are one of the research interests in systems biology (Gardner et al. 2000; Anderson et al. 2007; Siuti et al. 2013; Moon et al. 2012), which are extremely similar to electronic circuits in electronics. Several essential biological logic circuits have successfully been built to realize oscillator (Bintu et al. 2005) and synthesize fundamental biological gates (Bintu et al. 2005; Elowitz and Leibler 2000). In particular, some specific bio-combinational logic circuits and sequential logic circuits have been unveiled (Chuang et al. 2013; Lauria et al. 2004; Buchler et al. 2003).

An electronic computer consists of CPU, ROM, RAM and other integrated logic circuits. However, the concept of sequential and combinational logic hasn't been fully utilized in the current trend of developing biocomputer. In the computer construction, register is a major part of the central processing unit (CPU). It serves as an intermediate data storage unit while working with an accumulator. A register can reserve a command, a storage address, or any kind of data (such as a bit sequence or individual characters). With regards to the issue of construction of biocomputer, as a preliminary step toward the ultimate goal of implementing bio-CPU, we propose here four types of bioregisters which could be used for keeping information about molecule concentration. It is demonstrated that a multiple-bit register can be realized by cascading a series of combinational genetic logic circuits. The function is verified via simulation study.

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Model construction

In the electronic computer, a register serves as an intermediate data load-store section of the central processor. With which, the central processor fetches data from memory and temporarily puts data in the register for subsequent arithmetic or logic operations. After completing the operations, the resultant data are stored back to the memory via the register.

In general, a one-bit register is formed by an edge triggered D-type flip flop (FF), which forms as the fundamental unit of registers. It is a combinational circuit consisting of one NOT gate (INVERTER), two AND gates and four NAND gates as illustrated in Fig. 1.

To realize the idea in the biological sense, we establish the steps in the following sections.

Establishment of fundamental genetic gates

In the genetic systems, Boolean logic computing should be realized before conducting complicated logic or arithmetic operations. A genetic expression is composed of transcription and translation processes before generating genetic logic gates (Lin and Chuang 2014). DNA is transcribed into messenger RNA (mRNA) when an enzyme RNA polymerase (RNAP) restricts to the relevant promoter, the rate of transcription can be controlled by transcription factors (TFs). Employing standard biological parts (Marchisio and Stelling 2011), including reporter coding regions and transcription factor, RNA, promoters, one can build up fundamental genetic logic gates with specific functions.

We start by building mathematical models to describe biochemical reaction of the fundamental genetic systems. Typical reactions including a set of two differential equations can be described by Elowitz and Leibler (2000)

$$\begin{aligned}\dot{m}_i &= \alpha_i f_i(u) - \gamma_{m_i} m_i + \alpha_{i,0}, \\ \dot{p}_i &= \beta_i m_i - \gamma_{p_i} p_i, \quad i = 1, \dots, L\end{aligned}\quad (1)$$

where m_i and p_i represents, respectively, concentrations of mRNA and protein of the gene i , γ_{m_i} and γ_{p_i} represent,

Fig. 1 Logic diagram of the edge triggered D FF

respectively, degradation rates of mRNA and protein, α_i is transcription rate of mRNA, β_i is synthesis rate of protein, $\alpha_{i,0}$ is basal rate, $f_i(\cdot)$ denotes promoter activity function used to describe the non-linear transcriptional reactions, and u is concentration of TF from other inducers to control the gene expression.

A gene with an operator site can bind a repressor or activator TF, the promoter activity functions for the genetic logic NOT can be described as

$$f_{\text{NOT}}(u) = \frac{1}{1 + \left(\frac{u}{K}\right)^n} \quad (2)$$

where f_{NOT} is promoter activity functions of logic NOT, u is concentration of a repressor or activator TF, n is Hill coefficient denoting the binding cooperatively between TF and the corresponding operator, and K is Hill constant which is proportional to the lengths or affinities of the TF binding sites inserted in the promoter region of target genes. For the genetic expression of logic NOT, the gene is activated by unbinding with a repressor TF in the corresponding operator while its state is inactivated by binding a repressor TF. The framework is illustrated in Fig. 2a.

A gene with two operator sites can bind two repressor TFs or activator TFs, the promoter activity functions for the genetic logic AND gates can be described as

$$f_{\text{AND}}(u_1, u_2) = \frac{\left(\frac{u_1}{K_1}\right)^{n_1} \left(\frac{u_2}{K_2}\right)^{n_2}}{1 + \left(\frac{u_1}{K_1}\right)^{n_1} + \left(\frac{u_2}{K_2}\right)^{n_2} + \left(\frac{u_1}{K_1}\right)^{n_1} \left(\frac{u_2}{K_2}\right)^{n_2}} \quad (3)$$

where f_{AND} is promoter activity function for logic AND (Chen and Wu 2010), u_1 and u_2 are concentrations of

Fig. 2 Constructions of a class of genetic logic gates. **a** NOT gate, **b** AND gate, **c** NAND gate

repressor or activator TFs, K_1 and K_2 are Hill constants for u_1 and u_2 , respectively, n_1 and n_2 are the corresponding Hill coefficients. For the AND gate, the protein is produced only in the presence of both TFs. See Fig. 2b. The NAND gate can be realized by cascading a Not and a AND gates. See Fig. 2c.

Synthesis of NAND gate

A combinational biological register involving at least NOT, AND, NAND and other fundamental gates. One may acquire more details of biological gates from Wang and Kitney (2011). Using a hetero-regulation one may build AND gate in *Escherichia coli*. The AND gate comprises co-activating genes *hrpR* and *hrpS* controlled by promoter inputs, and output depend on $\sigma 54$ -dependent *hrpL* promoter. The ribosome binding site (RBS) is used to adjust the protein expression level when it was treated as a linear amplifier. The *gfp* reporter can be placed on the output as a marker. The next step is to cascade a NOT gate which is assembled with *cI*/Plam repressor module including λ gene *cI* and PR promoter.

With the above description, a modular combinational logic NAND gate can be produced in Fig. 3.

To build up the model, we consider the steady state behavior of mRNA in (1):

$$m_i = \frac{\alpha_i}{\gamma_{m_i}} f_i(u) + \frac{\alpha_{i,0}}{\gamma_{m_i}} \quad (4)$$

The protein concentration dominating the response is then given by

$$\dot{p}_i = \alpha_{p_i} f_i(u) - \gamma_{p_i} p_i + \alpha_{p_{0,i}} \quad (5)$$

where $\alpha_{p_i} = \frac{\alpha_i \beta_i}{\gamma_{m_i}}$ and $\alpha_{p_{0,i}} = \frac{\alpha_{i,0} \beta_i}{\gamma_{m_i}}$. Based on the simplified dynamic equation and Fig. 3, the dynamic equations characterizing behavior of the genetic NAND gate can be described as

$$\begin{aligned} \dot{p}_{AND} &= \alpha_{p_{AND}} f_{AND}(u_1, u_2) - \gamma_{p_{AND}} p_{AND} + \alpha_{p_{0,AND}}, \\ \dot{p}_{NOT} &= \alpha_{p_{NOT}} f_{NOT}(p_{AND}) - \gamma_{p_{NOT}} p_{NOT} + \alpha_{p_{0,NOT}}, \\ p_{AND}(u_1, u_2) &= p_{NOT} \end{aligned} \quad (6)$$

Synthesis of D-type flip flop

A D FF consists of several biological logic gates with a clock input as the triggering source. Implementation of the

Fig. 3 Combinational NAND gate

equivalent biological circuit to Fig. 1 is given in Fig. 4 (see the separate page containing the figure). As illustrated, by adding high or low protein concentration as inputs, one can expect the similar conclusion as that in the digital circuit.

The model of an edge triggered D FF can be formulated using the following Hill differential equations:

$$\begin{aligned} \dot{p}_{AND1} &= \alpha_{p_{AND}} f_{AND}(D, \bar{Q}) - \gamma_{p_{AND1}} p_{AND1} + \alpha_{p_{0,AND1}}, \\ \dot{p}_{AND2} &= \alpha_{p_{AND}} f_{AND}(p_{NOT}(D), Q) - \gamma_{p_{AND2}} p_{AND2} + \alpha_{p_{0,AND2}}, \\ Q &= p_{NAND3}(p_{NAND1}(p_{AND1}, clock), \bar{Q}), \\ \bar{Q} &= p_{NAND4}(p_{NAND2}(clock, p_{AND2}), Q) \end{aligned} \quad (7)$$

where

$$\dot{p}_{NOT} = \alpha_{p_{NOT}} f_{NOT}(\cdot) - \gamma_{p_{NOT}} p_{NOT} + \alpha_{p_{0,NOT}}$$

and D denotes concentration input, $p_{NOT}(\cdot)$ denotes output concentration of the NOT gate, output concentration of the NANT gate $p_{NAND}(\cdot, \cdot)$ is defined as in (6), *clock* is a periodic bio-clock signal. Conducting simulation confirms the result of the truth table of D FF in Table 1. Normalized output responses of the biological D FF are shown in Fig. 5, the leading edge trigger is reacted when the time is about the moment that the clock signal changes from low to high. The clock signal could be generated via a clock generator (Chen and Lin 2015). From this viewpoint, the stimulus' concentration level changes at the moment the clock signal triggering is enough to stimulate exact response. On the other hand, it was found that the signal exhibits a time delay while compared with the ideal response. This would be acceptable in the biological systems, because biochemical reactions are comparatively slow.

Realization of bioregister

The bioregister is formed on the basis of the D FF introduced above. We construct four types of bioregisters according to the way of data fetching and storage. These include the type of serial-in serial-out (SISO), serial-in parallel-out (SIPO), parallel-in serial-out (PISO) and parallel-in parallel-out (PIPO). At the beginning of operation, the statuses of all FFs are reset, i.e. their output Qs are set to zero. The bit information transmitted through one FF to the next within the register in which the output status changes, once it is triggered by the clock. The startup mechanism of an edge trigger in the 4 bit bio-register is illustrated next.

Type 1: SISO bioregister

The input message is applied sequentially to the input D of the first FF on the left (D0). When a clock pulse comes, one

Fig. 4 Schematic diagram of the biological D-type FF

Table 1 Truth table of D-type FF (1 (0) refers to normalized high (low) concentration), SET is determined by $P_{NAND3}(0)$ and RESET is determined by $P_{NAND4}(0)$

Set	Reset	D	CLK	Q	$\bar{Q}$
1	0	—	—	1	0
0	1	—	—	0	1
—	—	1	⌊	1	0
—	—	0	⌊	0	1

genetic message is transmitted from left to right for one bit. By this connection, the message is allowed to shift directly through the D FFs and out of the other end (Fig. 6). The message string is presented at ‘Data Input’, and is shifted to the right one stage with each clock. As the clock goes, the bit on the far left is shifted to the last FF’s output. The result demonstrates the same outcome as shift-registers in the digital logic circuit, see Fig. 7.

Type 2: SIPO bioregister

This type is similar to the SISO, except that the message was read in a parallel form from the outputs Qa to Qd. This configuration allows conversion of a “word” between serial to parallel format. The message is input serially. Once data have been clocked in, it may be either read off at

Fig. 5 Normalized response of the biological D FF

Fig. 6 Schematic diagram of the SISO register

Fig. 7 Normalized response of the SISO bioregister

Fig. 8 Schematic diagram of the SIPO bio register

each output at the same time, or it can be shifted out. For this configuration, each FF is edge triggered. Each subsequent FF halves the frequency of its predecessor doubling its duty cycle. Consequently, it takes twice as long for the rising/falling edge to trigger each subsequent FF; this staggers the serial input in the time domain while leading to the parallel output. See Fig. 8.

Type 3: PISO bioregister

This type plays the role on the contrary way to the SIPO above. The message is loaded-in in a parallel manner and all messages enter their input ports at the same time. The

Fig. 9 Schematic diagram of the PISO bioregister

Fig. 10 Schematic diagram of the PIPO bioregister

data are generated one bit at a time with each clock in a serial format. See Fig. 9.

Type 4: PIPO bioregister

This type also acts as a provisional storage device or like a time delay device. Data are presented in a parallel format to the parallel inputs and then transferred together to their respective outputs Qa to Qd. The data transfers are synchronized to the clock pulse. See Fig. 10.

Practical concern

Edge trigger will play an important role in biocomputers. Because a trigger determines whether a register output signal will go on to the next stage or not. Through the mechanism of strand exchange, the concentration of an mRNA molecule can be formulated as a condition to determine the time of transition. This mechanism could be used as signal conversion to implement on the edge trigger (Benenson 2009). This is reserved as an open issue to be investigated.

Conclusion

This paper presents an innovative idea for implementing a data register in biocomputer. The model for simulation study has been built. In silicon experiments have also been conducted to check correctness of the operation. The

results show feasibility of the proposed design. However, all the ideas mentioned here are just a beginning for possible realization of a functional biocomputer. We expect more successors to the current development.

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