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Synthesising gene clock with toggle switch and oscillator

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Abstract: The usefulness of a genetic clock lies in its role to stimulate a sequence of logic reactions for sequential biological circuits. A clock signal is a periodic square wave, its amplitude alternates at a steady frequency between fixed minimal and maximal levels. Transition between the minimum and the maximum is instantaneous for an ideal square wave; however, the function is unrealisable in physical bio-systems. This research develops a new genetic clock generator based on a genetic oscillator, in which, a sine wave generator is adopted as a signal oscillator. It is shown that combination of a genetic oscillator with a toggle switch is able to generate clock signals forming an efficient way to generate a near square wave. *In silico* study confirms the proposed idea.

1 Introduction

Many basic biological logic behaviours have recently been developed in the existing studies, such as oscillator [1], toggle switch [2] and NOT, AND, OR, NAND, NOR, XOR logic gates [3–5]. Moreover, some specific bio-combinational logic circuits and sequential logic circuits have also been developed [3, 6–9]. However, previous works focused in synthesising biological circuits cannot trigger bio-sequential logic circuits which required a square wave as the clock input.

Synchronisation of several logic circuits with a clock signal resort to the following requirements:

- The ability to memorise states is gained with synchronisation via clock signals.
- The possibility to design of biological circuits with higher complexity in time-related operational functions, such as a molecular computer.

In the previous study [7], the clock signal has been realised by combining a few fundamental genetic oscillators based on the Fourier series. Moreover, the clock signal has been applied in biological sequential logic circuits such as a JK flip-flop. Although the simulation results show satisfactory responses, it may resort to a large number of fundamental genetic oscillators to synthesise an ideal clock signal.

The genetic toggle switch developed in [2] is a benchmark achievement in synthetic biology, which is a synthetic, bistable gene-regulatory network in Escherichia coli. A toggle switch is a fundamental component for logic operation which is actuated by certain biochemical signal. In this research, we replace inducers (IPTG, aTc) in the traditional genetic toggle switch with a new input. Combining an oscillator with a toggle switch one is able to realise a square wave generator. This would be useful to simplify the circuit structure while preserving the ideal waveform.

Genetic oscillators have been widely studied by many researchers. For example, some researchers use an effective rate of synthesis of the repressor to couple an oscillator and a toggle switch in [10]. This construction was used to generate a permanent transition from a stable steady state to self-sustained oscillations after a transient external perturbation. In this study, the intention is to construct a genetic circuit which is constituted by an oscillator and a toggle switch to synthesise a genetic clock generator. Our previously developed sine wave generator [3] is adopted to form the oscillator. Referring to a class of frameworks of transcriptional regulation in [4, 5, 11] a dual repressor combines an oscillator and a toggle switch so that the toggle switch continuously changes its state when an exogenous input is presented. We observe and analyse the non-linear dynamic behaviour of this new biological network circuit through a linearised model. It is found that this biological network system exhibits the similar dynamic characteristics as a standard linear time-invariant second-order dynamic system. Comparing with the characteristic equation of a closed-loop second-order system, the damping ratio and the undamped natural frequency in the biological network can thus be determined. A clear relationship between biological parameters and natural frequency is successfully identified. On the basis of this result, the key parameters can then be tuned to control the natural frequency for specific applications.

2 Description of model

2.1 Genetic oscillator

The pioneers, Elowitz and Leibler, used three transcriptional repressor systems (*tetR*, *lacI*, *λcI*) to build a biological

oscillating network [1]. Its dynamics can be described by the following coupled first-order differential equations

$$\begin{aligned}\frac{\mathrm{d}m_i}{\mathrm{d}t} &= -\gamma_{m_i} m_i + \alpha_i b_i\left(p_j^n\right) + \alpha_0 + w_{i1}(t)\\ \frac{\mathrm{d}p_i}{\mathrm{d}t} &= \beta m_i - \gamma_{p_i} p_i + w_{i2}(t)\end{aligned} \tag{1}$$

where

$$b_i\left(p_j^n\right) = \frac{1}{1+p_j^n}$$

and $(i, j) \equiv$ (lacII, λcI), (tetR, lacI), or (λcI, tetR), $m_i \in \mathbb{R}_+$ is concentration of the mRNA, $p_i, p_j \in \mathbb{R}_+$ are concentrations of the proteins, $\alpha_i \in \mathbb{R}$ denotes transcription rate of the mRNA, α_0 is leakiness of the promoter, $\beta \in \mathbb{R}$ is ratio of the protein decay rate to the mRNA decay rate, $\gamma_{p_i} \in \mathbb{R}_+$ and $\gamma_{m_i} \in \mathbb{R}_+$ are decay rates of the proteins and mRNA, respectively, and $n \in \mathbb{R}$ is Hill coefficient, w_{ij}, $j = 1, 2$ denote effect of the environmental noises.

In [3], a genetic oscillator with the simplest structure was realised using the real structured genetic algorithm (RSGA) to facilitate oscillating behaviour with the desired amplitude, phase and frequency of a sinusoidal wave.

2.2 Toggle switch

Another pioneer work, toggle switch, proposed by Gardner *et al.* is constituted by two genes which mutually inhibit each other. Mathematically, the dynamic behaviour of this model can be described by two coupled first-order differential equations as

$$\begin{aligned}\frac{\mathrm{d}u}{\mathrm{d}t} &= \frac{a_1}{1+v^{h_1}} - u\\ \frac{\mathrm{d}v}{\mathrm{d}t} &= \frac{a_2}{1+u^{h_2}} - v\end{aligned} \tag{2}$$

where u is concentration of the lacI repressor, v is concentration of the tetR repressor, $a_1 \in \mathbb{R}_+$ is effective rate of synthesis of the lacI repressor, $a_2 \in \mathbb{R}_+$ is effective rate of synthesis of the tetR repressor, the Hill coefficients, $h_1 \in \mathbb{R}$ and $h_2 \in \mathbb{R}$ specify, respectively, the co-operativity of repression of the tetR and lacI promoters.

2.3 Combination of genetic oscillator and toggle switch

According to the inducer (IPTG, aTc) relationship with lacI reaction and the conjunctive way of C-element [6], we come to the most direct and simplest binding method for combination of a genetic oscillator and a toggle switch. The structural connection of the circuit model should be in the form of Hill function $a_2/(1+u^{h_2})$ and taking effect in the denominator according to the previous research works [4–8, 12, 13]. Without considering the affinity between proteins and time delay, we adopt the genetic oscillator's repressor protein as an input to construct a genetic circuit described by

$$\begin{aligned}\frac{\mathrm{d}u}{\mathrm{d}t} &= \frac{a_1}{1+v^{h_1}} - u\\ \frac{\mathrm{d}v}{\mathrm{d}t} &= \frac{a_2}{(1+u^{h_2})(1+p_{\mathrm{lacI}}^n)} - v\end{aligned} \tag{3}$$

where p_{lacI} is protein concentration of the lacI repressor of the genetic oscillator. Fig. 1 illustrates the architecture proposed herein which shows a genetic circuit with a dual repressor. Regulation factors have several different regulatory motifs. We adopt a dual repressor to connect two circuits, because the interaction of two transcription factors does not consider the impact of Boltzmann weight [5]. Moreover, this circuit is a single-input and single-output system so that it will not be affected by free energy of the DNA looping [5].

A dual repressor can enhance sensitivity in the repression exhibiting interaction. The $\mathrm{P}_{\mathrm{LtetO-1}}$ promoter [11] containing two tetR is a typical example. The tetR dimers do not physically contact with each other. The repression system of the Tn10-derived tetR resistance operon controls the promoter $\mathrm{P}_{\mathrm{LtetO-1}}$. Through tetR and anhydrotetracycline, one can control activity with $\mathrm{P}_{\mathrm{LtetO-1}}$ by combining this promoter with the tetR operators. Change of the concentration of aTc can obtain partial induction of $\mathrm{P}_{\mathrm{LtetO-1}}$. Partial induction can be achieved with all promoters because induction of the promoter $\mathrm{P}_{\mathrm{LtetO-1}}$ presents strong cooperative effect in the binding of the inducer aTc to the tetR repressor. Schematic diagram of the structure of the dual repressors with lacI repressors can be illustrated as in Fig. 1. In Fig. 2, the symbol of inverted 'T' represents repression. Longer yellow blocks are lacI repressors. Shorter yellow blocks are lacI promoters. Longer pale orange blocks are tetR repressors. In operating procedures of the entire circuit, the genetic oscillator produces oscillation on the lacI_1 repressor. The toggle switch consists of a lacI_2 repressor and a tetR_2 repressor. LacI_2 promoter is inhibited by the dual repressors-lacI_1 and lacI_2 repressors. Concentrations of lacI_2 and tetR_2 repressors change along with concentration of lacI_1 repressor. One can thus adjust concentration of the lacI_1 repressor to control concentrations of the lacI_2 and tetR_2 repressors.

Although the model under construction is straightforward, it is expected it is enough to generate acceptable results. We adopt the input signal $\sin \bar{\omega} t + 1$, $\bar{\omega} \in \mathbb{R}$ to the circuit for testing and conducting analysis from the system response.

In Figs. 3–5, the line indicated by "u" denotes concentration of the lacI repressor and the line indicated by "v" is concentration of the tetR repressor in the toggle switch. The input accepts concentration of the lacI repressor of the genetic oscillator. From Fig. 3, one can examine damping characteristics of the state responses. It can be observed from Fig. 4 that oscillation frequency of the circuit is consistent to the frequency of the input. However, it is observed from Fig. 5 that not all frequencies are feasible when frequency of the input signal is greater than a certain value.

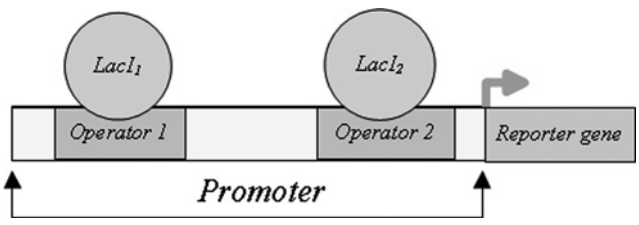

Fig. 1 *Schematic diagram of the dual processor's structure*

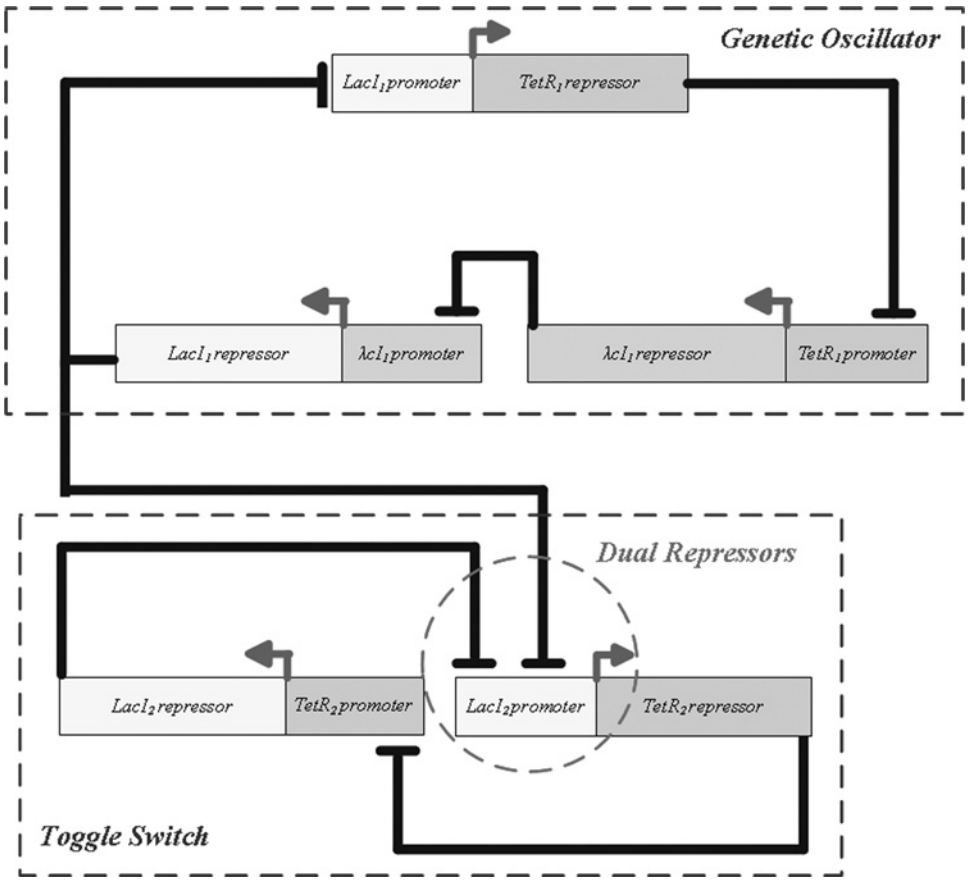

Fig. 2 *Schematic diagram showing connection of a genetic oscillator and a toggle switch to form a genetic clock generator*

To proceed the in-depth analysis, we regard this circuit as a second-order damped system with the input $c \sin \bar{\omega} t$

$$\ddot{y}(t) + 2\zeta\omega\dot{y}(t) + \omega^2 y(t) = c \sin \bar{\omega} t \tag{4}$$

where ζ and ω are, respectively, damping ratio and undamped natural frequency of the system with $\bar{\omega}$ denoting the input signal frequency. The full solution of the differential equation is given by

$$y(t) = y_c(t) + \tilde{c}\frac{1}{(1-\tilde{\beta}^2)^2 + (2\zeta\tilde{\beta})^2}\left[(1-\tilde{\beta}^2)\sin \bar{\omega} t - 2\zeta\tilde{\beta}\cos \bar{\omega} t\right] \tag{5}$$

where $\tilde{\beta} = \bar{\omega}/\omega$, the coefficients $c, \tilde{c} \in \mathbb{R}$ and $y_c(t)$ denotes

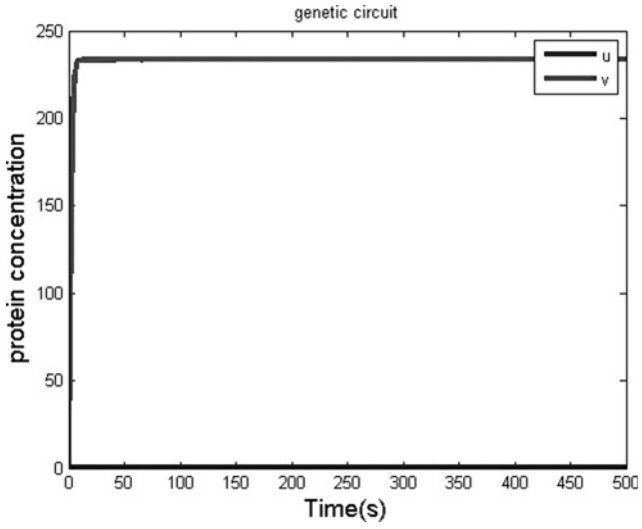

Fig. 3 *Output response of the circuit when there is a unit input with $a_1 = 156.25$, $a_2 = 234$, $h_1 = 2$, $h_2 = 1$ and $n = 4.728$*

u and *v* are, respectively, concentrations of the lacI and tetR repressors of the toggle switch

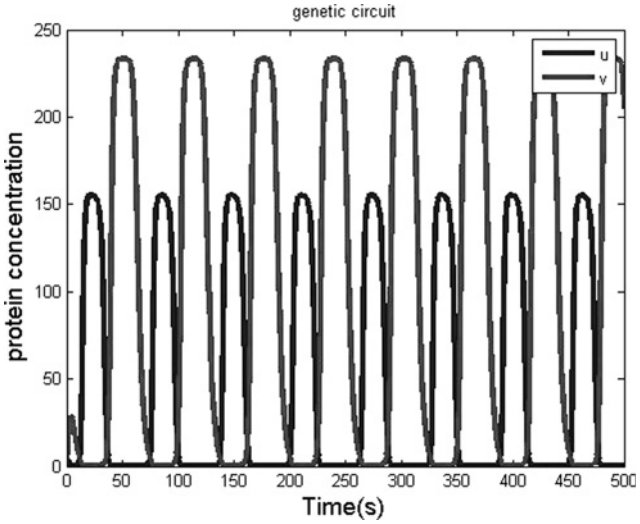

Fig. 4 *Output response of the synthetic gene circuit when its input is sin(0.1t) + 1, $a_1 = 156.25$, $a_2 = 234$, $h_1 = 2$, $h_2 = 1$ and $n = 4.728$*

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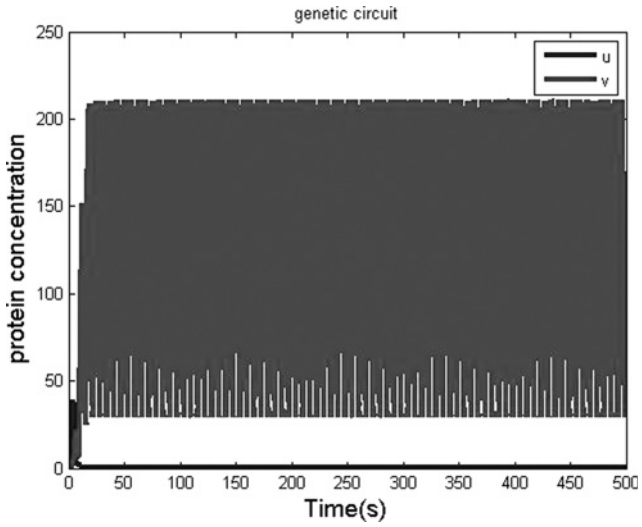

Fig. 5 *Output response of the synthetic gene circuit when its input is sint + 1, $a_1 = 156.25$, $a_2 = 234$, $h_1 = 2$, $h_2 = 1$, $n = 4.728$*

the term contributed by initial states. Since oscillation phenomenon is dominated by the particular solution, it is easily seen that when $\tilde{\beta} > 1$, amplitude of the oscillating signal would be smaller and interfered by the term $\cos \bar{\omega} t$. Therefore to concrete a desired resonance because of the input $c \sin \bar{\omega} t$ with the specific frequency $\bar{\omega}$ we impose the requirement of $\bar{\omega} < \omega$. Since ζ is positive, when $\tilde{\beta} \ll 1$, the output signal's frequency behaviour will then be dominated by the sine term alone.

3 Resonant frequency

The synthetic gene circuit presented above is a non-linear dynamic system. To investigate its steady state behaviour, we assume that the system operates in the vicinity of the equilibrium point. To further analyse its dynamic behaviour, we conduct linearisation around the equilibrium point and establish the relationship between the system output and parameters to manage the desired output response.

3.1 Equilibrium point

A linearised model is not to relate the original system state and its output, but to consider dynamic behaviour of the system around the equilibrium point (u_0, v_0). Consider the non-linear system dynamic equations for our synthetic gene circuit depicted in (3) with

$$\begin{aligned} f_1 &= \frac{a_1}{1 + v^{h_1}} - u \\ f_2 &= \frac{a_2}{(1 + u^{h_2})(1 + p_{\text{lacI}}^n)} - v \end{aligned} \tag{6}$$

Combining both equations by setting $f_{1,2} = 0$ yields

$$0 = (u_0 - a_1)((1 + u_0^{h_2})(1 + p_0^n))^{h_1} + a_2^{h_1} u_0 \tag{7}$$

Using the binomial theorem, we obtain

$$0 = (u_0 - a_1)(1 + p_0^n)^{h_1} \sum_{z=0}^{h_1} \frac{h_1!}{z!(h_1 - z)!} u_0^{h_2 z} + a_2^{h_1} u_0 \tag{8}$$

where the gamma function $z!$ is defined as

$$z! = \Gamma(z + 1) = \int_0^\infty t^z \mathrm{e}^{-t}\, \mathrm{d}t$$

One can use (8) to solve u_0 and obtain v_0 accordingly, which should be a real number, in terms of the parameter set $\{p_0, n, a_1, a_2, h_1, h_2\}$.

3.2 Linearised model

Given the parameter set $\{n, a_1, a_2, h_1, h_2\}$ and the equilibrium point $\{p_0, u_0, v_0\}$, we define the deviations from the equilibrium states as $\tilde{u} = u - u_0$, $\tilde{v} = v - v_0$ and $\tilde{p} = p - p_0$, respectively. Expanding Taylor series about the equilibrium point and ignoring the higher-order terms gives

$$\begin{aligned} \dot{\tilde{u}} &\simeq \left.\frac{\partial f_1}{\partial u}\right|_{u_0,v_0,p_0} \tilde{u} + \left.\frac{\partial f_1}{\partial v}\right|_{u_0,v_0,p_0} \tilde{v} + \left.\frac{\partial f_1}{\partial p}\right|_{u_0,v_0,p_0} \tilde{p} \\ \dot{\tilde{v}} &\simeq \left.\frac{\partial f_2}{\partial u}\right|_{u_0,v_0,p_0} \tilde{u} + \left.\frac{\partial f_2}{\partial v}\right|_{u_0,v_0,p_0} \tilde{v} + \left.\frac{\partial f_2}{\partial p}\right|_{u_0,v_0,p_0} \tilde{p} \end{aligned} \tag{9}$$

Let $\boldsymbol{X} = \begin{bmatrix} \tilde{u} & \tilde{v} \end{bmatrix}^{\mathrm{T}}$ and $\boldsymbol{Y}$ be the output. One can obtain the linearised equation of the state-space form as

$$\dot{\boldsymbol{X}} = \boldsymbol{A}\boldsymbol{X} + \boldsymbol{B}\tilde{p}, \quad \boldsymbol{Y} = \boldsymbol{X} \tag{10}$$

where

$$\boldsymbol{A} = \begin{pmatrix} -1 & \dfrac{-a_1 h_1 v_0^{h_1 - 1}}{(1 + v_0^{h_1})^2} \\ \dfrac{-a_2 h_2 u_0^{h_2}}{(1 + u_0^{h_2})^2 (1 + p_0^n) u_0} & -1 \end{pmatrix},$$

$$\boldsymbol{B} = \begin{pmatrix} 0 \\ \dfrac{-a_2 n p_0^n}{(1 + u_0^{h_2})(1 + p_0^n)^2 p_0} \end{pmatrix}$$

The corresponding characteristic equation is given by

$$s^2 + 2s + \left[1 - \frac{a_1 a_2 h_1 h_2 v_0^{h_1} u_0^{h_2}}{(1 + v_0^{h_1})^2 (1 + u_0^{h_2})^2 (1 + p_0^n) u_0 v_0}\right] = 0 \tag{11}$$

From which one can obtain the undamped natural frequency of the system as

$$\omega = \sqrt{1 - \frac{a_1 h_1 h_2 (a_2/(1 + u_0^{h_2})(1 + p_0^n))^{h_1} u_0^{h_2}}{\left[1 + (a_2/(1 + u_0^{h_2})(1 + p_0^n))^{h_1}\right]^2 \left[1 + u_0^{h_2}\right] u_0}} \tag{12}$$

Since a typical bio-system does not exhibit agile response in general, if one sets $\omega \leq 1$ then the damping ratio satisfies $\zeta \geq 1$ in (11). Therefore we can use the known parameter set $\{p_0, n, a_1, a_2, h_1, h_2, u_0\}$ to determine the natural frequency ω under the constraint $\bar{\omega} < \omega$. The above analysis forms a basis for adjusting an appropriate frequency of the input sine signal to produce a desired clock signal.

4 Design of genetic circuit

Given $\{h_1, h_2\}$, the complexity is determined by (8) in terms of u_0. Solving for u_0 is a difficult task for a high dimensional case. One can first determine the values of $\{h_1, h_2\}$ before solving (8) to get u_0 then proceeds to adjust other parameters $\{p_0, n, a_1, a_2\}$ to meet the requirement.

Design steps of the genetic circuit depicted here can be summarised as follows:

Step 1: Determine h_1 and h_2 which reflect cooperativity of the repression of the tetR and lacI promoters in the genetic toggle switch. Under the normal circumstances, the values of $\{h_1, h_2\}$ should be >1.
Step 2: Specify the Hill coefficient n of the lacI repressor of the genetic oscillator, a_1 and a_2 are effective synthesis rates of the lacI and tetR repressors, respectively, in the toggle switch.
Step 3: Substitute $\{h_1, h_2, p_0, n, a_1, a_2\}$ into (8) and obtain a real root u_0 which is the predicted equilibrium point of the lacI repressor of the genetic toggle switch.
Step 4: Substitute $\{p_0, n, a_1, a_2, h_1, h_2, u_0\}$ into (11) and (12) to obtain the damping ratio ζ and the natural frequency ω.

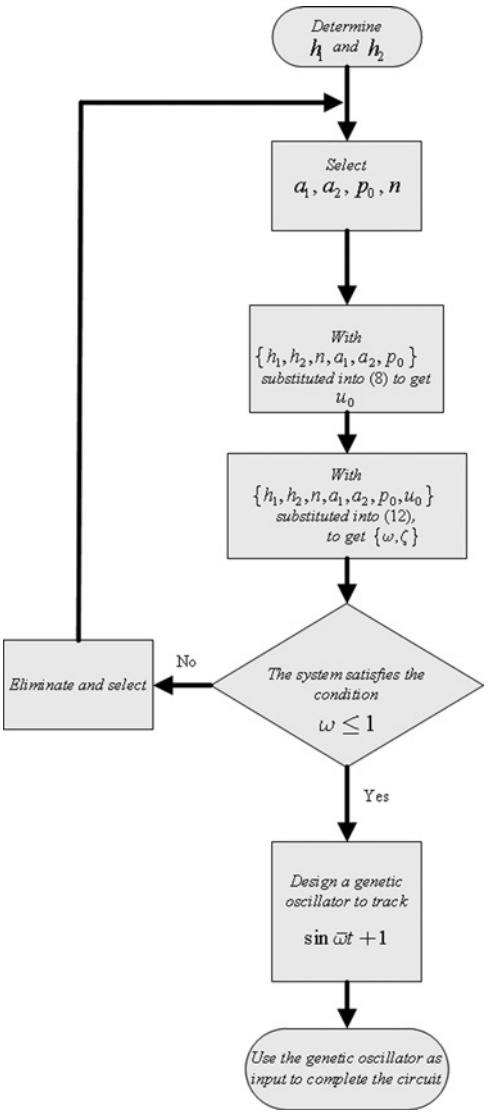

Fig. 6 *Operational flowchart for designing the gene circuit*

Step 5: If $\omega \leq 1$ is achieved, proceed to the next step, otherwise, repeat Steps 2–5 until $\omega \leq 1$ is reached.
Step 6: Implement a genetic oscillator (using the RSAG proposed in [3] or other approaches) to track $c \sin \bar{\omega} t + c$ with $\bar{\omega} < \omega$.
Step 7: Realise the oscillator by referring to Fig. 1.

Fig. 6 illustrates details of the design procedure.

4.1 Design of genetic circuit under constraints

To simplify the genetic circuit design, we may restrict the polynomial degree less than three. First select h_1 and h_2 under the condition (h_1, $h_2 > 1$) in [1] and restrict (8) to be a cubic polynomial. Choosing $h_1 = 2$, $h_2 > 1$ and $h_2 \simeq 1$ gives

$$0 = k_1 u_0^3 + (2 - a_1) k_1 u_0^2 + (k_1 + a_2^2 - 2a_1 k_1) u_0 - a_1 k_1$$
$$k_1 = (1 + p_0^n)^2 \tag{13}$$

From the cubic polynomial formulation, we can obtain the real solution given by

$$u_0 = \frac{1}{6}\left(\frac{k_2^{1/3}}{k_1}\right) + \frac{2}{3}\left(\frac{k_1 + 2a_1 k_1 - 3a_2^2 + a_1^2 k_1}{k_2^{1/3}}\right) + \frac{1}{3}(a_1 - 2) \tag{14}$$

where

$$k_2 = k_1^2(8k_1 + 24a_1 k_1 + 24a_1^2 k_1 + 72a_2^2 - 36a_1 a_2^2 + 8a_1^3 k_1 + 12\sqrt{3}a_2(4k_1 + 8a_2^2 + 12k_1 a_1 + 12k_1 a_1^2 - 20a_1 a_2^2 + 4k_1 a_1^3 - a_1^2 a_2^2 + (4a_2^4/k_1))^{1/2})$$

Substituting $\{p_0, n, a_1, a_2\}$ into (14) yields u_0. On the basis of the known $\{p_0, n, a_1, a_2, h_1, h_2, u_0\}$ one can determine the natural frequency ω. Design of the genetic oscillator with an appropriate input should meet the requirement of $\bar{\omega} < \omega$.

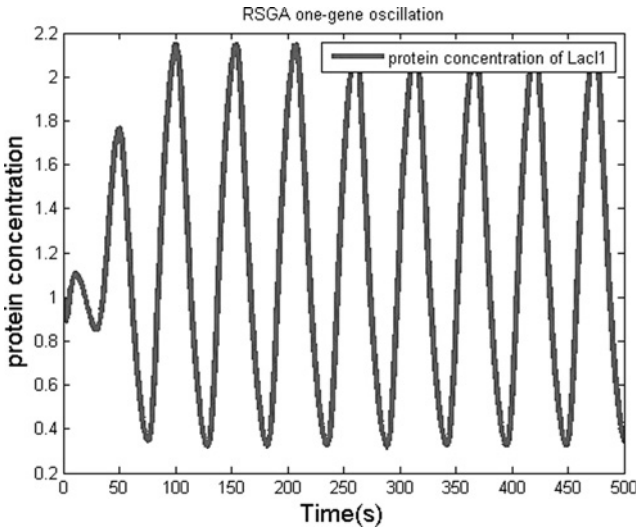

Fig. 7 *Protein concentration of the lacI repressor of the genetic oscillator with* $\bar{\omega} = 0.0375\pi$

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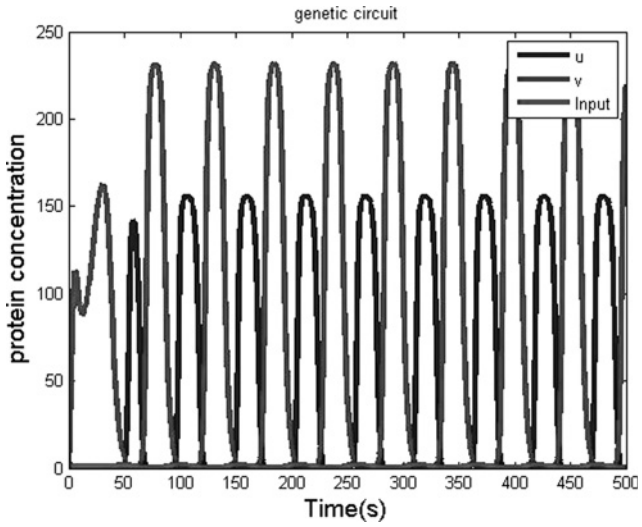

Fig. 8 *Output response of the circuit when the input accepts protein concentration of the lacI repressor of the genetic oscillator with $a_1 = 156.25$, $a_2 = 234$, $h_1 = 2$, $h_2 = 1.05$ and $n = 4.728$*

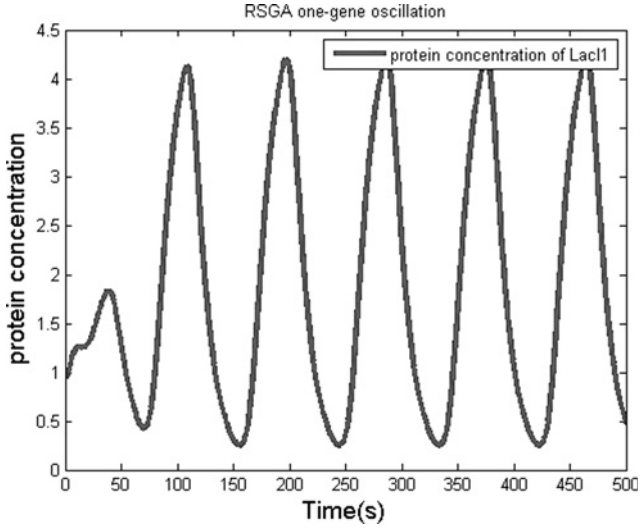

Fig. 9 *Protein concentration of the lacI repressor of the genetic oscillator where $\bar{\omega} = 0.0225\pi$*

4.2 Numerical experiments

For $h_1 = 2$, $h_2 > 1$ and $h_2 \simeq 1$, we select $a_1 = 156.25$ and $a_2 = 15.6$. Next, select $p_0 = 1$ and $n = 4.728$ because the initial value of the genetic oscillator is usually $0 \leq p_0 \leq 1$ and n is determined, for example, by the optimisation algorithm-RSGA [3]. Substituting $\{p_0, n, a_1, a_2\}$ into (14) gives $u_0 = 0.0117$. Next, substituting $\{p_0, n, a_1, a_2, h_1, h_2, u_0\}$ into (12) obtains the natural frequency $\omega = 0.9884$. We select $\bar{\omega} = 0.0375\pi$ which satisfies $\bar{\omega} < \omega$. The genetic oscillator is implemented to track the sine input in Fig. 7 where$\bar{\omega} = 0.0375\pi$. Finally, we add concentration of the lacI repressor of the genetic oscillator as the input to the toggle switch. The circuit output is shown in Fig. 8.

To proceed, we select $\bar{\omega} = 0.0225\pi$ which satisfies $\bar{\omega} \ll \omega$ and the amplitude of input becomes double to the original input to observe influences of these factors. We implement a genetic oscillator to generate the signal $2\sin\bar{\omega}t + 2$, as displayed in Fig. 9. Then, select $p_0 = 1$ and $n = 4.095$ because the oscillator has been changed, the optimal n was determined by the RSGA. The natural frequency is still $\omega = 0.9884$, because $p_0 = 1$ didn't change k_1 and u_0. The range of condition remains the same as the original one. Fig. 10 shows the resulting state (output) response of the toggle switch. It is seen that frequencies of the output and input responses are synchronised. In addition, the output signal's amplitude is much higher than the input while both of the input and output possess the same period. It can be observed that the output signal is closer to an ideal square wave. This demonstrates feasibility of the proposed design.

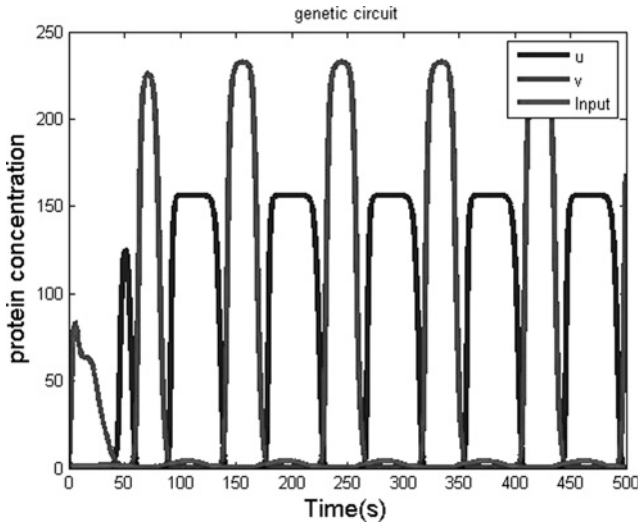

Fig. 10 *Output response of the circuit when the input accepts protein concentration of the lacI repressor of the genetic oscillator with $a_1 = 156.25$, $a_2 = 234$, $h_1 = 2$, $h_2 = 1.05$ and $n = 4.095$*

5 Conclusions

This research presents a novel approach to synthesise a genetic clock generator. The design is based on the architecture of a sinusoidal input accompanied with a genetic oscillator connected with a toggle switch. Relationship between clock frequency and natural frequency of the oscillator dynamics is analytically established, which provides a useful reference for tuning the combinational circuit with the desired clock frequency. *In silico* study shows feasibility of the presented approach.

6 Acknowledgment

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